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ENDOCRINE FACTORS RELATED TO TOXICITY OF OXYGEN AT ATMOSPHERIC PRESSURE

by
Charles W. Smith

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ENDOCRINE FACTORS RELATED TO TOXICITY OF OXYGEN AT ATMOSPHERIC PRESSURE

by Charles W. Elstead Smith

Earlier work has established that pituitary, adrenal, and thyroid principles enhance adverse response to oxygen at high pressures. The purpose of this investigation was to determine the possible effects of these endocrine factors upon the adverse response to oxygen in high concentrations at atmospheric pressure (OAP).

Young Sprague Dawley rats, maintained on a uniform diet, were chosen as experimental subjects. They were exposed to oxygen in especially constructed airtight chambers. Concentration of oxygen was, in general, maintained above 95 per cent by continuous flow of gas supplied from commercial cylinders. Carbon dioxide level was kept below 0.20 per cent by soda lime. Temperature was about 28°C.

In the first section, experiments were carried out to determine the effects of hypophysectomy upon rats exposed to oxygen at atmospheric pressure. It is well-known that animals continuously exposed to OAP for long periods exhibit gross pulmonary congestion, hyperemia, consolidation and massive hydrothorax. The extreme involvement is indicative of a major alteration of permeability in the vascular bed. Hypophysectomy was found to provide a very significant degree of protection from OAP adverse effects as indicated by lessened pulmonary pathology, decreased hydrothorax and increased survival time of the operated rats. The inference was made that this protection was attributable, in part at least, to elimination of adrenocorticotrophin and of adreno-cortical factors.

In carrying this investigation further, it was found that adrenalectomy also provided protection against OAP ill effects. This protection was evidenced by a decreased degree of pulmonary pathology, lessened hydrothorax and increased survival time of the operated rats. These experiments substantiated the inference that removal of adreno-cortical factors was responsible, at least, in part, for the protection from OAP effects conferred by hypophysectomy. The conclusion that removal of some adrenal principle imparted beneficial effects to rats in OAP was justified.

This conclusion was further supported by the results of experimental studies in which cortisone was administered to animals in OAP. This adreno-cortical factor significantly shortened survival time in oxygen and accentuated the typical pulmonary pathology.

Since adrenalectomy removes medullary as well as cortical hormones it is possible that part of the protection conferred by adrenalectomy might be due to removal of epinephrine. Experiments were undertaken, therefore, to determine what effect, if any, epinephrine would have upon animals exposed to OAP. Subcutaneous injection of epinephrine, in amounts too small to be injurious in air controls, brought about a significant enhancement of OAP adverse effects. This enhancement was evidenced by greatly decreased survival time and by accentuated lung pathology. The results of this study indicated that the medullary factor may act in mediating adverse response to OAP.

Part of the protection afforded by hypophysectomy might possibly be due to removal of thyrotrophin with consequent

diminishment of thyroid secretion; therefore, studies were undertaken to determine the effect of thyroid factors upon the toxic response of rats to OAP. It was found that thyroid hormone when administered as dessicated thyroid or as thyroxine decreased survival time and enhanced lung pathology of normal rats in OAP. In addition, thyroid factors were found to reverse the beneficial effects of hypophysectomy; this also was shown by decreased survival time in oxygen and increased degree of lung damage. These data indicate that the beneficial effects of hypophysectomy may be due, in part, to decreased thyroid function.

While it must be recognized that the conditions of stress which are present in oxygen at high pressures and in oxygen at atmospheric pressures are not identical, the data obtained from this investigation demonstrate that the endocrine factors which have been shown to be damaging in oxygen high pressure are also operative in enhancing the adverse effects of oxygen at atmospheric pressure.

ACKNOWLEDGMENTS

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TABLE OF CONTENTS

	Page
LIST OF TABLES	iv
LIST OF ILLUSTRATIONS	vii
I. INTRODUCTION	1
II. APPARATUS AND METHODS	7
III. EXPERIMENTAL PROCEDURES AND RESULTS	11
A. Hypophysectomy and the Adverse Effect of Oxygen at Atmospheric Pressure (OAP)	11
B. Adrenal Factors	23
1. Adrenalectomy	23
2. Cortical Principles--Cortisone	38
3. Epinephrine	48
C. Thyroid Factors	65
1. Dessicated Thyroid	66
2. Thyroxine	76
IV. DISCUSSION	87
V. SUMMARY AND CONCLUSIONS	92
LITERATURE CITED	95

LIST OF TABLES

Table		Page
1.	Number of Hypophysectomized and Normal Rats in OAP and in Air (Experiment I)	11
2.	Mortality, Survival, Lung Data and Hydrothorax for Hypophysectomized and Normal Rats in OAP	17
3.	Number of Hypophysectomized and Normal Rats in OAP and in Air (Experiment II)	18
4.	Number of Hypophysectomized and Normal Rats in OAP and in Air (Experiment III)	18
5.	Mortality, Survival, Lung Data and Hydrothorax for Hypophysectomized and Normal Rats in OAP (Experiment III)	20
6.	Number of Adrenalectomized and Normal Rats in OAP and in Air (Experiment I)	24
7.	Mortality, Survival, Lung Data and Hydrothorax for Adrenalectomized and Normal Rats in OAP and in Air (Experiment I)	26
8.	Number of Adrenalectomized and Normal Rats in OAP and in Air (Experiment II)	28
9.	Mortality, Survival, Lung Data and Hydrothorax for Adrenalectomized and Normal Rats in OAP and in Air (Experiment II)	31
10.	Number of Adrenalectomized and Normal Rats in OAP and in Air	33
11.	Mortality, Survival, Lung Data and Hydrothorax for Adrenalectomized and Normal Rats in OAP and in Air	35
12.	Number of Cortisone Treated and Normal Rats in OAP and in Air	40

Table Page

13. Mortality, Survival, Lung Data and Hydrothorax for Cortisone Injected Rats and for Controls in OAP and in Air 42

14. Number of Epinephrine Treated and Normal Rats in OAP and in Air (Experiment I) 49

15. Mortality, Survival, Lung Data and Hydrothorax for Epinephrine Injected Rats and for Controls in OAP and in Air (Experiment I) . . . 51

16. Number of Epinephrine Treated and Normal Rats in OAP and in Air (Experiment II) 56

17. Mortality, Survival, Lung Data and Hydrothorax for Epinephrine Injected Rats and for Controls in OAP and in Air (Experiment II) . . . 57

18. Number of Hypophysectomized and Normal Rats in Test and Control Groups in OAP and in Air (Experiment I) 66

19. Mortality, Survival and Lung Damage for Normal Thyroid Fed Rats and for Controls in OAP 70

20. Mortality, Survival and Lung Damage for Hypophysectomized, Thyroid Fed Rats and for Controls in OAP 71

21. Number of Hypophysectomized and Normal Rats in Test and Control Groups in OAP and in Air (Experiment II) 72

22. Mortality, Survival, Lung Data and Hydrothorax for Normal, Thyroid Fed Rats and for Controls in OAP 74

23. Mortality, Survival, Lung Data and Hydrothorax for Hypophysectomized, Thyroid Fed Rats and for Controls in OAP 75

24. Number of Hypophysectomized and Normal Rats in Test and Control Groups in OAP and in Air (Experiment III) 78

25. Mortality, Survival, Lung Data and Hydrothorax for Normal, Thyroxine Injected Rats and for Controls in OAP 79

Table

Page

26. Mortality, Survival, Lung Data and Hydrothorax for Hypophysectomized, Thyroxine Injected Rats and for Controls in OAP 82

LIST OF ILLUSTRATIONS

Figure		Page
1.	Cross-section Representation of Oxygen Exposure Chamber	9
2.	Photograph of Oxygen Exposure Chamber	10
3.	Lungs of Rat Exposed to OAP with Lungs of Air Control	13
4.	Effects of OAP on Lungs of Normal and Hypophysectomized Rats	16
5.	Effect of Hypophysectomy on Survival in OAP	21
6.	Effect of OAP on lungs of Normal and Adrenalectomized Rats	29
7.	Effect of Adrenalectomy on Survival in OAP	34
8.	Effect of OAP on Lungs of Normal and Adrenalectomized Rats	37
9.	Effect of Adrenalectomy on Hydrothorax (OAP)	39
10.	Effect of Cortisone on Survival in OAP	44
11.	Effect of OAP on Lungs of Rats Injected with Cortisone and on Lungs of Control	45
12.	Effect of Cortisone on Lungs of Rats in OAP and in Air	47
13.	Effect of OAP on Lungs of Epinephrine Injected Rat and on Lungs of Control	54
14.	Effect of Epinephrine on Lungs of Rats in OAP and in Air	55
15.	Effect of OAP on Lungs of Epinephrine Injected Rats and on Lungs of Control	60
16.	Effect of Epinephrine on Survival in OAP	61

Figure Page

17. Effect of Epinephrine on Hydrothorax (OAP) . . . 62

18. Effect of OAP on Lungs of Normal, Thyroid Fed Rat and on Lungs of Control 68

19. Effect of OAP on Lungs of Hypophysectomized, Thyroid Fed Rat and on Lungs of Control 69

20. Effect of Thyroid on Survival in OAP 73

21. Effect of Thyroid on Hydrothorax (OAP) 77

22. Effect of Thyroxine on Survival in OAP 80

23. Effect of OAP on Lungs of Hypophysectomized, Thyroxine Injected Rat and on Lungs of Control . 83

24. Effect of Thyroxine on Hydrothorax (OAP) 84

I. INTRODUCTION

In 1775 Priestly speculated that "dephlogisticated air" might be useful in medicine, but also that this same substance could be harmful: ". . . for," said he, "as a candle burns out much faster in dephlogisticated air than in common air so we might as may be said live out too fast and the animal powers be too soon exhausted in this pure kind of air." Lavoisier (1783) reporting the results of post mortem findings in guinea pigs which had succumbed in oxygen stated: "The flesh, upon inspection, was very red, the heart was livid and filled with blood, especially the right auricle and ventricle; the lungs were very flaccid but exceedingly red and engorged with blood even on the surface."

Following the initial experiments of Priestly and of Lavoisier on the essentiality of oxygen for oxidations within the body, its employment was widely advocated for therapeutic purposes. Beddoes, although an early advocate of this therapeutic use, realized that oxygen might cause pulmonary damage because he found that lungs of a kitten maintained in 80 per cent oxygen for seventeen hours were exceedingly red; those of a control were normal (Beddoes and Watt, 1796). During the early part of the nineteenth century, enthusiasm for indiscriminate use of oxygen was somewhat dampened due to an

awareness of its possible ill effects. At about 1850, there was a revival, however, of interest in oxygen as an agent beneficial in the treatment of pneumonia and of hypo-oxia in general. The actual damaging action of oxygen constituted the subject of continuous debate and, indeed, the problem is not completely resolved today.

The work of Paul Bert, reported in his La Pression Barometrique (1878) constituted a major landmark in the study of oxygen toxicity. Bert concluded from his experiments that oxygen in high pressures (OHP) was toxic to all living organisms. He observed the central nervous system effects which were indicated by the appearance of convulsions in birds, rats, frogs and dogs exposed to oxygen pressures of three atmospheres and greater.

Thompson (1889) noted that cold blooded animals were not so readily affected by oxygen at increased pressures as were higher animals. In addition to the CNS effects observed by Bert, Thompson described the pulmonary congestion which is an accompanying manifestation of OHP.

While Bert and Thompson studied the results of exposure to oxygen at pressures in excess of one atmosphere, a thorough study of lower tensions of the gas was not carried out until J. Lorrain Smith in 1899 set out "to find the effects which arise with tensions approaching by degrees that required to produce the toxic effect described by Bert." The lowest oxygen tension studied by Smith was 0.4 atmosphere; above this, at intervals, exposures were made until a tension of 4.5 atmospheres was reached.

The first experiment, which he regarded as a control, involved the exposure of two mice to an average tension of 41.6 per cent oxygen at atmospheric pressure for eight days; they showed no abnormal symptoms. In the second series of exposures mice were placed in 70 - 80 per cent of an atmosphere of oxygen and after four days two out of four of them were dead. Further exposures using increasingly higher tensions of oxygen, i.e., percentages of 130, 180, 230, 300, 357 resulted in progressively shorter survival of the mice. Autopsy of these animals revealed consolidation and congestion of the lungs which sank in fixing fluid; the pneumonic condition was universal and was compared, by Smith, to early stages of croupous pneumonia. Very few micro-organisms were found in the lung sections.

Smith concluded that there are two phases of oxygen effect. First there is the slowly developing pulmonary effect and second a rapidly developing action on the nervous system. He further suggested that the pulmonary damage caused by oxygen might be protective to the central nervous system by impeding absorption of excessive amounts of oxygen. Smith thought of oxygen as a direct irritant to pulmonary tissue.

Karsner (1916) substantiated these findings of Smith and held that oxygen by exerting an irritative action on lung tissue brought about the observed edema, congestion, epithelial degeneration, desquamation and finally pneumonia. He found edema and congestion in liver, stomach, adrenals and other abdominal viscera.

Studies on humans as experimental subjects have also indicated that oxygen in high concentrations exerts an effect comparable to that described for animals (Clamann and Becker-Freyseng, 1939). Some have claimed that the lungs of man are less readily damaged by oxygen than are those of other animals and that susceptibility may vary from one species to another.

Numerous studies on the effects of atmospheric pressure and of high pressure oxygen have been undertaken within the past years. The literature has been extensively reviewed by Stadie, Riggs and Haugaard (1944) and by Bean (1945).

The exact mechanisms of adverse action of oxygen are not well known. For the damage due to high pressure oxygen at least, a number of explanations have been suggested; for example, an accumulation of carbon dioxide at the tissue level due to a disruption of dual function of hemoglobin, poisoning of oxidative enzymes, and direct irritative action of oxygen on pulmonary tissue.

Since, as Smith showed, there is no real clear line of demarcation between the effects of high tensions of oxygen at atmospheric pressure and the effects of oxygen at pressures greater than one atmosphere, it is reasonable to assume that some of the factors which are responsible for effects of oxygen at high pressures are also active in pressures of approximately one atmosphere.

With the assumption that oxygen at high pressures constitutes a stressor of major importance, Bean (1951) studied the condition of adrenals of rats exposed to oxygen high

pressure (OHP) and found evidence of hyperactivity of these organs. Later studies (Bean and Johnson, 1952) showed that hypophysectomy confers a very pronounced protection against the deleterious effects of OHP. Subsequent studies using ACTH and cortisone which reverse the beneficial effects of hypophysectomy led to the conclusion that the normal defensive action of these agents against the more usual types of stress was absent in the stress of oxygen high pressure and that actually the presence of these pituitary-adrenal factors exaggerated rather than relieved the adverse effects of oxygen (Bean, Johnson and Smith, 1953).

It has been stated above that no clear line of demarcation exists between the effects of high concentrations of oxygen at atmospheric pressure (hereafter designated OAP) and those of high pressure oxygen (OHP); however, there are some differences between the actions of OHP and of OAP; for example, a small increase of carbon dioxide has peculiarly striking effects in OHP, whereas in OAP these are either entirely absent or much less pronounced. Similarly endocrine factors might not have the same effect in OAP as in OHP. The present study, therefore, was undertaken to determine the possible significance of these factors in the toxicity of oxygen at atmospheric pressure. The subject matter will be presented under the following headings:

A. Hypophysectomy and the Adverse Effect of Oxygen at Atmospheric Pressure (OAP)

B. Adrenal Factors

1. Adrenalectomy
2. Cortical Factors--Cortisone
3. Medullary Factors--Epinephrine

C. Thyroid Factors

1. Dessicated Thyroid
2. Thyroxine

II. APPARATUS AND METHODS

In these experiments young Sprague Dawley rats were chosen as experimental subjects. In all the experiments except one, males were used. They were exposed to oxygen in airtight chambers constructed especially for this purpose. Figures 1 and 2 show structural details, size and general significant features of these chambers.

A screen mesh floor was supported by an angle iron frame above wood shavings. Each chamber was divided centrally by means of a wire screen so that two groups of rats could be kept separate but exposed simultaneously to the same atmosphere. On the wooden top of the chamber provision was made for inlet and outlet of oxygen or air, and for entrance of a water fountain; lucite hatches sealed with rubber gaskets permitted introduction or removal from the chamber of rats and of food which consisted of bread, oranges, meat, lettuce and carrots supplemented with commercial dry laboratory animal rations. A centrifugal fan driven by an induction motor circulated the gas past water cooling coils and through soda-lime cartridges to remove carbon dioxide (Figure 1). Temperature was maintained at 28°C.

Oxygen was provided to the chambers in a continuous flow from cylinders of commercially pure oxygen and room air

where needed was furnished from a compressor. Quantitative analysis of gas was made at regular intervals using a Haldane-Henderson-Novy absorption type gas analyzer. With the exception of one instance, which will be mentioned in a later section, oxygen concentration was maintained above 95 per cent and carbon dioxide never rose above 0.20 per cent.

The experimental data derived from this investigation was statistically analyzed by Student-Fisher t test for difference between groups which were compared (Snedecor, 1950). Standard error (S.E.) was computed for all averages of survival time, hydrothorax and lung-heart weights. Tabulation of these data have been included in the text so that information is available for each exposed rat as to mortality (D., died in oxygen; S., sacrificed for evaluation of pulmonary effects), survival time, degree of lung damage and hydrothorax. For some of the experiments lung-heart weights have also been included.

