PULMONARY EMPHYSEMA:
an experimental study

A THESIS

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INTRODUCTION

This thesis is based upon a series of experiments designed to produce chronic vesicular pulmonary emphysema experimentally in rabbits.

The theoretical basis for the experiments is the hypothesis that chronic emphysema should be regarded as an atrophy of lung tissue possibly resulting from tissue ischaemia. To test this hypothesis rabbits were subjected to repeated intravenous injection of an inert particulate substance, Caledon Blue R.C., in the hope of producing enough vascular blockage to lead to ischaemia of the alveolar tissues and the production of emphysema.

The thesis has been divided into sections as follows:-

SECTION I.

An historical account of anatomical studies of human emphysema. This account shows that certain aspects of the histological features of the condition appear, largely, to have been forgotten and that there is scant evidence for the existence of primary lesions in the elastic tissue of the lungs.

SECTION II.

An historical review of the literature dealing with the pathogenesis of chronic emphysema. This review shows that most of the opinions expressed have been concerned with the remote mechanisms which lead to the development of emphysema and that few clear statements, and no direct evidence, exist as to the mechanism of intimate pathogenesis.

SECTION III.

An account of spontaneous emphysema in rabbits which establishes standards for the assessment of the experimental work and shows that there is no essential difference between the lesions in rabbits and human emphysema.

SECTION IV.

An historical review of experimental pulmonary emphysema and an account of the present experiments in which experimental emphysema was produced by the repeated intravenous injection of Caledon Blue R.C.

SECTION V.

Studies of right ventricular hypertrophy in spontaneous and experimental emphysema in rabbits which show that the significant factor in

the production of right ventricular hypertrophy is the vascular obstruction and not the emphysema.

SECTION VI.

Studies of the changes in mean right ventricular pressure in rabbits following the injection of Caledon Blue or Lycopodium spores which provide some support for the conclusions reached in Section V.

SECTION VII.

A discussion of the experimental results in relation to the pathogenesis of emphysema and the relationship between emphysema and cor pulmonale in humans.

Technical and other details are included in the Appendixes.

SECTION I

HISTORICAL REVIEW OF THE HISTOPATHOLOGY OF VESICULAR PULMONARY EMPHYSEMA

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HISTORICAL REVIEW OF THE HISTOPATHOLOGY OF VESICULAR PULMONARY EMPHYSEMA

Before attempting to review the literature on the anatomical changes in pulmonary emphysema it seems advisable to set limits to the scope of the work and to give some definitions of the terms employed.

Interstitial pulmonary emphysema will not be discussed. In spite of the fact that there is an overlap between what might be called pneumoconiotic emphysema proper, as described by Heppleston (1947 & 1953) and other forms of emphysema, pneumoconiotic emphysema will also be excluded. Thus "ordinary" vesicular emphysema remains for consideration.

No attempt will be made to trace the development of current terminology through the literature. A basic terminology evolved fairly early in the history of the disease and this is still in general use. However, frequent modifications of this basic terminology have appeared from time to time and, unfortunately, not all writers have given an exact definition of their meaning when they have used the word "emphysema" without qualification.

It has been found convenient, for the purposes of this thesis, to make the following distinctions:-

A. Acute vesicular emphysema.

B. Chronic vesicular emphysema.

Acute Vesicular Emphysema.

This may be local or generalised, is not associated with any anatomical changes other than abnormal distension of the parts involved and is a temporary condition, being reversible when the causative factors cease to operate.

Chronic Vesicular Emphysema.

This, too, may be local or generalised but is associated with definite tissue changes other than mere distension of the air spaces and is not reversible.

Chronic emphysema is capable of subdivision according to the distribution of the lesions and the nature of the underlying causative conditions. The main subdivisions are:-

a. Complementary (vicarious or compensatory).

b. Senile.

c. Hypertrophic or substantive (essential, idiopathic, obstructive).

From the anatomical point of view the important distinction is the separation of the acute and chronic forms. It will be seen that, if the original anatomical definitions are accepted, there is a fairly sharp dividing line between the two. However, the subdivision of the chronic forms is much more difficult as all three may be present in the same lungs. This difficulty, for the present purpose, is somewhat minimised as the basic histological changes are the same in all three subdivisions.

Most of the work which will be described and reviewed in this and subsequent sections of the thesis is concerned with the chronic forms of vesicular emphysema.

An effort has been made to avoid becoming involved with minute details of histological terminology, as it is believed that the essential nature of emphysema can be appreciated without this. Where reference to specific portions of the terminal respiratory tree is necessary, it has been found convenient to adopt a slight modification of the basic terminology of W.S.Miller (1925) and Hepple-ston (1953).

These authors regard the terminal bronchiole (non-respiratory) as dividing into two or three orders of respiratory bronchi which have

increasing numbers of alveoli as the final order is approached. The final order respiratory bronchus divides into alveolar ducts which are completely surrounded by subtended alveoli. There is only one order of alveolar ducts and they can be recognised by the presence of smooth muscle in their septa. The alveolar duct divides into atria which are very similar to alveolar ducts but have no smooth muscle. The atria divide and end in blind sacs subtending alveoli. These are the final division and are called alveolar sacs, but should not be confused with the alveoli themselves.

The distinction between alveolar ducts and atria is a fine one, depending in part on the recognition of smooth muscle in the alveolar duct. So, for the present purposes, this distinction will not be made. The following terminology will be used:-

Terminal bronchiole, respiratory bronchi, alveolar ducts and, finally, alveolar sacs.

The Greeks used the word emphysema (literally - to inflate), in medical literature, to indicate distension of the abdomen. Kountz & Alexander (1934) give a brief account of the early use of the term. Following Lovel in 1661 the word was used to describe the swelling of external wounds and later this usage became established to indicate the presence of gas in the subcutaneous tissues in surgical or traumatic emphysema.

In 1764 Watson described a case, at autopsy, where the lungs were said to be "truly emphysematous" and, after this period, the phrase emphysema of the lungs came to mean an abnormal distension of the lungs by air. Isolated autopsy reports subsequently appeared, e.g. the account given by Baillie (1812) of the lungs of Dr.Samuel Johnson, but no systematic account of the disease is available until 1819 when Laennec published his classical treatise.

The work of Laennec (1819)* was based on naked eye autopsy studies of cases previously observed during life. He distinguished between vesicular emphysema, where the distending air was contained within the air spaces of the lungs, and

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* The original was not available, and the English translation by J.Forbes (1834) was consulted.

interstitial emphysema, where the air escaped into the interstitial tissues. Laennec did not definitely distinguish between acute and chronic emphysema. He considered that emphysema was essentially a dilatation of the air cells; but in the larger lesions, at least, this dilatation was permanent and fusion of the air spaces occurred due to rupture of their boundary walls. He gave a good description of superficial bullae and noted that, if dried expanded lungs were sectioned, similar lesions could be seen in the deeper parts of the lungs. Though he did not subdivide his cases, as later became the custom, he distinguished between partial and generalised emphysema. He did not recognise senile emphysema as such, but he was aware that the senile lung had a different texture from that of the younger subject and described this in the chapter on the normal appearances of the lungs.

The first microscopic description of emphysema appears to be that of Rainey (1848). Although the description seems to be based on the appearances in one case, which he described as being "of the ordinary kind", it is a remarkably good account of the essential features of chronic vesicular emphysema to which little has been added since.

He described the capillary network of the alveolar walls and considered that the function of the alveolar walls was to support these capillaries. The earliest change was a dilatation of the alveoli and a widening of the meshes of the capillary net. Then gaps or pores appeared in the walls in the spaces between the capillaries. Progressive enlargement of these fenestrations led to destruction of the alveolar walls. Extension of the process caused fusion of neighbouring air sacs and further development of this process led to the formation of bullae. He noted that, in the formation of the larger lesions, there was condensation of the interlobular fibrous tissue around the periphery of the affected zone. He considered that the fenestrations arose out of a fatty degeneration of the alveolar walls and described refractile globules which could be seen in the sites where fenestrations later appeared. Subsequent observers have not substantiated this claim. Rainey stated that pores or fenestrations were not seen in normal lungs; but if one or two were present they should be regarded as evidence of incipient disease.

While views on the aetiology of the condition were expressed in the next decade, it remained for Rokitansky (1861) to publish the next significant anatomical account. Like Laennec, he recognised

interstitial emphysema and, though his distinctions are not always clear, he was the first to subdivide vesicular more or less into the categories recognised by later workers. He clearly stated that the basic micro-anatomical change was principally one of atrophy of the lung tissue and described the appearance of perforations or holes in the alveolar membrane which enlarged as the membrane was destroyed. This extended to the interlobular septa and in due course the larger bullous lesions were formed.

Rokitansky distinguished senile emphysema, though he called this, simply, senile atrophy of the lung. He noted that the lungs remained small in this condition and there was generalised rarefaction of the lung tissue without bullous development. He recognised vicarious or complementary emphysema which developed round areas of consolidation, but appeared to regard this as mainly an acute process, not attended by tissue changes other than distension. He next separated off substantive emphysema and gave a good naked eye description of what would be called chronic hypertrophic emphysema to-day. It should be noted that Rokitansky included, in this section, lesions which appear to be identical with those described as chronic vicarious or complementary emphysema by later authors.

Waters (1862), while he did not subdivide emphysema as clearly as Rokitansky, gave an excellent account of the lesions. He studied the normal anatomy and gave a good description of the relationship of the alveoli to the "air sacs" (the alveolar sacs and alveolar ducts of modern terminology). He considered that the earliest stage of the disease was simple dilatation of these air sacs with the result that the alveoli became wider and their septa reduced in height, until the alveolar walls became partly obliterated. The elastic fibres became more widely separated but showed no other changes.

At this stage there appeared "perforation of the walls themselves".

> "This is at first but slight. Here and there a circular or oval opening may be seen in the membrane: as the disease progresses, these openings become more numerous, and larger; in some instances the whole of the walls of the air sacs and the septa of the alveoli being perfectly riddled with small openings, so that a horizontal section of the lung substance has a general cribriform appearance. These openings are for the most part either circular or slightly oval. They exist in all parts of the walls, and are often seen in the septa between the alveoli, before the air sacs are sufficiently distended to obliterate the septa."

He went on to describe the extension of the process of fenestration till the boundaries between the air sacs were destroyed and fusion of neighbouring units occurred, with the ultimate formation of large surface or even pedunculated bullae.

He noted that, in the course of the fenestration, the capillary bed was destroyed but that vessels might persist in the strands of tissue traversing the larger cavities. No anatomical change could be seen in the lung which preceded the distension and the fenestration. He recognised a "lobular" form of emphysema where there might be much distension but much less perforation and a "lobar" form where often the fenestration was very marked but the distension less than in the lobular form.

The work of Waters has been quoted at length as it is the last detailed description of this type to appear in the English literature on emphysema.

Niemeyer (1864) gives a brief but good account of the anatomy and micro-anatomy of the disease which corresponds to that of Waters. However, Niemeyer clearly subdivides emphysema into most of the categories which are recognised to-day. Vesicular emphysema is classified as vicarious (complementary or compensatory) which may be either acute or chronic, and substantive (chronic hypertrophic). He emphasises that the anatomic changes in chronic vicarious emphysema are identical to those in the substantive variety. Niemeyer, however, does not mention senile emphysema.

Villemin (1866) confirmed the observations of the previous authors with regard to the destruction of the alveolar walls by fenestration. However, while his predecessors had considered that the fenestrations had arisen de novo in the alveolar membranes, Villemin described a proliferation of cells lying in the intercapillary spaces which cells then degenerated and fell out of the alveolar membrane leaving the perforation in its place. Villemin could detect no changes in the elastica which preceded the development of fenestration.

Isaaksshon (1871) agreed with the previous authors in his general description of the changes but also described the occurrence of capillary thrombi which originated as mural thrombi and ultimately occluded the lumen of the vessel. This caused loss of continuity of the capillary which ultimately disappeared.

Rindfleisch (1871) accepts the descriptions of previous authors but insists that simple dilatation of the air spaces is the first step and that fenestration follows this. However, he does not make a clear distinction between acute and chronic emphysema. Thierfelder (1872) illustrated fenestration in his atlas; he also illustrated elastic fibres but made no mention of lesions in them. Hertz (1874) in the course of a good review

of all aspects of the subject described the first stage in emphysema as a dilatation of the lumen of the infundibulum (alveolar sac) and its alveoli; the alveoli became flattened and later the walls became perforated. He insisted that there was no anatomical change which preceded the above events and that even in established lesions the elastica appeared normal.

Eppinger (1876) published his classical work which, while it covered most aspects of emphysema was concerned mainly with the anatomical lesions. Eppinger subdivided vesicular emphysema into acute and chronic. Acute vesicular emphysema he regarded as being a simple over-distension of lung tissue around a consolidated focus. There were no permanent anatomical changes. Chronic vesicular emphysema was divided into several sub-groups and, though his distinctions are not always clear, they agree, in general, with those of previous authors. It should be noted that he recognised senile emphysema as one of the varieties of chronic emphysema and distinguished this from the substantive type on the absence of large bullous lesions in the senile form.

He stated or implied that the micro-anatomical changes were essentially the same in all these forms; but details might vary in

different cases, especially with regard to complicating inflammatory lesions. He regarded chronic emphysema as consisting of dilatation of the air spaces plus an actual anatomical change in the alveolar septa. Eppinger's own words are as follows:-

> "Das chronische Emphysem besteht in einer abnormen Ausdehnung der Alveoli durch Luft mit gleichzeitiger Rarefaction der Alveolarwände, die bis zum völligen Schwunde derselben gesteigert wird, so dass dann mehrere ektasirte Luftzellen zu weiteren Luftraumen verschmeltzen."

His description of the histological changes is excellent and agreed, in all essentials, with those of earlier writers. In addition, however, he gave the first account of changes in the elastic fibres and fibre bundles in emphysema.

He used sections cleared with potassium hydroxide for the study of the elastic fibres and, in addition to bronchial, pleural and vascular elastic tissue, he distinguished three main types of normal elastic tissue bundles according to their size and distribution in relation to the alveoli.

a. Thick fibre bundles running round the mouths, sides and bases of the alveoli.

b. Medium fibre bundles running across the side walls of the alveoli and connecting with the thick bundles.

c. Fine fibres or bundles of fibres running between the others and the capillaries of the alveolar walls.

Eppinger described the process of fenestration of the alveolar walls in detail. Like his predecessors he stated that the fenestrations appeared first in the interspaces between the capillary net. This he called "primary dehiscence". The capillaries appeared stretched and the fenestrations became larger and ultimately fused, with obliteration of the intervening capillary. This was the stage of "secondary dehiscence" and an extension of the process led to the eventual destruction of the alveolar walls with the formation of the simple infundibular bulla, i.e. an alveolar sac denuded of alveolar septa and presenting as a rounded vesicle. Fenestration occurred next in the walls of the infundibula and ultimately caused fusion of these structures and the production of the larger lesions.

With regard to the elastica he was much less precise. In the later lesions the fine fibres were lost and all that remained were the coarser fibres which ran round the edges of the fenestrations but did not usually show any morphological changes. In such cases the edges of the

fenestrations were sharp. However, Eppinger thought he could detect more recently formed fenestrations, the edges of which were irregular. In these cases elastic fibres ran right up to the irregular edge and ended in an apparently frayed manner.

He considered that this appearance was the earliest lesion in chronic emphysema and, on the basis of it, he maintained that overstretching damaged the elastic fibres which then ruptured and that the fenestration appeared in the alveolar wall at the site of rupture of the fibres. It is important to remember that Eppinger did not claim to see any lesion in the elastica which actually preceded the appearance of fenestration.

He thought that further destruction of the fine elastic fibres occurred in this manner until, as the fenestration advanced, all the fine fibres were destroyed. But he admitted that some of the fibres are compressed peripherally around the developing lesions. In considering the larger bulla he stated that occasional ruptured fibres might be seen.

It should be noted that while Eppinger considered the process to be one of lung atrophy he remarked that, in certain circumstances, there was an increase in the amount of fibrous tissue

in the walls of the emphysematous lesions.

The work of Eppinger has been reviewed at length as, in large measure, it provides the factual basis for the commonly accepted view that the primary lesion in emphysema lies in rupture of the elastic fibres of the lung.

Kläsi (1886) using methods similar to Eppinger, in the course of an anatomical and experimental study of the problem, thought that the fenestration in emphysema was the result of the desquamation of alveolar epithelium and insisted that fenestration could be seen before there was any change even in the finest elastic fibres. Kläsi made the point that the elastic fibres were among the last structures to be destroyed in the emphysematous process.

Orth (1887) gave a good description of the anatomical changes which agreed with that of previous authors though there was no specific mention of changes in the elastic tissues. His article is of interest in that he emphasised that, morphologically, atrophy and loss of lung tissue was an essential feature of emphysema but he insisted that, in the absence of distension, the term emphysema should not be applied. This, perhaps, fine distinction was employed in his classification

of senile emphysema. He called the appearance of the senile lung senile atrophy when there was no trace of distension of the organ; but he pointed out that when a degree of distension was present the condition should be called senile emphysema. However, he freely admitted that it might be impossible, even anatomically, to make this distinction.

Virchow (1888) did not emphasise the microscopic changes but gave a very good account of the development of the larger lesions by fusion of the lobular units due to a slowly developing perforation of their boundary septa. He insisted that this was a slow atrophic process and not the result of mechanical tearing.

In England, Auld (1893) in a brief account of the essential features, described the process of fenestration which he considered was preceded by hypertrophy of both the alveolar epithelium and the capillary endothelium.

