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AMRL-TR-68-175

# PROCEEDINGS OF THE 4TH ANNUAL CONFERENCE ON ATMOSPHERIC CONTAMINATION IN CONFINED SPACES

10-12 SEPTEMBER 1968

DECEMBER 1968



AEROSPACE MEDICAL RESEARCH LABORATORIES
AEROSPACE MEDICAL DIVISION
AIR FORCE SYSTEMS COMMAND
WRIGHT-PATTERSON AIR FORCE BASE, OHIO

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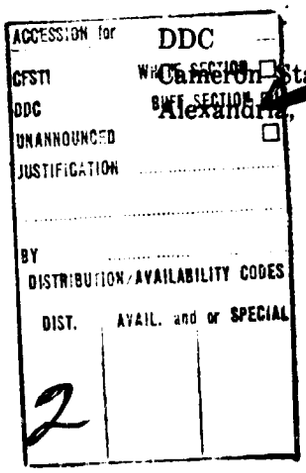


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The voluntary informed consent of the subjects used in this research was obtained as required by Air Force Regulation 169-8.

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# FOREWORD

The 4th Annual Conference on Atmospheric Contamination in Confined Spaces was held in Dayton, Ohio on 10, 11, and 12 September 1968. Sponsor was the Aerospace Medical Research Laboratories, Aerospace Medical Division, Air Force Systems Command. Arrangements were made by the Toxic Hazards Research Unit of SysteMed Corporation under the terms of Contract F33615-67-C-1025. The Toxic Hazards Research Unit is located at the Toxic Hazards Division, Wright-Patterson Air Force Base, Ohio and the papers presented at this Conference by personnel of SysteMed Corporation represent research conducted under the cited contract. Dr. Anthony A. Thomas, Director, Toxic Hazards Division, served as Conference Chairman. Mr. Edmond Vernot served as Conference Coordinator for SysteMed Corporation, and Mrs. Mildred Pinkerton for the Air Force.

Acknowledgment is made on behalf of the Aerospace Medical Research Laboratories to Major General Charles H. Roadman, Commander, Aerospace Medical Division, Brooks Air Force Base, Texas, for his Introductory Remarks and support, to the session chairmen and speakers, and to all those who actively participated in the discussions. Special thanks are due to MSgt W. F. Hunt, Jr., TSgt H. D. Stull, Sgt B. N. Holcombe, SSgt J. L. Hall, and Mrs. Linda Evans of the Toxic Hazards Division, and to Mrs. Lois Doncaster, Mr. Dean Carlson, and Mrs. Marilyn Collins of SysteMed Corporation.

## ABSTRACT

This report is a compilation of the papers presented and the Proceedings of the 4th Annual Conference on Atmospheric Contamination in Confined Spaces, sponsored by the Aerospace Medical Research Laboratories and held in Dayton, Ohio on 10, 11, and 12 September 1968. Major technical areas discussed included toxicological evaluation of atmospheres and contaminants, histopathological evidences of toxicity, evaluation of cabin materials, instruments and detection, and life support systems.

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# WELCOMING REMARKS

Clyde H. Kratochvil, Colonel, USAF, MC

Commander
Aerospace Medical Research Laboratories

Welcome to the 4th Annual Conference on Atmospheric Contamination in Confined Spaces. We are hosting a Fall meeting this year because we were shut down after the tragic Apollo and Brooks Air Force Base fires. However we have been on line since last September, and you will hear the data which we have developed since that time. We also have new construction and we are very excited about that. The new Thomas domes will be in operation, we think, this January, plus some new laboratory space.

The sessions look very good to me. I'm not going to go over them with you since you all have programs and can read. I would suggest, however, that you address yourselves to the subject of tolerance because this is a subject that has, if you will, "bugged" all of us. Tolerance can be defined as survival by some people, by other people as no detectable effect, and somewhere in-between there must be a pragmatic working definition. Those of us who are concerned with human exposure are very much involved in this, both from an operational point of view and an ethical point of view, so I would hope something would come out of this.

I am proud to be associated with Dr. Thomas and his group here because this is an outstanding Air Force effort. It's an effort that I personally feel deserves much more attention, and it's a subject which I think all of us need to work much harder on than we have in the past. So, let me wish you success for this session. Let me ask that if you have any comments, criticism, anything that you wish to direct to me as Commander, or to Dr. Thomas, please make these comments known to us and we will do our best to incorporate these into the future sessions. There has been more time allowed for discussion after the papers and in addition there is an open forum. Since I'm one of these people who comes to meetings with two guns on my hip, I hope you have come this way also and that there will be a hot and heavy discussion. I think this is a criticism all of us have at meetings. Papers are presented and they're shut off and you don't have enough time to really dig in and get to the meat of it. Dr. Thomas has deliberately built this into the schedule so I would hope you would make good use of this. We have the comparative morphological data from a group of animals

on enriched oxygen atmospheres. I think this is an acute problem to which I would hope you would turn your attention. We also have the electron microscopic findings from the reduced pressure 5 PSIA, mixed gas environment, and this, we think, will be of great interest to all of you. So, again, my welcome from the laboratory, from myself personally, and gentlemen, have at it!

# INTRODUCTORY REMARKS

Charles H. Roadman, Major General, USAF, MC

Commander
Aerospace Medical Division

As our plans for prolonged duration manned space flight missions become a reality, our concern for providing a habitable environment for the crew is ever-increasing. This concern is clearly evidenced by two recent publications from the Space Science Board of the National Academy of Sciences. The first, "Physiology in the Space Environment", Volume II, concerns itself with the problems of respiratory physiology and points out many gaps in our knowledge which must be filled and many questions resolved before we can commit ourselves to artificial atmospheres that are markedly different from our earthly environment.

The seond publication, titled "Atmospheric Contamination in Spacecraft", addresses itself specifically to the toxicology aspects in a perspective of 90 and 1,000-day mission durations and, again, points out the need for a vigorous research program to define the character and peculiarities of long-term continuous exposure to toxic substances in the atmosphere.

The most eloquent evidence of this concern is your presence here today. The Aerospace Medical Division, in sponsoring this conference for the fourth year, wants to provide you the opportunity to learn of all new developments brought about by research at the Aerospace Medical Research Laboratories and elsewhere on a timely basis, so that this knowledge can be applied toward the solution of our present problems in a most expeditious manner.

As our knowledge broadens in the area of toxic effects of basic cabin atmospheres and trace contaminants, our approach to research becomes more specific in order to solve existing and anticipated bottleneck areas which have major impact on the design of future systems. The Aerospace Medical Division has been, and is, a leader in the exploration and definition of human tolerance to all kinds of stress presented by the aerospace environment. The area of chemical stress is a relatively new one, and systematic research was started but five years ago. There are still many basic answers needed concerning chronic toxicity during continuous exposure and about the influence of other environmental stresses on the outcome of toxic effects. With the completion of several new facilities here at the Aerospace Medical Research Laboratories, the opportunity

and capabilities to study such combined stress effects will be greatly enhanced and facilitated. These unique facilities will represent a national capability to study the toxic effects of atmospheric contaminants, not only in the closed environment situation, but also from the standpoints of atmospheric pollution and public health, which is, as you know, a mainstream problem today.

There is a great deal of common interest among various Government agencies on the effects of such common air pollutants and pulmonary irritants as hydrocarbons, carbon monoxide, etc. not only from the toxicological standpoint, per se, but also from the preventive occupational medicine and public health effects. To mention only one such area of common interest, recently a great deal of attention has been focused on the role of carbon monoxide in eliciting cardiovascular changes in cigarette smokers. I'm sure that much of the information which the Air Force research program generates in this area will help to understand the impact of toxicological mechanisms on our everyday life and will further the wellbeing and overall health of the entire population at large.

I wish you a very profitable and productive meeting, and I hope that the information that will be disseminated here will help to mold your thinking when you approach your specific problems related to the closed environments of the new generation of space vehicles, and that it will stimulate the generous exchange of scientific information and discussion.

SESSION I

# TOXICOLOGICAL EVALUATION OF ATMOSPHERES AND CONTAMINANTS

Co-Chairmen

Dr. Harold C. Hodge
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Mr. Ralph C. Wands
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# PHILOSOPHY OF 100-DAY AND 1000-DAY LIMITS FOR SPACE CABIN ATMOSPHERES*

Ralph C. Wands

National Academy of Sciences
Washington, D. C.

Recommendations for conditions of exposure of people to potentially harmful circumstances are the result of a balanced judgment. Each of us arrives at hundreds of such balanced decisions every day of our lives. They are always based upon an evaluation of risk versus benefit. The decision may be merely one of whether or not to use cream and sugar in our coffee. The benefit might be improved palatability and the risk might involve considerations of whether the individual was overweight, diabetic, or had a cholesterol problem. These balanced judgments which we make routinely usually only affect the individual. However, when we make decisions or recommendations involving the exposure of other people to a risk we have an obligation to be particularly careful. It is this situation to which these remarks are addressed. It is intended that they should have special applicability to recommendations for atmospheric contaminants in space cabins but the basic philosophy will be the same for all problems whether they involve community air pollution, drug evaluation, food additives, or pesticides.

One limiting characteristic of air contaminants in space cabins is that their presence in the air is of no direct benefit to the exposed individuals. The contaminants arise from processes or sources which may be beneficial but their presence in the atmosphere is only incidental to that benefit.

The first step in recommending an acceptable concentration for exposure to an atmospheric contaminant is to describe the dose-response relationship. What effects will result from exposure to various concentrations for various periods of time? Such descriptions of exposure versus effect are sometimes called air quality criteria. In theory, with sufficient experimentation they

*The opinions expressed herein are those of the author and do not necessarily represent those of the National Academy of Sciences or any of its affiliated Boards and Committees.

can be determined quite precisely. Of course in practice, at any given moment in time, one must use the information available from a review of the literature, both published and unpublished.

The second step in recommending a concentration for human exposure to an atmospheric contaminant is to determine the acceptable level of effect which can then be matched against the dose-response curve to establish the concentration. The acceptable level of effect is almost completely dependent upon the circumstances of exposure. Will the exposure occur during bird watching in Central Park?--in which case an objectionable odor might be limiting--or will it occur during armed combat?--in which case reversible hypertension might be acceptable but temporarily decreased visual or auditory acuity would not.

Let us consider briefly how others have defined an acceptable effect and proceed to what might be acceptable for 100 and 1000-day space flights. After that we will return to the first step of dose-response relationship and discuss some of the critical variables.

There is a wide spectrum of acceptable effects from air contaminants. At one extreme of the spectrum are the Emergency Exposure Limits which are recommended by the Committee on Toxicology of the National Research Council or by a committee of the American Industrial Hygiene Association. Both of these committees accept any reversible effect that (a) will not interfere with the performance of tasks to be accomplished during the emergency, (b) will not significantly reduce vision or visibility or interfere with breathing or prevent self-rescue, and (c) will not expose the individual to additional risks such as fire and explosion.

The Threshold Limit Values of the American Conference of Governmental Industrial Hygienists accept the fact that an occasional individual may suffer some discomfort, aggravation of pre-existing condition, or even occupational illness. The recommendations of the NRC Committee on Toxicology for nuclear submarine atmospheres accept effects such as increased respiration, provided the effects are reversible and do not impair performance of normal duties. For high performance tasks of relatively short duration, as in the Mercury and Gemini earth orbital missions, no performance decrement, either direct or indirect such as slight eye or respiratory irritation, could be accepted. Even odors were kept to a minimum to avoid olfactory fatigue or confusion with other odors which might be first indicators of mechanical malfunctions. Thus as we shift from one end of the spectrum of acceptable effects to those of more stringent requirements, we find the definitions relating to what is not acceptable instead of what may be tolerated.

Acceptable effects for 100 and 1000-day space flights are certainly best described by what is excluded.

First, we cannot accept any permanent adverse health effects; second, we cannot accept any effects, even temporarily, which will impair the ability of the individual to carry out assigned tasks; and third, we cannot accept any effects which will interfere with the purpose of the mission, such as the effects of weightlessness on man.

With these broad specifications in mind, let us return to the first step and consider the dose-response data. As we review the literature we are looking especially for data on human exposures, most of which come from industrial or community air pollution studies. However, unlike the air pollution control people, we do not have to consider exposures to all of Shakespeare's seven ages of man. We can ignore the problems of "the infant mewling and puking in the nurses arms", and we can ignore the oldster in second childishness "sans teeth, sans eyes, sans taste, sans everything". We must concentrate on collecting dose-response data for that age of man described as "a soldier full of strange oaths and bearded like the pard". This is not really a very bad description of our astronauts, especially after their flight.

What we actually find in the literature is all kinds of data except that which would be most useful. We find extensive animal data, some of which is by inhalation exposure but with very little from long term continuous experiments. Information gathered from other routes of exposure may be useful especially if they reveal mechanisms of effects. We are seldom concerned with skin contact effects except where aerosols are possible in the spacecraft. One of the kinds of data for which we most frequently search in vain are doses producing no effects in humans. Industry could make a valuable contribution by providing hard data from industrial hygiene surveys showing actual levels which produce no effects. The Navy could make a highly significant contribution by a thorough documentation of air analyses during a submarine cruise accompanied by a detailed medical workup before, during, and after the cruise. The work of Dr. Schaeffer and his co-workers on carbon dioxide was an excellent start in this direction. We need many more such studies.

Having collected all the available data on an atmospheric contaminant, one must try to construct a dose-response graph or table which will relate directly to the astronaut and his exposure conditions. This involves a great deal of transference of data from several species to Homo sapiens. It involves conversion of such transferred data to hypobaric conditions. It involves estimating the respiratory rates and volumes. Ultimately one arrives at whole body dose rates or perhaps dose rates for a critical organ with the associated effect levels.

Before using this tabulation of dose rates versus effects, one must estimate its reliability or its shortcomings which arise from the estimates, conversions, transferences, etc. that went into its construction. One must then give consideration to such other variables as the unique responses of which humans are capable. None of the animals have told us they have a headache, and on the other hand, teratogenicity per se is not yet a problem for our astronauts.

One of the major deficiencies of the thinking process which I have described is that, so far, no consideration has been given to the effects of exposures to mixed contaminants. With a few very notable exceptions this reflects the real world of existing recommendations for atmospheric contaminants. For example, we know that physical or emotional stress, or carbon dioxide will increase the respiratory minute volume. This, in turn, will increase the rate at which the body becomes saturated with an air contaminant such as carbon monoxide. In

some of its recommendations the NRC Committee on Toxicology has taken such factors into account but there are practically no data for considering other potential interactions. A start on this problem has recently been made in the laboratories of the 6570th Aerospace Medical Research Laboratories and before that in the Public Health Service laboratories in Cincinnati by Dr. Stokinger.

Along this line an area in which research is sorely needed is whether or not mixtures of respiratory irritants act independently, additively, synergistically, or perhaps even antagonistically. It would seem to be a fairly straightforward task to develop the necessary quantitative data. The results would be of tremendous value in recommending levels for space cabin contaminants since so many of them are only primary irritants.

In conclusion, the process of recommending levels for air contaminants in space cabins is not unique. It involves the two steps of determining an acceptable effect and of matching this to a dose-response description. As the mission duration becomes longer and the opportunity for an abort or rescue diminishes, the acceptable effect of an air contaminant decreases. This coupled with the uncertainties of the dose-response relationship requires conservative judgment. Without more data from additional research, these judgmental processes may result in recommendations which are unnecessarily restrictive. Everyone involved in these processes is acutely aware and concerned about the problems of over-engineering and the associated delays and increased costs. Nevertheless, it is inconceivable in our society that the health of our astronauts should be secondary to engineering exigencies.

## DISCUSSION

DR. HODGE (University of Rochester): Now, we have had a fine example. We are ahead of time, and Mr. Wands has not used up his actual allotment as originally scheduled, so that we can begin by having an opportunity for comments or questions. The floor is open.

CAPT. SIEGEL (National Naval Medical Center): I would like to ask Ralph Wands what the difference between a 100-day and a 1000-day philosophy is. That's the subject of the paper.

MR. WANDS (National Academy of Sciences): Well, Jack, these two, in my thinking, were almost identical in that we have the actual experience of the Navy with 90-day submersions which probably can be directly applied to 100-day . When we go to 1000-day , however, we must become even more careful. With the Navy's experience in submarine activities, whenever they get into a really serious problem, (and to the best of my knowledge they have not had too many of these) short of a wartime situation, they can always get up to the surface very quickly and ventilate the submarine or do whatever else is necessary. With this difference in mind, it is not always easy to translate the Navy experience in submarines to a spacecraft situation. Even in an earth orbital mission, it is not always easy to come right back down in a hurry. So that we do have that additional restriction. Certainly, as we consider the longer flights of a thousand days and places where this will take us, the opportunity for a rescue or a return to a safe normal environment is terribly remote. Therefore, we cannot take very much of a chance, so from this standpoint, you just cannot take any chances on the health of these people.

QUESTION: If I'm not being too specific, may I ask you this question, something you mentioned just a few moments ago. You mentioned that you would recommend toxicity studies of mixtures of materials in order to determine if the effects are additive, synergistic or antagonistic. Would you have a few specific materials in mind at this time?

MR. WANDS: When I was thinking of this, I had primarily in mind things which are upper respiratory irritants, --the acid gases, and specifically the aldehydes. We have the feeling that there are some concentrations of each of these which do not produce any specific irritation, no response, no cough response or anything like this, but we don't really know whether half of the no-effect level of sulphur dioxide, for example, added to half of the no-effect level of HCL might produce an effect. We don't know this. It's the sort of thing which seems to be a fairly straightforward experiment to perform, one which would be most critical to the spacecraft.

As the Space Science Board Committee, under Dr. Norton Nelson, was reviewing its recommendations for space flights, about which you will hear later from Dr. Albert, they were impressed with the number of just simple primary irritants that had been observed in the atmosphere of these confined spaces, both the flight simulation studies and some of the earth orbital things, as well as some of the Navy experience. This was the sort of thing that I had in mind.

FROM THE FLOOR: Philosophy is an explanation of the universe and man's role in it, usually identified as idealism or pragmatism, etc., and I'm just kind of curious as to what is the philosophy recommended in this paper.

MR. WANDS: I think, basically, the philosophy is in the final statement. Let me reread that. It is inconceivable in our society that the health of our astronauts should be secondary to engineering exigencies. We cannot tolerate any injury to these astronauts.

DR. BENJAMIN (NASA): Considering the more than 300 contaminants that had been found in the Mercury program, and the permutations and combinations associated with a synergistic effect, I wonder whether, within this coming century anyhow, one might be able to determine their effect on man. It is more likely that if one were to find contaminants in a space cabin environment above background level, I sort of have the feeling that the engineer will simply attempt to remove them down to background level rather than attempt to rely on data which is probably not likely to exist. I was wondering whether you might comment on that.

MR. WANDS: I have tremendous faith in the engineering profession to come up with any sort of gadgetry that may be necessary to perform a given task that has been assigned. I think th is one of the functions that the Space Science Board report has served, that is to give the engineer a target toward which he may design. I have a great deal of faith that this will be achieved. I have tried to give you a little of the thinking that went into some of those levels. Dr. Albert will expand upon this in more detail. Does that cover the whole question?

DR. BENJAMIN: I have one more question as far as your acceptable level is concerned. We have the problem that sometimes you want to determine some unknown environmental factor, say, as the effect of weightlessness. In that case, in order to eliminate any possible effect of contaminants, you have to determine some level where they are quite sure there is no toxic effect, which is really probably lower than anything you specified so far.

MR. WANDS: Well, Dr. Benjamin, I don't think I have specified anything here. What you are saying, really, is that we must have a standardized, normal astronaut. Each of our astronauts is an individual and there are a lot of variations among them so that, in effect, each individual must be his own control. I'm not suggesting that we take each of our astronauts and put them in the Thomas Domes along with chimpanzees for company, but we do need a course, a very thorough definition of their own physiology, metabolism, this sort of thing, and we need to have sufficient knowledge so that we can be assured that their physiology

and metabolism will not be affected by the levels of contaminants which we are going to have, assuming that the engineers will be successful. This does not ignore the possibility of an emergency of any sort coming up. We do have to try to plan for these emergencies. We have to try to eliminate the possibility of sudden leaks, sudden malfunctions, etc., but on the other hand these are going to occur. Where these can be foreseen and emergency techniques developed to control those which are going to interfere with the medical aspects of the mission, (for example, weightlessness) we must of course plan for these. In any exploratory pioneering program like this, even when you go back to the days of Daniel Boone, he never knew when an Indian or a bear would come out of the bushes. Our astronauts don't know whether there's going to be an Indian or a bear in the woodwork either. There are some risks involved. We have to minimize these to the fullest extent of our abilities.

AMRL-TR-68-175

PAPER NO. 2

# REVIEW OF TENTATIVE ABORT, ALERT, 100 AND 1000-DAY LIMITS FOR SPACE CABIN CONTAMINANTS

Roy E. Albert, M.D.

New York University Medical Center
New York, New York

This presentation summarizes the report, "Atmospheric Contaminants in Spacecraft," which was made by the Panel on Air Standards for Manned Space Flight of the Space Science Board, National Academy of Sciences (October, 1968).

The Panel was asked to examine the likelihood of adverse effects of air contaminants on the health and performance of space crews on prolonged missions. Subsequently, the charge was expanded to include consideration of limits for exposure to air contaminants under emergency conditions. Realistic air quality guides are essential for prolonged spacecraft missions, and standards must be formulated early in mission planning because of the complexity of the operational systems. Contaminants in the spacecraft atmosphere may be life-threatening, others merely irritating. The contaminants may be produced by the occupants themselves, by structural materials making up the spacecraft, by equipment under normal or abnormal operating conditions, and from atmospheric regeneration systems.

The criteria used in establishing provisional limits were that the contaminants not produce significant adverse changes in the physiological, biochemical, and mental stability of the crew. The spacecraft environment should not contribute to a decrement in performance of the crew that would endanger the mission objectives nor interfere with physical or biological experiments or with medical monitoring.

The uncertainties in establishing even provisional limits for prolonged manned missions are many, involving engineering, environmental and toxicologic considerations. Since the materials to be used in future spacecraft construction or in the type of regenerative and environmental control systems to be employed have not been determined, there are major uncertainties regarding the kind and amount of air contaminants that might be present. There is also a major uncertainty as to how reduced atmospheric pressure may alter the toxicity of contaminants. These uncertainties are relatively minor compared to those due to inadequate or incomplete toxicologic information. Industrial threshold limits have not been established for many of the compounds identified in spacecraft tests and very few contaminants have documented 90-day limits values. Data relevant to continuous

exposure are few, and information on continuous exposure to multiple compounds is virtually non-existent. Most of the available toxicological data has been obtained on subjects in a "normal" physiologic state. The effect of stress, prolonged confinement, weightlessness, and other factors which might tend to alter normal physiology and thus change the responses to any given compound cannot be accurately predicted at this time. For all of these reasons the limits that have been recommended are provisional and subject to revision.

The Panel reviewed reports of the analyses of atmospheric contamination detected in a variety of closed environments: seven manned space flights (Mercury and Gemini series), seven ground-based simulated cabin atmospheres, both manned and unmanned, (SAM and MESA series, Mercury malfunction, and "Integrated Life Support System Test"), nuclear submarines and Sealab experience and analysis of off-gas products and cabin materials. About 200 compounds that could be considered as possible space contaminants were identified. While the total number of contaminants is impressive, relatively few reached levels of serious concern. Some of these contaminants had been subject to critical review by the National Academy of Sciences-Nation Research Council Committee on Toxicology, and limits have been recommended for 60-minute emergencies and 90-day continuous exposure in submarines. Because of the substantial differences in the conditions of exposure, the submarine standards were used as guides to the 90-day exposure but not for the 1000-day limits. Of the remaining compounds, there were about 50 of possible concern because of potential toxicity. The Biomedical Research Office of the NASA Manned Spacecraft Center reviewed these compounds and indicated ten as being of first-priority. The Panel was also asked to recommend emergency limits for 3 of the 10 compounds and 2 others.

The limit values were derived from the air concentration which produced the earliest significant toxicological response (threshold response), as determined by the most sensitive, reliable measure obtained for that contaminant to date. The nature of the threshold responses produced by the compounds under study is given in table I. The duration of exposures before the threshold effects occur differed greatly in the reports of investigations and in some cases, the "no effect" concentration for a particular response was not available. Differing sensitivity among individuals to the different contaminants was also common.

A safety factor was applied to each 90 and 1000-day limit value because of (1) the inadequacies of the data mentioned above, particularly the fact that most current toxicologic data are based on non-continuous exposures, (2) because of uncertainty as to synergism among chemicals, and (3) to allow for the possibility of minor excursions above the ceiling limit. The magnitude of the safety factor differs according to the toxicologic category of the contaminant. If the contaminant is an irritant at the threshold of response, an estimated factor of 5 was included in the limit. If the contaminant is capable of producing systemic, irreversible injury, a factor of 20 was included.

TABLE I

NATURE OF TOXICOLOGIC THRESHOLD RESPONSES TO "LIMIT" COMPOUNDS

| Compound | Nature of Threshold Response |
|---|---|
| n-Butanol | Irritation of mucous membranes (man) |
| 2-Butanone | Irritation of mucous membranes (man) |
| Carbon monoxide | COHb formation, decreased CNS performance (man) |
| Carbonyl fluoride | Pulmonary irritation (animals) |
| Chloroform | Fatty infiltration in liver (animals) |
| Dichloromethane | Reduction voluntary activity (animals) |
| Dioxane | Kidney injury (animals) |
| Ethylacetate | Objectionable odor (man) |
| Ethylene Glycol | CNS depressant (animals) |
| Formaldehyde | Irritation, increased airway resistance (animals) |
| 2-Methylbutanone | Irritation of mucous membranes (man) |
| Trichloroethylene | Growth depression (animals) |
| 1, 1, 2-Trichloro, 1, 2, 2-trifluoroethane and related congeners | Irritation, central nervous system depression and performance decrement (man) |

The duration of exposure to which a limit value applies was determined by the type of response induced by a given contaminant: if a local irritant (e.g., the butanones), the Panel felt that so long as the concentration was kept below the irritant level no cumulative effects would occur. In such cases, the 90-day limit applies equally to a 1000-day mission. When, however, the contaminant has the potential for cumulative action, albeit at an exposure level well above the provisional limit for 90 days, a reduction appropriate to the seriousness of the response was made for the 1000-day mission; in such instances a five-fold reduction in the 90-day limit was arbitrarily made (e.g., chloroform, dioxane).

Table II gives limits for continuous exposure for 90 and 1000-days to air concentrations of 11 contaminants for normoxic conditions. These limits are the result of informed judgments which often had to be made in the face of a paucity of reported toxicologic information. For this reason and the fact that the number and nature of the substances of concern may change, the limits are provisional.

The limits are expressed in millimoles of contaminant/25M[a]. This unit was selected since it gives a numerical value which at 1 atmosphere pressure and at 25 C is the equivalent of parts per million (ppm) by volume (the units used for submarine standards and by the American Conference of Government Industrial Hygienists). At the same time it expresses the molar concentration per unit of space volume and is, therefore, equivalent to partial pressure of the contaminant.

TABLE II

PROVISIONAL LIMITS FOR SPACE CABIN CONTAMINANTS
FOR 90 AND 1000 DAYS*

| Air Contaminant | Air Limit in Millimoles per 25 $M^3$ (ppm) | |
|---|---|---|
| | 90 Days | 1000 Days |
| n-Butanol | 10 | 10 |
| 2-Butanone | 20 | 20 |
| Carbon monoxide | 15 | 15 |
| Chloroform | 5 | 1 |
| Dichloromethane | 25 | 5 |
| Dioxane | 10 | 2 |
| Ethyl acetate | 40 | 40 |
| Formaldehyde | 0.1 | 0.1 |
| 2-Methylbutanone | 20 | 20 |
| Trichloroethylene | 10 | 2 |
| 1, 1, 2-Trichloro, 1, 2, 2-Trifluoroethane and related congeners | 20 | No Recommendation |

*Applicable to atmospheres with normal oxygen tensions.

These limits, except for carbon monoxide, refer to "ceiling" values of single substances below which all concentrations should fluctuate. They are, therefore, maximal values and efforts should be directed to keeping the contaminants as far below the limits as possible. The carbon monoxide limit is based on an integrated exposure over a 12-hour period.

Carbon monoxide, among all the spacecraft contaminants presently known or envisaged for the immediate future, held a preeminent place of concern to the Panel as possibly the limiting toxicant. Carbon monoxide (CO) is contributed by materials, by some regenerative systems, and by man himself. CO from materials arises from the oxidative degradation of organics, while in regenerative systems CO is associated with the incomplete reduction of $CO_2$. In man, CO is produced from normal degradation of hemoglobin at a rate of 0.4 ml/hr/man. Because of its capacity to interfere with oxygen transport to the tissues and thus to affect cardiovascular and central nervous system function, CO has a broad capacity to synergize or potentiate biologic responses by altering host susceptibility. Moreover, recent investigations indicate that exposures to very low concentrations of CO can cause a subtle but significant decrement in high-level performance.

TABLE III

PROVISIONAL EMERGENCY LIMITS FOR SPACE CABIN CONTAMINANTS*

| Air Contaminant | Air Limit in Millimoles per 25 $M^3$ (ppm) for 60 min |
|---|---|
| 2-Butanone | 100 |
| Carbonyl fluoride | 25 |
| Ethylene glycol | 100 |
| 2-Methylbutanone | 100 |
| 1, 1, 2-Trichloro, 1, 2, 2-Trifluoroethane and related congeners. | 200 |

*Applies to a single exposure during the mission.

Emergency Limits

Table III provides allowable limits for peak exposures, not to exceed 60 minutes, for five substances which might accidentally be released within the spacecraft. In developing these limits, the Panel followed the principles used by the NAS-NRC Committee on Toxicology in establishing emergency inhalation exposure limits for military and space chemicals. Foremost among these principles as applied here is that the exposure not seriously interfere with the performance of a task or result in irreversible injury, although transient effects may be experienced. The emergency limits contain no safety factor. They are considered to be tolerable for a single emergency during the duration of the mission. In addition to solvents (the butanones), carbonyl fluoride ($COF_2$) can provide an acute, short-term hazard from two material sources: pyrolytic decomposition of carboxy nitrosofluoride rubber at and above 450 F, and of polytetrafluoro ethylenes at and above 850 F. Accidental air contamination by ethylene glycol $(CH_2OH_2)_2$ can arise from leaks in heat-exchange fluid systems or from its projected use as a space-suit coolant. Accidental contamination of the air with 1, 1, 2-trichloro, 1, 2, 2-trifluoroethane and its pyrolysis products could occur from other uses. Trichloroethylene and 1, 1, 2-trichloro, 1, 2, 2-trifluoroethane pose special hazards in the event of subnormal operating temperatures of the catalytic burners. Dichloroacetylene (ClC=CCl), which is highly hazardous to health at extremely low levels, arises from the temperature degradation of trifluoroethane.

It is of interest to compare the recommended limits derived independently by this Panel with those suggested by others for different conditions. Table IV shows the limits recommended by this Panel for 90-day continuous exposures, the threshold limit values recommended by the American Conference of Governmental Industrial Hygienists (ACGIH, 1966) for industrial environments, and the Soviet maximum allowable concentrations for industrial premises. The Panel's limits are considerably lower than the ACGIH limits, but in a few cases they exceed the industrial limits established in the U.S.S.R.

TABLE IV

PANEL'S 90-DAY CONTINUOUS LIMITS COMPARED WITH INDUSTRIAL LIMITS FOR NON-CONTINUOUS EXPOSURES (mM/25 $m^3$ or ppm)

| | Committee Recommended 90-Day Limit | ACGIH[a] Industrial T L V's | U.S.S.R.[b] M A C's for Industrial Premises |
|---|---|---|---|
| n-Butanol | 10 | 100 | 67 |
| 2-Butanone | 20 | 200 | 68 |
| Carbon Monoxide | 15 | 50 | 18 |
| Chloroform | 5 | 50 | -- |
| Dichloromethane | 25 | 500 | 1.4 |
| Dioxane | 10 | 100 | 2.8 |
| Ethylacetate | 40 | 400 | 57 |
| Formaldehyde | 0.1 | 5 | 0.83 |
| 2-Methyl Butanone | 20 | 200[c] | 58[c] |
| Trichloroethylene | 10 | 100 | 9.3 |
| 1, 1, 2-Trichloro, 1, 2, 2-trifluoroethane and related congeners | 20 | 1000 | -- |

a. American Conference of Govermental Industrial Hygienists, 1966, threshold limit values.

b. Approximate conversions from mg/$m^3$ supplied by Dr. Herbert Stokinger for Soviet maximum allowable concentrations.

c. Based on limits for methyl propyl ketone rather than methyl isopropyl ketone (2-methyl butonone).

Soviet scientists ordinarily recommend (or report) "nominal concentrations" of space cabin contaminants by chemical groups rather than individual compounds. A "package" limit for total organics, although useful as an engineering guide, has not been set, owing to the large variation in toxicity among the organic space cabin contaminants: common practice, which places the limit for the package on the most toxic substance, often leads to unnecessarily severe engineering controls for some contaminants (e.g., formaldehyde vs. other saturated aldehydes).

These comparisons with the U.S.S.R. levels have no real value without full knowledge of the meaning of the term "nominal concentration," that is, whether these are measured average values in flight or whether they are recommended limit values. U.S.S.R. limits for air contaminants are generally lower than those used in western countries primarily because Soviet health scientists when establishing industrial limit values frequently appear to base their limits on subtle, often transitory, changes which do not necessarily reflect significant adverse effects on health or performance.

The Panel had a number of general and specific recommendations for research. The general recommendations included the following. It was considered essential that work now underway to identify the nature and concentration of spacecraft air contaminants be continued and expanded. Research is needed to validate the 90-day limits for continuous exposure and to verify projections of these limits to 1000-day exposure. Studies should be initiated to determine the long-term effects of continuous exposure to selected single and multiple contaminants at various altitudes and under different oxygen partial pressures.

A major difficulty in establishing permissible limits for air contaminants in prolonged space voyages is the undetermined effect of weightlessness on toxicological responses; for example, it has been suggested that the cardiovascular effects of weightlessness might indirectly but strongly influence the action of dichloroethylene in producing cardiac arrhythmias. It is therefore desirable to compare the action of selected toxicants in the weightless state with results obtained under ground-based conditions, particularly at the various atmospheric compositions to be used for space missions.

Another major problem in establishing permissible contaminant levels for prolonged space flights is the lack of detailed information on the mechanism of toxicity of many of the compounds of potential importance. In most cases, the information on which judgments are based is too restricted. Such data cannot support more than gross estimates of permissible limits. Intensive efforts should be made (1) to characterize the mode of action of some of the important toxicants under conditions of chronic low-level exposure, in order to determine the nature of injury, its rate of production, and the physiological mechanisms and rates of repair; from these data, it might be possible to evaluate the net rate of accumulation of damage for various exposure levels, and (2) to obtain much more detailed information on human exposures, particularly in industry, to relate exposure level to long-term toxic effects.

## DISCUSSION

DR. HODGE: Dr. Albert's paper is now under discussion.

QUESTION: There is a term mentioned in your slide--normoxic conditions. Could you explain the derivation of that term?

DR. ALBERT (New York University Medical Center): This refers to an atmosphere which maintains the partial pressures of oxygen and carbon dioxide within the alveoli at a sea level equivalent, resulting in normal oxygen saturation of hemoglobin.

DR. HODGE: I'm glad you asked. I thought it simply meant the atmospheric concentration or the usual percentage of oxygen.

FROM THE FLOOR: I noticed in the first priority compounds the absence of compound gases, particularly ammonia, and halogen acids. I was wondering--I imagine there might be some reasonable amount of toxicological data on these species in the literature. I was wondering why one example of each is not indicated in the priority compounds.

DR. ALBERT: The selection of the compounds was not made by the committee. The compounds were chosen in terms of their likely occurrence in spacecraft, and they weren't considered because they weren't thought to be of primary concern.

FROM THE FLOOR: I think it was Ray Saunders of the Navy Research Lab, about a year or two years ago, who pointed out the toxicity of dioxane, which gives rise to dioxyethylene toxicant that was really responsible for abort by one industrial company in a manned 30 day simulator mission. I notice your level is four times that of the Russians, and I wonder if the Committee feels that this particular compound is not as toxic as was discussed previously.

DR. HODGE: Do you want to comment on this, Roy, or does Ralph want to?

MR. WANDS: I wonder if, perhaps, we don't have both of the experts in the nation, or in the world, on this subject in our audience, and perhaps it might be better to refer the question to them. Ray Saunders from the Naval Research Laboratory is here, and so is Captain Siegel from the Navy Toxicology Unit, who has done some experimental work with this compound. Do either of you care to volunteer and stand up? Captain Siegel, would you care to comment on your experience with dichloroacetylene?

CAPT. SIEGEL: We've done quite a bit of work with dichloroacetylene. It's sort of an explosive material and there is difficulty in just generating it. The toxicity has been set at about a tenth of a part per million for 90 days. We are still working on it. We don't have a total answer.

DR. HODGE: May I ask Mr. Wands if he has some comments he'd like to make now, from the philosopher's standpoint, perhaps, on these specific considerations?

MR. WANDS: One of the things that has impressed me as I have had the privilege of sitting with Dr. Albert and the others of this Space Science Group is that there is a tremendous need for close liaison between the toxicologist and the engineer at the early stages of engineering design. You saw that one of the critical compounds that Dr. Albert mentioned was ethylene glycol. This was chosen as a conventional heat exchange medium in a mixture with water, such as most of us drive around with in the winter time in our radiators. It works beautifully, and they do have a heat exchange problem requiring an antifreeze. There are some unknowns associated with ethylene glycol which could probably be avoided if a propylene glycol had been used as the antifreeze. However, by the time this question became known to the toxicologist, the hardware was long since locked-in and one could not change the designs of the heat exchange fins and the pumps, so we were stuck at that moment, then, with a potential problem in the spacecraft. We do have to be extremely careful about leaks and we are concerned about the toxicology of ethylene glycol. There are at present some rather extensive studies underway which might have been avoided if we had had an opportunity to review this specific point in the beginning. There are any number of things which come up in the choice of materials for construction where toxicology can be of assistance, at least to the engineering personnel in their choice of materials of construction. I think, also, some of the problems in choosing means of controlling bacterial contamination of the drinking water, the choice of bacteriostatic or bactericidal agent which may be added can be varied fairly widely in terms of bacteriological effectiveness, but there must be some very critical choices made in terms of effects upon materials, and here is a direct instance where the engineer and the toxicologist, or in this case, the microbiologist, must work very closely in the early stages of design so that one does not end up with a piece of hardware that is so far down the road you can't back up and modify it in order to reach a particularly critical stage in the mission.

COLONEL KRATOCHVIL (Aerospace Medical Research Laboratories): May I make a point here? I'm not an engineer, but I would like to dispute this a little bit and defend the engineers in that ethylene glycol, I think it is 37% glycol in water, slushes at the specific temperature which they want in the cooling system. It allows for the design of a cooling system which is valve free. That is, when you reach this critical temperature, the formation of the slush shuts down the radiator tubes; so from an engineering point of view, it's a beautiful design. These gentlemen were limited by weight considerations, valving considerations, thermostats, and all the other things you can envision, and this is why they went to it. I agree with you completely that perhaps there should have been consultation earlier in the game because they didn't realize the trade-off between ethylene glycol and propylene glycol; but from the engineering point of view, they have got a lot going for them. We argued this point with them and lost completely because of the engineering considerations.

DR. HODGE: With regret, I feel we must leave this topic. Dr. Albert, there was the question about dioxane and I don't think it was answered. I think it may not have been apparent that--As I recall the point, the point was that the Committee's recommendations were substantially lower than the Russian levels--

FROM THE AUDIENCE: Higher. Higher.

DR. HODGE: Four times higher.

DR. ALBERT: And the question is what? Why? I can't put my finger on the answer. I will have to run through my notes and come up with the answer later.

QUESTION: I was unable to see the slides up there in their entirety. There was a limit for carbonyl fluoride I noticed. What was that limit? For how long a time? I think it was the list of five compounds, I'm not sure.

DR. ALBERT: Well, it was one of the emergency limits which is for 60 minutes.

QUESTION: And another thing, please, how would it be possible to obtain copies of these limits before the proceedings of this meeting are published? Will this be possible?

DR. ALBERT: Yes. The report of the Committee has already come out and there are a couple of minor corrections, mostly typographical, and I'm sure that it can be made available to you from the National Academy of Sciences.

FROM THE FLOOR: Could I comment just briefly on the Soviet limits? When our delegation visited the USSR to study the problem, about five years ago, the one thing we agreed on, I think, was that in general the Soviet limits are much closer to ours when you're dealing with primary irritants such as acids, and so forth. We are talking here about chronic toxic effects. It is in this area that their limits seem to be in general lower than ours for the various reasons which we explained in our report. I would like somebody to tell me what the evidence is that dioxane causes renal injury in humans at low levels. I'm not aware of any documentation of this.

DR. HODGE: Can you speak to this, Dr. Albert?

DR. ALBERT: We have the documentation which I can show you.

FROM THE FLOOR: All right. I'll see it later.

QUESTION: Can I come back to this normoxic problem? Did I understand it right that you defined it in terms of hemoglobin saturations, or was this the $pO_2$ prevailing in the atmosphere?

DR. ALBERT: It would be hemoglobin.

DR. HODGE: I would like to caution somewhat on that. We are told in these conferences all the time about oxygen toxic effects, mainly of the lung, but I would think one would also have to define normoxic in terms of the $pO_2$ in the atmosphere that was used, because even a higher concentration in the atmosphere would give you normoxic conditions in the hemoglobin.

FROM THE FLOOR: Just one real quick comment. Normoxic, of course, ideally, would be the partial pressure of oxygen delivered to the mitochondria in the cell at .04 millimeter.

# EFFECTS OF AIR POLLUTION ON ANIMALS EXPOSED TO STREET ATMOSPHERES IN DETROIT

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I must confess that I have wondered how the topic about which I'm going to speak would conform to your symposium title ("Atmospheric Contamination in Confined Spaces"), but after thinking about it, I guess Detroit could be considered a confined space. Many people spend 90 days there, and some have been known to spend up to 90 years in the city. Escape is not always easy or possible, so perhaps we have many problems in common with those in space cabins. Toxicologically, we are concerned with the net effect of many things acting together, usually in unknown quantities, varying with time, and containing a mixture at any given time of a great number of chemical compounds which, if sought, could be found.

Our purpose in conducting a study of the type I'm going to describe was to attempt to determine whether health effects, or indeed any effects, attributable to ordinary levels of air pollution could be detected by a study involving laboratory animals. I think you will agree that if one wishes to answer questions relating to air pollution and health, it can be approached several different ways. People living in cities can be studied directly. This is the epidemiological approach being used by many investigators. Laboratory animals can be exposed to controlled concentrations of pure substances or to mixtures such as Mr. Wands suggests are necessary, and certainly much more research of this kind is required. Animals may also be exposed to synthetic or naturally occurring mixtures, such as auto exhaust, which contains a large number of substances, not all of which are necessarily known. But, finally, if it is desired to determine whether urban air pollution such as is found in Detroit or other large cities exerts an effect on health, there is a certain obligation to try to measure the effect directly, and this is what we attempted.

Instead of creating the exposure conditions, our animals lived in normally contaminated air in the city of Detroit. We modified this air to some extent by enriching it with vehicular exhaust products from a nearby street. The exposure facility was maintained for approximately four years, and the animals breathed air from the street 24 hours a day throughout this period, with only minimum modifications which were required to keep the animals alive. Because Detroit is not located in the tropics, it was necessary to heat the air in the winter in order

to prevent the animals from dying, and it was also necessary to cool it somewhat in the summer to prevent overheating. Both of these functions, however, were accomplished in such fashion as to minimize the alteration of the aerosol or the gases present in the polluted air.

I should like to stress, however, that the insult, or challenge, to the animals was a minimal one, for the air was essentially the same as many people in Detroit breathe daily. The results of exposure, therefore, should be indicative of the kind of effects one might anticipate from exposure of humans to a similar environment. One major difference between human exposure and our animal study was that our experiment was limited in time. People may live an entire lifetime in city air, and we cannot necessarily extrapolate our findings to predict the results of 70 years of such exposure.

The Wayne State University Medical School laboratory from which we operated is located quite near the center of Detroit, and happened to be ideally located for a study such as this. Probably the fact that this area was reasonably well-polluted was one of the reasons why the U.S. Public Health Service selected our facility for such a study. Had we been an ivy league campus out in the country, we could never have performed it.

Figure 1. OCCUPATIONAL AND ENVIRONMENTAL HEALTH LABORATORY

Figure 1 is a picture of our laboratory and the facility erected across the nearby street to inhale somewhat increased concentrations of vehicular exhaust products. This rather dramatic-appearing truss system across the street had a number of air intakes which took in a large amount of air, as much as 10,000 cubic feet per minute when the temperature was optimal. The air tempering system was located in the small hexagonal structure, and directed air into our exposure chambers inside, which accommodated as many as 2000 small animals. The rate of change of air in the room was quite rapid, up to one air change per minute, or more under certain conditions, so that the air in the exposure rooms reflected changes on the street very quickly.

TABLE I

TOTAL SUSPENDED PARTICULATE MATTER
HIGH VOLUME SAMPLERS
W.S.U. - AIR POLLUTION STUDY
DETROIT, MICHIGAN

| Year | Mean | Maximum Daily Loading |
|---|---|---|
| 1962 | 179 $\mu g/m^3$ | 818 $\mu g/m^3$ |
| 1963 | 200 $\mu g/m^3$ | 457 $\mu g/m^3$ |
| 1964 | 263 $\mu g/m^3$ | 502 $\mu g/m^3$ |
| 1965 | 202 $\mu g/m^3$ | 381 $\mu g/m^3$ |

The animals were exposed constantly to air containing particulate matter and gases, and table I summarizes the degree of contamination by particulate matter as sampled by conventional high volume filters. The mean of more than two-hundred micrograms per cubic meter is typical for a large city, and maximum levels are considerably in excess of this, so exposure to particulate matter can be considered conventional for a big city.

TABLE II

AIR LEAD ANALYSES
MARCH 1960 - JUNE 1966

| | Number of Samples Analyzed | Range of Values | Mean Values |
|---|---|---|---|
| Weekday | 48 | 0.90-5.30 $\mu g/m^3$ | 2.67 $\mu g/m^3$ |
| Weekend | 15 | 0.20-2.03 $\mu g/m^3$ | 1.57 $\mu g/m^3$ |
| Total | 63 | 0.20-5.30 $\mu g/m^3$ | 2.46 $\mu g/m^3$ |

The lead content of the particulate matter is presented in table II, and it can be seen that the air contained approximately 2-1/2 micrograms of lead per cubic meter. Such a lead level is also typical of city air, and presumably the body burden of the animals would be expected to reflect this exposure in a manner similar to humans.

Table III summarizes some data resulting from the analysis of lung tissue of exposed animals. The "clean air" animals were the controls that lived in a room identical to that containing the exposed animals, except that the air supply was passed through absolute filters and activated carbon. This removed the particulate matter completely and decreased the concentration of some of the gases.

It might be expected that the difference in exposure to particulate matter would result in differing tissue levels of various metals, including lead, in the two groups of animals, and the analyses summarized in table III represent an effort to detect such differences in lung tissue. It does appear that some of the exposed animals contain increased quantities of several metals, but statistically the small number of samples and the large variability combine to make the observed differences of low significance. Additional analyses to permit more positive determination of significance are presently in progress.

Table IV is a brief summary of gas analysis data from the study resulting from continuous recording instruments of the type presently used in U.S. Public Health Service network sampling program. The air is typical of that found in a great many streets in Detroit and in many other cities, and contains the usual amounts of the several gases measured. Detroit air contains relatively little oxidant and average concentrations of most other gases. The air purification system did not remove carbon monoxide, so the findings cannot be considered related to carbon monoxide in any way.

The study was conducted for several years during which time animals breathed the atmospheres described, and every effort was made to measure any effects which could be attributed to air pollution. Presumably, any differences had to be air pollution-related because other environmental factors had been controlled. The handlers were the same, the food was the same, the water was the same, the surroundings were the same, insofar as possible, so that any effects which were found could be assumed to be air pollution-related. The problems were the same as those encountered in determining no-effect levels--what effects are to be expected and how can they be measured? This is very difficult, for it is not to be expected that severe damage to any organ will result from daily exposure to urban air. Hence, a number of variables were measured, including physiological and biochemical measurements which could be made on living animals, and, of course, histopathological examination of tissues after sacrifice. It seemed probable that the tissue studies would be most informative.

TABLE III

CONCENTRATIONS OF THE ELEMENTS
IN LUNGS OF TWO EXPOSURE GROUPS OF ANIMALS

| | Clean Air | | Ambient Air | |
|---|---|---|---|---|
| | Range ppm | Mean Ash | Range ppm | Mean Ash |
| Al | 40 -952 | 170.0 | 20 -925 | 249.0 |
| Ba | < 0.1- 7.1 | 2.9 | 0.4- 22 | 5.6 |
| Be | < 1 | < 1.0 | < 1 | < 1.0 |
| Bi | < 1 | < 1.0 | < 1 | < 1.0 |
| Cd | <10 | < 10.0 | <10 | < 10.0 |
| Cr | < 1 - 2.8 | 1.7 | < 1 - 4.5 | 1.9 |
| Co | < 2 - 2.2 | 2.0 | < 2 - 2.5 | 2.0 |
| Cu | 71 -200 | 117.0 | 52 -170 | 114.0 |
| Pb | 3.5- 19.5 | 10.4 | 3.8-103 | 23.5 |
| Mn | 6.1- 33.6 | 16.0 | 2.6- 20.7 | 13.4 |
| Mo | < 1 - 7.5 | 4.8 | 3.9- 10.7 | 5.5 |
| Ni | 2.5- 16 | 6.8 | < 1 - 11.5 | 5.1 |
| Ag | < 0.1- 0.9 | 0.15 | < 0.1- 0.75 | 0.25 |
| Sr | 1.6- 10.7 | 5.6 | 2.4- 7.9 | 4.8 |
| Sn | < 1 - 7.5 | 2.5 | < 1 - 4.5 | 0.9 |
| Ti | < 2 - 15.7 | 5.0 | < 2 - 9.2 | 5.2 |
| V | < 4 | < 4.0 | < 4 | < 4.0 |
| Zn | 725 -2550 | 1480.0 | 1125 -2475 | 1750.0 |
| | % in Ash | | % in Ash | |
| Ca | 0.35- 1.79 | 0.93 | 0.25- 1.41 | 0.72 |
| Fe | 0.31- 2.92 | 1.44 | 0.55- 1.91 | 1.16 |
| Mg | 0.37- 2.24 | 1.19 | 0.33- 1.90 | 1.00 |
| P | 6.2 -14.9 | 10.8 | 8.9 -12.3 | 10.6 |
| K | 9.7 -19.4 | 14.5 | 7.7 -16.6 | 13.0 |
| % ash in wet tissue | 0.90- 1.15 | 1.04 | 0.93- 1.32 | 1.11 |
| % ash in dry tissue | 5.14- 5.98 | 5.59 | 4.87- 6.35 | 5.63 |

Most results based on analysis of tissue from 12 animals.

TABLE IV

CONCENTRATIONS OF GASES CONTINUOUSLY MEASURED
WAYNE STATE UNIVERSITY
DETROIT, MICHIGAN
1962 - 1965

| | Mean Conc. (1 Hr. Average) | Highest Recorded Conc. (1 Hr. Average) | Highest Recorded Conc. Instantaneous |
|---|---|---|---|
| CO | 3.2 PPM | 33 PPM | 98 PPM |
| $CO_2$ | 305.0 PPM | 643 PPM | 851 PPM |
| NO | 8.6 PPHM | 209 PPHM | 215 PPHM |
| $NO_2$ | 6.1 PPHM | 68 PPHM | 102 PPHM |
| $SO_2$ | 5.1 PPHM | 85 PPHM | 198 PPHM |

Figure 2 is a combined mortality curve for all of the animals in the study, and is a plot of the number of deaths cumulatively plotted against time. The rates at which the animals died were practically identical, indicating no air pollution effect. The curves are also a tribute to the success of the efforts to prevent epidemics and diseases not air pollution-related.

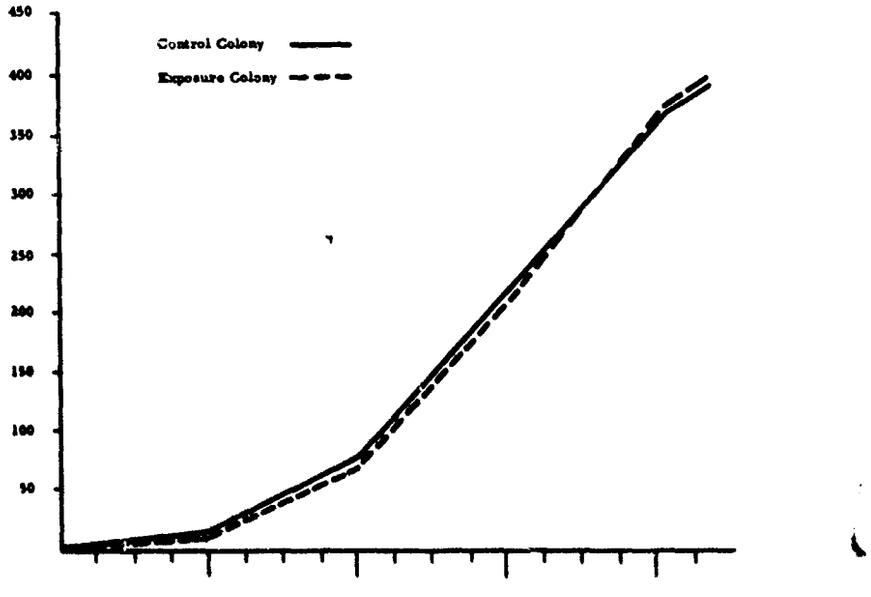


Figure 2. CUMULATIVE DEATHS ALL SPECIES

It is generally agreed that rate of weight gain, or growth, is a sensitive indicator of challenge to laboratory animals, and figure 3 presents a typical plot of data from a colony of male rabbits. Figure 4 is a similar plot for female rabbits, and variations due to pregnancies may be noted, but no evidence of reduced weight gain attributable to air pollution is apparent in either sex. Actually, if all curves were presented for each sub-group of animals in the study, it would be noted that some appear to exhibit an effect, but it is almost certainly not related to air pollution. Sometimes weight depression is observed, while in other groups there is an apparent enhancement in the weight of exposed animals.

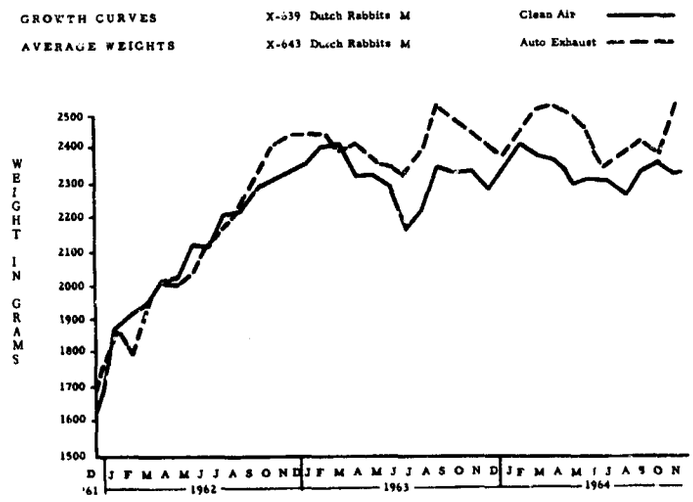


Figure 3. RATE OF GROWTH (MALE RABBITS)

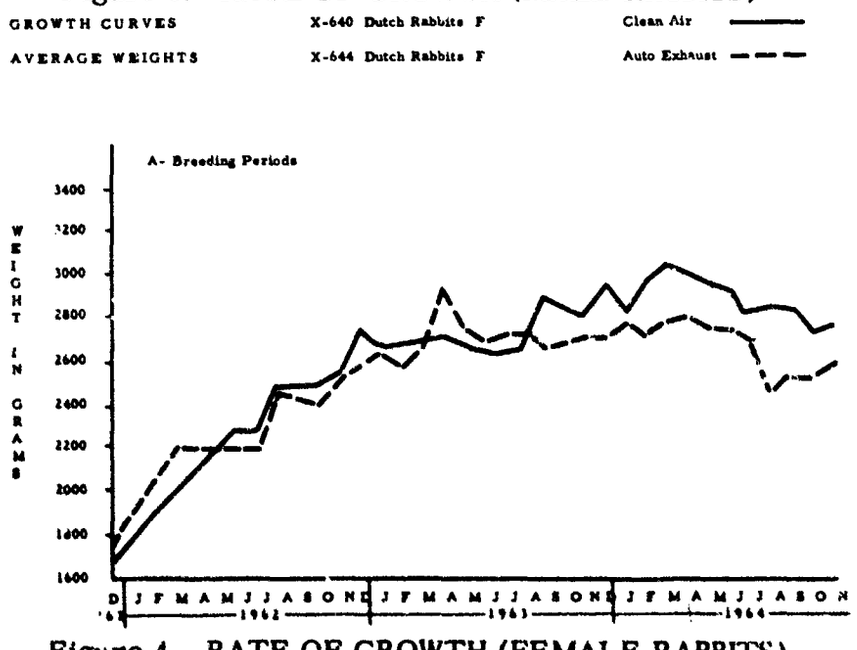


Figure 4. RATE OF GROWTH (FEMALE RABBITS)

Physiological testing during the study involved the following measurements: pulmonary function, electrocardiogram, cardiac function and body temperature. None of these parameters showed any valid differences. The pulmonary function studies of pulmonary compliance and tidal volume were made on our capacitance respirometer which was under development during the study. The calibration techniques were not perfected and, consequently, changes of pulmonary compliance or tidal volume with time are not available. However, comparisons between the two colonies for any particular time were probably valid and showed no statistical difference or trend.

The biochemical study program consisted of plasma acid and alkaline phosphatase determinations and paper and starch gel electrophoresis determinations of serum, pulmonary and tracheobronchial lymph node extracts for esterases and proteins. The electrophoretic work showed no statistical difference between the exposure colonies. The plasma alkaline phosphatase exhibited a rather debatable difference. Figure 5 shows plasma alkaline phosphatase levels against time for a Dutch rabbit colony. The decline with time is normal and is a result of aging. For no one time period is the difference between the means for the exposed and control groups statistically different, and yet nearly every time period shows a greater alkaline phosphatase plasma level in the exposed group. This is true for two generations of rabbits. The differences in mean acid phosphatase levels of the two animal colonies were not statistically significant. Furthermore, there was a completely random pattern of values with respect to which colony had the highest mean.


Figure 5. PLASMA ALKALINE PHOSPHATASE

Histopathological studies were conducted on lung tissue from rabbits, guinea pigs, rats, mice and hamsters. The tissues were prepared and presented to the pathologist without his knowledge of which colony they represented. Each tissue specimen was examined and graded for amount of particulate matter present, degree of emphysema, degree of chronic inflammation, degree of fibrosis and acute pneumonitis. An arbitrary grading scale was used, ranging from 0 for none evident to 4 for severe generalized change. The only parameter regularly higher in the exposed colony is the amount of particulate in the lung (figure 6). The amount of emphysema present is higher among the rats in the exposed colony, but not statistically different among the other species.

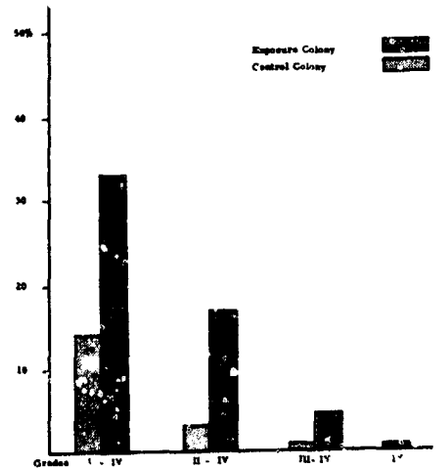


Figure 6. PARTICULATE IN LUNGS ALL SPECIES

Blood was drawn on a regular basis from rabbits, rats, and guinea pigs. The following tests were done routinely: hemoglobin, hematocrit, red blood cell count, white blood cell count and differential count. The latter test divided the white blood count into percentage neutrophils, eosinophils, basophils, lymphocytes and monocytes. The differential count was combined with the white blood cell count to give absolute counts per cubic millimeter of the various cell types. The only consistent difference between the colonies appears to be in the white blood cell count. This is nearly always higher in the exposed colony and usually significantly so. A study of the differential count shows that this is predominantly due to an increase in lymphocytes. The statistical difference in the lymphocyte count is sometimes more significant than the total white cell count. The neutrophil and eosinophil counts are consistently lower in the exposed animals, although the difference is not always statistically significant. The monocytes and basophils show no regular difference. An increased white cell count is general, and the lymphocyte portion in particular is usually a response of the organism to microbial infection. While the levels observed were not high enough to indicate acute infection with particle-borne microorganisms, it is also possible that they represent an increased phagocytosis in response to a higher particulate loading in the respiratory tract.

It should be pointed out that there may be as much importance in these tests which were performed and which gave no difference as in the others. It is of some interest to note that there is not a statistically provable difference in some of these things often associated with air pollution. This study was conducted on rodents for periods up to four years. The validity of extrapolating the findings to humans breathing a similar atmosphere for seventy years is hazardous. The problem of assessing the health effects of a combination of toxic agents, each present at a relatively low level, over a long period of time, is a very complex one. Epidemiological evidence, the toxic effects of these substances at higher levels, their possible potentiation on one another, and the differing biological responses of different species are only some of the considerations which must be kept in mind when considering the evidence presented here.

## DISCUSSION

FROM THE FLOOR: I have one comment and one question. The comment relates to the study which is presently being conducted in Los Angeles. I don't know whether you know about that.

DR. SMITH (Wayne State University): Yes, I do.

QUESTION: They had an interesting finding. They located rats above the freeways and I have heard recently that they found reduced incidence of malignancies in the rats above the freeways. The second question was, in your slides showing the slight elevation of the alkaline phosphatase activity, I noted your starting point on the ordinate. Is that the control value?

DR. SMITH: Yes, the green line was the control value.

QUESTION: Is this the starting line before exposing the animals, because this is significantly higher, considerably higher than the control animal level. Was this just a group difference?

DR. SMITH: No, we noticed this, too, of course. All I can tell you is that if you study this and all the data that go to make up the curve, you can toss a coin as to whether there is a significant difference. First of all, at any given point, there is no significant difference. It is well within the normal rate expected. The only thing that I pointed out is that the one line is consistently above the other. It can be, as you suggested, that for some reason we had a biased group and that the one group started out higher and simply stayed there but we have reason to think this isn't really the case, although the curve supports that point of view. In any event, we don't consider the finding very significant. This happened to be one of the few things that differed.

QUESTION: On the activity of the polluted animals versus those of the clean air, is it possible that this might be related to some pharmacological action of the pollutants? Do you think the difference is real enough in your estimation that this might be considered?

DR. SMITH: This, of course, was our hope in finding if there were a difference it would be so related. To my knowledge, the analysis of that curve and all the other data supporting it did not make the difference attributable to air pollution. It appears so at first. Again, there is not a large number of mice involved here, a couple of dozen, and one ought to make the animals be their own control. This is not the case here. We were attempting to compare one group

with another. We took certain measures to minimize the differences. I'm not sure we succeeded. I don't think the difference we saw is attributable to pharmacological or toxicological effects of air pollutants.

QUESTION: Did you do particle size analysis? If so, what were the ranges that you might have gotten there?

DR. SMITH: Yes, we did a fair amount of air sampling, including visual and electron microscopy. One finds the entire spectrum of sizes. The inhalation system, if I may call it that, was such that the aerosol was almost unchanged except for the very large particles. The room was 13 feet wide and if air continued to flow through it, the grosser particles, certainly 10, 20, 30 microns on up, succeeded in getting into the system. Usually they fell on the floor somewhere in getting from one side of the room to the other. Beyond that, one found a spectrum of sizes from the submicroscopic, as small as you can see, up to several microns. There is nothing any different from what street air would be expected to be.

DR. SMITH: There has been a similar study supported in Los Angeles, part of which was along side the freeway, if not over it, and identical in design to ours with the exception that they were dealing with photochemical smog. They ran into problems including one rather severe one. They weren't going to air-condition and the first heat wave killed the animals so they had to start over again in airconditioning.

QUESTION: Did you not say, Dr. Smith, that you had noticed an increase in weight in the animals that were exposed to the polluted air?

DR. SMITH: No, what I said was that of the many curves I could have shown you, I elected to show you second generation rabbits, male and females. I think the male Dutch rabbits appeared to increase in weight. This is a chance thing, because any small group of animals may show a difference. All of this added together, there was no difference, no.

MR. GISCLARD (Flight Dynamics Laboratory, W-PAFB): I thought it was significant. From the point of view that when you speak of physiological response to many of these gases and vapors, I wonder if the toxicologist has taken into consideration the possibility of gain in weight from the standpoint that you may have these solvent vapors acting as carbohydrate sparers. We have found in a study done long ago that men, for example, exposed to amyl acetate, all these men in the course of a year's time seemed to gain weight and the doctor could not attribute it to anything but the constant exposure to amyl acetate. He felt, when you got right down to it, amyl acetate actually acted as possibly a carbohydrate sparer. If that is the case, you have another response which you have to consider in 100-day or 1000-dav exposures. You may have some, to use the expression, "fat astronauts".

(Laughter)

I don't know if this is a particular point to bring out, but in long term exposure I believe that this is a possibility that should be considered, because you take a substance acetone, which we say, "Oh, there's no problem here," but the constant exposure to a high concentration of acetone may produce this effect.

DR. SMITH: Some of the handlers were exposed to ethanol, but the animals weren't.

MR. WANDS: I would like to respond to Dr. Slonim's comments on what fouled up on the MESA project. The MESA project was aborted primarily, as Ray Saunders showed, because there was a dichloroacetylene problem. However, as Ray also showed, there was some dioxene which presumably came by degradation of dioxane. Dr. Slonim was concerned about levels the Space Science Board recommended for dioxane and wondered whether or not basically dioxene had been considered at that time. Dioxene was not considered. It was not one of the 50 materials which the NASA administration felt was of prime concern to the Apollo flights. The recommendation for dioxane is for dioxane and dioxane only. It does not consider any problems of decomposition. This general consideration is true of all the recommendations of that committee. They did not concern themselves with possible degradation products of these unless they were specifically identified as compounds in their list of recommendations.

AMRL-TR-68-175

PAPER NO. 4

# A METHOD TO PREVENT AIR POLLUTION BUILD-UP IN POPULATED AREAS

Lester M. McKay

University of Nevada
Reno, Nevada

## INTRODUCTION

I would like to pay tribute to Dr. Luther L. Terry for his contributions in air pollution control and to use a portion of his speech presented to the National Conference on Air Pollution in 1962 while he was Surgeon General of the United States Public Health Service.

Quote: "Since modern man can determine the nature of his environment, he must learn to accept responsibility for its deficiencies in much the same way that he accepts responsibility for his individual acts. Otherwise, our repeated pleas for cooperative effort and shared responsibilities have a hollow sound. If a crime is committed, we are quick to bring the immense weight of civilization to bear upon the guilty one, but what about the crime that we commit as a group? These are offenses we commit against ourselves--often in good faith and with the most highly sanctioned of motives.

From this point of view, Donora was a crime. The deaths from chronic diseases associated with environmental factors which occur daily are also crimes. Who is to blame? Where are the culprits? What should we do to apprehend them? Where?--Everywhere. WE ARE ALL GUILTY--not health officials alone, nor legislators, nor businessmen--but ALL of us!

Certainly now, when we can and do determine--by chance or by choice--the structure of our environment, we cannot blame the vagaries of nature for its defects. The time is past. WE are responsible. Let's get on with it! Let's clean the air!"

End Quote

Thank you, Dr. Luther Terry!!

Hundreds of millions of dollars have been and are now being spent to research cause and effects of air pollution. Many facts have been documented and there is now no doubt that man must search diligently for the solution.

Senator Gaylor A. Nelson (D. -Wis.) told the Senate, when he introduced S-2410, bill providing $500 million a year to assist abatement of air pollution, "The majority of our state and local governments have done nothing in the past twelve years".

We know the causes and many of the effects. --It is imperative that we come up with a workable solution!

This proposal offers a plan, though unique, which could very well prove to be the "woods" we have failed to see because of the "trees"!

## CAUSE

Air is the most important substance in your life. You might exist many days without food and a few days without water, but without air you could not live long enough to read this paper.

Science is radiant with farflung successes and clouded by the ever-increasing difficulty in providing the most necessary of all commodities for life (clean air). As the outcry against pollution rises to its highest pitch we hear increasing demands for controls even where no control devices have been developed. The chalienge is great, for the problem of air pollution is an inseparable part of one of the most important needs of our time: the creation, in our era of accelerating change, of a healthful environment worthy of our high level of economic development and scientific achievement.

It is not necessary to recapitulate all of the contributors to air pollution. The better known sources in order of the magnitude of their contribution are: 1- TRANSPORTATION, 2- MANUFACTURING, 3- ELECTRIC POWER GENERATION, 4- SPACE HEATING, 5- BURNING OF REFUSE. These five major sources alone are dumping one hundred forty two million (142, 000, 000) tons of garbage into the American atmosphere every year. We might compare this to one train of standard 50-ton coal cars 36, 000 miles long--or 12 unbroken trains the length of the United States! (figure 1)

Nature has provided man with a limited amount of life sustaining air--No more and no less is available. Nature has also provided an atmosphere dispersion system which has handled the job of cleaning our air supply very well until the past few decades. However, we have arrived at a point in time when the capacity of the atmosphere to assimilate air-borne wastes is much too frequently overwhelmed.

With all of our technical know-how and ability to travel, communicate, and live and work in comfort during the most adverse of weather conditions we are, as of this date, ENTIRELY at the mercy of the elements to dispose of the aerial garbage we continue to dump into the atmosphere. If the yardstick of man's achievement is based on his degree of independence of the elements, our score in this one area is ZERO and falling even more.

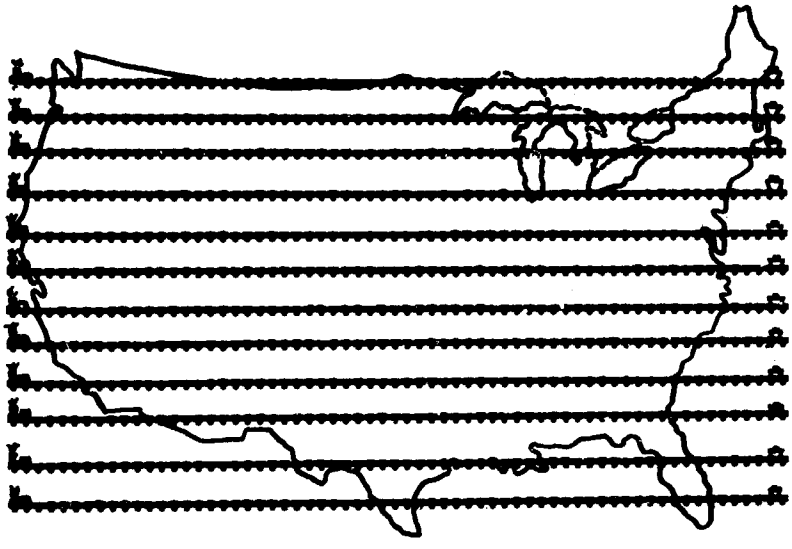

Figure 1

## EFFECTS

The adverse effects of air pollutants are by no means limited to the health of man. The most timid estimate of economic losses due to air pollution suggests that the cost to the United States alone exceeds Eleven Billion Dollars ($11,000,000,000) each year *($65.00 for every man, woman, and child). This estimate DOES NOT include costs of medical care for people who have respiratory diseases associated with air pollution, nor do they include factors such as lost earnings or reduced productivity, which are almost invariably associated with illness and absence from work.

In addition to its effect on health, air pollution causes extensive economic damage through its effects on animal and plant life, through corrosion and soiling of materials and buildings, depreciation of property values, interference with air and surface transportation through reduction in visibility and losses of unburnt fuel.

Damage to "Salad Crops" has actually forced many truck farmers out of business along the eastern seaboard. Los Angeles smog drifts into the fertile San Joaquin Valley blighting Ten Million Dollars (10,000,000) in crops annually. Crop damage in many industrial areas including Spokane, Washington; Anaconda, Montana; Ducktown, Tennessee; Chicago, Illinois; St. Louis, Missouri; Tampa, Florida, and others were well documented by Thos. L. Kimball in "Air Pollution" in 1966.

*U.S.P.H.S. Bulletin #1560

Dr. Morely Kare, a University of Pennsylvania researcher, indicates that entire species of animals may be lost as a direct result of air pollution. This is especially true for our wildlife where feeding, breeding and environment cannot be controlled. (figure 2)

THE JERSEY JOURNAL, TUESDAY, SEPTEMBER 10, 1968

## Could Kill Entire Species

# Air and Water Pollution Called Peril to Animals

ATLANTIC CITY (UPI) — A University of Pennsylvania researcher said today that air and water polution in our environment is causing animals to lose mating and eating instincts and could wipe out entire species.

Pollution is changing the taste and oder of air, water and is destroying the signals use to select food and mates, Dr. Morley Kare told the 156th annual meeting of the American Chemical Society.

"When we pollute the environment in this way we are in effect blinding some animals," Dr. Kare said. "Smell and taste are as important to some animals as sight is to humans."

Dr. Kare also said more work is needed on finding better foods for animals.

### Need New Sources

"We need to study the taste preferences of domestic animals so we can develop new sources of food for them—sources that will involve foods that are not eaten by humans," he said.

"Humans often assume their pets will like the same foods they do but animals often dislike foods that humans like—and will select foods that humans find unpalatable.

He said that eventually we may not be able to feed our animals on the "cereal grains and high-grade roughages we feed them today."

Figure 2

Air pollution soils and damages buildings and other structures, as well as clothing and home furnishings, thus adding to expenses for cleaning and replacement. Some things, however, cannot ever be replaced. During my recent tour of Europe, I sadly viewed the effects of airborne chemical attack on many of the beautiful buildings and statues. Many of these internationally famed treasures, which have thrilled millions for generations, will completely disintegrate and be lost to the world within a few years unless the rate of deterioration is checked substantially. This tragic loss could not be estimated in dollars.

In our own American cities the effects on buildings can best be realized by the frequent painting requirements and by comparison during the cleaning process as so evident in figure 3.

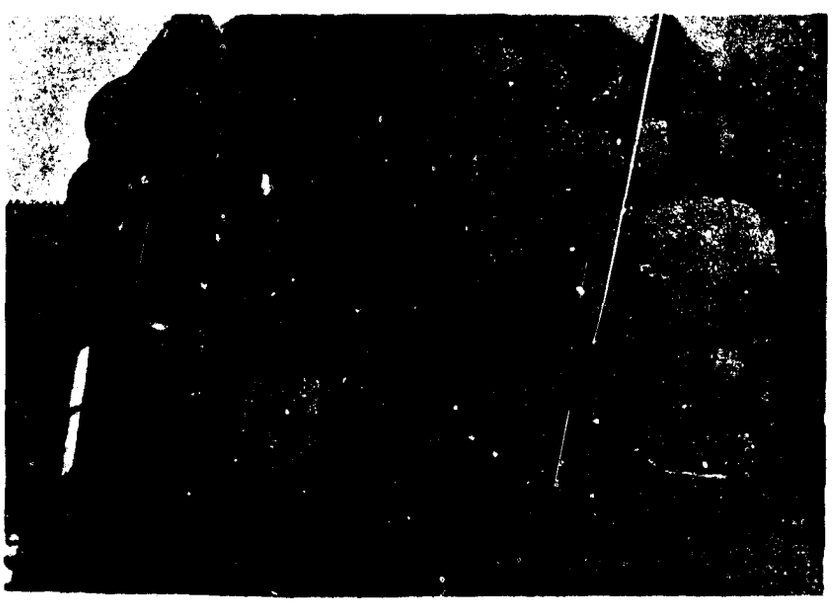

Figure 3

Nowhere is the paradoxical effect of scientific progress on the welfare of man more acutely revealed than in the problem of environmental pollution. Our knowledge of health effects of air pollution has been amplified considerably through three types of investigations:-

1. STATISTICAL STUDIES OF PAST ILLNESS AND DEATH AS CORRELATED WITH GEOGRAPHIC LOCATIONS AND OTHER FACTORS ASSOCIATED WITH AIR POLLUTION.

2. EPIDEMIOLOGICAL STUDIES OF DEATH AND RESPIRATORY FUNCTION AS RELATED TO VARIATIONS IN AIR POLLUTION.

## 3. LABORATORY STUDIES OF RESPONSES BY ANIMALS AND, IN SOME CASES, BY HUMAN BEINGS, TO EXPOSURE TO VARIOUS KNOWN POLLUTANTS OR COMBINATIONS OF POLLUTANTS.

There is no longer any doubt that air pollution is a hazard to health and that it is causally related to many chronic and acute cardiopulmonary diseases. Right heart failure is a direct result of pulmonary disease. *

Figure 4 shows what a normal lung should look like. This particular lung is from a mouse. The ideal pink color, free from discoloration, is not often seen in the human lung.

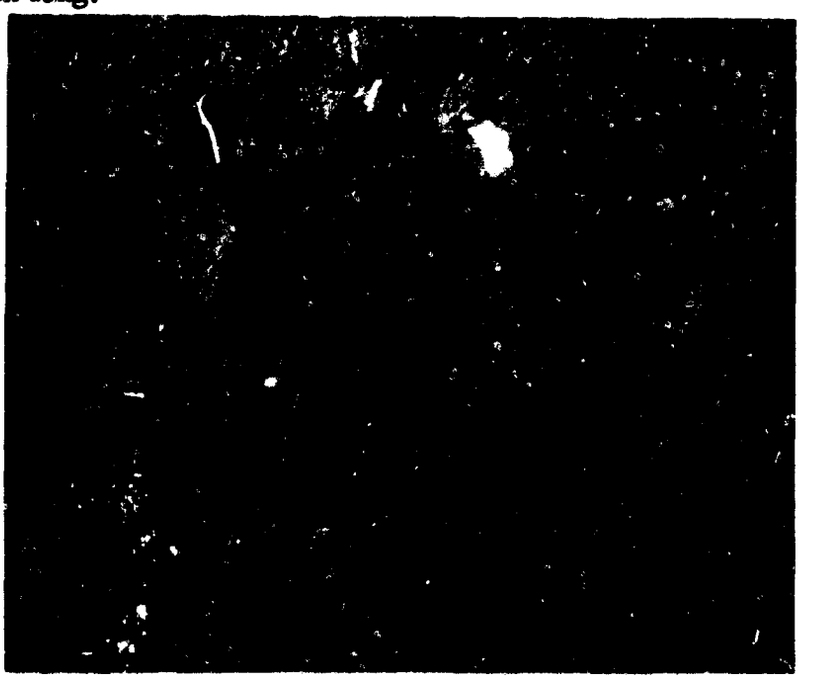

Figure 4

*U. S. P. H. S. Publication #1560

Figure 5. A MORE NORMAL LUNG OF AN ADULT HUMAN

Evidence that this person was a cigarette smoker is commonly accepted; however, this condition exists in most adult humans living in industrialized areas.*

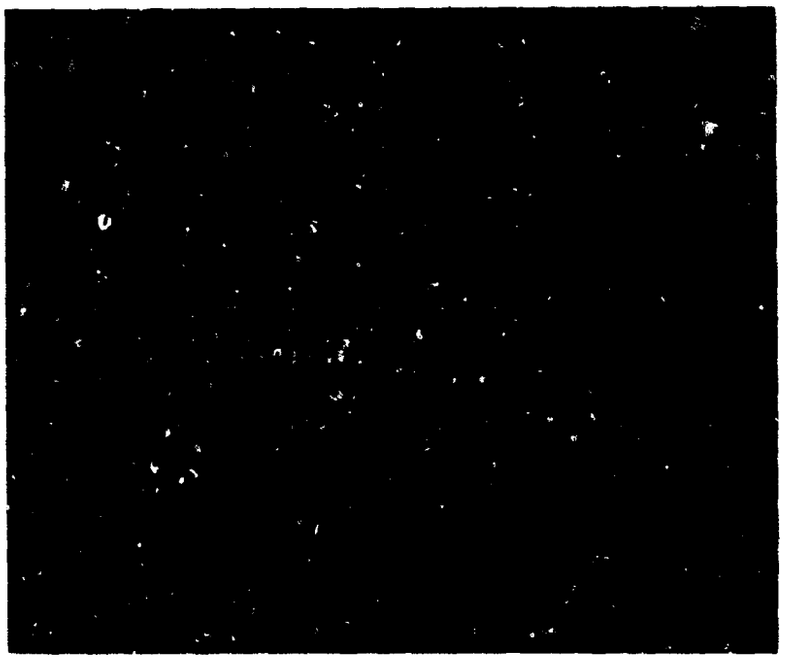

Figure 6. AN EMPHYSEMATOUS LUNG

*Eric J. Cassell, M.D. NTA Bulletin - January, 1965

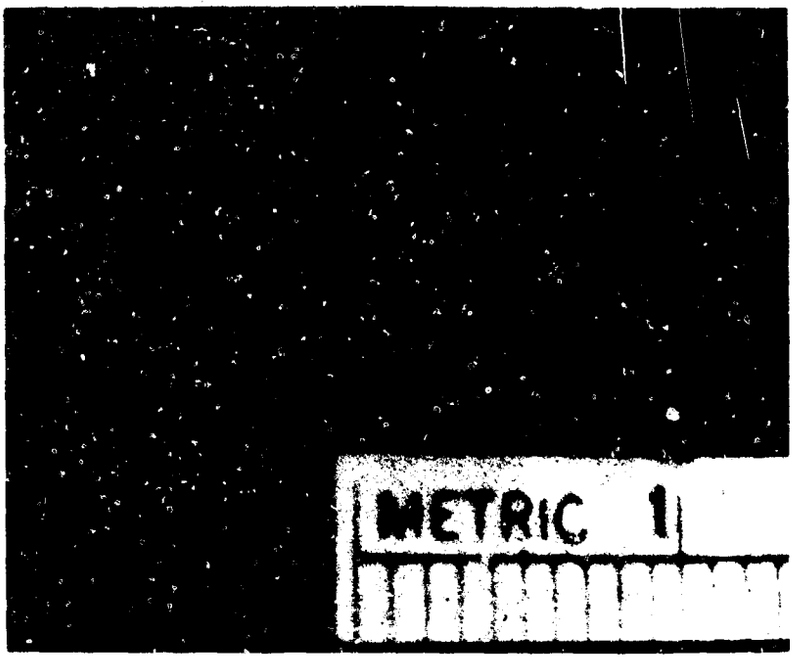


Figure 7. A CLOSE-UP OF THE SAME LUNG SHOWN IN FIGURE 6

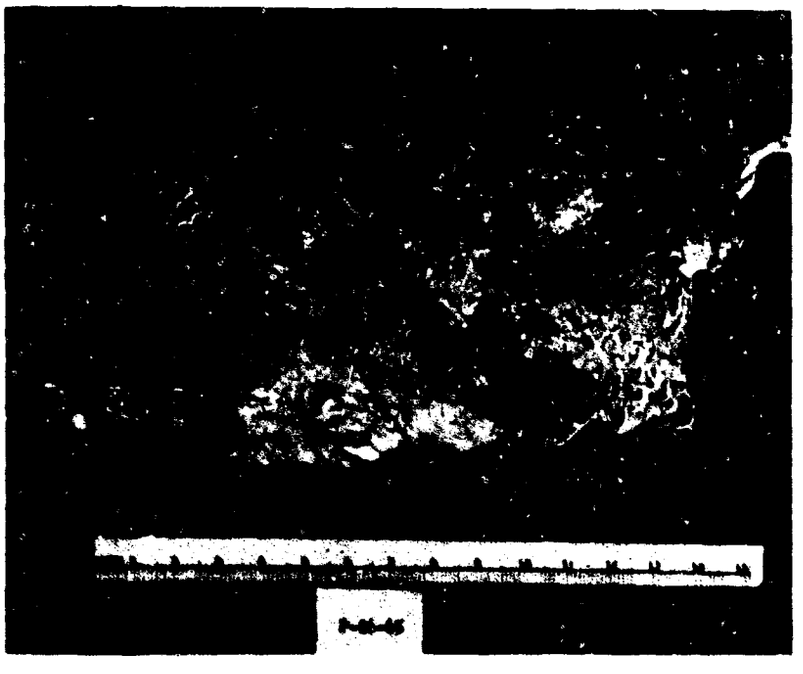

Figure 8. LUNG CANCER

Figure 9 shows a list of the more commonly known contaminants and their known effects on health. I would like to call special attention to the fifth item which is 3, 4 - Benzpyrene. This is the compound generally accepted as the carcinomatous producing by-product of cigarette smoke.* However, a nonsmoker, living in cities such as Birmingham, St. Louis, Chicago, and others, by merely breathing, inhales an equivalent of this compound as is present in the smoke from over 50 cigarettes, or $2\frac{1}{2}$ packs per day!

| Pollutant | | Health Effects |
|---|---|---|
| $SO_2$ Sulfur dioxide<br>$H_2SO_4$ Sulfuric acid | $SO_2$<br>$H_2SO_4$ | Sulfur dioxide and sulfuric acid irritate the nose and throat in concentrations as low as 15 parts per million. in the bronchial tubes, the lining membrane becomes swollen and eroded, and clotting may occur in the small arteries and veins. |
| CO Carbon monoxide | CO | Acute carbon monoxide poisoning results in lowered concentrations of oxygen in the blood and the body tissues. |
| $O_3$ Ozone | $O_3$ | Ozone irritates the air passages, causing chest pain, cough, shortness of breath and nausea. |
| $NO_2$ Nitrogen dioxide | $NO_2$ | Exposure to high concentrations of nitrogen dioxide can result in acute obstruction of the air passages and inflammation of the smaller bronchi. |
| 3, 4-benzpyrene |  | This and similar compounds are known to cause certain types of cancer under laboratory conditions. |
| Olefins | H H<br>H-C=C-H | These substances have an injurious effect on certain body cells, and are especially apt to cause eye irritation. |

Figure 9

Death and morbidity resulting from intense air pollution are well documented. The classic examples of intense air pollution "episodes" are well known. They include the episodes in the Meuse Valley of Belgium; Donora, Pennsylvania; in New York City and in London. During these periods of intense air pollution, brought about by stagnating weather conditions, the number of deaths attributed to air pollutants, not to mention the survivors which were affected, ranged from twenty fatalities in Donora to nearly 5, 000 in London.**

* Dr. Luther Terry's Report
**N. T. A. Bulletin - January, 1965, Franklin Field, O. D.

## SOLUTION

The solution to complete air pollution abatement cannot be resolved by one simple system. It will take a number of contributing factors working effectively together toward the same goal - CLEAN AIR FOR SURVIVAL!

Limiting the types and amounts of contaminants which can be discharged into the atmosphere by improved heating methods, more efficient engines, atomic power, electric autos, better methods of trash disposal and other means are necessary and very important steps in the right direction. However, merely placing limits on the amounts of contaminants is not the complete answer. This is especially true during prolonged inversion periods when the air is trapped in an area permitting even small amounts of contamination to build up to a dangerous level of concentration.

A system of evacuating significant amounts of low level contaminants at or near the source, and even more effectively during inversion periods, can be accomplished in most cities by modifying already existing facilities. Since 90% + of the pollutants are dumped into the atmosphere at, or below, 25 feet off the ground and most of this within one foot, as in the case with automobiles, it appears logical that an effective system would be one which would take advantage of the ideal time (when the total volume of affected air is minimal and close to the ground), and to dispose of the problem before it raised to contaminate such a great volume of air that we become entirely at the mercy of the elements and the dispersing mechanisms of nature. Figure 10 shows the problem during a frontal inversion period.

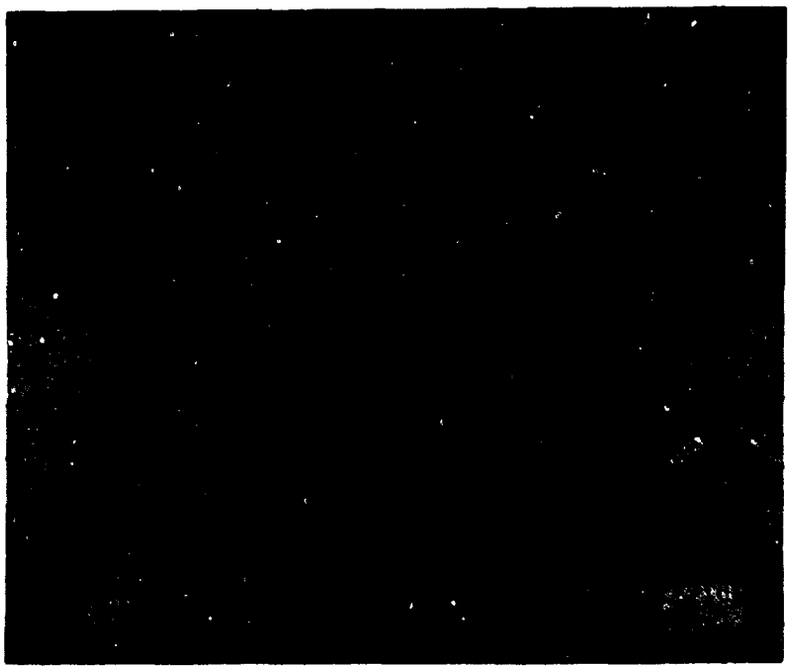

**Figure 10**

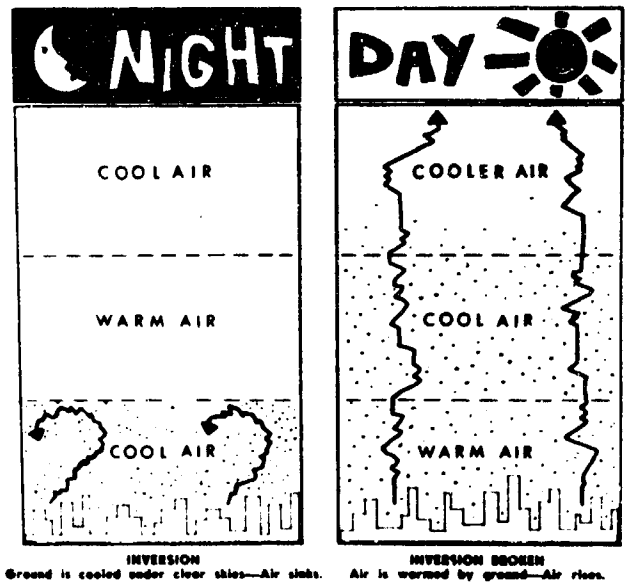


Figure 11. CONDITION DURING NIGHTTIME INVERSION

At nighttime, when the surface of the earth radiates its warmth out to space, the ground cools quickly. In turn, the air in contact with the earth's surface is chilled. By morning, the lowest layers of the air have been considerably cooled while aloft the air temperature has changed little. The result as shown in figure 11 is a temperature inversion which will begin to break up as the ground is once more warmed by the sun's heat. This type of inversion is usually one of lower altitude level than a frontal inversion.

The nighttime inversion holds all of the auto exhaust, heating exhaust and other contaminant buildup. We are entirely dependent upon the sun to come up to warm the ground which, in turn, warms the surface air causing an upward draft.

Figure 12 shows how the heaviest concentrations are held close to the ground, especially at street surface levels.

Now, let us make the picture even a little clearer by showing a container or tub for this lid of inversion air to cover. Figure 13 demonstrates the condition.

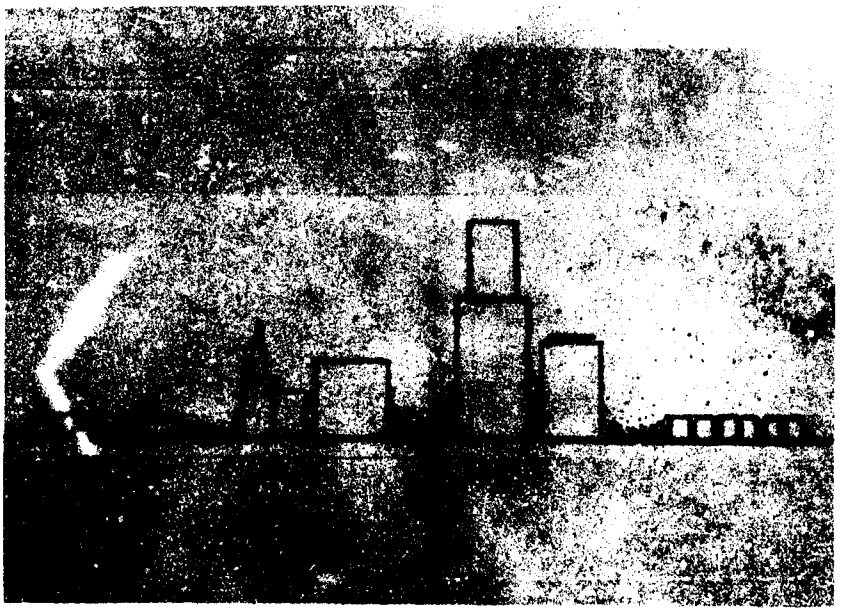

Figure 12

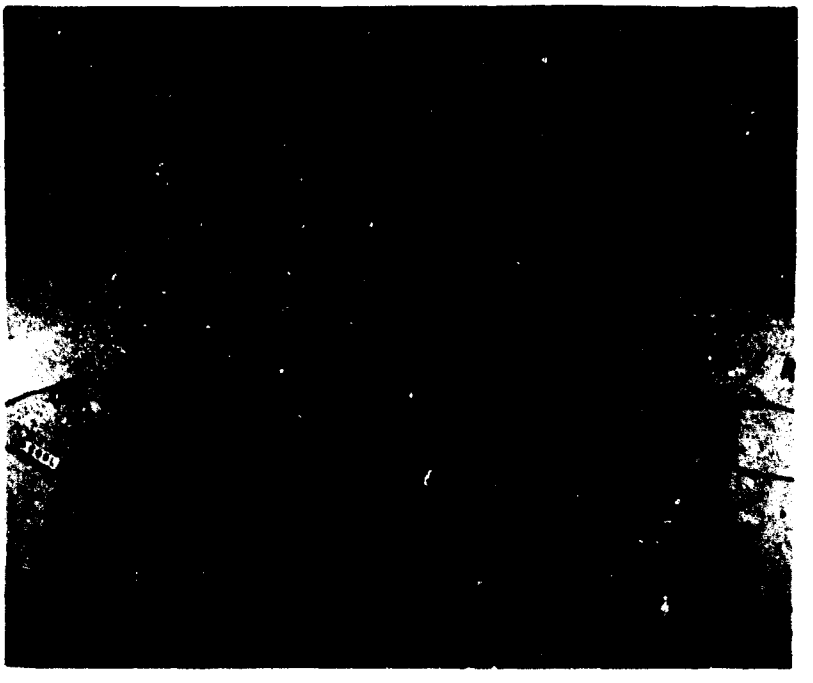

Figure 13

Assume, for the moment, that we have a tubful of dirty water and it is to our advantage to replace it with clean water. How would we do it? Would we boil the water, causing it to steam and eventually evaporate away? That is exactly the process which takes place when the sun comes up to warm the ground (bottom of the tub), which warms the air (water in the tub), causing it to become lighter and

rise upward--hopefully, taking the filth with it. Or, would we attempt to bail the dirty water out over the rim as we would a sinking boat? We would do neither of these. We would simply "PULL THE PLUG". Figure 14 shows this practice being put into use. How would this work since we are really concerned with air, not water?