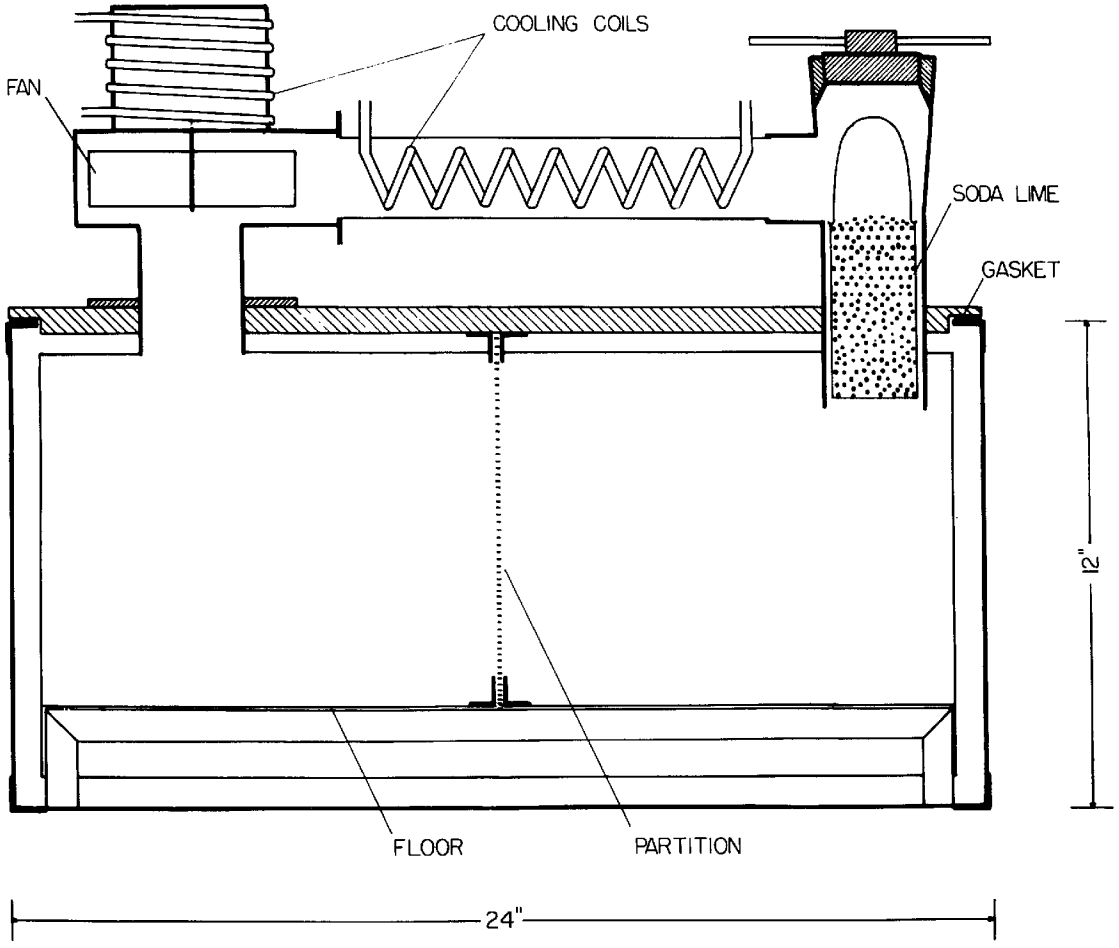


Figure 1.--Cross-section Representation of Oxygen Exposure Chamber. Circulating fan, cooling coils, and replaceable soda lime cartridge are indicated. Floor and central partition consist of open screen mesh. Beneath floor is room for layer of wood shavings.

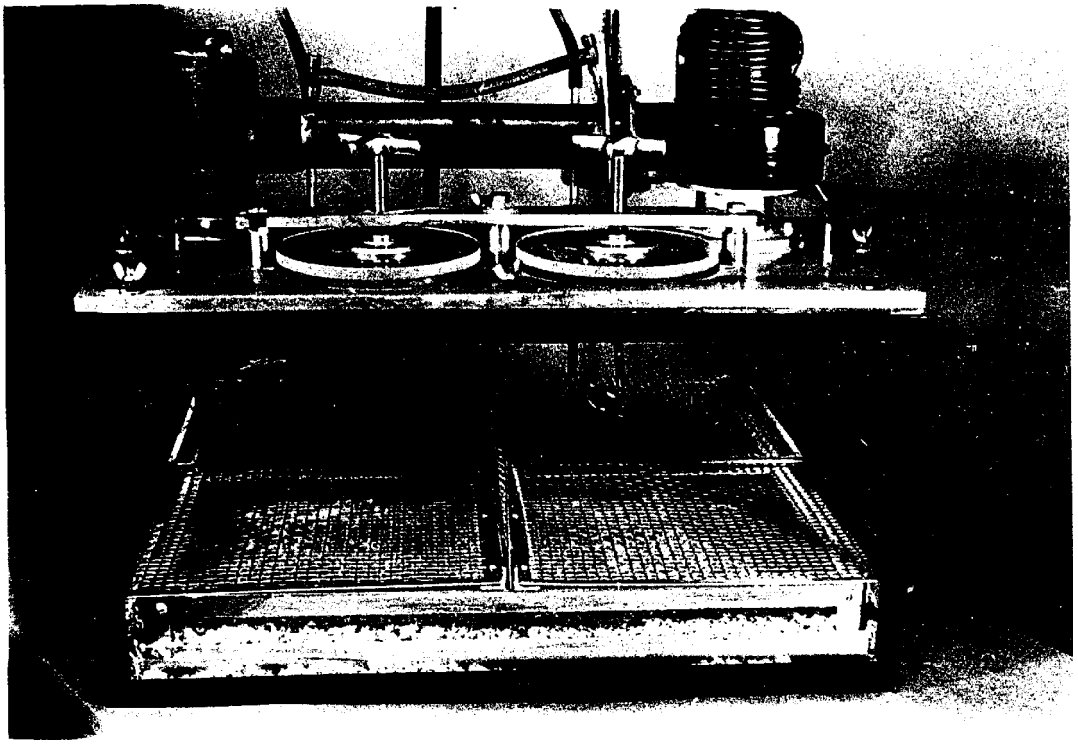


Figure 2.--Photograph of Oxygen Exposure Chamber. Lucite hatches for introduction and removal of rats and food are shown. Holes drilled in top allow for inlet and outlet of gas and for drinking fountains.

III. EXPERIMENTAL PROCEDURES AND RESULTS

A. Hypophysectomy and the Adverse Effect of Oxygen at Atmospheric Pressure (OAP)

It has been shown that hypophysectomy confers a measure of protection from oxygen high pressure effects (Bean and Johnson, 1952). In this section three experiments were carried out to determine what effects hypophysectomy might have upon rats exposed for long periods to oxygen in high concentrations at atmospheric pressure.

Experiment I.--In the first of these experiments 21 rats were used, 6 hypophysectomized and 5 normals were exposed in the chamber. Five hypophysectomized and five normals were maintained in air as controls. This division is summarized in Table 1.

TABLE 1

NUMBER OF HYPOPHYSECTOMIZED AND NORMAL RATS IN OAP AND IN AIR (EXPERIMENT I)

	Hypophy.	Normals
OAP	6	5
Air	5	5
Total	11	10

Frequent analysis of carbon dioxide and oxygen showed that carbon dioxide did not rise above 0.2 per cent and that the oxygen was maintained above 95 per cent except for two short intervals during the first twenty-four hours when, due to failure of the supply, it fell to 65 per cent for a short time. The animals were observed throughout the course of the experiment for indications of adverse effects.

Estimation of lung involvement (as shown in Table 2 and in succeeding tables) was based upon relative severity of observed macroscopic changes. Lungs which remained essentially normal were designated 0; those showing complete hemorrhagic congestion 4, while intermediate degrees of severity were indicated by 1, 2 or 3. Figure 3 which shows typical effects of OAP illustrates the contrast between lungs graded 0 and those graded 4. Degree of hydrothorax was also crudely estimated and has been included in the tabulation of these early experiments as much, some or little.

Criteria for evaluation of the OAP effects were: (1) time of onset of symptoms of oxygen's adverse effects, (2) mortality, i.e., the number of animals in each group which died in OAP, (3) condition of the lungs at death, (4) average survival of the animals in oxygen.

In the first experiment the animals were apparently unaffected until approximately the thirtieth hour of exposure. By thirty-six hours all the normal animals were dyspneic and at forty-five hours the first rat (non-operated) had succumbed; two of the same group expired in rapid succession at about



Figure 3.--Lungs of Rat Exposed to OAP with Lungs of Air Control. The animal whose lungs are shown on the right succumbed in oxygen; lungs of an air control on the left. This figure illustrates the involvement of lungs which is typically seen the result of exposure at atmospheric pressure.

fifty hours. The two remaining unoperated animals were sacrificed in terminal states at the end of seventy hours with intraperitoneal injection of sodium pentobarbital. In contrast to this, none of the hypophysectomized rats died in oxygen; all were sacrificed; at the death of each non-hypophysectomized animal an hypophysectomized rat was killed for comparison of pulmonary pathology.

Comparative examination of the lungs provided an effective criterion for evaluation of the protection which results from hypophysectomy. Lungs of the non-operated rats which died in oxygen were dark and liver colored; they sank in saline and appeared to have little or no air containing tissue. The thorax was found to contain a considerable volume of translucent yellowish fluid which clotted upon standing, indicative of a high protein content. The stomach contained large masses of undigested food; the intestines were empty or almost so. The liver was enlarged, darker than normal, and congested. The adrenals were dark, hyperemic, and enlarged.

In contrast to these effects every hypophysectomized animal sacrificed was relatively free of hydrothorax and of gross pulmonary damage. The stomach was normal; liver and adrenals were of normal size and color. The remarkable contrast between lungs of non-operated and of hypophysectomized rats which were simultaneously exposed is illustrated

in Figure 4; changes of the lungs seen in the non-operated animal are typical of the effects of OAP.

The procedure of this experiment in which a large number of control animals were sacrificed for comparison did not permit an exact appraisal of average survival time in the two groups. However, the fact that the non-hypophysectomized rats all died before their controls conclusively shows that hypophysectomy prolongs survival. The findings from examination of lungs also substantiate this conclusion. Table 2 outlines the results of this experiment in terms of the effect of OAP upon hypophysectomized and normal rats. Air controls were normal.

Experiment II.--The same procedures carried out in the second experiment on twenty rats as indicated in Table 3 resulted in data which are fully corroborative of those obtained in Experiment I. Onset of symptoms of OAP effects such as dyspnea and anorexia were delayed for the hypophysectomized rats. Comparison of mortality and lung pathology gave evidence that hypophysectomized rats enjoy at least a degree of protection from the adverse effects of OAP. The results of this experiment as summarized in Table 2 were equivalent to those of the first experiment and justify a similar conclusion; namely, hypophysectomy lends some protection from the characteristic harmful effects of oxygen at atmospheric pressure.

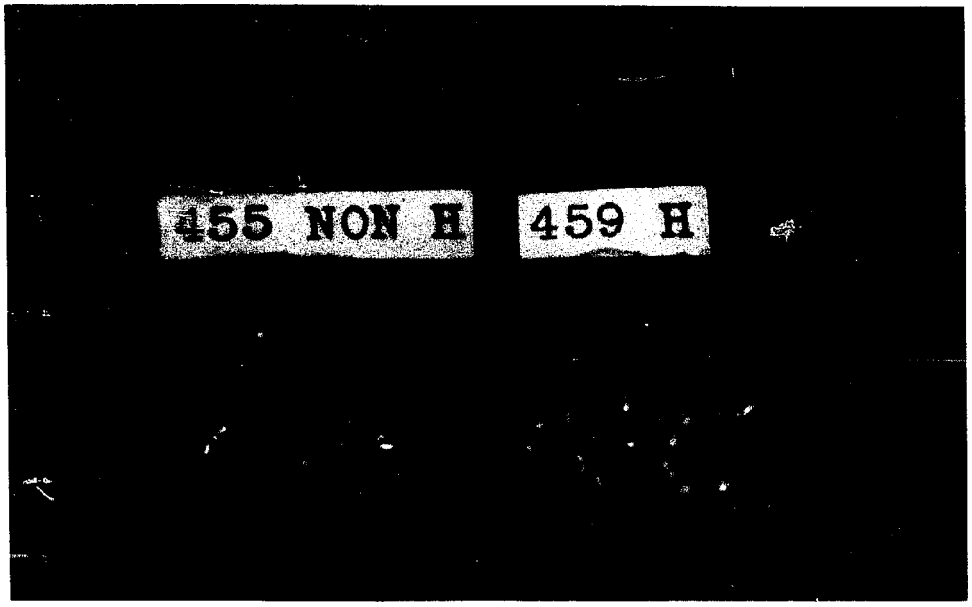


Figure 4.--Effects of OAP on Lungs of Normal and Hypophysectomized Rats. Lungs of normal, non-operated animal on the left show over-all congestion and hyperemia; those on the right--lungs of a sacrificed, hypophysectomized rat--are relatively unaffected.

TABLE 2

MORTALITY, SURVIVAL, LUNG DATA AND HYDROTHORAX FOR HYPOPHYSECTOMIZED AND NORMAL RATS IN OAP

Hypophysectomized					Normals				
Rat #	Mortality	Survival (hrs.)	Lung Damage	Thoracic Fluid	Rat #	Mortality	Survival (hrs.)	Lung Damage	Thoracic Fluid
Experiment I									
456	S.	50	0	Little	451	D.	45	4	Much
457	S.	45	0	Little	452	S.	70	4	Much
458	S.	50	0	Little	453	S.	70	4	Much
459	S.	50	0	Little	454	D.	50	4	Much
460	S.	70	0	Little	455	D.	50	4	Much
461	S.	70	3	Little					
Experiment II									
490	S.	40	0	Little	480	S.	60	3	Much
491	S.	60	2	Some	481	D.	40	4	Much
492	S.	45	0	Little	482	S.	52	4	Much
493	S.	60	3	Some	483	D.	45	4	Much
494	S.	52	2	Little	484	S.	60	3	Much

TABLE 3

NUMBER OF HYPOPHYSECTOMIZED AND NORMAL RATS IN OAP AND IN AIR (EXPERIMENT II)

	Hypophy.	Normals
OAP	5	5
Air	5	5
Total	10	10

Experiment III.--The third experiment was intended to provide further data on survival. In contrast to Experiments I and II none of these rats was sacrificed for observation of pulmonary effects until the end of the experiment. The procedure obviously did not permit so exact a comparison of lung involvement but did allow comparison of survival times in OAP. Division of the rats is summarized in Table 4.

TABLE 4

NUMBER OF HYPOPHYSECTOMIZED AND NORMAL RATS IN OAP AND IN AIR (EXPERIMENT III)

	Hypophy.	Normals
OAP	9	9
Air	5	5
Total	14	14

It was found that hypophysectomy again provided protection to the rats. All of the non-operated animals succumbed before any of the hypophysectomized. In every case their lungs were hemorrhagic, their thoraces were full of free fluid and the other manifestations of oxygen poisoning were present. However, during this experiment two of the hypophysectomized rats died late in the exposure and upon autopsy typical pulmonary pathology and some hydrothorax were found. Table 5 summarizes the data obtained for the two groups in terms of mortality, average survival, degree of lung damage and hydrothorax. Since all of the non-operated rats had succumbed before 64-1/2 hours, the remaining rats were sacrificed at 78 hours. The relatively minor pulmonary pathology as shown by post mortem examination indicated that the hypophysectomized sacrificed rats could have lived much longer; therefore, their average survival is indicated by arrows in Figure 5 as greater than 77 hours. The lungs of all air controls were normal.

Again, it can be concluded from this experiment (III) that hypophysectomy results in a delaying of manifestation of the adverse effects of oxygen at atmospheric pressure. These results thoroughly substantiate the findings of Experiments I and II.

The results of these three experiments clearly demonstrate that oxygen at atmospheric pressure is harmful to young non-hypophysectomized rats as evidenced from the pulmonary pathology, hydrothorax and early death.

TABLE 5

MORTALITY, SURVIVAL, LUNG DATA AND HYDROTHORAX FOR HYPOPHYSECTOMIZED AND NORMAL RATS IN OAP (EXPERIMENT III)

Hypophysectomized					Normals				
Rat #	Mortality	Survival (hrs.)	Lung Damage	Thoracic Fluid	Rat #	Mortality	Survival (hrs.)	Lung Damage	Thoracic Fluid
1475	S.	78	0	Little	1470	D.	$64\frac{1}{2}$	4	Much
1476	D.	67	3	Some	1471	D.	$42\frac{1}{2}$	4	Much
1477	S.	78	0	Some	1472	D.	$61\frac{1}{2}$	4	Much
1478	S.	78	0	Little	1473	D.	$64\frac{1}{2}$	4	Much
1484	S.	78	0	Little	1474	D.	$61\frac{1}{2}$	4	Much
1485	S.	78	0	Some	1480	D.	58	4	Much
1486	S.	78	0	Little	1481	D.	$61\frac{1}{2}$	4	Much
1487	S.	78	0	Little	1482	D.	$54\frac{1}{2}$	4	Much
1488	D.	78	4	Some	1483	D.	$64\frac{1}{2}$	4	Much
Av.	...	77 hrs.	...	...		...	59.2	...	...
S.E.	...	...	...	...		...	2.5 hrs.	...	...

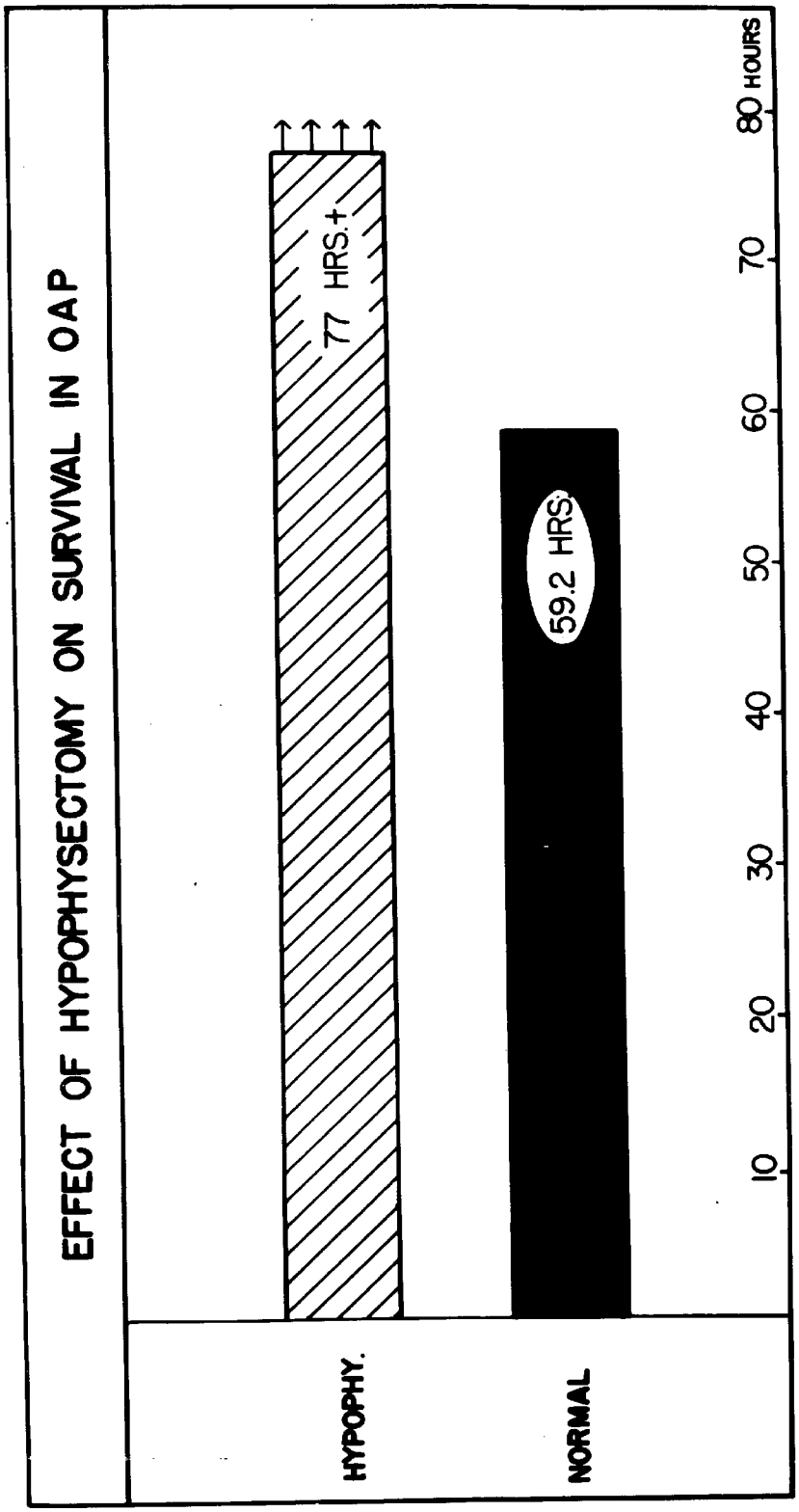

EFFECT OF HYPOPHYSECTOMY ON SURVIVAL IN OAP
77 HRS.+
59.2 HRS.
HYPOPHY.
NORMAL
10
20
30
40
50
60
70
80 HOURS

Figure 5

The results also show that hypophysectomy confers a large measure of protection from the adverse effects of OAP. The basis for this conclusion is (1) the lower mortality, (2) the decreased lung pathology, (3) the greatly increased survival time of hypophysectomized rats in OAP.