The next important work is that of Hansemann and his pupil Sudsuki. In order that this may be viewed in perspective it is necessary to consider briefly the subject of the alveolar pores: the pores of Kohn.

Henle (1873) in the course of his description of the micro-anatomy of normal human lungs noted that, occasionally, small rounded or oval fenestrations or pores could be seen in both the side walls and bases of the alveoli. He regarded these as communications between adjacent alveoli and between adjacent respiratory lobules. Henle credited the first description of these pores to Adriani in 1847 and noted that Kölliker and Schultz had denied that pores were seen in normal lungs. W.S.Miller (1892) failed to demonstrate the pores and thereafter stoutly denied their existence.

Kohn (1893) described communications in the alveolar walls through which fibrin could be seen to pass but inclined to the view that the communications were of inflammatory origin. Hauser (1894) considered that the pores were normal structures and suggested that Kohn's name should be applied to them. Hansemann (1895) employed a gelatine injection technique and also accepted the pores as normal structures.

Subsequent discussion as to the existence of the pores and dispute as to whether they should be regarded as pathological or not is not immediately relevant. This subject has been well reviewed by Macklin (1935, 1936) and Loosli (1937) who

regarded the existence of pores in man, rabbits and other mammals as indisputable. Macklin's illustrations are quite convincing and these appearances can be easily seen if thick sections are examined.

It appears that while much of the controversy concerns matters of interpretation with regard to the significance of the pores there can be little doubt about two things. Firstly, that the existence of the pores should be denied, arose out of the adoption of the method of serial section, using thin sections in preference to the study of thick sections, combined with the fact that pores are not present in every alveolus, and, secondly, that the structures described as alveolar pores are morpholigically indistinguishable from the fenestrations universally described as one of the earliest, if not the earliest, lesions of chronic pulmonary emphysema.

However, it is of interest to note that recent work on the subject of the alveolar lining, employing electron microscopy, as exemplified by that of Bertalanffy & Leblond (1955), while omitting specific mention of the pores, gives the impression that there is a continuous lining of the alveolar walls by attenuated epithelium and seems likely to

prolong the controversy as to the very existence of the pores.

The work of Hansemann and Sudsuki fell into two main parts: a study of the changes in the elastic fibres in emphysema and a consideration of the relationship of the alveolar pores to emphysema. Sudsuki (1899) gives most of the detail of this work. He employed paraffin sections of a thickness of 40 μ and used the resorcin-fuchsin technique of Weigert (1898) for staining the elastic fibres. His material consisted of "over 20" normal lungs, 30 cases of substantive emphysema, 13 vicarious and two acute emphysemas. He also compared the emphysematous portions with normal portions of the same lung.

Sudsuki agreed with Eppinger as to the distribution and types of elastic fibres in the normal lung but noted that there was wide individual variation in the numbers of fibres seen. (It is interesting to note that neither Sudsuki nor Eppinger mention the possibility of technical variations influencing the appearances.) Sudsuki concluded that, in emphysema, the largest fibres were more slender while both the medium and the fine fibres were variable. He thought that some of these

differences were the result of individual variation but that others were the result of the dilatation of the air spaces in emphysema which spread out the same number of fibres over a wider area giving an impression of diminution in numbers. In a comparable manner he thought that spatial re-arrangement accounted for those cases in which the fibres were apparently increased in numbers. Sudsuki did not see any of the fraying which Eppinger described as the stage preceding rupture of the fibres. While admitting that the elastica did disappear in certain lesions, he insisted that it did not do so before the other elements in the alveolar wall.

In the same paper Sudsuki gave a good description of the development of the lesions of emphysema on the basis of progressive fenestration of the alveolar walls as described by the older authors. However, he identified the initial fenestrations in the alveolar walls with the alveolar pores which he accepted as normal structures. He considered that a mechanical widening of these pores as a result of distension constituted the initial lesion in emphysema.

Hansemann (1899) accepted Sudsuki's conclusions both with regard to the histiogenesis of emphysema, in relation to the alveolar pores, and to the secondary role of any changes seen in the elastica.

Eppinger restated his own view in 1902 (Eppinger & Schauenstein, 1902) after employing Weigert's elastic tissue stain and did not retract his opinions of 1876.

In the same year Ribbert (1902) gave a good account of the development of the lesions of chronic emphysema, with especial regard to the so-called "infundibular bulla" which had been well described by Eppinger (1876). Ribbert sided with Sudsuki in noting that no specific changes could be seen in the elastic tissue until atrophy and disappearance of the alveolar walls were already in progress. A further point of interest is that Hibbert's descriptions and illustrations tended to be of a two-dimensional character in contrast to those of earlier writers. This was the first time that such a trend could be detected in the literature.

Tendeloo (1902), while concerned more with the theoretical aspects of lung elasticity, did study the anatomy of the elastic fibres in an attempt to assess the significance of the works of Eppinger and Sudsuki. He came to the same general conclusions as Sudsuki and could detect no specific changes in these fibres, but noted that there was considerable individual variation in the number and

apparent strength of the fibres. This was true in all types of emphysema. He noted briefly that, in some emphysematous areas, the fibres appeared to be more strongly developed than normal. He did not pursue this point.

Spalteholz, at the request of Hoffman (1903), studied an unstated number of cases of severe emphysema and was unable to detect the presence of lacerated or split elastic fibres. The changes were no more than could have been expected in consideration of the altered state of expansion.

Orsos (1907) gave a detailed account of the arrangement of the elastic and collagen fibres in normal lungs. His account of the elastica, in general, agreed with that of Eppinger (1876). However, Orsos subdivided the elastic fibres according to their origin. The thick and medium fibre bundles deriving from the bronchial system were designated "respiratory" fibres; while the finest fibres, related to the alveolar capillaries, and deriving from the elastica of the larger vessels were called "vascular" or "inter-capillary". The collagen fibres were more numerous and distinctly spiral in character but had the same course, distribution and origin as the elastic fibres.

In acute emphysema he considered there was simple dilatation of the air spaces with increased separation of the various fibres due to the expansion; but no other changes.

He divided chronic emphysema into senile and essential emphysema (chronic hypertrophic) and stated that in the chronic state atrophic and other permanent tissue changes were added to the simple dilatation of the acute state. These changes included fenestration of the alveolar walls, destruction of capillaries and changes in the elastic fibres.

In addition to the separation of the elastic fibres he considered that tearing of the fibres with their subsequent degeneration were the essential lesions. Sometimes he noted changes in the staining reaction of ruptured fibres, but more frequently no such change was seen. He did not see any change which preceded rupture. When only a few fibres ruptured they retracted in a serpiginous manner. If rupture was widespread, the retracted fibres appeared as scattered heaps of elastic tissue and degenerated into granular masses which ultimately lost their affinity for the elastic stain. The ruptured fibres, or the residual heaps of degenerate fibres, could be seen

on the alveolar walls or in the course of larger fibre bundles of which the ruptured fibres were a component. The changes were first seen in the fine intercapillary fibres but later in the larger bundles of respiratory fibres. Development of the process led to the destruction of a variable proportion of the elastica.

Orsos considered that these degenerative changes were principally seen in senile emphysema.

In the case of essential emphysema, though the above changes were seen, he considered that a reactive increase in the numbers of elastic and collagen fibres was a more prominent feature. He described an increase in the numbers of both respiratory and inter-capillary fibres and in larger lesions noted that the arrangement of the fibres differed from that in normal alveoli. This appearance he interpreted as evidence that, in the walls of the bullous lesions, formation of new fibres had occurred. In these lesions too he described an increase in the numbers of collagen fibres. He noted that where apparent regenerative changes had taken place the appearance of fenestration was much less prominent.

He gave a general description of the emphysematous process and emphasised that in the

wholly atrophic lesions all elements of the alveolar walls were ultimately destroyed. This description agreed with those of earlier writers.

He stated that all the changes were seen in progress at the same time and there was no definite sequence of loss of the various elements. The description of the elastic tissue changes was kept separate from that of the general changes and, at no time, did he specifically state that the changes in the elastic fibres preceded the other atrophic changes. He accepted that fenestration was one of the earliest manifestations of chronic emphysema and, like Eppinger, argued that extension of this process could only result from a loss of elastic and collagen fibres. In short, he did not show that the lesions in the elastica were primary but in his summary he inferred that this was so.

In a later paper, Orsos (1936) gave a more detailed account of the muscular and connective tissue systems of the lungs and reiterated his views on the primary nature of the elastic tissue changes in emphysema, without producing further evidence. He emphasised the occurrence of proliferative changes in chronic hypertrophic emphysema and noted that hypertrophy of the intimate musculature of the lungs also occurred.

Marchand & Laguesse (1911), while mainly concerned with a study of the alveolar pores in the normal human lung, maintained that the initial anatomical change in emphysema was dilatation and that the appearance of abnormal numbers of pores was a later and secondary phenomenon.

Loeschcke (1921) published his first paper devoted to morphological changes in the terminal respiratory lobule. Most of this paper is devoted to a study of the early changes at the level of the alveolar sacs, ducts and respiratory bronchi by injection of Wood's metal. However, Loeschcke checked his observations by the use of ordinary thick sections. He described dilatation and fusion of alveolar sacs and alveolar ducts due to the process of fenestration in the septal walls, as described by the older writers, and agreed that an extension of this process was responsible for the further development of the later lesions.

Loeschcke (1922, 1928a & 1928b) then published a series of articles which though concerned mainly with the aetiological aspects of emphysema contained good accounts of the histological development of the lesions. The article of 1928a is the most comprehensive but that of 1928b contains the most concise statement of his views on the anatomical changes. He was in agreement with

all previous writers on the destruction of lung tissue by fenestration. With regard to the elastic tissue he saw no changes which preceded fenestration and, indeed, regarded the elastic tissue as unchanged until later in the disease when advanced destruction of the alveolar walls had taken place. Even at this stage the elastica was remarkably persistent and any changes seen were in the nature of a strengthening and thickening of the individual fibres. Far from considering that the primary lesion resided in the elastica he considered that, of all tissues, this survived best the atrophic process of emphysema.

In contrast to Loeschcke, Laguesse (1927), without presenting any evidence, maintained that the increase in number of pores in emphysema was in fact due to loss of the finer elastic fibres.

Letulle (1928) accepted the views of the older writers with regard to fenestration and atrophy in chronic emphysema. He was non-committal on the subject of the elastic fibres, but noted that in addition to reduction in the numbers of fibres there was also some condensation of the surviving fibres in the walls of emphysematous lesions. He also drew attention to the fact that sclerosis is a frequent accompaniment of emphysematous lesions, not only in relation to tuberculous scars, but

also in other sites, even in very early emphysematous foci.

Antoniazzi (1934a & 1934b), first in a study of apical emphysema and later of other types of emphysema, drew attention to the accompanying sclerotic changes which he considered an integral part of the emphysematous process. Luisada (1934) was similarly impressed by sclerotic changes in all forms of emphysema.

Hartroft (1945) was concerned with the microscopic features of early emphysema as seen in thin sections. He pointed out the falsity of the, by now, popular idea that the free ends of alveolar septa projecting into the alveolar sacs and ducts were evidence of rupture of the alveolar tissues. He described emphysema as consisting of a dilatation of the alveolar sacs and ducts until the alveolar septa vanished, leaving a rounded vesicle. He considered that, apart from the laborious method of serial section and measurement, there was no practical way of recognising early emphysema.

Bezançon & Delarue (1947), in a study of the histogenesis of emphysematous bullae, also pointed out that rupture did not enter into the picture of the genesis of emphysema but they considered, as had older writers, that atrophy was responsible for the development of the lesions.

In addition to atrophic processes they stressed that interstitial inflammation with fibrosis, which might later progress to atrophy, played an important role in the genesis of the lesions.

Heppleston (1953) while not primarily concerned with hypertrophic emphysema, described the changes as consisting essentially of a dilatation of the air spaces distal to the respiratory bronchi. He stated that destruction of lung tissue was evident in the larger lesions but gave no details of the histological nature of the destructive processes.

DISCUSSION

Very little anatomical work on the subject of pulmonary emphysema has been published in the last thirty or forty years. During this period investigations have dealt mainly with the functional and aetiological aspects of the problem and references to the anatomy have been brief, adding nothing to the views of the older writers. In fact the original descriptions of the basic histological changes appear to have been forgotten. Textbook accounts and original papers give dilatation of alveoli, thinning and rupture of their walls and disappearance or rupture of the elastic fibres as the histological criteria for a diagnosis of emphysema. The ends of alveolar septa projecting into alvedar ducts have frequently been interpreted as evidence of rupture.

It has not been possible to trace these misconceptions to any particular source, but it appears that the paucity of anatomical work on emphysema published in English has been partly responsible. No detailed English description is available since that of Waters (1862). Another factor, which seems to be important, was the

adoption of the method of thin paraffin sections as a histological technique. The older writers employed thick sections and gave a three-dimensional account of the lesions. Most work in this century appears to have been done on thin sections in which the appearances can be most misleading.

Reverting to the original anatomical studies of emphysema, chronic emphysema was separated from acute at an early date, on the basis of the addition of atrophic changes in the lung tissues, which rendered the process irreversible; in contrast to the simple reversible distension that characterised the acute state. In chronic emphysema of all types, the atrophy manifested itself by the development of a process of fenestration of the alveolar walls. Extension of this led to the formation of the larger bullous lesions which, although expanded and apparently "hypertrophic" in character, were regarded as essentially atrophic lesions, in that they arose out of tissue loss.

In the more advanced lesions of chronic emphysema, attenuated strands of lung tissue have been described coursing across the bullae and, while it cannot be denied that rupture of such strands must occur, the descriptions indicate that this only happens when attenuation is extreme.

Final rupture of atrophic lung tissue is implied, rather than described, by the older writers suggesting that it is difficult to detect. This contrasts sharply with the general acceptance of rupture as an integral part of the process in those works where thin sections, only, have been studied.

Nearly all writers pointed out that as far as histological appearances are concerned there is no essential difference between the various forms of chronic emphysema. The distinction was made on the lack of large expanded lesions in the senile type, the relation to scars and other causative conditions in the complementary type, and the more generalised distribution with marginal bullous accentuation in hypertrophic or substantive emphysema, but not on the histological appearances.

It is important to remember that in practice it is not always easy to be certain which type of chronic emphysema is present in any given case for combination forms are common. This is particularly true of senile emphysema which receives very little attention in the literature. Orth (1887) is one of the few authors to discuss this in some detail; but nowhere is there a separate account of the changes in the senile lung. Indeed, Loeschcke (1928a, p.689) does not consider that this

is an entity. This will be discussed in the next section.

The problem of elastic tissue changes must be considered against this background of general agreement.

Eppinger (1876) was the first to claim that the primary defect which led to the fenestration lay in the finest elastic tissue fibres. It must be emphasised that he reached this conclusion by inference and he, himself, stated that no lesion could be seen in the elastic fibres before the appearance of fenestration.

Orsos (1907, 1936) also claimed that rupture and degeneration of fine elastic fibres occurred at an early stage in emphysema. In the text of his 1907 article he stated that these changes were seen at the same time as the appearance of fenestration; but in the summary of this article he gave the impression that the rupture and degeneration of the elastica preceded the fenestration; in fact, he did not make a categorical statement to this effect. In the 1936 paper he reiterated these views, but now stated that the 1907 article had shown that the elastic tissue changes preceded the fenestration, quite without adding any fresh evidence.

All other workers, who made a detailed study of this problem, denied the existence of the changes described by Eppinger and Orsos and concluded that there was no change in the elastica in the initial stages of the disease. Subsequent disappearance of the finest elastic fibres occurred in parallel with the disappearance of other components of the alveolar walls. In fact, it was noted that the elastic fibres appeared to be remarkably resistant to the atrophic process which was destroying the alveolar tissues.

In balance the evidence is against there being detectable changes in the elastic fibres in the initial and even the more advanced stages of chronic emphysema. Even Orsos emphasised that it was essential to employ thick sections to detect the changes he described. The frequent statement that loss or fragmentation of elastic fibres is one of the criteria for a histological diagnosis of emphysema is not a little surprising, in view of the fact that thick section technique was not employed.

One other point emerges from the anatomical survey. Rainey (1848) pointed out that there was condensation of the interlobular fibrous tissue around the larger lesions. From time

to time since then other authors have commented on this. Indeed, Orsos (1907 and 1936) laid more emphasis on apparent hypertrophic changes in the elastic and reticulin fibres, in hypertrophic emphysema, than he did on the degenerative changes, which he considered more characteristic of senile emphysema. Apart from Orsos, other writers have merely commented on such changes in passing. However, Letulle (1928), Antoniazzi (1934a and 1934b), and Bezançon and Delarue (1947) believed that sclerosis was an integral part of the emphysematous process. It is thought that this aspect of the problem might merit further study.

SUMMARY AND CONCLUSIONS

The authors of anatomical studies on chronic vesicular emphysema are, virtually, in complete agreement that it should be regarded as an atrophy of lung tissue.

The basic microscopic lesions are the same in all forms of the condition and consist of the appearance of fenestrations in the alveolar walls. The process of fenestration develops and leads, ultimately, to destruction of the alveolar tissues.

There is no acceptable evidence that changes in the elastic tissue precede the appearance

of fenestration, although a minority of authors have made this inference.

A few writers have suggested that sclerosis of inflammatory origin is an essential part of the emphysematous process.

SECTION II

HISTORICAL REVIEW OF THEORIES OF PATHOGENESIS OF PULMONARY EMPHYSEMA.

