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AN EXPERIMENTAL STUDY OF THE BREAKDOWN OF ERYTHROCYTES IN THE RAT LUNG

K. Fani, M.D.

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AN EXPERIMENTAL STUDY OF BREAKDOWN OF ERYTHROCYTE IN THE RAT LUNG

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AN EXPERIMENTAL STUDY OF THE BREAKDOWN OF ERYTHROCYTES IN THE RAT LUNG

PART I

INTRODUCTION

External respiration, the exchange of gases in the lungs with absorption of oxygen in the pulmonary circulation and elimination of carbon dioxide in the expired air, is dependent in part on intact capillaries in the walls of the pulmonary alveoli.

The blood in the alveolar capillaries is separated from the alveolar air by the vascular endothelium, the alveolar epithelium, and their basement membranes. Normally, only gaseous exchange occurs across this membrane. Alterations in pulmonary circulation, anoxia, various noxious agents and pathological changes in the pulmonary parenchyma may break the alveolar capillary barrier or increase the permeability and so lead to the accumulation of fluid, electrocytes, plasma proteins and formed elements of the blood in the pulmonary alveoli.

Accumulation of erythrocytes in the alveolar spaces is noted in a variety of pathological conditions, and here, as elsewhere, the extravasated erythrocytes are known to undergo breakdown with the formation of hemosiderin and hematoidin. However, experimental studies of the mechanisms of erythrocytic destruction in the lung are few. In the present work experiments have been designed to allow observation of the sequence of events taking place when

isologous and autologous blood is introduced into the tracheobronchial tree of the rat. Particular attention has been directed to

i) the rate and mode of breakdown of erythrocytes in the alveoli.

ii) the rate of elimination from the lung of the various products of erythrocytic destruction, particularly hemosiderin.

iii) the effect on the pulmonary parenchyma of the presence of blood in the alveolar spaces.

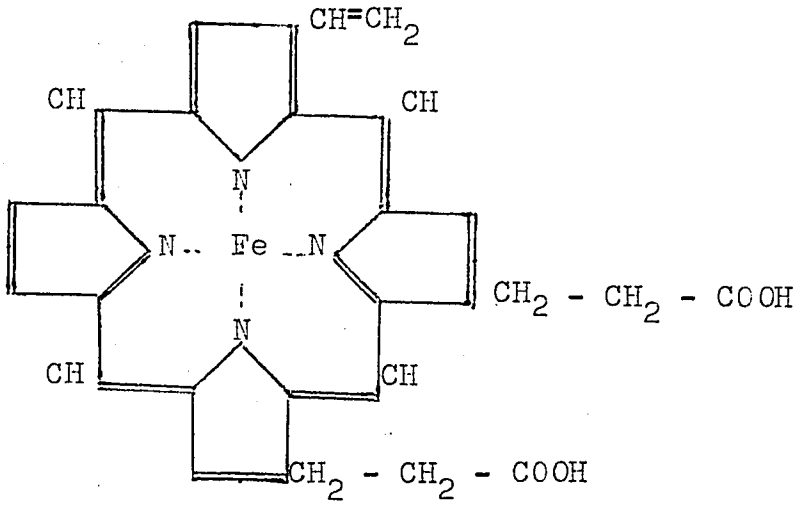
An attempt has been made to correlate certain of the changes observed in these experimental studies to those occurring in humans following intra-alveolar haemorrhage of various etiology.

PART II

STRUCTURE, FORMATION AND BREAKDOWN OF HEMOGLOBIN

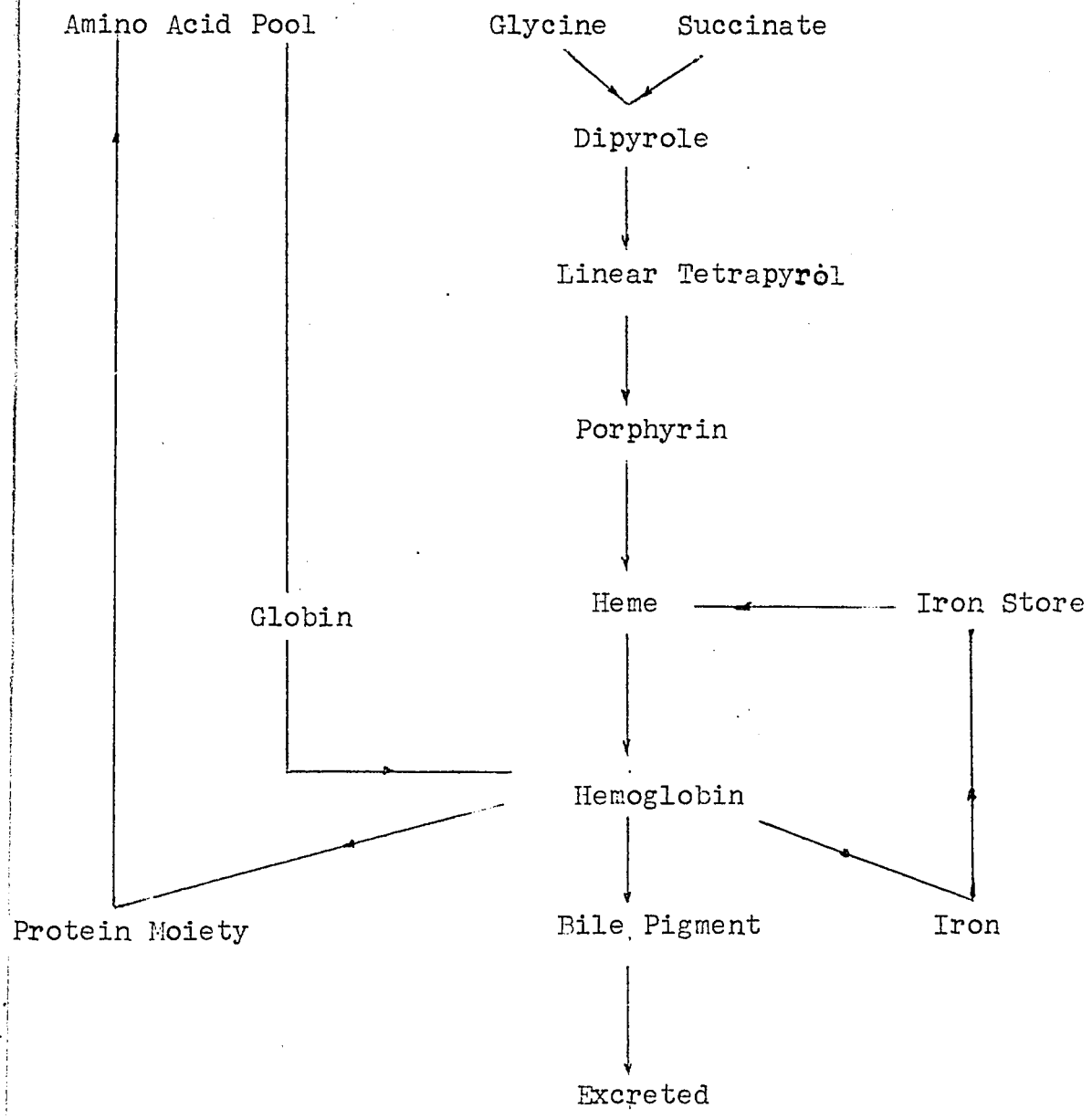
Cantarow[9] describes the formation and structure of hemoglobin as follows:

Hemoglobin is a conjugation of protein, globin and four molecules of heme. Heme is responsible for the colour and oxygen-carrying properties of hemoglobin. Each globin molecule consists of a peptide unit which may be further subdivided into pairs. Heme is a type of porphyrin (protoporphyrin) to which is attached an atom of iron in ferrous form (ferro-protoporphyrin). The difference between the hemoglobin molecules of various animal species resides in the difference between and sequence of the aminoacid molecules. Porphyrin is a cyclic tetrapyrole linked by a methyne (-CH=) bridge to form a large ring structure. Porphyrin contains many side chains attached to the carbon atom in the pyrole ring. Only protoporphyrin III is normally found in nature. Two other histologically important porphyrins are uroporphyrin and coproporphyrin. Protoporphyrins are able to form complexes with iron to form heme. Ingram[19] believes the formula of protoporphyrin to be:-



Insertion of an atom of ferrous iron into protoporphyrin III forms heme. Heme coupled with globin forms hemoglobin.

Over all production and destruction of hemoglobin (modified from Cantarow).



In the catabolism of hemoglobin, the molecule breaks down into iron, globin and protoporphyrin. The iron and globin are re-utilized for synthesis of hemoglobin, and protoporphyrin produces the bile pigment.

Granick[15] states the site of the destruction of the erythrocyte or hemoglobin molecule appears to be in the phagocytes resulting in the digestion of the globin, release of iron from heme and conversion of the ring porphyrin to open chain tetrapyrole (bilirubin). Reserve of iron is to be found in the liver, spleen, bone marrow and elsewhere. One part of the iron reserve is very easily mobilized (labile iron pool) but a greater part is held in a less mobile state. Iron is stored chiefly in the ferritin form. The ferritin molecule consists of a central nucleus of high iron content surrounded by a shell of protein. The electron microscope shows that the iron is held in six micelles of ferric hydroxide arranged at the corner of a regular octahedron. Ferritin is not detectible with iron stains because it is scattered too diffusely in the cells.

Shorr[37] found that ferritin is a strong vasodilatator material and an antidiuretic. Ferritin is a mixture of molecules of varying iron content and in presumably varying states of aggregation. According to Wintrobe[42] the term hemosiderin refers to clusters of iron visible with the light microscope. Among these clusters ferritin molecules and other substances, not yet defined, have been observed by electron microscopy.

The term hematoidin refers to the crystals within the phagocytes. They are chemically identical with the bile pigments.

Concerning the characteristics of hemosiderin Sheldon[36] and Cook[10] state that deposits vary in size from five to 20 microns in diameter. Hemosiderin has a deep yellow colour resembling ochre. Granules have sharp outline and are angular. It is extremely resistant to solvents and acids are the only chemical which attacks it. It is insoluble in alkali. Digestion of the tissue with caustic soda provides the best means of obtaining a pure suspension of hemosiderin. With potassium ferrocyanide and hydrochloric acid it stains dark blue. Iron becomes more firmly fixed with passage of time. The pigment consists of a ferric hydrate with the formular $Fe_2O_3(2H_2O)$ probably existing in the body in combination with organic matter. It is probably a special variety of ferric hydroxide. The granules of hemosiderin have a definite physical structure and are not an irregular particle of a homogeneous chemical compound.

Hemosiderin granules in a way resemble erythrocytes, since the pigment may be removed leaving substrate or stroma intact. The pigment contains a small amount of calcium and phosphate in addition to iron. With the ordinary light hemosiderin has no typical absorption spectrum but with ultraviolet light a secondary fluorescence is observed. The spectrum of the porphyrin is not seen in the component of the hemosiderin.

Sheldon gives the following account of hemofuscin. It is a yellowish-brown pigment and it occurs in a smaller and finer grain than hemosiderin. It is resistant to acid but affected by alkali and hydrogen peroxide. It is iron-free. In the process of breakdown of hemoglobin hemofuscin is formed first, changing later on into

hemosiderin with the lapse of time. Hemofuscin gives a negative stain for the fat and iron. Several methods of staining are proposed by various investigators and their usefulness denied by others because it is not certain that they were dealing with the same pigment.

PART III

REVIEW OF WORK CONCERNING a) THE BREAKDOWN OF ERYTHROCYTES IN TISSUE AND b) THE FATE OF ERYTHROCYTES IN THE LUNG

The history of the observation and investigation of the breakdown of erythrocytes starts with Virchow's[40] observation in 1847. In that year he observed and described hematoidin in tissues and he thought that it formed within and also outside of cells. Langhans[24] in 1869, studied the question experimentally in animals and described phagocytosis of erythrocytes. He observed the formation of hematoidin crystals in fowl. Cohnheim[8] in 1877 expressed the view that hematoidin is formed from free hemoglobin without the aid of cellular elements. Quincke[33] in 1884, injected dog's blood into dogs subcutaneously and observed the taking up of erythrocytes by cellular phagocytes and the formation of hemosiderin. He thought that when hemoglobin diffuses out from the cells a pigment (hematoidin) is produced and crystallizes. In 1888 Neumann[29] came to the conclusion that living cells form hemosiderin either from erythrocytes or hemoglobin, and formation of hematoidin represents a chemical process of destruction of erythrocytes independent of living cell activity.

Skrzeczka[38] in 1887 and 1888 supported Neumann's view and stated that hemosiderin is formed only in a comparatively large extravasation of blood, apart from the influence of the living cell. Rueck[18] in 1912, came to a similar conclusion.

An entirely new light was thrown on the subject by the observations of Rich[34,35] in 1924-25 using tissue culture. By this method he showed that erythrocytes added to cultures were taken up by mesodermal cells, that they underwent disintegration within their cells and that there appeared intracellular crystalline hematoidin both in rhomboidal form and as needles. The crystals gave a typical Gmelin reaction. Granular hemosiderin also appeared as a residue in the cell.

No crystals were formed outside cells before the time of their crystallization within cells. Rich divided pigments derived from hemoglobin into two divisions a) iron containing and b) iron free.

Muie and Young[27] and Muir and Niven[26], to investigate hematoidin formation, injected fresh homologous blood or a 60% solution of homologous hemoglobin in saline subcutaneously into rats, mice and rabbits. Tissues were examined microscopically after various lengths of time. Their conclusions were:

1) In mice and rats the formation of the granular and crystalline pigment from erythrocytes, injected subcutaneously, is intracellular.

2) Phagocytosis of erythrocytes is followed by the formation of hemosiderin within 24 hours and hemosiderin formation goes on until all of the erythrocytes are disposed of.

3) The formation of hematoidin is first seen in the seventh day. Crystals of hematoidin give the Gmelin reaction as for the bile pigment.

4) Changes following injection of hemoglobin are identical with the changes after injection of blood.

5) No formation of granular or crystalline pigments is observed outside of the cells.

6) In rabbits, formation of hematoidin is not observed.

7) Hemosiderin when first formed contains bilirubin constituents and at certain times and under certain conditions there occurs a change whereby iron is discharged from the cell and bilirubin is set free, thereafter crystallizing as hematoidin. According to Muir and Niven hemosiderin does not represent a definite compound but

merely a derivative of hemoglobin which gives the iron reaction. Iron may be in various conditions of combination and is present in various proportions. The hematoidin splits off from iron-reacting pigment.

Magarey[25] experimentally produced hemosiderosis of the lung. He drew blood from the inferior vena cava of rats and injected it intratracheally into other rats. Either fresh unchanged blood or heparinized blood was used. The blood was introduced through the larynx into the trachea, using a blunted needle attached to a syringe. In one set of experiments he gave a single intratracheal injection and killed the animals at various intervals up to 14 days. In other groups of animals he gave repeated injections of blood and killed the animals after several injections. The animals receiving a single injection of blood showed hemosiderin after two days and hematoidin after one week. The animals receiving multiple endotracheal injection of blood showed grouping of pigment-containing macrophages in the region of the alveolar ducts.

PART IV

EXPERIMENTAL PRODUCTION OF PULMONARY PHAGOCYTOSIS OF ERYTHROCYTES

MATERIALS AND METHODS

Rats of both sexes of the Sprague-Dawley strain weighing 300 to 400 grams were divided into 20 experimental groups, each group consisting of six test and three control animals. The groups were numbered one to twenty. In the test animals isologous (or autologus) blood was introduced by a catheter into the trachea or into a bronchus, as described below. Control animals received normal saline injected in the same fashion. Animals were killed at periods of from 12 hours to 44 days, and some animals died at various periods after the intrapulmonary injection. Animals in 19 groups received a single injection of blood. Animals in one group (Group 20) received multiple injections.

