THE OHIO STATE UNIVERSITY

RESEARCH FOUNDATION

1314 KINNEAR ROAD COLUMBUS, OHIO 43212

Report 1492-5

BIOLOGICAL EFFECTS OF PROLONGED EXPOSURE OF SMALL ANIMALS TO UNUSUAL GASEOUS ENVIRONMENTS

H. S. Weiss and R. A. Wright
Department of Physiology

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION
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R E P O R T

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THE OHIO STATE UNIVERSITY
RESEARCH FOUNDATION

Environmental Physiology Laboratory

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To: NATIONAL AERONAUTICS AND SPACE ADMINISTRATION
Washington 25, D. C.

Grant No. NsG-295-62

On: BIOLOGICAL EFFECTS OF PROLONGED EXPOSURE OF SMALL ANIMALS TO UNUSUAL GASEOUS ENVIRONMENTS

For the Period: 1 September 1964 to 28 February 1965

Submitted by: H. S. Weiss and R. A. Wright
Department of Physiology

Date: 1 March 1965

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I. INTRODUCTION

Our studies on the reaction of living systems to artificial atmospheres were stimulated by questions concerning the role of the inert gas component: specifically, whether prolonged decrease or absence of nitrogen in the breathing atmosphere had any adverse physiological effects. Nitrogen-free atmospheres capable of supporting life can be established in at least two ways: (1) by substitution of oxygen for the inert gas, i.e., creating 100% O_2 or hyperoxic atmospheres; and (2) substitution of another inert gas for the nitrogen, for example, creating helium-oxygen atmospheres. We have pursued both these avenues through a number of related subprojects. As in previous reports we are summarizing our findings under the several subproject headings. Subprojects 1 to 3 are concerned with hyperoxic atmospheres (i.e., oxygen toxicity), and subprojects 4 to 7 are concerned with 79% He - 21% O_2 environments.

II. SUBPROJECT SUMMARIES

1. Intermittent Exposure of Mice to Hyperoxic Atmospheres

In past reports, we showed that mice could survive the toxic effects of one atmosphere of O_2 for prolonged periods if their exposure was regularly interrupted by a period of air breathing. The actual proportion of time in air to time in 100% O_2 turned out to be about 1:2, regardless of whether the exposure cycle was calculated in parts of one day or multiples of days. Thus, whereas continuous exposure to 100% O_2 at one atmosphere killed 50% of the mice in 5-6 days (LT_{50}), there were no deaths in trials lasting 16-20 days if the exposure cycle was 16 hours in O_2--8 hours in air, or two days in O_2--one day in air. Shorter intervals in air changed the rate of mortality but did not prevent it; for example, four hours out of 24 in air moved the LT_{50} up to 16 days.[9]

The question we next addressed ourselves to was whether the benefit of the period in air lay simply in that the O_2 tension in the respired atmosphere was decreased to normal levels or whether the N_2 in the air played some specific protective role. To test this, one group of mice was exposed to 100% O_2 at one atmosphere in an altitude chamber. For four hours out of each 24, the pressure was reduced to 200 mm Hg, but still 100% O_2. Simultaneously, another group of mice was exposed to 100% O_2 at one atmosphere in the plastic isolators we use for most of our gaseous environment studies. For four out of every 24 hours, half of the mice in the isolator were moved out into room air; the remaining half stayed continuously in 100% O_2. Finally, a control group was observed in room air continuously. As an additional precaution to reduce extraneous variables, the movement of the mice in and out of the 100% O_2 isolator was designed to approximate that experienced by the mice in the altitude chamber; that is, the flush of O_2 or air in the gas lock was regulated to simulate the time-concentration changes in O_2 tension which occurred during the ascent to or descent from 200 mm Hg.

The results of this study (done in duplicate) are shown in Table I. Clearly, survival times are essentially the same for the two interrupted groups, whether in the altitude chamber or in the isolator. Both treatments extended the LT_{50} from the five days produced by continuous exposure to 16 days or better. In addition, weight changes were similar in the two interrupted groups. It seems safe to conclude, therefore, that the protection afforded against oxygen toxicity by interrupting an exposure in 100% O_2 with periods of air breathing is due solely to the reduced oxygen tension in the respired atmosphere.

