ON THE REACTION OF THE MAMMALIAN LUNG TO TRAUMA.

A thesis presented to Glasgow University
for the degree of Doctor of Medicine
by

George L. Montgomery,
T.D., M.B.,Ch.B., Ph.D.,(St. And.), F.R.F.P.S.G.

Vol. I - - - - Text.

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789 East Eisenhower Parkway
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INTRODUCTION

The purpose of this work was to study the reaction of lung tissue to injury. It has long been known that wounds of lung are not necessarily fatal and within recent years it has been possible to incise the lung surgically and remove portions of lobes with satisfactory results. Lung puncture, too, is an apparently harmless procedure commonly undertaken in ordinary clinical work, particularly in paediatrics. The inference is that wounds of lung heal, but little is recorded about the mechanism of this repair. The standard text-books of pathology and of general and thoracic surgery omit any reference to the healing process, and a search of the literature has yielded only one relevant paper (Olch and Ballon, 1929). These workers made linear incisions in the lungs of dogs at right angles to the main axes of the lobes and reported that the wounds healed with scars which did not differ materially from scars elsewhere in the body. The source of the scar tissue was not determined. It was noted, however, that phrenicotomy did not delay healing. In Maximow and Bloom's textbook of histology (1942) it is stated "there is no evidence that the pulmonary tissue can regenerate after destruction" and this represents the prevailing view on the subject.

The work which is presented in this thesis was governed largely by war conditions. Begun as a substitute for a

study of bronchiectasis which was interrupted by the war, it was felt that knowledge of the reaction of the lung to injury might possibly be of importance in war surgery, and that whether or not the information was of topical value, it would in any case fill a gap in general lung pathology. The first observations were made in a military laboratory where the only experimental animals were rats and mice. Thus the initial experiments were simple stab wounds into the chests of lightly anaesthetised rats and mice, using as weapons ordinary hypodermic needles sterilised by boiling. Transfixion experiments of this kind have two disadvantages. If the puncture is made towards the hilum of the lung immediate fatal haemorrhage may occur, and if the needle is directed to the periphery the lung may be missed; obviously this depends upon the stage of respiration at the moment of impact. Secondly, the wounds so produced are very small, and in animals where at post mortem no puncture mark could be seen, serial sections had to be prepared to ensure that a lesion was not being missed, a tedious and frequently unproductive labour. At best this was a haphazard and unsatisfactory type of experiment and the proportion of animals with lesions for examination was small.

It occurred to me then to try the effect of stab wounds with needles heated to redness in the bunsen flame. Experiment showed that although the immediate fatalities

were rather more numerous than when the unheated needles were used, the animals which did not die immediately seemed quite unaffected by the injury and behaved normally. This technique resulted in a great improvement in the size of the lesions; actual puncture of the lung was not required to produce a lesion and the stage of respiration affected the result less. The character of the lesions, however, was altered. By this technique actual burns of lung were produced, and the organisation of burned as distinct from punctured lung could be studied: indeed the type of injury resembled that due to the diathermy or cautery which coagulates rather than burns. Nevertheless, this method was still unsatisfactory. It proved exceedingly difficult to produce comparable lesions, the extent of the injury was a matter of chance, and although the mechanism of healing was clear, a comparatively large number of animals was required to obtain a few suitable for microscopic study, particularly of the later stages.

At this time, after completing these two groups of experiments, I had an opportunity of discussing the work with Professor James R. Learmonth, Professor of Surgery in the University of Edinburgh, and he very generously placed the facilities of the Wilkie Surgical Research Laboratory at my disposal. There I was able to use cats, and to proceed to open thoracotomy experiments under positive

pressure anaesthesia and to excise comparable wedges of lung tissue and produce wounds of similar extent.

Accordingly, the work which is described below consists of three groups of experiments each producing a different type of lung injury, and it is hoped to show that these experiments are complementary and that the reactions of the pulmonary tissue to different stimuli have many common features. It is proposed to present the work under four headings:-

The photographs have been bound in a separate volume.

I. PUNCTURED WOUNDS OF LUNG IN MICE AND RATS.

The number of animals used in this group of experiments approximated two hundred but after subtracting those which died from immediate fatal haemorrhage and those in which the needle missed the lung and failed to produce a lesion, the series which finally came to microscopic examination is enumerated in Table I.

Technique.

All the animals were young adults, the mice varying from 22 - 25 gms. and the rats averaging 120 gms. in weight, but otherwise unselected. Each animal was anaesthetised with ether, grasped firmly so that the skin was drawn tightly over the right half of the chest, and the hair over the fifth interspace anteriorly was removed by plucking. Puncture was performed by introducing the needle into the fifth interspace in the anterior axillary line, in a general direction backwards, downwards and slightly medially. Experiment showed that in this way lesions could be produced with a fair degree of certainty in the lower part of the right apical lobe, usually near the middle of its inferior margin but sometimes nearer the tip: the exact site depended on the stage of respiration at the moment of puncture. The puncture was made by a definite stabbing action and sharp hypodermic needles, mounted for convenience on a

TABLE 1.

List of lung punctures examined microscopically.

(rats and mice).

Time interval between lung puncture and sacrifice or animal.	List of animals.
30 minutes	R 21
3 hours	M 29
19 "	M 27
22 "	M 80
24 "	M 60
44 "	M 49
47 "	M 81, 82
48 "	M 32, 33
70 "	M 104 , 36.
72 "	M 61
96 "	M 37
5 days	M 40, 41, 42.
6 days	M 51
7 "	M 14
8 "	M 43, 44.

11 days	M 45
12 "	M 53
13 "	M 15, 47, 75
14 "	M 115
16 "	M 114
33 "	M 90, 91, 92
37 "	M 118.

syringe, were employed. Ordinary hypodermic needles, guages 12 to 14, were used for the mice while larger wider needles of the serum variety, guages 5 to 7, were used for rats. For the first group of experiments the needles were boiled in saline and used when cool: for the second and larger group, they were heated to redness in the bunsen flame and plunged into the chest immediately. As a rule the length of needle which entered the chest did not exceed 0.5 cm.

In the absence of fatal haemorrhage, the animals were not disturbed by their injuries. There was no apparent shock, they were lively and normal in their behaviour, they appeared to ignore the local burn of the chest wall and integument, and although the latter remained charred for a few days, healing was always uneventful. Occasionally an animal was noticed to have laboured breathing a few hours after the puncture. These were killed at once and invariably found to have haemorrhages which had developed more slowly.

At post mortem, the heart and lungs were removed together, searched carefully for puncture marks or other evidence of lesions, and fixed as a whole. To begin with, 6 hours fixation in Zenker-formol was used, but latterly formol-perchloride was employed (Lendrum, 1941). Paraffin sections were prepared, and in the case of puncture wounds

with the unheated needles, serial sections were frequently necessary to ensure that nothing was being missed. A variety of staining methods was employed, including haematoxylin and eosin; haematoxylin, phloxine and tartrazine (Lendrum, 1939); Mallory's connective tissue stain and its controllable modification (McFarlane, 1944); Masson's trichromic methods; and for elastic tissue, orcein, Weigert's resorcin-fuchsin and the Verhoeff-van Gieson methods; and Foot's ammoniacal silver carbonate for reticulum.

DESCRIPTION OF FINDINGS

The lesions arranged themselves naturally into two groups, representative respectively of the post-traumatic and proliferative stages of the reaction.

Post-traumatic lesions.

This term may be applied to animals killed prior to the onset of cellular proliferation, a stage which in general may be said to occur about 48 hours after the trauma, and indeed no animal whose injury was more than 48 hours old failed to show some proliferative change. It is of course impossible to lay down a time limit for these reactions and the criterion for including animals in the post-traumatic group has been the main characters rather than the duration of the lesion.

Rat 21 is the earliest lesion of the series, a lung puncture with a cold sterile needle in an animal killed

30 minutes later. In this instance the needle passed through and almost severed the tip of the right apical lobe anteriorly, producing a gap, 800μ wide, bridged only by strands of blood clot (fig. 1). Alveoli containing erythrocytes and having dilated, congested and tortuous mural capillaries, occupy both sides of the gap and these alveoli in turn are separated from normal lung by alveoli containing oedema but whose capillaries are not congested. The needle has punctured also the middle lobe where it lies behind the lower margin of the apical lobe, interrupting the pleura and leading to haemorrhage and some oedema of the superficial alveoli. In both lobes the reaction is local to the area of the puncture.

Mouse 60 was killed 24 hours after puncture with an unheated needle and shows a lesion involving only the tip of the middle lobe of the lung (fig. 2). Although the penetration appears to have been no more than 500μ in depth, the affected portion of the lobe is partially atelectatic, with considerable haemorrhage into the remaining alveolar spaces. Some degree of swelling of the alveolar lining cells and of the endothelium of the alveolar capillaries has occurred. Another example is to be seen in mouse 104, killed 70 hours after lung puncture with a cold sterile needle. There the lesion was in the right apical lobe and the injured area is indicated by a

pin-head yellowish area which looks ischaemic, and is surrounded in turn by a zone of congestion. Fig. 3 shows the microscopic appearance: the lung is atelectatic and has herniated above the pleural surface at the point of puncture. Extruded blood covers the surface of the pleura which has become detached around the puncture mark. The lung has been penetrated to a depth of 1 mm. (approximately one-quarter of the width of the lobe); fig. 4 is a micro-photograph of the needle track. In these lesions with cold needles there is no cellular infiltration apart from small collections of pus cells around the puncture marks: oedema and necrosis are absent.

It is however otherwise with the lesions produced by the heated needles where necrosis, congestion and oedema are the characteristics of the immediate post-traumatic reaction. To naked-eye examination the puncture mark was not always clearly seen, but when it was visible it appeared as a dark haemorrhagic point, frequently marked by carbon particles and surrounded by localised fibrinous pleurisy: adhesion of the pleural layers occurred in one animal, rat 29. As a rule the lung in the immediate neighbourhood of the puncture was swollen, protruded above the lung surface, and showed intense congestion which faded gradually to normal lung.

Microscopically the necrosis of the pleura and alveoli along the needle track is of the coagulative type, and although it varies in extent in different animals, in general in section it is triangular or crescentic in shape, i.e. the actual lesions are roughly wedge-shaped. The pleura is torn at the point of puncture, sometimes blood has been extravasated and adheres to the pleural surface, the serosal cells over the whole surface of the necrotic area have been lost, and the pleura stains poorly by haematoxylin and eosin although its elastic membrane retains its specific staining properties. Similar reactions are observed with the necrotic alveoli where nuclear staining is absent, and the walls in most instances are represented only as apparently acellular structures which, however, are coloured by specific elastic stains. The extent of the necrosis beyond the needle track varies considerably, but the tinctorial effect of the necrotising agent is remarkably clear-cut: for example it is not uncommon to see a bronchus or blood vessel with half its lumen lined by necrotic cells which have retained their shape but lost their nuclear-staining properties (fig. 5).

The necrotic tissue is enclosed by zones of congestion which vary in width from 200μ to 2 mms. (figs. 6, 7, 8 & 9). In these zones the capillaries of the alveolar walls are intensely congested and sometimes obscured by intra-alveolar

haemorrhage, particularly so at the junction with necrotic tissue, where the congestion is intense. In the lesions approximately 1 and 2 days old, red blood cells can be seen in many of the necrotic alveoli, while small blood vessels with necrosed walls contain blood which has been in circulation at the time of death. Thus in this stage (24 - 48 hours) there is evidence of re-establishment of a circulation through the necrotic lung, and examples can be seen of polymorphs pavementing the intima of necrotic vessels (figs. 10 and 11), although pus cells in the alveoli are uncommon.

The congested zones terminate sharply and are separated from the remainder of the lung tissue by oedematous alveoli with relatively collapsed alveolar capillaries, a comparative ischaemia in marked contrast to the congested tissue adjacent. Generally the zone of oedematous alveoli equals the congested zone in width, but examples were met with where the oedema has spread much further and involved a whole lobe, including the bronchi. Fibrin has not been detected microscopically in this fluid which, so far as staining qualities can indicate, is of uniform density (fig. 12).

Proliferative Reaction.

Cellular proliferation in the lung and pleura contiguous with the necrotic tissue commences very soon after

injury and evidence of this can be seen in the animals killed more than 40 hours after puncture, although the process is most active in the experiments of several days' duration.

Pleura.

The pleura covering the areas of lung which have been burned by heated needles is usually completely necrotic and appears as a rather featureless membrane. Occasional isolated pear-shaped serosal cells may be seen but they do not take part in the initial stages of the proliferative reaction. The earliest change is in the serosal cells of the pleura immediately adjoining the necrotic tissue and consists of swelling of the cells *in situ* (fig. 13). This is followed by cellular proliferation resulting in flattened elongated cells parallel to the lung surface (fig. 14). Sometimes more irregular cell types occur, as for example in fig. 15, where there are carbon particles embedded in the pleura, a frequent occurrence around the puncture mark.

Lung punctures by the cold sterile needles obviously produce much less damage. Although serosal cells are absent at the actual entrance wound there is no necrosis as in the burned lungs: consequently the cellular reaction is less, and usually limited to simple swelling of the serosal cells nearest to the puncture.

It may be said therefore that the pleural reactions

are essentially of the serosal cells: there is no vascular fibroblastic reaction and collagen is not excessive. The hyperplasia of the serosal cells persists for variable periods but certainly for two or more weeks, apparently without alteration, ultimately being restored to normal.

Alveolar Proliferation.

Almost two days after the trauma a change occurs in the alveoli enclosing the necrotic tissue, that is in the original haemorrhagic zone and particularly in the part of the zone nearest the normal lung. There the alveolar lining cells become visible as swollen cubical or cuboidal cells, usually with pale-staining cytoplasm and open vesicular nuclei. In collapsed or partially atelectatic alveoli these cells are arranged in groups or clusters of almost glomerular appearance, but more frequently they appear in columns or solid sheets. The pattern of this arrangement is in effect to produce a zone of demarcation or boundary zone, separating the injured and normal lung. But these changes are not limited to the hot needle experiments: they are to be seen also in the punctures with cold needles, where although the change is less easily recognised on account of the atelectasis, it is nevertheless characteristic. Figures 16 to 23 illustrate this reaction.

Frequently the boundary zone is found to have a bronchus situated near its centre. A portion of the bronchial

wall may be necrotic or more commonly the lumen contains some fibrin clot, insufficient to block it. At a very early stage however air channels can be traced from bronchi and bronchioles, channels which in some cases are no more than extremely narrow tortuous slits, frequently without any specific cellular lining, connecting the bronchi with partially collapsed alveoli (figs. 23 and 24). In particular air channels of this undeveloped type are to be seen leading from the boundary zones into the injured areas and sometimes ramifying within the boundary zones themselves. More significant, however, are proper bronchial buds or branches, lined by small cubical or cuboidal cells, and which are to be seen within the boundary zones at a slightly later stage. (figs. 25 and 26). The cellular lining of some of these minute air channels is morphologically very similar to the hyperplastic alveolar cells, but in others it is low or altered respiratory epithelium, non-ciliated and arranged as single rows lining channels which communicate on the one side with the bronchus or bronchioles, and terminate in the tortuous slits between the groups of altered alveolar cells.

Accordingly, it may be said that so far as the respiratory elements of the lung are concerned, the boundary zone is not only a zone of demarcation but also the starting line of bronchial proliferation, and of hyperplasia and

metaplasia of the alveolar cells. There seems little doubt that the latter are in fact alveolar lining cells rendered visible by the proliferative stimulus. In densely cellular areas and particularly where the degree of atelectasis is considerable, it is difficult to distinguish the endothelium of the alveolar mural capillaries from the altered swollen alveolar lining cells, and to separate the latter from the lining cells of the bronchial channels. In effect the boundary zone contains cells which have altered under stimulation to assume a more uniform appearance, and to resemble primitive respiratory epithelium. Examination of a large series of sections shows all degrees and graduations of the change which results in this barrier of pulmonary tissue.

In addition, however, to the metamorphosis of the respiratory elements, the vascular tissue of the lung immediately adjoining the necrotic portion shows marked activity. Capillary blood vessels in the alveolar walls and accompanying the bronchi have dilated, and are filled with red blood cells: the endothelial cells forming their walls have swollen and elongated into fleshy-looking bipolar cells, extending into the haemorrhagic zone and from it into the injured lung. Evidence of reestablishment of the circulation in the latter has already been noted, and in lesions two and three days old, cellular elements can be seen in what

was regarded originally as necrotic lung. In the main these cells are elongated endothelial cells arranged so as to form capillaries with walls one cell thick, but there are also isolated alveolar cells, similar in appearance to the swollen cells of the boundary zone. From their isolation in the necrotic tissue these cells are not the advanced elements of an infiltrative process, but rather cells which have suffered less severely from the trauma and ultimately re-developed in situ. Fibroblasts also can be seen extending out into the injured tissue, mainly from the adventitia of bronchi in the boundary zone. The tinctorial and morphological resemblance of these cells with the proliferating capillary endothelial cells is very close, and it is only by tracing each group of cells to its origin in the vascular or the adventitial tissue that they can be designated and identified with certainty.

Such then are the appearances met with in sections from animals killed up to 5 days after lung puncture. In so far as cold sterile needles lack a necrotising effect, the reaction is correspondingly less severe than that induced by the hot needles, and the subsequent fate of the two types of punctured wounds now differs considerably. Difficulty was experienced in obtaining evidence of the final stages of the cold needle experiments. Many animals were examined where the duration of the experiment varied

from 5 to 15 days, but it was impossible to identify the area of the injury despite serial sections of all the lungs. In one animal there was a tiny haemorrhagic point on the pleura, and in another a minute white circle thought possibly to be pleural thickening, both in the areas of lung which experience had shown to be most frequently involved, but sections failed to establish that these were of traumatic origin. Accordingly although it may be inferred that the lesions heal without leaving residual fibrosis - and from the results of the much more extensive wounds in cat lungs (vide infra) such indeed is most probable - the proof is of a negative character. Nevertheless the active formation of air channels from bronchi into atelectatic areas, and the apparent re-expansion of the marginal alveoli of these areas, are significant findings to be discussed fully later, but meantime it may be said that they are certainly important as heralding restoration of functional lung tissue.

Necrotic lung and pleura are the major complicating factors in the wounds with the hot needles, and it is from the boundary or reactive zone that the further organisation of the necrotic tissue is based. In the early stages the boundary zone varies from 300μ to 2000μ in diameter, varying in different specimens and according to plane of section, and animals killed at progressively longer intervals after

injury show that the boundary zone encroaches continuously on the necrotic area. The growth of capillaries becomes better developed, and fibroblasts and endothelial cells more numerous. Some of these endothelial cells have assumed polymorphous shapes, others are elongated spindle- or tadpole-shaped cells, staining more deeply than usual and obviously proliferating actively. The swollen alveolar lining cells have now split up into small groups separated by blood capillaries or air channels, and thus tend to assume an alveolar arrangement. Moreover, they can be seen to extend as short columns into the necrotic tissue to unite there with isolated cells of similar appearance. On their part, these isolated cells have proliferated, and a week after injury much of the damaged lung is no longer necrotic, but vascular cellular tissue.

Such a tissue cannot become functioning lung until it is in contact with the atmospheric air through the bronchial system. The bronchial channels or buds referred to above are the main agents in effecting this communication, and in the experiments of longer duration they can be seen to be more numerous and more complex. There is, however, considerable variation in the appearance of the later wounds. For example, mouse 47, duration of experiment 14 days, shows a wedge-shaped lesion where there has been ingrowth of bronchial branches, (fig. 27). The central pale portion

of the consolidated area, shown at greater magnification in figures 28 and 29, consists in fact of dilated bronchial branches embedded in cellular connective tissue. The branches are lined by single layers of flattened or cubical cells and contain coagulated mucus. A greatly dilated bronchus is to be seen at one lateral margin of the lesion, and in the other dilated alveoli. Experience of the cat wounds (vide infra) has shown that when new alveoli are formed in the margins of a scar, they are lined either wholly of in part by cubical cells. It is to be noted that in this mouse lung these alveoli lack a cellular lining. They are in fact emphysematous spaces, not to be interpreted as new alveolar formations.

Mouse 92, duration of experiment 33 days, is another example of bronchial proliferation into consolidated lung, where emphysematous alveoli also are to be seen. (fig.30). It is noticeable here, however, that the bronchial proliferation has been effective only near the hilum of the lung. The apical part of the lobe, despite the presence of a fairly large bronchus, is really not aerated at all, and that, be it noted, in the absence of any dense connective tissue, (fig. 31). On the other hand, it is possible to meet with lungs subjected to identical treatment and without any re-expansion of the lung. Mouse 118 (37 days) is an example, and a similar finding was met with

in mouse 113. Like the apical part of the lobe in mouse 92, these examples do not show fibrous or other local condition to which the failure of ventilation could be attributed.

It must be emphasised that Table 1 is no more than a list of the animals in which lesions were found: animals which died intercurrently from haemorrhage or other cause, and those in which no lesion was found have been omitted, although together they number many more than are included in the table. The wastage of animals was particularly noted in the experiments of more than a week's duration, a fact which in conjunction with the great variation in the character of the lesions, rendered this type of experiment unsatisfactory. This technique provides no more than a well-defined sequence of reactions to localised trauma, and indicates clearly the method by which final healing is accomplished. It is not possible, however, to state whether in fact complete restoration of anatomically and physiologically normal lung ever occurs in mice injured in this way. Where necrosis is absent, as in the punctures by the cold needles, there is every reason to believe that the result is a return of the lung to normal. A considerable number of lungs were cut serially without showing any abnormality, and it is unreasonable to assume that none of them had actually been penetrated by the needle. In the lungs

which had been burned it is highly probable that at least a very close approximation to normality can be achieved. This inference is based partly upon the experience of the cat wounds which are now to be described, and partly because in none of the animals has there been any excessive production of connective tissue. This is an important observation. Extensive fibrosis is bound to be a serious obstacle to the restoration of effective lung, and the absence of scarring is all the more significant.

II. EXPERIMENTAL WOUNDS OF LUNG IN CATS.

Open thoracotomy wounds in cats had great advantages over the stab wounds in small rodents. With open operation, comparable portions of lung could be removed and haemorrhage controlled, and there was the additional advantage that the lungs of larger mammals bear a closer similarity to human lungs. Cats, too, have a high resistance to microbial infection and their proverbial nine lives to their credit, points of some importance even where the operating facilities are good.

Experimental Method.

Adult cats were used, anaesthetised by intratracheal ether under positive-pressure anaesthesia, and thoracotomy was performed by resection of 2-3 cms. of the 7th rib on the left side in the axillary line, which exposed the upper part of the left lower lobe. The lower margin of this lobe was carefully withdrawn from the thorax on to gauze wrung out of hot saline, which was used also to pack the edges of the thoracotomy wound and prevent the lung from slipping back into the chest. With a pair of sharp Mayo scissors a triangular wedge of lung tissue, each side approximately 3 cm. long, was removed. In most instances there was considerable haemorrhage from one or more large vessels, and when a bronchus was involved, the blood frothed from mixture with air. As a rule, this bleeding was controlled by one or more

deep sutures of silk through the lung and tied firmly. In order that the tissues might not be crushed, clamping and ligation of vessels were avoided as far as possible; clamps for the wound margins were never employed. Thereafter the visceral pleural surfaces were carefully approximated by interrupted silk stitches, the lung returned to the chest, and the chest wall closed in layers after the pleural sac had been cleared of any fragments of blood-clot. As a rule, the operative procedure occupied 20 minutes.

The animals were killed at intervals between 36 hours and 115 days after operation. Usually death was induced by chloroform, but in some of the shorter experiments, the chest cavity was reopened under positive-pressure ether anaesthesia to observe the movement of the lung. After death the lungs were expanded in the chest with intratracheal formol-saline, fixed for 48 hours in a large volume of this fluid, and blocks taken. These were treated with a saturated solution of mercuric chloride in saline for 24 hours, dehydrated, and embedded in paraffin wax in vacuo. Thick (30μ) and thin (5μ) sections were prepared: the former, when examined by the binocular microscope are of particular value in lung histology. Staining was by the methods used for the mouse lungs (p. 9). Some frozen sections were cut also, but these were only of limited use.

Results.

The cats recovered speedily from this procedure and seemed little upset. In animals killed within the first week the scars were clearly defined as dark haemorrhagic bands protruding slightly above the pleural surface and surrounded by congested lung tissue, but later the result was a depressed or dimpled scar varying in colour with age from dark purple to light blue. As a rule, the scars within the first month were surrounded by a zone or halo of whitish pleura, (figs. 32, 33 and 34), and with two exceptions, cats L9 and L18, this was the limit of the pleural reaction. In these two animals, however, firm adhesions to the diaphragm and parietal pleura were present, and on consulting the operation protocols, it is seen that in one cat, ether vapour could be smelt and heard blowing from the lung after its return to the chest, while in the other, the lung was noted to be unusually friable and the superficial stitches tore out easily.

Accordingly it may be said that excision of wedges of lung tissue in cats results in depressed scars with local pleural thickening. The lack of pleural adhesions is to be attributed mainly to the thoracotomy opening being at a different level than the lung wounds, to the reasonably accurate apposition of the cut pleura, and to scrupulous haemostasis before the lung was returned to the thoracic cavity.

TABLE II.

List of experiments in cats.

1. Experimental excised wounds of lung.

Cat	L 25,	duration	of	experiment	36	hours.
"	L 26,	"	"	"	45	"
"	L 5,	"	"	"	48	"
"	L 34,	"	"	"	72	"
"	L 1,	"	"	"	4	days.
"	L 33,	"	"	"	5	"
"	L 17,	"	"	"	8	"
"	L 2,	"	"	"	10	"
"	L 3,	"	"	"	14	"
"	L 18,	"	"	"	16	"
"	L 10,	"	"	"	23	"
"	L 4,	"	"	"	28	"
"	L 12,	"	"	"	45	"
"	L 13,	"	"	"	51	"
"	L 14,	"	"	"	59	"
"	L 6,	"	"	"	60	"
"	L 7,	"	"	"	76	"
"	L 11,	"	"	"	115	"

TABLE II - cont.

2. Pleural wounds.

Cat	L 37,	duration of experiment	48	hours.
"	L 38,	" " "	5	days.
"	L 35,	" " "	8	"
"	L 47,	" " "	8	"
"	L 43,	" " "	8	"
"	L 46,	" " "	9	"
"	L 44,	" " "	9	"
"	L 36,	" " "	9	"
"	L 45,	" " "	12	"
"	L 41,	" " "	32	"

3. Fixed wounds of lung.

Cat	L 29,	duration of experiment	16	days.
"	L 39,	" " "	20	"
"	L 30,	" " "	28	"
"	L 40,	" " "	62	"

Microscopic Examination.

Table 2 provides a list of the material utilised in this work, and as in the case of the rat and mouse experiments, the observations group themselves into the immediate post-traumatic reaction and the regenerative process.

Immediate post-traumatic reactions.

These are the groups of changes which took place during the first five days after injury, and are exemplified in the cats L25, 26, 5, 34, 1 and 33.

The reaction of the lung to trauma of this type is of a local character, limited to the immediate neighbourhood of the wound. This is illustrated in figures 35-40 which show that the initial lesion has been haemorrhage into the gap in the lung, a space occupied at the time of the animal's death by fibrin clot containing in its meshes red blood cells with some leucocytes, although polymorphs are never numerous except around the sutures and ligatures. Obviously the amount of clot is variable, but it was not excessive in any of the sections of this group.

Except at the lung surface where the pleura has been inverted into the wound for 1 or 2 millimetres, the alveoli of the damaged lung are in direct contact with the blood clot. These alveoli have intensely congested capillaries in their walls, intra-alveolar haemorrhage is the rule, and many of the alveoli are partially collapsed. In the

shortest experiments, that is in animals killed 36 and 45 hours after operation, oedematous alveoli separate the congested area from the normal lung. Oedema is present too in the alveoli immediately under the pleura, (fig. 43): usually only a single row of alveoli are affected in this situation and it is noticeable that they contain coagulated fluid which stains much more intensely than in the alveoli bordering the clot deeper in the lung. It may be said, however, that the haemorrhage and congestion, rather than oedema, are the salient features of these early sections.

For two or more centimetres around a wound at the surface of the lung, the pleura is covered by fibrin continuous with that filling the gap in the lung. This surface fibrin has a cellular content which is never rich, and consists for the most part of mononuclear cells of macrophage and lymphocytic type: pus cells are few except in the vicinity of the sutures. Most of this pleura has lost its serosal cells, although small groups may remain beneath the fibrin. A vigorous serosal cell reaction can be seen, however, at the periphery of the fibrin on the lung surface, or beneath that fibrin wherever surviving serosal cells occur. These cells have proliferated markedly, and grown over and into the fibrin as sharply-staining polymorphous and spindle-shaped forms, many of them closely resembling fibroblasts. This reaction can be seen not only on the

external pleural surface of the lung, but also where the pleura has dipped into the wound, as it were lining the funnel-shaped surface opening, now blocked by fibrin. (figs. 46, 47, 48). It is this inversion of the pleura which constitutes the surface dimple of the wound, and there the pleural elastica can be traced on one or both sides of a wound (referring to the wound as though all in the plane of the section) to a depth of 1 or 2 mm., where it ends usually as a whorled mass. Sometimes it meets the pleura from the other side thus giving a V-shaped depression occupied by fibrin, vascular fibroblast tissue and serosal cells. (figs. 46, 59 and 60).

In addition to the serosal cell reaction external to the elastic membrane, there is also a cellular response on its pulmonary side. Normally the cat pleura consists of a single layer of serosal cells resting on an elastic membrane and separated from the pulmonary alveoli by a thin band of collagen and reticulum fibres. The reticulum and collagen fibres show an increase even in these early wounds, and the elastica is raised from the superficies of the lung by dilated capillary blood vessels, fibroblasts and proliferating endothelial cells.

For example in fig. 51, cat L33, duration of experiment 5 days, the surface alveoli of the lung have almost lost their alveolar structure and been replaced by a

vascular fibroblastic reaction originating partly from alveolar mural capillaries, and in part from the subpleural vascular network. These capillaries of subpleural origin appear to transgress the elastic membrane through minute gaps and take part in the organisation of the surface fibrin. (figs. 85 and 86). The surface alveoli contiguous with the pleura where it is inverted into the wound are involved in the same reaction, and deep to the termination of the pleura where the alveoli of the lung are in contact with the blood clot, a similar though less marked reaction can be seen. (figs. 40 and 45).

The lung alveoli are also affected by a cellular reaction of a different type, the pattern of which can be seen most easily in the superficies of the lung at the margins of the wound; it is particularly well developed in cat L5 (48 hours) fig. 41. In this lung the alveoli concerned have developed a cellular lining, somewhat irregular in its arrangement, of swollen hyperplastic cells, rather pale-staining with vesicular nuclei each with a single nucleolus. Many of these cells have assumed cubical and polygonal shapes; some are rather tadpole-shaped: although distinct from them and less deeply stained, they resemble in shape the proliferating endothelial cells of the subpleural alveolar capillaries. Moreover, they are closely similar to the alveolar cells of the early mouse

experiments, (figs. 21 and 22) and examples can be seen in the other cats of this group.

Bronchial and bronchiolar changes in this initial post-traumatic stage are less well developed. Where a bronchus has been occluded by clot or compressed by a deep stitch, the lining epithelium can be seen to alter at the point of stricture from tall ciliated respiratory cells to cubical or even flattened cells (fig. 49). Cubical epithelium may be heaped up at a point on the lumen, presumably preparatory to the development of a bronchial branch. Small air passages can be seen leading from the smaller bronchi (cat L5, fig. 42) and in cat L34 (5 days) air channels of bronchial origin are present in the margin of the clot (fig. 44). These changes, however, although chronologically part of this series of experiments, descriptively pertain to the regenerative stage next to be considered.

Accordingly it may be said that following the excision of a wedge from a cat's lung, there is local haemorrhage, congestion and oedema, although the latter is not a prominent feature. In wounds carefully apposed, the pleural gap was small, and occluded by fibrin clot. These immediate effects are followed by active cellular proliferation of the serosa and superficial alveoli, of which the lining cells, stimulated by the local reaction, undergo characteristic changes.

The regeneration of the lung.

Following the closure of the pleural gap, the subsequent stages in the healing are concerned with the restoration of functioning lung tissue. The speed and facility with which this is achieved depends upon whether the wound is distorted by ligatures and stitches, upon the amount of clot it contains, and the density of that clot. Two examples will illustrate this point.

Cat L2 (10 days), figs. 58-66, is a wound in which blood clot and connective tissue are minimal. The site of the excision is an area of relatively condensed lung tissue extending from the pleural surface almost to the large bronchus which traverses the field, and is marked on the surface by a dimpled pale area 1.4 cm. deep by 0.5 cm. wide. This cavity is occupied by vascular connective tissue continuous with that on the pleural surface around the wound, and lined by a continuation of the pleural elastica. There is nothing noteworthy about the surface reaction but on the pulmonary aspect of the elastic membrane in the dimpled area, the collagen is dense, forming a layer about the thickness of a normal alveolus: prolongations or extensions from the membrane traverse the collagen, separating it into alveolar-like compartments (figs. 61 and 62). Surrounding this pleural indentation, the lung is condensed into elongated narrow alveoli arranged radially from the wound and

separated one from another by alveolar walls thickened by collagen and reticulum fibres. Some alveoli are lined by a simple form of respiratory epithelium, low cubical cells with granular cytoplasm, and vesicular nuclei with prominent nucleoli. Deep to the apex of the wound, there are bronchi with cartilagenous walls from which bronchial buds lined by cubical cells extend towards the wound. In this animal, then, the healing process is almost complete, and has involved a simple closure of the pleural gap by vascular connective tissue, with the re-expansion of the alveoli which abut on the small amount of fibrin deeper in the wound.

Cat L17 (8 days), figs. 52-57, has a wound which although comparable in age with cat L2, presents a different picture. Fig. 52 is a low power photograph across the lobe showing the two pleural surfaces with scar tissue between. The thickness of the lobe (and depth of the wound) in the sections examined, varies from 0.8 cms. to 1.3 cms., and at the pleural surface it averages 4 mm. in width. On both sides of the wound in the plane of the section illustrated, the pleura is inverted towards the centre of the wound, and consists of thickened collagen with an elastic membrane. Around the surface of the wound, and occupying the space formed by the inverted pleura, there is vascular fibroblastic tissue with a single layer of serosal cells

covering it. The pulmonary tissue surrounding the scar is condensed to form long narrow alveolar spaces arranged more or less radially from it.

