**THE EFFECTS OF ATMOSPHERES ENRICHED WITH OXYGEN
UPON LIVING ORGANISMS, (a) EFFECTS UPON MICRO-
ORGANISMS, (b) EFFECTS UPON MAMMALS EXPERI-
MENTALLY INOCULATED WITH TUBERCULOSIS, (c)
EFFECTS UPON THE LUNGS OF MAMMALS, OR OXYGEN
PNEUMONIA**

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SECTION A. THE ACTION OF OXYGEN UPON MICRO-ORGANISMS

In the course of two previous investigations, published by Moore and Williams¹, in 1909 and 1910, it was shown that while certain micro-organisms are indifferent to amounts of oxygen lying between the atmospheric value and a full atmosphere of oxygen at ordinary barometric pressure, others, notably *Bacillus tuberculosis* and *Bacillus pestis*, are very sensitive to oxygen, and cease to grow when the percentage of oxygen exceeds about seventy per cent. of 760 millimetres.

It is remarkable how few organisms show this oxyphobic property, and how specific and definite the effect is when the property is present.

Out of twenty-six various organisms tested in a partial pressure of oxygen of 500 millimetres of mercury and over, only two showed complete stoppage of growth, viz., the bacilli of plague and tuberculosis, while those of the staphylococcic group were adversely affected and grew less rapidly than normally, and on some occasions failed to grow entirely, viz., *Staphylococcus pyogenes aureus*, and *albus*. All the remaining twenty-two organisms grew as well, so far as could be observed, in the increased oxygen as in air.

In the present investigation, these experiments have been repeated and amplified, and two additional and interesting members have been added to the oxyphobic group, namely, actinomyces and mycetoma from the streptothrix family.

1. *Bio-Chemical Journal*, Vol IV, p. 177, 1909; Vol. V, p. 181, 1910.

It was noticed by Moore and Williams that *B. pestis* was only arrested but not killed by exposure to the oxygen, for although no growth developed after some days' keeping in the enriched atmosphere, on afterwards transferring to ordinary air a good growth soon appeared. On the other hand, these observers found it impossible to revivify or sub-culture from *B. tuberculosis* which had been kept for a period of from two to three weeks in an atmosphere of 70 per cent. of oxygen, or over that amount.

In the present work it has been found that *B. tuberculosis*, while arrested completely in oxygen as they described, was found to grow again when transferred to air, as shown below. It was found in confirmation of their work that *B. pestis* was completely arrested during a stay in the oxygen-enriched air, but commenced to grow again as soon as the culture tubes were replaced in ordinary air.

It was otherwise, however, with the streptothrix organisms, which, in both instances, failed to grow again after exposure to the higher percentage of oxygen.

All experiments were carried out at atmospheric pressure of the total gases, the partial pressure of the oxygen being increased by complete or partial evacuation of the bell-jar under which the tubes containing the culture tubes were placed, so as to get varying percentages of oxygen.

The oxygen used was prepared most carefully in the laboratory from potassium permanganate, as the gas sold in the usual cylinders is contaminated with a good deal of nitrogen. It was most carefully washed, and stored until required in a large gas holder capable of holding about 100 litres.

The tubes with the cultures to be exposed to additional oxygen were placed in a large bell-jar fitting on to a ground glass-plate, and it was found necessary, on account of the long duration of each experiment, to prevent slow leakage by a mercury trap outside the flange of the bell-jar. The mercury was held in position by a circular mound of putty, and no mercury penetrated under the bell-jar. The bell-jar was evacuated after placing the desired inoculated culture tubes within, and then oxygen to the desired amount was allowed to enter, and analyses of the percentages of oxygen at beginning and end of the experiment were carried out by the usual gas analysis methods.

The control tubes were kept under similar conditions under a like bell-jar but in ordinary air, and both bell-jars were placed alongside of each other in the same incubator at 37° C.