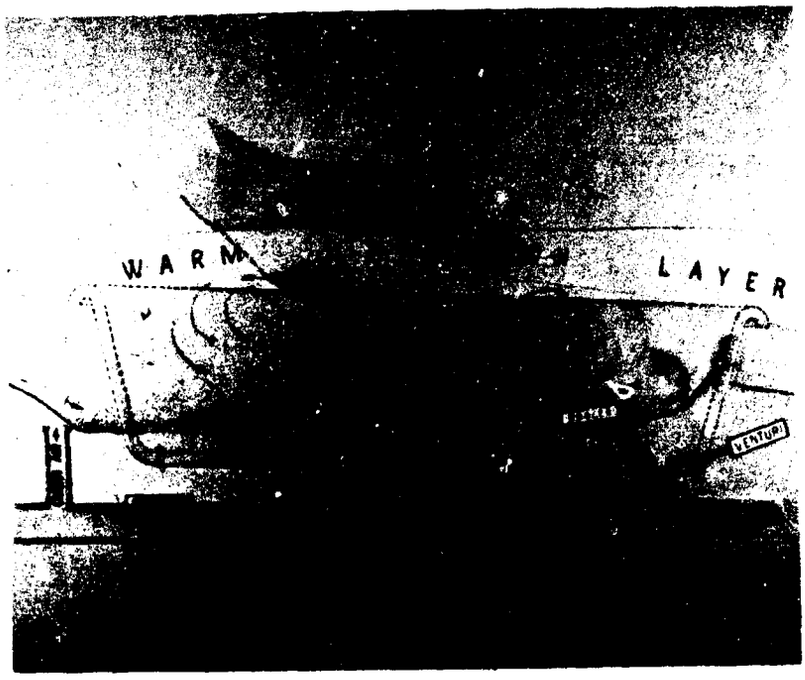


Figure 14

During an inversion period the air trapped under the inversion layer is quite stable and usually free from turbulence or wind currents. Since air has weight, the gravitational attraction of this stable air is toward the center of the earth. Figure 15 illustrates the condition which exists under most streets in planned cities. Note that the heaviest concentration of contamination is close to the street surface. The buildings between the streets act as barriers, funnelling the contaminated air toward the street. Note, too, the storm drains which have been installed to carry off rain and flushing water. These drain pipes range in sizes up to 20 feet in diameter in some cities. The drains are connected to the street surface by curbside catch basins and connecting pipes. Since the storm drains are closer to the earth's center than the street surface there is a greater natural gravitational attraction for the stable polluted air toward the lower space. HERE IS OUR DRAINPIPE FOR LOW LEVEL CONTAMINATION.

Unfortunately, most of the time this avenue of escape is plugged up with cool dormant air. So, how can we utilize this system? Nature, again, has shown us a way in a limited manner by providing rain. As the rain water enters the storm drains via the catch basin inlet and flows through the storm drains it provides the motivating power through surface tension, cohesion and adhesion, which causes the air in the drainpipe above the water level to move. As the air moves in the drainpipe, the air from the street surface rushes in to take its place carrying with it air-borne contaminants. Even this limited movement of air has a significant effect on the smog condition which existed before the rain.

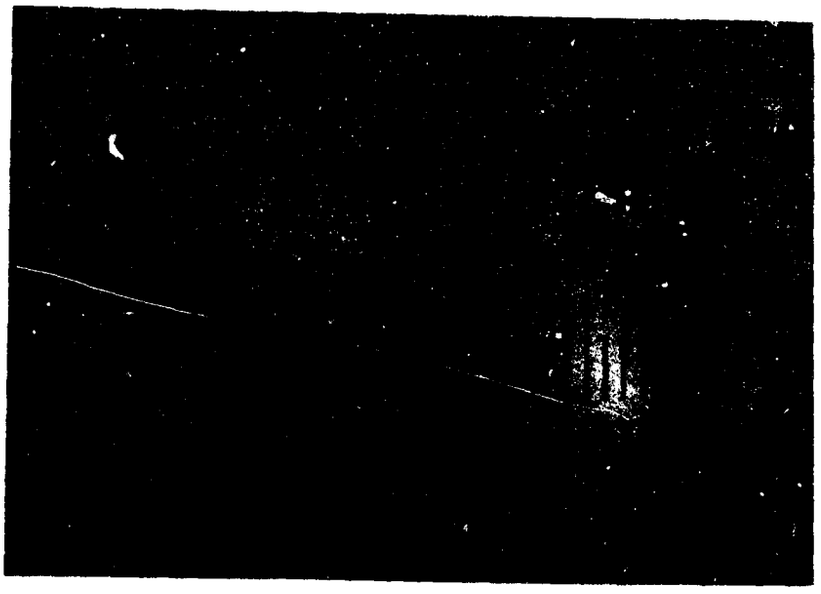

Figure 15

There are several additional factors which play important roles in the overall effect of this lower-than-street-level system.

1. As the air passes through the small catch basin opening and into the smaller connecting tube, the velocity of the air is INCREASED. When it then enters the larger opening of the drain pipe the velocity is DECREASED suddenly. This causes an immediate drop in temperature as a result of the "venturi" effect. Coincidentally, Daniel Bernoulli discovered this effect while working in the sewers of Paris in the 1700's. Bernoulli's venturi effect is one of the standby's of engineering today.

2. In addition to the drop in temperature which may result from the venturi effect, there is usually an additional drop in temperature in the underground tubes. This accelerates condensation which, in turn, enhances the coalescence of molecular bodies causing them to become heavier than air and sink.

3. When the contaminated air enters the storm drain, we now have the air, with the air-borne contaminants, contained where we may apply our skills toward reducing the contamination level through water baths, electronic precipitators, filters, or whatever. This was impossible to accomplish so long as the contaminated air remained in the atmosphere.

4. Reclamation of valuable components which, when permitted to be dispersed by the atmosphere were hazardous to animals and plants alike, now becomes an economic feasibility. Air pollution represents a prodigious waste of potentially valuable resources. The harmful sulfur dioxide that is vented into the American atmosphere, for example, contains well over three hundred million dollars ($300, 000, 000) worth of sulfur at today's prices. *

This engineering approach toward reducing significant amounts of life-threatening air-borne pollutants can be included in City, State and National planning at construction and power requirement costs consistent with our current demands for personal convenience, social advantages, and technological standards. In many metropolitan cities the existing storm drains would require minimal modification.

Our next step is to determine how we might achieve the most effective results utilizing underground conduits as combination storm and air pollution sewer systems. Two methods might be employed to effect control:

1. Since many cities confronted with an air pollution problem do not have access to lake, ocean or river water, we must, in those cases, depend on electricity to move the air. This is not the overwhelming task it might first appear to be.

A good-sized street (including the sidewalks) would be 100 feet wide. Assuming there are 10 city blocks to the mile, this means that the total street and sidewalk area in each block is equal to 100 feet x 528 feet or 52, 800 sq. ft.

We mentioned earlier in this presentation that automobiles emit their contamination within one foot off the ground. Domestic space heating wastes are emitted usually within 25 feet, and in Fall and Spring most of this is during the cool of night. Let us establish, as a starting point, that we desire to lower the street level air at the rate of one foot per minute. This means that every city block would require electric power enough to move 52, 800 cubic feet per minute. With this minimal amount we would be lowering the street level air 60 vertical feet per hour, or 720 vertical feet during the nighttime hours, or 1440 vertical feet in 24 hours.

Figure 16 demonstrates one possible method of installing the system in a city. Note in the drawing that we have installed the evacuating tube in an existing building, and that the space required is comparable to an elevator shaft.

*FORTUNE, November, 1965, Faltermayer, Edmund K.

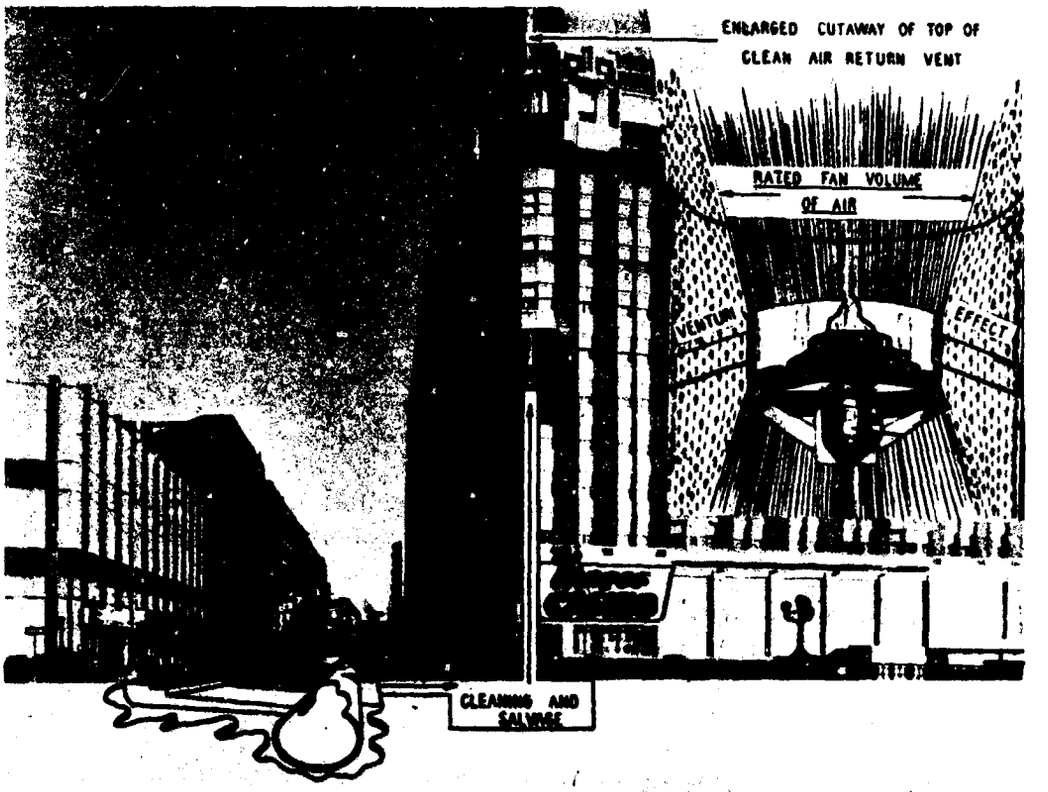


Figure 16

By placing the exhaust fan near the top of the evacuating tube as shown, we gain added air flow through venturi effect, i.e., a 72" fan enclosed in a 73" tube can move 44,900 C.F.M. of free air with a 2 h.p. motor (Robbins & Myers "Propellair" Model 6504-K as certified by A.M.C.A.). By suspending a short section of the 73" tube with the fan enclosed in an evacuating tube 12 feet in diameter, the fan still moves 44,900 C.F.M. through the short section of 73" tube. However, the air which is forced to move through the smaller (venturi) tube now becomes the force which caused the surrounding air in the larger tube to move. The rate at which the total volume is moved by this "venturi effect" is proportional to the velocity of the venturi tube air (the inner tube), the temperature of the air and the resistance within the complete system.

Between the storm drain and the exhaust end we have removed the troublesome contaminants and evacuated the air back into the atmosphere above the low level contamination. Again, note that we are not attempting to change all of the air in the city, but merely "collecting" the low level contaminated air where it originates, and staying ahead of the problem so it cannot contaminate too much air.

2. In those areas where water is plentiful it would be advantageous to pump water to the highest points in the storm drain system, permitting gravity to return the water to the lowest point, thereby supplying a form of water power. Creating turbulence in the water on its return trip would increase the movement of air. A combination of water and electric power would have the added advantages

of maintaining lower temperatures within the storm drains, accelerating condensation of the contaminated air, and supplying water baths where necessary.

In addition to supplying a movement of air, it will be necessary to redesign our catch basin inlets into the storm drain and to install a greater number than is usually installed to control storm water alone. The new design would necessarily include the most effective venturi features whereby we might take all advantages the venturi effect affords.

For you who might question the cost of this type of future city planning, consider what it could mean to the economy of the world and the number of jobs it would create which would not be dependent on war or peace: construction, fans and motors, cement and metal pipes, filters, precipitators--I could go on and on. In addition, atomic power is in our future to supply the added need for electricity. If we don't look ahead toward clean air, we will have nothing to look ahead to !!

## SUMMARY

A method for significantly reducing concentrations of low-level air-borne contaminants in populated areas has been described. Advantages leading toward the healthy well-being of man, plants and animals, and the influence toward economic improvement include the following:

1. THE ATMOSPHERIC PHENOMENA REFERRED TO AS FRONTAL AND NIGHTTIME INVERSION WHICH NOW PREVENTS THE NORMAL ATMOSPHERIC DISPERSION MECHANISM TO EFFECTIVELY MAINTAIN A CLEAN AIR CONDITION WOULD, IN THIS METHOD, BECOME AN ANCILLARY FORCE ENHANCING THE EFFECTIVENESS OF THE METHOD DESCRIBED.

2. THE CONTAMINANTS WOULD BE "COLLECTED" AT OR NEAR THEIR SOURCE. THIS WOULD PREVENT THE BUILD-UP OF CONTAMINATION AND THE SPREADING OF TOXIC MATERIALS.

3. VALUABLE COMPONENTS WOULD BE DEPOSITED IN STORAGE TANKS OR CONTAINERS MAKING SALVAGE OPERATIONS ECONOMICALLY FEASIBLE.

4. DETERIORATION OF BUILDINGS, HOMES AND STRUCTURES DUE TO AIR-BORNE CHEMICAL REACTION WOULD BE GREATLY REDUCED.

5. THIS SYSTEM, IF PUT INTO GENERAL USE, WOULD CREATE A NEW DEMAND FOR PRODUCTS AND SERVICES OF MANUFACTURERS, BUILDERS AND SUPPLIERS. THE MAGNITUDE AND DIVERSIFICATION OF THIS NEW DEMAND WOULD RESULT IN A PEACE-TIME ECONOMIC CONDITION NEVER BEFORE ENJOYED BY MAN.

6. MOST IMPORTANT--THIS SYSTEM WOULD HELP MAN IN HIS EFFORT TO MAINTAIN AN ATMOSPHERE IN WHICH HE, HIS PLANTS AND HIS ANIMALS COULD EXIST.

## DISCUSSION

QUESTION: I presume all of your pollutants are carried along in an airstream which blows the water stream, is that right?

MR. MC KAY (University of Nevada): Well, I think, primarily, what I'm bringing about, as long as somebody called it air pollution, I don't know why we are keeping it in the air, but for some reason we're keeping it up there. All I'm advocating is that we should get it in a confined area where we can do something with it. We are dumping in this country 150 million tons of pollutants into air every year. That represents a train of coal cars coupled, end to end, 36,000 miles long, or twelve times across the United States, so I may not be answering your question exactly the way you want me to but I want to get this underground, whether it's going to take water baths, electronic precipitators or a lot more power than we are now able to produce to clean the air up. At least we could put it in a confined area where we can do something with it.

QUESTION: All I was asking, sir, what happens to these air pollutants when they hit the water stream?

MR. MC KAY: It depends. It depends on what the pollutant is. Some of them are not water soluble and we have a problem there. We have to cross each bridge when we come to it. Water soluble pollutants, of course, are washed down, go through a sump or a common sump system, and we can capture them and recover them and use them for industrial fertilizer purposes, or whatever.

DR. PIERSON (Lockheed Aircraft Corporation): I'm kind of curious. This assumes that the air is being evacuated immediately over the city. However, I don't think the problem is quite that simple. A lot of plants which produce particulate matter have been built away from cities deliberately. Would this system not draw these types of smog back into the city? For example, the real problem now in the San Bernardino Mountains is the fact that the smog and the pollutants in that area are destroying the vegetation clear up as far as Arrowhead and Running Springs. Would you not be pulling that back into the Los Angeles basin itself?

MR. MC KAY: Bill, I think that as far as industrial plants are concerned that this is really no problem. It may be presently but legislation is being passed and it's becoming more and more necessary for each industrial plant to take care of its own problem, and we have very efficient scrubbers and types of mechanical and electronic devices so that they can be prevented from this. However, it's automobiles, and even domestic heating. We're using chimneys on our

housing that are as antiquated as those of the Indians. Why do we need a chimney on our house when with a controlled fan we can not only capture more heat from our fuel but we can run it through our precipitator and dump it down into a septic tank, which I'm doing at home, and never dump this into the air. This is our big problem, the automobiles and the homes and the type of things that we can't control. We are going to have to face facts; if we don't and we don't limit these industrial plants from dumping all this garbage in the air, we are not going to be around very long.

MR. WANDS: I think one of the important things about this proposal is that it is very apropos for the spacecraft. In the case of an unbearable condition of air pollution within the spacecraft, it has already been proposed that we simply pull the plug and evacuate the thing, that is to put on EVA equipment and open the hatch and let all the contamination out, so that this suggestion is directly applicable to our problem at hand here today, contamination in spacecraft.

Before I introduce our next speaker, I would like to make one other announcement. Earlier this morning there was some discussion following Dr. Albert's paper about the availability of the recommendations of the committee on which he sat and for which he spoke today. It was suggested at that time that this might be available from the National Research Council. I think it might be better for those of you desiring copies of the report on Air Pollution Criteria for 100 and 1000-day Space Flights to request this from NASA. Reports from the National Academy of Sciences are normally made to their sponsoring agencies and are the property of the sponsoring agency for distribution. On occasion, the Academy will undertake to issue these as publications from the Academy at the request of the agency, but until such time as that is done, I suggest you direct your requests for that committee's report to Dr. Walton L. Jones of the Office of Advanced Research and Technology, National Aeronautics and Space Administration Washington, D.C.

AMRL-TR-68-175

PAPER NO. 5

# A COMPARISON OF EXPERIMENTAL CONTROL ANIMALS HOUSED IN ALTITUDE CHAMBERS AND ANIMAL ROOMS

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and
Gary L. Fogle

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Wright-Patterson Air Force Base, Ohio

## INTRODUCTION

Thomas Domes are unique inhalation exposure chambers designed to study the toxicity of various atmospheric contaminants to animals in simulated space cabin atmospheres and pressures. Since their inception, these domes have been utilized for exposures ranging in duration from one week to several months. Dome atmospheres of pure oxygen, pure air, or oxygen-air mixtures have been used at pressures ranging from 260-760 mm Hg for these experiments. In those studies lasting more than three months, it was impractical to maintain control animals in altitude chambers since these were continually used for exposures. This then precluded the possibility of subjecting the control and test animals to identical environmental conditions. Among the environmental conditions are operational factors peculiar to the dynamic altitude chambers, such as elevated noise and odor levels and high animal population density which might influence the health status of the animals.

When a temporary halt was imposed on research conducted at elevated oxygen concentrations in order to establish rigorous fire safeguards, an opportunity arose to investigate the nature of the Thomas Dome environment as it affects animal growth and health. To this end, a long-term ambient study was carried out in a dome for a period of 165 days.

## MATERIALS AND METHODS

An altitude chamber, one of the Thomas Domes, in the AMRL facilities was used in this test. The altitude facilities were previously described in detail (Culver, 1966; Fairchild, 1967; and Thomas, 1965). In short, these specially designed chambers can be operated at reduced pressure with either a single gas or gas mixture of any desired oxygen-air mixture. The chamber can be automatically controlled to within 5 degrees of the desired temperature and 10% of the desired relative humidity. The chamber is operated in the dynamic mode with no attempt to recycle the exhaust gases.

Experimental Conditions

The operating conditions of the chamber used for the experiment are listed in table I.

TABLE I

EXPOSURE CHAMBER OPERATING CONDITIONS

| | |
|---|---|
| Atmospheric Composition | Ambient Air |
| $CO_2$ | 0.25 (0.12-0.78)% |
| Total Pressure | 720 mm Hg |
| Flow Rate | 37 (20-47) cfm |
| Temperature | 72 (67-76) F |
| Humidity | 51 (44-60)% RH |

Experimental animals used in these tests were Beagle dogs, Rhesus monkeys, rats, and mice. At the start of the study, their weights were: dogs, 3.9-5.5 kilograms; monkeys, 2.7-5.0 kilograms; rats, 154-186 grams; and mice, 20-25 grams. All animals were subjected to routine examination and placed in quarantine upon receipt from commercial sources. The appropriate biweekly clinical baseline measurements of hematology, blood chemistry and body weight were made 10 times before beginning the actual test. The large number of preexposure examinations resulted from the suspension of experimentation with oxygen-enriched environments and the subsequent reassignment of animals.

During the course of the experiment, all animals were observed routinely at 30-minute intervals. Mice were maintained for mortality and pathology information only. Body weights of rats, and blood chemistry determinations made on dogs and monkeys, were recorded biweekly. The blood chemistry examination included the following parameters: hematocrit, hemoglobin, red and white blood cell counts, sodium, potassium, calcium, total protein, albumin, SGOT*, SGPT**, alkaline phosphatase, and total phosphorus. Twelve blood chemistry determinations were made during the exposure period. At necropsy, tissue samples for histopathological examination were taken from all species except the mice. Organ weights of rats were recorded at necropsy and used in calculating organ/body weight ratios.

* Serum glutamic oxaloacetic transaminase
** Serum glutamic pyruvic transaminase

Table II is a summary of the animal population involved in this study.

TABLE II

| | Exposed | Control |
|---|---|---|
| Mice | 40 M | 40 M |
| Rats | 25 M, 25 F | 25 M, 25 F |
| Dogs | 4M, 4F | |
| Monkeys | 2M, 2F | |

## Experimental Results

The study was conducted without interruption and all dome atmospheric factors were maintained within the limits of the experimental protocol. No adverse effects due to exposure conditions were demonstrated by any of the animals in the test or control group. The health status of the experimental population remained as before the onset of the study with the exception of one male test rat that died 10 days following the initiation of the study.

## Organ and Body Weight Data

Mean body weights of male rats showed no significant difference between test and control groups. Female rat body weights for the control and test groups were comparable during the early stages of the test. As the experiment progressed (from the third month on) the growth rate of the control group was slightly above the test group. Comparison with similar groups of female rats indicated that the growth of the control group was above average while the growth rate for the test animals was identical to previous control groups. Growth rates of the dogs and monkeys were normal.

Examination of rat organ weights and organ to body weight ratios, shown in table III, exhibited no differences between the exposed and control groups with the exception of kidney to body weight ratio in the female rat. The small difference significant at the 0.05 level is unexplainable since there were no demonstrable pathologic differences.

# PATHOLOGY

Gross and histopathologic examination of the experimental animal tissues did not show any differences between the exposed and control groups. Although the rodents showed some evidence of chronic murine pneumonia, the incidence was identical in the two groups.

TABLE III

MEAN ORGAN/BODY WEIGHT RATIOS OF ALBINO RATS

| | | MALE | | FEMALE | |
|---|---|---|---|---|---|
| | | Test | Control | Test | Control |
| Heart | $\bar{x}$ | 0.299 | 0.311 | 0.338 | 0.337 |
| | S.D.* | 0.050 | 0.026 | 0.028 | 0.030 |
| Lung | $\bar{x}$ | 0.439 | 0.442 | 0.600 | 0.602 |
| | S.D. | 0.060 | 0.069 | 0.093 | 0.115 |
| Liver | $\bar{x}$ | 2.987 | 3.137 | 4.020 | 4.217 |
| | S.D. | 0.562 | 0.756 | 0.456 | 0.309 |
| Spleen | $\bar{x}$ | 0.283 | 0.263 | 0.292 | 0.268 |
| | S.D. | 0.053 | 0.083 | 0.067 | 0.068 |
| Kidney | $\bar{x}$ | 0.636 | 0.659 | 0.761 | 0.719 |
| | S.D. | 0.058 | 0.067 | 0.057 | 0.057 |

*= Standard Deviation

Dogs and monkeys housed in the chamber showed a lower than normal incidence of the usual enzootic pathologic changes seen in these species and were considered to be unaffected by the environmental factors to which they were exposed.

## Hematology and Blood Chemistry

A total of 13 different hematology and blood chemistry determinations were performed on each blood sample. Dogs and monkeys were bled biweekly prior to and during the exposure. The results of these examinations are shown in tables IV and V. Both hematologic and blood chemistry values remained within "normal limits" for the population and appeared more stable than the pretest values with a decreased variance about the mean.

TABLE IV

BLOOD CHEMISTRY DETERMINATIONS
(Mean Group Values)

| | TOTAL PHOSPHORUS (mg %) | | | | ALKALINE PHOSPHATASE (Units/ml) | | | |
|---|---|---|---|---|---|---|---|---|
| | DOG | | MONKEY | | DOG | | MONKEY | |
| Sample Period | Male | Female | Male | Female | Male | Female | Male | Female |
| | Preexposure Mean Values | | | | | | | |
| 1 | 6.7 | 5.6 | 5.5 | 6.5 | 3.0 | 2.8 | 22.8 | 22.0 |
| 2 | 6.1 | 5.9 | 5.3 | 5.4 | 1.7 | 1.9 | 21.8 | 17.0 |
| 3 | 5.4 | 6.1 | 4.9 | 5.5 | 1.3 | 1.8 | 17.8 | 15.3 |
| 4 | 5.2 | 5.2 | 4.7 | 5.8 | 1.3 | 1.6 | 17.8 | 12.5 |
| 5 | 4.9 | 5.2 | 5.5 | 6.4 | 1.3 | 1.1 | 16.8 | 19.9 |
| 6 | 4.9 | 5.2 | 5.7 | 4.9 | 0.9 | 1.3 | 18.8 | 17.1 |
| 7 | 4.8 | 4.7 | 5.6 | 4.9 | 1.3 | 1.5 | 20.0 | 23.8 |
| 8 | 4.7 | 4.4 | 5.2 | 5.0 | 0.4 | 1.3 | 18.0 | 27.8 |
| 9 | 4.6 | 3.9 | 4.5 | 6.3 | 0.9 | 1.1 | 19.5 | 23.9 |
| 10 | 4.4 | 4.3 | 5.1 | 5.2 | 0.4 | 0.7 | 17.5 | 22.0 |
| | Exposure Mean Values | | | | | | | |
| 1 | 6.0 | 5.3 | 6.6 | 7.8 | 1.1 | 1.2 | 15.8 | 20.3 |
| 2 | 5.1 | 5.1 | 6.7 | 7.0 | 0.8 | 1.3 | 14.5 | 21.8 |
| 3 | 5.7 | 5.3 | 4.5 | 6.7 | 1.0 | 1.1 | 19.5 | 22.8 |
| 4 | 5.7 | 5.1 | 5.5 | 5.7 | 0.6 | 0.6 | 15.6 | 19.0 |
| 5 | 5.1 | 5.4 | 5.1 | 6.5 | 0.8 | 1.1 | 17.5 | 23.0 |
| 6 | 4.4 | 5.2 | 5.3 | 5.8 | 1.1 | 1.2 | 9.5 | 17.0 |
| 7 | 3.7 | 4.5 | 5.1 | 5.7 | 0.8 | 0.7 | 17.9 | 22.0 |
| 8 | 3.7 | 4.6 | 4.9 | 5.7 | 0.4 | 0.4 | 10.1 | 9.0 |
| 9 | 5.0 | 4.4 | 5.9 | 6.2 | 0.5 | 0.7 | 9.8 | 11.8 |
| 10 | 3.8 | 4.2 | 5.3 | 5.5 | 0.6 | 0.7 | 10.9 | 8.7 |
| 11 | 4.1 | 4.2 | 4.9 | 5.5 | 0.9 | 1.0 | 10.1 | 13.1 |
| 12 | 3.5 | 3.9 | 5.1 | 5.0 | 0.4 | 1.9 | 13.0 | 11.1 |
| N= | 4 | 4 | 2 | 2 | 4 | 4 | 2 | 2 |

TABLE IV (Cont'd)

BLOOD CHEMISTRY DETERMINATIONS

| | SODIUM (mEq/1) | | | | POTASSIUM (mEq/1) | | | |
|---|---|---|---|---|---|---|---|---|
| | DOG | | MONKEY | | DOG | | FEMALE | |
| Sample Period | Male | Female | Male | Female | Male | Female | Male | Female |
| | Preexposure Mean Values | | | | | | | |
| 1 | 143 | 146 | 148 | 152 | 4.53 | 4.93 | 4.55 | 5.20 |
| 2 | 142 | 146 | 148 | 147 | 5.08 | 4.60 | 4.95 | 3.60 |
| 3 | 143 | 146 | 148 | 147 | 4.50 | 4.90 | 4.60 | 4.10 |
| 4 | 145 | 146 | 151 | 153 | 4.40 | 4.60 | 4.80 | 4.90 |
| 5 | 145 | 145 | 145 | 149 | 4.48 | 4.60 | 4.30 | 4.70 |
| 6 | 148 | 148 | 144 | 150 | 4.43 | 4.70 | 4.45 | 4.15 |
| 7 | 145 | 146 | 147 | 149 | 4.23 | 4.25 | 5.80 | 4.40 |
| 8 | 143 | 144 | 146 | 150 | 4.65 | 4.60 | 5.80 | 6.70 |
| 9 | 141 | 142 | 146 | 148 | 4.58 | 4.38 | 4.45 | 5.35 |
| 10 | 146 | 146 | 146 | 149 | 4.60 | 4.73 | 4.25 | 4.20 |
| | Exposure Mean Values | | | | | | | |
| 1 | 146 | 144 | 146 | 145 | 5.33 | 5.03 | 5.00 | 4.80 |
| 2 | 147 | 145 | 146 | 147 | 4.88 | 4.80 | 4.55 | 4.05 |
| 3 | 146 | 144 | 145 | 149 | 4.88 | 4.60 | 4.40 | 4.80 |
| 4 | 145 | 146 | 154 | 152 | 5.23 | 4.93 | 5.25 | 5.25 |
| 5 | 146 | 144 | 151 | 155 | 4.75 | 4.70 | 4.40 | 4.95 |
| 6 | 148 | 147 | 149 | 150 | 4.83 | 4.85 | 4.90 | 4.95 |
| 7 | 143 | 144 | 152 | 153 | 4.63 | 4.63 | 4.60 | 4.50 |
| 8 | 145 | 147 | 149 | 151 | 4.73 | 4.93 | 4.45 | 4.40 |
| 9 | 147 | 148 | 150 | 152 | 4.78 | 4.45 | 4.30 | 4.70 |
| 10 | 152 | 151 | 158 | 157 | 4.55 | 4.68 | 4.40 | 4.40 |
| 11 | 147 | 147 | 148 | 156 | 4.58 | 4.65 | 4.40 | 4.90 |
| 12 | 152 | 150 | 153 | 156 | 4.45 | 4.38 | 4.35 | 4.30 |
| N= | 4 | 4 | 2 | 2 | 4 | 4 | 2 | 2 |

TABLE IV (Cont'd)

BLOOD CHEMISTRY DETERMINATIONS

| | SGPT (Units/ml) | | | | SGOT (Units/ml) | | | |
|---|---|---|---|---|---|---|---|---|
| | DOG | | MONKEY | | DOG | | MONKEY | |
| Sample Period | Male | Female | Male | Female | Male | Female | Male | Female |
| | Preexposure Mean Values | | | | | | | |
| 1 | 23.0 | 24.3 | 28.0 | 26.5 | 24.0 | 35.3 | 33.0 | 45.0 |
| 2 | 26.5 | 23.5 | 30.5 | 29.5 | 19.8 | 32.8 | 42.5 | 45.0 |
| 3 | 22.8 | 19.8 | 25.0 | 26.5 | 22.3 | 25.3 | 27.0 | 35.5 |
| 4 | 25.0 | 27.3 | 33.0 | 25.0 | 20.8 | 25.3 | 31.0 | 40.0 |
| 5 | 21.8 | 21.8 | 32.0 | 30.5 | 18.3 | 20.5 | 40.0 | 55.0 |
| 6 | 22.5 | 20.8 | 31.5 | 29.5 | 23.3 | 23.0 | 35.5 | 42.5 |
| 7 | 24.0 | 24.3 | 35.0 | 28.0 | 22.3 | 25.0 | 33.0 | 40.0 |
| 8 | 29.3 | 23.5 | 37.0 | 26.5 | 21.5 | 18.3 | 43.0 | 37.5 |
| 9 | 25.0 | 23.5 | 26.5 | 23.5 | 24.0 | 26.3 | 24.0 | 11.5 |
| 10 | 28.0 | 23.5 | 28.5 | 26.5 | 23.5 | 23.5 | 38.0 | 40.0 |
| | Exposure Mean Values | | | | | | | |
| 1 | 28.8 | 28.0 | 31.5 | 28.5 | 28.8 | 26.0 | 40.0 | 42.5 |
| 2 | 35.3 | 32.5 | 28.0 | 33.5 | 23.0 | 32.0 | 37.5 | 47.5 |
| 3 | 46.0 | 36.8 | 42.5 | 29.5 | 38.8 | 41.3 | 62.0 | 50.0 |
| 4 | 32.5 | 30.8 | 33.5 | 23.5 | 21.5 | 15.3 | 26.0 | 24.0 |
| 5 | 31.8 | 30.8 | 33.5 | 25.0 | 34.3 | 32.8 | 37.5 | 40.0 |
| 6 | 32.0 | 34.0 | 31.0 | 25.0 | 25.3 | 27.3 | 28.5 | 26.0 |
| 7 | 37.8 | 38.8 | 33.0 | 31.5 | 28.5 | 33.3 | 37.5 | 37.5 |
| 8 | 39.5 | 36.0 | 44.5 | 31.0 | 30.8 | 32.0 | 40.0 | 38.0 |
| 9 | 47.8 | 34.0 | 38.5 | 29.5 | 35.3 | 34.3 | 45.0 | 42.5 |
| 10 | 30.8 | 30.0 | 31.5 | 23.5 | 23.5 | 25.3 | 35.5 | 27.0 |
| 11 | 39.5 | 34.0 | 38.5 | 37.0 | 34.3 | 34.0 | 37.5 | 42.5 |
| 12 | 37.8 | 41.8 | 40.5 | 33.5 | 42.5 | 60.0 | 62.0 | 42.5 |
| N= | 4 | 4 | 2 | 2 | 4 | 4 | 2 | 2 |

TABLE IV (Cont'd)

BLOOD CHEMISTRY DETERMINATIONS

| | TOTAL PROTEIN (gm %) | | | | ALBUMIN (gm %) | | | |
|---|---|---|---|---|---|---|---|---|
| | DOG | | MONKEY | | DOG | | FEMALE | |
| Sample Period | Male | Female | Male | Female | Male | Female | Male | Female |
| | Preexposure Mean Values | | | | | | | |
| 1 | 6.1 | 6.0 | 7.9 | 7.8 | 3.6 | 3.8 | 4.6 | 4.6 |
| 2 | 6.2 | 5.8 | 7.8 | 7.4 | 3.4 | 3.3 | 4.9 | 4.4 |
| 3 | 6.2 | 5.8 | 7.8 | 7.6 | 3.3 | 3.4 | 4.8 | 4.4 |
| 4 | 6.2 | 6.0 | 8.1 | 8.2 | 3.5 | 3.6 | 4.9 | 4.7 |
| 5 | 6.3 | 5.9 | 7.7 | 7.5 | 3.5 | 3.5 | 4.6 | 4.3 |
| 6 | 6.3 | 6.1 | 7.7 | 7.3 | 3.5 | 3.5 | 4.6 | 4.2 |
| 7 | 6.4 | 6.2 | 7.6 | 7.8 | 3.5 | 3.5 | 4.7 | 4.9 |
| 8 | 6.5 | 6.4 | 8.0 | 7.8 | 3.6 | 3.5 | 4.8 | 4.7 |
| 9 | 6.4 | 6.2 | 7.7 | 7.2 | 3.7 | 3.5 | 4.5 | 4.5 |
| 10 | 6.3 | 6.1 | 7.1 | 7.2 | 3.6 | 3.5 | 4.3 | 4.2 |
| | Exposure Mean Values | | | | | | | |
| 1 | 6.6 | 6.3 | 8.1 | 7.4 | 3.4 | 3.4 | 4.6 | 4.6 |
| 2 | 6.3 | 6.1 | 7.9 | 7.7 | 3.5 | 3.5 | 4.6 | 4.7 |
| 3 | 6.9 | 6.5 | 8.2 | 8.2 | 3.6 | 3.5 | 4.6 | 5.0 |
| 4 | 6.5 | 6.2 | 8.2 | 7.8 | 3.5 | 3.4 | 4.8 | 4.8 |
| 5 | 6.5 | 6.4 | 7.8 | 8.2 | 3.4 | 3.6 | 4.7 | 4.7 |
| 6 | 6.4 | 6.5 | 7.9 | 8.0 | 3.4 | 3.4 | 4.5 | 4.9 |
| 7 | 6.4 | 6.3 | 8.0 | 8.0 | 3.4 | 3.3 | 4.7 | 4.8 |
| 8 | 6.5 | 6.2 | 7.6 | 7.9 | 3.2 | 3.2 | 4.9 | 4.6 |
| 9 | 6.7 | 6.5 | 8.3 | 8.1 | 3.5 | 3.5 | 4.7 | 4.7 |
| 10 | 6.3 | 6.5 | 8.1 | 8.0 | 3.4 | 3.5 | 5.0 | 4.6 |
| 11 | 6.7 | 6.3 | 7.8 | 8.6 | 3.5 | 3.3 | 4.7 | 4.8 |
| 12 | 6.8 | 6.5 | 8.1 | 8.1 | 3.4 | 3.4 | 4.7 | 4.5 |
| N= | 4 | 4 | 2 | 2 | 4 | 4 | 2 | 2 |

TABLE IV (Cont'd)

BLOOD CHEMISTRY DETERMINATIONS

CALCIUM (mEq/1)

| Sample Period | DOG | | MONKEY | |
|---|---|---|---|---|
| | Male | Female | Male | Female |
| | Preexposure Mean Values | | | |
| 1 | 5.63 | 5.65 | 6.00 | 5.95 |
| 2 | 5.65 | 5.75 | 5.95 | 5.40 |
| 3 | 5.68 | 5.58 | 5.70 | 5.40 |
| 4 | 5.58 | 5.83 | 6.10 | 5.80 |
| 5 | 5.60 | 5.78 | 5.80 | 5.50 |
| 6 | 5.68 | 5.60 | 5.90 | 5.40 |
| 7 | 5.48 | 5.48 | 5.70 | 5.55 |
| 8 | 5.70 | 5.63 | 5.70 | 6.15 |
| 9 | 5.70 | 5.70 | 5.65 | 5.55 |
| 10 | 5.65 | 5.55 | 5.30 | 5.65 |
| | Exposure Mean Values | | | |
| 1 | 5.78 | 5.70 | 5.60 | 5.50 |
| 2 | 5.68 | 5.75 | 5.70 | 5.70 |
| 3 | 5.50 | 5.35 | 5.55 | 5.85 |
| 4 | 5.65 | 5.68 | 5.85 | 5.85 |
| 5 | 5.43 | 5.50 | 5.70 | 5.90 |
| 6 | 5.85 | 5.63 | 5.60 | 5.95 |
| 7 | 5.60 | 5.48 | 5.70 | 5.80 |
| 8 | 5.45 | 5.40 | 5.10 | 5.50 |
| 9 | 5.73 | 5.68 | 5.60 | 5.70 |
| 10 | 5.08 | 5.15 | 5.30 | 5.40 |
| 11 | 5.18 | 5.08 | 5.25 | 5.45 |
| 12 | 5.63 | 5.38 | 5.40 | 5.70 |
| N= | 4 | 4 | 2 | 2 |

TABLE V

BLOOD HEMATOLOGY DETERMINATIONS

| | RBC (x $10^6$) | | | | WBC (x $10^3$) | | | |
|---|---|---|---|---|---|---|---|---|
| | DOG | | MONKEY | | DOG | | MONKEY | |
| Sample Period | Male | Female | Male | Female | Male | Female | Male | Female |
| | Preexposure Mean Values | | | | | | | |
| 1 | 5.8 | 6.3 | 5.7 | 5.6 | 14.8 | 12.5 | 12.4 | 16.3 |
| 2 | 5.8 | 6.0 | 5.7 | 5.4 | 12.3 | 13.1 | 11.9 | 14.3 |
| 3 | 6.1 | 6.3 | 5.6 | 5.4 | 14.2 | 13.4 | 11.5 | 13.9 |
| 4 | 6.2 | 6.7 | 6.0 | 5.6 | 12.3 | 10.7 | 11.7 | 15.5 |
| 5 | 4.8 | 5.3 | 5.3 | 5.5 | 9.8 | 8.3 | 9.8 | 14.1 |
| 6 | 6.0 | 6.5 | 5.5 | 5.2 | 11.2 | 11.5 | 11.1 | 14.0 |
| 7 | 6.0 | 6.6 | 5.4 | 5.2 | 12.7 | 11.8 | 11.4 | 14.8 |
| 8 | 6.4 | 6.5 | 6.1 | 5.6 | 12.6 | 13.7 | 8.1 | 16.2 |
| 9 | 6.0 | 6.2 | 5.2 | 5.1 | 12.6 | 14.2 | 9.6 | 13.7 |
| 10 | 5.8 | 5.9 | 5.1 | 4.8 | 10.4 | 12.1 | 16.1 | 21.3 |
| | Exposure Mean Values | | | | | | | |
| 1 | 6.1 | 6.1 | 5.3 | 5.1 | 12.9 | 13.1 | 10.5 | 15.8 |
| 2 | 6.3 | 6.7 | 5.8 | 5.4 | 12.4 | 13.0 | 9.3 | 12.6 |
| 3 | 6.4 | 6.9 | 5.5 | 5.3 | 13.3 | 12.0 | 15.7 | 15.2 |
| 4 | 6.1 | 5.8 | 5.4 | 5.1 | 14.0 | 15.3 | 11.0 | 13.1 |
| 5 | 6.2 | 6.9 | 5.4 | 5.2 | 11.1 | 13.2 | 7.6 | 11.1 |
| 6 | 6.6 | 6.7 | 5.5 | 5.6 | 11.1 | 9.5 | 8.5 | 10.1 |
| 7 | 6.7 | 6.7 | 5.7 | 5.6 | 10.3 | 9.6 | 8.2 | 11.5 |
| 8 | 6.4 | 6.6 | 5.7 | 5.4 | 11.9 | 12.0 | 7.6 | 12.2 |
| 9 | 6.6 | 6.3 | 5.7 | 5.5 | 16.8 | 14.2 | 8.2 | 12.5 |
| 10 | 6.3 | 6.5 | 6.1 | 5.6 | 16.6 | 15.6 | 7.5 | 15.5 |
| 11 | 5.7 | 5.9 | 5.2 | 5.4 | 16.1 | 15.0 | 8.2 | 10.7 |
| 12 | 6.0 | 6.6 | 5.9 | 5.6 | 15.3 | 16.8 | 8.7 | 12.3 |
| N= | 4 | 4 | 2 | 2 | 4 | 4 | 2 | 2 |

TABLE V (Cont'd)

BLOOD HEMATOLOGY DETERMINATIONS

| | HCT (Vol %) | | | | HGB (gm %) | | | |
|---|---|---|---|---|---|---|---|---|
| | DOG | | MONKEY | | DOG | | MONKEY | |
| Sample Period | Male | Female | Male | Female | Male | Female | Male | Female |
| | Preexposure Mean Values | | | | | | | |
| 1 | 41.5 | 42.0 | 42.5 | 43.0 | 13.7 | 13.9 | 12.8 | 12.8 |
| 2 | 41.0 | 43.8 | 44.5 | 42.0 | 14.0 | 14.7 | 13.2 | 12.4 |
| 3 | 43.3 | 44.5 | 43.5 | 42.5 | 14.3 | 14.3 | 13.8 | 12.1 |
| 4 | 42.8 | 45.8 | 44.0 | 43.0 | 14.6 | 15.4 | 14.0 | 13.6 |
| 5 | 43.0 | 44.3 | 44.5 | 44.0 | 14.2 | 14.7 | 13.8 | 13.0 |
| 6 | 44.3 | 48.8 | 43.0 | 41.0 | 14.5 | 16.2 | 12.4 | 12.6 |
| 7 | 44.0 | 47.3 | 44.0 | 40.0 | 14.1 | 15.2 | 12.8 | 12.4 |
| 8 | 47.3 | 46.0 | 42.5 | 42.0 | 15.4 | 15.1 | 12.5 | 12.4 |
| 9 | 46.5 | 45.8 | 42.0 | 41.0 | 14.9 | 14.7 | 12.0 | 11.9 |
| 10 | 45.0 | 44.5 | 43.0 | 40.5 | 14.9 | 14.8 | 13.0 | 12.3 |
| | Exposure Mean Values | | | | | | | |
| 1 | 44.3 | 44.3 | 41.0 | 40.0 | 14.3 | 14.5 | 12.5 | 11.8 |
| 2 | 45.3 | 48.3 | 43.5 | 40.5 | 15.2 | 15.9 | 13.6 | 12.6 |
| 3 | 47.5 | 48.8 | 42.5 | 41.0 | 15.7 | 16.5 | 13.0 | 12.1 |
| 4 | 49.5 | 49.5 | 45.0 | 40.0 | 16.6 | 16.6 | 13.4 | 12.2 |
| 5 | 46.3 | 50.8 | 42.0 | 42.0 | 15.5 | 17.1 | 12.8 | 12.6 |
| 6 | 47.3 | 48.3 | 42.0 | 42.5 | 16.1 | 16.3 | 13.0 | 13.2 |
| 7 | 48.0 | 49.5 | 45.0 | 43.5 | 16.0 | 16.1 | 13.8 | 12.8 |
| 8 | 49.5 | 49.3 | 45.0 | 43.0 | 16.1 | 16.6 | 13.8 | 13.0 |
| 9 | 50.5 | 49.8 | 46.5 | 44.5 | 16.9 | 16.3 | 13.0 | 12.4 |
| 10 | 47.5 | 48.5 | 48.0 | 45.5 | 16.2 | 16.6 | 15.5 | 14.0 |
| 11 | 50.5 | 47.8 | 45.5 | 46.0 | 16.6 | 16.0 | 13.4 | 13.6 |
| 12 | 46.8 | 48.0 | 45.0 | 43.5 | 15.5 | 16.2 | 13.6 | 13.4 |
| N= | 4 | 4 | 2 | 2 | 4 | 4 | 2 | 2 |

## Discussion and Conclusions

Maintaining animals in the Thomas Dome does not appear to alter the health status of a mixed animal population. Gross and histopathological examinations have not revealed any significant differences between animal room and dome housed animals.

There was no measurable effect on rodent or large animal growth attributable to the Thomas Dome or its environmental parameters. Examination of rat organ weights and organ/body weight ratios also revealed the same no-effect pattern.

Statistical analysis of blood values for monkeys and dogs showed no significant difference between preexposure and exposure means.

Thus, the dome control experiment has shows that its environment does not adversely influence the health status of animals caged therein. Furthermore, the similarity between test and control animals, regardless of the biological parameter examined, supports the present procedure of maintaining control animals exterior to the dome.

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## DISCUSSION

DR. CAMPBELL (U.S. Public Health Service, Cincinnati): This may be an unfair question, but I wonder just how you might interpret similar differences were you studying an atmospheric contaminant?

MR. EGAN (SysteMed Corporation): In terms of the pathology reports that we have?

DR. CAMPBELL: Pathology, any of the other statistically significant. . .

MR. EGAN: Well, in the case of the pathology reports, there seemed to be a fairly close correlation between what we see in the control animals and what we saw in the test animals. In terms of blood chemistry determinations, there appears to be no significance in any of the parameters that were investigated. In the testing of a contaminant, we would have maintained control animals - dogs and monkeys I'm referring to - and would the test animals be significantly different than the control animals, regardless what the parameter, then at least we could possibly conclude effect of the contaminant, not just the dome itself.

FROM THE FLOOR: Did you control the time of day at which you sampled these animals? As you know, there's a well known circadian periodicity in most of the measurements you made and also there are some periodicities which are much longer than one day in length. This could account for some of the things that you saw.

MR. EGAN: I think you're referring to blood chemistry determinations. Generally speaking, all of the dome flights are made in the morning. The blood samples taken are run immediately thereafter, so I would be fairly sure of the fact it was a good control on the length of time between the actual obtaining of the samples and when they were run.

FROM THE FLOOR: You missed my point. The time they are drawn and the time they are run is unimportant, if, indeed, you don't have degeneration of samples. If you do not sample at the same time of day, day in and day out, and I mean not generally just in the morning. You have to be within let's say 30 minutes; otherwise, comparing one day to the next just cannot be done.

MR. EGAN: There are some limitations as to just when we can get up into the dome to do the sampling, and even with that, we do run into some problems actually in running the samples in the blood chemistry laboratory. I do think that many of the blood chemistry determinations--Perhaps Dr. Sopher could be

more helpful on this--that we analyzed don't really change that much. I might be wrong on that point. Dr. Sopher, do you care to comment there?

DR. SOPHER (Aerospace Medical Research Laboratories): The only thing I could really say is that, as Mr. Egan has alluded to, these animals are generally bled within about an hour of the same time in the morning. It's simply a matter of the routine. Occasionally, when something goes wrong, when you have an animal that doesn't want to be bled or something, then it can take longer, so as far as the circadian rhythm goes, I think that the vast majority of times the animals were bled were probably fairly close, and then again, we do either immediately separate the sample and run it, or separate the sample and freeze it until we are going to do the test. I don't think these variations would do it and I don't think the sample handling is significantly different either.

MR. HAUN (SysteMed Corporation): This isn't a question; rather, it's a confirmation. When the animals are bled in the domes, almost without exception, they are bled within an hour of the particular day every two weeks. It's very extraordinary when this isn't done. As Dr. Sopher mentioned, once in awhile we will have a tough animal and have to go back and get him later, but I feel safe in saying that in all the dome studies, 95% of the time the animals are bled within the same hour on the same day every two weeks.

AMRL-TR-68-175

PAPER NO. 6

# THE EFFECT OF ANTIBIOTIC PROPHYLAXIS IN OXYGEN TOXICITY IN RATS

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Many factors have been shown to influence the mortality rate of rats in a toxic oxygen atmosphere. These include partial pressure of oxygen (Felig, 1965), animal age (Dolezal, 1962), animal nutritional status, and environmental temperature (Cambell, 1937).

Robinson et al showed that the strain of rat used in $O_2$ toxicity experiments is also important (Robinson, 1967). In their study, Wistar, conventional Sprague-Dawley, and specific pathogen-free Sprague-Dawley rats were compared and differences in mortality rates among all three were found. One might expect differences between animals of differing strains such as Wistar and Sprague-Dawley but differences within the same strain would appear possibly to be due to an external variable rather than an intrinsic difference. One obvious difference between conventional and specific pathogen-free animals is their endogenous bacterial flora. This might influence infectious disease occurring secondary to the oxygen exposure. Bacterial pneumonia is common in rats exposed to high concentrations of oxygen for prolonged times, probably because of the protein-rich edema fluid in their lungs which provides an ideal culture medium. The present experiment was undertaken to determine if the mortality difference between SPF and conventional rats could be modified or prevented by antibiotic treatment to determine if pulmonary infection indeed played a significant role.

## MATERIALS AND METHODS

One hundred twenty-five conventional Sprague-Dawley (SD) rats and 125 Specific Pathogen Free (SPF) Sprague-Dawley rats weighing about 100 grams each were obtained. The animals were weighed and four weight-matched groups of each type rat were formed. Two days after the initial weighing the animals were again weighed and those failing to hold or gain weight were discarded and the groups were reduced to twenty-five animals each. Table I gives the mean weight and standard deviation of each of the eight groups of animals at the two weighing periods.

TABLE I

RAT GROUPS AND STARTING WEIGHTS*
(GRAMS)

| Group | Weights 15 Sep $\bar{X}$ | s | 17 Sep $\bar{X}$ | s |
|---|---|---|---|---|
| | | (Oxygen Exposed) | | |
| SPF-TET-$O_2$ | 123.8 | 8.8 | 135.2 | 7.7 |
| SPF-$H_2O$-$O_2$ | 123.8 | 8.8 | 133.1 | 10.0 |
| SD-TET-$O_2$ | 122.0 | 11.0 | 128.4 | 12.0 |
| SD-$H_2O$-$O_2$ | 123.6 | 10.4 | 129.4 | 11.7 |
| | | (Air Exposed) | | |
| SPF-TET-Air | 123.6 | 9.1 | 134.2 | 10.2 |
| SPF-$H_2O$-Air | 122.3 | 9.4 | 136.3 | 8.2 |
| SD-TET-Air | 122.4 | 11.4 | 128.5 | 11.9 |
| SD-$H_2O$-Air | 122.5 | 9.4 | 127.1 | 10.0 |

*These weights were taken before the animals were given tetracycline or exposed to oxygen.

Two groups of each rat type were placed in a Thomas dome in which the atmosphere was 100% oxygen at 760 mm of mercury pressure. One group of each rat type had 0.35 mg tetracycline per milliliter added to their drinking water while the other served as an oxygen exposed control. This was calculated to give a therapeutic dose if the animals took water at their usual rate, about 20 ml/day (E & S Livingston, Ltd., 1967). The other four groups, two each SPF and SD, were housed in the vivarium as non-oxygen exposed controls. These animals were treated in the same fashion as the exposed groups, i.e., one SD and one SPF group received 0.35 mg/ml tetracycline in their water while the other two groups received only water. All animals were fed a prepared commercial rat pellet diet ad libitum.

If an animal died during the exposure period it was removed from the Thomas dome at once and necropsied, or if at night, placed in a refrigerator and necropsied the next morning. The pleural effusion from animals that died was cultured by routine methods. At the end of the 28-day exposure period all of the oxygen exposed animals and two each of the air exposed animals were killed with an overdose of intraperitoneal sodium pentobarbital. The lungs from 10 animals randomly

selected from each $O_2$ exposed group (40 total) and the sacrificed air exposed animals were cultured by routine methods for aerobic and anaerobic bacteria as well as for pleuropneumonia-like organisms. If growth occurred on a primary culture appropriate subcultures were made to identify the organism(s) present. All of the exposed animals and the sacrificed air controls were necropsied, including histopathologic examination.

## RESULTS

Table II is a summary of the mortality in each group by exposure days. As can be seen, deaths occurred only in the $O_2$ exposed animals. The SPF-TET-$O_2$ group had three to five times as many deaths as did any other single group. At the end of the experiment all of the air control animals were weighed. Table III shows the mean weight of those animals by group. The SPF animals on tetracycline weighed considerably less than did those not on tetracycline. This phenomenon was not seen in the SD animals. The reason for the difference in standard deviation in both tetracycline fed groups as compared to the controls is another interesting finding but its significance is not known.

TABLE II

MORTALITY BY DAYS

| | Exposure Day | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Group | 1 | 2 | 3 | 4 | 5 | 6 | 7 to 28 | Total Dead |
| SPF-TET-$O_2$ | 0 | 0 | 8 | 1 | 1 | 0 | 0 | 10 |
| SPF-$H_2O$-$O_2$ | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 2 |
| SD-TET-$O_2$ | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 3 |
| SD-$H_2O$-$O_2$ | 0 | 0 | 1 | 2 | 0 | 0 | 0 | 3 |
| SPF-TET-Air | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| SPF-$H_2O$-Air | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| SD-TET-Air | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| SD-$H_2O$-Air | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

TABLE III

MEAN WEIGHTS OF AIR EXPOSED RATS AT 28th DAY
(GRAMS)

| Group | Weight | |
|---|---|---|
| | $\bar{X}$ | s |
| SPF-TET-Air | 246.2 | 27 |
| SPF-$H_2O$-Air | 271.1 | 62 |
| SD-TET-Air | 236.0 | 27 |
| SD-$H_2O$-Air | 227.6 | 57 |

## BACTERIOLOGY

The pleural fluid from the animals that died spontaneously during the exposure period was sterile. Table IV gives the results of the cultures from the animals killed at the end of the experiment.

TABLE IV

RESULTS OF LUNG CULTURES OF OXYGEN EXPOSED RATS

| Group | Rat No. | Initial Culture | Sens to Tet | Aerobic Subculture | Sens to Tet | Anaerobic Subculture | Sens to Tet | PPLO Culture |
|---|---|---|---|---|---|---|---|---|
| SPF-TET-$O_2$ | 33 | Alpha strep, Proteus sp., Staph Epid. | No | Proteus sp., Alpha strep. | No | NG | | neg |
| | 45 | NG | | D. pneumoniae | Yes | NG | | neg |
| | 59 | NG | | Alpha strep | Yes | NG | | neg |
| | 42 | NG | | Alpha strep | No | NG | | neg |
| | 54 | NG | | Hem Staph Epid | No | NG | | neg |
| SPF-$H_2O$-$O_2$ | 35 | Proteus sp., E. Coli Staph Epid, Aerobacter | Yes | Pseudomonas a. | No | NG | | neg |
| | 09 | D. pneumoniae | Yes | D. pneumoniae | Yes | D. pneumoniae | Yes | neg |
| | 13 | NG | | NG | | Anaerobic strep | Yes | neg |
| SD-TET-$O_2$ | 41 | Alpha strep | Yes | Alpha strep | Yes | NG | | neg |
| | 42 | Alpha enterococci | No | Alpha enterococci | No | NG | | neg |
| SD-$H_2O$-$O_2$ | 20 | Alpha strep, Proteus sp., B. subtilis, Staph Epid. | No | Alpha strep, Proteus sp., B. subtilis, Staph Epid. | No | NG | | neg |
| | 04 | Alpha strep. | Yes | Alpha strep. | Yes | NG | | neg |
| | 16 | NG | | Hem. Staph Epid. | Yes | NG | | neg |

The SPF-TET animals had more growth than did any other single group but the type of organism found was equivalent. Many of the organisms (e.g., the Bacillus species) were probably contaminants. Since tetracycline sensitive organisms were found in tetracycline fed animals, it raises doubts as to whether the dosage of the drug was sufficient.

## PATHOLOGY

All of the animals dying spontaneously had the typical gross and histologic lesions of acute oxygen toxicity, i.e., heavy, congested, edematous lungs with marked pleural effusion. No differences among the groups in type of lesion, incidence of bronchopneumonia, or murine pneumonia were seen.

The animals killed at the end of the exposure period typically showed atelectasis, emphysema (both destructive and compensatory), increased fibrous tissue, and varying degrees of murine pneumonia. A few animals also had bronchopneumonia. There was no set pattern of pathologic changes in any group and no real differences existed among the several groups.

We were unable to repeat the mortality curves of previously reported studies (Robinson, 1967; Felig, 1965; Roth, 1964). The reason for this is obscure but possibly resides in the fact that we used very young animals which are known to be much more tolerant to the toxic effects of oxygen (Dolezal, 1962). To test that hypothesis we placed our surviving air control animals in the Thomas dome when they averaged about 250 grams and had 100% mortality in about four days. This fits the established figures much more closely than the animals did at the younger age. In this exposure no difference was noted between TET and control groups.

From the weight data it would appear that the SPF rats were affected unfavorably by the administered tetracycline. This insult plus the oxygen environment possibly explains the three to five fold increase in death in the SPF-TET-$O_2$ group. Why the SPF animals were ill affected by tetracycline is not at all clear. They were ordered from a different dealer than the conventional animals. Kydd (1963) has reported differences in susceptibility to chronic oxygen effects in rats of the same strain when obtained from different sources. The results of the pathologic studies and bacterial cultures tend to show that antibiotic prophylaxis, at least at the level we administered, is ineffective in preventing pneumonia in oxygen intoxicated rats: a higher dose may have been effective.

Perhaps this experiment also once again shows that a rather innocuous drug used in abnormal environments can cause unexpected and untoward results.

## SUMMARY

Rats exposed to oxygen at 760 mm Hg and ambient air were given tetracycline prophylaxis in an attempt to modify the usual mortality pattern. This was not accomplished; the antibiotic appeared to be ineffective at the dose level used for that purpose. Indeed there is evidence that the combination of oxygen and tetracycline may cause increased mortality in some strains of rat.

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## DISCUSSION

DR. WEIBEL (University of Bern): I have a number of questions to Dr. Sopher. First of all, were these specific pathogen-free rats in the same dome with the other rats?

DR. SOPHER: Yes, they were, but they were caged separately.

DR. WEIBEL: Yes, we still have to ask how pathogen-free they will be when you have them together in the same dome, and these specific pathogen-free animals will not have the immunological baseline if you want to cope with all the infections that come from these regular Sprague-Dawley rats. This may be one of the factors you may have to take into account.

The next question is how does tetracycline influence metabolism of these animals? Does it interfere with protein synthesis or lipid synthesis, which would explain, perhaps, why these animals might be more damaged than the others and might die earlier than the others.

DR. SOPHER: Well, first of all, the animals were housed in the same dome and there very well could be a certain amount of interchange of bacterial flora. An experiment of this type was run in the domes and was reported out by one of our personnel a couple years ago where he reported he had a marker organism and he could follow this organism being transferred between dogs and monkeys. However, the dogs and the monkeys were all caged in the same area, the dogs all together in the pen, constantly biting each other and playing around and so one could expect to get an interchange there. Now, the flow in a Thomas Dome is approximately laminar so the amount of cross flow between the cages themselves is probably not very great so I don't think it's going to carry too many organisms to affect the immunologic status of the animals. These animals were not gnotobiotic, they weren't germ-free. They did have a bacterial flora but it was specific pathogen-free, so I don't think their immunologic status would have been affected as much as it would be in true gnotobiotic animals as you've seen in germ-free mice or this sort of thing. As far as the pharmacology of tetracycline is concerned, some of the effect, possibly the effect that might be most expected to be in play here, is that it has, in cellular suspension, been known to interfere with oxidative phosphorylation. Certainly interference has been shown to occur somewhat with oxygen toxicity as well. There is probably uncoupling of oxidative phosphorylation. These two together may well account for part of this. It has other unknown things, for example, it causes acute fatty liver in some pregnant women. No one knows exactly why that is. Degradation products can be quite toxic. In this case, we used fresh antibiotic, changed water

daily, so I don't think we are getting into that. I frankly don't know what the mechanism is. We didn't expect this result and I just really can't explain it to my satisfaction.

DR. ROBINSON (Aerospace Medical Research Laboratories): I'd just like to add a little more background as to why we got into this. In some of the earlier experiments the rats that we exposed and subsequently examined were quite obviously infected with, at least from a histopathologic standpoint, various bacteria, and we felt that by using the tetracycline we could possibly avoid some of this type of interference with the expression of oxygen toxicity.

DR. SMITH: I don't remember that slide showing mortality perfectly, but it seemed to me that you had eight deaths. Was that on one day?

DR. SOPHER: Yes, on the third day.

DR. SMITH: This looks more like an episode than a trend. I wonder if this could explain it, because it looks completely out of line with the records thereafter and of other groups being carried parallel with it.

DR. SOPHER: Well, Dr. Robinson can correct me on this if I'm wrong, but if I recall the data correctly, in a typical situation with oxygen toxicity, your mortality, your greatest mortality, generally, will occur very early, in approximately 72 hours, and this clustering effect was seen very well when we put the adult animals back in the dome, and we had the vast majority of animals dying at approximately two days, and then a few more filtered down over the next two days, and then they were all gone.

DR. RIESEN (IIT Research Institute): I could concur with the clustering of oxygen toxicity within two or three days after. The mass of evidence from many labs now, including Wright-Patterson data, supports that point of view. I think the cascade of oxygen from the atmosphere to the interior of the cell in relation to the cascade that you have from tetracycline to the interior of the cell should be considered in terms of the joint interaction between oxygen and tetracycline at the mitochondrial level. Certainly, we know that many antibiotics have very specific effects on either electron flow or coupling of phosphorylation, so that I would suggest some in vitro experiments in an attempt to determine how much tetracycline gets into the cell, if you wish to pursue that further.

AMRL-TR-68-175

PAPER NO. 7

# PSYCHOPHARMACOLOGY OF CARBON MONOXIDE UNDER AMBIENT AND ALTITUDE CONDITIONS

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## INTRODUCTION

During the past two or three years, it has become increasingly evident that carbon monoxide might become one of the major problems associated with long-term space flight. By long-term, I mean to imply missions of greater than 90 days duration. For longer missions, the space vehicles must be tighter to decrease leak and conserve the cabin atmospheres. In addition, present day thinking promotes the ideas of using regenerative oxygen producing systems such as the Sabatier reaction and others which utilize carbon monoxide (CO) in the cycle. These sources of CO plus our previous findings that almost all space cabin materials gas-off CO and the fact that man also produces CO make it imperative to study the effects of long-term exposure to relatively small amounts of the compound. There is a vast amount of literature pertaining to the acute effects of high doses of CO and even some on chronic effects of work-day exposures on man such as with personnel in traffic tunnels. However, there is little pertinent data on continuous exposures to either animals or man.

The experiments which I shall describe, then, are in a series designed to study the effects of carbon monoxide on the central nervous system in terms of the biochemistry involved at the cellular and subcellular levels, the effects on the electrical activity in the brain, and the total effects on performance. These specific experiments were designed to correlate performance with carboxyhemoglobin levels of exposed animals since there have been reports that exposures of CO to levels around the Threshold Limit Value of 50 ppm have caused performance decrements in human subjects.

## Methods

The subjects used in these experiments were 12 adult Macaca mulatta monkeys which were trained at Holloman Air Force Base and shipped to Wright-Patterson Air Force Base. All animals were trained on the performance tasks to a stabilized level during a period of several months.

Since most of the participants at this conference are familiar with our altitude facility, it is only necessary to indicate that the animals are placed in 12 individual working cages especially designed to fit in front of each of 12 windows within one Thomas dome. The psychopharmacology programming equipment is solid state and has been described in further detail in the 2nd Conference Proceedings by T. L. Wolfle and by K. C. Back in the 3rd Conference (Wolfle, December 1966; Back, December 1967).

The performance panel in each cage includes two stimulus lights over each of two response levers (left and right). These levers are used for a continuous avoidance task. Mounted between and slightly above these levers are two response push buttons. The upper push button contains a white lamp which flashes for a discrete visual response, while a lower button is the response object for a discrete auditory response to a 2800 cps tone mounted to the upper left of the panel.

The animals are programmed to work eight hours each day. They work for 15 minutes each hour starting on the hour, five days per week, for as long as the experiment is in progress. They do not work on weekends. The animals are fed at the end of the last session on each work day. When blood is obtained or when any other procedure involving the handling of the animals takes place, it is scheduled before the first work session in the morning (0800).

Each 15-minute work schedule includes three tasks. The monkeys must press each right and left continuous avoidance lever at least once each 15 seconds or they receive a light shock. In addition, they are presented 12 randomized visual and 12 randomized auditory cues during each 15 minute work period. The response to these cues must be made within two seconds or the animals receive shock.

In addition to carboxyhemoglobin levels, (Dominguez, 1957), the battery of tests accomplished on most of our chronically exposed animals is indicated in table I.

TABLE I

ROUTINE CLINICAL LABORATORY TESTS

| Hematology | Chemistry |
|---|---|
| WBC | Sodium |
| RBC | Potassium |
| Hemoglobin | Calcium |
| Hematocrit | Total Protein |
| Differential Count (when needed) | Albumin |
| | Total Phosphorus |
| | SGOT |
| | SGPT |
| | Alkaline Phosphatase |
| | A/G Ratio |

TABLE II

EXPERIMENTAL EXPOSURE CONDITIONS
USING TRAINED MONKEYS

| Atmosphere | Altitude | Ambient | Duration and Dates |
|---|---|---|---|
| 1. Air plus 55 mg/m³ CO | | X | 100 Days 14 Aug - 21 Nov 1967 |
| 2. 68% $O_2$/32% $N_2$ plus 55 mg/m³ CO | X | | 105 Days 4 May - 16 Aug 1968 |
| 3. 68% $O_2$/32% $N_2$ plus 110 mg/m³ CO | X | | 7 Days 16 Aug - 23 Aug 1968 |
| 4. 68% $O_2$/32% $N_2$ plus 220 mg/m³ CO | X | | 7 Days 23 Aug - 30 Aug 1968 |
| 5. 68% $O_2$/32% $N_2$ plus 440 mg/m³ CO | X | | 7 Days 30 Aug - 6 Sep 1968 |

As may be seen in table II, the experimental exposure conditions can be divided into 5 phases. In the first phase and following a baseline period of approximately one month in the dome, the animals were exposed to 55 mg/m³ CO in air under ambient conditions for 100 days. The ambient conditions consisted of an air flow rate of 25 cfm, 70-78F, and an RH of approximately 50%. Following this exposure, the animals were returned to the vivarium for about 5 months while the new water deluge system was installed and other repairs made to make the equipment safer to use. The animals were then returned to the unit and new baselines obtained under ambient conditions, after which they were taken to 27,000 feet altitude (5 psia) in a two-gas system of 68% $O_2$/32% $N_2$. The second phase of exposure, then, lasted for 105 days at 55 mg/m³ CO. Successive phases 3, 4, and 5 consisted of doubling the concentration of CO each 7 days following the last day of exposure to 55 mg/m³. On each successive Friday, and without stopping the exposure conditions, the animals were presented with 110 mg/m³ CO, followed the next week with 220 mg/m³ CO, and finally with 440 mg/m³ the last week. Blood was obtained on each Wednesday morning for carboxyhemoglobin levels during these last three weeks.

## Results

TABLE III

BLOOD CARBOXYHEMOGLOBIN LEVELS*

EXP 1 55 MG/M³ AMBIENT
6 SEP = 4.4 (4.0-5.0) % SATURATION
4 OCT = 3.1 (2.0-4.5)
9 NOV = 3.8 (2.0-5.0)
OVERALL = 3.7 (2.0-5.0)

EXP 2 55 MG/M³ MIXED GAS, ALTITUDE
23 MAY = 4.8 (4.0-5.5) % SATURATION
21 JUNE = 4.3 (4.0-5.5)
17 JULY = 4.8 (4.5-5.5)
14 AUG = 5.0 (4.5-6.0)
OVERALL = 4.7 (4.0-6.0)

EXP 3 110 MG/M³ MIXED GAS, ALTITUDE
21 AUG = 8.3 (7.0-10.0) % SATURATION

EXP 4 220 MG/M³ MIXED GAS, ALTITUDE
28 AUG = 19.5 (17.0-21.0) % SATURATION

EXP 5 440 MG/M³ MIXED GAS, ALTITUDE
4 SEP = 30.1 (27.0-34.0) % SATURATION

*Averages of all animals (Range)

The correlation between the dose of CO and blood carboxyhemoglobin levels is shown in table III. It is quite evident that at 55 mg/m³ (50 ppm equivalent), carboxyhemoglobin levels did not change appreciably during each of the three months and an overall average of 3.7% saturation was obtained. Likewise in phase 2 (55 mg/m³ mixed gas at altitude), carboxyhemoglobin levels averaged a very stable 4.7% saturation which is not significantly different from that found under ambient conditions. At the higher concentrations of CO, 110 mg/m³ CO produced 8.3% saturation, 220 mg/m³ CO produced 19.5% saturation, and 440 mg/m³ produced 30.1% saturation. This turns out to be almost a straight line relationship between dose and carboxyhemoglobin levels. Hemoglobin levels obtained at the end of phases 1 and 2 are shown in table IV.

TABLE IV

DATE 9 November 1967 SPECIES Macaca mulatta

| | ANIMAL NUMBER | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| TEST | M82 | M94 | M80 | V35 | 6P0 | E51 | 303 | 4P8 | F08 | 288 | 4P6 | E98 |
| HCT (vol %) | 51 | 46 | 48 | 43 | 53 | 35 | 42 | 42 | 52 | 43 | 47 | 46 |
| HGB (gm %) | 15.2 | 14.4 | 14.8 | 12.8 | 16.0 | 10.5 | 13.2 | 12.8 | 15.6 | 12.8 | 14.0 | 14.4 |
| RBC (x $10^6$) | 6.55 | 6.00 | 5.97 | 5.68 | 6.40 | 4.78 | 5.59 | 5.31 | 6.71 | 5.38 | 5.75 | 5.71 |
| WBC (x $10^3$) | 7.5 | 6.8 | 8.4 | 9.5 | 10.5 | 7.6 | 4.90 | 8.5 | 11.3 | 6.2 | 10.6 | 11.8 |

DATE 14 August 1968 SPECIES Macaca mulatta

| | ANIMAL NUMBER | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| TEST | M82 | M94 | F08 | M80 | V35 | 6P0 | E51 | 303 | 4P8 | 288 | 4P6 | E98 |
| HCT (vol %) | 52 | 49 | 53 | 47 | 42 | 47 | 41 | 42 | 40 | 42 | 44 | 41 |
| HGB (gm %) | 15.6 | 16.0 | 17.6 | 14.8 | 14.0 | 14.8 | 12.8 | 13.6 | 12.8 | 13.6 | 13.6 | 13.2 |
| RBC (x $10^6$) | 6.84 | 6.59 | 6.58 | 6.07 | 5.95 | 6.02 | 5.81 | 6.01 | 5.54 | 5.71 | 5.94 | 5.65 |
| WBC (x $10^3$) | 10.3 | 9.2 | 14.6 | 11.8 | 12.1 | 13.2 | 8.9 | 7.7 | 12.9 | 9.8 | 13.6 | 11.3 |

During all phases of these experiments, only 2 animals of the 12 showed any significant changes in performance levels. This was true at all dose levels of CO and despite the fact that at 440 mg/m³ the majority of the monkeys showed outward signs of toxicity during the first two days of exposure. In addition, most of the animals reduced their caloric intake, with 3 of the 12 animals eating less than 1/3 of their normal food intake during this exposure concentration. The remarkable finding was that although first appearing sick, by the end of the week a compensatory mechanism must have been instituted because the animals looked perfectly normal in all outward appearances though they maintained a blood carboxyhemoglobin saturation of 30%.

The two animals that showed changes during the experiments were designated E98 (Klutz), and M82 (Irving). Figures 1 and 2 depict the performance levels of Klutz at the times during the exposures when maximum changes had taken place. At the left of figure 1 can be seen the baseline lever presses and reaction times to visual and auditory discrete avoidance cues. The baseline represents the last five days of baseline work effort with the upper and lower limits of this animal described from the previous month's work. At 55 mg/m³ CO under ambient conditions, there was no significant change in visual or auditory reaction times and only a very slight change in lever presses during the final 5 days of exposure.

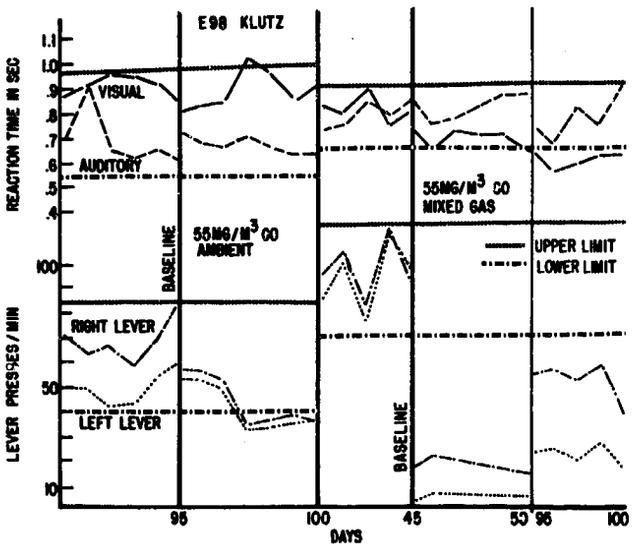


Figure 1. PSYCHOPHARMACOLOGY OF CO

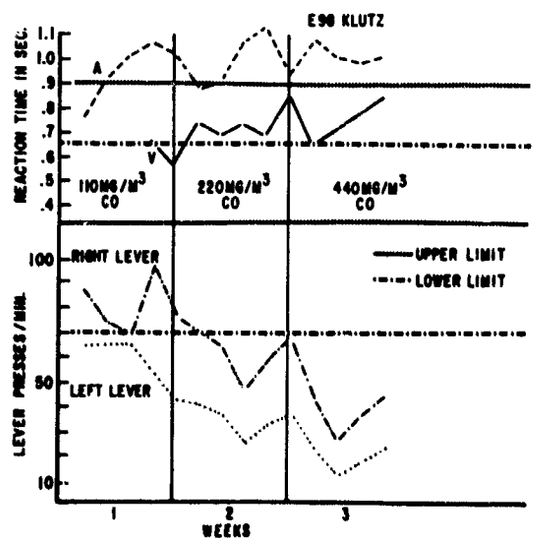


Figure 2. PSYCHOPHARMACOLOGY OF CO

All other data during his total exposure were within normal limits. During the second phase of the experiment and following the time in the vivarium, some changes had taken place in baseline activity (see the center portion of figure 1). This animal became quite sensitive to his environment and showed maximum changes in continuous avoidance parameters as shown between days 45 and 50. However, performance of lever pressing gradually increased steadily and finally reached the stage shown between the 95th and 100th day of exposure. Of interest is the fact that visual discrete performance (reaction times) became faster and actually bettered the test baseline times.

The reactions of this animal to 110, 220 and 440 mg/m$^3$ CO are depicted in figure 2. Remember that the 110 mg/m$^3$ dose was presented on the last day of the 55 mg/m$^3$ CO exposure. Therefore, work week #1 on this figure starts where day 100 left off in figure 1. As can be seen, right and left lever presses have markedly increased and auditory reaction times have slowed. This is followed by decreased lever presses at 220 mg/m$^3$ and further slowing at 440 mg/m$^3$.

Although E98 (Klutz) was an animal which had a more labile performance pattern, figures 3 and 4 represent the activity of M82 (Irving), an animal which has been one of the most stable workers of the 12. Figure 3 shows that there was absolutely no change in performance during the ambient exposure to 55 mg/m$^3$ CO. However, at 55 mg/m$^3$ CO at altitude, left lever presses suddenly peaked-out way off scale. The lever presses went up to about 150/minute but note that the right lever performance and discrete performance remain unchanged. The reaction to the higher concentrations is shown in figure 4. Of acute interest is the evidence of a relative slowly changing decrement of left lever presses while all other parameters remained perfectly stable. A possible explanation for this performance change is that the animal accommodated to an unpleasant situation by over-reacting. This is seen frequently in trained animals receiving shock by failure to perform one task and attempts to compensate by performing another task at a more rapid rate.

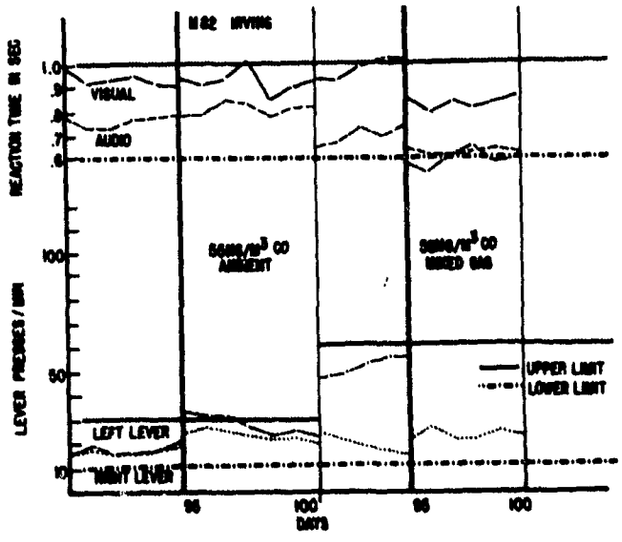


Figure 3. PSYCHOPHARMACOLOGY OF CO

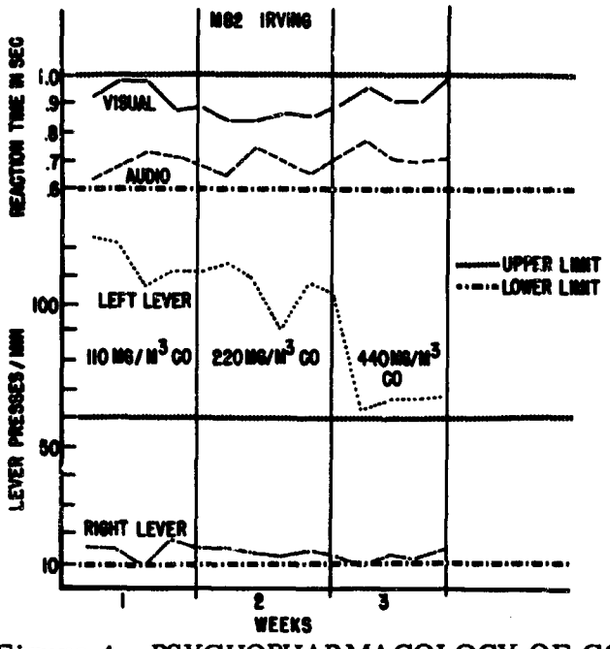


Figure 4. PSYCHOPHARMACOLOGY OF CO

Of particular concern here is that all animals could perform well even under conditions where carboxyhemoglobin attained levels of 30% saturation. Further, it is interesting to note that some sort of tolerance was apparent since the 440 mg/m$^3$ CO level produced outward signs of toxicity which tended to disappear with time.

## Summary

The Air Force has become very interested in the short-and long-term effects of carbon monoxide (CO) since studies of gas-off products of space cabin materials and processes (including man's contribution) reveal that the compound is likely to be found in space cabins if not properly scrubbed. Recent investigations have indicated that very low concentrations of CO can cause subtle decrement in high level performance. In order to test performance changes, two continuous 100-day CO exposure studies to 55 mg/m$^3$ in ambient air, and in a space cabin atmosphere of 5 psia total pressure (27,000 ft simulated altitude), mixed gas environment consisting of 68% oxygen and 32% nitrogen ($pO_2$ = 160 mmHg) were performed. At the end of the second experiment, and without stopping exposures, CO concentrations were increased to 110, 220, and 440 mg/m$^3$, respectively, at 7-day intervals. The experiments were performed on 12 trained monkeys in which operant behavior was conditioned to both continuous and discrete avoidance tasks by both audio and visual cues. The animals performed 15 min/hour, 8 hours/day and 5 days/week, during the continuous exposure period. Extensive clinical laboratory determinations were performed, including blood carboxyhemoglobin levels, throughout the test periods. In the two experiments conducted at 55 mg/m$^3$, carboxyhemoglobin per cent saturation plateaued after the first 48 hours' exposure at 3.7 and 4.7, respectively. There was no observable decrement in performance under either ambient or altitude conditions, nor were there changes in other

clinical parameters. Exposure to 110, 220, and 440 mg/m$^3$ caused mean carboxyhemoglobin saturations of 8.3, 19.5 and 30.1%, respectively. These levels produced performance changes in two of the 12 monkeys; the 440 mg/m$^3$ level also produced changes in appetite and in outward appearance in 6 of the 12 animals.

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## DISCUSSION

DR. ROBINSON: I think, Ken, that it may have gotten across that these monkeys hit the right lever with the left hand and vice versa and so on. Don't they, in fact, use the same hand for both levers?

DR. BACK (Aerospace Medical Research Laboratories): Some of them do and some of them don't. This particular animal is ambidextrous. Some of them stand at the console and press the left lever with the left hand and the right lever with the right hand. Most of them do, a few of them don't, this one happens to. Sometimes you can see, with other compounds an absolute changeover, and the lefthanded animal can become a right-handed animal; with exposure to decaborane, this has happened.

DR. THOMAS (Aerospace Medical Research Laboratories): This decrement of performance showing in some animals and not showing in others certainly points out the individual variabilities in these monkeys. Some are pretty tough. We've seen with other agents that you practically have to kill them before you can measure decrement in performance, which brings up the point of just how sensitive these criteria are.

DR. BACK: Yes. This is not a very complicated task. The task becomes practically automatic. This is a relatively gross test, no doubt about it. However, there are other data here that I have been unable to show and this is the number of shocks taken. I didn't show any shocks taken here, but a number of animals show an increase in the number of shocks that they will take, so that it is not as gross as that. We are toying with the idea that we would like a little more sophistication in the test, but one of the problems is that we are trying to keep it as simple as possible. We are not able to go in the dome on a moment's notice and fix a lever, so that we do have some logistics problems here to take care of. The more complicated the program gets, the rougher it is to maintain and monitor the equipment.

FROM THE FLOOR: Ken, do you feel that your carboxyhemoglobin levels for the monkeys were relatable to or correlate with man? I'm thinking of threshold limit values, in particular, where you mentioned 55 milligrams per cubic millimeter.

DR. BACK: In the first place, I think the carboxyhemoglobin levels are lower than those to be expected in man, under the same conditions. For instance, at 440 milligrams per cubic meter, we had about 29% saturation. It looks to me that they should be up around 40%, rather than 29%, a. ' I don't know whether

this is due to difference in methodology in the previous studies. I know the method Colonel Dominguez is using is more up-to-date than in the old data, but our carboxyhemoglobins are quite a bit lower than we could expect from previous human data.

QUESTION: It was my understanding from some while back that about 15 to 18% carboxyhemoglobin would begin to affect the oxygen saturation and you would be at a point where, with a man, he would not have enough oxygen. When you get up to 26%, where are you there?

DR. BACK: Well, we don't know yet. We're going to do a number of experiments in the near future. Miss George is setting up to do $pO_2$'s, brain $pO_2$'s, because we must know what's going on in oxygen transport. We are now getting animals ready with implanted brain electrodes to study the effect of these levels at altitude. We want to correlate the carboxyhemoglobin levels with mitochondrial changes and cell metabolism changes. Maybe we'll have the answers in the next ten years.

MR. WANDS: One of the things you will want to watch for is a marginal hypoxic condition of stress, particularly the physical stress. Work, exercise, becomes quite critical here.

DR. HODGE: It used to be said that the human was symptom-free if he had 10% or less carboxyhemoglobin. Is that still a reasonable value? If it is, it certainly must be reportable symptoms, and the tests, if he's like the monkey, show that he really was suffering a decrement within that limit.

DR. BACK: Beard's paper indicates that at 50 parts per million, he got decrement in temporal changes - a man's ability to judge the length of a tone. Fifty parts per million of CO is our threshold limit value. This would give about a 7% carboxyhemoglobin saturation, which means every cigarette smoker should have a decrement in performance.

FROM THE FLOOR: You must take into consideration compensatory hematocrit changes with smoking.

DR. BACK: Our hematocrit didn't change an iota in these animals. You must recognize that this is in a 5 PSIA mixed-gas atmosphere. This is an atmosphere in which you have an alveolar $pO_2$ quite similar to that which we have on the ground right here.

FROM THE FLOOR: Right. We're speaking of people in a normal environment, smoking.

DR. BACK: I can't really believe that people are that much different from monkeys as far as carboxyhemoglobin levels are concerned. I think there's something the matter with our past methods.

MR. WANDS: Dr. Back, it's quite true that literature on carbon monoxide is quite ancient and was obtained with an entirely different analytical procedure than we have available today. When we get into these fine points of differentiation between 7% and 10% saturation, these factors of analytical methods become quite important.

# RELATIONSHIP OF RESULTS OF LONG-TERM TOXICITY STUDIES TO THOSE OF SHORTER DURATION

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The testing of the toxicity of atmospheric contaminants in confined spaces for acute, subacute, and chronic exposure periods is a prime and essential function of the Toxic Hazards Division of the Aerospace Medical Research Laboratories. The inter-relationships of the toxicity results between these studies of varying duration is important for two reasons: first, so that the results of short term studies may be used to plan more accurately the dosage levels for longer term studies; and, secondly, to use short term studies in place of the long term if experience illustrates that a reliable relationship exists between such experiments.

It is obvious that atmospheric contamination in confined spaces will be a combination of low concentrations of several, or many materials. This paper is a report on two sets of experiments which bear on these aspects. A formula is presented which has proved valuable in the prediction of joint toxic action of combinations of materials by inhalation and by peroral dosing when their action is additive. Furthermore, to indicate a potential parallel line of attack for inhalation studies, the inter-relationship between the single peroral LD50, and the 7-day, 90-day, and 2-year feeding studies will be presented.

Approximately 10 years ago the Chemical Hygiene Fellowship started experiments to estimate the toxicity of mixtures. The results of these investigations testing 15 multicomponent mixtures by inhalation and by peroral routes and 36 paired mixtures inhaled by rats, 23 of which were also administered perorally were previously published (Pozzani, 1959). Prior to, and concurrent to these experiments, we predicted and tested the peroral joint action of all practical combinations of 27 materials (350 pairs). These latter results are in press (Smyth).

The formula used to predict joint-action is:

$$\frac{1}{M} = \frac{p1}{MED_1} + \frac{p2}{MED_2} \cdots\cdots + \frac{pn}{MED_n}$$

where: MED = median effective dose; LC50 or LD50 e. g.
M = predicted MED of mixture
p = proportion of component in mixture

An example of its use is presented in table I. The formula, which is equivalent to the calculation of a weighted harmonic mean, used to average rates (LD or LC50s are rates), is generally efficient for acute data. The coefficient of rank correlation between the predicted LC50 of 36 pairs of materials and that actually observed during vapor exposures was +0. 89, where 1. 0 is perfect correlation. Similar excellent agreement was usually obtained between the predicted and observed peroral joint-toxic tests, with the exception, of course, of those materials that produce reactions significantly greater or less than additive. An additional factor is worthy of note; the rank correlation coefficient between the observed inhalation LC50s and the peroral LD50s of 23 mixtures was +0. 86. This is an indication that, in general, acute inhalation and oral results are relatively similar in degree of toxic action. Because of this relative similarity, data collected on the relationship of single peroral LD50 and repeated dose feeding studies is herein presented, with the hypothesis in mind that these oral-route inter-relationships might be similar to those which could result from inhalation experiments of varying durations.

TABLE I

EXAMPLE OF CALCULATION OF PREDICTION OF JOINT TOXIC ACTION

| | p; Percentage by Weight | LC50; 8-Hour Inhalation, mg. /liter |
|---|---|---|
| Butyl acetate | 61. 6 | 40. 6 (28. 3 to 58. 3) |
| Butyl alcohol | 28. 2 | 29. 4 (26. 3 to 33. 0) |
| Isopropyl acetate | 10. 2 | 50. 6 (43. 0 to 59. 5) |

$$\frac{1}{M} = \frac{0.616}{40.6} + \frac{0.282}{29.4} + \frac{0.102}{50.6} = 0.02678$$

M = 37. 3 mg. /liter
observed LC50 of this mixture = 40. 0

In 1963 the results of a comparison between 33 subacute and long term feeding studies were presented (Weil, 1963). These involved materials tested during more than 15 years by the Union Carbide Fellowship at Mellon Institute and by the Dow Chemical Company Laboratories. The criteria of effect followed in these studies were enumerated in table III of that paper (Weil, 1963), as was the frequency with which these criteria were significantly altered at the lowest dosage level in which any effect was present in pertinent studies (ones in which at least one criterion of effect was significantly altered at some dosage level). It is apparent that many criteria are very inefficient in the determination of the minimum effect, maximum no ill-effect interval for materials. A summary presented in table VI of that paper (Weil, 1963) illustrated that body weight change, weight and pathology of the liver and of the kidney were the efficient criteria.

The ratios between the maximum no ill-effect levels obtained in the subacute (usually 90-day feeding studies) and the long term, 2-year studies were presented in tables I and V (Weil, 1963). Also illustrated in the latter table are the sensitive criteria at these minimum effect dosage levels in the studies of these durations. Of prime importance is that these ratios were low, indicating a favorable relationship between 90-day and 2-year studies.

Additional experiments were recently performed, using 22 chemicals, to determine if the relationship between studies of varying duration could be extended, i.e., whether the results of LD50s or 7-day feeding studies could be used to predict the outcome of 90-day feeding studies. The results of 20 of these 22 materials were previously summarized (Weil, in press). The materials covered a wide range of toxicity, as illustrated in table II. The criteria affected, table III, were similar to these previously presented as efficient for the subacute, 2-year feeding study experiments (Weil, 1963). The parameters of the ratios between these studies of varying duration are summarized in table IV.

The quantitative relationship between the LD50s and the 90-day minimum effect dosage level (MiE) was relatively poor; the median ratio was 10.0 and to encompass 95% of the cases, was 39.8. The semi-interquartile range, a measure of variability between individual ratios, was 9.8, which is approximately 6 times the variability of the one-week, 90-day ratios.

The LD50s and 7-day MiEs were somewhat better related, as three-fourths of these ratios were 11.4 or less, but to encompass 95% of them a ratio of 25.8 was needed. However, 95% of the ratios between the 7-day and 90-day minimum effect dosage levels were 6.8 or less, a 6-fold improvement from the 90-day estimate allowed by the LD50s. It is worthy of note that the parameters of the 90-day versus 2-year repeated oral ratios (Weil, 1963) were almost identical with the $MiE_7/MiE_{90}$ ratios. Therefore, one can predict 90-day results from a 7-day test (but not from LD50s) with the same confidence as he can the 2-year results from a 90-day test.

The coefficients of rank correlation summarize these relationships. They were all positive and significant with those for $MiE_7/MiE_{90}$ and $MiE_{90}/MiE_2$-year approaching the maximum coefficient of 1.0. The cumulative frequency percentage curves of these ratios are presented in figure 1.

TABLE II

DOSAGE RANGE OF THE TWENTY-TWO MATERIALS USED IN THE SEVEN OR NINETY-DAY STUDIES

| | Frequency of Occurence | | |
|---|---|---|---|
| | | Minimum Effect Level | |
| Dosage Range; ml. or gm./kg. | LD50 | 7-Day Study | 90-Day Study |
| < 0.1 | 7 | 7 | 12 |
| 0.1 to 0.99 | 4 | 10 | 7 |
| 1.0 to 9.99 | 8 | 4 | 3 |
| >10 | 3 | 1 | 0 |
| Range | 0.0009 to >64.0 | 0.0008 to 10.0 | 0.0002 to 5.3 |

TABLE III

SENSITIVITY OF CRITERIA AT MINIMUM EFFECT DOSAGE LEVEL IN SEVEN-DAY OR NINETY-DAY STUDIES

| | 7-Day | | | 90-Day | | |
|---|---|---|---|---|---|---|
| | No. of Pertinent | % of Times | | No. of Pertinent | % of Times | |
| Criterion of Effect | Studies | Criterion Affected | Sole Effect | Studies | Criterion Affected | Sole Effect |
| Body Weight | 22 | 86 | 41 | 22 | 64 | 45 |
| Liver Weight | 22 | 36 | 0 | 22 | 32 | 18 |
| Kidney Weight | 22 | 13 | 0 | 22 | 9 | 0 |
| Mortality | 22 | 4 | 0 | 22 | 4 | 4 |
| Cholinesterase | 4 | 100 | 75 | 7 | 71 | 29 |

## TABLE IV

## PARAMETERS OF RATIOS

| Parameter | Ratios | | | |
|---|---|---|---|---|
| | $LD50/MtF_{7}$-Day | $LD50/MtE_{\infty}$-Day | $MtE_{7}$-Day$/MtE_{\infty}$-Day | $MtE_{\infty}$-Day$/MtE_{8}$-Year |
| 25th Percentile = $Q_1$ | 1.2 | 2.8 | 2.0 | 1.1 |
| Median | 2.6 | 10.0 | 3.0 | 1.8 |
| 75th Percentile = $Q_3$ | 11.4 | 22.4 | 5.1 | 3.8 |
| 95th Percentile | 25.8 | 39.8 | 6.8 | 5.7 |
| Semi-Interquartile Range = $(Q_3-Q_1)/2$ | 5.1 | 9.8 | 1.6 | 1.4 |
| Coefficient of Rank Correlation | 0.860 | 0.799 | 0.935 | 0.946 |

Mtl = Minimum effect dosage level

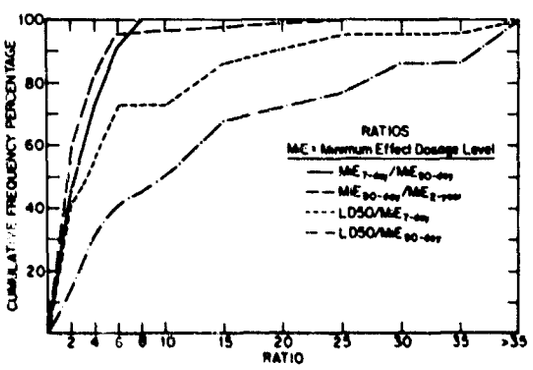


Figure 1. CUMULATIVE FREQUENCY PERCENTAGE CURVES OF ACUTE TO SUBACUTE AND TO CHRONIC FEEDING STUDY RATIOS

In summary, it has been demonstrated that joint toxic action of mixtures for acute inhalation and peroral routes can often be predicted by calculation. Also, the toxicities of these mixtures by these routes were positively correlated. Further studies should be instigated to determine if joint toxic action of mixtures over longer periods of exposure can also be accurately predicted from the repeated dose toxicities of their components.

Furthermore, the results of subacute feeding studies can often be predicted by a one-week test, as can long term results by a subacute test. These excellent relationships between feeding studies of varying duration indicate hope that similar correlations can be proved by experimentation between inhalation studies of short and long term durations. If these inter-relationships are good, dosage levels for the latter can be better predicted from the former, or short term studies might be used in place of long term exposures.

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## DISCUSSION

DR. FAIRCHILD (U.S. Public Health Service, Cincinnati): Carrol, I don't know whether I'm asking a naive question or not. I'll let you tell me. Doesn't mechanism of action have to be considered? For example, if you have an agent which produces its LD-50 within a few minutes--let's say strychnine sulphate, which would give an LD-50 at 12 minutes and be the same one at 7 days, your 7-day there is not a true 7-day, because what you measured was something that was happening in a very short period of time, so I think we have to be real careful in comparing agents. For example, in our own work we have something that's a little more recognizable, ozone. Practically everything occurs in the first 24 hours. Now, do you mean that perhaps the 7-day, which would probably be the same as the 24-hour, would be applicable to extending it to a 90-day extrapolation? Do I make myself clear?

MR. WEIL (Mellon Institute): Yes. First, I thought you were talking about predicting joint action of materials, the LD-50's, we'll say, of materials that kill at varying periods of time. You're talking about predicting the 90-day results from a 7-day test?

DR. FAIRCHILD: Yes. In other words, if someone takes data on something that has been reported for a 7-day mortality and then he says, "Well, there should be good correlation between the 7-day and the 90-day," but when, in fact, perhaps the mechanisms were different. Well, I go back to ozone-like pulmonary irritants which can have one effect which is acute and produces its response very fast, but if there is no pulmonary edema involved, you might have a completely different type mechanism.