Operative Procedure. The abdomen, chest and undersurface of the neck of the rats were carefully clippered. The skin was cleansed with 80% alcohol. Most of the groups were anaesthetized by injecting intraperitoneally, sodium pentothal. Some groups were anaesthetized with ether.

Blood was drawn aseptically from the heart of a rat lightly anaesthetized with ether. The blood was immediately placed in a heparinized tube. Some of the rats died a few minutes later of massive pericardial bleeding, but the majority of donor rats survived. The blood of one rat in each group was used for all experimental rats in the same group. If the donor rat died, a rat added to the group received intrapulmonary blood in its stead. Thus the majority of rats received isologous blood but if the donor rat survived this

animal received autologous blood. The time lapse between the preparation of the blood and the injection into the lungs varied from 10 minutes to two hours.

Intrapulmonary blood was administered as follows. After anaesthesia a midline longitudinal incision was made in the skin and subcutaneous tissue, the incision measuring about two centimeters in length and starting a little above the sternum. After separating the skin and subcutaneous tissue, the muscle of the neck covering the trachea was bluntly dissected. A longitudinal incision was made in the trachea about five millimeters in length. The heparinized blood was drawn into a syringe. A polyehtylene tube having a bore of approximately one millimeter was attached to the needle of the syringe. This was introduced through the tracheal opening and with gentle movement it was pushed down. The tube entered into a bronchus of a lobe, usually one of the lower lobes. At this time 0.2 milliliters of blood were injected and the tube withdrawn. The edges of the skin wound were brought together by the forceps and clamped with a No. 18 metallic clamp. Most of the animals went through the operation uneventfully, but some developed dyspnea and a few died of asphyxia. After varying periods of time, (as shown in the table), the rats were sacrificed in a tightly closed chloroform-containing jar. After removal of the rat from the chloroform jar the thorax and abdomen were opened. The thoracic organs were removed en bloc and fixed in 10% buffered formalin.

In Group 20 several injections were given in the same animal by a different method. With the aid of a speculum inserted through the rat's mouth the larynx was visualized and a polyethylene tube was introduced into it. The catheter was pushed into the

trachea only. Through the tube 0.2 milliliters of blood were injected. The respiratory movement of the rats drew blood into the bronchi. To each rat was given nine injections, one every third day, and the animals were sacrificed 20 days after the last injection.

Control animals received 0.2 milliliters of normal saline. The rest of the procedure including preparation, anaesthesia, time interval between operation and sacrifice, fixation of tissue and staining methods, was identical to that used in the experimental animals.

After fixation for 24 hours, multiple blocks were taken from the lungs, including particularly lobes seen to contain blood. After paraffin embedding sections were stained as follows: hematoxylin-phloxine and saffron, Gomori method for iron, amidoblack method for hemoglobin[6,32], Gmelin method for hematoidin, Wilder's reticulum stain. In addition some slides were stained by the Periodic acid-Schiff method and some were stained for elastic fibres by Weigert's method.

PART V

OBSERVATIONS

Rats in the same group generally showed lesions which were grossly and microscopically similar.

GROSS PATHOLOGICAL FINDINGS IN RATS GIVEN A SINGLE INTRABRONCHIAL INJECTION OF BLOOD

Twelve hours after the introduction of the blood into the lung, usually one of the lower lobes, the injected area is dark reddish brown in colour and rubbery in consistency. The overlying pleura is smooth. The area is relatively well delineated and varies in size with the amount of blood retained in the lung after injection. For the usual injected dose, 0.2 millilliters of blood, the area measures approximately 7 x 5 x 3 millimeters. The lesion cuts with slight resistance to reveal a homogeneous dark reddish-brown lustreless tissue. During the next 12 hours the gross appearance of the lesion does not change significantly. At 36 to 48 hours the lesion shows little further change. The overlying serosa is lustreless and at the junction of the blood-containing and aerated areas of the lung, there is a narrow zone of hyperemia. In the next three days the only significant change is a mild lightening of the colour of the lesion. At the fifth day the lesion is less firm and the lightening of the colour of the blood-containing area is more pronounced. The junction between the solid and aerated area become less distinct.

Between the sixth and the tenth day the colour changes from reddish brown to light brown. The consistency of the tissue becomes less firm and the injected area blends into the adjacent aerated area. After the tenth day the colour continues to lighten. At about the 14th

day it is difficult to distinguish the injected area of the lung and its consistency is approximately similar to the uninjected areas. (The changes are illustrated in Figures 1 to 10).

GROSS PATHOLOGICAL FINDINGS IN RATS GIVEN REPEATED INTRATRACHEAL INJECTIONS OF BLOOD

In the rats received multiple intratracheal injections the gross findings are quite different. The lungs are yellow and mottled pale brown. These changes are present more or less in all lobes. The consistency is not significantly different from the lung of the control group. The cut surfaces are also yellowish and mottled brown.

MICROSCOPIC FINDINGS IN RATS GIVEN A SINGLE INTRABRONCHIAL INJECTION OF BLOOD

At the 12th hour after the introduction of the blood into the lungs, microscopic sections from the injected lobe show the alveoli, alveolar ducts and small bronchi to be impacted with erythrocytes. The majority of erythrocytes are intact and well defined but some are smudged and have a scalloped border.

The formation of hemoglobin crystals is seen in various fields. The crystals are widely spread, small and needle-like and not larger than the diameter of an alveolus. They are randomly distributed in the various areas. The alveoli which did not receive the injected blood and are located distal to the areas of the injections contain edema fluid. The alveolar septa are congested and widened and the capillaries in the alveolar walls are packed with erythrocytes. Occasionally a few polymorphonuclear leukocytes are seen in the alveoli. Iron stains fail to show hemosiderin. The amidoblack stain for hemoglobin is positive. The erythrocytes and hemoglobin crystals are bluish-black. The Gmelin test for hematoidin is negative.

At 24 hours after injection sections of the injected areas of the lungs reveal portions to be impacted with the erythrocytes, hemoglobin crystals, polymorphonuclear leukocytes and macrophages (Figure 11). The density and proportion of these elements vary in the different areas of the injected lobe. Almost one third of the alveolar content is composed apparently of intact erythrocytes, one third smudged erythrocytes and one third hemoglobin crystals. Generally, the amount of erythrocytes and hemoglobin crystals have an inverse relationship. The breakdown of the erythrocytes is more advanced in the alveoli containing a moderate number of them and is slower in the alveoli tightly packed with erythrocytes. The hemoglobin crystals are located in the alveoli, alveolar ducts and occasionally in the lumens of small bronchi. The great majority of the crystals vary from two to 12 microns in width and from eight to 192 microns in length. The colour of the crystals is generally red in sections stained with hematoxylin, phloxine and saffron. The smaller crystals are more homogenous, denser and darker. The larger ones are paler and less homogenous and their central parts are reddish-yellow. The shape of the crystals is usually rectangular, occasionally fusiform and rarely diamond shaped. Both ends of the crystals are usually indented and each end looks like the end of a broken dry stick. However, some crystals have relatively smooth ends. The number of crystals varies greatly in the various areas. From one to a dozen may be present in one alveolus and in the same alveolus crystals of various sizes and shapes may be seen. When there is more than one crystal in an alveolus the long axis of each crosses the other. Occasionally they lie parallel like a bunch of match-sticks. The

formation of hemoglobin crystals as seen under the microscope seems to be as follows. The red blood cells become smudged with an irregular and hazy border and some become arranged into a straight column. The cells then seem to fuse together. Progressively, this fused column of erythrocytes becomes a distinct hemoglobin crystal. It appears that once a hemoglobin crystal is formed it does not increase in width but further increases in length may occur. The presence of macrophages does not seem necessary for formation of hemoglobin crystals because many of them are formed before the macrophages appear in considerable numbers. Crystals seem to form earlier in alveoli moderately packed with erythrocytes. In the alveoli densely packed with erythrocytes the hemoglobin crystals appear later but are larger and more numerous.

Further changes in the other structures of the lung have occurred at this time and alveolar walls are widened and congested. The interlobular septa and the peribronchial connective tissues are edematous. There is a considerable infiltration of these areas with polymorphonuclear leukocytes, which are also present in the alveolar walls and in the alveolar spaces. About 25% of nucleated cells seen in each high power field are polymorphonuclear leukocytes. Almost all of the polymorphonuclear leukocytes seem intact. The moderatly packed alveoli contain relatively more polymorphonuclear leukocytes than the alveoli which are densely packed with erythrocytes.

The majority of the alveoli contain a few macrophages. In this early phase they appear in close contact with the alveolar walls. Each macrophage has a relatively ovoid nucleus containing finely and uniformly dispersed chromatin. Some of the nuclei are vesicular and contain one or more red nucleoli. The number of macrophages in each alveolus is

approximately in inverse relationship to the number of polymorphonuclear leukocytes. The cytoplasm of most of the macrophages is pale pink and poorly outlined. Some macrophages seem well rounded, well demarcated and their cytoplasm is coarsely granular. The granules measure up to about half the diameter of a rat erythrocyte and are pink in colour. The granules seem to be the engulfed particles of erythrocytes. The iron stain shows early hemosiderin formation. In each high power field there are a few macrophages with bluish granules in their cytoplasm. They are presumed to be macrophages which also contain coarse particles of erythrocytes. No extracellular hemosiderin is seen. The hemoglobin crystals are negative with Gomori's iron stain. The amidoblack stain is positive for hemoglobin crystals, erythrocytes and their fragments.

At 36 hours (Figs. 12, 17, 18, 19) after the injection of the blood sections of the injected area reveal further breakdown of erythrocytes. At this time only about 10% of the erythrocytes remain intact. Hemoglobin crystals of all sizes are present within the alveoli, alveolar ducts and bronchioles. Some of the crystals are surrounded by polymorphonuclear leukocytes. The polymorphonuclear leukocytes do not seem to have increased in numbers in the last 12 hours but they appear in the centre of the alveoli, where the hemoglobin crystals are more numerous. Some polymorphonuclear leukocytes show degenerative changes. The macrophages are further increased in size and numbers. In many fields they now outnumber the polymorphonuclear leukocytes. Various numbers of mitotic figures are noted. The proportion of macrophages containing erythrocytes has increased. Occasional macrophages contain a whole and apparently intact erythrocyte.

The mesothelial cells of the pleura covering the involved area are enlarged and prominent. Iron stains show an increased number of hemosiderin containing macrophages and also an increase in the amount of hemosiderin within individual macrophages. The amidoblack stain is positive and the Gmelin test negative. The hilar lymphoid tissue is hyperplastic but the iron stain does not reveal hemosiderin in any of these cells.

At the 48th hour (Figs. 13, 14, 15, 16, 20, 22 and 23) the hemoglobin crystals are breaking down in most of the areas. Approximately 60 per cent are in the process of breaking down and in 10 per cent of the area they are so divided into minute fragments that they can hardly be recognized as hemoglobin crystals. About 30 per cent of the crystals seem intact. Most of the polymorphonuclear leukocytes show degenerative changes. The nuclear fragments of polymorphonuclear leukocytes are frequently mixed with the fragments of broken-down hemoglobin crystals. Almost all of the erythrocytes are turned into hemoglobin crystals or are phagocytosed by the macrophages. The macrophages are further increased in size and number and many of them are in mitosis. The nuclei of the macrophages are from two to five times larger than erythrocytes and many of the nuclei contain one to four nucleoli. The fragments of hemoglobin crystals are being taken up by the macrophages. Approximately 50 per cent of the macrophages contain, within their cytoplasm, fragments of hemoglobin crystals, fragments of erythrocytes and necrotic polymorphonuclear leukocytes. The iron stain shows a further increase of the hemosiderin pigment in the macrophages at this time. The Gmelin test is negative. The amidoblack stain is positive but it shows a quantitative decrease in hemoglobin either amorphous or in crystal form.

Sixty hours after the injection of the blood into the lung (Fig. 21) nearly all of the hemoglobin crystals are broken down and have been phagocytozed. Occasionally an intact hemoglobin crystal may be seen. The polymorphonuclear leukocytes are diminished in numbers and many show degeneration and necrosis. The macrophages are still increasing quantitatively. About 80 per cent of the nucleated cells seen in each field are now macrophages. They are either discrete or form small clusters. There are significant numbers of mitoses. Occasionally a multinucleated giant cell is seen. The iron stain shows a further increase of hemosiderin in the macrophages. The peribronchial and mediastinal lymphoid tissue seems hyperplastic but does not contain hemosiderin. The amidoblack stain is faintly positive and the Gmelin test is negative.

On the third day after the injection of the blood, there is a still further increase in the number of macrophages. The polymorphonuclear leukocytes, erythrocytes and hemoglobin crystals have practically disappeared. The iron stain shows a further increase in hemosiderin. The amidoblack stain does not reveal hemoglobin in the lesional tissue (except in erythrocytes in blood vessels).

On the fourth day the majority of the macrophages contain hemosiderin, but in varying amounts. In the hematoxylin, phloxine and saffron stain the cytoplasm of many macrophages are more or less brownish in colour and these cells resemble the siderophages of the human lung. The brown pigment contains hemosiderin and stains positively for iron. All of the macrophages are in contact with the alveolar walls or with each other. None appear "loose" in the alveolar spaces. At this time almost all of the alveoli in the injected area are impacted with

macrophages.

On the fifth day (Figs. 24 and 37) it seems that there is no further increase in macrophage numbers. Yet there is an increase in the percentage of macrophages containing hemosiderin and numerous macrophages seen more heavily laden with pigment. There is now no further evidence of proliferation of macrophages. Up to the fourth day the pulmonary tissue in the involved area is completely consolidated but now the macrophages begin to be separated by empty spaces and hence many seen to "lie free" in the alveoli.

On the sixth day (Fig. 36) after the injection of blood into the lung, one can detect no further increase in hemosiderin-containing macrophages. For the first time the Gmelin test becomes positive in scattered areas. With this reaction a yellowish-green dust becomes visible for a few seconds, or actual crystals may be seen. This substance is hematoidin which, chemically, is identical with bilirubin. In sections stained with hematoxylin, phloxine and saffron, hematoidin particles cannot be distinguished amidst brownish granules of hemosiderin within the macrophages. However, with the Gomori technique for iron, amorphous and crystalline material of golden-yellow to pale brown colouration is apparent in addition to the blue-staining hemosiderin granules. This material may perhaps be hematoidin; it appeared in macrophages in sections cut at a space of three microns from sections giving the positive Gmelin test. Crystals vary in size and shape but are mostly polygonal and are translucent. They are unevenly distributed in the cells and were not seen in an extracellular location.

At the seventh day (Fig. 28) there is a further increase of crystals thought to be hematoidin within the cytoplasm of individual

macrophages and also in each field there are more macrophages forming hematoidin. The number of the macrophages has decreased and spaces between macrophages seen for the first time on the sixth day, are becoming larger.

From the eighth to the fifteenth day (Figs. 25, 26, 27, 29, 31, 32, 33, 34, 35, 38 and 39) two major changes occur in the tissue. There is continued slow diminution in the number of macrophages and a relative increase of what I presume to be hematoidin crystals. There are clumps of hemosiderin-containing macrophages detached from the alveolar walls, and "lying free" within the lumens of the alveoli. Also aggregations of similar macrophages are present in the alveolar ducts where they open into bronchioles and within the lumens of the bronchioles. The alveolar lumens, choked with cells in earlier stages, now reappear and become more and more prominent. At about two weeks after the injection, the macrophages appear for the most part as if attached to the wall of the alveoli and only a few are "free" within alveolar spaces. Most of them contain more hematoidin than hemosiderin.