The enriched oxygen atmosphere was entirely without effect on the following organisms:—

Bacillus coli communis	Bacillus pyocyaneus
„ typhosus	„ of hog cholera
„ dysenteriae (Flexner)	„ diphtheriae
„ „ (Kruse)	„ anthracis
„ mallei	Vibrio cholerae
„ gaertner	Sarcina lutea
„ prodigiosus	„ aurentiaca
„ pneumoniae	Oidium albicans
Proteus vulgaris	

The staphylococcic group showed very fair growth, but not so marked as in the controls. The results with *B. pestis* were as stated above. *B. tuberculosis* showed a very small but definite growth at the end of a prolonged experiment lasting seven weeks. The organism was not killed but only inhibited by its treatment, and was able on further incubation in air to overcome to a slight degree the adverse circumstances under which it had been placed, and sub-cultures then made from it developed normally.

Actinomyces and mycetoma, on the other hand, were not only inhibited but killed, no growths appearing after the oxygen treatment.

These results are shown in the following protocols:—

Experiments I, II and III.—Six tubes of Glycerin-acid-agar were inoculated with a laboratory strain of Bacillus Tuberculosis (Human), and three were placed in an atmosphere of 90 per cent. of oxygen and the controls in the air chamber.

Results:—At the end of three weeks' incubation of the two sets under similar conditions, the three controls showed good growths and the tubes in oxygen, in which the percentage had diminished to 70 per cent., showed no appreciable growth.

The tubes were replaced in the oxygen chamber with oxygen of 85 per cent. strength and incubated for a further four weeks, when the oxygen had diminished to 68 per cent., and there was an appreciable amount of growth. The oxygen was renewed and incubation continued for a further two weeks, when the growth was about equal in amount to the growth at the end of three weeks only in the control tubes, but it was of a dark brown colour and consisted of raised discrete masses differing from the uniform growth in the controls.

One of the tubes was continued in the oxygen and three weeks later showed a very slight increase in growth of the same character.

Another was placed in the air bell-jar, and after three weeks showed a much more profuse growth, but still of the dark brown colour.

The remaining tube was sub-cultured, and incubated in the ordinary manner, and three weeks later, growths, normal in colour and other respects, were obtained.

Specimens from the various tubes were now stained and submitted to microscopical examination, but no appreciable difference could be discovered in the smears.

These results were confirmed later in all respects by two additional similar experiments.

Experiments IV and V.—Three agar tubes were inoculated with each of two different strains of actinomyces, and two tubes of each strain were placed in the oxygen chamber, which was then filled with oxygen and the percentage determined, and found to be 87 per cent. The remaining tubes were placed as controls in the air chamber.

After four days' incubation the control tubes showed a slight growth as minute white specks, whilst the tubes in oxygen showed no growth. The tubes were replaced in their respective chambers, and seven days later again examined, when the controls showed a good growth. There was no trace of growth in the tubes kept in oxygen.

A further seven days' incubation in the oxygen chamber was tried, without any trace of growth, and the tubes were then transferred to the ordinary incubator. Fourteen days subsequently there was no trace of growth in either of them.

This experiment was again repeated, with similar results throughout.

Experiments VI and VII.—Three agar tubes were inoculated with mycetoma from the original strain, and two of these were placed in the oxygen chamber, and one in the air chamber for control. Seven days later, on careful examination, the control tubes showed a growth, but the tubes in oxygen showed no trace of growth. After incubation for an additional seven days, under the same conditions, they were again examined, and the controls showed a good growth, while again the tubes in oxygen gave no growth.

This was repeated for a third week with a like result, and the oxygen tubes were then transferred to the ordinary incubator and examined fourteen days later, when no evidence of growth could be found in the tubes.

This experiment was then repeated twice with similar results.

It having been suggested to me by Professor B. Moore that the inhibiting effect of the oxygen on the *Bacillus tuberculosis* in vitro, as well as the well-known effects of fresh air on tubercular patients, might be due to the presence of traces of oxides of nitrogen in the atmosphere, the following experiments were carried out.

Experiment VIII.—Suitable culture media, in tubes as for the previous experiments, were inoculated with the following organisms:—

<i>Bacillus tuberculosis</i>	<i>Actinomyces</i>
„ <i>pestis</i>	<i>Mycetoma</i>
„ <i>typhosus</i>	<i>Pneumococcus</i>
„ <i>diphtheriae</i>	<i>Staphylococcus aureus</i>
„ <i>coli communis</i>	<i>Streptococcus pyogenes</i>
„ <i>anthracis</i>	<i>Staphylococcus albus</i>

The tubes to be tested were placed in a similar bell-jar to that used in the previous experiments, and this was exhausted to a pressure of ten millimetres of mercury. A sufficient quantity of nitric oxide gas, was prepared by action of nitric acid on copper, and washed and found to contain 90 per cent. of nitric oxide, was added so as to form 0.5 per cent. of the total capacity of the jar. Atmospheric air was now admitted, thus ensuring the entrance of the nitric oxide dilution into the tubes, and the jar was placed in the incubator, accompanied by the controls in a similar jar, but containing only atmospheric air.