MR. WEIL: Well, I didn't mean to imply that the 7-day or 90-day or 2-year will always hold, but on the same material, in our experience, the LD-50 (which might be somewhat similar to your acute work) was not well-related to the sub-acute. But even a 7-day--(these are feeding studies, of course)--even the 7-day sub-acute was generally well-related. All of the ratios, 8 or less, well-related to a 90-day. This doesn't mean all materials will behave this way. On the prediction of the joint action formula, remember the word "prediction". This is predicting what you would get. It's just that we found that we really predict very well what a 90-day test will be by doing a 7-day. We started this testing primarily because we wanted to choose our 90-day levels better than we were setting them just by getting LD-50's before we started.

DR. FAIRCHILD: Yes, that answers the question. I just wanted to point out, I think, that something that has a delayed response, and in ingestion this is very true, such as mercuric chloride, the one-day LD-50 is going to be considerably different from a one-week or two-week, so we have to think about the mechanism.

MR. VERNOT (SysteMed Corporation): If you say that a 7-day gives good predictability for a 90-day, and a 90-day gives good predictability for 2 years, does it necessarily follow that a 7-day test will give you good predictability for a two-year test?

MR. WEIL: Quite possibly. The reason why we didn't make this next step was that many of the materials that we have done for 7 days were not necessarily the same ones we had done for two years. It takes quite awhile to collect a group of two-year data. This is one reason why we merged with the Dow Chemical Lab in order to get a better collection and I wouldn't be a bit surprised, at least in our peroral experiments, that the 7-day will give you a fair prediction for 2 years. Of course we are not talking about carcinogenicity or anything else that might occur late in a two-year study, but generally, yes. Generally, I would guess that even a 7-day would predict a two-year with a fair degree of accuracy. Not one to one. If it's one to one, all the ratios would be one, but a definite relationship between the tests of varying durations.

MR. WANDS: I was interested, Carrol, in your comments that the weight gain was one of the most sensitive measures you could have of the chronic toxicity of these materials.

MR. WEIL: Very definitely. At least half of the dosage levels could be delineated if you measured only weight gain and nothing else. In our experience it is the best criterion of effect. Weight gain is altered, and here we are generally talking about a decrease as compared to controls, and is the most sensitive criterion of effect we have ever found.

MR. WANDS: You're talking entirely of ingestion studies.

MR. WEIL: That's correct.

DR. BACK: Carrol, what about a different end-point? We're talking here of LD-50's where death is the end point. Have you tried any correlations with other parameters for the end point, more subtle ones than death? Because death is a rather final thing and that's not really what we're looking for in the end.

MR. WEIL: Well, we were trying to compare with a single-dose study as usually done, and there death is the criterion. We are looking for no-effect levels just as you are and we wanted to find the best way we can predict no effect levels from chronic studies. We definitely found that LD-50's were not. Anything else that's worthy of suggestion should be tried.

QUESTION: Did your list of criteria reflect all of the criteria that you were testing, or were those chosen from a number of criteria, and could you tell us what types of criteria you did examine?

MR. WEIL: Yes. We published this paper. It's in a journal. Too many of you don't perhaps see that. It's going to be referred to in the publication of my paper, but it's in the American Industrial Hygiene Association Journal. We followed all of the criteria that anybody could ever follow, all of the organs that anyone would ever look at, any pathologist. We weighed all of the organs. We have done urine analysis, hematology, biochemistry, and all of these criteria, of course, are affected if your dose is high enough. We are talking here about sensitive criteria at the lowest dosage levels--those levels producing the least effect, the least difference from the controls.

DR. TEPPER (MSA Research Corporation): Was your comment with respect to general decreases in body weight also the case with respect to specific organ weight changes? Are these ordinarily reduced?

MR. WEIL: No, sir, these are usually just the opposite. They are generally increased, and these are in relation to body weight. If you get a body weight decrease, then you would expect the organ weight to be decreased also, but these organ weight changes, such as liver weight and kidney weight, are usually increased ratios of the organ weights as percentages of body weights. Relative liver and kidney weights increase significantly. In each case where we talked about a difference, we were talking about the statistical comparison to the controls.

DR. TEPPER: Because of the natural attempt of the body to purify what has been placed upon it?

MR. WEIL: Why is always a good question: Sometimes it just may be due to the excess work of detoxifying and may not be the toxic effect at all. Sometimes it is the indication of histological damage which will occur to those organs, if not at those levels, damage at higher dosage levels. It's sometimes merely work and sometimes it is an indication of organ damage.

AMRL-TR-68-175

PAPER NO. 9

# THE MEASUREMENT OF ANIMAL LUNG FUNCTION UTILIZING A CAPACITANCE RESPIROMETER

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and
Edward Domeier

Wayne State University
Detroit, Michigan

A new and simple method has been devised for measuring pulmonary volumes and inspiratory and expiratory flow rates without the added errors of general anesthesia and the trauma of endotracheal intubation or neck collars. This method eliminates incorrect data caused by the friction and air flow resistance of conventional respirometers and has the further advantage of reducing psychological errors to a minimum. The extremely fast response of the capacitance respirometer allows very small irregularities in lung ventilation to be observed and recorded.

The basic principle of the capacitance respirometer involves an oscillating capacitor circuit with a known frequency. Capacitance changes, in the magnitude of 1/100 to 1/1000 $\mu\mu$ farads, result in frequency changes, and ultimately as voltage changes, which are detected and calibrated as volume changes. If the test subject is one plate of the capacitor and the other plate is constant in distance and size, then during respiration the capacitor circuit frequency changes. These changes are converted to voltage changes and then recorded as respiratory volumes.

A schematic of the electronics involved in the capacitance respirometer is shown in figure 1. The cylindrical screen (S) represents the constant plate of the capacitor, and the animal (V) within represents the variable plate which changes in size and distance in respect to the constant plate during respiration. The screen (S) is grounded and an external electrode (E) in contact with an extremity (contact being made with electrode paste) is connected to the circuit of the variable capacitors (A) and (B). This circuitry has an oscillating frequency which is dependent upon how the instrument is constructed and can be varied with capacitors (A) and (B). Changes in the capacitance between the wire mesh cage and the test subject, as caused by surface area changes during respiration, cause a frequency change in this circuitry. This change in frequency is mixed with the constant frequency of a fixed oscillator (C) to produce what we call a "beat frequency". This beat frequency is then picked up by the discriminator (figure 1) where the change in frequency is converted into a change in voltage. This signal is then amplified, rectified, and filtered before it is recorded.

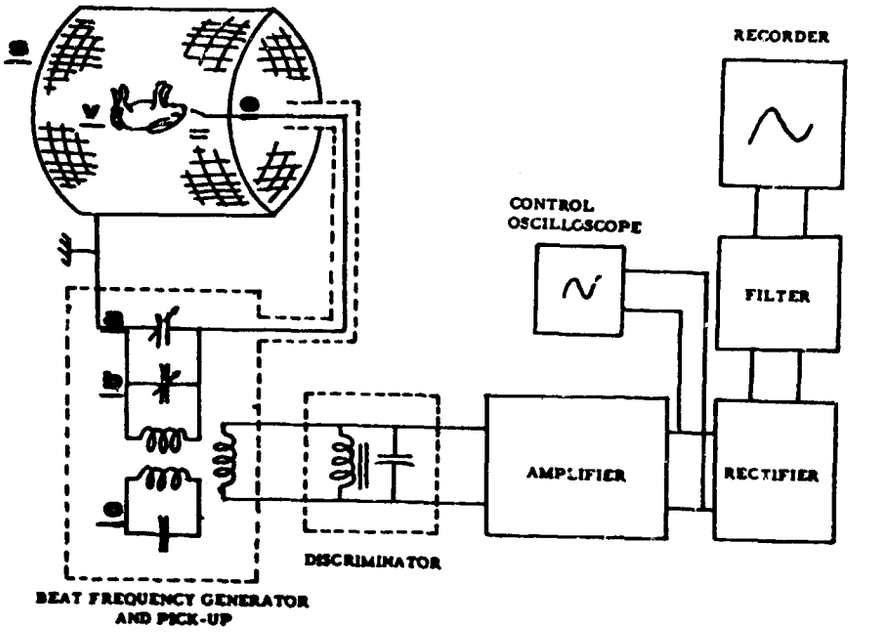


Figure 1. CAPACITANCE RESPIROMETER

The frequency of the variable oscillator, which again involves the wire cage and test animal, is adjusted to 200,000 cycles per second. The normal breathing of a test animal causes a variation of 1 or 2 cycles per second. This change in frequency during the respiratory cycle is caused by (1) a change in the body surface area with each respiratory volume, and (2) a change in the distance between the animal's body and the grounded wire cage. A change of 1 or 2 cycles per 200,000 cycles is not easily detected. For this reason the signal is conducted to the beat frequency generator, where the 200,000 cycles per second is distuned to give a resulting beat frequency of 300 cycles per second. The mixing of these two frequencies is simply a method of increasing the sensitivity of the instrument.

Many different techniques were employed in an attempt to calibrate this instrument. Both the direct and indirect methods were utilized, the indirect method being the one finally selected for calibration. The indirect method of collecting respiratory gas volumes using a 2-way valve proved to be the most satisfactory way of calibrating this respirometer. This involved the collection of approximately 20 tidal volumes in a gas collecting bag. This gas volume was then measured by water displacement at a pressure which was slightly less than normal atmospheric pressure and an average single tidal volume was calculated. The volume measured needs a correction of 7% due to body temperature and water vapor content.

Since the surface area of all solid objects does not increase in a linear manner with a volume increase, it was necessary to apply a correction factor to the volume curves. This correction factor was based on the weight of the test animal and was used as an estimation of body volume. Figure 2 is a general correction curve for rabbits, guinea pigs, and rats and was constructed by using many different weights for each animal type. From this curve it is possible to select a correction factor which is employed in the calculation of respiratory volumes.

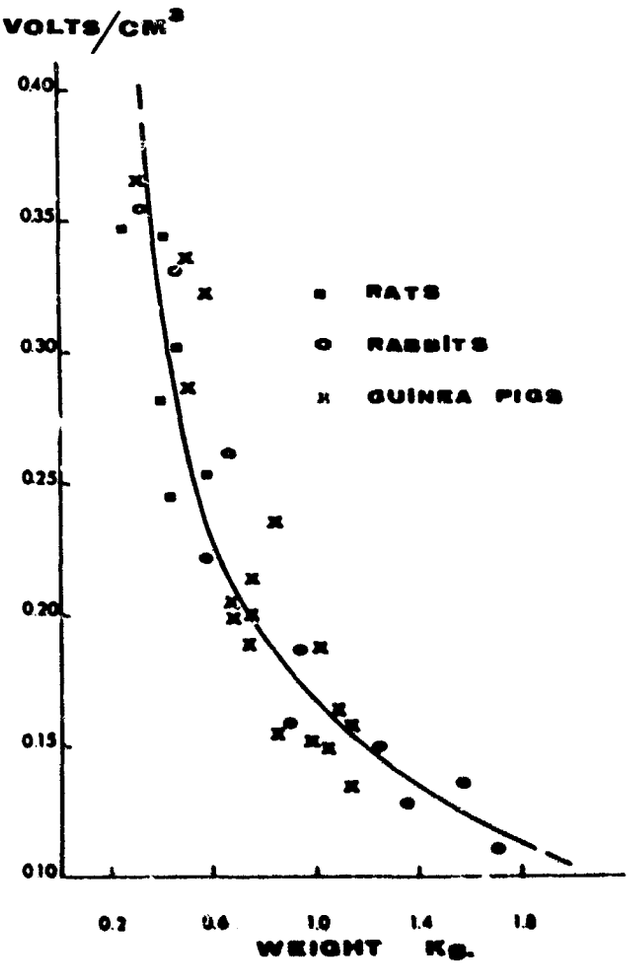


Figure 2. CORRECTION CURVE

The wire mesh cage (figure 3) seen inside the hexagonal chamber is a cylinder, 42" in diameter, and 62" in length. Two windows for entry into the chamber are located on either side; when closed and clamped, this provides an air-tight chamber in which to perform pulmonary function tests before, during, and after an exposure to a known gas atmosphere. This dynamic type chamber is kept under a slight negative pressure to insure no leakage during an exposure. The outer cage is supported by foam rubber to eliminate building vibrations, and is the support for the nylon mesh platform located in the center of the chamber. The test animal is placed spatially in the center of the wire mesh cage on the nylon grid (figure 4) and is secured to the outer wooden cage. A pick-up electrode is attached externally to an extremity, and skin contact is made with electrode paste. A coaxial cable connects the pick-up electrode with the electronic unit of this instrument. The electronic unit senses the changes in capacitance between the test subject and the surrounding cage resulting from breathing activity, and converts these capacitance changes into equivalent voltage changes.

Figure 3. AIR-TIGHT OUTER CHAMBER

Intrapleural pressures are approximated utilizing an esophageal balloon to indicate changes in the elasticity of lung tissue. For this measurement it is necessary to keep the animal's mouth open (figure 5) to prevent it from chewing on the fine polyethylene tubing connecting the esophageal balloon to a Strathsburg Pressure Transducer. The insertion of the esophageal balloon is done in such a manner as to give maximum pressure changes with a minimum of cardiac pulse pressure change. This area is generally found to be approximately 1-2 cm. above the cardiac end of the stomach. With the animal in this position, it is now possible to record simultaneously the tidal volumes and intrapleural pressures for periods up to 4 hours. During this time the animal can be exposed to fumes, dusts, or gases, and their effects directly observed. A typical recording of a normal rabbit's respiration is shown in figure 6. The curves are, from top to bottom, flow rates, esophageal balloon pressure changes, and tidal volumes. The average

tidal volume in this spirogram is 19 milliliters with a respiratory rate of 158 breaths per minute. From curves of this type it is possible to obtain data on: tidal volumes, respiratory rates, minute volumes, inspiratory and expiratory flow rates, and pressure-volume relationships.

Figure 4. SPATIALLY MOUNTED RABBIT

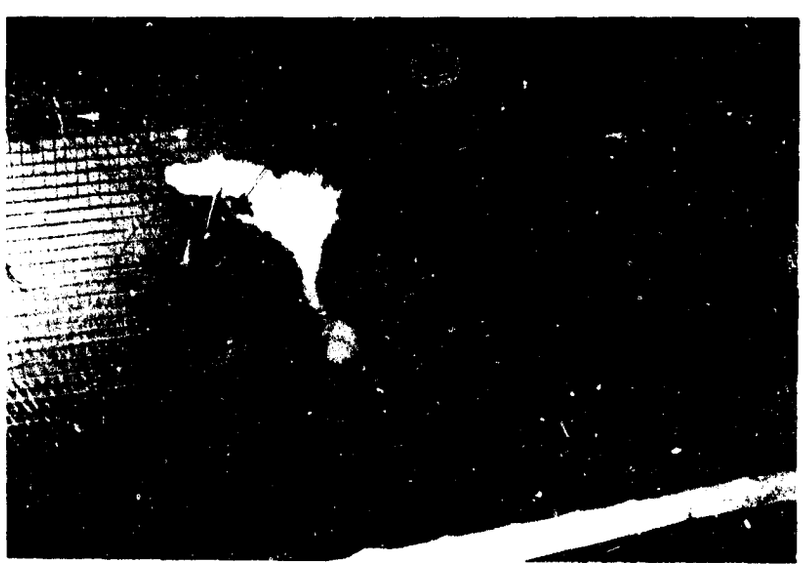

Figure 5. SPATIALLY MOUNTED RABBIT WITH ESOPHAGEAL BALLOON INSERTED

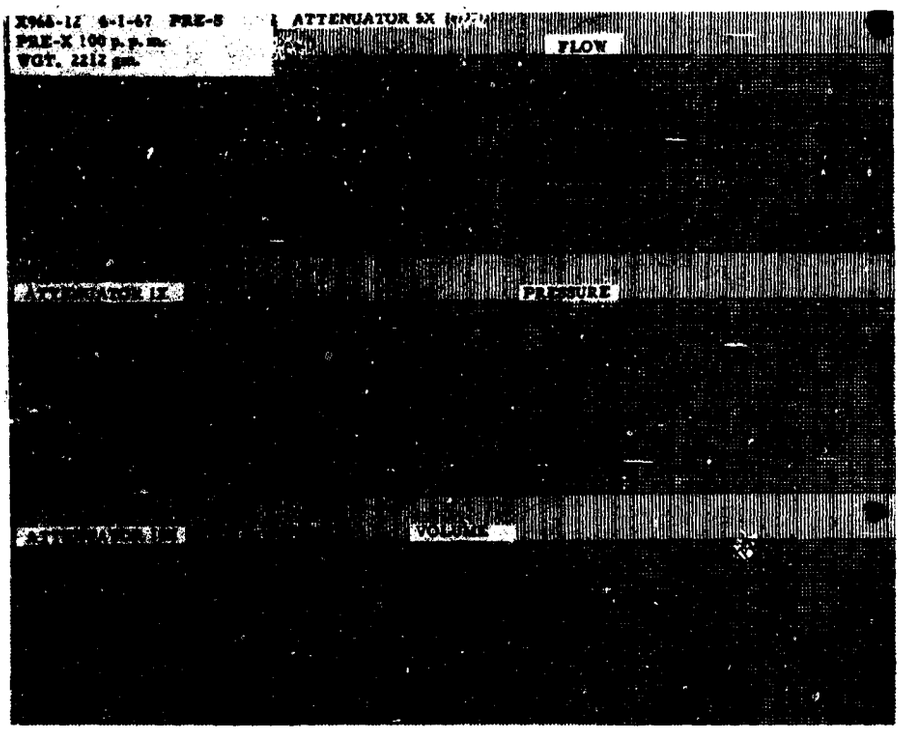


Figure 6

Some small doubt concerning what actually was being measured and recorded was removed when a rabbit, breathing normally (figure 7), voluntarily closed its epiglottis while maintaining the body movements of normal respiration. As figure 8 shows, normal intrapleural pressure changes continued while the volume recording shows almost no change (less than 1 cc.). The data presented in figures 7 and 8 shows that the normal average tidal volume for this rabbit was 34.6 cc., at a rate of 40 breaths per minute, requiring an intrapleural pressure of 5.4 cm. of water. During this breath-holding phenomenon, the intrapleural pressures were 4.8 cm. water, at a rate of 20 efforts per minute, with a volume change per effort of less than 1 cc. These recordings are evidence of the validity of the data from the capacitance respirometer in respect to body configuration changes under conditions of zero air flow and constant body volume. This type of data helps prove that the volume recordings are actually true tracings of the respiratory volumes and flow rates and not artifacts due to body configuration changes.

In summary, it appears that valid data concerning pulmonary function can be obtained on rabbits, guinea pigs, and rats before, during and after experimental exposures without traumatizing the animal, thus allowing the animal to continue in the experiment throughout its entire life span. The capacitance respirometer also eliminates errors caused by the friction and air flow resistance of conventional methods. This instrument has the further advantage of reducing psychological errors to a minimum.

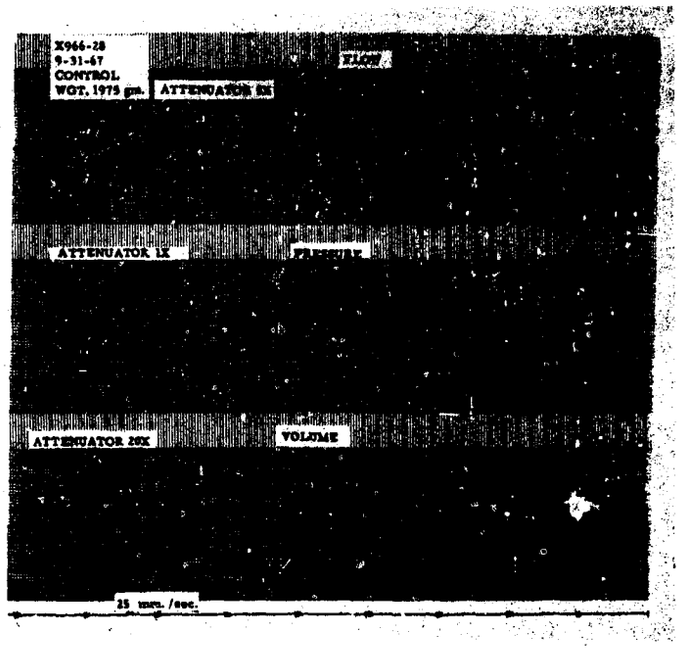


Figure 7

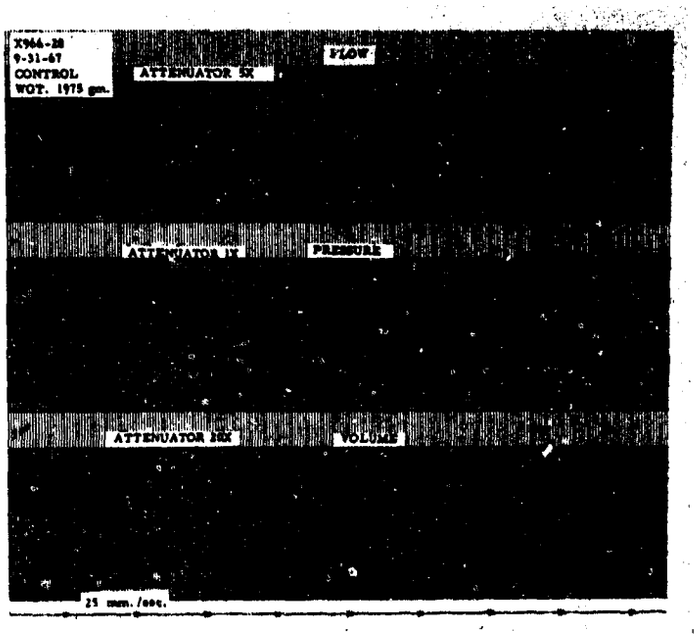


Figure 8

## DISCUSSION

QUESTION: Does the heart action have a trace on the recorded pattern?

DR. BARROW (Wayne State University): Yes. You can pick it up on the esophageal balloon pattern. You have to accommodate for that. On the respiratory curves themselves, you generally don't see the pulse pressure unless you're using a very high sensitivity on the machine. You can pick it up.

QUESTION: Could you amplify it?

DR. BARROW: Oh, yes. I might point out, we were looking at anywhere up to 15 to 30 cc tidal volumes here. This instrument, the one I used for the rabbit here, can measure down to a hundredth of a cc tidal volume.

QUESTION: How does it compare with the pneumograph system, which is a band around the chest measuring expansion of the lungs?

DR. BARROW: Well, I don't know; I haven't compared this with that at all. I couldn't answer that.

DR. LEWIS (U.S. Public Health Service, Cincinnati): I guess this is more of a comment than a question. I'm probably one of the few people in the audience who has used this technique, and it was developed under a USPHS contract. We did get a capacitance respirometer much more adaptable to small animals - rat, mouse, even rabbit and the guinea pig, but when you get to the monkey and the dog, you have a lot of different problems here. The dog obviously can't be tied on its back in an awake state, and we didn't want to put the dog under anesthesia. At first we were going to work with tranquilizers, but the problem with this technique is that you always have to present the same geometric shape. In other words, the animals are always elongated. The dogs would not settle in the same geometric pattern. One would lay out straight, another form a circle and things like this, so these are some of the logistical problems you run into. We finally abandoned it, and went to the pneumotachygraph, integrating flow to volume, measuring pressures within esophageal balloons and it enabled us to use other measurements like diffusing capacities at the same time, but always under anesthesia. I like your method for rodents, but for monkeys and dogs it's a little different problem.

DR. BARROW: I haven't done a dog, but we won't use the same instrument for monkeys or humans. We actually have an electrode which is about the size of a sheet of paper and we don't use the complete wire cage any more. It's just a single electrode which is variable. This works very nicely.

DR. FAIRCHILD: Would you expect to find with this method where you are using mouth-breathing a different response than in the normal animal which would be really nose-breathing, especially with particulates?

DR. BARROW: We used both. Actually, when I'm working with just tidal volumes, expiratory-inspiratory flow rates, the animal does not have its mouth forced open. Only for the pressure measurement do we force the mouth open.

MAJOR THEODORE (Aerospace Medical Research Laboratories): I was wondering, on the one trace there where you had essentially tracheal occlusion, you said that the volumes didn't change within the lung. You demonstrated that you had no volume change on your tracings. However, to generate negative pressures within the thorax, the diaphragms would have to descend and therefore you would have abdominal and thoracic changes even though the lung volumes are the same. Using body plethysmography you could have measured FRC in that fashion. I wondered why you don't get a volume measurement since the chest and abdominal walls changed position in the capacitance field.

DR. BARROW: I said the change was less than 1 cc. There is a change. You can see a change and it calculates out very nicely what you would expect for that pressure for the expansion of the gas in the lung. It does come out very nicely. You do get an expansion.

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PAPER NO. 10

# HYPOTENSION DURING BROMOTRIFLUOROMETHANE EXPOSURE

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## INTRODUCTION

Bromotrifluoromethane ($CBrF_3$, Freon 13B1) is of interest to the United States Air Force, the National Aeronautics and Space Administration, and the private aircraft industry for potential use in fire-extinguishing systems to be used in closed environments. $CBrF_3$ is a fully halogenated hydrocarbon of relatively low toxicity. Exposure of dogs and monkeys to atmospheres containing from 10% to 80% $CBrF_3$ was shown to cause a fall in mean arterial blood pressure, spontaneous cardiac arrhythmias, and lethargy (Van Stee and Back, in press).

These experiments were designed to elucidate the mechanism of the initial fall in mean arterial blood pressure which was seen prior to the onset of cardiac arrhythmias.

### Methods

Ten Beagle dogs of both sexes from 18 to 30 months of age weighing from 7.8 to 12.3 kg were used. The dogs were anesthetized by a single intravenous injection of 30 mg/kg of sodium pentobarbital. Endotracheal catheters were inserted and femoral venous and arterial cutdown performed. Stage III, plane 1 anesthesia was maintained by the slow intravenous drip of a mixture of sodium pentobarbital (2500 mg/liter) and tubocurarine hydrochloride (60 mg/liter). The animals were ventilated mechanically at a rate of 150 ml/min/kg. The respiratory dead space of the mechanical system was 30 ml which was added to the required tidal volume.

Electrocardiograms were obtained from which heart rate was calculated. Arterial blood pressure was determined with the use of a pressure transducer (Statham). Cardiac output was determined by the indicator-dilution technic using indocyanine green (Guyton, 1966).

Total peripheral resistance (TPR) was calculated by dividing the mean blood pressure drop ($\Delta P$) across the peripheral vascular bed by the cardiac output in ml/sec (Burton, 1965). Mean blood pressure was estimated from the central arterial blood pressure recordings according to the formula:

$$\frac{(\text{systolic pressure}) + 2 \times \text{diastolic pressure})}{3}$$

Venous pressure was assumed to be 5 mm Hg and thus $\Delta P$ was estimated to be equal to the mean arterial pressure minus 5 mm Hg. Since TPR varies inversely with cardiac output values for TPR were multiplied by body weight to provide values for TPR-kg which afforded a basis for comparisons among different subjects.

Figure 1 illustrates the design of the experiment. The 10 dogs were divided into two groups of five. Five determinations of cardiac output were performed at 10 minute intervals during each period. The treatments of the series of "treated" dogs differed from the series of "untreated" dogs only during the second period during exposure to 70% $CBrF_3$.

Comparisons of the mean values for mean arterial blood pressure, cardiac output, total peripheral resistance, heart rate, and stroke volume between the treated and untreated groups were made using Student's t-test (Freund, 1960).

The myocardial inotropic effect of exposure to bromotrifluoromethane was determined using 9 isolated cat and 2 isolated dog hearts prepared according to a method modified from that of Langendorf (Locke and Rosenheim, 1907). The hearts were perfused with continuously oxygenated Locke's solution containing, per liter, 9.2 gm NaCl, 0.42 gm KCl, 0.12 gm CaCl, 0.15 gm $NaHCO_3$, 1.0 gm glucose, and 5 units of regular insulin. The preparation was bathed in oxygenated Locke's solution. All experiments were performed at 32 C. The force of contraction was measured by inserting a 21 ga metal cannula through the myocardium into the left ventricle (De Geest, 1965). The cannula was connected to a transducer with a length of polyethylene tubing. The transducer chamber, tubing, and cannula were filled with Locke's solution. Locke's solution was saturated with 50% $O_2$ - 50% $CBrF_3$ by bubbling the gas through the solution for 60 minutes prior to its perfusion through the heart. Both oxygen and $CBrF_3$ were continuously bubbled through the perfusate during the actual perfusion.

Electrocardiograms were recorded from the isolated hearts by locating two electrodes on opposite sides of the heart within the chamber in which the heart was bathed.

The sequence of events in the preparation of an isolated heart was as follows: All solutions were equilibrated with the gases for one hour and brought to 32 C. A pan of Locke's solution was placed in an ice bath and reduced to 10 C. Animals

were anesthetized with intravenous thiamylal (20 mg/kg), the thorax opened and the heart excised as rapidly as possible including about 1 cm of aorta distal to the anastomoses of the brachial and left subclavian arteries. Excision of the heart seldom required more than one minute. The heart was then plunged directly into the cold Locke's solution and gently irrigated throughout with the cold Locke's solution to remove all blood from the chambers and coronary vessels. Contractions were arrested within one minute of excision. The cannula was inserted into the aorta with the tip thrust through the aortic valve and secured by ligation well above the ostia of the coronary arteries. The pressure probe was inserted through the left ventricular myocardium and the preparation placed into the 32 C. bath. The perfusate, flowing freely into the ventricle from a height of 45 cm, rapidly warmed the myocardium and resulted in a spontaneous resumption of the contractile cycle within a few seconds. The time which elapsed between anesthetization and re-warming the final heart preparation usually amounted to 15 to 20 minutes. The observation was made that the heart could be manipulated without hurrying once it had been chilled.

Seven dogs from 6.5 - 9.0 kg and five monkeys from 2.0 to 5.5 kg were prepared for the measurement of left intraventricular pressure. They were anesthetized with sodium pentobarbital, intubated, and placed on a mechanical respirator. The heart was exposed by making a left parasternal incision through the costal cartilages from T2 to the xiphoid process. The pericardium was incised and a plastic catheter placed in the left ventricle through the left ventricular myocardium. The catheter was connected to a pressure transducer and the recordings made on a direct writing oscillograph.

## Results

The effect of 70% $CBrF_3$ on mean arterial blood pressure is illustrated in figure 2. The blood pressure during $CBrF_3$ exposure was significantly lower than pre- and postexposure levels in the treated group and during the corresponding period (2nd) in the untreated group.

Changes in cardiac output are shown in figure 3. Cardiac output in the untreated group fell significantly from one period to the next throughout the course of the experiment. Cardiac output in the treated group followed the same general trend from the lst to 3rd period with a statistically insignificant increase during $CBrF_3$ exposure (2nd period). No significant difference was observed between the treated and untreated groups.

Changes in the calculated total peripheral resistance (TPR) are shown in figure 4. TPR rose throughout the course of the experiment in the untreated animals whereas it declined significantly during the $CBrF_3$ exposure and then rose again postexposure.

The effect of $CBrF_3$ on heart rate is illustrated in figure 5. Heart rate did not change significantly in the untreated group but was significantly lowered during exposure to $CBrF_3$ (2nd period) in the treated group.

The effect of $CBrF_3$ on stroke volume is illustrated in figure 6. The tendency in the untreated group was for the stroke volume to fall throughout the course of the experiment, but exposure to $CBrF_3$ in the treated group (2nd period) caused a significant rise in stroke volume during the exposure period.

Figure 7 illustrates the response of the isolated hearts to standard agents which produce a negative or positive inotropic effect. The upper left recording represents a control. The upper tracing is the left intraventricular pressure and the lower tracing is the time line (seconds). The upper right recording shows the negative inotropic effect following the injection of acetylcholine into the perfusate column just above the entrance of the catheter into the aorta. The lower left recording shows the reversal of the acetylcholine effect by the injection of atropine followed by 3 or 4 cycles of gentle cardiac massage. The lower right recording shows the positive inotropic effect of the injection of epinephrine into the perfusate column.

The effect of $CBrF_3$ on an isolated cat heart is illustrated in figure 8. The top tracing is the electrocardiogram, the center is the intraventricular pressure and the bottom, the time line (seconds). The lower recordings illustrate the negative inotropic effect reflected in the decreased pulse pressure switching from the $CBrF_3$-containing Locke's solution at a constant left ventricular end diastolic (LVED) pressure. The ECG indicated a marked negative chronotropic effect. The upper recordings show the results of switching from the $CBrF_3$-free to $CBrF_3$-containing Locke's solution and then raising the LVED pressure 5 cm $H_2O$, a procedure which would normally be accompanied by a marked increase in the force of contraction.

The results obtained from the cat and dog isolated heart preparations were similar on the basis of 9 cat and 2 dog hearts. Because of this observation further repetitions of the dog heart experiments were not considered necessary.

Figure 9 illustrates a progressive rise in left ventricular end diastolic pressure (bottom series of recordings) during exposure to 80% $CBrF_3$. The end diastolic pressure returned to preexposure values postexposure. As the end diastolic pressure rose the left ventricular systolic pressure (top series of recordings) fell significantly during exposure to $CBrF_3$.

## DISCUSSION

Blood pressure fell during exposure to $CBrF_3$. Cardiac output did not vary significantly between the treated and untreated groups. A decrease in blood pressure without a change in cardiac output indicated a fall in peripheral resistance. Cardiac output is the product of heart rate (HR) and stroke volume (SV). Since cardiac output did not change significantly during exposure to $CBrF_3$ and HR fell significantly, stroke volume apparently increased. The decrease in resistance to the outflow of blood from the left ventricle was sufficient, even in the presence of a decreased heart rate, to allow the stroke volume to rise sufficiently to maintain cardiac output during $CBrF_3$ exposure. If the blood pressure fall had been the result solely of impaired cardiac function without a concurrent relaxation of

the resistance vessels, the TPR would have been expected to rise sharply since the force tending to distend the arterioles would have been reduced which would have resulted in a decrease of diameter. This in turn would sharply raise resistance to the flow of blood (law of LaPlace, Burton, 1965). Finally, a decrease in TPR accompanied by no change in cardiac output implies a decreased myocardial contractility.

The experiments with the isolated hearts and the open-chested animals indicated that exposure to $CBrF_3$ reduced the myocardial contractility and possibly the heart rate also. When blood pressure falls, activation of the baroreceptor reflexes normally results in reduced vagal tone which has the effect of increasing both the heart rate and force of contraction. However, heart rate decreased significantly during the exposure of the intact dogs in the first experiment (figure 5). This supports the observation of a negative chronotropic effect in the exposed isolated hearts.

The rise in the left ventricular end diastolic (LVED) pressure associated with exposure to $CBrF_3$ was apparently the result of a combination of two factors: 1) decreased precapillary arteriolar sphincter tone and 2) decreased myocardial contractility. The initial effect of $CBrF_3$ appeared to be a relaxation of the resistance vessels which caused the TPR to fall. The capacitance vessels distended in response to the flow of high pressure arterial blood into the venous system and this shift of blood caused the pressure fall in the arterial system. Because of the greater compliance of the venous system, the venous pressure rises approximately 1 mm Hg for each fall of 24 mm Hg in the arterial side. As the right atrial pressure rose in response to the filling of the venous system the heterometric auto-regulatory response of the normal heart would increase the cardiac output. The exposure of the heart to $CBrF_3$ resulted in a reduced myocardial contractility which interfered with its ability to compensate entirely for the increased venous return and blood was pooled in the venous system resulting in an elevated LVED pressure. A balance between the two factors of decreased TPR and myocardial contractility was established. The increased venous return was not entirely accommodated by the weakened heart which continued to pump against a reduced resistance with the result that the cardiac output remained the same and the LVED pressure rose (Guyton, 1966).

## CONCLUSIONS

Exposure to bromotrifluoromethane ($CBrF_3$) caused a relaxation of the precapillary arteriolar sphincters which reduced the peripheral resistance. Simultaneously, the force of myocardial contraction decreased with the net effect that blood shifted from the arterial system to the venous system. The increased venous return was not accommodated by the weakened heart. Thus, no change in cardiac output accompanied the fall in peripheral resistance and the left ventricular end diastolic pressure rose.

| | PERIOD 1 0-40 MIN | PERIOD 2 40-90 MIN | PERIOD 3 90-140 MIN | |
|---|---|---|---|---|
| TREATED | AIR | 70% $CBrF_3$ | AIR | 5 DOGS |
| UNTREATED | AIR | AIR | AIR | 5 DOGS |

5 DETERMINATIONS PER DOG PER PERIOD

Figure 1. DESIGN OF EXPERIMENT TO DETERMINE IF EXPOSURE TO $CBrF_3$ ALTERS PERIPHERAL VASCULAR RESISTANCE. Five determinations of cardiac output were performed on each dog at 10-minute intervals during 3 consecutive exposure periods and the means for each period reported.

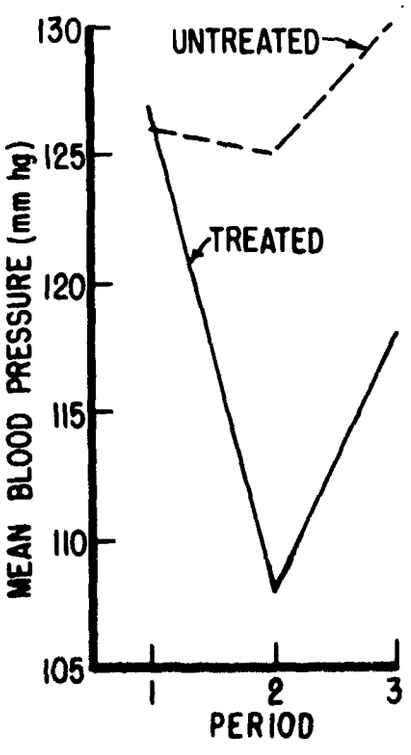


Figure 2. EFFECT OF $CBrF_3$ ON MEAN ARTERIAL BLOOD PRESSURE. Blood pressure fell significantly during exposure to $CBrF_3$ (2nd period, treated) and rose postexposure but remained significantly below preexposure and untreated group levels.

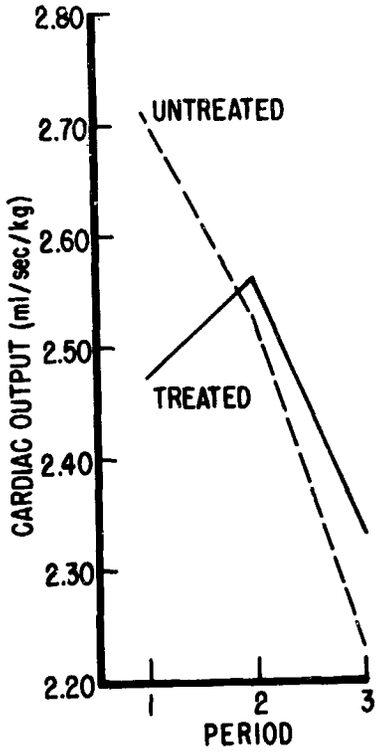


Figure 3. EFFECT OF $CBrF_3$ ON CARDIAC OUTPUT. Cardiac output fell during the experiments. No significant difference was observed between the treated and untreated groups.

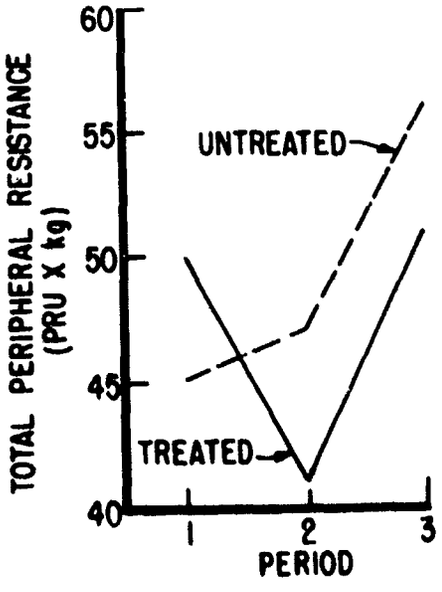


Figure 4. EFFECT OF $CBrF_3$ ON TOTAL PERIPHERAL RESISTANCE (TPR). TPR was significantly decreased during exposure to $CBrF_3$ (2nd period, treated group).

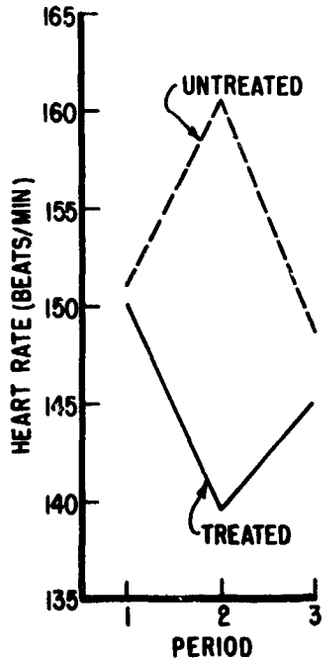


Figure 5. EFFECT OF $CBrF_3$ ON HEART RATE. The only statistically significant difference was between the treated and untreated groups during the 2nd period during which the treated group was exposed to $CBrF_3$.

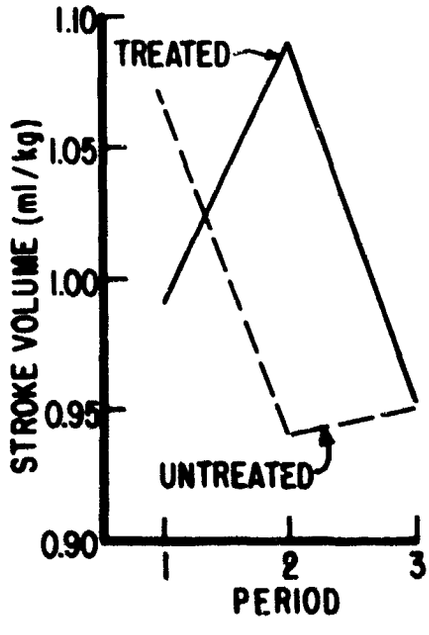


Figure 6. EFFECT OF $CBrF_3$ ON STROKE VOLUME. Stroke volume increased during $CBrF_3$ exposure (2nd period, treated) but returned to preexposure levels post-exposure.

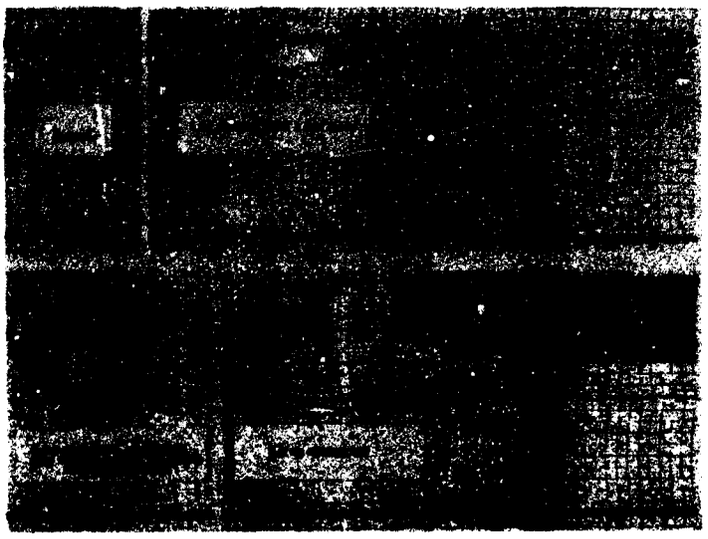

Figure 7. NEGATIVE INOTROPIC EFFECT (UPPER TRACINGS) AND POSITIVE INOTROPIC EFFECT (LOWER TRACINGS) OF ACETYLCHOLINE AND EPINEPHRINE, RESPECTIVELY, ON THE ISOLATED HEART PREPARATIONS.

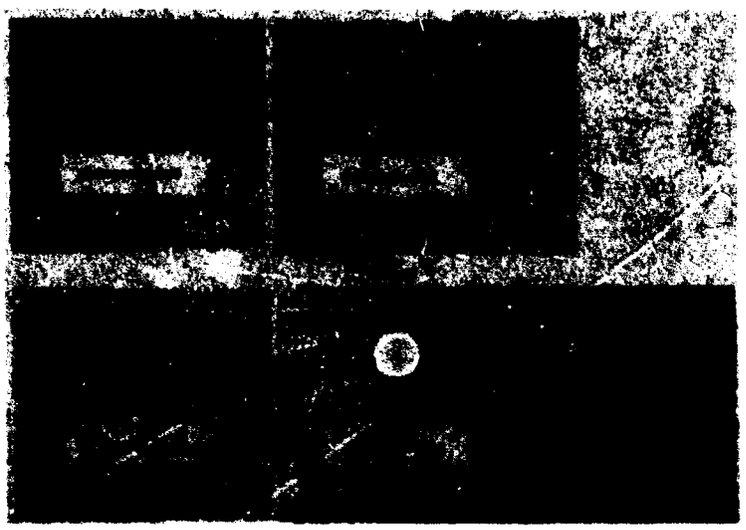

Figure 8. NEGATIVE INOTROPIC AND CHRONOTROPIC EFFECTS OF $CBrF_3$ ON ISOLATED HEART PREPARATIONS. The left ventricular end diastolic (LVED) pressure was held constant in the lower tracings and elevated by 5 cm $H_2O$ in the upper tracings. Elevating the LVED pressure in the untreated, isolated heart caused a marked increase in the force of myocardial contraction but was without effect in the $CBrF_3$-treated he

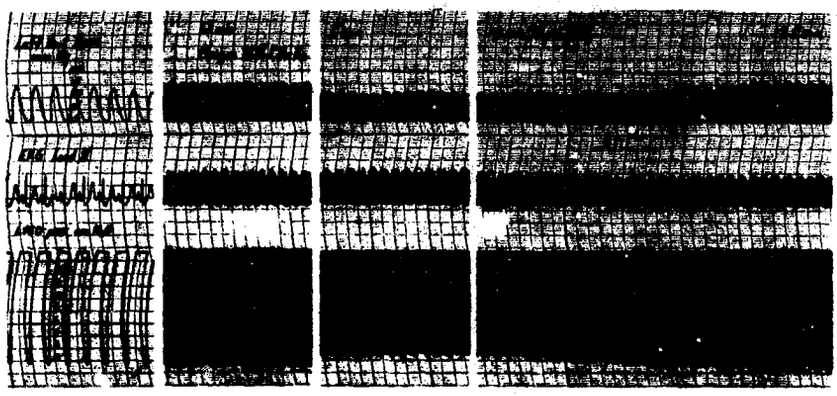

Figure 9. EFFECT OF $CBrF_3$ EXPOSURE ON LEFT VENTRICULAR BLOOD PRESSURE IN THE OPEN-CHESTED MONKEY. The top tracing shows the fall in systolic blood pressure during $CBrF_3$ exposure. The bottom tracing shows the same ventricular pressure curve amplified to show the rise in left ventricular end diastolic (LVED) pressure (systolic pressure is not shown in the bottom tracing).

ACKNOWLEDGEMENTS

The authors wish to thank Sgt. Doyle Manion for his assistance in performing these experiments.

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## DISCUSSION

DR. SMITH: I assume the freon is not very water soluble. I wonder how you got it in the Locke's solution? Was it saturated, emulsified, or how?

DR. VAN STEE (Aerospace Medical Research Laboratories): The control Locke's solution was saturated with 100% oxygen, and the test solution was saturated with a mixture by volume of 50% oxygen and 50% $CBrF_3$. You're right. Freon is very poorly soluble.

QUESTION: Do you have some idea of predictable air concentration which would have resulted in a similar tissue concentration of $CBrF_3$?

DR. VAN STEE: We have no way of evaluating this. Unfortunately, our methods have not evolved to the point where we are able to work with tissue concentrations. This is the problem. What we have to do is prepare our mixtures on the basis of volume and then administer them, and give our results in terms of what the animal was exposed to on a volume concentration basis. This is the best we can offer at the moment.

MR. WANDS: I was impressed with the speed of recovery of the isolated hearts as you switched $CBrF_3$ on and off.

DR. VAN STEE: This is very true. This is obvious in both the intact animal and in the isolated hearts. Very, very quickly there is recovery. This requires some elaboration. I'm now finding some changes in the CNS where the electroencephalogram changes persist for as long as ten minutes following removal, but most of the changes which are observed are resolved within two or three minutes of the removal of the gas.

DR. FAIRCHILD: Did I understand you to say there is a difference in species of the CNS stimulation and depression?

DR. VAN STEE: I did imply that, yes. I have moved from the cardiovascular system, working on the CNS problem right now. At the very beginning, when we were given this gas to work with, we had the results from a very limited number of dog exposures. The very first thing I did was to take some wide-awake animals and expose them to mixtures of the gas to see what would happen, to see what we had to do. One of the first things we noticed was that the dogs went into convulsions just like grand mal seizures in humans. Monkeys, on the other hand, appeared to go to sleep. We find they can be aroused. This is more a lethargic state. This is all I can say at the moment.

DR. BACK: I might point out some things Dr. Van Stee didn't mention. These are the changes that you see in the electrocardiogram which give some insight into the rapidity of onset. You can titrate in and out of a cardiac arrhythmia with certain amounts, like 50-60% of $CBrF_3$ relatively rapidly, within seconds certainly, and recovery within seconds. Dr. Van Stee didn't get into the blood pressure dependency of arrhythmias. He presented a paper in Minneapolis on this aspect. There are actually three aspects: inotropic effect on the heart; a change in peripheral resistance; and a change in total blood pressure. Actually, the animal can regulate himself if he is breathing $CBrF_3$. If you regulate it with the right amount, cause a drop in blood pressure, and the arrhythmia stops, as soon as it stops the blood pressure continues back up and it turns it on and off.

DR. FASSETT (Eastman Kodak Company): What was the rest of the gas mixture when they were breathing 70% freon?

DR. VAN STEE: Oxygen. In all of these experiments we performed periodic determinations of $pO_2$ in order to maintain these within normal limits. We have conducted other experiments under conditions of acidosis and alkalosis, which are the subject of another paper entirely.

DR. LAWTON (Naval Ordnance Environmental Health Center): I noticed that you used 50% as the minimum. Did you have a reason for selecting that level?

DR. VAN STEE: In this particular series of experiments, we picked this more or less arbitrarily. We have done a number of exposures at 10% increments, 10 through 80%. Since we had a large number of experiments to do, in order to evaluate this hypotensive response, we had to pick one blood pressure which we felt would be representative in order to work with it, since we do run into complications by varying the concentration of the gas. In a 100% oxygen environment, if a fire should start 50% $CBrF_3$ must be added in order to suppress the fire so this is another thing that went into the decision to use 50%, not mentioning the fact it's in the middle of the 10 and 80.

DR. KEITH (K-G Laboratories): Dr. Van Stee, have you tried exposure of animals who are vagotomized or atropinized in the series?

DR. VAN STEE: We did this work with relation to the arrhythmia study, not with relation to the blood pressure depression itself. No, not with this blood pressure depression. The most consistent observation we made in hundreds of exposures to various concentrations of this gas is the fall in blood pressure and we have used a lot of drugs to modify the response to the exposure to $CBrF_3$ and I do not know of anything which we have done which has modified this fall in blood pressure. The drugs, of course, will modify the arrhythmias and various things that have to do with that, but I believe I am safe in saying that drug effects will not alter this blood pressure response. I believe this to be a direct smooth muscle effect.

DR. BENJAMIN (NASA): Do I understand that your effect on cardiac output was negative in the intact animal and only in the isolated heart you found this negative inotropic effect?

DR. VAN STEE: The evidence for a negative inotropic effect was based upon the rise in the left ventricular diastolic pressure in the open chest of dogs. This is the only criterion we used in the intact animals. Apparently, in the whole intact animal, the total peripheral resistance goes down. The ability of the heart to pump also diminishes some. These things apparently occur at the same rate so that the weakened heart is able to produce a cardiac output which is, in the presence of $CBrF_3$, equal to the control values because of the decreased resistance to the outflow of blood.

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PAPER NO. 11

# CLINICAL TOXICOLOGIC STUDIES ON FREON, FE 1301*

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## INTRODUCTION

This study of the pharmacology and toxicology of "FREON", FE 1301, has special reference to possible myocardial sensitizing properties of the compound, and effects on the central nervous system. The investigation was done in two parts. The first part involved exposure of dogs to various levels of FE 1301 under fright-producing situations in order to ascertain whether endogenously released epinephrine would cause myocardial arrhythmias. The second part was concerned with exposure of human subjects to varying concentrations with measurement of mental alertness and acuity and monitoring of the electrical activity of the myocardium. Previous studies by McFarland (November, 1967) in animals had elicited signs of irritation not related to concentration. He found no significant alteration of hepatic function and no evidence of histopathologic changes in four species of animals exposed to concentrations as high as 25 volumes percent. Van Stee and Back (1968) had demonstrated a difference in response of rats and dogs. No significant central nervous system effects were produced in rats exposed to concentrations as high as 84 volumes percent, while dogs exhibited excitement and tremors at concentrations as low as 20 percent and convulsions at concentrations of 50 percent and higher. Convulsive effects were blocked by thiopental anesthesia. Ninety seconds after cessation of gassing, all animals appeared normal. Stopps (see reference 5) elicited multiple abnormal ventricular complexes

*This work was sponsored jointly by The Boeing Company and I. E. du Pont de Nemours and Company.

in 20 percent of dogs exposed to 10 volumes percent of FE 1301 and challenged with 0.008 mg/kg of epinephrine. Exposure of human subjects to concentrations as high as 10 volumes percent (Haskell Laboratory Staff) produced a slight disturbance in equilibrium of the three test subjects and subjective sensation of light headedness after 3.5 minutes exposure. The opinion was expressed that had exposures been prolonged, the subjects would have become semiconscious or that loss of consciousness might have occurred.

The intent of this study was to obtain data which might help define those levels which could be considered as safe for passengers and operative personnel in those situations where there might be need for exposure to FE 1301.

## EXPERIMENTAL APPROACH

### Material

The test material "Freon" FE 1301 was identified chemically as bromotrifluoromethane ($CF_3Br$). The material was furnished through the courtesy of E. I. du Pont de Nemours and Company. The material was described as typical of commercial production and free from impurities. FE 22B1 ($CHF_2Br$) and FE 12B2 ($CF_2Br_2$) were stated to be present at concentrations of less than 1 ppm though specifications on the product would allow up to 50 ppm of either compound. Free halogens or acids were stated to be absent. No further purification of the material was attempted and it was used as received.

### Exposure Facilities

All exposures took place in a cubicle exposure chamber of 8064 liter capacity. The chamber was lighted and equipped with a circulating fan. During some exposure of dogs, it was made totally dark by covering of the observation ports. All exposures were made in static environment. Exposure in animals lasted up to 50 minutes and those in man up to 25 minutes.

### Determination of FE 1301

An appropriate method was developed for the determination of air concentrations of FE 1301 utilizing an Aerograph 600 gas-liquid chromatograph. For this purpose we used a gas chromatograph with a hydrogen flame ionization detector and a stainless steel tube column, i.d. 4 mm and 15 cm in length, packed with 80-100 mesh Polapak "A". Operating conditions were: injection temperature 185 C; oven temperature 165 C; flow rate of nitrogen carrier gas 16.8 ml/minute and of hydrogen 25.8 ml/minute; attenuation x2 to x4; output sensitivity 1x; input impedance $10^7$. A volume of 1 ml of gas was injected. Under these conditions a full scale deflection represented 16 volumes percent. Standards were prepared from gas bottles containing 100% FE 1301. Appropriate dilutions were made of these to reach the concentrations 5, 10, and 20%.

### Provision of Desired Concentration

FE 1301 gas was allowed to flow from the cylinder at a metered rate into the chamber to give the desired nominal concentrations. Samples were taken repeatedly until the conditions within the chamber were stabilized. The air and FE 1301 input were adjusted to achieve the exact concentration.

## EXPOSURE OF ANIMALS

### Animals

Mongrel dogs used in the experiment were obtained from a local animal supplier who guaranteed them to be in good health and free from disease. They were held for a period of one week for acclimation and examination to determine fitness. A total of 29 dogs were used, 8 female, and 21 male. Their weight range was from 5.0 to 9.5 kilograms.

### Exposure Conditions

Animals were exposed to nominal air concentrations of FE 1301 varying from 5 to 40%. Buildup time to reach the desired concentration ranged from 10 to 50 minutes. Exposure times were for 5 minutes after reaching the desired concentration. In some experiments carbon dioxide was added to the chamber over a 10-minute period to give a final concentration of 10%.

### Production of Fright Situations

Anxiety and fright were produced in the dogs by stressing with a Strobe light and a low-pitched horn of approximately 90 decibel intensity irregularly over a period of 5 minutes while housed in the dark.

## EXPOSURE OF HUMAN SUBJECTS

### Test Subjects

Ten test subjects were used in assessment of the effects of FE 1301 on behavior and myocardial irritability. They were drawn from the population of research personnel, medical and dental students from the professional schools at the University of California at San Francisco. The nature of the test and the risks involved were explained to each subject emphasizing that the clinical pharmacology of the substance had not been fully ascertained and that this was part of the purpose of the experiment.

## General Procedure

Subsequent to acceptance into the program the following characteristics of the subjects were recorded: age, weight, body type, sex, race, occupation, past medical history, previous experience with anesthetics, allergies and sensitivities, drug ingestion, blood pressure, pulse, summary of physical defects and then mood and affect. They were examined, an electrocardiogram was taken and they were trained to acceptable levels of performance on tests measuring mental alertness and neuromuscular coordination.

## Chamber Exposure

The FE 1301 was administered at nominal concentrations of 5 or 10% to all subjects. Conditions of exposure were static. The subjects entered rapidly into the chamber. The air concentrations were analyzed at 5-minute intervals. While in the chamber subjects made observations as to sensory stimulation and carried out tests for mental alertness and neuromuscular coordination. All exposures were for 20 minutes or until the subjects completed the schedule given below:

| Time (Minutes) | Test |
|---|---|
| 0 | Record Sensory Experience |
| 1 | Record Sensory Experience |
| 2 | Record Sensory Experience |
| 5 | Record Sensory Experience |
| 6 | Perform Romberg |
| 7 | Perform Finger-to-Finger |
| 8 | Perform Maze XI |
| 10 | Record Sensory Observation |
| 11 | Perform Pursuit Rotor |
| 15 | Record Sensory Experience |
| 16 | Perform Simple Reaction |
| 20 | Record Sensory Experience |
| 21 | Perform Maze XII |

Leave chamber and narrate description of exposure experience.

## Tests for Mental Alertness and Neuromuscular Coordination

The following tests were administered by one of the test subjects to the other in rotation so that each was tested:

1. Adjective Check List: Descriptive word list was identified as pertinent to emotional and mental states.
2. Modified Romberg Test: Positive scores were maintenance of balance for 5 seconds; a negative score resulted from failure to do so.

3. Finger-to-Finger Test: The quantitative test described by Goldberg in which the subject wearing a target shield on his left hand and a stylus on his right hand opposes the stylus to the surface of the disc approximately 30 times at 2 second intervals. Scoring is done by measuring the length of lines which connect the two fartherest points and the diameter of a circle enclosing 20 of the points.
4. Porteus Maze: Tests XI and XII were used. Quantitative scores were determined by errors on an arbitrary scale such as entering a blind alley, bumping or touching the printed maze line, or lifting the pencil. The higher the score, the worse the performance.
5. Pursuit Rotor: This device measures manual dexterity by measurement of the time in which contact is made by a jointed probe held in the dominant hand with a 2 cm. spherical electrode on the surface of a disc rotating in the horizontal plane at 60 rpm.
6. Simple Reaction Time: Subjects are confronted with variables consisting of three different colored lights and three levers. The testee pushes consecutive levers until the light goes off. Each run consists of 10 randomly selected challenges. Time is recorded automatically to one-hundredth of a second.

## Measurement of Sensory Modalities

Tests of sensory recognition of eye irritation, nose irritation, pulmonary discomfort, intensity of smell and effects on the central nervous system were recorded by subjects in the exposure chamber at 7 time periods ranging from immediately on entrance to 20 minutes of exposure. They were graded according to a scale of absent, light, moderate, severe, and extreme in intensity.

## Narrative Description

At the conclusion of exposure, the subject wrote a narrative description of his sensations and an appraisal of his physical and mental condition during his exposure time. Twenty minutes later, he made a similar evaluation of his condition. This test was also performed in exposures using the Heidbrink.

## Exposure Using the Heidbrink

Different concentrations of FE 1301 were administered through a Heidbrink Kinet-O-Meter with a closed circuit system for $CO_2$ absorption. A dynamic system was used in some experiments to deliver air concentrations with pre-calibrated flow settings which were monitored to give precise concentrations. In addition, the gas was administered at 10% concentrations from a pre-mixed reservoir. In the evaluation of 6 subjects carbon dioxide was added directly to the inhalation mixture in concentrations of 10%.

Electrocardiograms

Electrocardiograms were obtained repeatedly at intervals during the exposure utilizing a Burdick Electrocardiograph Model EK 4, utilizing Lead II.

Control of Bias

The experiment was carried out on a single blind basis but without placebo control, the subjects being unaware of the concentrations that were being administered. Two selections of the particular level of exposure administered through the Heidbrink were arrived at through the agreement between the examiners based on results obtained as the experiment progressed. Starting at concentrations of 5%, there was a gradual increase over the study in rotation. Final interpretation of the electrocardiograms was made without knowledge of the concentration to which the examinee was exposed.

## RESULTS

Studies on Dogs

There were no deaths among the 24 dogs exposed to concentrations of 10 to 40 volumes percent of FE 1301 and challenged in the fright situations. No effects which could be related to the exposure to the FE 1301 were observed in 8 animals exposed to 10 volumes percent. When the stimulus was applied the animals showed an increased alertness, occasionally whined and paced about within their cages. The group at 20% exhibited tremors but no other toxic sign. At a concentration of 40%, dyspnea, salivation, episodes of howling or warbling, and tremors were observed in most animals. This data is summarized in table I. None of these 24 animals was exposed more than once. From 10 to 50 minutes was required to reach the desired concentration in the chamber so that the total exposure was as long as 55 minutes. At the highest level of exposure, salivation and dyspnea continued for approximately 30 minutes following exposure. Animals 25-29 were exposed to FE 1301 alone and in combination, on four occasions, with carbon dioxide. Observations made on these animals are summarized in table II. Hyperventilation, agitation, and trembling occurred in all animals exposed to carbon dioxide in the absence of FE 1301. On exposure to 10% carbon dioxide and 10% FE 1301, the picture was essentially similar except that one animal showed depression and trembling was abolished. Exposure to 20% FE 1301 produced no hyperventilation in any of the five animals, no effect of any kind in three, tremors in two, and convulsions in one. The convulsions were preceded by restlessness at 5 minutes and tremors at 6 minutes. Their onset was at 7 minutes; generalized convulsions persisted to the end of the experiment at 21 minutes. The second animal with tremors first exhibited them after a 7-minute exposure; these continued until the end of the experiment. When the dogs were re-exposed to 10% carbon dioxide and 20% FE 1301, hyperventilation again appeared in two of five dogs, but was absent in three. One additional dog exhibited agitation and a second dog convulsed but the picture was essentially no different than when the group had been exposed to 20% FE 1301 by itself. One animal again showed depression. Increased gastro-

intestinal activity was noted at the two higher levels in one dog, characterized by vomiting and forceful evacuation of stool. None of these animals died and all returned to normal approximately 20 minutes following the exposure.

## TABLE I

SUMMARY OF EFFECTS ON MONGREL DOGS (NOS. 1-24) OF EXPOSURE TO VARIOUS CONCENTRATIONS OF FREON 1301, TOGETHER WITH A FRIGHT SITUATION

| Dog Nos. | Sex: M | Sex: F | Body Weight | Concentration: Nominal | Concentration: Measured | Time to Reach Concentration | Observation |
|---|---|---|---|---|---|---|---|
| 1 - 8 | 4 | 4 | 5.8 - 9.1 | 10 | 10.5 | 10 minutes | Increased alertness without signs of toxicity |
| 9 - 16 | 7 | 1 | 5.0 - 9.4 | 20 | 18.9 | 22 minutes | No signs of alerted behavior except tremors |
| 17 - 24 | 5 | 3 | 6.0 - 9.5 | 40 | 38 | 50 minutes | Dyspnea, salivation, howling and tremors |

## TABLE II

SUMMARY OF EFFECTS ON MONGREL DOGS (NOS. 25-19) OF EXPOSURE TO CARBON DIOXIDE AND FREON 1301 SEPARATELY AND TOGETHER

| Carbon Dioxide: Present | Carbon Dioxide: % | FREON 1301: Present | FREON 1301: % | Observation |
|---|---|---|---|---|
| + | 10 | - | | Hyperventilation 5/5<br>Trembling 2/5<br>Agitation 1/5 |
| + | 10 | + | 10 | Hyperventilation 5/5<br>Trembling 0/5<br>Agitation 2/5<br>Depression 1/5 |
| - | | + | 20 | Hyperventilation 0/5<br>No effect 3/5<br>Tremor 2/5<br>Convulsion 1/5 |
| + | 10 | + | 20 | Hyperventilation 2/5<br>Tremor 1/5<br>Agitation 1/5<br>Convulsion 2/5<br>Dyspnea 1/5<br>Depression 1/5 |

## STUDIES ON HUMAN VOLUNTEERS

### Characteristics of the Ten Subjects

The age span was 22 to 33 years of age. Only one of the subjects was receiving chronic medication. Five had a past history of allergies and sensitivities. None of these was currently clinically active. No unusual physical defects were found. One subject had a systolic murmur accentuated by exercise and had a past history of congenital heart problem. The mood and affect of the subjects extended over a wide spectrum.

### Levels of Exposure

The concentrations determined by GLC analysis were close to nominal concentration in all runs. There was a slight dropoff in concentration during the 20 minutes of exposure in most instances. Concentration ranged from a high of 6.1% to a low of 4.7% at the nominal 5% concentration and from a high of 10.3% to a low of 8.2% at the nominal 10% concentration.

### Unusual Events

The experiment proceeded satisfactorily according to experimental design and it was not necessary to alter significantly any of the procedures during the course of the investigation. The subjects had no difficulty in performing the tests within the chamber and none asked to terminate his exposure although it had been indicated beforehand that they could do so if conditions were unpleasant. Three subjects breathing the higher concentrations of FE 1301 from the inhalator indicated that they wished to discontinue the experiment. Explanation was subsequently given of a feeling of impending unconsciousness. Subject 5 developed an unusual myocardial activity with dissociation of the pacemaker and his exposure was discontinued. Return to normal rhythm occurred within 3 minutes. It was not necessary to use any resuscitative devices and with all subjects recovery from the central nervous system depressing effects was prompt. Two subjects reported headache during or following exposure; one of these persisted for 12 hours.

### Mental Alertness and Neuromuscular Coordination

At the 5% exposure level, one of the four subjects showed no overall improvement in his performance. Two showed no significant changes and one showed a decrease in performance. When the total changes in the six trials given the four subjects were summated, there were seven trials showing improvement, nine showing no change, and eight showing a decrease in performance. Most of the subjects improved during testing on the rotor pursuit. In our experience this result frequently occurs with subjects taking central nervous system depressants in small quantities. The most likely explanation is a relaxation of tension and decrease in tendency to overcorrect in the test. All subjects had the impression that they were doing well and not significantly impaired at the 5% concentration.

At the 10% level, there were 24 decrements in performance. A typical record is shown in table III. All subjects had more tests with decreased performance than with increased performance. Out of the total of 36 possible changes in comparison with control values, there were 24 poorer performances, 6 with no change, and 6 with improvement. While hand steadiness was not scored in terms of decreased performance, the maze tests were revealing in that the drawn lines were wavy and irregular in all subjects at the 10% level in contrast to the smooth lines drawn in the control runs.

TABLE III

SUMMARY OF SCORES OBTAINED ON MENTAL ALERTNESS AND COORDINATION TESTS BY SUBJECT NO. 7 WHO WAS EXPOSED FOR 20 MINUTES TO 10% OF FE 1301

| Test | Control Value | Exposure Value | Effect |
|---|---|---|---|
| Romberg: | + | - | Decreased performance |
| Finger to Finger: | | | |
| Greatest Distance | 2.8 | 4.9 | Decreased performance |
| Circle Diameter (cm) | 1.3 | 2.5 | |
| Maze Test XI: (Errors) | 0 | 17 | Decreased performance |
| Rotor Pursuit: (Seconds) | 8.19 | 11.91 | Increased performance |
| Simple Reaction Time: (Seconds) | 6.22 | 7.11 | Decreased performance |
| Maze Test XII: (Errors) | 0 | 8 | Decreased performance |

SUMMARY: 5 Decreased performance
0 No change
1 Increased performance

Descriptive Words

This test indicated a clear pattern of change in self-appraisal of mood and mental clarity. There was a decrease in the adjectives describing mental acuity and alertness with an increase in words describing mental acuity and alertness

with an increase in words describing mild depression. Most subjects felt either drowsy or lightheaded during some of the exposures and there was an increase in sense of well-being. Typical response is given in table IV.

TABLE IV

DESCRIPTIVE WORDS USED BY SUBJECT NO. 4
TO INDICATE HIS FEELINGS

| Word Choice | Control | Chamber | Inhalation |
|---|---|---|---|
| alert | x | | |
| amazed | | | |
| careful | | | |
| cheerful | x | | |
| clear headed | x | | |
| cooperative | x | x | x |
| decisive | x | | |
| detached | | x | x |
| drowsy | | | x |
| dull | | x | |
| easy going | | | x |
| elated | | | |
| energetic | x | | |
| enterprising | x | | |
| forceful | | | |
| generous | | | |
| genial | x | | |
| groggy | | x | x |
| happy | x | | |
| industrious | x | | |
| irritable | | | |
| joyous | | | |
| kindly | | | |
| lackadaisical | | | |
| languid | | | x |
| lightheaded | | x | x |
| quiet | | | |
| relaxed | | | x |
| resourceful | x | | |
| sad | | | |
| satisfied | x | | |
| self-confident | x | | |
| sluggish | | x | x |
| talkative | | | |
| tired | | | |

## Sensory Appraisal

The majority of subjects did not experience eye irritation, nose irritation, or pulmonary discomfort during most of the period of testing at either the 5 or 10% level. A slight eye and nose irritation was indicated by some subjects. This was related to a material with a halogen-like odor and not to the FE 1301 which had a pleasant odor. The source of the odor was thought to be halogens formed by decomposition of the FE 1301 vapors by a space heater which was located near the area where the mixture from the anesthetic device was released into the air. There was a decreased olfactory cognition with time, though this was less pronounced at the higher exposure level. Onset of central nervous system effects was rapid and occurred at the higher concentration in some subjects immediately on entering the chamber. In half the subjects these were rated as only slight or moderate at the 5% level. The majority of the subjects felt that these were moderate at the 10% level and one subject described the effect as severe. Data is summarized in table V. While one of the subjects was really disturbed in his alertness and mental acuity at the 5% level, three of the six subjects at the 10% level showed definite deviation from normal. Judging from all factors, it would be concluded that they were in the beginning states of inebriation.

TABLE V

INTENSITY OF RESPONSE WITH TIME AMONG SIX SUBJECTS EXPOSED TO 10% FE 1301

SENSORY MODALITY

| TIME | Eye Irritation | | | | | Nose Irritation | | | | | Pulmonary Discomfort | | | | | Olefactory Conition | | | | | CNS Effects | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A | S | M | S | E | A | S | M | S | E | A | S | M | S | E | A | S | M | S | E | A | S | M | S | E |
| 0 | 1 | 4 | 1 | | | 3 | 3 | | | | 5 | 1 | | | | | 2 | 4 | | | 3 | 2 | 1 | | |
| 1 | 1 | 4 | 1 | | | 1 | 5 | | | | 5 | 1 | | | | 1 | 1 | 4 | | | 1 | 2 | 3 | | |
| 2 | 2 | 4 | | | | 2 | 3 | 1 | | | 4 | 2 | | | | 1 | 2 | 3 | | | | 3 | 2 | 1 | |
| 5 | 3 | 2 | 1 | | | 2 | 3 | | 1 | | 4 | 2 | | | | | 5 | 1 | | | | 2 | 3 | 1 | |
| 10 | 3 | 2 | 1 | | | 3 | 2 | | 1 | | 4 | 2 | | | | 1 | 4 | 1 | | | | 1 | 5 | | |
| 15 | 3 | 2 | 1 | | | 3 | 2 | | 1 | | 4 | 2 | | | | 1 | 2 | 3 | | | | 2 | 4 | | |
| 20 | 3 | 2 | 1 | | | 3 | 2 | | 1 | | 4 | 2 | | | | 1 | 2 | 3 | | | | 2 | 4 | | |

A (Absent) S (Slight) M (Moderate) S (Severe) E (Extreme)

## Narrative Descriptions

The narrative description of the experience following the chamber exposures and exposures to the higher concentration of the FE 1301 followed a similar pattern except for subject 9 who though exposed to a concentration up to 15.7%, indicated no significant central nervous system effect. Subject 1 was exposed to only 4.1% of FE 1301 and experienced only a mild tingling following the inhalation of carbon dioxide. Subjects 2 and 3, who were exposed to concentrations of about 9.6%, had mild lightheadedness. Subject 4, who reached a similar concentration,

experienced a buzzing in his ears in addition to tingling of extremities. Subject 5 at the level of 16.9% was suddenly aware of impending unconsciousness. Subject 6 felt drowsy and had a sensation of impending unconsciousness at 14.5% when breathing added $CO_2$. Subject 7 felt a sensation of numbness over his entire body which was extremely unpleasant at concentration of 14.1%. Subject 8 felt impending unconsciousness at a concentration of 14.8%. Subject 10 felt dull and confused at the concentration of 15.1%. A typical narrative description follows:

Chamber Exposure

> "I noted a slight burning of the eyes and nose, then tingling and progressive lightheadedness, followed by quite severe dizziness. There was a buzzing sound similar to that experienced in ether induction. Gradually these conditions improved as I became adjusted to the concentration, but I remained lightheaded throughout the exposure. My voice pitch increased almost immediately and remained elevated throughout the exposure. On leaving the chamber I was aware of strong odors in the room."

Inhalation Exposure

> "After commencing the breathing of the higher Freon concentrations, I felt lightheaded with a minimal spinning and a buzzing sensation. A little later I felt tingling of the fingers, toes, and lips and then of the tongue and limbs. I experienced a dermal flushing and felt weak about half-way through the exposure. Once these feelings began they continued while breathing the gas mixture but cleared with a few breaths of fresh air. No difficulty twenty minutes after the exposure."

Electrocardiographic Changes

Four of the ten subjects had sinus arrhythmia as a baseline finding. This was not unexpected in an age group represented here. One had a wandering pace maker at baseline and one had a predominant sinus rhythm. There was no change in seven of the electrocardiograms during the exposure period. Subject 7 had lowering of the T wave on two occasions. Subject 9 had a flattening of his T wave at one time and increased sinus arrhythmia later. The most marked changes were noted in subject 5, who developed a sinus tachycardia, followed by flattening of the T wave, premature ventricular contractions forming bigeminy and the A-V dissociation with no pace maker. Sinus rhythm was restored two minutes after cessation of the exposure. The summary of the electrocardiographer's reading of the ECG tracings at various time periods, together with some vital signs at the different concentrations, are set forth in subject 5 in table VI.

## TABLE VI

SUMMARY OF VITAL SIGNS AND EKG READINGS IN SUBJECT NO. 5

| Concentration | | Exposure Time | Blood | Pulse | EKG Readings |
|---|---|---|---|---|---|
| Nominal | Reached | (minutes) | Pressure | | |
| 0 | | 5 | 140/80 | 70 | Sinus rhythm |
| 5 | 4.5 | 5 | 130/80 | 60 | No change |
| 5 | 4.5 | 6[a] | 125/85 | 50 | No change |
| 10 | 7.0 | 7 | 130/80 | 60 | No change |
| 17 | 16.9 | 1 | | 40 | Flat T waves |
| | | 36 HOUR TIME LAPSE | | | |
| 0 | | 5 | 110/70 | 70 | Sinus rhythm |
| 13.2 | 12.8 | 5 | 130/80 | 110 | Sinus tachycardia, flattening of T waves |
| 16.2 | 13.0 | 2 | 150/88 | 120 | Premature ventricular contractions forming bigeminy |
| 16.2 | 14 | 1 | 150/100 | 65 | A-V dissociation with no pace maker |
| 10.2 | 14 | 2 | 130/80 | 80 | Premature beats from various foci |
| 0 | | 2 | 130/80 | 80 | Sinus rhythm |

[a] Rebreathing

## DISCUSSION

The toxicity and biology of the fluorocarbons has been extensively reviewed by Clayton (1967). He reported that on the basis of acute inhalation toxicity of fluoromethanes which contained bromine, $CBrF_3$ was the least toxic. A concentration of 83 volumes percent was required for 50% mortality in rats. The majority of overt pharmacologic signs resulted from effects on the central nervous system, the principal pharmacologic effects being narcosis and anesthesia. Except in unusual circumstances, death in animals is apparently due to respiratory depression. Convulsive reactions have been obtained by others(Van Stee, 1968) working with FE 1301 and our observations of tremors and convulsions in dogs were not unexpected.

Stopps (see reference 5) classified benzene, heptane, chloroform, and trichloroethylene as compounds which would be considered most active as cardiac sensitizing agents. Halothane (Fluothane) has caused cardiac sensitization in animals and man in 0.5-1 volume percent range. Anesthetic experience with this compound has been excellent because of close supervision and awareness of these sensitizing properties and anesthetic fatalities are relatively rare following its use. A number of halogenated aliphatic compounds and hydrocarbons are considered weak sensitizing agents to the myocardium. This group included some of the fluoroalkane and alkene derivatives. Some fluoro-compounds including tetrafluoroethylene and difluoroethylene were reported as not causing sensitization.

None of the 29 dogs which we exposed developed ventricular fibrillation since all survived the experiment. Ventricular fibrillation is usually a fatal event unless resuscitative procedures are instituted. We did not monitor the electrocardial activity of these animals and therefore could not state whether any arrhythmias were produced, but based on the work of Van Stee and Back (1968) this might be expected. Van Stee reported one fatality in a group of 9 dogs (11% incidence) exposed to a 40% V/V concentration of FE 1301 and not challenged with epinephrine. It would appear that at concentrations of 20% or higher of FE 1301 it is possible to induce cardiac arrhythmias and even ventricular fibrillation in animals challenged with epinephrine, but experience with several species at concentrations of 10% and less has not resulted in this effect. It is probable that ventricular arrhythmias are in part dose-related since increasing the level of cyclopropane and chloroform does increase the incidence and variety of cardiac arrhythmias. Arrhythmias introduced by this class of compounds, and presumably FE 1301, include a wandering pace maker, atrial extrasystoles, atrial fibrillation, A-V nodal rhythm, sporadic ventricular extrasystoles, monofocal or multifocal ventricular tachycardias, and bigeminal rhythm. According to Wollman and Dripps (The Macmillan Company, New York, New York) cyclopropane does not cause ventricular fibrillation in man. Experience by other anesthetists is not in keeping with this conclusion; given a large enough series it is probable that cyclopropane and an agent such as FE 1301 would do so in man. Therefore, the question revolves about relative degree of risk. In a cross-section of the population such as would be represented by passengers in an aircraft, one would expect persons with subclinical or clinical heart disease. These clinical states fortunately do not predispose to the arrhythmic effects. In fact, the age group most frequently involved is represented by the young, vigorous adult with high endogenous epinephrine release capacity. Respiratory acidosis increases the incidence of ventricular arrhythmias in anesthesia and presumably might be expected to do so in exposure to FE 1301. The administration of carbon dioxide or rebreathing of carbon dioxide did not precipitate any arrhythmias in our test group. This is of some significance since an increased carbon dioxide content in the air would be expected subsequent to fires.

We have no explanation for the increased olfactory perception that was stated to be present by most subjects. Equally surprising was the halogen odor which apparently resulted from the thermodecomposition of the FE 1301. Small concentrations of an irritant substance were noticeable but not unpleasant. It is our opinion that other contaminants such as difluorobromomethane or dibromodifluoromethane would not significantly contribute to irritating potential at concentrations of 50 ppm or less. Study of inhalation toxicity of the pyrolysis products indicated no effect at low levels from these substances (Haun, 1967).

We did not establish the anesthetic level for our human subjects since all the volunteers stopped short of this point. However, the majority of subjects approached Stage I (analgesia) characterized by the onset of paresthesias, tingling sensation, numbness, and dizziness with retention of the ability to obey commands. The stage of delirium would probably have been achieved were an additional 2-3 percent present in the air and it is our opinion that loss of consciousness would occur in most subjects between 20 and 25 volumes percent. At twice this level, exposed dogs did not lose coordination or consciousness and there is an obvious species difference.

At concentrations of 10% of FE 1301 and greater, all subjects experienced some degree of confusion, misinterpretation of their surroundings or feelings of unsteadiness and giddiness. The majority that were tested objectively could function though at a decreased level of ability. Clearly, one would have difficulty in sustaining complex operations at such a concentration and those tasks requiring precise timing and coordination would deteriorate. In a sitting position, however, the sensation was not unpleasant, did not cause any great distress and could be tolerated. Passengers would be expected to fare better. At the 5% level, three of the four subjects experienced sensory changes which varied from slight lightheadedness to a feeling of relaxation. Performance was affected to a minor extent, though the subject did not have any feeling of inability to perform. A no-effect level was not reached since the lowest concentration which was administered was 5%. The rapid fall off of subjective impressions of impairment on cessation of breathing the material is in agreement with the rapid decrease in concentration of this type of agent from the blood stream. Using halothane as an analogy, in two to three minutes of exposure the arterial blood gas tension approaches about 20% of that in the inspired air. The saturation curve rises sharply at this level and conversely falls off rapidly on cessation of inhalation. The fluoroalkanes have a blood:gas ratio of about two and partition coefficients in brain, liver, and muscle which exceed this. Partition coefficient in fat is 60 times that in blood. Small, but physiologically insignificant, concentrations of the agent probably remain in the body stores some time after inhalation; however, fat uptake is relatively slow and does not achieve any significant levels on short exposures. There is no reason from animal data or experience gathered from extensive anesthetic experience with halothane to suggest there is hepatotoxic property associated with this agent. After 20-minute exposures to concentrations in the 10-15% range achieved in our studies, there was no indication on subsequent followup of icterus, hepatic tenderness, or other clinical evidence which would suggest any liver dysfunction. Inhalation of the FE 1301 produces a sensation which in the inexperienced is slightly disturbing; however, those who previously had anesthetics or who had consumed moderate quantities of alcoholic beverages were familiar with the sensation produced and were not perturbed by exposure to concentrations of 10% and below. If persons are to be operative under conditions where sudden release might occur, it would be desirable to acquaint them with expected effects by furnishing an experience of inhalation of this material so that they could be prepared to cope with a slight giddiness which might occur at the proposed 6% level. The use of masks would obviate the problem. There is no reason based on our studies to believe that passengers would have any significant degree of confusion or be incapable of leaving their seats were rapid evacuation of the cabin area necessary.

This study has extended human experience with FE 1301 and indicates that inhalation over periods up to 20 minutes at concentrations as high as 10 volumes percent does not produce unconsciousness or critically impair muscular movements.

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## DISCUSSION

DR. BACK: I went over these data with Dr. Hine sometime ago. The blood pressure effect on these patients may well not have been seen because they were cuff pressures. The anesthesiologist became highly excited, of course, when he got an AV dissociation in this subject. He wasn't taking them very often, and besides that, he had too many other things to do, you know, looking at him, but he was taking them every couple or three minutes, and he may well have missed a drop in blood pressure. And the other thing, when you're using cuff pressures, you can automatically keep elevating. If you just take a pressure every 15-20 seconds, you automatically start increasing the blood pressure by this method. It's a poor way of doing it but it's the only way they had to do it.

DR. SMITH: Did I understand this was in air? These concentrations were in air?

DR. HODGE (University of Rochester): That is correct. The gas is metered into a chamber, administered by mask with air.

DR. SMITH: That's what I wondered, and in the anesthesia machine, was the remainder air or oxygen?

MR. WANDS: Can you answer this, Dr. Back?

DR. BACK: It was air.

MR. WANDS: It was air all the way.

QUESTION: I was interested to know what was the total length of time for recovery of all the men involved?

DR. HODGE: How long were they followed?

FROM THE FLOOR: That's right, and how long all these symptoms were going.

DR. HODGE: Dr. Hine didn't comment in numbers as to how long they were followed, but he did comment on the extremely rapid return to normal.

QUESTION: How long is rapid?

DR. HODGE: Two or three minutes.

QUESTION: And then all the effects were gone, or only the arrhythmias?

DR. HODGE: There was one subject who had a headache that persisted for 12 hours. This was the only comment that Dr. Hine made as to an extended symptom on the part of any of the subjects.

QUESTION: How about lack of alertness and the detachedness?

DR. HODGE: Very prompt recovery, in just a very few minutes. I don't know whether he said some one or two of these--He did say two or three minutes, but very promptly. Of course, this is characteristic of these very insoluble synthetic gases like halothane and cyclopropane. There was very rapid recovery.

MR. WANDS: Dr. Hine told me about his work, along with Dr. Back when the two of us chatted with him a few months ago on this, and that the patients recovered completely within ten minutes, and this was also verified in some human exposures at the DuPont Corporation.

FROM THE FLOOR: The reason I asked, I was concerned with putting out a fire in a spacecraft, and the men would have to be performing things all this time.

MR. WANDS: I think the conclusion that Dr. Hine reached was that this would not interfere with their motor performance significantly in the emergency situation. I believe that is correct from what you read.

DR. HODGE: This is just a confirmation.

DR. REINHARDT (E. I. duPont de Nemours and Company): I was one of the subjects and I can confirm that the recovery was quite rapid. We only were exposed to a maximum of 10% for a few minutes, but the effects wore off very quickly.

DR. HODGE: May I ask, Dr. Reinhardt, as long as we have a fellow who's made this "trip", would you be kind enough to say what your subjective sensations were?

DR. REINHARDT: Yes, they were sensations of lightheadedness for the most part, a little bit of dizziness and unsteadiness. It seemed we had to concentrate more on our task at hand to perform it, but essentially, they were the symptoms one would expect when being exposed to this sort of material.

AMRL-TR-68-175

PAPER NO. 12

# THE ACUTE INHALATION TOXICITY OF MONOMETHYLHYDRAZINE VAPOR

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## INTRODUCTION

In recent years, the broad search for new exotic chemical formulations with properties suitable as high energy rocket propellants has been replaced with increased emphasis on a selected smaller number of materials. As a consequence one of the fuels, monomethylhydrazine (MMH), has been employed extensively because of its high performance characteristics, thermal stability, and long term storability if contact with air is prevented.

Whereas the literature contains a substantial amount of inhalation toxicity data on hydrazine and its dimethylated derivatives (Rinehart, 1960; Weeks, 1963; Comstock, 1954; Shook, 1957), only one publication was found that reported information on MMH. Jacobson, et al conducted single, four-hour exposures on dogs, hamsters, mice, and rats to the vapors of hydrazine, 1, 2-dimethylhydrazine, 1, 1-dimethylhydrazine and monomethylhydrazine and found, in the order the compounds were mentioned, increasing toxic response (Jacobson, 1955). He concluded that MMH was the most toxic and also the most hazardous of the four hydrazine compounds tested.

The paucity of acute inhalation data along with the increased use of MMH clearly indicated the need for additional studies to provide the basis for improvement, if needed, of existing methods for hazard reduction and safe handling. Therefore, the experiments reported here were conducted to confirm and supplement the existing inhalation data. They also served to establish the experimental methodology for planned emergency exposure limit studies to verify current values (Smyth, 1969). These latter experiments are currently in progress and will be reported in subsequent publications and presentations.

## MATERIALS AND METHODS

Groups of 10 rats, 20 mice, 1-5 beagle dogs, squirrel monkeys, and 1 or 2 rhesus monkeys were exposed to various measured concentrations of MMH vapor for specified time periods. Rodents were exposed for 30-, 60-, 120-, and 240-minute periods; dogs and squirrel monkeys for 15, 30, and 60 minutes; and rhesus monkeys for 60 minutes only. The toxicity of MMH for the five animal species was defined by determination of $LC_{50}$ values, pathological examination of organs, observations of symptoms, measurements of body weight in rats and mice, and blood chemistry and hematology tests on dogs and rhesus monkeys. Rodents were sacrificed at 14 days postexposure. Dogs and rhesus monkeys were retained for several weeks in some cases to study blood changes, while squirrel monkeys were held only long enough to observe postexposure symptoms.

All animals that died during or following exposure were submitted for postmortem gross and histopathologic examination as were selected rodents and all dogs and monkeys at the termination of the postexposure observation period.

## EXPOSURE CHAMBERS AND VAPOR GENERATION SYSTEMS

### Rodent Chambers

The reactive nature of MMH necessitated the use of new and modified test systems designed to minimize its degradation during animal exposures. Figure 1 shows a schematic presentation of the apparatus constructed to accomplish this purpose for rodent toxicity studies.

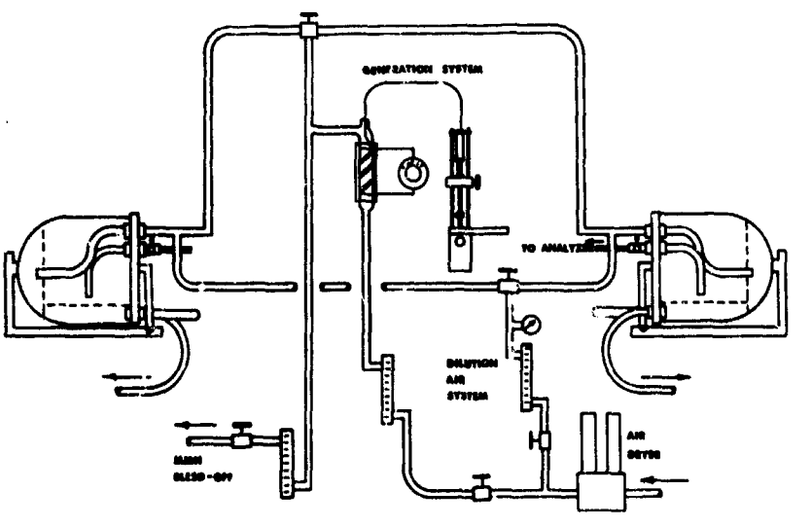

Figure 1. RODENT INHALATION TOXICITY EXPOSURE SYSTEM

An infusion pump was used to meter the correct quantities of MMH at the desired delivery rate for each exposure. A smaller version of the evaporator unit described by Carpenter, et al (Carpenter, 1949) was used to vaporize the test material. Exposures were carried out in a 30-liter bell jar chamber at an air flow of 30-40 liters per minute. Chamber MMH concentrations were first established in a parallel empty exposure system, then the gas stream was switched to the test animals in the alternate chamber to commence the exposure. To substantially reduce decomposition of MMH, water vapor in the metered air flows to the evaporator and chamber was reduced to -40 F dew point by means of an automatic regenerating air dryer using molecular sieve as the water vapor adsorbant.

A floor barrier and backwall made to 321 SS expanded wire mesh formed a cage for containing the animals and preventing their interference with the gas flow pattern. Effluent gas was discharged through an outlet at the bottom center of the chamber door. The interior of the door was lined with sponge rubber covered with a thin Teflon sheet. The chamber door was operated by a spring-loaded, snap-handle mechanism attached to the metal framework that supported the inhalation chamber.

## Large Animal Chambers

A modification of a standard Rochester Chamber (Leach, 1959; MacEwen, 1965) was used for the exposure of dogs and monkeys. Figure 2 shows this chamber, as well as the analytical and generation systems. Changes were made in the chamber air supply and exhaust systems to minimize decomposition of the test materials.

Equipment similar to that used for the bell jar exposure chamber system was used to meter and evaporate predetermined quantities of MMH.

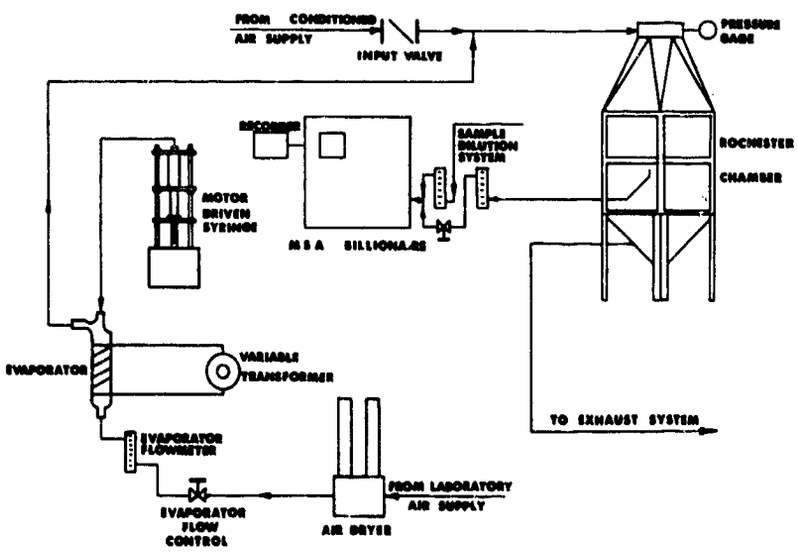


Figure 2. ROCHESTER CHAMBER SYSTEM FOR TOXICITY TESTS ON LARGE ANIMALS

## ANALYTICAL METHOD

Because of the reactive nature of MMH and the extremely small range of concentrations between the no-effect and lethal levels seen in preliminary experiments, a method of continuous analysis was required. The continuous monitoring of chamber MMH concentrations was accomplished by use of an electron capture instrument which measured the concentration of an aerosol formed by the reaction of MMH with trifluoroacetic acid vapor (Geiger, 1967).

## EXPERIMENTAL RESULTS AND DISCUSSION

### Control of Exposure Concentrations

The chamber air-conditioning system provided filtered input air controlled at approximately 72 F and 50% relative humidity. It was impractical from an engineering standpoint to predry the large volumes of air used in this system; therefore, tests were conducted to determine the effect of water vapor and air flow on MMH concentration. Figure 3 is a graph of the results. The tests were conducted in a Rochester Chamber at 40% RH and 100 cfm at an MMH delivery rate leading to a theoretical concentration of 200 ppm. Analysis, however, gave an actual chamber MMH concentration of 100 ppm, showing a 50% loss under these conditions. When the humidity was increased to 70% by water vapor injection, only 60 ppm was found in the chamber, a 70% loss. Decreasing the flow rate at any relative humidity level had little, if any, effect on MMH concentrations. It appeared, therefore, that the major factor leading to the disappearance of MMH from the chamber was relative humidity. This conclusion was strengthened by the initial animal work in small 30-liter bell jar chambers in which the air was predried to -40 F dew-point. In these experiments, the analyzed MMH concentration was reduced only 10% below theoretical. Since the relative humidity in Rochester Chambers cannot be readily decreased below 40%, the loss of contaminant could not be eliminated. Analysis, however, revealed that the desired MMH chamber concentration throughout the exposures could be satisfactorily controlled and was reproducible when relative humidity was maintained at a fixed level.

## MORTALITY

### Rodents

The only rat deaths during exposure occurred at the two highest concontration levels in the 240-minute studies. Two of three animal deaths were caused by exposure to 75 ppm MMH and one of nine succumbed during the exposure to 95 ppm. The CT products in these two exposures were higher than in any other, thus indicating a threshold for during-exposure mortality. Without exception, all other deaths occurred within four hours postexposure.

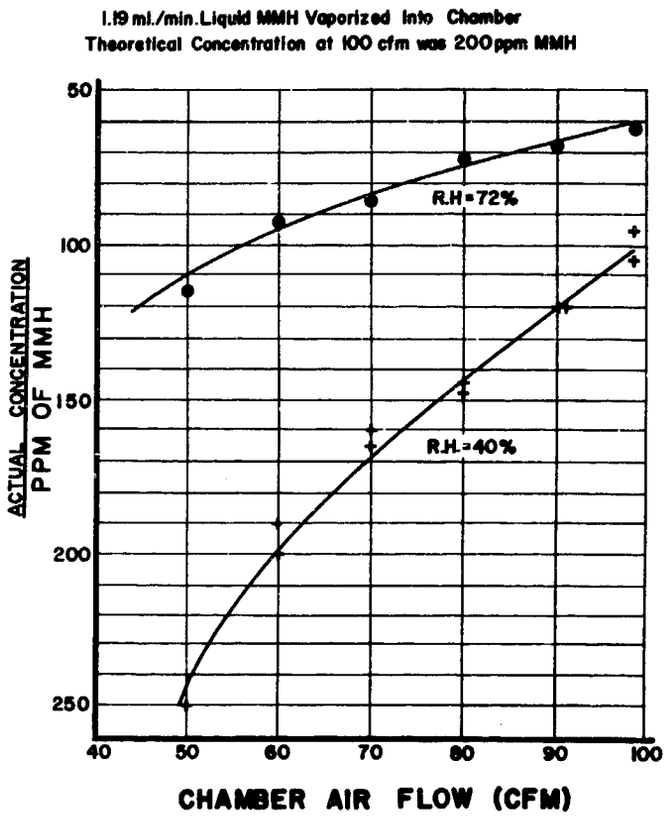


Figure 3. EFFECT OF RELATIVE HUMIDITY AND OUTFLOW ON MMH CONCENTRATION

Mouse deaths, too, were generally delayed until after exposure. Except for the higher concentrations at 120 and 240 minutes, which produced some mortality during exposure, practically all of the mice died within five hours after exposure. Here again, it appears that a CT threshold may have been exceeded to produce during-exposure deaths.