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HISTORICAL REVIEW OF THEORIES OF PATHOGENESIS OF PULMONARY EMPHYSEMA

As was the case in the anatomical review in Section I, this section will deal principally with chronic vesicular emphysema. Emphysema associated with the pneumoconioses and interstitial emphysema will not be discussed.

The publication of Laennec's treatise in 1819 not only laid the foundations of the systematic anatomical study of emphysema but also provided the first attempt to explain the pathogenesis of the condition. Laennec (1819) considered that the lesions were the result of a mechanical overdistension of the lung alveoli by air, a view with which few subsequent writers were to disagree. He noted that most cases were associated with bronchial catarrh and postulated that bronchial obstruction, due to the catarrh, called for an increased inspiratory effort in order to draw the air past the obstruction. Thus air could enter the lung against increased resistance, but passive expiration, dependent on the normal elastic recoil of the lungs,

would be unable to expel the air. Consequently, with successive respirations, the amount of air in the lungs increased and produced distension which in turn caused the lesions of emphysema. Laennec did not attempt to explain the exact mechanism whereby distension would damage the lungs. In this he was not alone.

This work established what was subsequently to become known as the inspiratory theory.

Louis (1837) attacked this theory on the ground that, although the bronchitis is most severe in the bases of the lungs, the major emphysematous lesions are seen in the upper portions; he did not, however, offer any satisfactory alternative proposal. Budd (1840) also attacked Laennec and maintained, without presenting any concrete evidence, that loss of elasticity was in fact the primary lesion in emphysema and that the distension followed this.

Mendelssohn (1845) propounded, apparently for the first time, what is now known as the expiratory theory. He pointed out that emphysema occurred mainly in those parts of the lungs which moved relatively little with inspiration. On the basis of his experimental experience he argued that, when expiration is obstructed, the accessory respiratory muscles are brought into operation.

These act mainly from below upwards and from behind forwards and tend, in the presence of obstruction, to drive the air into the upper and anterior parts of the lungs, causing distension of these parts with damage to the tissues and the production of emphysema. He suggested that this mechanism could operate in singing, shouting and the playing of wind instruments but emphasised that coughing was the main factor concerned in damaging the lungs by this means.

This statement of the expiratory theory by Mendelsshon in 1845 differs in no essential from the various modifications of this theory that have been advanced between his time and the present.

Rainey (1848), while he accepted that abnormal mechanical forces could distend the alveoli and perhaps injure the walls, considered that, in the presence of a primary degeneration of the alveolar walls, normal respiratory forces would be sufficient to cause the dilatation. He believed that this primary degeneration was fatty change of the alveolar walls.

Gairdner (1851) at the conclusion of a series of articles dealing with bronchial obstruction considered that the effect of this obstruction was to produce collapse of the portion of the lung

supplied. This meant that the remaining portions of the lung had to expand to compensate for the collapsed area. If considerable collapse were present then the remaining portions were expanded beyond their functional limit with the result that permanent tissue damage occurred and emphysema was produced. This compensatory or complementary mechanism was soon to become established as explaining local forms of emphysema; but never attained popularity in regard to the more generalised substantive form.

Freund (1858, 1859) was the first to postulate that the changes observed in the thoracic cage in emphysema were not secondary to the emphysema but, in fact, caused the development of the emphysema. He described degenerative changes, occurring first in the 2nd and 3rd costal cartilages, which led to swelling, elongation and ultimate calcification of the cartilage. Extension of the process led to the formation of the fixed barrel-shaped chest. Freund claimed that the degeneration preceded the emphysema and that it was the dilatation of the thorax which caused overdistension of the lung and hence emphysema. He restated his case later (Freund, 1902, 1906) but failed to produce convincing evidence that the thoracic changes were other than secondary, or at least unrelated, to the emphysema.

Rokitansky (1861) took a broader view of the causative conditions than did previous authors and it should be noted that most subsequent workers who made a comprehensive study of the condition followed his example. Rokitansky had no doubt that the most important causative condition was chronic bronchitis; but this could operate in several ways. Bronchial obstruction could impede expiration or prolonged explosive coughing could injure the lung tissues. On the other hand, if a bronchus was completely obstructed, collapse resulted and, in this area, lung tissue was destroyed with the formation of emphysema and the dilatation of the surviving bronchus. Finally, he considered that inflammatory changes could damage the lung tissues causing diminution in elasticity (he did not say "damage the elastic tissue") and the development of emphysema.

Waters (1862), who considered that dilatation of the air sacs was one of the earliest features of the disease, accepted this as evidence of loss of lung elasticity. He thought that many cases could be explained on the basis of distension of the lung following bronchial obstruction or coughing and favoured the expiratory theory. He considered that where external factors could not

be detected the lesions might result from underlying constitutional factors responsible for weakness of the lung tissue. Such hypothetical "constitutional factors" have been invoked as primary or ancillary mechanisms by not a few authors before and since. In discussing the intimate pathogenesis in cases where mechanical factors were operative, Waters believed that the sequence of events was as follows. Mechanical distension caused loss of elasticity, then followed capillary loss which led to impairment of nutrition and the development of the atrophic changes of emphysema. Waters did not develop this theme of capillary loss and on the whole appeared to regard it as an ancillary rather than a primary mechanism.

Niemeyer (1864) clearly stated that, in vicarious or complementary emphysema, whether acute or chronic, the lesions were the result of an over-expansion of lung tissue surrounding consolidated or scarred foci in the lung. This over-expansion was necessary to compensate for the lack of expansion in the consolidated or scarred foci. This view has been accepted without comment by almost every subsequent writer on the subject. In considering substantive (hypertrophic) emphysema, while Niemeyer

favoured the expiratory theory, especially with regard to the role of coughing, he stated that, whether due to inspiratory or expiratory forces, the tissue changes were brought about by raised pressure within the lungs. This produced an atrophy of lung tissue. He did not attempt to be more specific than this with regard to the intimate pathogenesis. Like Waters, Niemeyer thought that mechanical factors could not be implicated in every case and postulated that constitutional factors were operative. Niemeyer made no mention of senile emphysema.

Villemin (1866) was not satisfied that mechanical factors could explain all cases of emphysema but on the whole favoured the expiratory theory. He believed that emphysema should be regarded as a degeneration of the alveolar wall which was preceded by the hypertrophy of the epithelial cells; but preferred not to hypothesise as to the nature of the stimuli which were responsible for the changes he observed. In spite of his own restrained approach, Villemin is usually quoted as being one of the founders of the "nutritional" school of thought.

At this time Greenhow (1867) strongly favoured the expiratory theory but, like Waters and Niemeyer, believed that, in certain cases,

constitutional factors, possibly of an hereditary nature, played a large part in the development of the lesions.

Isaaksshon (1871) was the first to make a specific study of vascular changes in emphysema, employing injection techniques for his study of the capillary bed. He described the loss of capillaries from the alveolar walls as a result of mural thrombi. He believed that these changes were primary and that the subsequent atrophic changes followed the vascular loss.

Rindfleisch (1871) accepted both inspiratory and expiratory forces as being operative but also considered that the disappearance of the vessels was an essential part of the process and might form the connecting link between the mechanical and nutritional theories. He did not, however, develop this point.

Thierfelder (1872) favoured mechanical distension as the remote cause of the degeneration and disappearance of the connective tissues of the alveolar septa, and considered that obliteration of the vessels by pressure contributed to the impairment of nutrition of the affected parts.

Hertz (1874) agreed with Rindfleisch and noted that capillaries appeared to become obliterated presumably as the result of pressure and of

deformity of the alveolar walls. While he doubted that capillary obliteration was the sole explanation of the atrophic lesions, he could see no anatomical changes which preceded dilatation of the alveoli and capillary obliteration.

In the same year Knauthe (1874) published the most complete review, including theories of aetiology, that had hitherto appeared, but he did not add anything original to the problem. However, his bibliography included several references which have not been included in the present section as in general they add little to the subject.

Up to this date most workers favoured the mechanical theories of genesis; but several were inclined to what came to be called nutritional theories. In particular, the view that capillary obstruction played a part in the development of the atrophic changes was a fairly common one.

Eppinger (1876) published his account of the changes in the elastic tissues. In discussing pathogenesis, he maintained that all forms of acute emphysema and chronic complementary or vicarious emphysema were the result of inspiratory distension; but that active expiratory effort was needed for the development of the more generalised

substantive or idiopathic emphysema (chronic hypertrophic emphysema). Eppinger clearly stated that the immediate mechanism of pathogenesis was damage to the finest elastic fibres by the distending air pressure in the alveoli. This led to the dilatation of the alveoli becoming permanent and also to weakening of the alveolar walls with the appearance of fenestration and the subsequent development of the lesions. It is interesting to note that, with the exception of one or two cases of complementary emphysema developing near infarcts, Eppinger strongly denied the probability of primary nutritional changes being the cause of emphysema; but at the same time he admitted that capillaries could be stretched or occluded by the distension. In fact he put this forward, in one passage, as evidence for regarding chronic emphysema as a pressure atrophy. Further, although he had, on anatomical grounds, differentiated senile emphysema from other types of chronic emphysema, he accepted the senile form as an atrophy of the lung but did not include it in his discussion on pathogenesis.

In later works, Eppinger (1894) and Eppinger & Schauenstein (1902), he restated his views but did not modify the opinions expressed in his original article.

Orth (1887) discussed the problem under two headings, viz: atrophy and emphysema. He accepted that atrophy was an essential part of the emphysematous process but insisted that, before the term emphysema could be applied, the lung or part of the lung had to be distended in addition to showing atrophic changes.

Thus he regarded the condition of the senile lung as simple senile atrophy if there was no distension; but as senile emphysema if distension was present. In pure senile atrophy, he considered that the changes resulted entirely from senile involution or atrophy; but in senile emphysema external factors, which led to some degree of distension, were also operative.

With regard to other types of emphysema, he agreed with Eppinger and others that the common factor was distension of all or part of the lungs, due to mechanical upset of the respiratory function. He considered that inspiratory distension was more important in complementary emphysema but that expiratory effort was the main factor in substantive or hypertrophic emphysema. He discussed individual mechanisms, e.g. coughing, bronchial obstruction due to foreign body, spasm or peribronchial fibrosis, etc.

With regard to the intimate mechanism of lung injury, he considered that raised intra-alveolar pressure could cause a diminution of elasticity by direct mechanical action; but he also argued that capillaries were stretched and compressed and that this was likely to lead to local impairment of nutrition due to ischaemia, thus furthering the development of the atrophic changes. He also noted that some individuals appeared to develop emphysema without the operation of external factors and postulated that constitutional factors were responsible for this.

Grawitz (1892) objected to all mechanical theories on the assumption that all parts of the air spaces of the lungs should be at the same pressure. While his arguments have little factual basis, he advanced the view that the predominantly peripheral localisation of the larger emphysematous lesions could be explained on the basis of poorer blood supply and fewer anastomoses in the peripheral parts of the lung.

The next important contribution came from Hansemann and his pupil, Sudsuki. Hansemann (1895) studying the alveolar pores asserted that they were normal structures but that a mechanical widening of these pores in early emphysema provided an

explanation for the subsequent development of the lesions. Sudsuki (1899), on the basis of his anatomical and experimental studies, denied that there were primary changes in the elastic tissue and considered that the primary lesion was a mechanical widening of the alveolar pores. The forces responsible for this were of mechanical origin and principally of expiratory type. Hansemann (1899) extended these views and emphasised the part played by the expiratory effort of coughing which he believed to be the most important remote cause. As far as the intimate pathogenesis was concerned he thought that, in addition to simple mechanical widening of the pores, pressure necrosis occurred at their margins. However, he did not state precisely what he meant by this.

Ribbert (1902) accepted the operation of both inspiratory and expiratory factors and, like others before him, stated that the resistance of the lung could be weakened by bronchial inflammation, pneumonia, inhalation of dust or the senile state. He thought that these factors alone might enable even normal respiratory forces to produce emphysema.

Fischer (1902) found by clinical methods that no case of emphysema was present in 500 musicians playing wind instruments. Prettin &

Leibkind (1904) studied 230 glassblowers and found no increase in the incidence of emphysema in them. They concluded that respiratory effort had no effect on the healthy lung. Similar work by Lommel (1910) on glassblowers and Becker (1911) on musicians showed no increase in emphysema but only a slight elevation of the mid-position of respiration. Becker considered that respiratory obstruction by bronchitis was necessary before emphysema would be produced. Thus it appeared that at an early date the idea of strenuous respiratory effort damaging normal lungs had been disposed of. However, the notion lingered on till Christie (1939) drew attention to the above work. Nevertheless, it would be of interest if a controlled autopsy study could be performed on the lungs of glassblowers and the like.

Tendeloo (1902), on the basis of studies of the elastica and of the mechanism of lung expansion, maintained that emphysema should be regarded as a pressure atrophy dependent on mechanical causes. The atrophy was greatest in places in those areas where the lung undergoes the greatest degree of expansion in normal and abnormal respiration. He did not offer any explanation of the intimate mechanism of the pressure atrophy.

Orsos (1907) commented on the amount of theoretical writing on the subject of emphysema and confined himself fairly rigidly to a consideration of the anatomical changes. With regard to pathogenesis he considered that, in forms of chronic emphysema other than senile, inflammatory changes were of importance and appeared to be responsible for hypertrophic changes in both the elastica and reticulin elements of the lung tissues.

Bohr (1907), in sharp contrast, on the basis of spirometric studies put forward the theory that all emphysema should be regarded as truly compensatory, in the functional sense, on the ground that dilatation of the lung facilitated blood flow and thus increased the functional capability of the distended portions. His arguments were, in general, lacking in factual support.

Thornton & Pratt (1908) experimenting with isolated lungs argued that, in coughing, lung distension would be unlikely because of equilibration of the pressures in the lung. They also discounted the effects of coughing on the ground of intermittency and thought that bronchial stenosis per se was likely to be the most important factor. Like others, they pointed out the possibility of capillary occlusion by pressure as being a possible operative local factor.

Wiesel (1909) in the course of a study of arterial disease mentioned cases, presenting clinically as emphysema, where sclerosis was present in the finest branches of the pulmonary arteries. Without presenting any further evidence he postulated that much emphysema was the result of sclerosis of the pulmonary arteries.

Hofbauer (1910, 1912) on the basis of spirometric studies, while recognising the varied remote causes of emphysema, put forward the view that emphysema developed on the basis of dyspnoea which was the only common factor.

Tendeloo (1910) developed the ideas expressed in his earlier paper and accepted that any factor which caused distension of the lung could thereby produce emphysema. He argued, by analogy from rubber models, that the distension must be marked in order to produce narrowing of the capillaries. In order to obviate the difficulty of his failure to observe morphological changes in the elastic fibres, Tendeloo postulated that, when distensive forces were operative, the fibres were stretched beyond their elastic limit and did not return to their original length, exactly as happens when a rubber band is overstretched. Though this had been implied by previous authors, Tendeloo appears to be the first to state the thesis precisely.

It appears that this pronouncement was welcome and provided a convenient explanation for the general failure of anatomists to see changes in the elastica and probably helped in dispelling lingering doubts as to whether damage to the elastic tissue was in fact the primary lesion in emphysema.

Loeschcke (1911) published the first of a series of articles dealing with the pathogenesis and other aspects of emphysema. For convenience, all his work will be reviewed at this point although the papers extended over a number of years (Loeschcke, 1922, 1928a, 1928b).

In 1911 he first expressed the view that certain cases of emphysema were the result of primary changes in the thorax. Unlike Freund (1859), however, Loeschcke considered that lesions of the vertebral column (osteoarthritis in the elderly and tuberculosis in the young) caused the development of kyphosis. Depending on the site of the kyphosis there was variable deformity of the thoracic cage which caused fixation of all or part of the chest in the position of maximum inspiration. This caused distension of the related portions of the lungs and led to the development of emphysematous change in those parts.

This view was elaborated and well illustrated in the papers of 1928a & 1928b but it must be emphasised that Loeschcke did not consider that all emphysema was produced by this mechanism. He freely accepted that remote factors of pulmonary origin such as bronchial stenosis could lead to lung distension and the production of emphysema in the absence of thoracic changes and also accepted the standard view with regard to the pathogenesis of complementary emphysema.

Apart from dealing with the remote causes of emphysema, he also expressed definite views on the nature of the intimate pathogenesis of the lesions. These views are somewhat scattered throughout all his papers but are most succintly expressed in the 1922 article. In all his works he maintained that distension, however caused, is the essential factor in all forms of chronic emphysema. The distension caused obstruction to the flow of blood through the capillaries, either by mechanical deformity or by direct occlusion due to raised intra-alveolar pressure. The diminished blood supply was responsible for the tissue atrophy seen in the lesions of chronic emphysema.

Loeschcke considered that the large peripheral lesions in emphysema were, in part, the result of poorer blood supply at the periphery of

the lungs. While he admitted that primary vascular changes could produce emphysema and quoted vascular occlusion leading to infarction as an example of this, he did not consider that the emphysema in these cases was the direct result of tissue ischaemia. The ischaemia led to infarction and overdistension of the surrounding lung led to the development of emphysema of the complementary type.

Loeschcke made little mention of senile emphysema but in the 1928a article (p.689) considered that cases of emphysema in the aged fell into the group of cases resulting from primary thoracic deformity. It should be noted that he did not consider that all cases of so-called thoracogenic emphysema were in fact senile emphysema; but it is evident that he did not regard senile emphysema as an entity.

