THE PATHOLOGICAL EFFECTS OF ATMOSPHERES RICH IN OXYGEN.

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Plates 20 to 23.

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Studies of the pathological effects of oxygen administered under various conditions have not been so numerous or exhaustive as the investigations of its chemical and physiological relations. The fact that oxygen is being used freely as a therapeutic agent, as a prophylaxis against asphyxia in anesthesia, as a protective agent in certain industries, in some forms of deep sea diving apparatus, and in high ascents into the air, makes the study one of practical importance. Even today there is much difference of opinion as to how far oxygen plays a part in the production of some of the manifestations of caisson disease. Furthermore, oxygen is used extensively in respiration chambers, such as those of the Carnegie Nutrition Laboratory, but the percentage of oxygen in the atmosphere of the chamber is rarely that of out-door air; i.e., 21 per cent, yet it is rarely below 18 or above 24 per cent. The present study deals with the effects on the organism of high oxygen partial pressures (80 to 96 per cent) under ordinary barometric pressure, particularly from the point of view of pathological anatomy and histology. The brief extracts from the literature are taken from the papers that bear directly on the problem in hand.

According to Schmiedehausen (1), Lavoisier shortly after his discovery of oxygen in the atmosphere demonstrated that pure oxygen produces congestion and even inflammation of the lungs. With the introduction of ether and chloroform, the use of oxygen to combat asphyxia began and in later times deep sea diving and the use of oxygen in various industries turned the attention of physiologists and pathologists to studies of the effects of oxygen inhalation. Paul Bert
(2) emphasized the effects on the nervous system of high atmospheric pressures, but apparently overlooked the effects on the lungs. In contrast to this, Lorraine Smith (3) pointed out the inflammatory reaction in the lungs of mice and also called attention to the appearance of moderate congestion in the abdominal viscera. Smith claims, however, that fibrin and leukocytes play no part in inflammation. He found that 40 per cent oxygen for 8 days did not produce pneumonia, but that 80 per cent killed 2 mice in 2 days, while 2 others survived unharmed. An average pressure of 125.3 per cent oxygen killed mice in an average of 64 hours; 180 per cent oxygen killed in 24 hours; and 300 per cent oxygen produced pneumonia in 5 hours. Heller, Mager, and von Schrötter (4), in their extensive studies, ignore pneumonia as a factor in caisson-disease, but Hill and Macleod (5) confirm the findings of Lorraine Smith in regard to pneumonia; they agree, however, with Paul Bert and von Schrötter that in caisson-disease the liberation of compressed gases in the tissues is the most important pathological effect. Hill and Macleod call attention to a considerable variation in individual resistance to oxygen poisoning, "but the larger animals seem just as susceptible as mice." They also state that in regard to production of pneumonia their animals were somewhat more resistant than those of Lorraine Smith.

Schmiedehausen (6) in a series of experiments with 13 animals (2 mice, 3 rabbits, 8 guinea pigs) used pure oxygen by tracheal cannula in 2 animals and high percentages of oxygen in chambers for the rest. His pathological findings are not critically described, but he finds in a general way hyperemia, more or less marked atelectasis, focalized edema, and inflammatory processes. He lays much stress on minor changes after short exposures, but demonstrates no true pneumonia in less than 69 hours' exposure. Schmidt and David (7) caution against the too free or prolonged use of oxygen in chloroform anesthesia. They state that percentages of oxygen as low as 40 to 60 may produce inflammatory changes in the lungs after 70 hours' exposure. David (8) confirms the work of Schmiedehausen and suggests the use of high oxygen partial pressures for the purpose of inducing a pulmonary hyperemia for therapeutic purposes. Bornstein and Stroink (9), working principally with rats under increased barometric pressure, in order to determine the advisability of the use of oxygen by divers, state that high pressures (5 atmospheres of oxygen) for periods as short as 2 to 4 hours produce alveolar edema, desquamation, and slight hemorrhage. They call attention to the fact that the lung findings are similar to those of slightly irritating gases, such as ether, and state that in their animals no organs except the lungs show changes. They state that lower pressures of one to three atmospheres for 20 to 48 minutes are harmless. Schmidt and David (10) regard Bornstein and Stroink's work as confirmatory of their own.

The purpose of the present study is to examine the thoracic and abdominal viscera, as well as the hematopoietic system, in an effort
to explain the more obvious results in the lungs. There is included also a critical study of the pathological histology of the lung changes in comparison with the lungs of control animals. Throughout the study the most careful attention has been given controls, instead of placing any great reliance on the theoretical condition called normal. As will be seen from the reports on different organs, this precaution, particularly in studies on the rabbit, is of extreme importance.

For the purpose of the study the animals were placed in the small animal apparatus devised especially for this investigation by Benedict (11). A supply of carrots sufficient for three days was placed in the chamber and replenished in those cases where the experiments were considerably prolonged. No animal at autopsy showed an empty stomach. Water was supplied freely at all times. Brünig (12) claims that the effects reported by Bornstein and Stroink (13) are due to excessive dryness of the atmosphere rather than its rich oxygen content, but the Benedict apparatus provides for moistening the air.