Within the third week (Figs. 30, 40, 41 and 44) the clearance of the macrophages from the lung continues and at the end of this period the parenchyma of the lung has returned almost to the normal state. However, still the tissue is studded with macrophages in contact with alveolar walls.

MICROSCOPIC FINDINGS IN RATS GIVEN REPEATED INTRATRACHEAL INJECTIONS OF BLOOD

In the rats which received multiple intratracheal injections (Figs. 42 and 43) the change in the lungs was different from that occurring in animals receiving a single injection into a lobe. There was a diffuse and mild infiltration of all lobes by macrophages. The majority of the alveoli contain a few macrophages apparently attached to their walls. Hemosiderin and hematoidin was present in abundance in most of the macrophages. The general pattern of the lung was unchanged and the shape of the alveoli was well preserved. No polymorphonuclear leukocytes, hemoglobin crystals or intra-alveolar erythrocytes were seen.

GROSS AND MICROSCOPIC FINDINGS IN THE CONTROLS

The lungs of the control groups, grossly, do not show any significant pathological changes. Some of the lungs of the control groups, sacrificed after 12 and 24 hours were mor pinkish than normal. Microscopically, some of the sections taken after 12 and 24 hours after injection of the normal saline reveal a mild congestion of the capillaries of the lung. However, most of the control sections were unremarkable. Generally the Gomori iron stain, the amidoblack stain for hemoglobin and the Gmelin tests were negative. However, in occasional sections scattered macrophages gave a positive reaction for iron.

OBSERVATIONS ON RATS a) DYING DURING THE TEST PERIOD AND b) SURVIVING TO THE END OF THE TEST PERIOD WITH BRONCHOPNEUMONIA

As shown in the table, each test group consisted of nine rats, six of which received intrapulmonary injection of blood and three of which received normal saline. In 16 of the 20 groups some rats died during the experiment (one rat in each of eight groups, two rats in each of five groups and three rats in each of three groups). At autopsy these animals revealed severe bilateral bronchopneumonia, often with pulmonary abscess formation and sometimes with evidence of organization of the inflammatory process. Approximately 20 per cent of those animals surviving to the end of the test period showed bronchopneumonia of moderate intensity. Because of the complicated microscopic picture in which bronchopneumonic changes were mixed with changes attributable to reaction to injected blood, these animals were not studied intensively. Macrophage reaction and erythrocytic breakdown appeared to be irregular and it is my impression that clearance of the lung from phagocytes was retarded or halted.

SUMMARY OF OBSERVATIONS

After a single intrabronchial injection of 0.2 milliliters of isologous blood, the blood-containing area of the lung grossly resembles a pulmonary infarct. Microscopically hemoglobin crystals are seen as early as 12 hours and they rapidly increase quantitatively reaching a peak about 36 hours after injection of the blood. By the end of the third day almost all of the hemoglobin crystals have been phagocytized by macrophages. A polymorphonuclear leukocytic infiltration of the involved lung is concurrent with the hemoglobin crystal formation. The polymorphonuclear leukocytes became degenerated and are also phagocytized by macrophages. The macrophages appear in the pulmonary alveoli sometimes between 12 and 24 hours after injection of blood and they are a main factor in the clearance of the injected erythrocytes from the lung and the formation of the various substances resulting from the breakdown of the hemoglobin. The phagocytes take up the fragmented hemoglobin crystals and red blood cells. Hemosiderin appears in the cytoplasm of the macrophages approximately 24 hours after the intrabronchial injection of blood. The quantity of the hemosiderin in the macrophages rapidly increases and in the meantime the amount of hemoglobin-containing substance in the macrophages decreases. Pale brown to golden yellow amorphous or crystalline material, thought to be hemotoidin is seen for the first time on the sixth day after injection of blood. "Free" macrophages containing blood pigment appear on the fifth day in the alveolar ducts, bronchioles and larger bronchi, and they are present in diminishing numbers until the end of the experiment. Approximately three weeks after injection of blood the parenchyma of

the lung has returned to normal, save for the presence of scattered pigment-filled macrophages.

In cases of multiple intratracheal injections of blood, the pulmonary reaction and the process of the breakdown of the hemoglobin is essentially similar to that taking place when a single intrabronchial injection of blood is given. However, in cases of intratracheal injection, the pulmonary reaction is diffuse and all lobes are proportionately involved.

Control animals receiving intrapulmonary injections of normal saline show no pulmonary abnormality except for the fact that occasional hemosidern field macrophages, closely applied to alveolar walls occurred in some sections of the lungs of some animals.

A number of animals developed bronchopneumonia; of these some died during the test period. Some survived to the end of the experiment. The presence of bronchopneumonia altered considerably and seemed to retard the sequence of events occurring following introduction of blood into the lungs.

NOTE: These observations are shown on a graph. See PART X.

TABLE SHOWING NUMBERS OF TEST AND CONTROL ANIMALS IN EACH EXPERIMENTAL GROUP AND THE LENGTH OF SURVIVAL AFTER INTRAPULMONARY INJECTION OF ISOLOGOUS BLOOD

Group	No. of Rats	Died During Test Period	Survived to End of Test	Length of Survival	No. of Controls Surviving
1	6	1	5	12 hours	3
2	6	0	6	24 hours	3
3	6	0	6	36 hours	3
4	6	1	5	48 hours	3
5	6	1	5	60 hours	3
6	6	0	6	3 days	2
7	6	2	4	4 days	3
8	6	0	6	5 days	3
9	6	2	4	6 days	3
10	6	1	5	7 days	3
11	6	1	5	8 days	3
12	6	1	5	9 days	3
13	6	3	3	10 days	3
14	6	1	5	12 days	3
15	6	2	4	14 days	3
16	6	1	5	16 days	3
17	6	2	4	18 days	3
18	6	3	3	20 days	3
19	6	2	4	3 weeks	1
20	6	3	3	44 days	2

PART VI

BRIEF REVIEW OF THE PRINCIPAL PATHOLOGICAL CONDITIONS IN HUMAN LUNGS WHICH ARE ASSOCIATED WITH ALVEOLAR PHAGOCYTOSIS OF ERYTHROCYTES AND HEMOSIDERIN FORMATION

When erythrocytes gain entrance into the alveoli of the lung they are taken up by macrophages and hemosiderin is formed with resultant hemosiderosis of pulmonary tissue. In pathological states erythrocytes may gain entrance into the alveoli when the pulmonary circulation is disturbed or the pulmonary tissue diseased. A simple classification of causes of pulmonary hemosiderosis based on standard text-book accounts (1,2,3) follows:

Table - Classification of Types of Pulmonary Hemosiderosis

I - Secondary pulmonary hemosiderosis

1 - Following passive venous congestion

a) brown induration of lung

b) hypostatic congestion of lung

2 - Following pulmonary embolism and infarction

3 - Following pulmonary arteriosclerosis

4 - Following pulmonary arteriovenous fistula

II - Primary ("idiopathic") pulmonary hemosiderosis

Passive venous congestion of the lungs occurs in two main types

a) Brown induration. This is always associated with hypertension in the pulmonary circuit. The cause of the increase of the pressure in the lungs is usually mitral stenosis but emphysema, long standing cardiac failure, aortic insufficiency, pleural adhesions, marked diminution in the lung volume, silicosis, some types of congenital heart disease are among the pathological conditions which

may be associated with pulmonary hypertension. The lungs are brown, indurated and difficulat to cut. Under the microscope, dilated, tortuous, congested and thickened alveolar capillaries may be seen. The most characteristic finding is the presence of many macrophages laden with hemosiderin granules. These are the so-called "heart failure cells".

b) Hypostatic congestion. This is a very common condition. In a minor degree it may be found in almost every autopsy. It is characterized by accumulation of blood in the lower and posterior parts of the lung. This is due to relaxation of the vessels and weakness of the heart's action. The posterior portion of the lung may appear consolidated If it lasts more than a few days diapedesis of the erythrocytes occurs.

Pulmonary embolism and infarction. Any physical mass which enters the systemic venous circulation, if it has a diameter of more than about 20 microns, may be caught in the pulmonary vessels. Most commonly this physical mass is an embolus coming from thrombotic material formed in the veins of the leg but clumps of bacteria, tumour cells, fat, air and amniotic fluid behave in a similar way. Normally, the parenchyma of the lung is supplied with a double circulation (bronchial and pulmonary). For the development of an infarct in the lung, prior disturbances of both circulations are required. Thus, in association with pulmonary arterial obstruction, concommitant cardiac failure is necessary for the production of pulmonary infarction. If the infarct develops capillary walls in the area of the infarction cannot retain the erythrocytes and blood enters the alveoli. The part of the lung which has lost its circulation becomes nectoric and is eventually replaced by hemosiderin-laden scar tissue. Even though the heart is not in failure, intra-alveolar hemorrhage may still develop. This is due

to the disturbance of circulation distal to the blocked area, back pressure within the capillaries and relative anoxia of tissue. Microscopically, in cases of pulmonary infarction, necrosis of the alveolar wall does exist. In the absence of an infarct, the alveoli contain erythrocytes but the alveolar walls are viable. Because the parenchyma of the lung in this area is not necrotic phagocytosis of the erythrocytes occurs faster and if the other conditions are favourable the involved area is cleared of the products of blood destruction.

Pulmonary arteriosclerosis. This may be secondary or primary. The causes of the secondary type is increase of the blood pressure within the pulmonary artery and its branches. Many of the diseases of the parenchyma of the lung and the valves of the heart may increase blood pressure in the lungs. The primary type (Ayerza's disease) is characterized by dyspnea cyanosis, polycythemia and diffuse pulmonary arteriolosclerosis with or without associated pulmonary fibrosis.

Pulmonary arteriovenous fistula. Produces a shunt and short circuit of blood. The shunt facilitates transmission of the arterial pressure into the pulmonary vein and consequently an increase in capillary pressure, so that alveolar hemorrhage occurs.

Idiopathic Pulmonary Hemosiderosis

According to Harrison[16] idiopathic pulmonary hemosiderosis is a relatively uncommon pulmonary disease that results from recurrent hemorrhage into the lung. The etiology of the hemorrhages are known. Ceelen[17] in 1931 described the pathological findings in two children with the disease which, in 1948, was called by Wyllie[43] idiopathic pulmonary hemosiderosis. By 1958, Branson[4] was able to collect from the literature 93 cases, 56 in children under 16 years of age and 37 in adults.

According to Branson the patient is usually pale and hemoptysis, persistent cough and dyspnea are common clinical findings. An intermittent fever may be present. The acute symptoms last from a few hours to several days and subside slowly. There follows a symptom-free interval except for general weakness and pallor. This free interval may last from several weeks to several months. However, acute exacerbations are repeated after various lengths of time. Physical findings may be scanty. In some cases hepatosplenomegaly and jaundice may occur and signs of pulmonary fibrosis and cor pulmonale with cyanosis, orthopnea and clubbing of the fingers may appear. Laboratory investigation shows hyperchromic microcytic anaemia. The hemoglobin level characteristically lies in the range from 2 grams to 7 grams per cent. The bone marrow is normal or may show erythroid hyperplasia. The serum iron is low and the iron binding capacity of the serum is high. The erythrocyte count is usually in the vicinity of 2 to 3 million per cubic millimeter. The most significant laboratory finding is the presence of hemosiderin laden macrophages in the sputum or in gastric aspirations. The majority of adult cases are clinically diagnosed during the second or third decades of life but the disease is seen at all ages and in both sexes. In approximately two-thirds of the cases the first chest X-ray shows the disease. The typical pattern of the lesion is described as "diffuse fine grainy" or "ground glass infiltrate" or "fine stippling". The lesion is most prominent in the hilar areas and bases of the lungs. The greatest infiltration occurs during clinical exacerbations and there is partial or complete clearing in intervals between attacks. The size of the consolidations varies from a few millimeters to two or three centimeters in diameter. Ellman[12] has mentioned the following in

the differential diagnosis of idiopathic pulmonary hemosiderosis: miliary tuberculosis, sarcoidosis, pneumoconiosis, lymphangitis carcinomatosa and collagen diseases involving the lung. Occasionally mitral stenosis produces clinical, radiological and pathological findings very similar to those of idiopathic pulmonary hemosiderosis. Ellman has described two cases of pulmonary hemosiderosis in patients known to have had mitral stenosis for several years. The chest X-ray showed a miliary pattern in the lung. At autopsy, despite the presence of many hemosiderin-filled macrophages in both lungs, there was minimal fibrosis. This author concluded that chronic pulmonary congestion and hemosiderosis do not always produce a pulmonary fibrosis or brown induration.

Regarding the prognosis of the disease in a series of 34 cases reported by Branson, 13 were alive at the end of 10 years after the clinical diagnosis. The longest duration of the disease was in the neighbourhood of 20 years and the shortest was 18 months. The patient usually dies of intercurrent pulmonary infection.

Wyllie described the pathological findings in idiopathic pulmonary hemosiderosis as follows: Most of the alveoli contain edematous fluid or masses of pigment-laden macrophages or erythrocytes as a result of a more recent hemorrhage. The alveolar walls are thickened from increase in reticulum and collagen and the alveolar ducts show hypertrophied smooth muscle bundles. The elastic fibres have completely disappeared from the walls of many alveoli. In other areas they show varying degrees of degeneration. They are often torn, fibrillated or appear as plump, star-shaped bodies. The intracellular septa are moderately thick and formed by edematous loose connective

tissue with numerous distended capillaries. Small and medium sized vessels are also elongated and stretched in appearance and they show fibrillation and disruption of elastic fibres in their walls, with foreign body giant cell reactions about them. The bronchopulmonary superior and inferior tracheobronchial lymph nodes show sinus catarrh and the presence in the peripheral and central sinuses of hemosiderin-laden macrophages. Some foreign body type giant cells with degenerated iron-coated elastic fibres are found in the sinuses. The other organs do not reveal significant pathological change. Concerning the pathogenesis of idiopathic pulmonary hemosiderosis there are several hypotheses.

1. Nancekievill[28] believes that the primary change may be degeneration of the elastic tissues of the lung. The majority of investigators consider these changes to be the effect and not the cause of the disease. The degree of elastic change is proportional to the duration of the illness.
2. Steiner[39] introduced the concept of auto-sensitization in this disease. He thinks that the permeability of the pulmonary capillaries is altered by an antigen-antibody reaction. He considers that the spleen has a role in auto-sensitization as a member of the reticulo-endothelial system The lung reacts as a shock organ.
3. Ferguson et al[13] suggest that the intrapulmonary hemorrhage stems from the anastomosis present between branches of pulmonary arterial and bronchial arterial tree. Following the elevated pressure in the pulmonary arteries, these vessels become congested, varicose and widened similar

to the varices in portal hypertension.

4. Lendrum et al[21,22,23] believe diapedesis of erythrocytes to be due to congestion between the branches of the pulmonary and the bronchial arterial tree. They think that marked pulmonary hypertension must exist as in cases of mitral stenosis and other types of heart disease.

5. Herzog[17] believes the essential change in the disease to be a proliferation or hyperplasia of capillary argyrophilic fibrils. He demonstrated numerous tortuous or sinuous capillary proliferations, about which there was an abnormal increase in argyrophilic reticulum.

6. Propst[31] showed an increase in acid mucopolysaccharides in the wall of the small blood vessels in the intra-alveolar septa, thought to cause weakening of elastic fibres. According to him, as the result of this, vascular dilatation and bleeding by diapedesis occurs. He compares idiopathic pulmonary hemosiderosis to senile elastosis and states that it may be a form of arachnodactyly.