The $LC_{50}$ values and the 95% confidence limits for rats and mice exposed to MMH for 240, 120, 60, and 30 minutes are shown in table I. A comparison of $LC_{50}$ values obtained for the four-hour exposures to mice and rats was made with Jacobson's data (Jacobson, 1955). His values are essentially the same as those obtained in this laboratory (74 ppm versus 78 ppm in rats and 56 ppm versus 65 ppm in mice).

## Dogs and Monkeys

No deaths occurred during exposure of large animal species regardless of the duration or MMH concentration. The pattern of postexposure deaths observed in dogs was similar to that of rodents since the dogs died within two hours following the conclusion of exposure. Squirrel monkeys exhibited delayed deaths to a much greater degree than was the case for dogs. Although a few squirrel monkeys died as early as 2 and 4 hours, most deaths were observed between 10 to 24 hours after exposure. The number of rhesus monkeys tested was too small to give a precise measure of time to death.

TABLE I

$LC_{50}$ VALUES AND 95% CONFIDENCE LIMITS
FOR RATS AND MICE EXPOSED TO MMH

| Animals and Sex | Duration Exposure (min.) | $LC_{50}$ ppm | Confidence Limits - ppm |
|---|---|---|---|
| Male Rats | 240 | 78 | 71-86 |
| | 120 | 127 | 119-134 |
| | 60 | 244 | 219-275 |
| | 30 | 427 | 398-458 |
| Male Mice | 240 | 65 | 63-66 |
| | 120 | 92 | 88-96 |
| | 60 | 122 | 116-128 |
| | 30 | 272 | 258-287 |

The $LC_{50}$ values and the 95% confidence limits for the three large animal species tested can be seen in table II. Although Jacobson did not expose dogs for less than four hours, his 4-hour data may be compared to that obtained in these experiments by analysis of the CT values. His 4-hour data permits estimation of a CT value of 6000 ppm - minutes compared to the 5860 CT calculated from our 60-minute dog exposure. This comparison appears to be valid since dogs in our experiments exhibited a consistent CT relationship throughout the time range tested.

TABLE II

$LC_{50}$ VALUES AND 95% CONFIDENCE LIMITS FOR DOGS,
SQUIRREL AND RHESUS MONKEYS EXPOSED TO MMH

| Animals and Sex | Duration Exposure (min.) | $LC_{50}$ ppm | Confidence Limits - ppm |
|---|---|---|---|
| Beagle Dogs (Male and Female) | 60 | 96 | * |
| | 30 | 195 | 174-218 |
| | 15 | 390 | 337-404 |
| Squirrel Monkeys (Female) | 60 | 82 | 67-101 |
| | 30 | 145 | 115-182 |
| | 15 | 340 | 298-390 |
| Rhesus Monkeys (Male and Female) | 60 | 162 | 118-222 |

*Insufficient data to calculate range

In addition to the $LC_{50}$ values determined for different species, the results of these experiments provide other useful information. Variable mixing of sexes in tests conducted on rhesus monkey and dog exposure groups provided evidence that no measurable difference due to sex could be demonstrated as a result of exposure to MMH. The experiments further show that a steep dose-mortality response curve is obtained for all the species tested regardless of length of exposure. A comparison of the $LC_{50}$ values obtained during 60-minute exposure of the five species tested is shown in table III in which the species are ranked in order of their decreasing response to the acute effects of MMH. Rats were the most resistant while squirrel monkeys were found to be the most susceptible to MMH.

TABLE III

ACUTE TOXICITY RANKING BY SPECIES

| Species | 60 Minute $LC_{50}$ (ppm) |
|---|---|
| Squirrel Monkeys | 82 |
| Beagle Dogs | 96 |
| Mice | 122 |
| Rhesus Monkeys | 162 |
| Rats | 244 |

## RESPONSIVE SIGNS AND SYMPTOMS

The degree of symptomatic response of rats and mice appeared to be dose related. That is, as the MMH concentration was increased for each series of experiments, the number and degree of toxic signs progressed according to the following sequence from mild to severe:

1. Irritation of nose and eyes.
2. Diarrhea, abnormally frequent urination, and rapid labored breathing.
3. Increased alertness; piloerection; hyperactivity, interrupted by periods of inactivity characterized by rigid posture and exophthalmos.
4. Tonic-clonic convulsions and tremors, mucus discharge from mouth and nose and frequent biting.

The last two categories of symptoms occurred either during exposure or within a few hours postexposure. The rodents that developed all the toxic signs except convulsions survived, whereas those that convulsed died during or following exposure. The mice that succumbed to MMH usually died immediately after a single convulsive seizure.

The general pattern of symptoms observed in dogs and monkeys was similar to that seen in rodents. However, some additional symptoms were noted in the larger animals. These may have been due either to physiological or biochemical differences, or possibly to their larger size. The signs of toxicity, in the general order of occurrence during and after exposure, were as follows:

1. Eye irritation.
2. Salivation and licking.
3. Emesis (occurred earliest in dogs).
4. Diarrhea, frequent urination and pupil dilation. Ataxia in dogs.
5. Hyperactivity, convulsions, tremors and cyanosis (dogs only).
6. Prostration and apparent unconsciousness.

Although the emetic response occurred later in monkeys than in dogs, its severity was greater and it reoccurred frequently. The more acute response of dogs to MMH, in comparison with both monkey species, was noticeable not only in the early postexposure mortality (within two hours), but in the rapid onset of symptoms. Furthermore, convulsions were produced in dogs during exposure but not so early in either squirrel or rhesus monkeys.

Convulsions in large animals did not inevitably lead to death. Some dogs as well as monkeys were able to withstand the stress and survive until completion of the postexposure observation period.

Gross evidence of renal and intestinal damage was seen in two dogs exposed to MMH when blood was observed in their urine and feces on more than one occasion after exposure.

## CLINICAL DETERMINATIONS

### Body Weight

Rats surviving MMH exposures lost weight or showed subnormal gains on the first and second days after exposure. Near-normal gains occurred in all of the survivors on the third day, and growth rates had returned to normal by the fourteenth postexposure day except for animals exposed to the highest dose levels where subnormal gains were noted.

At one day postexposure, surviving mice showed mean weight losses of 10-15%. A trend toward weight recovery was observed in most of the groups at the second and third postexposure weighings. A comparison of the third and seventh day weights showed a sharp increase in body weight during this time period for the majority of the survivor groups. Examination of weights taken 14 days after exposure showed that the mice had made up all losses and were essentially identical to controls.

## Hematology

Of the various blood parameters selected to indicate possible effects of MMH on dogs and rhesus monkeys, only the hematologic factors reflected positive evidence of deleterious change. Comparison of hematocrit, red blood cell, and reticulocyte values obtained from blood samples taken before, immediately after, and twice weekly postexposure from surviving dogs and rhesus monkeys clearly indicated a red blood cell hemolysis induced by exposure to MMH. Moderate to severe anemia occurred in all surviving dogs, while mild to moderate hemolytic effects were produced in all surviving rhesus monkeys. Decreasing hematocrit and increasing reticulocyte values were the obvious indicators of this reaction in rhesus monkeys, while pronounced changes in all four hematologic determinations occurred in dogs.

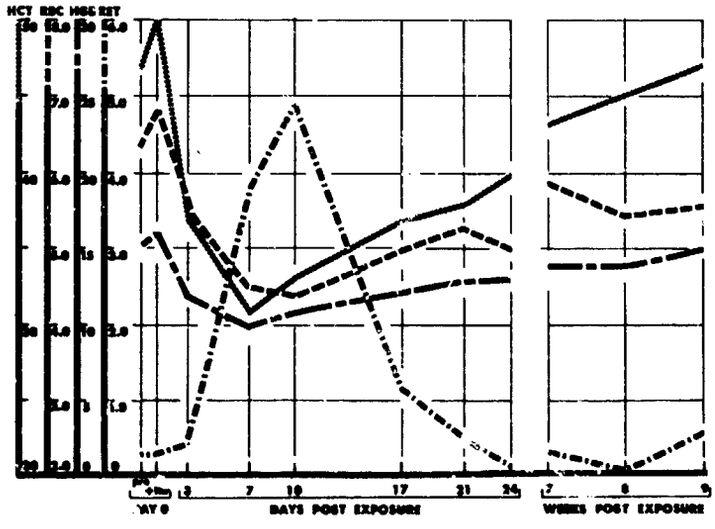


Figure 4. HEMOLYTIC RESPONSE IN DOGS THAT SURVIVED A 60-MINUTE EXPOSURE TO 92 PPM MMH. PLOTTED VALUES ARE ARITHMETIC MEANS.

Figure 4 is a graph of the hematocrit, hemoglobin, red blood cell, and reticulocyte values for three dogs that survived a 60-minute exposure to 92 ppm. As shown on the graph, the hematocrit, hemoglobin and red blood cell values from blood samples taken immediately postexposure were slightly elevated. This elevation may have resulted from mild dehydration due to emesis rather than from any specific effect of MMH. These values were markedly lowered three days postexposure, while reticulocyte counts showed a modest increase. A rapid compensatory rise was seen in reticulocytes by the seventh day, at which time the other three determinations had fallen to the lowest values recorded for the entire observation period. By ten days, reticulocyte counts reached their maximum value

and the hematocrit and hemoglobin values had begun to increase slightly. Reticulocytes declined rapidly thereafter, evidenced by measurements made on the seventeenth day, and returned to near-normal levels by 24 days. The hematocrit and hemoglobin values showed a gradual rise approaching preexposure levels nine weeks later. The red cell counts fluctuated during recovery without reaching the preexposure value at the time the experiment was terminated.

A graph of the same four hematology determinations made on one female rhesus monkey that survived a 60-minute exposure to 170 ppm is shown in figure 5.

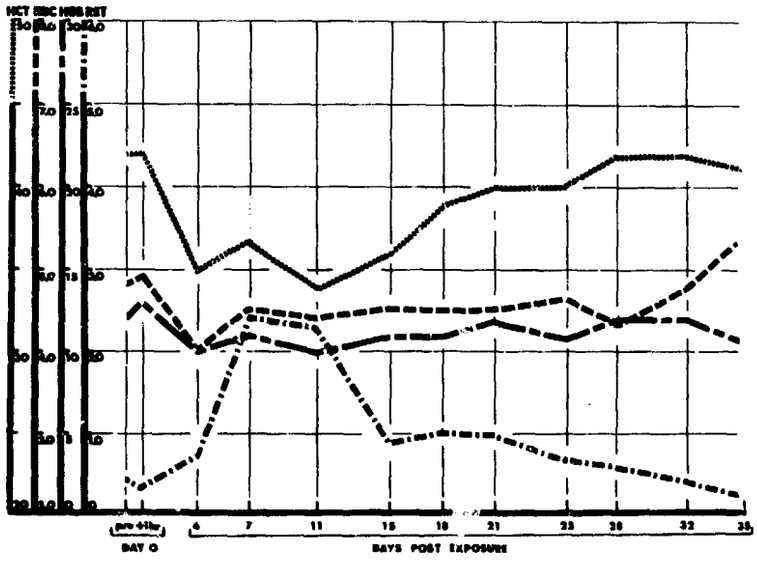

Figure 5. HEMOLYTIC RESPONSE IN ONE FEMALE RHESUS MONKEY THAT SURVIVED A 60-MINUTE EXPOSURE TO 170 PPM MMH.

The moderate decline in hemoglobin and red blood cell values, and particularly the sharp drop in the hematocrit level 14 days postexposure, as well as the characteristic rise in reticulocytes between the fourth and seventh days, presents evidence that the hemolytic response also occurs in rhesus monkeys as a result of exposure to MMH. Other monkeys showed milder responses. Recovery appears to be complete 35 days later when the last blood samples were taken.

Manifestations of cyanosis observed in dogs suggested the possibility of methemoglobin formation. During convulsive seizures, the tongues of the exposed animals were blue to dark purple in color, while mucous membranes appeared dusty brown. The color of blood samples taken immediately postexposure from those dogs that survived the higher dose levels of MMH were rusty brown rather than the characteristic dark red color of normal venous blood. Methemoglobinemia has been produced in anesthetized dogs one hour after intravenous injection of MMH

(Fortney, 1967). Jandl et al (Jandl, 1960) present a theoretical model for the production of methemoglobin by aromatic compounds having N-N groupings. Since no analyses for methemoglobin were performed on our test dogs, no definite conclusions can be drawn concerning its presence. However, it is plausible that cyanosis was the result of a combination of the following factors: (1) involuntary respiratory arrest during repeated and sustained convulsive episodes resulting in oxygen depletion; (2) accumulation of fluids in the respiratory passages interfering with respiration and oxygen transport; (3) transformation of normal hemoglobin into methemoglobin.

## PATHOLOGY

The pathologic evaluation of tissues from animals exposed to lethal or near-lethal concentrations of MMH is the subject of the next presentation and, therefore, needs little mention here.

The amount of visceral congestion and pulmonary hemorrhage observed was not sufficient to produce death which could only be attributed to CNS damage as previously reported by Jacobson et al (Jacobson, 1955).

## SUMMARY AND CONCLUSIONS

In terms of mortality, squirrel monkeys proved to be the most sensitive and rats the least sensitive to the lethal effects of MMH. The descending order of sensitivity found in comparable experiments was squirrel monkeys, dogs, mice, rhesus monkeys, and rats. Signs of toxicity occurred earlier in dogs and rodents than in monkeys. The symptoms of MMH intoxication were irritation, emesis (seen only in large animals), ataxia, and convulsions, which always proved to be fatal in the case of the rodents. The postexposure period was characterized by nausea in dogs and especially monkeys and by anorexia in all species. Indicative of the pronounced acute effects of MMH was the time pattern of postexposure mortality. Rodents and dogs exposed to lethal MMH concentrations died within a few hours, while monkeys survived for a longer period. Weight losses seen in surviving rodents were regained, and the exposed animals were not significantly different from their unexposed controls after fourteen days.

MMH has been shown to be an active hemolytic agent most notably in dogs and to a lesser extent in rhesus monkeys. This effect was temporary, however, with blood values returning to within normal range a few weeks postexposure. The cyanosis observed in dogs and the color of blood specimens taken from these animals suggests that MMH may produce methemoglobin in this species. The phenomenon may warrant further investigation.

The results of these acute inhalation experiments classify MMH as a highly toxic compound. The toxicity information and experience gained in these investigations was used in planning further studies to determine the level at which MMH produces no irreversible injury.

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## DISCUSSION

QUESTION: Can I ask you what the biological significance of your nice linear line of factors might be?

MR. HAUN (SysteMed Corporation): Oh, on the table of descending order of sensitivity?

FRCM THE FLOOR: Yes.

MR. HAUN: Absolutely none. It was just interesting to me, so I thought I would show it. That's all.

QUESTION: What is the extent of the decomposition of MMH at high moisture, high humidity? Is there any effect of light or UV radiation on the rate?

MR. HAUN: On the latter I can't answer, the former I can. As I mentioned in the paper, at 40-50% relative humidity, if we put in 200 parts per million of MMH, we lost half of it, it goes that quickly, and it gets worse the higher the relative humidity goes.

MR. WANDS: What are the decomposition products, Charlie?

MR. HAUN: I knew somebody was going to ask that. Ed Vernot can correct me if I'm wrong. I believe it is methane and nitrogen. Am I right, Ed?

MR. VERNOT (SysteMed Corporation): That's true. Methane and nitrogen are major products along with formaldehyde and the condensation products of MMH.

QUESTION: Any hydrolysis?

MR. VERNOT: No, sir, so far as we know, we haven't found any. However, it's difficult for me to see that water enters into the decomposition of MMH. I think it does have an effect, as Charlie has shown, but I think this effect may very well be instead of a decomposition effect, it may be the formation of a hydrate, a hydrate which is much more easily adsorbed on the walls of the chamber as it goes into the chamber and effectively removes it from the vapor phase. I don't believe that water itself has an active part in decomposition of MMH. As far as the UV is concerned, the little work that we did on that score seems to indicate oxidative decomposition of MMH. It's heterogenous, not a free radical type of thing. We didn't get any difference between a brown bottle and a clear bottle.

MR. WANDS: I was thinking more in terms of the decomposition which might take place in the presence of moisture within the lungs so that you would have an entirely different toxicological agent being absorbed. Any comment on that, Ed?

MR. VERNOT: I don't know what takes place inside a lung, of course. I just believe that the decomposition, if it takes place, is a surface phenomenon that may very well take place in the lung, but whatever the active principle is in the lung, it remains quite toxic at that point.

MR. HAUN: May I add something to that. Needless to say, we have found quite a bit of difference in the sensitivity of various animal species. This could be a factor that influenced the different toxicities found with the five animal species.

MR. WANDS: I am happy to know that Dr. Back has some thoughts on the therapeutic measures associated with MMH as compared with some of the other hydrazines. Ken, would you care to stick your neck out publicly on your thoughts on this, please? I think there is a significant factor here.

DR. BACK: Therapeutically, we have to stick with pyridoxine. It works, but it doesn't take care of the whole picture. There's a change in metabolism as far as glucose is concerned that you see with MMH that you don't see with UDMH, and whereas you get an increase in blood glucose with UDMH, you get a marked decrease with MMH. In fact, Dr. Carter has done some work on this recently. This also has to do with the activity of the animal, so there are a lot of factors going on here. If you chair a monkey and keep him relatively quiet, his glucose doesn't drop nearly as fast as if you just let him loose in the cage. If you let him loose, then his blood glucose drops out of sight, down to maybe 40 milligrams percent. If you keep him in the chair, it takes a much greater dose to get the same effect. But at any rate, the pyridoxine does not protect against this phenomenon at all. It just protects against the convulsions. That's about all I can say about the use of pyridoxine. It has to be the drug of choice at the moment. Barbiturates probably help, but you must recognize that after the convulsive effects you get a decrease in central nervous system activity, and if you over-barbitalize a person, you're liable to throw him into a deeper depression. If you remember last year, Sterman and Fairchild and the co-workers at Yale also found out that some of this activity was due to sensory motor changes. In other words, we're almost opening up the whole sensory side of the central nervous system, provoking convulsions, and we are just in the beginning of examining some of this with pyridoxine. At least, I hope we are.

AMRL-TR-68-175

PAPER NO. 13

# THE EFFECT OF METHYLHYDRAZINE BY INHALATION OR INJECTION IN DOG'S KIDNEYS

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Alfredo R. Esparza, M.D.
and
Farrel R. Robinson, Major, USAF, VC

Methylhydrazine (MMH) has been shown to be a strong central nervous system (CNS) stimulant as are the other hydrazine derivative propellants (Back, 1963; Back, 1963; Dost, 1964; Fortney, 1967; Patrick, 1964) but it is also a renal toxin and hematotoxin (Sopher, in press). The CNS effects, except for emesis, may be prevented or aborted by the administration of a large dose of pyridoxine hydrochloride (vitamin B6) (Weir, 1964). The renal and blood untoward effects cannot be prevented by pyridoxine prophylaxis or treatment (Sopher, in press).

MMH is toxic when administered by the intravenous (IV), intraperitoneal (IP) or inhalation (IH) routes. When given parenterally 15 mg/kg MMH is required to cause clinically evident renal damage in 100% of Beagle dogs. Ten mg/kg will cause sporadic clinical renal damage in a group of dogs (Sopher, in press). When administered via inhalation, without pyridoxine prophylaxis, 90 ppm for one hour or 190 ppm for 30 minutes is required to approximately mimic the 10 mg/kg parenteral dose (Haun, verbal communication, June, 1968). Experiments utilizing pyridoxine prophylaxis have not been accomplished at the time of this writing in the inhalation toxicology of this propellant and, therefore, the effects of significantly higher inhaled concentrations of MMH on the kidney are not known. This is because the animals die rapidly due to the severe CNS stimulatory effects of MMH at high concentrations.

The results described are based on a series of 12 Beagle dogs that received 15 mg/kg MMH plus 200 mg pyridoxine HCL. The lesions seen in animals with renal damage following inhalation are similar in all respects.

## Pathologic Changes

The first clinical evidence of renal damage is gross hematuria and hemoglobinuria at 12 to 24 hours postexposure. This continues for about 14 hours and the animal is somewhat oliguric for several days. By six days postexposure the animal is clinically recovered.

At 12 hours postexposure the kidneys are markedly swollen and are deep purple-red with a somewhat greenish sheen. On section the parenchyma bulges from the capsule and the demarcation between cortex and medulla is lost. The capsule strips easily, revealing a smooth swollen surface. Microscopically the glomeruli are essentially normal but somewhat bloodless. The epithelium of the proximal tubules is swollen and contains numerous deeply eosinophilic hyaline droplets. Often the cells have burst and the droplets are spilled into the tubular lumina. The loops of Henle, distal tubules, and collecting ducts are usually spared. The interstitium may be slightly edematous and the vessels are unremarkable. Hemoglobin casts and sometimes hemoglobin crystals may be found in tubular lumina.

The gross appearance at 24 hours postexposure is similar to that seen at 12 hours. Microscopic examination shows the hyaline droplet change of the proximal tubules to be accentuated. The picture is otherwise very similar to that seen at 12 hours.

At 48 hours the kidneys are somewhat less swollen and hyperemic. The greenish sheen is no longer present and corticomedullary demarcation is possible. Microscopically the hyaline droplets in the proximal tubular epithelial cells have regressed markedly and only few cells are still so involved. Many proximal epithelial cells are necrotic and desquamating into the tubular lumen. Mitotic figures are frequently seen in the lining cells of the proximal tubules. This change seems most marked in the pars recta of the proximal tubules. Many of the proximal tubular lining cells have apparently lost their cohesiveness resulting in partial separation of the cells from each other. This causes the tubular epithelial lining to take on a "cobble-stone" appearance.

At 72 hours the kidneys are essentially normal size and show only slight brownish pigmentation. Microscopic examination shows the hyaline droplets to be completely absent and the desquamation of cells to be nearly absent. Mitotic figures are still frequent.

At 6 days the kidneys are virtually normal both grossly and microscopically.

Special stains show the hyaline droplets to be strongly positive for hemoglobin and at 48 hours and beyond the proximal tubular epithelium contains abundant stainable intracellular iron.

The liver at 12 and 24 hours shows marked erythrophagocytosis by the Kupffer cells in the sinusoids. By 48 hours the ingested red cells have mostly been broken down into hemosiderin and this process is essentially complete by 6 days.

## DISCUSSION

There are at least two separate phenomena occurring in MMH renal toxicity. First, the MMH causes an episode of severe intravascular hemolysis, hemoglobinemia and hemoglobinuria. This is reflected in the kidney by hyaline droplet accumulation in the renal tubules which represents, at least in part, resorption of protein from their lumina (Oliver, 1954; Fisher, 1964; Straus, 1964; Miller, 1960; Reger, 1961). This, however, should not cause the degree of damage seen in the epithelium of the tubules. Numerous experiments have shown that hemoglobin, by itself, is relatively innocuous although it does cause rather marked changes in renal histology. Paroxysmal nocturnal hemoglobinuria in man causes little effect in the renal tubules, but after a number of years can cause interstitial fibrosis (Reger, 1961). Secondly, there is frank necrosis of proximal tubular epithelial cells with the cells sloughing into the lumen and with active cellular regeneration. This type of change is probably not related simply to the hemoglobinuric episode. It would seem then, that MMH does have definite primary nephrotoxicity. The mechanism of this nephrotoxicity is not known. The prominent erythrophagocytosis gives ample evidence to the fact that MMH is hematotoxic.

Renal toxicity is definitely dose-dependent. Animals receiving less than 10 mg/kg MMH do not show physical or pathologic signs of hemoglobinuria although erythrophagocytosis is seen (Sopher, in press). Nephrotoxicity is seen as swelling and increased eosinophilia of the proximal tubular epithelium but necrosis and sloughing of cells is absent. At doses greater than the 15 mg/kg used in this study the renal changes are amplified. At 30 mg/kg large hemoglobin crystals may be seen in the renal tubules (Sopher, in press). We do not know why there is no inflammatory component in the face of obvious tissue necrosis. Perhaps since the process is restricted to the tubular epithelium chemotactic material is not released in the surrounding tissue. The renal effects of MMH are obviously divorced from its CNS effects, or so it would seem, since pyridoxine does not ameliorate the renal toxic or hematotoxic effects. When administered by inhalation rather than injection, the effects of MMH are more variable. Only two of the more than 20 exposed dogs were noted to have blood-tinged urine. Both of those animals were in rather high concentrations of MMH, 92 ppm for 1 hour and 188 ppm for 30 minutes, respectively (Haun, verbal communication, June, 1968). A large number of dogs exposed to up to 60 ppm for 1 hour showed neither hematotoxic nor renal toxic changes.

Methylhydrazine-induced renal toxicity in its full-blown form seems restricted to dogs. We examined numerous rhesus and squirrel monkeys that received doses of MMH equivalent to those in the dogs, and the only renal effect seen was swelling of the proximal tubular epithelium. Hemoglobinuria has not been seen in monkeys, even with massive doses. Erythrophagocytosis has been seen but is not especially prominent.

## SUMMARY

Methylhydrazine (monomethylhydrazine, MMH) produces red cell damage, nephrotoxic changes, and hemoglobinuria in dogs despite prophylactic treatment with pyridoxine. The mechanism of action in these various toxic manifestations is not as yet known.

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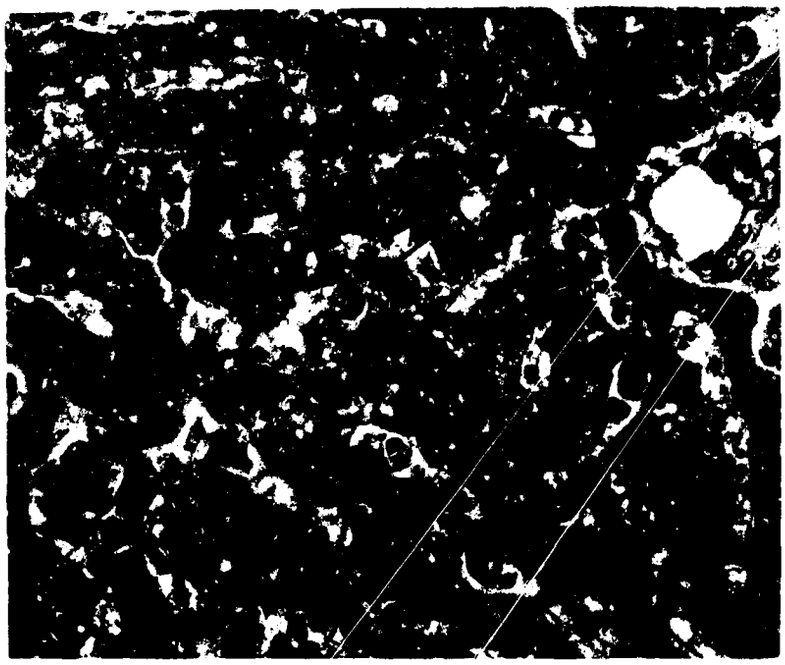

Figure 1. LIVER, 24 HOURS POSTEXPOSURE TO MMH. Note the prominence of the reticuloendothelial cells. The arrows point to Kupffer cells containing numerous ingested erythrocytes. x 252

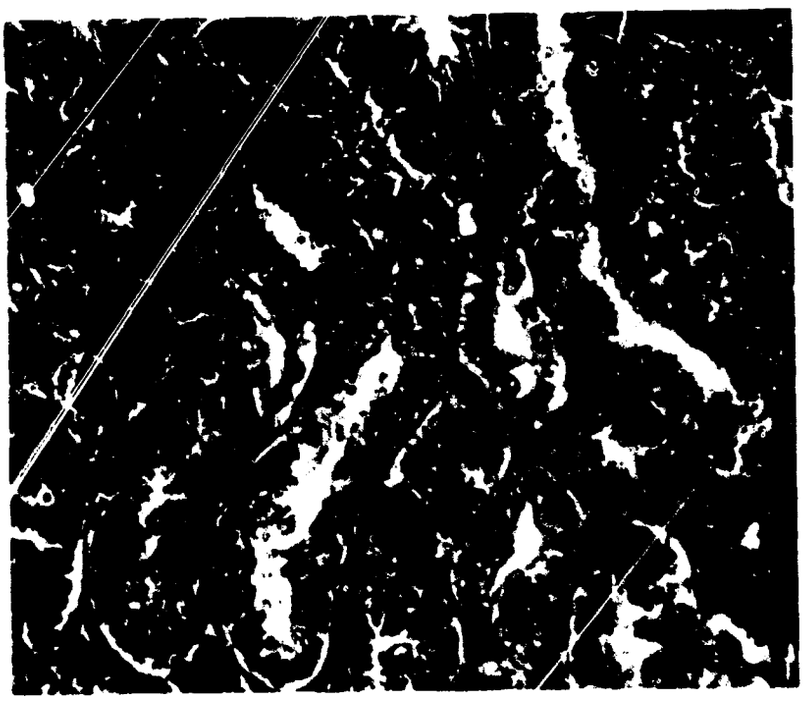

Figure 2. KIDNEY, 24 HOURS POSTEXPOSURE TO MMH. Note the prominent hyaline droplets in the proximal convoluted tubule (PT). The distal tubule (DT) in the center of the picture is virtually unaffected. x 252

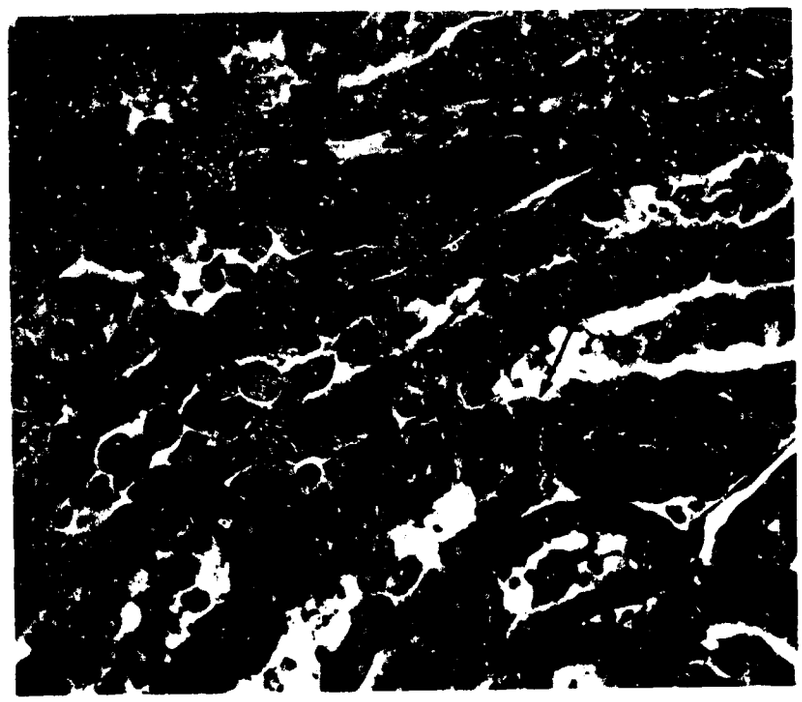

Figure 3. KIDNEY, 48 HOURS POSTEXPOSURE TO MMH. Note the numerous desquamated cells in the tubular lumen, many of which show marked nuclear pyknosis and/or karyorrhexis. The arrows point to mitotic figures characteristic of the active regeneration of tubular epithelium. x 400

## DISCUSSION

DR. KLION (Mount Sinai Hospital): This is more by way of confirmation than a question. We have had opportunity to study, primarily in monkeys exposed to much smaller dosages of MMH, kidney changes by electron microscopy. There was no hemoglobin that we could see, at least in these small, tiny pieces of tissue. There were, however, substantial changes and they were limited to the proximal tubular cells just as Dr. Sopher reported. I think there is very definite evidence that this drug is nephrotoxic and particularly to the proximal tubules.

MAJOR CARTER (Aerospace Medical Research Laboratories): There is one thing that stands out. If you are a dog, don't get around monomethylhydrazine! The dog is much more susceptible to convulsions. In other words, a dose of 7.5 milligrams per kilo in the monkey or any of these others, doesn't bother them. In addition, with doses of 5 milligrams per kilo you can get a tremendous increase in osmotic fragility of the red cells within four hours in a dog. You don't see this in the other species. The third aspect is the renal pathology which you see in the dog but not in the others. It seems then that there must be one basic mechanism, some subcellular mechanism that's common to all three of the systems. Hopefully, if we can find this then we can find the answer to all these questions.

DR. THOMAS: This just points out the need for better relationships between the field people and the toxicologists. If any of you can find human exposures, start looking for blood in the urine. We would like to know where a man stands in this game. Is he more like the monkey or the dog? So please, whoever works with monomethylhydrazine, start looking for any possible kidney changes.

DR. HODGE: Someone recently reported an increase in the plasma of free fatty acids in rats treated with monomethylhydrazine and this reminded me of the fact that Wong, a couple years ago, described an increase in plasma free fatty acids in cats treated with carbon tetrachloride. Now, these cats sustained proximal tubule injury, which is the bridge, maybe. Dr. Recknagle in Cleveland has been on the trail of an auto-oxidative mechanism for carbon tetrachloride injury. I just wonder if by any chance this might be a key to start looking for a monomethylhydrazine mechanism.

DR. SOPHER: I don't know. The hydrazines, of course, are strong reducing agents rather than oxidizers.

DR. HODGE: Carbon tetrachloride isn't an oxidizing agent but it triggers auto-oxidative phenomena among some fatty acids.

MR. VERNOT: With regard to the oxidative properties of monomethylhydrazine, although it is classically considered to be a reducing agent, it does form Heinz bodies and methemoglobin, product of the oxidation of the iron, of course, so there is some oxidizing going on there. I'm not sure if anybody has satisfactorily explained why it comes about, but it does.

DR. MEDINA (USAF School of Aerospace Medicine): Commenting on this business of oxidation by monomethylhydrazine, there might be a possibility also of some peroxide formation on fatty acids, and this question of an increase, was it an increase in fatty acids?

DR. HODGE: Free fatty acids.

DR. MEDINA: We have been looking at the hydrazines for about 7 years and so far every system that we've looked at--transaminase, amino acids, epinephrine, GABA, serotonin, histamine, --all are affected by the hydrazines. It appears as if it's a very, very strong poison. To delineate the specific site of action of the hydrazines will certainly be a tremendous undertaking.

DR. WEIBEL: I wonder whether Dr. Sopher would like to comment on where the primary site of damage indicated would be, because what he showed in the proximal tubule would be the sign of reabsorption of hemoglobin, which, in the first place, has to get into the nephron. Is this glomerular damage? Did you see anything? How does this come about?

DR. SOPHER: I should have made this clear. The glomeruli in all the kidneys I have seen, unless there was pre-existing disease, were relatively unaffected by this. One found protein in Bowman's capsule and that was about all. Sometimes you could see evidence of a good proteinuria there. I believe that the hemoglobin comes from severe intravascular hemolysis, filtered and reabsorbed by the proximal tubule, and as a matter of fact, just off the top of my head, from what I have seen, it looks to me like the pars recta of the proximal tubule is the part most affected compared to the convoluted portion.

DR. WEIBEL: Then I wonder whether you may call this a renal intoxication because this is a secondary effect due to overloading of the urine with hemoglobin, so it's actually a blood intoxication.

DR. SOPHER: As far as colloid bodies, or hyaline changes go, that's true. However, the desquamating cells can be found from the exit of the glomerulus all the way down to the proximal tubule. It seems to me with the degree of renal damage seen--I didn't show higher doses because the picture is complicated considerably by the hemoglobin crystals and reaction to the crystals, so you can't tell what's a pressure effect and what's irritation from the crystals, and what's a true nephrotoxic effect by this compound. I really think that the hemoglobin is not toxic and that it is the monomethylhydrazine that's doing the severe damage, that is causing the cellular death with the desquamation. Why we see

a cell here, a cell there, a cell somewhere else dying, and not widespread necrosis of the epithelium of a whole tubule, I don't know. These cells, for some reason, apparently are more susceptible.

DR. TOWNSEND (San Antonio Tuberculosis Hospital): Is there any alteration in the coagulation in any of these animals?

DR. SOPHER: I don't know. I haven't done any coagulation studies on the animals. We did especially look at a lot of animals for other investigators in a series reported elsewhere. Whether coagulation studies were done on those or not, I don't know. I don't believe so. It might be a fruitful area to investigate, especially because we did find there was evidence of intact red cells passing through the kidney. Otherwise, how would you get the blood clots in the bladder? In examining the urine, one sees innumerable red cells, too many to count. You'd have to use a coulter counter to count them. Plus free hemoglobin. Certainly one does see passage or bleeding from the kidney when patients are over-anticoagulated. It's a classic finding. There might be some deficit in coagulation here. I don't know.

## GENERAL DISCUSSION

DR. HODGE: Mr. Egan, you discarded discrepant data in the case of significant difference because you expected no difference between control and experimental group. Would you do the same in the study of contaminants where you're not trying to prove a point?

(Laughter)

MR. EGAN: I think I tried to explain this morning that in comparing either blood chemistry results, gross or histopathology results, comparing control to test, that the similarities were very pronounced, and the actual findings per se weren't really biologically significant. Now, in the case where we were examining a contaminant, if we found results to be the same between the test and the control, regardless of the incidence of murine pneumonia or changes in hematology, I don't think you could induce or deduce there was any effect from the contaminant. I don't know whether that is just begging the question or not.

DR. HODGE: Another question along the same vein. You rationalized statistically significant differences as biologically meaningless. Why, then, conduct the statistical test?

MR. EGAN: Well, to begin with, we have an entire accumulation of data which is analyzable, if you will, and I don't know how sophisticated some individuals are, whether they can look at data and eyeball it and determine whether or not there is a biological difference or not. Now, certainly, I wasn't trying to rationalize away significance here. We do have changes, but, for instance, in the case of hemoglobin, there was definitely a mathematical difference between the pre-exposure levels and exposure values. However, in looking at these values biologically, the hemoglobin values are down around 14 gms % or so pre-exposure and for dogs this is fairly low, but it doesn't necessarily indicate that the animal is in good health. During exposure it increases slightly for one reason or another. These values are going back up again to what we think are more normal values. Now, in the case of the enzymes, again mathematically, these things are significant, but in some cases, enzymes can have enough of a variable range where you're not really getting any kind of a biological indication.

DR. HODGE: That sort of hinges on the question that toxicologists keep themselves up late at night over. Because there is a change, is this an evidence of a toxic response? I have had the privilege of sitting in on some of these discussions and, Mr. Egan, you're on the trail of a very juicy one, you can chew on this for a very long time. It's real, it's statistically real, but is it adverse?

Is it meaningful? And this is, as you say, a question of judgment. A physician friend of mine was discussing with a medical school class at grand rounds the treatment of burns. These were huge burns, involved the entire thorax, for example, and there came a time when the surgeon snipped up this burned sheet and slipped it right off the patient, what used to be his skin, and one of the medical students said, "Well, how do you know when to take that off?" And the doctor said, "Well, that comes from good clinical judgment." (Laughter)

QUESTION: Captain Van Stee, how do the levels of bromotrifluoromethane, which caused blood pressure drops, relate to the concentrations necessary to prevent and/or extinguish a fire?

DR. VAN STEE: I believe I stated that the engineers tell us that in a 100% oxygen atmosphere, when fire is ignited, sufficient $CBrF_3$ has to be put in the vicinity of a fire to reach a 50% by volume concentration. Now, the question is what is the degree of hypotension associated with a 50% exposure? At 10%, there is very little change in blood pressure, up to perhaps 10 millimeters of mercury change, and at 80%, this may range from 20 mm to 60 mm. Ordinarily, it will be on the order of 40 to 50 millimeters of mercury drop, so in this middle range, at 50%, the nominal amount required to extinguish a fire, you would expect a blood pressure drop of 15 to 40 or 45 millimeters of mercury. I hope that answers that.

DR. HODGE: The last question is addressed to Dr. Hine. I'll be glad to mail it to him. How does the dose of epinephrine given dogs relate to endogenous concentrations?

At the moment, I'm not real sure that I remember what the norepinephrine content of normal myocardium is, but I think it's about a tenth of a microgram per hundred grams. Is that a ballpark figure?

DR. BACK: About ballpark. Circulating is about what? About a microgram per liter? Isn't that what we are told?

DR. HODGE: And the doses that were used in the study at duPont were 8 micrograms per kilo.

DR. BACK: Which is a fantastic amount. That's why Dr. Hine probably had a hard time scaring these dogs into a fibrillation.

DR. HODGE: He didn't.

DR. BACK: Normally, you can't and even with Dr. Van Stee's animals we did a number unanesthetized and they were convulsing all over the place, obviously convulsing hard enough to squeeze out all the epinepherine from the adrenal gland. We only had one death, I think. Is that about it?

DR. VAN STEE: I might say, I would like to make a very careful statement about these convulsions, since we are off into the realm now. We observed convulsions in the dogs during the period of exposure, but we don't know what caused

the convulsions yet. If you listen very carefully to that, I cannot say without qualification that $CBrF_3$ causes convulsions because the physiology of a conscious animal exposed to a dose of $CBrF_3$ required to produce a convulsion is really something to behold. The respiratory mechanics are completely altered. The acid-base balance of the body is completely altered. We haven't come to the point where we have been able to evaluate this thing very carefully, and so far all we can say is that these animals convulse, some of them will convulse in the presence of the exposure to the gas, but how this is brought about I don't know. It's entirely possible that these might be alkalotic convulsions having nothing to do with the $CBrF_3$ directly. That is where it stands.

DR. HODGE: On the other hand, what is Indoklon? Indoklon was the convulsant gas being used here, and it's a hexafluorodiethyl ether. There is a primary convulsant belonging to the same family.

DR. BACK: Right. I think that was discussed in Wesley Clayton's review of that.

DR. FAIRCHILD: There are convulsant barbiturates, too.

DR. HODGE: There are convulsant barbiturates. Can we throw this open to general discussion?

DR. REINHARDT: I have been involved in doing some experiments on cardiac sensitization, and in discussing this concentration of adrenalin, we found with another compound we were testing, namely, trichlorotrifluoroethane, that when we exposed dogs to 2% by volume of this compound, and this was just two dogs, after breathing it about five to ten minutes, they began to struggle and had what we termed multiple ventricular beats, which is something that may readily convert itself into ventricular fibrillation. So they developed this serious abnormal ventricular arrhythmia without any exogenous adrenalin. That was while being exposed to 2% of that compound.

DR. CAMPBELL: From what I have heard today about this Compound 1301, particularly the human work that was done out West, I'd be inclined to think that there would be pretty dangerous effects under conditions where this compound might be used in a space cabin atmosphere. He was getting definite effects, it seemed to me, between 10 to 15% out there. You're telling me this compound is going to reach 50% under conditions of use, and this would concern me a great deal.

MR. WANDS: I think we have to remember that the 50% figure is for 100% oxygen conditions. I'm not sure that this is planned for present flight conditions for spacecraft. Certainly, it is not planned for many of the other applications of this compound in fire control. In normal ambient atmospheres, the percentage volume figure is much less, on the order of 4 to 6%.

DR. BACK: That's about what I was going to say. No one has picked this compound or any compound like it for extinguishment in a spacecraft. However, it may be the one that they ultimately have to pick, and obviously we'll have to

go to protective measures in order to use it. The other thing is that this compound is a very quick acting one as far as fire extinguishment is concerned. It can put out a fire in milliseconds if you have a high enough concentration. The work that Dr. Hine did was done at the insistence of some people at Boeing. They were interested in its use in aircraft in which you don't have 100% oxygen, and as Ralph said, under these conditions, about 7% is necessary, but there are lots of problems that Boeing and the FAA and others have to think about because you have to get that 7% somewhere and if we were sitting in a 747 here, you'd have to put out a fire in the center of the room as quickly as you put one out over there. Now, if you have the ducts leading in from all over the place, somebody is going to be exposed to very high concentrations, and some others to relatively low concentrations, and so it's a time problem that we may be working with. However, look at the alternative. The alternative is certain death, so there may be trade-offs and it may be respiratory protective devices. The other thing is the rapidity of the reversibility of the action in the body. Maybe this is a saving factor, too; if you can get the fire out quickly enough, people won't be exposed for very long periods of times, and it depends upon the use, certainly. A man in a space cabin, if we were to use it that way, might have to put his face mask on and breathe on a closed system for a short period of time, but that may be a matter of engineering solution rather than anything else.

QUESTION: I have just a brief question for Dr. Albert from the presentation this morning. I was wondering what sources of information he used to determine which compounds would be present in the space cabin in significant concentrations?

DR. ALBERT: The committee was provided with measurements obtained from a series of tests, measurements that were done in capsules and also from ground tests, a series of about 7 tests as I recall, and there were several hundred compounds that had been measured under conditions of various sorts, and it was a question of winnowing from these the ones that might be really significant.

MR. WANDS: I might add that the first winnowing from over 200 compounds down to 50 was done by NASA on the basis of things which might be expected to be found in these longterm flight craft. After that, then, the committee winnowed these down to ones which would be of toxicological signficance and established their recommendations for those.

DR. AZAR (Ohio State University): I would like to ask Dr. Van Stee if he would mind commenting on the possible interaction of the Freon 1301 with the anesthetic used on the dogs. For example, was Freon 1301 accentuating the hypotensive effect of the barbiturate he used to anesthetize his animals? And the second question--Correct me if I'm wrong on this--I had the impression that he said the cardiac output did not change in the animals. Yet with a negative inotropic effect, decreased myocardial contractility, decreased stroke volume and slow heart rate, I would expect a fall in the cardiac output.

DR. VAN STEE: As far as the anesthetic is concerned, we anticipated problems in this, and if you will remember the design of the experiment which had a parallel experiment with three periods in which no exposures to $CBrF_3$ were made. We played around with this anesthesia problem quite awhile and

finally ended up with a mixture of pentobarbital, 2500 milligrams per liter, and 600 milligrams per liter tubocurarine hydrochloride by intravenous drip, in order to maintain or to prevent unnecessary fall of the blood pressure during the course of the experiment, and in our control experiment, the blood pressure was maintained quite well over a period of 150 minutes by this method. Now, as far as interaction between this anesthetic and the $CBrF_3$, I can't think of any comments I can make on that at all. I guess there is no way to know if there was a synergistic effect or not.

DR. BACK: In the monkey there was a slight synergistic effect. In the dog--

DR. VAN STEE: Well, yes, synergism as far as prolongation of the hypnotic state is concerned. If you put an animal down on pentobarbital or put him on a gas, the apparent recovery from anesthesia is greatly protracted to the extent that a single injection of 30 milligrams per kilo of pentobarbital which would normally be expected to put it down into Plane 2 or Plane 1 of a stage 3 anesthesia, you would expect the animal to be trembling, somewhat coming up from that within a period of 30-40 minutes. They will lie there absolutely still for two hours while they're breathing the $CBrF_3$ in the presence of pentobarbital and when you stop the gas they come right up. We thought at first maybe we had something that was going to compete with halothane as an anesthetic gas but it doesn't have anesthetic properties by itself. True, you would expect cardiac output would go down; however, apparently, the entire reserve component of the heart was not used up by the effect of the $CBrF_3$. There was also a reserve component left in the peripheral vascular smooth muscle, and the way I look at this thing is that there was a sufficient decrease in resistance to the outflow to compensate for the weakness created in the heart by exposure to the gas. Therefore the net effect was that the cardiac output did not change. Remember that the heart rate went down and stroke volume went up during this period of exposure, because the weakened heart was pushing against a lower resistance. Do you buy that?

DR. AZAR: I have some doubts.

DR. BENJAMIN: I would just like to make some comments regarding something that was raised previously and that is the pure oxygen atmosphere. Actually, in the present spacecraft, there is practically no flammable material and under those conditions the condition of needing 50% freon for suppression of open flame is practically impossible. If you have just some slight smoldering fire, then the requirement for freon is much lower. We consider something like 10-15% about the maximum required in actual flight and under those conditions, it's pretty safe.

DR. HODGE: Thank you. Gentlemen, I think I'd like to call this discussion to a close. On behalf of Mr. Wands and myself, I'd like to make two very brief comments. As Dr. Thomas has said, the objective of this meeting is providing a medium for the exchange of information, ideas, experiences, by the people who are actively engaged in the field of inhalation toxicology and the allied sciences, and I submit that we have today achieved this objective, at least so far.

SESSION II

# HISTOPATHOLOGICAL EVIDENCES OF TOXICITY

Chairman

Dr. Frank M. Townsend
Laboratory Service
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AMRL-TR-68-175

PAPER NO. 14

# ELECTRON MICROSCOPIC OBSERVATIONS ON THE KIDNEYS OF ANIMALS

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## INTRODUCTION

Alterations in the ultrastructure of renal tubular cells after exposure to 100% oxygen at 15 PSIA and 5 PSIA have been reported previously (Mautner, 1966). These changes consisted of irregularities in size and shape of the mitochondria of the proximal tubules and of changes in the structure of the mitochondrial cristae. There was also an increase in microbodies in the proximal tubular cells.

In order to determine the time relationship and the possible reversibility of these changes, serial biopsy studies were undertaken. Besides oxygen, animals were also exposed to a mixed gas atmosphere and to monomethylhydrazine.

## MATERIALS AND METHODS

Monkeys and dogs were subjected to unilaterial translocation of the kidney to a subcutaneous site for the purpose of facilitating the serial biopsy procedure (Kaplan, 1967). One to two months after translocation, baseline renal function tests and renal biopsies were performed. Thereafter, groups of animals were exposed to one of the following:

1) 100% oxygen at 15 PSIA for 3 - 12 days
2) 68% oxygen, 32% nitrogen at 5 PSIA for 3 and 7 months
3) Monomethylhydrazine injected intraperitoneally in varying amounts, and for varying periods of time (George, 1968)

In addition, rats were exposed to oxygen at 15 PSIA for periods of 15 to 360 minutes.

Appropriate control animals were included in each group. Methods of exposure, biopsy, tissue processing and electron microscopic investigation were reported previously (Mautner, 1966; Felig, 1965).

Following exposure, monkeys and dogs were sacrificed either immediately or within a few months. Complete autopsies were performed, and, in a few instances,

ante mortem biopsies of the translocated as well as the in situ kidney were obtained at the time of sacrifice.

## RESULTS

1) Baseline studies: Renal function tests and renal biopsies performed one to two months after translocation showed no significant alterations.

2) Oxygen, short term: Rates exposed to 100% oxygen at 15 PSIA for up to six hours showed no significant changes. In the previous study, rats similarly exposed for 24 hours showed prominent alterations.

3) Oxygen long term: The severity of the changes in monkeys and dogs exposed to 100% oxygen at 15 PSIA is as follows:

| | |
|---|---|
| 3 days | +++ |
| 4 days | ++++ |
| 7 days | ++++ |
| 12 days | ++ |

The comparable findings in the previous study (without serial biopsy) performed at 5 PSIA were as follows:

| | |
|---|---|
| 2 weeks | + |
| 3 months | +++ |
| 7 months | + |

The nature of the changes was the same in both series. The proximal tubular mitochondria had irregular profiles and showed smudging and focal disappearance of their cristae (figure 1). There was also an increase in the number of microbodies.

4) Mixed gas atmosphere: Animals exposed to the mixed gas atmosphere for 3 and 7 months showed some, but not all, of the changes found in the oxygen exposed animals. There was some irregularity of mitochondrial profiles, with areas devoid of mitochondrial cristae (figures 2, 3). The degree of change was less severe than in the animals exposed to pure oxygen. A significant increase in the number of microbodies could not be detected, nor was there evidence of a reversal of these changes.

5) MMH: Acute tubular alterations following exposure to monomethylhydrazine have been reported (George, 1968). Whether these changes leave a residual has not yet been determined, largely because of the changes due to translocation described below.

6) Changes due to translocation: Autopsy tissues from the kidneys of 17 animals have been studied. Thirteen of these showed alterations in the translocated,

as opposed to the in situ, kidney, irrespective of the type of exposure (or lack of exposure). All of these were autopsied seven months or more after translocation. The nature of these changes was remarkably uniform in all animals examined. Bands of fibrous tissue extended from the medulla to the cortex, always perpendicular to the plane of the capsule, and frequently extending all the way from the pelvis to the capsule. When such a band did extend to the capsule, there usually was a V-shaped indentation of the capsule of the type commonly attributed by pathologists to atherosclerosis of the larger arteries ("benign nephrosclerosis"). In addition to varying in length (which may of course be due to the plane of sectioning) the bands varied in thickness, some extremely narrow, about the diameter of a tubule, some obviously involving a large number of adjacent nephrons. Another important variable was the degree of inflammation. While the inflammatory component was always of a chronic nature, consisting primarily of lymphocytes and macrophages, the number of inflammatory cells varied markedly, sometimes almost completely absent, sometimes almost obscuring the connective tissue. The distribution of these bands varied from one to 6 or 7 per section. In view of the large number, the possibility that they are healed needle biopsy tracts can be ruled out.

## DISCUSSION

The finding that animals exposed to 5 PSIA oxygen-nitrogen mixtures with a partial pressure of oxygen approximately equal to that of earth atmosphere show some of the changes of animals exposed to 5 PSIA pure oxygen raised the question of whether these changes were in fact due to oxygen, or to some other factor. Also, since the early experiments required a different animal for each biopsy, the suggestion of reversibility of changes due to oxygen was open to question; in a small series, biologic variation might account for these differences. The experiments reported here attempt to answer both questions.

The serial biopsy procedure has made it possible to compare the changes after different exposure times in the same animals. The findings in the 15 PSIA 3 - 12 day experiments strongly support the hypothesis that oxygen has a toxic effect which is at least partly reversible. Animals kept in the Thomas Dome for similar periods, but without similarly high pressures of oxygen, do not show these changes.

Two questions remain unresolved by the present studies. The first is the reason for the reversal of these changes; it is not yet clear whether this reversal is due to an adaptive mechanism on the part of the tubular cell organelles, or to a vascular mechanism, or to decreased oxygenation due to lung damage. The second is the cause of the alterations found in the animals exposed to mixed gas atmospheres. These might be due to a contaminant in the Thomas Dome, or to the low ambient pressure, or to another factor as yet unsuspected. It must be stressed that the changes described above are nonspecific and do not implicate any specific toxic agent.

The cause of the alterations due to translocation is also not yet clear. The appearance of the changes suggest a vascular etiology. However, blood vessel alterations have not yet been substantiated; the degree of inflammation suggests

the possibility of an infectious component; and the previous handling and exposed position of these kidneys requires that trauma be considered as a cause. A thorough study of the nature and development of these lesions is important in order to assess the value of this procedure in toxicologic studies.

## SUMMARY

1) Serial biopsy studies of kidneys of animals exposed to 100% oxygen at 15 PSIA show maximal changes at 4 to 7 days with a return towards normal by 12 days.

2) Earliest changes develop between 3 and 24 hours.

3) Animals exposed for long periods of 68% oxygen, 32% nitrogen at 5 PSIA show some, but not all, of these changes, and to a lesser degree.

4) The translocation procedure required for serial renal biopsies induces chronic changes which may interfere with the interpretation of changes due to exposure within a few months after the translocation procedure.

## ACKNOWLEDGMENT

This work was sponsored by the Aerospace Medical Research Laboratories, Aerospace Medical Division, Air Force Systems Command, Wright-Patterson Air Force Base, Ohio, under Contract AF 33(615) - 3464.

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Figure 1. PROXIMAL TUBULE OF MONKEY, 100% OXYGEN AT 15 PSIA, 7 DAYS. Brush border lower left, basement membrane upper right. Mitochondria show smudging and loss of cristae. Microbodies (dark bodies near center) increased in number. Electron micrograph, x 25,000.

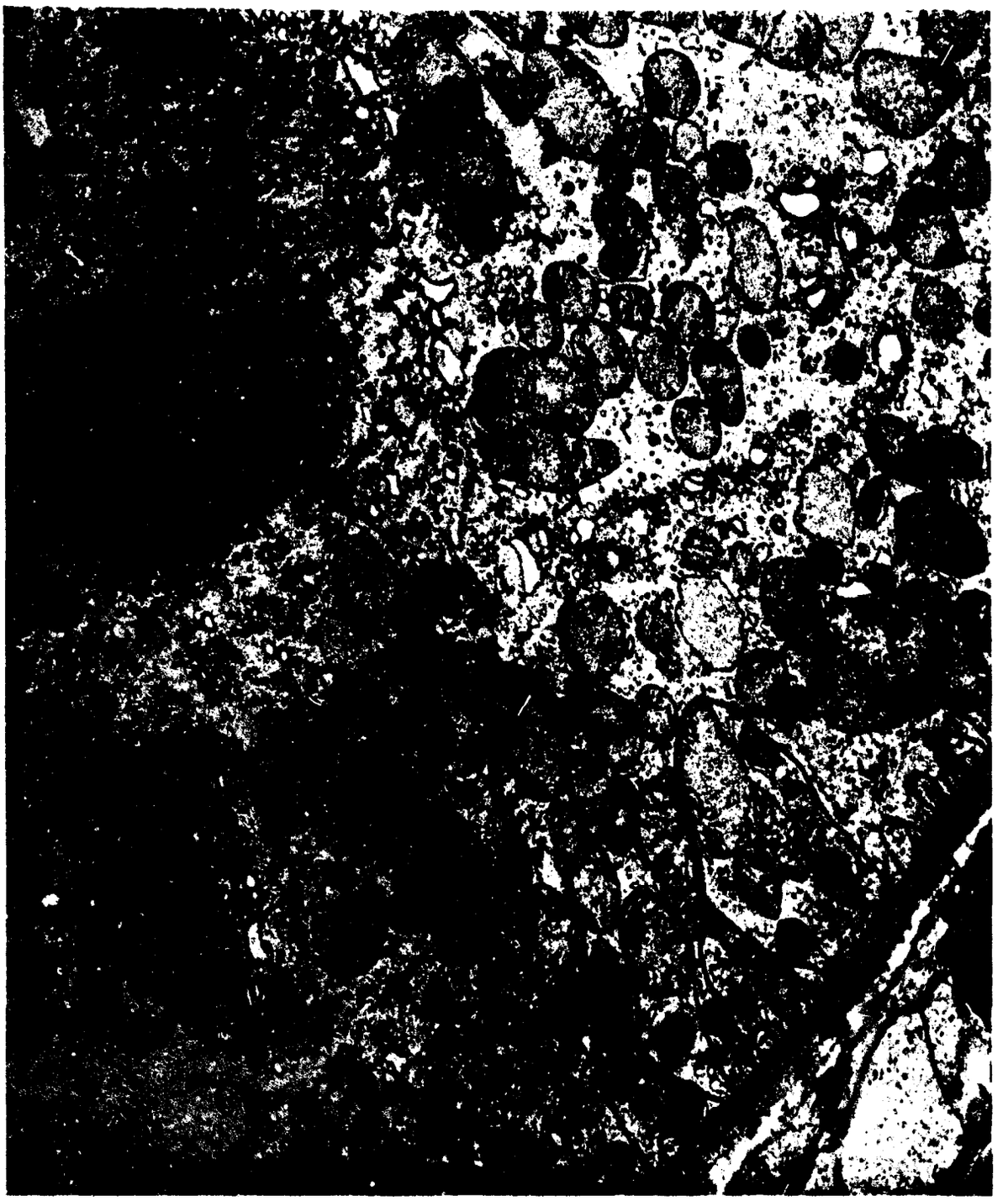

Figure 2. PROXIMAL TUBULE OF MONKEY, 68% OXYGEN, 32% NITROGEN, 5 PSIA, 7 MONTHS. Brush border upper left, basement membrane lower right. Some irregularity of mitochondria, no significant increase of microbodies. Electron micrograph, x 16,000.

Figure 3. SAME AS FIGURE 2, HIGHER POWER. Mitochondrial structure is altered, but not as severely as in figure 1. Electron micrograph, x 35, 000.

## DISCUSSION

DR. BACK: Dr. Mautner, those last two slides were monkey kidney, were they not?

DR. MAUTNER (Mount Sinai Hospital): Yes they were. The monomethyl-hydrazine work was on monkeys. Dr. Sopher's was mainly dogs. Monkeys are less susceptible, I understand, and the exposures were less in amount. These were at seven and a half milligrams per kilo. Dr. Sopher's were twice or more this amount.

DR. BACK: I think this shows something that we argued a little bit yesterday, the effect of changes in blood constituents in the proximal tubules, and that the monkey doesn't show this effect; but you still did see it. So I think this does clear up the point of the need for blood changes to produce an effect on the kidney tubules.

I think the question was raised by Dr. Weibel yesterday. This unequivocally shows there can be a real direct effect without a change in blood constituents.

DR. MAUTNER: I tried to answer Dr. Weibel's question before he raised it. I am convinced from these findings that there is a direct effect on the tubular cells, irrespective of any hematological changes.

DR. WEIBEL: Dr. Mautner, you have referred repeatedly to microbodies. How do you define microbodies?

DR. MAUTNER: Well they are little bodies and cells which nobody knows what they do or where they come from. They are supposedly, as I am sure you know, packets of enzymes.

DR. WEIBEL: Well, that's the feeling I had but you may know that the term "microbodies" actually is restricted to what is also called peroxysomes; mainly, they are like little lysozyme like organelles containing only two enzymes, uricase and catalase, but there are quite considerable specie differences in the appearance of these.

DR. MAUTNER: Organ differences. The kidney microsomes look quite different. You may remember from the picture that they have very sharp edges, which is an unusual thing; and I have seen it only in certain species, and only in the kidney.

DR. WEIBEL: Did you see the crystalline structure inside?

DR. MAUTNER: No, I did not define that, and I was very cautious not to say too much about them because I am not convinced that we are dealing with the same thing that you see in other organs.

DR. SCHAFFNER (Mount Sinai Hospital): There is a good deal known about these peroxysomes. They do contain more than two enzymes. They contain quite a number of them. Their function does seem to be an oxidizing one. They are not lysosomes at all. They are not digestive in the sense that the lysosomal enzymes all have an acid pH maximum. The crystalline structure that you asked about, in those species and in those organs where the bar is off to the side, is apparently identical in function at least to the nucleoid in those where it is in the middle as in the livers of rats and dogs, but not in hamsters where there is a central bar and then there is a side bar as in some other animals. It is always interesting to come a good many miles to a meeting and find that a colleague in the same institution, with whom we meet quite frequently, has turned up work which shows similarities to work we have done in the liver, and is working in an area that we are very much interested in; namely, that adaptive changes occur apparently on exposure to oxygen. We are now convinced that all species, and probably all organs, are capable of adapting also to changes when exposed to various chemicals, and these adaptive changes, the capability of performing the adaptive changes and maintaining them, is the very phenomenon that we have all been talking about. The inability to maintain this adaptive response or develop it fully is toxicity, and we have had to learn whole new sets of parameters to measure this, and the degree of sophistication in this area is enormous. I wonder, Willy, since we haven't communicated on this before, if you have any information as to the functional significance of some of these changes that you have shown--I mean in terms of modern-day toxicology?

DR. MAUTNER: The functional data that we have are primarily from renal function tests of the classic sort. They do not show any changes but I think that these tests that I am talking about--glomerular filtration rates, blood flow transfer maximums so forth--these types of tests are relatively crude I think compared to electron microscopy; so I am not surprised you will find electron microscopic changes without any functional support. Biochemical data I do not know about on these animals. I don't believe there was any done on them.

DR. LEON (Ames Research Center): This question refers to the work that you showed, and some of the work yesterday. As was discussed yesterday, usually rats in one hundred per cent oxygen at one atmosphere will die in three days. If they are young enough, they will survive or sometimes if they are not young they will survive even in the pure oxygen. Of course you had some that had been in oxygen for 12 days. The thing that kills them on the third day is not the oxygen, but the lack of oxygen since their lungs are so damaged that there is no oxygen getting into the periphery. So the point I would like to know is: Are you sure that these massive changes that you show at four and seven days are in fact due to the oxygen or to the lack of oxygen?

DR. MAUTNER: If I may ask Miss George to reply to that. I have some remarks to make about that also, but she has the data.

MISS GEORGE (Aerospace Medical Research Laboratories): All the animals at four days were hyperoxic, 300 to 500 mm arterial $pO_2$. At seven days they had $pO_2$ of 200 to 300, and there were several that were down to 50-100. There was a great variability at seven days, but they were all lower than they were at four days. At four days they were all between 300 and 500.

DR. MAUTNER: I would say that we cannot with any certainty say that this is oxygen. It may be hypoxia. The changes, as I pointed out, are not specific and I could not be at all sure that either one or the other may produce the same alteration. There is a suggestion that oxygen does this. On the other hand, the lung damage is such that the reversal at 12 days particularly suggests that hypoxia may also play a role. Incidentally, I should have pointed out that I was talking about monkeys, not rats. I could not get into the problem of species. There are differences and in brief they are that the dogs reverses the most and the rat is the most unreliable experiment animal for this purpose. We like the monkeys. They all look the same.

DR. BENJAMIN: Dr. Mautner, your findings regarding mixed atmosphere are especially alarming since we always considered this mixed atmosphere as physiologically inert. I wonder whether you have any indication that there could possibly be some other contaminant present. Do you have some analysis of the atmosphere?

DR. MAUTNER: I don't know about the analysis of the atmosphere. What we need here and have just recently received, a series of animals which were recently put in the domes under normal ambient air atmosphere, and the big question is whether these will show changes. Because if they show changes then there is something in the dome. It may be contaminants. I don't know, but it was disturbing to us also. But I think it is quite unequivocal that the mixed gas animals do show changes.

DR. THOMAS: There is something in the dome, yes, the animals are in there. You have been out there and seen these experiments. You must realize that there are 40 rats, 50 mice, 8 to 10 dogs and four monkeys in there. We have four complete air changes once an hour but it does smell, and if you can smell something there is something in the air. We agree to that. We weren't bothered about this fact for awhile because we found that, as the systems were at that time, the air smells in the capsule too. However, to be puristic about this, we do have dome controls now, which have been running for five months, and if we find that there are any changes, I suspect the next thing will be to increase the airflow. We have a capability of going up to 125 cfm and we are only using 20 at the present. By increasing the airflow rate or the gaseous flowrate through the dome to the maximum amount, we can get rid of the odors.

DR. RIESEN: I was interested in the mitochrondial changes that were shown, Dr. Mautner, and I am curious regarding the biochemical functionality of such mitrochondria and I was wondering if there was any data on that. I would presume that when the inner membrane which constitutes these crystae, structurally falls

apart like that, that this would reflect either an intra-mitochondrial media change, i.e., the so-called intra-mitochondria matrix, or a change in the structure or an occlusion of some kind in the membrane, thereby changing the conformation of the membrane subunit which aggregates to form this crystae. The thing that I want to just mention here is that the disaggregated crystae can still be biochemically functional. That is, they will not have all of the capability of control in response to the cytoplasmic environment but they will function. Just because the crystae are not visible does not mean there is not some kind of phosphorylation going on. And the second point is that the reversibility may well be feasible intra-mitchrondially without any real membrane damage. That could be a reversible thing and so from what we know about mitochrondia, this kind of reversibility is entirely feasible.

DR. MAUTNER: I would like to say we have had many discussions as to possible significance of these morphologic changes which are essentially only pictures. The function tests are too crude to show the capacity of the kidney. Certainly the proximal tubules are such that a great deal of damage could be done before it would show up with our present day function tests. We don't try to say that this means any interference with function. What we do say is that the mitochondrion in the proximal tubules is an extremely important organelle, and whether its function is interfered with because of the changes, we cannot say.

DR. KLION: This is in the way of a comment. You may recall that last year we presented electron microscopic findings of the liver with the two-gas system, and there we found non-specific changes primarily involving microbodies that were more pronounced than in the oxygen studies. This suggested then that in the two-gas system, there is some phenomenon going on that is different from the oxygen atmosphere.

DR. TOWNSEND: Dr. Mautner, would you just contrast for us the changes between MMH kidney and what you would see in a so-called anoxic kidney?

DR. MAUTNER: I think the striking thing about MMH, and the simplest thing to pick up, is the pure involvement of distal tubules. With all other exposures we are talking essentially about proximal involvement and here we have, and can show a rather substantial amount of, distal involvement. It is, as I said, not easily seen by light microscopy. This makes a very simple distinction. There might be mitochondrial changes in all of these, and whether the mitochrondion is smaller or whether it is shortened and loses its crystae I don't know whether this is significant. I would be very reluctant to ascribe any specific significance to the type of change. All we can say is there is a change.

AMRL-TR-68-175

PAPER NO. 15

# ELECTRON MICROSCOPIC AND MORPHOMETRIC STUDY OF RAT, MONKEY AND DOG LUNGS EXPOSED TO 68% $O_2$ AND 32% $N_2$ AT 258 TORR FOR EIGHT MONTHS

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## INTRODUCTION

Previous studies have shown that breathing pure oxygen leads to a cellular damage which causes thickening of the alveolo-capillary air-blood barrier. In rats and monkeys this change was found after two or four days respectively if the gas was applied at 760 mm Hg; this atmosphere was lethal for rats while 60% of monkeys survived (Kistler, 1967; Kapanci, 1968). Exposure of rats to pure $O_2$ at 258 mm Hg led to no apparent pathology after two weeks (Kistler, 1966), while after eight months in oxygen at 258 mm Hg dogs and monkeys showed alterations in the tissue barrier similar to those observed at 760 mm Hg. This damage was largely repaired after recovery for 40 days in room air (Schwinger, 1967).

These studies have revealed that long-term breathing of pure oxygen may have deleterious effects on the lung even at pressures reduced to 1/3 atm., as used in space travel. The purpose of the present investigation was to establish whether long-term breathing of a mixed gas atmosphere (68% $O_2$, 32% $N_2$) at 258 mm Hg would also have adverse effects on lung tissue.

## MATERIALS AND METHODS

Four monkeys (Rhesus), four dogs (Beagle) and five rats (Sprague-Dawley) spent eight months in an atmosphere of 68% $O_2$ and 32% $N_2$ at 258 mm Hg. Exposure was done in Thomas domes (Thomas, 1966) at 6570th AMRL at Dayton, Ohio. General experimental procedures and the conditions of chamber operation for this experiment are presented elsewhere (Fairchild, 1968). Two dogs, two monkeys and five rats were kept in room air as controls.

Sacrifice of the animals and fixation of lungs by instillation of glutaraldehyde were done at 6570th AMRL, Ohio; then the fixed lungs were shipped to Berne (Switzerland) for further processing and analysis.

Lung volume measurements and black and white photographs were taken of each lung before further processing of the specimens for light and electron microscopy.

Fixation and preparation of the lungs for electron microscopy as well as morphometry followed the standardized methods described in detail elsewhere (Weibel, 1966; Kistler, 1967).

A random sample of six Epon embedded tissue blocks per animal was sectioned and studied in an EM 200 electron microscope. For morphometric evaluation six micrographs per section were recorded on film at a magnification of 2400 x, yielding a random sample of 36 micrographs. These were analyzed by point counting procedures in a table projector unit (Weibel, 1966); the data was analyzed by computer.

The material had been fixed in glutaraldehyde followed by $OsO_4$, both buffered with potassium phosphate. Long intermediate storage in glutaraldehyde, due to shipping of specimens from the USA to Switzerland, resulted in granular artifacts as described by Gil and Weibel (1968). This aesthetic defect did not affect the usability of these lungs for morphometry.

## RESULTS

### Comparison of Ultrastructure and Morphometric Data of Control Animals

The basic composition of the pulmonary air-blood tissue barrier is comparable in the three species used in this study. Compared to the rat, the larger animals, monkey and dog, show a focal accentuation of collagen and elastic fibers; this probably is related to the larger lungs of these animals.

On the whole it appears that the air-blood barrier of rat lungs is generally slightly slimmer than that in dogs and monkeys. As figure 1 shows, this is expressed in species-dependent differences in the mean barrier thickness of control animals. The focal or strand-like accentuation of connective tissue leads to slight differences in the arithmetic mean. The proportion between the barrier constituents epithelium, interstitium, and endothelium is approximately equal in

all three species. The general slight thickening of tissue elements throughout the barrier expresses itself in higher values for the harmonic mean thickness in monkeys and dogs; this parameter essentially measures the contribution of thin barrier portions.

The size of the gas exchange apparatus of the lung is estimated by the total alveolar surface area $S_a$. Figure 2 reveals that $S_a$ correlates well with body weight. The ratio of capillary to alveolar surface $S_c/S_a$ is about 1.0 in rats, 0.9 in monkeys, and 0.85 in dogs.

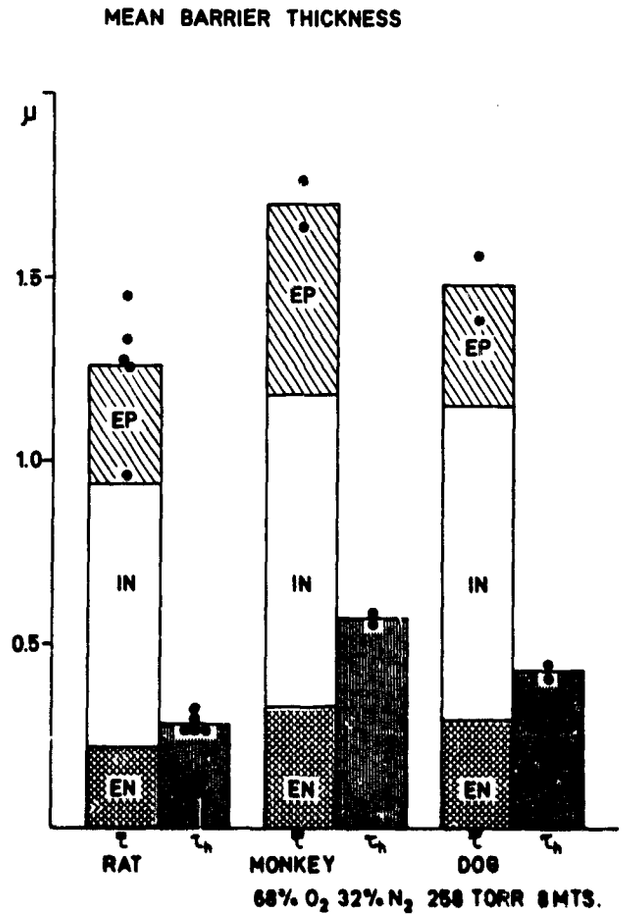


Figure 1. COMPARISON OF MEAN BARRIER THICKNESSES IN RAT, MONKEY, AND DOG. Control animals.

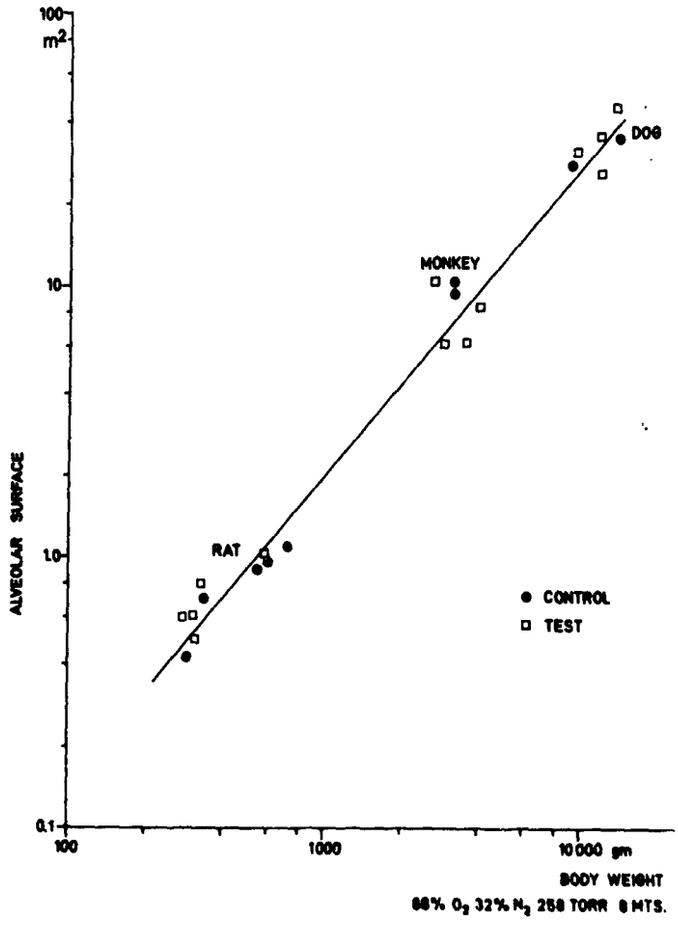


Figure 2. RELATION BETWEEN ALVEOLAR SURFACE AREA AND BODY WEIGHT.

In conclusion, the basic morphometric parameters characterizing the pulmonary gas exchange apparatus show appreciable interspecies differences. The small number of control animals investigated does not justify any more specific conclusions. The main consequence for this report is that the findings in exposed animals have to be discussed with respect to species.

## CHANGES OBSERVED IN ANIMALS EXPOSED FOR EIGHT MONTHS TO 68% $O_2$ AND 32% $N_2$

### Morphometric Findings

Table I presents a synopsis of a number of morphometric parameters characterizing the animals and their lungs.

# TABLE I

SYNOPSIS OF MORPHOMETRIC DATA

| | | Start Weight g | Final Weight g | Lung Volume ml | $S_{CT}$ $m^2$ | $S_{AT}$ $m^2$ | $\mu$ | ep $\mu$ | en $\mu$ | in $\mu$ | h $\mu$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Control Rats | 1 | 124 | 294 | 7.6 | 0.43 | 0.43 | 1.28 | 0.30 | 0.28 | 0.70 | 0.29 |
| | 2 | 142 | 600 | 13.0 | 1.11 | 0.95 | 1.27 | 0.30 | 0.22 | 0.74 | 0.27 |
| | 3 | 105 | 342 | 9.1 | 0.68 | 0.70 | 1.45 | 0.38 | 0.19 | 0.87 | 0.27 |
| | 4 | 138 | 720 | 13.5 | 1.19 | 1.04 | 0.96 | 0.27 | 0.20 | 0.49 | 0.31 |
| | 5 | 131 | 551 | 11.9 | 0.88 | 0.90 | 1.34 | 0.35 | 0.22 | 0.77 | 0.27 |
| Test Rats | 1 | 113 | 310 | 8.2 | 0.57 | 0.53 | 1.89 | 0.43 | 0.26 | 1.20 | 0.30 |
| | 2 | 100 | 283 | 10.4 | 0.67 | 0.62 | 1.81 | 0.47 | 0.19 | 1.14 | 0.35 |
| | 3 | 142 | 332 | 9.2 | 0.74 | 0.78 | 1.30 | 0.36 | 0.22 | 0.73 | 0.31 |
| | 4 | 133 | 309 | 8.8 | 0.72 | 0.66 | 1.16 | 0.24 | 0.23 | 0.69 | 0.30 |
| | 5 | 129 | 572 | 14.4 | 1.12 | 1.07 | 1.25 | 0.37 | 0.21 | 0.66 | 0.29 |
| | | Start Weight kg | Final Weight kg | Lung Volume ml | $S_{CT}$ $m^2$ | $S_{AT}$ $m^2$ | $\mu$ | ep $\mu$ | en $\mu$ | in $\mu$ | h $\mu$ |
| Control Monkeys | 1 | 2.72 | 3.18 | 150 | 7.98 | 9.48 | 1.64 | 0.41 | 0.31 | 0.92 | 0.56 |
| | 2 | 3.18 | 3.18 | 163 | 9.29 | 10.11 | 1.76 | 0.62 | 0.35 | 0.79 | 0.59 |
| Test Monkeys | 1 | 2.72 | 2.95 | 124 | 4.84 | 6.20 | 1.82 | 0.58 | 0.22 | 1.01 | 0.68 |
| | 2 | 2.72 | 2.72 | 166 | 11.32 | 10.49 | 1.60 | 0.29 | 0.33 | 0.97 | 0.59 |
| | 3 | 3.18 | 4.08 | 171 | 7.18 | 8.55 | 2.19 | 0.64 | 0.48 | 1.08 | 0.73 |
| | 4 | 3.18 | 3.63 | 180 | 6.48 | 6.30 | 2.50 | 0.60 | 0.40 | 1.49 | 0.56 |
| Control Dogs | 1 | 10.0 | 14.1 | 432 | 29.4 | 34.1 | 1.56 | 0.34 | 0.33 | 0.90 | 0.41 |
| | 2 | 8.6 | 9.1 | 327 | 21.9 | 27.4 | 1.38 | 0.31 | 0.25 | 0.83 | 0.44 |
| Test Dogs | 1 | 10.9 | 13.6 | 576 | 49.5 | 47.8 | 2.00 | 0.58 | 0.33 | 1.10 | 0.56 |
| | 2 | 10.0 | 12.3 | 401 | 32.9 | 34.5 | 2.16 | 0.52 | 0.24 | 1.39 | 0.51 |
| | 3 | 10.4 | 12.3 | 432 | 23.8 | 25.5 | 2.79 | 0.64 | 0.30 | 1.85 | 0.49 |
| | 4 | 8.2 | 9.6 | 373 | 27.6 | 32.1 | 1.40 | 0.39 | 0.26 | 0.76 | 0.42 |

Figure 2 reveals that the alveolar surface area $S_a$ of test animals was in the same range as that of controls. The ratio of capillary to alveolar surface area did not show any changes, nor did the capillary volume.

Consistent changes were observed in the arithmetic mean barrier thickness $\bar{\tau}$ (figure 3). In the rats two of the five animals had a $\bar{\tau}$ of the order of 1.9 $\mu$ which is clearly above normal. A more distinct mean barrier thickening was found in monkeys and in dogs where two and three animals respectively were clearly outside normal range. As revealed by figure 4 this barrier thickening was essentially due to an increase in the thickness of the interstitial space. That this was largely but not completely confined to the thick fibrous portions of the barrier is shown by figure 5: the harmonic mean barrier thickness appeared also to be consistently increased in the test animals of all three species, but this increase was slighter than the increase of the arithmetic mean thickness (figure 3).

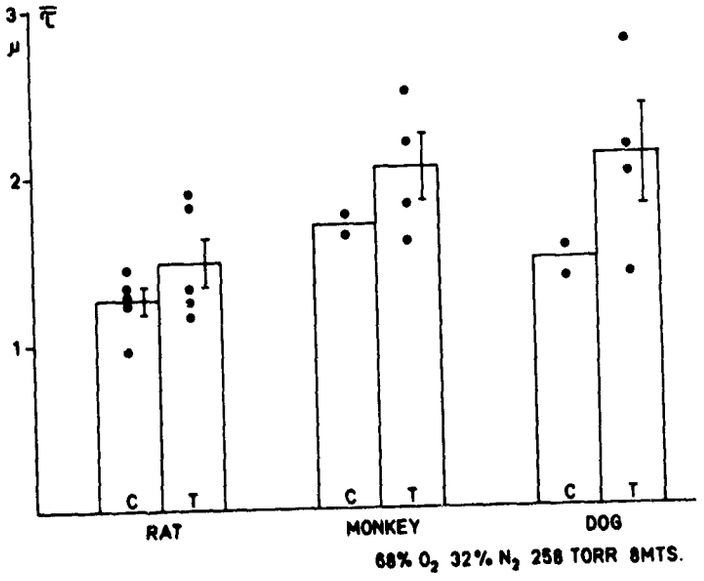


Figure 3. CHANGE IN ARITHMETIC MEAN BARRIER THICKNESS BETWEEN CONTROL (C) AND TEST (T) ANIMALS. Individual values (dots), group means, and standard errors.

In view of recent findings (Kapanci, 1968) the mean thickness of alveolar epithelium may be of interest. In spite of considerable scatter of the data figure 6 reveals a distinct trend towards a thickening of epithelium in exposed animals. This is particularly marked in the dogs.

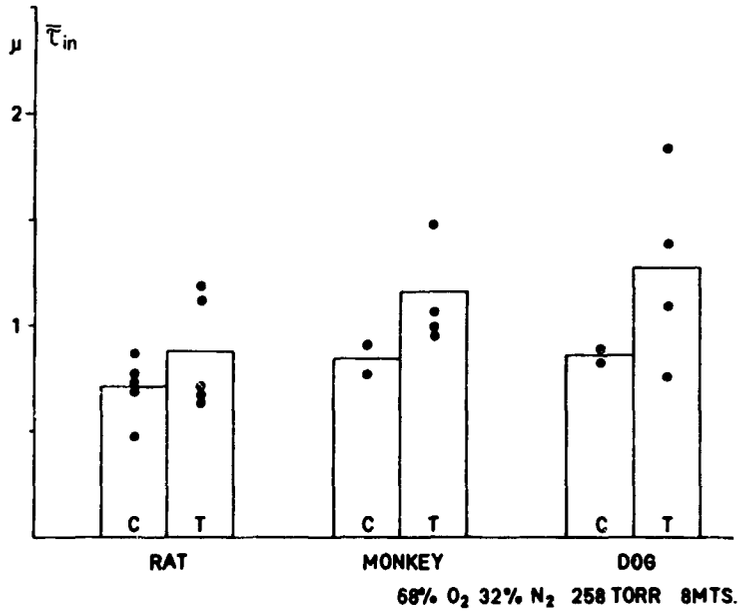


Figure 4. CHANGE IN MEAN THICKNESS OF INTERSTITIAL SPACE

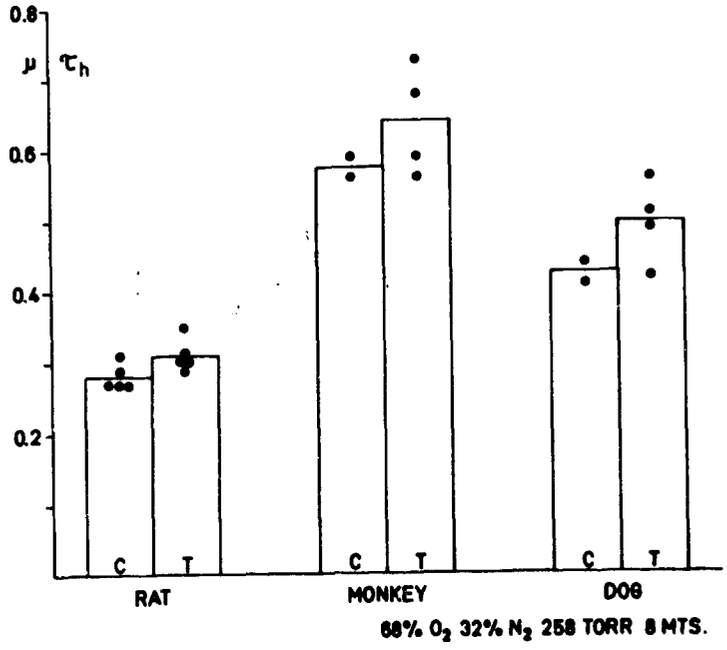


Figure 5. CHANGE IN HARMONIC MEAN THICKNESS

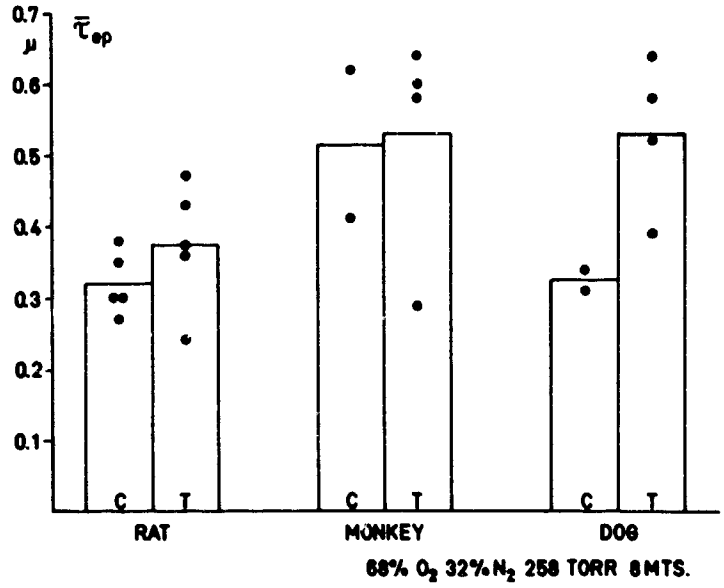


Figure 6. CHANGE IN MEAN THICKNESS OF ALVEOLAR EPITHELIUM

Ultrastructural Findings

The fine structure of the air-blood tissue barrier was not dramatically altered, on the whole. In accordance with the morphometric findings some edematous swelling of the interstitial space could be observed in all species. This was most marked around connective tissue fibers and caused a separation of epithelium and endothelium (figure 7). These enlarged interstitial spaces sometimes contained an unusual number of free cells, such as mast cells and plasma cells (figure 8), or macrophages (figure 9). Focally, interstitial cellular proliferation was very marked, as in figure 10.

The majority of alveoli appeared clean. In some regions, however, signs of alveolar exudation were noted. Figure 11 shows one part of the alveolar surface of a dog lung to be lined by a proteinaceous layer of exudate up to 8 μ thick which, at the surface, is in contact with a surfactant film (Weibel, 1968). Occasional alveoli were completely stuffed with tubular myelin figures (figure 12) which presumably are a derivate of surfactant (Weibel, 1966; Weibel, 1968). Others contain fibrin strands (figure 13) and numerous macrophages or desquamated cells. All in all, these findings suggest exudation into the alveolar lumen which must be considered pathological.