As the study progressed the details of the examinations varied somewhat. In a few of the earlier experiments animals from stock were placed in the chamber and autopsies performed as soon after death as possible, or the animals were removed from the chamber on exhibition of marked dyspnea, killed by a blow, and the autopsy was performed. It was soon considered advisable to examine the urine before exposure in the chamber and to exclude from the study all animals with albuminuria. The examinations increased in complexity, however. Each animal before entering the chamber was examined for albuminuria, complete blood counts were made, clotting time was determined, hemoglobin estimated, and the percentage of reticulated erythrocytes estimated. The animals that were removed alive from the chamber were examined in the same way, and in addition a sufficient amount of blood was removed from the femoral artery to permit determination of the erythrocyte resistance to hypotonic salt solutions. In spite of the fact that frequently examinations for

\[1\] Bornstein later wrote to the *Deutsch. med. Wchnschr.* (1912, xxxviii, 2035) maintaining that his controls were sufficient to overcome the objection made by Brünig, to the effect that dryness of the atmosphere in the oxygen chamber produced the pneumonia.
ATMOSPHERES RICH IN OXYGEN

albumin were made on two successive days preceding the exposure to high oxygen, the autopsy on several occasions showed chronic nephritis. This matter will be discussed later.

Twenty-one of the 55 rabbits studied were control animals. Seven of these were in room air in a cage the same size as the special chamber and under the same conditions as to food as those in the chamber. The other controls were in the chamber, with oxygen kept at a low percentage; 6 animals at 21 per cent, 2 at 23 per cent, 1 at 27 per cent, 2 at 28 per cent, and 2 at 31 per cent. One control animal was taken directly from stock.

The pathological examination includes gross and microscopical examination of the heart, lungs, liver, kidney, adrenal, spleen, lymph nodes, and bone marrow, as well as gross examinations of the aorta, stomach, and intestines. Blocks were placed in Zenker fluid and in 10 per cent formalin solution. The routine stain was hemalum and eosin, and in addition the lung sections were stained with the Mallory connective tissue stain for fibrin; numerous frozen sections of the heart, kidney, and liver were stained with Scharlach R for fat.

The pathological findings were at first grouped according to finer grades of difference in oxygen percentage; namely, 70 to 75 per cent, 76 to 80 per cent, 81 to 85 per cent, etc. It was found, however, that these minor changes in percentage were not of apparent importance and that a grouping of 60 to 80 per cent and 80 per cent and higher gave results that were easily distinguishable. The following description of organs is confined to animals which had been exposed for varying periods of time to atmospheres containing 80 per cent and more oxygen. Further studies of lower percentages are now in progress. As will be seen, the examination of these organs is constantly compared with that of the control animals.

Heart.

The examination of the hearts of 19 control animals showed numerous departures from the normal, particularly in the presence of fat in the muscle, foci of chronic interstitial myocarditis, chronic fibrous pericarditis, and in 2 of the 19 cases slight dilatation.

Hearts of Animals in High Oxygen Partial Pressure.—The results of the examination of 26 hearts in this series can be condensed into
the following statements, with the reservation that the changes noted in the control hearts were also found in the oxygen hearts in practically the same proportions.

First, of 9 animals that died in the oxygen chamber the hearts of 7 showed notable dilatation either of the right or of both sides. Two other animals that survived respectively 2 and 5 days' exposure to high oxygen atmospheres also showed bilateral cardiac dilatation. Second, as exposure to high oxygen becomes prolonged, cloudy swelling, meaning loss of transverse striations and coarse granulation in the protoplasm of the fiber cell, becomes practically uniform. Several cases of acute non-suppurative interstitial myocarditis are found in the hearts of high oxygen animals, but these are not sufficiently frequent to be of significance. Of 16 examinations for fat in the high oxygen animals 14 resulted positively as compared with 7 in 11 normal animals,—a difference probably within the margin of error of biological experimentation, but possibly associated with the practically constant cloudy swelling noted above. Litten, Naunyn, Nasaroff, Werhovsky, and Welch have shown that exposure of animals for several days to a temperature of 40° C. results in fatty degeneration of the heart muscle (14). In none of our experiments did the environmental temperature rise above 25° C.

Aorta.

The aortas of 24 animals were examined grossly and arteriosclerosis was found in 3 cases, all of which were controls. It can therefore be stated that no notable effect is produced in the aorta.

Lungs.

Grossly the lungs of the so called normal animals have the normal color and consistence, and crepitate throughout. Occasionally areas of slight congestion are observed posteriorly at the base of one or both lungs (3 animals). One animal showed marked distention of the lung, and a few minute subpleural hemorrhages.