From this point onwards the general trend of work on emphysema was directed more towards functional investigation of the various haemo-respiratory abnormalities associated with the condition rather than the immediate problems of pathogenesis. Views on pathogenesis were on

the whole concerned with remote mechanisms rather than intimate pathogenesis and very little was added to the opinions already expressed by the older writers. In the main, emphysema was considered to be the result of mechanical over-distension of the lungs. This led to damage to the elastica or to impairment of the elasticity but few writers concerned themselves with the details of this alleged damage. Thus, if the interval between 1910 and the present day appears to be inadequately covered it is simply because very little was added to the fundamental knowledge of the pathogenesis of chronic emphysema in this period.

Münzer (1913) in the course of a long paper on vascular sclerosis maintained that emphysema should be regarded as a sclerosis of the arterio-capillaries of the lungs but he presented no evidence to substantiate this claim. He reasserted this view (Münzer, 1923), but again provided no supporting evidence. Engelen (1923) repeated Münzer's opinion, but H.R.Miller (1925), who found that pulmonary vascular sclerosis was associated with certain cases of emphysema in a study of 800

unselected autopsies not restricted to emphysema, considered that the sclerosis was secondary to the emphysema.

Winter (1913) accepted, on theoretical grounds, that bronchitis could cause distension by increased inspiratory effort and on equally theoretical grounds considered that prolonged or oft-repeated distension would cause permanent tissue changes, not by damaging the elastic tissue, but by tearing and obliterating the capillaries.

Stähelin (1915) published a comprehensive review but added nothing to the basic knowledge, although he favoured the purely mechanical theories of genesis. Ribbert (1916) restated his earlier views and drew attention to two local mechanisms which he thought played a part in the development of the larger lesions. He pointed out that bullae themselves could press on bronchial walls thus preventing egress of air in expiration and that peri-bronchitis with fibrosis could form a valve-like bronchial obstruction which would cause distension distal to the valve.

Hoover (1922), as a result of clinical observation and acute animal experiments, claimed that bronchial stenosis did not cause lung distension

in the absence of excessive respiratory demands and, even although he did produce lung distension in the presence of expiratory obstruction and hyperpnoea, he considered that the results invalidated the expiratory theory.

Bard (1925, 1928) discussed the problem in a general manner and while he accepted the operation of mechanical factors he maintained, without corroborative evidence, that cases occurred where these factors did not operate. Such cases, he considered, depended on constitutional weakness of the lungs. Letulle (1928) who had drawn attention to the role of inflammatory changes in emphysema inclined to the view that both mechanical strain and inflammatory weakening of the lung tissue could play a part in the genesis of the lesions but considered that in individual cases it was virtually impossible to decide which factor was the more important of the two. Like Bard, he thought that underlying constitutional factors might be responsible.

Kountz & Alexander (1933) considered the problem of senile emphysema and came to the conclusion that what was called senile emphysema on clinical grounds, was merely a senile malformation of the thoracic cage depending initially on degenerative changes in the intervertebral discs. They

did not give anatomical details of the state of the lungs but stated that these may be normal although in some cases they may be distended. They called this state "non-obstructive" emphysema.

Antoniazzi (1934a & b) on the basis of his anatomical studies believed that local inflammatory factors were more important than the remote mechanical causes. Luisada (1934) considered that the importance of inflammation lay in causing incoordination of the terminal musculature of the lungs. This in turn caused distension of the distal air-spaces with resultant hypertrophic changes in both muscles and connective tissue. Later the degenerative atrophic changes of emphysema became superimposed on the hypertrophic changes. Luisada applied this reasoning to all forms of chronic emphysema but admitted that pure atrophic changes could be responsible for senile emphysema.

In the same year, Kountz & Alexander (1934) published their review. In this they restated their views on senile emphysema and proposed that the syndrome should be called postural emphysema. With regard to hypertrophic emphysema, they considered that the most important factor in remote pathogenesis was the mechanical stress of expiratory effort in association with partial bronchial

obstruction. This led to permanent distension and rupture of the alveoli. They proposed, therefore, that hypertrophic emphysema should be called obstructive emphysema.

Christie (1934), on the basis of studies of intrapleural pressure in emphysematous subjects, considered that the primary functional defect in hypertrophic emphysema was a loss of pulmonary elasticity; but at this stage he did not express an opinion as to the pathogenesis of the condition. His views on pathogenesis were given later and appear, in most detail, in his Goulstonian Lectures (Christie, 1944). He rejected the idea that, in hypertrophic emphysema, the whole lung was over-distended. It should be noted, however, that the original exponents of the expiratory theory, which Christie now attacked, had insisted that local overdistension was required to produce the lesions in the distribution commonly seen. He also rejected vascular theories on the ground that the total flow of blood through the lungs was not reduced. At the same time he rejected the idea that emphysema should be considered as an atrophy of lung tissue inview of the fact that the aetiological factors were too diverse.

In place of these views, Christie, on the basis of the almost invariable association between chronic hypertrophic emphysema and chronic bronchitis and asthma, considered that the stresses and strains involved in the expiratory effort of coughing, and also in the spasms of asthma, damaged the alveolar walls by frequent violent changes in pressure with the result that there was diminution in elasticity. He argued that the forces were more likely to be compressive than distensive in nature. When loss of elasticity had occurred, this led to distension of the affected parts.

In other words, distension was the consequence and not the cause of the diminution in elasticity. However, Christie did not specify the exact mechanism whereby the compressive forces damaged the alveolar walls, though he appeared to regard this as a direct mechanical effect.

It should be noted that Christie specifically excluded senile and complementary emphysema from discussion and that he had had experience of three cases of emphysema where shortness of breath had preceded the cough. One of these was confirmed at autopsy.

Orsos (1936) restated his earlier views and re-emphasised that, in hypertrophic emphysema, toxic or inflammatory changes were more important than purely atrophic lesions. In addition, he considered that individual variations in the strength and amount of elastic tissue present in the lungs might have some influence on the liability of the individual to develop emphysema.

Korol (1938) in a discussion of emphysema in tuberculosis considered that ischaemia, due to endarteritis or thrombosis, was a major factor in its production.

Owren (1943) discussed three cases of giant bullae. He believed that the bullae increased in size due to pressure within the bullae resulting from valve-like action of degenerate bands of lung parenchyma. Unlike Ribbert (1916), Owren found no evidence of inflammatory change to explain the initiation of the process. Amberson & Spain (1947) described a similar case but they agreed with Ribbert in finding inflammatory stenosis of the bronchioles connecting with the bulla.

Gordon (1944) revived the theory, first suggested by Gairdner (1851), that hypertrophic emphysema is really complementary in nature. The portions of lung, served by the obstructed bronchi, remained poorly expanded or even collapsed,

while the surrounding areas became overdistended during inspiration. If this condition were prolonged or oft-repeated, permanent overdistension, i.e. emphysema, resulted. Gordon also admitted that check valve action, e.g. by a foreign body, could produce overdistension and emphysema.

Fleischner (1950) supported the view of Gordon, or more correctly, Gairdner, and considered that distension produced by this means led to a loss of elasticity and so became permanent. Fleischner also remarked, incidentally, that the capillaries appeared to be collapsed or occluded and thought that ischaemia produced by this means might lead to atrophy of the alveolar walls. Similarly, the recent functional studies of Mead et al. (1955) lend some support to Gairdner's theory as to the role of bronchial obstruction in the genesis of emphysema.

Korol (1947), without presenting any convincing evidence to support his case, asserted that sclerotic vascular lesions noted in emphysema were, in fact, primary and that emphysema should be considered as an atrophy of lung tissue due to inadequate blood supply. He accepted that mechanical respiratory factors might enhance the vascular effects.

Mayer & Rappaport (1952), in discussing the similarity between certain features of cystic lung and bronchiectasis as seen in children and emphysema in the adult, considered that both the childhood and adult lesions arose on the basis of a developmental defect with or without the operation of external factors. They did not, however, specify the nature of the developmental defect.

Cudkowicz & Armstrong (1953) employed injection techniques in a study of the bronchial arteries in emphysema and described occlusive changes in these vessels. They considered that ischaemia thus produced might be responsible for the fibrosis noted in emphysematous lesions.

Abbott *et al.* (1953) were impressed by the association of bronchial spasm and clinical hypertrophic emphysema. They commented that emphysema, histologically, is an atrophic process but did not think that sclerotic changes seen in both pulmonary and bronchial arteries were primary lesions. They accepted that a variety of irritants could cause bronchial spasm which in turn led to distension of the lung. They argued that direct occlusion of capillaries occurred in the distended portions and, at the same time, bronchial obstruction led to a reflex vaso-

constriction in the related portion of the lung. They considered that emphysema should thus be regarded as an ischaemic atrophy.

Spain & Kaufmann (1953) reporting on ten cases of hypertrophic emphysema found inflammatory changes and fibrosis round the terminal bronchioles and considered that bronchial narrowing, thus produced, caused overdistension of the distal portions of the lung with subsequent atrophy of the tissues.

Rappaport & Mayer (1954) discussed the confusion which surrounds the concept of senile emphysema and accepted the older anatomical definition of emphysema as an atrophy of lung tissue. The atrophy has been regarded by others as being a purely involutionary process but they considered that disuse was also of importance. They then restated the view, originally expressed by Orth (1887), that simple senile atrophy should not be considered pathological per se and that the term senile emphysema should not be employed unless, in addition to atrophy, distension is also present. They considered that the forces responsible for distension in senile emphysema were generalised in nature and dependent on senile changes in the thoracic cage which fixed the chest in the

position of inspiration; whereas in other types of emphysema the distensive forces were the result of pulmonary and bronchial pathology.

DISCUSSION

No attempt has been made, in the above review, to include all references to the pathogenesis of chronic pulmonary emphysema. It is believed that the review includes all of the work necessary to give a fair picture of the present state of knowledge of the remote and intimate pathogenesis of the condition. It must be pointed out, however, that emphasis has been placed on opinions concerning the intimate mechanism of pathogenesis and in this sense, only, the review is biased.

CHRONIC HYPERTROPHIC EMPHYSEMA.

It is not infrequently stated that the pathogenesis and aetiology of chronic hypertrophic (substantive) emphysema are obscure and that there are many factors involved. In spite of this, there is a fair measure of agreement with regard to certain aspects of this condition:-

a. There is little or no convincing evidence that a true idiopathic emphysema exists, i.e. emphysema developing without the operation of remote factors (e.g. bronchitis, asthma, etc.).

b. Nearly all authors accept that remote factors are responsible for the development of hypertrophic emphysema.

c. While some disagreement exists concerning the relative importance of the various possible remote factors, chronic bronchitis is accepted, in general, as the most important of these.

d. The remote factors operate by disturbing the mechanics of respiration. Thus, the lungs or parts of the lungs are subjected to over-distension or abnormal stresses and the emphysema develops as a result of these.

Much of the literature is devoted to discussion of what might be termed the intermediate pathogenesis of emphysema, i.e. the mechanism by which the remote factor produces over-distension of the lung. It appears that a reasonable case can be made for almost any of the mechanisms which have been put forward, although the classical "expiratory" theory has gained most supporters.

Much less attention has been devoted to the intimate pathogenesis, i.e. the exact manner in which over-distension or abnormal stresses damage the lung tissues and produce the emphysematous changes.

Most authors belong to the "mechanical" school of thought and have accepted that the distensive forces, however produced, damage the alveolar tissues mechanically. Following the work of Eppinger (1876), Orsos (1907) and Tendeloo (1910) it became accepted that the mechanical forces affected the elastic tissue causing ultimate rupture and disappearance of this tissue. However, as was shown in Section I, there was little or no confirmatory anatomical evidence that lesions in the elastica were present and this caused the more discerning writers, e.g. Christie (1944), to be more guarded in their views. Such authors considered that the mechanical damage produced a loss of lung elasticity but did not express a definite opinion as to the exact nature of the tissue changes involved. Sudsuki (1899) and Hansemann (1899) believed that the tissue changes were the result of a widening of pre-existing pores in the alveolar walls but this view did not account for the increase in the numbers of fenestrations seen in the development of the emphysematous lesions.

On the other hand, various writers minimised the mechanical factors and advanced what may be termed "nutritional" theories. These views never gained general acceptance but it should be noted that, commencing with Isaakssohn (1871), occasional attempts

to explain the pathogenesis on the basis of primary vascular changes have appeared in the literature. Korol (1947) and Cudkowicz & Armstrong (1953) are the most recent exponents of such views. In general, however, these authors have presented little convincing evidence in support of their claims.

Although there is little support for the view that hypertrophic emphysema is the result of primary vascular changes it should be noted that, from the time of Waters (1862), several authors have mentioned that capillary occlusion, in the course of mechanical distension, could play a part in the development of the lesions. Even Eppinger (1876) accepted this as an ancillary mechanism. Such references are made in passing and do not appear to have attracted the attention of the majority of workers. However, Loeschcke (1922, 1928a and 1928b) stated quite clearly that capillary occlusion due to distension was the mechanism which produced the atrophy of the alveolar tissues. Abbott *et al.* (1953) arrived at a similar conclusion and regarded emphysema as an ischaemic atrophy but their work was much less convincing than that of Loeschcke.

CHRONIC COMPLEMENTARY (COMPENSATORY) EMPHYSEMA.

Since the review of Niemeyer (1864) very little has been said about chronic complementary emphysema. Nearly all authors have accepted that this results from over-distension of aerated lung around a scar or consolidated focus. This subject has usually been excluded from discussion, but those who have mentioned it considered that the distensive forces were inspiratory in origin. It has been accepted that the distensive forces damage the tissues in the same manner as in hypertrophic emphysema.

SENILE EMPHYSEMA.

As was pointed out in Section I, there is very little published anatomical work on this condition. Discussion of pathogenesis is complicated by the lack of a generally acceptable definition of the term. Loeschcke (1928a) did not regard senile emphysema as an entity. However, Orth (1887) and Rappaport & Mayer (1954) considered that the senile lung should be regarded as showing simple senile atrophy if distension were absent and that the term senile emphysema should be applied only if distension were present. Distension could be produced by any of the remote factors considered above, though Rappaport & Mayer favoured the thoracogenic theory.

It thus appears that "senile emphysema" can be regarded as a "senile" atrophy of lung tissue which merges imperceptibly with hypertrophic emphysema if remote factors such as bronchitis or thoracic changes are present. It is a matter of pure speculation as to where simple senile atrophy ends and atrophy due to other mechanisms begins.

SUMMARY AND CONCLUSIONS

CHRONIC HYPERTROPHIC EMPHYSEMA.

It is generally accepted that chronic hypertrophic emphysema is the result of disturbances in the mechanics of respiration which lead to the operation of distensive forces or abnormal stresses in the lung.

Most authors agree that distension injures the lung mechanically but there are few clear statements and no direct evidence as to the precise mechanism by which the alveolar tissues are damaged.

Attempts to explain the pathogenesis on the basis of primary vascular changes have not been generally accepted.

The suggestion has been made, from time to time, that capillary occlusion in the course of mechanical distension plays a part in the genesis

of the lesions. This view has attracted little attention in spite of the convincing arguments advanced by Loeschcke.

CHRONIC COMPLEMENTARY (COMPENSATORY) EMPHYSEMA.

Complementary emphysema has received little attention and, in general, views on its intimate pathogenesis are similar to those discussed in relation to hypertrophic emphysema.

SENILE EMPHYSEMA.

It is doubtful if this should be considered as an entity. If so, it appears that it should be regarded as the result of a "simple senile atrophy" with or without the additional effect of external factors as discussed in relation to hypertrophic emphysema.

SECTION III

SPONTANEOUS PULMONARY EMPHYSEMA IN RABBITS

SECTION III

SPONTANEOUS PULMONARY EMPHYSEMA IN RABBITS

In the course of the first attempt to produce pulmonary emphysema experimentally in rabbits it became obvious that emphysematous lesions occurred spontaneously in these animals. While this is probably known to individual workers, no records of such lesions could be found in the available medical and veterinary literature apart from Zahn's personal statement to Kläsi (1886) that emphysema occurred spontaneously in old rabbits. For this reason it is felt desirable to give a detailed account of the lesions especially as this has a direct bearing on the controls for the experimental series. At the same time it was found that inflammatory changes were frequently present in the lungs of the rabbits and that, in certain circumstances, the inflammation and the emphysema were related. In view of this, a brief account of the inflammatory lesions is also included.

MATERIALS

The description which follows is based on the study of the lungs of 155 rabbits of various ages and breeds.

METHODS

Some practical details of the histological techniques are given in Appendix A. Exactly the same methods have been employed in the case of all rabbit lungs considered in this and subsequent sections of the thesis.

The lungs were removed from the chest and inflated gently through the trachea by Bouin's fluid at a head of pressure not exceeding 18" until expansion, as judged by inspection, was complete. The trachea was then tied and the lungs stored in 10% formol-saline till blocks were selected for histological examination.

In the case of lungs showing no naked eye abnormality two blocks were taken, one from an upper lobe including the anterior margin near the apex and the other at random from the lower lobe of the same side. Additional blocks were taken as indicated by the naked eye appearances. The blocks were embedded in paraffin at a pressure of -250 mm. Hg.

Ordinary thin sections were not employed routinely. The optimum thickness for a thin section of rabbit lung was found to be 20 microns; but even this was too thin for the study of the emphysematous lesions and the elastic fibres. Therefore thick paraffin sections of 100 microns were cut in every case.

Sections from each block were stained by Haemalum and Eosin and by Hart's modification of Weigert's resorcin-fuchsin elastic tissue stain, using picric acid as a counterstain. Serial sections were employed as required and to examine for organisms 4 micron sections were stained by Gram's or Giemsa's stain.