This wound differs from L2, however, in its central part being occupied by fibrin and fairly dense collagen fibres. Irregular spaces of various sizes, some quite large, have appeared in this collagen and are lined by a form of respiratory epithelium, prominent cubical cells with clear cytoplasm and vesicular nuclei. Occasionally, these spaces are beside the larger blood vessels at the periphery of the wound, and contain some red blood cells, but most of them are air spaces, related either to the adjacent alveoli or to bronchial buds. The latter are very numerous and have originated in two large bronchi near the centre of the wound: all transitions can be seen between the bronchial buds, the air spaces in the collagen and the alveoli of the contiguous lung. Thus in addition to the vascularisation of scar tissue, there is in this wound a pulmonary reaction directed to the restoration of the scar as functioning lung tissue.

To this process of organisation I have applied the term "aeration of the scar": it consists of the dispersal and reduction of scar tissue by air and blood channels which transform it into functioning lung tissue. Two mechanisms

are involved in the growth of air channels. First in importance and most arresting in its microscopical appearance, is the proliferative activity of the bronchi leading to the formation of bronchial buds or new outgrowths from the parent stems. In the larger bronchi, the earliest sign of the reaction is the development of cubical cells replacing the tall ciliated epithelium at one or more points in the lumen: this is succeeded by the appearance of these cells external to the membrana propria, and subsequently, the formation of bronchial buds characteristically in the form of canalised narrow channels of cubical cells. A similar appearance has been noted in connection with smaller bronchial branches as early as 2 days after operation (fig. 42), and figure 72 shows the complexity to which the process can develop (cat L4, killed 28 days after operation). The reaction affects large and small bronchi simultaneously and is limited only by direction, in that the proliferation occurs from the side of the bronchus next to the wound.

In wounds where the scar tissue is more dense and hyaline, however, the sections show that the hyaline tissue undergoes splitting or cleavage in advance of the bronchial penetration. Irregular slits and spaces appear in the tissue: usually these have no cellular content and they communicate directly with a bronchial branch or system of bronchial buds, and can be assumed to be air-containing spaces.

Other spaces less numerous than the air spaces are seen to be in contact with blood vessels, and contain red blood cells. Sometimes the spaces lack a cellular lining, but as a rule they are lined by flattened, elongated or cubical cells. The latter are small and closely aligned cells with deeply-staining vesicular nuclei, and clear or sometimes slightly granular cytoplasm: the flattened cells are usually squat, but may be elongated almost to a bipolar shape. Both types of cell, however, have the staining reactions of bronchial epithelium, and although cilia can not be identified with certainty, both are clearly varieties of respiratory epithelium.

Aeration of this type is to be seen also in most of the cats of this series and particularly in L10, duration 23 days, (fig. 70) and L13, duration 51 days, (figs. 74-77). This cat lung presents a most remarkable appearance in the central part of the scar, where there is a dense collagenous area containing numerous air spaces lined not by respiratory cells but by stratified squamous epithelium containing prickle cells. Most of the spaces are lined by two or three rows of squamous cells, but where the plane of section is oblique, they are arranged several rows thick, and even in clumps without lumina and appear like an epithelioma. In the periphery of this collagen are bronchial buds lined by normal or cubical-cell respiratory epithelium, and by

following the buds into the central part of the scar, the metaplasia to squamous cells can be seen to occur where the tissue is densest and relatively avascular: it is of a local nature, confined to this part of the scar.

There is, however, another type of aeration which involves all the wounds with scar tissue whether dense or cellular, namely the appearance of new alveoli in the margins of the scar. In its simplest form this process consists of an indenting or scalloping of the edge of the scar by existing lung alveoli, so that one wall of each alveolus is formed by the connective tissue. It is more correct to say that the wall rests on the connective tissue, because in fact the alveolar wall proper consists of a single row of small cuboidal cells, which are similar but smaller than the metaplastic respiratory epithelium which lines the bronchial buds. Entire alveoli can be seen lined by these cells, and thus they resemble foetal alveoli, but not infrequently only the alveolar wall resting on the connective tissue is of this cellular nature. (figs. 56, 57). In addition to this indentation of the margin of the scar, groups of alveoli can often be seen deeper in the tissue. They are identified by their complete lining of small cubical cells. Sometimes their communication with existing lung alveoli is obvious: in other cases the spaces appear isolated in the scar and serial sections may be required to

establish their connection with pre-existing alveoli. Where a bronchial branch has aerated a portion of the scar and at the same time there is evidence of alveolar infiltration from the margin, difficulty may be experienced in deciding whether a particular group of air spaces are of alveolar or of bronchial origin. In general it may be noted that the small cubical cells are more regularly associated with new alveoli, and larger less regular cells with the spaces apparently of bronchial origin, but the distinction is not significant.

It is in these various ways then, by bronchial budding, by the expansion of collapsed alveoli, by the splitting of collagen into air spaces and by the formation of alveoli in the scar, that the regeneration of the lung depends and may proceed until the whole wound is aerated and the scar reduced to a thin band of collagen with elastic fibres. Figs. 71 and 83 show the patchy reduction of scar tissue in wounds 28 and 76 days old, and figure 84, from a cat killed 115 days after operation, demonstrated how remarkably little evidence of the injury may persist.

Two points remain to be mentioned to complete this description, the ultimate return of the pleura to normal, and the behaviour of the lung around the sutures.

The initial reaction of the pleura has already been described as the proliferation of the capillaries and

fibroblasts from the potential pleural plexus, and from the superficial pulmonary alveoli, accompanied by a vigorous serosal cell reaction around the fibrin on the lung surface. The result of this process is the formation of vascular fibroblastic tissue covered by a single layer of rather darkly-staining flattened serosal cells, and the fate of this tissue is similar to vascular fibroblastic tissue in healing wounds anywhere in the body. The fibroblasts assume a direction parallel to the lung surface, reticulum fibres and subsequently collagen are laid down, the tissue becomes progressively less vascular, more compact and reduced in depth to 60μ and less. In this manner the serosal cells are progressively apposed to the lung surface and assume their normal relation to the pleural elastica.

In excising wedges of lung tissue the pleural elastic membrane is of course cut twice, and on the release of tension, assumes a sinuous or wavy shape frequently terminating in a coiled tortuous mass. As a general rule inversion of the pleura into the margins of the wound occurs, and thus the coiled elastica is often to be seen 1 or 2 mm. below the lung surface. Elastic membrane rendered untaut in this way shows not only a wavy outline, but it appears like a ribbon seen alternately on edge and on the flat, the width of the ribbon being the thickness of the section. Whether as a whorled mass or as a ribbon, the elastic tissue persists

practically unaltered in the lung or the scar for long periods, certainly for two months. (fig. 87). Only slight variation in the staining properties of the elastica can be observed, in that whereas the normal taut elastic membrane stains sharply with specific stains, the undulating and broader ribbon stains less intensely and not so uniformly: it appears also to be more readily stained by watery eosin and orange G. Thus the elastica persists apparently without stimulating any response in the surrounding tissue, or in the body phagocytes, and without undergoing any important modification itself.

It seemed a reasonable assumption that in the reconstitution of the pleura some new formation of pleural elastica might take place, although in a carefully apposed wound the gap in the elastica is very small. It is true that there was little evidence of new elastic tissue in the wounds themselves. Careful examination of specifically stained sections showed localised areas where there was apparent reduplication of elastica, and in cat L4 (28 days) a palisade arrangement of elastic fibres can be seen where the surface pleura is inverted into the wound. It was felt, however, that more might be learned if larger pleural wounds could be made, and maintained sufficiently shallow to obviate an extensive involvement of the underlying lung where the wealth of elastic tissue is confusing. An initial experiment

showed that it was possible to remove between 1 and 2 sq. cms. of pleura in cats without producing a pneumothorax, provided the wound was drawn together in the centre by a superficial stitch. A wound of this pattern was not ideal, but it was sufficiently encouraging to warrant the expenditure of a further group of eight cats noted in Table II under the heading "pleural wounds."

The operative technique of this group involved thoracotomy under positive pressure anaesthesia with the removal of a shaving of pleura by a razor blade. Oozing from the lung was controlled by pressure of a swab wrung out of hot saline and by a single superficial stitch, and the lung was returned t o the chest which was closed in layers. The animals were killed at intervals from 48 hours to 32 days after operation, and the pleural wounds had healed as rather opaque white thickenings without adhesions.

The protocols are in the appendix,but it may be said at the outset that the experiments failed to supply the information for which they were designed. One animal only, cat L43, killed 8 days after operation showed newly-formed elastic fibres and these are around the termination of the severed existing pleural elastica at one edge of the wound (figs. 88-91). There the elastica ends amongst cellular connective tissue with fibroblasts arranged tangentially to it in the parallel formation called "palisade." The

fibroblasts are situated both externally to the elastic membrane and also separate it from the underlying lung. In the latter situation are numerous very fine fibres staining specifically for elastica. Similar fibres, also specifically stained, can be seen parallel to the elastic membrane of this wound in other places (fig. 92). Apart from these examples, however, no unequivocal evidence of reformation of pleural elastica was obtained from these experiments which nevertheless corroborated the description of the changes seen in the pleura and superficial alveoli in the earlier experiments already recorded.

For a purpose to be discussed later, a further group of cat experiments ~~were~~ was undertaken (Table II, "fixed wounds") where after excising wedges of lung in the normal way, an attempt was made to immobilise the lungs by stitching them firmly to the chest wall at the thoracotomy level. Thus adhesions were formed with the pleura reflected around them. These wounds are mentioned at this stage, and in parenthesis, because they also lack any sign of new formation of elastic tissue. The existing elastic tissue, although divided, extended almost completely across the adhesion in a more or less unaltered condition, and no elastic tissue at all can be seen in the pleural reflections.

Finally, mention must be made of the pulmonary reaction to the silk stitches employed in the cat experiments as a

whole. It is admitted at once that silk is probably unsuitable suture material to use in lungs, but it was convenient, and is usually employed for animal work. It stimulated a polymorphonuclear reaction in lung wounds which otherwise were very free from pus cells, and produced ultimately whorled connective tissue around the stitches. In other words it behaved as a foreign body, and experiments where stitches were placed in otherwise normal lungs showed that these reactions were independent of the lung wounds themselves, and that black (or dyed) and white (undyed) silk affected the tissue equally. The principal reason why this subject is mentioned, however, is because the connective tissue around the sutures formed distorting factors in the wounds, and resulted in most remarkable air channels being formed around them (fig.68). Most commonly these airways communicated directly with bronchial branches and were lined by respiratory cells, but occasionally squamous cells formed the lining. Thus these apparently irritant materials afford further evidence of the persistence with which the aeration of pulmonary wounds is undertaken.

ANIMAL PROTOCOLS.

R.21. Duration of experiment 30 minutes.

Large white male rat.

31.10.40. Ether anaesthesia. A cold sterile serum needle (guage 6) was passed through into the right chest at the level of the 4th costal interspace anteriorly. There was no shock and the animal made a good recovery. It was killed 30 minutes later by breaking the cervical spine under light ether anaesthesia. Post mortem, there is recent blood clot over the right lung and pericardium. The puncture is on the tip of the anterior margin of the right apical lobe: it passes completely through that lobe and enters the anterior edge of the middle lobe which lies behind the apical lobe there.

Microscopic examination. Fig. 1 refers.

At the puncture area the apical lobe measures 700μ in depth. The puncture gap is 800μ wide and has been bridged by blood which contains air vacuoles but nevertheless has been sufficiently adhesive to retain the tip of the lobe *in situ* not only for 30 minutes of life but throughout fixation and embedding. The pleura has been inverted into the wound on the medial side of the gap; the pleural cells are slightly swollen and stain deeply, but neither within the wound nor around it do they show any other abnormality.

The alveoli on each side of the gap to a depth of 200 - 300μ are filled with erythrocytes. In the walls of these

alveoli there are tortuous capillaries dilated with blood. Flattened alveolar cells can be seen to form an incomplete lining to the alveolar spaces, but they are not noticeably altered in appearance although they stain more intensely than in other parts of the lung. Leucocytes are scanty in the alveoli and in the haemorrhagic area: they are mostly monocytic. Separating the haemorrhagic alveoli from the normal lung and also along the free margin of the lung on either side of the puncture mark there are oedematous alveoli, and these lack congested walls. Owing to the way in which the serial sections were mounted it was not possible to stain any particular section specifically for fibrin in addition to the normal stains, but the appearance of the haematoxylin and eosin sections does not suggest that fibrin is present.

The puncture needle has passed no more than half-way through the upper part of the middle lobe and has caused a depression or gap in one margin measuring 80μ. The pleura has been interrupted, haemorrhage is present in the superficial alveoli lining the wound, and also into the wound itself. Oedematous fluid can be seen sometimes mixed with the blood and oedematous alveoli are present around the puncture. In them phagocytic cells can be seen to have mobilised from the alveolar wall.

The larger bronchi contain muco-purulent exudate and

there is evidence of generalised bronchitis in this lung with iron pigment in the bronchial walls.

M.60. Duration of experiment 24 hours.

Adult white mouse, 23 gms.

16.6.43. Ether anaesthesia. Puncture of right chest at level of 5th interspace with sterile needle, guage 12, boiled in saline and allowed to cool. Recovery uneventful.

17.6.43. Killed by breaking neck. Post mortem, small lesion 2 mm. in diameter at lower free margin of right basal lobe. Puncture area raised slightly above the lung surface and covered with blackish-red clot: there is some diffuse congestion of the surrounding lung. Fixation Zenker-formol, 10 hours.

Microscopic examination. Figs. 2 & 21 refer.

This is a lesion at the tip of a lobe measuring in depth 1200μ. The puncture mark of the needle can be seen as an interruption and indentation of the pleura, and the needle has obviously penetrated the lung only for a short distance (500μ), passing through a peribronchial lymph node. The whole of the affected portion of the lobe shows considerable atelectasis with haemorrhage into the partially collapsed alveolar spaces. Some degree of swelling of the alveolar lining cells and of the endothelium of their capillaries has occurred. A small collection of pus cells is present around

the puncture mark but otherwise there is no cellular infiltration. Oedema is absent. The congestion of the endothelial capillaries involves to some extent the aerated lung adjacent to the collapsed tip of the lobe, but the reaction is remarkably local. The pleural serosal cells have been lost only at the point of puncture, while the cells which remain nearest the puncture mark show swelling.

M.104. Duration of experiment 70 hours.

Adult male mouse, 23 gms.

19.1.46. Ether anaesthesia. Puncture of right lung at level of 5th interspace with sterile needle, guage 14, boiled in saline and allowed to cool.

22.1.46. Killed by blow on head. Post mortem, the lesion appears as a pale yellow area surrounded by a zone of haemorrhage in the anterior part of the right apical lobe adjacent to the right auricle.

Microscopic examination. Figs. 3, 4 and 22 refer.

The lesion averages 1 mm. in depth and 800μ in width. At the point of puncture the pleural membrane is detached artificially and there is a protrusion of lung tissue above the normal lung surface. The lesion is atelectatic and alveolar structure is lost in some places and indistinct in others, and there is considerable alteration in the appearance of the alveolar cells. They are swollen and tend to

be oval in shape: the nuclei are pale and vesicular with prominent nucleoli. This metamorphosis affects mainly the subpleural alveoli although it can be seen also deeper in the lung where the alveoli contain fibrin. At the point of puncture the serosal cells are slightly swollen. There is some congestion of the alveolar capillaries of the lung generally, but the reaction to the lesion is remarkably local.

M.61. Duration of experiment 72 hours.

Adult male mouse, 24 gms.

16.6.43. Lung puncture immediately following M.60 and by same technique - needle guage 14.

19.6.43. Killed by breaking neck. Post mortem, small greywish-pink area of collapse present at the lower margin of the right upper lobe - puncture mark not defined. Fixation: Zenker-formol, 10 hours.

Microscopic examination. Figs 19 and 20 refer.

Serial sections examined microscopically reveal a lesion of wedge or triangular shape measuring at its maximum 600μ long X 400μ wide. The major portion of this tissue has lost all alveolar structure: it is homogeneous faintly eosinophilic tissue of sparse content of oval and bipolar cells. They have relatively faintly-staining cytoplasm with vesicular nuclei containing many chromatin knots and a single prominent nucleolus, conforming morphologically to

endothelial cells. In addition small numbers of bipolar fibroblasts occur throughout this damaged tissue.

A well-defined boundary zone separates this tissue from the remainder of the lobe and consists of hyperplastic endothelial cells arranged irregularly, sometimes in clumps as it were in a glomerular fashion and occasionally in an alveolar pattern, the whole being 200 μ deep. The hyperplastic cells are proliferated alveolar lining cells. In the centre of this reactive zone is a bronchus from which small tortuous branches can be traced and these in turn are lined by cubical cells similar in character to the proliferated alveolar cells described above. Serosal cells are absent both over the damaged lung and the reactive zone but their absence is probably artificial.

Well-aerated lung is proximal to the reactive zone.

M.29. Duration of experiment 3 hours.

Fawn mouse, 20 gms.

23.11.40. Ether anaesthesia. Puncture of right lung by hot guage 14 needle at 11 a.m. Good recovery. Killed 3 hours later by breaking neck. Post mortem, the upper lobe of the right lung is of dark plum colour with fibrinous flakes adhering to it, and producing adhesions to the chest wall at that point. Fixation of whole lung Zenker-formol for 8 hours.

Microscopic examination.

The area of lung damaged by the needle is triangular in the sections and measures 450 μ in depth by 1700 μ in diameter at the pleura. Over this area the pleura has been interrupted and the superficial alveoli at the point of puncture lack their pleural wall but some specks of carbon adhere to them; they and the alveoli situated centrally around the needle track have lost all nuclear staining in their walls, although this is not true of all the alveoli of the damaged area. At the apex of this triangular area there is a blood vessel of dilated capillary type, partially thrombosed and with red blood cells in part of the lumen: some leucocytes have entered the thrombosed contents. The alveoli of the necrotic lung sometimes contain pale, slightly eosinophilic coagulated fluid but others are empty: there is no loss of elastic staining by orcein.

A zone of alveoli with haemorrhagic contents and congested

walls and measuring three or four alveoli in depth separates the triangle of necrotic lung from the rest of the lobe. The whole lobe shows some degree of atelectasis and the bronchi contain globules of coagulated fluid with some phagocytes.

M.27. Duration of experiment 19 hours.

Adult white mouse, 20 gms.

2.11.40. Under ether anaesthesia a red hot needle, guage 14, was passed into the right side of the chest. The animal made a good recovery.

3.11.40. Killed at 11 a.m. by breaking neck. Post mortem, there is a bruised scarred area in the right middle lobe. Fixed Zenker-formol for 6 hours: serial sections cut.

Microscopic examination.

This lung is disappointing in that, despite serial sections, no point of entrance of the needle can be seen. In the section, both lobes of the lung are oedematous and partially collapsed. The external lobe in the section shows only a wedge-shaped area affected, but the other lobe is completely involved. There is a considerable degree of collapse of many alveoli and the others stain fairly deeply with coagulated fluid.

M.80. Duration of experiment 22 hours.

Mouse, adult, male, 25 gms.

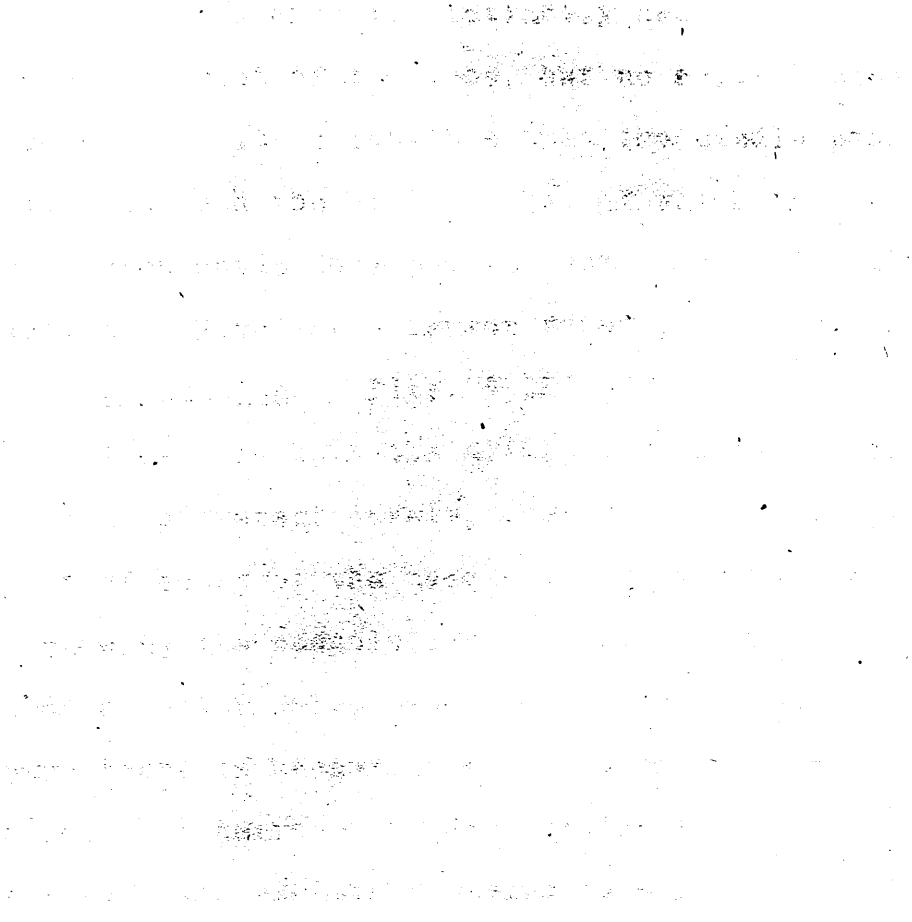
1.7.42. Under ether anaesthesia, a red hot needle, guage 14, passed into right side of chest and withdrawn at once. Good recovery. Killed 22 hours later by blow on head. Post mortem, the upper part of the right apical lobe is an acutely congested area with flakes of fibrin adhering to it. No puncture mark can be seen. Fixation, Zenker-formol, 6 hours.

Microscopic examination. Figs. 6 & 12 refer.

Transverse sections across the lung show that the lesion is limited to the adjacent portions of the apical lobe. At the level of the section, the apical lobe measures 1200 μ across and contains a crescentic necrotic area 250 μ deep in its centre, bordered by a zone of haemorrhage and congestion. The alveoli of the necrotic area have retained their outlines and walls, but no nuclear staining can be seen. In the haemorrhagic zone the alveolar structure is largely lost and replaced by haemorrhage, while the remainder of the lobe is oedematous. Most of the remaining alveolar spaces of this lobe are occupied by very pale-staining coagulated fluid, and a narrow band of more densely-staining fluid separates the haemorrhagic and necrotic areas from the remainder of the lobe. Some flakes of fibrin are present, especially in the lumen of divided bronchioles and in the tissue surrounding

the blood vessels, where also polymorphonuclear and mononuclear cells of the blood have aggregated.

Over the necrotic lung the pleura is necrosed and at one point separated from the lung by a type of haemorrhagic blister: some fibrin adheres to the pleural surface covering the congested area.



M.49. Duration of experiment 44 hours.

Male mouse, 22 gms.

26.5.41. Ether anaesthesia. A red hot guage 14 needle was passed into the right chest and withdrawn at once. The animal made a good recovery.

28.5.41. Killed by blow on head. Post mortem, the lesion is mainly in the middle lobe of the right lung. The lobe is of light red haemorrhagic appearance but covered by loose fibrin. The injured area is distinctly swollen and protrudes beyond the rest of the lobe, but no actual puncture mark can be seen. It is possible that the needle passed between this lobe and the apical lobe, of which the inferior margin has a crescentic dark plum-coloured area of collapse and congestion. Fixation - Zenker formol, 6 hours.

Microscopic examination. Figs. 5,10, 14 refer.

The sections show both the middle and apical lobes but although cut at different levels, they do not include the actual point of entry of the needle. The middle lobe is necrotic, showing the coagulative type of necrosis with retention of alveolar walls except at one point beneath the pleura where there is haemorrhage: this is presumably just below the point of entrance. Most of the alveoli are filled with haemorrhage and nuclear staining is almost entirely lost, except for serosal cells of the pleura and the walls of the

larger vessels. The pleural cells are of the "pear-shaped" pattern, and sometimes form a double layer: pleural capillaries are prominent also. The larger blood vessels retain their nuclear staining, but thrombosis has occurred in some and polymorphonuclear leucocytes in their lumina line the endothelium: there are good examples of pavementing of leucocytes.

Similar changes are present in the adjacent portion of the apical lobe: there is alveolar necrosis and haemorrhage, very few serosal cells remain. Separating this necrotic area, which measures 200μ in diameter, from more normal lung is a zone also 200μ in diameter of alveoli filled with homogeneous pink-staining material. The alveolar walls themselves are mainly necrotic but between the alveoli spindle and curved cells with nuclei are present and a few cubical respiratory cells can be seen on the respiratory surface of these alveolar walls. Here also is a bronchus with one necrotic side while the other shows normal nuclear staining. The remainder of this lobe has a normal alveolar pattern with nuclear staining but most of the alveoli contain pale staining coagulated fluid: polymorphonuclear leucocytes can be seen in the alveolar capillaries and occasionally in the alveoli themselves.

M.81. Duration of experiment 47 hours.

Mouse, adult, male.

1.7.42. Under ether anaesthesia, red hot needle, guage 14, passed into right chest at level of 5th interspace, and withdrawn at once. Good recovery. Killed 47 hours later by blow on head. Post mortem, the basal lobe of the lung was found to be deeply congested with a blistered surface. Fixation Zenker-formol, 5 hours.

Microscopic examination. Fig. 13 refers.

Transverse sections show the lesion to affect the entire basal lobe, the tip of the middle lobe and the lower free margin of the apical lobe. The basal lobe in the sections examined measured 3.4 mm. from root to lateral border and has been increased in width by a blood-containing pleural bulla, 1200μ in diameter. The pleura appears to have split to retain this blood: both layers are necrotic and granules of carbon are present in the external layer.

Within the lobe proper, there is a lateral crescentic area of haemorrhage and necrosis measuring 1200μ in diameter. A similar appearance is to be seen in a small area of the lobe posteriorly and in the tip of the middle lobe: in these regions the area involved averages 500μ deep. The condition in the basal lobe conforms to the appearance repeatedly seen in these experiments, the superficial alveoli and vessels show coagulative necrosis while deeper there is haemorrhage. In

this particular instance the haemorrhage is extensive and it is difficult to discern the necrosed alveolar walls in the mass of red blood cells.

Most of the remainder of the lobe contains oedematous alveoli filled with pale staining fluid and fibrin.

The lower free margin of the apical lobe, which is in close contact with the haemorrhagic bulla of the basal lobe, shows the early stages of serosal cell and endothelial hyperplasia. The immediate tip of this lobe is necrotic but the contiguous tissue shows columns of swollen endothelial cells enclosing alveolar spaces which contain fibrin, while the pleural serosal cells protrude as oval swollen cells. Both varieties of cells have similar characteristic nuclei with prominent nucleoli.

M.82. Duration of experiment 47 hours.

Mouse, male, adult.

1.7.42. Under ether anaesthesia, red hot guage 14 needle passed into right chest and withdrawn at once. Godd recovery. Killed by blow on head 47 hours later. Post mortem, the whole of the apical lobe is deeply congested with a crescentic red swollen area at its lower free margin. Fixation, Zenker-formol, 5 hours.

Microscopical examination.

The section is transverse across the apical and middle lobes of the right lung, and the apical lobe measures 6 mm. from pleura to lung root, of which approximately two-third represents the lesion itself. The latter is typical of this acute type of lesion; there are the necrotic superficial lung alveoli, then the zone of haemorrhage and finally the oedematous lung with fibrin in some of the alveoli. Over the necrotic lung the pleura shows coagulative necrosis with some haemorrhage on its surface at the point of entry of the needle, marked by carbon particles. The coagulated alveolar tissue is 800 μ deep and includes some large vessels while the haemorrhagic or congested zone is some 1200 μ wide. There is a very considerable portion of this lobe which shows congestion and oedema of groups of alveoli and the aerating tissue of the lobe generally is small.

M.32. Duration of experiment 48 hours.

Male white mouse, 20 gms.

28.11.41. Under ether anaesthesia a red hot needle, guage 14, was passed into right side of chest and withdrawn at once. Good recovery: animal killed 48 hours later by breaking its neck. Post mortem, the needle-puncture is in the anterior surface of the middle lobe which is deeply congested and of dark plum colour. The apical lobe is oedematous and lightly adherent to the chest wall: the basal lobe is normal. Fixation Zenker-formol, 8 hours. Serial sections.

Microscopic examination. Fig. 7 refers.

The sections which include the puncture and needle track show that the needle penetrated to an average depth of 1 mm. producing a V-shaped area of damaged lung tissue: the base of the V is almost 3 mm. wide. The most superficial alveoli of this damaged lung are necrotic, sometimes retaining their walls intact but frequently with ruptured walls. This necrotic area is enclosed by alveoli filled with haemorrhage and there is also much interstitial haemorrhage where alveoli have ruptured.

The alveoli of the remainder of the lobe are filled with homogeneous dark eosin-staining coagulated oedema. In the haematoxylin and eosin sections it is difficult to distinguish alveolar walls against this dark material and the

alveolar capillaries are mainly collapsed. Where the traumatised and oedematous lung meet, at the junction of the haemorrhagic and oedematous areas, however, marked proliferation has taken place. The proliferative activity is partly from the adventitial tissue and the bronchi and larger blood vessels: many spindle-shaped fibroblasts can be seen extending out towards the necrotic zone. There is also proliferative activity of the alveolar lining cells which are swollen and prominent, sometimes as cubical cells, but also in polymorphous forms, - tadpole and pear-shaped cells. Many of the smaller arteries have necrotic walls and are partially thrombosed, showing red blood cells only in their centres.

The pleura around the needle track is intact but with few and widely separated serosal cells. Black carbon pigment external to the elastic layer indicates the point of entry of the needle, and dilated and congested capillaries are present in this space. The serosal cells which remained have proliferated into spindle and polymorphous forms with deeply staining cytoplasm and nuclei. Where there has been some exudation of fibrin, a portion of the serosa has become partially detached and in it also similar changes have taken place.

M.33. Duration of experiment 48 hours.

12.12.40. Ether anaesthesia. Puncture of right chest at 5th interspace with hot guage 14 needle. Post mortem, the apical lobe was adherent to the chest wall by acute fibrinous pleurisy. Fixation, Zenker-formol, 6 hours.

Microscopic examination.

The lesion affects mainly the tip of the lobe, and consists of a necrotic area measuring 2.4 X 1 mm. enclosed by a well-defined zone of demarcation. Covering the necrotic tissue the pleural serosal cells have proliferated and are three and four cells thick. The necrotic lung has retained its alveolar structure, and although devoid of nuclear staining the capillaries contain blood. Separating the damaged lung from the remainder of the lobe there is a boundary zone of more deeply staining cells, many of them cubical, others spindle and tadpole shaped. They are derived in part from the alveolar lining cells, in part from the fibroblasts of the bronchial and vascular adventitia and in part from the alveolar capillaries. In the centre of this boundary zone there are two bronchi of approximately equal size, one necrotic without nuclear staining and the other normally stained. Small channels of cubical cells can be traced in the boundary zone from these bronchi where they unite with the cells of alveolar origin.

M.36. Duration of experiment 70 hours.

Male mouse, 23 gms.

3.2.41. Ether anaesthesia. Punctured hot guage 14 needle in right lung. Good recovery. Killed by breaking neck 70 hours later. Post mortem the lesion is at the base of the right lung, and is of butterfly haemorrhage type, paler in the centre. No puncture mark is visible. Zenker-formol, 6 hours.

Microscopic Examination.

The lesion is of crescentic type and shallow, measuring 1000μ long and 450μ deep in the sections examined. The necrotic lung is of characteristic appearance with the alveolar pattern only faintly visible and many of the alveolar walls torn. Haemorrhage has occurred into many of these alveoli. The reactive zone is about 200μ deep and clearly marked as a deeply-staining, densely-cellular zone. Many fibroblasts extend from the blood vessel and bronchial adventitia. Swollen alveolar cells line the alveoli of this zone and endothelial hyperplasia is also visible. Hyperplasia and hypertrophy of the respiratory epithelium of the bronchi has occurred and some polymorphs are present in the bronchial lumen. Oedematous alveoli separate the zone of reaction from the normal lung.

M.37. Duration of experiment 5 days.

Male mouse, 20 gms.

3.2.41. Under ether anaesthesia, a red hot needle, guage 14, was passed into the right side of the chest at level of 5th interspace and withdrawn at once. Good recovery.

7.2.41. Killed by breaking neck. The lesion is a small irregular haemorrhagic area at the lower margin of the middle lobe of the right lung. A few flakes of fibrin adhere to this but there are no adhesions to the chest wall. No puncture is visible. Fixation Zenker-formol, 6 hours.

Microscopical examination. Figs. 8, 15, 18 and 24 refer.

Serial sections show that the lesion is confined toward one portion of a lobe and comparatively shallow. The lesion itself (1200 μ wide X 600 μ deep) consists of a narrow surface area of necrotic alveoli with partially ruptured walls and filled with red blood cells, the whole walled-off from the remainder of the lobe by a band of cellular proliferation about 200 μ wide. The proliferation is mainly on the part of alveolar lining cells and the endothelium of alveolar capillaries. In appearance the proliferated cells are similar to those described for M.36 and their shapes are equally diverse. Many small aggregations of cells forming solid sheets can be seen, some are bipolar and others of tadpole shape: they extend towards and into the haemorrhagic and necrotic tissue. Fibroblasts are remarkably few, but

some have developed from the adventitial tissue of the bronchus and larger blood vessels.

There is a most striking proliferation of serosal cells external to the pleural elastica. They are mostly elongated flattened cells, but some of them are almost polygonal in shape and these appear to be continuous with the pulmonary endothelium in places but the pleural elastica is intact. Carbon particles mark where the hot needle has grazed and are embedded in the pleura.

The bronchial epithelium is altered in two ways. At the end of the bronchus nearest the lesion, the normal respiratory epithelium has been replaced by a single layer of cubical cells: at the end farthest from the lesion, there is obvious proliferation of the respiratory epithelium, to form cubical cells several layers thick.