2. Reaction of Aves to Hyperoxia

We have noted in our previous reports the interesting fact that the growing White Leghorn chick is markedly resistant to the lethal effects usually expected from exposure of homeotherms to 100% O_2 at atmospheric pressure (OAP). Although there is some depression in growth rate and feed intake, chicks in the age range of 3-7 weeks exhibit no mortality or even morbidity on exposures lasting four weeks, the longest we have attempted so far.[5,11]

At first, we were inclined to attribute this resistance to O_2 toxicity to the unique anatomy of the bird's respiratory system. The bird's lung, for example, is considered to be a semi-rigid structure, with continuous air capillaries rather than blind alveoli, and with numerous diverticula or air sacs throughout the thoracic and abdominal cavities. Such a system might not respond to hyperoxia with the atelectasis, congestion, and other pathology typical of the mammalian lung. Further, the bird's lung was reported to be deficient in surfactant, thought by some to be the major site of action of high oxygen tensions.

Though the arguments for an anatomical-surfactant basis for the bird's resistance to O_2 toxicity appear valid, there are reasons for questioning such a conclusion. For one, with modified extraction procedures, surfactant has been demonstrated in the bird's lung. Secondly, a few workers have looked for but failed to find any change in the quantity or composition of surfactant in lungs of mammals suffering from O_2 toxicity. Third, the young rat also can live for weeks in OAP, although the LT_{50} for adults is between four and six days. Thus what we have seen in the chick may perhaps be nothing more than an example of resistance to O_2 toxicity which is common to the young or immature of a number of species.

Clearly, to further resolve this problem, one of the first questions to be answered concerns the susceptibility of the adult chicken to OAP. Such tests are now under way but initially required considerably more mechanical manipulation than had been estimated. This was because the isolators in which we have done most of our gaseous environmental work, and the devices in which our embryos, chicks, rats, and mice were held were simply not designed to accept 2-3 kg animals requiring a cage of about 1 x 1 x 2 feet in size. As it is, only one adult chicken can be run in an isolator at a time, and accumulation of results is painfully slow. Too few animals have

Table I. Mortality Pattern in Mice Exposed to Various Hyperoxic Atmospheres

Days of exposure	Accumulative % mortality (20 animals/group)			
	100% O_2 at 1 atm continuously	100% O_2 with 4 hrs/day at PO_2 of 200 mmHg	100% O_2 with 4 hrs/day in room air	Room air continuously
1	--	--	--	
2	--	--	--	
3	--	--	--	
4	5	--	--	
5	60	5	5	
6	80	--	15	
7	90	20	--	
8	95	--	--	
9	--	30	30	None
10	--	--	--	
11	--	35	35	
12	100	40	--	
13	--	--	--	
14	--	--	--	
15	--	45	--	
16	--	50	40	

been run so far for definitive results, but we can say that some adult chickens also show surprising resistance to the lethal action of one atmosphere of O_2, surviving, though morbid, for as long as two weeks. It may therefore still be necessary to utilize at least some aspects of the anatomical-surfactant concept to fully explain the birds' behavior in a hyperoxic atmosphere.

3. Lung Lipids in Oxygen Toxicity

Although there is much speculation that surfactant is an important site of action of high oxygen tension, the reports which have appeared in the literature presenting actual data are few in number and tend to be contradictory. Part of the problem may lie in the difficulty of applying quantitative and controlled methods in extracting and measuring surfactants. Accordingly, we have decided to investigate the lung lipids in our chicks and rats exposed to one atmosphere O_2, using procedures which appear to be reproducible and reliable.

Surfactant is being obtained from the rat lung by saline perfusion via the pulmonary artery. The lungs and heart are removed with the major pulmonary vessels intact, and a catheter inserted through the right ventricle into the pulmonary artery. A known amount of saline is introduced through the catheter at a known pressure. As the saline reaches the capillaries, it perfuses through the alveolar wall, out through the trachea. and is collected quantitatively. This flow of saline presumably carries with it all the saline-soluble lipid lining the alveoli. Exactly how quantitative the procedure is will be investigated by a series of repeat flushings, using different volumes and pressures of saline.

The saline extract is then re-extracted with chloroform-methanol in a separatory funnel. This extract should contain only that material lining the alveoli which is soluble both in saline and in chloroform-methanol, i.e., surfactant phospholipid. The extract is then dried, re-dissolved, and spotted on glass plates coated with silica gel for thin-layer chromatography. Three primary fractions have been identified: sphyngomylin, lecithin, and cephalin, by comparison with standards run simultaneously. Separation is accomplished by developing the plates two hours in chloroform-methanol, and spots are identified by iodine spray or rhodamine 6-G.