Figure 7. ELECTRON MICROGRAPH OF TEST DOG LUNG SHOWING EDEMATOUS ENLARGEMENT OF INTERSTITIAL SPACE. x 7'000

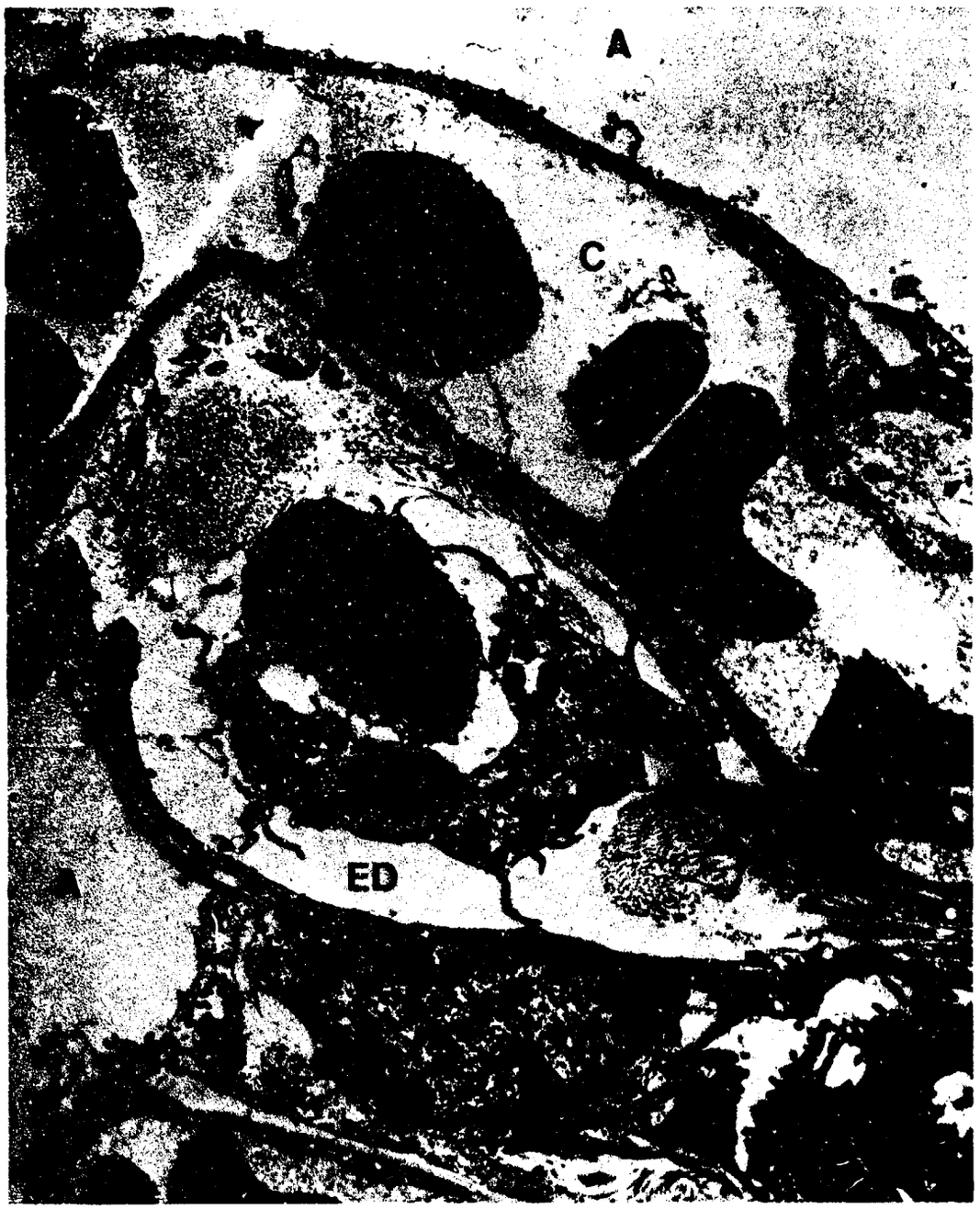


Figure 8. ELECTRON MICROGRAPH OF TEST DOG LUNG. Enlarged interstitium contains plasma cell and mast cell. x 7'000

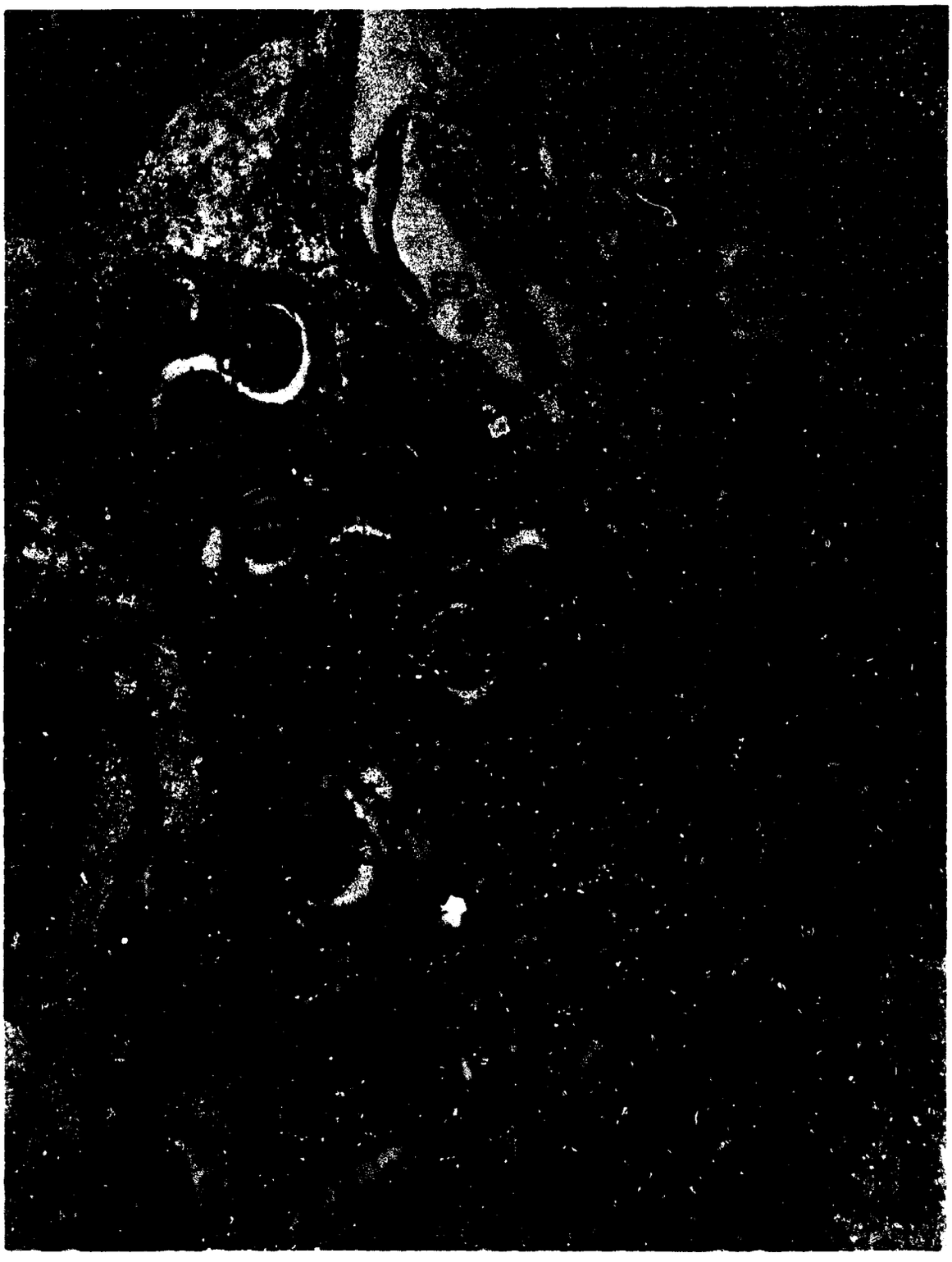

Figure 9. ELECTRON MICROGRAPH OF TEST DOG LUNG WITH MACROPHAGE IN CAPILLARY LUMEN. x 15'000

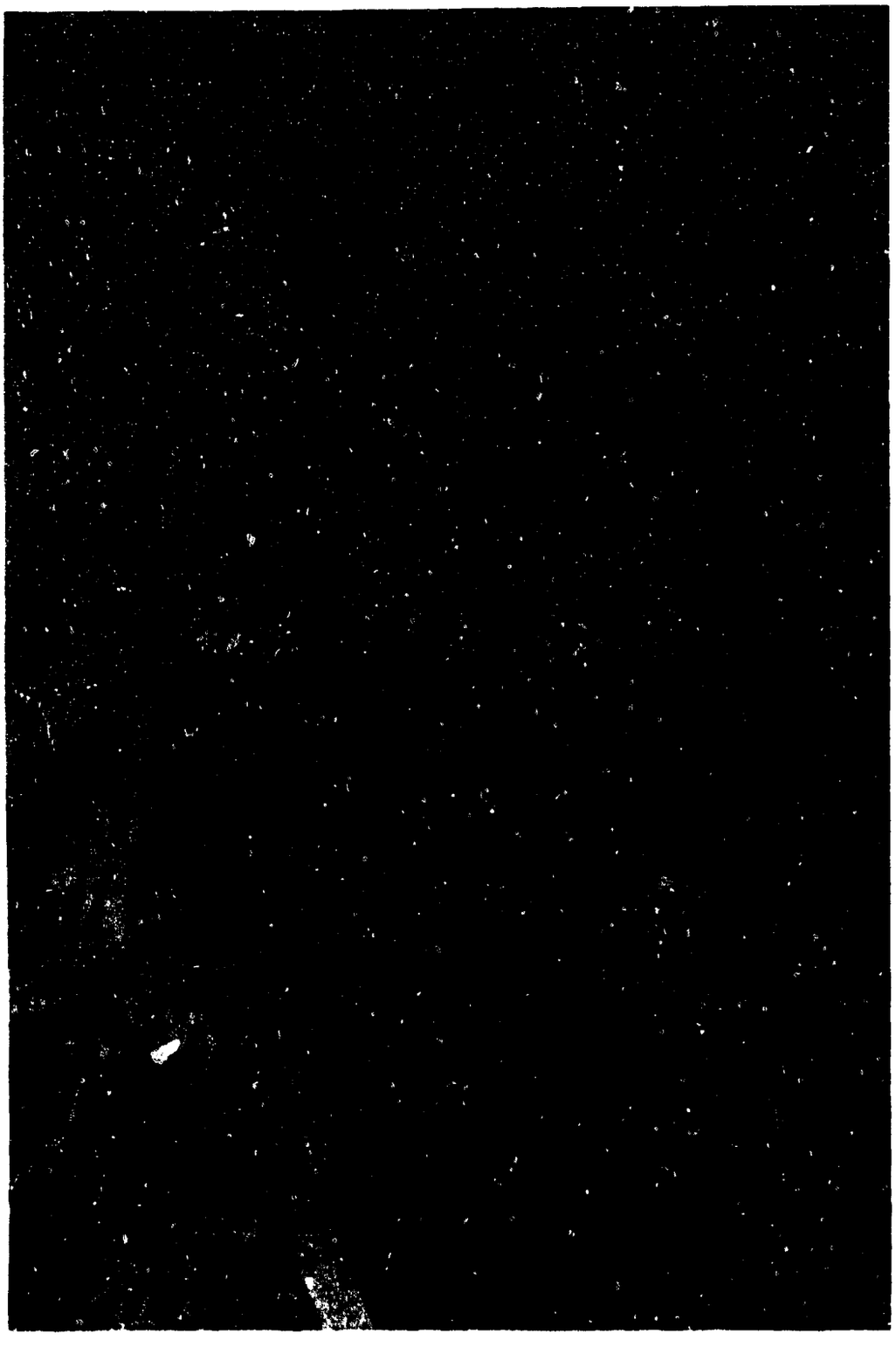

Figure 10. ELECTRON MICROGRAPH OF TEST DOG LUNG SHOWING MASSIVE CELLULAR INFILTRATION OF INTERSTITIAL SPACE. x 5'000

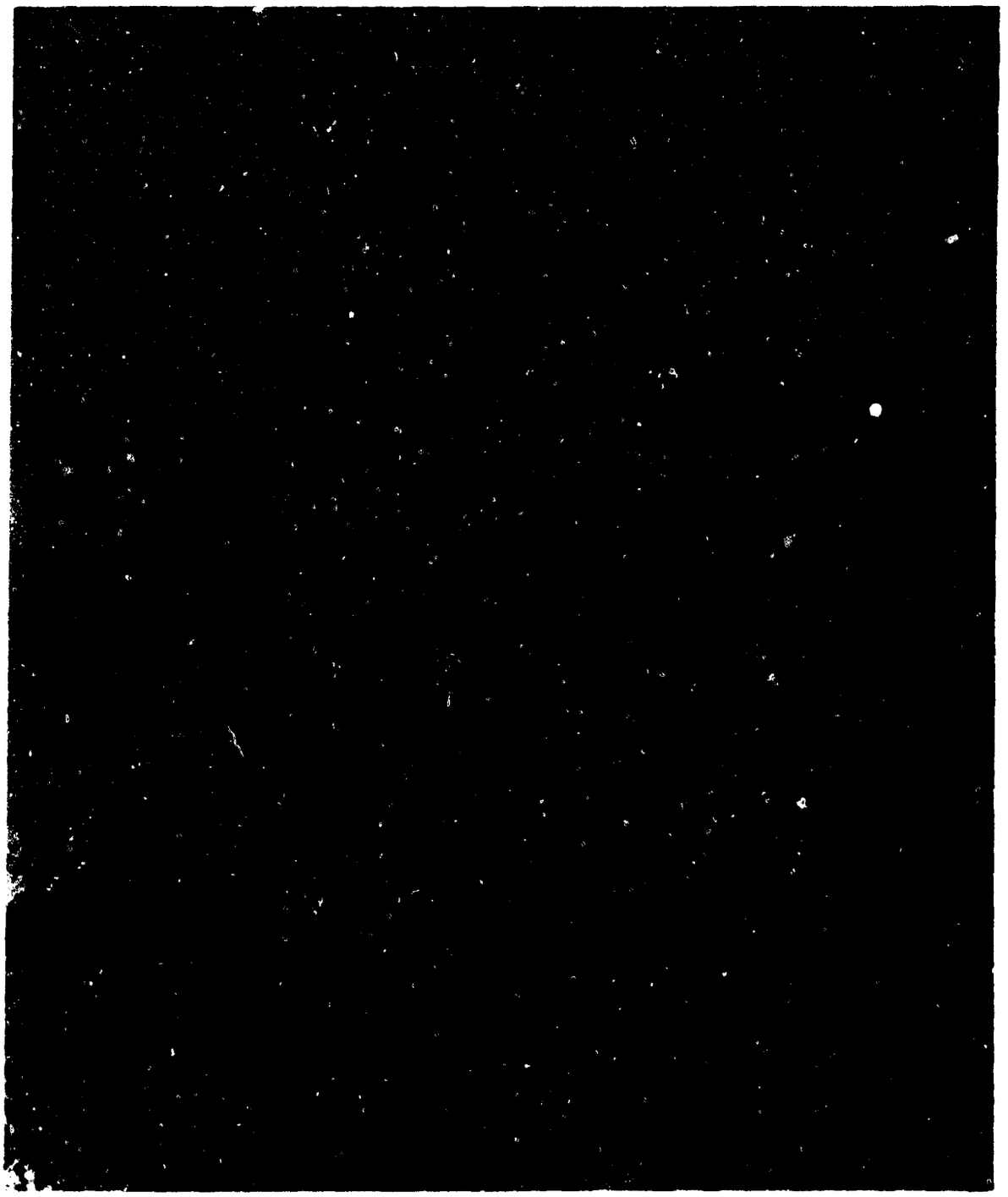

Figure 11. ELECTRON MICROGRAPH OF TEST DOG LUNG. Alveolar surface is covered by thick floccular fluid layer in contact with fragments of surfactant material at the surface. x 15'000

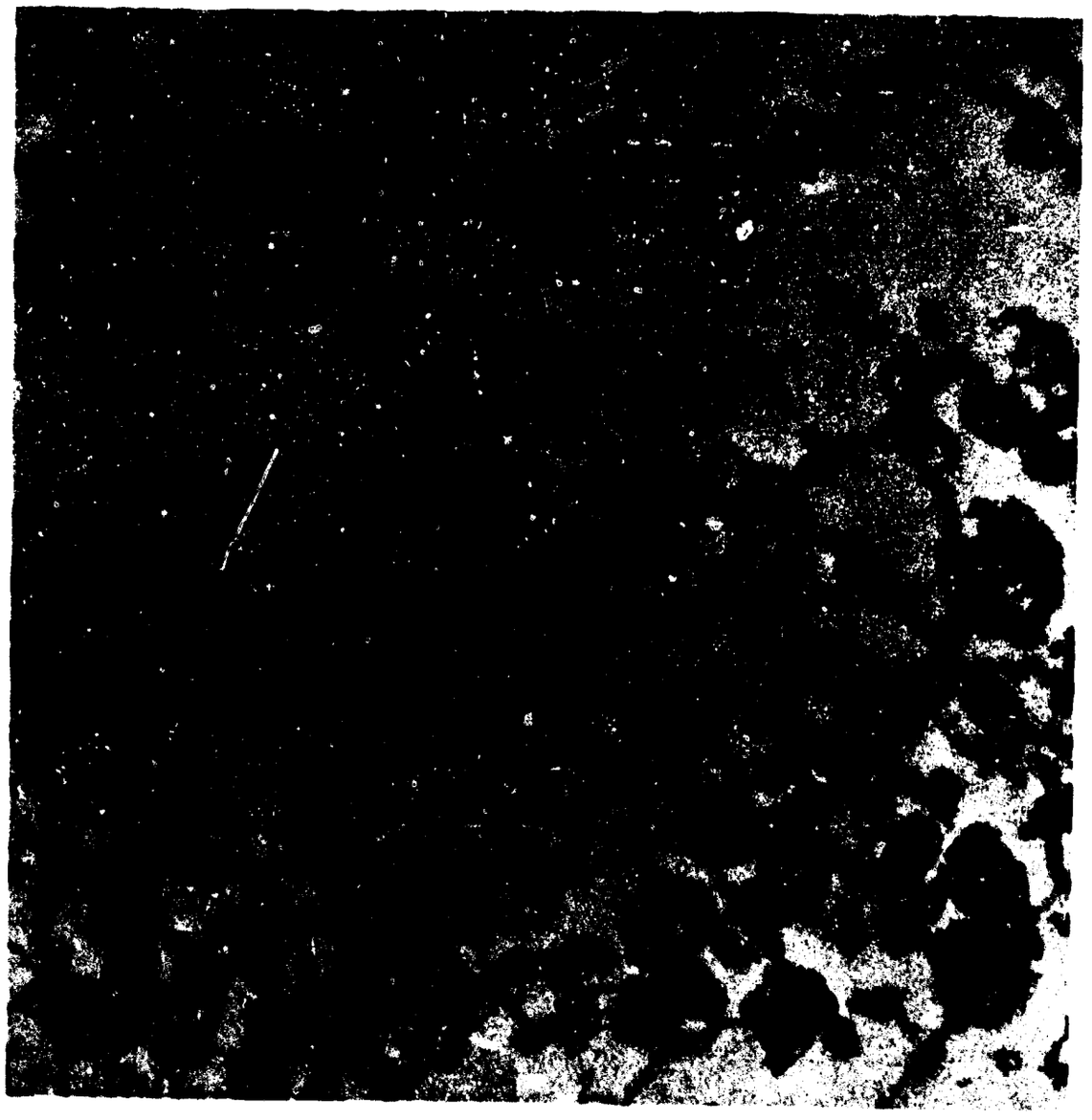

Figure 12. ELECTRON MICROGRAPH OF TEST DOG LUNG. Alveolus contains large mass of (tubular) myelin figures, probably derivates of surfactant. x 15'000

Figure 13. ELECTRON MICROGRAPH OF TEST MONKEY LUNG. Alveolar space contains threads of fibrin which here cross through pore of Kohn. x 10'000

## DISCUSSION

Compared with the previous studies of this program (Kistler, 1966; Schwinger, 1967; Kapanci, 1968) the morphometric and ultrastructural findings of this experimental series were less striking. Nevertheless, the present results strongly suggest that breathing a mixture of 68% $O_2$ and 32% $N_2$ at 25$^p$ Torr does not leave the lung unaltered. This conclusion may be particularly relevant in view of the clinical and pathophysiological data recorded by Fairchild (1968) on these animals: although the data did not show statistically significant differences between control and test animals, there was a consistent suggestion of reduced health in the experimental animals.

The same can be said for the present results. The pathological changes in test animals were not striking, but they were consistent in all three species. Although the small number of animals did not allow statistical significance tests to be performed the trend of the data is so consistent that it cannot be disregarded.

Edematous swelling of pulmonary interstitium has been one of the primary events in the development of pulmonary signs of oxygen toxicity (cf. Kistler, 1967). At 760 mm Hg it is a transient episode which is replaced after a few days by cellular infiltration and scarring (Kapanci, 1968). At 258 mm Hg of pure oxygen no interstitial edema was observed within two weeks of exposure of rats (Kistler, 1966), but it was present in dogs after eight months (Schwinger, 1967). The interstitial edema observed in the present study is somewhat less marked after eight months than in pure oxygen at 258 mm Hg.

The wide scatter of data in the test animals is noteworthy: it may reflect different levels of susceptibility, or resistance, to toxic oxygen levels.

In previous studies it had been noted that an elevated oxygen tension caused the specific gas exchange surface to be reduced (Kistler, 1966; Schwinger, 1967). In the present study no such change could be recorded.

Kapanci (1968) has shown that monkeys exposed to pure oxygen at one atmosphere over a prolonged period of time react with alveolar epithelial hyperplasia. A similar observation has been made by Nash (1967) on human lungs, and Kistler (1967) had observed a slight but significant thickening of alveolar epithelium in rats after three days of pure oxygen at one atmosphere. It is noteworthy that in this series an epithelial thickening is also apparent.

## CONCLUSIONS

The present morphometric study with the electron microscope has revealed differences in lung structure between the groups exposed to 68% $O_2$ at 5 psia in the Thomas dome and the control animals. These differences could not be established with statistical significance, mainly because of the small number of control animals available. But the trend towards increased air-blood barrier thickness was consistent in all three species.

The changes observed were qualitatively compatible with those previously observed under conditions of oxygen toxicity. But they may well be attributed to other causes, such as the reduced absolute pressure, duration of anesthesia or return to ambient pressure prior to sacrifice, or to some contaminant in the Thomas dome.

The minimum statement derived from this study as a conclusion is the following: It has not been possible to prove the absence of lung damage when animals breathe 68% $O_2$ and 32% $N_2$ at 5 psia. On the contrary there is a strong suggestion of real damage although it is slight and variable. Such slight damage requires a rigorously controlled experiment if it is to be detected with certainty; but the present study did not meet this requirement because of a number of shortcomings:

(a) The controls were not kept in a Thomas dome under the same conditions as the experimental animals.

(b) The animals were brought to ambient air at 720 mm Hg prior to sacrifice.

(c) The duration of anesthesia preceding lung fixation was not rigorously controlled and may have had an adverse effect on the lungs.

(d) The use of three different species, which had to be considered separately, reduced the number of animals per group so that no statistical tests were applicable which could have established the observed differences with acceptable confidence limits.

If the observed changes are considered sufficiently alarming then a systematic study appears indicated in which two parameters are independently varied: total pressure and oxygen partial pressure, for example according to the following scheme (table II).

TABLE II

| | BAROMETRIC PRESSURE | | |
|---|---|---|---|
| | 760 mm | 258 mm | 190 mm |
| * $P_IO$ 143 mm - 149 mm | ** .209 O (ambient air) | ** .680 O .320 N | 1.000 O |
| $P_IO$ 211 mm - 214 mm | .300 O .700 N | 1.000 O | |
| $P_IO$ 713 mm | 1.000 O | | |

* $P_IO = (BP-47) \times (F_IO)$
The 47 mm = vapor pressure of H O at 37 C.
** $F_{AT}O + F_{AT}N$ ---------- $+ F_{AT}X = 1$
AT = atmosphere

This table is derived from table III which compares the $pO_2$ in various phases as related to barometric pressure, vapor pressure, and estimated alveolar pressures. The latter is estimated assuming the RER and $pCO_2$'s.

TABLE III

| BAROMETRIC PRESSURE | FRACTION OF $O_2$ IN ATMOSPHERE | $pO_2$ IN ATMOSPHERE (Frac. O x BP) | PRESSURE OF INSPIRED GASES (BP-47) * | $P_IO_2$ (INSPIRED $pO_2$) (BP-47) x ($F_IO_2$) | ** ALVEOLAR $pO_2$ (estimated) RER = (RQ or $\frac{VCO_2}{VO_2}$) 0.8 | 1.0 | 1.2 |
|---|---|---|---|---|---|---|---|
| 760 mm Hg | .209 (ambient) | 159 mm | | 149 mm | ASSUME $pCO_2$ = 40 mm | | |
| | | | | | 99 mm | 109 mm | 116 mm |
| (Sea Level) | | | 713 mm | | | | |
| | 1.000 | 760 mm | | 713 mm | 663 mm | 673 mm | 680 mm |
| 258 mm Hg | .209 | 54 mm | | 44 mm | ASSUME $pCO_2$ is 35 mm 0.3 mm | 9 mm | 15 mm |
| (approx 27,000 ft) | .680 | 175 mm | 211 mm | 144 mm | 100 mm | 109 mm | 115 mm |
| | 1.000 | 258 mm | | 211 mm | 167 mm | 176 mm | 182 mm |
| 190 mm | .209 | 40 mm | | 30 mm | ASSUME $pCO_2$ is 35 mm -- | -- | -- |
| (approx 33,000+ ft) | .680 | 129 mm | 143 mm | 97 mm | 53 mm | 62 mm | 68 mm |
| | 1.000 | 190 mm | | 143 mm | 99 mm | 108 mm | 114 mm |

* 47 mm = $H_2O$ vapor pressure when dry gases are saturated at 37 C (body temp.)

** Calculated using alveolar air equation, i.e.: $P_AO_2 = P_IO_2 - P_ACO_2 \left(\frac{F_IO_2 + 1 - F_IO_2}{RER}\right)$

In the above we are setting estimated $pCO_2$'s + RER's. However, to get an accurate estimate of $P_AO_2$, one must measure the $pCO_2$ + RER, which are variable.

All animals are to be kept under identical conditions in the Thomas domes with identical air circulation times, etc.

The study should be restricted to one species only, preferably the dog (Beagle) because the dog lung is least affected by disease and because much physiological data is available on this species. The number of animals should be such that at least 7-10 animals are available for morphometric study from each group including controls.

Duration of the experiment could again be eight months with blood studies performed once a month. From each experimental group about five animals should be kept in ambient room air for several months to allow recovery from any possible damage.

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## DISCUSSION

DR. THOMAS: Thank you very much. You just outlined our work for us for the next three years.

These findings came as a big surprise to us, and I really don't have any questions to you specifically. I want to be a little philosophical for a moment. You have seen what it takes to run this type of experiment. Every time we complete one we discover with a 20/20 hind vision what we did wrong. Now, eight months is a long time, and it is a lot of work, it is a lot of investment of time, money and manpower. However, these findings look scarey enough to me that we will have to find out and the sooner the better, just what is going on. We are considering at this meeting 1000-day missions. These animals were exposed for only eight months. We don't know whether these changes would be more pronounced if the animals were exposed for a longer time. Would they be reversible? Obviously, if we want to be of any help to the design of those future systems, which are luckily not designed yet, we should make up our minds whether we should go, like the Russians, with ambient pressure air and forget all the contingencies of an exotic environment. This complicates toxicity studies tremendously. We are blessed anyhow with an interaction between a mixture of contaminants which is a hard enough nut to crack. Now we are superimposing an unnatural environment on it. Well, if we have to do it because of engineering reasons, because of propulsion reasons, we want to know as soon as possible whether we should plan a 1000-day space mission in an exotic atmosphere or should we plan it in an earthly atmosphere? That is just food for thought.

DR. SCHAFFNER: I have a question and a comment. First the question: In the measurement of the mean barrier thickness, is this a one dimensional measurement around the whole perimeter of the capillary, or is it only in the exposed area? The reason I asked this question is, when we studied the lungs of the animals exposed at 700 millimeters pure oxygen, I think for ten days, we found that the most striking difference was not in the thickness, that is, in the distance between the alveolus to the capillary, but in the surface area of capillary exposed. This seemed to be rather greatly reduced. Instead of the capillary bulging into the alveolus, the interstitium seemed to be swollen, that is, enlarged with collagen tissue, as you too have seen, and less of the capillary bulged into the lung, so the surface area of the capillary was reduced.

Now we got into the discussion one night with a compatriate of yours and he got very excited about this because he said that you had taught him that it was the mean barrier thickness that counted, and he apparently disagrees with you and felt that it was surface area. Now it isn't clear from your description pub-

lished or spoken, which we are dealing with. Now if you are talking about surface area, and you multiply changes to bring it into two dimensions, the question comes up, perhaps the most important question, not what happens to the animals that are in the new atmosphere, but what happens when you quickly change them from one atmosphere to another. Supposing an astronaut returns and opens up his capsule when he is bobbing around in the ocean and suddenly breathes good clean earth air, and he has to perform tasks at this time, is he going to be short of breath? Or, as some of the animals did, is he going to turn cyanotic and drop dead? I think these are the questions that we are going to have to answer. Now you have raised the issue, I think semantic or philosophic, and maybe we should have Mr. Wands here to referee this, as to what is adaptation and what is injury. Surely a change in the milieu of anything, a change in the task of anything is going to introduce a change into the animal. Now this initial change we may call injury, but the response of the animal is adaptation, and that response protects the animal from further injury from the same change. There is no question that the oxygen causes a change in the animals. If we call this initial injury, fine. This is an appointed, an arbitrary definition; but then the response of the host is really adaptation to protect it from further injury. As long as the animal can do that, or a cell, or a society for that matter, then that is adaptation and the function then becomes perfectly normal under the new circumstances.

By the way, one more comment, back to anatomy. A dog is an unusual animal as far as his vasculature is concerned both in terms of lung and liver. The dog is one of the few animals where the primary shock organ is not the lung as it is in man, but it is the liver, because the dog has very large muscular sphincters in his hepatic veins, and consequently dogs react considerably differently than either rodents or primates to changes in epinephrine and steroid contents in their blood stream. And while I agree with you that dogs are easier to work with in many respects than rats, I would make a plea that if we are going to concentrate on species, we either control the dogs with primates or do just what they are doing here, namely use three species.

DR. WEIBEL: Dr. Schaffner has just given such a long lecture that I have to answer now; otherwise, I'll forget it. Well, I think we have been talking since 1962 or '63 about what the meaning of mean barrier thickness is. I have shown you two types of measurements of mean barrier thickness and we are fully aware of the meaning of these parameters. Professor Rossieux may also perhaps not have understood me. The mean barrier thickness measures the tissue mass. It is essentially defined as the volume of tissue per surface, and by surface we mean alveolar and capillary surface area. So that is the actual meaning of the mean barrier thickness, and this does not discriminate between the thin portions which are where the capillary is most closely exposed to air or the thick portions, where the capillary is exposed to air but with thicker layer in between. I think we have to get away from the idea that there are two regions in the lung, one region where the capillary is exposed to air and the other where it is not exposed to air. It is exposed all over. It has even been shown that in the arteriole, the blood is exposed to air; that for example, hydrogen can pass through the wall of the arteriole into the blood. The only question is how much of it. So we have a second measurement which is the so-called harmonic mean. I think it was Mr. Weil

who yesterday argued about the use of harmonic means instead of arithmetic means if you are dealing with rates. Now the transport of oxygen from air to blood is a classical example for a rate and if you want to estimate the inhibition imposed on this transport rate by the tissue you will have to take the harmonic mean thickness of this barrier; and by the way, I might say here that the lung is a wonderful machine. It has a ratio of three between the tissue mass and the harmonic mean thickness. That is, if by the arrangement of the tissue in the lung, by having thin and thick portions to the tissue, the lung is imposing only one third of the resistance to oxygen diffusion than it would do if the tissue were homogeneously spread out over the entire surface area. I don't want to go into the methods of measurement. These have been published repeatedly. Now the question of adaptation and injury: I think, let's be very clear about it. If we find damage, the lung will have to repair this damage, and it is doing so in the monkey lung by regeneration of a cellular layer which has not been damaged. The damage in these monkey lungs at pure oxygen is in the Type I alveolar cell. Those are the thin extensions, and I believe there are reasons for that. The Type II cells with the lamellar bodies are apparently not injured and from there on they can regenerate. When the animal lives in one hundred per cent oxygen at one atmosphere pressure, it does not really need such a thin barrier as it needs under ordinary circumstances where it is given only 20 per cent oxygen to breathe; and there I agree with you. You might call this adaptation. Maybe the lung is just too lazy to make this extensive very thin layer and it keeps it at the acceptable thickness, and this thickness is then secondarily reduced to the very thin layer after cessation of the exposure to oxygen. So what you term adaptation is actually a repair process which just goes as far as is necessary, and if you agree with this definition then I agree with your calling this adaptation.

Well now about the use of the dog: I'm very glad that the dog is a good animal for you because it is especially good for studies on liver, but as for the lung, the dog has one major advantage and this is that the majority of physiological data that has been obtained on the respiratory system has been obtained on the dog. It is just a classical experimental animal for respiratory physiologists. The lung behaves quite similar to the human lung, much more similar than the rat lung, or the rabbit lung, or the cat lung which is terrible.

MR. VERNOT: I know nothing at all about lung physiology and when changes of this sort are discussed it is pretty difficult for me, more difficult perhaps than for people who are familiar with it, to understand what the significance of the changes are. Could you describe the severity of these changes compared to the difference between the lungs of city dwellers and country dwellers, desert dwellers, high humidity dwellers? In other words, to a man who is familiar with electron microscopy of the lungs, would these be considered serious changes or would they be considered minor changes--the sort of changes that we see in a population whenever they are exposed to many different things?

DR. WEIBEL: No, I don't think they are very serious changes. If you keep the space missions below eight months, and if we may extrapolate from these data to man, I would almost say that is just one of the risks that are involved, and maybe not even the largest that are involved in space missions. But if you are talking about 1000-day missions, I don't know how we can extrapolate to that, or

what will happen. I think they are rather mild changes, the mildest we have ever seen but there is something there.

DR. THOMAS: Dr. Weibel, would you hazard a guess, that if we would do the most sensitive in vivo pulmonary function measurements in these animals, do you think that any of our in vivo pulmonary tests are sensitive enough to discriminate this 30 and 50 per cent increase?

DR. WEIBEL: No, they are not.

AMRL-TR-68-175

PAPER NO. 16

# AN INQUIRY INTO THE PROBLEMS AFFECTING EVALUATION OF RAT ORGAN WEIGHT DATA

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For many years, pharmacologists and toxicologists have utilized organ weight as a tool in determining the relative toxicity of various compounds. The basic assumption underlying this approach concerns itself with the reaction of tissues to chemical or drug insult. Subtle toxic response may be mild edema or slight cellular proliferation which are not always histologically detectable but may produce small measurable changes in organ weight. If organ weight increases do occur, it is important to be certain that they are due to chemical insult and not the sacrifice technique. Highly vascularized organs may display marked weight variations in response to the influence of barbiturates because of blanching or engorgement of the organ's vascular system. Also, the liver may undergo weight changes resulting from pre-sacrifice diet habits. Therefore, it was decided to examine the effect on organ weight of overnight fasting versus feeding, and exsanguination versus barbiturate overdose. Four combinations of diet and euthanasia technique were selected: Fasted-exsanguinated, fed-exsanguinated, fasted-barbiturate overdosed, and fed-barbiturate overdosed.

The absolute values of the organ weights and ratios were not as important as the relative precision of the results in any group. Therefore, statistical comparisons of the variances were made to determine the degree that each sacrifice technique affected organ weight. In the event that one technique was significantly different from the other three, and gave the lowest error estimate, it would then be selected as the optimum sacrifice technique.

Sprague Dawley strain, male Charles River rats with an initial weight of 80-100 grams were used in the experiment. All rats were fed ad libitum and caged to allow for uninhibited body growth.

The rats were randomly grouped into 40 sets of 7 rats each, with 4 sets sacrificed each week for 10 weeks in one of the following ways:

1. Deprived of food for 12 hours and sacrificed with pentobarbital.
2. Fed ad libitum prior to sacrifice with pentobarbital.
3. Deprived of food for 12 hours and sacrificed by exsanguination.
4. Fed ad libitum prior to sacrifice by exsanguination.

All rats were weighed weekly. Gross pathology observations and organ weights were recorded for all animals submitted for necropsy.

Pentobarbital sacrifice was accomplished by intraperitoneal injection of 325 mg sodium pentobarbital/kilogram body weight. Prior to exsanguination, rats were anesthetized with 65 mg sodium pentobarbital/kilogram body weight. Exsanguination was accomplished by cutting the femoral artery and vein bilaterally.

The investigation was carried on without incident during the ten-week test period. Animals were eliminated from subsequent statistical considerations when murine pneumonia was detected at the time of necropsy in order to prevent masking of differences by this uncontrolled variable.

Variances of absolute organ weights are presented in table I. Bartlett's test of variance homogeneity indicated that the precision of the four sacrifice techniques was essentially equal for the heart, spleen, and kidney. Significant differences in the precision of the lung and liver weights suggested that an optimum sacrifice method existed for these organs. However, by removing the largest lung error estimate, that corresponding to the fed-pentobarbital sacrificed group, the remaining three became statistically equivalent. The same process of elimination did not change the results of the liver analysis at the P. 05 level confirming the existence of an optimum method which appeared to be fasting-exsanguination.

Table II lists the variance estimates ($S^2_{y,x}$) for organ data assuming linear regression of organ weight on body weight. Bartlett's test of homogeneity resulted in acceptance of the hypothesis for the heart and spleen data only. As before, removal of the fed-pentobarbital sacrificed lung data resulted in the acceptance of the hypothesis of equality for the other three test groups. Likewise, when the kidney data from the fasted-exsanguinated group were removed from consideration, the variances of the other three sacrifice groups were statistically equal. Liver data could not be equalized by similar techniques. A third approach used was the analysis of linear regression of organ weight on heart weight (table III). This approach did not indicate an optimum sacrifice procedure for the kidney and the spleen. The precision of lung weight data treated in this fashion was influenced by sacrifice technique but, again, removal of the fed-pentobarbital sacrificed group eliminated statistical differences. Analysis of liver data showed that only fasting improved the precision of measurement and that the method of sacrifice had little or no effect.

## TABLE I

## VARIANCES OF ABSOLUTE ORGAN WEIGHTS

| | | Fed-Pentobarbital Killed | Fasted-Pentobarbital Killed | Fed-Exsanguinated | Fasted-Exsanguinated |
|---|---|---|---|---|---|
| Heart | $S^2_y$ = | 0.076 | 0.051 | 0.065 | 0.050 |
| Lung | $S^2_y$ = | 0.249 | 0.134 | 0.095 | 0.108 |
| Liver | $S^2_y$ = | 13.383 | 8.725 | 11.371 | 3.638 |
| Spleen | $S^2_y$ = | 0.062 | 0.051 | 0.064 | 0.039 |
| Kidney | $S^2_y$ = | 0.257 | 0.175 | 0.206 | 0.185 |

TABLE II

LINEAR REGRESSION OF ORGAN WEIGHT ON BODY WEIGHT MODEL

| Organ | | Fed-Pentobarbital Killed $n_1 = 65$ | Fasted-Pentobarbital Killed $n_2 = 56$ | Fed-Exsanguinated $n_3 = 64$ | Fasted-Exsanguinated $n_4 = 56$ |
|---|---|---|---|---|---|
| | | Mean Body and Organ Weights (grams) | | | |
| Body | | 305.0 | 304.0 | 295.0 | 307.0 |
| Heart | | 1.1 | 1.2 | 1.0 | 1.1 |
| Lung | | 1.8 | 1.7 | 1.5 | 1.5 |
| Liver | | 14.2 | 12.0 | 11.6 | 9.8 |
| Spleen | | 0.8 | 0.8 | 0.8 | 0.7 |
| Kidney | | 2.4 | 2.5 | 2.2 | 2.3 |
| | | Regression Line Parameters | | | |
| Heart | $S^2_{y.x}$ = | 0.014 | 0.028 | 0.016 | 0.022 |
| | a = | 0.229 | 0.415 | 0.326 | 0.409 |
| | b = | 0.030 | 0.025 | 0.024 | 0.024 |
| | $\sigma$(b) = | 0.002 | 0.004 | 0.002 | 0.003 |
| | r = | 0.902 | 0.688 | 0.873 | 0.753 |
| Lung | $S^2_{y.x}$ = | 0.199 | 0.080 | 0.056 | 0.082 |
| | a = | 0.915 | 0.501 | 0.830 | 0.806 |
| | b = | 0.028 | 0.038 | 0.022 | 0.024 |
| | $\sigma$(b) = | 0.007 | 0.006 | 0.003 | 0.005 |
| | r = | 0.475 | 0.654 | 0.654 | 0.523 |
| Liver | $S^2_{y.x}$ = | 2.709 | 3.959 | 1.803 | 0.844 |
| | a = | 2.314 | 1.252 | 1.732 | 2.508 |
| | b = | 0.391 | 0.353 | 0.335 | 0.236 |
| | $\sigma$(b) = | 0.024 | 0.042 | 0.018 | 0.017 |
| | r = | 0.897 | 0.750 | 0.920 | 0.881 |
| Spleen | $S^2_{y.x}$ = | 0.062 | 0.048 | 0.062 | 0.038 |
| | a = | 0.662 | 0.487 | 0.576 | 0.462 |
| | b = | 0.006 | 0.011 | 0.007 | 0.008 |
| | $\sigma$(b) = | 0.004 | 0.005 | 0.003 | 0.004 |
| | r = | 0.197 | 0.301 | 0.243 | 0.278 |
| Kidney | $S^2_{y.x}$ = | 0.045 | 0.062 | 0.033 | 0.072 |
| | a = | 0.768 | 0.831 | 0.877 | 0.850 |
| | b = | 0.055 | 0.054 | 0.045 | 0.048 |
| | $\sigma$(b) = | 0.003 | 0.005 | 0.002 | 0.005 |
| | r = | 0.910 | 0.813 | 0.919 | 0.790 |

TABLE III

LINEAR REGRESSION OF ORGAN WEIGHT ON HEART WEIGHT MODEL

| Organ | | Fed-Pentobarbital Killed $n_1 = 65$ | Fasted-Pentobarbital Killed $n_2 = 56$ | Fed-Exsanguinated $n_3 = 64$ | Fasted-Exsanguinated $n_4 = 56$ |
|---|---|---|---|---|---|
| | | Regression Line Parameters | | | |
| Lung | $S^a_{y.x}$ = | 0.189 | 0.098 | 0.056 | 0.087 |
| | a = | 0.724 | 0.627 | 0.651 | 0.751 |
| | b = | 0.927 | 0.884 | 0.791 | 0.703 |
| | $\sigma$(b) = | 0.195 | 0.184 | 0.115 | 0.176 |
| | r = | 0.513 | 0.547 | 0.657 | 0.477 |
| Liver | $S^a_{y.x}$ = | 3.348 | 2.538 | 3.344 | 1.614 |
| | a = | 1.164 | -0.095 | 0.051 | 2.426 |
| | b = | 11.520 | 11.051 | 11.171 | 6.470 |
| | $\sigma$(b) = | 0.821 | 0.939 | 0.896 | 0.761 |
| | r = | 0.870 | 0.848 | 0.846 | 0.756 |
| Spleen | $S^a_{y.x}$ = | 0.056 | 0.051 | 0.063 | 0.036 |
| | a = | 0.488 | 0.613 | 0.571 | 0.366 |
| | b = | 0.311 | 0.172 | 0.194 | 0.294 |
| | $\sigma$(b) = | 0.107 | 0.133 | 0.123 | 0.114 |
| | r = | 0.344 | 0.173 | 0.196 | 0.331 |
| Kidney | $S^a_{y.x}$ = | 0.055 | 0.084 | 0.063 | 0.085 |
| | a = | 0.592 | 0.899 | 0.662 | 0.684 |
| | b = | 1.634 | 1.347 | 1.491 | 1.439 |
| | $\sigma$(b) = | 0.105 | 0.171 | 0.123 | 0.175 |
| | r = | 0.890 | 0.731 | 0.838 | 0.747 |

The manner in which the organ weight data was obtained for this experiment was extremely important. Care was taken to minimize experimental error by standardizing the sacrifice procedure, removal of organs, and weighing of these organs. Any of these factors could affect the quality of the data.

In addition to standardization of techniques, an attempt was made to employ the best and most suitable statistical methodology. Application of modern techniques has made the mathematical analysis of biological data more formalized and comprehensive. The use of regression models is one example. No attempt is made in this paper to compare the merits of ratio and regression analysis, although this appraisal is currently underway in our statistics department; and, although the inclusion of absolute organ weight analysis may provide some area for comparison, no recommendations are offered regarding the selection of the best statistical technique.

All four sacrifice procedures resulted in equal organ weight variation at the P. 01 significance level in the case of the spleen and the heart regardless of the statistical method employed. Kidney weight variances were shown to be equal if the absolute organ weight or regression on heart weight analysis was used. This was also true in the case of regression on body weight if the largest error estimate corresponding to the fasted-exsanguination sacrificed group was removed. Although not significantly different, the fed-exsanguinated group had the lowest error estimate for this organ.

The sacrifice techniques tested generated the same degree of variation in lung weight with the exception of the fed-pentobarbital sacrifice method which had significantly poorer precision. Error estimates of liver weight, employing absolute organ weight or regression on body weight analysis, revealed that the fasted-exsanguinated method produced the smallest variation. Use of regression of liver weight on heart weight analysis demonstrated that both fasting techniques were statistically equal (P. 01 level) with regard to variances, giving the lowest error estimates. Physiological mechanisms may be involved in such a finding. The liver is the one organ studied that is most sensitive to the presacrifice food regimen and this experiment has demonstrated that presacrifice fasting results in the highest precision. Indeed, any change in liver weight, as a response to a toxic insult, could easily be masked by the experimental error resulting from the sacrifice method.

In conclusion, variation of spleen, heart and kidney weight are not significantly influenced by the method of sacrifice. The precision of liver and lung weight measurements are, however, significantly improved by the use of the fasted-exsanguinated technique.

## DISCUSSION

DR. MAC EWEN (SysteMed Corporation): Before we get into the same question about your reasons for rejecting sick animals from statistical consideration, I presume that you are referring to those that had acute pneumonia, and a resultant or accompanying congestion, is that right?

MR. EGAN: That is right.

DR. SCHAFFNER: I wonder if you could clarify a point. Those of us who are doing ultra structural studies are very concerned to receive tissue least affected by hypoxia, and handling and congestion and all these other things that occur at the time of sacrifice of the animals. In the examination, when you do remove the organs and when do you get a specimen that you weigh? After the anesthesia, how long do you wait, --I mean, is it as soon as the animal falls asleep and you zip in and take out what you want? Certainly as far as we are concerned for electron microscopy, we would prefer all things done on conscious, living, peaceful animals, and although we may not get that, obviously we should come as close to this as possible, and it seems to me that at least for our purposes, exsanguination would not be acceptable.

MR. EGAN: In terms of the actual experimental procedure used in exsanguination, the animals were anesthetized and we used as our criterion for level of anesthesia a simple pinch reflex or absence thereof. Once this was ascertained the femoral artery and vein was cut bilaterally, and the animal was bled until actual blood droplets stopped coming out. Immediately thereafter the animal was removed to the pathology laboratory where the organs were removed and weighed. We tried as best as possible to standardize the time element involved here.

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PAPER NO. 17 A

# COMPARATIVE OXYGEN TOXICITY IN SUB-HUMAN PRIMATES

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The effects of breathing high concentrations of oxygen have been of considerable interest in recent years especially with regard to the national space effort. Several species of animals have been used in studying the space-cabin atmospheres of 100% oxygen at 5 psia and 68% oxygen - 32% nitrogen at the same pressure for extended periods. Ultrastructural and minor light-microscopic changes have been seen (Back, 1966; Hagebusch, 1966; Harper, 1967; Kaplan, 1968; Lawerenz, 1967; Patrick, 1967) and the significance of these changes is still under study since there was little or no clinical effect on the animals.

Even though these space cabin environments have been nominally validated as habitable for man, there is still an interest in the basic problem of oxygen toxicity at ambient pressures. This is true not only because of the need for complete understanding of potential aerospace problems but also because of the direct application of this research to current human medical problems.

Animals exposed to 100% oxygen at near-ambient pressures (600-760 mm Hg) have been seriously affected with deaths occurring in 2-3 days in acute cases exposed at 750-760 mm Hg (MacEwen, 1966; Robinson, 1966). With decreases in pressure, mortality has dropped but characteristic pulmonary lesions have developed, especially in rhesus monkeys (Macaca mulatta) (Robinson, 1967). The subacute lesions in monkeys have been of the proliferative type characterized by hyperplasia of the granular pneumocytes that make up a major portion of the alveolar epithelium, thereby significantly increasing the blood-air barrier. Interstitial edema and fibrosis also contribute to the potential diffusion problem (Kapanci, submitted for publication; Kaplan, submitted for publication).

The characterization of oxygen toxicity in the rhesus monkey (M. mulatta) has been paralleled by reports of oxygen toxicity in man (Fuson, 1965; Hawker, 1967; Nash, 1967; Northway, 1967; Pratt, 1965; Roth, 1964; Shanklin, 1967). Acute cases were reported as a result of hyperbaric therapy (Fuson, 1965) and subacute cases were linked with normobaric oxygen therapy of the newborn and adults requiring increased alveolar partial pressures of oxygen (Hawker, 1967; Pratt, 1965).

Lesions produced experimentally in rhesus monkeys are quite similar to those reported for man. But since there are differences in the response to oxygen toxicity between various species of animals, and some variation between individuals within a species, the question arose as to what type of response would develop in other sub-human primates. Therefore, cynamolgus monkeys (Macaca irus), baboons (Papio sp.), and squirrel monkeys (Saimiri sciureus) were exposed to high concentrations of oxygen in order to compare the effects with those seen previously in rhesus monkeys (M. mulatta).

## MATERIALS AND METHODS

Only male primates were used in this experiment. They had been stabilized in a vivarium for at least 90 days before the experiment was started and had shown no response to three successive tuberculin tests. Numbers of primates per species and weight data are summarized in table I.

TABLE I

NUMBERS AND WEIGHTS OF PRIMATES USED IN COMPARATIVE OXYGEN TOXICITY EXPERIMENT

| | No. exposed/ control | Mean Weight | Weight Range |
|---|---|---|---|
| Baboon (Papio sp.) | 7/2 | 3.47 kg | 2.41-4.0 kg |
| Cynamolgus (M. irus) | 6/2 | 2.02 kg | 1.81-2.38 kg |
| Squirrel monkey (Saimiri sciureus) | 12/2 | 0.48 kg | 0.40-0.62 kg |

The primates were exposed in the Toxic Hazards Research Unit of the Aerospace Medical Research Laboratories at Wright-Patterson Air Force Base, Ohio. The exposure chamber, a Thomas dome, has been previously described (Thomas, 1965) and typical environmental parameters and their degrees of variation have also been reported (Robinson, 1967). To summarize, they are unique dynamic-flow experimental altitude chambers in which the environmental conditions are automatically controlled within narrow ranges.

In this experiment, pressure was maintained at 720 mm Hg and the mean oxygen concentration remained between 99% and 100% at flow rates near 20 cu ft/min (CFM). The temperature was maintained at 72 F and the relative humidity at 50%. Daily entries were made through an airlock to care for the primates and to perform necessary experimental procedures. The primates were fed monkey chow and water ad libitum.

All primates were exposed during a two-week period. Those scheduled for a 14-day exposure were placed in the chamber with those scheduled for a 4-day exposure and exposed to 99%-100% oxygen at 720 mm Hg pressure until they died or reached the end of the scheduled exposure. The primates scheduled for 7-day exposure were placed in the same environment after those exposed for four days were removed.

The baboons and M. irus were caged separately and the squirrel monkeys were caged in pairs. Additional light was provided the squirrel monkeys for 12 hours a day so that they would maintain an adequate amount of spontaneous activity.

Heparinized blood samples were taken on all baboons and M. irus before the experiment started and the following determinations were made: RBC, WBC, hematocrit, and hemoglobin. Similar determinations were made on samples drawn just prior to the primate's entry into the dome and three times per week until the animal died or was euthanatized. Blood samples were drawn from the squirrel monkeys, including the controls, for the same purpose at the time they died or were euthanatized. When it became obvious that handling the primate just for the purpose of drawing a blood sample might precipitate its death, that sample was not taken. Blood samples from the control baboons and M. irus were taken at the same intervals as those in the high oxygen environment. At the termination of the experiment when the control baboons and M. irus were euthanatized, two blood samples were taken from each animal for blood-gas and pH determinations, the first before they were given anesthesia, and the second when they reached a light surgical plane of anesthesia.

All control blood-gas measurements were made on an IL pH/Gas Analyzer, Model 113*. Control femoral arterial blood-gas values on each baboon and M. irus were taken the week prior to the first exposure and on exposure days 4 and 7 when possible, in addition to samples taken terminally or at the end of the scheduled exposure.

Samples for erythrocyte osmotic fragility studies were also taken on all baboons and M. irus the week prior to the first exposure and terminally or at the end of the scheduled exposure unless the primate died unexpectedly, in which case a sample could not be taken. Erythrocyte fragility was determined using the Fragilograph** by the osmotic fragility technique (Danon, 1963).

At the termination of the scheduled exposure or when it became obvious that the primate was near death, the dome was entered and sodium pentobarbital was administered intravenously or intraperitoneally to give light surgical anesthesia and a laparotomy was performed. Arterial blood samples were taken from the abdominal aorta for hematology on all primates and for fragility studies on baboons and M irus. Additional anesthesia was given for euthanasia and the lungs were perfused intratracheally with 3% buffered glutaraldehyde after they were allowed to

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collapse following bilateral incision of the diaphragm. The primate was removed through an air-lock and taken to the necropsy laboratory where a complete gross examination was made. The perfused lungs were removed and submersed in 3% buffered glutaraldehyde for at least 2 hours before blocks were taken for electron microscopic (EM) procedures. Tissue samples from all organ systems, including the lungs after EM samples were taken, were placed in 10% buffered formalin and processed using conventional histologic techniques. Hematoxylin and eosin sections were prepared on all tissues and special stains were used to demonstrate connective tissue elements in the lungs. Sections, one micron-thick, were cut on an MT-2 Porter-Blum ultramicrotome* and stained with toluidine blue for high magnification light microscopy and photographic purposes.

## RESULTS

### Clinical

The baboons showed no signs of discomfort during the first two days and were in apparent good health. Starting on the third or fourth day they began to become anorectic and seemed to lose body condition, although when aroused they responded well. With the passage of time, they became lethargic, carefully maintained an upright condition and became indifferent to attempts to arouse them. When removed from their cages for blood sampling procedures, they had to be held as nearly upright as possible to avoid serious respiratory distress. Within a day or two of death, they began to struggle more and more to breathe, and just prior to death they were very weak and exhausted.

The M. irus maintained an aggressive attitude and did not appear to be as sick as the baboons during the course of the experiment. They did become somewhat anorectic and the two that died were found near death without prior signs of marked deterioration.

The squirrel monkeys huddled together from the time they were put into the cage until they were removed. In one case a live squirrel monkey was holding a dead one upright, apparently not knowing or caring that the other was dead. When the supplemental lighting was turned on at 0600 each moring, the amount of spontaneous activity did increase and they did eat and drink until they became very ill. When one of the cage mates died, the remaining animal was put in another cage with a mate. The characteristic position of the squirrel monkeys was of particular interest, since most of the time they maintained a huddled position with their heads tucked down and between their hind legs. Even though they were in a 100% oxygen atmosphere, the question of the protective effects of this position arose.

*Ivan Sorvall, Inc., Norwalk, Connecticut 06852

## Hematology

Hematological results are given in Table II. There were no marked hematological changes seen in any of the groups although there were trends indicating hemoconcentration in the 7-day baboons and the 4-day M. irus.

TABLE II

SUMMARY OF HEMATOLOGICAL DATA FROM BABOONS, MACACA IRUS AND SQUIRREL MONKEYS EXPOSED TO 100% OXYGEN AT 720 mm Hg FOR NUMBER OF DAYS INDICATED

| | | Exposed (Days) | | | | | |
|---|---|---|---|---|---|---|---|
| BABOONS | Controls | Before | 0 | 2 | 4 | 7 | 14** |
| | n = 14* | n = 6 | n = 7 | n = 6 | n = 6 | n = 3 | n = 1 |
| Hematocrit | 43.1+ | 48.1 | 45.0 | 47.5 | 47.0 | 48.3 | 45.0 |
| (Vol. %) | 3.0 | 1.9 | 1.9 | 2.1 | 3.1 | 2.3 | -- |
| Hemoglobin | 13.5 | 14.9 | 13.4 | 14.1 | 14.2 | 15.0 | 12.0 |
| (Gm %) | 1.0 | 0.3 | 0.5 | 0.9 | 1.0 | 0.9 | -- |
| Erythrocytes | 5.32 | 5.82 | 5.55 | 5.71 | 5.62 | 6.18 | 4.71 |
| ($x10^6/mm^3$) | 0.57 | 0.23 | 0.46 | 0.33 | 0.34 | 0.27 | -- |
| Leucocytes | 10.2 | 12.7 | 12.9 | 10.6 | 11.7 | 8.6 | 12.4 |
| ($x10^3/mm^3$) | 3.2 | 4.5 | 4.4 | 3.2 | 1.6 | 2.7 | -- |
| M. IRUS | n = 14* | n = 6 | n = 6 | n = 5 | n = 5 | n = 3 | n = 1 |
| Hematocrit | 37.3 | 39.2 | 39.5 | 42.6 | 44.9 | 41.7 | 38.0 |
| | 2.6 | 3.0 | 1.9 | 2.1 | 4.1 | 1.5 | -- |
| Hemoglobin | 11.0 | 11.2 | 11.0 | 11.9 | 12.7 | 11.8 | 11.1 |
| | 0.7 | 1.0 | 0.5 | 0.5 | 1.2 | 0.2 | -- |
| Erythrocytes | 5.93 | 5.95 | 6.29 | 6.49 | 6.70 | 6.20 | 5.71 |
| | 0.31 | 0.71 | 0.37 | 0.37 | 0.70 | 0.52 | -- |
| Leucocytes | 13.1 | 16.0 | 12.8 | 11.1 | 13.0 | 12.1 | 13.2 |
| | 3.7 | 2.9 | 6.0 | 3.4 | 3.8 | 2.0 | -- |
| SQUIRREL MONKEYS | n = 2* | -- | -- | -- | n = 4 | n = 4 | n = 1 |
| Hematocrit | 41.5 | | | | 43.2 | 44.2 | 38.0 |
| | 3.5 | | | | 4.2 | 4.6 | -- |
| Hemoglobin | 13.6 | | | | 13.9 | 13.9 | 12.0 |
| | 1.1 | | | | 1.0 | 1.3 | -- |
| Erythrocytes | 7.25 | | | | 7.28 | 7.25 | 7.81 |
| | 0.24 | | | | 0.79 | 1.09 | -- |
| Leucocytes | 8.4 | | | | 11.6 | 8.2 | 6.8 |
| | 3.5 | | | | 3.0 | 1.5 | -- |

* All samples from 2 animals
** Last sample was on day 8 for the baboons
\+ Top number is the mean and the standard deviation is given just below

Erythrocyte Fragility

Blood samples were obtained from two baboons and two monkeys after four days exposure (table III). One of the baboons and both monkeys demonstrated slight increases in erythrocyte osmotic fragility of questionable statistical significance. One baboon showed no alteration in osmotic fragility from control values. The baboon and cynamolgus monkey available for sampling after seven days exposure both showed significant increases in erythrocyte fragility. The sodium chloride concentration (in grams/liter) inducing 50% hemolysis increased from 4.3 to 4.8 in the baboon and from 4.4 to 4.9 in the monkey.

When these curves were plotted on normal probability paper (figures 1 and 2), a shift to the left is evident in the fragility of the entire erythrocyte population of each subject. This indicates an increase in osmotic fragility of the youngest, as well as the oldest, erythrocytes of both subjects.

TABLE III

RESULTS OF OSMOTIC FRAGILITY STUDIES ON THE ERYTHROCYTES OF BABOONS AND CYNAMOLGUS MONKEYS EXPOSED TO 720 mm Hg OF OXYGEN FOR NUMBER OF DAYS INDICATED.
Data are given in grams of sodium chloride per liter that cause 50% hemolysis.

| SUBJECT | PRE-EXP. | POST EXP. | EXP. TIME |
|---|---|---|---|
| Baboon | 4.3 | 4.3 | 4 day |
| Baboon | 4.3 | 4.6 | 4 day |
| Monkey | 4.4 | 4.6 | 4 day |
| Monkey | 4.4 | 4.7 | 4 day |
| Baboon | 4.3 | 4.8 | 7 day |
| Monkey | 4.4 | 4.9 | 7 day |
| Baboon | 4.4 | 5.0 | 8 day |
| Monkey | 4.2 | 5.1 | 14 day |

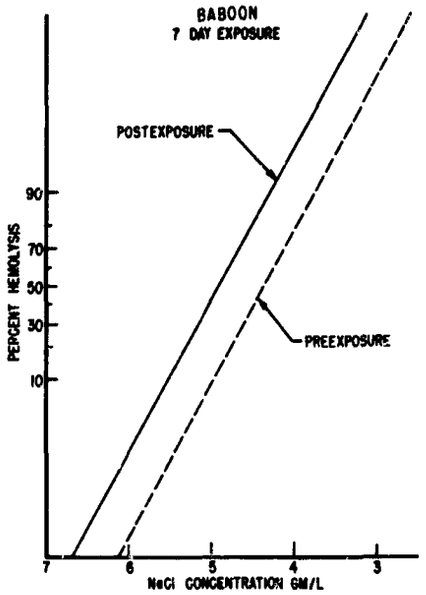


Figure 1. GRAPH OF PRE-EXPOSURE AND 7 DAY EXPOSURE CURVES OF THE OSMOTIC FRAGILITY OF ERYTHROCYTES FROM A BABOON EXPOSED TO 720 mm Hg OF OXYGEN ON NORMAL PROBABILITY PAPER. There is a shift to the left indicating increases in fragility of the entire erythrocyte population.

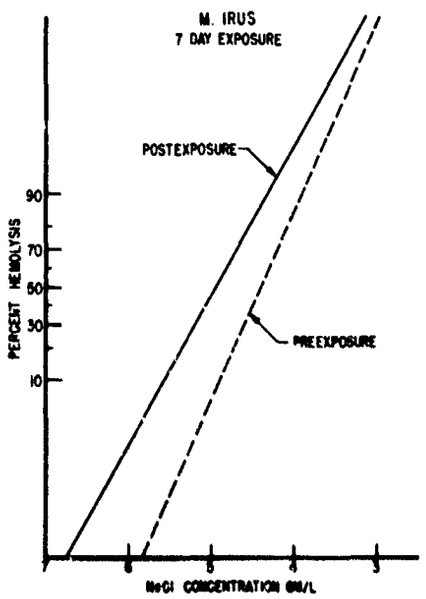


Figure 2. GRAPH OF PRE-EXPOSURE AND 7 DAY EXPOSURE CURVES OF THE OSMOTIC FRAGILITY OF ERYTHROCYTES FROM A CYNAMOLGUS MONKEY EXPOSED TO 720 mm Hg OF OXYGEN ON NORMAL PROBABILITY PAPER. There is a shift to the left indicating increases in fragility of the entire erythrocyte population.

One baboon sampled after eight days exposure showed a similar increase from 4.4 to 5.0 grams per liter. The one cynamolgus available for sampling after 14 days exposure exhibited a large increase from 4.2 to 5.1 gram per liter.

## Blood Gases

A summary of the blood-gas data is shown in table IV. Arterial partial pressures of oxygen ($pO_2$) were generally below 100 in the control samples.

TABLE IV

BLOOD-GAS DATA ON PRIMATES EXPOSED TO 100% OXYGEN AT 720 mm Hg

| | | Baboons | | M. Irus | | Squirrel Monkeys | |
|---|---|---|---|---|---|---|---|
| | | $pO_2$ | $pCO_2$ | $pO_2$ | $pCO_2$ | $pO_2$ | $pCO_2$ |
| Control - | mean | 95.1 | 22.8 | 87.4 | 27.5 | 75.0 | 37.0 |
| | range | 87-107 | 19-27 | 70-93 | 24-30 | 74-76 | 34-40 |
| | n | 9 | 8 | 8 | 8 | 2 | 2 |
| 4 day - | 1 | 457 | 38 | 465 | 26.5 | 460 | 46 |
| | 2 | 485 | 31 | 265 | 25 | 470 | 56 |
| | 3 | 540 | 27.5 | | | 450 | 47 |
| | 4 | 425 | 34 | | | 460 | 37.5 |
| 7 day - | 1 | 445 | 30 | *76 | 57 | 475 | 59 |
| | 2 | *52 | 100+ | | | 485 | 47.5 |
| | 3 | | | | | 457 | 42 |
| | 4 | | | | | 470 | 58.2 |
| 8 day - | 1 | | | | | *515 | 100+ |
| 9 day - | 1 | | | | | *515 | 73 |
| 13 day - | 1 | | | | | *[1] 120 | 38 |
| 14 day - | 1 | | | 470 | 37 | *365 | 100+ |

*Terminal

[1] Focal hemorrhages in basal ganglia

The baboons had the highest mean value followed in decreasing order by the M. irus and the squirrel monkeys. Conversely, the squirrel monkeys had the highest mean partial pressure of carbon dioxide ($pCO_2$) and the baboons the lowest.

All primates maintained 3-5 times the control amounts of arterial $pO_2$ until they were near death when the $pO_2$ decreased sharply except in the squirrel monkeys. Partial pressure of $CO_2$ did not exceed two times the control values until the primates became moribund when the levels often increased to over 100 mg Hg. The apparent exception was the squirrel monkey that died on day 13 wherein death was probably related to numerous hemorrhages found in the basal ganglia of the brain during necropsy.

Since the control values were taken from restrained awake animals, a comparison of the values obtained from the awake control baboons and M. irus was made with samples taken from the same animals while under light sodium pentobarbital anesthesia (table V). There were small differences which were not predictable since there were increases in some cases and decreases in others.

TABLE V

COMPARISON OF BLOOD-GAS AND BLOOD pH VALUES FROM AWAKE AND LIGHTLY ANESTHETIZED BABOONS AND M. IRUS

| | Baboon | | | M. Irus | | |
|---|---|---|---|---|---|---|
| | $pO_2$ | $pCO_2$ | pH | $pO_2$ | $pCO_2$ | pH |
| Arterial | | | | | | |
| Awake | 97-88 | 23.5-23.5 | 7.26-7.41 | 85-80 | 27 -27.5 | 7.37-7.36 |
| Anes. | 90-86 | 38 -31 | 7.41-7.42 | 100-63 | 36.5-30.5 | 7.38-7.38 |
| Venous | | | | | | |
| Awake | 41-24 | 34.5-40 | 7.15-7.24 | 30-24 | 34 -41 | 7.32-7.29 |
| Anes. | 28-28 | 36 -37.5 | 7.38-7.40 | 47-33 | 34.5-41.5 | 7.37-7.35 |

Gross Pathology

Mortality is given in table VI. Two baboons died on days 6 and 8 of the experiment, two M. irus died on days 4 and 9, and three squirrel monkeys died on days 8, 9, and 13. One M. irus and one squirrel monkey survived the 14 day exposure, but 8 days was the longest survival time for a baboon. Pulmonary lesions of edema, congestion, and increased consistency were evident in the primates that died but these lesions were difficult to assess because the lungs were perfused before removal from the chest cavity. Generalized visceral congestion was usually seen in those animals that died following a prolonged course. Punctate gastric ulcers were seen in two squirrel monkeys and in one of the same monkeys there were multiple hemorrhages up to 1 mm in diameter scattered in the basal ganglia of the brain.

TABLE VI

MORTALITY DATA ON BABOONS, M. IRUS, AND SQUIRREL MONKEYS EXPOSED TO 720 mm Hg OF 100% OXYGEN

| Days | Baboons | M. Irus | Squirrel Monkeys |
|---|---|---|---|
| 4 | 0/2 | 0/2 | 0/4 |
| 7 | 0/3 | 1/2 (4)* | 0/4 |
| 14 | 2/2 (6, 8)* | 1/2 (9)* | 3/4 (8, 9, 13)* |

*Number in parenthesis indicates the day the primate died.

Histopathology

Both baboons killed on day 4 showed moderate diffuse congestion of the lungs as well as mild focal septal edema. Alveolar edema was scant in one baboon; and in the other, some perivascular lymphatics were moderately distended with edematous fluid. In the lungs of the baboon dying on day 6, there were scattered groups of alveoli containing fresh edematous fluid and a few neutrophiles superimposed on resolving fibrinous exudate (figure 3). There was a moderate degree of septal inflammation with focal edema and scattered inflammatory cells including neutrophiles. Individual widely dispersed granular pneumocytes were enlarged. In the lungs of the two baboons killed on day 7, there were extensive exudative changes with severe acute edematous exudation predominating in one and marked interstitial reaction with extensive resolving alveolar fibrinocellular exudate in the other. Hyperplasia of granular pneumocytes was evident in both lungs, but this change was greater in the lung with the interstitial inflammation. In the lungs of the baboons killed terminally on days 7 and 8, much the same type of reaction was seen; the 7 day animal exhibited severe acute alveolar edema, and the one killed on day 8 had marked thickening of the septa with resolving fibrinous exudate in the alveoli, sometimes taking on a layered appearance (figure 4). Hyperplasia of the granular pneumocytes was also quite prominent. Detectable increases in connective tissue elements were first noted in the baboons exposed for 7 and 8 days.

Other isolated microscopic lesions not related to oxygen toxicity in baboons included mild focal subacute hepatitis and pericholangitis, a mineralized granuloma in the liver, mild focal interstitial nephritis and pyelitis, mild focal adrenal hemorrhage, a submucosal caseated abscess in the colon, and a small mast-cell tumor.

Figure 3. LUNG OF A BABOON EXPOSED TO OXYGEN AT 720 mm Hg FOR 6 DAYS. Scattered groups of alveoli containing fresh edematous fluid and scattered neutrophiles superimposed on resolving fibrinous exudate. (H & E - x 96)

Figure 4. LUNG OF A BABOON EXPOSED TO OXYGEN AT 720 mm Hg FOR 8 DAYS. Marked thickening of the septa with resolving exudate in the alveoli in which hyperplastic granular pneumocytes are prominent. (H & E - x 96)

Microscopic pulmonary lesions in both M. irus killed on day 4 consisted of mild diffuse congestion with marked edematous distention of the perivascular lymphatics and mild focal septal edema in one. The monkey dying on day 4 exhibited similar lesions in addition to regional moderate alveolar edema. In the monkey killed on day 7, there was moderate edematous and cellular thickening of the septa with groups of alveoli containing fresh edematous fluid superimposed on resolving fibrinocellular exudate which was present in most alveoli (figure 5). Individual granular pneumocytes were enlarged. Lesions in the lungs of the monkey dying on day 9 were similar but more severe, with the amount of apparently functional tissue severely reduced. In addition, focal emphysema was seen and there was a marked diffuse hyperplasia of granular pneumocytes lining the alveoli. In the lungs of the monkey killed on day 14, the septal thickening was marked but fairly uniform

throughout most parts of the lung (figure 6). There was some general congestion and scant amounts of organized fibrinocellular exudate in most alveoli. The alveoli were almost entirely lined with enlarged granular pneumocytes. Detectable increases in connective tissue elements were discernible by day 7 and were quite prominent by day 14.

Figure 5. LUNG OF A CYNAMOLGUS MONKEY EXPOSED TO OXYGEN AT 720 mm Hg FOR 7 DAYS. Edematous and cellular septal thickening with resolving alveolar exudate. (H &E - x 96)

Figure 6. LUNG OF A CYNAMOLGUS MONKEY EXPOSED TO OXYGEN AT 720 mm Hg FOR 14 DAYS. Uniform marked septal thickening with alveoli lined with enlarged granular pneumocytes. (H & E - x 96)

Other isolated microscopic lesions in M. irus not related to oxygen toxicity included mild to moderate subacute nephritis and pyelitis, mild proteinuria, mild focal subacute hepatitis and pericholangitis, mild centrilobular fatty changes, a hepatic parasitic granuloma, and moderate subacute inflammation around a pancreatic duct.

Mild focal histopathologic lesions were seen in squirrel monkeys killed on day 4 and consisted of focal distention of the perivascular lymphatics with edematous fluid and mild general congestion. Focal septal and alveolar edema was evident in one. Lesions in monkeys killed on day 7 were only slightly more severe with congestion being more prominent and there was detectable hypertrophy of isolated granular pneumonocytes (figure 7). Alveolar exudate was scant and there were mild focal pneumonic foci discernible in two. Lesions seen in the monkeys sacrificed terminally on days 8 and 9 were quite similar to those seen on day 7 in that there was mild congestion, scant fibrinous alveolar exudate and hypertrophy of individual granular pneumocytes. In the monkey dying on day 13, the lesions were similar but more advanced in that the septa were thicker and there was more fibrinocellular alveolar exudate. Focal hemorrhages in the basal ganglia of the brain appeared to be acute with little destruction of brain parenchyma. Lesions in the lungs of the monkey killed on day 14 were considerably advanced over those described above. There was extensive fibrinocellular exudate in the alveoli with only small amounts of fresh edema (figure 8). Clusters of alveoli contained, in addition to fibrin and small inflammatory cells, many large macrophages similar in character to the very large granular pneumocytes which were scattered as part of the epithelial lining of most alveoli. These granular pneumocytes were increased in number but not to the point of lining the alveoli. There were no detectable increases in connective tissue elements in any of the lungs of squirrel monkeys.

Other isolated microscopic lesions in squirrel monkeys not related to oxygen toxicity included mild focal subacute hepatitis and pericholangitis, mild focal myocarditis and endocarditis, Sarcocytis cysts in the myocardium and skeletal muscle, mild focal subacute nephritis and pyelitis, mild microurolithiasis, a fluke in a pancreatic duct, and mild hemosiderosis in a spleen.

## DISCUSSION

Known toxic effects of breathing high concentrations of oxygen were for years restricted to symptoms and lesions becoming evident after 48-72 hours exposure. These signs and lesions are now recognized as the acute exudative phase of oxygen toxicity in man and experimental animals, especially the rhesus monkey (M. mulatta) which has been the species of sub-human primate most extensively studied. Recent reports have described the subacute phase of oxygen toxicity which is characterized by proliferation of the granular pneumocytes which eventually line the alveoli and by fibrosis of the interstitium (Kapanci, submitted for publication; Kaplan, submitted for publication). Two rhesus monkeys were studied that were returned to ambient air after exposure to high concentrations of oxygen for 8 and 13 days, respectively. Marked interstitial fibrosis persisted in the 13-day animal but not in the one exposed for only 8 days, implying that complete recovery could be made following the acute exudative phase without permanent damage, but not after the subacute proliferative phase (Kapanci, submitted for publication; Kaplan, submitted for publication).

Figure 7. LUNG OF A SQUIRREL MONKEY EXPOSED TO OXYGEN AT 720 mm Hg FOR 7 DAYS. Scant alveolar exudate and focal distention of perivascular lymphatics with edematous fluid. (H &E - x 96)

Figure 8. LUNG OF A SQUIRREL MONKEY EXPOSED TO OXYGEN AT 720 mm Hg FOR 14 DAYS. Fresh and resolving exudate in alveoli containing isolated very large granular pneumocytes. (H & E - x 96)

In earlier studies using the rhesus monkey, individual variation and susceptibility have been noted as were the variations of response of other species, i. e. dog, rat, and mouse (MacEwen, December 1966; Robinson, December 1966). Therefore, it was not too surprising to find differences in response of the three other species of subhuman primates to oxygen. Even though the numbers exposed were small, the experience of this laboratory with oxygen toxicity in the rhesus monkey (M. mulatta) is quite extensive and therefore the comparative response of these three additional species appears significant.

Laboratory animals in pure oxygen atmospheres ranging from 258 mm Hg to 750 mm Hg have been studied in our laboratory and pressures of 650 mm Hg and greater have been considered toxic within a 14-day experimental period. A pressure of 720 mm Hg was selected for this study with the hope that there would

be a spread of toxic response over the entire 14-day period. This proved to be true for the M. irus and the squirrel monkeys but not for the baboons since the longest surviving baboon died on day 8.

Both the acute exudative and the subacute proliferative lesions were seen in all three species, although appearing at different time intervals and in varying degrees. The acute exudative phase occurred at both the 4 and 7 day intervals and was more severe in the baboons than in the M. irus. Of the four baboons that died or were killed on days 7 and 8, two had characteristic acute lesions while the other two showed the early proliferative response. Acute lesions were milder in the M. irus killed at 4 days than in the baboons, although one M. irus did die on day 4 of the acute disease. The M. irus were more resistant to the acute disease and proliferative lesions predominated from days 7 to 14. Mild exudative lesions were common in all of the squirrel monkeys except for the one surviving the 14-day exposure. This monkey exhibited an early proliferative response consisting of some hyperplasia of the granular pneumocytes but no detectable increase in interstitial connective tissue.

Interpretation of the data indicates that the baboon is more susceptible to oxygen toxicity than the M. irus, squirrel monkey, or M. mulatta. Clinical observations of the marked respiratory effort and subsequent physical exhaustion of the baboons, the time-sequence development of pathologic lesions, and the survival time support this thesis. Further studies would be required to substantiate this contention but, if true, the baboon would then be the susceptible animal of choice in the study of normobaric and hyperbaric oxygen environments.

The M. irus responded in a manner similar to the M. mulatta as was expected, and there is no reason to prefer one species over the other except that the lungs of the M. irus are usually not as heavily infested with lung mites as are those from the M. mulatta.

Squirrel monkeys gave an entirely unexpected reaction to the pure oxygen environment in that the pulmonary lesions in this species were minimal when compared to the others. One cannot forget, however, that they did die in this environment, which poses a problem in determining the cause of death. There were no diagnostic lesions to explain the cause of death in those that died spontaneously with the exception of the one having multiple hemorrhages in the brain. Squirrel monkeys are different in many ways from the macaques, and this observation was borne out by their requirement for supplemental lighting, the apparent need for the company of a cage mate, and their characteristic huddling position. Their attitude seemed to be one of defeatism; and once they began to feel ill, they visibly refused to make an effort to survive. At the time of necropsy, they were quite dehydrated and emaciated; and even though they were observed drinking from the automatic waterers and nibbling on their food, they evidently did not take adequate amounts of either. So the question remains as to the significance of the rather mild pulmonary disease as the primary cause of death.

Hematological studies were generally unrewarding since the only change seen was hemoconcentration in some primates prior to death. In the limited number of samples analyzed, significant increases in erythrocyte osmotic fragility occurred after 7 days exposure. Blood-gas studies showed that arterial $pO_2$ remained very high in the baboons and M. irus until the primate was near death, at which time the $pO_2$ fell precipitously and the $pCO_2$ was significantly increased. From the histologic picture, it was obvious that there was sufficient increase in the thickness of the blood-air barrier to affect the diffusion of gases. In the squirrel monkeys high arterial $pO_2$ was maintained until death except in the one with the brain hemorrhages. The only squirrel monkey showing significant amounts of alveolar exudate and proliferation of the epithelial lining was killed on day 14 and still had an arterial $pO_2$ of 365.

There are only a few reports from other laboratories of oxygen toxicity in subhuman primates. Two of 10 rhesus monkeys survived 20 days in a 90-95% oxygen environment at ambient pressure. The eight that died had acute lesions of congestion and edema along with small foci of pneumonia and emphysema (Friedrich, 1944).

Squirrel monkeys were used in early oxygen toxicity experiments; one died and the other was moribund on day 16 after exposure to 622 mm Hg of oxygen. Both showed moderate lung damage and it was doubtful, according to the authors, whether the lung damage alone was sufficient to cause death. The same authors add a word of caution regarding the necessity of providing an adequate diet for squirrel monkeys (Cook, October 1960). The squirrel monkeys used in our experiment received the same diet on which they were subsisting in the vivarium.

Two Sooty Mangabey monkeys were exposed to 98% oxygen at ambient pressure and they died in 70 and 75 hours. They exhibited acute exudative lesions including alveolar wall thickening, fibrinous alveolar exudate and emphysema (Weir, 1965).

## SUMMARY

Baboons, Macaca irus monkeys and squirrel monkeys were exposed to 100% oxygen at 720 mm Hg pressure for periods up to 14 days. Mortality occurred from days 4 through 13 with one each M. irus and squirrel monkey surviving the 14 day exposure. The last baboon died on day 8. The M. irus reacted similarly to Macaca mulatta that have been studied rather extensively in our laboratory; they went through the classical early exudative response which later resolved and was followed by the proliferative phase which was marked by day 14. The squirrel monkeys were remarkable in their apparent resistance to oxygen as measured by their pulmonary response which was considerably less than in the other species. The baboons were more susceptible to the acute disease than the M. irus. Blood-gas studies in all three species showed that during exposure they maintained arterial oxygen levels 3-5 times their normal concentrations and dropped in baboons and M. irus only if the animal was near death. There was a significant increase in erythrocyte fragility in exposed animals.

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PAPER NO. 17 B

# PULMONARY LESIONS IN MONKEYS EXPOSED TO 100% OXYGEN AT 258 AND 750 mm Hg PRESSURE

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The toxic effects of breathing high concentrations of pure oxygen at pressures from 258 to 760 mm Hg have been extensively investigated in the Toxic Hazards Research Unit of the Aerospace Medical Research Laboratories in recent years. Two questions have remained unanswered in these studies and are the subject of this report.

Of the several species studied (monkey, dog, rat, mouse), most interest has been in the response of the rhesus monkey (Macaca mulatta) because of its phylogenetic relationship to man and consequently to the validation of confined aerospace environments. Until the past year there have been no gross and only minor microscopic pulmonary lesions found in monkeys that were possibly related to exposure to 258 mm Hg of pure oxygen; these findings have been reported for monkeys exposed for 14 to 92 days (Harper, 1967) and for 235 days (Hagebusch, December 1966; Kaplan, 1968). In addition, in the lungs of monkeys, there were no ultrastructural changes related to the 235-day exposure although morphometric analyses were not done (Lewerenz, December 1967). During the past year, rhesus monkeys exposed to a pure oxygen atmosphere at 258 mm Hg in a study of hematological effects (to be reported later) were submitted for routine pathologic studies. The results of these examinations are given in Part A of this report.

Secondly, subacute proliferative changes have been reported in the lungs of rhesus monkeys exposed to pure oxygen at 600 to 760 mm Hg for two weeks (Robinson, 1967). Some of the monkeys surviving the two-week exposure, even at the higher pressures, were in apparent good clinical health, and the question arose as to whether they could survive indefinitely in this hyperoxic environment. It was known and demonstrated that they became rapidly cyanotic upon return to ambient air, supposedly because of the physical increase in the pulmonary blood-air barrier as later supported by blood-gas studies. This possibility of extended survival was theorized to be an adaptive effect and not necessarily pathologic. This concept was challenged on the basis that ultrastructural studies of monkeys

exposed to pure oxygen at ambient pressures for two weeks showed changes which were definitely pathologic and not adaptive (Kapanci, submitted for publication; Kaplan, submitted for publication). To answer this question, rhesus monkeys were exposed to pure oxygen at 750 mm Hg until death, the results of which are discussed in Part B of this report.

## PART A

## MILD PULMONARY LESIONS IN MONKEYS EXPOSED TO PURE OXYGEN AT 258 mm Hg FOR PERIODS UP TO TWO WEEKS

### MATERIALS AND METHODS

Nineteen rhesus monkeys (Macaca mulatta), 7 males and 12 females, with a mean weight of 3.01 kg (range 1.8 - 4.4 kg) were used in this study. Five monkeys each were exposed to 258 mm Hg of 99-100% oxygen for 4, 7, and 12 days in a Thomas dome (Thomas, 1965) which is a unique dynamic flow altitude chamber in which the environmental parameters can be closely controlled and is capable of extended continuous operation at a given pressure. All monkeys had been stabilized for at least 90 days before the experiment started and had shown no response to three successive intrapalpebral tuberculin tests. Four monkeys were used as controls.

All test monkeys were exposed over a period of four weeks and were fed monkey chow and water ad libitum. All exposed monkeys survived their scheduled exposures with no overt clinical signs of disease resulting from the exposure. They were euthanatized with an overdose of sodium pentobarbital before being presented for necropsy. A routine gross examination was made including the intratracheal perfusion of the lungs with either 10% buffered formalin or 3% buffered glutaraldehyde. Tissues from all major organ systems were processed through routine histologic procedures for the production of 5-micron thick sections stained with hematoxylin and eosin.

### RESULTS

There were no gross lesions in any of the monkeys that indicated a response to the exposure. Significant microscopic lesions were limited to the lungs from which multiple sections representing different lobes from each monkey were examined.

#### Four-Day Exposure

Occasional atelectatic foci up to 2 mm in diameter were present in 3 of the 5 monkey lungs. These foci were usually widely scattered in all lobes of the lung although occasionally two foci were confluent. There was apparent thickening of the septa, probably due to lack of normal distention rather than pathologic processes, with scant hemorrhage into the atelectatic alveoli (figure 1). In the lung

of one monkey there was mild edema and lymphocytes, small phagocytes and occasional neutrophiles accompanying the erythrocytes in the alveoli. The remainder of the lungs was unremarkable.

Figure 1. ATELECTATIC FOCUS IN THE LUNG OF A MONKEY EXPOSED TO 100% OXYGEN AT 258 MM Hg FOR 4 DAYS. Apparent thickening of the septa with scant exudate in the alveoli. (H & E - x 96)

Figure 2. ATELECTATIC FOCUS IN THE LUNG OF A MONKEY EXPOSED TO 100% OXYGEN AT 258 mm Hg FOR 7 DAYS. Scant hemorrhage with small amounts of organizing fibrin and phagocytes in the alveoli. (H & E - x 96)

Seven-Day Exposure

Three of the five monkey lungs exhibited atelectatic foci up to 2 mm in diameter and the other two showed a diffuse type of reaction classed as a mild diffuse exudative pneumonitis without atelectasis. There was mild to moderate congestion in two of the lungs with atelectasis and scant hemorrhage with small amounts of organizing fibrin and small phagocytes in the atelectatic alveoli (figure 2). One monkey's lung had mild widespread hemorrhages throughout one lobe, which in some instances extended up to 2.5 mm of frank hemorrhage.

Twelve-Day Exposure

All five of these monkey lungs had partially atelectatic foci less than 2 mm in diameter. In most cases the alveoli were only partially collapsed, or teleologically, partially expanded, containing organizing fibrin and small phagocytic cells, rarely neutrophiles (figure 3). In one lung scant amounts of this same type of organizing exudate were diffusely scattered throughout the lung.

Figure 3. RESOLVING ATELECTATIC FOCUS IN THE LUNG OF A MONKEY EXPOSED TO 100% OXYGEN AT 258 mm Hg FOR 12 DAYS. Organizing fibrin and phagocytic cells in partially expanded alveoli. (H & E - x 96)

## DISCUSSION

The lesions seen at all three time intervals were mild and never appeared to be severe enough to compromise the respiratory functional capability of the monkey. They seemed to be developing in the 4-day monkeys and were near their fullest expression in the 7-day monkeys. Even though all 5 of the 12-day monkeys had mild lesions, they were in stages of resolution with re-expansion of the atelectatic alveoli, suggesting that they were reversible and that they would have completely resolved eventually.

The visualization of the atelectatic foci reported above is probably the result of standardized routine intratracheal perfusion of the lungs of the larger laboratory animals at necropsy. This technique provides lung specimens that are much easier to analyze pathologically than are lungs that are not perfused. In previous reports where monkeys were exposed to 5 psia of pure oxygen for 14 days (Harper, 1967), the lungs were not perfused and lesions of this type and severity may not have been readily seen and interpreted. Also, results of the present experiment indicate that these lesions, if present, might have been resolved by 14 days, or nearly so.

Atelectasis is believed to occur in the distal alveoli in man and animals breathing pure oxygen because of the lack of nitrogen to keep the alveoli expanded and the rapid absorption of oxygen (Roth, 1964). Morphologically, the atelectatic foci were not immediately associated with air passages, but serial sectioning was not done to confirm this supposition. Since there was some inflammation and exudation, although mild, it would appear that there might be local toxic injury to the alveolar septa. It is impossible to say whether this mild injury occurred before or after the atelectasis. Taking the conventional approach that absorptional atelectasis occurs because the absorption rate exceeds the ventilatory rate, then inflammation and exudation would follow the atelectasis. On the other hand, high concentrations of oxygen present in the distal alveoli for an extended period may have caused acute inflammation and exudation which preceded and was possibly responsible for the atelectasis. Because of the mild degree of inflammation in the 4-day lesions, the conventional absorptional atelectasis explanation is favored.

## PART B

## SEVERE PULMONARY LESIONS IN MONKEYS EXPOSED TO PURE OXYGEN AT 750 mm Hg FOR PERIODS UP TO 22 DAYS

### MATERIALS AND METHODS

Four male rhesus monkeys (Macaca mulatta), with a mean weight of 4.86 kg (range 3.06 - 6.01 kg) were exposed to 99-100% oxygen at 750 mm Hg continuously for periods up to 22 days in a Thomas dome (Thomas, 1965). These four monkeys were standardized and met the same qualifications as those used in Part A. Two of the four monkeys were native-born and the other two were imported.

All four monkeys were placed in the dome on the same day and were fed monkey chow and water ad libitum. They remained in this environment undergoing no handling or clinical procedures of any kind during the course of the experiment. After they died they were removed as quickly as possible from the dome and their lungs were immediately perfused with 3% buffered glutaraldehyde. The lungs were then removed and submersed in the same fixative until samples were cut for light microscopy. A complete gross examination was done on each monkey, and tissues from all major organ systems were placed in 10% buffered formalin for subsequent production of routine sections stained with hematoxylin and eosin.

## RESULTS

All four monkeys died as a result of the exposure to oxygen, with deaths occurring on days 6, 17, 20, and 22. The two native-born monkeys were the last to die. None of the monkeys showed signs of discomfort until after 48 hours exposure. Soon after, variable amounts of respiratory distress became evident and progressed to death of one monkey on day 6. The other three seemed to accommodate to the environment during the 7th to 12th day although they never fully regained their appetites. Deterioration in their health became obvious after day 12 when they became lethargic and again markedly anorectic, seeming to lose body weight and condition until death. They did not exhibit the great respiratory effort previously seen in baboons dying after acute phases of oxygen toxicity (Robinson, 1968).

The perfused lungs showed varying degrees of redness grossly, and lung mite lesions were visible in one imported monkey. Microscopic lesions in the monkey dying on day 6 were typical pulmonary lesions for that length of exposure consisting of widespread alveolar edema superimposed on resolving fibrinocellular exudate and small foci of neutrophiles (figure 4). Isolated granular pneumocytes were enlarged. Kidney lesions consisted of severe proximal tubular necrosis with proteinaceous tubular casts and hyaline droplets.

**Figure 4.** LUNG OF A MONKEY THAT DIED AFTER EXPOSURE TO 100% OXYGEN AT 750 mm Hg FOR 6 DAYS. Fresh alveolar edema superimposed on resolving fibrinocellular exudate in the alveoli. (H & E - x 96)

Microscopic pulmonary lesions in the monkeys dying on days 17, 20, and 22 were characterized by advanced interstitial inflammation with resolving fibrinocellular exudate in the alveoli appearing as focal areas of consolidation. In the somewhat emphysematous alveoli outside of these consolidated areas (figure 5), granular pneumocytes were numerous, lining the alveolar walls but rarely more than one cell thick (figure 6), nests of neutrophiles were conspicuous in fresh alveolar edema.

Figure 5. LUNG OF A MONKEY THAT DIED AFTER EXPOSURE TO 100% OXYGEN AT 750 mm Hg FOR 17 DAYS. Emphysematous area adjacent to septa with severe interstitial inflammation. (H & E - x 96)

Figure 6. LUNG OF A MONKEY THAT DIED AFTER EXPOSURE TO 100% OXYGEN AT 750 mm Hg FOR 22 DAYS. Moderate amounts of organized exudate in alveoli lined with large granular pneumocytes. (H & E - x 240)

Mild but definite degenerative renal tubular changes were present in the two monkeys dying on days 17 and 20, while severe tubular changes were seen in the monkey dying on day 22. Renal changes in the latter monkey consisted of advanced tubular degenerative changes with a pronounced inflammatory cell response made up primarily of neutrophiles affecting the tubules of the medullary rays.

## DISCUSSION

There is now little doubt that the proliferative lesions reported in the lungs of monkeys exposed to 100% oxygen at ambient pressures are pathologic and do progress irreversibly until death if the monkey is continucusly exposed. Questions arising from the exposure of these four monkeys are intriguing. For instance, is decreased resistance and resultant infection the ultimate end in oxygen exposed animals? And are the kidney lesions seen in these four monkeys related to the oxygen exposure? And what would be the ultimate cause of death if the monkeys were treated prophylactically with broad-spectrum antibiotics?

The answers to these questions and others are unknown at this time and speculations can only be made on the basis of current information. Kidney lesions were not seen in over 50 monkeys previously used in oxygen toxicity experiments in our laboratory before 1 January 1968. In more recent experiments, the incidence of renal changes similar to those described above has been significant enough to merit consideration. At this time, the etiology of these changes is not known. It is a fact that there has been no clinical disease in the monkey colony indicating renal problems and monkeys used for other purposes in our laboratory have not shown similar lesions.

It has been demonstrated that exposure to lung irritants will heighten the susceptibility of animals to infections of the lung by pathogenic organisms (Coffin, December 1967). Even so, lung disease directly related to oxygen exposure was probably adequate to cause death by anoxia in these four monkeys even in the presence of the nephritis and the focal purulent pneumonia that was developing. Even if these animals had been on antibiotics to suppress possible opportunistic infections, it seems improbable that they could have lived for a much longer time.

## SUMMARY

Mild atelectatic foci have been demonstrated in monkeys (M. mulatta) exposed to pure oxygen at 258 mm Hg. These foci rarely exceeded 2 mm in diameter and appeared to be resolving by day 12 of the exposure. In previous studies extending from 14 to 235 days, this type of lesion was not found, indicating that it was probably transitory.

Monkeys exposed continuously to pure oxygen at ambient pressures will eventually succumb to the insult. Four monkeys (M. mulatta) so exposed died on days 6, 17, 20, and 22. The first monkey to die had pulmonary lesions previously described for that exposure. The other three had severe interstitial inflammation of the septa of the lungs with focal consolidation and a mild purulent pneumonia.

## ACKNOWLEDGEMENTS

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## DISCUSSION

DR. TOWNSEND: I would like to make a comment from the Chair about the presence and the number of these studies that have involved the various animals with resulting hemorrhages which sometimes can be explained purely by trauma and otherwise they don't seem to have a good explanation. Amongst the armamentarium of laboratory tests it perhaps would be helpful in these studies, as we discussed yesterday afternoon to have some coagulation studies, which I have not heard mentioned as a part of the routine. Certainly there are methods of rapid screening that would give some clue as to whether we are dealing with a heparin-like process or with some fault in the first stage of coagulation, or other factors that might be playing a role here. Perhaps this would be something to consider.

DR. WEIBEL: I was impressed with Major Robinson's study and I would like to comment that Dr. Kapanci has recently obtained a considerable amount of human material from people exposed to pure oxygen in chambers for therapeutic reasons not related to lung disease and he has found exactly the same findings as you have found in the monkeys and, which is most interesting, with the same time course. I have a philosophical question to the forum. Is death of the animal adaptation?

(Laughter)

DR. SCHAFFNER: Death is the ultimate failure of adaptation and the various degrees in between good adaptation and poor adaptation are just the areas that we have to concentrate in.

DR. AZAR: In regard to adaptation and death, I'm not a pathologist but I am a physician, and I would like to ask the pathologists if they have noticed the similarity in the light microscopy to that seen in Hyaline Membrane Disease in infants which may represent a failure to adapt, in that those babies that survive three days with the disease go on and do quite well. Wouldn't it be of value to do a study where you progressively increase the oxygen concentration, starting off at 20 per cent on animals for a week; then raise it next week by ten per cent to 30 per cent, etc.? If there is adaptation, and if we assume there is poisoning of an enzyme system and the body needs time to compensate for this, by making new enzymes or whatever the adaptive process is, see if this would be of benefit. This may have been done but I'm not aware of it.

MAJOR ROBINSON: On this question of Hyaline Membrane Disease, Drs. Nash or Northway and associates discussed this entity in infants a year or so ago in the New England Journal of Medicine, and my impression of their article was that there is more to Hyaline Membrane Disease than oxygen toxicity. But their approach to the problem was that arterial $pO_2$ has to be constantly monitored in these patients so that just an adequate amount of oxygen is provided so that they are not hyperoxygenated.

On this business of increasing the oxygen content, there are literally hundreds of different approaches that we would like to take in the study of oxygen toxicity but I was awfully happy when Dr. Thomas and Dr. Back saw fit to give me two weeks in the Dome to work with these three subhuman primates, because Dome time is at a premium.

DR. FAIRCHILD: Relative to Dr. Azar's question. Most of you realize, I am sure, that the relative buildup type of thing has been done with many types of respiratory irritants and of course oxygen is a respiratory irritant under certain conditions. What you suggest has been done, and there have not been any biochemical changes that could be correlated. The adaptation becomes extremely great, and this proliferation of the alveolar septum becomes thicker and thicker so that they are very tremendously resistant to challenging doses. I'm referring now to some work that has been done with ozone, nitrogen dioxide, nitrosyl chloride, phosgene and a few other irritants. They have not been able to find biochemical alterations which correlate with this type of adaptation, and presumably it is very similar in oxygen.

DR. LEON: Dr. Kydd in Pennsylvania has been doing some adaptation studies and we are collaborating with him somewhat on this. And what he has found is this: if he exposes rats to 520 to 550 Torr oxygen for at least 15 days, they can withstand subsequent exposure to 760 Torr much better than if they were simply exposed for the first time. I would like to ask Dr. Robinson about these four monkeys that survived up to 22 days. It didn't look to me from the slides as though they might have died from extensive lung damage. Was that your feeling--that they did die from extensive lung damage?

MAJOR ROBINSON: I think the lung damage was adequate. This complicating factor of the renal lesion is something that has to be remembered. I possibly didn't show you the worst area; you know when you look through the slides you pick out only the pretty ones. But you've got a glob of stuff there, what can you show? But there were areas of actual consolidation in some of these lungs.

DR. LEON: The reason I asked is that we are doing long term exposures with rats on oxygen pressures up to 760 Torr and if you pick a rat young enough, you can have a rat survive in one atmosphere of pure oxygen for at least eight weeks. We have never carried it longer than that, but what happens at pressures above 450 Torr is that their food consumption drops off inversely proportional to the oxygen partial pressure. Now in a rat this isn't too bad because a rat would simply slow up its growth rate or its metabolism and in fact at 600 Torr the rat is not growing at all. It is just eating enough food to maintain a very minimum metabolism. So I was thinking that perhaps you did check the food consumption on these monkeys. And if you did, is it possible that part of the cause of death might have been voluntary starvation?

MAJOR ROBINSON: Well of course I don't know whether these animals can starve to death in two weeks or not. I suppose it is possible. Those squirrel monkeys gave me the most thought-provoking problem, and I actually watched these little rascals eat and drink, and I know they were consuming food and water, but how much of course we weren't in any position to tell. But when we finally necropsied these animals they did appear to be quite dehydrated and emaciated, quite dried out as a matter of fact.

DR. WEIBEL: I was struck by the difference between the squirrel monkeys and the baboons and macaca and by the similarity in the behavior of response of the squirrel monkeys to what we find in rats. We also find that if you put rats in pure oxygen atmosphere they also huddle together, become lethargic, and they don't eat or drink too much.

Now what is the size of the squirrel monkey? What is the metabolic rate, the basal oxygen consumption? Is it similar to the rat?

MAJOR ROBINSON: The weight is approximately 500 grams and I really don't know about the metabolic rate. Ken, have you got an answer there?

DR. BACK: No, not to that specific question, but it is in the Biological Handbook. As you know, I think that rats are the wrong animals to look at for oxygen, for any pulmonary irritant, really. These animals can live in anything for a long time I am sure. If you look at the susceptibility to oxygen toxicity, as we looked at it for the last three or four years, you see that the dog and the large monkey, the baboon, are the most susceptible. The larger the animal gets, the more susceptible. It may be that it has to do with something phylogenetic, I don't know. But the only point against that is that the mouse sits up there with the dog and the monkey, they are quite susceptible, whereas the rat is the least susceptible. If you remember, we did 760, 720, 700, and then 600 millimeters of mercury $pO_2$ and the dog and the monkey were the most susceptible and next came the mouse. The rat was much more resistant. I mean he could live for 18, 20 days at 760. So I don't know, it certainly could be phylogenetic, with the mouse being out of kilter, or it could be size.

DR. WEIBEL: I don't know, this perhaps brings us too far this morning. This goes into the mechanism of oxygen toxicity. It is utterly surprising that we are not killed by oxygen as it is in the atmosphere. This is pretty potent stuff. But we are protected against oxygen effects by the system of enzymes, like glutathione. There are strains of mice which are deficient in these enzymes and maybe we should once try to expose some of these. Now we have in Europe an entirely different strain of rats. I think it is the Charles River Rats(by the way, we have almost no murine pneumonia) but they just die at 72 hours. We have repeatedly done experiments at 760 and we have never had any extensive survival rate. By 80 hours they were all dead.

QUESTION: What weights did you use?

ANSWER: The ones we used were around 200 grams, but the Sprague Dawley rats we used in 1964 in the study at Wright-Patterson were 120 grams I think.

DR. SMITH (Federal Aviation Administration): I am surprised at what you say about the squirrel monkey. We have found them to be beautiful resistant little animals. We have had them living for over a year with five pairs of implanted electrodes in the brain, once we got them parasite-free. They were dying quite unexpectedly when we first began to receive them and we had to cure them of filariasis. We have not found one yet that didn't come in infected.

DR. WEIBEL: Is this in pure oxygen?

DR. SMITH: No I'm not talking about susceptibility to oxygen. The intimation was that they were a rather delicate animal, non-specifically, but this I don't believe is quite true. What you say about strains of rats is interesting to us because we have been doing chronic toxicity studies on organophosphorus pesticides and as long as we use Holzman or Sprague Dawley rats we got rather uniform effects, but when we changed to Charles River Rats we found that they are a completely different animal.

MAJOR ROBINSON: I think we belabored the disease problem in animals to extremes last year, but I do want to admit at this point that those squirrel monkeys are a parasitologists dream, no doubt about it. I don't know whether I pointed out or not but this experiment on the three species was at 720 mm rather than 750 mm and we reduced this pressure in the hopes that we would get animals surviving the entire 14-day period. The baboon didn't. The last baboon didn't die at eight days. These baboons are not entirely free of lung disease. We have found occasional lung mites in these animals, but from what little I have seen I would certainly like to experiment with the baboon on oxygen toxicity for a couple of years.