Microscopically the picture with three exceptions is practically constant. The larger blood vessels exhibit wide open lumina containing blood corpuscles; in some of the smaller vessels the lumina
contain fine acidophilic granules, probably the precipitated protein of the serum. The bronchi are of normal size and contain a small amount of granular precipitate, which does not stain for fibrin, but which immediately overlies the epithelium. The epithelium shows well marked granulation of the protoplasm and in many cases contains mucin. Desquamation of epithelium is frequently seen and occasionally there occur small mononuclear cells, probably lymphocytes, in the lumina of the bronchi. In small foci the alveoli are distended and the walls thin, but the lungs for the most part show well marked capillary congestion, even to a point where the capillaries appear as small bullae projecting into the alveolar spaces. Leukocytes are numerous in the capillaries and in some cases form a striking part of the picture; they sometimes appear within the alveoli. The alveolar epithelium is normal except that occasional desquamated cells showing cloudy swelling are seen. Alveolar edema cannot be demonstrated. In three animals the alveoli are seen to be markedly distented, but the bronchi remain the same as in the other lungs. Throughout the series small lymph nodes are found in relation to the bronchi, made up principally of small lymphocytes with a few large mononuclears included.

Lungs of Animals in High Oxygen Partial Pressure for Approximately Twenty-Four Hours.—These 6 lung specimens show little material departure from the normal except that congestion is slightly more marked (Fig. 1, Rabbit 10). Leukocytes are apparently not more numerous than in many normal lungs. Cloudy swelling and desquamation of alveolar epithelium are seen in all these lungs, and in one lung (Rabbit 13) there is a small area of consolidation made up largely of desquamated alveolar epithelium, which has fused in places to form giant cells. The bronchi show no material changes, but the lymph nodes are enlarged and contain many large mononuclear cells.

High Oxygen for Approximately Forty-Eight Hours.—Of the 9 lungs studied in this series, 5 (Nos. 19, 29, 30, 44, 54) show no more change than do the lungs of the preceding group. Rabbit 18 shows in addition a small focus of desquamative bronchopneumonia similar to that seen in Rabbit 13, but showing more fibrin and leukocytes. In Rabbits 20 and 21 alveolar and bronchial desquamation are marked and numerous alveoli contain a small amount of fine granular acidophilic precipitate in addition to a moderate deposit of fibrin strands. In Rabbit 4, which died in the oxygen chamber, the edema and fibrin are richer, occasional alveoli being almost filled with fibrin. Numerous polymorphonuclear leukocytes appear in the alveoli. As has been already indicated, these differences cannot be accounted for by minor differences in oxygen percentage.
High Oxygen for Approximately Three Days.—Of the 6 animals forming this series the lungs of all save one show serious alterations. In Rabbit 23 intense congestion is present, but the changes are not more marked than those pictured in Fig. 1. All the others show somewhat varying degrees of edema, epithelial desquamation, exudate of leukocytes, and fibrin formation,—in other words, varying degrees of pneumonia. Of the 5 lungs showing this inflammation only one shows complete filling of the alveoli, and in this case the edema occupies most of the space. The bronchi show cloudy swelling of the epithelium, but little mucin formation; there is infiltration of polymorphonuclear leukocytes into the wall and into the lumen; there is marked epithelial desquamation and some of the bronchi show fibrin formation and an occasional erythrocyte. The peribronchial and perivascular lymphatics are frequently the seat of edema. The blood vessels of all kinds and sizes are considerably distended. The alterations in the alveoli are shown in Figs. 2, 3, and 4. They consist of cloudy swelling, and occasionally fatty degeneration of the attached epithelium, desquamation, advanced cloudy swelling, and necrosis; there is moderate infiltration of polymorphonuclear leukocytes and lymphocytes; edema appears as a fine acidophilic precipitate, occupying usually only small areas in the alveoli, but in one case (Rabbit 49) filling the alveolar spaces; fibrils, nodules, and networks of fibrin appear usually in irregularly disposed foci, occupying sometimes a small part, sometimes all of an alveolar space. The fibrin has the typical histology, is acidophilic, and takes the red stain by the Mallory connective tissue method.

High Oxygen for Approximately Four Days.—Of the 4 animals comprising this series one (Rabbit 56) shows only slight changes in the lungs, two (Rabbits 1 and 2) show exudation as seen in most of the lungs of the preceding series, and one (Rabbit 5) shows marked edema in addition to the inflammatory exudate and is similar to the lung of Rabbit 49 of the preceding series. It can safely be said that exposures of approximately 4 days produce practically the same alterations in the lungs as are seen at the end of 3 days.

Rabbit 6 survived 4 days and 20 hours in 83 per cent oxygen. Grossly the lungs were mottled with small areas of congestion. Histologically the lungs show the same marked congestion, epithelial desquamation and degeneration, leukocyte infiltration, moderate edema, and fibrin formation observed in lungs of shorter exposure to high oxygen atmospheres. Two notable additional changes are observed; namely, slight clumping of fibrin in a few alveoli and in a few instances perivascular edema with leukocyte infiltration.

Rabbit 3 survived 7 days’ exposure to an atmosphere of 82 per cent oxygen. Grossly the lungs showed mottled areas of congestion and well marked edema. Histologically the lungs show the same changes as Rabbit 6, including the clumping of fibrin and the perivascular edema. The alveolar edema is somewhat more marked than in the preceding animal.