The remainder of the lobe has many areas of oedema, congestion and collapse.

M.40. Duration of experiment 5 days.

Male mouse, 20 gms.

31.3.41. Ether anaesthesia. Punctured red hot needle, guage 14, in right chest and the needle withdrawn immediately. Good recovery. Killed by breaking neck 5 days later. Post mortem, the needle has damaged only a small crescentic area at the inferior margin of the right lower lobe, where the tissue is deeply congested and plum coloured. Recent blood clot occupies the right pleural sac. Fixation Zenker formol, 6 hours.

Microscopic examination. Fig . 23 refers.

Transverse sections show that the lobe has a mean diameter of 1200μ, the area of necrosis at its central and deepest part measuring 1000μ. The necrotic lung is typical: it is a coagulative necrosis with intact alveolar, bronchial and blood vessel walls, all devoid of nuclear staining. The superficial necrotic alveoli are mainly empty, but the deeper alveoli contain red blood cells and are contiguous with fairly large blood vessels filled with red blood cells: Pavementing of leucocytes is evidence of at least a slow circulation.

There is a fairly sharp margin between the necrotic, haemorrhagic lung and the reactive area of the undamaged lung. The reaction is mainly on the part of the alveolar cells which have proliferated and developed the hyperplastic

appearance already described. (M.36). In this tissue alveolar structure is lost, although here and there are spaces surrounded by altered endothelium and containing fibrin clots. The remaining small portion of the lobe which has retained its alveolar structure has many areas of collapse. A transverse section of a bronchus in the boundary zone between the cellular and the necrotic tissue shows flattened endothelium next to the necrosis with normal or hypertrophic respiratory epithelium on the other side.

Fibrin has been deposited on the pleural surface in long strands separated from one another and from the pleural elastica by capillary blood vessels. This exudation of fibrin has lifted the serosa from its underlying elastic membrane and considerable serosal proliferation has taken place.

M.41. Duration of experiment 5 days.

Male mouse, 20 gms.

31.3.41. Ether anaesthesia. Chest punctured on right side at level of 5th interspace by hot guage 14 needle. Post mortem showed that this was a low puncture and that the animal had a cystic liver, and consequently only a narrow crescentic area at the base of the lower lobe of the lung was damaged. Fixation: Zenker-formol, 6 hours.

Microscopic examination.

This is a shallow lesion measuring 600 μ in depth X 3 mm. in length. It consists of a superficial area where the alveolar structure is replaced by blood enclosed in spaces lined by scanty endothelial cells which have obviously grown from the contiguous tissue. The latter is a zone 400 μ wide of alveoli lined by deeply staining endothelial and alveolar cells. Most of these alveoli contain fibrin and although there is an alveolar structure, this zone is in fact similar to the barrier or boundary zone of the shorter experiments. Over the lesion as a whole the pleural elastica is intact but the serosal cells are missing.

M.42. Duration of experiment 5 days.

Male mouse, 20 gms.

31.3.41. Under ether anaesthesia a red hot needle, guage 14, was passed into the right side of the chest in the 5th interspace and withdrawn. Good recovery. Killed 5 days later by breaking neck. Post mortem, only a narrow crescentic area at the free margin of the lower lobe is involved and is deeply congested. Fixation Zenker-formol, 6 hours: embedded to cut lesion transversely, i.e. in the long axis of the lobe.

Microscopic examination.

This is a small roughly triangular lesion on section, measuring 250μ in length, necrotic and separated from the remainder of the lobe by a comparatively broad band (400μ) of reactive lung. In this reactive area are all the varieties of proliferated alveolar and endothelial cells with dilated capillaries which are in other animals of this duration and there is nothing specially noteworthy about this lesion except that there has been no pleural reaction and that the pleura is included in the local necrotic process.

M.51. Duration of experiment 6 days.

Male mouse, 22 gms.

26.5.41. Ether anaesthesia. Hot needle, guage 14, passed into right chest at level of 5th interspace and withdrawn

immediately. Good recovery.

1.6.41. Killed by breaking neck. Post mortem, the lesion measures 0.3 X 0.7 cm. and occupies the free lower margin of the basal lobe of the lung. Fixation Zenker-formol, 6 hours.

Microscopic examination. Fig. 25 refers.

A transverse section across the lobe shows a necrotic lesion separated from reactive lung tissue by a zone of congestion: each of these areas - necrotic, haemorrhagic and reactive, measures 200μ in mean diameter.

In the necrotic congested areas it is just possible to identify alveolar structures in some places but mostly the tissue is of homogeneous structureless appearance, split into small spaces containing red blood cells. There appear also to be some irregular air spaces, especially where a bronchus communicates with the lesion. Pleomorphic endothelial and alveolar cells are present in the congested area, extending out from the reactive zone in characteristic fashion. The reactive tissue itself is a mass of endothelial cells together with some spindle cells from the adventitia of the bronchus and pulmonary vessels.

A large bronchus has been occluded by the hyaline tissue at the margin of the necrotic area, and shows a transition from the tall respiratory epithelium to a cubical and finally flattened cellular lining. The necrotic tissue is covered by pleural cells only in part and the serosal cells have

assumed flattened spindle shapes and are heaped up, forming a serosa several cells thick. The remainder of the necrotic area has no pleural covering and particles of carbon are adhering to the necrotic lung.

R.14. Duration of experiment 7 days.

Large male rat.

9.10.40. Under ether anaesthesia a red hot serum needle was passed into the right chest at the 5th interspace and withdrawn immediately. Killed 7 days later by breaking neck under ether anaesthesia. Post mortem shows that the right middle lobe is firmly adherent to the chest wall and is clearly the seat of the lesion. Sections have been prepared from a block which includes the muscle and cartilage of the chest wall.

Microscopic examination. Fig. 26 refers.

The lung lobe in transverse section is oatshaped, measuring 2 mm. in length and 600 μ in width. A bronchus is in the centre of the lobe with reactive lung tissue extending for 1 mm. on each side. The remainder of one half of the lobe is fairly normal lung tissue: the other half contains a partially vascularised necrotic lesion, sandwiched between two zones of reactive tissue.

The necrotic area occupies 2 mms. of the length of the lobe and retains its alveolar walls without their nuclear staining. Some of the alveolar capillaries, however, are

clearly patent and have been maintaining circulation at least immediately before ~~the~~ death, and most of the alveolar spaces contain red blood cells supplied by the many dilated capillaries and blood vessels of the reactive tissue. All shapes of alveolar and endothelial cells, together with fibroblasts and leucocytes are extending into this necrotic area from the reactive zone.

The reactive lung tissue shows the hyperplasia of endothelial cells and fibroblasts, and the metaplasia met with regularly under the experimental conditions. In addition, bronchial budding has taken place and many newly-formed bronchial channels can be recognised by their densely-staining cubical cells. These have obviously developed from the bronchi in the centre of the lobe and epithelium of a similar type (recently formed) lines the bronchioles in the necrotic area.

An adhesion 2 mm. long unites the central part of the lobe with the parietes of the chest wall, but this adhesion is external to the elastica of the visceral pleura: the elastica is undisturbed and continuous. The adhesion is of loose vascular tissue, largely of serosal origin: flattened metaplastic serosal cells line one end of the adhesion in a fashion analagous to the growth of vascular endothelium over a thrombus. The fibres of the intercostal muscle are separated by masses of endothelial cells and

fibroblasts; great fibroblastic activity has taken place also around the costal cartilage.

M.43. Duration of experiment 8 days.

Adult male mouse, 20 gms.

31.3.41. Ether anaesthesia. Red hot guage 14 needle introduced into right chest at level of 5th interspace and withdrawn at once. Good recovery. Killed by blow on head 8 days later, when examination showed a small dark haemorrhagic area in the centre of the right middle lobe. Fixation Zenker-formol, 4½ hours.

Microscopic examination.

This lesion is roughly triangular in section and measures 1400μ X 1600μ. The apical portion is necrotic presenting a structureless appearance without recognisable alveolar walls, although spaces of alveolar type are present and contain red blood cells. A boundary zone of congestion and haemorrhage separates the necrotic tissue from the reactive lung, and many cells of blood, endothelial and alveolar origin can be seen extending into the congested area, sometimes with reformation of vascular channels.

The reactive lung is similar to that described already in experiments of shorter duration. There is a barrier some 200μ thick of proliferated and metaplastic alveolar cells of elongated, bipolar and tadpole shapes, arranged sometimes in irregular fashion, although many of the

elongated forms have clearly been directed towards the necrotic lung. Some alveolar spaces have been retained in this tissue and these are occupied by fibrin plugs. Nests and rosettes of darkly stained cuboidal cells can be seen and identified as newly-formed bronchial buds, some of them canalised and others solid. Direct extension from the nearest bronchus or bronchiole is not always clear in a single section but there is no doubt of their nature. Hyperplastic epithelium is present in some of the bronchi adjacent to the reactive zone.

The pleura also shows hyperplasia of its serosal cells, but this reaction is of patchy type. Most of the necrotic area is covered by a single layer of flattened and elongated serosal cells but in some places the serosa is three and four cells thick.

M.44. Duration of experiment 9 days.

Male mouse, 25 gms.

31.3.45. Under ether anaesthesia, red hot needle, guage 14, introduced into right chest at level of 5th interspace and withdrawn at once: good recovery. Killed by blow on head at 9th day. Post mortem, there is a comparatively large congested lesion affecting the basal lobe and lower part of the middle lobe of the right lung: puncture marks can be seen anteriorly and posteriorly to indicate the route taken by the needle.

Microscopic examination.

The sections include portions of all three lobes of this lung, and transverse the basal lobe at the level of the lesion. The latter is a more or less square area beneath the pleura and measuring 2 mm. in diameter; it has been necrotic and now is largely vascularised. Considerable haemorrhage and congestion occupy the irregular spaces which have developed in the homogeneous eosinophilic necrotic tissue. Many of the spaces are lined by elongated flattened endothelial cells but some are unlined. Altered endothelial cells are very numerous in this tissue and can be seen extending into it from the reactive lung which surrounds it. The section shows a more advanced stage of the process of revascularisation already described in the experiments of shorter duration. There has been a re-establishment of the circulation in the necrotic blood

vessels of the area but no nuclei can be detected in these walls. In the pleura covering the lesion, a considerable degree of hyperplasia is evident, both serosal cells and fibroblasts being involved.

The remainder of this lobe is atelectatic and consists of a solid mass of alveolar and endothelial cells without any well-defined reactive zone around the lesion itself. There is a large non-cartilaginous bronchus in the centre of the lobe and many canalised bronchial buds, some of stellate section, lined by cubical epithelium can be seen.

Half the middle lobe is atelectatic but does not show any other noteworthy changes, while the lower margin of the apical lobe in the section closely adjacent to the necrotic lung shows hypertrophy of the pleural serosal layer.

M.45. Duration of experiment 11 days.

Male mouse, 22 gms.

11.12.41. Anaesthetised with ether. Lung punctured by hot needle, guage 14. Good recovery. Killed by breaking neck. Post mortem: small wedge-shaped lesion in lower free margin of middle lobe.

Microscopic examination.

This is a small triangular lesion involving the apex of a lobe. It is necrotic in character and has lost all alveolar structure. The boundary zone is almost twice the width of the lesion and consists of bronchial channels lined sometimes by cubical and sometimes flattened cells, separating groups of alveolar and endothelial cells. A bronchus in the centre of this zone is the origin of the respiratory channels. The pleura has been detached and is absent from most of the lobe.

M.53. Duration of experiment 12 days.

Mouse, 22 gms.

19.6.41. Ether anaesthesia. Hot guage 14 needle passed into right side of chest. Killed by blow on head 12 days later. Post mortem: small greyish area of collapse at lower margin of right middle lobe.

Microscopic examination.

This does no more than confirm the naked-eye findings.

There is a small superficial area of collapse and it is possible that the needle has not actually entered this lung. Considerable subacute bronchitic change is present in the affected lobe.

R.15. Duration of experiment 13 days.

Adult male rat.

9.10.40. Ether anaesthesia. Puncture of right chest by hot serum needle.

22.10.40. Killed by breaking neck under ether. Post mortem, there is a small adhesion uniting the right basal lobe to the chest wall. The lung around the adhesion is rather dark in colour, but otherwise the lungs are normal. Fixation with piece of chest wall in formol saline followed by decalcification in 10% nitric acid with phloroglucin.

Microscopic examination.

The damaged lung is small, triangular in area and measures 800μ X 400μ. It is united to the parietal pleura by a vascular fibroblastic adhesion and is separated from the remainder of the lobe by a boundary zone of condensed lung tissue containing bronchial channels lined by cubical cells. Decalcification has impaired the staining of the lung tissue.

R.47. Duration of experiment 14 days.

Adult large white male rat.

15.4.41. Ether anaesthesia. Red hot serum needle passed into right chest at level of the 5th interspace.

28.4.41. Killed by breaking neck under ether anaesthesia. At post mortem there is a wedge-shaped rusty brown lesion at

the base of the upper lobe. Fixation Zenker-formol and formol-saline.

Microscopic examination.

There is a wedge of consolidated tissue extending from the hilum to the periphery of the lung and measures 2.5 cm. in depth and 2.5 mm. in width at the pleura. This tissue consists of collagen with fibroblasts which enclose bronchial branches. The latter are oval spaces lined by single layers of flattened cells and containing coagulated eosinophile fluid. Other bronchial branches are lined by cuboidal and tall ciliated cells. This is a partially aerated lesion where the bronchial buds have become occluded by oedema. Many iron-containing phagocytes are present in the connective tissue.

Surrounding this scar there are pulmonary alveoli of varying size separated by small areas of collapsed lung, and the appearance suggests that the lesion originally must have occupied a much larger portion of lung and have undergone reduction in size through resolution. The pleura over the point of puncture is considerably thickened by fibroblasts and serosal cells many layers thick.

M.75. Duration of experiment 13 days.

Male white mouse, 23 gms.

27.10.41. Ether anaesthesia. Hot guage 14 needle passed into right chest. Good recovery. Animal found dead 13 days later. Post mortem: crescentic area of collapse at base of the middle lobe.

Microscopic examination.

This is an atelectatic lesion with the bronchi filled with pus. There is an acute bronchitis with bronchiolitis and suppurative broncho-pneumonia masking any change which might be attributed to the trauma.

M.113 - 125.

A group of 12 adult mice punctured with hot guage 14 needles in the right chest under ether anaesthesia. They were killed at intervals from 14 to 60 days and serial sections cut of all the lungs. Two only, nos. 114 and 115, showed lesions.

M.114. Duration of experiment 16 days.

Post mortem there is a shallow lesion at the base of the right apical lobe. Fixation formol perchloride - serial sections.

Microscopic examination.

There is a narrow superficial area (300μ or 400 μ deep) of collapsed lung where the alveolar structure is replaced by columns of small oat-shaped cells arranged more or less at right angles to the pleura. The lung adjoining this consolidated area is of normal structure but congested and there is no evidence of any attempt to organise the injured area. There is some pleural thickening over the consolidated lung, the cells being flattened serosal cells arranged parallel to the lung surface.

M.115. Duration of experiment 14 days.

Post mortem there are some haemorrhagic areas throughout the right middle lobe but no obvious injured area.

Microscopic examination.

The haemorrhagic areas are of recent origin and probably related to the type of death. Transversely across the lobe, however, there is a narrow area of lung tissue (200 μ wide) where the alveolar structure is replaced by blood capillaries with swollen endothelial cells. These capillaries border spaces of which some are filled with blood and others aerated. The pleura on the external surface of the lobe is indented as a narrow V-shaped depression without pleural thickening but which appears to represent the point of entrance of the needle; similarly the transverse lesion across the lobe is the remains of the needle track.

R.90. Duration of experiment 33 days.

Young male rat.

21.8.42. Under ether anaesthesia, heated serum needle was passed into right chest at the level of the 5th interspace and withdrawn immediately. Good recovery. Killed by chloroform 33 days later. Post mortem, there is a small linear depressed scar of greyish-pink colour running vertically down the lateral aspect of the lobe. Fixation Zenker-formol, 6 hours.

Microscopic examination.

This tissue is disappointing. There are a few areas of collapsed lung throughout sections which are otherwise normal, but these lesions are obviously recent and probably unrelated to the experimental injury.

R.91. Duration of experiment 33 days.

Punctured similarly to Rat 90 and at same time. At post mortem there is a shallow depressed area on the lateral aspect of the right middle lobe. Fixation Zenker-formol, 6 hours.

Microscopic examination.

Transverse sections of the right middle lobe show a linear area extending from the hilum to the periphery where the lung tissue is replaced by young connective tissue. This

consists of loosely-packed fibroblasts in parallel arrangement with some small capillaries and numerous iron-containing phagocytes. Near the base of this area is the main bronchus to the lobe with a large branch, but there is no evidence of bronchial budding. Alveoli and large air spaces lined by flattened cells are present in the loose scar tissue, however, and are in communication with the alveoli of the surrounding lung.

R.92. Duration of experiment 33 days.

Punctured similarly to rat 90 and at same time. Post mortem, there is a collapsed middle lobe and one small depressed area in the mid line of the basal lobe. Fixation Zenker-formol, 6 hours.

Microscopic examination. Fig. 30 refers.

The sections are transverse across all three lobes of the lung. In the basal lobe there is a broad wedge-shaped area of consolidation extending from the hilum to the lateral aspect of the lobe. This consolidated area is of vascular connective tissue containing bronchi and their branches. Many of the latter are lined only by a layer of flattened cells, but others have true respiratory epithelium. Numerous iron-containing phagocytes and many small capillaries are to be seen in this area which contains also alveolar structures which can be seen to communicate with the normal

lung alveoli at the margins of the scar, It may be said that this lobe has been undergoing aeration both by bronchial budding and alveolar formation.

The middle lobe presents a different appearance. At the base of the lobe, near the hilum, there is connective tissue with bronchial branches similar to those described above, but the remainder of this lobe is not expanded. It shows a more or less solid cellular structure of connective tissue and blood cells, with here and there small syncytial sheets of paler cells like hypertrophied pulmonary alveolar cells. Some slight thickening of the pleura has occurred over this lobe but this is mainly of a fibrous nature, there is no serosal cell proliferation.

The apical lobe shows some small atelectatic areas but nothing noteworthy.

L.25. Duration of experiment 36 hours.

Adult cat.

18.3.43. Under positive pressure ether anaesthesia, a left thoracotomy was performed at the level of the 7th rib in the usual way. The bleeding from the wound was controlled by deep through and through sutures of black silk, and interrupted black silk was employed for the visceral pleura. The chest was closed by interrupted white cotton sutures in layers. The animal made a good recovery.

20.3.45. Killed by chloroform 36 hours later. Post mortem, there was a puckered dark-purple pulmonary scar surrounded by congested lung. No noteworthy pleural change. Fixation intratracheal formol saline and followed by mercuric mordant.

Microscopic examination. Fig. 35 refers.

Transverse sections across the wound demonstrate the dimpling of the lung surface to a depth of 2-3 mm: the wound itself extends half across the lobe and measures 1.5 cms. in depth. In its centre, it is occupied by haemorrhage with fibrin containing scattered leucocytes, partly polymorphonuclear and partly mononuclear, and bordering the haemorrhage there are many alveoli filled with blood and with dilated, congested capillaries in their walls. External to this congested zone and separating it from the normal lung, are alveoli containing eosin-staining oedema fluid.

The pleura for a distance of approximately 1 cm. around

the wound shows its serosal layer separated from the underlying elastica by fibrinous exudate. Many of the serosal cells are missing, and those which remain are swollen and irregular in shape, for the most part bipolar with their long axes parallel to the pleural surface. In some sections the pleura dips into the wound in a V-shaped space occupied by fibrin; in other sections the space contains an island of lung tissue which, due to the puckering of the wound, protrudes into the V-shaped gap.

L.26. Duration of experiment 45 hours.

Adult cat, 4 kilos.

18.3.45. Under positive pressure ether anaesthesia, a left thoracotomy was performed at the level of the 7th rib in the mid-axillary line and a triangular wedge of lung tissue was excised from the lower margin of the left lower lobe. There was considerable pulmonary bleeding and a large vessel had to be ligated in the posterior side of the wound. The pleura was carefully apposed with interrupted black silk sutures, and the chest wall closed in layers using white linen thread. The animal made a good recovery.

20.3.45. Killed by chloroform, 45 hours later. The wound was a puckered dimpled scar with dark red congested lung on either side and black sutures crossing the line of the wound. There were no adhesions and no obvious pleural thickening or loss of gloss. The lung was fixed by formol saline

intratracheally, the blocks being mordanted in a saturated aqueous solution of mercuric chloride.

Microscopic examination. Fig. 36 refers.

Sections show that the wound is approximately 0.5 cms. wide and in depth measures 1.5 cms., extending almost across the width of the lobe. It consists of a central area of haemorrhage bounded by alveoli with congested walls and occupied by intra-alveolar haemorrhage. The alveoli surrounding the deepest part of the wound are atelectatic or partially so, some with walls swollen with dilated and congested capillaries but in general not showing a visible cellular lining.

External to the zone of congestion there are oedematous alveoli filled with coagulated fluid staining lightly with eosin: in contrast to the congested area the capillaries of the oedematous alveoli are almost empty of blood.

The serous and elastic layers of the pleura are separated from one another by a narrow layer of coagulated oedema across which a few elongated fleshy fibroblasts extend. Some fibrin is present on the serosal surface, sometimes replacing the serosal cells. The pleural elastica is inverted into the wound, at one point to a depth of 2 mm.

L.5. Duration of wound 48 hours.

Large adult cat.

12.8.42. Under positive pressure ether anaesthesia a left thoracotomy was performed by the standard procedure at the level of the 7th rib on the left side. A wedge of lung was excised in the normal way, bleeding was not serious and easily controlled by two deep through and through sutures of black silk. The anterior pleural margins of the wound were neatly apposed with black silk stitches. In view of the good alignment, no sutures were placed in the posterior margin. The animal made a good recovery.

14.8.42. Killed by chloroform. On naked-eye examination the wound is of dark linear shape, with slightly puckered swollen edges, and bordered by a narrow zone of partially collapsed lung. There is a light deposit of fibrin on the pleural surface around the wound, but no pus. The lobe, not distended artificially, was placed in formol-perchloride overnight. In the morning three blocks were cut at right angles to the line of the wound and placed for a further 48 hours in a saturated solution of perchloride of mercury in physiological saline.

Microscopic examination. Figs. 37 - 42 refer.

Sections transversely across this wound show that it consists of collapsed or partially collapsed alveoli bounding an irregular mass of blood clot which contains numerous

leucocytes, mostly polymorphs, arranged diffusely and in small clumps around the sutures. Fibrin strands transverse the clot and a definite line of fibrin separates the torn lung alveoli from the blood clot.

The gap in the pleural surface of this wound measures 170 μ in diameter in the sections principally used for this description and is occupied by fibrin clot continuous with that filling the wound in the lung. Mononuclear macrophages and lymphocytes are present in the fibrin: pus cells surround the sutures. This fibrin extends over the pleural surface around the wound for approximately 2 cms., separating and lifting the serosal cells from the underlying elastica and covering small groups of serosal cells which remain in situ. There is a vigorous serosal cell reaction at the periphery of the wound. At the margins of the wound the surface alveoli are separated from the pleura by a subserous cellular zone of dilated capillary blood-vessels, collagen fibrils, and spindle-shaped fibroblastic cells; mononuclear and macrophage cells are not numerous and pus cells are rare except in the immediate vicinity of the sutures. The cellular lining of the superficial alveoli is visible, the cells are swollen and hyperplastic, many have assumed cubical and polygonal shapes, and all have large vesicular nuclei with prominent nucleoli. Like the subpleural capillaries, the alveolar mural capillaries have dilated and are

lined by swollen endothelium, and from these capillaries, as from the hyperplastic alveolar endothelium, spindle-shaped cells stretch out into the subserosa and augment its cellular content.

On the pulmonary aspect of the inverted pleural membrane there is an alveolar reaction similar to and continuous with that taking place in the subserous tissue of the lung surface. Deep to the termination of the elastica, however, the fibrin of the clot is in immediate contact with the fibrin deposit on the pleural surface and beneath the fibrin wherever surviving serosal cells remain. The latter have proliferated and grown over and into the fibrin as polymorphous and spindle-shaped forms, many of them morphologically indistinguishable from fibroblasts: especially prominent are the vesicular nuclei and sharply-staining nucleoli of these proliferating cells.

In this cat the edges of the pleural elastica are inverted into the wound for about 1 cm. terminating in an undulating membrane. The elastica itself is for the most part a normal linear structure with microscopic interruptions only where subpleural capillaries abut against it, and there is considerable thickening of the collagenous layer of the pleura, both on the surface of the lung and where it has been inverted into the wound. Thus hyperplastic pleural collagen separates the elastica proper from the reacting lung alveoli.

L.34. Duration of experiment 72 hours.

Adult black and white cat.

8.9.43. 11 a.m. Under positive pressure anaesthesia, left thoracotomy at level of 7th rib, with excision of triangular wedge of lung of base and side 1 inch long. Lung very friable and stitches tore out easily: considerable bleeding from large vessel near apex of wound: no ligatures used. Stitches all white silk.

11.9.43. 11 a.m. Killed by chloroform. The pulmonary wound is a dark puckered scar without adhesions: the lung around the scar is atelectatic. Fixation by intratracheal formol saline.

Microscopic examination. Figs 43 and 44 refer.

The area of pulmonary consolidation averages 6 mm. X 8 mm. and microscopically the lesion is essentially one of haemorrhage, the entire centre of the wound being occupied by blood clot. The alveoli which border this clot are somewhat distorted in shape and their walls are thickened from congested vessels, but the degree of collapse is only partial. Occasional oedematous alveoli can be seen in the lung but oedema is not a feature except in the alveoli immediately beneath the pleural surface: these spaces contain dark-staining coagulated fluid (fig. 43), the subpleural capillaries are greatly distended with blood while the

endothelial cells of their walls have grown out as elongated and spindle-shaped structures. The pleura is inverted into the wound for approximately 1 mm. producing a dimpled appearance, and fibrin extends over the pleural surface for a short distance around the wound; serosal cells are scanty and a few polymorphs are present in the fibrin although they are numerous around the white linen ligatures both in the lung and under the pleura.

At the periphery of the clot air spaces are to be seen and these are in direct communication with bronchial branches: the latter are elongated narrow channels, extensions of bronchi, and lined by flattened endothelium.

L.1. Duration of experiment 4 days.

Adult cat.

17.7.42. Under positive pressure anaesthesia a left thoracotomy was performed by the standard procedure at the level of the 7th rib on the left side and a wedge of lung tissue 1 inch deep and approximately 1 inch base was removed. Considerable bleeding from a large vessel on the anterior margin of the pulmonary wound was controlled by two deep black silk sutures. The pleura was approximated anteriorly and posteriorly by interrupted black silk sutures, and the chest was closed in layers, using white linen thread.

18.7.42. Wound slightly wet with serous discharge. The animal otherwise well.

21.7.42. In order to inspect the lung moving in the chest

the animal was ~~killed~~ anaesthetised by positive pressure ether anaesthesia. On reopening the thoracotomy wound the lung was seen to move normally and without adhesions. The wound was a puckered scar of purplish and bluish colour without particular characters: it was mobile and the lung expanded well on both its sides. Death was induced by chloroform and the affected lobe was fixed in mercuric formol for 2 days followed by saturated aqueous mercuric chloride overnight; no intratracheal distension was employed.

Microscopic examination. Figs. 45 - 50 refer.

The central part of this wound is occupied by blood-clot containing considerable masses of yellow pigment. In the clot there are some small groups of phagocytes with dark blood pigment and some isolated pus cells.

The shape of the wound is shown in fig. 45. There is a narrow indentation forming a space 3.5 mm. deep lined by pleura: fibrin surrounds the wound at the lung surface and occupies the wound gap. Both on the lung surface and in the wound gap there is a vigorous serosal cell reaction similar to that described for cat L.5. On the pulmonary side of the elastic membrane there has been some increase in collagen fibres beneath the pleural elastica, and the superficial alveoli have mural capillary blood vessels so dilated and congested as to distort the spaces, making them partially atelectatic. Elongated swollen and bipolar endothelial

cells line these capillaries but evidence of an alveolar cellular lining is lacking. In the central clot of the wound itself there are fibrin strands which are in contact with the mass of congested dilated capillary blood vessels representing pulmonary alveoli in this area. These capillary vessels, packed with erythrocytes, are continuous on the one hand with the subpleural congested zone of the more superficial parts of the wound, and on the other hand can be followed to less distorted and finally normal alveoli, Collagen fibres mingle with these dilated capillaries and in places separate the fibrin clot from them.

The bronchi surrounding the wound are mostly terminal or respiratory bronchioles, widely dilated and showing no alteration in their mucosa. One bronchus which has been partially obliterated by the deep suture when the wound was drawn together shows metaplasia of respiratory epithelium into cubical epithelium just where the stricture begins: at one point, presumably the site of a subsequent bronchial bud, the cubical cells have proliferated to form a layer three or four cells deep.

There is an aggregation of pus cells around the black silk ligatures but the strands of silk are unaltered.

L.33. Duration of experiment 5 days.

Adult cat.

8.9.43. Under positive pressure ether anaesthesia, left thoracotomy at level of 7th rib with excision with Mayo scissors of triangular wedge of lung, of base and side each one inch. Some bleeding occurred from the apex of wound but was controlled by through and through sutures of white silk. The pleura was approximated carefully by white silk and the lung returned to the chest cavity.

19.3.43. Killed by chloroform. A dimpled lung wound deeply congested and surrounded by less congested lung: some fibrin has been deposited on the pleural surface around the wound but there is no adhesion.

Microscopic examination. Fig. 51 refers.

Transverse sections across this wound show that the area of damage measures 5 mm. wide X 8 mm. deep. The wound at the pleural surface is dimpled to a depth of 2 mm. and the pleura itself inverted into the wound for a similar distance. Much of this dimpled area, external to the elastica, is occupied by recent haemorrhage and older fibrin clot: neutrophile polymorphs are aggregated round a white silk suture. Fibrin clot occupies also the central area of the wound. The clot in itself has no particular features but on both sides of it, in the plane of the section, there are numerous elongated air spaces separated from one another by rather

thick-walled capillaries filled with blood. These spaces conform to alveoli but many of them have no cellular lining other than that provided by the thick-walled capillaries. Spaces of this nature can be seen to communicate directly with alveoli proper and with bronchial branches, and these spaces are lined by elongated flattened cells directly continuous with similar cells lining the dilated bronchus or bronchiole.

The superficial alveoli immediately beneath the pleura contain darkly-staining coagulated fluid and there is considerable proliferation of the endothelial cells of the mural capillaries which have elongated and become bipolar or tadpole-shaped. They extend in a network across the subserous alveoli and between them and the pleura, forming a type of vascular granulation tissue.

L.17. Duration of experiment 3 days.

Adult cat, 2.5 kilos.

14.12.42. Under positive pressure ether anaesthesia a left thoracotomy was performed at the level of the 7th rib and a transverse wedge of base 1 inch excised from the basal lobe of the left lung. The cuts were made with the Mayo scissor. Considerable bleeding occurred from both sides of the wound; the large vessels were ligated with black silk and the lung approximated by three through and through

silk sutures. The chest was stitched with linen in layers. 21.12.42. Killed by overdose of anaesthetic ether. The wound in the left lung was a clean, depressed scar occupied by blood clot; there was no naked-eye pleural reaction. The lung was fixed in formol saline and mordanted in mercuric chloride.

Microscopic examination. Figs. 52 - 57 refer.

The sections have been cut transversely across this lobe and show two pleural surfaces with the wound between. The width of the lobe (and depth of the wound) in the two blocks examined measures 0.8 and 1.3 cm. respectively, and in width the variation is from 4 mm. at the pleura to 900μ in the centre. The pleura has been inverted towards the centre of the wound to a depth of 2 mm. This inverted pleura consists of thickened collagen and elastic membrane. Microscopic gaps, measuring 300μ to 500μ in extent, occur in the elastic membrane at points where fibroblasts and capillaries from the lung immediately beneath the pleura are continuous with the serosal reaction external to the pleural elastic membrane. On the surface of the elastica around the wound and also in between the layers of inverted pleura there is vascular fibroblastic tissue with a layer of serosal cells extending across the wound.

The pulmonary tissue surrounding the wound itself consists of alveolar walls thickened by capillaries and

fibroblasts, forming long and narrow air spaces arranged in a radiating fashion from the wound. The central part of the wound is occupied by fibrin surrounded by fairly dense collagen fibres. Irregular spaces of various sizes, some quite large, have appeared in this collagen and are lined by prominent cubical cells with clear cytoplasm staining like respiratory epithelium. Occasionally these spaces in proximity to the larger blood vessels at the periphery of the wound contain some red blood cells but most of them are empty and are related to bronchial buds. The latter are very numerous and can be traced from two large bronchi near the centre of the wound. Transitions can be seen between the bronchial buds, the air spaces in the collagen and the alveoli of the adjacent lung.

L.2. Duration of experiment 10 days.

Adult cat.

17.7.42. Under positive pressure ether anaesthesia the chest was opened by left thoracotomy at the level of the 7th rib and using Mayo scissors a wedge of lung 1 inch deep and of 1 inch base was excised. Troublesome bleeding from a vessel deep in the wound was controlled by black silk sutures passed round the region. The pleura was approximated anteriorly by interrupted black silk sutures; this seemed to give a good result and the lung margins posteriorly

adhered together without stitches. The chest was closed in layers. Duration of operation 20 minutes.

18.7.42. Cat well.

27.7.42. Killed by ether anaesthesia, using positive pressure to allow inspection of the wound in the chest before death. The lung moved normally and showed a dimpled scar without adhesions. The animal was killed by increasing the anaesthesia and the lungs fixed in formol saline saturated with perchloride of mercury. Intra-tracheal fixation was not employed.

Microscopic examination. Figs. 58 - 66 refer.

The site of the excision is an area of relatively condensed lung tissue extending from the surface almost to the large bronchus which traverses the field. The wound site at the surface is marked by a dimpled pale area 1.4 cm. deep and 0.5 cm. wide. This is a cavity lined by elastic membrane continuous with the pleural elastica and occupied by vascular connective tissue continuous with that external to the pleural elastica on the surface of the lung.