Striking as this contrast between hypophysectomized and normal animals is, it is noteworthy that the protection is by no means complete, since two operated animals succumbed in OAP with extensive pulmonary damage. The hypophysectomized rats which were sacrificed also showed varying degrees of lung pathology which may be attributed to oxygen effects.

Demonstration of this protective effect of hypophysectomy raises the question of its mechanism of action. Extirpation of the pituitary removes, among other things, adrenocorticotrophin and thyrotropic hormone. The protection may be the result of lowered adreno-cortical hormone production, of lowered metabolic activity, of a combination of these factors or of some other factors related to hypophyseal function. Conversely it appears that presence of these factors permit or contribute to the occurrence of adverse effects of oxygen, particularly to pulmonary involvement including increased vascular permeability as evidenced by extensive hemorrhage into tissue spaces and the massive effusion of free fluid into pleural areas. The observation of the high protein content of this hydrothorax fluid as reported earlier (Bean and Smith, 1952) has been more recently confirmed by Aschan and Wallenius (1953) who, working with guinea pigs,

reported that the edema fluid which causes hydrothorax during oxygen poisoning has an electrophoretic pattern such as to indicate that the protein composition of the fluid is similar to that of blood serum of the animals studied.

It can be concluded from the experiments of this series that hypophysectomy renders young male rats less susceptible to oxygen at atmospheric pressure as well as to high pressure as discussed in the introduction. It may be inferred that this protection like that in oxygen at high pressure is attributable in part at least to elimination of ACTH and of adreno-cortical factors.

Since, as has already been mentioned, the adrenal cortex becomes hyperactive (Bean, 1951; Bean, Baker, and Johnson, 1953; Bean and Johnson, 1954) under the influence of OHP, studies were undertaken to determine, if possible, the part played by the adrenal glands in the response of rats to OAP. These will constitute the subject of the following section.

B. Adrenal Factors

1. Adrenalectomy

The data of the previous section have shown that considerable protection from oxygen's ill effects results from hypophysectomy and at least part of this protection was ascribed to removal of ACTH and adreno-cortical factors. If this suggestion be correct, removal of the adrenals should grant a comparable protection to animals exposed to oxygen.

The experiments reported in this section of the investigation are concerned, therefore, with effects of adrenalectomy on the toxic action of oxygen at atmospheric pressure (OAP).

Ninety-nine young male albino rats (125-150 grams body weight) were the experimental subjects of three separate experiments in this series. The general treatment of these animals and the manner of their exposure was similar to that described above. The adrenalectomized rats were maintained on one per cent saline drinking water.

Experiment I.--In the first of these experiments, 23 animals were used and were grouped as shown in Table 6. Seven adrenalectomized rats and 7 normals were exposed to oxygen while 5 adrenalectomized and 4 normals were maintained in air as controls.

TABLE 6

NUMBER OF ADRENALECTOMIZED AND NORMAL RATS IN OAP AND IN AIR (EXPERIMENT I)

	Adr'ect.	Normals
OAP	7	7
Air	5	4
Total	12	11

During exposure to oxygen, the subjects were observed carefully for indications of adverse effects of OAP. The criteria for evaluation of the effects were essentially the same as those used in the previous section: (1) survival, (2) comparison of lung damage, (3) degree of hydrothorax.

The findings of Experiment I are summarized in Table 7. The notation used to express lung damage is the same as that outlined in the section on hypophysectomy, i.e., 0 represents no observable abnormality while 4 represents maximal congestion, hepatization and edema; intermediate degrees of involvement have been recorded 1, 2, or 3.

In this first experiment of the adrenalectomy series, volume of free thoracic fluid was expressed by the notation much, some, or little. Much refers to an overflowing (as great as 10 cc.) while little indicates a very small volume of fluid present (perhaps less than 1.0 cc.).

The results of Experiment I indicate that adrenalectomy affords some protection against OAP. This is summarized in Table 7 which shows (1) that the adrenalectomized rats lived longer in OAP, (2) that lung damage was appreciably less for the adrenalectomized rats than for the normals, and (3) that less free fluid was present in the operated rats' thoraces. The criteria upon which most weight should be placed, however, are degree of lung damage and degree of hydrothorax. The effect of adrenalectomy upon survival was not particularly striking because of the limited number of animals considered; however, the adrenalectomized rats did live longer on an average (51-1/2 hours) than the normals (46 hours).

TABLE 7

MORTALITY, SURVIVAL, LUNG DATA AND HYDROTHORAX FOR ADRENALECTOMIZED AND NORMAL RATS IN OAP AND IN AIR (EXPERIMENT I)

OAP

Adrenalectomized					Normals				
Rat #	Mortality	Survival (hrs.)	Lung Damage	Thoracic Fluid	Rat #	Mortality	Survival (hrs.)	Lung Damage	Thoracic Fluid
680	D.	55	1	Little	687	S.	43	1	Some
681	S.	$43\frac{1}{2}$	1	Little	688	S.	58	3	Some
682	S.	$33\frac{1}{2}$	1	Little	689	S.	55	1	Some
683	D.	56	1	Little	690	D.	56	4	Much
684	D.	$43\frac{1}{2}$	3	Little	691	D.	46	4	Much
685	S.	48	1	Little	692	D.	$51\frac{1}{2}$	4	Much
686	S.	58	1	Some	693	D.	$32\frac{1}{2}$	4	Much
Av.	...	51.5	1	...	...	...	46.5	3	...
S.E.	...	4.0 hrs.	...	...	...	...	5.8 hrs.	...	...

TABLE 7-Continued

AIR										
Adrenalectomized					Normals					
Rat #	Mortality	Survival (hrs.)	Lung Damage	Thoracic Fluid	Rat #	Mortality	Survival (hrs.)	Lung Damage	Thoracic Fluid
694	S.	68½	0	None	699	S.	68½	0	None
695	S.	68½	0	None	700	S.	68½	0	None
696	S.	68½	0	None	701	S.	68½	0	None
697	S.	68½	0	None	702	S.	68½	0	None
698	S.	68½	0	None	703	S.	68½	0	None

The effect of adrenalectomy was very strikingly shown, however, from comparison of the lungs. Only one adrenalectomized animal manifested any marked degree of pulmonary injury; this in contrast to five of the unoperated rats which evidenced hemorrhagic edematous, congested lungs. A typical contrast between lungs of an adrenalectomized rat and those of an unoperated animal is represented by Figure 6.

Consistently less pleural effusion was observed in the thoraces of the adrenalectomized animals again indicating protection due to adrenalectomy.

Results of Experiment I indicate that adrenalectomy renders young male rats more resistant to the adverse effects of oxygen at atmospheric pressure.

Experiment II.--The procedure in the second experiment was essentially similar to that described for the first. Grouping of the 36 rats employed is shown in Table 8.

TABLE 8

NUMBER OF ADRENALECTOMIZED AND NORMAL RATS IN OAP AND IN AIR (EXPERIMENT II)

	Adr'ect.	Normals
OAP	12	12
Air	6	6
Total	18	18



Figure 6.--Effect of OAP on Lungs of Normal and Adrenalectomized Rats. The unoperated rat whose lungs are shown on the left succumbed after only 32-1/2 hours in oxygen; the adrenalectomized rat (lungs on the right) was sacrificed shortly after death of its partner. The over-all hemorrhagic pulmonary congestion of the unoperated rat is clearly contrasted to the relatively clear and normal lungs of the adrenalectomized animal.

The results of the second experimental study of this series are in general agreement with those obtained previously. In this experiment also, the criterion of survival cannot be emphasized greatly because of the limited number of rats considered and because of the variance within groups. But it may be pointed out that average survival for the adrenalectomized rats which died was five hours greater than that for the normals.

Comparison of effects on lungs clearly shows, however, that those of the adrenalectomized rats which succumbed were much less severely affected than were the lungs of their controls. Maximum involvement of the lungs was seen in only four adrenalectomized rats whereas lungs of all seven of the normals which died were involved. Without exception the non-operated animals manifested the over-all hepatization, edema and congestion which is typical of OAP action.

Rough comparison of the volume of free fluid which was present in the thoraces of the autopsied rats left little doubt that absence of adrenal factors provides some protection from fluid loss into the thorax which is so characteristic of OAP damage (Summary of results in Table 9).

Experiment III.--Procedure for the third experiment was similar to that used in the first two experiments. Table 10 shows the division of the forty animals into test and control groups. The criteria of OAP effects were, as formerly, survival time, lung damage, and degree of hydrothorax. In

TABLE 9

MORTALITY, SURVIVAL, LUNG DATA AND HYDROTHORAX FOR ADRENALECTOMIZED AND NORMAL RATS IN OAP AND IN AIR (EXPERIMENT II)

OAP

Adrenalectomized					Normals				
Rat #	Mortality	Survival (hrs.)	Lung Damage	Thoracic Fluid	Rat #	Mortality	Survival (hrs.)	Lung Damage	Thoracic Fluid
1400	D.	68½	2	Little	1410	S.	119	3	Some
1401	D.	64½	4	Little	1411	S.	119	3	Some
1402	D.	105	4	Little	1412	D.	68½	4	Much
1403	D.	70½	4	Little	1413	D.	68½	4	Much
1404	D.	92½	2	Little	1414	D.	92½	4	Much
1405	D.	74½	3	Little	1415	D.	80	4	Much
1420	D.	69½	3	Little	1430	S.	119	3	Some
1421	D.	44½	1	Little	1431	S.	45	0	Little
1422	D.	53½	1	Little	1432	D.	60	4	Much
1423	D.	81½	4	Little	1433	D.	58	4	Much
1424	S.	119	1	Little	1434	D.	64½	4	Much
1425	D.	105	3	Little	1435	S.	119	2	Some
Av.	...	75.4	2.7	...		...	70.3	3.3	...
S.E.	...	5.8 hrs.	...	...		...	4.6 hrs.	...	...

TABLE 9-Continued

AIR

Adrenalectomized					Normals				
Rat #	Mortality	Survival (hrs.)	Lung Damage	Thoracic Fluid	Rat #	Mortality	Survival (hrs.)	Lung Damage	Thoracic Fluid
1406	S.	119	0	None	1418	S.	119	0	None
1407	S.	119	0	None	1419	S.	120	0	None
1408	S.	119	0	None	1426	S.	120	0	None
1409	S.	119	0	None	1427	S.	120	0	None
1416	S.	119	0	None	1428	S.	120	0	None
1417	S.	119	0	None	1429	S.	120	0	None

order to obtain more objective information on hydrothorax, the volume of free fluid was recorded for each rat.

TABLE 10

NUMBER OF ADRENALECTOMIZED AND NORMAL RATS IN OAP AND IN AIR (EXPERIMENT III)

	Adrʼect.	Normals
OAP	15	15
Air	5	5
Total	20	20

The results convincingly illustrate that adrenalectomy affords an appreciable degree of protection from the harmful effects of OAP. The adrenalectomized rats lived on an average significantly longer than their unoperated controls (adrenalectomized, 71.6 hours; normals, 57.1 hours). These survival averages take into account only animals which died during exposure; sacrificed animals are not included (Figure 7).

Comparison of pulmonary effects shows that adrenalectomized rats were not nearly so severely affected by OAP as their controls. Pulmonary pathology was evident in every case of the normal animals observed (Table 11); in contrast six of the adrenalectomized rats presented at autopsy lungs which were to all appearances normal. This contrast between lungs is shown in Figure 8.

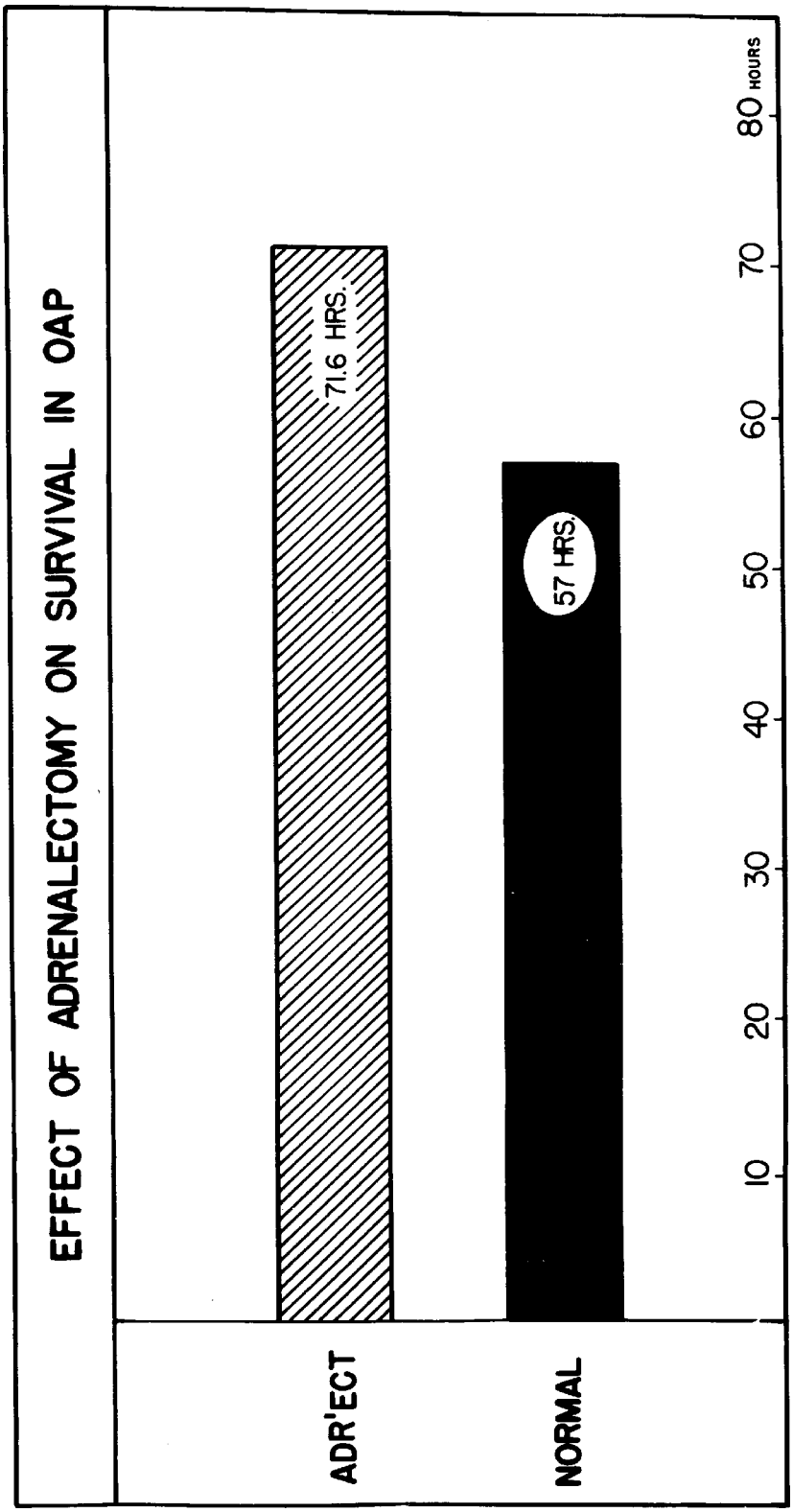

EFFECT OF ADRENALECTOMY ON SURVIVAL IN OAP
ADR'ECT
NORMAL
71.6 HRS.
57 HRS
10
20
30
40
50
60
70
80 HOURS

Figure 7

TABLE 11

MORTALITY, SURVIVAL, LUNG DATA AND HYDROTHORAX FOR ADRENALECTOMIZED AND NORMAL RATS IN OAP AND IN AIR (EXPERIMENT III)

OAP

Adrenalectomized						Normals					
Rat #	Mortality	Survival (hrs.)	Lung Damage	Lung-Heart Wt. (gm.)	Thoracic Fluid (cc.)	Rat #	Mortality	Survival (hrs.)	Lung Damage	Lung-Heart Wt. (gm.)	Thoracic Fluid (cc.)
2750	D.	85	4	3.3	0.0	2755	S.	86½	2	2.8	1.5
2751	S.	86½	0	2.9	1.2	2756	S.	86½	2	2.9	0.0
2752	D.	82½	1	3.0	0.0	2757	D.	57	4	2.8	4.8
2753	S.	86½	0	3.0	0.0	2758	S.	86½	2	3.1	1.0
2754	D.	83	4	3.1	0.0	2759	D.	52½	4	2.3	5.4
2760	D.	58	4	2.8	5.0	2765	D.	60	4	2.8	7.0
2761	D.	65	2	2.8	0.5	2766	S.	85½	3	3.3	0.0
2762	S.	85½	0	2.7	0.0	2767	S.	86	1	2.7	0.0
2763	D.	72½	4	3.1	3.6	2768	D.	59½	4	2.8	6.2
2764	D.	68	4	3.0	3.6	2769	D.	63	4	3.2	5.4
2770	D.	57	2	2.8	1.6	2775	S.	86	2	2.8	0.5
2771	S.	33	0	2.5	0.0	2776	D.	32	4	3.0	4.2
2772	D.	60	4	2.9	2.0	2777	D.	73½	4	3.7	2.0
2773	S.	85½	0	2.8	1.0	2778	D.	57	4	2.9	5.6
2774	D.	85	0	2.9	0.0	2779	D.	59½	4	3.0	6.4
Av.	...	71.6	2	2.9	1.6		...	57.1	3	2.9	5.2
S.E.	...	1.2 hrs.	...	0.1 gm.	0.6 cc.		...	3.6 hrs.	...	0.1 gm.	0.5 cc.