To summarize the examination of the lungs it can be said that exposure for 24 hours to atmospheres containing 80 per cent and
more oxygen produces little material change in the lungs. Exposure of approximately 48 hours may show nothing of moment or may lead to the development of marked congestion, edema, and early fibrinous bronchopneumonia. Exposure of approximately 3 days leads almost constantly to fibrinous bronchopneumonia which shows little change after 5 or 7 days.

The inflammatory process in the bronchi, the marked desquamation of alveolar epithelium, associated with fibrinous exudation into the alveoli, establishes the diagnosis of fibrinous bronchopneumonia. At no stage that we have observed does there appear the rich infiltration of leukocytes seen in true croupous or lobar pneumonia or in the advanced bronchopneumonia of man.

Grossly the pneumonic lungs showed an extremely variable distribution of the consolidated areas. Sometimes the process was diffuse, but even here the sharp edges of the lung frequently escaped the process; more commonly the process was irregularly distributed in small foci, 4 or 5 to the lobe. The color of the pneumonic areas was usually a deep red or bluish red, but a few areas were reddish gray. The cut surface was constantly moist and from it could be expressed frothy, salmon colored, limpid fluid. Sometimes small blocks sank in water, and others floated.

Liver.

The livers of 19 control animals were examined; 7 are absolutely normal, and 7 more show such slight changes as to be regarded as practically normal. The pathological alterations in the controls include perilobular fibrosis, coccidiosis, focal necrosis, hemosiderosis, hydric degeneration, and in one case distinct central congestion.

Livers of Animals in High Oxygen Partial Pressures.—These organs also show the changes enumerated above in several instances, but the general picture can be presented in the statement that the principal change in the livers of animals exposed to high oxygen atmospheres is passive congestion, which may or may not be associated with hemosiderin pigmentation. This change appears in 24 hours, but is not marked until about 72 hours have elapsed, and thereafter is associated with intercellular edema, indicated by a deposit of albuminous granules between liver cells and between cell columns and sinusoidal endothelium.
Stomach and Intestine.

The gross examination of these organs showed the presence of food in all stomachs. Congestion was noted in 4 of the oxygen animals and in none of the controls. Submucous hemorrhages were found in the stomachs of 4 oxygen animals and in one control. An additional oxygen animal showed submucous hemorrhages in the upper ileum.

The gastro-intestinal tract, therefore, takes part in the general congestion found in the animals exposed to the high oxygen atmospheres.

Kidneys.

The histological examination of the kidneys of the control animals shows normal glomeruli except that in 8 of the 19 cases the loops of the glomerular tuft are unusually well filled with erythrocytes. In 4 kidneys congestion is more extensive. In 11 kidneys examined for fat, 9 show it in the loops of Henle, and in 4 of these there is additional involvement of the convoluted tubules. In 2 kidneys no fat can be demonstrated microscopically. Seven kidneys show chronic interstitial nephritis, and 4 show foci of lymphoid and plasma cells in the interstitial tissues, these changes being combined in two cases (15). Sixteen animals were examined for albuminuria before being used as controls and also after they had been in the open cage or in the chamber with practically normal oxygen content at the Nutrition Laboratory, and 5 of these on later examination showed albuminuria. Histologically, the kidneys from these 5 show chronic interstitial nephritis. Two kidneys showing chronic interstitial and one showing subacute interstitial nephritis excreted urine which was negative for albumin by the tests employed.

Kidneys of Animals in High Oxygen Partial Pressure for Approximately Twenty-Four Hours.—Six kidneys were studied in this series, and 5 show well marked general congestion. All show glomerular tufts well filled with blood. Of 3 animals examined for albuminuria, 1 was positive, the same animal being the only one of the 6 which shows a well marked chronic interstitial nephritis. Two kidneys (1 of them from the case of chronic interstitial nephritis) show albuminous degeneration to a slightly more marked degree than was found in the controls. Five were stained for fat, 4 show fat in the loops of Henle, and in 1 of these the fat appears throughout the cortex.
High Oxygen for Approximately Forty-Eight Hours.—Of the 9 kidneys in this series all but 1 show moderate or marked congestion. The subcapsular spaces of one animal show albuminous precipitate. Cloudy swelling of the epithelium of the convoluted tubules is present in all but 2 specimens. Eight specimens were examined for fat, 7 show fat in the loops of Henle, and in 2 of these the fat is found also in the convoluted tubules. The kidney of 1 rabbit shows hyaline casts in the tubules, but there is a coincident chronic interstitial nephritis. Three kidneys in this series show chronic interstitial nephritis, and 3 show subacute interstitial nephritis, the two being combined in two instances, thus making a total of 4 kidneys showing interstitial changes in this series. Albuminuria appeared in 6 of 7 examinations, in 4 instances associated with interstitial changes, in 2 cases with no marked change other than congestion.