The vascular connective tissue on the surface of the pleural elastic membrane contains fibroblasts arranged more or less parallel to one another and to the elastic membrane. Occasional polymorphs are present and capillaries with young fibroblasts can frequently be traced from the pulmonary

side of the elastic membrane where the superficial lung tissue, although containing some air spaces of irregular shape, is largely collapsed.

Covering the surface of this vascular connective tissue is a single layer of flattened deeply-staining serosal cells obviously recently formed and not quite continuous across the surface of the wound.

On the pulmonary side of the elastic membrane in the dimpled area of the wound is a layer of dense collagen about the thickness of a normal alveolus. The elastic membrane in this area is much thicker and more nodular than normal, and prolongations from it dip into the collagen, thereby separating it into alveolar-like compartments. Much of the tissue which stains specifically for elastica impregnates heavily with silver carbonate and by this method also the richness of the reticulum in the vascular connective tissue of the scar is clearly demonstrated.

The lung tissue surrounding the pleural indentation of this wound consists of elongated narrow alveoli arranged radially from the wound and separated one from another by alveolar walls thickened by collagen fibres. Some alveoli have a lining of small cubical cells with granular cytoplasm and vesicular nuclei with prominent nucleoli.

Bronchi with cartilagenous walls are present deep to the apex of the wound and from them bronchial buds lined also by cubical cells extend toward the wound.

L.3. Duration of experiment 14 days.

Adult cat.

6.8.42. The animal was anaesthetised by positive pressure ether, and the lung exposed by left thoracotomy at the level of the 7th rib. The exposure was rather too far forward and the most accessible portion of the lung was the thin anterior margin of the upper lobe. A triangle of it was removed by a straight cut across the lobe with Mayo scissors. The margins of the wound were clamped with pressure forceps prior to stitching although there was no serious haemorrhage. The wound was approximated by black silk sutures.

21.8.42. Killed by chloroform. The left lobe at the area of excision showed a pale-blue scar about 0.5 cm. in diameter. The lobe was fixed by intra-tracheal formol solution (10%), cut next day into blocks across the scar and mordanted for 48 hours in mercuric chloride.

Microscopic examination. Figs. 67 and 68 refer.

The whole topography of this wound is dominated by two black silk sutures, each 700μ in diameter, inserted one on either side of the scar, while a suture is external to the pleural elastic membrane at the point of its invertion into the wound. External to the elastic membrane too, on both surfaces of the wound, there is a layer of flattened connective tissue cells arranged more or less parallel to the lung surface. This cellular layer is seldom more than 85μ

in thickness and the whole is covered by a single layer of flattened and elongated serosal cells. The wound extends completely across a tongue of the lung lobe measuring 2 mm. across, the scar being 250 μ wide and the wound itself represented by a narrow strip (85 μ wide) of connective tissue cells. On one side of this scar the pulmonary alveoli are of normal shape but with walls thickened by fibroblasts and collagen and lined in the main by flattened cells although occasional cubical cells can be seen. The other side of the scar, however, is in contact with cellular connective tissue which has split irregularly into air spaces lined by flattened and cubical epithelium or respiratory type. Alveoli are present in the free margin of this connective tissue giving it rather a scalloped appearance and these alveoli communicate either through other alveoli or through unlined air spaces with a small bronchus adjacent. The alveoli in the scar margin are lined by cubical cells and have walls thickened by connective tissue: they appear like alveoli in lung resolving after inflammation.

The strands of black silk ligature are unaltered apart from tearing by the microtome knife, but they are heavily infiltrated with neutrophile polymorphs. In addition the pulmonary tissue surrounding the ligature shows an endothelial reaction with sheets of cells, almost syncytial, but resembling the cells of the superficial alveoli of the lung

in the experiments of shorter duration. Strands of connective tissue enclose the sutures and some imperfect alveolar spaces also can be seen: around the large suture are two air channels lined by cells mostly of squat cubical type but some have developed the characters of true respiratory epithelium.

Iron-containing phagocytes are very numerous in the scar tissue and in the lumina of some of the newly-formed alveoli.

L.18. Duration of experiment 16 days.

Adult male cat.

14.12.42. Under positive pressure ether anaesthesia a left thoracotomy was performed in the usual way and a triangular wedge of 1 inch base and depth removed from the lower margin of the basal lobe. Considerable bleeding occurred from two large vessels which required ligation. The lung was approximated by interrupted black silk sutures in the pleura. The stitches tore out and the operation was slower than usual. A small portion of the pleural surface above the scar was torn with forceps and ether vapour could be heard and smelt blowing from the wound as the lung was returned to the chest, but the animal's condition necessitated closing the thoracotomy at once. The chest wall was closed by linen thread in layers.

30.12.42. Killed by chloroform. A horse-shoe-shaped adhesion united the lung to the diaphragm. The wound above the adhesion was healed satisfactorily as a congested linear scar. Fixation of lung adherent to diaphragm in formol saline.

Microscopic examination.

The sections transversely across the scar and the adhesion show that the latter is of vascular fibroblastic type external to the pleural elastic membrane and does not present any features of special interest. The interruption in the pleura measures only 500μ and has been closed by fibrin and fibroblasts. Beneath the pleura the lung scar is of densely cellular connective tissue of fairly vascular type, and the margins of this scar have been in the process of being invaded by alveoli situated at its margins. These present the appearances which are characteristic, the air space of irregular shape lined wholly or in part - and always on its "scar" wall - by cubical cells. There is no evidence of aeration by bronchial branch penetration. Necrosis almost amounting to caseation has developed at the sites of the sutures, an unusual feature in these experimental wounds.

L.10. Duration of experiment 23 days.

Adult cat.

3.10.42. Under positive pressure ether anaesthesia, a left thoracotomy was performed at the level of the 7th rib on the left side and a wedge of lung of 1 inch base removed from the upper lobe of the left lung. The excision was done with a sharp B.P. blade: some bleeding deep in the anterior side was caught by a suture of black silk and the lung stitched by interrupted sutures of the same material. Difficulty was experienced in removing all the blood from the chest cavity. The chest was closed in layers with white linen stitches. Time 30 minutes. The animal made a good recovery.

26.10.42. Killed by chloroform. There is a depressed puckered pulmonary wound, dark pink in colour with black markings where the stitches show through the pleural lining. The scar is a wavy line of collapsed lung, and the whole area surrounding the wound shows thickened white pleura.

Microscopic examination. Figs. 69 and70 refer.

The section of this wound shows that its depth is approximately 1 cm. and that it consists of a track of condensed lung tissue leading to main central mass.

The pleura is thickened (90-100μ) fairly uniformly for some distance away from the wound, and external to the elastic membrane consists of flattened compressed fibrocytes. The serosal layer itself exists only as elongated flattened

cells. Inversion of the pleural elastica is not marked and on each side of the wound gap the elastica terminates as a coiled membrane.

Four separate black silk stitches have been cut transversely in this section. Many of the silk fibres have disappeared artificially but the polymorphonuclear infiltration has remained. Numerous dilated capillary blood vessels with fibroblasts surround the stitches.

The main central part of the wound is of recently formed alveoli lined by cubical and elongated cells. These alveoli are very numerous and are all richly surrounded by fibroblasts and capillary blood vessels, but separated into groups and sometimes from one another, by homogeneous pink-staining collagen. This collagen appears to be residual and air spaces have formed in it. Some of the larger bronchial branches in the periphery of the wound contain pus cells although there is no desquamation of mucosa and all the newly-formed air spaces in the scar also are filled with pus cells, and there is no doubt that they communicate directly with the bronchial branches. There has been no detectable new formation of elastica around the newly-formed bronchial branches, or air spaces, in the wound itself, but many small broken elastic fragments are to be seen in the central area and around the black silk ligatures.

L.4. Duration of experiment 28 days.

Adult male cat..

12.8.42. Under positive pressure ether anaesthesia a left thoracotomy was performed at the level of the 7th rib exposing the basal lobe of the lung. A triangular wedge of tissue of base and side at least 1 inch was removed by scissor. Some troublesome bleeding occurred from the depth of the wound near the apex of the incision and a vessel had to be ligated with black silk. Two deep through and through sutures were employed to control the general oozing from the wound, and the lung stitched together by interrupted black silk sutures. Clot had to be removed from the pleural cavity and the chest closed in layers using linen sutures. Good recovery.

9.9.42. Killed by chloroform. In the base of the left lower lobe there is a large broad bluish scar but the lung surrounding the scar is well-expanded. Fixation by intratracheal formol saline in situ.

Microscopic examination. Fig. 71 and 72 refer.

This is a V-shaped wound, of depth approximately 2 mm., rather wider at the lung surface. The diverging limbs of the V are really the elastic tissue of the pleura, enclosing vascular connective tissue which is dense near the apex of the V but much looser superficially where it is covered completely by a single layer of rather deeply-staining

serosal cells. At the actual apex of the V there is a mass of connective tissue with collagen whorled around two black silk ligatures.

The elastic tissue forming one limb of the V is sinuous and turned on itself with undulations of varying thickness, as though it were a ribbon seen alternately on edge and in the flat. It terminates in a whorled mass at one of the apical sutures. On the other side, the elastica is re-duplicated many times, as a collection of parallel fibres, all staining deeply with specific stains, and is more or less in direct contact with the underlying lung. In fact, it practically replaces the pleural collagen layer, which is thin on this side although well-developed on the other. A similar appearance is present near the surface of the wound where the elastica becomes parallel to the lung surface. There, many elastic fibres can be seen in palisade arrangement extending into the vascular fibrous tissue.

The lung tissue bordering the scar is condensed and consists of narrow elongated alveoli more or less at right angles to the wound, all lined by somewhat cubical cells, and having walls thickened by dilated capillaries and collagen fibres.

Some large bronchial branches with cartilage in their walls are present near the apex of this wound, and from them well-developed bronchial buds extend towards the wound.

These buds are more or less stellate on section, and are lined by cubical cells for the most part, although where the buds are wider, the change to respiratory epithelium is clearly seen. Elastic tissue and smooth muscle accompany the better-developed branches. Alveoli having a cubical cellular lining have formed in the collagen of the wound both at the apex and around the wound margin. In these as in the bronchial buds iron-containing phagocytes are numerous.

The black silk ligatures are infiltrated with polymorphonuclear leucocytes, but their strands are unaltered. Around one ligature there is an air space lined by bronchial epithelium, communicating directly with a bronchus.

L.12. Duration of experiment 45 days.

Adult cat.

3.10.42. Positive pressure ether anaesthesia. A left thoracotomy was performed by the standard procedure and a wedge of lung 1 inch deep X 1 inch broad at its base removed from the inferior free margin of the lower lobe. There was considerable bleeding from the anterior side of the incision and frothing of air. Three deep black silk sutures were used to control the bleeding and a further stitch to appose the pleura. Wound closed in layers using linen thread.

18.11.42. Killed by chloroform. Post mortem showed a small white scar on the diaphragmatic lung surface with one black silk suture showing on the surface. Fixed formol saline with mercuric mordant.

Microscopic examination.

Through an error the blocks include only a small portion of the scar and are unsatisfactory for general purposes. There are some good examples of the appearance of sutures in the lung. The stitches are surrounded by pus cells and enclosed in connective tissue which contains dilated capillary vessels, but the strands of silk are themselves unaltered.

L.13. Duration of experiment 51 days.

Adult cat.

23.10.42. Under positive pressure ether anaesthesia left thoracotomy was performed at the level of the 7th rib and the left lower lobe exposed. A triangular wedge of tissue, 1 inch wide and 1 inch deep, was excised from the free margin of the lobe anteriorly and the margins stitched together by black cotton. The skin and muscle were closed in layers by linen thread. Good recovery.

13.12.42. Killed by chloroform. There was a puckered scar adherent to the diaphragm and parietal pleura with evidence of inflammatory pleural thickening. Fixation by intratracheal formol-saline mordanted by aqueous saturated mercuric chloride.

Microscopic examination. Figs. 73 - 77 refer.

This wound consists of a central collagenous mass canalised by many air channels and surrounded by partially distended alveoli which separate the collagen from normal lung. The whole area of the scar measures 0.6 X 0.8 mm. in the plane of the section.

The usual inversion of the pleura has taken place, more marked on one side than on the other, and to a depth of about 800μ. Serosal cells are absent from parts of the pleural surface, where, for example, the pleural surface is represented by loose vascular connective tissue enclosing a black silk ligature. The absence of serosal cells is clearly related to the adhesion of the lung to the parietal pleura at this point.

Partially bounded by the fibro-elastic membrane of the inverted pleura, and partially in direct contact with the lung, the central collagen or hyaline mass presents several different types of structure. There is the eosin-staining material itself, a relatively avascular tissue but containing phagocytes and fibroblasts. The most striking feature is the large number of bronchial branches of divers shapes and which canalise the material, and these bronchial canals have obviously direct communication with a large bronchus containing cartilage in the wall adjacent to the hyaline central mass. The cellular lining of the bronchial branches

is of particular interest. Normal ciliated epithelium lines the main bronchus and also the wider part of the main branch of this bronchus. As this branch enters the denser part of the mass, however, it narrows markedly, and the epithelium lining the narrower part consists of elongated, flattened cells without cilia, and arranged in several layers, three or four cells deep. Similar cells line many of the smaller branches and some of the large air channels which surround the sutures. In the majority of the small air channels or bronchial branches which canalise the collagen, the lining epithelium is true squamous epithelium with well-developed prickle cells. In general the lumina of these buds are empty, but some contain a cluster of typical lung phagocytes, thus establishing the continuity of the lumen with the newly formed alveoli, which have similar contents.

At its edges, where the hyaline central mass is in direct contact with the lung alveoli, the margin of the collagen presents a scalloped appearance, indented by the alveoli. The alveolar wall which abuts on the collagen is lined by closely packed cubical cells, while the walls remote from the collagen have the normal cellular lining. Groups of alveoli can be seen, almost completely within the collagen, and most of these are lined entirely by cubical cells. These are undoubtedly reformed or newly-formed structures, and

frequently have phagocytes in their interior; they are always in contact with distended capillary blood vessels.

The black silk sutures are frequently isolated by large air channels surrounding them. Pus cells are numerous, and collections of iron-containing phagocytes can be seen in the collagen.

L.14. Duration of experiment 59 days.

Adult male cat, 3.8 kilos, old.

23.10.42. Under positive pressure ether anaesthesia a left thoracotomy was performed and the left lower lobe of the lung exposed. The lung tissue was friable and tore easily, bleeding from the margins of the wound was serious and difficult to control. Linen ligatures were put on the deep vessels on both sides and the lung stitched with black silk. The chest wall was closed in layers with linen sutures.

21.12.42. Killed by ether anaesthesia. The wound in the left lung was a slightly-puckered scar with a good deal of pleural thickening around. Beyond the scar, the surrounding lung was very dark with haemorrhage, probably terminal.

Microscopic examination. Figs. 78 and 79 refer.

Extending to a depth of 4 mm. below the lung surface and measuring 7 mm. in width there is a mass of collagen which has developed in relation to the sutures, and embedded in its substance there is a well-developed bronchus with

cartilagenous walls.

From this bronchus, well-formed wide bronchial channels lined by cubical epithelium extend into the collagen which has split into many elongated air spaces. These are lined by rather squat epithelium which connect with alveoli of irregular shape and frequently abnormally large: for the most part the alveoli lack visible lining cells but where they infringe on and scallop the collagen they have a cubical cell lining. It is evident that considerable aeration of this scar has occurred.

The scar is distorted by the sutures which are infiltrated with polymorphs and surrounded by vascular fibroblastic tissue. Air spaces too, have developed in relation to the sutures and there is no evidence of their absorption.

One lip of the scar has protruded over the other as a senile protrusion which contains lung alveoli and a well-developed bronchial branch. There is no cartilage in the wall of this bronchus, but in appearance it suggests that it was carried forward in this protrusion of lung rather than that it has newly developed. The pleura after entering the very shallow cleft of the scar, is reflected over this protrusion of lung. The serosa is continuous except where there is a suture.

A noteworthy feature of this section is the mass of elastic fibres which it contains. In addition to the

somewhat thickened pleural elastica, a mass of elastic fibres, tortuous and sinuous, surrounds the new bronchial branches and run irregularly in the collagen generally near the pleura. Near another suture there is a mass of elastic tissue in the form of a sinuous membrane. This is clearly not newly-formed elastica and appears rather like a mass of original pleural elastica which has become detached and coiled.

The lung on either side of the scar shows many alveoli of normal appearance, although some near the surface are large and almost emphysematous. Some groups of alveoli have walls thickened by fibroblasts but this is not common, although congestion is the rule. Oedema can be observed also and it is probable that the oedema and the congestion together are related to the open ether anaesthesia used for killing.

L.6. Duration of experiment 60 days.

Adult black cat.

18.8.42. A left thoracotomy was performed in the usual way at the level of the 7th rib on the left side but on exposure the lung expanded poorly even under positive pressure anaesthesia. A wedge of approximately 1 inch base and depth was removed. A small amount of bleeding occurred at the apex of the wound and this was controlled by a deep suture: the lung was n ot touched by pressure forceps. The pleura was

sutured by black silk, only one stitch being used posteriorly. The chest wall was closed in layers with linen; the animal made a good recovery.

16.10.42. The animal was killed by chloroform. The wound is represented by a small horn-shaped protrusion, rather hard, and jutting above the surface of the lung for about 1 cm. There has been some puckering of the lung towards this protrusion. Fixation: intratracheal formol-saline followed by perchloride mordant.

Microscopic examination. Figs. 80 - 82 refer.

The lesion is of remarkable appearance: it is a firm congested protrusion above the lung surface, constricted at its base within the lobe by two closely apposed ligatures proximal to which there is a large bronchus. The tissue in the protruding area has lost its alveolar structure and consists of a central hyaline mass, containing whorls of elastic tissue, which has evidently surrounded large vascular and air channels when the lung was expanded, large numbers of irregularly arranged connective tissue cells, and haemosiderin, both extracellular and enclosed in phagocytes. Clearly this is the end-result of lung which has been severed from its vascular and air supply by ligation.

The pleural surface of this lobule is of course continuous with that of the adjacent lung tissue, and is covered by a single row of serosal cells which are separated from

the elastic membrane by flattened connective tissue cells arranged in a layer several cells deep. Beneath this pleural elastica, over the whole surface of the protruding lobule, is a zone about the width of two normal alveoli, occupied by greatly dilated capillary blood vessels and air spaces. Similar capillaries, more numerous, larger and better developed, are spreading into this subpleural area at the constricted neck of this lobule from the adjacent lung. Greatly dilated capillary vessels are present both in this adjacent lung and its pleura. This is an example of vascularisation and aeration of a portion of necrotic lung from the adjacent lung tissue.

L.7. Duration of experiment 76 days.

Adult cat, 4.8 kilos.

18.8.42. Under positive pressure anaesthesia a left thoracotomy of the usual type was performed. The lower edge of the left upper lobe was heavily marked by anthracosis and on cutting out a triangular wedge with scissors, the lung was felt to be tough. Troublesome bleeding was experienced from a divided vessel near the apex of the wedge and the vessel had to be clamped on both sides: the haemorrhage was controlled finally by a deep suture and ligation was unnecessary. The pulmonary pleura was stitched with black silk, and the chest wall closed in layers. Good recovery.

26.10.42. Killed by chloroform. The wound in the free margin of the lung appeared as an indented bluish scar surrounded by a halo of white pleural thickening.

Microscopic examination. Fig. 83 refers.

A transverse section across the wound includes both pleural surfaces and a somewhat dilated bronchus. Above the bronchus the wound scar is represented by comparatively dense collagen split near its margin into alveolar spaces of fairly normal appearance: many elastic fibres are present in this collagen. The walls of these alveolar spaces are slightly thicker than normal with collagen but the lining cells are few and flattened. Beneath the bronchus the scar remains as three septa which extend from the centre of the lung to the pleura. The central strand is the thickest and at its sides are spaces bordered by the thinner lateral septa and containing normal and emphysematous alveoli. It is difficult to say now whether all these alveoli have reformed but some certainly have.

The pleura shows the later stages of its involution after the proliferative reaction. Fibrocytes and blood capillaries form a layer 60μ wide between the serosal cells and the pleural elastic tissue which is complete over the width of the wound. There is a detached whorled elastic mass in the centre of the wound surface region and an inversion of the surface pleura (elastica and collagen) extends

on one side towards the centre of the wound, almost to the central bronchus. Some dilatation of this bronchus appears to have occurred but its structure is otherwise normal. Plugs of pus cells are present in the two smaller bronchi although their mucous membranes are undisturbed.

This is an example of a partially regenerated wound.

L.11. Duration of experiment 115 days.

Adult cat.

3.10.42. Under positive pressure ether anaesthesia left thoracotomy was performed and a wedge of lung of 1 inch base X 1 inch depth removed by the Mayo scissor from the basal lobe of the left lung. Very little bleeding occurred and there was no escape of blood into the chest cavity. The lung was stitched by deep through and through black silk sutures, and the pleura apposed by interrupted silk stitches. The chest wall was closed in layers by linen sutures. Good recovery.

26.1.43. Killed by chloroform. There is a pale-bluish dimpled scar in the upper anterior margin of the basal lobe. The lower margin of the upper lobe adhered lightly to this scar both on its anterior and posterior aspects, but there were no other adhesions. Fixation: intratracheal formol saline.

Microscopic examination. Fig. 84 refers.

The figure shows the appearance of this lung on section

and it is clear that little scar tissue remains. There is a narrow central septum of connective tissue richly vascularised with blood capillaries. Lung alveoli border this connective tissue and although many are of normal appearance, others are of the long narrow shape so frequently seen in relation to healing wounds. Small multinucleated cells are to be seen in the septum and these appear to be related to foreign material, difficult to identify but possibly strands of suture.

The adhesion between the lobes is of loose open connective tissue lined at each side by a thin elastic membrane with serosal cells over part of its external surface. The pleura over the superficial part of the scar contains a few fibres of collagen external to its elastica but otherwise is normal.

III. PLEURAL WOUNDS.

The operative technique of this group of animals was in general similar to that described for excised wounds of lung (p. 24-25), positive pressure anaesthesia was used and the lungs removed from the chest in the same way on to towels wrung out of hot saline. Instead of excising portions of lung tissue, however, by means of a razor blade small areas of pleura were shaved off the lateral surfaces of the lobes. The cuts were as shallow as possible and the centres of the wounds, where the cuts were deepest, appeared no more than two or three alveoli deep: at the margins, where the cuts began and ended, pleura only was removed. The areas of pleura removed were more or less alike in all the animals and measured approximately 1.5 sq. cms. Oozing from the lungs was controlled by pressure with a dry swab but the centres of the wounds where the gaps were widest were drawn together with a fine silk suture. Thus hour-glass wounds were produced with two small bare areas separated by apposed lung. The lobes were returned to the thoracic cavities and the chest walls closed in layers using white linen sutures. The animals were killed by chloroform at intervals from 48 hours to 32 days after operation. In none was there evidence of pneumothorax and the pleural wounds had healed as rather opaque white

thickenings without adhesions. After fixation in formol saline and suitable mordanting, paraffin sections were prepared and stained by orcein and by Weigert's resorcin fuchsin.

L.37. Duration of experiment 48 hours.

Adult male cat.

Operated on 29.9.43.

Killed on 1.10.43.

Macroscopically there is a small dimpled scar with white fibrotic thickening of the pleura extending radially from the stitch. The unstitched area remained raw and red in appearance but without noticeable haemorrhage. The pleural sac was dry. Fixation of lobe in mercuric-formol.

Microscopic examination.

In the sections examined the area denuded of pleura measures 1.8 mm. in length and the depth of the lung reaction 1500μ. The space so formed is occupied by haemorrhage with fibrin clot where it is in contact with the torn alveoli. The alveolar cells show less reaction than might have been expected and such change as has occurred affects principally the endothelial cells of the alveolar capillaries. The gap in the pleura is thus filled by clot but some degree of inversion of the elastica has occurred at one side.

L.38. Duration of experiment 5 days.

Post mortem, the pleura showed some opacity and radial thickening around the scar.

Microscopic examination.

In the sections the linear surface from which the pleura has been removed measures 1.5 cms., and is divided into two equidistant portions by the suture. There the lung reaction is 680μ deep and is represented by a collagen scar terminating at a vessel with hypertrophied fibrotic walls. The whole surface of the wound is covered by a vascular fibroblastic layer varying from 33μ to 330μ in depth and which presents the features already described in the earlier series of operation cat wounds. No evidence of elastic tissue formation can be seen, the existing elastica being situated about 40μ from the lung surface as an undulating membrane varying in thickness and terminating abruptly on each side of the suture leaving a gap of 2300μ.

L.35. Duration of experiment 8 days.

Post mortem, there is a patch of white thickened pleura at the wound site.

Microscopic examination.

The length of pleura removed measures 5 mm. in the section examined and the region is occupied by fibrin clot. There is a reaction on the part of the capillary endothelium

of the superficial alveoli similar to that described previously. The gap in the elastic measures 1200 μ in the sections examined.

L.47. Duration of experiment 8 days.

Post mortem, slight pleural thickening at the site of the wound.

Microscopic examination fails to show more than a small gap in the elastica. Fibrin has covered the pleural surface for some distance on both sides of the wound but neither the fibrin nor the underlying lung present any noteworthy features.

L.43. Duration of experiment 8 days.

At operation considerable oozing from the lung surface was encountered and three stitches were required to control it, leaving a rather puckered wound.

Post mortem, the wound was covered and surrounded by a fine layer of thickened white pleura. There was no evidence of haemorrhage.

Microscopic examination. Figs. 88 - 92 refer.

The wound in the sections examined is approximately 1 cm. long and 4 mm. deep in the centre where there is a suture surrounded by pus cells. Deep to the suture the lung tissue is condensed with the alveolar walls thickened by congested capillaries and swollen septal cells, but

the main cellular increase is due to fibroblasts. These are particularly numerous also at the margins of the wound where the gap in the elastic membrane occurs. The pleural thickening begins some distance around the gap and consists of collagen fibres and fibroblasts with dilated capillaries separating the elastic tissue from the superficial alveoli of the lung and, external to the elastic membrane, from its serosal covering.

The gap in the elastic membrane varies from 3.5 to 7 mm. in different sections and although at one margin of the wound the elastic membrane terminates abruptly without special characters, at the other margin the membrane ends amongst densely cellular connective tissue. There the fibroblasts are arranged parallel to one another in a palisade arrangement both internal and external to the elastica (fig. 88), on the pulmonary side of which are numerous very fine fibres staining specifically for elastic tissue by orcein and Weigert's methods. These are apparently newly formed elastic fibres and it is to be noted that they have developed in the collagenous and relatively acellular tissue in immediate contact with the divided original membrane.

L.46. Duration of experiment 9 days.

Microscopic examination.

This is a shallow wound. The pleural reaction extends over 1.8 cms. but measures 100μ deep except in the central part where it penetrates the lung to a depth of 3000μ. The reaction is of vascular fibroblastic tissue with the connective tissue cells arranged parallel to the lung surface: serosal cells are absent. Collagen with a few fibroblasts separate the elastic membrane from the lung alveoli. The gap in the elastica is no more than 300μ and the sections fail to show regeneration of elastica.

L.44. Duration of experiment 9 days.

Scar adherent to the chest wall, puckered and useless for examination.

L.36. Duration of experiment 9 days.

Post mortem the wound in the pleura is represented only by the shallowest dimple and with very little pleural thickening: the scar is identified mainly by the stitch.

Microscopic examination.

The pleural thickening measures 1.5 cm. in length and is very shallow, most of it measuring only 100μ in depth although the central part is 800μ deep. That thicker portion corresponds with the gap in the elastic membrane,

a gap which measures 3000μ: no evidence of elastic tissue can be seen in this gap. The membrane on each side of the gap is a normal sinuous structure terminating in a whorled end. Fibroblasts are aligned along the external surface of the membrane, and collagen with fibroblasts separate it from the lung alveoli. The latter show cellular lining on their external or pleural surface and where the wound has involved a bronchus, considerable proliferation of its lining cells has taken place with some metaplasia of squamous type. Air spaces in the lower part of the connective tissue scar are lined by cubical and flattened cells similar to those of the bronchus from which they have obviously arisen.

Serosal cells can be seen on the surface of the vascular-fibroblastic tissue but they are scanty, are swollen elongated cells and are arranged singly or in small groups.

L.45. Duration of experiment 12 days.

Post mortem, the wound is of dimpled type surrounded by opaque thickened pleura.

Microscopic examination. Fig. 87 refers.

Sections show the length of the pleural thickening to be 1.8 cms. and the depth in the centre 4 mm. where there is a white silk suture surrounded by polymorphs and fairly vascular connective tissue. The most superficial part of

the centre of the wound is occupied by fibrin which has undergone some organisation. The gap in the elastica is 3 mm. wide and no evidence of new formation of elastica can be seen.

L.41. Duration of experiment 32 days.

At post mortem, the pleural wound appears as a dimpled scar, rather white and thickened, but smooth.

Microscopic examination.

The p leural reaction measures 1 cm. in length and averages 100 μ in depth. It consists entirely of vascular connective tissue. The gap in the elastica is 1 cm. wide and the membrane ends sharply without evidence of regeneration.

IV. FIXED WOUNDS OF LUNG

L.29. Duration of experiment 16 days.

Black and white adult cat.

23.6.43. 3 p.m. Difficult to anaesthetise, became very shocked during the operation. Under positive pressure ether anaesthesia the left side of the chest was opened at the level of the 10th rib exposing the lower lobe of the lung about 2 cms. above its inferior margin. An excised wound was made in the usual way and stitched by black silk: very little bleeding was encountered. The lung was returned to the thorax and fixed to the chest wall by two linen sutures, one on each side of the wound, passed through the lung and the thoracic muscles, and tied off external to the muscle layers. The thorax was closed in the normal way: at 5 p.m. the animal was recovering normally.

9.7.43. Killed by chloroform. The left basal lobe was adherent to the diaphragmatic and lower thoracic parietal pleura at the site of exposure. The portion of the lobe so involved appeared as a lingula or process of collapsed lung, and was unfortunately not recognised immediately at post mortem; consequently the affected lobe was thus not removed with its attached parietes and was slightly torn. On examination, the lung wound was found to have been adherent to the parietes in a crescentic manner along the

sides of the black silk stitches which look remarkably undisturbed. Fixation was by intratracheal formol-saline followed by mordanting in saturated aqueous solution of mercuric chloride. Blocks were prepared by sectioning the lung transversely across the wound (i.e. parallel to the lung surface) and to naked eye examination these show considerable fibrosis around vessels and bronchi.

Microscopic examination.

Sections across the wound show a consolidated area measuring roughly 1 cm. in width and extending across the total width of the lung, here 0.7 cm. This area is occupied largely by dense, almost structureless hyaline material surrounded or enclosed by cellular connective tissue. In the latter some irregular air spaces lined by flattened or cubical epithelium have developed, and communicate directly with adjacent pulmonary alveoli. The alveoli which indent the connective tissue have a single layer of cubical cells lining their borders adjacent to the connective tissue, while their other margins, although showing an occasional swollen alveolar cell, as a rule lack cellular linings. Apart from this marginal activity, however, there is no evidence of aeration of the hyaline tissue: most of the nearby bronchi have been occluded completely or partially by the sutures used to fix the lung.

The usual fibroblastic pleural thickening extends for

some distance around the wound although interrupted by the tearing of the lung on removal. Inversion of the elastic membrane has occurred and many portions of whorled membrane can be recognised in the depths of the wound. No evidence of new formation of elastica can be seen.

L.39. Duration of experiment 20 days.

Adult cat.

21.10.43. Under positive pressure anaesthesia, left thoracotomy was performed at the level of the 7th rib, exposing the interlobar space. To ensure apposition of the lung wound to the chest wall material, a wedge was excised from the upper part of the lower lobe. Ligatures were not required and the wound was stitched by fine white silk through and through sutures: there were no pleural stitches. The wound was then apposed to the chest wall by deep white linen sutures which did not go completely through the lobe and were placed one on each side of the wound. The chest wall was closed in layers, the deep sutures being tied off over the muscle: interrupted sutures were used for the skin.

11.12.43. Killed by chloroform. The adhesion between the left basal lobe and the chest wall is quite firm but smaller than had been expected. It is of pink fleshy colour and appears well aerated. The chest wall tissue adherent to the lung was removed and fixed with the lung by formol

saline. Small abscesses are present in the centre of this adhesion and at its margins the pulmonary and thoracic pleurae are connected by reflexions of loose connective tissue containing serosal cells. No elastic tissue can be seen in these unions. The pulmonary pleural elastica continues with only slight interruptions across the pulmonary aspect of the adhesion and its only abnormality is reduplication. The elastica of the thoracic wall is interrupted by the adhesion but otherwise undisturbed.

Many dilated capillaries are present in the fibrotic lung and also vessels of similar size but with thicker fibrous walls.

L.30. Duration of experiment 28 days.

Black and white adult cat.

23.6.43. Anaesthetised with positive pressure ether, had left thoracotomy performed at the level of the 10th rib. A wedge of lung was excised in the usual way: there was considerable bleeding from two vessels in the anterior part of the lung, controlled by a deep suture. The pleura was approximated by black silk sutures and the lung apposed to the chest wall by white linen sutures, one on each side of the wound.

21.7.43. Killed by chloroform. On opening the chest there is a fan-shaped area of collapsed lung, 3 cm. across, firmly adherent to the parietal pleura. Long slender adhesions

from both mediastinal and diaphragmatic pleura extend to the scar. The lungs are fixed in situ with intratracheal formol-saline and removed together with the scar in the chest wall, and 9th and 11th ribs.

Microscopic examination.

Sections across the scar show that the width of the adhesion is 2.2 cms. Dense fibrous tissue forms the union with the thoracic wall and although most of this has formed external to the line of the existing pleura there is a shallow (0. 5 cm. deep) fibrotic scar in the lung itself. In the scar there are a few isolated and dilated spaces lined by flattened or cubical epithelium, obviously communicating with a bronchus at the periphery of the scar, and like the bronchus containing pus cells. Alveoli have formed also in the borders of the connective tissue and are lined by cubical epithelium in the usual way, but much of the adjacent lung is poorly expanded, alveoli are distorted and partially atelectatic and many have thickened fibrosed walls. The scar tissue as a whole is well supplied with capillary and dilated blood vessels and there is some degree of medial hypertrophy in the few pulmonary or bronchial vessels adjoining the lesion.