Twenty-four hours later there was no appreciable difference in the results obtained from the two sets of tubes.

The incubation was repeated for one week, when there was no apparent difference between the controls and those tested, and at the expiration of three weeks the tubes containing the more slowly growing organisms, viz., *Bacillus tuberculosis*, *Actinomyces*, and *Mycetoma*, were identical in amount of growth.

Experiment IX.—The following organisms were inoculated on suitable media, viz.:—

<i>Bacillus coli communis</i>	<i>Bacillus diphtheriae</i>
„ <i>typhosus</i>	„ <i>anthracis</i>
„ <i>dysenteriae</i> (Flexner)	„ <i>pestis</i>
„ „ (Kruse)	<i>Staphylococcus pyogenes aureus</i>
„ <i>mallei</i>	„ „ <i>albus</i>
„ <i>gaertner</i>	„ „ <i>citreus</i>
„ <i>prodigiosus</i>	<i>Vibrio cholera</i>
„ <i>pneumoniae</i>	<i>Sarcina lutea</i>
„ <i>pyocyaneus</i>	„ <i>aurentiaca</i>
<i>Proteus vulgaris</i>	<i>Oidium albicans</i>

One set of the inoculated culture tubes was placed in the oxygen chamber, and by evacuation and introduction of oxygen the oxygen concentration was raised to 90 per cent. A series of controls in the ordinary air bell-jar in the same incubator were compared at the expiration of twenty-four hours with the tubes being tested in the oxygen. On examination, *Bacillus pestis* showed no growth on the oxygen tube, while the control tube showed a commencing growth, and two days later the control showed a good growth but the oxygen tube showed nothing.

Staphylococcus pyogenes aureus on the oxygen tube gave a very minute growth, while the *S. albus* and *S. citreus* showed no growth. All three of the controls of the staphylococci gave good growths.

All the remaining organisms gave practically identical growths on the oxygen and control tubes.

The tubes of the *Bacillus pestis* and the staphylococci were replaced, and again examined in a further forty-eight hours, when the staphylococcus tubes all showed growth, but definitely less in those exposed to the oxygen than in the controls. The *B. pestis* was quite free of growth in the oxygen tube, the control showed good growth.

CONCLUSIONS

1. That the action of oxygen in increased percentages on the *Bacillus tuberculosis* is to arrest its growth, but the action is not bactericidal but inhibitory, as in the case of those other oxyphobic micro-organisms, *Bacillus pestis*, and in a lesser degree the members of the staphylococci group.

2. That an atmosphere enriched with oxygen not only arrests the growth of actinomyces and mycetoma but kills these organisms. This may be the explanation of the differences in the literature as to the description of actinomyces, which some authors describe as aerobic, while others state that it is anaerobic. The probability is that it lies on the border line; a small increase in oxygen turns the scale and prevents its growth.

3. That the action of the oxygen is not due to oxides of nitrogen contained as an atmospheric impurity is shown by Experiment VIII.

SECTION B. EXPERIMENTS UPON MAMMALS EXPERIMENTALLY INOCULATED WITH TUBERCULOSIS

The effects of oxygen in stopping the growth of the *Bacillus tuberculosis* obviously suggests an attempt at treatment of the disease by respiration of oxygen, and with this object in view Moore administered oxygen of about 80 per cent. at ordinary atmospheric pressure to three patients with pulmonary tuberculosis for one hour daily over a period of some weeks. The oxygen inhalation was enjoyed by the patients, but did not show any clear effect upon the course of the disease.

It was thought that an extension of the period of oxygen administration, by keeping animals for a prolonged period in oxygen in a respiratory chamber, might have an effect, and accordingly guinea-pigs were introduced into the chamber described below after subcutaneous inoculation with a strain of *Bacillus tuberculosis*, and retained there for some days.