To quantitate the individual lipids, the silica gel in the areas where a lipid spot has been identified is scraped off with a razor blade into a centrifuge tube containing a sulfuric acid-potassium dichromate solution. The mixture is heated allowing the solution to oxidize the lipid, then it is centrifuged and the absorbance of the supernatant determined. Standard curves have been run for each lipid with this system, permitting the optical density to be converted to a weight of lipid which can then be presented as a ratio to the weight of tissue from which it was originally obtained.

This work is being pursued by David L. Beckman, who hopes, among other things, to utilize the technique to probe the mechanism of increased resistance to O_2 toxicity observed in young rats and chicks.

4. Fluid Balance of Rats in an He-O_2 Atmosphere

Previous work in this laboratory suggested that rats living in an atmosphere of 79% He - 21% O_2 increased their urine volume and water consumption.[3] Presumably this is an aspect of the higher metabolism expected in He, and may therefore not be of any specific physiologic importance. However, it could complicate the weight and space requirements in He - O_2 atmospheres, and therefore, further experiments were undertaken to define the problem more quantitatively.

Sixteen Wistar rats were maintained in a closed environment for a period of 24 days. Eight rats were in 79% He - 21% O_2, and the remaining eight in air. Humidity was kept between 50 and 60%, temperature at 70-80°F, and CO_2 at less than 0.5%. The following parameters were measured: water and food consumption, urine volume and urine osmalarity. In two repeat trials no significant changes in urine volume or water consumption were observed, but there were significant increases in food consumption. These increases are probably caused by increased metabolism as a result of greater heat loss in He-O_2.

Analysis of the experimental protocols indicated that the discrepancy in urine volume and water consumption between this study and Tarsitano's work[3] may have been the result of differences in technique. In the previous study the parameters were measured on a group basis, whereas in the present work measurements were done on individual rats permitting a more precise statistical comparison. In addition, in the previous study, contamination of the urine with feces and drinking water was possible, but was eliminated in the present work with the aid of a new style metabolism cage. The isolator and metabolism cage setup in which these experiments were run are shown in Fig. 1.

It would appear that no special precautions need be taken with regard to water stores or urine disposal in He-O_2. However, it would be well to consider that both water intake and urine output are likely to be higher in any situation where metabolic rate and food intake are elevated.

5. Metabolic Changes Following Transfer of Animals to and from a Helium-Oxygen Environment

We are most intrigued with some metabolic changes observed in rats and chicks which have lived for a period of time in a 79% He-21% O_2 environment. On the first exposure of such animals to air, oxygen consumption falls below that of controls maintained in air, suggesting that animals living in an N_2-low environment may be unusually sensitive to the depressing (narcotizing?) action of N_2. The experiments on which these observations are based may be described briefly as follows:

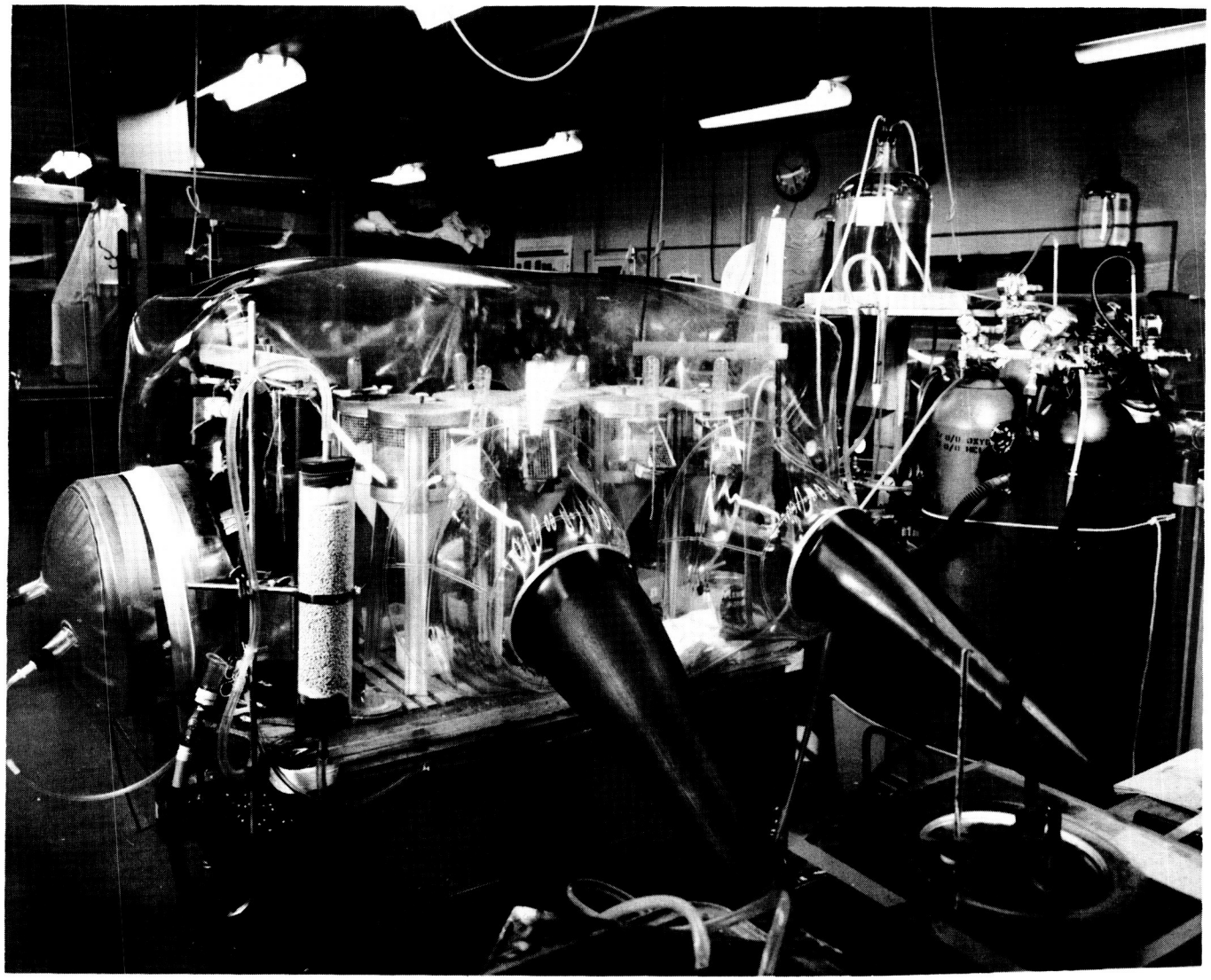

Fig. 1 - Isolators set up to determine water intake and urine output of rats in a He-O_2 atmosphere

The experimentals consisted of one-day old chicks, hatched from eggs incubated continuously in 79% He - 21% O_2 and 120-gram rats, living for two weeks in 79% He - 21% O_2. Controls were equivalent animals incubated or living in air. Both controls and experimentals were maintained in plastic isolators. In any one test, equal numbers of controls and experimentals were removed from the isolators and placed in a closed-type respirometer in which individual O_2 uptakes could be measured nearly simultaneously. In the respirometer the animals were exposed in succession to three atmospheres: First, O_2 uptake was determined in the gas in which the animals had come from, i.e., experimentals in He-O_2, controls in air (for the experimentals there was no intervening exposure to air). Next both groups were tested in 100% O_2, and finally the He group was exposed to air. The exposure interval in each gas was 60 minutes for the chicks and 30 minutes for the rats. This allowed for flushing and temperature equilibration between gases. The actual O_2 uptakes were measured over 15-minute intervals.

The clearest results of this study have been obtained in chicks, as presented in Table IIA. While metabolic rates for the control chicks were relatively unaffected by the transfer from one gas to another, the basic comparison with which we are concerned is between the experimentals and controls within each gas. Thus, compared to their controls, metabolism of the He-raised animals was elevated about 10% in the first step, i.e., in He - O_2. While not significant, this difference is believed to reflect an increased heat conduction in He at the time of the measurements, rather than any residual effect of their having lived in He-O_2. This conclusion is supported by the fact that in the second step, with both controls and experimentals in 100% O_2, metabolism of the He-raised animals is essentially the same as that of the controls. In the last step, where both controls and experimentals are in air, and in which the He-O_2 animals received their first exposure to N_2, the O_2 uptake of the experimentals is depressed a significant 25% below that of the controls.