The pulmonary pleural elastic membrane extends across the scar in the line of the original pleura with no more than minute interruptions. Although this membrane stains well by orcein and Weigert's stains, it demonstrates the

sinuous ribbon-like appearance, alternately thick and thin, already described in other wounds. At the borders of the adhesion there is an excess of vascular fibroblastic tissue external to the pulmonary pleural elastica and this tissue is reflected over the edges of the adhesion in the plane of the section, to join with the thoracic pleura which shows less evidence of reaction. This reflexion of pleura, however, consists only of serosal cells and vascular fibrous tissue: the elastic membrane is not involved and no new elastica has formed there. Accompanying the original pleural elastica across the scar however are thin fibres staining specifically for elastic tissue, but their significance is doubtful.

L.40. Duration of experiment 62 days.

Adult cat.

21.10.43. Under positive pressure anaesthesia, thoracotomy at level of 10th rib on left side. The usual wound was made in the base of the left lower lobe: there was considerable bleeding from frothing vessels in both sides of wound, controlled by pressure forceps but not ligated. Stitched with three deep sutures and pleural stitches, all of white silk. The lung was then apposed to the chest with two deep linen sutures tied over the muscle layers. The skin was closed by interrupted sutures.

23.12.43. Killed by chloroform. A fixed adherent wound, puckered and collapsed, adherent to parietal pleura over a length of approximately 2 cms. The lung appears poorly aerated. Fixation of lung with adjacent chest wall in formol saline.

Microscopic examination.

The adhesion measures only 1.2 cms. It consists of fibrous tissue, in parts dense, and in other areas vascular: in the centre of this tissue there is a purulent focus around a suture.

Aeration has been proceeding at the margins of this tissue, both by bronchial branch penetration and by the formation of alveoli from pre-existing alveoli. But for a scar of this age, the degree of aeration is much less than had been anticipated. Deep to the scar there is a large and dilated bronchus, from which, however, surprisingly few bronchial buds have arisen. The main aeration of the scar has been by alveolar formation, and it is to be noted that the alveoli at the margin of the scar are elongated and narrow, only partially expanded.

As with the other wounds of this series, the elastic membrane can be detected throughout the scar in its normal position on the pulmonary side. Considerable deterioration in the structure of the membrane has taken place: it is broken, tortuous and of irregular thickness: in places it

appears duplicated. There is, however, no sign of new formation of elastica either on the visceral or thoracic side of the wound. Nor is there any evidence of elastica in the reflexion of the pleura, where it forms the boundary of the adhesion. There the structure is of connective tissue carrying a single layer of serosal cells.

DISCUSSION.

It is claimed that the foregoing experiments have demonstrated the repair mechanism of mammalian lung following punctured and excised wounds, and after burns. Emphasis must be laid upon the fact that this work is concerned solely with the pattern or manner of the reaction, and except in the most general way, the time factor has been ignored; that is to say, no attempt has been made to compare the rate of healing of different wounds. Young et al (1941) exposed the fallacies of comparative experiments in the rate of healing even of relatively simple surface wounds, and it was clear that nothing was to be gained by interpreting the lung wounds from that aspect. Moreover, it is difficult to produce closely similar wounds even when the lung is exposed and comparable wedges of tissue excised. The lungs themselves differed in texture, some were friable and bled freely, others merely oozed slightly; in some, large vessels were cut, large bronchi in others. Thus repetitive experiments were realised to be of limited value, true biological controls could hardly be expected, and attention was directed rather to securing a series of lesions which would cover all the stages of the repair process. Not infrequently it was possible to learn much from the different blocks of one lesion and to apply these observations to the interpretation of subsequent sections.

In the case of the punctured wounds, and particularly of the burns, comparative experiments were partially successful, although there also, as was pointed out in the introduction, the nature of the lesion after any single experiment was in many respects unpredictable.

Nevertheless, from this series of observations it has been possible to describe a process which falls naturally into defined stages, some of which are common to healing wounds in any situation. Amongst these common factors is the general response to injury, and in the lung, whether or not there is a traumatic factor, the effect of trauma is local and limited in extent. In this category too, may be placed the initial haemorrhage with exudation of fibrin, the local congestion and slight oedema, and the development of capillaries. But in other respects the injured lung manifests organ peculiarities which are related to its structure and function; examples of these are the reconstitution of the pleura, the reaction of the alveolar cells and the regeneration of functioning lung tissue in the scar.

The reconstitution of the pleura.

The reformation of the pleura is in fact the least individual of the organ peculiarities referred to in the preceding paragraph, for the reason that the general pattern of the process in the pleura is common to repair as a whole. Following the superficial haemorrhage and exudation of

fibrin, the potentialities of the subpleural and pleural vascular network are revealed in the dilatation and congestion of the capillaries. From the lung itself, and from the pleura, active proliferation of endothelial cells occurs, and the resulting aggregation of fibroblasts, endothelial and serosal cells presents problems of cytological interpretation. It is difficult to avoid the conclusion that in this response to trauma the endothelial cells undergo true metaplasia in Virchow's sense of the term, connoting alteration of function as well as of form. The endothelial cell is regarded by many authorities as a multipotent cell, and in inflammatory exudates it may act as a fibroblast (Maximow and Bloom, 1942). So far as morphological evidence can be assessed, a similar change appears to occur in the superficies of the lung after trauma, and instances can be observed where endothelial cells appear to become serosal in character. It is not easy to be certain of this, but there is no doubt that the study of what are in effect comparatively simple processes, repair and response to injury, induces a more liberal attitude of mind at least towards the less specialised cells of the body.

Particular interest was directed to the pleura on account of its elastic membrane, and reference has been made to the experiments by which it was hoped to observe

the process of reformation of elastic tissue. Unfortunately the results of these experiments were unsatisfactory and inconclusive, probably for two reasons. Owing to an error in interpretation of the earlier experiments of short duration, the majority of the cats with pleural wounds were sacrificed within ten days of the operation, too early to observe elastic tissue formation. This was compensated by examining the wounds of other groups of cats, including those in which adhesions had been induced (vide infra), all of several weeks' duration, in which elastic tissue might have had time to form. Secondly, the portions of the pleura removed were probably too small to require replacement of their elastic membrane. As pointed out by Hass (1937), whatever the source or sources of elastic tissue may be, it is probably formed principally in relation to functional demands, and it can hardly be claimed that the removal of one or two square centimetres of pleura constitutes such a stimulus. Bunting (1939) observed that although elastic tissue was developed in the lines of tension of myocardial scars, it did not develop in general repair, for example of the skin, in the absence of tension.

Such evidence as can be gleaned from the cat experiments tends to support the view that elastic tissue in the cat pleura is no more than a special type of collagen, and is derived from collagen. The fine specific-staining

fibrils in the relatively acellular part of the pleural reaction, e.g. in cat L 43 (figs. 88-92) must be interpreted in this way, and similar examples, although less well-developed, can be seen by careful search in localised areas of the pleural scars throughout the whole series of cat experiments. It is not contended that the evidence is other than slight, but such as it is it supports Virchow's view, expressed in 1851, that elastic tissue is formed by the direct transformation of collagen. Nowhere in my experiments was direct cellular activity observed to take part in the formation of elastic tissue. In parenthesis and as a corollary, it is to be noted that elastic tissue which had been severed and isolated frequently became surrounded in the lungs by connective tissue and collagen; it was not treated as foreign material, and did not stimulate leucocyte or macrophage reaction.

In the final healing of the pleura, the serosal cells were apposed to the elastica by the organisation and absorption of the cellular proliferative tissue which separated them in the early stages.

The alveolar reaction.

Although it is unnecessary at this time to recapitulate work which has formed the basis of many articles and theses, unfortunately no discussion of pulmonary cellular reactions can fail to refer to the long-disputed question of the pulmonary alveolar lining.

This controversy has ranged around the two problems of whether an alveolar lining does in fact exist, and secondly, the nature and origin of the alveolar phagocytes. Evidence from embryological studies has demonstrated that a continuous cellular lining exists in the pulmonary alveoli in the early stages of foetal life but becomes discontinuous in the later stages. (Stewart, 1923; Bensley and Bensley, 1935; Clements, 1937; Cooper, 1938; Ham and Baldwin, 1941). The cause of this change has been attributed variously to gasping foetal inspirations in utero, to the failure of the epithelium to develop proportionately to the rapidly expanding capillary network of the alveoli, and to hydropic and other degenerative changes in the epithelium. Experimental and observational work by Cappell (1929) supported the view that the lining consists of small groups of nucleated cubical cells in the alveolar angles and occasional flattened nucleated cells on the remainder of the alveolar wall, which was covered

mainly by non-nucleated squames. This has not been accepted by the anatomists and histologists, (Lang, 1925; Fried, 1934; Policard, 1938; Maximow and Bloom, 1943) who believed that the alveoli consist only of capillaries supported by reticulum and elastic fibres, with scattered histiocytes. In an attempt to settle this question, a round table conference of anatomists and histologists was held at Ann Arbor, Michigan, in 1936, under the chairmanship of C. C. Macklin, to discuss the problem of the "presence or absence of a continuous epithelial lining in the mammalian adult pulmonic alveolar wall." Considering the notable names of those who took part, the conclusions of the conference were singularly disappointing. Agreement was reached that the idea of non-nucleated plates forming the pulmonary lining was erroneous, but that before the conception of a continuous epithelial layer in the human adult alveolus could be accepted, more evidence must be adduced.

To my mind such evidence was provided experimentally in animals by Gazayerli (1936), who, using rabbits vitally stained and injected subsequently intratracheally and intravenously with particulate substances, showed conclusively not only that the lung alveoli of the rabbit possess an epithelial lining of flat nucleated cells, but that these are additional to the alveolar phagocytes

grouped at the angles of the alveoli. In this instance it is permissible to argue from rabbit to man; there is general agreement that mammalian lungs of different species are similar in histological structure.

Pathologists, of course, have long been familiar with the appearance of a continuous alveolar lining in morbid conditions . This has seldom been disputed, and most modern textbooks of pathology accept the inference that the continuous lining exists normally, and is merely rendered more easily visible by disease. Miller (1937) used sections from a case of pneumonia in man in which pulmonary oedema had developed, to demonstrate a continuous alveolar lining in the oedematous alveoli. There are those who disagree with the practice of deducing the structure of healthy tissue from morbid conditions. For example, Barnard and Day (1937) in denying the presence of an alveolar epithelial lining in health, referred to this point, and claimed that it would be equally justifiable to study the normal histology of the kidney from sections of nephritis. Be that as it may, so far as my observations are concerned in the pulmonary reaction to trauma, alveolar lining cells are present, and although normally the arrangement and number of these cells may vary considerably, their acceptance as entities in experimental circumstances is beyond doubt. They are distinct from the alveolar

phagocytes and have great potential proliferative power. Whether the cells are epithelial, endothelial or mesenchymal is unsettled, and so far as this work is concerned, of less importance. Indeed the position has been summarised wisely by Cowdry (1934), who writes "to assert categorically that the lung is altogether epithelial in nature may not be justified, but the evidence is satisfactory that it is at least partly epithelial, for in certain conditions it proliferates and forms tumours which unmistakably consist of epithelium."

The proliferative reaction which was noted in the stab injuries and in the experimental wounds affected the endothelium of the alveolar capillaries and the alveolar lining cells alike. In point of fact, the capillary response was first; initially it consisted of dilatation and congestion, followed by capillary outgrowth. The congested area bordered the injured lung in the stab wounds and adjoined the clot in the experimental wounds, and in both types of injury was separated from the normal lung by oedematous alveoli. Oedema was not a marked feature of the lung wounds, probably because anoxia was limited to the area of slow circulation produced by the local congestion. Drinker (1945) has emphasised that in general with capillaries throughout the body, the pulmonary capillaries are very sensitive to anoxia. The local nature of the lesion

in these experiments is indicative of the maintenance of the general pulmonary circulation, an essential factor in preventing more generalised oedema of the lung. So far as the actual proliferation of capillaries is concerned, this is noteworthy on account of its vigour, and because when the endothelial cells become bipolar and grow out from the alveolar walls, their resemblance to fibroblasts is close. Indeed there is a stage in the sections of this reaction where it is difficult and sometimes impossible to identify these bipolar cells, or to determine their origin with any certainty. The endothelial cells have been regarded as potential sources of fibroblasts in inflammatory conditions, and there is no doubt that they contribute similary in the response of the lung to trauma.

More interest, however, is attached to the alteration in the alveolar lining cells themselves. In the fully developed reaction, the cells have increased in number and in size, and although somewhat polymorphous, tend on the whole to assume cuboidal and cubical shapes like respiratory epithelium. The stab wounds with the cold needles show the reaction in its purest form, but it is more extensive where necrosis has been produced by the hot needles. There, the altered cells tend to enclose the lesion as by a barrier or zone of demarcation, from which at a later stage the organisation of the necrotic tissue is developed. On the

other hand in the experimental wounds in cats, the subpleural alveoli mainly are affected, and this includes not only the superficial alveoli of the lung --- alveoli which are subpleural in the ordinary sense of the term --- but also those which accompany the pleura on its inversion into the lung at the margin of the wounds.

There is a striking resemblance between the hyperplastic alveolar cells and those described by Young (1928), following the intrapleural injection of bile-salts in liquid paraffin, and of aqueous solutions of neutral salts of calcium, strontium, and aluminium. It will be recalled that in these experiments Young showed that the hyperplasia of the cells of the marginal alveoli of the lung could be produced in varying degrees by these agents, and that so far as neutral salts were concerned, increased in intensity proportionately with the valency of the cation. The reactions reached their maximum 48 to 72 hours after injection, returning to normal within a few days. Young noted, moreover, that a small accidental trauma, whether situated superficially or more deeply, and any recent focus of necrosis, also results regularly on or before the third day in active proliferation of the surrounding epithelial and connective tissue cells; an observation amply confirmed in my experiments, where the mere pinching of the pleura with forceps was sufficient to provoke an alteration in the cells of the

underlying lung alveoli. Trauma alone probably accounts for the effect in the stab wounds with the cold needle, and the presence of necrotic tissue is obviously a factor where the lung was burned. In the experimental wounds, however, the stimulus was probably the presence of fibrin which would account for the change affecting the alveoli and portions of alveoli nearest to the clot and nearest the pleura. Fibrin on the pleural surface had a remarkably stimulating effect on the serosal cells, and it would be strange if it did not affect also the superficies of the lung.

This alteration in the alveolar cells is apparently of a transient nature. In my experience it was seen in animals 2-5 days after injury, but it was absent in those 7 days after injury: it is therefore fair to infer that it is of a reversible nature. Young also noted that the change was cyclical. He offered a physico-chemical explanation of the facts, and in my experiments the evidence suggests that the causative stimuli are trauma, necrosis and fibrin, but similar and sometimes more extensive cellular metamorphoses have been noted by other observers in connection with many bacterial and other agents. For example, in infections due to micro-organisms, although alveolar cellular proliferation is not seen in the acute stage, it is common in chronic cases and in delayed resolution (Bell, 1943). Proliferative characteristics have been described

also repeatedly in viral infections, e.g. in pertussis, measles, varicosis and psittacosis in human subjects (Güthert, 1938), and in toxoplasmosis in animals (Olafson and Monlux, 1942). Active foci of pulmonary tuberculosis commonly exhibit perifocal septal and alveolar cell proliferation in the surrounding lung, and in silicosis a similar change is frequently observed in alveoli surrounding the silicotic scars. Although true lining cell proliferation is rare in chronic venous congestion, it is frequently seen in alveoli around infarcts. Probably the most impressive examples of the condition are met with in veterinary medicine, particularly in the South African sheep disease known as Jagziekte, and the progressive pneumonia of sheep which occurs in Montana. (Cowdry and Marsh, 1927). These, although distinct infections of unknown etiology, both have as characteristic lesions proliferation of pulmonary epithelium to a degree which simulates adenomatosis. There is some evidence that in general the alveoli of the superficies of the lung are affected more readily by these changes than those deeper in the lungs, and although where the irritant is pleural it is reasonable to expect that the subpleural alveoli should be involved, this explanation is not always acceptable. The suggestion has been made that the subpleural alveoli are less stable than other parts of the lung: for

example both experimental and spontaneous mouse carcinoma is known to begin in the subpleural alveoli (Wells, Slye and Holmes, 1941; McDonald and Woodhouse, 1942; Grady and Stewart, 1940).

Alveolar cellular hyperplasia when of microbial or viral origin, appears to me to have a more permanent character than the condition described by Young and met with in my experiments. It is difficult to imagine the infective changes to be reversible although doubtless milder degrees of them exist in patients who recover. Nevertheless, the metamorphoses provoked by infection are to my mind more akin to the visible and sometimes prominent alveolar lining of cubical cells met with in relation to pulmonary scars of various sorts. Pathologists are familiar with this appearance and it is referred to by writers on this subject generally. The change is seen in and around apical scars whether or not their tuberculous nature is certain, around the connective tissue masses of silicosis and in scars of undetermined origin, where pavements of small cubical and cuboidal cells line the air spaces. Accordingly it is noteworthy that in the experimental work described in this thesis, and in particular in the experimental wounds of lung, the marginal alveoli usually have a visible lining of cuboidal cells. Sometimes this is continuous around an entire alveolus, or it

may affect only the wall of the alveolus directly contiguous with the scar; in this position the alveolar lining rests directly on the connective tissue.

The cause of this epithelialisation, as it has been termed, is uncertain, although there is little doubt that its nature is a proliferation of the alveolar lining cells themselves. According to Geever, Neuberger and Davis (1943) the reaction can develop in a few hours and in the early stages the cells are closely apposed, small, dark cuboidal cells like those lining the alveoli of foetal lung. The condition is in many ways a recapitulation of intra-uterine pulmonary development, where the terminal air-passages are provided with an epithelial lining which begins to disappear in the human foetus about the end of the 5th month. Barnard and Day (1937) suggest that the presence of lined spaces in diseased lungs, and in lungs after experiments, may be due to absence of movement of the alveoli in contact with the scars, and in support of this they advance the fact that it is sometimes only the alveolar wall adjacent to the scar, presumably the less mobile wall, which is affected. It is true that swelling of the alveolar cells has been reported in massive pulmonary collapse and in atelectasis generally, but this is different from the formation of a true alveolar lining. Moreover, although the wall of the alveolus which rests on

the connective tissue may be less mobile than the other walls, it is still very actively moving tissue, a fact which will be elaborated more fully in discussing the aeration of the lung. In my opinion the relative vascularity of the alveolar walls may play a more important role. For instance, it is noticeable that the alveolar walls which abut on connective tissue and which have a cellular lining, lack the normal relationship to the alveolar capillaries: they are in fact directly in contact with the scar tissue, and no obvious blood supply can be seen. Microscopic fields showing these appearances can be seen from time to time in sections of pathological conditions in human lungs, e.g. in fibrosis, and even where alveoli adjoin oedematous septa. Moreover, it is known that in the development of the human foetus, the diminution of the alveolar lining cells which occurs at the end of the 5th month corresponds with a marked increase in the development of the pulmonary capillary blood vessels. Accordingly it is not improbable that a relative and temporary local ischaemia may be an important factor in the reversion of the alveolar lining cells to a foetal type.

In summary it may be said that in this experimental work two types of alteration were noted in the character of the alveolar cellular lining. First in point of time is the cyclical change in the cells of the subpleural

alveoli which occurs within a week of the trauma, and secondly, there is the development of a foetal type of lining cell in the alveoli which form within or partly within the connective tissue of the older wounds in the stage of repair. It has not been difficult to distinguish in the latter type of alveolar lining between bronchogenic and autochthonous cells. The cells of alveolar origin are of fairly uniform appearance, whereas the bronchogenic cells are sometimes tall columnar, occasionally ciliated, and frequently metaplastic in type, sometimes even becoming squamous. As a rule the topography of the section is a good guide in tracing the communication between air spaces and pre-existing alveoli, or adjacent bronchial branches.

Aeration of the scar.

The transformation of scar tissue into aerating lung has been seen to be the result of two processes, the growth of bronchial branches and the reduction of the margins of the scar by the action of the adjacent alveoli.

In the mechanism of repair as seen in general pathology the development of branches from pre-existing channels is familiar enough, particularly in the vascular system. The regeneration of a highly specialised organ like the liver, too, is accompanied by the proliferation of bile-ducts, and there is some evidence that bile-duct epithelium may undergo metaplasia into parenchymatous liver cells.

Observation shows, however, that whereas bile-ducts grow initially as solid columns which ultimately become canalised, the bronchial buds are canalised from the beginning. It is my belief that this constant patency of the bronchial buds is highly significant: it must be directly attributable to the fact that these channels contain air, and are in communication with the atmospheric pressure transmitted through the respiratory passages. Thus they must contain air under the pressure normally met with in the smaller bronchial branches, and they must be subjected to the variations in pressure which are so important in normal respiration, and are dilating factors in the bronchi in appropriate disease conditions. In other words, the bronchial buds must be influenced by respiratory movements.

The effect which this will have on the ultimate ventilation of the scar tissue must be considerable. First it is to be emphasised that bronchial budding begins very early in the course of the reaction to injury, and develops only in the direction of the wound; thus the pressure of a non-expanding scar appears to be the stimulus. As they extend from the parent trunk, the bronchial branches not only transmit air under pressure, in common with the bronchial system, they undergo rhythmic dilatation and elongation with each respiratory movement. The penetrating power of these combined forces not only will aid the growth

of the buds, it will also have an expanding or splitting effect upon the tissue in advance of them. This, then, is my interpretation of the clefts or irregular spaces in the hyaline scars. It will be recalled that these spaces sometimes have a cellular lining and sometimes are unlined clefts. Geever, Neuberger and Davis (1943) commenting on air spaces around pulmonary scars of undetermined origin write "The presence of a cellular lining in and around the pulmonary scars is fairly common, and is similar to that observed in tuberculosis and silicosis. Within the larger scars one cannot be certain whether the lined spaces are really distorted alveoli, terminal bronchioles or tissue clefts. The latter possibility is quite an interesting one. We have never been able to find a discussion of this in the literature." One has but to visualise the scar tissue undergoing penetration by bronchial buds rhythmically widening and lengthening, transmitting repeated variations in air pressure, to realise that no better tissue-splitting combination can be found.

It has been shown that the nature of the cells lining the bronchial buds is in the main the low type of respiratory epithelium described as cubical. There is no reason to doubt that as the bronchial channels become more complex in structure, this epithelium develops into

tall columnar respiratory cells, but although this change may justifiably be inferred, it is difficult to say at what stage it occurs. That is, from examination of a wound 'x' days old, it is not possible to deduce what the particular bronchus was like 'y' days earlier. Reference has been made to cat L 13 where the bronchial buds in the centre of the scar were lined by squamous cells, presenting a remarkable appearance. The cause of this local metaplasia is not clear. It is known that deficiency of vitamin A will produce this change in the guinea pig throughout the whole respiratory tract from nares to bronchi (Wolbach and Howe, 1928), and Condon (1942) found that in experimental tracheal wounds in rats, deficiency of vitamin A led to epithelialisation by squamous rather than respiratory cells. Keratin formation, however, was never seen in these experiments, and as Condon points out, keratin is an attribute of a cell of definite physicochemical structure. He argued, therefore, that the squamous cells which grew over the injured area were not truly metaplastic, but merely more rapidly growing cells which reverted to their normal form after epithelialisation was complete. He noted that the success of this reversion was directly related to the quality of the underlying submucosa: where the latter was packed with inflammatory products, no reversion of the epithelium was observed. Epithelial

structure appears thus to be dependent on the underlying stroma; a scaffolding is necessary for its growth, and the epithelium itself plays only a passive role. In the particular instance of cat L 13, it is difficult to avoid the opinion that environment was an important factor: the squamous area is confined to the hyaline tissue, and probably is a reaction to the presence of this tissue and to the local ischaemia. Similarly the development of squamous cells in bronchiectatic cavities is also a local rather than a general manifestation. Occasionally in the experimental cats, small areas of stratified squamous epithelium lined the spaces in the lung occupied by sutures or ligatures of silk.

The combined factors of pulmonary motility and respiratory air pressure exert their influence also in the complementary process of reduction of scar tissue through alveolar action. Most of the scars show indenting of their margins where the alveoli of the aerated lung adjoin the hyaline tissue, giving the connective tissue a scalloped margin. In addition, new alveoli deriving their air supply from pre-existing normal alveoli can be seen to have formed in the margins of the connective tissue. These methods of aerating the scar, as it were by attrition, are consistent only with the conception of intermittent pressure being maintained against the connective tissue. When

the pulmonary wounds were examined under positive anaesthesia prior to the sacrifice of the animal, the margins of the wound were seen to balloon out and collapse rhythmically with respiration, and there is no doubt that this activity is the main factor in reducing the margins of the scar by indentation and alveolar formation.

Such then is the deduction to be drawn from the observations described above, that in the healing of wounds of lung, and particularly in the stage of re-ventilation or aeration of scar tissue, the motility of the lung is of primary importance, and it must be recognised that this motility is not merely the movement of the lung as a whole in the thoracic cavity, but also the inherent movements of the bronchi and their branches. In this way variations in intrabronchial air-pressure are transmitted to the new bronchial branches and so to the scar itself. To complete this argument it would be desirable to show that in wounds in which the mobility of the lung is impaired, either generally or locally around the wound area, the healing of wounds had been imperfect or delayed. To this end a series of five cats was subjected to operation and an attempt made to form fixed wounds of lung. Open thoracotomy was performed in the usual way, except that it was at the 10th interspace and not at the 7th as before, thus exposing the base of the left lower lobe. A wedge of lung tissue was removed and

stitched as in the previous experiments, and then two deep stitches were passed into the lung, one on each side of the scar, and parallel to one another. These sutures were passed through the thoracic wall muscles, the pleural and muscular wounds closed, and then the edges of the sutures tied firmly to draw the lung wound against the wound in the parietal pleura: finally the skin was closed by interrupted stitches. The animals made good recoveries and were killed off at intervals from 16 to 62 days. In three of them there was a fairly firm adhesion between the pleural layers, but in the last two animals killed, there were signs that the motility of the lung as a whole had overcome the adhesion, and that there had been some loosening of the sutures. Indeed, if the animals had been allowed to live longer, the adhesions would probably have been reduced to fibrous bands and probably undone completely. Like many biological control experiments, the results of this group of animals fulfilled the expectations only partially. Aeration of the scar tissue had occurred to some extent, but it is fair to say that it appeared to have failed to attain the degree of completeness seen in comparable wounds where the movement of the lung had not been restricted. Moreover, the aeration of the fixed wounds, as they may be termed, was mainly by alveolar action and not by bronchial branching, and this

despite the fact that in these wounds the connective tissue was cellular and not of the dense hyaline type. It is obvious that to produce satisfactorily immobility of a lung wound would require the fixation of an entire lobe and possibly interruption of its nerve supply, introducing many complicating factors.

Second only in importance to the movement of the lung and bronchi in accelerating the reformation and regeneration of lung is its vascularity. It is a truism that wounds heal in proportion to their blood supply (Learmonth, 1944) and the lung ranks high as a vascular organ: with the exception perhaps of the adrenal medulla, no tissue has a richer blood content than the pulmonary capillary system (Policard, 1938). Accordingly, it would be proper to expect the lung to heal as well as it does. Emphasis has already been laid on the remarkable and speedy proliferation of the lung capillaries, in particular those of the subpleural network, much of which was rendered visible by the reactive process, but the most interesting examples of the effect of an adequate blood supply are to be seen in those wounds where the necessary vascularisation was obtained by what may be termed a collateral route. In cat L 6, the lesion found at post mortem consisted of a small protrusion from the centre of the scar of tissue like a polyp

(fig. 80). The sutures which had been used to control haemorrhage had in fact so constricted the tissue that the polypoid portion was cut off from its blood supply. Microscopically, this tissue was seen to be necrotic, and to consist of homogeneous rather structureless hyaline material from which all appearance of alveolar formation had vanished. Some dilated vessels had appeared at the neck of the polyp but they had not succeeded (in 60 days) in penetrating the necrotic tissue. Nevertheless, this tissue had a surface blood supply derived from the subpleural and superficial alveolar capillaries of the contiguous healthy lung. Over the surface of the necrotic material, this vascular supply had maintained alive the cellular connective tissue in which alveolar spaces had formed, and these in turn derived their air supply from adjacent normal lung (figs. 61 and 62). Thus the devitalised tissue had been invested superficially by vascular and aerated lung from the contiguous healthy lung, and provides a remarkable example of the combined action of the aeration and vascularisation processes.

Having observed the process of regeneration and reformation of mammalian lung in cats following injury, theoretically there is no reason to doubt that, given an adequate blood supply and freedom of motility, a complete structural and functional result may be attained in time

in all similar wounds. Cat L 11 is an example of an almost perfect result in 115 days. On the other hand, so far as the mouse experiments show, it is less certain that results of such a high order can be achieved after a burn of lung. Reference has already been made to the experimental difficulties of that work and there is no doubt that healing after a burn anywhere is less satisfactory than after a surgical wound. In the experimental lung wounds, the factors which govern the result are the extent and density of the scar tissue (which is probably related to the amount of haemorrhage), and the presence or absence of foreign bodies such as sutures and ligatures. No one who has examined the sections of these experimental wounds, however, can fail to be impressed by the methods whereby air channels form around these obstacles.

That lung tissue can undergo this type of regeneration is, however, not generally recognised, and examples of the response of human lung to injury are difficult to obtain. It was my hope to make some observations on battle casualties, but most thoracic wounds were infected by the time they reached thoracic centres, and empyema was a constant finding in those who died. Blast injuries, or pulmonary concussion, have been studied extensively both by experiment and in autopsy

material, but they form a different group not comparable to my work. In infections in human lung, however, it is possible to trace the processes which are to be seen so clearly in experimental pulmonary repair. Examples are to be met with in tuberculosis and in silicotic scars, although the ischaemia of these lesions undoubtedly retards their organisation. Occasionally, however, organisation can be seen around partially fibrosed primary tuberculous foci in children, and also in the scarring of chronic bronchiectatic lesions. The influence of lung motility is unquestionably a factor there also, for just as in the therapy of tuberculosis, fibrosis is achieved by immobilisation, so in the rehabilitation of patients after chest wounds, re-expansion is sought by exercises. There is nothing novel in this allusion or this practice. My aim has been to show the mechanism whereby the results are achieved, and to record the fact, so far as I am aware hitherto undescribed, that lung tissue can reform in pulmonary wound scars in animals.

SUMMARY.

1. Mouse and rat lungs punctured by cold sterile needles show local atelectasis with a transient and reversible swelling of the alveolar lining cells. No evidence of the injury can be seen, by naked-eye or microscopically, a week later.

2. Burns of lung produced by heated needles are organised from a boundary or demarcation zone of lung tissue and converted to functioning lung by the ingrowth of bronchial branches from pre-existing bronchi.

3. Excision of wedges of lung tissue in cats is followed by local scarring and the ultimate reformation of lung tissue in the scar. This process occurs through bronchial proliferation together with alveolar formation in the margins of the scar, processes which depend primarily on the active motility of the lung.

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ON THE REACTION OF THE MAMMALIAN LUNG TO TRAUMA.

A thesis presented to Glasgow University
for the degree of Doctor of Medicine
by

George L. Montgomery,
T.D., M.B.,Ch.B., Ph.D.,(St. And.), F.R.F.P.S.G.

Vol. II - - - - Figures.

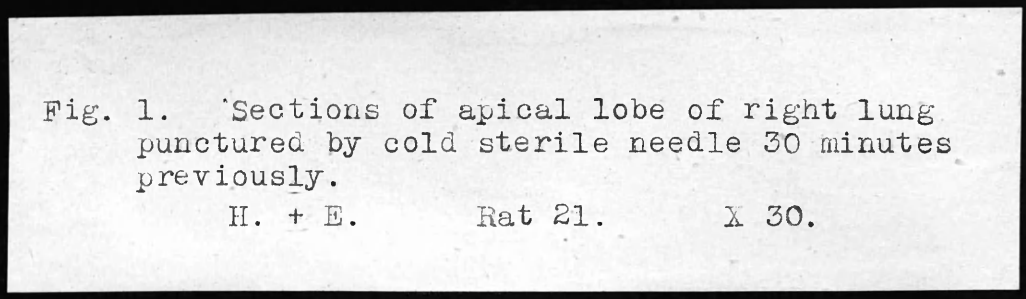


Fig. 1. Sections of apical lobe of right lung punctured by cold sterile needle 30 minutes previously.
H. + E. Rat 21. X 30.

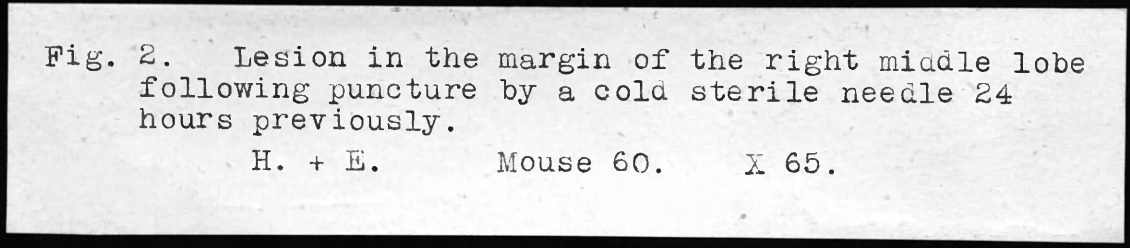


Fig. 2. Lesion in the margin of the right middle lobe following puncture by a cold sterile needle 24 hours previously.
H. + E. Mouse 60. X 65.