ARTEFACT

It might be thought that over-energetic tracheal inflation of isolated lungs could produce appearances similar to those of emphysema. During preliminary work this was investigated in rabbit, mouse and human lungs. These lungs were variously overdistended by intratracheal fixative or air followed by fixative. In no case was any artefact resembling emphysema produced. It should also be noted that these procedures did not increase the number of alveolar pores seen.

Shrinkage during all stages of preparation was a very variable factor and it was found impracticable to control this absolutely. In theory, the method of fixation should have ensured the production of sections all of the same degree of expansion; but in practice this was not so. Thus, it was concluded that exact measurement of alveolar size would serve no useful purpose and that extreme caution would have to be exercised in assessing even general impressions. A further complication was the fact that the alveolar size varies roughly in proportion to the size of the rabbit. Exact assessment of this was not possible. In spite of these variables it seemed legitimate to make comparisons between different portions of the same section.

MACROSCOPIC APPEARANCES

The lesions of naked eye dimensions fall into two main groups:-

a. Minor congenital anomalies.

b. Acquired lesions which are frequently associated with some degree of emphysema.

CONGENITAL ANOMALIES

Presence of a Left Middle Lobe.

A complete left middle lobe was present in 20% of cases. In 17% the lobe was absent. The remaining cases showed some degree of partial separation of the left middle lobe. This anomaly is of no importance unless a minimal degree of separation produces a small notch or cleft in the pleura of the anterior margin of the upper lobe and simulates a marginal lobule, one of the lesions to be described later.

Crenation of the Sharp Margins of the Lungs.

The sharp margins of the lungs showed some degree of crenation in 75% of cases. A marked degree of crenation (Fig. 1) may be confused with emphysematous lesions confined to the sharp margins of the lungs. However, such a degree of crenation was present in only 7% of cases.

Smooth Pleural Clefts of Developmental Origin.

In 33% of cases developmental clefts in the pleura occurred in the lower lobes, on the lateral diaphragmatic margin one or two centimetres from the anterior tip of this margin. Identical clefts occurred at various sites in other lobes in 7% of cases. These clefts are smooth and regular,

and, being unassociated with any emphysematous change, are of no importance. However, they must not be confused with pleural fissures of inflammatory origin.

Marginal Lobules and Pleural Fissures.

The name marginal lobule has been given to a lesion occurring on the sharp margin of the lungs. Many of these resemble the large hypertrophic bullae seen in human emphysema (Fig. 2) but there are all gradations between such frankly emphysematous lesions and non-emphysematous marginal lobules which, at first glance, appear to be no more than minor congenital anomalies of the pleural margin (Fig. 3).

Non-emphysematous lobules are simply portions of a sharp margin partially isolated by clefts or depressions in the pleura above and below the lobule. They are not over-expanded, the curved free margin of the lobule being sharp and the base of the lobule the same thickness as the parent margin. Indeed, it is sometimes difficult to be sure that this grade of lobule is not merely a local accentuation of the crenation which is not infrequently present on the sharp margins of the lungs.

When marginal lobules are grossly emphysematous they are striking, rounded, bullous

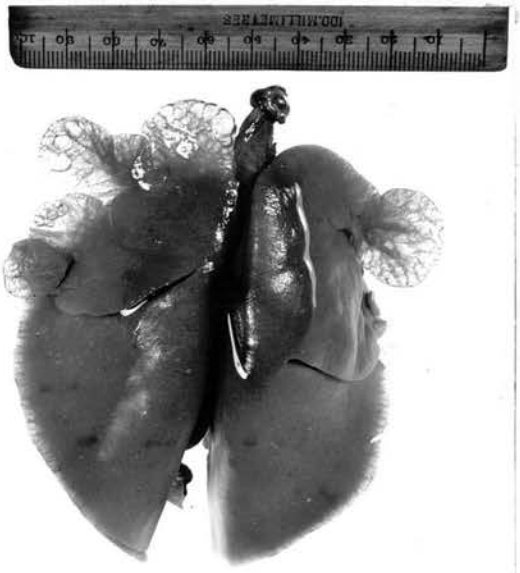


Fig. 4 (RC/51)
Multiple emphysematous marginal lobules.
Scale: mm.

Fig. 5 (RO/22)
Infolded marginal lobule.
Scale: mm.

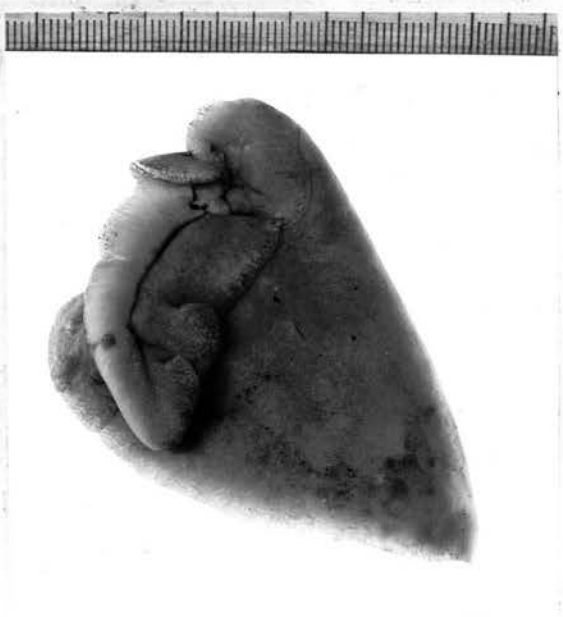


Fig. 6 (RO/36)
Pleural fissuring associated with a marginal lobule turned at right angles to parent margin. Scale: mm.

structures of various sizes. Some are attached to the parent margin by a narrow pedicle of lung tissue: others are less obviously pedunculated. There are many gradations between these two extremes. In general, the more prominent the lobules the more emphysematous they are and vice versa.

The lobules are seen on the sharp margins only. The most frequent site is the antero-medial margins of the upper and middle lobes on either side. In rare cases they do occur on other sharp margins but never on the diaphragmatic margins. They may be single or multiple (Fig. 4). Their diameter varies from a centimetre or less to even four centimetres. The larger lobules are not infrequently turned outwards on to the lateral aspect of the lung or folded on to the medial aspect (Fig. 5).

Pleural fissures may be present on the medial or lateral aspects of the lungs. They may be associated with marginal lobules or occur independently. When lobules and fissures are both present in the same lobe they may be quite separate or the fissures may be continuous with the pleural depressions that isolate the lobules from the parent margin (Fig. 6). The fissures may be easily distinguished from the smooth developmental pleural clefts in that they are much more irregular.

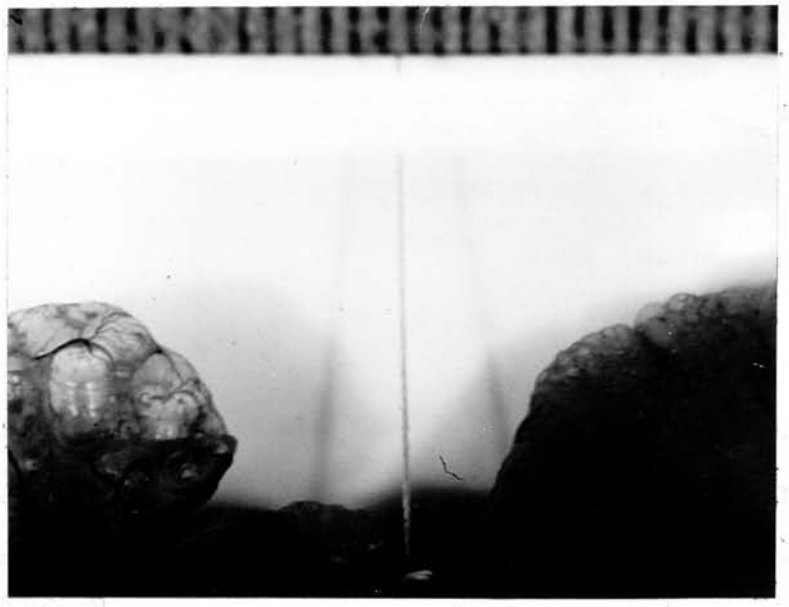


Fig. 7 (RO/4)
Vesiculation (R.U.L.) and a marginal lobule (L.U.L.)
Scale: mm.

Marginal lobules and pleural fissures have been described together as they are both believed to be the result of inflammation.

Vesiculation.

Macroscopic emphysematous lesions other than those described above as marginal lobules also occur on the sharp margins of the lung. These are comparable, in general, with non-pedunculated marginal bullae seen in man but are very small. They appear as tiny vesicles, like dew drops or froth, on the affected margin and the term vesiculation has been adopted for these lesions.

Vesiculation is most commonly seen at the apex or on the sharp antero-medial margins of the upper lobes. The lesion may occur on other sharp margins but this is unusual. As in human emphysema, a small crop of vesicles often occurs at the upper end of the aortic groove of the left lung. Vesiculation may be the only lesion present or it may be associated with marginal lobulation (Fig. 7).

It is important to note that vesiculation, visible to the naked eye, is always a manifestation of underlying microscopic emphysema; whereas a marginal lobule may be non-emphysematous.

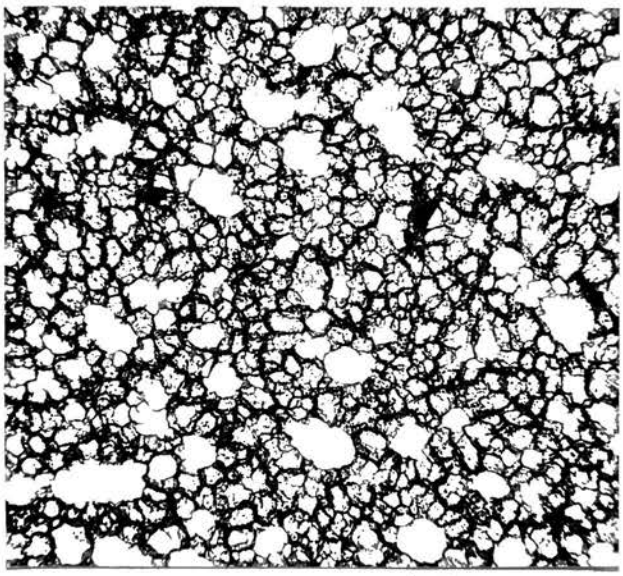


Fig. 8 (RO/19)
Normal alveolar pattern in
a thick section.
100μ. H&E. x 30.

The vesicles are generally very tiny, rarely exceeding 1.0 mm. in diameter, although they may reach 2.0 to 3.0 mm. They occur in groups and, in the more marked lesions, it can be seen that they are compound structures.

Generalised Emphysema.

While the presence of generalised emphysema may occasionally be suspected on macroscopic examination, for all practical purposes it can only be diagnosed, with certainty, microscopically.

MICROSCOPIC APPEARANCES

NORMAL HISTOLOGICAL APPEARANCES.

Thick Sections.

Most of the description which follows is based on the appearances in thick sections as this enables the alveolar walls to be seen as a whole and the lesions to be studied in three dimensions (Fig. 8).

General.

There is no material difference between the histological arrangement of the rabbit and the human lung.

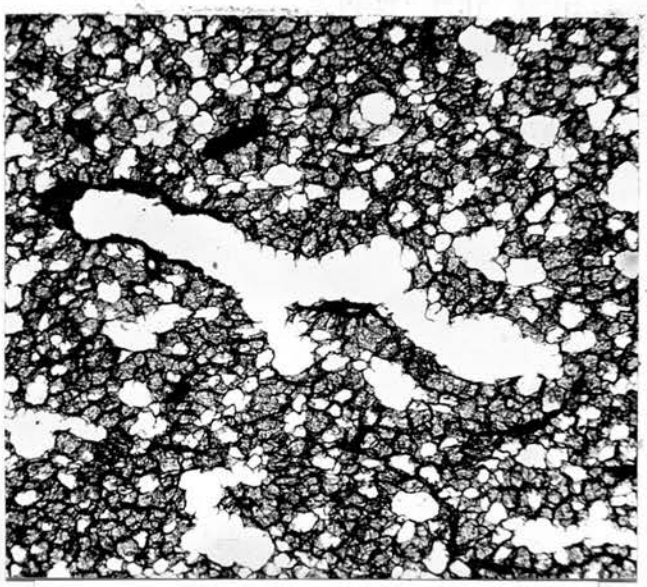


Fig. 9 (RC/99)
Alveolar ducts branching from terminal bronchiole the epithelium of which shows black.
100μ. H&E. x 30.

However, all structures are more delicate in the rabbit. The interstitial fibrous tissue is not conspicuous even in relation to large bronchi and blood vessels, while the interlobular boundaries are, for all practical purposes, non-existent. Bronchi and blood vessels bear the same general relationship to each other as in man. The arrangement of the terminal respiratory lobule is somewhat different. At most, only one order of respiratory bronchus can be detected and this is not a constant feature. In practice, the terminal bronchiole can be regarded as communicating directly with the alveolar ducts (Fig. 9). As in man, there is usually a single order of alveolar duct which communicates with the atria, which in turn communicate with the alveolar sacs. However, the term atrium will not be used as it is doubtful if this can justifiably be regarded as a separate structure in the rabbit.

The terminology employed will be as follows: Terminal Bronchiole, Alveolar Ducts, Alveolar Sacs.

Alveolar Pores.

The alveolar pores (pores of Kohn) have already been discussed briefly in Section I. In the present connection it is sufficient to state that, if thick sections are examined, there is no

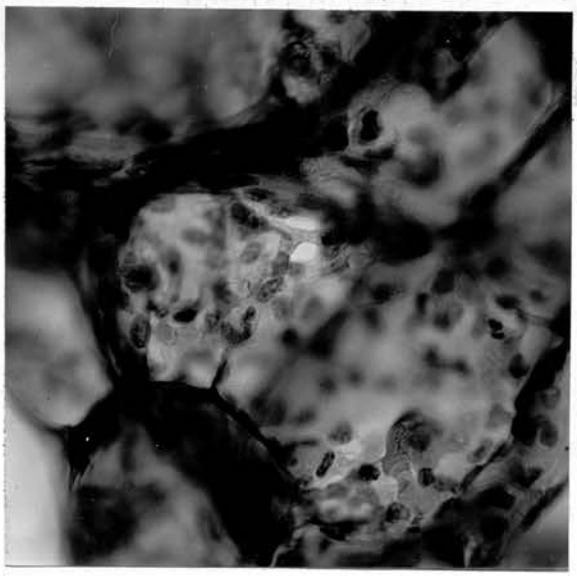


Fig. 10 (RC/138)
Alveolar pores in a young rabbit.
100μ. H&E. x 480.

Fig. 11 (RC/93)
Alveolar pores in an old rabbit.
100μ. H&E. x 480.

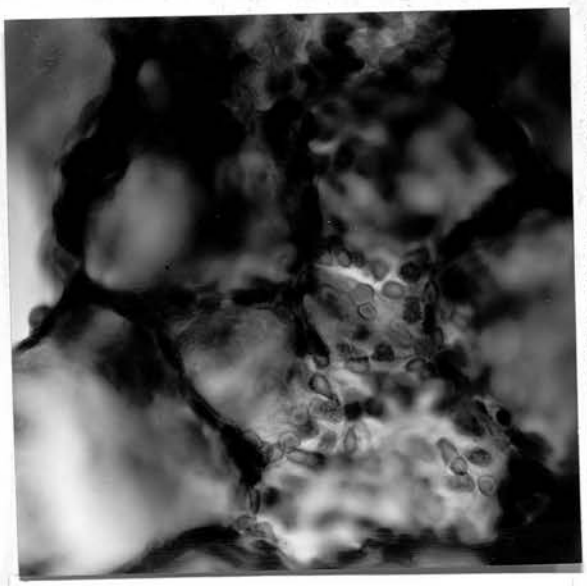


Fig. 12 (RC/50)
Artificial tear in alveolar wall.
100μ. H&E. x 480.

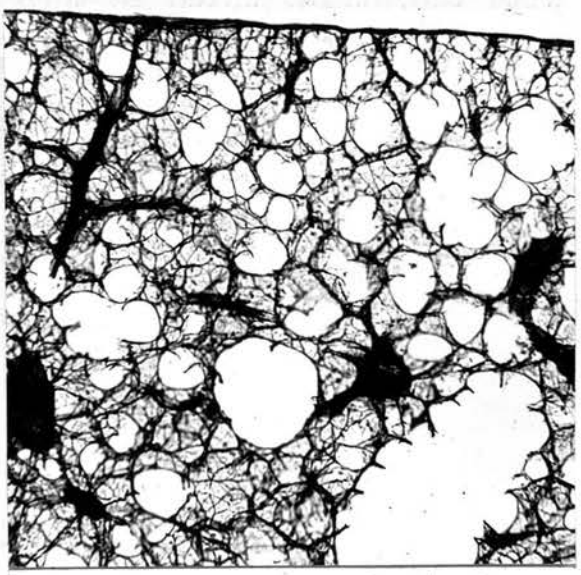


Fig. 13. (RC/136)
General pattern of elastic tissue in normal rabbit lung.
100μ. Elastic stain. x 60.

doubt that small rounded or oval fenestrations can be seen in the side walls and bases of the alveoli. Pores are not visible in every alveolus and there is considerable variation in the numbers present, but they have been seen in every rabbit lung in this series (Figs. 10 & 11).

Broadly speaking, they are very infrequent in very young rabbits and increase in numbers as the age of the rabbit increases. There is no difficulty in distinguishing a pore from an artificial tear in the alveolar wall (Fig. 12).

Elastic Tissue.