TABLE 11-Continued

AIR

Adrenalectomized					Normals				
Rat #	Mortality	Survival (hrs.)	Lung Damage	Lung-Heart Wt. (gm.)	Thoracic Fluid (cc.)	Rat #	Mortality	Survival (hrs.)	Lung Damage	Lung-Heart Wt. (gm.)	Thoracic Fluid (cc.)
2780	S.	51	0	2.1	...	2800	S.	52	0	2.4	...
2781	S.	86	0	2.5	...	2801	S.	60	0	2.5	...
2782	S.	53	0	2.7	...	2802	S.	86½	0	2.4	...
2783	S.	87	0	2.3	...	2803	S.	88	0	2.5	...
2784	S.	60	0	2.0	...	2804	S.	55	0	2.0	...
Av.	...	...	...	2.3	...		...	...	...	2.3	...
S.E.	...	...	...	0.1gm.	...		...	...	...	0.2gm.	...

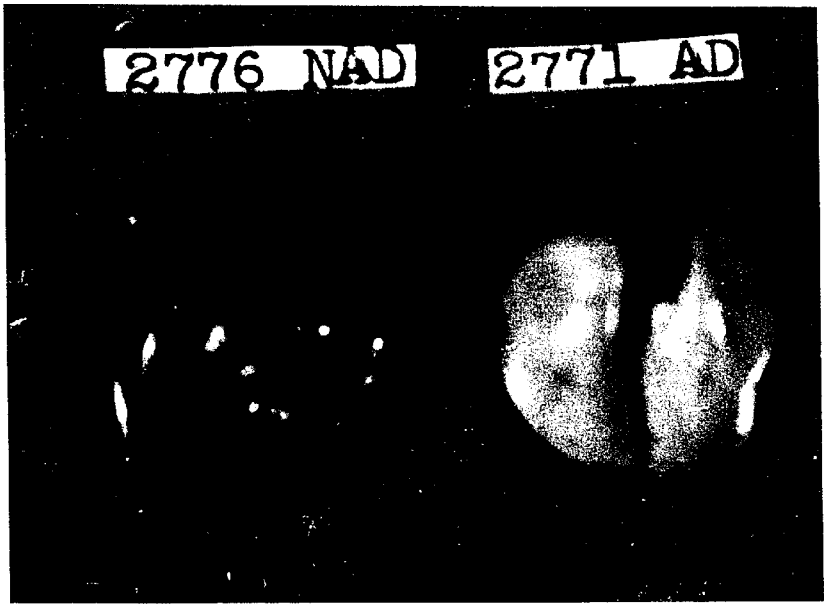


Figure 8.--Effect of OAP on Lungs of Normal and Adrenalectomized Rats. The unoperated rat whose lungs are shown on the left succumbed after 32 hours in oxygen; the adrenalectomized rat (lungs on the right) was sacrificed shortly after. Lungs of the adrenalectomized rat are completely clear and normal as contrasted to the very dark, hyperemic aspect of the lungs of the non-operated rat.

Degree of hydrothorax for the adrenalectomized rats (1.6 cc.) was significantly less than that of the normals (5.2 cc.). See Figure 9.

The results of these three experiments on adrenalectomy justify the conclusion, therefore, that removal of the adrenals confers upon young male rats an increased resistance to the adverse effects of oxygen at atmospheric pressure. This conclusion is evident from the fact that adrenalectomized rats live longer in oxygen, develop less hydrothorax, and manifest strikingly less lung pathology than do their controls in oxygen at atmospheric pressure. The data of this experimental series also support the conclusion that the resistance which hypophysectomy provides against OAP effects is due, at least partly, to removal of adrenocorticotrophic principles. Furthermore the conclusion is warranted that some of the endocrine factors which are active in oxygen at high pressure are also operative in mediating the adverse response of rats to oxygen in high concentrations at atmospheric pressure.

2. Cortical Principles--Cortisone

In the last section it was demonstrated that adrenalectomy provides a measure of protection from adverse effects of OAP and it was pointed out that this protection like that seen in OHP might be due to removal of ACTH with consequent decrease of cortical steroid production.

The present experimental study on cortisone was undertaken to determine the effect of adreno-cortical factors--

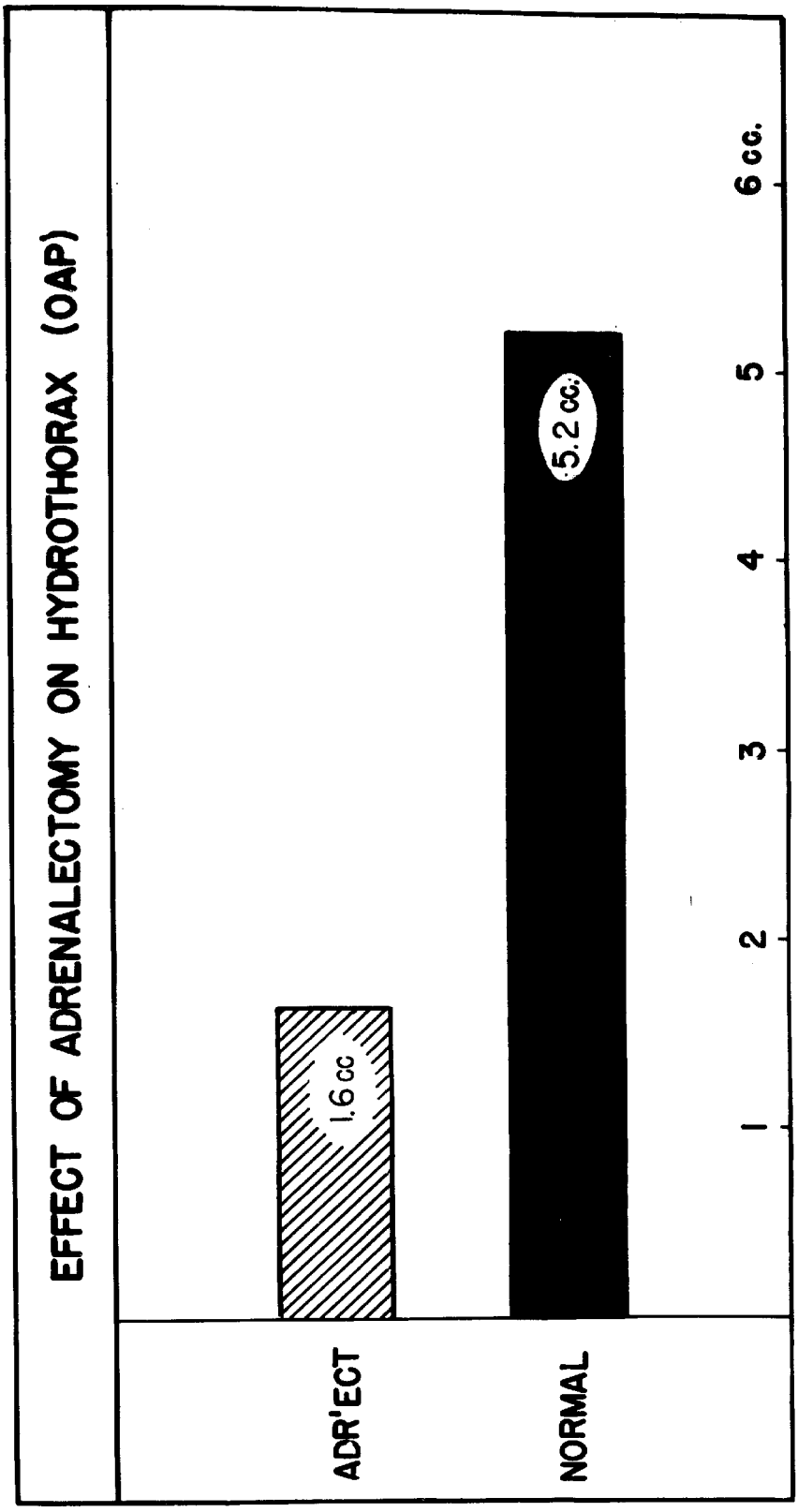

EFFECT OF ADRENALECTOMY ON HYDROTHORAX (OAP)
1.6 cc
5.2 cc.
ADR'ECT
NORMAL
1
2
3
4
5
6 cc.

Figure 9

cortisone in particular--upon the adverse reaction of rats to oxygen in high concentrations at atmospheric pressure.

Twenty-three young male albino rats of approximately 200 grams body weight were used as experimental subjects for this series. They were divided into test and control groups as shown in Table 12.

TABLE 12

NUMBER OF CORTISONE TREATED AND NORMAL RATS IN OAP AND IN AIR

	Cortisone	Normals
OAP	8	8
Air	4	3
Total	12	11

A dose of two milligrams of cortisone acetate (Merck, 25mg/cc) was administered subcutaneously daily for three days prior to exposure and two such doses were given daily during exposure which was carried out in the chambers described. Temperature and gas concentrations were maintained as previously outlined. Controls were injected with saline.

This series was undertaken primarily for comparison of survival times; but in addition to this, time of onset of symptoms of pulmonary damage and post-mortem macroscopic pathological changes constituted other criteria of OAP adverse effects. The animals which succumbed or were sacrificed for comparison of lung pathology were immediately autopsied; the

time of death, the volume of free thoracic fluid, the condition of the lungs and the heart-lung weights were recorded. The results of these observations are shown in Table 13.

The results convincingly illustrate that cortisone acts to enhance the deleterious effects of OAP. The cortisone treated rats manifested symptoms of oxygen effects earlier than their non-treated controls in OAP; that is, they became anorexic and hyperpneic at approximately 30 hours of exposure while these same manifestations did not appear in the non-cortisone treated controls until approximately 40 hours. These data are confirmatory of short preliminary experiments in this laboratory which indicated that cortisone acted to enhance the adverse effect of OAP on the lungs.

The cortisone rats succumbed after an average of 61 hours in OAP as contrasted to 71 hours for their saline injected controls. This contrast is illustrated graphically in Figure 10. Statistical treatment of the data shows that the survival difference between these groups is highly significant.

Examination of the lungs revealed that cortisone had accelerated the pulmonary pathology typical of oxygen poisoning; i.e., edema, congestion, hemorrhage, and consolidation. A striking contrast between lungs of cortisone rats and those of a non-treated rat in OAP is shown in Figure 11. While this contrast is remarkable it should be pointed out that the pulmonary changes which are seen in the cortisone rats are essentially the same as would appear in the controls after longer exposure.

TABLE 13

MORTALITY, SURVIVAL, LUNG DATA AND HYDROTHORAX FOR CORTISONE INJECTED RATS AND FOR CONTROLS IN OAP AND IN AIR

OAP

Cortisone						Controls					
Rat #	Mortality	Survival (hrs.)	Lung Damage	Lung-Heart Wt. (gm.)	Thoracic Fluid (cc.)	Rat #	Mortality	Survival (hrs.)	Lung Damage	Lung-Heart Wt. (gm.)	Thoracic Fluid (cc.)
2850	D.	58	4	4.2	6.6	2862	D.	79	4	3.3	5.4
2851	D.	63	4	4.0	7.0	2863	D.	79	4	3.3	9.0
2852	D.	65	4	3.3	7.0	2864	S.	59	4	4.2	3.8
2853	D.	68	4	4.0	6.6	2865	D.	79	4	3.5	7.8
2854	D.	58½	4	3.4	8.2	2866	D.	62	4	4.3	10.6
2855	D.	59½	4	3.1	8.8	2867	D.	63½	4	3.5	6.8
2856	D.	55½	4	4.3	7.2	2868	D.	69	4	3.9	9.8
2857	D.	61	4	3.4	7.0	2869	D.	67½	4	3.4	8.8
Av.	...	61.0	...	3.7	7.3		...	71.0	...	3.6	8.3
S.E.	...	1.5hrs.	...	0.2gm.	0.3cc.		...	2.9hrs.	...	0.1 gm.	0.7 cc.

TABLE 13-Continued

AIR

Cortisone						Controls					
Rat #	Mortality	Survival (hrs.)	Lung Damage	Lung-Heart Wt. (gm.)	Thoracic Fluid (cc.)	Rat #	Mortality	Survival (hrs.)	Lung Damage	Lung-Heart Wt. (gm.)	Thoracic Fluid (cc.)
2858	S.	59	0	2.3	...	2870	S.	59	0	2.6	...
2859	S.	60½	1	2.5	...	2871	S.	60½	1	2.7	...
2860	S.	97½	0	2.2	...	2872	S.	60½	0	2.7	...
2861	S.	97½	0	3.0	...						
Av.	...	...	...	2.5	...		...	...	...	2.7	...
S.E.	...	...	...	0.2gm.	...		...	...	...	0.1 gm.	...

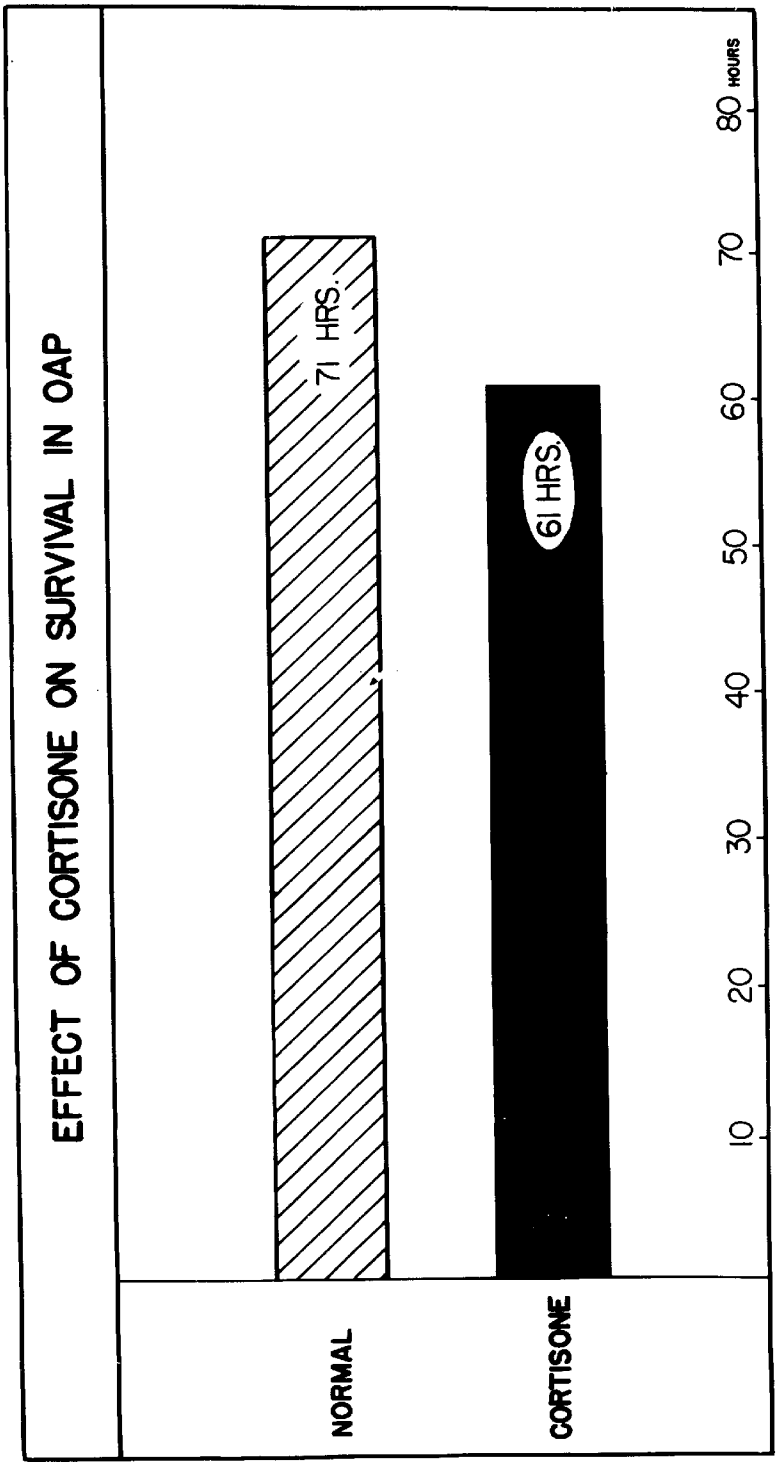

EFFECT OF CORTISONE ON SURVIVAL IN OAP
71 HRS.
61 HRS.
NORMAL
CORTISONE
10
20
30
40
50
60
70
80 HOURS

Figure 10

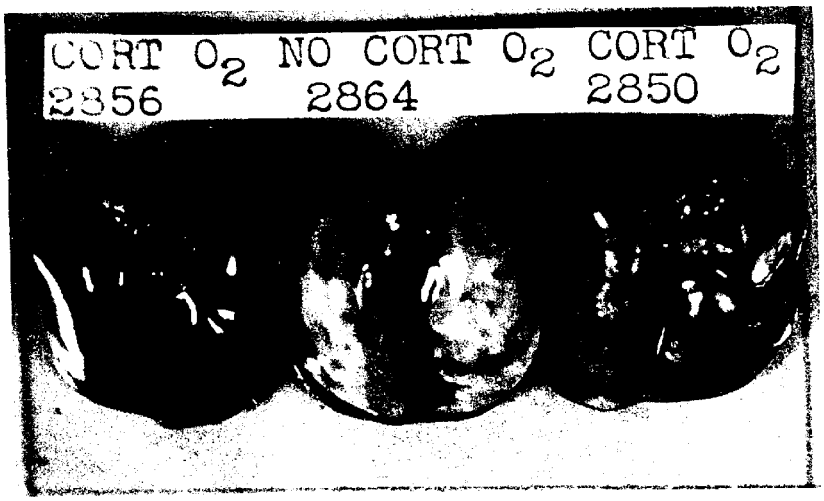


Figure 11.--Effect of OAP on Lungs of Rats Injected with Cortisone and on Lungs of Control. Lungs which are shown on the extreme right and extreme left manifest congested, hemorrhagic, liver-like appearance which is characteristic of OAP effect. Control animal whose lungs are shown in the center was relatively unaffected at the time of sacrifice.

Cortisone in the dosage administered in this experiment was without effect in the air controls; this absence of effect was manifest by appearance (Figure 12) and by lung weight of air controls (Table 13).

Examination of the thoraces showed that cortisone had no appreciable augmentative effect on hydrothorax induced by oxygen. The volumes of free thoracic fluid removed from both cortisone treated and normal rats were large. Table 13 shows the magnitude of these volumes. The degree of hydrothorax was not greater for the cortisone animals but, indeed, was slightly less. The protein content of this fluid as already pointed out in the preceding section was again demonstrated by its formation of a clot upon standing.