High Oxygen for Approximately Three Days.—In this series the glomeruli are normal except for well marked congestion. The convoluted tubules show slightly more cloudy swelling than do those of the control animals. Two kidneys show fat in the epithelium of the loops of Henle. Very slight chronic interstitial nephritis is present in 3 kidneys and marked chronic interstitial nephritis in 1 other kidney. General congestion is marked in 5 kidneys and moderate in 1. Three animals had no urine in the bladder on return from the oxygen chamber. Of the other 3 only 1 showed albuminuria, and its kidney shows very slight chronic interstitial nephritis.

High Oxygen for Approximately Four Days.—Three kidneys of this series were examined histologically and all show marked filling of the tuft capillaries by blood. In 2 of these, albuminous granules are present in the subcapsular space; all 3, however, are the seat of chronic interstitial nephritis, and of these, 2 are also the subjects of subacute interstitial nephritis. Cloudy swelling is distinct in all 3, and 2 examined for fat show it in the loops of Henle. Congestion is marked in all. The one animal with urine in its bladder after return from the oxygen chamber showed no albuminuria.

The kidneys of the animals that survived 4 days', 20 hours', and 7 days' exposure show no additional changes. Congestion is marked in both and the connective tissue is normal in both. The 7 day animal shows fat in the loops of Henle; the other was not examined for fat, and neither had any urine examination.

The important change in the kidney is the appearance of congestion. Cloudy swelling appears to be more distinct in the kidneys of the experimental animals than of the controls, but this is such a fine change that its importance can easily be overestimated, particularly when the controls show minor degrees of the same process, evidently as the result of the histological technique employed. Albuminuria appeared in 8 cases, and in 6 of these the corresponding kidneys show some degree of interstitial change. Two animals showed albuminuria
after exposure of 48 hours to high oxygen. Their kidneys histologically are free from interstitial change and the greatest probability in so far as the kidney is concerned is that the albuminuria was probably due to passive congestion. Seven negative examinations following exposures of 24 hours or more would indicate that the high oxygen atmospheres, if responsible at all for albuminuria, produce it only occasionally, provided the kidney is normal at the start of the experiment, and then probably as the result of passive congestion. The 6 positive cases of interstitial nephritis are probably to be explained by the superaddition of passive congestion to a kidney already diseased, but diseased so that under ordinary conditions albuminuria did not exist.

Adrenals.

The adrenals of 16 controls were examined, and those of 14 were found to be plump organs well filled with lipoid material. Two were small with almost solid cells very poor in lipoids.

The adrenals of 16 oxygen animals show no changes sufficient to justify their grouping into sequential series. Six of the 16 show congestion in somewhat more marked degree than the controls, and 1 of these shows a small area of hemorrhage into the middle of the cortex. Fourteen show rich lipid content and 2 do not. Two show focal necrosis, 1 in the same degree as exhibited in a control animal, and 1 shows somewhat larger and more frequent foci. This cannot be ascribed to postmortem change, as the control and the 2 oxygen animals were received alive and the organs placed in fixatives immediately after killing; furthermore, the reaction is characteristic of that taking place in living tissues.

It is therefore to be concluded that the adrenals show no change other than congestion as the result of exposure of the animals to high partial pressures of oxygen.

Spleen.

Comparatively little variation is found in the spleens of the controls except that some are more richly pigmented than others, the connective tissue content differs slightly, and the germinal centers of some spleens show more active mitosis than others.
Spleens of Animals in High Oxygen Partial Pressures.—Twenty-three spleens were examined in this series and 16 show well marked congestion. Moderate hyperplasia of endothelial cells in the pulp and sinuses is a frequent finding, but it is not more frequent than in the controls. Phagocytosis of pigment granules and of nuclear fragments is found in both controls and oxygen animals. Eleven of the 23 spleens show a few endothelial cells containing a single or only a few erythrocytes. This change is found in 2 of the control spleens; the same 2 spleens are the only controls to show congestion, and in both series this change does not occur independently of congestion. With the exception of a rabbit which was in the chamber for 2 days and 22 hours, whose spleen shows very active phagocytosis of erythrocytes, the degree of phagocytosis does not appear to be any greater in the oxygen spleens than in the controls. According to the same arguments that will be advanced in consideration of the changes in the lymph nodes and also in the general discussion, the phagocytosis of erythrocytes is regarded as part of the phenomena of congestion rather than specifically the result of the high oxygen exposure. It is therefore concluded that no specific alterations occur in the spleen as a result of the high oxygen exposure other than congestion and its incident changes.

Lymph Nodes.