The experimental design used in the rat study (Table IIB) does not lend itself to as direct a comparison of the effects of air exposure as with the chicks, because in the last step the air-raised animals were transferred to He-O_2 rather than air. What is brought out quite dramatically, however, is the rather immediate nature of the stimulating effect on metabolism of exposure to He, for O_2 uptake of the rats rose about 28% over their previous level. By contrast, the O_2 uptake of the He-raised rats fell some 16% in this step which marked their first exposure to air.

In either the chick or the rat study it is difficult to see how any mechanical factor like washout of gases or equilibration of chambers could produce the depression in metabolism observed when the He animals were transferred to air. For the present, therefore, we are using as a working hypothesis the idea that animals raised in He-O_2 (i.e., in the absence of N_2) have become sensitized to N_2. The depression in metabolism seen on first exposure to N_2 might then be considered similar to the nitrogen narcosis observed at much higher partial pressures of N_2 in animals accustomed to living in air.

Table II. Oxygen Uptakes in Animals Raised in Air (controls) or in 79% He-21% O_2 (experimentals) and Transferred in Succession to Air, 100% O_2, or 79% He-21% O_2

A. Chicks	No.	Body Weight (gms)	ml O_2/min/kg BW		
			in atmosphere in which raised	in 100% O_2	in air
Controls	9	36.7	21.2 (air)	21.0	21.4
Experimentals	9	34.7	23.0 (He-O_2)	20.9	16.4
Pooled S.E.		1.797	2.533	2.612	2.096

B. Rats	No.	Body Weight (gms)	ml O_2/min/kg BW		
			in atmosphere in which raised	in 100% O_2	reversal of atmospheres in which raised
Control	9	236.9	32.1	29.4	37.4(He-O_2)
Experimental	8	222.8	35.0	32.7	27.7(Air)
Pooled S.E.		6.398	2.624	2.572	2.126

We might note that this phenomenon of depression by N_2, or something similar to it, was observed before in our laboratory by Dr. Wright, and described in our last report. Dr. Wright, while working with the Warburg on homogenates of 8-day embryos incubated in He-O_2, noted that O_2 uptake was depressed if the tissues were homogenized in air and elevated if prepared in an He-O_2 atmosphere.[6,8,10]

While the degree of depression does not appear to be serious, the fact that it exists at all may be a matter of concern enough to warrant further exploration. For example, it would seem desirable to know how long an exposure is required in a N_2-free environment for the air effect to be manifested; whether prolonging the stay in He-O_2 increases the depression or whether it plateaus at some point; how long the depression lasts once air breathing has been resumed, etc., etc. It would also seem important to ascertain if deprivation of N_2 through use of a 100% O_2 atmosphere might lead to similar effects.

Mr. Rodney A. Rhoades, a NASA trainee and Ph.D. candidate in the Department of Physiology, is planning to present this work at the annual meeting of the Federation of American Societies for Experimental Biology, at Atlantic City, April 9-15, 1965.[13]

6. Hatchability and Metabolism of Chick Embryos in 79% He-21% O_2

Continuing efforts have been made to determine whether the decreased number and smaller size of chicks obtained from eggs incubated in He-O_2 are the result of a direct biochemical effect of He on embryogenesis or indirectly to helium-related changes in the physics of the incubation environment. To date we have completed ten trials, with apparently normal hatches in He-O_2 in only two. At this time it is still not possible to single out any factor(s) which would account for the poor hatches in He-O_2.

After our first few trials, we speculated that the lower isolator surface temperature and the higher egg weight losses we observed in He might be responsible for the poor hatches.[2,11] Both of these effects could be a result of the higher heat conductivity of He. We have tried to compensate for this in a number of ways, including injecting water into the isolators, placing coverings over the isolators, reducing fan speeds, etc. In our last trial, we managed, by virtue of a thick insulating layer around the entire isolator and stoppage of almost all leaks, to obtain a system for which surface T was actually higher in He than in air and egg weight loss was well below possible adverse levels. Nevertheless, the hatch in He was still about 1/2 that in air.

We are now considering the possibility that our system may have been too good, that is, hatchability may have been adversely affected by either too little evaporative loss or accumulation of toxic by-products or both. Incidentally, it was still necessary to keep relative humindity 15-20 percentage points higher in He-O_2 than in air in order to maintain the same

evaporative loss. Maintenance of relative humidity and control of evaporative loss has been a consistent problem for us in He-O_2 atmospheres.