The elastic tissue of the rabbit lung, as seen in thick sections, constitutes a skeleton that links bronchi, blood vessels and pleura with the alveolar framework of the lung (Fig. 13). The alveolar elastica consists of various bundles of elastic fibres that are in continuity with the elastica of the other structures, and may be roughly divided into three main types according to distribution and thickness:-

"Thick" - Large, thick, strong-looking bundles of fibres that run round the mouths of alveoli outlining the lumina of the alveolar ducts and sacs

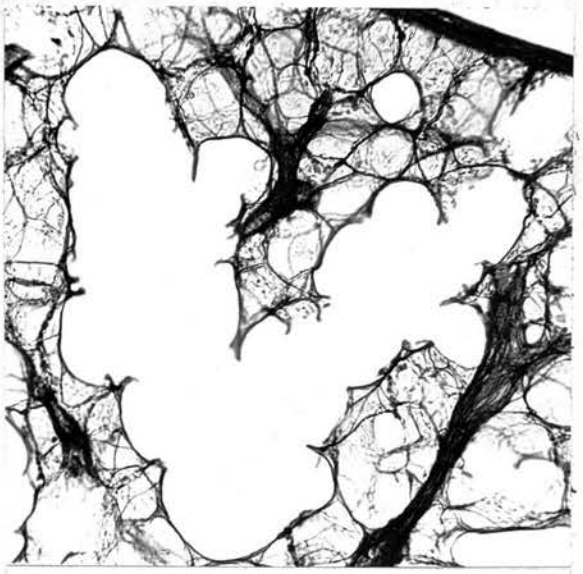


Fig. 14 (RC/84)
Thick elastic bundles outlining alveolar sacs.
100μ. Elastic stain. x 120.

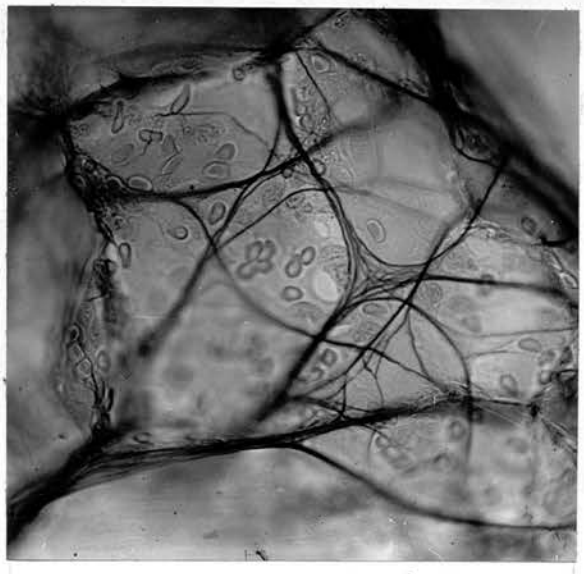


Fig. 15 (RC/44)
Crossing of medium elastic bundles and redistribution of fibres at base of alveolus.
100μ. Elastic stain. x 480.

(Fig. 14). The individual fibres of the thick bundles rearrange themselves and run down, mainly in the corners of the alveoli, as the medium bundles, to cross and rearrange themselves at the bottom of the alveolus (Fig. 15). Then these fibres run up in the corners of the contiguous alveoli which belong to the neighbouring alveolar sac or duct and continue on at the mouth of these alveoli as part of the thick bundles which outline this alveolar duct or sac.

"Medium" - These have been described as deriving from the thick bundles. In addition to running directly down in the corners of the alveolus, however, fibres or fibre bundles run off from the main medium bundles and course obliquely across the alveolar walls. These bundles or fibres may be very thin indeed but always run right across the alveolar wall to reunite with the main system of medium or thick bundles.

"Fine" - These bundles are usually, but not always, as thin as the thinnest of the medium bundles. Some of the fine bundles are derived from the medium type, some from the elastica of the terminal arterioles and others have no demonstrable connection with the remainder of the elastica. Many of these fine

bundles are remarkably tortuous, unlike the straight or only slightly sinuous thick and medium bundles. They appear to terminate in relation to the capillary walls by a fanning-out of their individual fibres over the capillary. In the rabbit the fine bundles are extremely scanty and not constantly demonstrable. Even in the same section they may be visible in some places but absent in others. In general, they are more plentiful in relation to the termination of small vessels and are more easily demonstrable in the old than the young rabbits.

The arrangement of the elastica is essentially the same as that described in man by Eppinger (1876) and Orsos (1907 and 1936) but the fine fibres are very much more scanty in the rabbit than in man. It should be noted that there is no regular spatial relationship between any of the elastic bundles and the alveolar pores. Occasionally, however, a bundle may run round the margin of a pore.

INFLAMMATORY CHANGES.

These changes constituted a subacute or chronic interstitial pneumonia and histological evidence of this was present in the lung parenchyma in 84% of cases.

In most cases the inflammation is of a very trivial extent and consists of small areas of

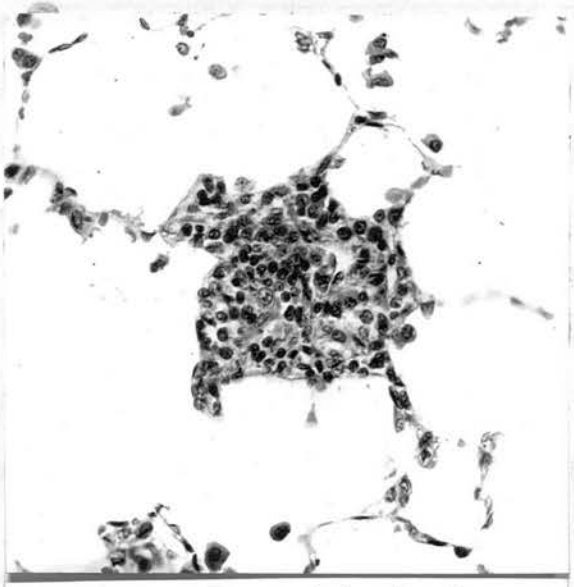


Fig. 16 (RC/146)
Minute focus of interstitial pneumonia showing mononuclear reaction. 4μ. H&E. x 300.

hypercellularity in the alveolar walls or perivascular connective tissue. These focal areas occur in all parts of the lungs but frequently only one or two foci were present in any given section. They consist of an interstitial infiltration by mononuclears and occasional lymphocytes (Fig. 16).

In other cases the lesions are not only larger but also more numerous. The infiltration is accompanied by alveolar catarrh and, in association with extreme and obvious interstitial infiltration, there are occasional small areas of alveolar consolidation. The appearances indicate that the inflammation commences as an interstitial lesion and spreads to involve the alveolar spaces only in cases of extreme activity.

When alveolar consolidation is present the exudate consists mainly of mononuclear cells with occasional giant cells and scanty lymphocytes. Polymorphs are rarely seen except when necrosis of the exudate occurs. The alveolar septa, around the area of consolidation, are hypercellular and identical in appearance to those in foci where no actual consolidation is present. There is no involvement of the lumina of bronchi except in larger consolidated areas.

All the more active lesions were examined in Gram and Giemsa stained sections but no organisms

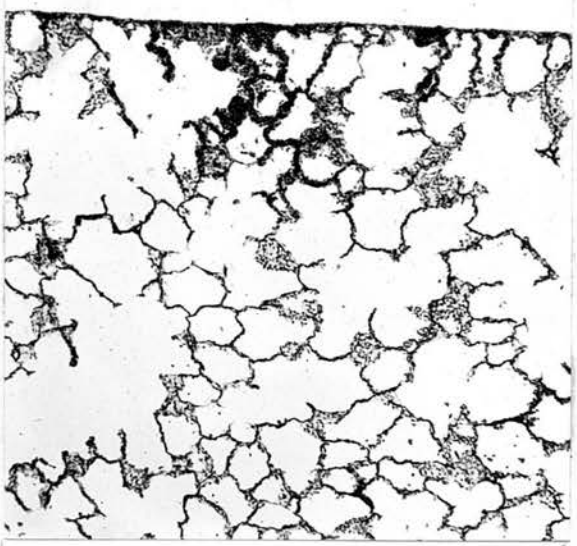


Fig. 17 (RO/19)
Thickening of alveolar septa
by interstitial pneumonia.
20μ. H&E. x 60.

or parasites could be identified. No further bacteriological studies were carried out.

When the process is of minor or moderate severity, as in most cases, there is no obvious change in lung architecture beyond the thickening of the septa (Fig. 17). But when the inflammation is severe, definite architectural changes may be produced. These arise in two ways. Firstly, there is a shortening and thickening of the alveolar septa in the inflammatory focus and secondly, when a large focus of consolidation occurs there is actual destruction of lung tissue within the area. Destruction of lung tissue arises in the course of the inflammatory reaction with or without the occurrence of necrosis and in such an area several terminal bronchioles may be seen grouped together in the inflammatory exudate, while the alveolar tissue that would normally have been associated with these bronchioles has vanished.

In the subpleural regions, deformity thus produced led to the formation of pleural fissures or marginal lobules but no signs of post-inflammatory deformity could be detected in the depth of the lung.

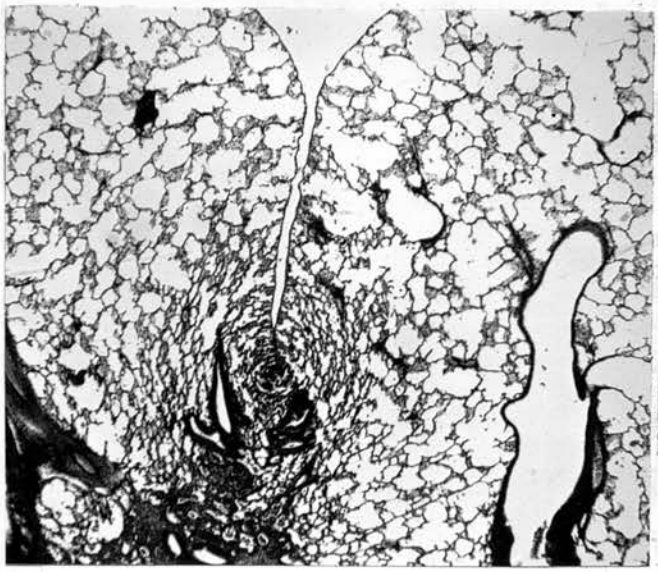


Fig. 18 (RC/146)
Pleural fissure caused by underlying interstitial pneumonia.
20μ. H&E. x 30.

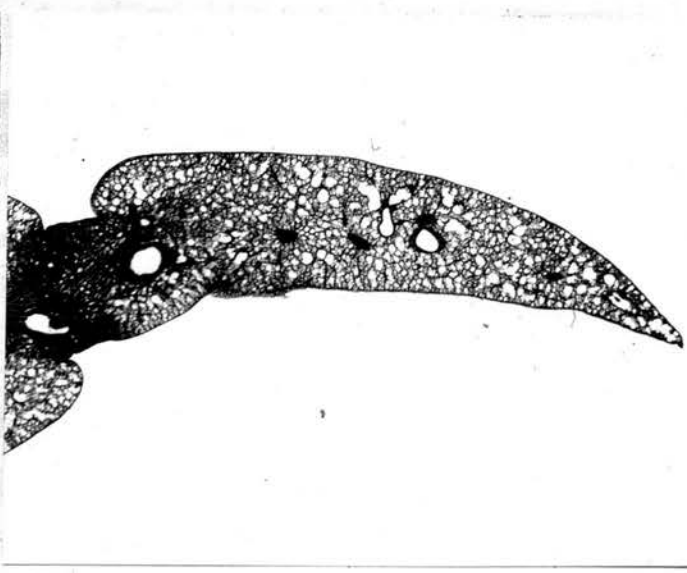


Fig. 19 (RC/146)
Gross interstitial pneumonia at base of marginal lobule.
100μ. H&E. x 7.5.

Fig. 20 (RC/146)
Detail of inflammatory focus shown in Fig. 19.
20μ. H&E. x 30.

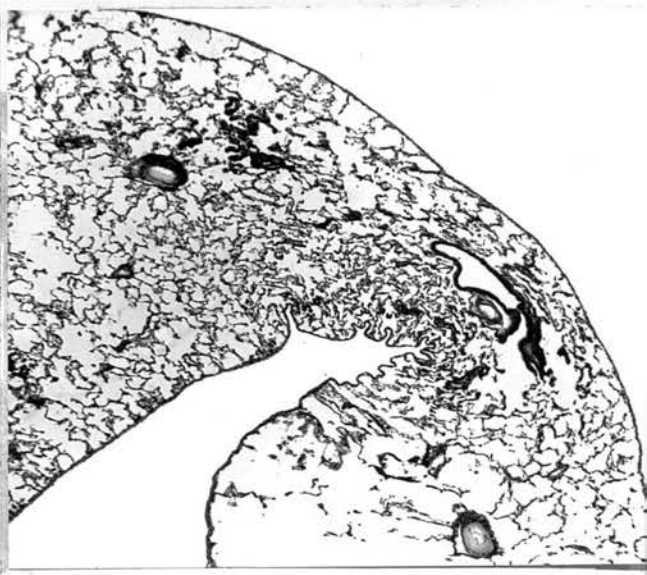


Fig. 21 (RO/6)
Trivial residual interstitial pneumonia in base of marginal lobule. 20μ. H&E. x 30.

MARGINAL LOBULES AND PLEURAL FISSURES.

The changes seen in marginal lobules fall into two groups: firstly, those concerned with the formation of the lobule itself and secondly, the development of emphysema in the lobule. The emphysematous lesions will be described later.

The lobules and fissures are both the result of the interstitial pneumonia described above. The architectural deformity associated with the severe subpleural lesions pulls down the pleura so that a fissure is formed (Fig. 18). If this happens at a sharp margin of the lung, the fissure partially cuts off a portion of the sharp margin to form a marginal lobule.

In early cases, marked interstitial pneumonia may be seen at the base of the lobule (Figs. 19 & 20) but at this stage there is no change in the lung tissue within the lobule. There is no dilatation of air spaces, fenestration or other change which could be interpreted as emphysematous. In time the causative inflammatory changes become less conspicuous and may be difficult to detect (Fig. 21). Fibrosis is not a prominent feature and usually there is only slight residual thickening of the shortened septa.

When emphysema develops in these marginal lobules it does so subsequently and bears no relation

to the degree of activity of the inflammatory changes. The emphysema does not differ histologically from emphysema in other parts of the lung.

EMPHYSEMA.

While the microscopic changes of emphysema are the same in all parts of the lung, differing only in grade of severity, it is convenient to classify the lesions according to distribution as follows:-

A. Marginal Emphysema
   i. Vesiculation
   ii. Emphysema in marginal lobules.

B. Generalised Emphysema.

Marginal Emphysema.

The term vesiculation, originally employed to describe a macroscopic appearance, has been retained to distinguish local foci of emphysema confined to the sharp margins of the lung, but not within a marginal lobule, from emphysema within these lobules. Either type of marginal emphysema may be the only lesion present or both may occur in the same lung. Either, or both, may be associated with generalised emphysema.

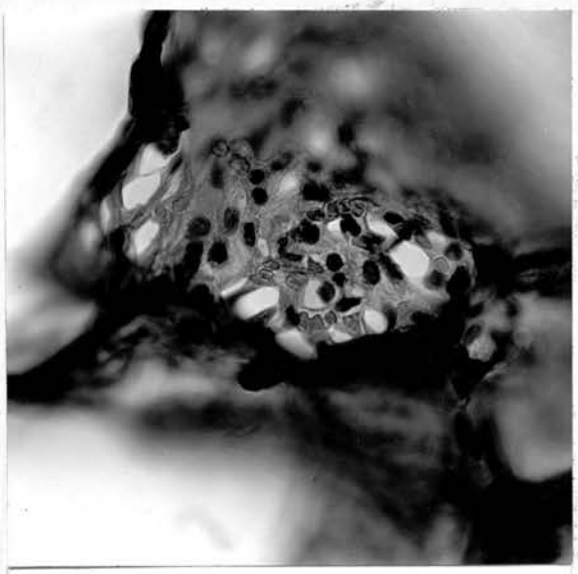


Fig. 22 (RC/129)
Small fenestrations in spontaneous emphysema.
100μ. H&E. x 480.

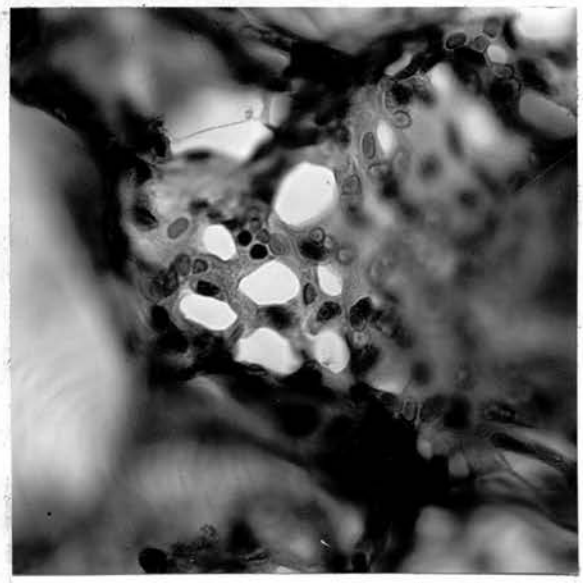


Fig. 23 (RC/129)
Large fenestrations in spontaneous emphysema.
100μ. H&E. x 480.