Attention was not directed to the lymph nodes until after the experiments were well under way. Consequently, the number studied is smaller than is the case with other organs. Fifteen controls show large active germinal centers in all but 1 case. The sinuses show large numbers of endothelial cells, and in 10 of the nodes active mitosis is to be seen in many of these cells. In 8 of the nodes amphophilic leukocytes are to be seen in considerable numbers. Pigment is constantly present and in 4 instances large amounts are found; it occurs principally within large mononuclear and multinuclear cells of the sinuses and to a lesser degree in the large mononuclears and occasional multinuclears in the follicles. In 1 lymph node there is marked phagocytosis of erythrocytes on the part of the endothelial cells of the central sinuses. Five other lymph nodes show after careful search an occasional endothelial cell in which a few erythrocytes have been englobed.
Lymph Nodes of Animals in High Oxygen Partial Pressure for Varying Periods of Time.—Of animals exposed to high oxygen for from 24 to 72 hours 9 groups of lymph nodes were examined. Of these all show the same large proliferating germinal centers as the controls except 2 (Rabbits 24 and 49), but as these animals died in the chamber the appearance of necrosis in the germinal centers and in many of the cells in the sinuses leads one to suspect that this is largely due to postmortem change. All the nodes show considerable amounts of pigment and 5 show markedly large amounts of pigment, distributed in all cases as in the controls. Six cases show phagocytosis of erythrocytes on the part of sinus endothelium, and in 3 of these the process is marked. The most marked case of pigmentation fails to show such phagocytosis.

It would appear, from the material available, that following exposure to high oxygen atmosphere there is somewhat more marked phagocytosis of erythrocytes in the lymph nodes. Three of these cases are associated with pneumonia and 3 are not; 3 are associated with notable cardiac dilatation and 3 are not. All are associated with general congestion as observed in the kidney and spleen, and 3 are associated with similar phagocytosis in the spleen. The process appears to be more clearly associated with passive congestion in other organs than with any other demonstrable change.

Bone Marrow.

In examining histologically the bone marrow of 15 control rabbits, variations are noticeable, particularly in the proportionate amounts of fat and functionating cellular tissue. Eight of the 15 show in the fat spaces an acidophilic granular mass; in the others the fat spaces are clear. General cellular hyperplasia is found in all but 3 animals, in only 1, however, to any considerable degree. Only 1 presents a strictly normal picture, and 2 others are aplastic. One specimen shows slight general hyperplasia and also small foci of necrosis. Mitotic figures are commonly seen and fine golden brown granules of pigment, sometimes intracellular, again in extracellular position, are frequent. All but 3 specimens show a rich blood content.

The leukoblastic and erythroblastic centers, as described by Bunting (16), and the platelet formation as described by Wright (17), can be made out easily. The number of megakaryocytes appears to be extremely variable.
Specimens of marrow from 10 animals exposed to oxygen atmospheres of 80 per cent and above show no distinct departure from the pictures seen in the controls. Two are quite normal. Six show the acidophilic granular mass in the fat spaces. Hyperplasia of all the elements or of either the leukoblastic or erythroblastic centers does not exceed that of the controls. Megakaryocytes and mitotic figures are present in normal numbers. Congestion is marked in 3 specimens (Nos. 23, 24, and 49).

**Erythrocyte Counts.**

Erythrocyte counts were made on 12 controls before and after the experiments. The counts before experiment varied between 5,512,000 per cmm. and 8,460,000 per cmm., but were largely in the neighborhood of 6,000,000. After the experiment 7 showed increases of from 300,000 to 1,990,000 (average 1,012,000), and 4 showed decreases of 456,000 to 1,312,000 (average 768,500). One animal showed no change. The counts were made under as nearly similar conditions as possible, but these variations made it seem unlikely that the oxygen animals might show much that would be distinctive.

Five oxygen animals, the blood of which was counted before the experiment, showed variations of from 5,120,000 to 7,320,000 erythrocytes per cmm. Four animals showed an increase after exposure in the chamber of from 488,000 to 2,788,000 (average 1,313,000), and 1 showed a decrease of 1,080,000. This last animal was in the high oxygen for approximately 4 days, whereas the others varied between approximately 48 and 72 hours.

It would appear that prolonged exposure to oxygen produces no material changes in the erythrocyte count that are not observed in control animals living for similar periods under the same general conditions.

**Resistance of Erythrocytes to Hypotonic Salt Solutions.**

The resistance of the erythrocytes to hypotonic salt solutions was determined by the technique described in a previous communication (18). It was, of course, impossible to obtain sufficient blood for the test before the experiments, hence the control of each test was either
an animal from stock, killed for the purpose, or in several cases where 2 animals were observed simultaneously, 1 in the chamber and 1 in the room air, the latter served as a control. Of 10 control animals tested against an animal from stock, 5 showed the same corpuscular resistance, 5 showed increases in resistance corresponding to 0.050 per cent sodium chloride, and 1 showed an increase corresponding to 0.100 per cent sodium chloride. Of 5 high oxygen animals tested against an animal from stock, 4 showed an increase of resistance equal to 0.050 per cent sodium chloride, and 1 an increase equal to 0.100 per cent sodium chloride. Of 3 animals tested with those that had been for the same time under similar conditions no change in corpuscular resistance could be demonstrated.

*Reticulated Erythrocytes.*

Attempts were made to determine the percentage of reticulated erythrocytes in the circulating blood, but in control animals this was found to vary between 2 and 25 per cent, a variation too wide for satisfactory work. The ground covered by this procedure is also covered by the work with resistance of the erythrocytes to hypotonic salt solutions.