There can be little doubt that cortisone augments the adverse action of oxygen at atmospheric pressure as indicated by the earlier onset of symptoms of oxygen's effects, the decreased survival time, and the augmented pulmonary damage of cortisone injected rats. This confirms the report of Warshaw and co-workers (1952) that cortisone augmented pulmonary involvement of mice exposed to oxygen at atmospheric pressure.

The occurrence in OAP of engorged and hemorrhagic lungs and of hydrothorax, the protein composition of which is probably similar to that of blood serum, indicates severely increased capillary permeability in the case of both cortisone treated rats and normals.

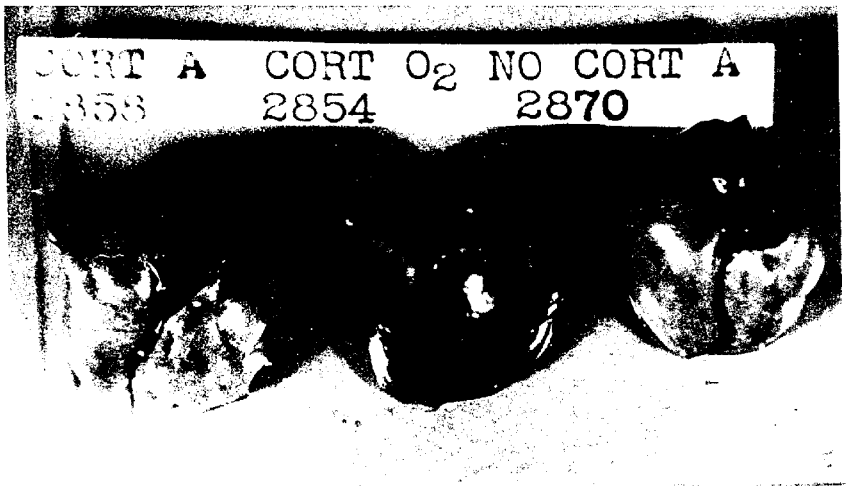


Figure 12.--Effect of Cortisone on Lungs of Rats in OAP and in Air. On the left are lungs of cortisone injected control maintained in air; in the center, lungs of cortisone injected rat in OAP; on the right lungs of non-cortisone treated air control. This serves to illustrate that lungs of cortisone treated rats in air were essentially normal in appearance.

Since cortisone is generally conceded to decrease capillary permeability one might expect that in this experiment the hydrothorax, extravasation and lung damage of the cortisone treated animals would have been less than that of the normals; however, this was not the case, in fact only a slight difference in volume of fluid was noted for cortisone treated animals as compared to normals. This absence, in OAP, of the capillary permeability decreasing characteristics of cortisone is exceedingly interesting.

These data have demonstrated that cortisone, in doses noninjurious in air, strikingly enhances the toxic action of oxygen at atmospheric pressure. This enhancement is manifest (1) by hastened onset of symptoms of oxygen's toxicity, (2) by a shortened average survival, and (3) by augmentation of pulmonary pathology.

These data add further weight to the conclusion that the beneficial effects of hypophysectomy in OAP as represented, in the last section, are due at least in part to removal of adrenocorticotrophic principle and that adreno-cortical factors permit acceleration and accentuation of pulmonary damage resultant from exposure to oxygen at atmospheric pressure.

3. Epinephrine

Results of the preceding experimental studies have shown that adrenalectomy brings about a diminished reaction to oxygen at atmospheric pressure and that a cortical factor--cortisone--enhances the pulmonary pathology induced by oxygen

at atmospheric pressure (OAP). However, the fact that adrenalectomy removes not only adreno-cortical elements but also medullary tissue suggests the possibility that the protection which adrenalectomy affords may result from removal of epinephrine also.

If this be so, administration of the medullary factor--epinephrine--should enhance the lung damage characteristic of OAP. The experiments described in this section were, therefore, carried out to investigate the effects of epinephrine administration upon the response of rats to OAP.

This series consisted of two separate experiments involving a total of 66 young albino rats of approximately 200 - 235 grams body weight.

Experiment I.--For the first experiment 27 female rats were used, 14 epinephrine injected and 13 maintained as controls; they were divided as outlined in Table 14. Exposure methods were similar to those for the preceding series; concentration of oxygen was maintained above 95 per cent, carbon dioxide below 0.20 percent and temperature at about 28°C.

TABLE 14

NUMBER OF EPINEPHRINE TREATED AND NORMAL RATS IN OAP AND IN AIR (EXPERIMENT I)

	Epinephrine	Normals
OAP	10	10
Air	4	3
Total	14	13

Epinephrine in peanut oil (for slow absorption) was injected subcutaneously in three equally spaced doses over a period of thirty-six hours. An equal volume of peanut oil was administered to the controls.

The rats were observed carefully during the experiment; as soon as an animal succumbed, it was autopsied and the following data were recorded: (1) length of survival in OAP, (2) an estimate of lung damage, (3) heart-lung weight, (4) volume of free fluid in the thorax. These data are presented in Table 15.

In order to obtain information concerning survival time, the majority of the rats were allowed to remain in OAP until death but, for comparison of lung effects, a few were sacrificed during the course of exposure. The data from these sacrificed animals have, therefore, not been included in the computed averages of survival, lung weight or hydrothorax volume (Table 15).

The results of the first experiment definitely indicate that epinephrine accelerated and accentuated OAP effects. The basic criterion for this augmentative action was the greatly reduced survival time of the tests in oxygen. The first animal (epinephrine injected) succumbed at ten hours, the next one at 39-1/2 hours and all epinephrinized animals were dead after an average of 47.3 hours. All the control rats in OAP survived until 54-1/2 hours when the first one succumbed; the average for this group was nearly 62 hours (Table 15). Statistically this difference is shown to be highly significant.

TABLE 15

MORTALITY, SURVIVAL, LUNG DATA AND HYDROTHORAX FOR EPINEPHRINE INJECTED RATS AND FOR CONTROLS IN OAP AND IN AIR (EXPERIMENT I)

OAP

Epinephrine						Controls					
Rat #	Mortality	Survival (hrs.)	Lung Damage	Lung-Heart Wt. (gm.)	Thoracic Fluid (cc.)	Rat #	Mortality	Survival (hrs.)	Lung Damage	Lung-Heart Wt. (gm.)	Thoracic Fluid (cc.)
2679	D.	43½	4	3.5	1.5	2684	D.	54½	4	4.1	7.2
2680	D.	52	4	5.1	2.0	2685	D.	62	4	3.4	6.0
2681	D.	45	3	5.8	2.0	2686	D.	59	4	4.7	7.2
2682	D.	58	4	5.1	5.8	2687	D.	62	4	3.4	8.2
2683	D.	51½	4	4.4	0.5	2688	D.	62	4	3.5	7.0
2691	D.	67	4	4.0	4.0	2696	S.	54½	3	3.5	2.5
2692	D.	52½	4	5.1	1.5	2697	D.	67	4	4.0	6.0
2693	D.	12	2	3.7	0.0	2698	D.	62	4	4.0	8.4
2694	D.	52½	4	5.1	2.0	2699	D.	65	4	4.5	6.8
2695	D.	39	3	2.7	0.0	2700	S.	44	2	3.9	0.5
Av.	...	47.3	...	4.5	1.9		...	61.8	...	3.9	7.1
S.E.	...	4.6 hrs.	...	0.3 gm.	0.6 cc.		...	1.3	...	0.2 gm.	0.3 cc.

TABLE 15-Continued

AIR

Epinephrine							Controls					
Rat #	Mortality	Survival (hrs.)	Lung Damage	Lung-Heart Wt. (gm.)	Thoracic Fluid (cc.)		Rat #	Mortality	Survival (hrs.)	Lung Damage	Lung-Heart Wt. (gm.)	Thoracic Fluid (cc.)
2689	S.	55	0	2.2	...		2708	S.	107½	0	2.7	...
2690	S.	108	0	2.3	...		2709	S.	107½	0	2.7	...
2701	S.	108	0	3.1	...		2710	S.	107½	0	2.8	...
2702	S.	108	0	3.1	...							
Av.	...	...	...	2.7	...			...	...	...	2.7	...
S.E.	...	...	...	0.3 gm.	...			...	...	...	0.1 gm.	...

The augmentative action of epinephrine on OAP effect was also shown from comparison of lungs of the test and control animals. Lungs of the epinephrine treated animals were much larger and heavier than those of their non-treated counterparts in oxygen (Figure 13; Table 15). This difference between lungs of the two groups was evidently due to large quantities of blood and edema fluid within pulmonary tissue. Macroscopic comparison of the lungs of treated rats with those of sacrificed controls showed a striking contrast. Lungs of the epinephrine rats were hemorrhagic, edematous, congested and sank in saline; while lungs of the non-epinephrine treated rats were much less severely affected (Figure 13). Lungs of air controls remained unaffected (Figure 14).

In view of this contrast, one might expect that epinephrine would also increase the hydrothorax commonly induced by OAP; but contrary to this expectation, the free thoracic fluid of the epinephrine rats was less than that of the controls (Table 15). The average volume of free fluid for the controls (7.1 cc.) was about four times that for the epinephrine treated rats (1.9 cc.).

From the results of this experiment, it may be concluded that epinephrine enhances adverse effects of oxygen at atmospheric pressure.

Experiment II.--The procedure for this experiment was essentially the same as that for Experiment I. Thirty-nine male rats, 20 injected with epinephrine and 19 maintained



Figure 13.--Effect of OAP on Lungs of Epinephrine Injected Rat and on Lungs of Control. Lungs of an epinephrine injected OAP exposed rat (on the left) present a striking contrast to those of the non-injected control which was exposed a similar time to oxygen. The difference in size of lungs is well illustrated in this figure.

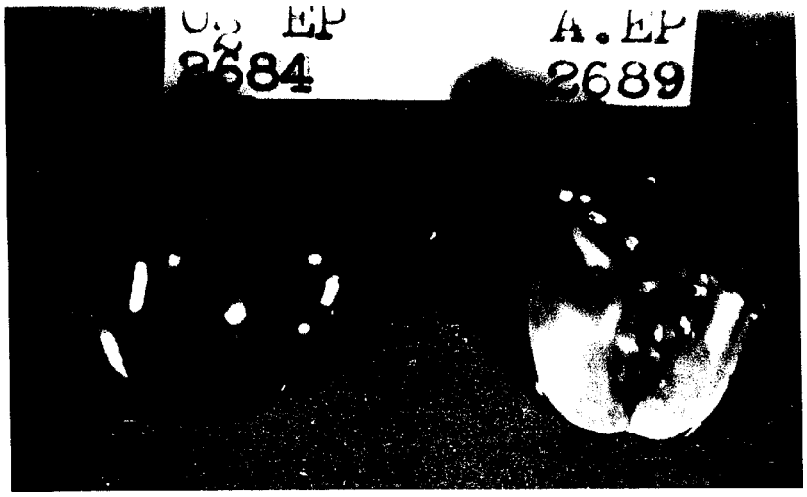


Figure 14.--Effect of Epinephrine on Lungs of Rats in OAP and in Air. Lungs on the left (those of rat in OAP) show typical engorgement and hemorrhage. Lungs of epinephrine injected air control are quite normal in appearance.

as controls, were divided for exposure as outlined in Table 16.

TABLE 16

NUMBER OF EPINEPHRINE TREATED AND NORMAL RATS IN OAP AND IN AIR (EXPERIMENT II)

	Epinephrine	Normals
OAP	15	15
Air	5	4
Total	20	19

The results were similar to those of the first experiment; they showed that the epinephrine injected rats succumbed at an average of 39.6 hours as contrasted to 53.8 hours for the controls (Table 17). The first test rat died after 10 hours of exposure, the next after 16 hours, another after 19 hours; while the first oxygen control succumbed in the 47th hour of exposure.

Examination of the lungs yielded an equally striking contrast. Lung-heart average weight for the epinephrine treated rats was greater (5.6 gm.) than for the controls (4.1 gm.). Comparative macroscopic observation of lungs gave reason to believe that survival was decreased due to augmentation of pulmonary effects. Lungs of the test rats which were large, edematous, hemorrhagic and congested, sank in saline. Those

TABLE 17

MORTALITY, SURVIVAL, LUNG DATA AND HYDROTHORAX FOR EPINEPHRINE INJECTED RATS AND FOR CONTROLS IN OAP AND IN AIR (EXPERIMENT II)

OAP

Epinephrine						Controls					
Rat #	Mortality	Survival (hrs.)	Lung Damage	Lung-Heart Wt. (gm.)	Thoracic Fluid (cc.)	Rat #	Mortality	Survival (hrs.)	Lung Damage	Lung-Heart Wt. (gm.)	Thoracic Fluid (cc.)
2711	D.	24½	4	6.1	1.0	2731	D.	53½	4	3.6	5.0
2712	D.	49½-50½	4	5.7	2.0	2732	D.	56	4	4.0	8.2
2713	S.	59½-60½	4	5.1	1.0	2733	D.	49½-50½	4	4.5	5.0
2714	D.	49	4	4.5	3.6	2734	D.	53½-54½	4	4.3	8.0
2715	D.	42	4	7.7	0.5	2735	D.	50	4	4.3	6.8
2716	D.	19	4	6.2	1.0	2736	S.	21	0	2.4	0.0
2717	D.	16	4	6.5	1.0	2737	D.	52	4	4.0	7.0
2718	D.	52	4	4.9	2.8	2738	D.	59½	4	3.6	5.0
2719	D.	50	4	4.5	3.8	2739	D.	47	4	3.9	6.2
2720	D.	52	4	6.2	4.0	2740	D.	59½	4	4.9	6.0
2721	D.	46½	4	5.1	3.0	2741	D.	52	4	4.2	5.8
2722	D.	42	4	6.7	0.5	2742	D.	51	4	4.0	5.6
2723	D.	10	3	3.0	0.0	2743	S.	11	1	2.4	0.0
2724	D.	51	4	5.3	3.0	2744	D.	59	4	4.4	5.4
2725	D.	52	4	5.5	3.2	2745	D.	56½	4	3.6	6.2
Av.	...	39.6	...	5.6	2.1		...	53.8	...	4.1	6.2
S.E.	...	4.1 hrs.	...	0.3 gm.	0.4 cc.		...	1.1 hrs.	...	0.1 gm.	0.3 cc.

TABLE 17-Continued

AIR

Epinephrine						Controls					
Rat #	Mortality	Survival (hrs.)	Lung Damage	Lung-Heart Wt. (gm.)	Thoracic Fluid (cc.)	Rat #	Mortality	Survival (hrs.)	Lung Damage	Lung-Heart Wt. (gm.)	Thoracic Fluid (cc.)
2726	S.	73½	0	2.6	...	2750	S.	120	0	2.9	...
2727	S.	121½	0	2.8	...	2751	S.	121	0	2.7	...
2728	S.	121½	0	3.2	...	2752	S.	75	0	3.0	...
2729	S.	121½	0	3.2	...	2753	S.	119	0	3.1	...
2730	S.	121½	0	2.7	...						
Av.	...	...	...	2.9	...		...	...	...	2.9	...
S.E.	...	...	...	0.1 gm.	...		...	...	...	0.1 gm.	...

of the non-epinephrine oxygen controls were pink and by contrast reasonably healthy in appearance. This is illustrated in Figure 15. Lungs of the epinephrine injected air controls were also unaffected.

Values for amount of free thoracic fluid were consistent in this experiment with the findings of Experiment I. An average 6.2 cc. of fluid was collected from the thoraces of the non-epinephrine treated rats as compared to 2.1 cc. average volume collected from the epinephrine tests.

The results of these two experiments, therefore, conclusively show that epinephrine accelerates and accentuates adverse effects which are typical of OAP. This is attested to primarily by shortened survival time (Figure 16 which represents a combination of results from Experiments I and II) and secondarily by increased lung pathology.

The unexpected finding mentioned above--that the hydrothorax of epinephrine treated rats was less than that of their controls--deserves special consideration (Figure 17 illustrates average hydrothorax for the two experiments). In view of the marked adverse effect of epinephrine upon the lung as shown by hemorrhage, edema and congestion, one might expect that the volume of free thoracic fluid of epinephrine rats would be greater than that of normal untreated controls in OAP. It is conceivable that this unexpected result might be due to a rapid development of pulmonary congestion causing death before sufficient time could elapse for massive effusion into pleural spaces. It is perhaps noteworthy that a similar



Figure 15.--Effect of OAP on Lungs of Epinephrine Injected Rats and on Lungs of Control. Lungs on the right and on the left are from epinephrine injected rats; the contrast between these and the lungs in the center (non-epinephrine, OAP exposed) is evident. Note again size difference.