In another series of trials we attempted to test the theory that the reason embryos develop poorly in He is because they have difficulty maintaining a higher than ambient intra-egg temperature during the last week of incubation. It has been found that in air metabolic heat is not conducted away as fast as it is produced by the embryo as hatching time approaches, and consequently intra-egg temperature rises above ambient. In the He atmosphere, metabolic heat presumably is conducted away rapidly and intra-egg temperature would not rise above ambient. However, all attempts on our part to follow intra-egg temperatures during incubation by means of thermocouples or thermistors have failed to show any difference related to incubation atmosphere. Furthermore, we failed to produce a normal batch in a test in which we progressively elevated ambient temperature in the He isolator during this last week of development in proportion to the rise expected in intra-egg temperature.

We are presently carrying out a test in which groups of eggs are being switched back and forth between the He and air isolators at various times during incubation. The hope here is that we might at least determine more precisely when the He effect takes place. At the same time we have made further modifications in our system, with the goal of eliminating from the comparison all possible factors relating to temperature, humidity, and contamination. It seems to us that until all adverse effects of He atmospheres are explained, regardless of the biological system in which they occur, some doubt must remain regarding the prolonged use of He with man.

In somewhat related work, we are exploring the metabolism of avian embryos incubated in an He-O_2 atmosphere and x-irradiated in the same gas without intervening air exposure. When this was done previously, preparation and irradiation of the embryos were carried out in air. The results were equal depression of metabolism, whether embryos were grown in air or He-O_2. Theoretically, we might have expected to see more of a depression induced in the He group by x-rays because of a smaller quenching effect of He compared to N_2. It is possible that any difference due to inert gas was masked by a prior depression in metabolism which occurred when the He incubated embryos were suddenly exposed to air.[6,8,10]

Plans are also evolving to follow cellular metabolism of the developing chick embryo day by day with the hope that a clue will be found as to why development in He-O_2 sometimes ceases before hatch. The Gilson Respirometer was to be used for these studies; however, it was found that gases would diffuse through the tygon tubing used to connect the reaction flasks to the manometers. This equipment is being replaced by Gilson for similar equipment with glass connections, since most of our work will require the use of oxygen, helium-oxygen, or other highly diffusible gases.

7. Heat Loss and Temperature Control of Men in an He-O_2 Atmosphere

Body heat loss may be higher in an He atmosphere, not only because of the 6-1/2 fold greater rate of heat conduction but also because of a faster rate of evaporation. This could be of practical value in the design of closed systems and space suits, particularly where the occupant must engage in physical activity. To establish how effective such a system might be in controlling heat balance in man, we have set up a large plastic chamber capable of holding a bicycle ergometer, and which can be filled with 79% He-21% O_2. Temperature relative humidity, and CO_2 can be controlled within the chamber, and various measurements, physical and physiological, transmitted to recorders on the outside. A photograph of this facility was included in our last report.

To date, the tests we have made with this system involve a 75-minute exposure to an He-O_2 environment. The 75 minutes are divided into three parts: rest--15 minutes; exercise--15 minutes; and recovery--45 minutes. In the first series, the plan was to hold ambient temperature of the isolator at 95°F and the relative humidity at approximately 93%. In the second series, temperatures were to remain at 95°F, but relative humidity between 40 and 60%. A third series of runs (at present, in progress) calls for an isolator temperature of 70°F with a 40-60% range in relative humidity. Parameters measured during rest, exercise, and recovery are: (1) mean skin temperature, (2) mean body temperature, (3) rectal temperature, (4) heart rate, and (5) sweat loss. Identical measurements were made on the same subjects in air under the same environmental conditions of temperature and humidity for a basis of comparison. Each series is based on duplicate runs made on each of three subjects.

The results of the first series of tests are difficult to evaluate. While there were virtually no differences in any of the measurements made on the subjects in the two environments, temperature and humidity could not be kept identical in the two systems. Relative humidity was consistently about 3% higher and ambient temperature about 1°F higher in He-O_2, despite similar settings on all controlling equipment. At the elevated temperatures and humidities of this first series even increases as small as these may very well result in considerably greater heat stress on the subjects. It is of interest to note that we have experienced similar difficulties in the control of relative humidity in egg incubation studies in He-O_2 which are also run at elevated temperatures.