7. The hematologic pattern is that of a recurrent loss of blood in the absence of external hemorrhage, associated with an increase of hemopoeisis. Some cases in the acute stage have cold agglutinines. Wyllie et al suggest agglutinins represent a secondary antibody response to a destroyed erythrocyte.

PART VII

DISCUSSION

The gross and microscopic changes occurring in the lungs of rats following intrabronchial and intratracheal injections of blood seems to depend in part on the following.

1. Volume of injected blood. Each rat received a measured volume of 0.2 milliliters of isologous or autologous blood but the exact amount of blood reaching the lung retained there is not known. It seemed that in intrabronchial injections that the major portion of the blood reached one lobe of the lung but some may have been dispersed more widely into other lobes and some may have escaped from the lung entirely. Furthermore, in some instances, the volume of intra-alveolar blood may have exceeded the volume of injected blood due to intrapulmonary bleeding resulting from trauma during catheterization. In most of the animals sacrificed 12 and 24 hours after intrabronchial injection of blood, approximately one third of the volume of a lower lobe of the lung was occupied by the blood. If a lobe of the lung contained blood up to one third of its volume the process of the breakdown of erythrocytes was relatively uniform throughout the blood-containing area. In cases in which more than about one third of the lobe contained blood the various stages of erythrocytic and removal took place in a more irregular manner.

2. Time. In the majority of the animals about three weeks were necessary for breakdown of the injected erythrocytes and almost complete clearance of the products of blood cell disintegration from the lung. However, scattered pigment-filled macrophages were still present even three weeks after the injection.

3. Superimposed Bronchopneumonia. Superimposed bronchopneumonia appeared to hinder the macrophage response, phagocytosis and disintegration of erythrocytes. When in repair following inflammation fibrosis

takes place, hemosiderin-containing macrophages remain in the fibrotic zones.

4. Method of introduction of blood into the lung. The parenchyma of the rat lung seems to be able to rid itself faster from the same amount of blood if it is relatively uniformly distributed in the lung. This situation takes place when blood is injected intratracheally. The respiratory movement of the lung draws the blood into the alveoli and the blood is uniformly distributed throughout the lobes. If an equal amount of blood is accumulated in one lobe more time is required for the clearance of the injected blood. This is the case even when the blood is introduced into a lobe through intrabronchial catheterization.

For the sake of systematic consideration of pathological changes taking place in pulmonary tissues in this experiment hemoglobin crystal formation, polymorphonuclear leukocytic infiltration, macrophage activity, hemosiderin and other pigment formation and changes taking place in the pulmonary parenchyma will be considered separately. The relevance of the experimental findings to certain problems related to human pulmonary hemosiderosis will also be discussed.

Hemoglobin Crystal Formation. Generally the injected erythrocytes form hemoglobin crystals but some of the red blood cells are phagocytozed as such or after degeneration and fragmentation without hemoglobin crystal formation. The formation of hemoglobin crystals does not seem to require the presence of polymorphonuclear leukocytes or macrophages. The crystals begin to form in the centre of the alveoli when no other cells except erythrocytes are present. The crystals form in the lumens of bronchi, as well as in alveoli. It seems that the

formation of hemoglobin crystals is a physicochemical rather than a biological reaction. The hemoglobin crystals do not provoke more reaction than intact or degenerated erythrocytes. Once within the cytoplasm of the macrophages, the intact and degenerated erythrocytes and the hemoglobin crystals are treated and processed in an identical manner.

Polymorphonuclear leukocytes. These are first to appear in response to the presence of intra-alveolar blood. However, the erythrocytes and the hemoglobin crystals are not phagocytosed by the polymorphonuclear leukocytes. In ordinary cases the polymorphonuclear leukocytes become necrotic and are later phagocytosed by macrophages. In cases where there are histological evidences of infection, the polymorphonuclear leukocytes markedly increase quantitatively and become the dominant cell in the tissues.

The Macrophage. There are a number of hypotheses about the origin of the macrophages which appear in the alveoli of the lung, but none is proven definitely. The possible origin of alveolar macrophages are [41] 1) Monocytes from the circulating blood pass through the alveolar wall and enter the alveoli.[14] 2) Histiocytic cells of the interstitial tissue and pulmonary septa[20] are the origin of the intra-alveolar macrophages 3) The living epithelial cells of the alveoli are able to become phagocytic cells.[5] 4) Extrapulmonary reticuloendothelial cells and pulmonary vascular endothelium are the origin of intra-alveolar macrophages.[30]

The macrophage, whatever its origin, is the most important cell in pulmonary phagocytosis of erythrocytes and their products. Sections taken from the injected area of the lung, after various intervals of time show the appearance of macrophages between 12 and 24 hours after the injection of blood. The macrophages aggregate around the hemoglobin

crystals. Later, hemoglobin crystals lose their shape and become fragmented. In the meantime in the cytoplasm of the macrophages fragments of the hemoglobin crystals appear. From these observations it seems possible that the macrophages have some role in causing fragmentation of hemoglobin crystals prior to phagocytosis.

Hemosiderin and Hematoidin Formation. Hemosiderin appears in the cytoplasm of the macrophages 4 to 7 hours after they have taken up the hemoglobin crystals and approximately 24 hours after injection of blood into the lung. Gomori's iron reaction shows formation of the hemosiderin only in the macrophages which contain fragments of hemoglobin crystals or erythrocytes. Probably the enzymatic activity of some intra cytoplasm structure of the macrophages is responsible for the breakdown of the hemoglobin into its moeity.[11] The hemosiderin is one of the products of the breakdown. In the earlier stages of its formation hemosiderin, as seen with Gomori's iron reaction, appears as a faintly bluish and diffuse substance in the cytoplasm of the macrophages. With the passage of time, the amount of hemosiderin within the macrophages increases and the hemosiderin forms coarse, dark blue and irregular clumps. No extracellular formation of hemosiderin is observed. It does not seem that hemosiderin is transferred from the macrophages to other cells. From the observations made on the phagocytes it seems that, if the amount of hemoglobin crystals taken up by the macrophages is within the limit of the functional capacity of the enzymatic system of the macrophages, the breakdown of the engulfed substance goes on. As a result, various elements including hematoidin are formed.

One may speculate that when the macrophage is loaded beyond its capacity to handle the substance which it has engulfed the intra-

cellular breakdown of hemoglobin slows down and later on ceases. As seen with the light microscope, there are macrophages in the lumen of the alveoli, alveolar duct and bronchioles with no visible attachment to adjacent structures. The majority of these macrophages have a cytoplasm packed with hemosiderin and sometimes with hematoidin or a mixture of both. Possibly they are the overloaded macrophages, which are detached from the alveolar wall and become wandering phagocytes in the alveoli and alveolar ducts.

Pulmonary Parenchymal Changes. I did not find any pathological changes in the stroma of the lung and Wilder's reticulum stain, periodic-acid-Schiff stain and Weigert's elastic tissue stain also did not reveal abnormalities. I believe that the blood per se has no particularly permanent harmful effect on the parenchyma of the lung and within certain limits the lung is able to clear itself completely in about three weeks without sequela. In cases in which there was superimposed bronchopneumonia, as mentioned above, the whole process of destruction of blood and clearance of it from the lung is interfered with.

Pulmonary Lymphoid Tissue. The peribronchial and mediastinal lymphoid tissue of the lung becomes hyperplastic from the first day of the experiment and remains so until about the 20th day by which time the lung has almost been cleared of injected blood. At no time are hemosiderin-containing macrophages seen in the lymph nodes.. Occasionally a slight amount of iron-positive material is seen free in the lymphoid tissue but a similar observation is made in control groups. This would seem to indicate that, ordinarily, the lymphatic system does not take part in clearing hemosiderin-containing macrophages from the lung.

Hemoglobin crystal formation was a striking feature in the lungs of the rats, occurring as early as 12 hours after the injection of blood and disappearing by the end of the third day. This phenomenon, as far as I know, is not recorded following pulmonary hemomorrhage in man nor have I observed it in some 1500 autopsies. Magarey noted hemoglobin crystal formation in the lungs of rats receiving intratracheal injection of blood but did not discuss this finding in his paper.

One obvious difference between the situation in these experiments and in most conditions of pulmonary hemorrhage in human subjects is that in the experimental animals the pulmonary parenchyma and status of the pulmonary circulation is presumably normal prior to the entry of blood into the pulmonary alveoli. In most states of intrapulmonary hemorrhage occurring in humans this is not so, abberrations of pulmonary blood flow or alterations of lung parenchyma or both antedating and frequently bearing a causal relationship to the intra-alveolar hemorrhage. The possibility must be considered, therefore, that lack of hemoglobin crystal formation in man is due to the fact that in most conditions in which pulmonary hemorrhage occurs a pathological process pre-exists in the lung which may in some way interfere with the formation of the crystals. However, when hemorrhage occurs in the upper respiratory tract in previously healthy humans, and blood is aspirated into the lungs, the experimental situation is more closely reproduced, and pulmonary contusion resulting from closed chest injury also provides an example of intra-alveolar hemorrhage into previously healthy lungs. In neither of these conditions has hemoglobin crystal formation been noted. It is true that the crystals in rats are evanescent, not appearing until 12 hours after entry of blood into the lungs and disappearing by the end of the third day. Nevertheless, it appears likely that they would have been

observed and recorded in some humans cases did they occur.

It seems possible that a species difference in the structure of the hemoglobin molecule may explain the occurrence of hemoglobin crystal formation in intra-alveolar blood in rats and their absence in man. Further work is obviously required to solve this problem.

In primary and secondary pulmonary hemosiderosis in man aggregations of hemosiderin-laden macrophages in the alveolar ducts have aroused speculation. Lendrum believed these to be accounted for by hemorrhage from varices at sites of bronchopulmonary anastomoses in the mucosa of terminal bronchioles. He further postulated that soluble iron reached adjacent pulmonary stroma producing damage and reactive changes leading to lymphatic obstruction, which "tended to perpetuate the accumulation" of hemosiderin. Magery however, on the basis of his experimental work was of the opinion that focal macrophage aggregations could follow diffuse hemorrhage and were not necessarily the result of focal hemorrhage from dilated anastomoses in bronchiolar walls. He discussed possible mechanisms to explain the macrophage aggregations but did not come forward with a definite hypothesis.

The present work confirms that of Magarey, that bronchiolar mural varices resulting from cardiac failure are unnecessary for the occurrence of focal marrophage aggregations, since there is no reason to believe that the previously healthy experimental animals had bronchiolar varices. Likewise there was no evidence of lymphatic blockage.

In my opinion focal macrophage aggregations can be explained on the basis that since an alveolar duct drains many alveoli it may

constitute a "bottle neck" for hemosiderin-filled cells, proceeding from alveoli. Such a "bottle neck" is relieved when the cells finally reach bronchioles with ciliated epithelium, thus explaining the absence of accumulations in air passages of larger calibre.

In human pulmonary hemosiderosis occurring in states of chronic heart disease fibrous thickening of alveolar walls about accumulations occurs. Lendrum considers this and other stromal changes (degeneration of elastica and reticulum, granuloma formation) to be due to the presence of iron emanating from an intra-alveolar source. No such changes were observed in the lungs of the rats used in this experiment, even in those receiving several intrapulmonary injections of blood. However, the absence of interstitial changes may well be related to the time factor because the longest duration of life following instillation of blood in these experiments was 44 days.

PART VIII

SUMMARY

Isologous and autologous blood was introduced into the lungs of rats by bronchial catheterization. The sequence of events that took place after a single injection may be summarized as follows:

1. Erythrocytes packed in alveoli began to show degenerative changes at about 12 hours, and at this time formation of hemoglobin crystals also began. The erythrocytes seemed to fuse into columns out of which crystals appeared. Crystal formation reached a peak at approximately 36 hours. Fragmentation of crystals commenced at about 24 hours and phagocytosis of fragmented crystals began to occur at this time. By the end of the third day almost all crystals had undergone phagocytosis.
2. A transient polymorphonuclear leukocytic infiltrate appeared between the 12th hour and the end of the third day, by which time the polymorphonuclear leukocytes had degenerated and undergone phagocytosis by macrophages.
3. Macrophages dominated the cellular response to injected intra-alveolar erythrocytes. They were present in recongnizable numbers at 24 hours, were at a numerical peak by the fifth day and progressively decreased thereafter. After three weeks only scattered macrophages remained. The macrophages engulfed degenerated erythrocytes, fragmented hemoglobin crystals and degenerated polymorphonuclear leukocytes.

4. Hemosiderin was first demonstrated within macrophages at 24 hours after the injection of blood. Hemosiderin increased in quantity progressively as hemoglobin-containing material decreased within macrophages. By the fifth or sixth day the majority of macrophages were heavily laden with hemosiderin granules.
5. A pigment considered to be hematoidin was demonstrable within macrophages from the sixth day after the injection of blood.
6. Three weeks after the injection of blood in the lungs were grossly normal and showed no microscopic abnormality save for the presence of scattered pigment-containing macrophages.

A number of rats developed bronchopneumonia. This interfered considerably with the sequence of changes described above and in general seemed to retard them.

In rats receiving multiple intrtracheal injections of blood, killed 44 days after the first and 20 days after the last injection, all lobes of both lungs contained scattered aggregations of pigment-filled macrophages but otherwise were normal.

PART IX

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Idiopathic Pulmonary Hemosiderosis(essential brown induration of lungs)
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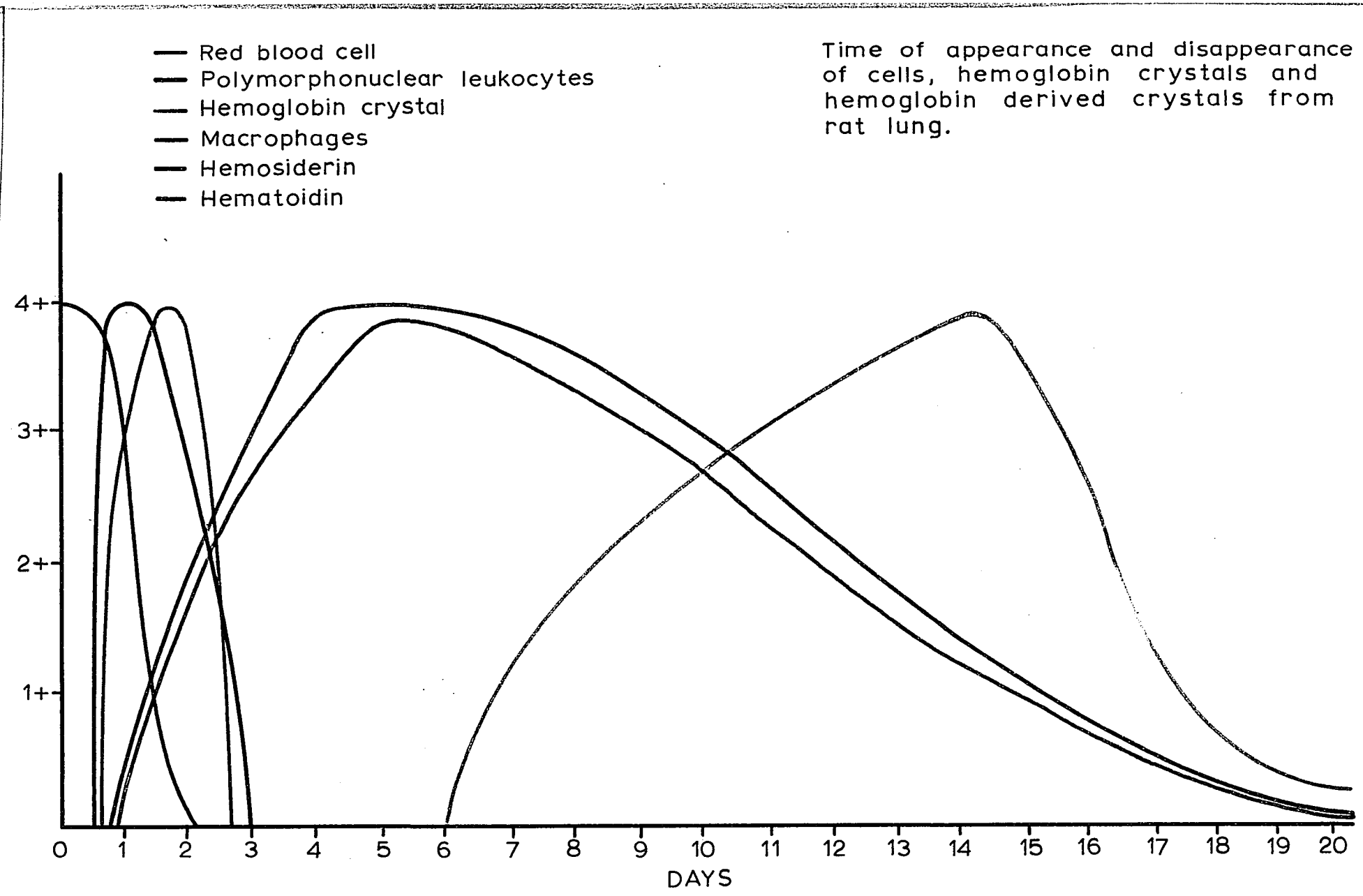

Time of appearance and disappearance of cells, hemoglobin crystals and hemoglobin derived crystals from rat lung.