Unfortunately it was found that with the more prolonged periods in oxygen, that peculiar form of simple exudative pneumonia, which has been termed oxygen pneumonia, made its appearance when over 70 per cent. of oxygen was employed, and below this level no action on the development of the bacilli as shown by arrest of the tubercles was obtainable.

The lung effects are described in Section C. For the sake of completeness, the history of the inoculation experiments is here recorded.

A suitable dose for inoculation which would show definite pathological lesions in a guinea-pig in a reasonable time was determined after a considerable number of experiments with various strains. This dose ranged from 0.1 to 10 milligrams, according to the virulence of the particular strain, and was so selected that definite and fairly advanced lesions appeared in about six weeks. The injections were made subcutaneously in the thigh. A number of animals were inoculated at the same time, and of these a portion were kept in the animal house as controls, while the rest were placed in the oxygen respiratory chamber.

The oxygen used was prepared by heating permanganate of potassium in an iron retort. After all the air had been expelled, and a steady stream of oxygen was coming off, this was conveyed through wash-bottles into a gasometer of a capacity of 100 litres, with suspension weights so adjusted that there was a constant pressure sufficient to cause the gas to bubble through a wash-bottle placed between the gasometer and the

respiratory chamber, where it was stored for use, a sufficient quantity for four or five days being made at once.

By this means an oxygen of a strength of 95 to 96 per cent. was obtained, the remaining 4 to 5 per cent. consisting of nitrogen. The solid and soluble impurities were removed by the washing process.

The respiratory chamber used consisted of a cylindrical vessel (see fig. 1) made of galvanized iron, with a conical bottom ending in a

A Respiration Chamber. B Water Seal. C Soda Lime Tray. D Excreta Outlet. E Gasometer.
F Wash Bottle. G Indicator Wash Bottle. H Outlet to Vacuum Pump.

rubber tube which dipped into a vessel of water for about three centimetres, and served to convey away the urine. Its roof was formed by a glass dome,¹ made airtight by a circular water-seal in the tinware under-portion. This dome was about fifteen centimetres in diameter, and through it the animals could be observed during the experiments. The capacity of the chamber was about forty litres.

The animals rested on a floor formed by a perforated zinc plate, and suspended round the chamber was an annular gallery filled with soda lime, of which it contained about 500 grams when loosely charged. The inlet and outlet for the gases to the chamber were situated just above the floor, the former attached by indiarubber tubing to the wash-bottle from the gasometer, and the latter to a small vacuum pump, with a thumb-screw on the tube by which the flow could be easily regulated, and a small wash-bottle served as an indicator of the rate of flow.

1. The glass-cover was such as are made for protecting memorial flower wreaths.

The inoculated guinea-pigs were placed in this chamber, with an adequate supply of food for three days. Three pigs were used at each test, it being found impossible to store food for more for the required time in the chamber, owing to their increased appetites, due possibly to the raised oxygen percentage. The outlet was then attached to the vacuum pump and the inlet to the oxygen container and the chamber flooded with oxygen until the required percentage was obtained, as shown by analysis.

Specimens of the gas were obtained by disconnecting between the vacuum pump and the wash-bottle indicator, and tested in Hempel's bulbs for carbon-dioxide and oxygen. Once the required concentration was reached, it was found that a flow of about twenty bubbles per minute from a drawn-out tube of about one millimetre diameter through the wash-bottle was sufficient to preserve the standard. This outflow is necessary because it is impossible to prepare oxygen quite free from nitrogen in such large quantities.

Specimens of the gas were taken morning and evening for the first week, and later daily, and by regulating the thumbscrew on the exhaust, it was found that fairly constant oxygen percentage could be retained, the variation not exceeding five to ten per cent. over the periods between the changing of the food supply. The carbon-dioxide percentage with fresh soda-lime did not rise above three per cent. and was usually much lower. Any excess over this showed the necessity of a fresh supply of the absorbent.

At the end of each period of three days, the chamber was cleaned out and the food supply renewed. It was found advisable on each occasion to wash it out thoroughly with a dilute solution of Lysol, and the use of this preparation on the glass dome was most advantageous in preventing condensation of moisture in drops, thereby obscuring vision.