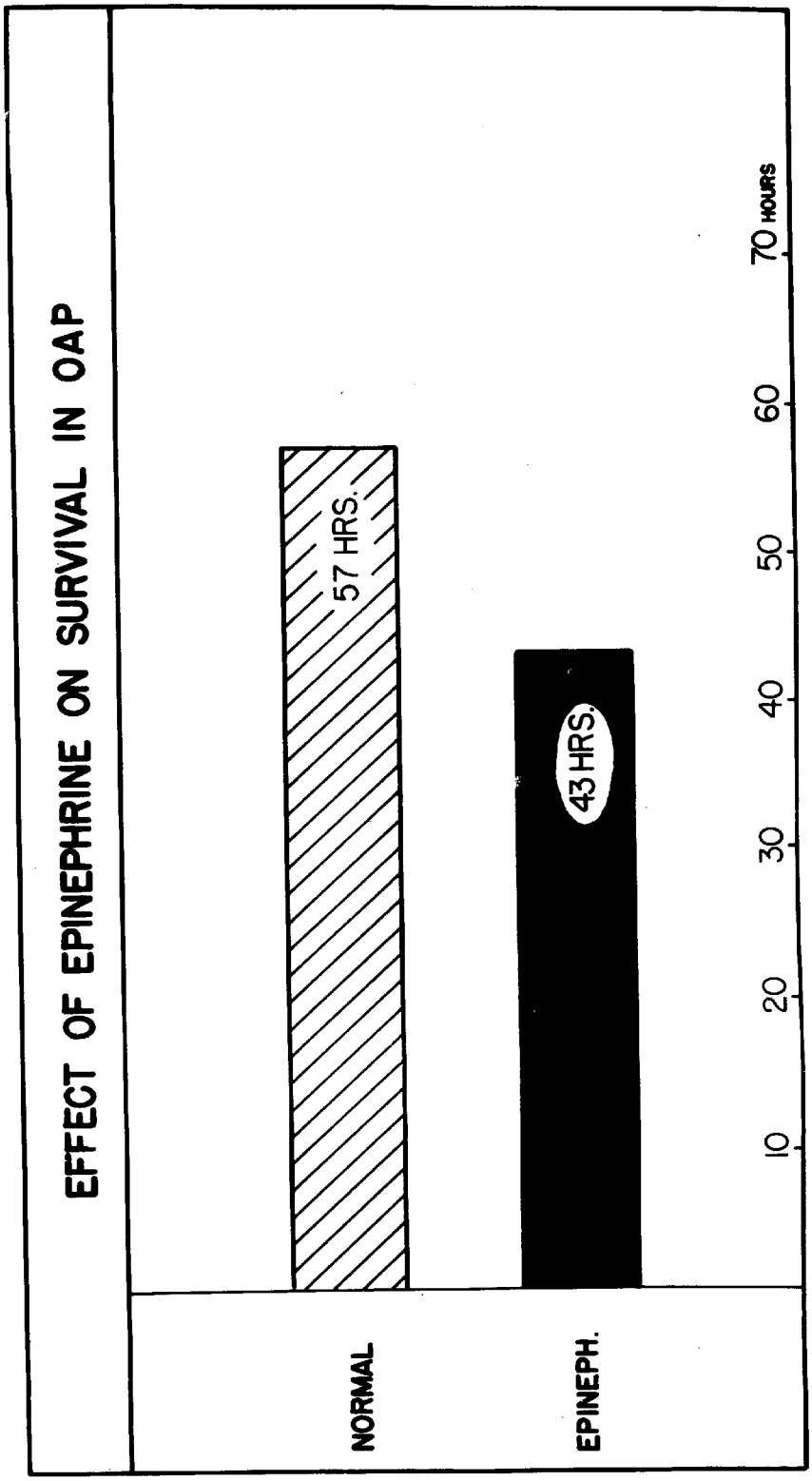

EFFECT OF EPINEPHRINE ON SURVIVAL IN OAP
57 HRS.
43 HRS.
NORMAL
EPINEPH.
10
20
30
40
50
60
70 HOURS

Figure 16

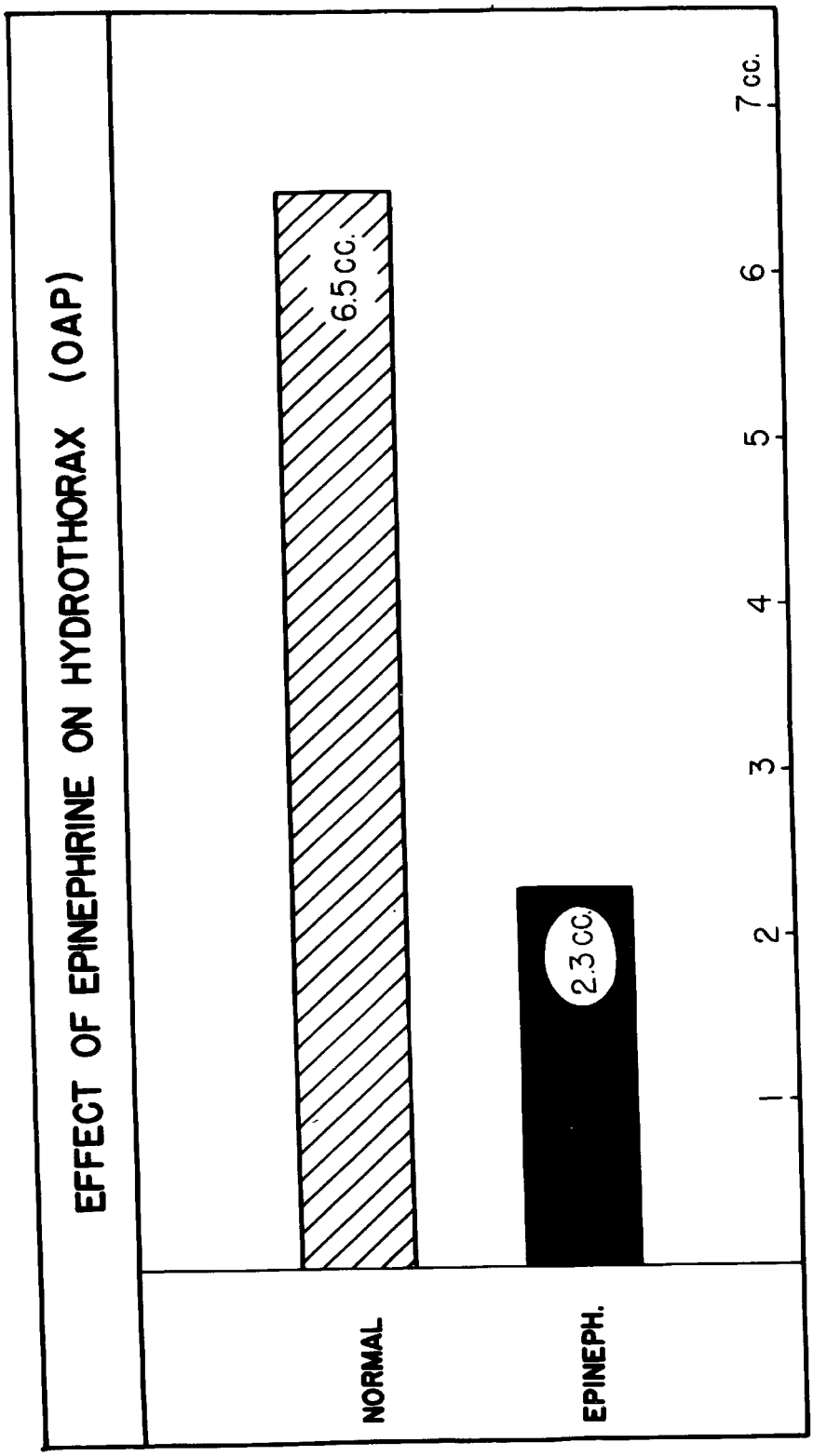

EFFECT OF EPINEPHRINE ON HYDROTHORAX (OAP)
6.5 cc.
2.3 cc.
NORMAL
EPINEPH.
1
2
3
4
5
6
7 cc.

Figure 17

though not so highly significant difference existed for volume of pleural effusion of cortisone and non-cortisone treated rats; cortisone treated animals evidenced less fluid than their controls.

Since epinephrine itself, when given in large amount, results in pulmonary edema (Meltzer, 1904), and since pulmonary edema can also be induced by OAP alone, it cannot be categorically stated that epinephrine augments oxygen effects. It may be that these extreme pulmonary manifestations are in reality an augmentation, by oxygen, of epinephrine effects. It would appear that the effect of epinephrine is the result of a synergistic action.

The exact mechanisms by which epinephrine induces edema are not known although a number of suggestions have been offered; for example, (1) a circulatory effect involving high systemic blood pressure and "backward failure" of the left ventricle, (2) an effect upon capillary permeability within the lungs, and (3) a combination of these two actions.

Similarly there is a lack of definite information concerning the means by which oxygen incites pulmonary damage. Here also a number of suggestions have been offered: (1) direct local action by oxygen upon capillaries of the lungs, (2) action of high carbon dioxide levels within the blood, (3) irritative action upon the alveolar wall, and (4) some general systemic effect, such as excitation of the central nervous system and possibly epinephrine secretion.

The results of these experiments indicate that knowledge of the exact mechanisms by which epinephrine exerts ill effects upon pulmonary tissue would facilitate our understanding of the edema induced by OAP.

From these experiments it may be concluded that epinephrine when administered to rats exposed to oxygen at atmospheric pressure results in an enhancement of the lung damage ordinarily associated with OAP toxicity. This is borne out (1) by a decrease in average survival time of the epinephrine rats, (2) by an acceleration of typical pulmonary pathology.

In summarizing the effects of adrenal factors which have been discussed in this section, it may be concluded that adrenalectomy renders rats much more resistant to oxygen toxicity and that cortisone administration enhances the deleterious effects of oxygen at atmospheric pressure. These results confirm and extend the suggestion as given above in the section on hypophysectomy (page 23).

Experiments on epinephrine have shown that medullary factors may also enhance pulmonary pathology typical of oxygen poisoning. This finding does not positively define the adrenal medulla as a factor in provoking manifestation of lung effects; since no increased epinephrine secretion has been demonstrated for animals in oxygen at atmospheric pressure. However, since OAP constitutes a stressor condition it may be safely assumed that there is an outpouring of epinephrine during exposure to oxygen at atmospheric pressure. These findings would justify the inference that medullary factors act in OAP even as they

have been shown to be operative in OHP (Bean, Johnson and Smith, 1954).

C. Thyroid Factors

In the experiment described above it was shown that hypophysectomy confers a measure of protection from the deleterious effects of exposure to oxygen at atmospheric pressure. This protection was ascribed to the elimination of adrenocorticotrophic principle; subsequently it was demonstrated that adreno-cortical factors are indeed active in mediating an adverse response to OAP.

However, hypophysectomy also removes thyrotropic hormone and brings about an appreciable decrease of thyroid gland secretion. It is reasonable, therefore, to assume that a part of the protection which hypophysectomy affords might be ascribed to diminished thyroid hormone production. That this may be so is suggested by the findings of several investigators (Campbell, 1937; Grossman and Penrod, 1949; Bean et al., 1953) who have reported that thyroid factors are instrumental in enhancing the toxicity of oxygen at pressures of several atmospheres.

Preliminary experiments in this laboratory (Bean and Bauer, 1952) indicated that administration of thyroid hormone would result in an augmentation of the pathological changes induced by oxygen at atmospheric pressure. The experiments to be described in this section were performed to provide further data concerning the possible influence of thyroid factors on oxygen toxicity. These studies were carried out

in three separate experiments using dessicated thyroid and thyroxine. A total of 199 young male albino rats were employed as experimental subjects.

1. Dessicated Thyroid

Experiment I.--In this first experiment, the effect of thyroid plus oxygen at atmospheric pressure was investigated using 75 hypophysectomized and normal rats; 37 of these were fed dessicated thyroid (20 mg./rat/day) while 38 were maintained as controls. Table 18 outlines the division of these animals into test and control groups.

TABLE 18

NUMBER OF HYPOPHYSECTOMIZED AND NORMAL RATS IN TEST AND CONTROL GROUPS IN OAP AND IN AIR (EXPERIMENT I)

	Thyroid		Controls	
	Hypophy.	Normals	Hypophy.	Normals
OAP	7	13	8	13
Air	6	11	6	11
Total	13	24	14	24

The procedure of exposure was essentially similar to that described in previous sections; temperature was maintained about 28°C., oxygen above 95 per cent, carbon dioxide below 0.20 per cent and the diet was the same as that described earlier.

The criterion for evaluation of oxygen effect in this experiment was that of lung pathology; since this necessitated sacrifice of many animals the criterion of survival was less significant.

The results of this experiment show definitely that administration of thyroid increases the severity of OAP lung effects in normal, non-operated rats, and reverses that partial protection provided by hypophysectomy. Lungs of the thyroid fed rats, both hypophysectomized and normal, were typically engorged, edematous, and consolidated; whereas lungs of the controls were essentially normal. This striking contrast is illustrated in Figures 18 and 19.

Survival data, although limited due to the large number of rats sacrificed, support these findings. The thyroid fed rats succumbed earlier than the non-thyroid fed controls (Tables 19 and 20).

From the data of this experiment, it may be concluded: (1) that hypophysectomy affords protection against adverse effects of OAP and (2) that thyroid enhances the deleterious effects of OAP in normal and hypophysectomized rats.

Experiment II.--With the exception of the fact that few rats were sacrificed, the procedure for this experiment was similar to that employed above. In this experiment, in contrast to Experiment I the criterion for evaluation of effects was that of survival time rather than lung damage.

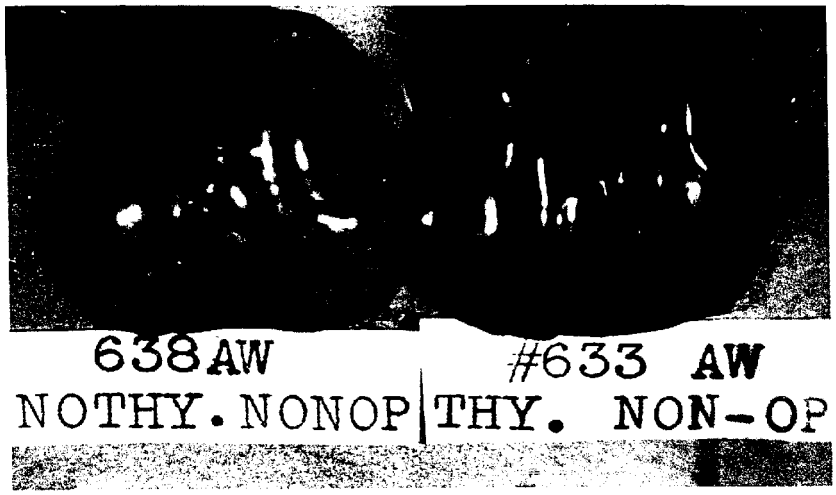


Figure 18.--Effect of OAP on Lungs of Normal Thyroid Fed Rat and on Lungs of Control. Lungs on the right which show gross hemorrhagic congestion are from rat exposed to oxygen but fed desiccated thyroid prior to and during exposure; those on the left are of OAP exposed, non-thyroid fed control. Some congestion is evident in lungs of the control but certainly the contrast is striking.

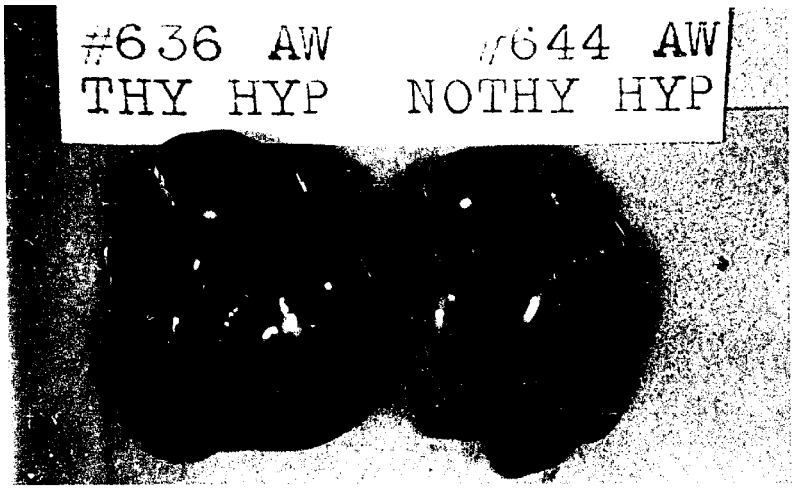


Figure 19.--Effect of OAP on Lungs of Hypophysectomized, Thyroid Fed Rat and on Lungs of Control. Lungs of thyroid fed, OAP exposed rat (left) show marked contrast to those of non-thyroid fed, OAP exposed control.

TABLE 19

MORTALITY, SURVIVAL AND LUNG DAMAGE FOR NORMAL, THYROID FED RATS AND FOR CONTROLS IN OAP

Thyroid				Controls			
Rat #	Mortality	Survival (hrs.)	Lung Damage	Rat #	Mortality	Survival (hrs.)	Lung Damage
550	D.	54½	4	565	D.	60	4
551	S.	60½	3	566	S.	60½	1
552	D.	54	4	567	D.	58½	4
553	D.	60	4	568	S.	54	1
554	S.	58½	3	569	S.	60½	2
618	S.	72	3	570	S.	55	1
619	S.	72	3	624	S.	72½	2
620	S.	69	3	625	S.	55½	3
604	D.	56	4	626	S.	69½	2
630	D.	62½	4	638	S.	48	2
631	D.	54	4	639	D.	54	3
632	D.	54	4	640	D.	55	3
633	D.	46	4	641	D.	62½	3
Av.	...	55.1	3.6		...	58.0	2.4
S.E.	...	1.7hrs.	...		...	1.6hrs.	...

TABLE 20

MORTALITY, SURVIVAL AND LUNG DAMAGE FOR HYPOPHYSECTOMIZED, THYROID FED RATS AND FOR CONTROLS IN OAP

Thyroid				Controls			
Rat #	Mortality	Survival (hrs.)	Lung Damage	Rat #	Mortality	Survival (hrs.)	Lung Damage
615	S.	72½	2	621	S.	29	2
616	S.	72½	4	622	S.	72½	2
617	D.	29	4	623	S.	72½	3
634	S.	70½	3	608	D.	59	4
635	D.	50½	4	642	D.	70½	4
636	D.	56½	4	643	S.	50½	0
637	S.	82	1	644	S.	56½	2
				645	S.	82	1
Av.	...	45.3	3.1		...	64.8	2.1
S.E.	...	8.5 hrs.	...		...	5.8 hrs.	...

Sixty-one young male rats were used; 31 of these were fed thyroid (30 - 40 mg./rat/day) for seven days prior to exposure while 30 were maintained as controls. Grouping is outlined in Table 21.

TABLE 21

NUMBER OF HYPOPHYSECTOMIZED AND NORMAL RATS IN TEST AND CONTROL GROUPS IN OAP AND IN AIR (EXPERIMENT II)

	Thyroid		Controls	
	Hypophy.	Normals	Hypophy.	Normals
OAP	10	11	10	10
Air	5	5	5	5
Total	15	16	15	15

The results show clearly that thyroid feeding significantly decreased the survival time of rats exposed to oxygen at atmospheric pressure. This was true for both operated and non-operated animals. In the case of the non-operated rats the decrease of survival time due to feeding of thyroid was approximately 6 hours; for the hypophysectomized rats it was nearly 11 hours. These survival times are represented in Figure 20. The results for individual animals are shown in Tables 22 and 23.