*Hemoglobin.*

Most of the hemoglobin determinations were made with the Tallquist scale, as it was believed that to be of any importance in the rabbit, the hemoglobin difference must be large enough to show on this scale. No such differences could be demonstrated. The Dare hemoglobinometer was used with 2 controls and 2 high oxygen animals. One control showed an increase from 90 to 98 per cent, and the other a decrease from 80 to 72 per cent. Two high oxygen animals showed decreases respectively from 95 to 88 per cent and from 93 to 88 per cent, the first being in the chamber for 2 days, the second for 4 days. The hemoglobin determinations are therefore no more conclusive than the erythrocyte counts.

*Leukocyte Counts.*

The leukocyte count of rabbits is very variable within certain limits, and although all the counts were made at noon or early in the
afternoon (so as not to follow the late afternoon feedings), the 9 controls showed primary counts of from 5,400 to 13,200. After the experiment 4 showed increases of from 1,600 to 7,400 (average 3,950), 4 showed decreases of from 500 to 2,800 (average 2,175), and 1 remained unchanged.

Of the high oxygen animals, 1 (No. 25) showed 19,800 before and 15,100 after the experiment. No. 26, not counted before, showed 4,800 after the experiment. Of 4 others 2 showed slight increases (600 and 1,400) and 2 showed distinct increases (3,000 and 6,800) in the number of leukocytes. These may be tabulated as follows (Table I):

<table>
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<th>Rabbit No.</th>
<th>Before experiment</th>
<th>After experiment</th>
<th>Increase</th>
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<td>7,400</td>
<td>8,000</td>
<td>600</td>
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</tbody>
</table>

Rabbit 25 is excluded as being unsatisfactory because of the high primary count. It is safe to say, however, that Rabbit 26 shows no increase even though no primary count had been made; this animal had a distinct pneumonia. Rabbits 29 and 56 show a slight increase and were free from pneumonia. Rabbits 30 and 48 show marked increases; No. 30 had no pneumonia and No. 48 had pneumonia. It would appear that the increases are not dependent upon the presence of a pneumonia, and with the wide variations seen in control animals, variations which are not exceeded by the oxygen animals, the differences in leukocyte count are probably to be explained only as accidental variations so frequently seen in the rabbit.

**Clotting Time.**

Clotting time was determined by the Brodie-Russel Boggs coagulometer. Thirteen controls and 5 high oxygen animals failed to show any material differences in the clotting time as observed before and after the experimental periods.
DISCUSSION.

A discussion of the results of this work cannot be complete without calling attention to the appearance in rabbits, with a fairly high degree of frequence, of lesions not the result of experimental procedures, the so called spontaneous lesions. Without the study of numerous controls the present study might have led to false conclusions, notably in the matter of fatty degeneration of the heart. Fortunately, most of the non-experimental lesions are of subacute and chronic nature and could not reasonably be attributed to the relatively short exposure in the chamber. There is in rabbits a distinct individual variation in resistance to oxygen, as pointed out by Hill and Macleod (19), and also shown in the present series; these variations might well be accentuated by living through the diseases leading to these non-experimental lesions. There is, however, no final and conclusive evidence to support this view. The chances of error in biological experiments include this individual variation and can only be overcome by the use of a considerable number of animals.

The accuracy of study of the changes in the lungs is favored by the fact that non-experimental lesions of the lungs are rare, but on the other hand individual variations in the development of the pneumonia are fairly well marked and in the study of the lesions in this series of experiments are favored by the number of animals included. As has been shown, the lesion is a fibrinous bronchopneumonia in which edema and desquamation play a prominent part. Lorraine Smith (20) stated that fibrin plays no part in the inflammation, but our studies controvert this statement in so far as the rabbit is concerned. His statement in regard to the unimportance of leukocytes in the process is borne out in our work. The resemblance of this pneumonia to that produced by irritant gases has been noted by Bornstein and Stroink (21), and the resemblance to the earlier forms of pneumonia following nitric oxide poisoning is also to be mentioned. The absence of any well marked leukocytic infiltration in the pneumonic area and the absence of demonstrable leukocytosis in the circulating blood point toward a pneumonia of irritative rather than of bacterial origin. The lesion in oxygen poisoning develops in from two to three days and the individual variation is such that the state-
ment of Schmiedehausen and others, that the pneumonia appears in a certain number of hours, cannot be supported. The German workers particularly call attention to changes appearing in a few hours, but when compared carefully with controls the significance of these minor changes has certainly been overestimated and the percentages of oxygen used in the present series cannot be said to have produced any important changes in less than 24 hours; and exposures of approximately 24 hours have not in our experience produced any notable change other than congestion. The fact that numerous animals showed dilatation of either the right side or both sides of the heart must be considered in reference to the lung changes and the appearance of congestion of the abdominal viscera.