The controls and the animals undergoing the tests received alike the same food. Bran moistened with boiling water, carrots, and straw were the staple articles of diet.

The percentage of oxygen used in the tests was regulated by the amount the animals were able to bear. It was found, as already stated, that a continuous exposure for prolonged periods to a concentration greater than 70 per cent. caused death by pneumonia. Below this strength the animals seemed to be very little affected by their surroundings; the only observable difference being that they ate much more greedily, and on increasing the concentration they were for a time much more lively, but soon settled down to their normal condition again. The sudden increase

from the normal atmospheric percentage of oxygen to the increased concentration used, showed no ill effects, and on opening the chamber for cleansing purposes, the guinea-pigs, after their three days in the stronger oxygen, seemed unaffected by the change back to atmospheric air, and greedily attacked the fresh food supplied to them.

Experiment X.—A number of guinea-pigs were inoculated with a virulent strain of *Bacillus tuberculosis* (Human) under the skin of the thigh. Three, weighing together 1230 grams, were placed in the respiration apparatus, and the oxygen passed in until a sample of the gas withdrawn at the exhaust showed a strength of oxygen of 64 per cent. The thumbscrew on the exhaust was then screwed down so as to allow only a small bubble at a time to pass. The suspension weight on the gasometer was so regulated for this purpose, that the water-seal round the glass dome showed the water to be about one centimetre lower inside the dome than outside.

The remaining guinea-pigs were placed as controls in the ordinary animal house.

Next morning an analysis of the gas gave Carbon dioxide, nil; Oxygen, 60 per cent.

The current from the exhaust was slightly increased, and two days later an analysis gave Carbon-dioxide, 1·4 per cent.; Oxygen, 59 per cent.

The weight of the guinea-pigs on taking out showed an increase to 1,345 grams. They were again placed in the oxygen chamber, and the oxygen percentage raised to 66 per cent. The gas analysis next day gave Carbon-dioxide, 0·8 per cent.; Oxygen, 60 per cent.

The succeeding day, the percentages were Carbon-dioxide, 1·2 per cent.; Oxygen, 61·0 per cent.

The chamber was again cleansed next morning and the experiment restarted. This course of treatment was continued during the whole period with short intermissions for cleansing, the percentage of oxygen not rising above 68 per cent. or falling below 50 per cent., and the Carbon dioxide not rising above 2·8 per cent. on any occasion.

During the third week one of the guinea-pigs in the chamber died of natural causes. Small glands in the groin were found, post mortem, also *Bacillus tuberculosis* at the site of inoculation.

The lungs were healthy. This animal was replaced by one of the controls in the latter part of the experiment.

At the end of the fifth week, the guinea-pigs were showing signs of

loss of weight, and were killed and examined and compared with the controls kept in the animal house.

All three animals kept in the oxygen chamber showed definite tubercular lesions, consisting, in two cases, of sinuses discharging pus, enlarged caseating glands in the groins and axillae, and general emaciation. The controls also showed definite lesions of a similar character.

No definite difference could be observed between the stages of the lesions in the controls and treated guinea-pigs, and it was impossible to say that either was the more advanced or serious.

A number of other experiments were carried out under the same conditions, but none were continued for so prolonged a period, owing, usually, to accidental death of the tested animals, but all the results pointed to the same conclusions.

CONCLUSIONS

1. That the inhibiting effect of the increased oxygen percentage on the growth of the *Bacillus tuberculosis* in vitro cannot be extended to treatment in vivo.

2. That the beneficial effects of fresh air and ventilation on strumous diseases are not due to the small increase of oxygen over that of the interiors of dwellings, or to any chemical condition of the air, but rather to some physical stimulus.

SECTION C. ACTION UPON THE MAMMALIAN LUNG OR OXYGEN PNEUMONIA

As stated in the previous section, it was found that while oxygen up to a partial pressure of about 500 millimetres of mercury could be readily tolerated for days together without any observable ill effects, a slight increase to about 550 millimetres always led to the animals perishing from a condition resembling lobar pneumonia. The limit bearable without injury for prolonged periods thus lies just below seventy per cent. of oxygen in an atmosphere at 760 millimetres of total pressure.