From these results one might expect that feeding of thyroid would increase hydrothorax; surprisingly enough

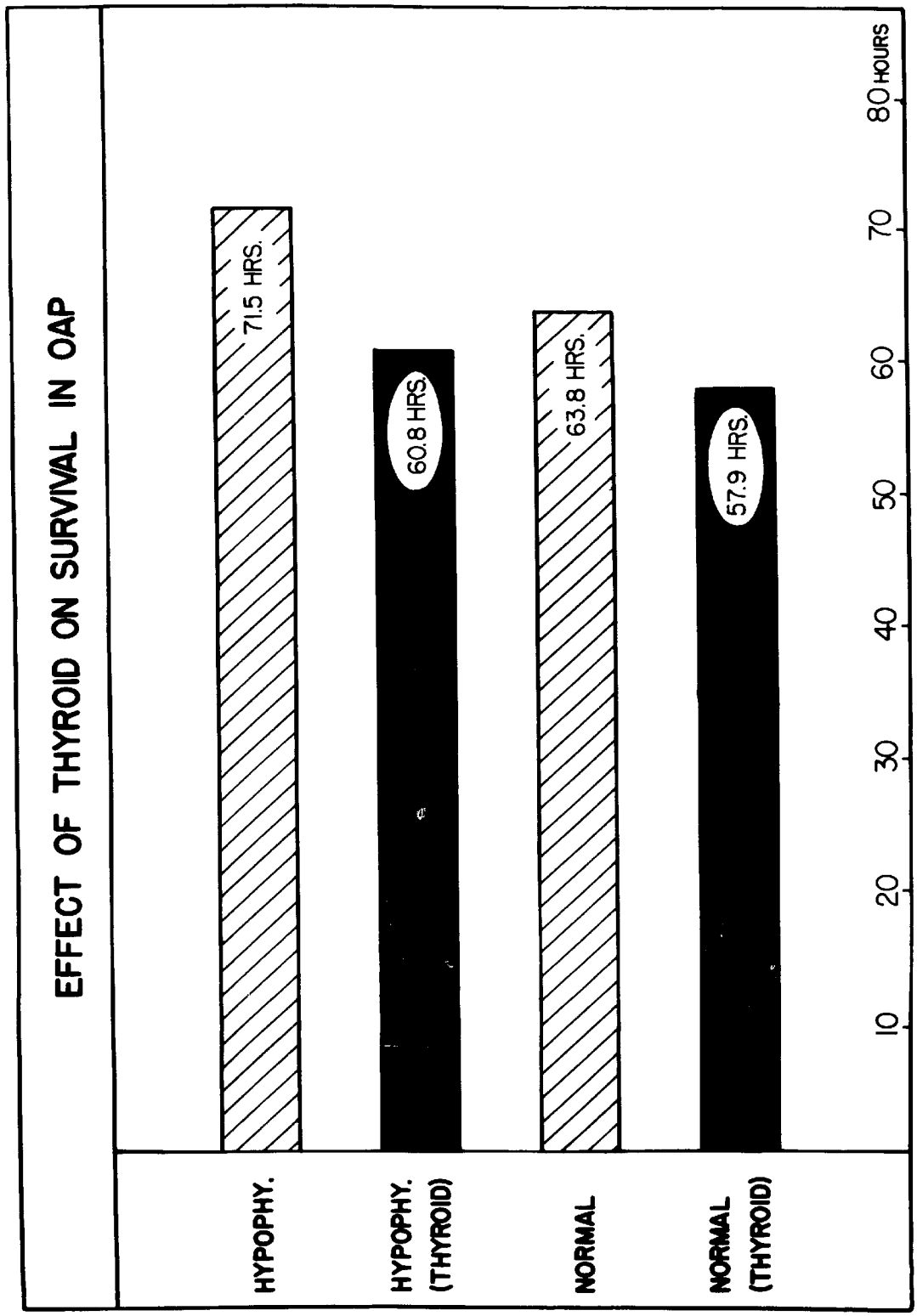

EFFECT OF THYROID ON SURVIVAL IN OAP
71.5 HRS.
60.8 HRS.
63.8 HRS.
57.9 HRS.
HYPOPHY.
HYPOPHY. (THYROID)
NORMAL
NORMAL (THYROID)
10
20
30
40
50
60
70
80 HOURS

Figure 20

TABLE 22

MORTALITY, SURVIVAL, LUNG DATA AND HYDROTHORAX FOR NORMAL, THYROID FED RATS AND FOR CONTROLS IN OAP

Thyroid					Controls				
Rat #	Mortality	Survival (hrs.)	Lung Damage	Thoracic Fluid (cc.)	Rat #	Mortality	Survival (hrs.)	Lung Damage	Thoracic Fluid (cc.)
1988	D.	45½	4	0.0	2004	D.	59	4	6.6
1994	D.	66	4	4.2	2005	D.	60	4	5.2
1995	D.	63	4	6.6	2006	D.	65½	4	6.8
1996	D.	67	4	5.0	2007	D.	64	4	7.0
1997	D.	59	4	5.8	2008	D.	61	4	5.0
1998	D.	61	4	4.8	2009	D.	69½	4	7.0
1999	D.	51½	4	2.2	2010	D.	70	4	7.4
2000	D.	51½	4	2.8	2011	D.	61½	4	4.8
2001	D.	55½	4	3.8	2012	S.	101	2	0.0
2002	D.	56	4	3.0	2013	D.	64	4	7.0
2003	D.	60	4	5.6					
Av.	...	57.9	...	4.0		...	63.8	...	6.1
S.E.	...	2.2 hrs.	...	0.6 cc.		...	1.3 hrs.	...	0.3 cc.

TABLE 23

MORTALITY, SURVIVAL, LUNG DATA AND HYDROTHORAX FOR HYPOPHYSECTOMIZED, THYROID FED RATS AND FOR CONTROLS IN OAP

Thyroid					Controls				
Rat #	Mortality	Survival (hrs.)	Lung Damage	Thoracic Fluid (cc.)	Rat #	Mortality	Survival (hrs.)	Lung Damage	Thoracic Fluid (cc.)
1970	D.	64	4	2.2	1950	D.	$77\frac{1}{20}$	4	2.2
1971	D.	61	4	2.0	1951	D.	$45\frac{1}{20}$	2	0.6
1973	D.	61	4	2.2	1952	D.	65	4	1.6
1975	D.	$58\frac{1}{20}$	4	2.0	1953	D.	77	4	1.4
1976	D.	74	4	1.2	1954	D.	74	2	2.8
1977	D.	63	4	1.8	1955	D.	71	4	4.0
1978	D.	97	1	0.5	1956	D.	84	1	1.5
1980	D.	$57\frac{1}{20}$	4	2.2	1957	D.	80	4	3.2
1981	D.	$55\frac{1}{20}$	4	2.4	1958	D.	65	4	2.2
1982	D.	$52\frac{1}{20}$	4	0.0	1959	D.	76	4	3.4
Av.	...	60.8	...	1.6		...	71.5	...	2.3
S.E.	...	2.1 hrs.	...	0.3 cc.		...	3.5 hrs.	...	0.3 cc.

quantitative measurement of free thoracic fluid revealed that the contrary was the case in both normal and hypophysectomized rats; for example, in the normal rats fed thyroid, free fluid averaged 4.0 cc. while that of their controls was 6.1 cc.

The data also show that, on the whole, the thoracic fluid was less for hypophysectomized than for normals. This difference held for both thyroid fed and control animals (Figure 21).

This experiment demonstrates conclusively that thyroid hormone enhances the severity of the effects of oxygen as manifest by shortened survival time in OAP, and that thyroid reverses the partial protection conferred by hypophysectomy. It indicates further that part of the protection which results from hypophysectomy may be attributable to loss of thyroid function.

2. Thyroxine

Experiment III.--The procedure of this experiment was essentially similar to that of Experiments I and II except for the use of thyroxine instead of dessicated thyroid powder. Subcutaneous injection of thyroxine (0.14 mg./rat/day) was employed in order to control more accurately the dose of thyroid hormone. Controls were injected with saline. Grouping of the sixty-three animals is shown in Table 24.

In this experiment, as in Experiment II, the primary criterion for evaluation of thyroid's effects upon the adverse

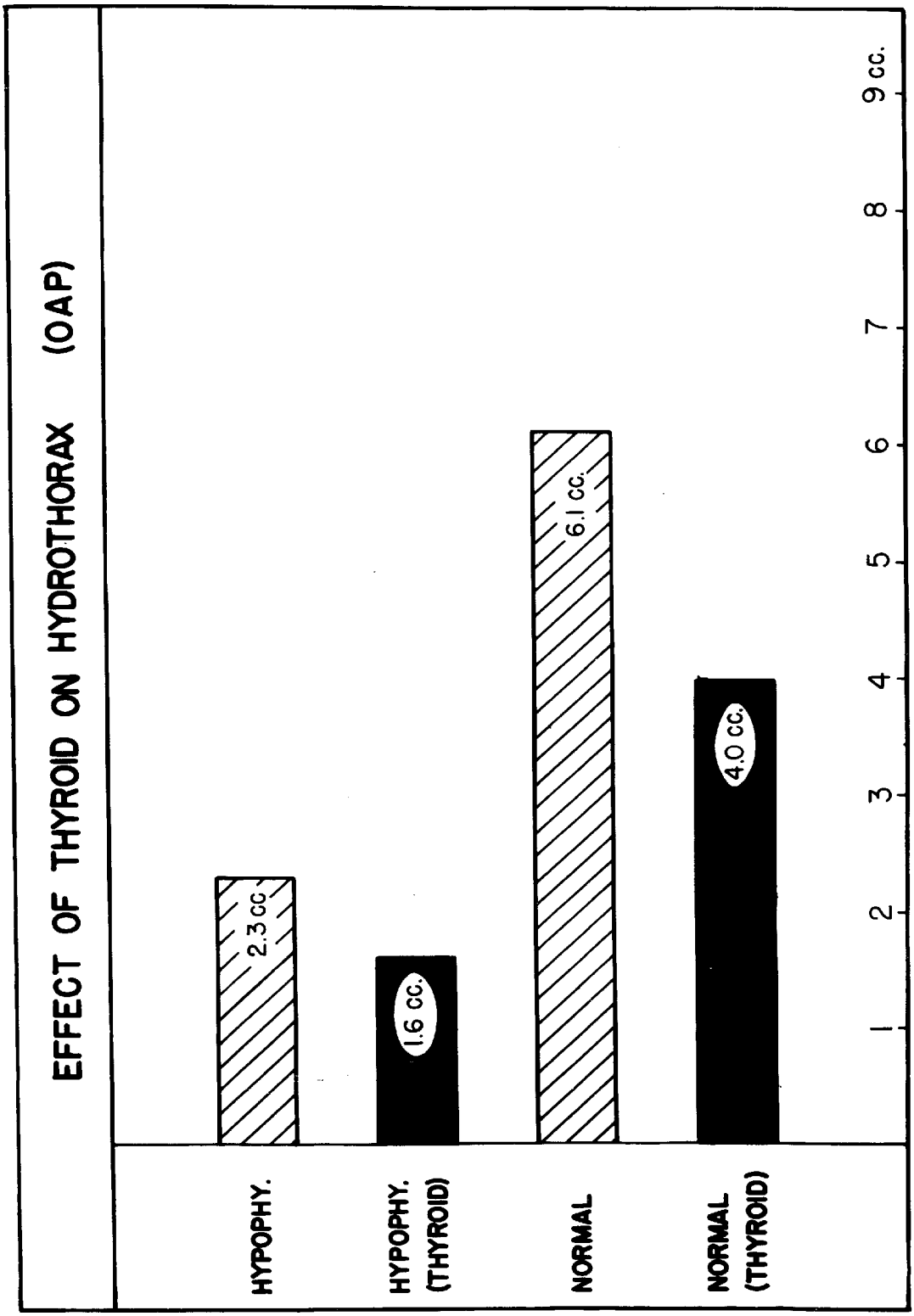

EFFECT OF THYROID ON HYDROTHORAX (OAP)
2.3 cc.
1.6 cc.
6.1 cc.
4.0 cc.
HYPOPHY.
HYPOPHY. (THYROID)
NORMAL
NORMAL (THYROID)
1
2
3
4
5
6
7
8
9 cc.

Figure 21

action of oxygen was that of survival time. Since the greater proportion of the animals were allowed to succumb in the chambers, the use of lung damage as a criterion was limited, particularly for the normal rats. Comparison of lungs was, however, possible in several of the hypophysectomized animals.

TABLE 24

NUMBER OF HYPOPHYSECTOMIZED AND NORMAL RATS IN TEST AND CONTROL GROUPS IN OAP AND IN AIR (EXPERIMENT III)

	Thyroxine		Controls	
	Hypophy.	Normals	Hypophy.	Normals
OAP	11	11	11	10
Air	5	5	5	5
Total	16	16	16	15

The results convincingly show that thyroxine administration enhanced the adverse effects of oxygen; it decreased survival time of both intact and hypophysectomized rats. The average survival of the non-operated rats was decreased from 57 hours to 51 hours; that of the hypophysectomized from 69.9 hours to 60.3 hours (Table 25; Figure 22).

Comparison of lungs of the hypophysectomized rats showed that thyroxine enhanced pulmonary damage due to OAP. Lungs of all the thyroxine injected rats were extensively involved; whereas only six out of eleven of the non-thyroxine

TABLE 25

MORTALITY, SURVIVAL, LUNG DATA AND HYDROTHORAX FOR NORMAL, THYROXINE INJECTED RATS AND FOR CONTROLS IN OAP

Thyroxine						Controls					
Rat #	Mortality	Survival (hrs.)	Lung Damage	Lung-Heart Wt. (gm.)	Thoracic Fluid (cc.)	Rat #	Mortality	Survival (hrs.)	Lung Damage	Lung-Heart Wt. (gm.)	Thoracic Fluid (cc.)
3000	D.	54	4	3.4	8.0	3011	D.	55½	4	3.7	9.6
3001	D.	50	4	3.9	2.8	3012	S.	84½	3	3.5	5.0
3002	D.	50	4	3.7	5.6	3013	D.	57	4	3.5	8.4
3003	D.	51	4	3.4	4.4	3014	D.	54½	4	4.4	10.4
3004	S.	84½	3	3.4	0.2	3015	D.	65	4	3.4	8.2
3005	D.	51	4	3.8	5.0	3016	D.	54	4	3.2	9.6
3006	D.	45	4	3.7	3.6	3017	D.	52½	4	3.7	9.0
3007	D.	52	4	3.3	3.8	3018	D.	52	4	3.2	7.6
3008	D.	52½	4	3.9	3.4	3019	D.	62	4	4.2	9.0
3009	D.	55	4	3.5	7.4	3020	D.	63½	4	3.9	6.0
3010	D.	53	4	3.9	6.0						
Av.	...	51.3	...	3.7	5.0		...	57.3	...	3.7	8.6
S.E.	...	0.9 hrs.	...	0.1 gm.	0.6 cc.		...	0.5 hrs.	...	0.5 gm.	0.4 cc.

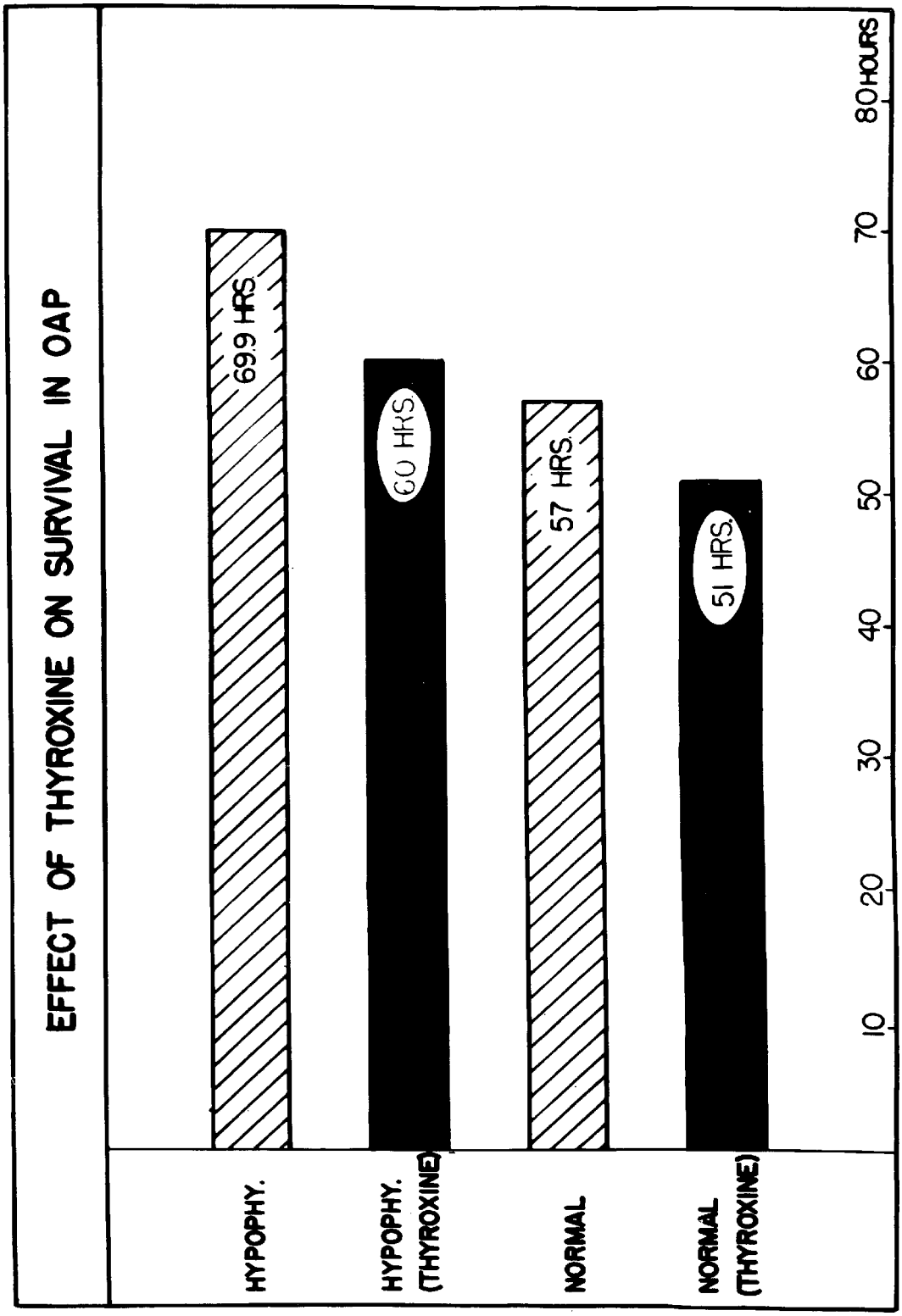

EFFECT OF THYROXINE ON SURVIVAL IN OAP
69.9 HRS
60 HRS.
57 HRS.
51 HRS.
HYPOPHY.
HYPOPHY. (THYROXINE)
NORMAL
NORMAL (THYROXINE)
10
20
30
40
50
60
70
80 HOURS

Figure 22

controls were similarly affected (Table 26; Figure 23). Control animals in air were not adversely affected by thyroxine.

The results of this experiment, as in Experiment II, show that the average volume of free fluid in the thoracic cavities of the hypophysectomized rats (2.4 cc.) was less than in those of the normals (8.6 cc.). Furthermore, the volume of thoracic fluid in the thyroxine injected rats (hypophysectomized and normals) was consistently less than in their controls. These findings are illustrated in Figure 24.

The results of this experiment show that thyroxine augments the deleterious effects of oxygen at atmospheric pressure; it shortens survival time and enhances pulmonary pathology. Furthermore, they confirm the results of earlier experiments that hypophysectomy imparts protection from OAP adverse effects.

In summary, the data from all these experiments on thyroid (I, II and III) confirm again the finding that hypophysectomized rats are less affected in OAP than are normal, unoperated animals. This is borne out by the following observations: (1) hypophysectomy delays onset of lung involvement, (2) it prolongs survival, and (3) at the time that these animals do succumb, the degree of congestion is less in the hypophysectomized rats as shown by volume of free thoracic fluid and by macroscopic examination of the lungs.