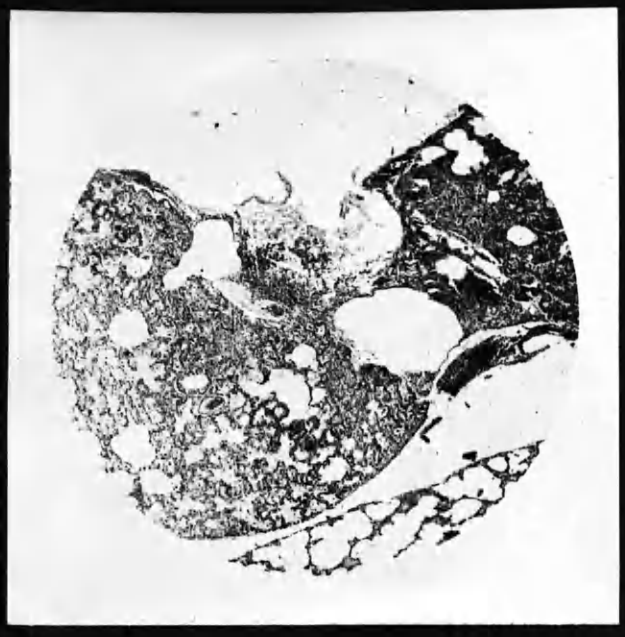
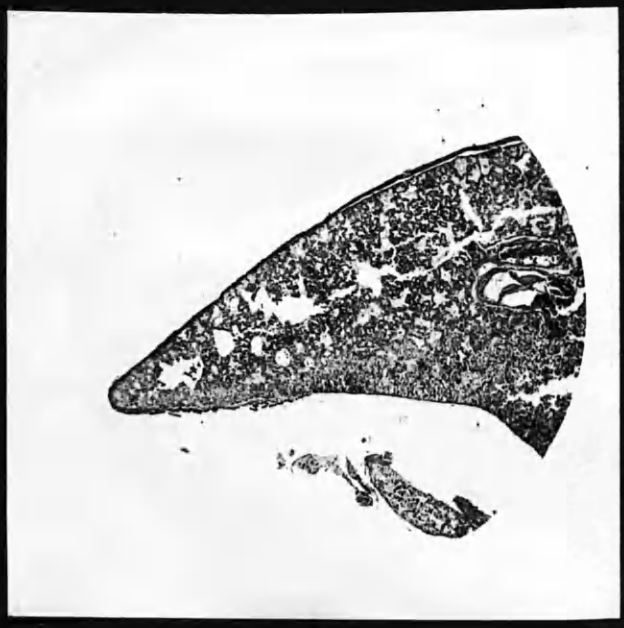


Fig. 5. Mouse lung 44 hours after puncture by hot needle. The area of necrosis includes a portion of the bronchial wall.
H. + E. - Mouse 49. X 50.

Fig. 6. Mouse lung 42 hours after puncture by hot needle. There is a crescentic area of necrosis enclosed by a zone of congestion.
H. + E. Mouse 80. X 35.



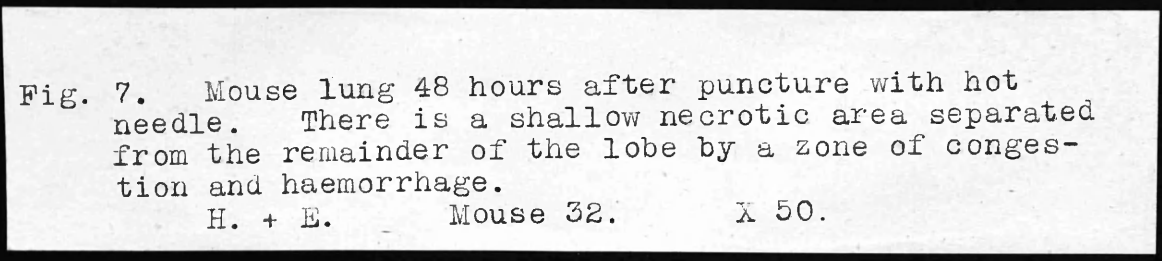


Fig. 7. Mouse lung 48 hours after puncture with hot needle. There is a shallow necrotic area separated from the remainder of the lobe by a zone of congestion and haemorrhage.
H. + E. Mouse 32. X 50.

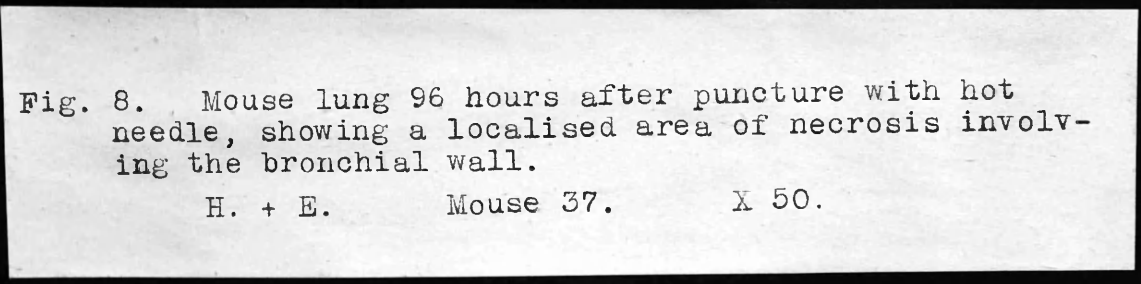


Fig. 8. Mouse lung 96 hours after puncture with hot needle, showing a localised area of necrosis involving the bronchial wall.
H. + E. Mouse 37. X 50.

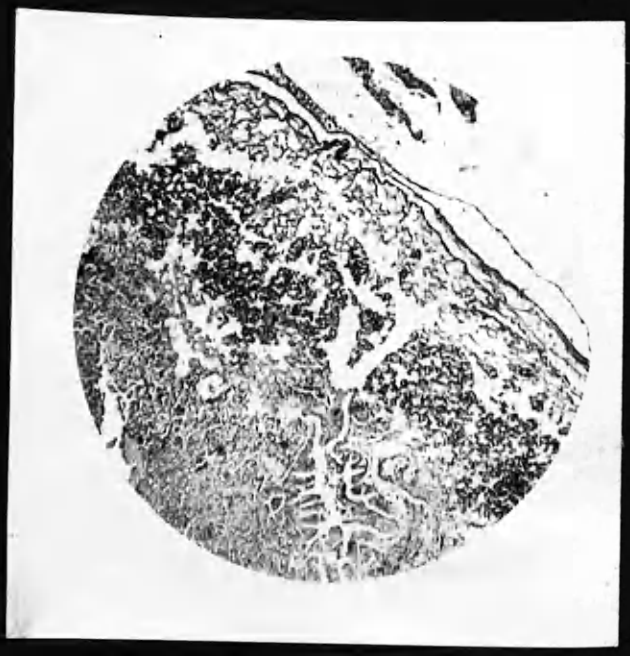


Fig. 9. Mouse lung 72 hours after puncture with hot needle to show boundary zone.

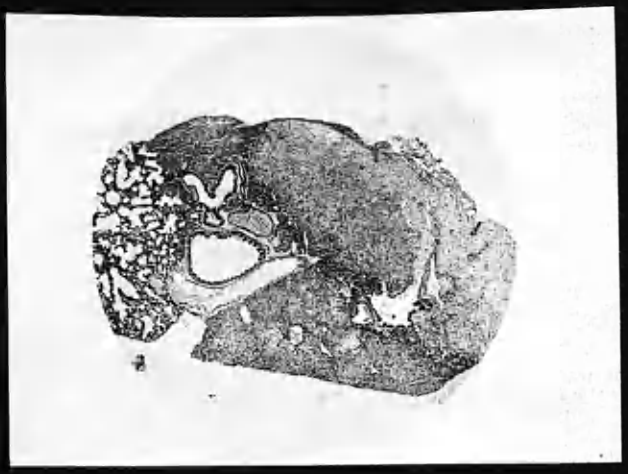
H. + E. Mouse 36. X 25.

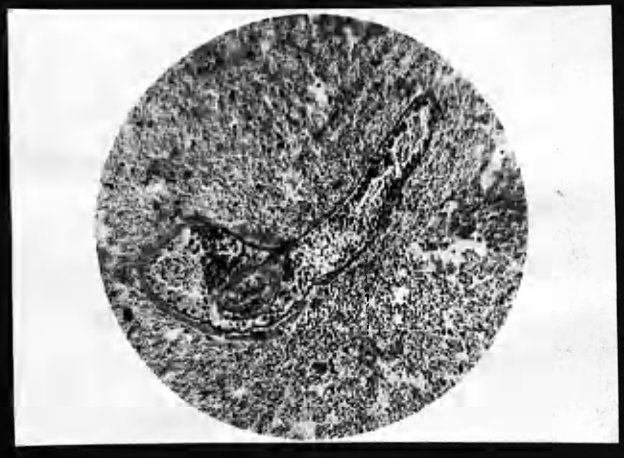
Fig. 10. Mouse lung 44 hours after puncture with hot needle to show circulation through a partially necrosed vessel.

H. + E. Mouse 49. X 200.

Fig. 11. Mouse lung 72 hours after puncture with hot needle. The photograph shows the margin of the lesion with a partially necrotic blood vessel where pavementing of leucocytes has occurred. The alteration of the character of the bronchial mucosa on the side next the lesion can be seen.

H. + E. Mouse 36. X 200.





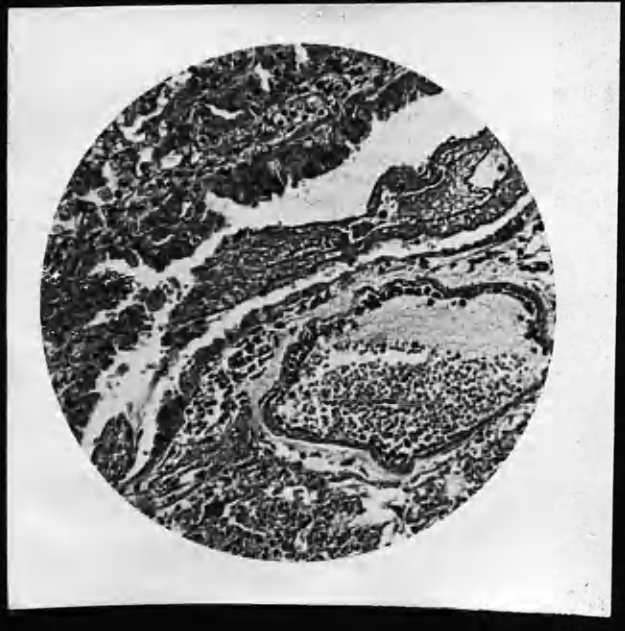


Fig. 13. Serosal cells and superficial alveoli of mouse lung 47 hours after puncture with hot needle. The section is from tissue adjoining the puncture area and shows the swelling of the serosal cells. Pale-staining swollen alveolar lining cells can be seen in the atelectatic lung tissue.

H. + E. Mouse 81. X 500.

Fig. 12. Mouse lung 22 hours after puncture with hot needle to show the alveolar and bronchial oedema adjacent to the congested zone.

H. + E. Mouse 80. X 35.

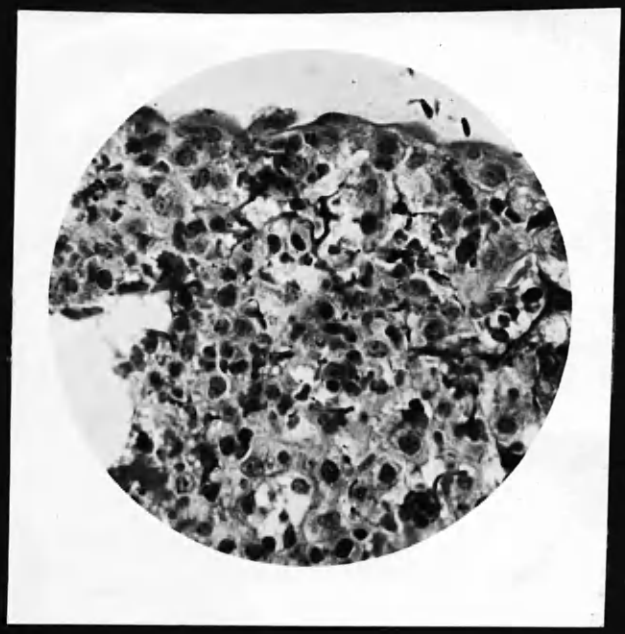


Fig. 14. Mouse lung 44 hours after puncture with heated needle, to show a later stage of the pleural proliferative change.

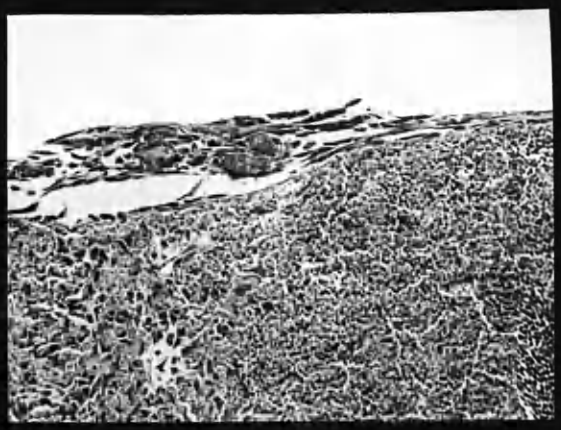
H. + E. Mouse 49. X 60.

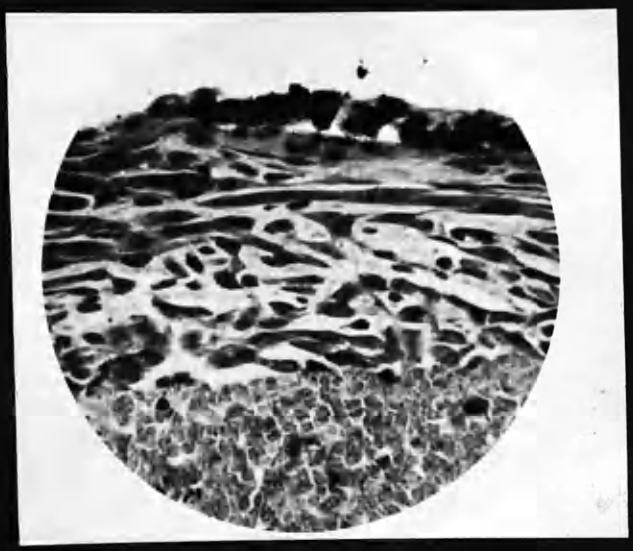
Fig. 15. Mouse lung 96 hours after puncture with heated needle to show the proliferation of pleural serosal cells in relation to carbon particles near the puncture.

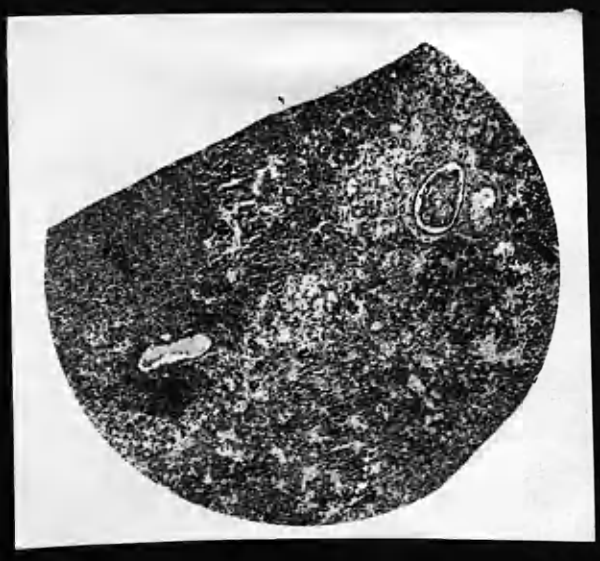
H. + E. Mouse 37. X 500.

Fig. 16. Mouse lung 72 hours after puncture with heated needle to show the beginning of the barrier of pale-staining alveolar cells.

H. + E. Mouse 36. X 50.







This is the same as fig 16 at same magnification.

Fig. 17. From the same section as fig. 15, magnified 120 times.

H. + E. Mouse 36. X 120.

Fig. 18. Another field of fig. 15, magnified 200 times.

H. + E. Mouse 37. X 200.

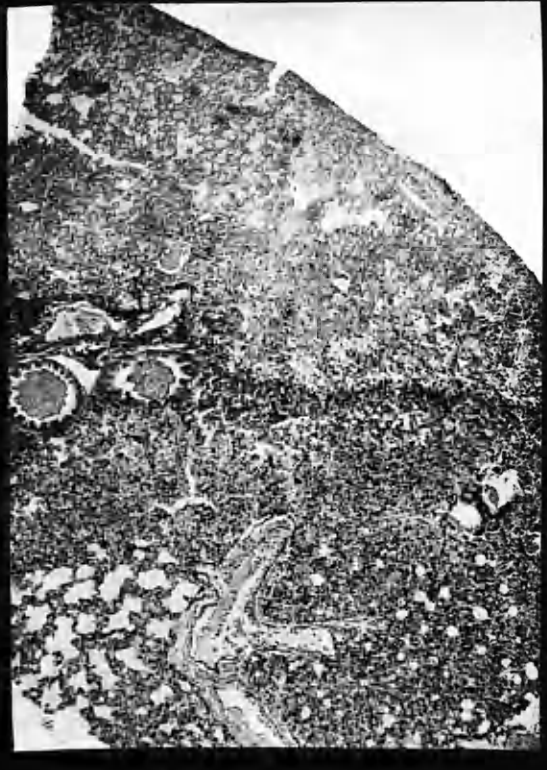


Fig. 19. Mouse lung punctured by cold sterile needle 72 hours previously, to show boundary zone appearance.
H. + E. Mouse 61. X 65.

Fig. 20. Higher magnification of boundary zone.
H. + E. Mouse 61. X 150.

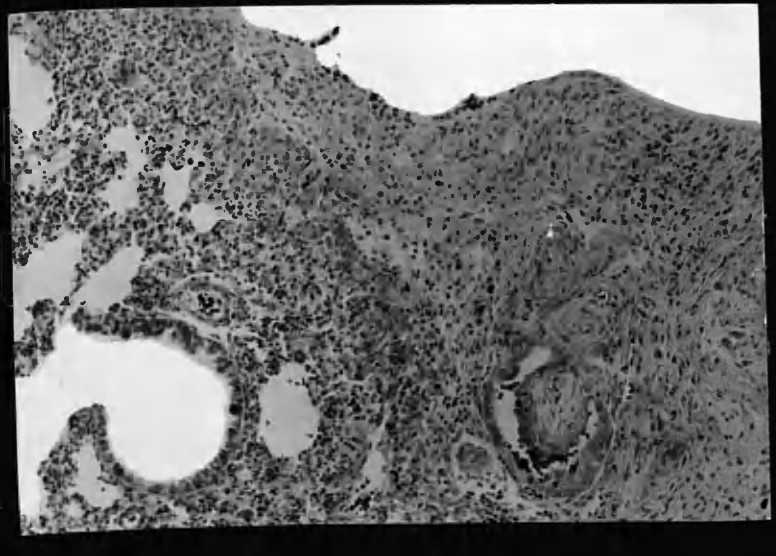


Fig. 21. Mouse lung punctured by cold sterile needle 24 hours previously to show swollen pale-staining alveolar lining cells in atelectatic lung from the puncture area.

H. + E. Mouse 60. X 200.

Fig. 22. Mouse lung punctured with cold sterile needle 70 hours previously showing pale-staining, swollen and proliferated alveolar lining cells at the puncture area where the lung tissue has herniated.

H. + E. Mouse 104. X 300.

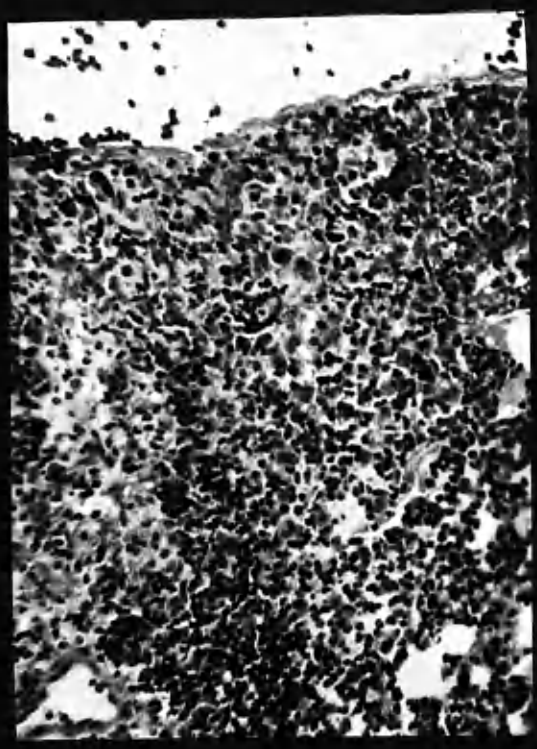
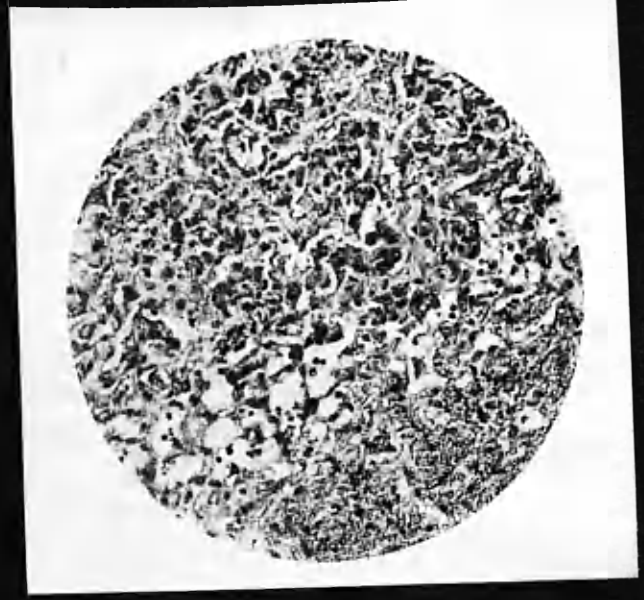
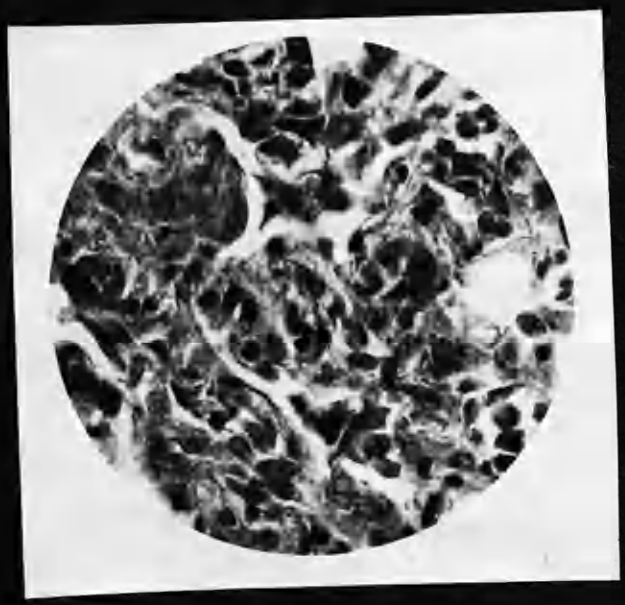


Fig. 23. Mouse lung 5 days after puncture with hot needle to show the blood capillaries, endothelial and alveolar cells of the reactive zone.
H. + E. Mouse 40. X 110.

Fig. 24. Mouse lung 4 days after lung puncture with hot needle to show unlined air channels in the reactive zone.
H. + E. Mouse 37. X 500.

Fig. 25. Mouse lung 6 days after puncture with hot needle to show further stage in the development of air channels.
H. + E. Mouse 51. X 60.





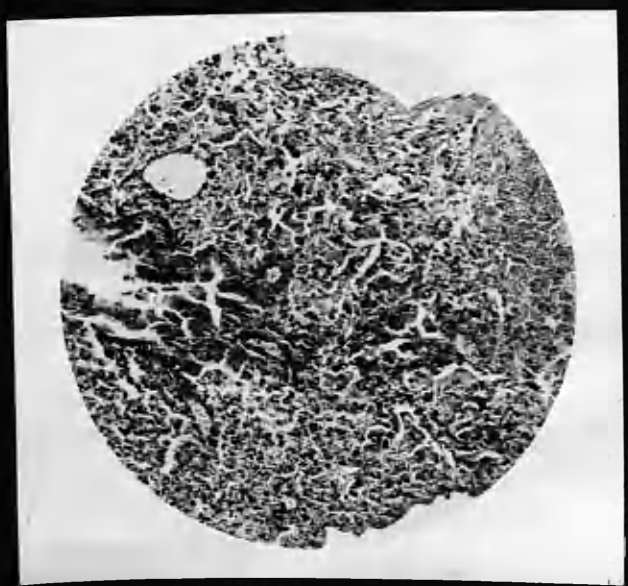
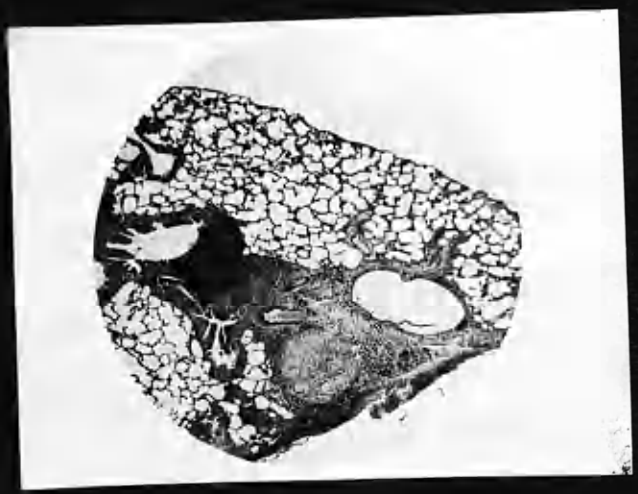


Fig. 26. Mouse lung 7 days after puncture with hot needle to show later stages in the development of bronchial buds.
H. + E. Rat 14. X 65.

Fig. 27. Mouse lung 14 days after puncture with hot needle to show wedge-shaped lesion.
H. + E. Mouse 47. X 25.

Fig. 28. Higher magnification of fig. 27 to show bronchial branches in the scar tissue.
H. + E. Mouse 47 (14 days). X 35.



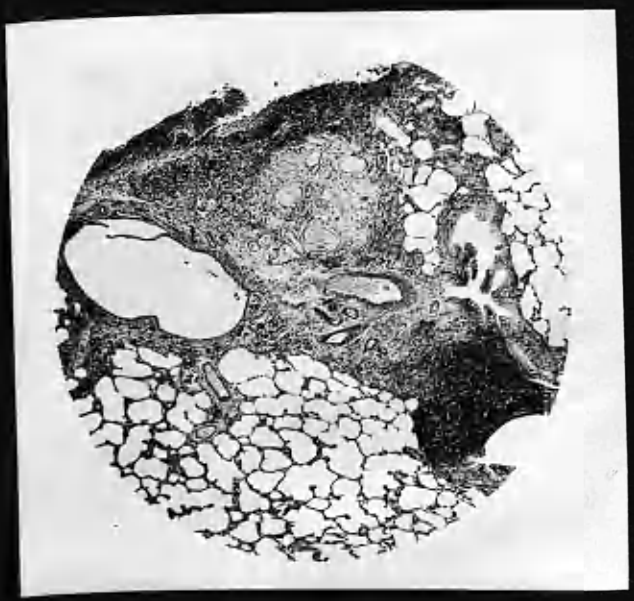
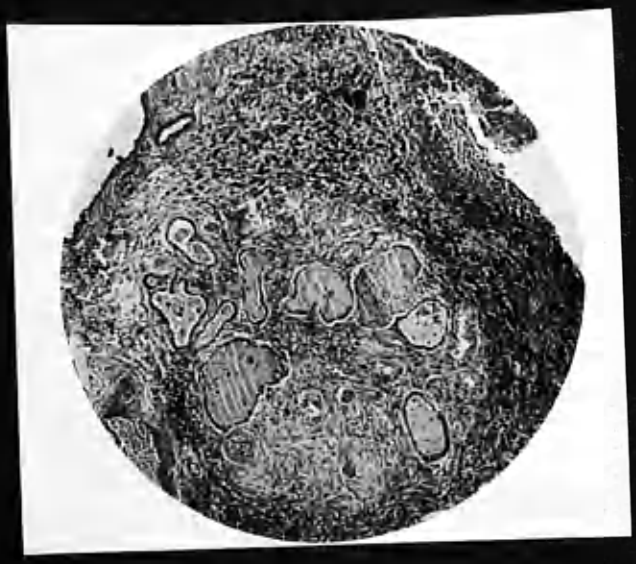
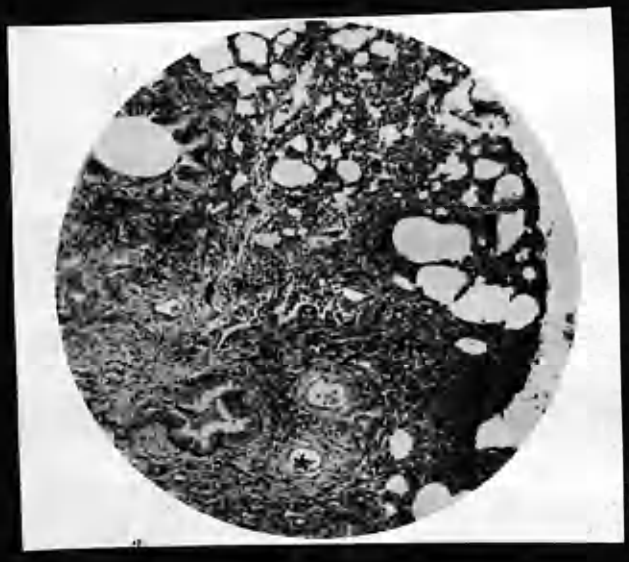


Fig. 29. Bronchial branches containing mucoid material.
H. + E. Mouse 47 (14 days). X 60.

Fig. 30. Mouse lung 33 days after puncture with hot needle to show a further stage in the organisation and aeration of the scar.
H. + E. Mouse 92. X 65.

Fig. 31. Photograph of middle lobe from same section as fig. 30, to show that the organisation is less developed.
H. + E. Mouse 92. X 25.





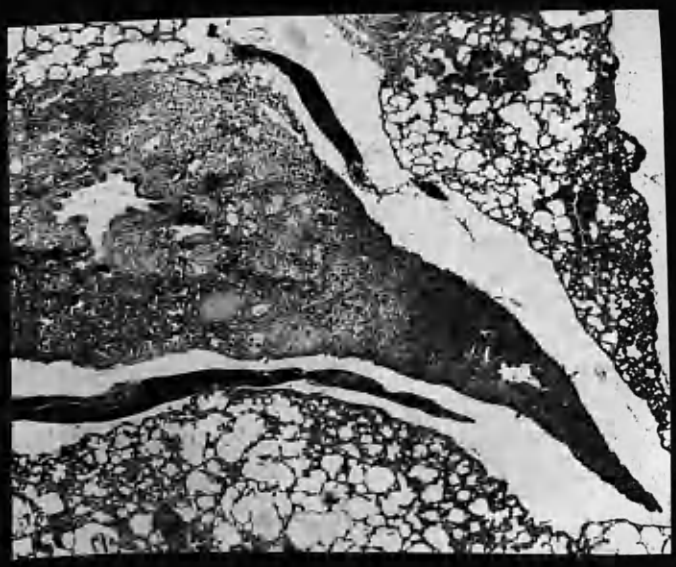


Fig. 32. Cat L 10 (23 days).
Photograph of pleural wound.

Fig. 33. Cat L 4 (28 days).
Photograph of pleural wound in lower lobe.

Fig. 34. Cat L 7 (76 days).
Photograph of pleural wound.

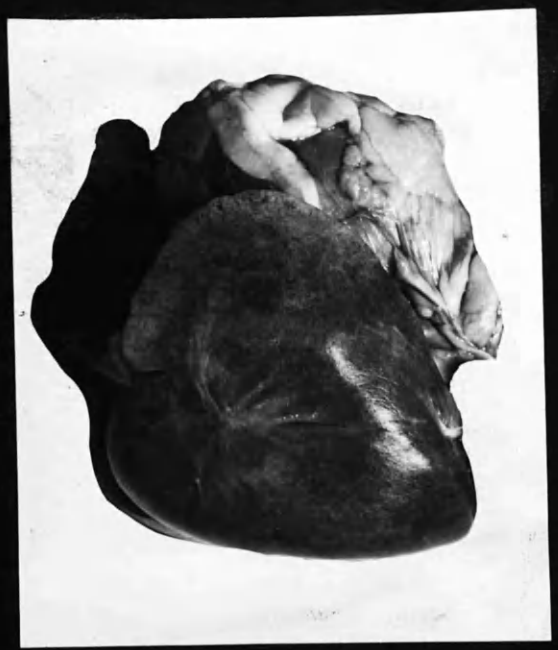
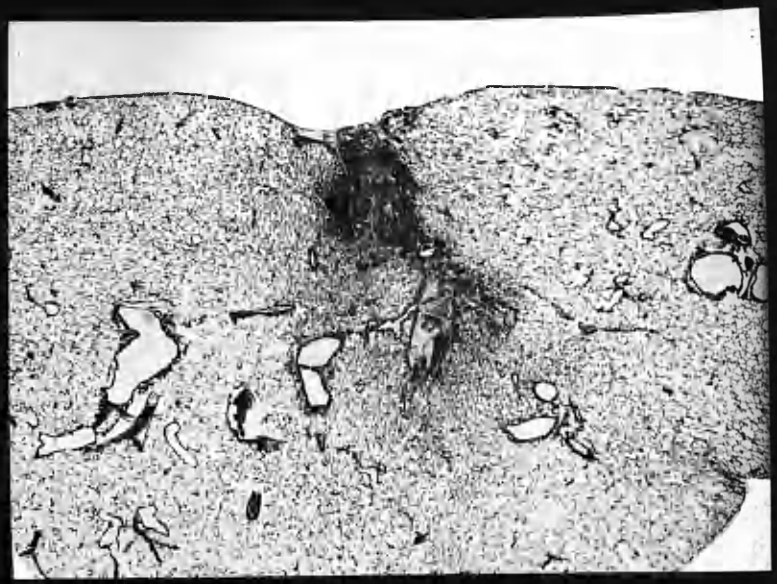


Fig. 35. Cat L 25 (36 hours).
Transverse section of lesion to show the local haemorrhage and congestion.
H. + E. X 5.

Fig. 36. Cat L 26 (45 hours).
Transverse section of wound.
H. + E. X 4.