SESSION III

# EVALUATION OF CABIN MATERIALS

Chairman

Mr. Edmond H. Vernot
Toxic Hazards Research Unit
SysteMed Corporation
Dayton, Ohio

AMRL-TR-68-175

PAPER NO. 18

# THERMAL DECOMPOSITION PRODUCTS OF CARBOXYNITROSO RUBBER (CNR)

F. Neil Hodgson
and
John V. Pustinger

Monsanto Research Corporation
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Carboxynitroso rubbers (CNR) are potentially valuable materials for use as non-flammable coatings in manned space vehicles. In addition to flammability, however, consideration must be given to the possibility of the material thermally degrading to toxic products. For this reason, the thermal degradation of a selected CNR was studied, and the products obtained under various conditions were measured. Work was performed under a contract sponsored by the Aerospace Medical Research Laboratory, Wright-Patterson Air Force Base. The particular CNR studied, MSC 1549, was selected on the basis of thermogravimetric curves of various carboxynitroso rubbers.

A carboxynitroso rubber (figure 1) is a copolymer of tetrafluoroethylene (I), a perfluoro alkyl or aryl nitroso compound (II) and a nitrosoperfluorocarboxylic acid (III). The mole ratio of I:II:III would typically be 50:48:2.

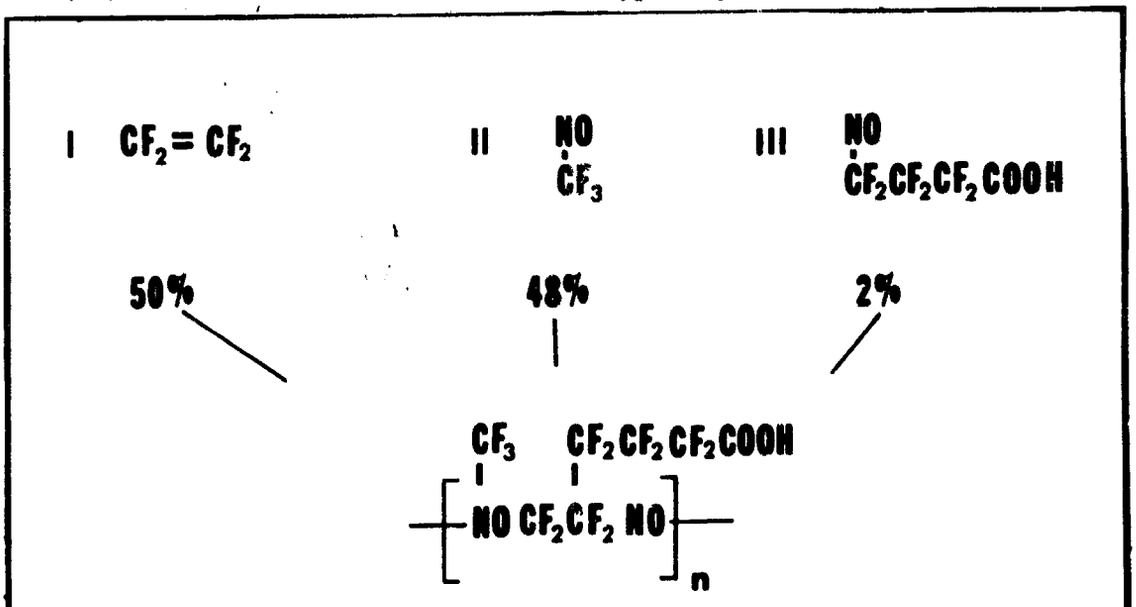


Figure 1. A TYPICAL CARBOXYNITROSO RUBBER

Figure 2 shows the thermogravimetric analysis (TGA) pattern of the CNR specimen to be studied. It should be noted that weight loss at 125 C is minimal, weight loss at 250 C is moderate, and at 350 C almost complete decomposition has occurred. These three temperatures were selected for the study, with initial tests being performed in 5 psia of pure oxygen. Additional tests were made in 5 psia nitrogen and in a complete vacuum at 350 C in order to establish the influence of a non-oxidizing atmosphere.

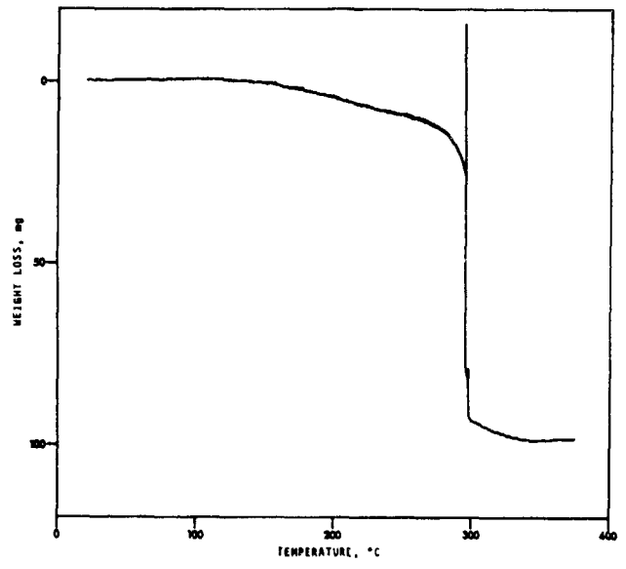


Figure 2. TGA PATTERN OF CNR MSC 1549

## EXPERIMENTAL

Material weighing 500 mg was sealed in an all-glass (Pyrex) test chamber having a volume of 500 cc. The test vessel is illustrated in figure 3. As shown in this figure, the specimen (A) was sealed in the chamber by actually fusing the two glass components together (at B) to form a single, completely enclosed envelope. The sample tube was sufficiently long that the sample could be positioned at one end and thus remain cool while the glass was being sealed at the other end.

A pure oxygen atmosphere was introduced through tube C and adjusted to a pressure of 5 psia. Tube C was then removed by touching the constricted point with a torch, thus completely encapsulating the sample in glass. The entire chamber with specimen enclosed was placed in an oven at the desired temperature and heated for one hour.

Volatile decomposition products were withdrawn through the standard-taper joint affixed to one end of the vessel. Inside the joint was a glass break-seal (D) which was broken by means of a small steel bar placed inside the tube for that purpose. With the tube in a vertical position, the bar was raised by means of an external magnet and then allowed to fall on the break-seal.

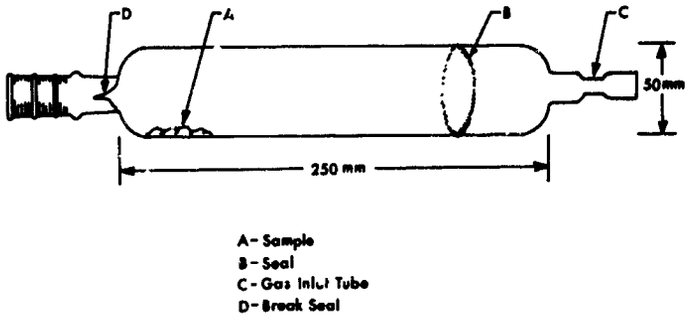


Figure 3. DIAGRAM OF BREAK-SEAL FLASK

In addition to the glass system, several experiments were performed at 350 C using a stainless steel bomb. The purpose of these experiments was to study the effect of the glass system on the products obtained.

## RESULTS

Approximately the same products appear at each of the three temperatures, the chief difference being in the amount formed. Figure 4 lists the volatile products obtained in the tests along with their mole ratios. Carbon dioxide is taken as unity. Except for the oxides of nitrogen, these same products are formed in a nitrogen atmosphere at 5 psia and also in a complete vacuum. The results of the test, which was performed in a stainless steel bomb at 350 C, are shown in the last column of figure 4. With the glass system, at 350 C large amounts of $SiF_4$ were obtained. Use of the stainless steel vessel showed that although a considerable amount of $SiF_4$ comes from reaction with the glass, much of the $SiF_4$ is formed from silicon present in the rubber. The presence of a moderately high silicon level in the rubber has been confirmed by emission spectrographic analysis. In the test at 250 C, the amount of silicone tetrafluoride formed is somewhat less, and at 125 C, none is detected. Another fluorosilicon compound is formed at 350 C, and it too is less in the test performed in stainless steel.

The 350 C test performed in the steel bomb shows one other important difference from the one performed in glass. One component, hexafluorodimethylamine, $(CF_3)_2NH$, was detected only in the steel bomb. A trace was found to be formed in glass at 250 C but none at 350 C.

| | Components | Mole Ratios of Components Relative to Carbon Dioxide Tested for 1 Hour at 125 C | 250 C | 350 C | 350 C* |
|---|---|---|---|---|---|
| Carbon Dioxide | $CO_2$ | 1.0 | 1.0 | 1.0 | 1.0 |
| Carbon Monoxide | CO | 0.01 | 0.4 | 0.5 | 0.6 |
| Nitrogen Dioxide | $NO_2$ | N.D. | trace | 0.1 | 0.1 |
| Nitric Oxide | NO | N.D. | trace | 0.02 | 0.01 |
| Silicone Tetrafluoride | $SiF_4$ | N.D. | 0.2 | 0.3 | 0.1 |
| Carbonyl Fluoride | $COF_2$ | 0.06 | 0.2 | 0.02 | 0.02 |
| Trifluoromethyl isocyanate | $CF_3N=C=O$ | 0.009 | 0.04 | 0.04 | 0.02 |
| Perfluoro-n-methyl methylenimine | $CF_2=N-CF_3$ | 0.08 | 0.3 | 0.05 | 0.04 |
| Uncharacterized | Silicon Component | N.D. | N.D. | 0.006 | trace |
| | Perfluorocarbons | 0.06 | 0.005 | 0.03 | 0.006 |
| Hexafluorodimethylamine | $(CF_3)_2NH$ | N.D. | trace | N.D. | 0.02 |
| | TGA Results | <1% wt. loss | ~10% wt. loss | >95% wt. loss | |

*Performed in stainless steel bomb.

Figure 4. VOLATILE DECOMPOSITION PRODUCTS OF CNR NO. 1549

Carbonyl fluoride is present at all temperatures with a relatively higher amount being observed at 250 C. Perfluoro-n-methyl methylenimine ($CF_3-N=CF_2$) and perfluoromethyl isocyanate ($CF_3N=C=O$) are also present in relatively higher amounts at 250 C and appear in tests at the other two temperatures as well.

Although temperature does not drastically affect the kinds of products formed, the duration of heating does have a marked influence on certain of the volatile products. If a specimen is heated in the test chamber at 350 C for 72 hours rather than for one hour, no trace of the imine or isocyanate can be detected. Apparently these components are converted to nonvolatile products. This observation can be made regardless of whether the test chamber contains nitrogen or oxygen at 5 psia or whether it has been completely evacuated. If the specimens are heated for only one hour and then analyzed after storing at room temperature for 72 hours, both the isocyanate and imine are present.

Each of the decomposition products was identified by obtaining the mass spectra of the pure components by a combined gas chromatograph-rapid scan mass spectrometer. These data, combined with infrared absorption measurements, are the basis for assignments. Major infrared bands for some of the compounds are shown in figure 5.

| Compound | Wavelength (microns) |
|---|---|
| $COF_2$ | 5.2 |
| $CF_3N{=}CF$ | 5.55 |
| $(CF_3)_2NH$ | 2.85 |
| $CF_3N{=}C{=}O$ | 4.35 |

Figure 5. MAJOR INFRARED ABSORPTION BANDS FOR DEGRADATION PRODUCTS

## DISCUSSION

An overall reaction for the degradation of nitroso copolymers, which yields equimolar amounts of carbonyl fluoride and perfluoro-n-methylmethyleimine, has been proposed (1) as follows:

$$\left[-CF_2-\underset{\displaystyle CF_3}{N}-O-CF_2-\right]_{(A)} \rightarrow \underset{(B)}{CF_2{=}\underset{\displaystyle CF_3}{N}} + \underset{(C)}{O{=}CF_2}$$

Minute amounts of water, which are undoubtedly present either on the specimen or in the sample chamber, may cause these compounds to react further (2), accordingly:

(a) $COF_2 + H_2O \rightarrow CO_2 + HF$

(b) $CF_3N{=}CF_2 + H_2O \rightarrow CF_3N{=}C{=}O + HF$

This accounts for the presence of the perfluoromethyl isocyanate. Hydrofluoric acid formed, of course, can react with the glass chamber to form silicon tetrafluoride. The reaction goes stepwise:

$$SiO_2 + 6HF \rightleftarrows H_2SiF_6 + 2H_2O$$

$$H_2SiF_6 \rightleftarrows SiF_4 + 2HF$$

The last reaction proceeds to the right on heating. This may explain the absence of $SiF_4$ at the lower temperature even though carbonyl fluoride and the imine are present. Hydrofluoric acid may also react with the imine (1):

$$CF_3N{=}CF_2 + HF \rightarrow (CF_3)_2NH$$

The resulting compound, hexafluorodimethylamine, was observed in appreciable amounts only in the test which was performed in the stainless steel bomb. In the glass system, this reaction does not proceed, since hydrogen fluoride apparently reacts more readily with the glass.

Much of the $CO_2$ and CO that was observed is certainly due to decarboxylation of the acid moiety, since these components are formed even in a $N_2$ atmosphere and in a complete vacuum.

## CONCLUSION

This study has shown that decomposition of the carboxynitroso rubber specimen at 125 C is very slight and the major product formed at this temperature is carbon dioxide, presumably due to decarboxylation. Also, a trace amount of carbonyl fluoride is formed. However, at 250 C the specimen loses as much as 10% of its weight, with a significant amount of the highly toxic carbonyl fluoride being formed. A further increase in temperature to 350 C causes complete decomposition, releasing approximately the same products as at 250 C, except that the oxides of nitrogen now appear.

The purpose of this paper has not been to judge the advisability of using carboxynitroso rubbers in manned space vehicles, but rather to produce data upon which a toxicological evaluation of the material can be made. Hopefully, these data can aid in defining conditions in which the use of the material is indicated and, conversely, in establishing a limit to its utility.

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1. Barr, D. A., and R. N. Hazeldine; J. Chem. Soc., pg 3416 and pg 3428, 1956.

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## DISCUSSION

MR. VERNOT: Perhaps a word on the genesis of this carboxy nitroso rubber problem might be in order here. For those who aren't familiar with it, the first time I heard about carboxy nitroso rubber was as a nonflammable material with potential use in space craft; following the first Apollo tragedy and the tragedy at Brooks when everybody went full force to try to find nonflammable materials. And it was quite true that carboxy nitroso rubber was a rubber that had all the qualities of elasticity that they wanted in rubber and it was nonflammable. But it was rather soon noticed that once you got to a certain temperature, which is pretty close to 300 degrees centigrade, the thing would just go like that--a puff of smoke and the carboxy rubber disappears and you just have fine grey powder. We happened to smell the effluent gas that came off one time, and it was pretty potent. I happened to smell it and for three days my nose, throat and bronchi were hurting a little bit.

MR. MOBERG (Aerojet-General Corporation): Did you not notice $CF_4$ in any of the decomposition products in CNR? There could have been an intermediate there. It might have been rather high concentration. Did I miss that in one of the listings?

MR. HODGSON (Monsanto Research Corporation): We listed it in the perfluorocarbon grouping. We saw C-1, to C-3, perfluorocarbons. This is included in that figure.

MR. MOBERG: I see, and it was not the predominant material in that group?

MR. HODGSON: No, it was not.

DR. MAC EWEN: I noticed in one of your charts, one of the first charts where you had the three different combinations and the additional one temperature combination in stainless steel, that the two compared, glass and stainless steel at 350 degrees were not very much different in your silicon tetrafluoride content; that is to say, one was 0.3% and the other was 0.2% in the stainless steel. I don't think it is necessary to postulate that the silicon tetrafluoride was formed because of the use of the glass apparatus. There was an adequate amount of silicon dioxide used as one of the materials in the formulation, coated with Silstone 110 to be responsible for almost all of the silicon tetrafluoride formed.

MR. HODGSON: Yes, I think that is true.

AMRL-TR-68-175

PAPER NO. 19

# THE ACUTE TOXICITY OF THERMAL DECOMPOSITION PRODUCTS OF CARBOXY NITROSO RUBBER (CNR)

J. D. MacEwen, Ph. D.
and
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The Toxic Hazards Research Unit (THRU) provides toxicologic investigations of potentially hazardous materials to the Air Force. These investigations, conducted by SysteMed Corporation personnel, are designed to characterize the acute or chronic toxic effects of materials to which military or civilian personnel may be accidentally or unavoidably exposed. The toxicologic research of manned space flight problems is concerned with defining the risk of breathing trace air contaminants resulting from outgassing of agents incorporated in cabin construction materials and of chemicals used for propulsion and life support systems. The toxicity of the pyrolysis products of individual space cabin materials are also evaluated by the THRU.

Carboxy nitroso rubber (CNR) is a synthetic polymer with rubber-like properties and has the added property of being noncombustible. Due to its noncombustible property CNR was selected as a candidate material for use in spacecraft cabins as a replacement for combustible materials with similar properties of softness and stretch.

Carboxy nitroso rubber was first examined for determination of its flammability in a 100% oxygen environment and for chemical analysis of any pyrolysis products formed. Insofar as can be determined from information supplied by the manufacturer, the formulation of CNR is:

| | Compound | Formula | Weight % | |
|---|---|---|---|---|
| CNR gum | Nitrosotrifluoromethane | $CF_3NO$ | ? | 80 |
| | Perfluoroethylene | $C_2F_4$ | ? | |
| | Perfluoronitrosobutyric acid | $HOOC(CF_2)_3NO$ | 2 | |
| | Silstone 110 (10% Silicone oil coated silica) | $SiO_2$ | 14.4 | 16 |
| | | $(R_2Si\text{-}O)n$ | 1.6 | |
| | Chromium trifluoroacetate | $CrOOCCF_3$ | | 4 |

A portion of CNR was placed in a small chamber filled with oxygen at ambient pressure. Ignition was attempted using a nichrome resistance wire heated to 800 C. No burning occurred; instead the wire melted through the rubber releasing visible, acrid fumes.

One gram of CNR was pyrolyzed in a glass stoppered 250 ml flask filled with 100% oxygen. The flask was slowly heated, using a low flame, until the beginning of reaction, signalled by physical decomposition of the rubber into a frothy gray powder and the evolution of dense white fumes. The reaction continued spontaneously for about a minute after initiation. The temperature necessary to initiate pyrolysis was determined to be approximately 300 C.

Mass spectrographic analyses were performed on the pyrolyzate of the CNR and five compounds were tentatively identified as shown in table I.

TABLE I

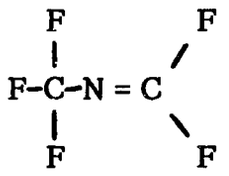
PYROLYSIS PRODUCTS OF CNR IDENTIFIED BY MASS SPECTROMETRY

| | |
|---|---|
| Carbon dioxide | $CO_2$ |
| Silicon tetrafluoride | $SiF_4$ |
| Perfluoroethyleneimine | $CF_2CF = NF$ |
| Carbonyl fluoride | $COF_2$ |
| Trifluoromethyl isocyanate | $CF_3NCO$ |

These are listed in the approximate order of their concentration in the pyrolyzate vapors, although no attempt was made to achieve quantitative estimates. It must also be emphasized that these identifications are made solely on the basis of mass spectrometric examination and cannot, therefore, be regarded as unambiguous. However, formation of these compounds appears to be plausible given the formulation of the carboxy nitroso rubber.

Infrared spectra were obtained on the rubber before and after pyrolysis. The prepyrolysis spectrum was obtained using the multiple internal reflectance accessory and the postpyrolysis spectrum by transmittance using a Nujol mull. The main point of interest was the essential disappearance in the spectrum of the pyrolyzed material of bands due to nitroso, carboxy and silica groups, indicating that these had largely reacted and escaped in the vapor. Absorption bands due to C-F and CrO groups were seen which indicated that the chromium remained nonvolatile and that the pyrolysis residue was still highly fluorinated.

The more complete analysis conducted by Hodgson and Pustinger (Hodgson, 1968) has shown that the compound we identified as perfluoroethyleneimine is more likely to be its isomer perfluoro-n-methylmethyleneimine:



One might expect a gaseous mixture containing the substances listed in table I to be irritating to the mucous membranes and to the entire respiratory system. In truth, one sniff of the pyrolyzate vapor did produce irritation of the nasal passages and deep chest pain in one experimenter. In view of the observed irritative property of the CNR pyrolyzate, animal experimentation was conducted to define the acute toxicity of the pyrolyzate.

The pyrolysis of CNR was extremely rapid (it literally went off in a puff of smoke) and consequently did not lend itself to the continuous generation techniques desirable for dynamic animal exposure systems. The continuous feeding of CNR to a pyrolyzer system such as described by Kupel and Scheel (Kupel and Scheel, 1968) in their studies of polytetrafluoroethylene (PTFE) was not practical due to the small amount of sample CNR submitted for initial testing and to its reported high cost. The rodent exposures to the pyrolyzate of CNR were, therefore, conducted in a 30-liter chamber under static conditions.

Based on information that approximately 65 grams of CNR would be used in a space cabin having a free volume of 8.3 $M^3$ an inhalation exposure concentration of 7.65 mg/l was selected for testing. A group of five male Charles River CD rats was placed in a 30-liter chamber which was then purged with $O_2$ until a 100% $O_2$ environment was achieved. A sample of CNR placed in a nichrome wire basket was heated to 300 C at which temperature it pyrolyzed and dense white fumes filled the static chamber in which the rats were caged. Four of the five rats died during the 30-minute exposure period and the last animal expired immediately upon removal to ambient air.

Samples of chamber atmosphere were analyzed at the end of the 30-minute exposure period to determine $CO_2$ concentration. The $CO_2$ levels did not exceed 0.5% by volume. The expression of chamber concentration of mg/l of pyrolyzate is related to the amount of CNR used and as such is a nominal figure for the maximum possible exposure. The actual exposure environment consisted of the gases identified and also a white finely dispersed aerosol. This aerosol which accounted for approximately 2% of the weight of the original sample, was believed to consist of chromium and unreacted silica.

During exposure the animals displayed signs of extreme pulmonary eye irritation. Profuse lacrimation and sniffling was followed by prostration during which diaphragmatic breathing was observed. Gross examination of the tissues of the animals showed a uniform picture of massive pulmonary hemorrhage and edema as shown in figure 1.

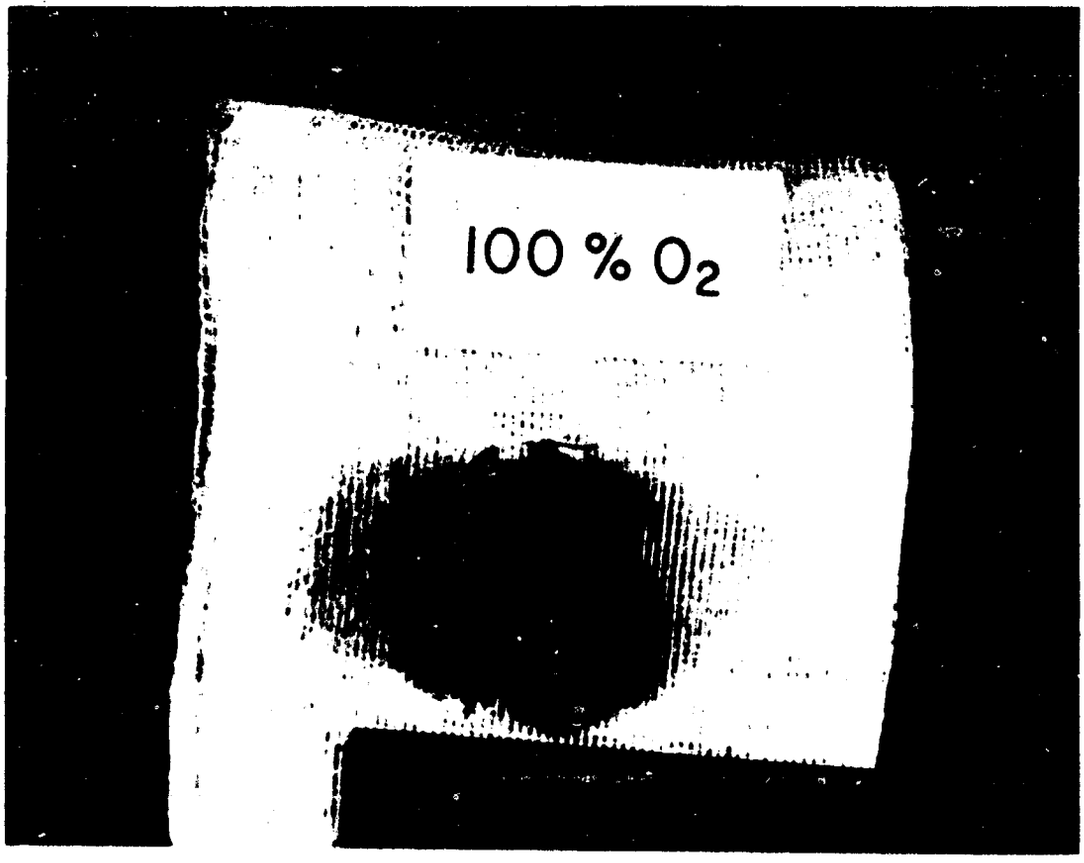



Figure 1. RAT LUNG AFTER EXPOSURE TO THE PYROLYSIS PRODUCTS OF CARBOXY NITROSO RUBBER.

A series of experiments was conducted to find the "no-effect" concentration for albino rats exposed to CNR pyrolysis products for 30-minute and 2-minute periods. During 30-minute exposures a concentration of CNR pyrolyzate as low as 0.37 mg/l produced in excess of 80% mortality. A concentration was ultimately reached, 0.20 mg CNR pyrolyzate/l, that did not result in fatalities and further, produced only minimal signs of pulmonary and eye irritation. The lowest concentration producing mortality during 2-minute exposures was determined to be 1.80 mg CNR/l. This value is less accurate than the 30-minute concentration value due to the inherent problems associated with complete termination of animal exposure in 2-minute duration experiments.

Inhalation exposure concentrations selected for testing the "no-effect" level for 2-minute and 30-minute exposures to CNR pyrolyzate were 0.66 mg/l and 0.20 mg/l respectively. Twenty-five male rats were exposed to each concentration time period, during which only occasional animals displayed signs of mild irritation such as blinking or sniffing. The exposed animals were serially killed, as were both positive and animal room controls, over a 14-day postexposure period. All animals were weighed at each necropsy period and then the organs of those killed were weighed and submitted for histopathological evaluation.

At the two selected time-concentrations tested the CNR pyrolyzate did not produce any effect on rat growth rate or upon organ to body weight ratios. There were also no apparent gross pathologic differences between the exposed groups of rats and their sham exposed or animal room controls.

Histopathologic examination of the tissues of all animals exposed to the tested concentrations showed only mild pulmonary irritation during the first few post-exposure days and had been resolved by the 14th day. The signs of irritation consisted of slight fluid accumulations in the bronchi and increased phagocytic activity which disappeared seven days postexposure. The mild changes seen may have been due to exacerbation of endemic murine pneumonia present in both exposed and control rats.

The observed acute toxic response to CNR pyrolyzate cannot readily be explained with respect to a specific causative agent. A search of the available literature has not revealed any information on the toxicity of either trifluororsocyanate or trifluoromethyldifluoromethyleneimine. The work of Scheel et al (Scheel, 1968; Scheel, 1968) defines the one hour $LC_{50}$ for $COF_2$ as 360 ppm and for $SiF_4$ as 922 ppm using the 14-day mortality data. Qualitatively neither $SiF_4$ nor $COF_2$ occurred in excess of 25% of the pyrolyzate mixture and thus were not present in concentrations greater than 25 or 40 ppm respectively in an environment that produced more than 50% mortality in 30 minutes. Again a 2-minute exposure of rats to concentrations of $SiF_4$ and $COF_2$ lower than 100 and 150 ppm respectively produced mortality. Thus the observed toxicity of the pyrolyzate cannot be due to either $COF_2$ or $SiF_4$ nor can it be explained as an additive response of both compounds.

A calculation of lethal concentrations for either time period studied using actual space cabin volumes indicates that the heating and pyrolysis of as little as 3 grams of CNR could prove fatal if inhaled for 30 minutes. Furthermore, the pyrolysis of 15 grams of CNR could produce a lethal concentration of gases if breathed for 2 minutes, a time period less than that required for astronauts to put on their pressure suits and to vent the cabin to space. The pyrolysis products of carboxy nitroso rubber must, therefore, be considered extremely toxic and appropriate safety measures considered with its use. This material would represent a serious potential hazard to both householders and to fire fighters if it finds use in domestic or commercial applications.

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## DISCUSSION

DR. TANNENBAUM (Thiokol Chemical Corporation): We make carboxy nitroso rubber. I have a few things to say to make the record a little more understandable, to interpret whether this has an application or not. There is nothing which rules out proper use of CNR because we have conducted studies at different temperatures, of course, and if we have not decomposed the material, if you heat it for example to 200 degrees Fahrenheit, your rats survive very well. There is, of course, a temperature threshold, which was pointed out when the material was first made available to NASA, and we knew this before, because it was under development for other purposes. If you heated it to the point at which it catastrophically decomposed you had a toxic material. Although it had that property, the suggestion was made that because it could not ignite in oxygen up to several hundred pounds pressure of oxygen you had a material which might be quite useful. Now this matter of ignition is important because the tests we just witnessed certainly are a very graphic illustration of what will happen if you expose animals for 60 minutes or thereabouts, or even much less to material of this sort. One reference that you made, Dr. MacEwen, would be difficult for me to understand, and that is if somebody dropped a match on CNR no fire can result and so you cannot have a dangerous condition. The only way to maintain decomposition of CNR is to provide an external heat source. It hasn't got so much to do with the temperature as it has to do with maintaining that temperature; and CNR, of course, cannot maintain the temperature by itself, in that it cannot burn. The question then arises, and I imagine it arises frequently in toxicological studies of this sort, as to whether a material can be used for a specific mission. You made this point quite clear in your talk, and I just wanted to make reference to that one item of a match dropping, because in order to sustain the decomposition of CNR you must have a fire going on, or a source of heat. The theory in the early development of CNR or early consideration of its application was that you could not have a source of heat unless you had a major catastrophe already going on. One property of CNR which is interesting and relates to this business of having to have a constant source of heat is this: If you have a wire which is coated with CNR or a source that can be heated, once you decompose the material in the immediate proximity of the wire, no further decomposition occurs because you have a heat transfer problem. So, I think the conclusion that we reached and the conclusion we have suggested in the past is, of course, if you have a situation where CNR can be continually heated to a temperature above five hundred degrees Fahrenheit, you must not use that material if any human is going to be in the environment. However, you have here a nice question because it is an elastomer which cannot ignite, which may prevent many tragedies. I merely wanted to, as I say, clarify a couple of points in terms of how you might still use CNR despite this toxicity problem.

DR. MAC EWEN: I didn't mean to imply that it was completely unusable. If I did, I'm sorry. However, when you do put a match to it, it does decompose quite instantaneously. However, that is a local area, and the resultant expansion of gases and the large quantity of decomposition products that come off will put the match out. If you have another heat source that isn't quite that neatly operating, such as a hot wire that falls upon a surface of it, it will continue to pyrolize as long as there is any material near it. The point I was trying to make was that it doesn't take very much to be very irritating. A very little bit of this goes a long way.

DR. THOMAS: We didn't mean to imply that as toxicologists, there is no understanding of use conditions at all. As you recall, there was a scare about teflon which was started many years ago. I believe there is a wonderful booklet on this--"Anatomy of a Rumor". We have been using Teflon insulation in great quantities, but there were years when engineers were anxiously calling us: "Hey, that thing is so toxic and it decomposes, can we use it?" And, of course, you can under the proper use-condition if you can guarantee that that material will never be heated to decomposition temperature. As a matter of fact I would like to have a safety factor around the temperature. According to that slide we have seen, the decomposition products were qualitatively similar between 200 and 300, but lesser in quantity--Is that so, Mr. Hodgson?

MR. HODGSON: Yes.

DR. THOMAS: Was that you conclusion?

MR. HODGSON: Yes.

DR. THOMAS: So I think we should put a safety factor on temperature limits it can be heated to without decomposing.

DR. MAC EWEN: If I could add just one point. There is a difference between CNR and Teflon-like products by approximately one order of magnitude of toxicity. Therefore it does represent a more serious hazard, should combustion occur.

DR. FASSETT: Could I ask how you ignited the sample and how you measured the total quantity in the air? Was this a calculation for weight loss?

DR. MAC EWEN: As I said earlier in the paper, the sample was placed in a nichrome wire basket, the nichrome wire basket was heated to 300 degrees. Now this did give essentially a hundred per cent decomposition of the material--rapid; it happens right now. As I also said the concentrations were nominally based on the weight of the amount of material placed in the chamber, into the nichrome wire basket. We couldn't sample each of the exposures without removing all of the decomposition products from the exposure chambers.

DR. FASSETT: The nichrome wire basket was heated electrically?

DR. MAC EWEN: Electrically.

DR. TANNENBAUM: As a matter of relative hazard, I don't know how many of you realize it, but teflon, although it is a fluoro-carbon can burn in oxygen. Therefore the problem that you face in a situation like that is that if an ignition source exists, then the material can consume itself. It requires no external fire to feed it. This means that you can create rather massive quantities of material in an oxygen environment. The difference again here in terms of actual use-problem, or use situation, is that if no fire or no over-heating sufficient to raise the temperature of this material exists, it cannot of itself provide the heat source; therefore you must have an external fire going at the same time. This is the case with CNR, but not the case with teflon.

MR. VERNOT: I don't think we were trying to make the point that CNR rubber was a dangerous material to have around during normal use. I don't believe that that is the point the toxicologist tries to make. What we were trying to do was to say that communication about these things among toxicologists, engineers, design engineers and manufacturers ought to be established rather early in the game during the selection of these things.

QUESTION: One slide showed that at 125 C you had the same decomposition products if there was decomposition--this is aimed at Mr. Hodgson. You had the same decomposition products at 350. Now I have heard that below 300 C you had small chain polymers coming off from CNR, now can you clarify? Did I have that slide right that you had the same products coming off?

MR. HODGSON: There were some products that were given off at a higher temperature, which were not detected at the 125 degree temperature. Again, we are only measuring products that are volatile; perhaps some of the short chain polymeric groups are formed, but they may not be volatile enough to be included in the measurements we are making.

FROM THE FLOOR: The reason I asked this is that TGA showed a very sharp break right around 300 C, but it is very sharp and went all the way.

MR. HODGSON: Right.

QUESTION: How do you reconcile the amount of these other decomposition products at a much lower degree?

MR. HODGSON: Well, the data we give in the table is relative composition of the vapors. Of course you have to go to the TGA curve to find out how much vapor is really formed. We are giving you relative composition of vapors.

QUESTION: In other words, you may have had a small amount of each of those there, but the amount, not being specified for all practical purposes, why is it significant then?

MR. HODGSON: Perhaps our table is misleading. I don't mean to imply because there would be, say, 0.1 mole ratio of a given component at two different tests, that this means there is the same amount in each test. These are mole ratios so you would have to convert mole percent times the weight loss.

AMRL-TR-68-175

PAPER NO. 20

# CONTAMINANT TRAPPING AND EFFECT OF SURFACE ADSORPTION ON ATMOSPHERIC CONCENTRATION OF CONTAMINANTS

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## INTRODUCTION

The subject of trapping volatile contaminants from confined atmospheres is of great interest to space scientists generally and to inhalation toxicologists particularly. This interest derives from the necessity of continuously purifying the atmosphere so that it remains nontoxic to astronauts or aquanauts and also from the desirability of isolating and concentrating vaporous contaminants for subsequent analysis. At the present time, there are three basic methods of removing and concentrating impurities from the atmosphere; cryogenic trapping, gas-solid adsorption and gas-liquid adsorption. This report represents the results of a study into the theoretical bases upon which the three trapping techniques rest. The analysis has been kept as simple as possible in the hope that workers from all disciplines will be able to understand it without the need to refer to a physical chemistry textbook. In addition, the final portion of this paper includes initial data obtained from a closed-loop life support system designed for testing the possible toxic effects of the offgassing products of space cabin materials. Examination of the variation in concentration of both gas-off products and pure contaminant vapors leads to the conclusion that the surfaces of a closed-loop life support system may act as a buffer in controlling the contaminant concentration.

## PART I

## CONTAMINANT TRAPPING

The phase rule is a physicochemical rule of thumb which permits prediction of the number of thermodynamic factors which have to be fixed in order to define a system completely. These factors include pressure, temperature, molar volume, and concentration. Tabe I lists the phase rule in its general form.

TABLE I

PHASE RULE

$F = C - P + 2 + j$

P = Number of phases
C = Number of components
F = Degrees of freedom
$j$ = Number of interfaces

For a vapor in equilibrium with its liquid or solid phase
P = 2, C = 1, $j$ = 0, F = 1

For a vapor absorbed on a solid or liquid surface
P = 3, C = 2, $j$ = 1, F = 2

## CRYOGENIC TRAPPING

Of the two examples listed, the first illustrates the situation in the case of cryogenic trapping. Here, for a vapor in equilibrium with its own liquid or solid phase: the number of phases is two - gas and condensed liquid or solid, the number of components is one and there are no adsorbed interfaces. It follows, therefore, that the system has one degree of freedom. This means that if the temperature is held constant in such a system, the partial pressure above the condensed phase will remain constant, and addition of more gas will merely result in further condensation. Thus, in a cryogenic trap, if the gaseous contaminants entering have a pressure above their vapor pressure at the trapping temperature, the contaminant concentration, as measured by its partial pressure, will be reduced to a constant value by condensation. If the partial pressure of a contaminant is below that of its vapor pressure at the trapping temperature, no condensation will take place, and the contaminant concentration will not be reduced.

One of the advantages of cryogenic trapping is that there is no loss of efficiency over a trapping cycle; the contaminant concentration in the air leaving the trap is as low at the end of a trapping period as it was at the start. Recovery of the trapped material is also advantageous; heating the trap revaporizes the contaminants completely for characterization and analysis.

The disadvantages of cryogenic trapping can be summed up as follows:

1. Cooling the air stream too quickly may result in solid or liquid aerosols being formed. These are not easily deposited on surfaces and may be carried through the trap back into the atmosphere.

2. Portions of the trapping system with small annular spaces may tend to plug if condensation occurs there.
3. Usually, an accessory heat removal system such as a cryogenic liquid or refrigeration system is necessary. In solar space, however, the radiative and absorptive properties of a black body might be used advantageously to cool an exterior trap on the dark side of a space vehicle while warming it on the sunny side to expel trapped contaminants.

The use of cooling coils to condense water vapor and maintain the relative humidity of atmospheres at comfortable levels is an example of "cryogenic" trapping as explained in this paper.

## GAS-SOLID ADSORPTION

Adsorption is the most common technique presently used for the removal of gaseous impurities in atmospheres. Molecular sieves are used for $CO_2$ removal and activated charcoal for organic impurities. Although these two substances differ greatly with respect to the type of material trapped most efficiently, they both act by holding vapor molecules to their surface. The phenomenon of surface adsorption is a general one, but in commercially designed adsorbents the surface is increased to such an extent that remarkable quantities of gas can be held.

Reference to table I illustrates that a gas-solid adsorbing system has the following phase rule parameters:

1. There are three phases - gas, adsorbed gas and solid.
2. There are two components - solid and adsorbate (adsorbed and unadsorbed).
3. There is one interface between adsorbed gas and solid adsorbent.

The system, therefore, possesses two degrees of freedom which signifies that, at constant temperature, the partial pressure of a contaminant above the adsorbent will not be constant as in the case of cryogenic trapping, but will depend on the amount of contaminant already adsorbed. This is illustrated by the Langmuir Isotherm reproduced in figure 1. The isotherm illustrates the constant temperature variation of the amount (weight or volume) of gas adsorbed at equilibrium with increasing partial pressure of the gas and is a plot of the equation shown in the figure. Although investigators have shown that many of the assumptions upon which the equation is based are not valid (principally the assumption of monomolecular adsorption), the isotherm still describes the adsorption phenomenon better than any other. The curve can be divided into three sections, each of which is delineated in figure 1 along with the mathematical expression describing that section. In the first or Henry's Law section, the partial pressure of the gas increases only slightly and in linear fashion as the amount adsorbed increases.

Contaminant trapping is usually not carried past this adsorption region. In the section where the Freundlich Isotherm applies, the partial pressure increases exponentially with the amount adsorbed and, finally, the adsorbent becomes saturated, and no more adsorption takes place no matter how high the contaminant partial pressure rises.

$$a = \frac{k_1 p}{k_1 p + 1}$$

a = Amount of gas adsorbed per unit weight adsorbent

p = Partial pressure of gas

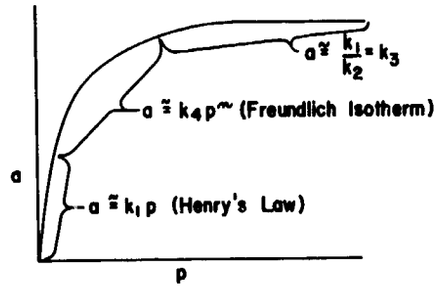


Figure 1. LANGMUIR ISOTHERM

The advantages of a gas-solid adsorption system may be listed:

1. No matter how low the concentration of a contaminant, some of it is trapped.
2. No accessory energy source is needed unless regeneration in situ is planned.
3. In the case of charcoal, there is not much affinity for water vapor and, therefore, low concentrations of organic impurities in an atmosphere of normal relative humidity may be effectively trapped.
4. Molecular sieve has a particular affinity for $CO_2$ because of the polar nature of the adsorptive forces involved.

Possible disadvantages in adsorptive trapping include:

1. The concentration of contaminant in the atmosphere leaving the trap will increase with time. This will be minimized, however, by two factors - a) in a flowing system the last part of the trap will have the smallest amount of adsorbed contaminant and, b) adsorption traps

are usually never utilized beyond the Henry's Law portion of the isotherm where only small increases in contaminant concentration occur.

2. It has been suggested that recovery of contaminants from adsorbents for analysis is not complete. However, Saunders (1965) has shown that all contaminants identified in atmospheres directly or after cryogenic trapping have also been found after desorption from charcoal.

## GAS-LIQUID ADSORPTION

The base of the system is an inert support whose only function is to provide a large surface. A liquid with a very low vapor pressure is coated on the support, and adsorption (or in this case, possibly absorption) and solution of the gas in the layer of liquid occurs. Reference to figure 1 shows that, since the system possesses two degrees of freedom, the amount of contaminant adsorbed is a function of both temperature and partial pressure and is described by an isotherm. In this case the isotherm is much simpler than that for gas-solid adsorption. Figure 2 illustrates the equilibrium behavior of gas-liquid systems. Theoretically, Henry's Law applies throughout the range of solubility of the gas in the liquid substrate. Moreover, the Henry's Law constant can be evaluated as the reciprocal of the contaminant's vapor pressure at the temperature of trapping. Thus, the partial pressure of a contaminant over a gas-liquid trapping system rises linearly with the amount of material trapped (expressed as mole fraction of the liquid substrate). One can expect positive or negative deviations from this ideal situation depending on whether the solvent has less or more affinity for the contaminant than for itself. These deviations lead to curves similar to those shown in figure 2.

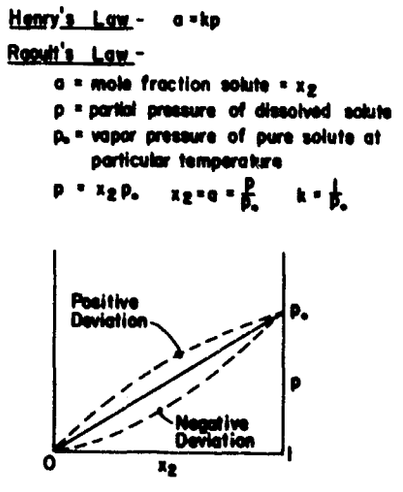


Figure 2. SOLUTIONS OF GASES IN LIQUIDS

The one advantage of gas-liquid trapping appears to be high specificity. Since the efficiency depends upon solubility, contaminants of high solubility tend to be trapped in higher concentration than sparingly soluble ones. The selection of a liquid substrate then can be made on the basis of which types of contaminants are desired to be trapped. Conversely, one would not expect to use gas-liquid systems for the general trapping of all possible contaminants. It would also appear that the capacity of gas-liquid systems to attain low effluent contaminant partial pressures is not as great as that of gas-solid or cryogenic systems.

## PART II

## CONTAMINANT ADSORPTION AND ITS EFFECT ON ATMOSPHERIC CONCENTRATION

In past years, an equation dealing with the increase in atmospheric concentration of contaminants in space cabins has been used extensively (Thomas; Jones, 1923). It was derived theoretically from certain basic assumptions (which will be discussed later) concerning rate of generation and rate of leak. There was little, if any, information available concerning the effect of animals or possible removal of contaminants by water condensing in the humidity control system.

We recently constructed a cabin materials testing system (Johnson, 1968) designed to simulate as closely as possible the atmospheric conditions prevailing in a space cabin. Figure 3 is a diagram of the system. Candidate cabin materials are placed in the oven through which a portion of the total atmosphere flow passes, carrying any volatile gas-off products into the animal chamber containing mice and rats. The atmosphere is split to give a variable flow ratio through or around lithium hydroxide canisters to control $CO_2$ at any desired level. Humidity control is effected by a condenser on the oven bypass. The atmosphere is continuously monitored for $O_2$ and $CO_2$, and samples for gas chromatography may be removed from the system periodically.

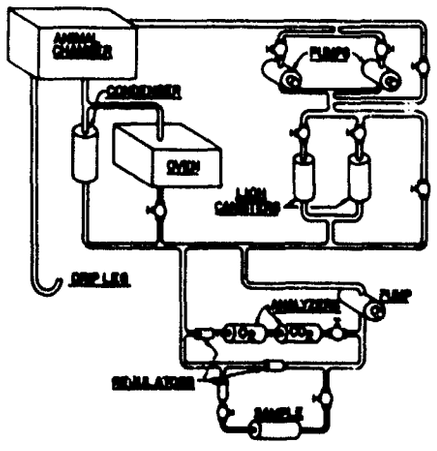

Figure 3. CABIN MATERIALS TESTING SYSTEM

In order to begin our experiments at the least complex level, we decided to test the chamber contaminant buildup and loss in the following way. Running our experiments at atmospheric pressure, the chamber filled with air, surrounded by pure oxygen a leak was established in the system by punching a small hole in the animal chamber. Samples of four volatile contaminants, isopropanol, trichloroethylene, methylisobutylketone and toluene, were placed in the oven in quantities calculated to give 1000 ppm in the system. The atmospheric concentrations of the contaminants were monitored by periodic removal of samples for gas chromatography. The system contained no animals, nor was the water condenser being operated. The total air supply passed through the lithium hydroxide in the normal manner without bypass. Figure 4 illustrates the calculations used to determine the leak rate. These calculations apply under the conditions of the experiment - chamber filled with air, chamber surrounded by pure oxygen and inboard leak equal to outboard leak to maintain constant pressure.

Chamber filled with air
Chamber surrounded by 100% oxygen
$O_2$ = oxygen concentration
a = total volume of chamber
b = leak rate

$$\frac{d\,O_2}{d\,t} = \frac{b}{a}\,(1 - O_2)$$

$$\int \frac{a}{b}\,\frac{d\,O_2}{1-O_2} = \int d\,t$$

$$-\frac{a}{b}\,\ln\,(1 - O_2) = t + k$$

$$k = -\frac{a}{b}\,\ln\,(1 - O_{2\,t=o}) = -\frac{a}{b}\,\ln\,0.8$$

$$\frac{a}{b}\ln\frac{0.8}{1-O_2} = t$$

$$b = \frac{a}{t}\,\ln\frac{0.8}{1-O_2} = 2.303\,\frac{a}{t}\,\log\frac{0.8}{1-O_2}$$

Figure 4. DERIVATION OF EQUATION FOR CHAMBER LEAK RATE

$$-\int \frac{dc}{dt} = \int \frac{b}{a} c$$

$$\ln c = -\frac{b}{a} t + k$$

$$\ln c = \ln c_o - \frac{bt}{a}$$

$$\log c \times 2.303 = \log c_o \times 2.303 - \frac{bt}{a}$$

$$\log c = \log c_o - \frac{bt}{2.303\, a}$$

Figure 5. DERIVATION OF LEAK OF CONTAMINANT INTRODUCED AT ONCE.

Once an atmosphere leak rate had been determined in this manner, it was possible to calculate the theoretical concentrations of the contaminants at anytime after maximum concentration had been attained under the assumption that the only process affecting them was leakage out of the chamber. Figure 5 illustrates the calculations. $C_o$ is the maximum concentration attained, at which point the time is taken to be zero.

Table II lists the average daily concentrations for the pure materials placed in the cabin materials testing system for five days. The first striking aspect of the data is that the theoretical concentration of 1000 ppm was not achieved for any contaminant, and, in the cases of isopropanol and methylisobutylketone, only four and nine percent respectively of the theoretical concentrations were measured at maximum. The only explanation for this loss appears to be that adsorption of the vapors on the internal surfaces of the chamber life-support system took place and that the more polar alcohol and ketone adsorbed to a much greater degree than the less polar toluene and trichloroethylene. Figure 6 is a semilog plot of the data in table II along with the theoretical relative concentration drop calculated using the equation from figure 5. As previously noted the assumption underlying this equation is that the only process operating on the contaminant is an outboard leak with and at the same rate as the atmosphere. Obviously, this assumption is invalid since all contaminant concentrations decreased at a much slower rate than that predicted by the figure 5 equation. The contaminant vapors appear to be adsorbed on the system surfaces and then slowly desorbed when equilibrium is disturbed by the chamber leak.

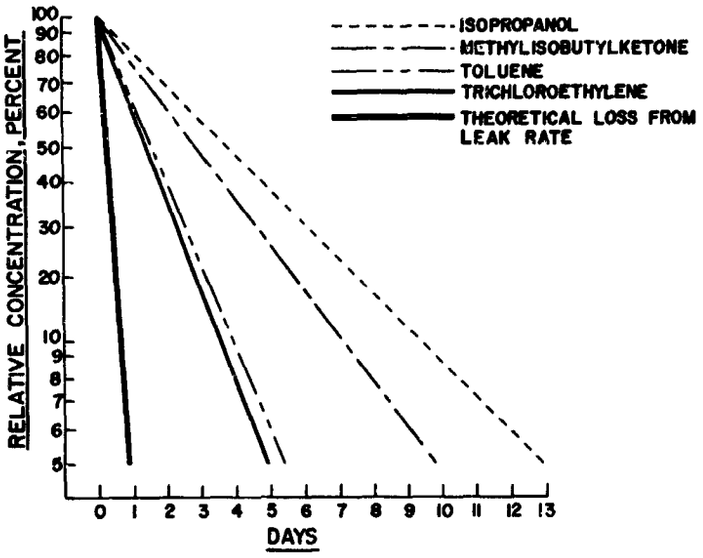


Figure 6. RATE PLOT-CONTAMINANT LOSS IN CABIN MATERIALS SYSTEM

TABLE II

CONTAMINANT LOSS IN CABIN MATERIALS SYSTEM

Theoretical concentration of each contaminant = 1,000 ppm

Maximum concentrations achieved in three hours

| | Concentration (ppm) | | | |
|---|---|---|---|---|
| Day | IPA | TCE | MIBK | TOL |
| Start | 43 | 475 | 89 | 410 |
| 1 | 27 | 275 | 52 | 232 |
| 4 | 24 | 57 | 30 | 43 |
| 5 | 14 | 16 | 21 | 31 |

IPA = Isopropanol, b.p. - 82.4
TCE = Trichloroethylene, b.p. - 87.0
MIBK = Methylisobutylketone, b.p. - 116.9
TOL = Toluene, b.p. - 110.6

The log of the concentration, however, does appear to be a linear function of time which would not be the case if the classical concentration-time relationship, $c = w/b\,(1 - e\,a^{\frac{-bt}{a}})$, held since, on converting to logs the expression $\log (1 - b/w\ c) = \frac{-bt}{a}$ is obtained, which is non-linear. Figure 7 details the derivation of the classical equation. The one assumption which appears to be invalid in the case of adsorbed contaminants is that of constant contaminant generation. Certainly, if there is no leak in a chamber, equilibrium will be established between adsorbed and vaporous forms of contaminant, and no further gassing-off will take place. It also seems reasonable to assume, therefore, that rate of contamination generation or gas-off from adsorbing materials will be a function of the difference between adsorbed and vapor concentration. When this assumption is made and the further restriction of quasi-equilibrium is postulated, the derivation in figure 8 follows. Under these conditions the semilog plot of contaminant vapor concentration versus time is linear and can be expected to be dependent on the free vapor-adsorbed vapor concentration equilibrium of each individual contaminant. This combination of effects will lead to exactly what was obtained in our experiments, linear plots of different slope for each individual contaminant.

Assume constant rate of contaminant generation
w = wt. of contaminant generated per time unit
a = total volume of cabin
b = leak rate

$$\frac{dc}{dt} = \frac{w}{a} - \frac{bc}{a} = \frac{w-bc}{a}$$

$$a\,\frac{dc}{w-bc} = dt, \quad -\frac{a}{b}\,d \ln (w-bc) = dt$$

$$\int d \ln (w-bc) = \int -\frac{b}{a}\,dt$$

$$\ln (w-bc) = -\frac{bt}{a} + k$$

When $t = o,\ c = o,\ k = \ln w$

$$\ln (w-bc) - \ln w = -\frac{bt}{a}$$

$$\ln \frac{(w-bc)}{w} = -\frac{bt}{a}$$

$$\frac{w-bc}{w} = e^{-\frac{bt}{a}}$$

$$\frac{bc}{w} = 1 - e^{-\frac{bt}{a}}$$

$$c = \frac{w}{b}\,(1 - e^{-\frac{bt}{a}})$$

Figure 7. DERIVATION OF CLASSICAL CONTAMINANT BUILDUP EQUATION.

Assume rate of contaminant generation proportional to some function of the difference between adsorbed & vapor concentration

$$\frac{dc}{dt} = k_1 x - k_2 c - \frac{b}{a} c$$

x = weight of adsorbed contaminant per unit surface

Then: If equilibrium conditions are almost attained

$$\frac{x}{c} \cong k_3, \quad x \cong k_3 c$$

$$\frac{dc}{dt} \cong k_4 c - k_2 c - k_5 c \text{ where } k_4 = k_1 k_3 \text{ and } k_5 = \frac{b}{a}$$

$$\frac{dc}{at} \cong c (k_4 - k_2 - k_5) = kc$$

$$\int \frac{dc}{c} \cong \int k \, dt$$

$$\ln c \cong kt + k_6$$

$$k_6 = \ln c_{t=o} = \ln x_{\frac{t=o}{k_3}}$$

$$\ln \frac{k_3 c}{x_{t=o}} \cong kt$$

$$c \cong \frac{x_{t=o}}{k_3} e^{kt}$$

Figure 8. LOSS OF ADSORBED CONTAMINANT THROUGH LEAK

## GAS-OFF EXPERIMENT ON CABIN MATERIALS

In an experiment designed to test adsorption processes under more realistic conditions, twelve materials of the types listed in table III were placed in the oven for seven days. Our usual complement of 20 rats and 25 mice was housed in the exposure chamber, the water condensing system was operating, and the recirculated atmosphere was directed through the lithium hydroxide canisters. Gas chromatography was used to measure the number and relative concentration of contaminant circulating through the exposure system. Five major peaks were observed. These peaks were not identified, but their heights were measured throughout the experiment and plotted on log graph paper against time in days.

TABLE III

GROUP N MATERIAL TYPES

Polyester - Glass Laminate
Epoxy Adhesive
Primer Residue
Silicone Rubber - Glass Laminate
Silicone Grease, Light
Silicone Grease, Medium
Silicone Adhesive
Silicone Oil
Epoxy Potting Compound
Dry Film Lubricant - Unknown Composition
Polyester Coating
Epoxy Paint

Figure 9 shows the plots obtained for each of five peaks. The initial line rising at the start of the exposure depicts the increase in peak heights of all contaminants during the first four hours of exposure. The maximum concentration was attained during the first night when no analyses were made. However, examination of the plotted data after the first day showed that the situation was much more complex than in the case of simple liquids without the presence of animals or moisture. Linear plots were obtained in two cases, but the other three were obviously curved. This may be a result of different forces holding the vapors to the cabin materials, or it may represent interaction with the animals or moisture in a non-linear fashion. Future experiments are being planned to investigate the effect of each potential interacting factor with a view towards illuminating the fate of any contaminant in the system and, eventually, in a space cabin.

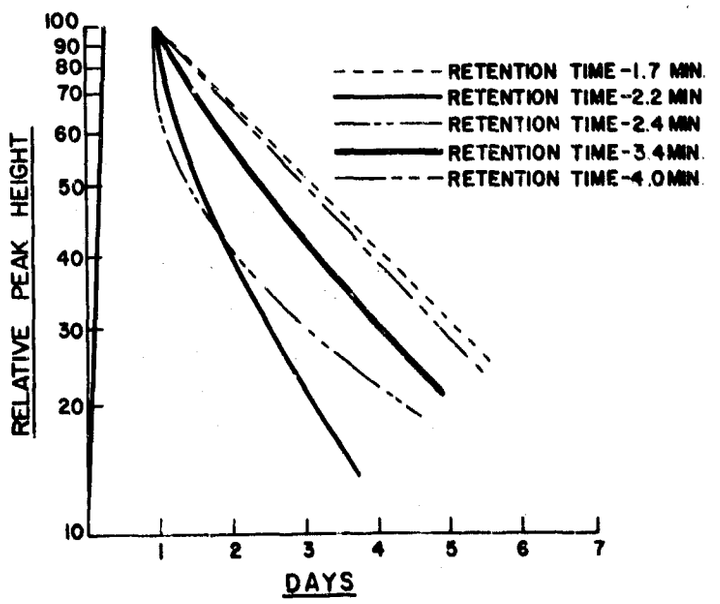


Figure 9. GAS CHROMATOGRAPHIC PEAKS OF APOLLO CANDIDATE MATERIALS GROUP N

## SUMMARY

The basic physicochemical parameters involved in cryogenic, vapor-solid, and vapor-liquid adsorption trapping have been investigated. Cryogenic trapping is seen to be a univariant system, i.e., temperature alone determines effluent partial pressure. Both types of adsorption are bivariant, i.e., temperature and concentration of adsorbed vapor determine effluent partial pressure. The method subject to fewest uncertainties appears to be cryogenic trapping. The simplest and most efficient method appears to be vapor-solid adsorption.

Both experimental and theoretical analyses indicate that gassing-off of adsorbed volatile contaminants in a space cabin atmosphere cannot be treated using the classical equation: $c = w/b\,(1 - e^{\frac{-bt}{a}})$. Instead, a simpler equation, $c = k_1 e^{-k_2 t}$ best describes the actual process occurring. The implication of the first equation is that an equilibrium contaminant concentration will eventually be obtained in a space cabin with a leak, whereas, the second predicts eventual zero concentration.

Initial experimentation in a space cabin material testing system has shown that the gas-off contaminant concentration decrease need not be a linear function of time, but will approach zero concentration with time.

## REFERENCES

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## DISCUSSION

MR. BIBIE (General Electric Company): I wonder if you would tell us what your oven temperature was and if you ever repeated this at other temperatures?

MR. VERNOT: No, we always run our ovens at 150 degrees, although we can achieve other temperatures. We can go up as high as the boiling point of water, no higher.

Oh, you mean below 150?--We've never done that.

DR. THOMAS: Well we didn't pick this temperature; we were advised to use it because the general philosophy in spacecraft design is that with solid state circuits, transistors and so forth, you don't want to go over 155 degrees. They just assume that this would be the hottest operating temperature for any part before a malfunction occurs. I have a question on the last graph. Were there any animals in the chamber?

MR. VERNOT: There were animals in the chamber. This was a regular run.

DR. THOMAS: All right, my next comment is that we should assume the animals scrubbed out some of these contaminants. The lung is a very efficient scrubbing device.

MR. VERNOT: I'm sure that is true. The controlled experiments, that is without the animals, the only process working beside leak that I could figure was surface absorption.

DR. THOMAS: Lithium hydroxide was in that run or not?

MR. VERNOT: The lithium hydroxide canister was not in that run. We by-passed it completely.

DR. FAIRCHILD: Ed, could you relate this possibly to one of the problems we used to think about in terms of a seven-day exposure to gas-off materials? Whether the animals were actually receiving equal portions of these materials over the seven-day period, or whether they might have gotten all the material in the first days and nothing six days later?

MR. VERNOT: The last slide showed that the concentration was going down at all times once it achieved the maximum. We have done some work since then on a 60-day study. After 13 or 14 days we can hardly see any contaminants at all, except what the animals produced.

DR. BACK: Ed, you checked the materials that you started out with. Did you ever find different peaks than what was there to begin with? Did you ever look for other materials by other methods? In other words, did any of these five materials ever change to Compound X, Y, or Z?

MR. VERNOT: No, we didn't find any evidence of this. Of course this is just one experiment, and it is possible that we didn't have the right materials in there. I assume that what you are leading up to is the production of contaminants by reaction with the atmosphere or something of this sort. In this particular experiment we did not find that; however, we did find something very interesting. I showed you the results of one experiment but we ran two experiments and we tried to mix up our materials so that we would get a fair distribution of whatever is available in each chamber, and it worked out that, of course, by gas chromatography alone it is hard to say absolutely that they are the same thing, but the peaks, the four or five peaks had exactly the same retention times. An indication to me of what you will see in a space cabin atmosphere from this type of material is a very limited number of compounds which have to do with solvent behavior matrices, this sort of thing; the things they mix up in their paints, silicons, etc.

DR. MAC EWEN: We might point out that the very small sample that you took in no way concentrated the materials, and that although they look like they are approaching zero, they are not approaching zero. We couldn't run an animal experiment and remove enough samples to concentrate by freezeout or something like that, and still expose animals. So we were only allowing 500 ml samples a couple of times a day, from a 200 liter atmosphere.

MR. VERNOT: Yes, that is certainly true. We have to take the atmosphere as it is for analysis.

QUESTION: Were these concentrations relatively high concentrations;--how high?

DR. THOMAS: Do you have anything at a 1000 PPM?

MR. VERNOT: We were down in the order of a tenth of a part per million in that last analysis. This is on the adsorbed material. Now as far as the cabin material themselves are concerned, on the 60-day study, by the 13th or 14th day we were below one part per million.

## DISCUSSION

MR. WANDS: There is one question arising out of the first session. This is a question addressed to Dr. Roy Albert. Is Dr. Albert in the audience to answer it himself? If not I'll have to try to field it for him. Let me read the question first of all: "Among the dozen or so contaminants decreed by NASA to be of most concern in a spacecraft atmosphere, dichloracetylene was not included (although its precursor trichloroethylene was). Why was DCA omitted in view of the fact that it is the only so called exotic type of contaminant that has been encountered in any closed atmosphere to date which has actually produced serious toxic symptoms and the additional fact that allowing for the presence of trichloroethylene or any other precursor, it is a compound which can easily be produced in some future spacecraft atmosphere?"

MR. WANDS: Dr. Albert?

FROM THE FLOOR: Dr. Albert is not here.

MR. WANDS: Does Dr. Favorite from the Space Science Board wish to try to field this one?

DR. FAVORITE: No, he doesn't.

MR. WANDS: I know, Frank, that you did not sit with that particular committee of Dr. Nelson's. A couple of us that are in the audience, myself and one or two others, were involved on a peripheral basis with this committee although of course we were not members of it, and thus I refuse to accept any responsibility for what the committee may have said. But I think perhaps I can explain what has happened in response to this question. You will recall that in the recommendations of the committee, they first of all picked up the recommendations of the National Research Council/Committee on Toxicology for nuclear submarine atmospheres. Included in that is a recommendation of the committee, but I think I can paraphrase it reasonably well. That dichloroacetylene should be kept to as near absolute zero as is analytically and engineeringly possible because of its high toxicity. And they further pointed out or reminded the Navy of the information that had been submitted by the Navy that dichloroacetylene is produced by pyrolysis, thermal degradation etc.; whether or not it is over an alkaline catalyst is somewhat important here; but for the Navy situation the recommendation was, that since dichloroacetylene is produced essentially by pyrolysis of trichloroacetylene and similar precursors, the temperatures of operation of the catalytic burners which are used for purification of the air should be controlled within those limits that will prevent the formation of dicholoracetylene. Now let me expand on that a

little bit further for those of you who are not familiar with the operation of the nuclear submarine. The catalyst is an hopcalite catalyst which operates at a fairly elevated temperature and its purpose is to remove combustibles of one kind or another, particularly carbon monoxide and hydrocarbon type materials. Studies have shown that when this catalytic furnace is operated at low temperatures, below the recommended range, then dichloroacetylene may be produced. But when it is operated at the normal range, the dichloroacetylene itself is also decomposed. Therefore, even though it might be made momentarily during the passage of an atmospheric sample through the furnace, by the time it came out at the far end, it too would be decomposed. As long as the engineer on board the submarine is on the ball and keeps his furnaces operating the way they are designed to be operated, it is feasible to keep the atmosphere in the submarine, as far as dichloroacetylene is concerned, at a pure zero level. The committee recognized when they made that recommendation that there was no very good or sensitive analytical method for sampling the air, as Captain Seigel mentioned yesterday. The stuff is explosive when you get very much of it in the air and it is a tricky thing to handle. So this is kind of ducking the issue to some extent, but I think that for the same reasons the Space Science Board Committee on a 100 and 1000-day space flights had to do the same thing, so as far as dichloroacetylene is concerned for space cabins that committee's recommendation is the same as the Navy's.

DR. PIERSON: I'm kind of curious as to why they went to the submarine atmospheres when there are available, I'm sure they were not used, four multi-million dollar projects concerning space station atmospheres and space station design, as well as a complete report on the toxic contaminants to be generated in a larger rotating space station.

MR. WANDS: The Space Science Board Committee reviewed not only the results of these cabin inflight simulation studies, several of them, they also reviewed the available data from earth orbital missions of both Mercury and Gemini, and from those they developed their list of materials of potential contaminants in the Apollo and post-Apollo craft. In terms of reviewing the toxicological literature about the significance of these materials and what levels might be acceptable, they went to the prime source of human experience--those recommendations that the Navy was accepting for operation of their submarines which had not resulted in any difficulty. Therefore, we knew that at least these levels would be safe for a period of up to 90 days in a confined environment, and it was the intent of the committee that since we do have at least this as a baseline, this should be a safe recommendation for these materials for 100 days. You will note also that the Space Science Board Committee's recommendation did not extrapolate the Navy's submarine experience to 1000 days.

DR. PIERSON: I was thinking not so much of short mission flights like Apollo and Gemini, but of studies conducted on large rotating space stations where materials are going to be different and the toxic contaminants due to be generated would be somewhat different. The reports that came out of these three large rotating space stations studies as well as the toxic contaminant studies that were to be generated along with it would indicate a somewhat different list of materials than were shown here. And this is primarily for long, large rotating space station missions.

MR. WANDS: I think, Dr. Pierson, all I can say to that is that the Committee looked at all of the potential contaminants, generated a list of over 200 or so of these things, said to NASA: Which of these do you consider most likely to be encountered in the flight of concern to you for a hundred and a thousand days? Who made that decision in NASA and who made that selection and on what basis I cannot say. Perhaps Dr. Benjamin or someone else from NASA would care to comment on that, but this came back then to the Space Science Board Committee as a list of 50 materials, and out of those then, Dr. Nelson's Committee, represented here at this meeting by Dr. Albert, picked ten of these which were of significance from the toxicological standpoint and added one or two others of their own generation such as carbon monoxide which was an obvious potential problem. This is the history of it and I am sorry I cannot give you the basis of this screening down from 200 or so down to 50.

DR. FAVORITE: I am quite sure that the selection of the ten or so compounds from the long list was done within OAR, with Dr. Jones and his group, rather than over in Manned Spaceflight even though there may have been coordination between the two. I don't know, but would like to have identified the reports that Dr. Pierson is speaking of--could you identify those?

DR. PIERSON: There were three contracts that came out about '63 to '65 on the large rotating space station. These were NASA contracts. There was the logistics and support part of it, there was the design of the craft itself and there was a ballistics resupply study, all three of which tied into the same general type of craft. There was also a report on the toxic contaminants to be generated on these three various crafts. If you wish, a little later on I can actually give you the numbers on these.

FROM THE FLOOR: That would be helpful. I think, in the meanwhile, it is a rather circular discussion because neither Mr. Wands nor myself sat with this group one hundred per cent of the time, although Ralph did participate to a great extent, so I don't know whether they considered these reports or not. They may well have.

MR. WANDS: Dr. Back, do you have any recollection of these specific reports in any session that you sat on?

DR. BACK: No, they sound like arm-chair toxicology to me. These reports that were done back in '63 must have been arm-chair toxicology. I don't know of any reports that are factual.

MR. WANDS: I think these were intended to be predictive reports rather than reporting reports.

DR. PIERSON: Any report of 1000-day missions is bound to be arm-chair toxicology. We are talking now about toxic contaminants to be generated on 1000-day mission, and that is what many of these papers have been about. You have to go into arm-chair toxicology and then verify it later on in actual flight.

MR. WANDS: This, of course, was one of the purposes that NASA had in mind when they came to the Space Science Board with: What things do we have to look at? What things do we need to do additional research on? This was a first approach to the thing. I would like to ask also if Dr. Johnson or Mr. Saunders have any further comments upon my review of the nuclear submarine atmosphere control. Did I make any misstatements or misleading remarks there, Enoch?

DR. JOHNSON (U.S. Naval Research Laboratory): No, I think you stated it pretty well.

MR. WANDS: Thank you. Ray?--Ray Saunders spoke to you last year about some of these atmosphere studies.

MR. SAUNDERS (U.S. Naval Research Laboratory): In omitting dichloroacetylene from that list you gave us a reason--the fact that it should be maintained at zero concentration and I agree that it should be. But in omitting it, it tends to take it out of the picture so that people forget that it exists. It can be produced in any atmosphere when the conditions are right and the necessary precursors are present, and if it were produced it would be exceedingly dangerous.

MR. WANDS: The compound was not eliminated from the Space Science Board Committee's recommendations. It was incorporated lock, stock, and barrel along with all of the recommendations for the Navy for submarines. In addition to that then, this committee added some other recommendations so that dichloroacetylene is included in the Space Science Board recommendation for 100-day flights. They made no recommendations for 1000-day flights for anything, even CO.

DR. CULVER (SysteMed Corporation): I wonder if Dr. Hodge would comment on the appropriateness of setting any material at zero. Is this really a toxicologist talking?

MR. WANDS: While he is thinking, I will say some had their tongue in cheek with that recommendation, realizing the ridiculousness of a zero tolerance level of anything. This is patterned after the problems with pesticides, of course.

DR. HODGE: Is this what you had in mind, Dr. Culver? After a great deal of blood, sweat, and tears, so to speak, the people who are trying to get a workable statement for handling really dangerous contaminants, oh let's say powerful carcinogens that might find their way into our food supply, have wrestled for a long time until they got members who sat on some of teams right here in the audience. Perhaps we should hear from them. Now the problem of the zero tolerance or no effect level, that is below measurable limits. The catch, as I dare say all of you know, is that as we continue to refine our analytical techniques, we keep talking about a sliding number in the past ten years. Some of that legislation written in '38 and some a little bit later which sounded pretty good then because our analytical methods were pretty much standardized for one part per million or a tenth of a part for million which was a pretty small amount to detect. Then all of a sudden we had a flood of new analytical techniques--gas chromatography and atomic absorption, and activation analysis, and all of a sudden now we can do fragments of certain things, way under parts per billion; so all of a sudden what are we talking about? And all this means is that it is an amount that is undetectable by present analytical procedures.

MR. WANDS: And that was the nature of the report to the Navy, the recommendation for the Navy of dichloroacetylene--zero by the best analytical procedures.

CAPTAIN SIEGEL: It is true that they suggested that we approach zero if possible, but they also said that for the time being we think a tenth of a part per million would be pretty good and our general philosophy is to try to approach zero anyway, so this is acceptable.

DR. HODGE: It seems that there isn't any substitute for informed judgment, and this judgment should take into account more factors than an analytical method.

MR. MOBERG: We have been involved in some analytical work on these contaminants found in simulator cabins as well as some tests recently at the Cape, and I think that the philosophy, as I understood what Ray was driving at, was that if any of these might even remotely be possible, we shouldn't exclude them from the list because there is a tendency to use this list from the expertise of this room generated to other areas and say: "Well if it is not on the list don't worry about it and don't design instruments to detect it" and therefore it gets screened out inadvertently. I am sure you are conscious of it and being concerned about it, but unfortunately the engineers will say well we won't build detectors for it because it is not on the list; so on that basis it should have been left on the list.

MR. WANDS: I'm sorry, Mr. Moberg, I don't think that I made myself clear. Dichloroacetylene is included in the list of the Space Science Board Committee's recommendations for a 100-day space flight by virtue of its presence in the recommendation for submarine atmospheres and all of those recommendations were incorporated as a total entity by the Space Science Board Committee, so it is on the list.

MR. MOBERG: Well it wasn't one of the 12, 15 or 25 mentioned.

MR. WANDS: It was one of the additional 12 or so that the Space Science board added to the Navy's list.

MR. MOBERG: I've probably seen the list but I don't remember seeing that compound on the list. Maybe the list I saw was an abbreviated one circulated in NASA.

MR. WANDS: I'm sorry I don't have a copy of the report with me so I can't show it to you, but it is there.

DR. BACK: There are about five pages of compounds.

MR. WANDS: Do you remember that large Table A in the Appendix of the Report which listed a lot of compounds, and some of those were marked with a particular symbol? I don't remember what it was as being Navy Recommendations, and those then are automatically a part of the recommendations of the Space Science Board Committee for 100-day space flights.

DR. BACK: I would like to bring up the point of "units" for airing here, because it was aired at Langley--the units that were picked by the Board and using millimols per 25 cubic meters. My point is that it may well be misconstrued by some people. We brought up at Langley that any engineer worth his salt could use his slide rule and he didn't care what units you used in this area, and I have to agree with them. On the other hand, some people will say O.K. if I can use 25 millimols per 25 cubic meters, can I use four times that much for a hundred cubic meters? It is a poor system, I think, because it depends on the amount of free space you have in your cabin; so what if I've got four times that amount, can I quadruple the concentration?

MR. WANDS: You may not quadruple the concentration but you may quadruple the total amount if you quadruple the volume. This is simple arithmetic and that is all that you can do, and anybody doesn't even need a slide rule.

DR. BACK: I think that is the way most people are taking it.

MR. WANDS: I don't think I quite understand the problem. I didn't understand why they were having difficulties with it at Langley either for that matter. It is a number. It is a convenient number. It is probably more meaningful than parts per million/cubic feet or milligrams per cubic meter, or even parts per million which all of us deal with continually.

DR. BACK: I beg to differ--if you don't have the molecular formula you can't derive it.

DR. THOMAS: Ralph, why is it more meaningful than milligrams per cubic meter--why?

MR. WANDS: It is not more meaningful, it is more convenient because it is a more convenient number, particularly to spacecraft engineers who wanted to convert a number produced by a group of toxicologists to a different atmospheric pressure, whether it was to be an elevated hyperbaric or hypobaric system. They wanted to be able to take numbers such as PPM and convert these then to hyper and hypobaric systems and it was pointed out that a numerical system such as this, a unit such as this, would be directly convertible without a lot of slide rule manipulation; that is all--simply a matter of convenience.

MR. VERNOT: It is a molecular unit and is therefore directly relatable to such things as partial pressure; millimols per 25 cubic meters essentially says if you have a pure material in 25 cubic meters you will have one million millimols. Therefore you can tell exactly what the partial pressure is by multiplying the number of millimols per 25 cubic meters by $7.6 \times 10^{-4}$. It has this advantage over $mg/m^3$--unless you know the molecular weight, $mg/m^3$ does not tell you what the partial pressure is. But it is a cumbersome figure, there is no doubt about it, and I think that is what probably has led to most of the problems--it is hard to say millimols per 25 cubic meters.

DR. BACK: If you don't know the molecular weight of the compound?

MR. WANDS: If it is particulates, the term is useless, there is no molecular weight.

DR. BACK: This is the problem that I have. I don't have to know the molecular weight to know how many milligrams are involved, but I sure have to know the molecular weight if I want to go to millimols, and what if I have an unknown?

MR. VERNOT: No you don't have to know the molecular weight because a mole of any gas occupies the same volume no matter what this molecular weight is. You can still express it as partial pressure.

DR. HODGE: Dr. Thomas pointed out that this will require us to put into a new phase of translocated values all our usual concepts of threshold limits which are parts per million or milligrams per cubic meter. We've been talking, and gotten sort of used to magnitudes expressed in these numbers. Isn't this going to do toxicologists no favors?

MR. WANDS: I think it was pointed out sometime, --perhaps at the Langley meeting, that the figure of millimols per 25 cubic meter is numerically equivalent to parts per million at ambient temperature and pressure. So that parts per million in this atmosphere, right here, is identical numerically to millimols/25 cubic meters. You see, I haven't gotten used to it either. I'm not used to it because I did not have to deal with these hypo and hyperbaric systems but for this new breed of cats that are coming along that have to work with these unusual atmospheres, this is a convenience.

DR. THOMAS: The point that we were pushing here, Ralph, during the last meeting and the meeting before that is as we explained, that we converted in space cabin toxicology from the use of parts per million to milligrams per cubic meter space. Because it is not dependent on the pressure in the cabin (whether you fly 5 psi, 7 and one-half, or ambient) as long as you give an absolute quantity it is meaningful and it is a familiar number to most of us. Now, when you say there is a convenience because this new expression is numerically equivalent to parts per million figures at ambient pressure that doesn't really help us in space cabin toxicology because parts per million figures are misleading.

MR. WANDS: It does not help the space cabin toxicologist, but it does help the conventional toxicologist who is the guy that used the TLVs. I don't think we've got any industrial operation at hypo or hyperbaric conditions yet, so this then is a bridge between the two operating situations, and it is a measure of concentration.

DR. BACK: We just need a third column, that's all, just put down the other figures, and you can use what you want, and I'll use what I want.

MR. WANDS: Ken, I don't think it is a problem for either you or me. We both have slide rules.

DR. BACK: I've forgotten how to use mine.

MR. WANDS: The committee was specifically requested to produce some more convenient terminology for the purpose of space engineers. This was what the space science board committee endeavored to do. I'm sorry that the man who requested this from NASA particularly isn't here to defend his request.

DR. FASSETTE: I just feel that I would like to agree with Dr. Thomas, that from a point of view of the toxicologist, there really are some advantages in talking about milligrams per cubic meter. Since we are interested in the dose to the person, we can think about this more clearly if we are talking about the dose deposited or absorbed, and as many of us are interested in biochemical assays of exposures, we really need to know this no matter what the ambient pressures and so forth are, and I think that this is one reason for not dropping this particular unit.

MR. WANDS: The only thing I would add to that, it was proposed at one time--and I'll just throw it out for information--It was pointed out that as toxicologists, we are becoming more and more biochemists in studying lesions and thus one has to be concerned with moles of material, how many molecules of material are coming in, not how many milligrams, but what is your molar dose in terms of number of moles available at the site of the biochemistry lesion. This is looking a long way down the road for toxicology, but it will probably take a long time before this new unit gets adopted.

DR. THOMAS: This might be a real simple question, but what happens when we find in operational experience, like the Navy did, that we have dust problems, aerosol problems--how will this problem work out?

MR. WANDS: I think that any recommendations for particulates or other materials which do not have a definite molecular weight or a measurable vapor pressure will have to be reported in terms of milligrams per cubic meter, cubic feet or some such term as that. We already have this problem with some of our threshold limit values, where we deal with materials which are known to be associated in a bi-molecular, tri- or tetra-molecular state, and yet we still speak of them as parts per million as though they were a unit molecule. Hydrogen fluoride is a classic case in point here.

DR. CULVER: Is the millimole per 25 cubic meter equivalent to parts per million at ambient pressure at 25 degrees centigrades or at zero degrees centigrade?

MR. WANDS: I believe it is at 25 degrees centigrade and 760 millimeters mercury.

QUESTION: I wonder how this new unit will work out in the case of nitrogen dioxide. Are you going to run into the same problems as with PPM, where you have a drop of temperature, and the equilibrium between $NO_2$ and $N_2O_4$ shifts so that milligram per cubic meter would be a much more meaningful measure in this case at least?

MR. WANDS: Or if one could measure the partial pressure of the contaminant at whatever temperature you are concerned with. You have to decide which, the $NO_2$ or $N_2O_4$, you want to measure.

DR. THOMAS: No system is perfect, but this means from now on you will not only carry around detectors for the agent you are looking for, but you had better carry around other instruments to measure vapor pressures under existing conditions.

MR. WANDS: One might also just try to educate space engineers to use parts per million. There is something, I've forgotten the exact relation between the 25 cubic meters and something like a 24-hour inhaled volume or something like this. It has a fairly close relationship to a normal daily inhaled volume. I've forgotten what the relationship is here. Dr. Nelson did mention it, do you recall that, Dave?

DR. FASSETTE: I think I can remember it since I first proposed that formula about 20 years ago. Parts per million times molecular weight times 2 is the milligrams of material which you will put into your lung per week if you assume ten cubic meters of breathing in an eight-hour day in five days a week. But actually you only retain half of it, so when you say parts per million times molecular weight you come out about right.

DR. HODGE: I wonder if I could ask Captain Siegel if he would be willing to tell us, off the cuff, some of the things that are going on at his place nowadays?

CAPTAIN SIEGEL: Regular ir rmation work on 90 days for a vast number of materials plus work under pressure at 25 and 50 psi, particularly carbon monoxide. Still working on dichloroacetylene. Found a new way to make dichloroacetylene where we can now make it by the ton, I think, and not blow up the place. So we are now ready to go to large chamber work on dichloroacetylene.

MR. WANDS: I had one question that was presented to me informally several times by a variety of people in the audience. It deals with the conditions of operation of the Thomas Domes. It is a 2-part question, I guess. First of all, do you have any data on the atmospheric composition in the normal control, Thomas Dome full of animals? In other words, what is your baseline atmosphere? Is there ammonia in it, for example?

MR. VERNOT: We don't have much information. The only work that we've done on what might be called metabolic contaminants that the animals themselves produce were taken rather early in the game, using these squeeze bulb-type detectors where you had so much ammonia, it turned so much of the tube green. We were never able to find anything that was above the threshold limit of these sensors. We tested, I think, for ammonia and for hydrogen sulfide. I'm not sure just exactly what else, but this is what it smelled like so we tested for them, and although the odor was fairly strong, we were never able to find detectable amounts of contaminants by this technique. However, in this work that we have done recently on the cabin materials gas-off products, there are peaks in the closed recirculating system that we see which are the result of animals being in the small

chambers, that is peaks that we get when we have no contaminants, just animals that we don't get from an empty chamber. So it must be coming from something that the animals are doing. We don't know what they are.

CAPTAIN SIEGEL: In our chambers at ambient where we have an air change every two minutes versus your every 20 minutes, we have between ten and twenty parts per million ammonia. I would suspect that at your air change rate you might have a much larger quantity, and what effect this might have on your exposure I don't know.

MR. VERNOT: What's the loading capacity of your chamber? Do you have more animals per unit volume? Perhaps that's where you are getting it.

CAPTAIN SIEGEL: Possibly so. In the Rochester chambers we probably have about 25 per cent of our area inhabited with animals.

MR. VERNOT: We have much more free space than that.

DR. CAMPBELL: This is more of a comment than a question, but we did notice in our chronic inhalation studies with nitrogen dioxide at about 25 parts per million in one hundred cubic foot chambers ventilated at about one change every two minutes that we detected, with dogs in the chambers, an alien particulate material on which more work has been done. I don't have much information on it now, and we call them "doggy nitrates" because they happen only with dogs in the chamber, and incidentally the loading was about, well, between 4 and 8 dogs in one of these chambers. I understand this has been reported in the literature, but I have not sought or seen this kind of thing in the literature. The nature of this material is being worked on, and I believe some reports have been submitted.

DR. THOMAS: We would be grateful if you could write us a letter when you find out where those reports are.

DR. CAMPBELL: I would be happy to.

DR. THOMAS: We have been thinking lately about increasing or doubling the flow rates through the chamber. There is one problem. It costs money. Remember that we are using oxygen which is very expensive. I think however, that this would be a good idea to try. Didn't we take some environmental samples out of the domes?

DR. BACK: We took freezeout samples, as I remember, and the amounts were relatively small of anything that we could detect at 25 CFM flow.

DR. THOMAS: As mentioned yesterday, you've got to keep in mind that we have almost perfect laminar air flow in the domes and as a result you could probably find ammonia and these things only if you would sample right at the exhaust of the dome, but not if you sample at the animal breathing level. We saw this when we ran some fire test. The way that smoke cleared out was dramatic. Very straight horizontal separating line of air pushing the smoke front down. This might indicate that it makes a difference where you squeeze your bulb.

DR. MAC EWEN: For those of us who have been in these chambers either at altitude conditions or at ambient conditions, we would not really identify these odors as animal metabolic waste products. The odors are not like animal feces or urinary smell. In fact, they do not smell to one who would like to think he has an analytical nose like ammonia or any of the low molecular weight materials; it is more of a fruity or sweet smell. It is not pungent. It's really not unpleasant, just has an odor.

MR. WANDS: This amplifies the remark that I was going to make. It sounds to me very wise, before Dr. Thomas spends a lot of money on additional oxygen, to get some good analytical values, using techniques a little more sensitive than a MSA sniffer, and looking for other things besides the obvious $H_2S$, ammonia and things like that.

The other part of the question has been posed to me by the audience in general, and let me throw it out at this point also: There was a great deal of concern after today's papers particularly in electron microscopy, that there seems to be a "Thomas dome effect" in controlled animals or perhaps in the experimental animals which is not yet identified. Can you comment further on that?

DR. MAC EWEN: Let me make a few minor comments on this. When we say there is an effect, there is a minimal effect. The effect appears to be one of stabilizing the animals more to a better stability level than we do have in an ambient control room or an animal room. That is, there is less variation around the normal values; there is less variation from day to day, or week to week, in most of your clinical determinations. They follow the same kind of growth patterns as they do outside the dome. The growth rate is approximately normal, but they seem to be more stable animals because probably their environment is held much more uniform continuously. There is less than a two-degree Fahrenheit variation in temperature from day to day, week to week, year to year. The humidity is held at 50 RH with $\pm$ 10%. This is considerably different than your environment and mine and the environment that the animal normally has in a vivarium.

MR. WANDS: Is constancy of this sort going to produce histopathological changes, or histological changes?

DR. MAC EWEN: Not expected to be.

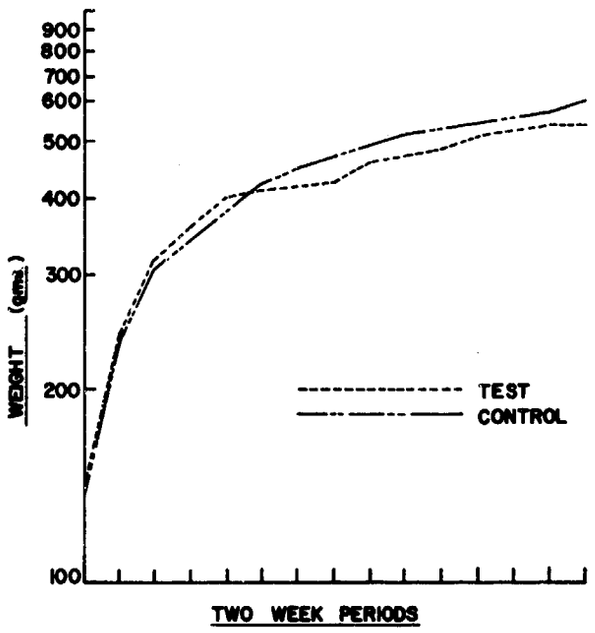
MR. WANDS: This is the question about the Thomas Dome effect.

DR. MAC EWEN: Well the effect would be then to not really produce any change relatable to the Thomas Dome itself.

DR. THOMAS: Dr. MacEwen, this is the time for those slides. I think right now we are getting back to that mixed gas study.

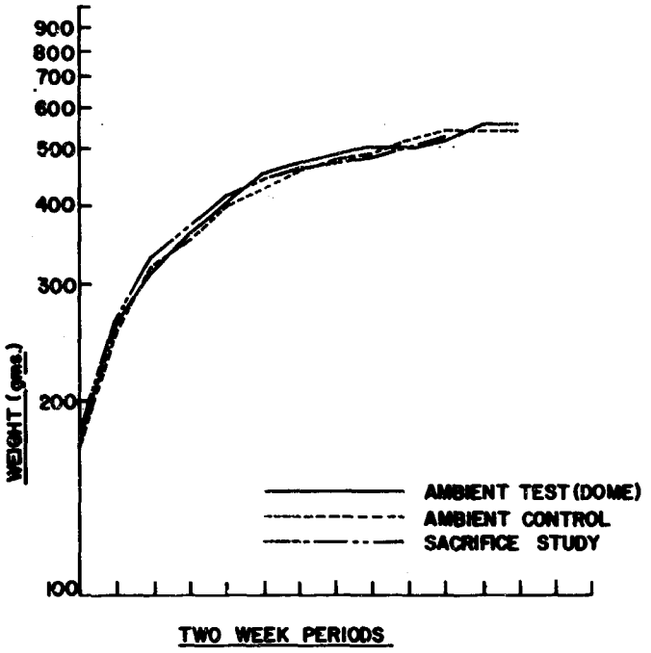
DR. MAC EWEN: Last year Dr. Fairchild, now with the Public Health Service, presented some information on our first eight month study with the 68 per cent oxygen, 32 per cent nitrogen environment at 5 psia. We have since repeated that experiment. One of the most important factors that we noticed in the first run

was an inverted AG ratio in almost every dog that was put in the chamber. We deliberately biased the repeated experiment in trying to select dogs that had unusually high AG ratios, that is, high normals. In rats, particularly in male rats, we saw a decreased growth rate over the eight month period. I might also mention that in the dogs that exhibited the inverted AG ratios there were significant increases in absolute weight of the liver mass and of the liver to body weight ratio. If I could have the first slide?



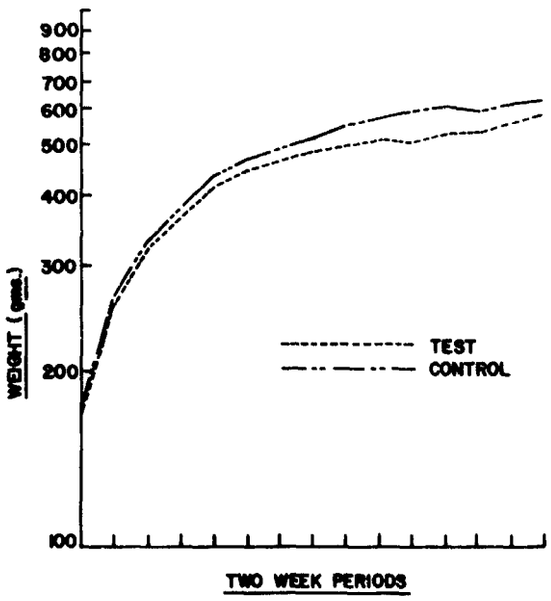

GROWTH CURVE FOR MALE RATS--8 MONTH MIXED GAS STUDY
(68% $N_2$ - 32% $O_2$; 5 PSIA)

This is the first mixed-gas exposure. The upper line represents the control rats housed in the animal room. The lower line is the growth curve for male rats exposed in the Thomas dome for eight months to the mixed gas environment at 5 psia. You will see that the exposed animals have outgrown their controls. The difference was not statistically significant, although it looks like it should be. The lack of statistical significance was due primarily to one unusually large control rat. He didn't change the average, but he affected the standard deviation, which kept the weight difference from being statistically significant. Now if I can have the next slide:




GROWTH CURVES FOR MALE RATS UNDER VARIOUS ENVIRONMENTAL CONDITIONS

The three curves shown here are groups of male rats again, all of which Mr. Egan talked about yesterday. The solid line at the top represents the male rats that were used in the ambient exposure study in the domes for six months. The dotted line represents the ambient controls that were used in the same study and the dotted bar line was another group of rats that came in at the same time from the same large group and were used in a sacrifice study. All of the growth rates shown here are identical with that of the control rats shown in the previous slide. Now if we can have the third slide:




GROWTH CURVE FOR MALE RATS--REPEAT 8 MONTH MIXED GAS STUDY (68% $N_2$ - 32% $O_2$; 5 PSIA)

This is the latest eight month mixed gas study that was just completed about three or four weeks ago from which the pathology has not been evaluated. We've taken the samples but have not begun to work on them. Again, you can see the significant difference which is statistically significant point by point. About the second month the exposed male rats begin to show a depression in growth rate. The growth rate for exposed female rats was depressed slightly, but the difference was not statistically significant. This slide shows that we have reproduced essentially identically, the rat growth rates shown in the first experiment and as Carrol Weil pointed out yesterday, one of the most sensitive tests in many toxicological studies is growth rate rather than one of the multitude of chemical or clinical chemistry parameters measured. I should also say, in the repeat experiment the AG ratios, of the dogs which we selected for exposure in a biased manner, were not significantly altered. However, we did get an occasional inversion of AG ratios in some dogs but they did not persist like they did in the first study. This information is just eye-balling of the preliminary data as we just said. The experiment was just finished.

DR. THOMAS: I want to point out that in repeated eight-month,5 psia,mixed-gas study, we don't have the pathology yet, but we duplicated one result and that it is consistent:--decreased growth rate in male rats. This is a fact of life. Now as for the "Thomas dome effect": those growth curves which we had up there--they overlap exactly. So we know that we aren't dealing with artifacts due to the dome per se. The outside dome controls for five months in ambient air, and the six months ambient control in the dome, overlap perfectly. So by the most sensitive definition, rat growth rate, we don't see any effect from the dome environment, but we sure do see effects from the 5 psia mixed-gas environment.

DR. FAIRCHILD: Doug, I'm really interested in knowing--this is the first time I've heard: Would you mind telling me, on your AG ratio, albumin, globulin, did you use a salt precipitation or electrophoresis or both in the determination?

DR. THOMAS: We tried both methods and I want to talk to you about that, but I'm not ready to discuss it in the open--no statistical analysis yet.

DR. LEON: I would like to make a comment on the differences reported yesterday with regard to the higher hematocrits of dogs in the chamber as compared to the dogs outside the chamber. We are working on blood, so we have looked into this a little bit. In the late twenties Barcroft showed that in the dog, the spleen particularly is very responsive to manipulation, and if you don't handle the dog right you can cause up to a 15 per cent change in hematocrit by causing splenic contraction. This can happen in a matter of seconds or minutes. In our blood studies that we are performing now, we are not working with dogs, we are working with rats; we have endeavored to use splenectomized animals to avoid this complication.

DR. THOMAS: For your information, the way we draw blood in the Dome is not an ideal procedure at all. We will have it solved in the new dome facility where we will have an inter-connecting operating room to do this type of thing, but at the present we have a small aluminum flip-up table attached to the entrance bar

which is usually a little bit too small for the large Beagles and maybe we are squeezing the spleen while restraining these dogs. Of course in the control room studies, the outside controls, there is no such problem because we have all the facilities and the comforts to draw blood from the control animals. This might be a good point.

DR. LEON: I would say that is the basis for your differences then.

DR. THOMAS: It very well could be. This would be true with the monkey too, since they are even more difficult to get blood out of.

DR. SOPHER: Just a comment on this. Certainly autotransfusion is a good possibility; the spleen is rich in cells and relatively deficient in the fluid fraction. From the day that they are bought are bled at specified intervals so that the animals are really used to being handled, used to having a needle put in their neck. We certainly do have some disadvantages in drawing in the dome, there is no doubt about that, but the dogs aren't particularly wild; vicious dogs are usually excluded from the experiment; all are rather docile. I think your point is valid but I really don't know how significant it would be in our situation.

DR. LEON: You just said there was a difference in the time involved in the blood withdrawal.

DR. SOPHER: Part of the reason for this is, if you have four people trying to bleed a dog in the space that I'm sitting in which is roughly the amount of room you've got in the dome to do this, you are running into each other, and it is just as far as the manipulations go--it makes it much more difficult, plus you've got on a beta cloth suit. Your tools are setting half way across the dome on a monkey cage, perhaps you have to reach for a needle and you might drop the fool thing. It is not an ideal situation. But as far as the handling of the dogs goes, these dogs are used to being handled. They are fairly well trained to accept being bled.

DR. WILSON: I'm not working with dogs now, but a number of years ago in Berkley I was in a lab where they were working with dogs, and it was a high altitude study at that time. It was a blood study also; and in this case also it came across to me and to the investigators that you had to be extremely careful because the slightest amount of epinephrine release will cause this contraction; so I would simply suggest, and I think it would be an easy thing to do, just handle the dogs in the same way in the chamber and outside the chamber, and of course the simplest thing to do is to stress them, either by noise or by slapping them in some fashion, get a little bit of epinephrine flowing. In this way you will get maximum contraction in both cases in which case you should get the higher hematocrit in both cases.