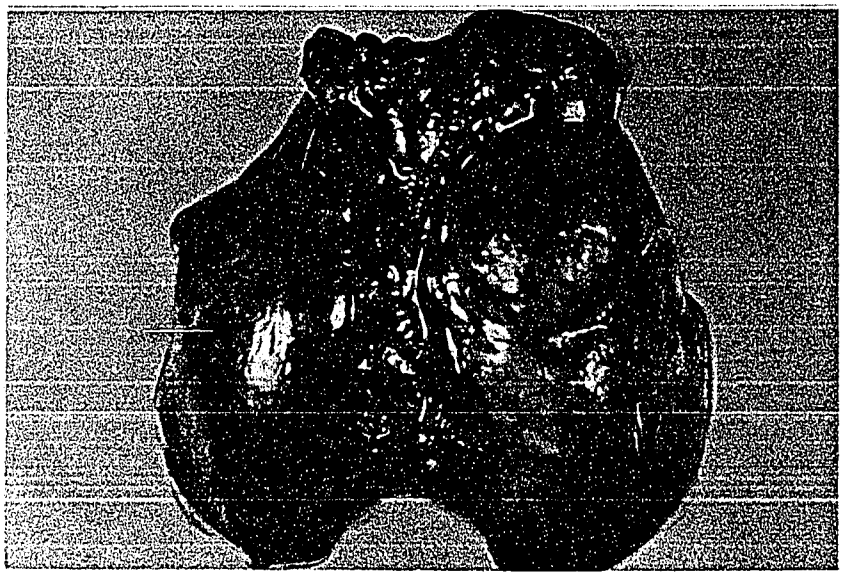

Figure I- Lung. I2 hours after intratracheal injection of blood into the right lower lobe. The blood-filled area is sharply demarcated and dark reddish-brown. The overlying plura is dulled.

Figure 2- Lung. 24 hours after intratracheal injection of blood into the left lower lobe.

Figure 3- Lung. 48 hours after intratracheal injection of blood into the right lower lobe.

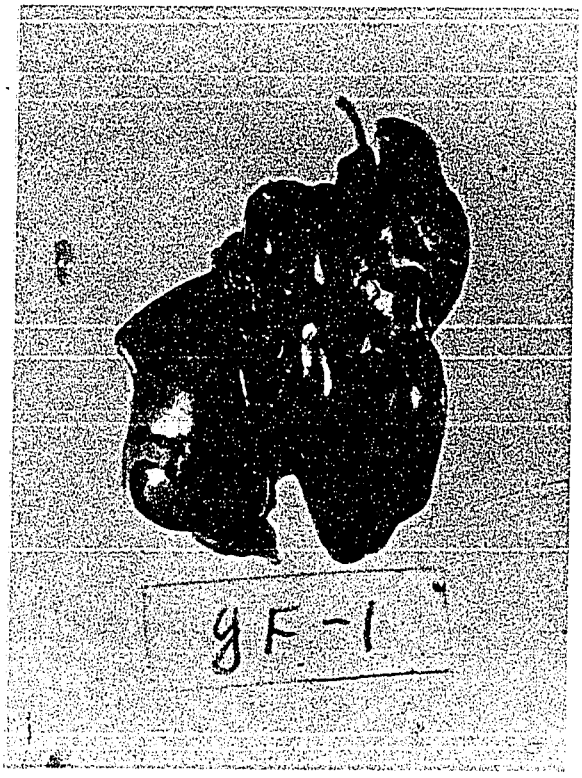

Figure 4- Lung. 3 days after intratracheal injection of blood into right lobe. The colour of the blood-filled area is slightly lighter than the corresponding area in previous photograghs.

Figure 5- Lung. 4 days after intratracheal injection of blood into the right lower lobe. The blood-filled area is less sharply demarcated and less dark in colour.

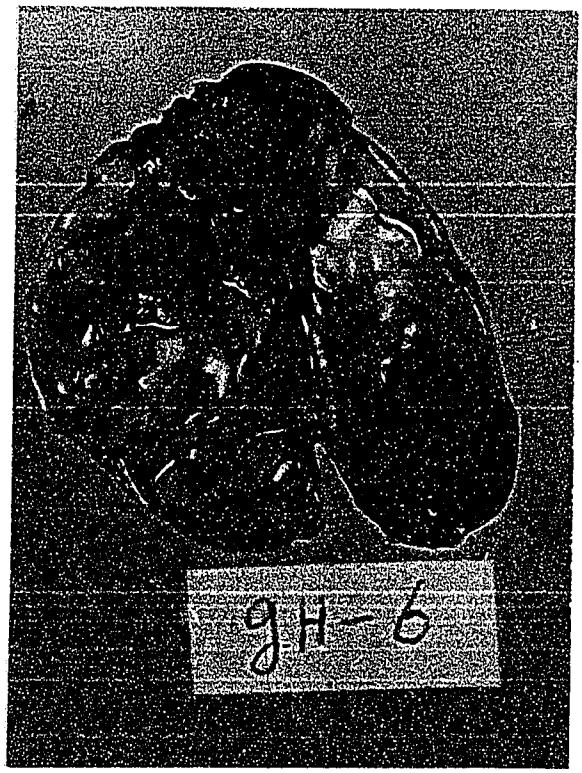

Figure 6- Lung. 5 days after intratracheal injection of blood into the right lower lobe. The blood-filled area is of a light brown colour.

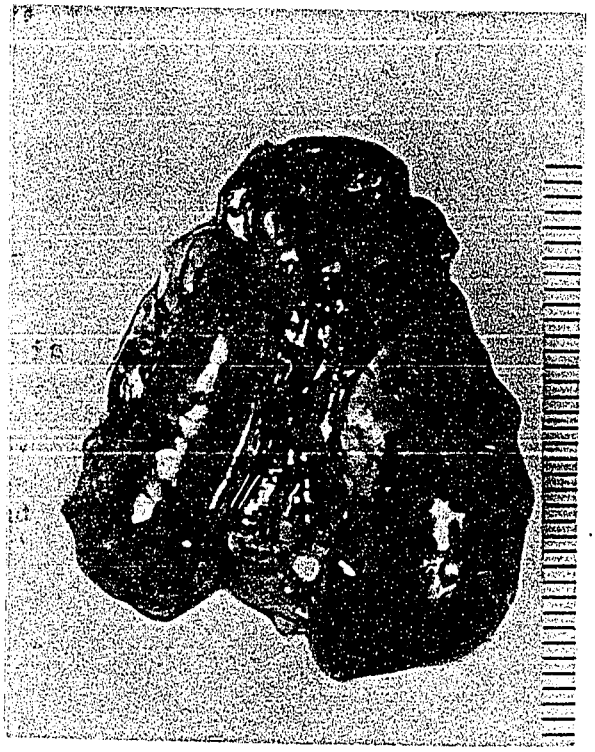

Figure 7- Lung. 7 days after intratracheal injection of blood into the right lower lobe. The blood-filled area is now a light yellowish brown and undistinctly demarcated from the surrounding tissue.

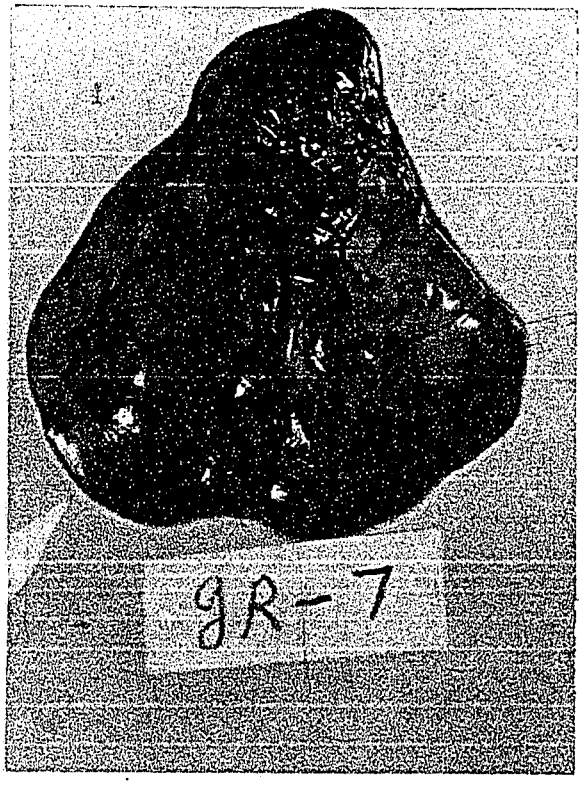

Figure 8- Lung. 9 days after intratracheal injection of blood into the lower lobe of left lung. A light greenish-yellow zone can be made out at the lower left of the photograph.

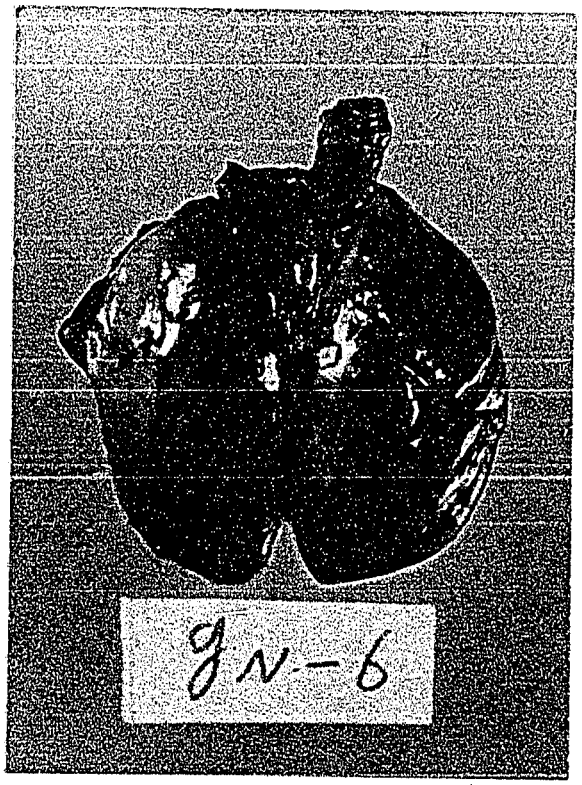

Figure 9- Lung. I2 days after intratracheal injection of blood into the left lower lobe. An area at the lower left of the photograph is faintly yellowish and represents the area which contained blood.

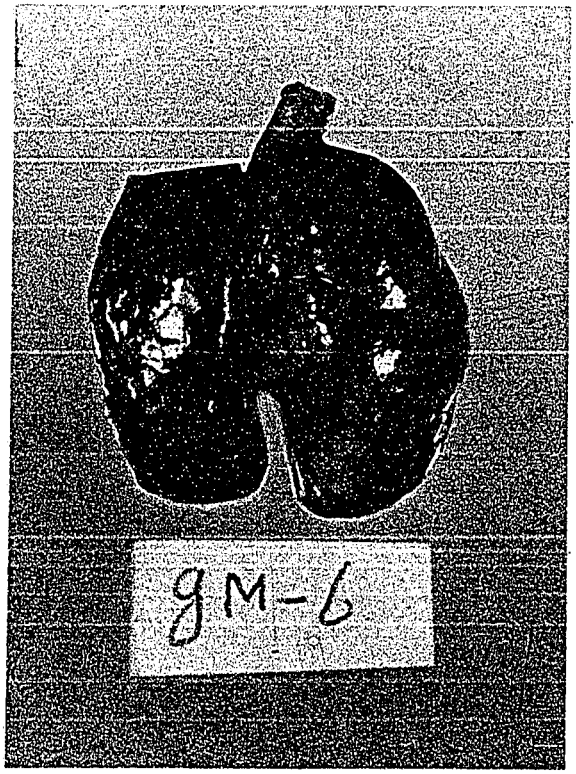

Figure I0- Lung. I4 days after intratracheal injection of blood into the left lower lobe. Scarcely any colour difference can be perceived to differentiate the area which received blood (at the lower left of the photograph) from the remainder of the pulmonary tissue.