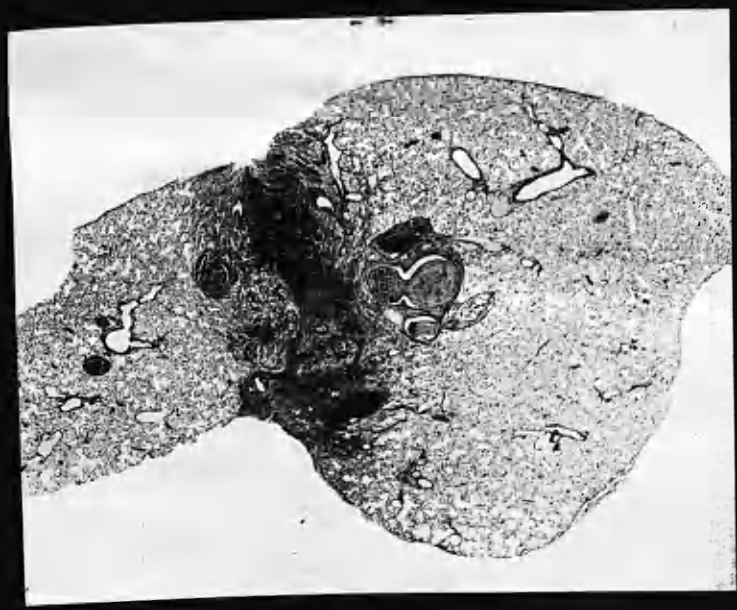


Fig. 37. Cat L 5 (48 hours).
Transverse section of wound.
H. + E. X 4.5

Fig. 38. Cat L 5 (48 hours)
Superficial area of wound showing blood clot and local reaction. A suture has been cut transversely twice.
H. + E. X 25.

Fig. 39. Cat L 5 (48 hours).
Blood clot deeper in the wound showing fibrin strands bordering the alveoli.
Celestin blue, fuchsin-ponceau, aniline blue.
X 35.

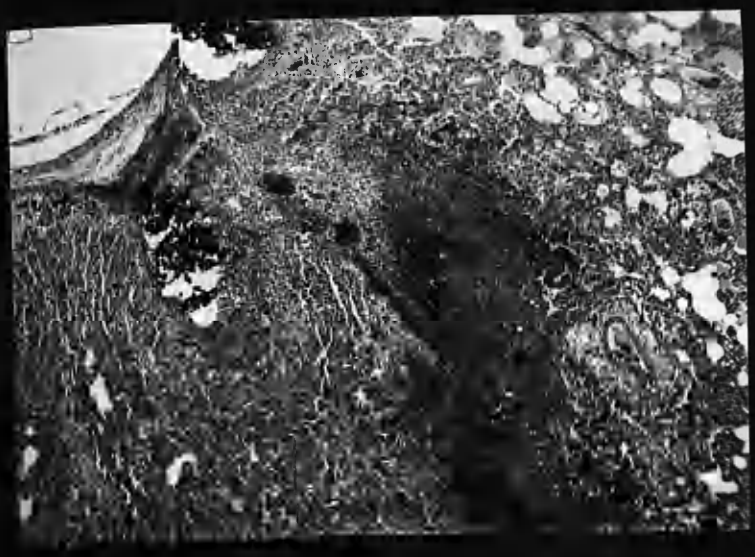
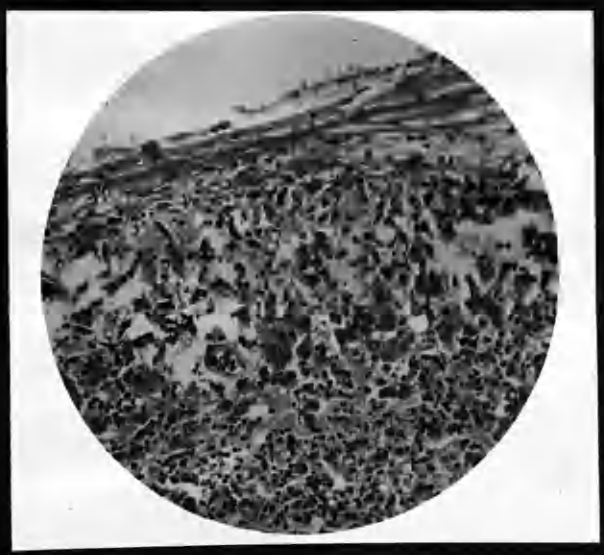


Fig. 40. Cat L 5 (48 hours).
Another view of the depth of the wound showing the local type of reaction.
H. + E. X 55.

Fig. 41. Cat L 5 (48 hours).
Reaction of the subpleural alveoli showing the proliferation of the alveolar cells.
H. + E. X 125.

Fig. 42. Cat L 5 (48 hours).
Early stage of bronchial budding. The small air channels are lined by cubical cells.
H. + E. X 75.



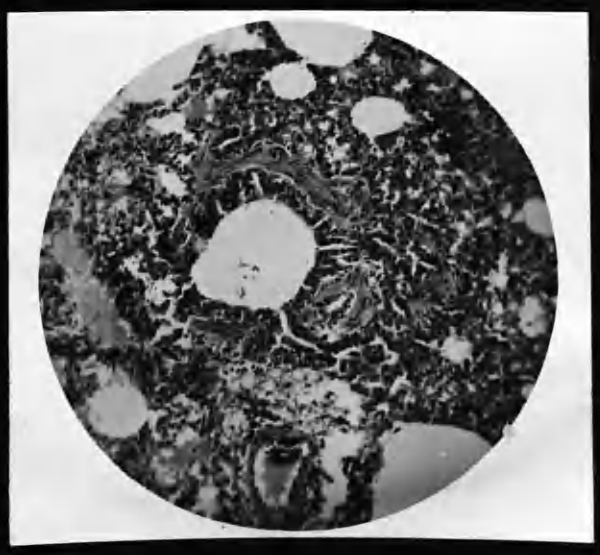


Fig. 43. Cat L 34 (72 hours).
Pleura and subpleural alveoli from the wound margin, showing oedema of the subserous alveoli.
H. + E. X 60.

Fig. 44. Cat L 34 (72 hours).
To show the development of an air channel from a bronchial branch at the margin of the scar.
H. + E. X 120.

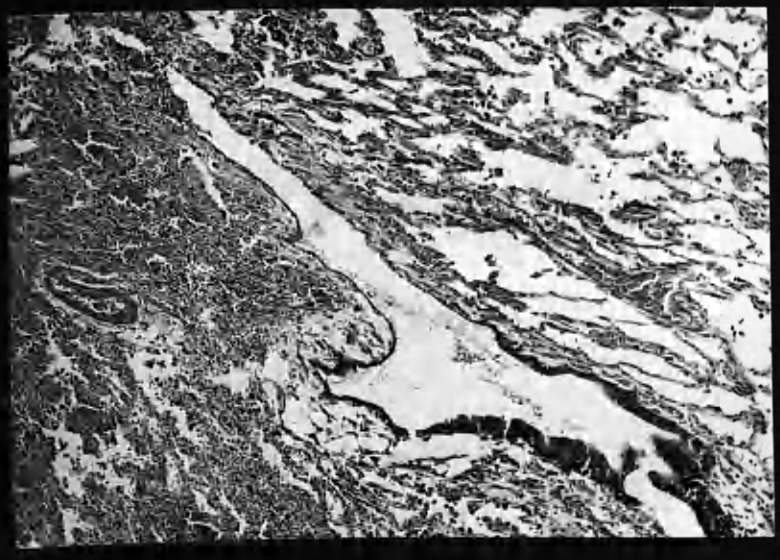
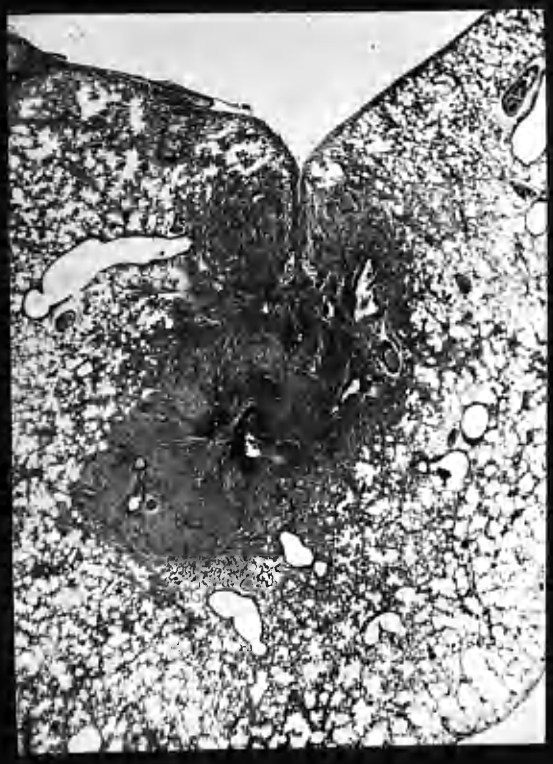


Fig. 45. Cat L 1 (96 hours).
Low power view of wound.
H. + E. X 6.

Fig. 46. Cat L 1 (96 hours).
The superficies of the wound with the pleural reaction.
H. + E. X 55.



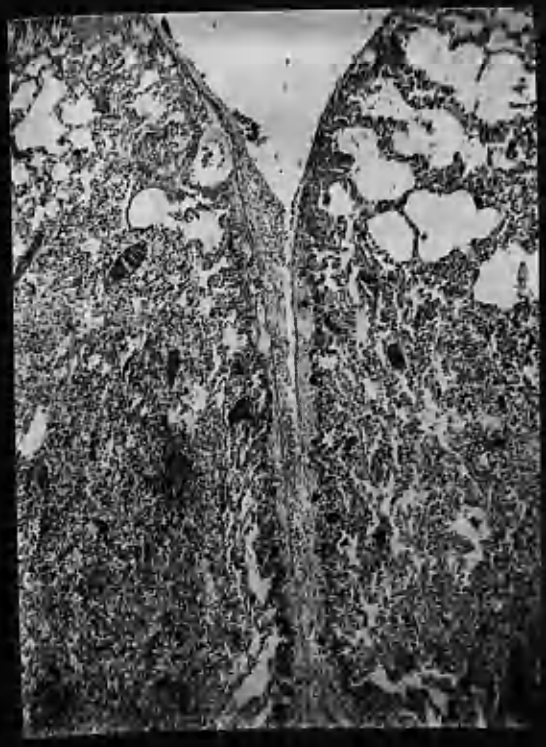


Fig. 47. Cat L 1 (96 hours).
Serosal cell reaction to fibrin in the indentation of the wound.
H. + E. X 100.

Fig. 48. Cat L 1 (96 hours).
Serosal cell reaction around surface fibrin.
H. + E. X 100.

Fig. 49. Cat L 1 (96 hours).
Alteration in the character of the bronchial respiratory epithelium following constriction of the branch near a ligature.
H. + E. X 100.

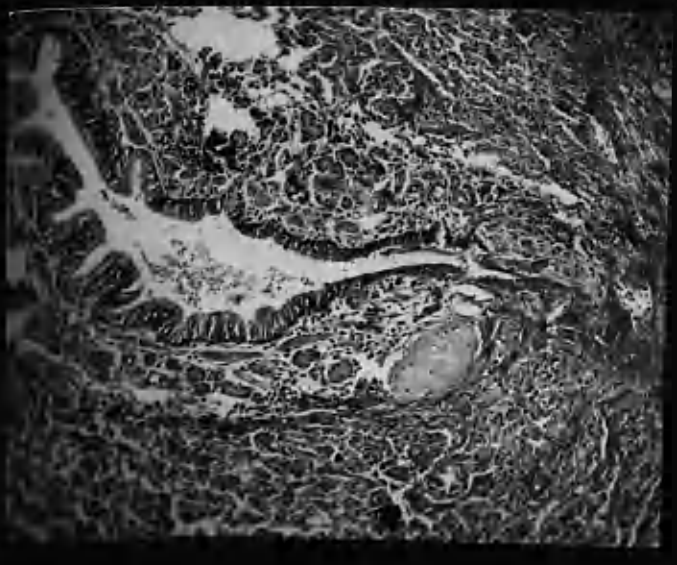


Fig. 50. Cat L 1 (96 hours).
Deepest part of wound showing the dilatation of the lung capillaries bordering the scar. X 100.
Haematoxylin, orange G, fuchsin-ponceau, aniline blue.

Fig. 51. Cat L 33 (5 days).
Pleura and subserous tissue from margin of wound to show the vascular capillary proliferation.
H. + E. X 300.

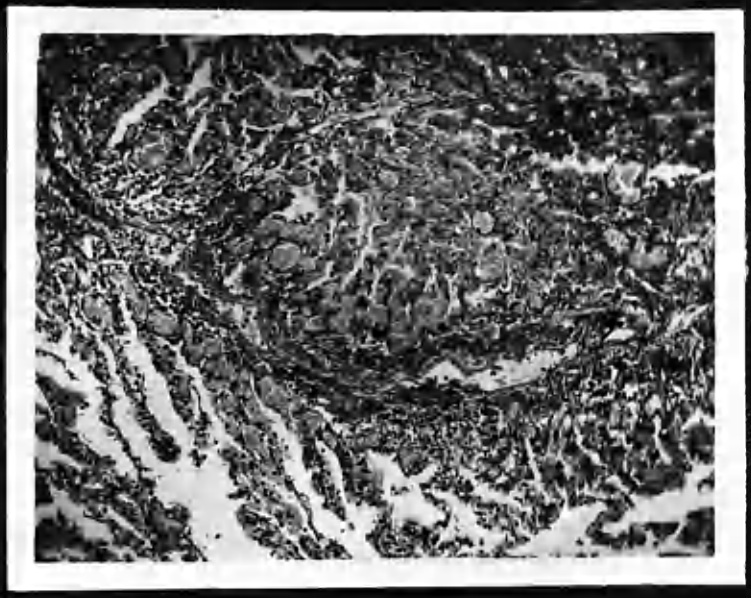
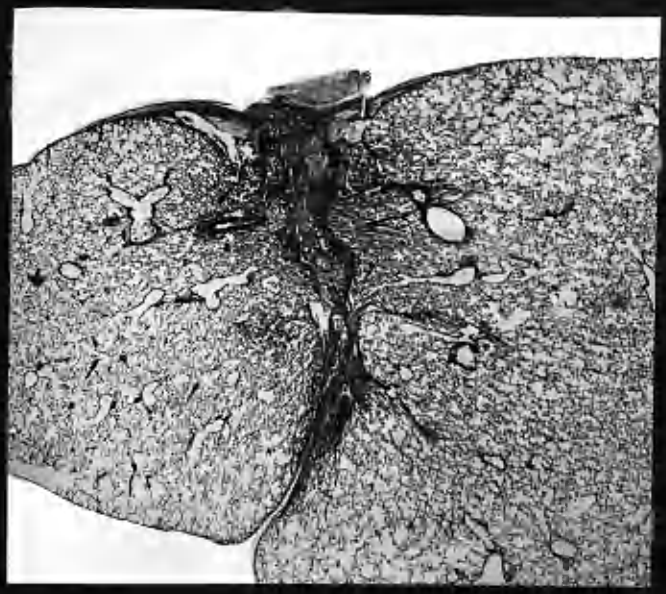


Fig. 52. Cat L 17 (8 days).
Low power photograph to show the general structure of the wound.
Orcein and safranin. X 5.

Fig. 53. Cat L 17 (8 days).
Scar tissue showing the appearance of the irregular spaces lined by respiratory epithelium of flattened and cubical type.
H. + E. X 60.



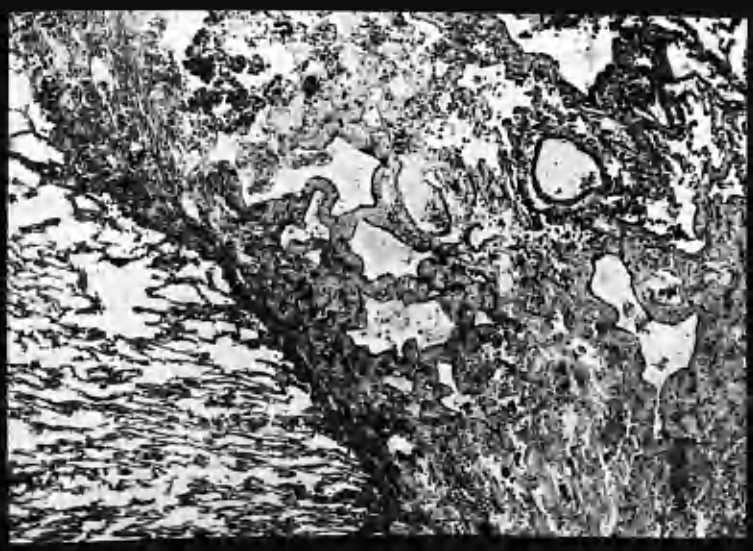
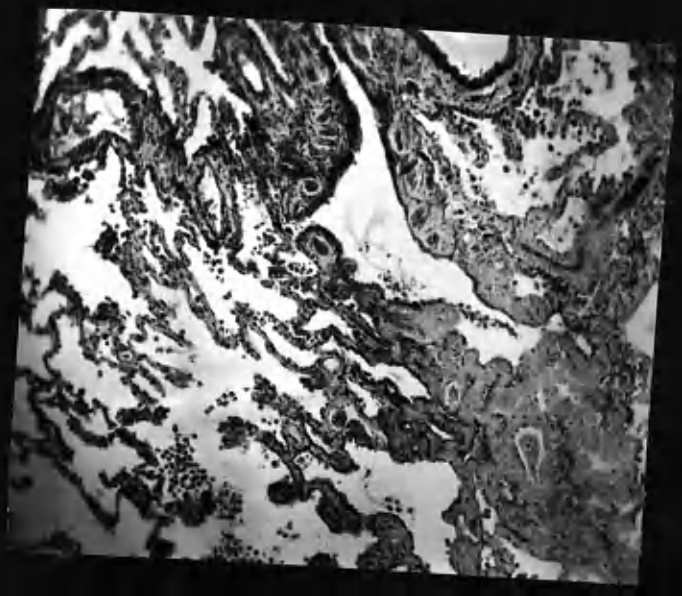
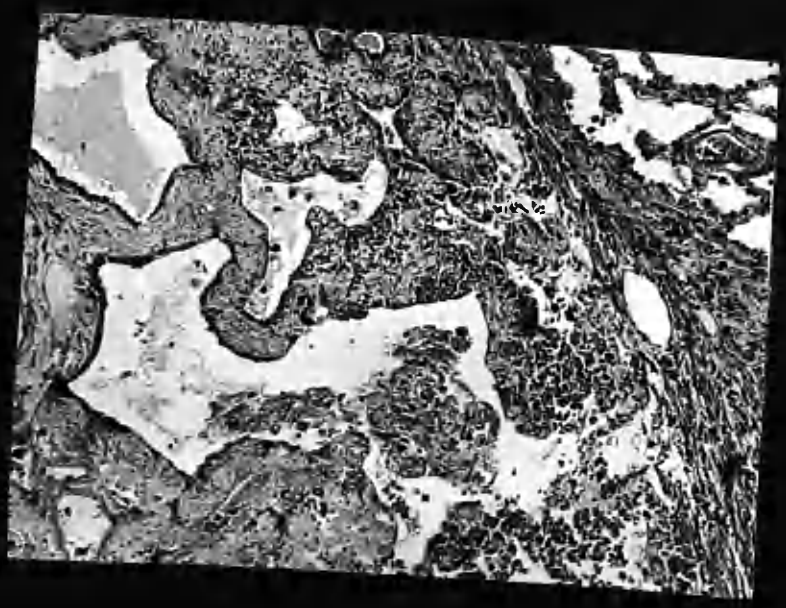


Fig. 54. Cat L 17 (8 days).
Portion of fig. 51 at higher magnification to show the lining of the irregular spaces and that some of them contain coagulated fluid with blood cells.
H. + E. X 120.

Fig. 55. Cat L 17 (8 days).
Margin of scar to show the development of air spaces from bronchial branches. Other air spaces and alveoli have formed in relation to the aerated lung contiguous with the scar.
H. + E. X 100.





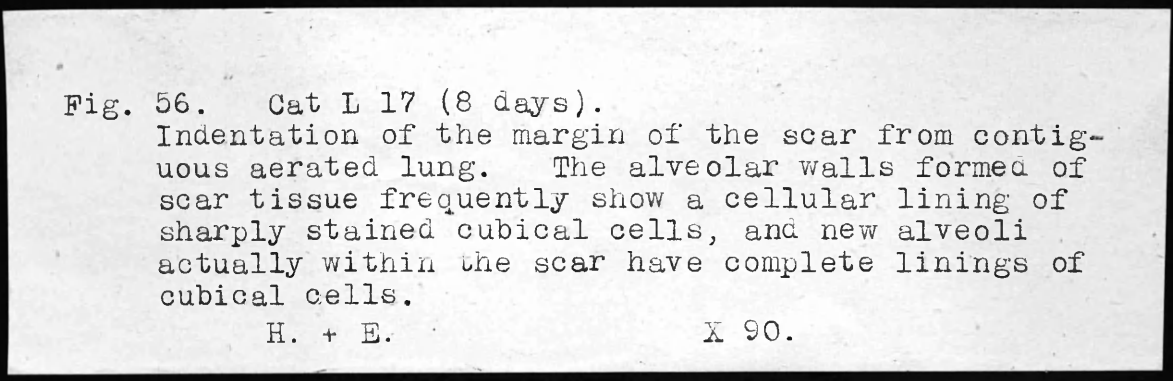


Fig. 56. Cat L 17 (8 days).
Indentation of the margin of the scar from contiguous aerated lung. The alveolar walls formed of scar tissue frequently show a cellular lining of sharply stained cubical cells, and new alveoli actually within the scar have complete linings of cubical cells.
H. + E. X 90.

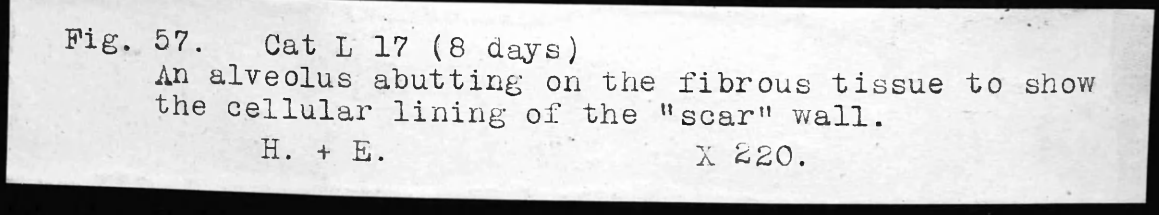
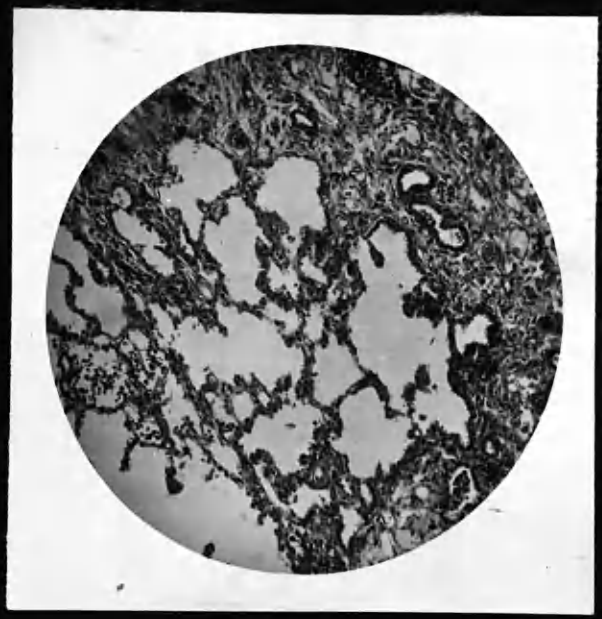


Fig. 57. Cat L 17 (8 days)
An alveolus abutting on the fibrous tissue to show the cellular lining of the "scar" wall.
H. + E. X 220.



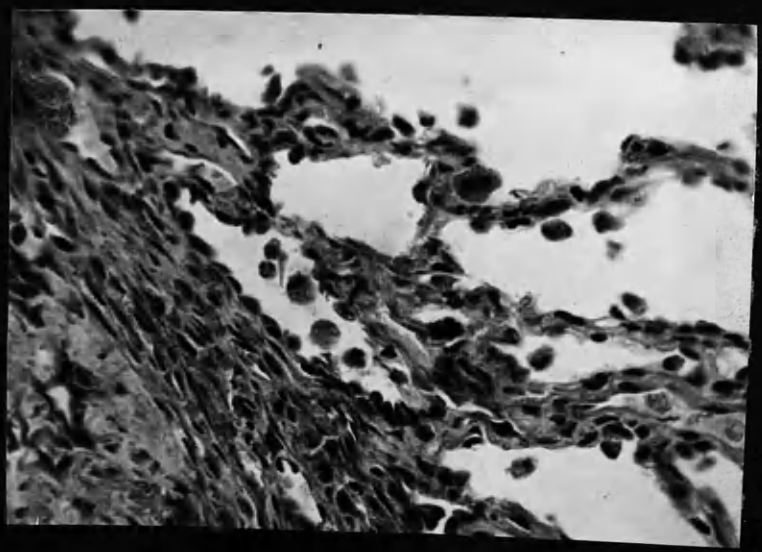


Fig. 58. Cat L 2 (10 days).
Low power view of wound (block 1).
Orcein and safranin. X 4.5.

Fig. 59. Cat L 2, (10 days).
Superficies of wound.
Orcein and carmalum. X 35.

Fig. 60. Cat L 2 (10 days).
Low power view of wound (block 2).
H. + E. X

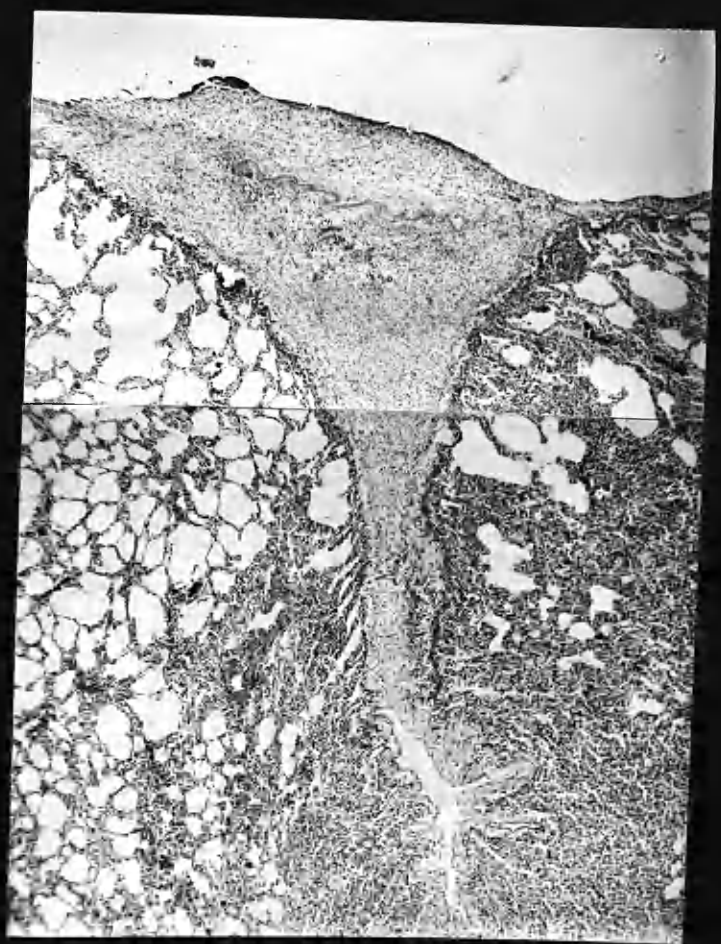
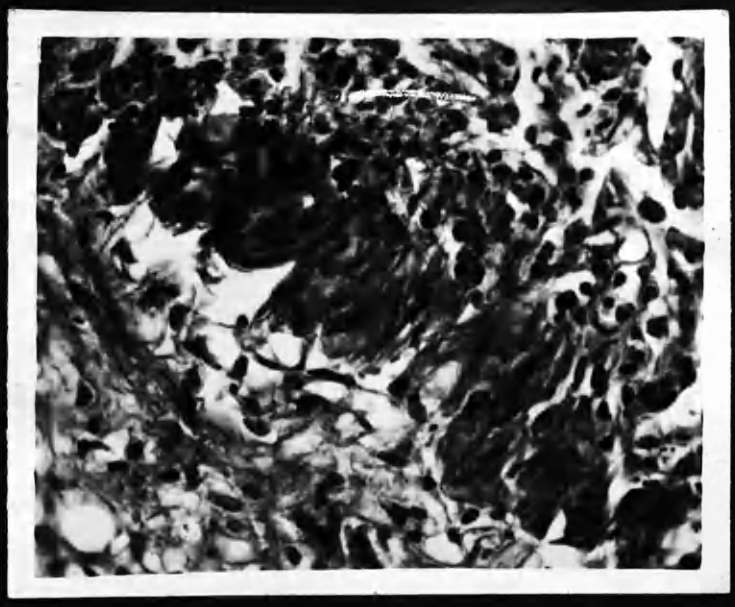


Fig. 61. Cat L 2 (10 days).
Margin of surface indentation to show thickened pleural collagen.
Picro-Mallory method. X 400.
(Photographed through orange filter).

Fig. 62. Cat L 2 (10 days).
Similar field to fig. 59 to show elastic tissue masked by collagen.
Orcein and safranin. X 400.
(Photographed through blue filter).



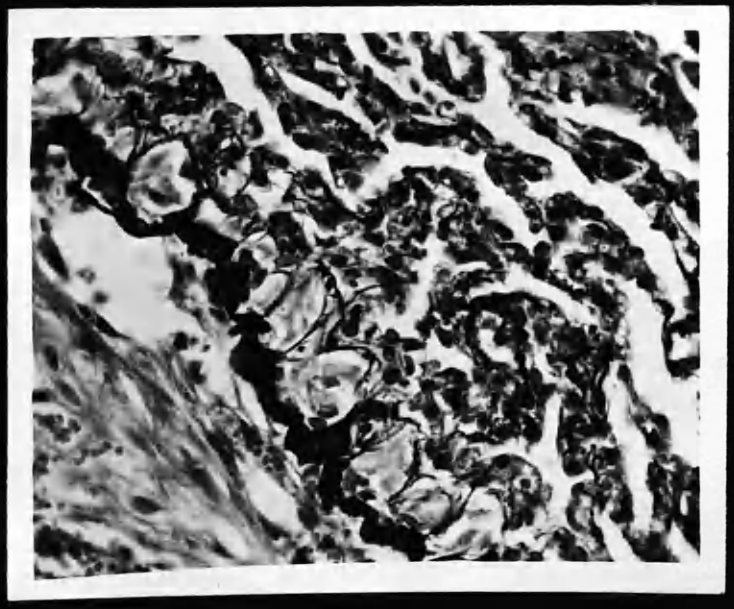
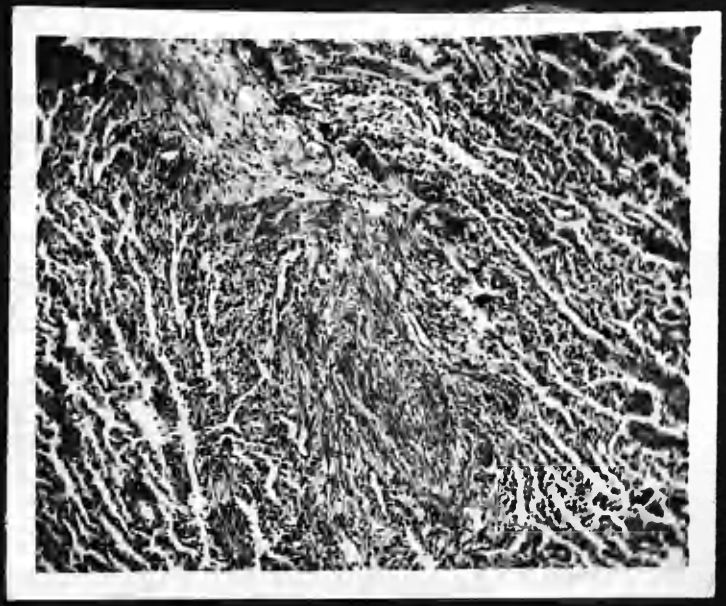


Fig. 63. Cat L 2 (10 days).
Deepest part of wound to show narrow elongated air channels.
Modified picro-Mallory method. X 100.

Fig. 64. Cat L 2 (10 days).
Bronchial buds at base of wound.
Modified picro-Mallory method. X 100.



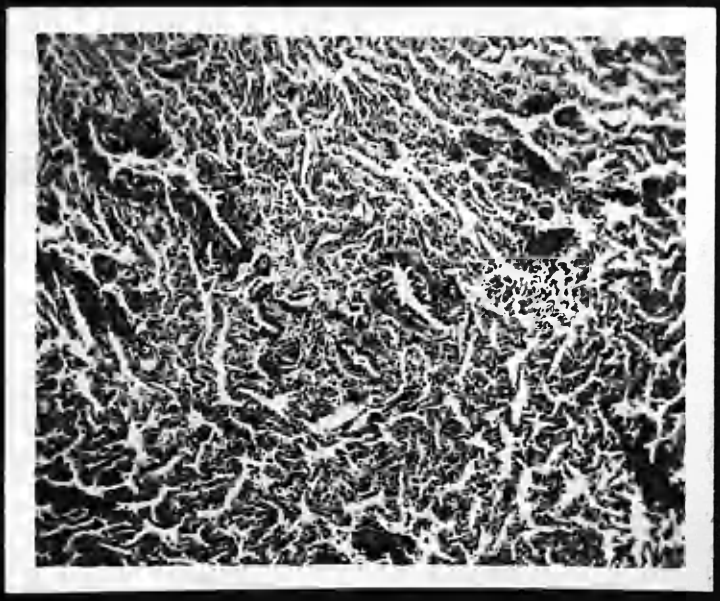


Fig. 65, Cat L 2 (10 days).
Subserous and pleural reactions at periphery of wound.
H. + E. X 100.

Fig. 66. Cat L 2 (10 days).
Similar to fig. 63.
H. + E. X 170.