DR. TOWNSEND: On this business of hematocrits, I can't recall the particulars of the paper at the moment, but it was in this last week's issue of the Journal of the Americal Medical Association, as I recall a clinical article in there depreciating the value of the hematocrit anyway as being a reliable guide in clinical surgery to give you any indication of what your blood volume is really doing. It didn't occur to me that it might come up here, but I would recommend that you look at that as a re-evaluation of what the hematocrit is going to tell you in the first place.

DR. FAIRCHILD: I just want to ask one thing before we get away from this subject. We are talking about domes and smells and this and that and the other. Some people are concerned probably that maybe they are influencing something. I would like to know, ask NASA, do they have something on the atmosphere in a space cabin with two to three smelly astronauts after quite some time insofar as odors and analysis--anyone volunteer some information?

MR. WANDS: Ray Saunders, can you comment on that from your analyses of some of the charcoal samples or anything of that nature?

MR. SAUNDERS: I've asked on two or three occasions people who should know at Manned Spacecraft Center whether or not there was any smell in one of the space cabin atmospheres and they say there is none; at least that was what I understood them to say. I couldn't tell what odor might be present from a charcoal analyses. The compounds that we do find, of course, do have odors, but how you could relate that back to any odor in the cabin, I don't know.

MR. WANDS: Thank you, Ray. I suspect by the time one has lived in a spacecabin one doesn't notice them any longer anyway; this may be the source of the comment that no, it doesn't smell.

MR. SAUNDERS: I can say on the basis of personal experience that a submarine atmosphere has a distinctive characteristic odor not necessarily unpleasant, but it does have an odor that you can recognize, and I would assume that a spacecraft atmosphere would also.

DR. HODGE: This may be a naive question completely out of place here, but are there plans for, or have there been conducted simulated 300 day and a thousand day spaceflights on earth with good medical supervision, air analyses and so forth? If that's a wrong question, just forget it.

DR. LEON: We have just written up budgetary type experiments to get a ball park figure for the budgets in the next five years, and the experiments were written around a 90-day simulated flight.

DR. THOMAS: I believe that it becomes increasingly difficult to get volunteers beyond 90 days. As a matter of fact, I don't think that you can pay them enough to do this for 300 days. It seems like 300 years to them, I'm sure. You really wonder whether with that little in it for the experimental subjects, you can ever find people who are willing to go into simulators for such a long time.

DR. BACK: I wanted to know from Dr. Barrow how much voltage or amperage is used in this capacitance respirometry, because we are certainly interested in the possible application of this at altitude and we need to worry about how much electronic gear we put in the domes.

DR. THOMAS: No problem, it was, I believe, ten microamps or something like that. No fire hazard involved, besides we have shielded cables, so we are in good shape.

DR. CAMPBELL: There has been considerable discussion concerning the monkeys versus dogs as study subjects in these experiments, and particularly, in regard to histopathology of the lung, I am wondering if there has developed any consensus regarding the suitability or preferability of one or the other as to extrapolation to inhalation toxicity in man?

DR. THOMAS: That's good because that takes us into Session II.

DR. CAMPBELL: I'm sorry, I thought we were on Session II but it could have happened in either session. Well, before you field that one, anyone, we have been doing a little fragilograph work down in Cincinnati, and while none of this is published yet, we have seen fairly consistent results with nitrogen dioxide at an admittedly elevated level, 25 parts per million in dogs. We have noted a decrease of fragility. Now I make no attempts to explain this as yet, but I think it is in the opposite direction of your results in this area with oxygen toxicity.

DR. BACK: Dr. Carter has found increased fragility with monomethylhydrazine, so did the work done over in Israel with oxygen.

DR. CAMPBELL: And there was some English work some years ago that got me on this in the first place which reported spherocytosis of red blood cells in mice exposed to ozone. From this I felt there might be an increased fragility in these cells. We did not follow up with the mice and all I have is dogs, and we did not follow up with ozone, but we followed up with $NO_2$ which is also an irritating oxidizing compound. We found the opposite results and we are still puzzling over this one.

DR. BACK: I have to refer back to Dr. Carter when he gets here tomorrow, but he did a comparative study and I don't have the data here but it seems to me that in general the dog has a peculiar red cell that is more fragile than any other species that he has tested so far. So we have a peculiarity here that we must worry about. I think that he studied the monkey, the rat, the mouse, and every other species he could get his hands on. I think that he found that the dog has the most fragile red blood cell of all the species that he worked with.

DR. TOWNSEND: On the business of extrapolation to man, I gathered from the rather philosophic discussion this morning that no one was prepared to take a stand on this at this moment, that it was the consensus that perhaps we should continue to work with all three animal species for a while to get a better fix on just what changes were being seen. Now that was my interpretation of that.

DR. THOMAS: Of course we don't even know whether we are looking at oxygen toxicity in the case of mixed gas; we might even be looking at hypoxia.

DR. TOWNSEND: You are seeing mitochrondial changes, whether they mean anything or not I'm not prepared to say. But certainly mitochrondia are where enzymes are made, and I think somewhere along the line we are going to have to do a lot more, know a lot more about the enzymology of these animals. Of course this gets us into a tremendous field, because in the case of the human, we find besides the glucose-6-phosphodehydrogenase and other enzyme deficiencies we have in red cells that it can make a difference in how people react to different stresses, drugs, as well as other things in their environment. So without knowing some of these things about these animals, we still will have to answer those questions. It would be good if there were a body of knowledge on the enzymology of these animals, especially the enzymes in the Emden-Meyerhoff pathway, because there might be some clue to interpret these mitochrondial changes, if indeed they can be interpreted.

DR. MAUTNER: May I make a remark about the relationship of these various species to man? As far as the kidney is concerned, I have had the opportunity to see mice, rats, rabbits, monkeys, dogs, and others. There is no question but that the monkey kidney resembles in appearance the human kidney more than any other. Whether it functions in a similar way that of course we cannot tell. Nonetheless, there are substantial differences in the appearance of several of the organelles between the various species and the monkey and the man are virtually indistinguishable electron microscopically.

DR. CAMPBELL: I just wondered if someone could comment in the same way about the lung?

DR. THOMAS: I would like Dr. Weibel to tackle that. Before we do this, if you recall the conclusions from the eight-month, 5 PSI oxygen study, light microscopy indicated that the dog was the most sensitive species as far as the lung damage goes with oxygen. So if we want to decide whether we have oxygen toxicity in the lung with the mixed gas atmosphere I think the choice of the dog is quite superior. Besides, it is about the cleanest lung, when you do morphometry. I think Dr. Weibel would like to say a few words as to why he selected the idea of looking at lungs only in dogs.

DR. WEIBEL: May I show a few slides.

DR. THOMAS: Absolutely.

DR. WEIBEL: Well I have made some comments already on that this morning. I am first talking about the pure oxygen at one atmosphere, 760 millimeters of mercury. We have studied three species the rat, first, and then the monkey, and now also man. Actually the human studies are done by Dr. Kapanci who is partly with us and partly in Geneva. I can show you one slide of a human lung. This is a human lung from a patient which has been in an external respirator under pure oxygen for 12 days. You see the airspace on the left and the red cell in the capillary, and you may note that here (pointing) we have instead of the usual very

thin epithelial lining, we have a rather thick epithelial lining. You see microvilli on the surface and you see these osmiophilic bodies lots of organelles, lung mitochrondia, which are characteristic of Type II epithelial cells. Now this is 12 days of pure oxygen breathing at one atmosphere. This other slide shows the picture from the monkey at 12 days, pure oxygen, one atmosphere. As you can note, exactly the same picture. So in that respect we can say that the monkey is apparently quite similar to man also in terms of the time course. Now we have not done any morphometry yet on human lungs with oxygen; this is underway. This is more difficult because of the preparation problems in getting good human lungs for these studies.

Now with respect to the rat: We have an entirely different situation there. The rat never survives in our hands, at least with our breed, to a state where it achieves this type of hyperplasia in the epithelial layer. But it is our experience in the rat first there is destruction of capillary endothelial cells and then as a consequence, leakage and formation of the interstitial edema. Destruction of epithelial lining is secondary, and then exudation into the alveolae and then they just die. Now the monkey does exactly the same thing with a difference that it survives the destruction of a large number of alveolar epithelial lining cells. I think in Dr. Kapanci's work there were about 30 per cent of the alveolae still patent, 70 per cent being completely clogged up with exudate, as Major Robinson has shown to you in his study which was exactly identical; but then you get this regeneration. And with the regeneration with the formation of these epithelial cells, the alveolar content is resorbed and you have again functioning alveolae, and this thickening of the air barrier, which is about four or five times thicker than normally, but which apparently is still adequate for gas exchange. These monkeys are quite happy after a certain time, although apparently they get sick again. We just went up to 12 days. So the rat certainly is not a suitable animal to extrapolate to man. The monkey is and I don't know what the dog does. We have never studied dogs in that respect. Maybe one should do that, I don't know. The reason why I would prefer dogs instead of monkeys for detailed, quantitative studies in pathology, is because you can obtain dogs that are clean in terms of their lungs while this is very difficult with monkeys; and secondly because there is a wealth of data on respiratory physiology of the dog whereas we do not have any comparable amount of data for the monkey.

DR. TOWNSEND: Does that help resolve our problem of extrapolation?

DR. RIESEN: I don't want to get into any arguments as to what constitutes a normoxic condition, other than to say this: I think a lot of the species problems in the area of oxygen toxicity in the lungs, whether we have toxicity or whether we have hypoxic conditions, can be arrived at by considering one thing. Namely, how much oxygen is really getting to the tissues? There are techniques now that are available for getting some measure of the amount of oxygen delivered peripherally to the brain, liver, kidney, and certain other tissues. This certainly is important because of the differences in the hemodynamics and in the lung structure. But then, when you get down to the cell, Dr. Chance, in a talk he presented at the International Congress last Saturday in Washington, pointed out that there is now a very good physical index of the intracellular oxygen concentration. He has in fact determined the level of oxygen that is compatible with the life of a cell, intra-

cellularly and this comes out to about $7 \times 10^{-8}$ molar oxygen or 0.04 millimeters of mercury of partial oxygen pressure. This is arrived at in the case of the brain of the rat by at the same time measuring EEG as a measure of cellular activity and measuring NADH by fluorescence, remotely from the outside of the perfused organ in the live animal. Now NADH is in equilibrium extra-mitochondrially with the NADH intra-mitochondrially and intracellularly. This in turn is then in equilibrium with the flavones and all the other redox systems that finally go to oxygen. The interesting thing is that there is an exact coincidence between the falling out or the flattening out of the EEG, which I am sure we all recognize as a major measure of cortical activity in the brain, and the level of NADH. He says in his case when he got 80 per cent reduction, the EEGs were flattened out completely. I think here we have a fairly precise measure of the intracellular oxygen level which is compatible with life. Here we have defined a maximum degree of hyperoxia which can be tolerated by the cell. Now in these cases, even in the mixed gas atmosphere where we have some question as to whether we are having indeed hyperoxic conditions or a hypoxic condition at the cellular level, I do think that beyond measuring the $pO_2$ in the blood, possibly some kind of measure of the cellular $pO_2$ would be highly desirable. These newer techniques may not be applicable necessarily in all kinds of environmental circumstances, but I think they can be incorporated into some of these dome experiments.

Regarding the freons that have been discussed, this area is very related to the problems of halothane anesthesia. The work of Pauling and the work of P. G. Cohen at the University of Pennsylvania, and others, has suggested that anesthetic levels do inhibit reversibly the acceptance of hydrogen by NAD. Not only that, by X-ray diffraction it has actually been shown that the actual oxygen dissociation curve of myoglobin can be affected by things like Xenon, and if you extrapolate this to things like inert gases, anesthetics, halothane, and halogenated hydrocarbons, it is entirely conceivable that these compounds are actually affecting the oxygen dissociation curve not only of cytochromes intercellularly but of myoglobin in muscle and of hemoglobin in the transport of oxygen to the cells. These are two thoughts that I wanted to leave with you

DR. WEIBEL: I was just going to comment we have to carefully differentiate what we are talking about. I was only talking about the oxygen toxic effect in the lung and maybe in the blood. These are the first target organs of oxygen. Dr. Riesen is completely right that if we want to talk about the kidney or the liver changes, then we have to take an entirely different point of view, because we have some kind of buffer inbetween: the blood and maybe even the lung. When you retain only about 30 per cent of the lung functioning, and this 30 per cent is still impaired as we found it, you are bound to get relatively low oxygen out at the periphery. I think Dr. Kaplan has made some measurements of $pO_2$ in the monkeys that we have been studying. I don't have the figures right with me. Is Major Robinson here? Do you have the figures?

MAJOR ROBINSON: No, not with me. I can get a slide of mine that I didn't show this morning.

DR. THOMAS: There was more work done in that area which has not been completed yet. It's going on.

DR. WEIBEL: That was on the same animals that I showed.

DR. THOMAS: We are adding more data. You are referring to the initial uncoupling of oxidative phosphorylation in the liver?

DR. WEIBEL: Yes, I would like to differentiate these two aspects. First, I think the cleanest signs of oxygen toxicity at one atmosphere are in the lung and the blood perhaps. These are the places we have exposure without anything in between and what we get in the kidney and the liver may be much more complicated in its genesis.

DR. THOMAS: I'm kind of inclined to believe now that with oxygen at one atmosphere pressure we pretty much can say that the primary target organ is the lung because anything biochemical which happens in the liver and the kidney at the subcellular level is of only academic interest. It is of no practical concern, because the animal dies of the lung damage and he still has, for clinical purposes, a functional liver and a functional kidney when he dies. Now this is not true at 5 PSIA or somewhere inbetween, but when you go to one atmosphere oxygen, then the story is very simple. Major Robinson has a slide.

MAJOR ROBINSON: I just wanted to show you this slide on one atmosphere oxygen that I didn't get to show you this morning. It shows the tremendously high arterial $pO_2$ in the neighborhood of 400-500 millimeters of mercury. I think these roughly parallel those figures that Dr. Kaplan had in his animals. I don't believe his figures are quite as complete and possibly our interpretation is not quite the same. These arterial $pO_2$s were maintained at a very high level until the very terminal illness of the animals.

Those low values are in those animals that were terminally ill and were sacrificed before they could die so that we could get some of this information. On those terminally ill animals the $pO_2$ was low, the $pCO_2$s were over a hundred. We could only measure up to a hundred and they were well over a hundred indicating that the perfusion was completely inadequate in that there was retention of $CO_2$ and very little diffusion of oxygen into the blood. These samples were taken from animals in the chamber but the measurements were made outside. The squirrel monkeys were different from the irus and the baboons as I mentioned this morning. They maintained high arterial $pO_2$ clear to the end with that one exception, the one that Ken is pointing to. That was the animal with the brain hemorrhages.

DR. BACK: That was a 120. All the rest were 515, well up to 515. These are terminal at 515. Here is one terminal at 365, the squirrel. The terminal was at 52 in the baboon and 76 in the irus.

DR. TOWNSEND: Are there any other questions or comments then on the areas of histopathology that we have covered?

DR. BACK: I had one question that has to do with where we are going from here on carbon monoxide. This is sort of directed at Dr. Robinson. He did this work on baboons because we were interested in knowing whether we ought to go towards the baboons rather than the monkey. We have a few more baboons which

we have had around for quite some time. We have some good baseline data on them and we are debating on the next 90-day carbon monoxide study as to whether the baboon would be a good choice over other species, the Macaca irus for instance? Would he have any comments on this?

MAJOR ROBINSON: Well, my experience with baboons is what you have seen this morning, but my subjective feelings are that these animals are probably superior to the other three species of subhuman primates that we have used--The Macaca irus, Macaca mulatta or the squirrel monkey, and subjectively it seems to me that they were more susceptible. I hasten to add that these were very young baboons and the fact that they were young may have some influence on their susceptibility. I did mention that we did find lung mites in these baboon lungs but there certainly weren't very many of them, and just on the basis of the natural occurring diseases in the lung I would certainly prefer the baboon to any of the other three. They were, I would say, very clean generally speaking. Phylogentically I don't know how they stand. These little baboons that we used were only six, seven, eight pounds, and I hear rumblings from my cohorts in the rear that we should put a really big baboon in there. Of course these rascals do get quite large and difficult to handle; but on the other hand, the younger baboons are really fairly accessible, they are not hard to come by and they could very well be a very decent type of laboratory animal to use, keeping in mind that they are young, and naturally as with any of the other subhuman primates in our laboratory they have to go through about a 90-day standardization process before they are ready to be used. I think it is just a matter of using them to the point of where we are either convinced they are good or are not. I personally was quite impressed with the few that we looked at here.

DR. TOWNSEND: Another point in favor of this is that there has been accumulated quite a bit of baseline data on the baboon, particularly at Southwest Research where they have been working with baboons for many years. Is there a baboon expert in the audience to defend or condemn this animal?

DR. THOMAS: I believe we have a rabbit expert--Jac, do you still hate rabbits?

CAPTAIN SIEGEL: Hate them like poison.

DR. SOPHER: I can't say that I'm a baboonologist or anything like that, but I did have the opportunity to do necropsies on a dozen or so adult baboons that were being used on another project, and all I can do is reiterate what Major Robinson has said--that these animals wild, caught in Africa, and brought over; and as a wild caught animal, standardized here, had very good lungs, very little endogenous disease at all. On the other hand, the brutes do get to be 50 or 60 pounds and they are approximately five times as strong as any men, so that can lead to some problems.

DR. TOWNSEND: That would reduce the number of hematocrits, wouldn't it?

(Laughter)

DR. SOPHER: As a matter of fact, one of the technicians said that if we start using baboons, they are going to ask the animals if they want to be bled!

DR. LITTLEFIELD (Hazleton Laboratories): I don't know if any of you have seen the article I saw about a month ago; unfortunately I can't remember the author or the source. It was one of those times when I was in a hurry but this author classified animals according to anatomical structures and he showed according to this classification their susceptibility and relationship to each other. Unfortunately the monkey, and most of the laboratory animals, weren't in the same classification as man. It was very interesting to note that one animal did fall in the same classification as man--the horse, on which for obvious reasons inhalation studies are unreasonable. One other comment in relation to species. We use the cynamolgous monkey quite extensively in our laboratory and we've had very good success with this species.

MAJOR ROBINSON: I can't share this gentleman's enthusiasm for the Macaca irus. We have seen a good bit of spontaneous lung disease in the ones that we have handled, as Dr. Sopher says, including tuberculosis.

DR. FAIRCHILD: Dr. Back asked a question a while ago, and I don't know whether he got a direct answer. In view of the fact that there is a well known discrepancy in oxygen as well as irritant toxicity, there is an age related factor, it looks to me as though you should go ahead and use your old baboons and try to get in immediately some young ones and do a little comparison right there. This would be one way of starting to use these animals because I think Dr. Sopher mentioned a very great difference in the young animals and the older animals in oxygen toxicity. In relation to the use of horses, people are beginning to use the burro to quite some extent, as you probably know, in the study of emphysema.

DR. HODGE: I can see the next one is going to be: "The Dome on the Range".

DR. BACK: Would you like to sing that for us?

DR. CAMPBELL: I wouldn't touch that one. But since emphysema has been brought up, I recall that Dr. Robinson mentioned emphysema among his findings this morning, and I wondered if he found that fairly consistently in all species, or whether it was limited to one; and also, what type of emphysema it was?

MAJOR ROBINSON: What I was talking about this morning was seen in the rhesus monkey and to a fairly limited extent in these oxygen toxicity animals exposed for lesser periods of time. I frankly haven't seen much emphysema. I don't really know how you would classify it. Are you talking about a destructive emphysema?

DR. CAMPBELL: Yes.

MAJOR ROBINSON: I presume this would be called a destructive type, and I think there is good reason for it, because the lung volume is quite limited there and I am sure there are tremendous pressures brought about to cause this sort of thing.

SESSION IV

# INSTRUMENTS AND DETECTION

Chairman

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PAPER NO. 21

# EXPERIENCE WITH THE SMA-12 SIMULTANEOUS MULTIPLE ANALYZER IN ANIMAL TOXICOLOGY

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Among many advances in clinical chemistry is the development of instrumentation for multiple analyses on a single serum specimen. These instruments are designed, by and large, for screening large populations for a set battery of chemical determinations. One such instrument is the SMA-12-30 produced by the Technicon Corporation. We became interested in the possibility of using such an instrument for screening large numbers of animals in toxicological experimentation. The purpose of this report is to share some of our experience in using the SMA-12-30 in such a context.

The SMA-12-30 is an instrument designed to perform 12 simultaneous analyses on a single serum specimen at a rate of about 30 specimens an hour. The machine we purchased performed analyses for total cholesterol, calcium, inorganic phosphorus, total bilirubin, uric acid, blood urea nitrogen, glucose, alkaline phosphatase, lactic acid dehydrogenase, total protein, serum albumin, and serum glutamic-oxaloacetic transaminase. Descriptions of the principles of operation and individual chemical analytical methodologies are available elsewhere.

In using the SMA-12 it became evident that some problems exist in adapting this instrument for use in an animal-oriented toxicology laboratory. These problems fall into three major areas. First, two of the twelve channels are essentially useless when the specimen is from animals which we routinely use, i.e., Beagle dogs, Rhesus monkeys (Maccaca mulata), Irus monkeys (M. cynomologus), baboons (Papio sp.) and domestic pigs; these channels are uric acid and serum albumin. The uric acid channel is not usable because in the species mentioned the end product of nitrogen catabolism is allantoin, not uric acid (Fruton, 1958.) The albumin channel fails because the chemical reaction is, apparently, not adaptable to non-human serum proteins. This procedure is based on the selective binding of a dye molecule to the serum albumin molecule. Since animal albumins differ from human albumin in both amino acid sequence and secondary structure the dye binding to the molecule is affected. Rhesus monkeys average about 75% of the dye binding capacity found in human serum and Beagle dogs about 55%. At first we thought that a simple correction factor might be applied so as to give a

"true" reading. However, when several series of monkey and dog sera were analyzed in parallel by the dye binding and a standard salting out method the degree of scatter was such that no correction factor could be determined. An example of such a comparison is given in table XVIII.

The second general category rests in methodology of analysis. Again two channels can present problems. These are the alkaline phosphatase and lactic acid dehydrogenase (LDH) channels. The problem here is not that the methodology does not work but rather that in some animals the serum activity of these enzymes may be so great as to go off the chart. This was often true of alkaline phosphatase when serum from young monkeys was analyzed. High LDH results could occur with any animal and reflects, among other things, the degree of activity and trauma the animal underwent when it was captured for bleeding purposes. Also, red cells contain several thousand times the LDH activity of serum and if there is a significant degree of hemolysis a spuriously high result will be obtained. We read of a modification that Hazelton Labs have used in the alkaline phosphatase procedure for use with monkeys and found it to solve our problem as well (Busey, 1967.) Briefly, the method is to set the chart print-out at half scale with the standard (e.g., if the standard contains 18 KA units, one would set the machine to read 9 KA units). At the same time, double the usual amount of substrate is used. This is done to ensure that the reaction rate is limited by the amount of enzyme activity present and cannot go to completion. We were able to verify that the results do remain linear and that multiplying by two will give the "correct" answer. With the LDH we elected to leave the instrument as it is. The reason for this is that the results are highly variable and scattered even among normal controls. For that reason we feel that one must be suspicious of single animals having increased LDH activity. In the majority of cases the LDH results will fall within the normal range of the instrument. The third problem group that we found might be called operational difficulties. These have to do with the peculiarities of operation of the instrument. First, there is a very short wash cycle between specimens. This will allow a specimen which contains a large amount of some constituent to affect the result obtained on the next specimen if its content is markedly lower. For this reason we feel it is important to group the specimens by species, in some cases by age (e.g. monkeys) and by clinical history when processing them. This will tend to minimize the problem as the several specimens in a single group will usually be similar in their chemical content. Should a marked difference occur then the results obtained on the following sample must be considered suspect. For this reason it is advisable to run more controls than are suggested in the operations manual. We used pooled serum of the species being run as every 5th to 10th specimen. Since these should all assay about the same, a significant difference would indicate either an instrumental malfunction or follow-on contamination by a preceding specimen. As a check on the precision of the SMA-12, we took the results from the replicate analysis of pooled monkey and pooled dog serum and determined the mean and standard deviation on each of the 12 tests done. The results, shown in tables I and II, were determined from 41 to 49 separate aliquots of a monkey serum pool and 39 to 56 specimens of dog serum pool. As can be seen, the instrument, in general, functions with great precision. Because we use plasma rather than serum as a specimen, the reasons for which are discussed below, another problem may occur. This is the precipitiation or formation of small coagula of protein. These can block a line, especially in a dialysis plate.

To guard against this, one must wash the system completely, following each day's run, for a minimum of 30 minutes.

Another problem is in the preparation of a specimen suitable for analysis. Since this instrument requires 3 ml of fluid this would mean about 6-8 ml of blood is required for that purpose alone. If other analyses outside the SMA-12 battery are contemplated then even more blood is required. Fifteen milliliters of blood is not an unusual requirement if an exhaustive battery of clinical laboratory procedures is to be done. This is a large amount of blood when taken from a 3 kilogram monkey, especially if the battery is to be repeated several times in a short period of time as in a pre- and post-exposure series. For that reason, the amount of blood to be drawn should be minimized. Also if a single sample could be taken and all routine tests run from it, the glass loss factors, etc. would be decreased as well. Also, a single sample is easier for the technicians to draw. This can be important when the animals are housed in a hazardous environment due either to a dangerous atmospheric contaminant or reduced pressure, not to mention it is less traumatic for the animal being bled. For those reasons we decided to see if plasma could be substituted for serum. The report from Hazelton Labs declared that they did successfully use plasma. When plasma is utilized a 10 ml sample of blood will generally suffice where a 15 ml sample was previously required. The reason for this is twofold; first, one does not require a separate sample for routine hematologic tests, about 2 cc; instead the minute amount required (less than 0.5 ml) may be drawn from the tube before it is centrifuged to harvest the fluid phase. Second, we obtained from 10-20% more fluid when plasma was used as compared to clotted blood. The question came up as to whether the results from plasma would be the same as from serum. We ran a small experiment to see what differences might be found. Since prior experience showed that dog blood was the most fragile and because we could safely bleed dogs for a larger amount of blood we used large mongrel dogs as our donors. Thirty milliliters of blood was drawn from each of ten dogs. Each specimen was separated into aliquots of 10 ml each. One aliquot was allowed to clot, one was heparinized with sodium heparin and the third was heparinized with ammonium heparin. The samples were then analyzed for the SMA-12 battery and for several other parameters included in our routine screening procedure. The results of the analyses are given in tables III through XVII. As can be readily seen there are certain differences which are major in proportion. These occur in plasma/serum hemoglobin and lactic acid dehydrogenase. Individual differences but not especially significant are seen in potassium. The explanation for this resides in the fact that these are constituents found in large amounts in red cells and increased serum/plasma values reflect red cell damage. The data show that in dogs the red cells are more stable in plasma than in serum. So from that viewpoint plasma is possibly more desirable as a specimen when dogs are the experimental subject.

Lastly, if something goes wrong with the machine while the specimen is being processed, all 12 results are often lost as well as the 3 ml specimen. This usually means that no data can be had for that run for the specimens involved as the animals would probably not tolerate another bleeding.

These then are the major problem areas of which we feel one should be aware if use of such an instrument is contemplated. On the plus side of using an instrument of this type, a limited number of technicians can produce more data in a given period of time than has ever before been feasible. In a single day well over 1000 individual analyses have been made on our SMA-12. Because of the amount of serum/plasma required the instrument is not particularly well suited to following a group of animals through an experiment. It comes into its own, however, in screening programs where one is following the health of the large number of animals found in a vivarium for future use or in processing specimens from a preliminary experimental run so as to see which biochemical parameters may be affected. One can then easily follow those results by appropriate micro methods before, during, and after an exposure. The SMA-12-30 when used in the proper context is a most valuable instrument, but it does have limitations.

TABLE I

MEAN RESULTS AND STANDARD DEVIATION ON POOLED MONKEY SERUM REPLICATE ANALYSIS

| Determination | n | Mean | SD |
|---|---|---|---|
| Cholesterol | 41 | 114.8 mg % | 5.86 |
| Calcium | 41 | 11.5 mg % | 0.25 |
| Inorg Phosphorus | 49 | 5.7 mg % | 0.10 |
| Bilirubin | 49 | 0.31 mg % | 0.06 |
| Total Protein | 49 | 7.7 g % | 0.09 |
| BUN | 49 | 35.3 mg % | 0.6 |
| Glucose | 46 | 97.6 mg % | 3.86 |
| Alk. Phosphatase | 49 | 24.8 KAU | 1.06 |
| SGOT | 48 | 56.9 KU | 6.71 |

TABLE II

MEAN RESULTS AND STANDARD DEVIATION ON POOLED DOG SERUM REPLICATE ANALYSIS

| Determination | n | Mean | SD |
|---|---|---|---|
| Cholesterol | 56 | 195.5 mg % | 4.23 |
| Calcium | 39 | 10.1 mg % | 0.30 |
| Inorg Phosphorus | 56 | 6.1 mg % | 0.12 |
| Bilirubin | 56 | 0.5 mg % | 0.08 |
| Total Protein | 56 | 6.9 g % | 0.08 |
| BUN | 56 | 16.2 mg % | 0.6 |
| Glucose | 56 | 67.7 mg % | 5.13 |
| Alk. Phosphatase | 52 | 12.8 KAU | 1.04 |
| SGOT | 55 | 32.2 KU | 5.75 |

TABLE III

PLASMA HEMOGLOBIN (MANUAL)

| Animal | Serum | Na Heparin Plasma | $NH_4$ Heparin Plasma |
|---|---|---|---|
| FE68 | 32 mg % | 4 mg % | 8 mg % |
| FE66 | 24 mg % | 4 mg % | 8 mg % |
| FE70 | 16 mg % | 8 mg % | 8 mg % |
| FE74 | 8 mg % | 4 mg % | 4 mg % |
| FE76 | 24 mg % | 4 mg % | 0 mg % |
| E45 | 16 mg % | 4 mg % | 0 mg % |
| E49 | 4 mg % | 0 mg % | 4 mg % |
| E64 | 8 mg % | 8 mg % | 4 mg % |
| E65 | 4 mg % | 4 mg % | 4 mg % |
| F80 | 12 mg % | 4 mg % | 8 mg % |
| Mean | 14.8 mg % | 4.4 mg % | 4.8 mg % |

TABLE IV

SODIUM (IL FLAME)

| Animal | Serum | Na Heparin Plasma | $NH_4$ Heparin Plasma |
|---|---|---|---|
| FE68 | 143 meq/L | 141 meq/L | 140 meq/L |
| FE66 | 143 meq/L | 142 meq/L | 142 meq/L |
| FE70 | 142 meq/L | 144 meq/L | 144 meq/L |
| FE74 | 144 meq/L | 142 meq/L | 144 meq/L |
| FE76 | 143 meq/L | 143 meq/L | 143 meq/L |
| E45 | 144 meq/L | 139 meq/L | 141 meq/L |
| E49 | 141 meq/L | 142 meq/L | 141 meq/L |
| F64 | 145 meq/L | 142 meq/L | 141 meq/L |
| E65 | 145 meq/L | 142 meq/L | 141 meq/L |
| F80 | 141 meq/L | 139 meq/L | 140 meq/L |
| Mean | 143 meq/L | 142.4 meq/L | 142 meq/L |

TABLE V

POTASSIUM (IL FLAME)

| Animal | Serum | Na Heparin Plasma | $NH_4$ Heparin Plasma |
|---|---|---|---|
| FE68 | 3.8 meq/L | 3.5 meq/L | 3.2 meq/L |
| FE66 | 3.7 meq/L | 3.4 meq/L | 3.3 meq/L |
| FE70 | 2.9 meq/L | 2.7 meq/L | 2.6 meq/L |
| FE74 | 3.8 meq/L | 3.3 meq/L | 3.2 meq/L |
| FE76 | 3.8 meq/L | 3.5 meq/L | 3.5 meq/L |
| E45 | 3.0 meq/L | 3.6 meq/L | 3.6 meq/L |
| E49 | 3.1 meq/L | 2.5 meq/L | 2.4 meq/L |
| F64 | 4.3 meq/L | 4.0 meq/L | 4.0 meq/L |
| E65 | 3.5 meq/L | 3.2 meq/L | 3.2 meq/L |
| F80 | 3.4 meq/L | 3.1 meq/L | 3.0 meq/L |
| Mean | 3.5 meq/L | 3.3 meq/L | 3.2 meq/L |

TABLE VI

CHOLESTEROL (SMA-12)

| Animal | Serum | Na Heparin Plasma | $NH_4$ Heparin Plasma |
|---|---|---|---|
| FE68 | 175 mg % | 170 mg % | 175 mg % |
| FE66 | 175 mg % | | 170 mg % |
| FE70 | 140 mg % | 140 mg % | 140 mg % |
| FE74 | 145 mg % | 150 mg % | 145 mg % |
| FE76 | 145 mg % | 145 mg % | 140 mg % |
| E45 | 210 mg % | 200 mg % | 200 mg % |
| E49 | 105 mg % | 115 mg % | 115 mg % |
| F64 | 145 mg % | 145 mg % | 140 mg % |
| E65 | 130 mg % | 130 mg % | 130 mg % |
| F80 | 115 mg % | 115 mg % | 115 mg % |
| Mean | 148.5 mg % | 145 mg % | 147 mg % |

TABLE VII

CALCIUM (SMA-12)

| Animal | Serum | Na Heparin Plasma | $NH_4$ Heparin Plasma |
|---|---|---|---|
| FE68 | 9.2 mg % | 9.7 mg % | 9.3 mg % |
| FE66 | 9.2 mg % | | 9.4 mg % |
| FE70 | 10.0 mg % | 10.1 mg % | 10.0 mg % |
| FE74 | 11.2 mg % | 11.5 mg % | 11.0 mg % |
| FE76 | 9.5 mg % | 9.6 mg % | 9.5 mg % |
| E45 | 9.5 mg % | 9.6 mg % | 9.5 mg % |
| E49 | 9.7 mg % | 9.9 mg % | 9.7 mg % |
| F64 | 10.0 mg % | 10.4 mg % | 10.1 mg % |
| E65 | 10.0 mg % | 10.3 mg % | 10.1 mg % |
| F80 | 9.4 mg % | 9.7 mg % | 9.5 mg % |
| Mean | 9.8 mg % | 10.0 mg % | 9.8 mg % |

TABLE VIII

INORGANIC PHOSPHORUS (SMA-12)

| Animal | Serum | Na Heparin Plasma | $NH_4$ Heparin Plasma |
|---|---|---|---|
| FE68 | 4.8 mg % | 4.5 mg % | 4.3 mg % |
| FE66 | 4.0 mg % | | 3.7 mg % |
| FE70 | 1.7 mg % | 1.6 mg % | 1.5 mg % |
| FE74 | 3.2 mg % | 2.7 mg % | 2.6 mg % |
| FE76 | 3.2 mg % | 3.0 mg % | 2.9 mg % |
| E45 | 5.1 mg % | 4.5 mg % | 4.5 mg % |
| E49 | 2.2 mg % | 1.8 mg % | 1.7 mg % |
| F64 | 5.4 mg % | 5.0 mg % | 4.9 mg % |
| E65 | 3.0 mg % | 3.8 mg % | 2.8 mg % |
| F80 | 1.9 mg % | 1.7 mg % | 1.8 mg % |
| Mean | 3.4 mg % | 3.2 mg % | 3.1 mg % |

TABLE IX

TOTAL BILIRUBIN (SMA-12)

| Animal | Serum | Na Heparin Plasma | $NH_4$ Heparin Plasma |
|---|---|---|---|
| FE68 | 0.3 mg % | 0.2 mg % | 0.2 mg % |
| FE66 | 0.3 mg % | | 0.2 mg % |
| FE70 | 0.2 mg % | 0.2 mg % | 0.2 mg % |
| FE74 | 0.2 mg % | 0.2 mg % | 0.2 mg % |
| FE76 | 0.2 mg % | 0.2 mg % | 0.2 mg % |
| E45 | 0.4 mg % | 0.3 mg % | 0.3 mg % |
| E49 | 0.2 mg % | 0.2 mg % | 0.2 mg % |
| F64 | 0.2 mg % | 0.2 mg % | 0.2 mg % |
| E65 | 0.3 mg % | 0.2 mg % | 0.2 mg % |
| F80 | 0.3 mg % | 0.2 mg % | 0.2 mg % |
| Mean | 0.26 mg % | 0.21 mg % | 0.21 mg % |

TABLE X

TOTAL PROTEIN (SMA-12)

| Animal | Serum | Na Heparin Plasma | $NH_4$ Heparin Plasma |
|---|---|---|---|
| FE68 | 6.4 gm % | 6.5 gm % | 6.5 gm % |
| FE66 | 7.8 gm % | | 7.9 gm % |
| FE70 | 7.2 gm % | 7.3 gm % | 7.3 gm % |
| FE74 | 6.8 gm % | 6.8 gm % | 6.9 gm % |
| FE76 | 5.7 gm % | 6.0 gm % | 6.0 gm % |
| E45 | 7.0 gm % | 7.2 gm % | 7.2 gm % |
| E49 | 8.2 gm % | 8.2 gm % | 8.2 gm % |
| F64 | 6.7 gm % | 6.8 gm % | 6.8 gm % |
| E65 | 7.1 gm % | 7.0 gm % | 7.1 gm % |
| F80 | 7.2 gm % | 7.3 gm % | 7.3 gm % |
| Mean | 7.0 gm % | 7.0 gm % | 7.1 gm % |

TABLE XI

BLOOD UREA NITROGEN (SMA-12)

| Animal | Serum | Na Heparin Plasma | $NH_4$ Heparin Plasma |
|---|---|---|---|
| FE68 | 15 mg % | 16 mg % | 15 mg % |
| FE66 | 14 mg % | | 14 mg % |
| FE70 | 20 mg % | 20 mg % | 20 mg % |
| FE74 | 21 mg % | 21 mg % | 21 mg % |
| FE76 | 18 mg % | 19 mg % | 19 mg % |
| E45 | 12 mg % | 12 mg % | 12 mg % |
| E49 | 14 mg % | 14 mg % | 14 mg % |
| F64 | 20 mg % | 20 mg % | 20 mg % |
| E65 | 16 mg % | 16 mg % | 16 mg % |
| F80 | 13 mg % | 13 mg % | 13 mg % |
| Mean | 16.3 mg % | 16.7 mg % | 16.4 mg % |

TABLE XII

GLUCOSE (SMA-12)

| Animal | Serum | Na Heparin Plasma | $NH_4$ Heparin Plasma |
|---|---|---|---|
| FE68 | 125 mg % | 125 mg % | 125 mg % |
| FE66 | 120 mg % | | 120 mg % |
| FE70 | 135 mg % | 135 mg % | 135 mg % |
| FE74 | 165 mg % | 165 mg % | 160 mg % |
| FE76 | 115 mg % | 115 mg % | 120 mg % |
| E45 | 110 mg % | 110 mg % | 115 mg % |
| E49 | 185 mg % | 185 mg % | 180 mg % |
| F64 | 110 mg % | 100 mg % | 105 mg % |
| E65 | 110 mg % | 110 mg % | 110 mg % |
| F80 | 175 mg % | 170 mg % | 175 mg % |
| Mean | 135 mg % | 135 mg % | 134.5 mg % |

TABLE XIII

LACTATE DEHYDROGENASE (SMA-12)

| Animal | Serum | Na Heparin Plasma | $NH_4$ Heparin Plasma |
|---|---|---|---|
| FE68 | 135 tu | 65 tu | 75 tu |
| FE66 | 70 tu | | 45 tu |
| FE70 | 60 tu | 30 tu | 25 tu |
| FE74 | 185 tu | 40 tu | 40 tu |
| FE76 | 75 tu | 30 tu | 25 tu |
| E45 | 195 tu | 40 tu | 45 tu |
| E49 | 150 tu | 110 tu | 50 tu |
| E64 | 40 tu | 60 tu | 30 tu |
| E65 | 35 tu | 40 tu | 30 tu |
| F80 | 35 tu | 25 tu | 30 tu |
| Mean | 98 tu | 49 tu | 40 tu |

TABLE XIV

ALKALINE PHOSPHATASE (SMA-12)

| Animal | Serum | Na Heparin Plasma | $NH_4$ Heparin Plasma |
|---|---|---|---|
| FE68 | 6 KAU | 6 KAU | 5 KAU |
| FE66 | 5 KAU | | 4 KAU |
| FE70 | 10 KAU | 9 KAU | 8 KAU |
| FE74 | 5 KAU | 4 KAU | 4 KAU |
| FE76 | 6 KAU | 4 KAU | 4 KAU |
| E45 | 6 KAU | 5 KAU | 5 KAU |
| E49 | 4 KAU | 2 KAU | 2 KAU |
| E64 | 5 KAU | 5 KAU | 4 KAU |
| E65 | 4 KAU | 3 KAU | 3 KAU |
| F80 | 4 KAU | 3 KAU | 3 KAU |
| Mean | 5.5 KAU | 4.6 KAU | 4.2 KAU |

TABLE XV

GLUTAMIC OXALOACETIC TRANSAMINASE (SMA-12)

| Animal | Serum | Na Heparin Plasma | $NH_4$ Heparin Plasma |
|---|---|---|---|
| FE68 | 30 KU | 30 KU | 30 KU |
| FE66 | 25 KU | | 25 KU |
| FE70 | 25 KU | 20 KU | 20 KU |
| FE74 | 25 KU | 20 KU | 20 KU |
| FE76 | 20 KU | 20 KU | 25 KU |
| E45 | 25 KU | 20 KU | 20 KU |
| E49 | 20 KU | 20 KU | 20 KU |
| E64 | 35 KU | 35 KU | 35 KU |
| E65 | 25 KU | 25 KU | 25 KU |
| F80 | 25 KU | 25 KU | 25 KU |
| Mean | 26 KU | 24 KU | 25 KU |

TABLE XVI

CREATININE (SINGLE CHANNEL A. A. )

| Animal | Serum | Na Heparin Plasma | $NH_4$ Heaprin Plasma |
|---|---|---|---|
| FE68 | 0.5 mg % | 0.5 mg % | 0.5 mg % |
| FE66 | 0.6 mg % | 0.5 mg % | 0.5 mg % |
| FE70 | 0.7 mg % | 0.65 mg % | 0.65 mg % |
| FE74 | 0.8 mg % | 0.75 mg % | 0.75 mg % |
| FE76 | 0.45 mg % | 0.55 mg % | 0.5 mg % |
| E45 | 0.4 mg % | 0.4 mg % | 0.45 mg % |
| E49 | 0.5 mg % | 0.5 mg % | 0.5 mg % |
| E64 | 0.6 mg % | 0.55 mg % | 0.55 mg % |
| E65 | 0.55 mg % | 0.5 mg % | 0.55 mg % |
| F80 | 0.5 mg % | 0.45 mg % | 0.5 mg % |
| Mean | 0.56 mg % | 0.53 mg % | 0.55 mg % |

TABLE XVII

CHLORIDE (SINGLE CHANNEL A. A. )

| Animal | Serum | Na Heparin Plasma | $NH_4$ Heparin Plasma |
|---|---|---|---|
| FE68 | 115 meq/L | 117 meq/L | 115 meq/L |
| FE66 | | 117 meq/L | 119 meq/L |
| FE70 | 119 meq/L | 118 meq/L | 117 meq/L |
| FE74 | 120 meq/L | 119 meq/L | 115 meq/L |
| FE76 | 120 meq/L | 118 meq/L | 115 meq/L |
| E45 | 120 meq/L | 116 meq/L | 120 meq/L |
| E49 | 119 meq/L | 120 meq/L | 117 meq/L |
| E64 | 121 meq/L | 123 meq/L | 120 meq/L |
| E65 | 114 meq/L | 119 meq/L | 114 meq/L |
| F80 | | 115 meq/L | 114 meq/L |
| Mean | 119 meq/L | 118 meq/L | 117 meq/L |

TABLE XVIII

COMPARISON OF MONKEY SERUM ALBUMIN VALUES
SMA-12 VERSUS MANUAL METHOD

| Specimen | SMA-12 | Manual | SMA-12/Manual |
|---|---|---|---|
| 1 | 3.9 g% | 5.1 g% | 76% |
| 2 | 3.3 g% | 4.4 g% | 75% |
| 3 | 3.3 g% | 4.6 g% | 72% |
| 4 | 3.6 g% | 4.4 g% | 81% |
| 5 | 3.8 g% | 4.8 g% | 79% |
| 6 | 3.5 g% | 4.8 g% | 72% |
| 7 | 3.2 g% | 4.4 g% | 72% |
| 8 | 3.5 g% | 4.6 g% | 76% |
| 9 | 3.3 g% | 4.4 g% | 75% |
| 10 | 3.2 g% | 4.6 g% | 69% |
| 11 | 3.9 g% | 5.0 g% | 78% |
| 12 | 3.5 g% | 4.6 g% | 76% |
| 13 | 3.7 g% | 4.8 g% | 77% |
| 14 | 3.9 g% | 4.8 g% | 81% |
| 15 | 3.7 g% | 4.8 g% | 77% |
| 16 | 3.2 g% | 4.4 g% | 72% |
| 17 | 3.8 g% | 4.8 g% | 79% |
| 18 | 3.7 g% | 4.6 g% | 80% |
| 19 | 3.6 g% | 4.4 g% | 81% |
| 20 | 3.0 g% | 4.2 g% | 71% |
| $\bar{x}$ | 3.5 g% | 4.6 g% | 75% |

## ACKNOWLEDGEMENTS

We gratefully acknowledge the technical assistance of MSgt. William Leonard, USAF; TSgt. Donald Cunningham, USAF: TSgt. William Hunt, USAF; and TSgt. Harold Stull, USAF.

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## DISCUSSION

MR. WEIL: Dr. Sopher, on the comparisons that you made between the plasma and the serum, while the means on certain ones look like they were directly comparable, on some of them they look like they weren't. Even those that weren't, looked like in some cases you had very good correlation. Some of those where it looked like you had very poor correlation, where you had high values in serum, might have been low on plasma. I wonder if you did more than just compare the means to see if they were similar or whether you also tried something like a rank correlation test?

DR. SOPHER: No, we didn't get very sophisticated in a statistical treatment of the data. What we did was to compare, animal by animal, what the three results were, and then calculated the means just a quick and dirty comparison for this Conference. Where there was significant scatter, the two plasma values would parallel each other closely and it would be the serum values that were quite different. In the situations where the serum value was different, it was generally in a component that was rich in the red cell. If nothing else comes out of this, I have convinced myself that if one is using dogs and is doing clinical chemistry, for the vast majority of tests plasma is a superior substrate to use, for the simple reason that you don't have this variability in content of certain enzymes, of potassium, of hemoglobin, that can interfere with things like bilirubin, because you don't have to worry about damage to the red cells. We found, for example, you don't dare let dog blood sit around when you're doing routine hematology. If you let the blood sit for a couple of hours, the red cells swell, many of them burst and the results you'll get, for example on the red count using the coulter counter, will be significantly different than if you run it immediately after having bled the animal. Several other people have found the same thing and there's been a fair bit of work on what the proper dilution solution should be when you're doing cell counts on dog blood. We found that we could get away with just using standard isotonic solutions if we get right at the blood and counted it immediately after bleeding. If it sits around, you might as well forget it.

DR. FASSETT: I think I would just like to comment that I would agree completely with your findings about LDH and dog blood, particularly. As a matter of fact, I wonder whether it's worth doing it in this case, but I wanted to ask a question. Are you satisfied that the morphology of all the elements in the blood smear is as good with heparin as with regular procedures?

DR. SOPHER: I think probably there are changes, some changes in morphology from heparinized blood specimens compared to a direct smear. However, it's sort of a trade-off between having a lot of slides cluttering up the dome and

making a smear from the heparinized blood. You can do an adequate differential, which is the main thing we'd be interested in. The red cells don't change too much, possibly some of the white cell morphology does. Really, the trade-off is such that it doesn't pay to make a direct smear in the dome. Now if we had a situation where it was an absolute necessity, that is something else again, but for routine screening, I don't think it's really particularly necessary.

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PAPER NO. 22

# PRACTICAL USE OF MICROWAVE SPECTROSCOPY FOR TRACE CONTAMINANT DETECTION

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## INTRODUCTION

Microwave spectroscopy has several potential advantages which render the technique especially attractive as a tool for gas analysis. Highly specific identifications can be made by measurements of the frequencies of the microwave absorption lines of molecules. Compounds may be unambiguously identified, even in mixtures. Molecules are not fractured since ionization is not necessary. No carrier gas or temperature programing is needed. The technique is all electronic, making it versatile and amenable to automation. The sensitivity of microwave spectroscopy to trace amounts of gases depends upon the strengths of the microwave absorption lines. The strengths of absorption lines vary among molecules, but some gases can be detected in concentrations of a few parts per million.

Currently, a program is underway at the NASA-Langley Research Center to develop microwave spectroscopy as a means of detecting trace components of gas and vapor mixtures in space cabin simulators. A basic description of the technique and its application to trace contaminant analysis is contained in this paper. The unique capabilities of the method are discussed. In particular, the versatility is stressed and illustrated with a series of spectra of the same molecule run under different conditions. Considerations of special importance in quantitative analysis are described and illustrated. The paper concludes with a brief discussion of automated analysis by microwave spectroscopy.

## BASIC CONSIDERATIONS

The theory of microwave spectroscopy is similar to other types of absorption spectroscopy, except that in this case the spectra normally arise from changes in molecular rotation states. The frequencies of the simplest type of spectrum, that of a linear molecule, are as follows:

$$f = \frac{h}{4\pi^2 I}(J+1) = f_o(J+1)$$

f = frequency
h = Planck's constant
I = moment of inertia
J = angular momentum quantum number

Of particular significance is the dependence of the frequency on molecular moment of inertia. This means that microwave spectra depend upon molecular structure. For nonlinear molecules, the above expression is complicated by additional terms.

It should be noted that a molecule must possess an electric dipole moment in order to absorb microwave energy. Nonpolar molecules such as methane, benzene, nitrogen, and carbon tetrachloride cannot be detected. However, a large number of toxic gases are polar and can be detected by microwave spectroscopy. The lack of detectability of nitrogen is an advantage in trace contaminant analysis in air because no spectral interference will result from the much larger concentrations of nitrogen.

Figure 1 shows a block diagram of a microwave spectrometer. The microwave power entering the absorption cell from the source is regulated by the attenuator and, thus the power entering the absorption cell can be varied for purposes of quantitative analysis. The detector is seen to be behind the cell. Signals resulting from absorption of microwave energy are amplified and displayed on the recorder. The absorption spectra then appear as intensity versus frequency on the recorder chart as the source is swept through its frequency range.

Microwave spectrometer systems have been developed to a high degree of sophistication using modern microwave technology, and highly useful systems are now commercially available.

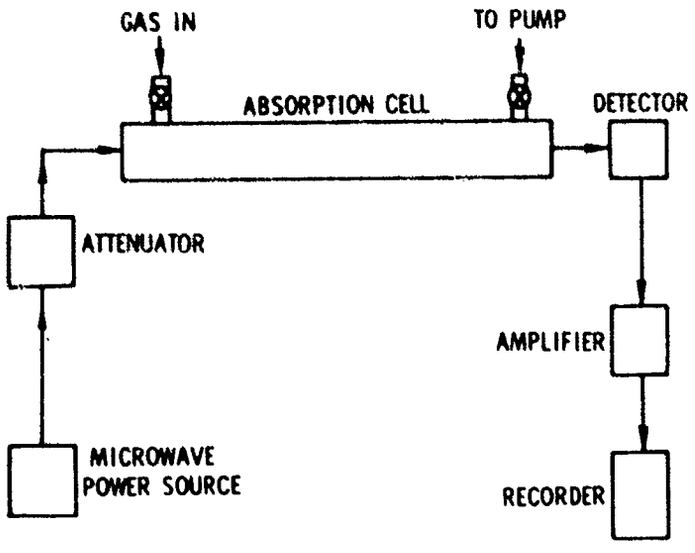



Figure 1. BLOCK DIAGRAM OF MICROWAVE SPECTROMETER

Figure 2 is a photograph of the microwave spectrometer at Langley Research Center.

Although microwave spectroscopy has strong capability for future use in routine chemical analysis, the application of the technique is currently limited by the lack of adequate catalogs of spectral data. This limitation is, however, being overcome by programs now in progress.

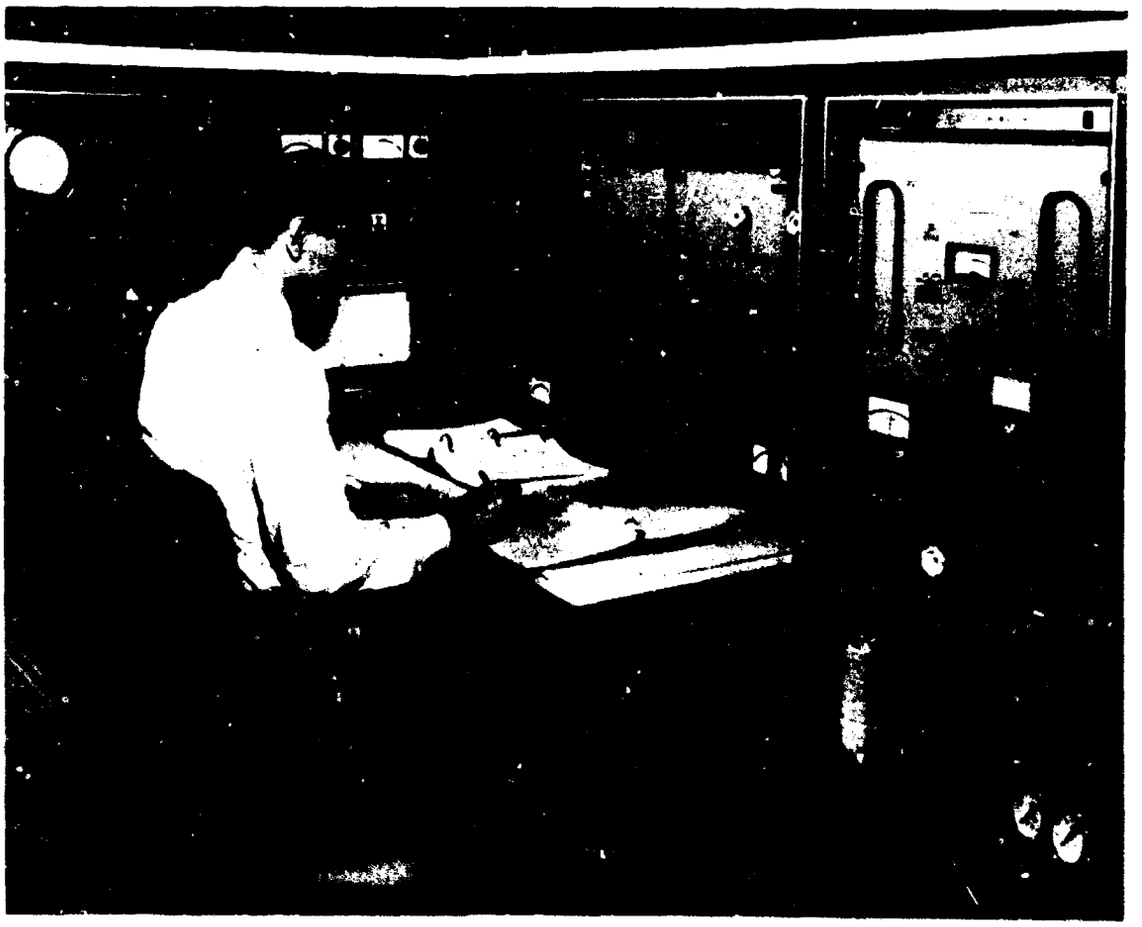


Figure 2. MICROWAVE SPECTROMETER

## QUALITATIVE ANALYSIS

The microwave absorption spectrum of each gas is unique. The average number of lines per molecule is about 400. The uniqueness of its spectrum, when combined with high resolution and highly accurate frequency measurements, usually permits identification of a gas in a mixture by measurement of the frequencies of a relatively small number of its absorption lines. However, due to the large number of lines, interferences do exist, making it necessary to have available for reference the entire spectrum of every molecule.

Modern microwave spectrometers are quite versatile because they permit continuous variation of both the total scan time and the frequency range which is scanned. The sample composition and pressure determine the maximum scan rate which may be used in a given case. Higher pressures permit the fastest scan rates, but also result in the poorest resolution and frequency measurement accuracy. Figures 3-7 illustrate the effects of pressure on resolution using a methylene chloride sample as an example. Progressively smaller portions of the range are covered to keep the scan times comparable in each case.

Figure 3 shows a spectrum of methylene chloride at about 2 torr. This spectral region extends from 26.5 to 40 GHz (R-band). There are about 1200 discrete lines for methylene chloride cataloged in this region. However, the spectrum shown in figure 3 lacks distinctive features. Resolution and accuracy are obviously absent, but the scan can be done in as little as 1 minute. For many gases, a more discrete line spectrum can be observed, even at this high pressure.

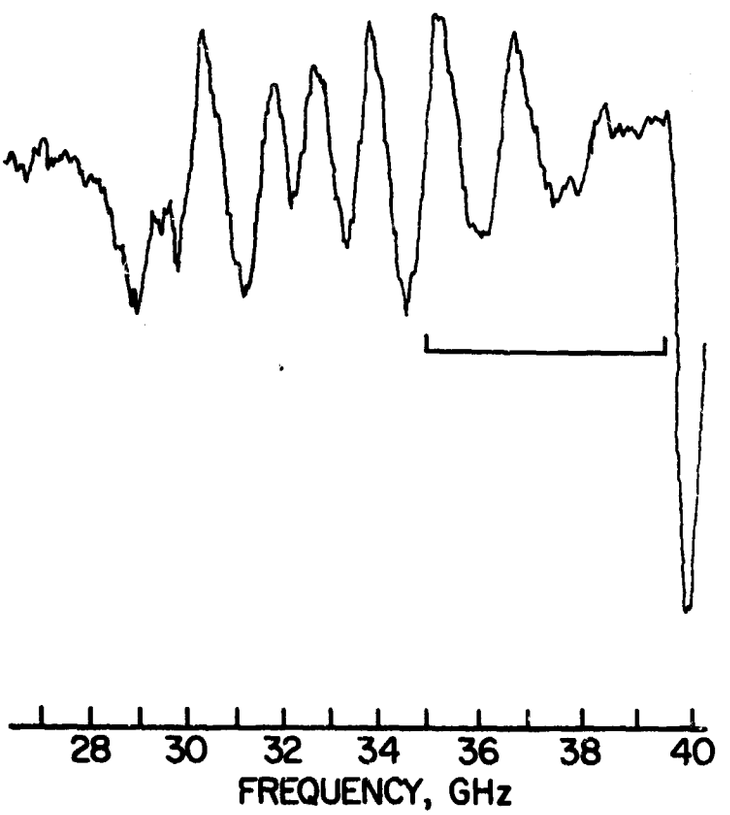



Figure 3. SPECTRUM OF METHYLENE CHLORIDE AT 2 TORR

As the pressure is decreased, the broad bands of figure 3 split up into resolvable sharper lines, as shown in figure 4. This is the portion of the methylene chloride spectrum indicated by brackets in figure 3, run at 0.15 torr pressure. Resolution and frequency measurement accuracy are greatly improved here, at the expense of speed. Corresponding values of wave number are shown for the purpose of resolution comparison.

Figure 5 shows the portion of the methylene chloride spectrum indicated by brackets in figure 4. Here the pressure is 0.03 torr. The single strong line is now seen to be a triplet. Frequency measurements can readily be made to 5 or 6 digit accuracy at pressures of this order, but several hours would be required to scan the entire band at this scan rate.

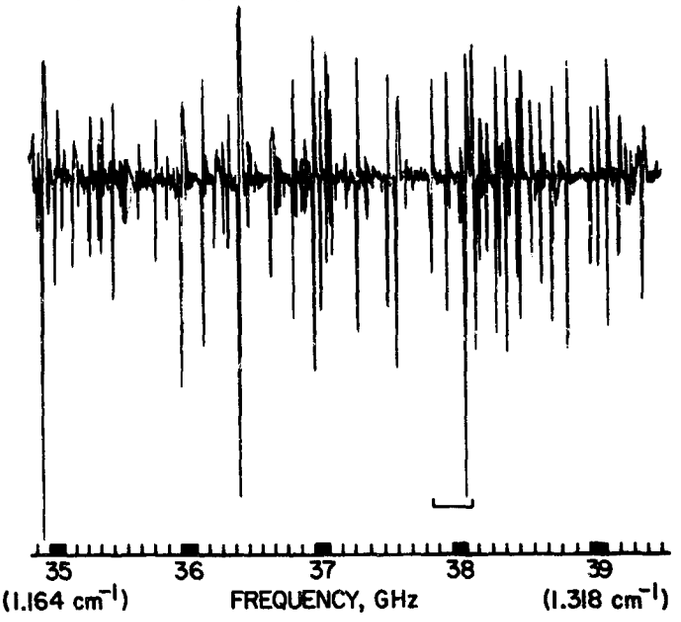



Figure 4. PORTION OF METHYLENE CHLORIDE SPECTRUM AT 150 MICRONS PRESSURE

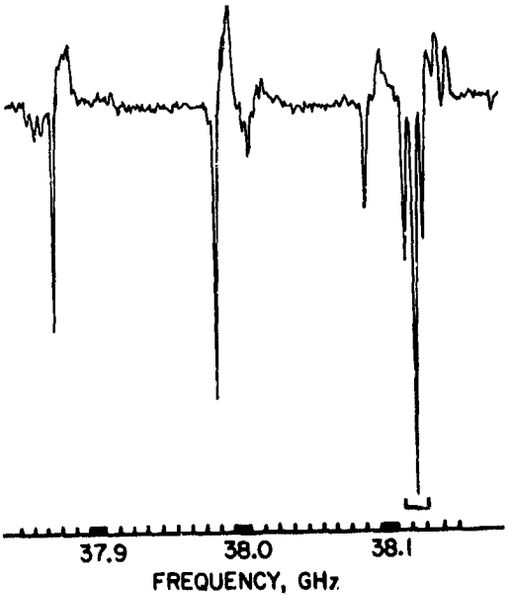



Figure 5. PORTION OF METHYLENE CHLORIDE SPECTRUM AT 30 MICRONS PRESSURE

Further reducing the pressure to 0.01 torr and correspondingly slowing the scan rate shows each component of the triplet in figure 5 to be a triplet itself, as shown in figure 6. Frequencies can be measured to 7-digit accuracy under these conditions, but one or more days would be required to cover the entire band at this scan rate.

The maximum resolution which can be achieved with ordinary microwave spectrometers is shown in figure 7. The mean free path of molecules in the gas is such that, at these low pressures, collisions with the cell walls predominate and further reduction of pressure reduces the signal strength without affecting line width. Again, the wave number increment is shown for resolution comparison.

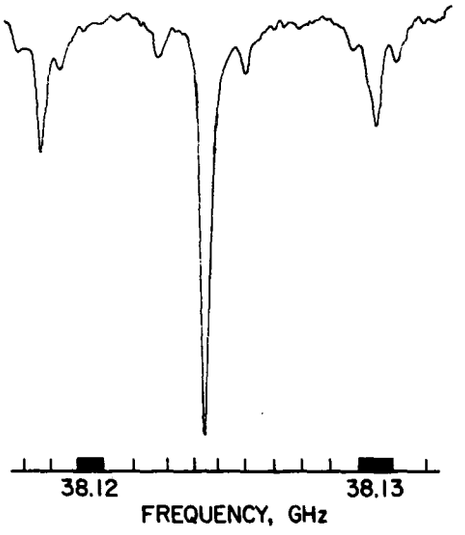
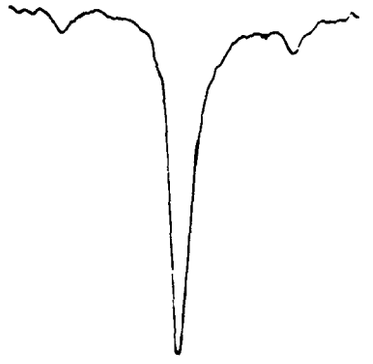



Figure 6. $11_{0,11}$ $11_{1,10}$ TRANSITION OF $CH_2{}^{35}C_2$ AT 10 MICRONS PRESSURE

←0.0001 cm⁻¹→



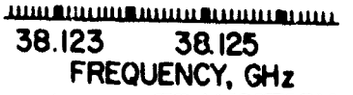



Figure 7. $11_{0,11}$ - $11_{1,10}$ TRANSITION OF METHYLENE CHLORIDE ($Cl^{35}$) AT 5 MICRONS PRESSURE

## QUANTITATIVE ANALYSIS

In addition to its application to qualitative analysis, microwave spectroscopy is also useful for quantitative analysis. The output signal from the spectrometer is the important consideration here. Figure 8 illustrates the behavior of the spectrometer output signal as a function of the microwave power in the absorption cell. It is seen that the signal rises to a maximum and then decreases as the power increases. This maximum signal is proportional to partial pressure.

Figure 8 also illustrates the effect of sample composition on signal strength. It is seen that the value of the maximum signal itself is the same in each case, but that the maximum signal occurs at a different value of power. It may, therefore, be concluded that the value of the maximum signal obtained from a given partial pressure of a gas in a mixture does not depend upon the composition of the mixture, although the signal at an arbitrary power level will, in general, vary with sample composition.

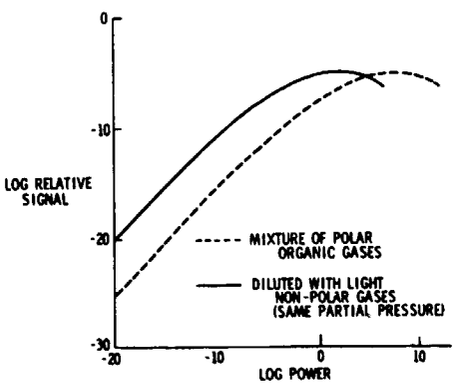



Figure 8. SIGNAL VERSUS POWER

Figure 9 (Harrington, 1967) illustrates some other important features of the maximum signal produced by a gas. In this example ethylene oxide is used. The solid line in figure 9 shows that the intensity of the maximum signal of an ethylene oxide line increases linearly with increasing pressure of ethylene oxide in the sample. Another feature of importance in quantitative analysis is illustrated by the broken lines in figure 9. The lowest broken line shows the behavior of the intensity of the maximum signal of the ethylene oxide line when increasing pressures of water are added to a given partial pressure of ethylene oxide. It is seen that the line intensity does not change. When increasing pressures of p-chlorotoluene are added to a different partial pressure of ethylene oxide, it is again seen that the intensity does not change. As a third case (top broken line), air is added in increasing pressures to a third given partial pressure of ethylene oxide. Again, the observed intensity of the ethylene oxide line is unchanged. In these examples the total pressure of the sample is increased, but the partial pressure of ethylene oxide remains constant.

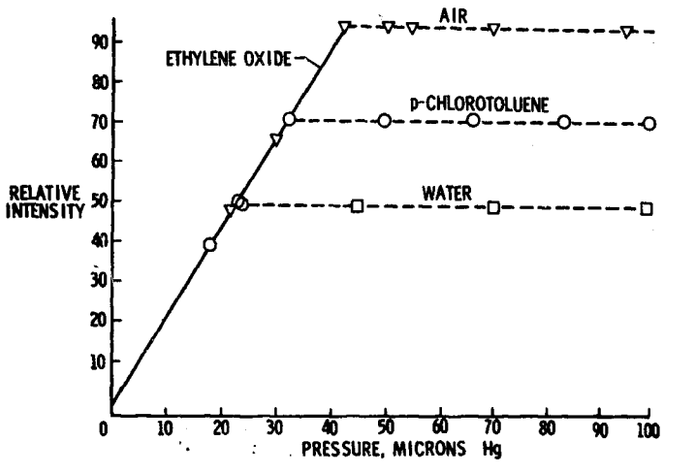



Figure 9. EFFECT OF SAMPLE COMPOSITION ON MAXIMUM SIGNAL

The important characteristics of the maximum signal are: (a) the value of the maximum signal obtained from a particular partial pressure of a particular gas is independent of the sample composition and (b) the maximum signal varies directly with the partial pressure of the gas in the sample.

## CONCLUDING REMARKS

That microwave spectroscopy is a valuable tool for analysis of gas and vapor mixtures has been established. Versatility is one of the most important advantages because scan time and spectral resolution can be adjusted to many analytical requirements. Reliable quantitative analysis by microwave spectroscopy is possible because the maximum signal obtained from a particular gas depends only upon the partial pressure of the gas. Microwave spectrometers are all electronic and amenable to automation.

The microwave spectrometer at Langley Research Center employs a paper tape programmer which permits selected frequencies to be scanned at high resolution. The output spectra appear on the recorder chart and manual data interpretation is required. However, for the large amount of data that will be needed to confirm the presence (or absence) of many molecules, use of a computer for data reduction is desirable. Automated spectrometry would also be highly desirable, and an automated spectrometer is currently being purchased by Langley Research Center. This automated spectrometer will incorporate an adaptive program which will provide only those high resolution scans needed to resolve ambiguities of the rapid scans. Versatility is being stressed in developing the program to permit continuous modification of spectrometer operation.

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PAPER NO. 23

# AN IMPROVED CONTAMINANT SENSING TECHNIQUE FOR LABORATORY AND SPACECRAFT APPLICATION

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## INTRODUCTION

The Langley Research Center of NASA has, for the past several years, conducted an intensive research and development program in the area of trace contaminant sensing. This effort has been primarily directed at the detection of trace contaminants in air, at some fraction of their recommended Threshold Limit Value (TLV). This effort, in support of the manned space program, is expected to provide a laboratory sensor with increased capability for sensing all contaminants present in a short time period. There are several requirements for such sensors, such as monitoring atmospheres within manned chamber tests, measurement of performance of contaminant control systems, measurement of rates of contaminants produced from both materials and man, and development of techniques for flight sensors for intermediate and extended-duration manned space missions. This paper will outline the progress that has been made to date on a concept that shows considerable promise for improved sensitivity for measuring many compounds that may be present simultaneously, in a breathable atmosphere.

## SENSOR DISCUSSION

In April 1967, the Langley Research Center undertook a study and test program with the Perkin-Elmer Aerospace Systems Company of Pomona, California to develop a sensing technique which would combine the best features of both gas chromatography and mass spectrometry.

The weaknesses of the mass spectrometer, when used by itself, are a lack of sensitivity and an inability to provide accurate data for complex mixtures of contaminants at trace levels. Most mass spectrometers use energetic electrons to accomplish the ionization of molecules, which invariably results in the

fracturing of these molecules into smaller fragments. A large number of these fragments, resulting from the fracturing of different contaminants, appearing at the same places in the spectra, will result in a very complex data analysis problem. In addition, there are many instances of contaminants that are very toxic, occupying the same, or nearly the same, place on the mass spectra as other gases (carbon monoxide and nitrogen, for example, or nitrous oxide and carbon dioxide). Mass spectrometers that are capable of resolving these doublets or triplets are very large and expensive.

Gas chromatography, on the other hand, is a technique that requires much expert care and operation. Accurate temperature and flow control are critical, and these parameters must be maintained constant in order to obtain repeatable data. Furthermore, some of the most sensitive detectors, such as flame ionization, are relatively insensitive to inorganic compounds. Sorber materials are subject to aging and degradation, such that regular reconditioning or replacement is necessary. In addition to these shortcomings, gas chromatography does not provide a positive means of contaminant identification and, for the most part, the identity of a chromatogram peak can only be determined using a lengthy calibration procedure.

However, by combining these techniques in a particular manner, it is felt that most of the shortcomings of both the mass spectrometer and the gas chromatograph for measuring trace contaminants can be overcome. The approach of this "hybrid" sensor program is to use gas chromatograph column sorber materials, such as the Porapaks, charcoal, and molecular sieves, to preconcentrate the trace contaminants, and thus increase the sample quantity available to the mass spectrometer. In addition, it is expected some additional data would be made available concerning the identification of the contaminants by utilizing several sorbers, rather than one. Because of the increased sample quantity from these preconcentrators, it was possible to use a very low ionization potential in the mass spectrometer; in this case, 9 to 12 electron volts (eV), and by this means, greatly reduce the molecular fracturing that normally takes place with the usual 70 to 80 eV ionization potentials. Finally, in order to aid in the gathering and analysis of the data, a digital computer program was written which will greatly simplify the use of this instrument.

A preconcentration system was designed, tested, and installed in the inlet system of a relatively simple mass spectrometer that was originally intended as a residual gas analyzer. In figure 1, two of these preconcentrators are shown diagramatically. The preconcentrators are connected in a series fashion, the first containing Porapak Q and the second a palladium-treated charcoal. The incoming air containing the trace contaminants of interest is passed through the preconcentrator cell with both the flow rates and the time of collection being known. It is desirable that the amount of each contaminant collected not exceed the linear loading capacity of the concentrator material, Porapak or charcoal, in order that a linear relationship may be maintained between the quantity of contaminant sorbed and the time of adsorption. Further, in this manner practically all of the contaminant will be collected in the preconcentrator, and little or no interaction between contaminants will be experienced.

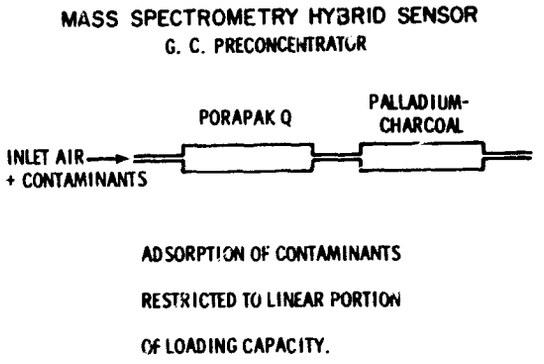


Figure 1. PRECONCENTRATOR FOR MASS SPECTROMETER HYBRID SENSOR

Following the adsorption of contaminants, each preconcentrator is individually desorbed by a combination of heat and vacuum. As shown in figure 2, the air contained in the preconcentrator is removed (or precut) from the cell by connecting it directly to a vacuum pump. After a short time, the inlet of the mass spectrometer is opened and a heater element in the sorber cell is activated. As the temperature within the sorber cell increases (a linear temperature increase with time is followed) the contaminant with the weakest adsorption characteristics will leave the cell and enter the inlet of the mass spectrometer. If the concentration of the contaminants were to be measured as they entered the inlet of the mass spectrometer, they would appear similar to a gas chromatogram, as illustrated in this figure. The separation of these compounds is not as discrete as that obtainable in a gas chromatograph, since no carrier gas is used, and only heat and vacuum are utilized as driving forces for the molecules. Noted on this figure, for purposes of identity, are different mass numbers for the different peaks. With increasing time, the temperature continues to increase and the desorption process is continued, until all of the adsorbed contaminants have been desorbed into the mass spectrometer. The cell is then allowed to cool and may again be used for preconcentration. Each of the preconcentrators is cycled in this fashion; a process that currently takes about 30 minutes, at a temperature program rate of 7 C per minute. It is expected that this rate can be increased by a considerable degree without data loss, if required.

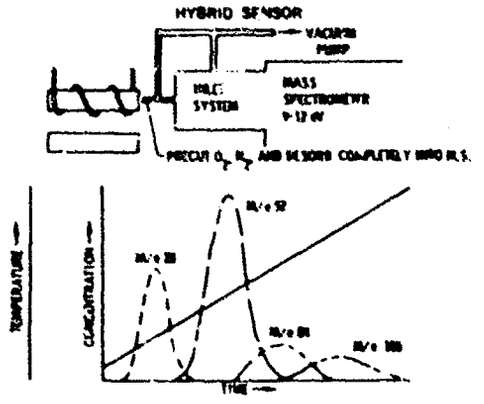


Figure 2. MATCHING PRECONCENTRATOR AND MASS SPECTROMETER INLET SYSTEM

Within the mass spectrometer, the various molecules that have been desorbed are sensed, not just in terms of concentration, but also in terms of their mass-to-charge ratio or M/e ratio. Thus, the data is given a third dimension in addition to concentration and time. As illustrated on figure 3, as the M/e spectrum is scanned by the mass spectrometer, peaks will appear at the appropriate mass number, indicating the presence and quantity of contaminant having that M/e ratio. The scan rate of the mass spectrometer can be set to about three cycles per minute, thus each preconcentrator cell output can be scanned about 90 times.

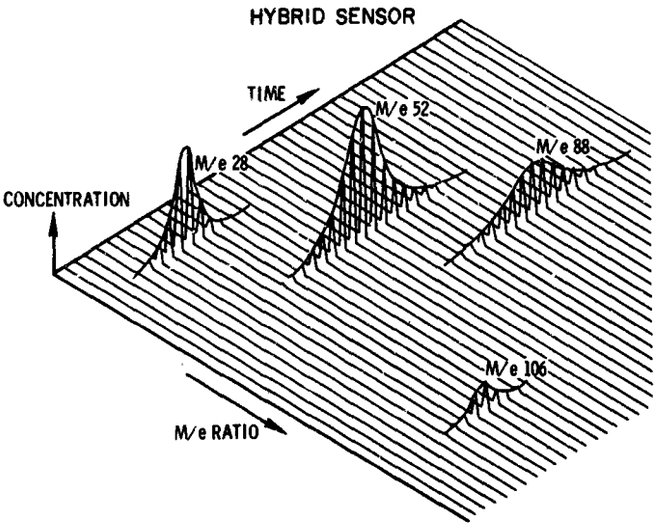


Figure 3. PARAMETERS OF THE HYBRID SENSOR

In the previous figure, M/e 106 was shown to come off the preconcentrator last. In figure 3, this peak (M/e 106) has arbitrarily moved to coincide with M/e 28, to illustrate that coincidental desorption from the preconcentrator (unlike a gas chromatograph) presents no difficulty with this instrument. In the opposite case, compounds having like M/e ratios (nitrogen, carbon monoxide, and ethylene at M/e 28; and nitrous oxide and carbon dioxide at M/e 44, for example) have, in all cases to date, either different desorption times or will be adsorbed on different adsorption cells altogether. Thus, in order for this hybrid instrument to be unable to resolve two compounds, they must have very similar chemical properties; adsorbing on the same material and desorbing at the same time with equal mass-to-charge ratios.

One other advantage of the present instrument is that by deliberate use of a high ionization potential, additional information can be obtained to aid in the identification of a molecule. If an unidentified compound appears, the instrument can be switched to the high ionization potential mode of operation and the fragmentation pattern noted. Since a great deal of catalog data is available to identify compounds by their fragmentation pattern, this information can be used for identification.

The present capability of this instrument for sensing trace contaminants is given on table I. Shown is a list of 26 compounds, and a number indicating the desired sensitivity goals for the instrument in parts per million. Also shown is

the present capability of the instrument, as demonstrated by calibration, also in parts per million. This last number is the lower limit of detectability of the instruments for these compounds in air as of about 6 months ago. Work is currently underway to increase the sensitivity and to determine the capability of the sensor to detect an additional 40 compounds, in concentrations from 100 ppb to 50 ppm.

TABLE I

LABORATORY CONTAMINANT SENSOR
PRESENT SENSITIVITY

| CONTAMINANT | GOAL, PPM* | ACHIEVE, PPM |
|---|---|---|
| Acetone | 100.0 | Yes |
| Acetaldehyde | 20.0 | Yes |
| Allyl Alcohol | 0.2 | No, 2.0 ppm |
| Ammonia | 5.0 | No |
| Benzene | 2.5 | Yes |
| Butene - 1 | Not established | 2 |
| Carbon Dioxide | 500.0 | 300 |
| Carbon Disulfide | 2.0 | Yes |
| Carbon Monoxide | 5.0 | Yes |
| 1, 4 - Dioxane | 10.0 | Yes |
| Ethylacetate | 40.0 | YR |
| Ethylene Dichloride | Not established | 10 |
| Formaldehyde | 0.5 | No |
| Freon - 11 | Not established | 7 |
| Hydrogen Chloride | 0.5 | No |
| Hydrogen Sulfide | 1.0 | No |
| Methane | Not established | No |
| Methyl Alcohol | 20.0 | 5 |
| Methylene Chloride | 50.0 | Yes |
| Nitric Oxide | Not established | 10 |
| Nitrous Oxide | 1.0 | No, (5.0) |
| Phenol | 0.5 | No |
| Sulfur Dioxide | 0.5 | No, (2.0) |
| Toluene | 20.0 | Yes |
| Vinyl Chloride | 50.0 | Yes |
| m-Xylene | 10.0 | Yes |

*Goals are 1/10 TLV, where established

## CONCLUSIONS

In this paper is outlined a laboratory contaminant sensing technique that shows considerable promise for the measurement of many toxic compounds, in combination, in a background of air. Work on this technique is continuing, with improvements in sensitivity and detection capability receiving the most attention. The potential of this approach for the development of a flight sensor is quite good. None of the components making up this instrument are extremely complex, nor particularly critical, as far as stability and repeatability are concerned. A double-focusing mass spectrometer has been developed which will provide the same capability as the laboratory spectrometer now in use, but which has been designed as a flight-qualifiable instrument that weighs less than 10 pounds. It is expected that the total package, consisting of preconcentrators, the mass spectrometer, and a microminiaturized computer-controller would be feasible, with weight and power requirements tentatively estimated at about 15 pounds and 20 watts, respectively. If this concept proves feasible, it would provide the capability for sensing a large number of contaminants at well below their present TLV, onboard a manned spacecraft.

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## DISCUSSION

DR. SLONIM (Aerospace Medical Research Laboratories): I would like to address my question to Mr. Easley. In view of what Dan's paper had to say, I'm not quite sure I know what the advantage of this expensive microwave spectroscopy is. Is it sensitivity or a combination of factors here?

MR. EASLEY (NASA): Well, the sensitivity is not actually any better. The versatility, in most cases, is the factor, because you have the capability to continuously vary the pressure in the absorption cell and, also, the scan time; and again, the resolution is quite good, too. In many cases, if you have a complex mixture of compounds, the microwave spectrum is resolved to the extent that many lines are only about a quarter of a megacycle per second wide, out of 13,500 megacycles per second in one band of the spectrum. So, versatility and resolution are the factors here which enabled us to do things that so far we don't have anything else to do with.

MR. POPMA (NASA): I might add one word to that. I seriously suspect there are going to be compounds for which we will be unable to find a good preconcentrator material, especially the extremely complex molecules.

MR. MOBERG: I think the instruments do complement one another. If you were to spend the same type of money and get mass spectrometer and get a double focusing consolidated 21-110, you're spending about the same amount and you have just about the same complexity in resolving the curves, so that one can favor either one.

MR. WANDS: Dan, I'd like to compliment you on the excellent progress you're making in air analysis for your system. I wonder what you're doing with particulate materials?

MR. POPMA: Mr. Wands, nothing.

MR. WANDS: It's not an easy thing to do. I was hoping you might have been able to devote some igenuity to this.

MR. POPMA: The programs we are conducting in trace contaminant sensing are about one-third of our total effort. As you are aware, we are also interested in some other things and again, the perennial cry, we don't have the manpower to do all the things we would like to do.

MR. VERNOT: A question to Wes Easley. I didn't hear a detection limit, or sensitivity figure for the microwave technique. Did you mention one or did I miss it?

MR. EASLEY: No, I think I probably did leave that out. The sensitivity is not something that can be given a blanket value because this does depend on the intensity of the spectral line. Ammonia has strong lines, some have weak lines, such as mercaptans, they do have spectra but they are weak. For ammonia, we can probably detect less than 5 parts per million; whereas for ethyl mercaptan, 500 parts per million. I did mention that it does vary with the individual molecule. This is something that has to be determined experimentally.

MR. VERNOT: Is your sensitivity greatest at the highest pressure of measurement?

MR. EASLEY: Yes, it would be--The sensitivity would be, but the qualitative identification would be hampered due to wide lines. There's a trade-off there so you have to compromise.

MR. VERNOT: The advantage of the accumulator mass spectrometer system over that of, say, conventional gas chromatography, hooked up to a mass spectrometer, seems to be that you've gotten rid of your carrier gas. The material, if you will, in this case the carrier gas, is atmosphere.

MR. POPMA: I might point out that in addition we got rid of oxygen, nitrogen, and water vapor.

FROM THE FLOOR: Yes. Right. While you've done this, it also suffers because the separation of the materials is not quite as good as in gas chromatography.

MR. POPMA: The separation isn't as good; however, looking at it in a different dimension. We're looking at mass to energy ratio spectra, rather than differences in concentration with time.

FROM THE FLOOR: Yes, that's true. That is the mass spectrometric portion of the problem, you could do this no matter how you put your sample in.

MR. POPMA: If you can retain the sensitivity in the face of the helium background or sample loss with various concentrator systems subsequent to separation in a gas chromatograph, yes.

MR. VERNOT: Okay. That's my point.

MR. MOBERG: I have a question, Dan, or at least a point I'd like you to emphasize a little bit. Can you give me some idea of the recoveries during calibration studies? You must have made some rather detailed recovery studies. Did I miss part of that on the slide?

MR. POPMA: Define what you mean by recoveries.

MR. MOBERG: I should say, if one puts in five or ten milligrams of a component like ethyl mercaptan, do we recover this amount of material upon heating and evacuation?

MR. POPMA: Yes, we have repeatedly absorbed and desorbed known amounts of these kinds of molecules from the preconcentrator cell and we see no variation in the preconcentrator. That is, we are desorbing into the mass spectrometer all of the contaminants which are absorbed from the air stream.

MR. MOBERG: I was thinking not necessarily of the qualitative aspects but the quantitative. Here, again, would you repeat this?

MR. POPMA: Again, in order to make an easy calibration for the instrument, we have maintained a linear relationship between the peak height as displayed on the mass spectrometer and the total quantity of contaminant initially absorbed.

MR. MOBERG: So you can calibrate on the basis of 80% or 90% of absolute recovery, and it still comes up to give you the proper number.

MR. POPMA: In any mass spectrometer, unfortunately, you never ionize a hundred per cent of the molecules.

MR. MOBERG: Near 1%?

MR. POPMA: That's about right, especially at 90 EV that we're running, rather than the 80 EV.

MR. MOBERG: And even less than that, even a tenth of a per cent?

MR. POPMA: Yes, but this is why we are restricting ourselves to the linear portion of the loading capacity of the preconcentrator in order to keep the picture simple.

MR. STEVENS (National Air Pollution Control Administration): I'm wondering about the application of the accumulator mass spectrometer system to sub parts per million level of $SO_2$, nitric oxide and perhaps other air pollution materials.

MR. POPMA: I'm not sure I understand the question. This is what I addressed myself to in the closing minutes of the paper wherein we see a disappearance of these molecules at these very low concentrations, somewhere either in the inlet system to the mass spectrometer or in the preconcentrator. We don't see spectra in the mass spectrometer resulting from known quantities being put into the preconcentrator cells. If we increase the quantity sufficiently, yes, we will see it. But the threshold sensitivity of the instrument hasn't been established for these materials.

FROM THE FLOOR: I'd like to address a question to Mr. Easley in regard to the sensitivity of the microwave technique. In a recent article in Analytical Chemistry by Funkhouser, Harrington, and others on working at power saturation high pressure conditions. Now, I'm sorry I didn't hear your talk. I was wondering

about conditions under which you did your work. Did you work at low pressure Beer's Law region, or did you try some of these conditions?

MR. EASLEY: Well, you're talking about the power saturation intensity. That was next to the last slide. This maximum in the curve was the power saturation intensity although I didn't mention it here bu.. that's what Harrington did. He is the one that has pretty well been the pioneer in the intensity work and we have tried to do what he has done. Harrington is from Hewlett-Packard, and they are the ones who built the instrument we have.

MR. MOBERG: I might add, as you see some of these instruments and some of the data displayed, in our analytical review of our problems, we should consider this word "art" in the more sophisticated standards, as we do in the medical field and the clinical laboratories. You see, everything is not sunshine. When you give the sample to the chemist and say, "Please give me a number", we have ten minutes, and there's a possibility of five million compounds present as contaminants. Also you would like the values down to the parts per billion level. Those, of course, are exaggerated terms, but I'm sure they are the dreams of the other scientific professions to request this of the laboratory. The chemist and physicist wrestle with these kinds of problems as the medical people wrestle with the meanings of the different pieces, the interpretation of data to see if there really was adaptation or if it was complete failure.

AMRL-TR-68-175

PAPER NO. 24

# GENERAL INSTRUMENTAL APPROACHES TO TRACE CHEMICAL ANALYSIS IN CLOSED ECOLOGICAL SYSTEMS

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Trace analysis studies of Closed Ecological Systems include the performance in three important specialties. These are: (1) sampling, (2) instrumentation, and (3) data collation, correlation, and presentation. Each of these requires meticulous care and knowledge of the other tasks if reliable information is to be generated without added anomalies.

In a 1967 monograph prepared by the Department of Commerce National Bureau of Standards, several authors discussed preconcentration for trace analysis; one was Dr. Josey Menzuski from the University of Poland whose efforts were mainly directed toward solids and liquids. He used complexing agents and chromatographic exchange techniques with primary emphasis on contamination in the reagents and equipment used for the test. Another piece of work described in a paper by Dr. George Morrison of Cornell University certainly complements that of Dr. Menzuski. He divides all methods into three component steps, those of sampling, chemical and/or physical pretreatment, and instrumental measurement. Certainly these steps are interrelated and require different degrees of emphasis depending on the individual analytical situation. Morrison emphasized trace analysis primarily in solids and liquids, and used adsorption photometry, fluorescence, flame, atomic adsorption, neutron activation, emission spectrography, mass spectrometry, microprobe, and X-ray fluorescence. Each of the methods proposed by these authors certainly is valid and the complexity of instrumentation continuously increases. I think that it would be very fine if each of our laboratories were equipped with a total complement of the instruments described before and some of the instruments described in the papers we are going to hear today. One of the problems, obviously, is that unless one has the NASA or Air Force budget behind us to manage and equip our laboratories with more than a million dollars worth of tools, one must find more moderate methods that will provide acceptable trace contaminant information.

Sampling or sample collection from a closed ecological system (for this presentation only atmospheric samples will be discussed), and resampling of the first sample for distribution to the analytical devices will be described. A third sampling procedure, which relates to continuous atmospheric monitoring, will be discussed under instrumentation. For the space programs, two sample collection

systems that have been extensively evaluated and used rely on cryogenically isolating and concentrating the condensable sample components. One device, designed and constructed by the School of Aviation Medicine, Brooks Air Force Base, utilizes three stainless steel collection bottles serially aligned and submerged in three progressively colder baths of wet ice, dry ice-Dowanol, and liquid nitrogen. Each sample cylinder contains a center tube and inlet and exit lines with valve closures. Special temperature control for -75 and -175 C trapping is provided at the operating console. This collection system is completely described by Mr. Conkle in an article appearing in Aerospace Medicine (Conkle, 1965). A second system, constructed by the Atlantic Research Corporation for NASA Langley, cryogenically isolates the condensable components and also provides for on-stream sampling (grab samples) to monitor permanent gases and/or other non-condensables. The second collection system is described in a report by Aerojet-General (Report 3570, 1968). Both methods use a positive gas flow technique provided by a diaphragm pumping system. This latter system was used at NASA-MSC and NASA-KSC. Volumetric capacities of these two cryogenic systems were 200 cc cylinders for the Brooks unit and 500 cc cylinders for the NASA one. The gas flow through these systems averaged 300 cc/min and 500 to 800 cc/min, respectively. Other preconcentration methods include the one just described by Mr. Popma of NASA-Langley Research Center, charcoal canisters used in the Apollo and Gemini Vehicles, and chromatographic columns described by Dr. Courtney Phillips of Oxford University. Operating and space requirements usually influence the choice of collection systems as well as ease and simplicity in recovering the collected sample components from the original sampling device. One must also consider the possibility of altering the chemistry of the collected components during sampling and sample redistribution to the measuring devices.

The second phase of sampling identified as "sample splitting" requires a high-vacuum manifold system with pressure measurement. The vacuum system must be thermally controlled and capable of providing homogeneous and representative sample fractions that can be used for multiple instrumental analysis. Mixtures of gases containing known micro amounts of compounds must be used to verify material recovery from the sample splitter. To insure cleanliness in the sample splitting system and cylinders used for isolating fractions of the respective samples, an extensive heating and evacuation procedure is used. Following repeated heating, evacuation and flushing with inert carrier gas, fractions are taken from this system with an inert carrier gas and the gas itself analyzed by the respective instruments to establish background levels of contaminants that may have resisted the vigorous purge procedure.

Instrumentation has continuously improved in sensitivity to yield lower detection limits, but the potential loss of sample components by adsorption on the hardware remains constant. If the analyses depend on straight or non-preconcentrated samples, the quantities of material or number of components lost by adsorption on the sampling cylinder walls might be greater than the observed values. By comparison, a concentration method offers several-hundred-fold more material with essentially the same sample losses through non-recoverable adsorption in the hardware system. Other advantages lie in the area of instrumental calibration for the identified contaminants. Identification and quantification of elements or compounds such as hydrogen, oxygen, argon, nitrogen, carbon monoxide, methane, ethane and ethylene can and should be made by continuous monitoring or high sensitivity analytical tools specifically designed to handle straight or grab samples.

## INSTRUMENTATION

The more common tools used for measuring small quantities of volatile materials are gas chromatography and mass spectrometry. Microwave spectrometry was presented as a new and most capable device for resolving and measuring micro amounts of atmospheric contaminants. Non-dispersive infrared spectrophotometry has also been used extensively for continuous monitoring of selected compounds in gases. For example, the Mine Safety Appliance's LIRA Models 300 and 500, and Beckman's IR-215 spectrometers are useful for measuring carbon monoxide, carbon dioxide, nitrogen dioxide, methylene chloride, and certain hydrocarbons in the parts-per-million region. The L & M tap gas analyzer depends on nondispersive infrared and thermal conductivity for measuring air pollutants in industrial services. Some mass spectrometers are used for continuously monitoring low level atmospheric contaminants. The Bendix Time-of-Flight RF instrument is one example of this capability. Monopole and quadripole spectrometers also provide rapid scanning with reasonably good sensitivity but each of these instruments lacks the required spectrum reproducibility to serve as a good quantitative tool. Also, published spectra from sector type instruments cannot creditably be used for unknown identification except for special cases and with highly qualified operators. In general, the combination of gas chromatography and sector-type mass spectrometry is most suitable for atmospheric trace contaminant analysis.

Currently, gas chromatography, used as a quantitative tool for trace contaminant measurements, offers the best services considering its relative simplicity, versatility, and cost. Our laboratories believe that multiple chromatographic analyses (2 to 4 chromatographs containing selected columns for different types of compounds) provide the greatest confidence and data reliability. For example, Poropak columns are used for low molecular weight hydrocarbons, esters, and Freons; a Carbowax 1000 column is used for alcohols, aldehydes, and esters of moderate molecular weight; and a high temperature SE 30, Apiezon L, or a silicone column has been used for the higher molecular weight species. Each chromatograph should have dual ionization detection capabilities, e.g., flame and electron capture ionization. Four mass spectrometers would be unrealistic and, therefore, combining the high temperature, high molecular weight chromatographic system with one M.S. appears to be the choice for state-of-the-art trace analyses. A carefully designed M.S. sample introduction system following chromatographic exhaust splitting with or without separators of the Biemann or Llewellyn type provides the required analytical capabilities.

The above analytical approach to trace analysis has consistently provided reliable information on more than 100 trace contaminants found in controlled closed environmental systems.

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## DISCUSSION

DR. BACK: Of all the instrumentation that you know about, what instrument or series of instruments would you put aboard a space cabin if you needed to look at half a dozen important components like carbon monoxide, and you had to consider weight and power requirements?

MR. MOBERG: That's a loaded question. You always must face weight penalties when you have sophisticated instruments. I think that mass spectrometry along with some kind of preconcentration technique is as close as one could come to answering this kind of a problem. We have been working on gas chromatography for years and I think that it has a strong possibility of providing results for ten to twenty components. The problems really are: do you get the resolution of the ten to twenty components and not have interference from other components that are present? And how do you manage it? Because if you cut down on column length and you streamline the instrumentation, you have too much of a chance of getting artifacts, two or three, or possibly four components covering under one signal and giving you falsely high results. The quadruple and mass spectrometer is starting to come into its own. I just briefly mentioned it. It has its own family of problems but as for the size and weight it can offer advantages. Consolidated has worked on a quadruple mass spectrometer for space craft since 1962, and I still don't know of one flying today, so there are problems there too. There isn't an answer to covering all of these problems of analysis to accurate levels. I would only offer a small mass spectrometer or sophisticated chromatograph.