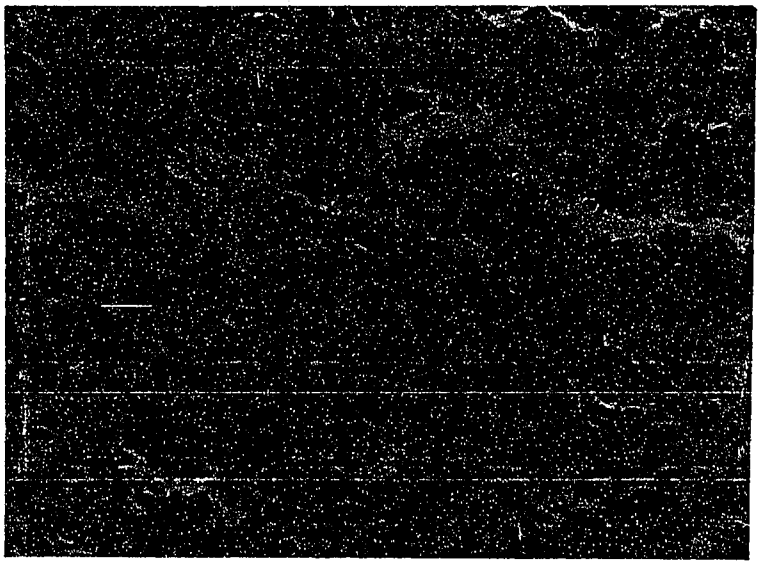

Figure II- Lung. 24 hours after injection of blood. Alveoli contain intact and degenerating erythrocytes. Haemoglobin crystals are seen at centre and upper left of the microphotograph.
Haematoxylin-phloxine-saffron. x 290

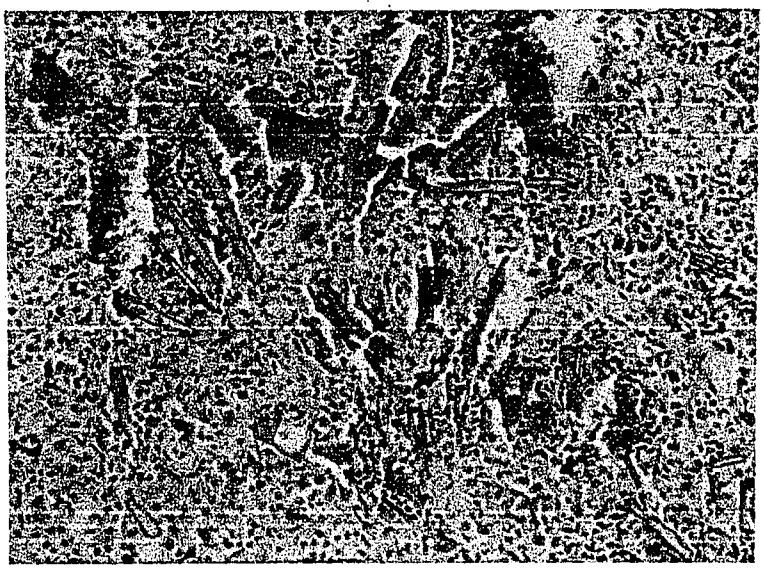

Figure I2- Lung. 36 hours after injection of blood. Haemoglobin crystals of varicus sizes and shapes are very numerous. There is an infiltration of pulmonary tissue with macrophages and polymorphonuclears.
Haematoxylin-phloxine-saffron. x I30

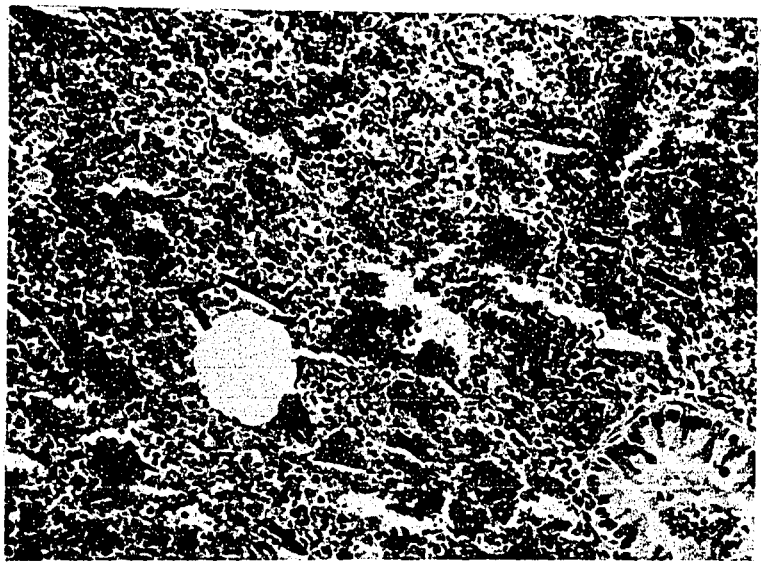

Figure I3- Lung. 48 hours after injection of blood.
Almost all the injected erythrocytes have been transformed into hemoglobin crystals. There is a polymorphonuclear and macrophagic accumulation.
Haematoxylin-phloxine-saffron. x 90

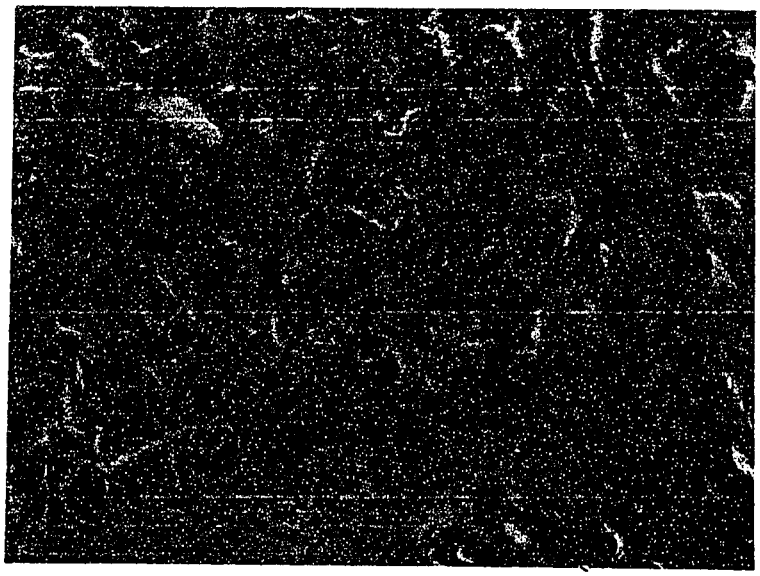

Figure I4- Lung. 48 hours after injection of blood.
Note crystals present in the lumen of a bronchiole.
Haematoxylin-phloxine-saffron. x I20

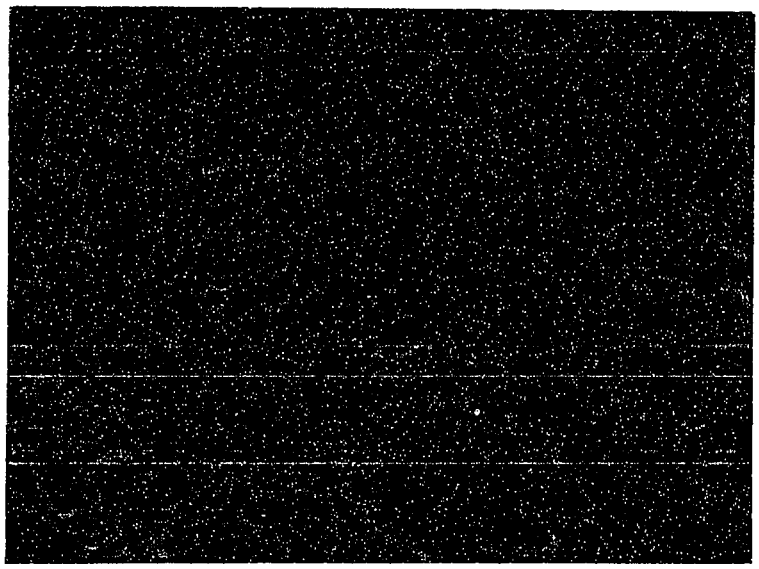

Figure I5- Lung. 48 hours after injection of blood. Macrophages are very numerous. Note cell in mitosis in lower left corner of microphotograph. Hemoglobin crystals are fragmentory and the fragments are being taken up by macrophages.
Haematoxylin-phloxine-saffron. x 350

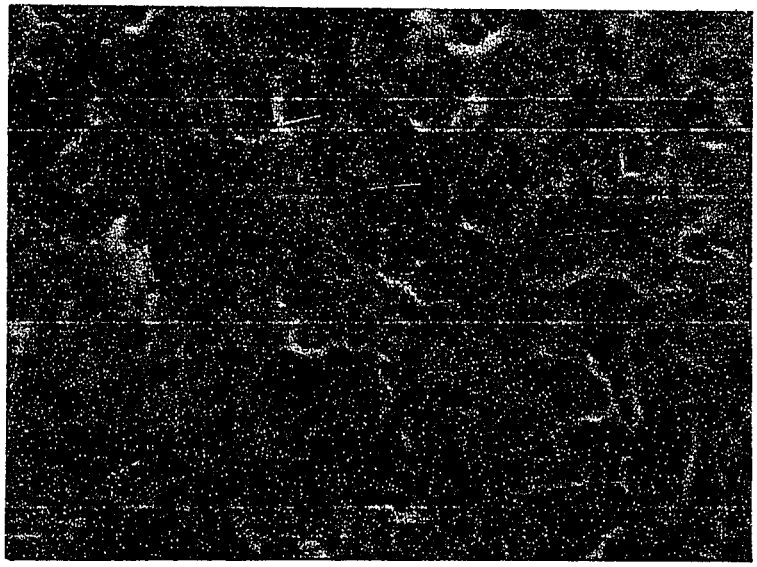

Figure I6- Lung. 48 hours after injection of blood. Break down and phagocytosis of hemoglobin crystals is shown. Break down of polymorphonuclear leukocytes is also seen.
Haematoxylin-phloxine-saffron. x 3I0

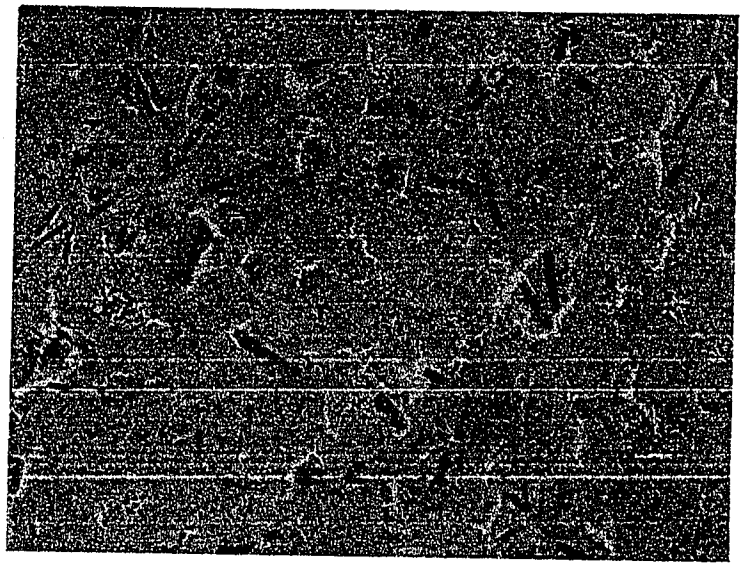

Figure I7- Lung. 36 hours after injection of blood. Hemoglobin crystals are bluish-black when stained with Amidoblack. x 90

Figure I8- Lung. 36 hours after injection of blood. Hemoglobin crystals are seen. Reticulum is faintly visible but it does not show pathological changes. Wilder reticulum stain, x I30

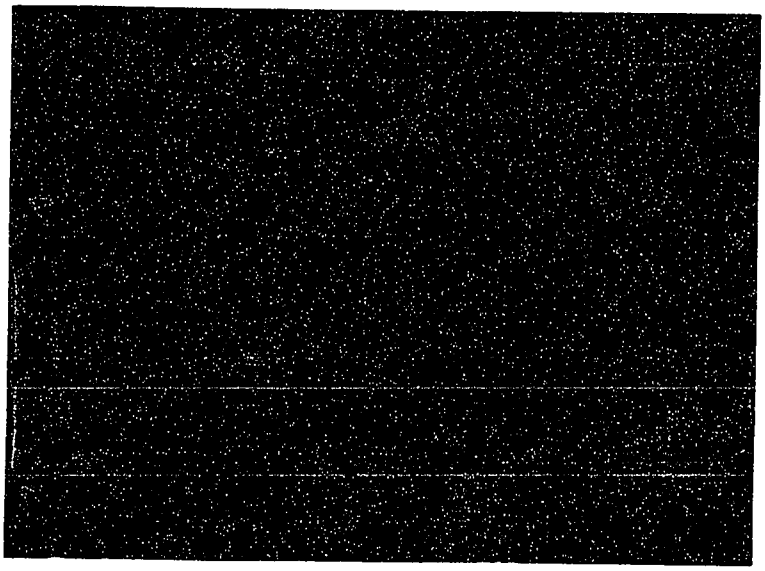

Figure I9- Lung. 36 hours after injection of blood.
A macrophage contains phagocitized erythrocytes and fragment of hemoglobin crystals.
Haematoxylin-phloxine-saffron. x II50

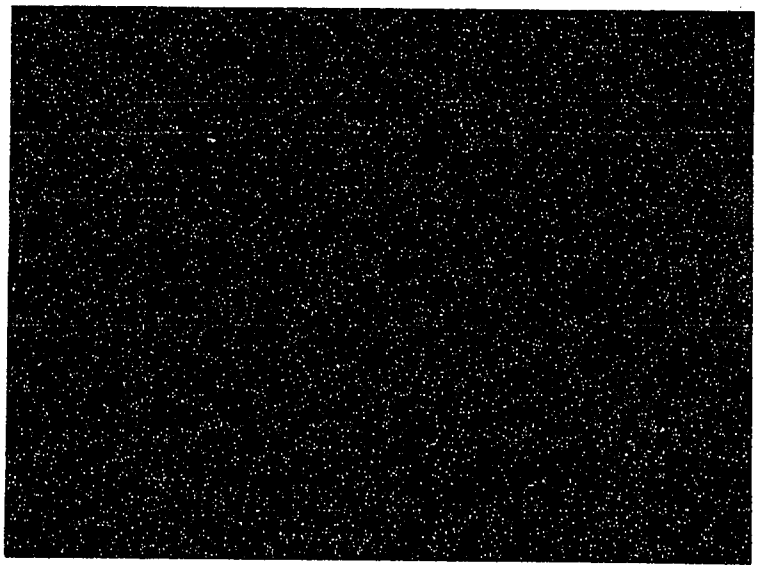

Figure 20- Lung. 48 hours after injection of blood.
Another macrophage containing phagocitized material.
Haematoxylin-phloxine-saffron. xII00

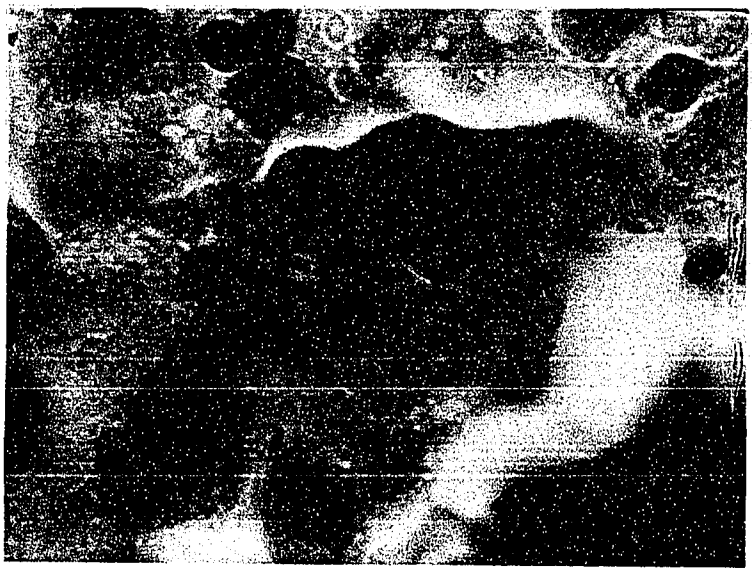

Figure 21- Lung. 60 hours after injection of blood. Macrophages are surrounding a partially fragmented hemoglobin crystal. Some cells contain phagocytized crystallin particles.
Haematoxylin-phloxine-saffron. x 950

Figure 22- Lung. 48 hours after injection of blood. Macrophages in contact with hemoglobin crystals.
Haematoxylin-phloxine-saffron. x 850

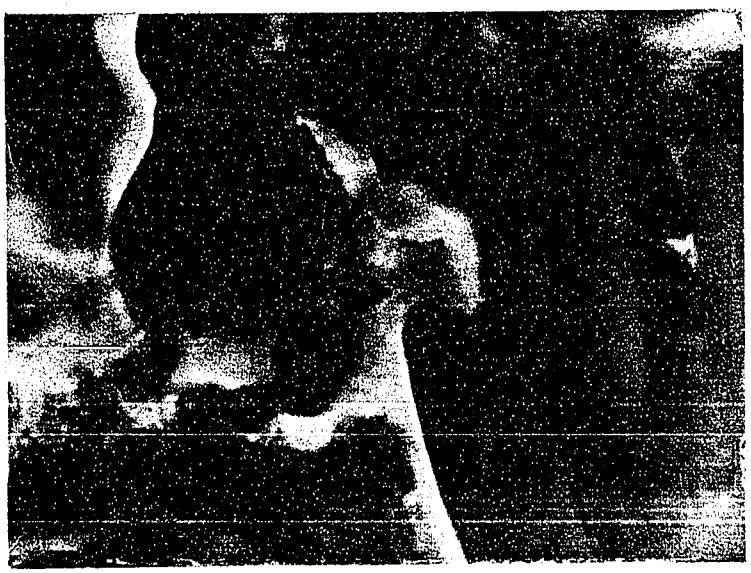

Figure 23- Lung. 48 hours after injection of blood. Macrophages appear to be phagocytizing hemoglobin crystals causing them to fragment. At the bottom of the microphotograph there is a macrophage loaded with crystallin material. Haematoxylin-phloxine-saffron. x 1150