This pneumonic effect has been observed by several previous observers, but in most cases it has been studied in cases where the total pressure has been raised to many atmospheres, and the duration of the experiment has been a short one.

As the subject has some importance now that oxygen is becoming popular in the treatment of disease, and also has relationship to caisson work, rescue work with oxygen apparatus where oxygen may be breathed for long intervals and similar uses of the gas for respiration in confined or vitiated spaces, it was determined to experiment more fully and find the limits of causation, show freedom from any bacterial causation, and obtain micro-photographs showing the nature of the lesions and the minute pathology. It was found by several observers in early days after the isolation of oxygen that respiration of the gas produced inflammatory effects. The respiration and circulation were both found to be increased, in time the lungs became congested, and in certain cases inflammation and death occurred. The adherents of the school of Lavoisier set these effects down to the effects of increased pulmonary combustion, but this was controverted by Regnault and Reiset, who showed that no increased oxidation took place. It was Bert who introduced the fact that the pathological effects due to high pressures were dependent on the partial pressure of the oxygen only, by demonstrating that as much effect was obtained with four atmospheres of pure oxygen as with twenty atmospheres of compressed atmospheric air.

Bert worked chiefly with very high pressures, and found that these induced a status epilepticus in birds, and also in mammals at a higher pressure. He compared the effect to that of tetanus or strychnine poisoning, and thought it due to a toxic action on the nervous system either directly by the oxygen or indirectly by some nerve intoxicant formed by the action of the oxygen on the blood. The symptoms persisted for hours after brief exposures to high oxygen pressures, the animals remaining in an epileptic condition and having fits of tetanic nature at short intervals. Blood transfused from such animals did not, however, induce the condition in normal animals.

The animals in Bert's experiments succumbed to the nervous effects before the lungs became pneumonic, and he does not appear to have investigated the effects of lower concentrations of oxygen acting for longer periods. After a considerable interval, this was taken up by Lorrain Smith¹, who also worked with a pressure chamber, but used much lower pressures than Bert. In Bert's experiments the convulsions occurred at about 3 atmospheres of oxygen with birds, and 4 atmospheres with dogs; in Lorrain Smith's work while some experiments were carried

1. *Journal of Physiology*, Vol. XXIV, p. 19, 1899. We are also indebted to this paper for the materials for the brief resumé of the literature given above.

out at 3 atmospheres or slightly higher, the greater number were carried out at a level lying between 1 and 2 atmospheres of oxygen, and two were performed at pressures between 70 and 80 per cent. of an atmosphere of oxygen.

An important point made out by Lorrain Smith was that previous exposure to an intermediate pressure (1·4 atmosphere) acted to a certain extent as a preservative against the onset of convulsions, which did not occur at the later stage until a higher concentration for a longer interval. This is ascribed by Lorrain Smith to the previous lower exposure reducing or upsetting the normal secretory activity of the lung epithelium, which when uninjured causes the blood to be more highly and rapidly charged with oxygen.

In the first experiment recorded by Lorrain Smith, the percentage of oxygen was only 41·6 of an atmosphere, and this caused no injury within eight days, our own results confirm this finding. In his second and third experiments the percentages were 73·6 and 79·9 respectively. In each two mice were employed, and in each one succumbed in four days with more or less marked congestion of the lungs, while two of the animals survived for a week.

All the other experiments recorded in Lorrain Smith's paper are with amounts of over an atmosphere of oxygen, and more results seemed to us desirable between 70 per cent. and one atmosphere of oxygen.

The fact of working with a small pressure chamber precluded also the use of large animals, and with the exception of one short experiment at a fairly high pressure on a guinea-pig, and a brief one on a rat, all the experiments recorded by Lorrain Smith were made upon mice or small birds.

The pneumonia produced in these experiments with over one atmosphere of oxygen pressure, as described by Lorrain Smith, was identical with that described in this paper. Bacteria in many cases were entirely absent. The alveoli were filled with an exudate which was granular and fibrillated in appearance, but did not give the fibrin test by Weigert's method. There were no leucocytes in the exudate. The pneumonic condition was universal, and could be compared only with the earliest stages of catarrhal pneumonia.