DR. BACK: As a biologist, I know nothing about this, but for my own edification, what are the problems inherent in reducing a signal that you get on a mass spectrometer to something that you can send back to earth for on-line, on-time answers?

MR. MOBERG: The management of the signal is quite simple. It has been simplified. If the signal can be obtained and it is meaningful to the instrument, then it can be amplified and telemetered back to earth in real time. One to five-volt signals which are usually required in spacecraft output are very reasonable for many of the instruments.

MR. WANDS: This question brings up another point that I don't think has been mentioned at all this morning regarding analytical systems, and this is the equipment reliability. As one gets way out yonder without any technicians handy with little pockets full of screwdrivers, this is most important, and I'm wondering how the boys down at Langley have been faring with their very elaborate setup in terms of system reliability.

MR. EASLEY: Well, that's a very good point. As far as the microwave spectroscopy equipment is concerned, we have given no thought to flying what equipment we now have because it's too complex electrically, and occasionally we do have to use screwdrivers and wrenches in order to get it going. So, I wouldn't recommend anybody trusting it in flight, even if it were small enough to do it, so we are quite a ways off from a flyable microwave spectrometer. I think we are much closer in the case of the small mass spectrometer with the preconcentrators or the chromatographs that Mr. Popma and Mr. Moberg talked about, but as far as microwave, we can't fly one now.

MR. MOBERG: I might comment on reliability. If you are familiar with the data that can be obtained from the animals that you look at and find that a single animal is pretty unreliable in giving you either toxic information or body function information, I am almost convinced, particularly with Mr. Murphy sitting at my shoulder, that the sophisticated instrumentation we use has become human, and just about the time when you want it to perform, and you feel it can perform because there is no obvious thing wrong with it, it decides not to perform. We have looked at reliability for chromatography and requests have been made that the instrument must be completely functional for somewhere near 30 months without any attention whatsoever and producing completely repeatable numbers, and we haven't seen the instrument that can do this yet. Mass Spectrometry also suffers from the same type of plague.

MR. VERNOT: I don't really have any evidence to support this belief of mine, but in answer to Dr. Back's question, I think that if you know what you want to analyze for, that is, if you set up six discrete things you want to analyze for, it seems to me quite possible the direction that will be taken will be specific instruments. This is happening all the time, particularly in the inorganic field where we find potentiometric methods, specific ion detection and measurement, things we never heard of ten years ago, fluoride ion, all the calcium ion electrodes, this sort of thing. I think it's perfectly possible to apply this technique to the organic molecules or the specific molecules you're thinking of using on a space cabin. I don't know what properties of the materials to use for detection and measurement, but these materials have to have specific physical-chemical properties or you wouldn't be interested in them.

DR. BACK: What instrument may or may not be dependent upon gravity? Are there any of them that can not work under a weightless condition?

MR. MOBERG: I think they can all be made to function under weightless conditions. This is where my nose may fail but the instruments may not.

MR. ADAMS (Brooks Air Force Base): I'd like to prevail upon Dr. Johnson to bring us up to date on the Mark V analyzer.

DR. JOHNSON: What Mr. Adams is referring to here is a submarine analyzer based on gas chromatography. All I can say at the moment is that it's onboard several submarines. We are having difficulty with them and the experience we have had in the past with automatic analyzers, in all of our submarine work, including the Mark II through IV, would indicate to me that this problem of reli-

ability is difficult and is going to be awfully hard to handle. The Mark V analyzer actually handles some very simple gases. I'm trying to think of the components that we are looking for, oxygen, $CO_2$, hydrogen and the like. Even with those simple gases, infrared and other methods such as gas chromatography, we're still having trouble keeping these on line, and I don't think that is very helpful, but that's the fact.

DR. COX (McDonnell Douglas): I'd like to ask Dan a couple of questions. One is with respect to an unknown, dynamic changing atmosphere. What is the solution to the problem in this matter of loading capacity on your accumulator?

MR. POPMA: Here again, in the face of an unknown atmosphere you're going to have to take two or three cuts at it at a different flow rate or with different sensitivities in order to come up with an answer that you feel you can trust. If you do exceed the linear loading capacity, obviously, you're going to have some errors. The cycling time for this instrument is fairly short and I don't think we're going to see rapid changes in the atmospheric contaminant level, that is, over a period of just minutes.

DR. COX: I hope not. Certainly, there is always a potential and I think this is a basic problem with respect to your instrumentation. I don't think it's insurmountable. I hope we can solve it. One more question with respect to the specific analysis of CO in a nitrogen containing atmosphere with your system. Do you think that has a real potential?

MR. POPMA: I believe CO was one of those listed on the chart this morning. I don't recall the numbers, but I think that I showed a capability of one part per million and I think with some continued examination in this area we can get well below that.

DR. COX: It will require a specific accumulator for CO?

MR. POPMA: The accumulator we're using now is palladium-treated charcoal which shows good specificity for CO in the presence of nitrogen.

AMRL-TR-68-175

PAPER NO. 25

# MONOMETHYLHYDRAZINE (MMH) ABSORBING CAPACITY AND INDICATOR EFFICIENCY OF THE MSA ROCKET PROPELLANT CANISTER GMN-SSW

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In the preceding sessions of this conference, a considerable amount of data has been presented to demonstrate the high toxicity of monomethylhydrazine (MMH). This high toxicity, coupled with the use and storage of MMH in confined spaces such as missile silos, requires that personnel who may be exposed to this compound be afforded protection from its vapors.

The problem of personal protection in regard to MMH can be considered in two parts. First, in cases of high concentrations of MMH, such as might be encountered in a serious leak or spill, both respiratory and skin protection would be required. In such cases a complete protective clothing ensemble, such as a "Scape Suit" would be required. In the second case, where only low concentrations of MMH were present, respiratory protection only, such as that provided by a gas mask should be sufficient.

A number of gas mask canisters which hold promise for this purpose are commercially available although none of these is designed specifically for use with MMH. The canister which appears to be best suited for this application is the model GMN-SSW Rocket Propellant Gas Mask Canister marketed by the Mine Safety Appliance Co., Pittsburgh, Pennsylvania. The federal designation of the canister is M15A1.

This canister has been previously tested by the Regional Environmental Health Laboratories, McClellan AFB, California to evaluate its usefulness in removing nitrogen dioxide ($NO_2$), unsymmetrical dimethylhydrazine (UDMH), and $NO_2$ followed by UDMH. These tests determined that the breakthrough value for $NO_2$ was $283 \times 10^3$ ppm-min with a flow rate of 25 l/min and $300 \times 10^3$ ppm-min for UDMH at the same flow rate. The canister also effectively removed UDMH and hydrazine even after saturation with $NO_2$.

The GMN-SSW canister is designed to afford protection against red fuming nitric acid, UDMH, hydrazine, hydrogen peroxide, and other organic vapors such as aniline, kerosene, and alcohol. The interior of the canister is divided into three sections by the use of perforated metal baffles and filter paper (figure 1). The layer nearest the inlet contains calcium carbonate, the second layer is made up of granular silica gel, and the last layer is of activated charcoal. A window at the silica gel layer allows the wearer to view a portion of the silica gel which has been impregnated with an indicator substance. A flapper valve is attached to the inlet. Corrugated filter paper is also present at the inlet to act as a particle filter.

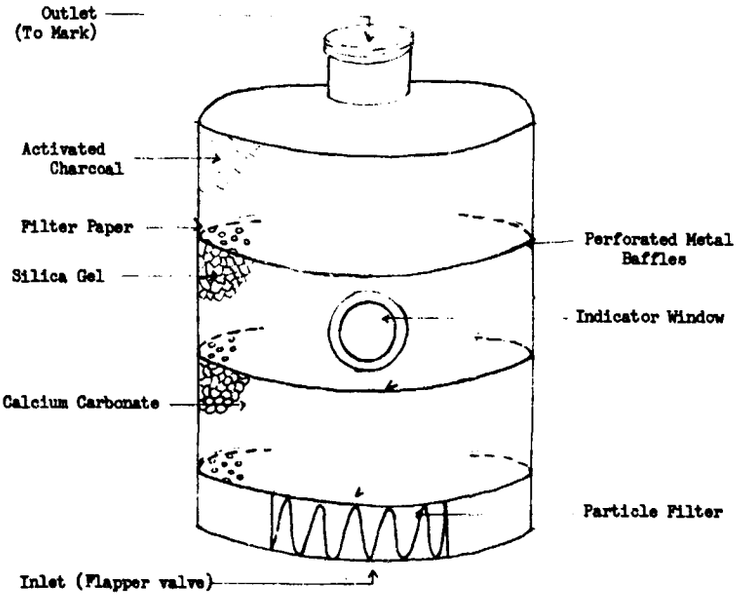


Figure 1. CANISTER CONSTRUCTION

A dynamic test system was fabricated for the study. This system consisted of four basic parts: 1) a vacuum pump and valves to maintain a constant flow rate of 50 l/min through the canister; 2) a Harvard infusion pump and mixing chamber to obtain the desired concentration of MMH in air; 3) a MSA "Billionaire" trace gas analyzer for monitoring the concentration of the influent air stream into the canister; and 4) a Technicon Autoanalyzer for measuring the concentration of MMH in the canister effluent to determine time of breakthrough. This test system is shown in figure 2. A flow rate of 50 l/min was chosen for the study since this rate approximates the respiratory rate of an individual undergoing strenous exercise (Altman, October 1964). A continuous flow rather than an intermittent flow was chosen so that the results obtained would indicate minimum protection afforded. All canisters tested, with the exception of canister #1, used the infusion technique for preparing a constant concentration of MMH. Sufficient liquid MMH (Olin-Matheson Co., Lake Charles, La.) was added to the mixing flask so that when the air stream was passed through the flask, the desired concentration range was obtained. Fine adjustment of the concentration was made with a T-joint and micrometer valve in the MMH input line. Sufficient liquid MMH was infused into the mixing chamber to maintain a constant liquid level in the chamber.

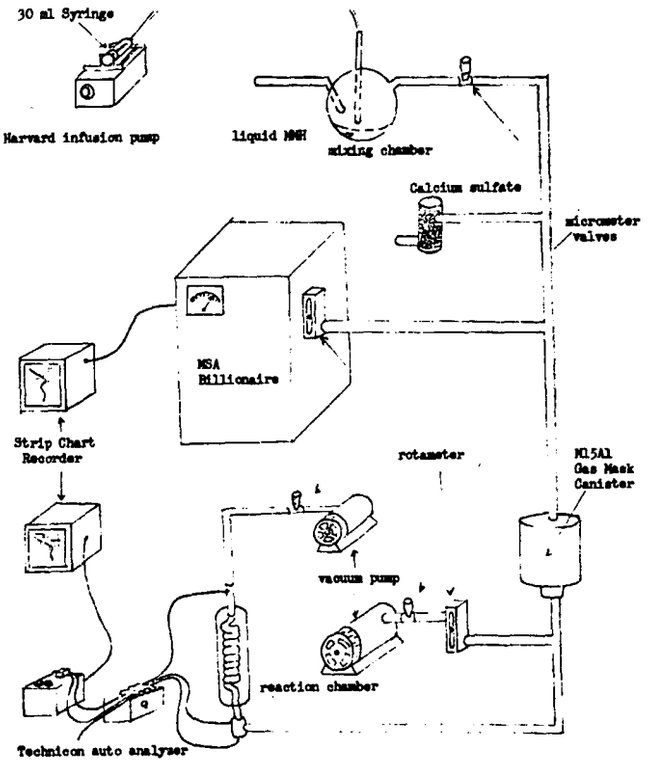


Figure 2. EXPERIMENTAL SYSTEM

For the test of canister #1, the mixing chamber and infusion pump were replaced with a 210 liter mylar bag containing a nominal concentration of 3,000 ppm MMH in nitrogen. Input concentrations were monitored by passing a small part of the sample flow through the MSA "Billionaire" which had been previously calibrated for the desired MMH concentration range. Calibration was made by the use of standard bag samples of known concentration. The effluent stream of the canister was split by a T-joint and a portion of it was passed through a reaction chamber used in conjunction with the Technicon Autoanalyzer which measured the MMH concentration colorimetrically by the method of Vernot and Geiger (in press). This method is based upon the reaction of MMH with iodine according to the equation:

$$CH_3NHNH_2 + 2I_2 \rightarrow N_2 + 3HI + CH_3I$$

The decrease in absorbance at 466 mμ of a standardized $I_2$ solution is used to measure the concentration of MMH passing through the reaction chamber according to the following formula:

$$\text{concentration MMH (ppm)} = \frac{A \times K\,(ABS. - ABS_s) \times 24.5 \times 10^6}{4B}$$

A = flow rate of $I_2$ solution (ml/min)
K = normality $I_2$ solution/abs. of $I_2$ solution
ABS = Initial absorbance of $I_2$ solution
$ABS_s$ = Abs. of $I_2$ solution after reaction with MMH
B = sample flow rate (cc./min)

The autoanalyzer and the "Billionaire" were equipped with strip chart recorders so that a time record of both input and effluent concentration was made. A lag time of 11 minutes existed in the autoanalyzer due to the time necessary for solutions to be pumped through the colorimeter. Since previous studies had indicated heating of the canister due to reaction with hydrazine vapors, the temperature of the canister surface was monitored with a 0-600 C pyrometer during all of the tests.

Six canisters were used in this study. One was intermittently exposed to 30 ppm MMH, one intermittently exposed to 1,000 ppm MMH, one continuously exposed to 1,000 ppm MMH, one continuously exposed to 900 ppm MMH, and two were continuously exposed to 1,200 ppm MMH.

Table I is a summary of the data collected on the six canisters tested. Results show that the canisters varied greatly in concentration x time to breakthrough values and that a large amount of heat was generated in all canisters which were exposed continuously. Canister surface temperatures from 150-200 C were often experienced. This heating was generally found to be a prelude to breakthrough of MMH.

## TABLE I

SUMMARY OF RESULTS OBTAINED ON ALL CANISTERS TESTED

| Canister # | Input Conc (ppm) | Flow Rate (l/min) | Time to Indicator Change (min) | | (ppm min x 10 ) CT for indicator | | Time to Penetration .2 ppm (min) | CT for Penetration x 10 ppm min |
|---|---|---|---|---|---|---|---|---|
| | | | Ycl Gr | Full devel | yel-gr | full devel | | |
| 1 | 30 | 50 | 2370 | 4380 | 7.1 | 13.2 | -- | -- |
| 2 | 1000 | 50 | 300 | 480 | 30.0 | 48.0 | 630 | 6.3 |
| 3 | 1000 | 50 | 150 | 210 | 15.0 | 21.0 | 207 | 2.07 |
| 4 | 900 | 50 | 210 | 360 | 10.8 | 3..4 | 482 | 4.34 |
| 5 | 1200 | 50 | 75 | 130 | 9.0 | 15.6 | 110 | 1.32 |
| 6 | 1200 | 50 | 60 | 165 | 7.2 | 19.8 | 360 | 4.32 |
| Avg(3, 4, 5, 6) | -- | -- | -- | -- | 9.8 | 23.4 | -- | 3.01 |

The area of the canister surface which was heated to the highest temperature, as evidenced by blistering and burning of the paint, was adjacent to the internal baffle separating the silica gel and charcoal layers. This heating may result from an air oxidation of the MMH catalyzed by the large surface area of the activated charcoal. Heat generated in the canister interior would be effectively transferred by the metal baffle to the canister surface and would be expected to be most severe at the surface point in contact with the metal baffle. The amount of heat generated would be expected to affect the useful lifetime of the canister since an extreme rise in canister temperature would tend to desorb MMH which had previously been adsorbed on the silica gel layer.

Variability in the amount of internal heating and consequent variability in canister lifetime may be due to a number of factors. Physical factors such as grain size of the chemical components and channeling in the layers could vary the time necessary for a large concentration of MMH in air to reach the charcoal layer. Surface phenomena would also be expected to affect the degree of heating and time of commencement of heating. Probably the most important factor involved is the degree of hydration of the Ca $CO_3$ layer of the canister. It has been shown previously that MMH is decomposed much more rapidly in humid air than in dry air (Vernot, 1967). It would therefore be expected that as the water content of the Ca $CO_3$ layer is increased, the amount of MMH that is decomposed rather than adsorbed will also be proportionately increased. Since it is assumed that heating is governed by the concentration of MMH in the air when it reaches the activated charcoal and not the concentration of decomposition products such as nitrogen and methane, the hydration level would contribute significantly to the heating phenomenon and therefore vary the useful lifetime of the canister. Although the canister manufacturer has stated that the Ca $CO_3$ used contains approximately 15% water, storage conditions could greatly change this level and therefore lead to the variations in canister lifetime shown in this study. It should also be noted that the highest CT to breakthrough values were obtained on the two canisters which were exposed intermittently rather than continuously. (Canister #1 and #2.) It is postulated that some decomposition of the adsorbed MMH may occur during periods of non-exposure and therefore that the lifetimes of such canisters would be appreciably longer than those in which no "regeneration" time was allowed. While the variations experienced make generalization difficult about the safe lifetime of the canister for removing MMH, certain conclusions can be drawn from the data. At low concentrations of MMH (on the order of 30 ppm) the canister can be expected to offer adequate respiratory protection for a minimum of 73 hours. This figure was arrived at by extrapolating the minimum concentration x time value obtained (canister #5), to 30 ppm. Since canister #1, which was tested at 30 ppm, exceeded this figure by more than 24 hours, this should be considered a safe level. The window indicator also appears to be a reasonably accurate indicator of canister usefulness if the yellow-green color change is used as an end point rather than the dark-blue-green color of the fully developed indicator. Canisters should be discarded after this first color change occurs due to the possibility of lung injury which may occur from the rapid and intense heating of the air stream which may occur either before or after the color is fully developed. Storage of the canisters in areas of relatively high humidity is also to be recommended, since this will tend to keep the hydration of the Ca $CO_3$ layer at a maximum. Shorter periods of use (one hour or less at a time) will also tend to increase the useful lifetime of the canisters.

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## DISCUSSION

MR. BILLINGS (Kennedy Space Center): How did these UDMH or MMH tests compare?

CAPTAIN ARNOLD (Aerospace Medical Research Laboratories): Really, it looks better for MMH than it did for UDMH. They got a figure of somewhere around 300 x $10^3$ ppm minutes to break through at a flow rate of 25 liters per minute. We got a very similar figure. It was about 300 x $10^3$, but we were using twice the flow rate, 50 liters per minute. I might explain why we use this high flow rate. The biology data handbook gives a respiratory rate of 42 liters per minute for a man breathing under strenuous exercise, and we felt that we wanted to put this canister to the test under the most adverse circumstances, so we went to the high flow rate to give it really a difficult test, and then we figured we could extrapolate to what would normally be expected.

MR. BILLINGS: I think you'd be lucky to get that flow rate through that mask.

CAPTAIN ARNOLD: It took some doing to get it pumped through at this rate.

MR. BILLINGS: Also, have you attempted to draw any curves that would indicate useful life for higher concentrations? In case there is a spill we would like to use them for emergency evacuation.

CAPTAIN ARNOLD: Due to the variance in the canister it was really rather difficult to draw a meaningful curve. However, I think you can safely state that anywhere up to 1000 ppm concentration this canister is going to be good for at least two or three hours.

MR. BILLINGS: Would you care to give me a ballpark figure, how many ppm's, say for 15 minutes, something like that?

CAPTAIN ARNOLD: Well, we didn't test it at this. If it follows a similar pattern, though, for 15 minutes, I would think that you would have to get the concentration up five, ten thousand ppm before you would break through in 15 minutes.

MR. WANDS: Captain Arnold, in your flow rate of 50 liters per minutes, is this a steady flow rate, or is this pulsed?

CAPTAIN ARNOLD: This was continuous. There was no attempt made to pulse the rate.

MR. WANDS: This, incidentally, is going to work against the canister.

DR. CULVER: At the five or ten thousand parts per million level, would you anticipate a serious heating problem of the canister?

CAPTAIN ARNOLD: Yes. I wouldn't want to wear that canister at the five to ten thousand ppm. I feel I might end up getting my lungs fricasseed.

LIEUTENANT COLONEL WESTLAKE (Space and Missile Systems Organization): At high level, where you might be close to loading capacity, if you used it with the UDMH or MMH, and you then used it for $NO_2$, might you anticipate hypergolic effects?

CAPTAIN ARNOLD: Well, on the canister that was tested at the Regional Environmental Health Laboratory, when they loaded it with UDMH and $NO_2$ together, they got a severe heating effect. They blamed this on the fact that the $NO_2$ and the UDMH are reacting, and this is what is causing the heat. However, in our experience with MMH, we didn't even need $NO_2$ to get it quite hot.

AMRL-TR-68-175

PAPER NO. 26

# THE DETERMINATION OF CHLORINATED HYDROCARBONS AT LOW CONCENTRATIONS IN CLOSED ATMOSPHERES

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and
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## INTRODUCTION

The industrial and commercial applications of volatile halogenated hydrocarbons, particularly in the $C_1$ to $C_3$ range, have greatly increased in recent years. These compounds are being used in many new products and processes which increase the likelihood of their presence in the atmospheres of closed systems such as nuclear submarines, spacecraft, and hyperbaric chambers. Chlorinated hydrocarbons, for example, are often used to impart flammability resistance to solvent formulations, and in addition they are good solvents for polymers and other organic materials. Chlorofluorocarbons are very commonly used as refrigerants, aerosol propellants, fire extinguishing agents, degreasers, cleaners, and general solvents. In addition a few bromofluorocarbons are in general use. The increasingly wider use of chlorinated and other halogenated compounds is of special concern in closed atmospheric situations because of their potential contribution to corrosion problems, deterioration of organic materials, and the inherent toxicity of some compounds in this group. In addition, halogenated hydrocarbons can decompose under some conditions, such as catalytic combustion, to yield compounds of greater toxicity and corrosivity (Johnson, May, 1967; Johnson, July, 1967). Therefore, it is very important to control and remove these compounds from closed atmospheres. This need imposes a requirement for a good analytical method which can be used to determine the presence and distribution of halogenated hydrocarbons in samples of these atmospheres.

Identification of specific compounds present in a given atmosphere and determination of the concentration of each compound of interest are very difficult because of the many hundreds of individual compounds and the very low concentrations of each compound. In the past, special techniques have been used to concentrate the organic contaminants, separate out certain types of compounds, followed by detailed analyses of individual compounds. For

example, adsorption of organic compounds from the atmosphere on activated carbon, followed by desorption and analysis in the laboratory has been a powerful tool for learning what compounds were present. However, the data obtained is usually considered semi-quantitative at best, and often should be treated only as qualitative indications of the compounds in the atmosphere.

It is much more desirable to make analyses for organic compounds on the atmosphere itself, or where necessary, to obtain compressed samples of the atmosphere from the closed environment and return them to the laboratory. By working directly on gas samples, some of the problems which occur when a separate concentration step must be used can be avoided.

Detection and identification of the chlorinated compounds in gas mixtures containing dozens or even hundreds of organic compounds was our immediate goal. Gas chromatography was turned to because of its enormous power for separating in time and space individual compounds in the separation column. It would be doubly powerful if the detector unit of the gas chromatograph could be used as a second dimension. In other words, if after the separation the detector responded to the molecules of halogenated hydrocarbons only, and ignored all the rest, identification of a given peak would be more certain and its quantitation much easier.

For the detection of chlorinated hydrocarbons, three selective detectors were available: electron capture, stacked thermionic flame, and microcoulometer. All three were examined to some extent. The electron capture detector has the following disadvantages: very narrow linear range, separate calibration factor required for each halogenated compound, insensitivity to many chlorinated compounds, and response to certain other types of electron-attracting compounds. The stacked thermionic flame detector has not been successfully applied to routine determinations due to difficulties in stabilizing this composite flame for certain obscure reasons.

The microcoulometer held promise of being the best detector for our purposes; first of all, because it was specific to compounds containing chlorine, bromine, and iodine. Secondly, the detector response should be directly related through Coulomb's law to the number of chlorine atoms under each chromatographic peak. Two drawbacks in this detector were also apparent: slow response and lack of sensitivity. These drawbacks were compensated for by special techniques described in this report based on temperature programming and on-column concentration.

## EQUIPMENT AND PROCEDURES

The column packing materials used for developing the on-column concentrating technique were Porapak Q and Porapak S obtained from Waters Associates, Inc. The chromatograph used was a Microtek Model MT-220 equipped with a Dohrmann microcoulometer. The chromatograph was also equipped with a Microtek multifunction temperature programmer. Carle 8-port sampling valves were added later.

The chlorinated and brominated standard mixtures were prepared in 1.7 liter stainless steel bottles obtained from Alloy Products Corporation. The fluorocarbons were obtained from the Dupont Company and the other halogenated hydrocarbons were purchased from Chem Service Inc.

The gas mixtures were usually prepared by dilution techniques. For liquid halogenated compounds, the calculated volume of liquid was injected with a microsyringe, through a septum, into an evacuated gas bottle. The bottle was then pressurized to 300 psia with air or helium. For lower concentrations of these compounds, aliquots of the original mixtures were diluted further.

## DEVELOPMENT OF THE METHOD

The Dohrmann microcoulometric detector with a silver cell and operating in the oxidative mode is very specific, responding only to chlorine, bromine, and iodine atoms. It does not respond to fluorine. As the halocarbon components of the mixture are eluted from the chromatographic column, they are pyrolyzed in an oxygen stream at 950 C, so that the halogen ions can be titrated with silver ions. The coulometric analysis is of particular value since the response of the detector is related through Coulomb's law to a direct quantitative measure of the chloride ions present in a given peak. This quantitative response of the detector makes it possible to calculate the quantity of each compound without precalibration with that particular compound.

The specificity of the detector to the halogenated compounds in practical mixtures is illustrated in figure 1. In this case a gas sample taken in a closed atmosphere was injected into the chromatograph. After passing through the separation column, the effluent gas stream was split, with a portion of the carrier gas going to a flame ionization detector and the rest of the carrier gas going to the microcoulometric detector, the two detectors feeding into a two-pen recorder. The flame detector responds to almost any organic compound present in the atmosphere as shown in the upper chromatogram in figure 1. The microcoulometric or lower chromatogram is much simplified because this detector responds only to the chlorinated compounds in the mixture and ignores all the other compounds. It may be noted also that the baseline of the flame chromatogram changes due to degradation of the polymeric material. The microcoulometer does not respond to the degradation products.

Inherently, the microcoulometric detector is not sensitive enough to detect compounds at such low concentrations without the use of a concentrating technique. Fortunately, at this time a new class of chromatographic column packing materials became available. These were the porous polyaromatic polymer (Porapak) beads. A particularly unique advantage of these polymer beads is that at room temperature even the volatile contaminants in gas samples can be concentrated on such a column at room temperature. Even the lower molecular weight halogenated hydrocarbons have very long retention times on these columns. These volatile contaminants apparently remain concentrated in a very narrow zone at the head of the column until the column temperature is raised. In this manner, atmosphere samples as large as 500 ml have been concentrated on a porous polymer column at 50 C and then chromatographed successfully. A detailed discussion of this on-column concentrating technique will be published soon (Williams).

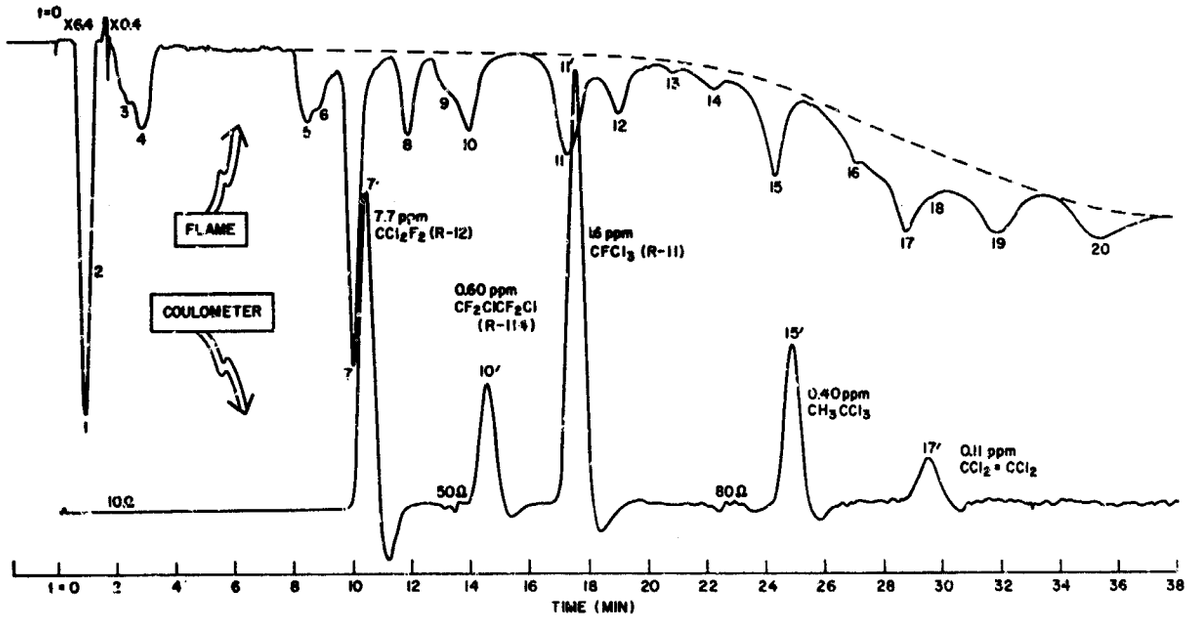


Figure 1. SIMULTANEOUS CHROMATOGRAMS OF AN ATMOSPHERE BY HYDROGEN FLAME AND MICROCOULOMETER DETECTORS

After concentrating the organic contaminants on the head of the column, these compounds are eluted and separated by using an appropriate temperature program. A typical analysis procedure is described below.

## Typical Analytical Procedure

The type of Porapak and the particular temperature program that is used depends on the mixture to be analyzed. A procedure that has been used for a number of submarine atmosphere samples is as follows: a portion of the gas sample to be analyzed is put in a 100-ml or 500-ml sample loop. Care is taken to equilibrate the column oven at a selected temperature between 30 and 50 C. In this case 50 C was chosen. This equilibrium temperature must be the same for each analysis or the retention times of the sample components will vary slightly in repetitive runs. The sample valve is opened and the sample is moved from the sample loop onto the column over a 5-minute period by the helium carrier gas. The flow rate of the carrier gas must be high enough to transfer the sample from the loop to the column in the allotted time. The organic contaminants are eluted from the column by raising the temperature according to the temperature program shown in figure 2. After the 5-minute sample sweep at 50 C, the

temperature is raised at the maximum heating rate to 100 C and held at this temperature for 5 minutes. The temperature is then raised to 215 C in three steps. After holding at 215 C for 12 minutes, the column oven is cooled at the maximum rate to bring the oven temperature back to 50 C and equilibrate there for 10 minutes in preparation for the next sample.

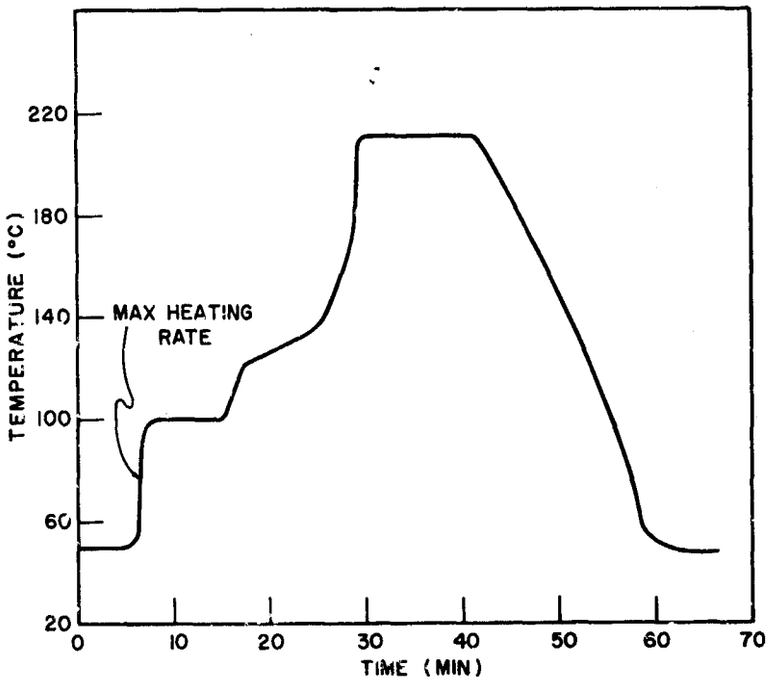


Figure 2. A TYPICAL TEMPERATURE PROGRAM

## DISCUSSION

By following a procedure such as the one described above, a large number of halocarbons commonly encountered can be separated and identified as discrete peaks. Table I lists the compounds studied which cover a range of boiling points from -81 C to 132 C. By careful selection of the temperature program, all of these compounds have been separated as discrete peaks at concentrations as low as 10 ppb by volume in air samples. These separations are shown in figure 3, with the vertical lines indicating the retention times of each compound.

Just as in the case of many other analytical techniques, certain characteristics are inherent to this method and should be understood for best results. These peculiarities are perhaps best explained by examining some actual chromatograms. For example, let us first refer to figure 4. If we look at peak 8 in this chromatogram, we see an overshoot below the baseline, followed by recovery to the baseline. For quantitative analysis, it is necessary to subtract the area of the overshoot from the area of the peak, to get the true area for the chloride ions.

TABLE I

HALOGENATED HYDROCARBONS ANALYZED BY MICROCOULOMETRIC GAS CHROMATOGRAPHY

| COMPOUND | FORMULA | B. P., °C |
|---|---|---|
| Chlorotrifluoromethane | $CF_3Cl$ | -81.4 |
| Bromotrifluoromethane | $CF_3Br$ | -57.8 |
| Chloropentafluoroethane | $CF_3CF_2Cl$ | -38.7 |
| Chlorodifluoromethane | $CF_2ClH$ | -40.8 |
| Dichlorodifluoromethane | $CCl_2F_2$ | -29.8 |
| Chloroethene | $CH_2=CHCl$ | -13.9 |
| 1, 2-dichloro-1, 1, 2, 2-tetrafluoroethane | $CF_2ClCF_2Cl$ | 3.8 |
| Dichlorofluoromethane | $CCl_2FH$ | 8.9 |
| Trichlorofluoromethane | $CCl_3F$ | 23.7 |
| 1, 1-Dichloroethene | $CH_2=CCl_2$ | 37 |
| cis-Dichloroethene | $CHCl=CHCl$ | 60 |
| 1, 1, 2-Trichloro-1, 2, 2-trifluoroethane | $CCl_2FCClF_2$ | 47.6 |
| trans-Dichloroethene | $CHCl=CHCl$ | 48.3 |
| 1, 1, 2, 2-Tetrafluoro-1, 2-dibromoethane | $CF_2BrCF_2Br$ | 47.3 |
| Trichloromethane | $CHCl_3$ | 61.2 |
| 1, 2-Dichloroethane | $CH_2ClCH_2Cl$ | 83.5 |
| 1, 1, 1-Trichloroethane | $CH_3CCl_3$ | 74.0 |
| Trichloroethene | $CCl_2=CHCl$ | 87.2 |
| 1, 1, 2-Trichloroethane | $CHCl_2CH_2Cl$ | 113.5 |
| Tetrachloroethene | $CCl_2=CCl_2$ | 121.2 |
| Chlorobenzene | $C_6H_5Cl$ | 132 |

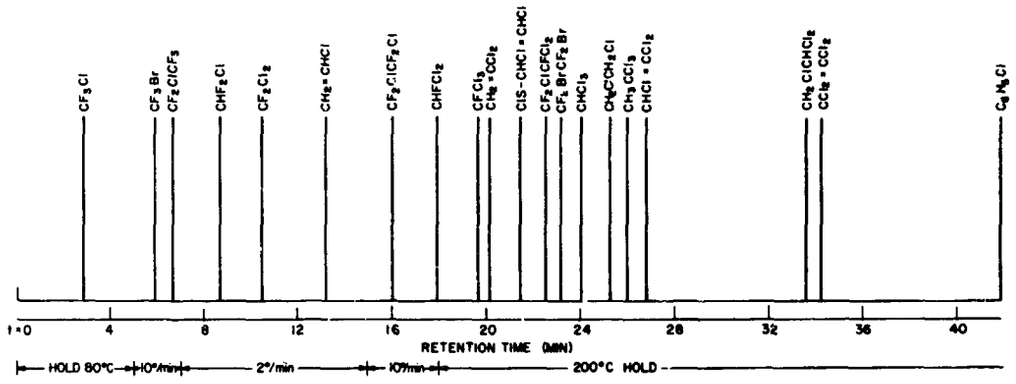


Figure 3. RETENTION TIMES OF CHLORINATED COMPOUNDS

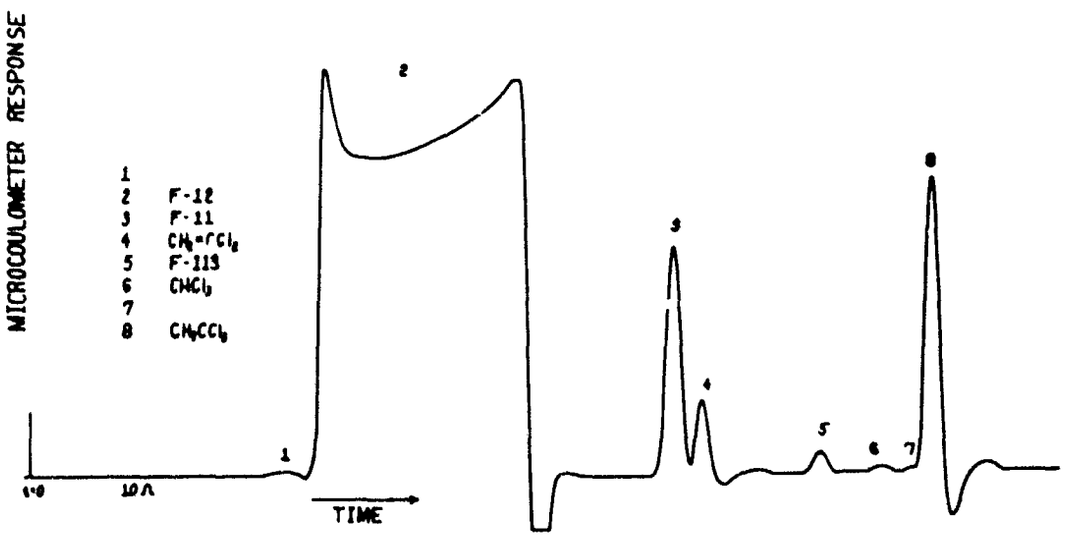


Figure 4. CHROMATOGRAM OF ATMOSPHERE SAMPLE WITH LARGE PEAK

A second unique phenomenon observed with the coulometric detector for chlorine is also illustrated in figure 4. Peak 2, which appears badly misshapen, can be a quantitative representation of the amount of chlorine compound under the peak. The response of the microcoulometer is somewhat different from other chromatographic detectors, in that a peak may appear to be flat at its maximum, but the area is still a direct quantitative measure of the chloride ions present.

Quite frequently a practical atmospheric sample will contain a component at a very high concentration relative to other peaks. This figure is a case in point. Peak 2 was identified as dichlorodifluoromethane, $CCl_2F_2$, which is a common refrigeration gas. With a peak as broad as Peak 2, there is no way to tell what other chlorinated compounds may be hidden in it. A technique was developed in which the large interfering peak could be vented after the separation column so that it did not pass through the detector, allowing us to look for other compounds. This procedure was followed with this sample, and the chromatogram obtained is shown in figure 5. The R-12 peak was eliminated and the new Peak 2, which has a longer retention time, was identified as R-114, $CClF_2$-$CClF_2$.

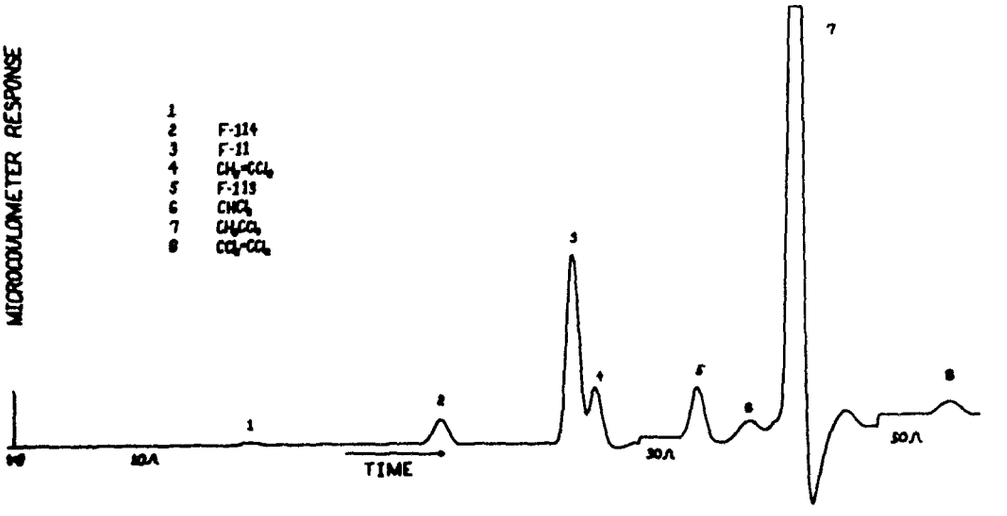


Figure 5. CHROMATOGRAM OF ATMOSPHERE SAMPLE WITH PEAK VENTED

Up to now, we have been dealing with compounds containing chlorine. We would like to mention the usefulness of this detector for bromine compounds also. Figure 6 is a chromatogram of an air sample containing 8 compounds, each at a concentration of about 2 ppm by volume, represented here by Peaks 2-9. All of these compounds are detected by the coulometer because of their chlorine atoms except Peak 5. This peak represents R-114B2 with the empirical formula, $C_2Br_2F_4$. It is detected by virtue of its bromine atoms. The detector has been used for bromine compounds in this manner, although such compounds are not nearly as common in use today as chlorine compounds. For quantitative work it is necessary to calibrate the instrument carefully for each bromine compound, because a fraction of the bromine atoms appear in the titration cell as hypobromite ions which are not precipitated by silver ions. We have not used this detector for compounds containing iodine, although it is reported to respond to them (Coulson, 1960).

The analytical method described here has been very useful in this Laboratory for studying trace contaminants in atmospheres of closed environmental systems, gas samples from many sources, and product mixtures from studies of chemical reactions.

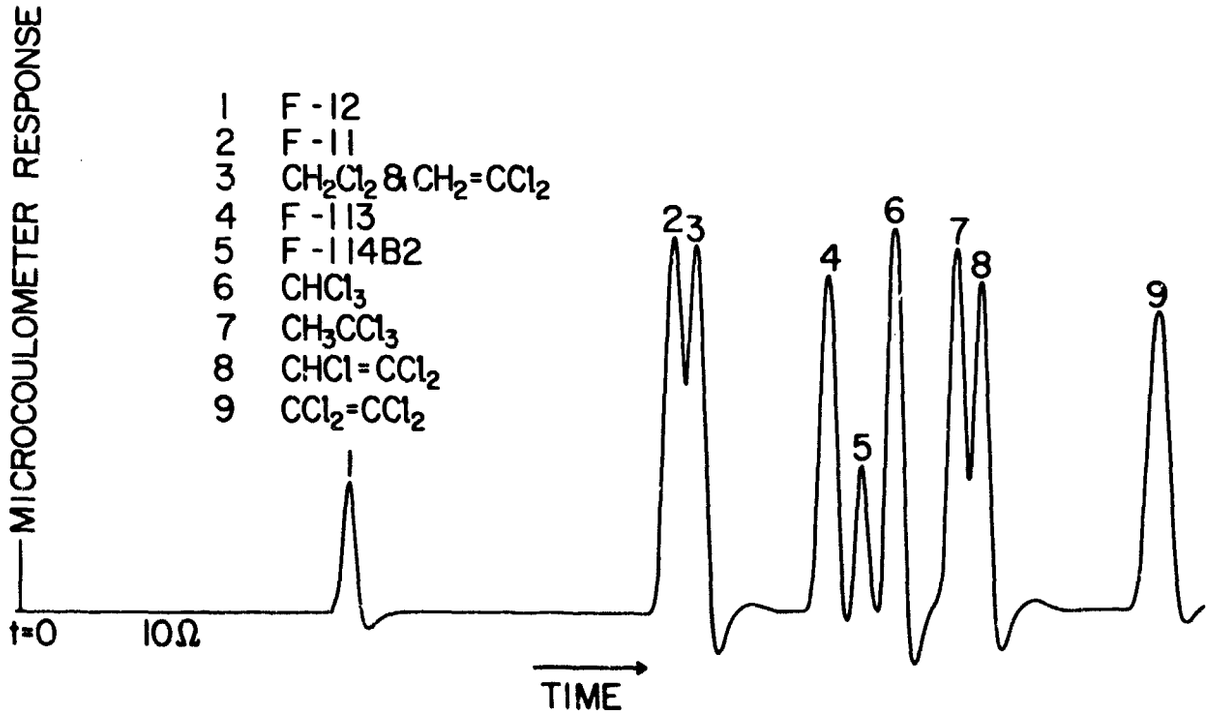


Figure 6. COULOMETRIC RESPONSE FOR HALOGENATED HYDROCARBONS IN 2 PPM RANGE USING 100 ml SAMPLE LOOP

## SUMMARY

It has been demonstrated that a chromatographic detector specific for certain elements can be used to great advantage for analyzing gaseous or liquid mixtures. The on-column concentration on Porapak eliminated the use of cold traps and enhanced the sensitivity of the microcoulometer so that gas samples could be analyzed quantitatively for chlorinated contaminant compounds at concentrations as low as 10-20 parts per billion by volume. In the case of the microcoulometer with a silver cell operating in the oxidative mode, a quantitative picture of the chlorinated hydrocarbon compounds present can be obtained without the confusion of the other compounds in the total mixture. From a qualitative standpoint, the microcoulometer greatly narrows the number of possible compounds to be considered. At the same time, the output of the microcoulometer is a direct quantitative measure of the chlorinated hydrocarbons present.

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## DISCUSSION

MR. MOBERG: Lest any of you get bothered, you know we are trying to go from parts per billion range into per cent levels, and if you think about it for a moment, it's a change of 9 orders of magnitude or more. It becomes a little difficult. We have problems.

DR. EKBERG (General Electric Company): How does this compare in sensitivity with the electron capture?

DR. JOHNSON: Well, it's almost impossible to answer that because with electron capture detectors for this same list of compounds, the sensitivity varies from extremely good to very poor, over a range of possibly $10^6$. We did work with it, but gave it up in preference to this system.

MR. MOBERG: I might mention that for something like carbon tetrachloride the electron capture is almost impossible to beat on sensitivity. You probably could look at concentrations in the picogram range with ease; and here, I think, the micro coulometric method is shy on sensitivity, but for other materials, for example, CF4, we can't see that in the electron capture because of the high electronegative forces surrounding the carbon atom.

DR. JOHNSON: I might mention that we can't see that one either because it doesn't have any chlorine, or fluorine. The same thing is generally true of something like $CF_3CL$. We can't see that.

MR. STEVENS: Dr. Johnson, did you try to stack thermionic planes in any of this work?

DR. JOHNSON: We did, but possibly we didn't give it enough time. We were never able to stabilize that detector to our liking, and it's been brought out before, one of the big things to look for is reliability in instrumentation. The stacked flame is sensitive to halogenated compounds and some others, but as I said, we were just not able to stabilize the particular one we had.

QUESTION: What is the volume of the gas sample put on the column?

DR. JOHNSON: The gas samples we used ranged from a single cc to 500 cc. If we are working at the less than a tenth of a part per million level, we usually use a 500 cc sample.

DR. BACK: Is there a way of using this method for picking up $CBrF_3$ in blood or body tissue?

DR. JOHNSON: Yes, there certainly would be. I wouldn't want to try to describe the technique here, but if you can get the material out of the tissue into a gaseous sample, you could certainly analyze it.

DR. BACK: It has to be a gaseous sample?

DR. JOHNSON: Not really. Actually, you can titrate sodium chloride in water solution if you like with this gadget. It is an actual titration itself. Of course, that would be another approach. You have to convert that bromine into the compound you mentioned to the bromide ion because we are depending on precipitation with silver, so I would be reluctant to take a tissue sample without working with it quite a bit and without discarding all of the foreign material and feel I was doing much good.

DR. BACK: It's so sensitive, we're looking for a good sensitive method and this seems so sensitive to this compound.

DR. JOHNSON: Well, if you could get it out from liquid samples, such as in blood, techniques have been worked out for getting gas samples from blood now, such as CO.

DR. BACK: Well, of course, that't the way we do our CO.

DR. JOHNSON: Right. I'm just guessing a little bit, but if it's not combined to the point where it's almost a molecular combination, you should be able to get it.

DR. BACK: This compound is pretty obviously not combined at all. I don't think it is. I think it's really quickly metabolized and pushed out, probably dissolved in blood, or floats around in it almost as a free molecule.

DR. JOHNSON: I'm almost going to bet we would have trouble, though, because we pushed the sensitivity of this detector to the limit by using all sorts of crazy things, like 500 cc samples. For CO, which is usually converted to methane and looked at by the flame, the inherent sensitivity of the detectors is something like a factor, it must be 10 to the 3.

MR. MOBERG: I think there's one possibility, to titurate the sample into a liquid and inject the whole sample in the chromatograph and the volatile materials then would go into the gas phase and we could measure by one of the other detectors. I think there's a strong possibility of getting a compound of your interest by gas chromatography.

DR. JOHNSON: I neglected a very important part of this equipment in discussing this. We think of course of the Dohrmann microcoulometer or anything like that as a detector, just as you might think of a mass spectrograph as a detector. A basic part of the equipment that is included is a pyrolyzer which is

used in almost a hundred per cent oxygen to convert these halogenated compounds which are molecular organic-type over to chloride ion, and this would have to be done in this case.

DR. HODGE: This is coming back to Dr. Back's question about bromotrifluromethane. In our laboratories we have very recent evidence that somewhat similar compounds, like halothane for example, in traces are fairly strongly bound to something in blood.

SESSION V

# LIFE SUPPORT SYSTEMS

Chairman

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AMRL-TR-68-175

PAPER NO. 27

# DESIGN OF A CLOSED RECIRCULATING SYSTEM FOR TESTING SPECIFIC CABIN MATERIALS IN A 5 PSIA MIXED-GAS ATMOSPHERE

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## INTRODUCTION

A life support system for exposing experimental animals to the resultant gas-off products of space cabin materials heated to 155 F within a closed loop, reduced pressure, oxygen-nitrogen or oxygen-helium environment was designed and installed in the THRU facility.

The life support system, similar in function and equipment to that previously designed for testing Apollo space cabin construction materials, was constructed according to new design criteria to permit two-gas system operation, better utilization of dome area and to provide more efficient chamber operations. Specific equipment changes were (1) removable animal cages, (2) separate environmental support consoles and (3) simplified control and monitoring equipment.

### Animal Chambers

The animal chambers were designed with two main sections, the outer framework with input and output flow connections and three removable mesh cages. Probes for wet-bulb/dry-bulb readings for RH, a water flush ring, and a drain line are installed on the outer section. Also included on this structure is a static pressure gauge for monitoring relative chamber pressure. The three animal cages (figure 1) were constructed of stainless steel mesh with angle supports and with a hinged door for access. Normal cage loading is 25 mice or 10 rats. Installed in the door is an automatic watering valve. Each cage is individually mounted permitting easy removal. Cage volume is approximately 0.8 cubic feet with a usable floor area of 200 square inches. As the animals are completely enclosed in each cage, each group may be removed without problem. Figure 2 shows the position of the animal cages during experimentation.

There is some loss of visibility of the animals but this was not considered to be excessive. An additional advantage of the removable cages is the resultant freedom in completely replacing a group of animals, if necessary, without extensive refitting. Cages of different or special configurations may also be constructed for utilization on special problems.

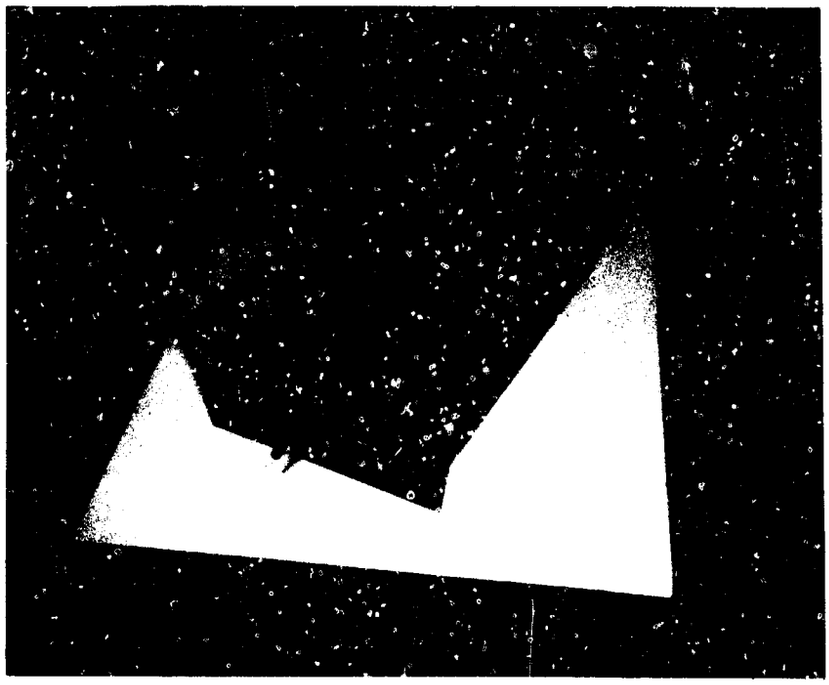

Figure 1. ANIMAL CHAMBER CAGES

Figure 2. ENVIRONMENTAL SUPPORT CONSOLE

## Environmental Support Console

The environmental support console includes the mounting for the animal chambers and associated atmospheric control equipment (figure 2). The environmental support console flow diagram is shown in figure 3. The chamber atmosphere is exhausted by means of two direct-driven dry-air pumps. These pumps are alternately activated to achieve a one-half hour duty cycle to reduce heat buildup. Each pump is provided with a manual input valve and a check valve in the output. Total system flow may also be varied by means of a manual valve inserted in the pump bypass line. The gas flow from the pumps then enters a dual LiOH scrubber arrangement for removal of $CO_2$. This system consists of two LiOH canisters manually selected by valves in the input lines. By this means canister replacement may be achieved without interruption of flow. Flow through the LiOH canisters may be varied from 10 to 100% of total system flow by means of a manual bypass valve. Downstream of the LiOH canisters bypass line reentry are sample ports for monitoring of the $CO_2$ and $O_2$ concentrations of the atmosphere.

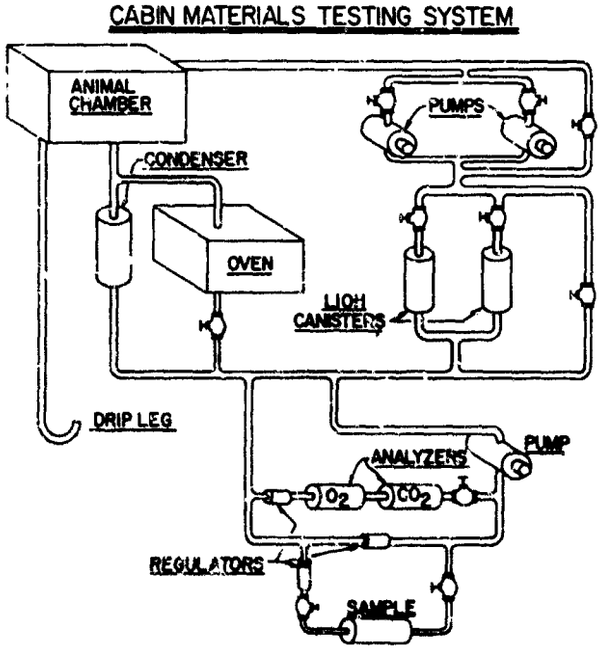


Figure 3. ENVIRONMENTAL SUPPORT CONSOLE FLOW DIAGRAM

Continuous sampling of the system atmosphere is achieved by sample pumps located outside of the Thomas Domes and will be explained in detail subsequently. Flow then enters a laminar flow element which monitors total flow. Readout of differential flow pressure is made from a water manometer located on the front of the environmental support console. System flow is indicated on this instrument. System flow can be varied between 0 and 6 cfm. System flow is then routed through a dehumidifier and water chiller which simultaneously cool and reduce the moisture content of the gas. The chiller is equipped with a continuous drain condensate trap. A manually adjusted bypass line is installed to route a portion of the gas flow around the laminar flow element and the water chiller. Located in this bypass

line is the cabin materials oven. This oven is operated at 155 F + 5 F. Gas flow through the oven is normally adjusted to approximately 10% of the total flow. Oven flow is combined with main flow after the water chiller and is then routed to the animal chamber. For two-gas systems requiring diluent gas replacement, the gas is introduced through the atmospheric sampling return line. Gas is introduced by means of a solenoid and valve arrangement controlled by the $O_2$ concentration monitoring system which will be described.

## System Monitoring and Control Panel

The system monitoring and control panel contains apparatus for monitoring and controlling all phases of the cabin materials screening system (figure 4). System requirements may be subdivided into the following areas:

a. Pump power switching and protection

b. $O_2$ - $CO_2$ concentration analysis

c. Oven power and temperature control

d. $O_2$ - $CO_2$ concentration, wet-bulb/dry-bulb temperature recording

e. System malfunction alarm

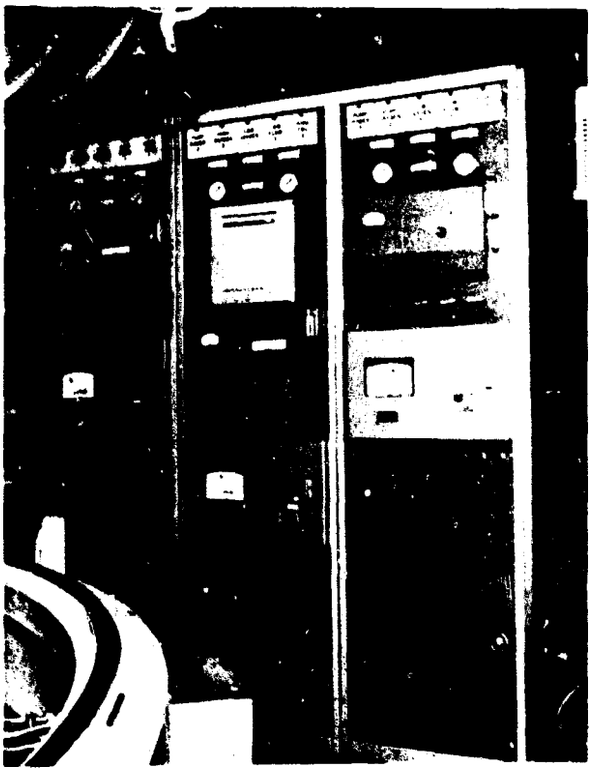

Figure 4. SYSTEM MONITORING AND CONTROL PANEL

The pump power switching and protection system has provisions for continuous operation of either pump or automatic one-half cycling of the pumps. Normally the system is operated in the cycling mode to prevent heat buildup, but in case of either pump failing, the remaining pump may be operated continuously until the malfunction is corrected. In addition, circuitry is provided to remove power from the pump motor and trigger the trouble alarm system in case of pump freeze-up or excessive motor current. Controls for selecting either pump and for selecting continuous or automatic cycling operation are located on the front panel. A pump overload light and circuit breaker are also located on the front panel.

Figure 5 describes the $O_2$ - $CO_2$ analyzing system. A diaphragm vacuum pump is located external of the Thomas Dome. The sample is removed between the LiOH canister discharge and bypass reentry as mentioned earlier. This sampling port serves to monitor both $O_2$ and $CO_2$ concentrations. A refrigerated dryer and pressure regulators at the pump discharge, provide a dry, pressure regulated signal for introduction to the analyzers. A paramagnetic oxygen analyzer and an infrared carbon dioxide analyzer continuously monitor the oxygen and $CO_2$ concentrations. The sample introduction system maintains the sample conditions at a constant flow and pressure. Analyzer sample requirements are approximately 2 cfh at 50 mm Hg positive pressure. After routing through the analyzers the sample is returned to the animal chamber flow. Alarm meters located in the electrical outputs of each analyzer provide contacts for triggering the $O_2$ - $CO_2$ concentration alarms. Provision is made for High and Low $O_2$ concentration alarms and for a high $CO_2$ alarm. Also in the $O_2$ analyzer electrical output circuit is an alarm meter used for indicating the per cent concentration of diluent gas. This alarm meter is provided with a set point which will activate a system to maintain the diluent gas concentration by means of an external gas supply. Electrical outputs from the analyzers also feed 6 points of a 12 point recorder for recording $O_2$ - $CO_2$ concentrations from each chamber. Valves for selecting sampling operation or calibration are located on the front panel. Sample gas pressure, analyzer pressure, and analyzer flow readouts are also available on the front panel.

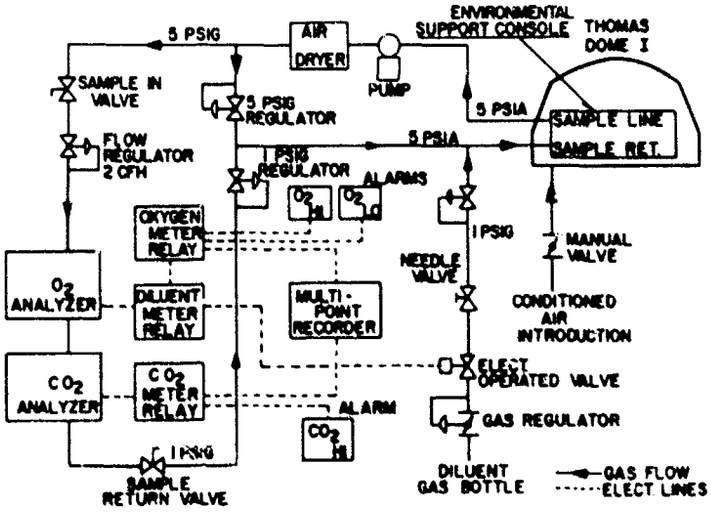


Figure 5. $O_2$ - $CO_2$ ANALYZING SYSTEM

Set points for the high and low $O_2$ concentration alarms and the high $CO_2$ concentration alarm are adjustable over the full range of concentration levels. $O_2$ concentration alarms are nominally set at $\pm$ 5% of the desired concentration and $CO_2$ concentration is alarmed at 2% $CO_2$. As stated before, sample gas pressure of 5 psig is indicated on the front panel. In addition, this pressure is monitored by an alarm switch, and if sample pressure drops below a preset value, indicating sampling system malfunction, an alarm will be activated.

A portion of the gas sample from the chamber sampling pump, as shown in figure 3, is routed through a regulating system and is available for collecting gas chromatographic samples. Quick disconnects are provided for insertion of a tonometer for sample collection.

The oven power and temperature control system is shown in figure 6. The vacuum ovens utilized in the environmental support console were modified to accept an additional temperature sensing probe. A stainless steel sheathed thermistor probe was inserted into the oven through a sealed fitting. The electrical leads from this probe were routed through the existing Thomas Dome cabling system and then to the system monitoring and control panel. A thermistor bridge circuit was designed to provide a range of 0 to 200 F. Output signal from this circuit is directed to an API optical meter relay. Oven temperature is indicated on a scale range of 0 to 200 F. Relay set-point is available over the total range and provides oven temperature control. Circuitry is also included for removal of oven power in case of oven overload or system malfunction. In addition, the internal oven thermostat control is set at a higher temperature to provide redundant oven control in case of primary control system malfunction.

The following parameters of the cabin materials toxicity screening exposure system are monitored:

1. $O_2$ concentration (0-100% $O_2$)
2. $CO_2$ concentration (0-1% $CO_2$ or 0-3% $CO_2$)
3. Wet-bulb temperature (0-100 F Wet-Bulb)
4. Dry-bulb temperature (0-100 F Dry-Bulb)

These parameters are recorded for each of the three environmental systems. The total of 12 parameters to be recorded are displayed on a Leeds-Northrup Model H - 12 point recorder. Electrical outputs are available from both the paramagnetic oxygen analyzer and the infrared $CO_2$ analyzer for recording. These outputs, which are in the millivolt range, are connected directly to the input of the recorder on the first six points. Thermistor probes, used for the wet-bulb and dry-bulb readings, were connected to a modified switching input of the multipoint recorder for points seven through twelve. A thermistor bridge circuit, designed with 0-100 mv output, was installed in the recorder chassis. The modified recorder switching circuit sequentially connects each probe to the bridge circuit on points seven to twelve.

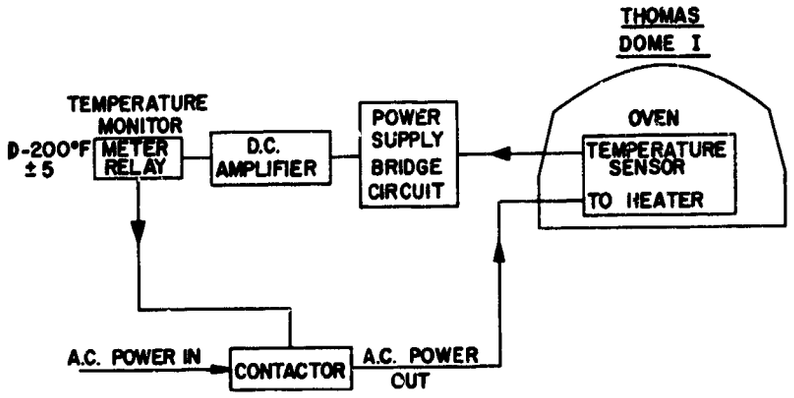


Figure 6. OVEN POWER AND TEMPERATURE CONTROL SYSTEM

Alarms are provided on the monitoring and control panel for high and low oxygen concentration, high $CO_2$ concentration, pump power failure and low system gas flow. Both visual and audio alarms signal the operator of system malfunctions. In addition, any alarm from the monitoring and control panel triggers the facility alarm system resulting in indication of system malfunction throughout the building.

## Thomas Domes

Modifications to the Thomas Domes selected for the cabin materials screening study consisted of installing the necessary sealed feed through fittings for both the electrical and mechanical requirements. Electrical power inputs were installed using a welded slug arrangement in the base of Thomas Dome 1 (figure 7). Three seven-conductor mineral-insulated cables were used; the end fittings providing sealed connections to the exterior of the dome. These three fittings supply the required 110-volt power to the system ovens and pumps. Two additional welded-slugs were installed; one for the six sampling discharge and return lines and one for the chilled water input to the environmental support console water chillers and to an auxiliary cooling coil located at the dome input flow outlet. This latter coil was installed to compensate for the additional heat load resulting from the added equipment.

Electrical signal lines consisting of the temperature probe wiring and low flow alarm switch wiring utilize the dome signal connector wiring which provides 24 pairs of signal lines connected from inside the dome through hermetically sealed connectors to a connector panel located outside of the dome.

Figure 7. THOMAS DOME PENETRATIONS

Considerable attention was given in the design of the system to equipment protection required due to the 100% $O_2$ atmosphere. Fire protection is provided by the automatic sprinkler system installed in the Thomas Domes (figure 8).

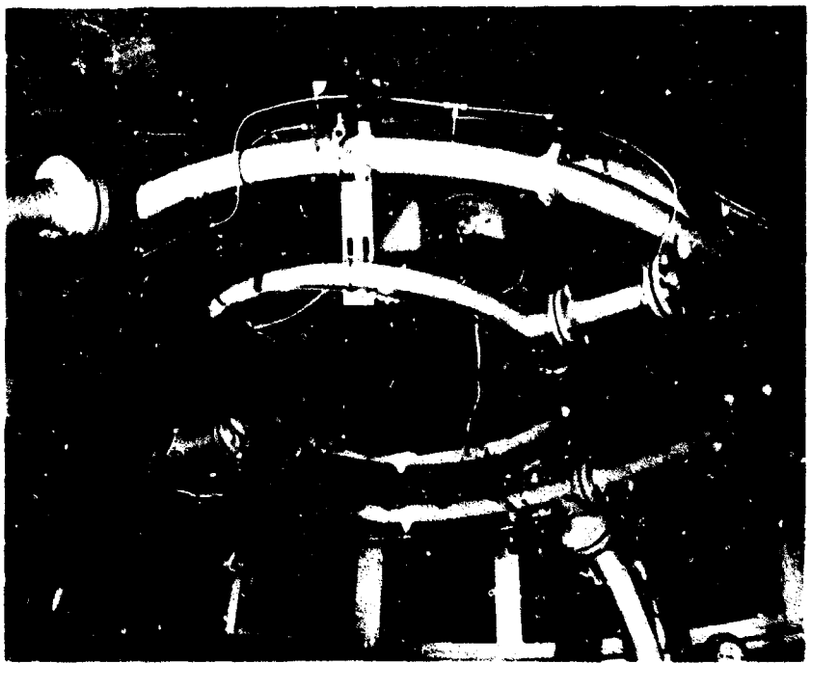

Figure 8. AUTOMATIC SPRINKLER SYSTEM

This system is activated by UV sensors installed in the upper portion of the dome cap. All electrical power to the cabin materials screening system is protected by a contactor, interlocked with the sprinkler system, to remove all electrical power in case of fire. Electrical wiring in the dome is either mineral insulated or teflon where practical. The wiring is protected by rigid conduit and enclosed metal junction boxes. In addition, power circuits are protected by circuit breakers installed in the monitoring and control panel. These breakers were selected with a rating approximately 10% in excess of normal operating current.

## SUMMARY

A life support system for exposing experimental animals to gas-off products of space cabin construction materials was designed and installed in a Thomas Dome at the Toxic Hazards Research Unit. The system may be operated under 100% oxygen or mixed-gas conditions. While similar to equipment previously utilized for Apollo space cabin materials, several design changes were incorporated. Life support equipment was grouped with each animal chamber resulting in an increase in usable dome floor area. Removable cages provide easier chamber maintenance and handling of experimental animals.

Protective circuitry was added resulting in increased safety and reliability during equipment operation. System parameters monitored and/or recorded are $O_2$ concentration, $CO_2$ concentration, wet-bulb/dry-bulb temperatures, oven temperature and system flow. Provision is also made for regulating and controlling the diluent gas concentration in two-gas systems. An alarm system is provided to monitor critical areas of operation.

## DISCUSSION

DR. EKBERG: I noticed there was a UV sensor on the sprinkling system. Could you clarify that, please?

MR. JOHNSON (SysteMed Corporation): Yes, that UV sensor, the sprinkler system installed in the Thomas domes, is an automatically activated system using UV sensors, Thomas A. Edison type sensors.

DR. EKBERG: What is the UV sensor looking at?

MR. JOHNSON: It was hard to see on the slide, but there are five sensors spaced around the top of the dome. They are looking down at the bottom of the chamber, so spaced to cover the complete volume of the chamber.

DR. EKBERG: What compound are they really looking at, is what I mean?

MR. JOHNSON: They are looking at a fire.

DR. EKBERG: Originally--the reason I wondered, I would think an IR sensor for heat rather than UV. That's why I brought it up.

MR. ADAMS: Have you made any attempt to analyze the water from the condenser for contaminants to see if they're washing any contaminants?

MR. JOHNSON: I'd like Mr. Vernot to answer.

MR. VERNOT: Yes, we do that routinely, as a matter of fact, and so far, we haven't seen anything, and it doesn't seem as though the materials we have put in there thus far are being washed out with the water.

LIEUTENANT COLONEL WESTLAKE: Have you analyzed for ammonia?

MR. VERNOT: No, we haven't. As a matter of fact, we can't see ammonia with the flame ionization detector we use. Our original rationale was to see what would happen to the gas-off products from the cabin materials, and we didn't consider ammonia in that particular philosophy. However, I'm sure that the philosophy will change as we go along and we will probably be looking for metabolic products, too.

AMRL-TR-68-175

PAPER NO. 28

# FIRE EXTINGUISHING SYSTEMS FOR SPACE CABIN SIMULATORS

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and

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The ignition point of materials in 100% oxygen at 1 atmosphere is much lower than the same material in air at 1 atmosphere. The burning rate of filter paper in air at 1 atmosphere is approximately 1 cm/sec as compared to approximately 4.3 cm/sec in 100% oxygen at 1 atmosphere (Cook, 1967). A unique characteristic of fire in 100% oxygen is the extremely rapid surface burning characteristic or "nap" fire which instantaneously spreads over the surface of the combustible material and initiates multiple point sources (Denison, 1967). The Aerospace Medical Division conducts many experiments in which man is exposed to oxygen enriched atmosphere either as a human test subject or during the course of tending experimental animals or equipment (Swan, 1967). These potential hazards have caused the Aerospace Medical Division to modify significantly the fire extinguishment aspects of its simulators which use these atmospheres. This paper will describe some of the modifications which have been accomplished.

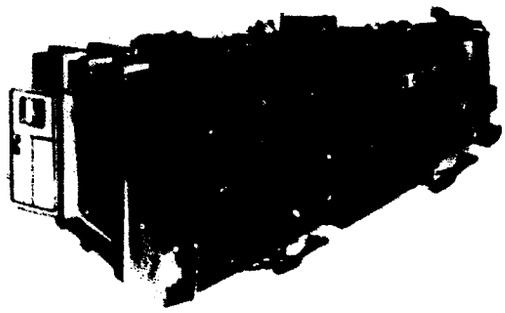

Figure 1. TYPICAL SPACE ENVIRONMENT SIMULATOR

The fire extinguishing system consists of three basic integrated subsystems--fire detection, control circuitry and extinguishing. The detection subsystem uses an ultraviolet (UV) light detector which sends a signal into the control subsystem when light in the UV (1900Å - 2900Å) region is emitted by the fire. Smoke detectors using either the ionization or the light path occlusion technique are also used to sound an alarm. The UV detector is less sensitive to false activation than other rapid response detectors (such as infrared detectors) and the smoke detector could give an alarm of an overheat and impending fire condition. Other types of detectors such as preset temperature or rate of temperature rise do not have the needed response time.

Figure 2. UV DETECTOR

Figure 3. SMOKE DETECTOR

The signal from the smoke detector is routed through the control circuitry to energize only visual and audible local alarms due to the possibility of false activation of the detector from fog (produced as a result of rapid decompression of the chamber) or from several other nonfire conditions. The signal from the UV detector is routed through the control circuitry and performs several functions.

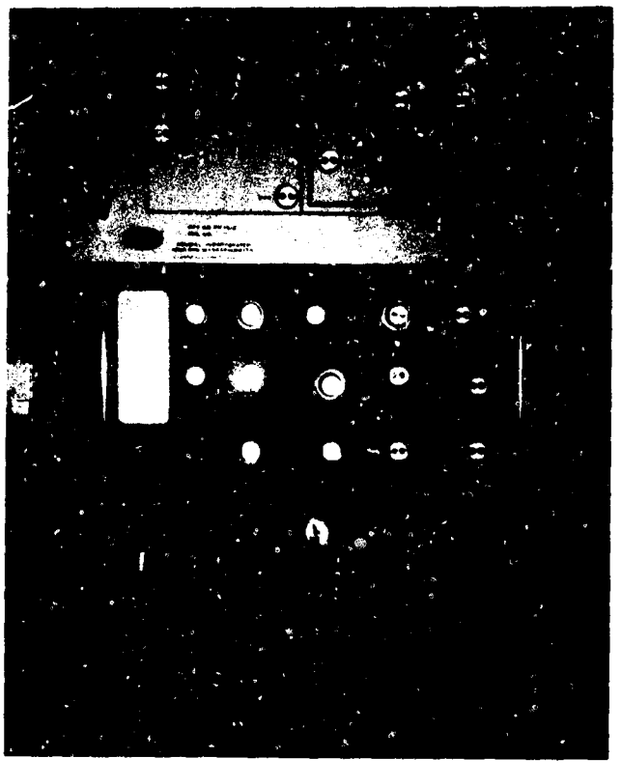

Figure 4. FIRE EXTINGUISHING SYSTEM MONITORING CONSOLE

If the control circuitry is in the "manual-electric" mode, visible and audible local alarms are energized and a light which designates the "sensing" detector is energized. The chamber operator or the chamber occupants can then decide whether or not to flow the water. If the water flow is actuated, the functions described in the "automatic" mode occur. If the control circuitry is in the "automatic" mode, electrical power to the chamber (other than emergency illumination, communications and fire detectors) is deenergized, normally closed solenoid valves are opened to initiate the water flow, local audible and visual alarms are energized, the Base Fire Department and Dispensary are summoned and a 20 second timer is energized. After 20 seconds have elapsed the water is automatically turned off and the system resets itself within 5 seconds for a second application, if necessary. The control circuitry has other alarms which alert the chamber operator to such conditions as low water pressure, inoperative fire detectors, low battery power (backup power mode), valves in the wrong position, etc. "Manual-electric" mode switches for initiating the water flow are located at the chamber operator's console as well as within each compartment of the chamber. The control circuitry is designed so that the detectors and alarms can be tested without flowing water.

The water extinguishing subsystem is supplied either from the base water distribution system or, if the demand cannot be satisfied, from a pressurized tank. The system is designed to flow at the rate of 7.5 gallons per minute per square foot of chamber floor area (Botteri) through a series of nozzles to disperse the water in a spray/fog so as to cover the horizontal and vertical planes from both directions. If a pressurized tank serves as the source, it should have sufficient capacity for at least two and preferably three 20 second applications.

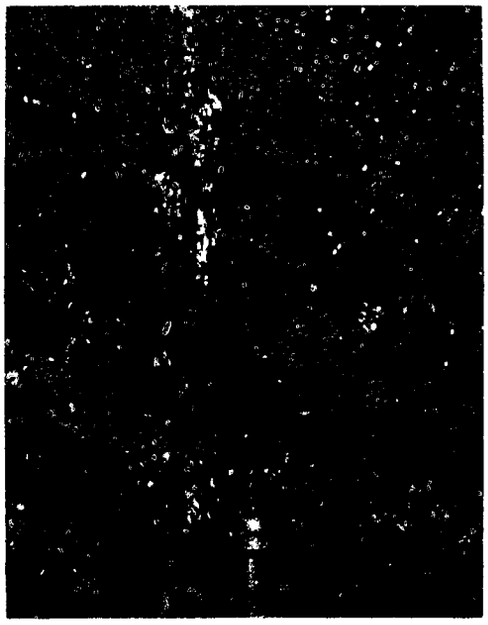

Figure 5. EXTERNAL WATER SUPPLY SYSTEM

The water extinguishing subsystem is integrated with the detectors and control circuitry so that initial water flow occurs within 0.2 seconds subsequent to the initial appearance of flame within the chamber. The water flow is stabilized at maximum rate within 0.5 seconds. To meet this criterion the water is piped from the source into the chamber and through the distribution system inside the chamber to differential hydraulic pressure valves which are located adjacent to each nozzle.

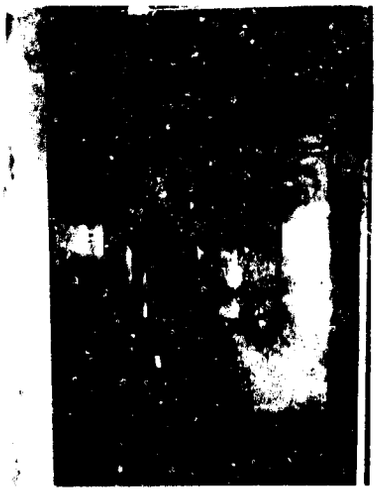

Figure 6. INTERNAL WATER DISTRIBUTION SYSTEM

Locating the valve adjacent to the nozzle decreases the distance the water must flow subsequent to opening the valve and thus decreases the reaction time. The control pressure on the opposite side of the valve is taken from the same water source so that fluctuations in the supply pressure will not falsely activate the system. The water flow is initiated by a signal from the control circuitry subsystem energizing a normally closed solenoid valve in the control line. This decreases the control pressure and the hydraulic valve opens permitting the water to flow. When the solenoid valve is deenergized the control line pressure increases and closes the valve thus stopping the water flow. The control line pressure can also be released by either of several strategically located, manually activated, quick-opening ball valves so that the chamber can be protected even if all electrical power is lost.

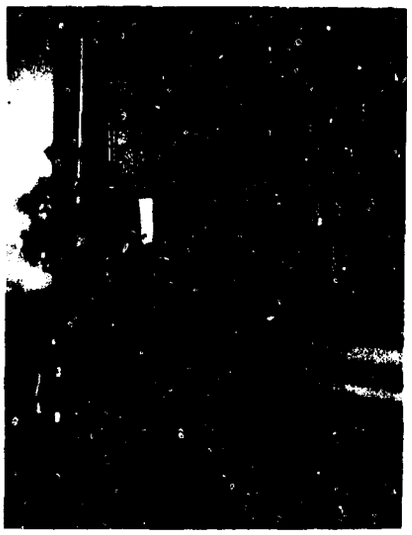

Figure 7. VALVE AND NOZZLE

Several fire extinguishing systems of this basic design have been tested subsequent to the initial research and development in 1967. The reaction times and water flow rates have proven effective in extinguishing fires in 100% oxygen at pressures up to 600 torr. Although the water flow rate is approximately 10 times as much as is designed for extreme fire hazards in commercial warehouses no difficulty with human compatibility has been experienced.

Thirteen Aerospace Medical Division chambers have been modified as described above and have returned to commissioned status. Attached is a list of general requirements each of these chambers had to meet to be recommissioned. The modifications have proven effective.

## GENERAL REQUIREMENTS FOR "MANRATED" CHAMBERS USING OXYGEN-ENRICHED ATMOSPHERES

1. Potential ignition sources should be minimized.
2. All electrical wiring should be protected from cuts, abrasions, or other damage by conduit, metal troughs, etc.
3. All electrical equipment should be grounded.
4. No convenience electrical outlets should be allowed in the chambers.
5. Insure that fuses are of proper rating and type.
6. All electrical switching should be accomplished external to the chamber. If this is not possible, switches should be hermetically sealed.
7. All unnecessary combustibles should be removed.
8. Clothing and bedding for personnel in the chambers should be noncombustible.
9. All internal wiring should be insulated with Teflon or equivalent.
10. Fire extinguishing systems should be provided.
11. Definite responsibility for the chambers should be assigned at the daily chore level for operations and maintenance procedures.
12. Updated schematics, flow diagrams and wiring diagrams for each chamber should be maintained.
13. SOP's should be short, to the point and verified for each experiment.
14. Standard procedures for keeping and maintaining operations, maintenance and training logs should be established.
15. Medical review of chamber operation and medical surveillance should be definitized.
16. An emergency medical treatment area should be provided.
17. Emergency procedures and systems should be short and easily accomplished.
18. Back-up emergency power should be provided.
19. Provisions should be made for both internal and external emergency recompression.
20. A communications system should be provided.
21. Each experiment protocol should be reviewed and approved by a laboratory board prior to implementation.

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## DISCUSSION

LIEUTENANT COLONEL WESTLAKE: I had a question myself with regard to your smoke sensor. What is the active principle of that smoke sensor? What does it see that makes it respond to smoke?

MAJOR MABSON (Surgeon General's Office): To preface my remarks, one of the reasons I mentioned that the smoke sensor does not turn on the water, obviously, is because it is sensitive to many non-fire conditions such as any aerosol. This particular one happens to have a light beam and photocell, 90 degrees to one another so that you end up with a reflection of light. You could have light occlusion, or ionization device and many other kinds, but basically, it is an aerosol smoke particle which either reflects or occludes the light from the photocell. Fog, on a rapid decompression of the chamber, could conceivably turn one of these on. The point is, once you're up and stabilized, if you had an overheat condition, perhaps with some combustion products being involved, smolder type of fire, there's a good chance one of these may warn you of an overheat situation and you could then look into it before you had an overt fire.

MR. VERNOT: We have a system which is almost identical to yours, as you know, and one thing we did rather early in the game was just to disconnect the electrical manual control to prevent it having any effect on the system. We thought that if we had to go to manual, the simple pneumatic manual control which was available to us was just as good, and we kept having visions of somebody poking that thing on the panel and deluging the chamber with water, so we don't use it.

MAJOR MABSON: I can certainly appreciate your fears as well. I often worry about somebody putting an elbow on this quick-acting valve. Obviously, with the three types of mechanisms, the automatic, semi-automatic, and the electrical, (fully manual being the valve), once it's in, you can do with it as you please; but obviously, the primary mode is the automatic one. This is the quick one, and the secret to saving someone's life, if you were in an oxygen fire, obviously, you need the speed of response, and this is the reason that the UV detector was selected in reference to the previous remark. I agree there are certain cases where, as an example, we have some UV light sources in the vicinity of the chamber and it's most convenient to put it on manual electric and still have the hand right there to turn on the water if something should happen while the chamber door is opening, so the sensors see the UV but they don't turn on the water.

LIEUTENANT COLONEL WESTLAKE: Mr. Vernot, recalling your particular application with all that glass in those domes, how much shielding do you have to use to cut out the ultra violet that may be outside the chamber?

MR. VERNOT: The glass is quite thick, an inch thick, and it's an effective UV filter. However, we have had problems with UV inside the chamber. We had a short circuit on a low voltage communications wire that sparked to a monkey cage. The monkey pulled the cord out and chewed the wire, short-circuited it and the UV sensor saw it. Fortunately, when nobody is in our chambers, we just have it on manual mode. We don't deluge. We get all the signals and alarms, or else we would have a lot of wet monkeys and dogs.

MAJOR MABSON: We have flashed a flash bulb right in front of the sensor and it did not turn on the sensor because of the glass envelope around the flash bulb. Almost any amount of glass will suffice but you get in trouble if it's a quartz envelope. Some of the high intensity lights which the Press tend to use have quartz envelopes that will definitely turn the sensor on.

DR. SOPHER: I noticed one thing in your movie that was similar to the movies we took in the Thomas domes, before the new fire protection system was installed. The nap fire went up and that was fine, but if a fire got started, once it got inside the overalls, the water deluge was relatively ineffective in putting that out. I suppose the Beta cloth suit is going to take care of that.

MAJOR MABSON: Well, I'd have to disagree with your statement that it won't take care of it. I think the situation that Dr. Thomas had, he adequately explained at the time, and as I mentioned, having been in the water deluge, having the Beta suit on, and underneath having on the hospital greens, I was totally wet all over. There was no place on my body that was not wet.

DR. SOPHER: Right, but you weren't on fire.

MAJOR MABSON: That's correct, but if the clothing is wet outside and on the inside, I have to presume my skin is wet as well.

DR. SOPHER: Well, no question, but the thing is, as I remember on the mannikin, the fire was started down about one boot and this thing went up on the inside with the water pouring down, went up on the inside of the leg of this suit and burned to the outside. There was a 100% oxygen atmosphere.

MAJOR MABSON: I think the people that were here at the time of the test had better comment on it.

MR. VERNOT: The water deluge we had at that time was not comparable to what we have now. I think it was probably 1/20th of the water volume which now pours down. However, even with that rather inadequate water supply we put the fire out before too much damage was done to the mannikin.

DR. SOPHER: Agreed. It would have been just a pretty good blistering burn on the leg; you saved the man, no doubt. I just wonder if some people who are kind of hairy would have a "body nap" fire in the oxygen atmosphere within the suit, too.

MR. VERNOT: As I say, Brooks has done experiments with pigs, as I recall, in which they did put coveralls on them and they started them burning. The pigs were not alive, of course. The pig burns much better than the man, has a large reservoir of fat to go once it starts, yet if I remember correctly, the system put the fire out perfectly satisfactory before any real damage was done.

MAJOR MABSON: That is true. I think one thing to be remembered here is that the system is coupled together to the point that it responds extremely rapidly to any source of fire and you must presume that the man is not going to be the source of fire because he doesn't have a source of ignition on him when he's inside the chamber. The point is that you try to protect him with the Beta uniform and you also give him a very rapidly responding system which in the case just pointed out a short circuit occurred, and if it had occurred when the man was inside the chamber the water would have been on before he could have moved back from it, and it would have been put out.

DR. PIERSON: I'm kind of curious about the action of the smoke sensor in the automatic mode.

MAJOR MABSON: Even in the automatic mode, the only thing the smoke sensor does is turn on a visual and an audible alarm; that's all it does. It does not start the water. It also sends it to the fire department. We felt if the sensor sounds the alarm there's a possibility something might be going on and if it was, let's get help as quick as we can. It's much better to have them roll a few miles for nothing than to sit back while you have a fire.

MR. JOHNSON: I wonder if you could comment on the relative effectiveness of water versus Freon 1301?

MAJOR MABSON: I thought I could get away without having to comment on that.

MR. JOHNSON: You might not want to use the Freon in simulators, but . . .

MAJOR MABSON: I might state in the test program we ran three tests with Freon starting at 258, 380 and 600 millimeters of mercury, all hundred per cent oxygen and then we repeated these with water. In all cases fire was adequately extinguished. There's no question about it. Freon certainly did put the fire out.

DR. THOMAS: Yesterday afternoon in the open forum we didn't have a chance to cover the last session, and if there are any questions pertaining to that one, we can handle it now. That would be, then, questions on the session on Evaluation of Cabin Materials, the papers on Thermal Decomposition Products of a Carboxy Nitroso Rubber; Acute Toxicity of Thermal Decomposition Products of Carboxynitroso Rubber; and the Theory of Retention of Various Contaminant Types on Adsorbents. Are there any questions on those three papers?

DR. HODGE: Dr. Thomas, is this the place to bring up again the question that was raised and not concluded yesterday about discussing the importance of all sorts of variations in space cabin materials, the selection of a different catalyst?

DR. THOMAS: The question pertains to how do we cope with the variations from batch to batch of cabin materials. We don't. It's a very simple answer. There is no way. With many polymers, every time you make them, the lengths of the chain may be different. We are trying to get from the subcontractors who build these systems the material which they use in manufacturing the particular part or gadget, but this is not always possible. Sometimes it's hard enough to get hold of a material which is on the list, because of insufficient identification of where it came from, and when it was made. So, what we use is a representative commercial sample. I would guess we are working with these conditions about 75% of the time. If it's a Teflon coated wire, made by a certain company, we can go to the same company and we may get the same type of wire, but whether we are lucky and get it from the same manufacturing batch, that is really impossible to find out or to guarantee. It may affect the testing results; however, I do not think that toxicologically it would be very different. It would be a real, real happenstance type of situation that quality control would slip so badly that one batch would show significantly different components. I think most of these processes now are pretty well controlled.

I wanted yesterday to prevail on Major Carter to get up here. He happens to have a few slides in his pocket pertaining to some of the red blood cell changes which we have been following up for a couple of years now with Dr. Mengel at Ohio State.

DR. CARTER: We have two different sets of data. The work that we are referring to is with Dr. Mengel at Ohio State. Dr. Kaplan, who left a year ago, has designed an experiment wherein 15 monkeys would be placed in a hundred per cent oxygen, around ambient pressure, and they would be sacrificed at 4, 7, and 12 days. Blood samples would be sent to Dr. Mengel and he would run them through his battery of tests--blood enzymes, peroxidases, and so forth, and then we were to repeat this at 5 PSI, mainly for the sake of comparison. Well, of course, since we were shut down for a year, it fell my lot to do this. Unfortunately, at ambient pressure oxygen we didn't get enough animals to survive. The animals that would have been sacrificed at 7 days would die at 5 and 6, and we couldn't really draw conclusions except that we had a lot of dead animals. Now, in the 5 PSI oxygen all of them lived and there, I think, two main results have importance.

First: at around 7 days, you get a drop in red cell count; all five animals dropped off. The 12-day animals were back up to normal. These 7-day animals also showed what may be a biologically significant increase in reticulocyte count. But when you have only five animals, how can you say that an average of 1.4 before and an average of 1.6 or 1.7 after is significant? Probably not, but when you look at the fact that there was a drop in red cell count, maybe this is significant. I don't know whether to use the word adaptation or not. Maybe the animal is adapting somewhere around 6 or 7 days to this oxygen-rich atmosphere, and

then, maybe coming back to a normal red cell levels. That, really, is all I have to say on this.

About the RBC fragilities with 100% oxygen at ambient pressure I have two slides that Dr. Robinson didn't get to show yesterday. These are fragilities that we did on the animals, with a characteristic sigmoid curve. If you plot these values on normal probability paper, you straighten the line so you have a linear relation, and get a fairly straight line function with a slope. You notice that you have the pre-exposure at which 50% hemolysis falls somewhere around maybe 4.2 or 4.3 grams, and then the whole cell population moves over, which you would really expect. You might have a comment on this one animal, up at the top, where the most resistant cells are not as changed. You really have a different slope of the line here. The second slide shows the baboon at 7 days where you notice a nice parallel shift in the fragility. Control represents, oh, at 50% hemolysis, around 4.4. Over here postexposure would be around 4.9.

DR. THOMAS: Any questions?

DR. BACK: Not to this, Vern, but to fragility. The question was brought up about differences in susceptibility to fragility between species of red cells, and I told them that you probably had the data on the top of your head as to which animal was most susceptible to fragility and which animal was least susceptible. You did monkeys, dogs, rats, mice and rabbits, didn't you?

MAJOR CARTER: All red cells are fragile. You mean which animal has the most fragile population?

DR. BACK: Right.

MAJOR CARTER: They all fall right around the same line.

DR. BACK: Didn't you tell me the dog was more susceptible?

MAJOR CARTER: All right, I know what you're talking about now. The dog red cell has notoriously been thought of as very fragile. Nobody likes the dog red cell because mechanically it seems to fall apart, and many people will tell you this is a high sodium, low potassium cell, although this has never really been shown to be the cause and effect relation as far as I know. In our work with methylhydrazine, the dog red cell is much more fragile at very small doses of MMH. You get this large shift in fragility in the entire population that you don't get with the monkey, rat, and so forth. In the beginning your baseline fragilities will follow 50% hemolysis somewhere 4.2, 4.3, 4.4, etc. Why, then, the dog red cell, if you stress it, by drugs, whether you roll it around a bunch of glass beads, etc. why it's more fragile, I have no idea. I thought I might have the answer one time, but it didn't work out.

DR. THOMAS: When you said that there was a drop in red blood cell count, wouldn't that be perhaps the factor that this blood had to be shipped up to Ohio State, which takes an hour and a half?

MAJOR CARTER: The only thing I can say to that is that the control animals were also shipped up.

DR. THOMAS: That settles that. Any more questions along this line?

DR. COX: I'm wondering if this shift in hemoglobin has been observed at a mixed gas atmosphere study, at 5 PSA total pressure.

DR. THOMAS: We didn't study this, because when we get into the mixed-gas game, we kind of assumed no oxygen effect, assuming that this physiological $pO_2$ would have no effect.

DR. COX: I'm wondering if we are dealing with oxygen toxicity or pressure effect?

DR. THOMAS: I think that is our job for the next five years to describe.

## CLOSING REMARKS

DR. THOMAS: I just want to leave with you some food for thought in closing. What you heard here in the past three days shows really how little we know. We have barely scratched the surface when it comes to toxicity of exotic atmospheres. There will have to be a great deal of thought going into selection and habitability of artificial atmospheres for long-term space missions. The philosophy which Mr. Wands presented is a very good one. We certainly cannot afford to expose our astronauts to unknown risks. The way the picture looks today with mixed-gas, 5 PSIA atmosphere I wouldn't be hesitating to use it for 60 days and, Dr. Weibel, I'm sure can correct me if I am wrong. Pulmonary changes, if any, would not be measurable in man by pulmonary function tests, so we would never see them. But still we are getting changes in animals exposed for 8 months and I would be very cautious about recommending such an atmosphere for a thousand-day mission unless we know exactly what is going on. Those changes could be progressive. We really do not know whether they develop initially in the first couple of weeks, perhaps as an adaptation mechanism, and then taper off to where nothing more happens after that forever; or whether there would be reversibility and readaptation to earthly atmospheres if the animal exposures are carried to a thousand days. I'm leading you up to the thought that some of these exposure studies on the toxicity of basic cabin atmosphere will have to be carried to the actual mission lengths, and perhaps longer since you cannot construct a curve by only one point. We have got to go beyond and settle on adaptation and readaptation. We hope that by next year we will know a little more about carbon monoxide and mixed-gas exposure. Some of the papers which you heard were hot off the press and not everything was completed. I think that the real purpose of this conference is to get these data to you right then and there when they are coming in. As you know, publication takes some time, so I think we are more helpful to you if you are up to date and up to speed even before the publications come out.

Thank you very, very much for being with us--it's been our pleasure!

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13. ABSTRACT

This report is a compilation of the papers presented and the Proceedings of the 4th Annual Conference on Atmospheric Contamination in Confined Spaces, sponsored by the Aerospace Medical Research Laboratories and held in Dayton, Ohio on 10, 11, and 12 September 1968. Major technical areas discussed included toxicological evaluation of atmospheres and contaminants, histopathological evidences of toxicity, evaluation of cabin materials, instruments and detection, and life support systems.

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