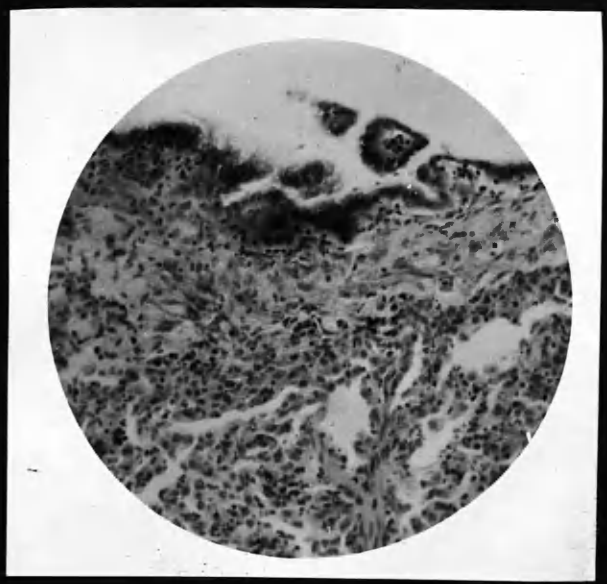
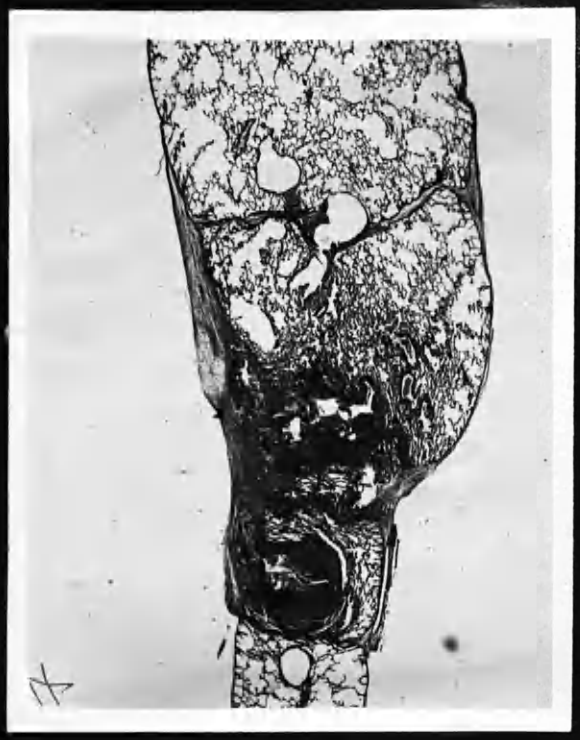


Fig. 67. Cat L 3 (14 days).
Transverse section of wound to show the distortion produced by sutures and the development of air channels around them.
H. + E. X 5.

Fig. 68. Cat L 3 (14 days).
Silk suture cut transversely to show the polymorph infiltration and condensation of lung tissue around the fibres. Air channels of alveolar and bronchial origin can be seen around the infiltration.
H. + E. X 50.



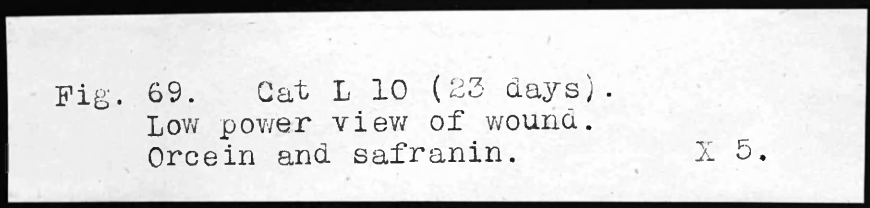
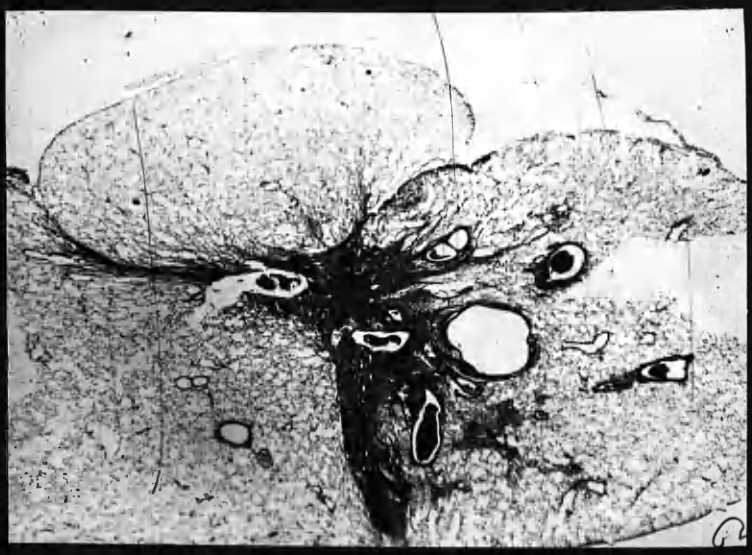
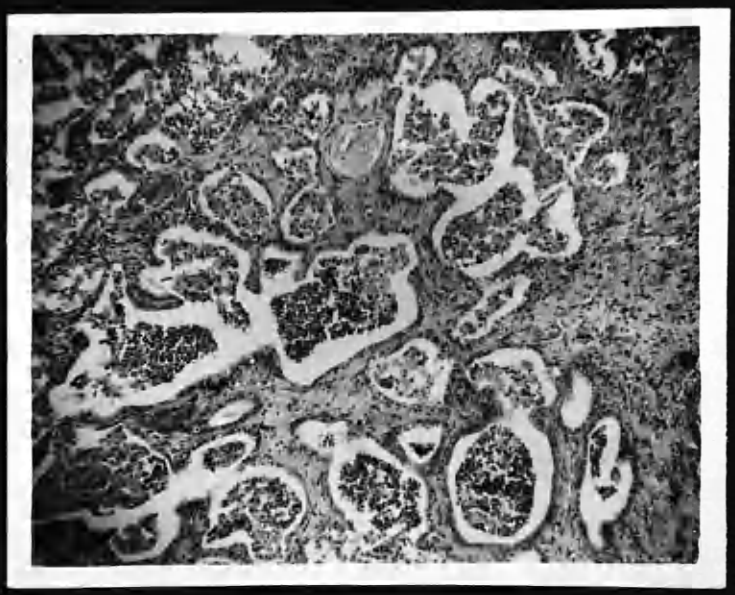


Fig. 69. Cat L 10 (23 days).
Low power view of wound.
Orcein and safranin. X 5.

Fig. 70. Cat L 10 (23 days),
To show bronchial buds in the scar. They are lined by a flattened type of epithelium and contain pus cells due to a terminal respiratory infection.
H. + E. X 90.





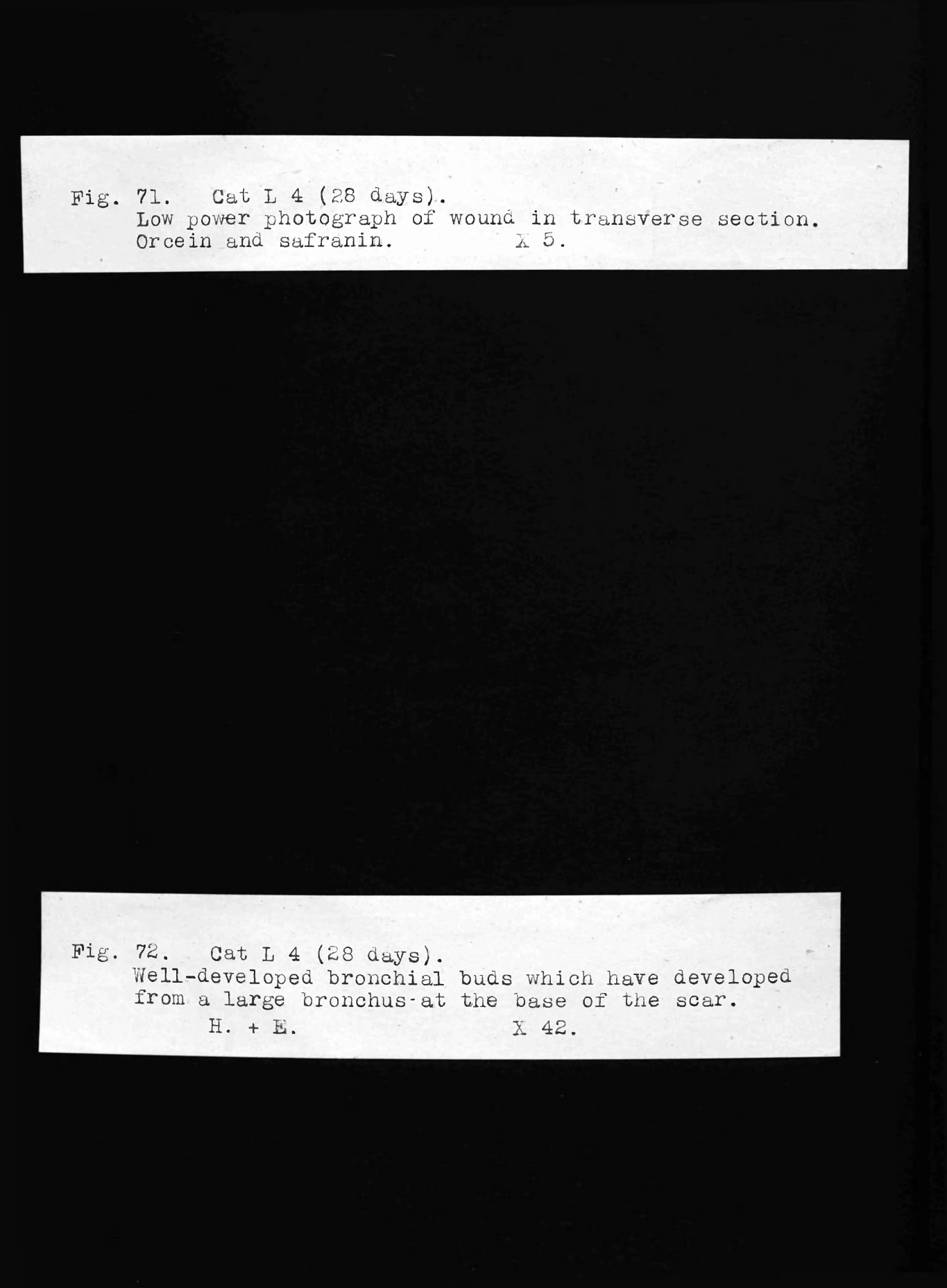
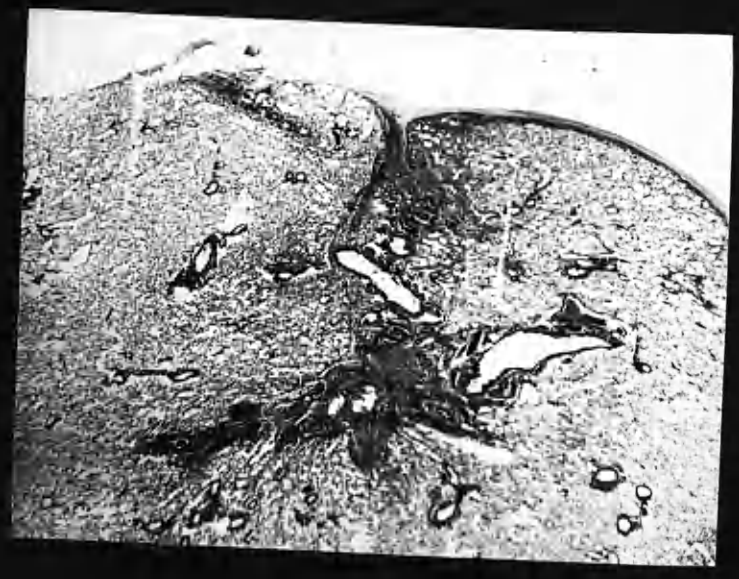


Fig. 71. Cat L 4 (28 days).
Low power photograph of wound in transverse section.
Orcein and safranin. X 5.

Fig. 72. Cat L 4 (28 days).
Well-developed bronchial buds which have developed from a large bronchus at the base of the scar.
H. + E. X 42.



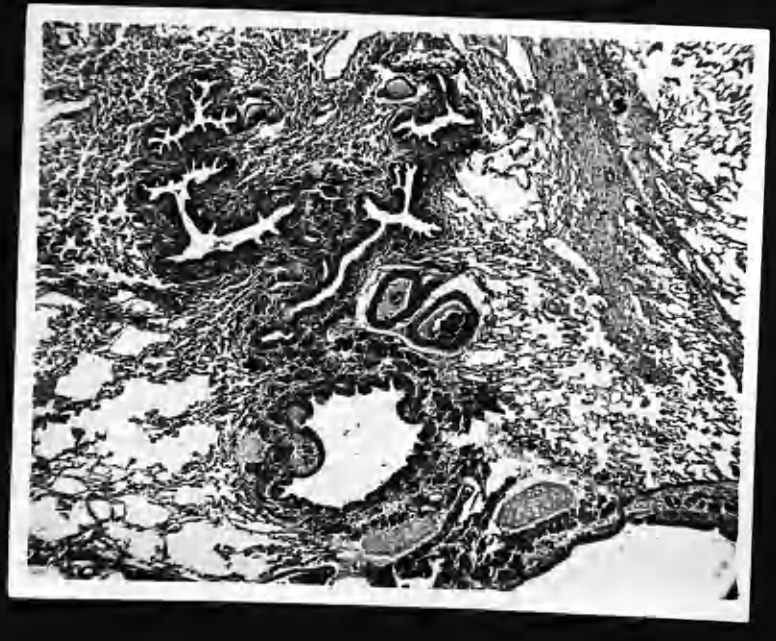
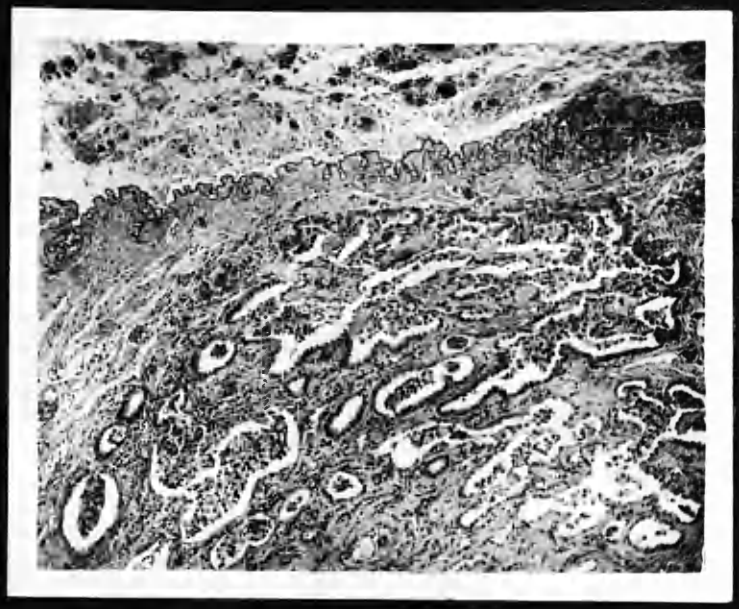


Fig. 73. Cat L 13 (51 days).
Alveoli-which have formed in the subpleural scar tissue at the margin of the wound.
H. + E. X 90.

Fig. 74. Cat L 13 (51 days).
Cellular lining of a new alveolus in the scar. This is a cubical type of respiratory epithelium; a ciliated border can be seen on some cells.
H. + E. X 300.



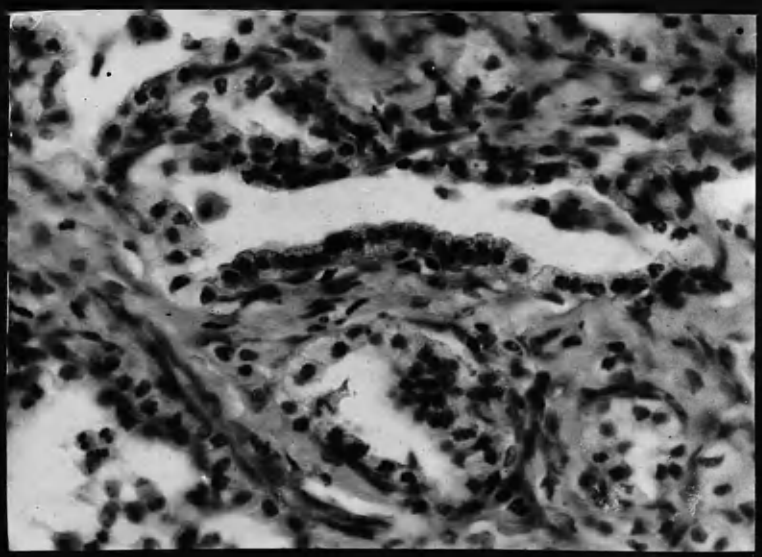
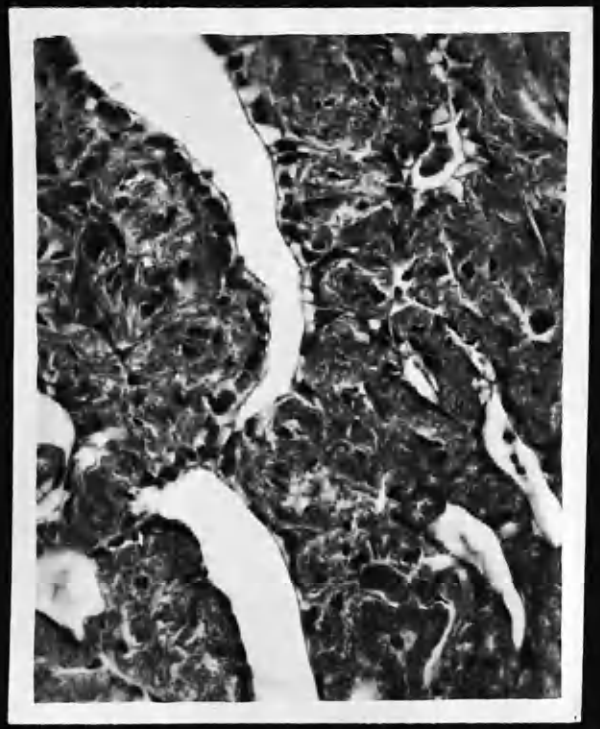


Fig. 75. Cat L 13 (51 days).
Bronchial buds lined by squamous epithelium in dense scar tissue.
H. + E. X 90.

Fig. 76. Cat L 13 (51 days).
Bronchial bud at point of penetration of hyaline tissue showing alteration in type of cellular lining.
H. + E. X 100.

Fig. 77. Cat L 13 (51 days).
Air spaces lined by cubical type of respiratory epithelium.
Modified picro-Mallory method. X 400.



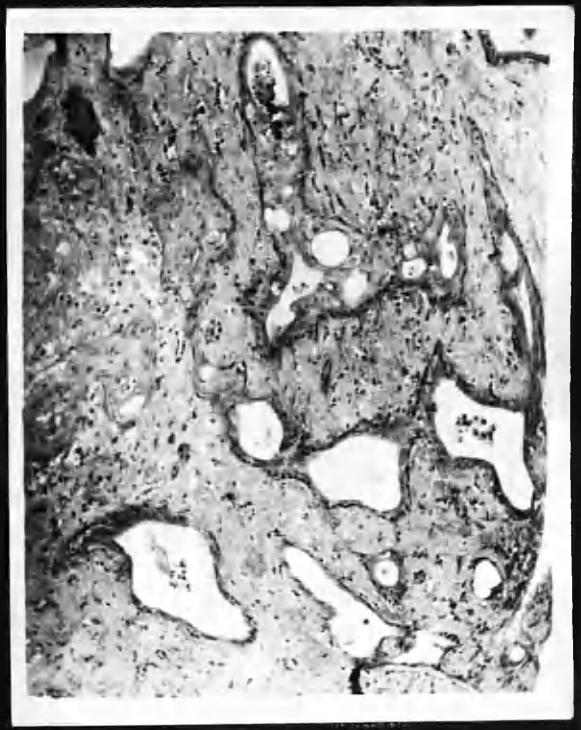
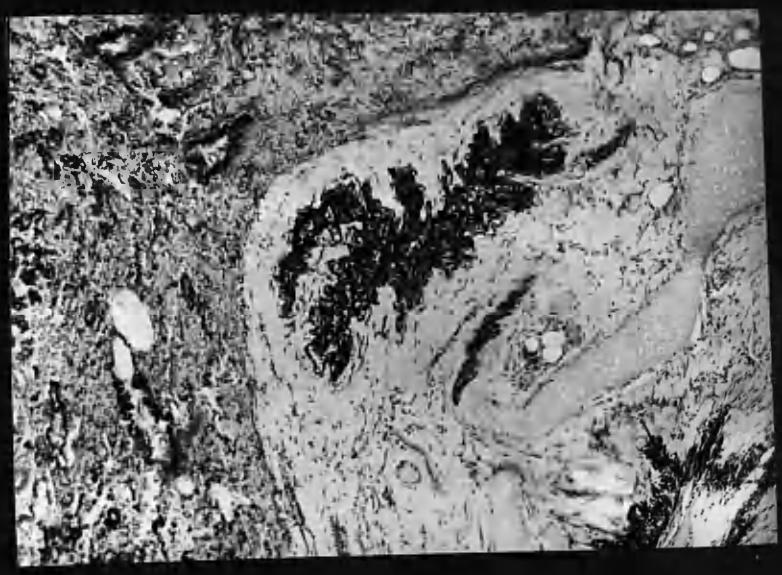


Fig. 78. Cat L 14 (59 days).
Low power of wound.
Orcein and safranin. X 5.

Fig. 79. Cat L 14 (59 days).
Stain of severed elastica representing site of collapsed and atrophied bronchial branch.
H. + E. X 60.

Fig. 80. Cat L 6 (60 days).
Low power photograph of section along the semi-necrotic protrusion.
H. + E. X 6.



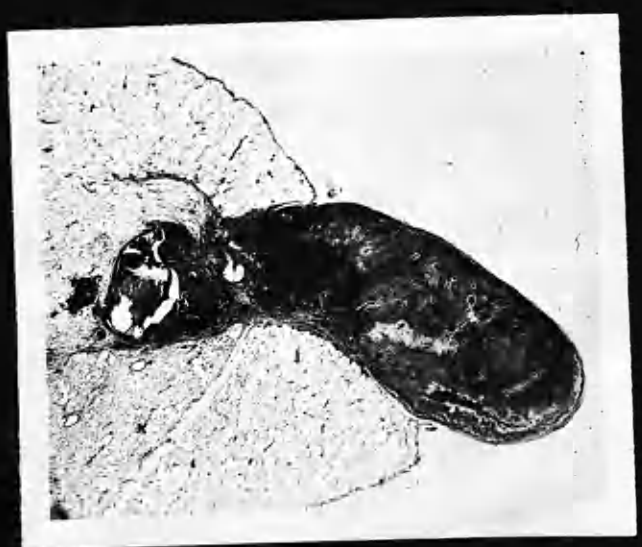
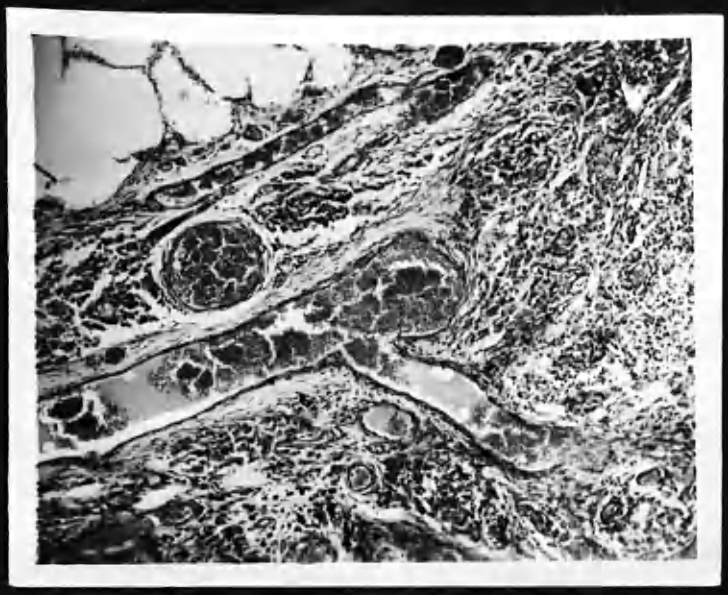


Fig. 81. Cat L 6 (60 days).
Dilated capillary blood vessels at the junction of normal and injured lung.
H. + E. X 90.

Fig. 82. Cat L 6 (60 days).
To show the development of subpleural air spaces on the surface of the necrotic tissue. (The connective tissue band separating the necrotic tissue traverses the photograph obliquely across the right lower corner).
H. + E. X 90.



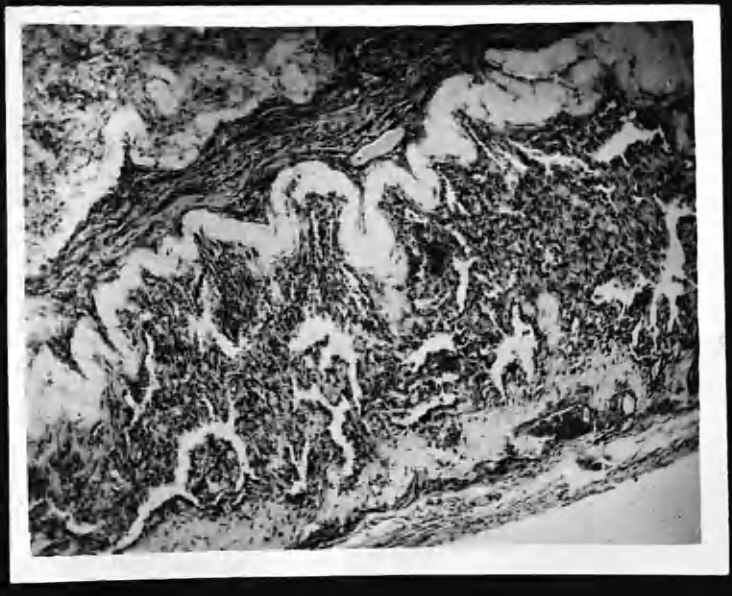
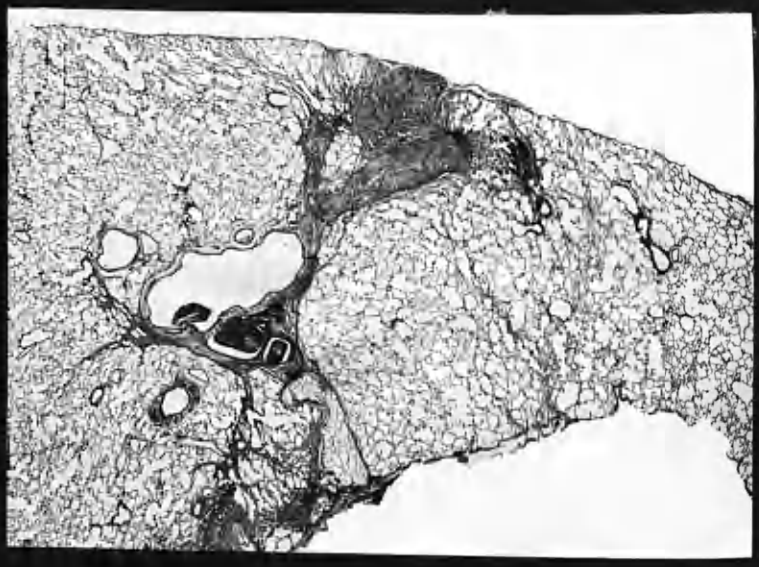


Fig. 83. Cat L 7 (76 days).
Low power photograph to show the general structure of the wound.
Orcein and Van Gieson. X 5.

Fig. 84. Cat L 11 (115 days).
Low power photograph illustrating a good restoration of normal lung structure after an excised wound. The site of excision is represented now by the linear scar and slight pleural adhesion.
Orcein and safranin. X 5.



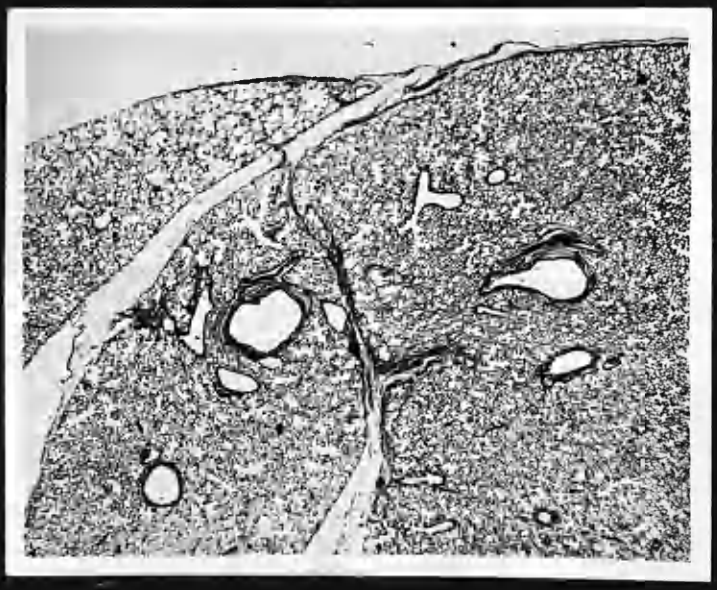


Fig. 85. Cat L 47 (pleural wound, 8 days).
To show relation of pleural elastica to vascular fibroblastic reaction.

Orcein and safranin. X 100.

Fig. 86. Cat L 47 (pleural wound, 8 days).
To show minute break in pleural elastica where it is penetrated by a capillary of the pleural or sub-pleural vascular network.

Orcein and safranin. X 400.

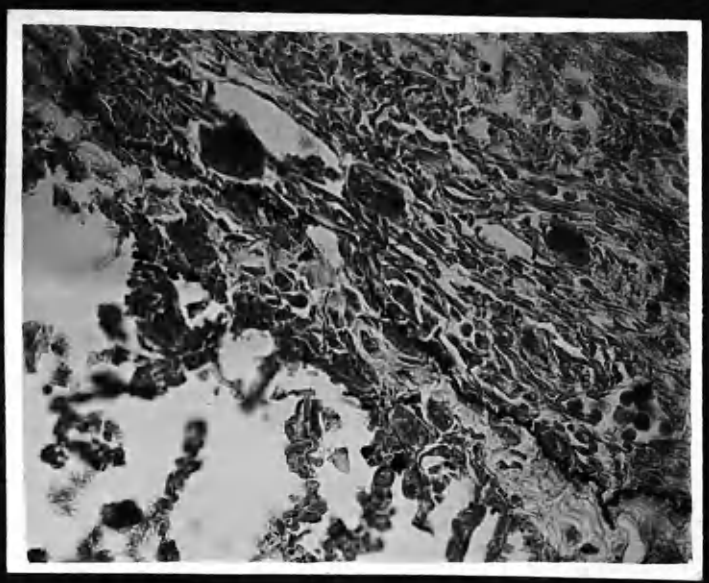
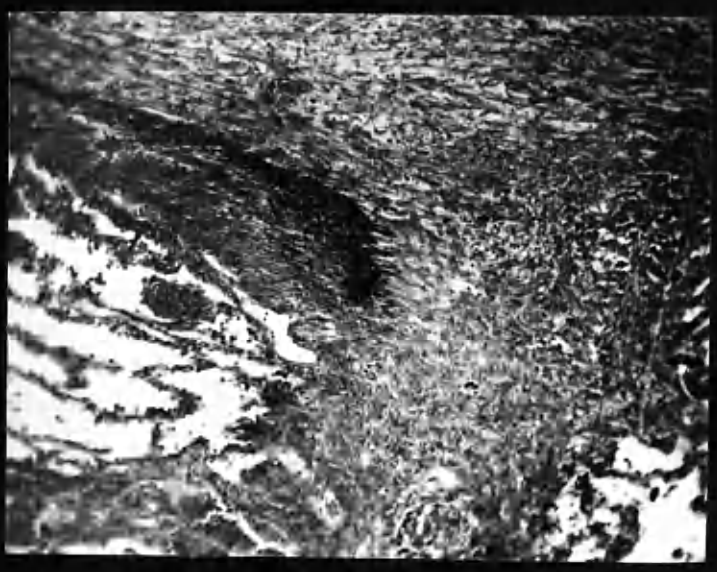


Fig. 87. Cat L 45 (12 days).
To show appearance of severed pleural elastica. The membrane appears like a ribbon seen alternately on edge and in the flat.
Weigert and van Gieson. X 100.

Fig. 88. Cat L 43 (8 days).
Pleural wound to show termination of severed elastic membrane.
Weigert and van Gieson. X 30.

Fig. 89. Cat L 43 (8 days).
Higher magnification of portion of fig. 88 with tortuous elastic membrane.
Weigert and van Gieson. X 100.



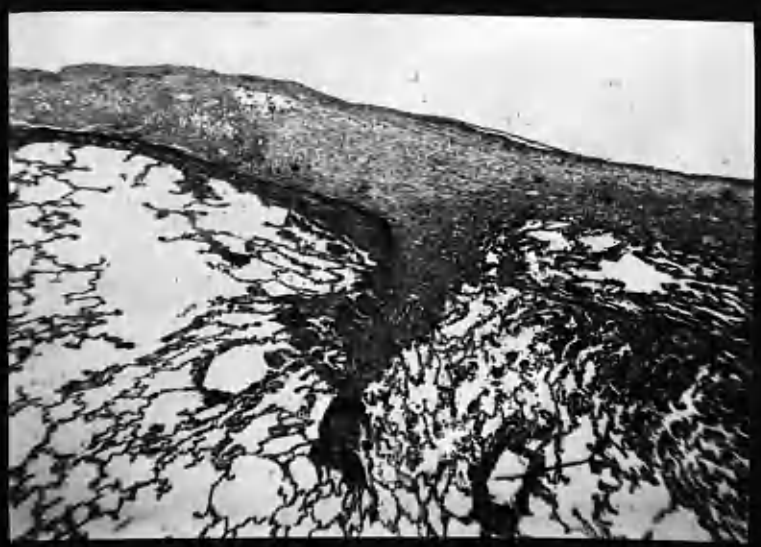


Fig. 90. Cat L 43 (8 days).
Haematoxylin and eosin section to demonstrate the relatively acellular nature of the collagen on one side of the elastic membrane and the palisading of fibroblasts next to the superficial lung alveoli.
H. + E. X 150.

Fig. 91. Cat L 43 (8 days).
The termination of the pleural elastica as a sinuous membrane with specific-staining fibres in the collagen.
Weigert and van Gieson. Orange filter. X 400.

Fig. 92. Cat L 43 (8 days).
Sinuous pleural elastic membrane with parallel specific-staining fibres arranged in the collagen beneath the membrane.
Weigert and van Gieson. Orange filter. X 400