The onset of the pneumonia and time of death, varied with the percentage of oxygen, as stated above in his two experiments with between 70 and 80 per cent., death occurred, in two cases out of four only, in 4 days; at 120 to 130 per cent., sluggish in 48 hours and found dead

at 90 hours, lungs pneumatic; at 128·9 per cent., sluggish in 48 hours, died in about 69, lungs congested and consolidated; at 129·7 per cent., dead in 40 hours, similar appearances; at 114 per cent., dead in 60 hours, consolidated lungs; at 182·9 per cent., one death in 23 and one in 27 hours; at 176·6 per cent., deaths within 24 hours; at 188·5 per cent., death in 7 hours; at 285 per cent. death in $8\frac{3}{4}$ hours; at 357 per cent., death in 5 hours with the characteristic consolidation and congestion of the lungs.

The filling of the lungs with exudate in such a short interval as five hours appears to us to be a most remarkable and interesting result, and seems to point to the exudate being a kind of pathological secretion by the lung epithelium, rather than an irritative exudation due to injury of that epithelium.

Observations have also been made by Leonard Hill¹ and J. J. R. Macleod showing that prolonged exposure of mice to a partial pressure of oxygen amounting to 70 to 80 per cent. of an atmosphere produces inflammation of the lungs, but 80 to 90 per cent. of oxygen used for two hours or more with the Fleuss rescue apparatus by miners in training for rescue work produced no ill results whatever. This has also been our own experience in this laboratory, in breathing oxygen of 80 to 90 per cent. from a large gas-holder before giving it to patients as mentioned above. The carbon-dioxide was fully taken up by a series of four annular trays in the lower part of the gas-holder, into and out of which the patient breathed by a very wide tube without the interposition of any valves, and no inconvenience whatever was ever experienced.

In the experiments here to be recorded with guinea-pigs, no total pressure greater than atmospheric was used throughout, the animals had plenty of food in a large respiration chamber sufficiently roomy for them to move about, the carbon-dioxide was efficiently removed by the soda-lime, and as much oxygen as required was sucked in as the animals used it, and enough extra supply was drawn in as described in a previous section by the suction pump to keep up the desired oxygen percentage. The oxygen appeared to increase appetite in the earlier stages, and the animals ate their food greedily and seemed to suffer from no inconvenience.

EXPERIMENTS XI, XII, XIII

Three guinea-pigs were placed at the same time in the respiratory chamber under the usual conditions described already. The history of each animal is separately given in the following account:—

1. See *British Medical Journal*, Vol. I, p. 71, 1912.; *Proc. Roy. Soc.*, Vol. LXX, p. 455, 1902; *Journal of Physiology*, Vol. XXIX, p. 492, 1903.

Experiment XI.—The guinea-pig was placed in the chamber and the oxygen was slowly raised to 79 per cent. Next day the oxygen stood at 88 per cent., the carbon-dioxide at 1·5 per cent. The animal seemed quite well, and was eating and lively. On the third morning the oxygen was 79 per cent., and carbon-dioxide 1·6 per cent. The chamber was cleansed out, the guinea-pig was in the air outside for thirty minutes during this operation, and the oxygen was raised to 70 per cent. On the fourth morning the animal was found dead. The oxygen stood at 75 per cent., and carbon-dioxide at 0·76 per cent.

A post-mortem examination was at once made with the following findings:—

(a) *Microscopic Examination.*—The lungs showed a markedly acute active congestion, which was in patches in certain places. All the lobes were so affected, and the tissue sank in water. The heart showed the distended and engorged condition of an asphyxial death.

(b) *Microscopic Examination.*—The sections of the lung when cut and stained showed the extraordinarily acute active congestion demonstrated by the first micro-photograph accompanying this paper. The capillaries are seen to be dilated to three or four times their normal diameter. There is a slight exudate in the alveoli, and a few leucocytes. The epithelium of the bronchi is somewhat desquamated, and there are traces of proliferation. By Weigert's stain traces of fibrin were found in some of the alveoli.

Bacteriological smears and cultures on various suitable media were made from the cut lung, as well as from the exudate in the smaller bronchi, and all these were found to be negative.

The cause of death was an acute lobar or catarrhal pneumonia, suppressing the respiratory function of the lungs.