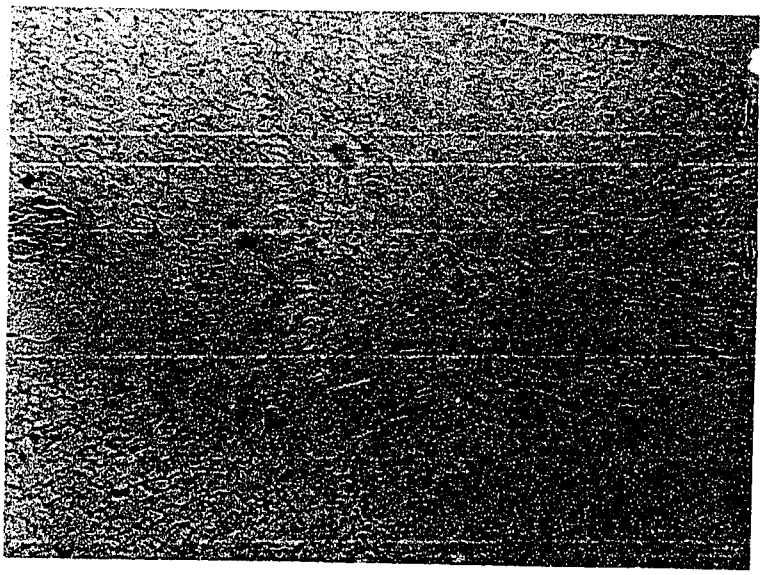

Figure 24- Lung. 5 days after injection of blood. Hemosiderin-containing macrophages are shown as blue dots. Gomori's iron reaction. x 50

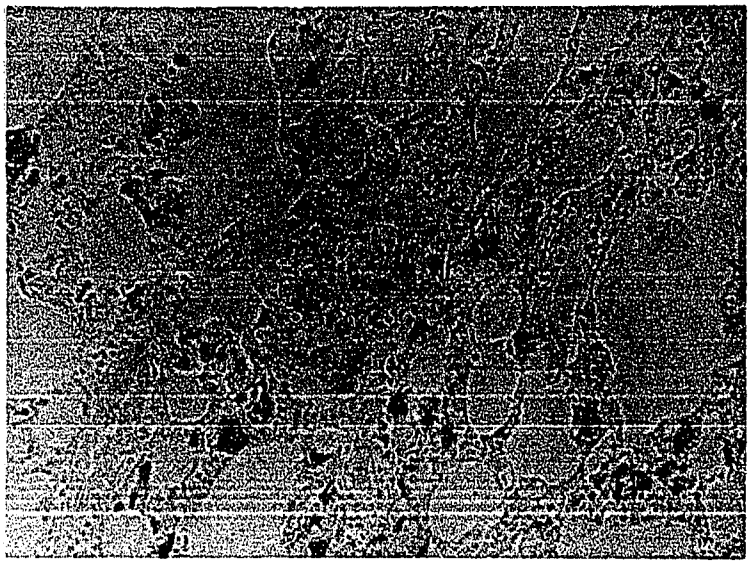

Figure 25- Lung. I0 days after injection of blood.
Hemosiderin-containing macrophages show blue cytoplasmic granules.
Gomori's iron reaction. x 90

Figure 26- Lung. I2 days after injection of blood.
Hemosiderin granules in macrophages are rusty-brown in colour.
Haematoxylin-phloxine-saffron. x I50

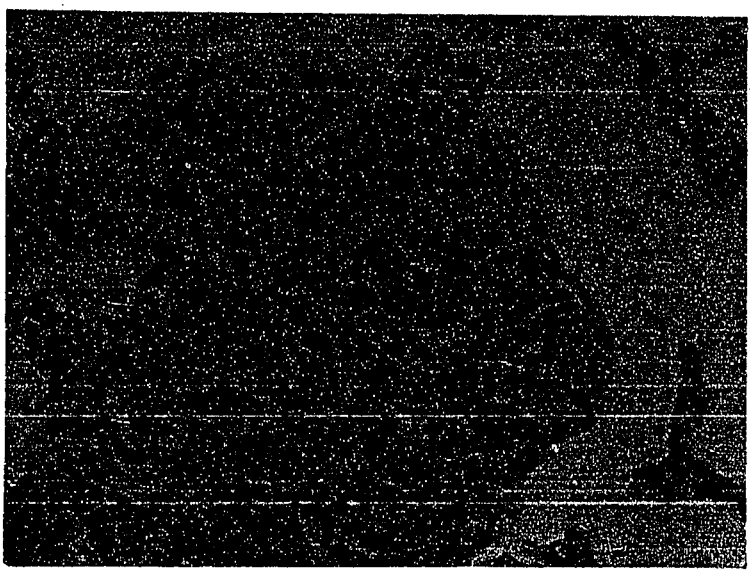

Figure 27- Lung. 12 days after injection of blood.
Macrophages containing hemosiderin granules which are rusty-brown in colour.
Haematoxylin-phloxine-saffron. x 205

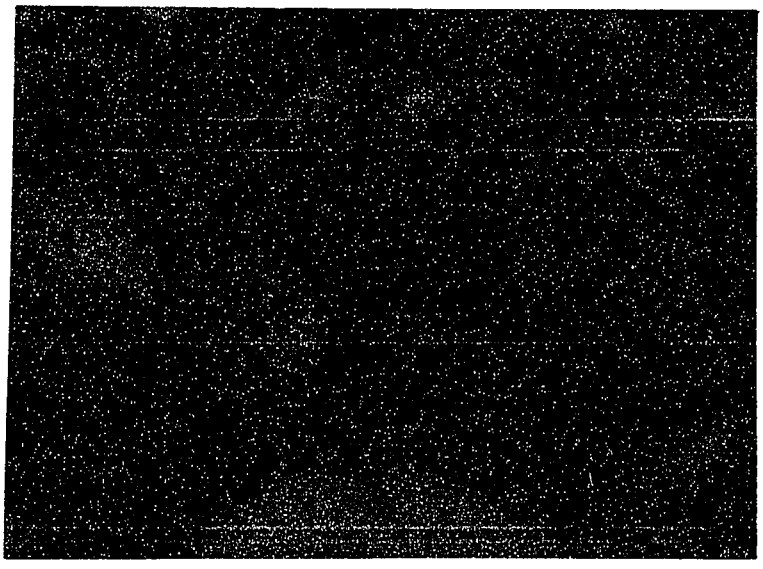

Figure 28- Lung. 7 days after injection of blood.
Brownish staining of cytoplasm of macrophages due to hemosiderin granules.
Haematoxylin-phloxine-saffron. x 750

Figure 29- Lung. I4 days after injection of blood.
The hemosiderin filled macrophages shown tobe attached to the alveolar wall.
Haematoxylin-phloxine-saffron. x I050

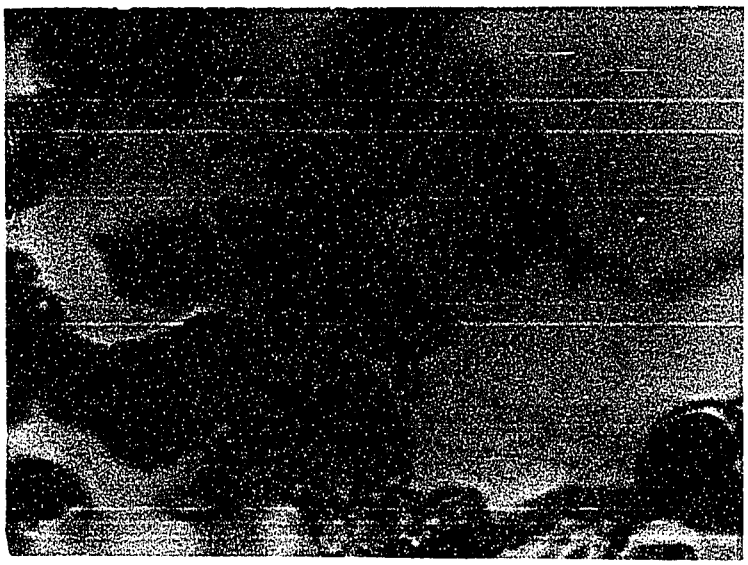

Figure 30- Lung. I6 days after injection of blood.
Hemosiderin containing macrophages are free in alveolar space.
Haematoxylin-phloxine-saffron. x 750

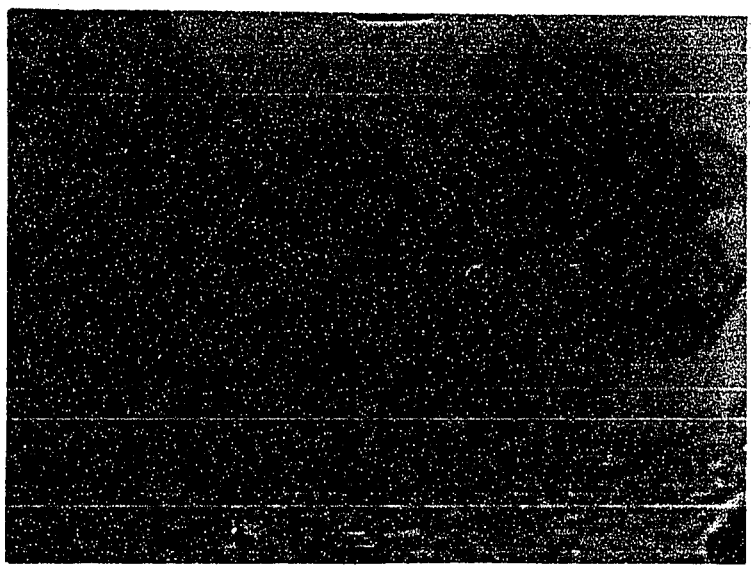

Figure 3I- Lung. I4 days after injection of blood.
A macrophage in the centre of the microphotograph containing yellowish-brown hematoidin crystals. Dark blue granules are hemosiderin. In the three other macrophages smaller amounts of hematoidin shown as hazy greenish areas.
Gomori's iron reaction. x I000

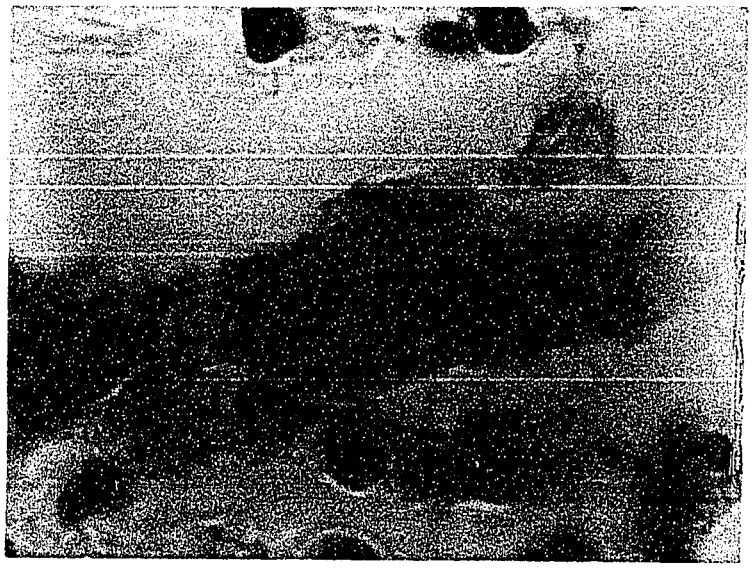

Figure 32- Lung. I2 days after injection of blood.
Macrophages containing crystalline hematoidin.
Gomori's iron reaction. x 900

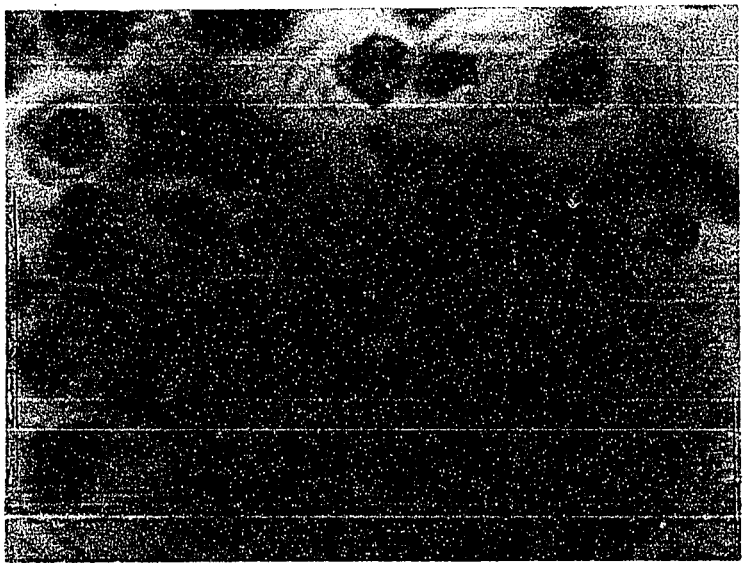

Figure 33- Lung. 8 days after injection of blood.
A macrophage containing a small quantity of amorphous hematoidin is shown in centre of microphotograph.
Gomori's iron reaction. x 910

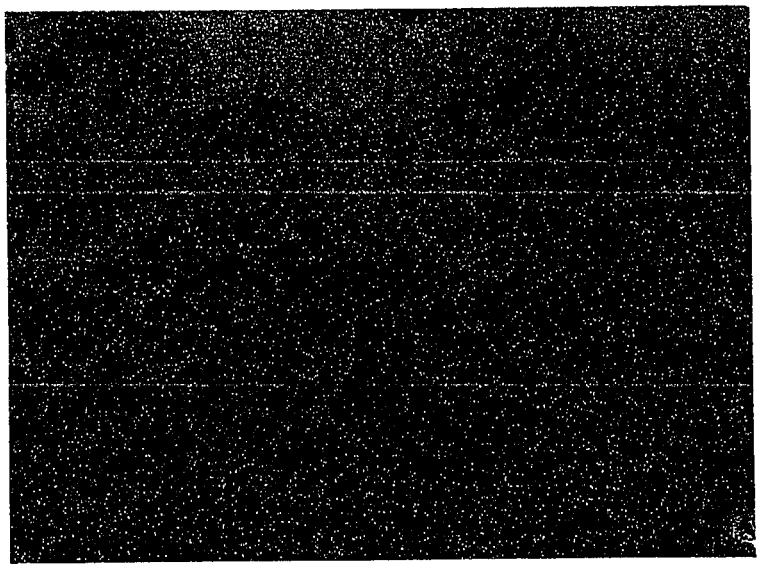

Figure 34- Lung. 10 days after injection of blood.
Macrophage containing crystallin hematoidin.
Gomori's iron reaction. x 950