In the second series of tests, also at high temperature but with lower humidities, environmental conditions were better controlled (Fig. 2). During the exercise portion of this test, mean skin temperature of the subjects tended to be lower in He-O_2. While this may suggest some benefit in He-O_2, the differences appear to be too small to be of much practical significance. Data from the third series of experiments are still being evaluated.

This work is being conducted by Edward L. Fox who hopes to use it for a Ph.D. dissertation.

Fig. 2 - Isolator temperature (IT), and relative humidity (RH), mean skin temperature (MST), and mean body temperature (MBT), versus time for three subjects exposed to air (solid line), or helium-oxygen (dashed line), during 15 minutes of rest, 15 minutes of exercise on a bicycle ergometer with a work load of 140 watts,* and 45 minutes of recovery. Each 5-minute plot represents an average of six values (three subjects, two trials in air, two trials in helium-oxygen). Temperatures are in degrees F, relative humidity is in per cent, and time in minutes.

*100 watts equals 10.19 KgM/sec equals 0.134 H.P.

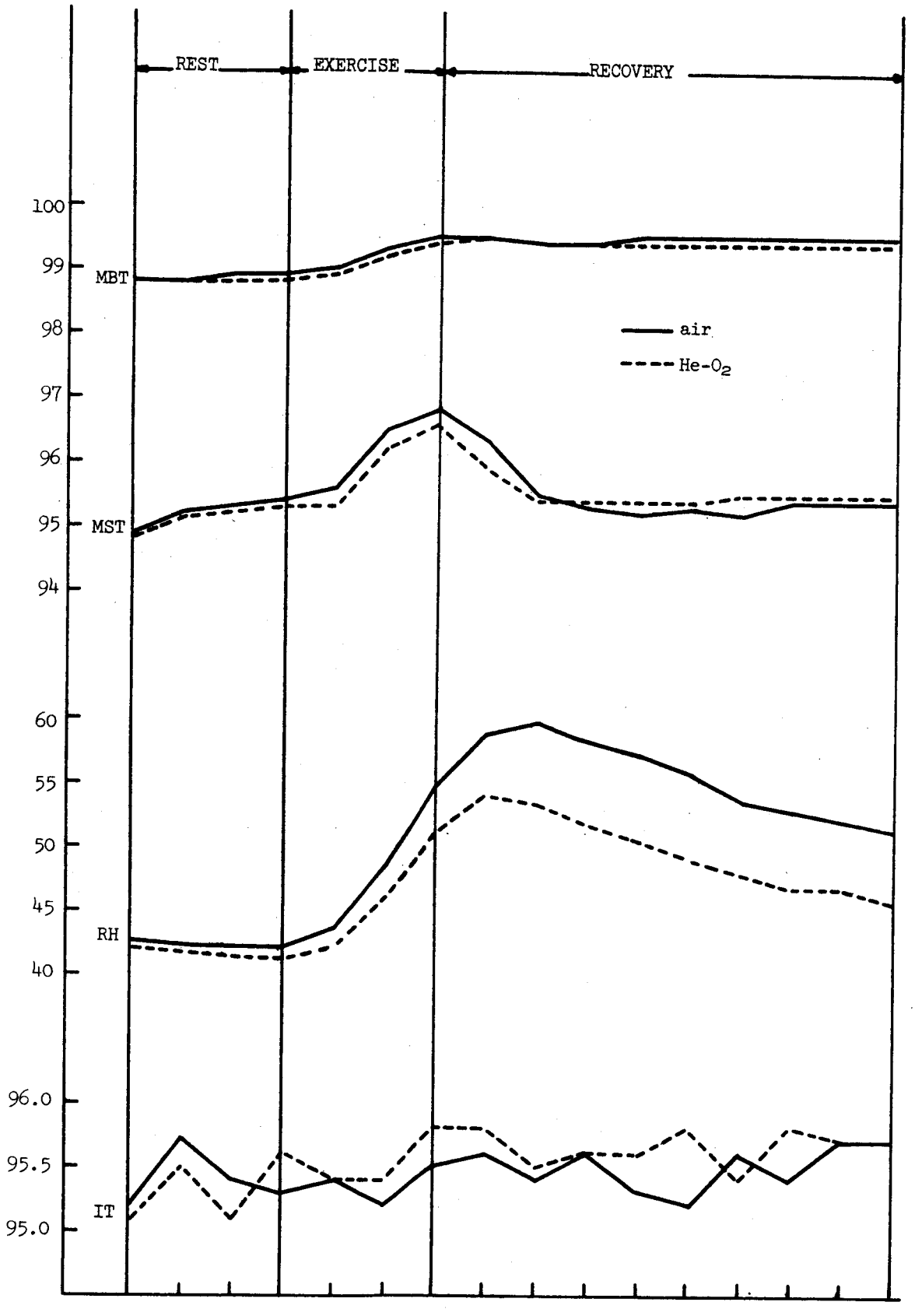
REST
EXERCISE
RECOVERY
100
99
MBT
98
97
96
95
MST
94
air
He-O2
60
55
50
45
RH
40
96.0
95.5
IT
95.0