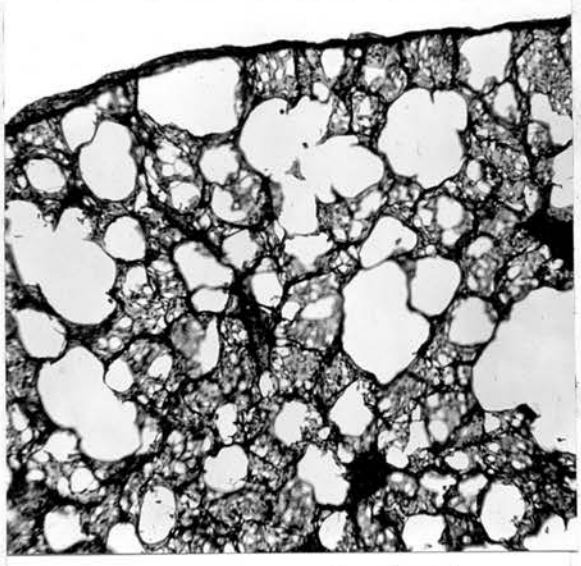


Fig. 24 (RC/129)
Marked fenestration with some loss of alveolar septa in area of marginal emphysema.
100μ. H&E. x 60.

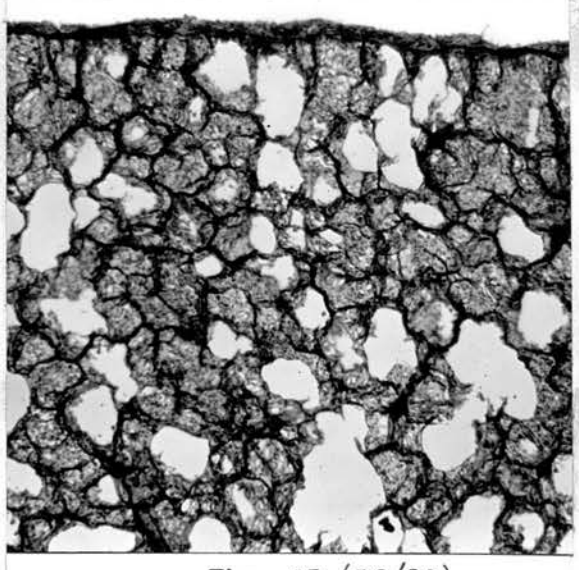


Fig. 25 (RO/22)
Normal alveolar pattern for comparison with Fig. 24.
100μ. H&E. x 60.

The earliest microscopic changes comprise a group of events. There is dilatation of the alveolar sacs and of the alveoli opening into these sacs, with widening of the mouths of the alveoli. The dilatation is readily appreciated in the marginal lesions where the rest of the lung acts as a control. At this stage another striking change is seen. Structures indistinguishable from the alveolar pores are much more numerous in the affected area than in the surrounding normal lung (Fig. 22). In addition to an increase in the numbers of the pores there is a definite increase in their size (Fig. 23). It is not possible to determine whether the dilatation of alveoli and alveolar sacs or the increase in the number of the pores comes first as the two phenomena are always seen together.

The next change is a progressive enlargement of the pores till adjacent pores merge to form larger gaps in the alveolar walls causing a striking fenestrated appearance (Figs. 24 & 25). As the fenestration develops the alveolar septa become lower and lower and finally disappear completely, leaving the alveolar sac as an expanded vesicle.

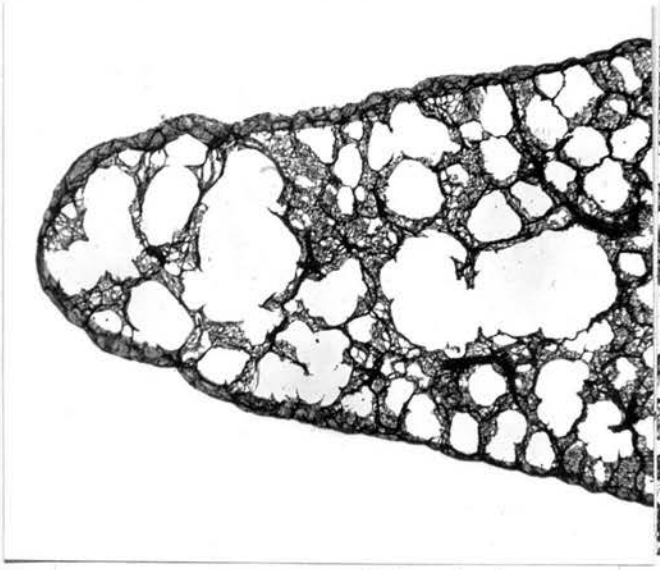


Fig. 26 (RO/22)
Vesiculation with loss of alveolar septa from alveolar sacs and ducts.
100μ. H&E. x 30.

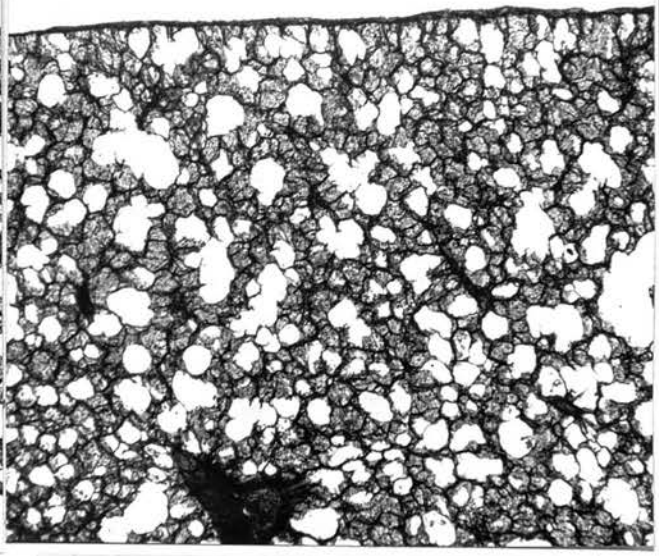


Fig. 27 (RO/22)
Normal portion of same section as Fig.26 for comparison.
100μ. H&E. x 30.

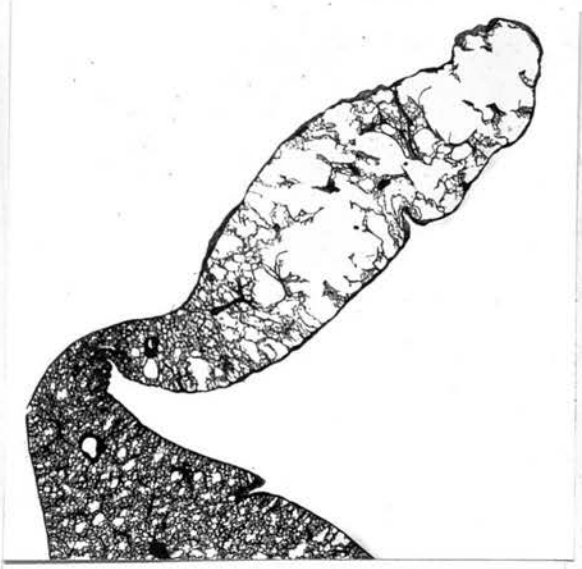


Fig. 28 (RO/6)
Marked emphysema in marginal lobule.
100μ. H&E. x 7.5.

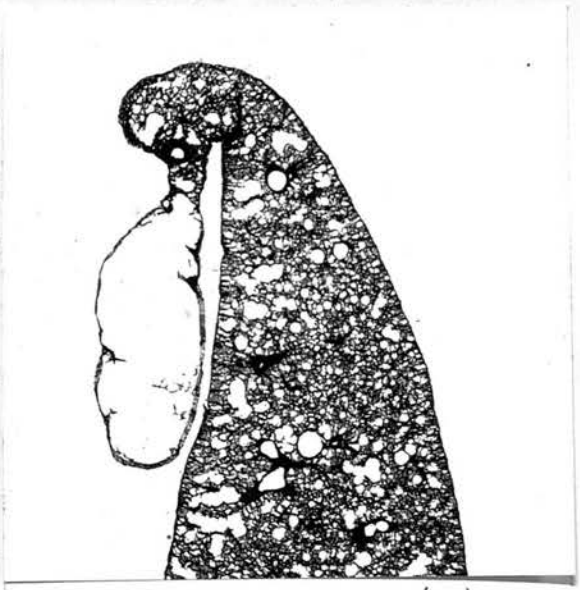


Fig. 29 (RC/21)
Virtual disappearance of tissue from small marginal lobule.
100μ. H&E. x 7.5.

When two or three neighbouring alveolar sacs are devoid of their alveolar septa they constitute a very obvious local lesion. Simultaneously, fenestration occurs in the alveoli subtended by the alveolar ducts and also in the boundaries between the neighbouring alveolar sacs. Usually this proceeds parallel to the fenestration of the alveolar walls and by the time the alveolar septa have disappeared the boundaries between the alveolar sacs and alveolar ducts show marked fenestration. Extension of the process leads to the fusion of neighbouring alveolar sacs to form a single larger vesicle which communicates with the dilated remains of the alveolar duct and presents macroscopically as vesiculation (Figs. 26 & 27).

In vesiculation the changes rarely develop beyond this point. In marginal lobules, however, the emphysematous lesions commonly continue to enlarge by further fenestration of the boundaries between the now dilated alveolar ducts so that, ultimately, the whole lobule consists of a few very large vesicles imperfectly divided from each other by sheets and strands of residual lung tissue (Figs. 28 & 29). The vesicles communicate with the

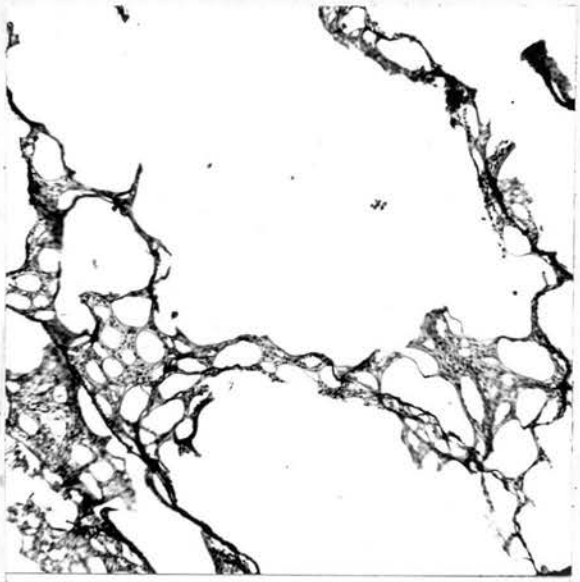


Fig. 30 (RO/6)
Fenestration in residual tissue in marginal lobule. Same section as Fig. 28.
100μ. H&E. x 60.

Fig. 31 (RC/131)
Medium elastic bundles intact and unrelated to fenestration.
100μ. Elastic stain. x 720.

terminal bronchiole while the residual sheets of lung tissue usually show a marked degree of fenestration (Fig. 30) and contain small arteries or veins which supply what remains of the capillary bed in the lobule.

During the process of fenestration the capillary bed is gradually obliterated. In fields where red blood corpuscles happen to remain as markers indicating the line of the capillaries, an appearance of stretching or narrowing of the capillary can sometimes be seen. A stretched capillary may be the only observable structure separating two pores which are on the point of fusing. In severe lesions the capillary bed diminishes as the alveolar septa and the boundaries between the alveolar sacs and ducts are destroyed until the only capillaries which remain are those in the residual sheets of lung tissue that course across the emphysematous vesicles.

So far, the elastic tissue has not been mentioned. In the earliest emphysematous lesions, no change in the elastica can be detected which precedes the appearance of fenestration of the alveolar walls. The elastic fibre bundles bear no constant relationship to the fenestrations (Fig. 31) and while bundles may run round the margins of

Fig. 32 (RC/131)
Tortuous fine elastic bundles in area of fenestration.
100μ. Elastic stain. x 720.

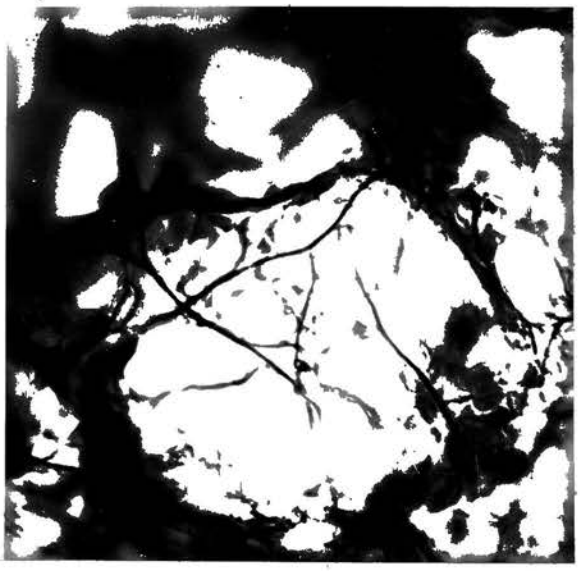


Fig. 33 (RC/131)
Normal medium elastic bundles in a fenestrated alveolar wall.
100μ. Elastic stain. x 720.

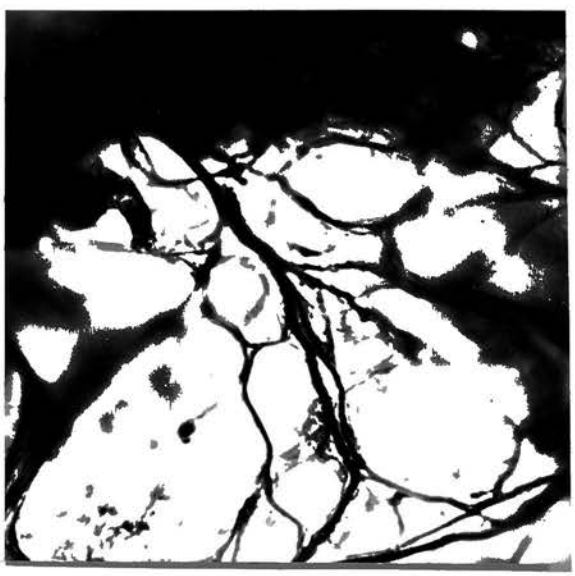


Fig. 34 (RC/130)
Displacement of medium bundle in severe fenestration. The bundle runs sinuously instead of straight.
Elastic stain. 100μ. x 720.

fenestration, or even separate two pores which are on the point of fusing, there is no evidence that the fenestrations are the result of rupture or other observable change in the elastica.

Changes in the fine bundles are extremely difficult to assess because of the extreme scantiness and variable staining properties of these elements. They vanish from the alveolar walls during the process of fenestration but even in severely fenestrated walls occasional fine bundles persist in the residual tissue (Fig. 32). Apart from the impression that they may retract and fuse with the medium bundles, no statement as to the mode of disappearance of the fine bundles can be made.

Initially the medium bundles show no change (Fig. 33) but as fenestration progresses they may be displaced by the enlarging fenestrations (Fig. 34). This leads to fusion of some of the medium bundles with each other so that fewer but thicker bundles now run across the alveolar wall. Superficial examination reveals no obvious signs of rupture of these bundles; but when careful search is made, occasionally, near a fenestration, the continuity of one of the finest of the medium

Fig. 35 (RC/130)
Appearance suggesting rupture of fine medium bundle to right of fenestration in upper part of alveolar wall.
100μ. Elastic stain. x 720.

fibres becomes interrupted and the ends appear to be retracted (Fig. 35).

It is only when the position in the alveolar wall is such that artefact can be excluded that such an appearance can be accepted as a genuine lesion. It must be emphasised that these changes indicative of rupture of the elastica are rare and there is no suggestion of a widespread loss or rupture of the medium fibres.

The thick fibre bundles initially, and even in markedly fenestrated alveoli, show no change. There is no hint of rupture. However, as the alveolar septa are destroyed the thick bundles become augmented by the addition of other bundles displaced in the process of fenestration and look thicker than corresponding bundles in less severely affected areas.

Both the thick and the medium bundles appear to be responsible for the maintenance of relatively normal architecture even in markedly fenestrated areas. However, as the destruction of the alveolar septa proceeds all the elastic bundles are displaced towards the periphery and when the septa have disappeared from an alveolar sac the elastic bundles remain intact in the rim of tissue that separates the sac from its neighbour. In this tissue there is an increased number of thick

Fig. 36 (RC/131)
Two-dimensional arrangement of elastic bundles outlining alveolar ducts after loss of alveolar septa.
100μ. Elastic stain. x 120.

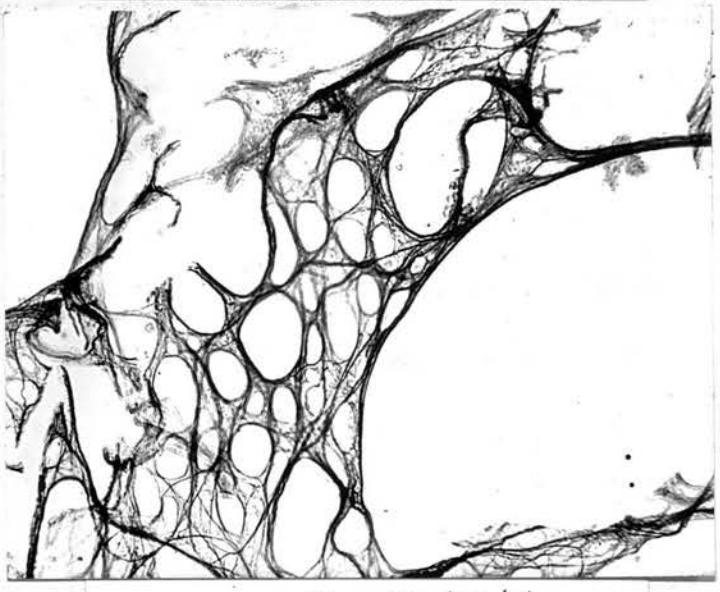


Fig. 37 (RO/6)
Intact elastic bundles running round fenestrations in residual tissue in marginal lobule.
100μ. Elastic stain. x 120.

and medium bundles per unit area, as compared with normal lung, while, in addition, the bundles are thicker and compounded of more fibres than normal.