These data also show that thyroid factors (administered as dessicated thyroid or as thyroxine) hasten onset of typical reactions to oxygen at atmospheric pressure.

TABLE 26

MORTALITY, SURVIVAL, LUNG DATA AND HYDROTHORAX FOR HYPOPHYSECTOMIZED, THYROXINE INJECTED RATS AND FOR CONTROLS IN OAP

Thyroxine						Controls					
Rat #	Mortality	Survival (hrs.)	Lung Damage	Lung-Heart Wt. (gm.)	Thoracic Fluid (cc.)	Rat #	Mortality	Survival (hrs.)	Lung Damage	Lung-Heart Wt. (gm.)	Thoracic Fluid (cc.)
3031	D.	60½	4	2.6	1.4	3042	D.	68½	4	2.1	2.8
3032	D.	58½	4	2.6	1.2	3043	D.	57	0	2.1	3.4
3033	D.	68	4	3.3	1.0	3044	D.	79	4	2.1	3.4
3034	D.	58½	4	2.1	2.4	3045	D.	56½	2	2.0	3.6
3035	D.	83	4	2.6	0.0	3046	S.	84½	2	2.5	3.2
3036	D.	62½	4	2.6	1.0	3047	D.	79	4	1.9	2.0
3037	D.	58½	4	2.2	2.4	3048	D.	65	2	2.1	2.0
3038	D.	61	4	2.6	1.6	3049	D.	62½	2	2.0	1.2
3039	D.	56	4	2.7	1.2	3050	D.	71	4	4.0	0.2
3040	D.	60	4	2.8	1.0	3051	D.	81	4	2.1	3.2
3041	D.	60	4	2.6	2.4	3052	D.	79	4	2.2	2.4
Av.	...	60.3	...	2.6	1.4		...	69.9	...	2.3	2.4
S.E.	...	1.0hrs.	...	0.1gm.	0.2cc.		...	3.0hrs.	...	0.2gm.	0.3 cc.

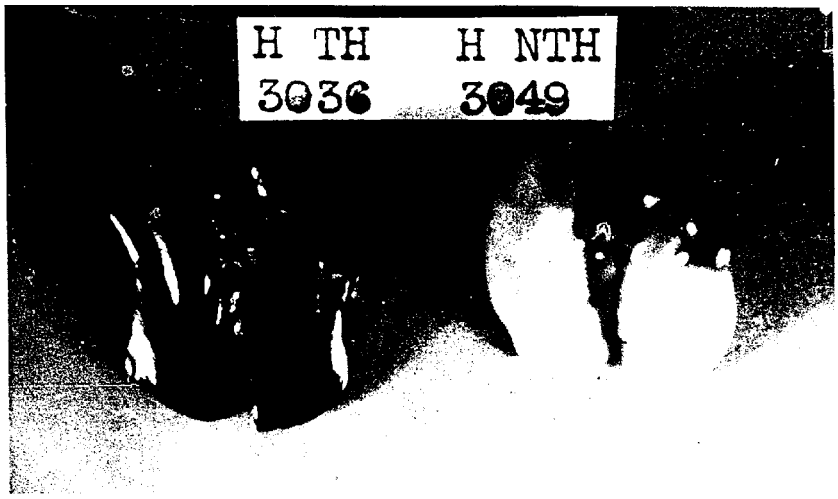


Figure 23.--Effect of OAP on Lungs of Hypophysectomized, Thyroxine Injected Rat and on Lungs of Control. A very striking contrast between lungs of thyroxine injected rats (left) and those of OAP exposed, non-thyroxine treated control is illustrated.

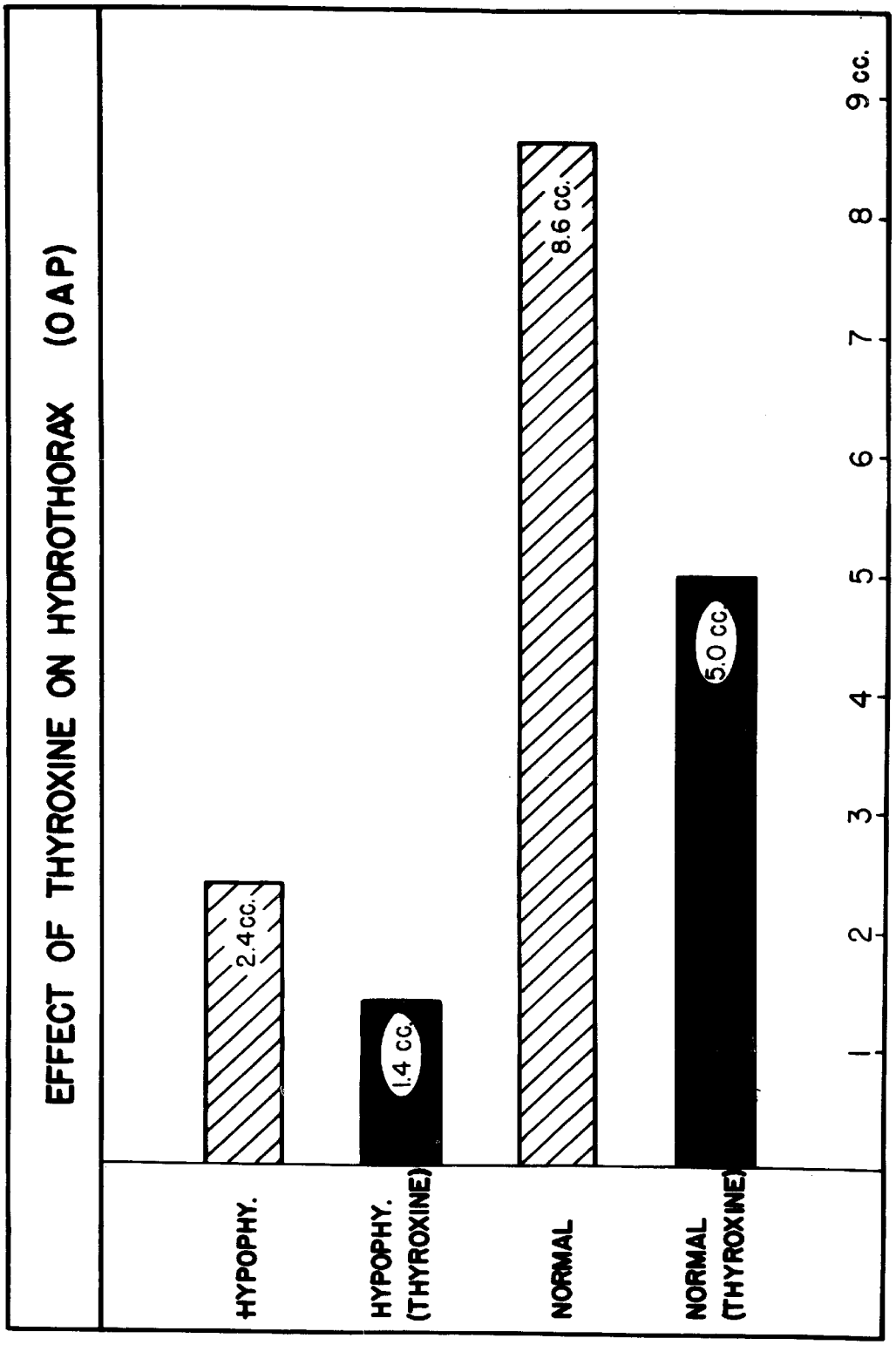

EFFECT OF THYROXINE ON HYDROTHORAX (OAP)
2.4 cc.
1.4 cc.
8.6 cc.
5.0 cc.
HYPOPHY.
HYPOPHY. (THYROXINE)
NORMAL
NORMAL (THYROXINE)
1
2
3
4
5
6
7
8
9 cc.

Figure 24

Since thyroid hormone augments the adverse lung effects of OAP it might reasonably be expected that it would also increase the hydrothorax. Experiments II and III indicated that this was not the case; for, there was considerably less free fluid in the thoraces of the thyroid treated animals--both hypophysectomized and normals--than in their non-treated controls. These results are reminiscent of those seen in connection with administration of epinephrine; i.e., the treated rats, which died earlier than the non-treated animals, evidenced less hydrothorax than their controls.

The finding that thyroid principle enhances toxic effects of oxygen at atmospheric pressure leads to consideration of some of the factors which may be responsible for this enhancement. One of the most prominent effects of thyroid is on metabolism. Campbell, who reported (1937) enhancement of high pressure oxygen effects by thyroid, attributed this action to an increased rate of oxidations and accompanying increased metabolite levels.

It is conceivable that this contributes to the hastening of adverse oxygen effects; nevertheless there appear to be other actions of thyroid in bringing about deleterious consequences of exposure to oxygen. It is well known that carbon dioxide very appreciably enhances the toxicity of oxygen high pressure (Thompson, 1889; Gesell, 1923). Ohlsson (1947) working with rabbits reported that this enhancement by carbon dioxide could be carried to the effects of oxygen at atmospheric pressure. There is, therefore, evidence which would

appear to implicate carbon dioxide; however experiments of our own indicate that the question of carbon dioxide involvement is much more complicated than Ohlsson's data suggest.

Thyroid may also act to hasten adverse effects of oxygen through stimulation of the adrenal medulla. The resultant increase in production of epinephrine would then, as our experiments have shown, augment toxic effects of OAP. This interpretation finds support in the literature concerning the relationship between thyroid and adrenal medulla (Eppinger, Falta and Rudinger, 1908; Harrington, 1933; Means, 1948).

It is also probable that thyroid may act through the adrenal cortex to bring about an increased production of adreno-cortical hormone which as our data have shown enhances deleterious effects of OAP. The report of Selye and Stone (1950) that thyroxine greatly potentiates the corticotrophic effect of lyophylized anterior pituitary tissue lends credence to this interpretation.

The evidence indicates that no one factor is operative to cause enhancement of oxygen toxicity by thyroid factors.

The data from the three experiments of this series conclusively show that thyroid factors enhance the toxicity of oxygen at atmospheric pressure; this is evidenced by shortened survival and accentuated pathological lung effects.

IV. DISCUSSION

The well-known harmful effects of continuous exposure to high concentrations of oxygen at atmospheric pressure (OAP) have been conclusively demonstrated by the above results. These effects include gross pulmonary hemorrhagic congestion, massive protein containing hydrothorax and finally death evidently due to anoxia caused by lung damage. Involvement of a large number of factors contributing to this toxic response has prevented complete understanding of the etiology of the pathology which results from exposure to OAP. As stated earlier, several explanations have been suggested; for example, accumulation of carbon dioxide at tissue level, poisoning of oxidative enzymes, and direct irritative action of oxygen on pulmonary tissue.

In addition to these factors, the data of this investigation have shown that endocrine factors are also contributory to the adverse effects of OAP. It has been demonstrated that hypophysectomy and adrenalectomy lend some protection from the extremely unfavorable effects of oxygen at atmospheric pressure and that cortisone administration enhances these adverse effects. The conclusion, therefore, that adreno-cortical factors exert unfavorable effects upon oxygen exposed animals is in order.

There are other principles which must also be considered. The administration of epinephrine very appreciably augmented the adverse effects of oxygen as manifest by decreased survival time and increased lung pathology. This finding gives reason to believe that at least part of the lung damage seen in OAP may be due to production of epinephrine. Some caution must be exercised regarding this assumption since increased epinephrine levels in animals exposed to OAP have not been demonstrated; although it is reasonable to assume that at some time during exposure epinephrine is liberated.

The finding of this experimental study that thyroid hormone accentuated the toxic response of rats to OAP introduces several considerations. They are: (1) relation of thyroid to metabolic effects; (2) relation of thyroid to adreno-medullary secretion; (3) relation of thyroid to adrenocortical secretion.

Relation of thyroid to metabolic effects.--Most prominent of thyroid's actions is that on metabolism. Associated with rapid metabolism are accelerated oxidations and increased production of carbon dioxide. Campbell (1937) when he described the enhancement of oxygen high pressure ill effects by thyroid attributed this augmentation to increased rate of oxidations. There is the possibility that this may be carried over to explain,at least in part, the enhanced effect, after administration of thyroid, of oxygen at atmospheric pressure.

The increased carbon dioxide production associated with thyroid hormone treatment brings up the suggestion that thyroid may be deleterious because it results in a greater production of carbon dioxide by the organism. Early studies of Thompson (1889) and later of Gesell (1923) showed that carbon dioxide greatly enhances adverse effects of oxygen high pressure. Ohlsson (1947) laid considerable stress upon carbon dioxide as etiological for the pulmonary pathology typical of exposure to oxygen at atmospheric pressure. He believed that carbon dioxide was the damaging factor because, in his experiments, rabbits in oxygen (at atmospheric pressure) were more severely affected when small percentages of carbon dioxide were present in their exposure chamber. The assumption appears valid in many ways; however experiments in this laboratory (on rats) indicate that the relationship of carbon dioxide to OAP toxicity is more complicated than Ohlsson suggested.

Relation of thyroid to adreno-medullary secretion.--Thyroid factors may also act to increase adverse response to OAP by bringing about potentiation of epinephrine which has been conclusively shown to enhance ill effects of OAP. The potentiation of epinephrine by thyroid factors is entirely reasonable in view of the findings of Kroneberg and Huter (1951) that mice pretreated with thyroxine were more susceptible to epinephrine poisoning than were non-pretreated animals.

Relation of thyroid to adreno-cortical secretion.--The report of Selye and Stone (1951) that thyroxine greatly potentiated the corticotrophic effect of lyophylized anterior pituitary tissue suggests that the adverse effects of thyroid, in OAP, may be due to adreno-cortical influences.

These varied effects and interrelationships of thyroid hormone afford considerable interest in connection with the oxygen toxicity problem; they also suggest that no one factor is operative to cause enhancement of OAP toxicity by thyroid factors.

In view of the present philosophy that secretions of the pituitary-adrenal axis protect against stressor agents it is somewhat unique that, in OAP, absence of these endocrine factors is beneficial while their presence is detrimental. The extreme pulmonary involvement as shown by congestion, extravasation and protein containing pleural effusion is indicative of serious alteration of permeability in the vascular bed. These endocrine factors--pituitary, adreno-cortical, adreno-medullary and thyroid--evidently affect pulmonary vascular balance to enhance rather than alleviate the damage.

Evidence exists which leads to the belief that oxygen itself may act upon lung capillaries to cause--apart from known reflex action or chemical mediation--vasodilatation and slowing of lymph flow from the lungs (Euler and Liljestrand, 1946; Nisell, 1948; Beznak and Liljestrand, 1949). If vasodilatation occurs but if at the same time lymph drainage from

the area is decreased then there is every likelihood that pulmonary edema will result.

With edema fluid leaking into alveoli, anoxia of that particular area will occur. One of the outstanding features of oxygen lack is its damaging effects upon the structures involved; so anoxia will cause capillary endothelium to become more and more permeable; even greater volumes of fluid will be released to surrounding areas. Thus, if these factors be operative in the manner outlined, one can visualize how oxygen may cause edema, congestion, extravasation and finally death from general anoxia.

Two factors which are usually associated with pulmonary edema are: (1) anoxia of capillaries and (2) sustained increase in pulmonary capillary pressure. Suggestion has already been made that oxygen may bring about the first of these--anoxia of capillaries; and it seems reasonable to suggest that cortisone, epinephrine, and thyroid factors may act to facilitate lung damage through their effect on pulmonary circulation.

Whatever the mechanism may be, at least there is a synergism of some kind between the action of oxygen itself and the endocrine factors which have been shown to enhance adverse effects of OAP.

V. SUMMARY AND CONCLUSIONS

Earlier work has established that pituitary, adrenal, and thyroid principles enhance adverse response to oxygen at high pressures. The purpose of this investigation was to determine the possible effects of these endocrine factors upon the adverse response to oxygen in high concentrations at atmospheric pressure (OAP).

Young Sprague Dawley rats, maintained on a uniform diet, were chosen as experimental subjects. They were exposed to oxygen in especially constructed airtight chambers. Concentration of oxygen was, in general, maintained above 95 per cent by continuous flow of gas supplied from commercial cylinders. Carbon dioxide level was kept below 0.20 per cent by soda lime. Temperature was about 28°C.

In the first section, experiments were carried out to determine the effects of hypophysectomy upon rats exposed to oxygen at atmospheric pressure. It is well-known that animals continuously exposed to OAP for long periods exhibit gross pulmonary congestion, hyperemia, consolidation and massive hydrothorax. The extreme involvement is indicative of a major alteration of permeability in the vascular bed. Hypophysectomy was found to provide a very significant degree of protection from OAP adverse effects as indicated by lessened

pulmonary pathology, decreased hydrothorax and increased survival time of the operated rats. The inference was made that this protection was attributable, in part at least, to elimination of adrenocorticotrophin and of adreno-cortical factors.

In carrying this investigation further, it was found that adrenalectomy also provided protection against OAP ill effects. This protection was evidenced by a decreased degree of pulmonary pathology, lessened hydrothorax and increased survival time of the operated rats. These experiments substantiated the inference that removal of adreno-cortical factors was responsible, at least in part, for the protection from OAP effects conferred by hypophysectomy. The conclusion that removal of some adrenal principle imparted beneficial effects to rats in OAP was justified.

This conclusion was further supported by the results of experimental studies in which cortisone was administered to animals in OAP. This adreno-cortical factor significantly shortened survival time in oxygen and accentuated the typical pulmonary pathology.

Since adrenalectomy removes medullary as well as cortical hormones it is possible that part of the protection conferred by adrenalectomy might be due to removal of epinephrine. Experiments were undertaken, therefore, to determine what effect, if any, epinephrine would have upon animals exposed to OAP. Subcutaneous injection of epinephrine, in amounts too small to be injurious in air controls, brought

about a significant enhancement of OAP adverse effects. This enhancement was evidenced by greatly decreased survival time and by accentuated lung pathology. The results of this study indicated that the medullary factor may act in mediating adverse response to OAP.

Part of the protection afforded by hypophysectomy might possibly be due to removal of thyrotrophin with consequent diminishment of thyroid secretion; therefore, studies were undertaken to determine the effect of thyroid factors upon the toxic response of rats to OAP. It was found that thyroid hormone when administered as dessicated thyroid or as thyroxine decreased survival time and enhanced lung pathology of normal rats in OAP. In addition, thyroid factors were found to reverse the beneficial effects of hypophysectomy; this also was shown by decreased survival time in oxygen and increased degree of lung damage. These data indicate that the beneficial effects of hypophysectomy may be due, in part, to decreased thyroid function.

While it must be recognized that the conditions of stress which are present in oxygen at high pressures and in oxygen at atmospheric pressures are not identical, the data obtained from this investigation demonstrate that the endocrine factors which have been shown to be damaging in oxygen high pressure are also operative in enhancing the adverse effects of oxygen at atmospheric pressure.

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