FIGURE 2

III. CONTINUING BIBLIOGRAPHY OF PUBLICATIONS RELATED TO RESEARCH SUPPORTED BY NASA GRANT NO. NsG-295-62 (listed in chronological order)

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13. Rhoades, R. A.; H. S. Weiss; R. A. Wright; and E. P. Hiatt, "Depression of metabolism in animals transferred from a helium-oxygen environment to air," (Abstract submitted to the American Physiological Society for inclusion in 1965 meeting of the Federation of the American Societies for Experimental Biology).

IV. PERSONNEL CURRENTLY ASSOCIATED WITH THE PROJECT AND THE ENVIRONMENTAL PHYSIOLOGY LABORATORY (listed in alphabetical order)

1.	Bartels, Robert L.,	Ph.D., Assistant Professor of Physical Ed. (Consultant)
2.	Beckman, David L.,	M.Sc., Ph.D. candidate, Dept. of Physiology, (Research Assistant)
3.	Fox, Edward L.,	M.Sc., Ph.D. candidate, Dept. of Physiology, (Research Assistant)
4.	Hiatt, Edward P.	Ph.D., M.D.; Professor of Physiology, (Project Supv. and Director of Env. Physiol. Lab.-on leave)
5.	Hitchcock, Fred A.,	Ph.D., Prof. Emeritus of Physiology, (Research Associate and Consultant)
6.	Kreglow, Edith S.,	Registered Nurse, (Research Assistant)
7.	Lessler, Milton A.,	Ph.D., Professor of Physiology, (Consultant)
8.	Pilmer, Richard A.,	M.A., Capt. USAF; Ph.D. candidate, Dept. of Physiol., (Research Assistant)
9.	Pitt, Joseph F.,	(Research Assistant).
10.	Porterfield, Susan F.	(Technical Assistant).
11.	Pratt, Phillip C.	M.D.; Chief of Pathology, T.B. Hospital, (Consultant)
12.	Rhoades, Rodney A.,	M.Sc., NASA Trainee and Ph.D. candidate, Dept. Physiol., (Research Assistant)
13.	Smith, Charles W.,	Ph.D.; Associate Prof. of Physiology, (Consultant)
14.	Weiss, Harold S.,	Ph.D.; Assoc. Prof. of Physiology, (Acting Project Supv. & Assoc. Director of Env. Physiol. Lab.)

15. Wright, Ronald A., DVM, M.Sc.; Research Assoc., Dept. of Physiology, (Investigator and Ass't Director of Env. Physiol. Lab.)

V. ADMINISTRATIVE ORGANIZATION

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2. College of Medicine, The Ohio State University
   Dean.....Richard L. Meiling

3. Department of Physiology, College of Medicine
   Chairman.....Robert C. Little

4. Environmental Physiology Laboratory, Department of Physiology
   Director.....Edward P. Hiatt (on leave)
   Assoc. Director....Harold S. Weiss
   Ass't. Director....Ronald A. Wright

5. NASA Project: Grant NsG-295-62, Research Foundation No. RF 1492

   Supervisor.....Edward P. Hiatt (on leave)
   Investigator (acting Supervisor)...Harold S. Weiss
   Investigator.....Ronald A. Wright

Investigator Ronald A. Wright **Date** March 11, 65

Supervisor (acting) Harold S. Weiss **Date** March 10, 1965

For The Ohio State University Research Foundation

Executive Director Robert C. Stephenson **Date** 10 March 1965