Experiment XII.—The second of the three guinea-pigs in the chamber being treated in the same manner, was noticed, when the preceding one was dead, to be suffering from dyspnoea. At 10 a.m. on the fourth day from the beginning of the experiment, the respirations were 120 per minute, the breathing was laboured, and the animal did not eat and avoided movements. At 2 p.m. this animal succumbed, and it was found that the oxygen was then 76·3 per cent., and the carbon-dioxide 0·5 per cent.

The examination gave identical results with those described in the previous case, the micro-photograph shows the extreme condition of congestion in the lungs.

Experiment XIII.—The third guinea-pig in the chamber with the preceding two was removed from the oxygen chamber at 2 p.m. on this same fourth day. It was then very dyspnoeic; respirations, 120 per minute, with heavy respiration, but not quite so pronounced as in the last experiment. It was removed to the animal house and placed in a straw bed. Next morning it was somewhat recovered, but still had not eaten and remained still. In the afternoon it began to eat a little, and was moving about freely in two days' time, having made a rapid recovery. Four days later, this animal was killed in order to examine the condition of the lungs. These showed, microscopically, a congestion in patches, but a considerable amount of air-space was present, and pieces of the lung now floated in water, instead of sinking as in Experiments XI and XII.

Microscopical Examination.—The lungs showed patchy congestion, alveoli generally full, capillaries distended, epithelium of bronchi desquamating and proliferating. Catarrhal cells show many fat vacuoles in the cytoplasm; the nuclei stained well and were displaced to one side. (See Micro-photograph No. 3.)

Experiment XIV.—Three guinea-pigs were placed in the respiratory chamber, and the percentage of oxygen was raised to 69. The next morning the oxygen had risen to 74 per cent., and the carbon-dioxide 0·6 per cent. The guinea-pigs were active and well on the third day, when analysis showed: oxygen 71 per cent., and carbon-dioxide 1 per cent.; and on the fourth day, before cleaning out the chamber, it was found that the oxygen was 68 per cent., and carbon-dioxide 1·3 per cent.

The chamber was now cleansed and the guinea-pigs, which were not so active or feeding so well as usual, were replaced, and the oxygen raised to 72 per cent. Next morning (fifth day), one animal was found dead: the oxygen standing at 69 per cent., and the carbon-dioxide at 0·3 per cent. During this fifth day the two remaining guinea-pigs showed evident signs of the effects of the oxygen in their respiration and general appearance, and the second one died next morning (sixth day), and the third one succumbed in the same afternoon (sixth day.) The oxygen on analysis showed now a strength of 72 per cent., and the carbon-dioxide stood at 0·6 per cent. The examinations of the tissues showed a pneumonia of a slightly more chronic type than that of the previous experiment (see Micro-photograph No. 4, from the second of these three animals).

From the results of these experiments it was concluded that

about 70 per cent. of oxygen was the minimum which was necessary to cause this pneumonic flooding of the lungs, and this was found in practically every succeeding experiment to be the result; for if the strength of the oxygen dilution rose over 70 per cent. for a day, the guinea-pigs invariably showed symptoms of lung mischief; whereas, with the lower dilutions, leaving out accidental causes of death, the guinea-pigs progressed favourably and were free from symptoms. Such results were obtained in four similar experiments to the above, in which about 70 per cent. of oxygen was used, while in an equal number with 60 to 65 per cent. of oxygen no pneumonic effects were observed.

CONCLUSIONS

1. That oxygen in any percentage at atmospheric pressure can be inhaled for *short* periods without ill effects.
2. That at percentages below 70 per cent. it can be inhaled for prolonged periods without giving rise to any symptoms or pathological signs.
3. That above 70 per cent. its use for a prolonged period is attended with serious risk of causing an irritative pneumonia, and if persisted in, produces death.
4. That the pneumonia is of a lobar and catarrhal character and due to the effects of the oxygen and not to any micro-organism.

In conclusion, I desire to express my thanks to Professor Benjamin Moore for the suggestion of the research, and his kindness and unfailing assistance at all times during its progress; also to Professor Ernest Glynn for much valuable advice on the pathological changes in the lungs, and to Dr. Stenhouse Williams for his assistance and advice.

EXPERIMENT XI

EXPERIMENT XII

EXPERIMENT XIII

EXPERIMENT XIV