Studies of the influence of oxygen inhalation on circulation have not been numerous. Benedict and Higgins (22) have demonstrated a slowing of the pulse following the breathing of atmospheres containing 40, 60, and 90 per cent oxygen, and the work has been confirmed by Parkinson (23), but no studies were made directly of the pulmonary circulation. Retzlaff (24), by plethysmographic studies, has shown that oxygen produces vasoconstriction in the lungs, and he therefore argues improved pulmonary circulation. He does not show how long the vasoconstriction may persist and hence gives no conclusive evidence as to the effect of prolonged oxygen inhalation on the right ventricle.

If vasoconstriction were prolonged, it is conceivable that the right heart might fail under the demand for increased work, and in this event the pneumonia could be explained as of hypostatic origin. From the pathologist's point of view, the wide-spread involvement of the lungs argues against such an origin for the lesion. The experiments here reported give no ground for assuming that the heart dilatation precedes the pneumonia; indeed the opposite seems to be the case. The appearance of the pneumonia is more constant than of the heart dilatation, and it would appear accordingly that the heart dilatation depends upon the same circulatory difficulties which determine the same complication in human pneumonias, perhaps in these animals also influenced by the vasoconstrictor effect of the oxygen inhalation.

The general passive congestion noted in practically all the abdominal viscera is such as is seen in cases of heart failure and is to be attributed in these animals to the failure of the heart as a whole or
of its right side. Secondary changes such as cloudy swelling and even fatty degeneration can easily be accounted for by the passive congestion. The appearance of pigment in spleen and lymph nodes is so common in the controls and so inconstant in the oxygen animals that it is not to be regarded definitely as a part of the phenomena of the oxygen poisoning.

The appearance of phagocytes of erythrocytes in the lymph nodes and spleen is more frequent in the oxygen animals than in the controls. This might depend upon some disturbance of the hematopoietic balance such as is supposed to take place in splenectomized animals (25), or it may be the result of the passive congestion in these organs. The studies of the resistance of the erythrocytes to hypotonic salt solutions, the studies of the other features of the blood and of the bone marrow fail to show any distinct general disturbance of the hematopoietic system. The livers of these animals failed to show phagocytosis in the proportion of cases which would be expected were the hematopoietic system disturbed as in blood destruction in splenectomized animals. These facts and the fact that controls showing passive congestion also showed similar phagocytosis of erythrocytes in the lymph nodes and spleen leads to the conclusion that in this series of experiments this phenomenon is due to local blood destruction as the result of passive congestion and perhaps also of the small hemorrhages which frequently are seen in cases of passive congestion. It seems improbable, however, that the pigmentation of these organs, which frequently is so extensive, can be accounted for in the same way because of the short duration of the experiments.

CONCLUSIONS.

In spite of numerous abnormalities or non-experimental lesions in the rabbit certain facts can be considered as established. It has been known for many years that pneumonia is produced by the more or less prolonged inhalation of high partial pressures of oxygen. The studies herein reported show that atmospheres containing 80 to 96 per cent oxygen under normal barometric pressure produce in 24 hours, or more commonly 48 hours, congestion, edema, epithelial degeneration and desquamation, fibrin formation, and finally a pneu-
monia, probably of irritative origin and to be described as a fibrinous bronchopneumonia. The important new points are the time relations of these changes and definition of the type of the pneumonia.

Other studies have noted slight passive congestion, but it is now established that this is to be accounted for in most cases by dilatation of the right side or of both sides of the heart. This congestion affects all the abdominal viscera and is accompanied by certain secondary changes such as cloudy swelling of the parenchymatous organs and phagocytosis of erythrocytes by endothelial cells of the mesenteric lymph nodes.

Although deficiency of oxygen may affect the hematopoietic system, the animals subjected to high oxygen percentages failed to show any demonstrable pathologic changes in blood, spleen, lymph nodes, or bone marrow, except for the presence of congestion.

This study is the first of a comprehensive series projected in and under the direction of the Carnegie Nutrition Laboratory. Different animals and various methods of attack will be employed in the investigation.

BIBLIOGRAPHY.

EXPLANATION OF PLATES.

PLATE 20.

Fig. 1. Photomicrograph of the lung, showing marked distension of the capillaries with peripheral position of the leukocytes, but without exudation into the alveoli. This condition is not uncommonly seen in control lungs, but it constitutes also the stage of early congestion in the lungs of animals exposed for a short time to atmospheres rich in oxygen.

PLATE 21.

Fig. 2. Photomicrograph of the lung, showing swelling and granular degeneration of attached alveolar epithelium, also moderate desquamation and granular precipitate within the alveoli.

PLATE 22.

Fig. 3. Photomicrograph of the lung, showing advanced desquamation of alveolar epithelium with slight infiltration of lymphocytes and an occasional leukocyte.

PLATE 23.

Fig. 4. Photomicrograph of the lung, showing rich fibrin formation, enclosing in the mesh desquamated epithelium, lymphocytes, and leukocytes.
(Karauer: Atmospheres Rich in Oxygen.)
Fig. 2.

(Kaiser: Atmospheres Rich in Oxygen.)