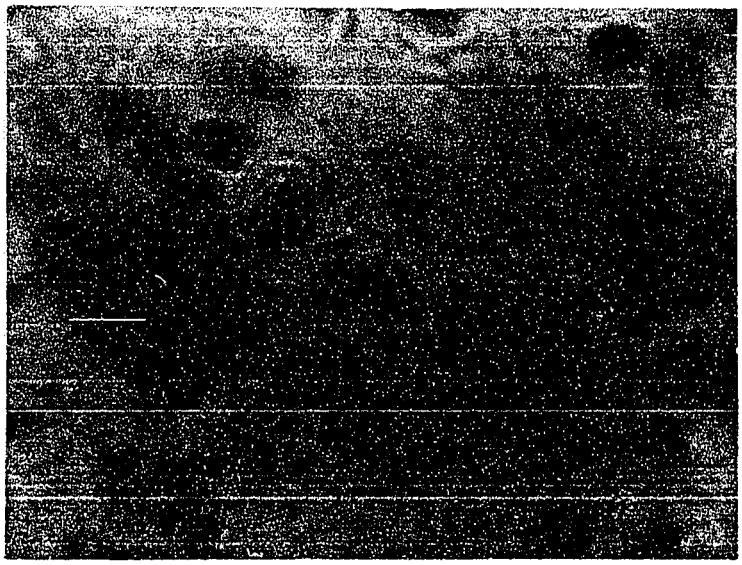

Figure 35- Lung. I0 days after injection of blood.
Macrophages containing crystalloid hematoidin.
Gomori's iron reaction. x 880

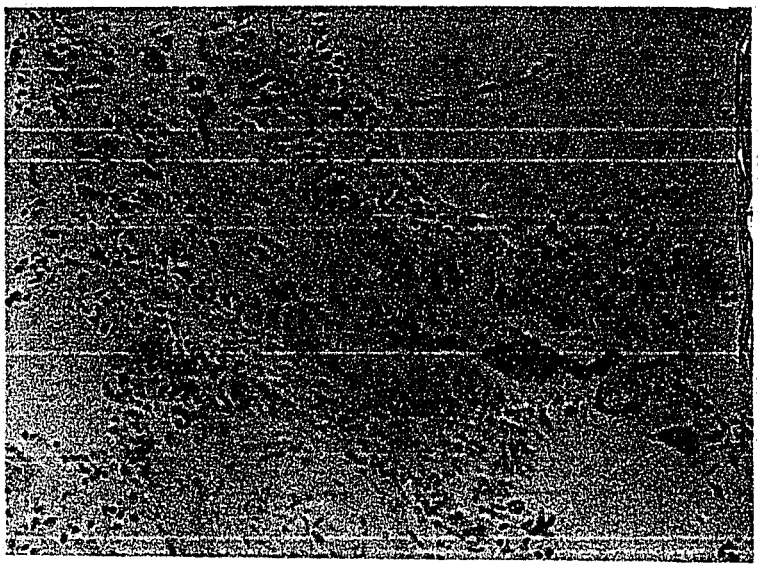

Figure 36- Lung. 6 days after injection of blood.
Hemosiderin containing macrophages are seen within the lumen of a small bronchus.
Gomori's iron reaction. x II0

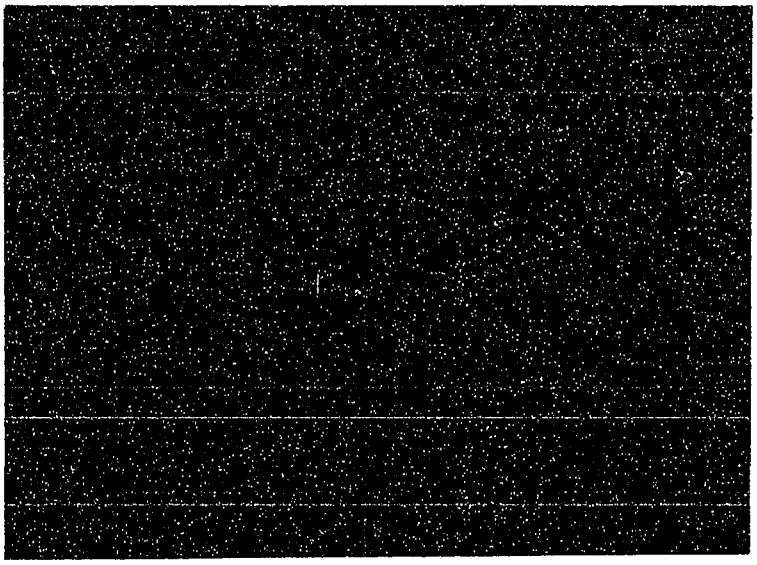

Figure 37- Lung. 5 days after injection of blood.
An alveolar duct opening from a bronchiole.
Hemosiderin containing macrophages are present in the mouth of bronchiole.
Gomori's iron reaction. x 240

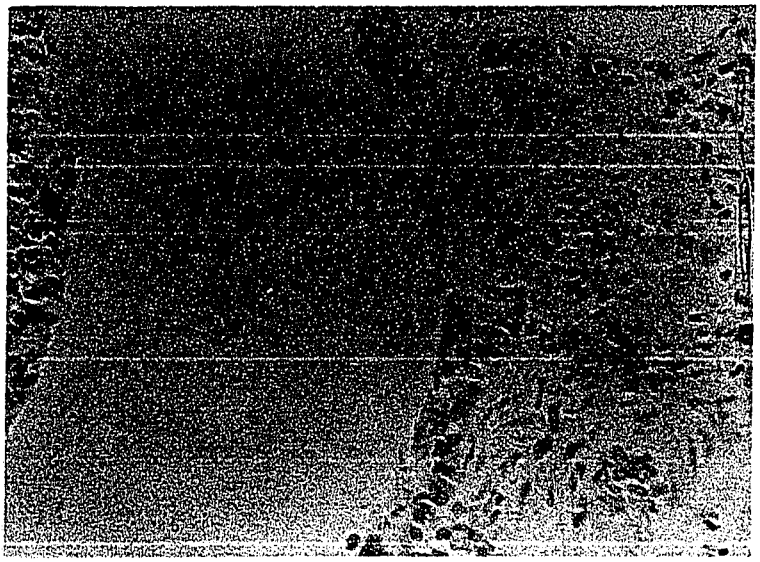

Figure 38- Lung. I0 days after injection of blood.
Hemosiderin-filled macrophages are present on the bronchial epithelium.
Gomori's iron reaction. x I50

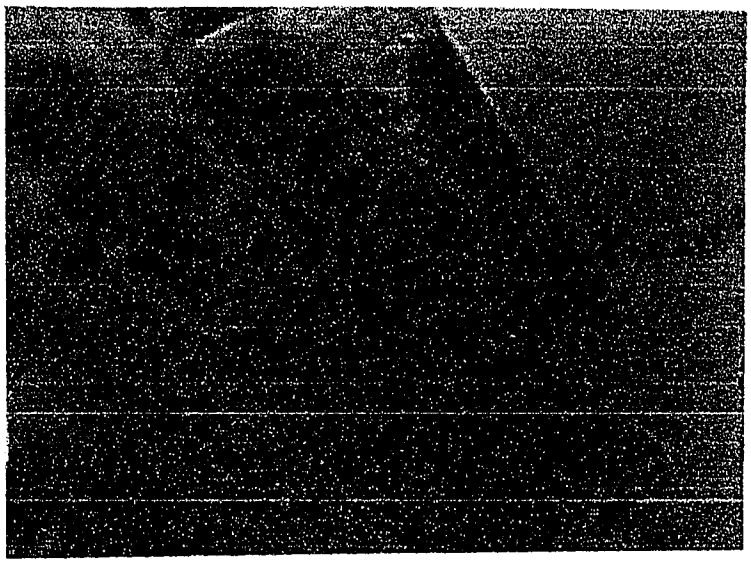

Figure 39- Lung. I0 days after injection of blood.
Two macrophages containing hemosiderin lie on the bronchial mucosa. The cell at the top of the microphotograph also contains yellow hematoidin crystals.
Gomori's iron reaction. x 7I0

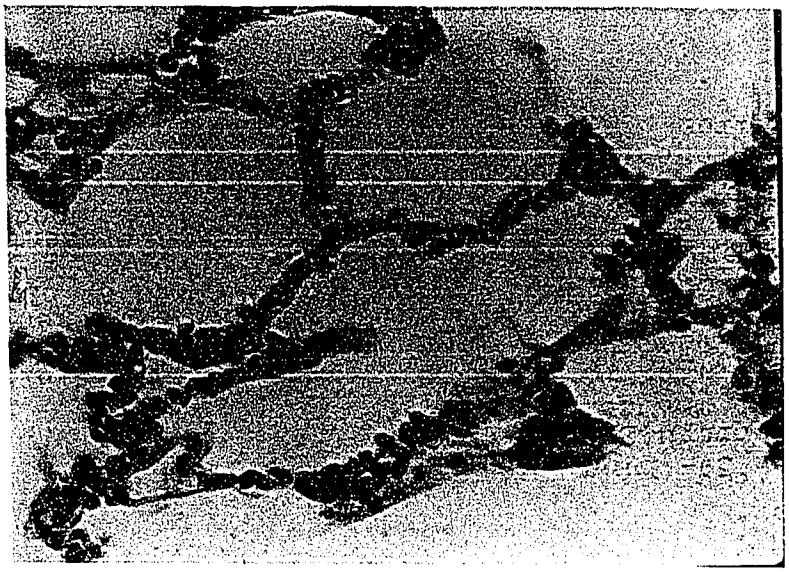

Figure 40- Lung. 20 days after injection of blood.
The parenchyma is unremarkable save for the presence of occasional scattered hemosiderin-containing macrophages.
Haematoxylin-phloxine-saffron. x 220

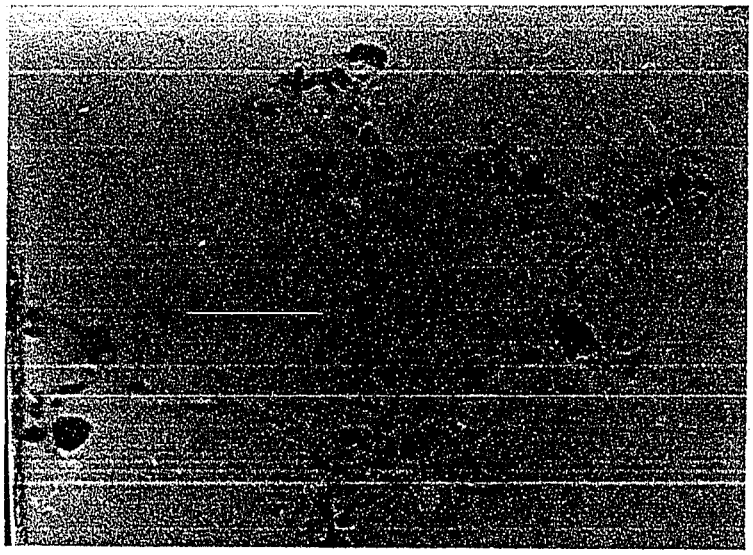

Figure 41- Lung. 20 days after injection of blood.
Hemosiderin-filled macrophages cling to alveolar wall.
One macrophage at the left of the microphotograph is loose in alveolar space.
Gomori's iron reaction. x 220

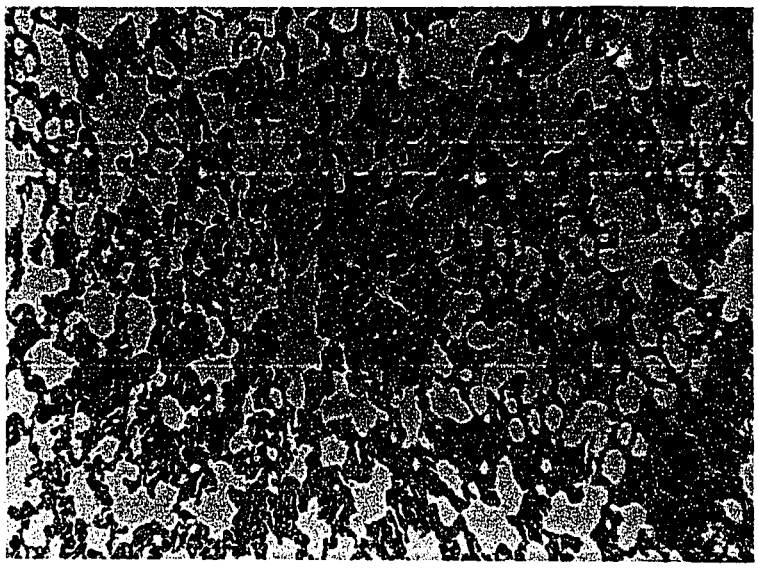

Figure 42- Lung. The rat received 8 injections of blood intratracheally every third day and was killed 20 days after last injection. The cellular areas are composed of macrophages containing brown hue.
Haematoxylin-phloxine- saffron. x 75

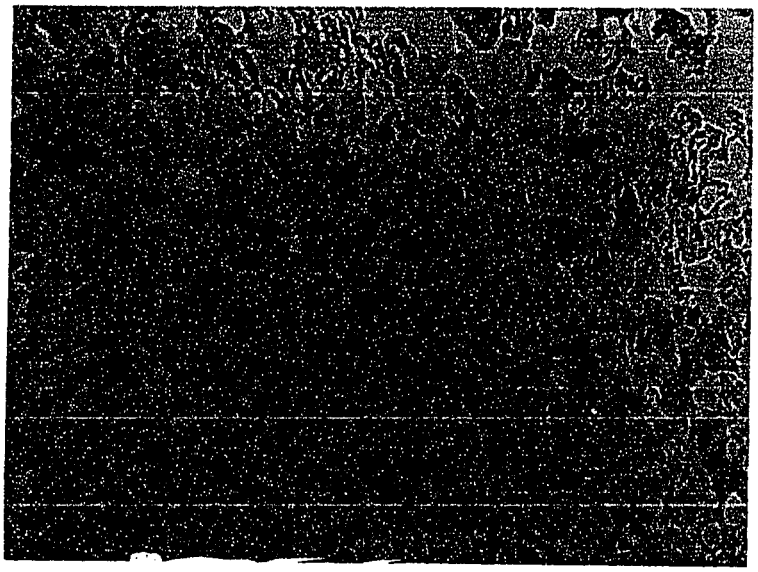

Figure 43- Lung. From same animal as in figure 42 showing the same area approximately. The clumping of hemosiderin filled macrophages are clearly seen.

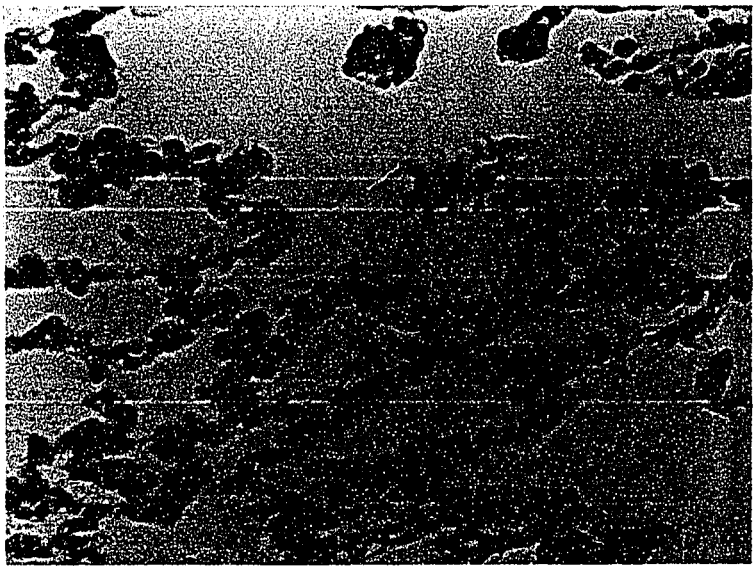

Figure 44- Lung. I8 days after injection of blood.
The microphotograph shows absence of pathological change in elastic fibers.
Weigert's elastic stain. x I60