Due to the loss of alveolar septa, the arrangement of the bundles is abnormal. The bundles no longer run in three dimensions from the mouth of one alveolus to cross at the base and then on, upwards, to the mouths of the alveoli of the adjacent alveolar sac, but are now rearranged into a simpler, virtually two-dimensional, network that outlines the simple ovoid vesicle of the alveolar sac. The individual fibres of the larger bundles re-distribute themselves at the crossing points of the network in the same manner as did the medium bundles at the base of a normal alveolus. At the same time, the alveolar ducts also lose their alveolar septa and an identical re-arrangement takes place at this level (Fig. 36).

As the marginal lesions develop, the spatial re-arrangement of the elastic bundles around the fenestrations continues, apparently reduplicating the process just described at the level of the alveolar sacs and ducts but at no stage is there evidence that rupture plays a part in the development of the lesions (Fig. 37).

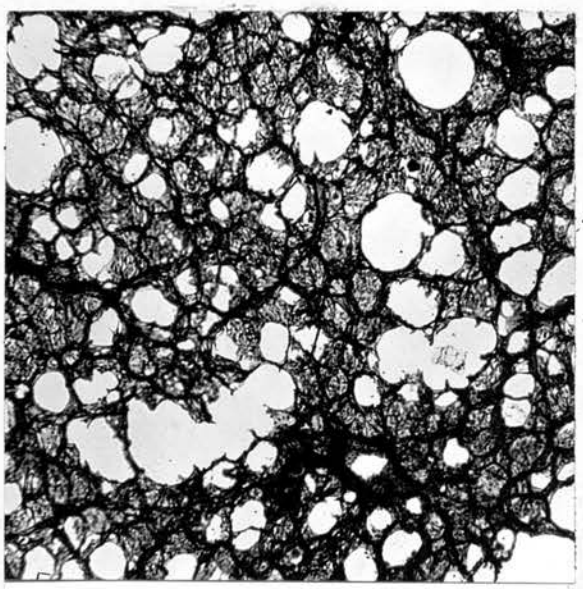


Fig. 38 (RC/72)
Early fenestration in generalised emphysema.
100μ. H&E. x 60.

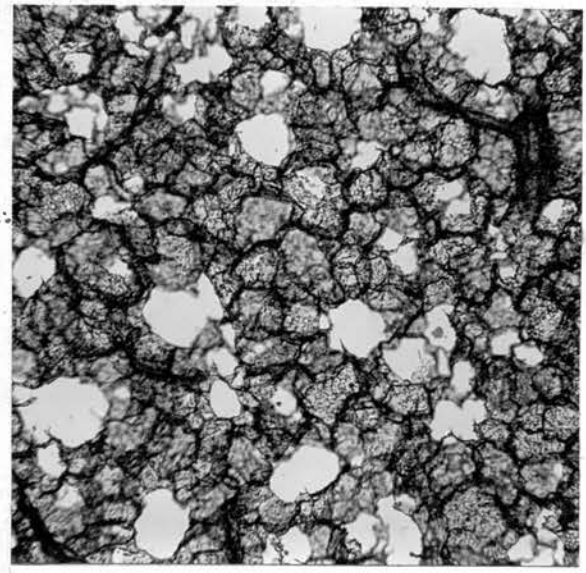


Fig. 39 (RC/99)
Normal lung for comparison with Fig. 38.
100μ. H&E. x 60.

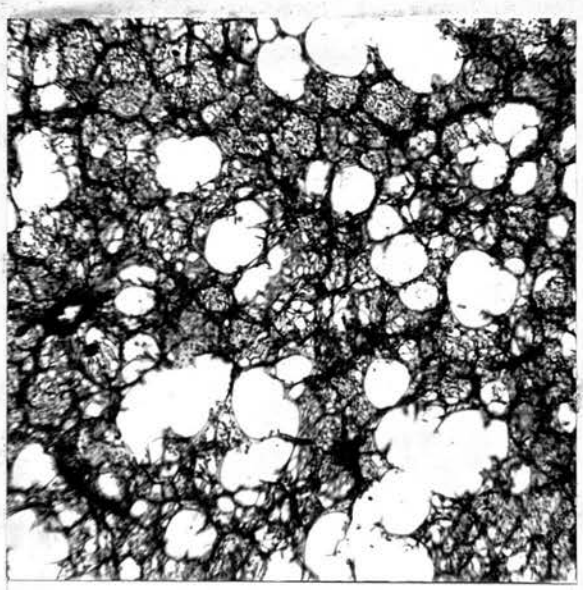


Fig. 40 (RC/90)
Later fenestration in generalised emphysema.
100μ. H&E. x 60.

Generalised Emphysema.

This may be present with or without any of the various types of local lesion. The changes in generalised emphysema are basically the same as in the marginal forms but differ in the extent to which they develop. There is destruction of the alveolar walls by fenestration but this is usually only partial.

In the marginal lesions the process of fenestration leads to fusion of neighbouring alveolar sacs and ducts with the production of large abnormal vesicles; however, in the deeper parts of the lungs this usually does not occur and there is preservation of the outlines of the alveolar ducts and sacs.

In the least severe cases of generalised emphysema there is a fairly widespread fenestration of the alveolar walls which may be of a patchy character, leaving areas of alveolar tissue that are within normal limits (Figs. 38 & 39). The fenestration increases in severity and extent until, in the most marked cases, there is widespread and obvious fenestration in nearly every part of the lung (Fig. 40). In the most advanced lesions the solidity of the alveolar walls as seen in thick sections is lost and, although the framework of

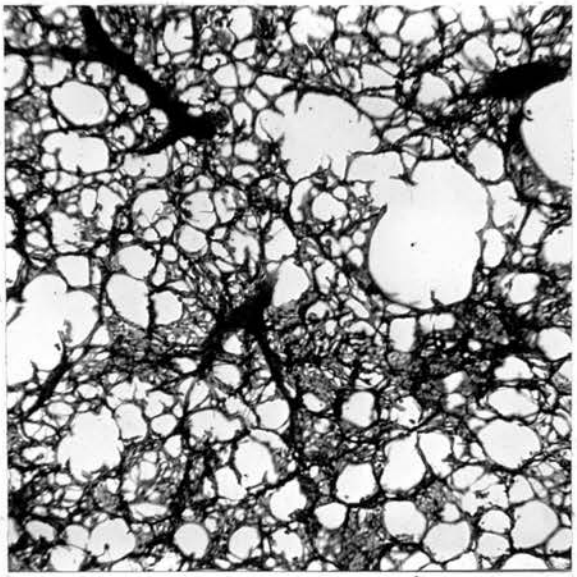


Fig. 41 (RC/92)
Gross fenestration in generalised emphysema with extensive destruction of alveolar walls.
100µ. H&E. x 60.

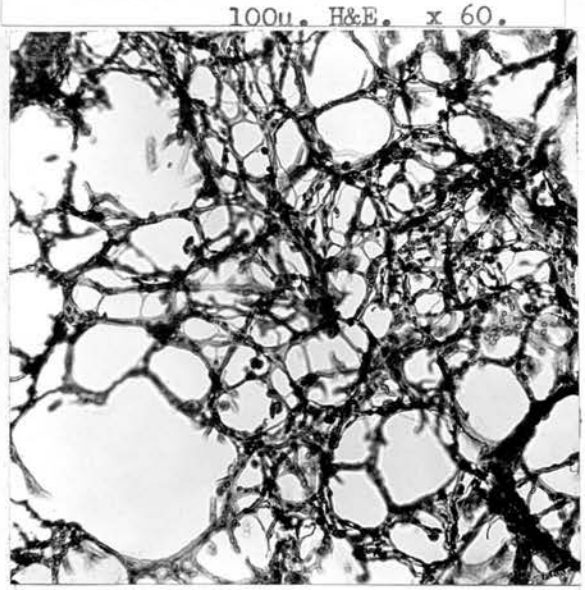


Fig. 42 (RC/92)
Detail of an area similar to that shown in Fig. 41.
100µ. H&E. x 60.

the alveolar sacs and ducts remains, the lung tissue is converted into an open network (Figs. 41 & 42). The remains of the capillary bed run in the residual strands of alveolar tissue.

In the absence of normal lung in the same section to act as a control, it is not easy to assess dilatation of the alveoli and alveolar sacs but the appearances give the impression of distension. This, added to the fenestration, makes the 100 micron sections of a lung with generalised emphysema look thinner than a similarly cut section of a normal lung.

The large expanded vesicles seen in marginal emphysema do not occur but in some cases there is loss of alveolar septa and even fusion of alveolar sacs. This occurred from time to time in the subpleural regions or in relation to larger bronchi or blood vessels in the deeper parts of the lung. When a sharp margin is thus involved the appearances previously described as vesiculation are produced.

It must be emphasised that it is necessary to employ thick sections for the certain diagnosis of generalised emphysema, especially in the less severe grades, in which there may be no obvious change in lung architecture in a thin section.

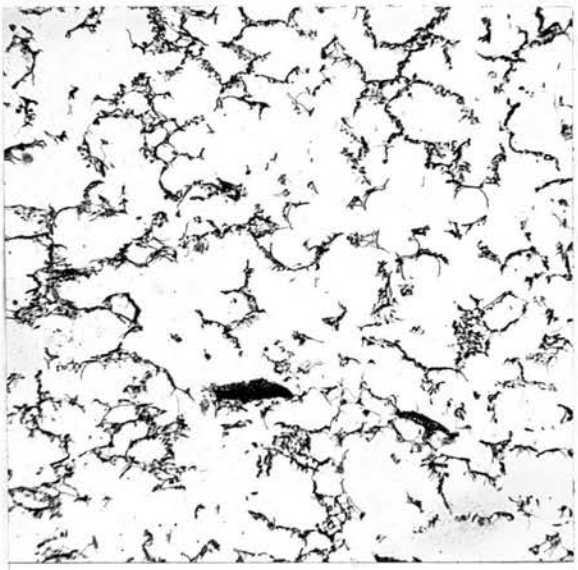


Fig. 43 (RC/92)
Apparent fragmentation of alveolar septa due to marked fenestration, as seen in a thin section.
20μ. H&E. x 60.

In the more severe grades of emphysema, however, gaps begin to appear in the line of the alveolar septa, even in thin sections (Fig. 43). The more severe the emphysema the more numerous and prominent the gaps become, until in places nearly all appearance of continuity is lost.

Broadly speaking, the changes in the elastic tissue in generalised emphysema are the same as in the marginal forms. In those cases where the emphysematous lesion consists of fenestration of the alveolar walls without complete loss of the septa, the changes are restricted to loss of the fine bundles and to re-arrangement and fusion of some of the medium bundles, as previously described. Only very occasional evidence of rupture of some of the thinner medium bundles may be seen. Usually the thick and medium bundles remain apparently unchanged and, unless there is loss of alveolar septa, they do not show the peripheral displacement seen in the more advanced marginal lesions. Indeed, these bundles appear to be largely responsible for the maintenance of the reasonably intact architecture. A lung which shows considerable fenestration in the Haemalum-Eosin stained section may show little obvious change

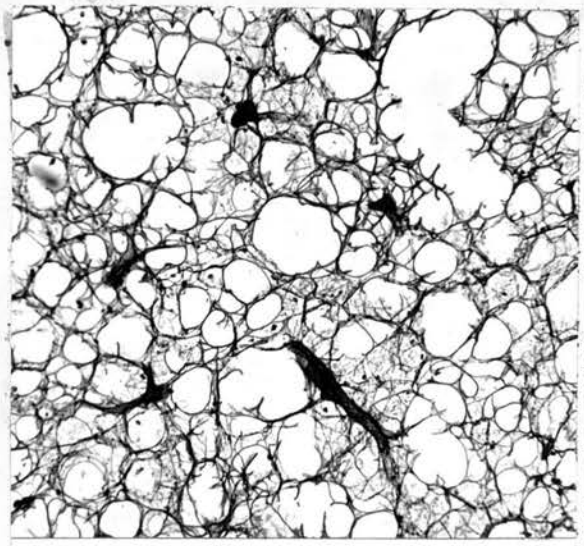


Fig. 44 (RC/92)
General pattern of elastica in severe generalised emphysema.
100μ. Elastic stain. x 60.

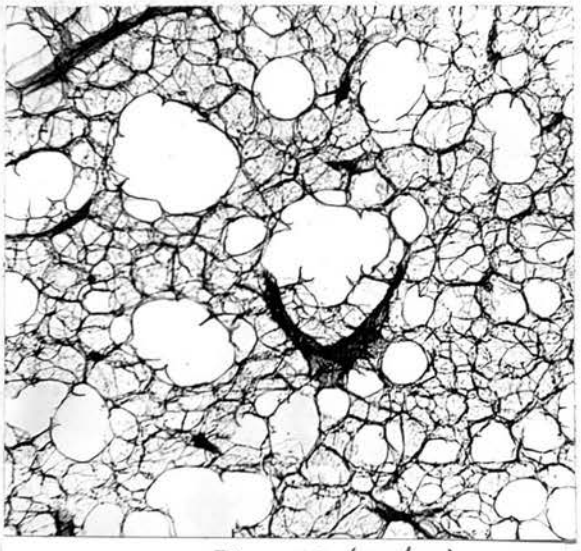


Fig. 45 (RC/49)
General pattern of elastica in a normal lung for comparsion with Fig. 44.
100μ. Elastic stain. x 60.

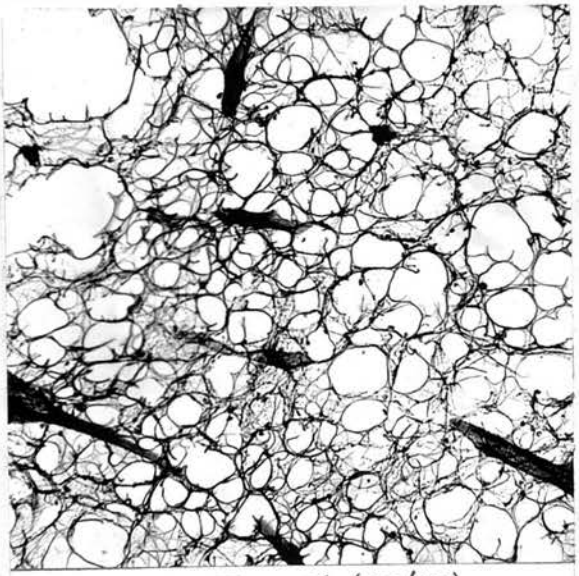


Fig. 46 (RC/92)
Elastic bundles are thicker in some areas, i.e. where destruction of alveolar walls by fenestration is most advanced.
100μ. Elastic stain. x 60.

in the elastic fibres (Figs. 44 & 45). However, when comparison is made between areas of gross and minor fenestration in the same lung, it will be seen that the thick and medium bundles are thicker in the grossly fenestrated areas, due to displacement and fusing of some of the bundles in the course of destruction of the alveolar wall by fenestration (Fig. 46). As in the marginal forms of emphysema, signs of rupture of fibres are very inconspicuous and there is no evidence of any degenerative change in the elastica.

INCIDENCE AND PATHOGENESIS OF THE LESIONS

The description is based on the lungs of 155 rabbits which have been divided into four groups:

Group A: Normal rabbits known to be between 5 and 11 weeks old.

Group B: Rabbits which had been killed in the course of experiments unconnected with the respiratory system, e.g. the administration of Synthalin A or Alloxan intravenously.

Group C: Normal rabbits known to be over 2½ years old.

Group D: Rabbits killed in the course of acute experiments involving thoracotomy with or without pneumothorax and direct puncture of the heart.

The rabbits in Groups B and D were adults of miscellaneous ages, apparently between 9 and 18 months old but their exact ages were not known. Groups A and C were collected to collate the incidence of the lesions with age.

CONGENITAL ANOMALIES.

These are unimportant except where the possibility of confusion with emphysematous lesions arises. The overall incidence has been given already.

ACQUIRED LESIONS.

The incidence of these is shown in Tables I and II. It should be noted that, in Table II, some of the lungs included in the non-emphysematous column have marginal lobules which did not show emphysema microscopically. Further, marginal lobules, vesiculation and generalised emphysema or any combination of the three may be present in the same lung. The tables give merely the overall incidence of the individual lesions. Full details

of all major lesions are given in Appendix A, Table 1.

Table I

Incidence of Macroscopic Lesions

Group	No. in Group	Macroscopic Appearances		
		"Normal"	Marginal Lobule	Vesiculation
A	20	17 (85%)	3 (15%)	0 (0%)
B	96	76 (79%)	18 (19%)	6 (6%)
C	21	12 (57%)	9 (43%)	7 (33%)
D	18	9 (50%)	8 (44%)	4 (22%)
Totals	155	114 (74%)	38 (25%)	17 (11%)

Table II

Incidence of Microscopic Emphysema

Group	No.in Group	Non-Emphysematous	Emphysematous		
			Marginal Lobule	Vesiculation	Generalised
A	20	20 (100%)	0	0	0
B	96	78 (81%)	11 (12%)	8 (8%)	8 (8%)
C	21	8 (38%)	8 (38%)	7 (33%)	11 (52%)
D	18	11 (61%)	6 (33%)	4 (22%)	1 (6%)
Totals	155	117 (75%)	25 (16%)	19 (16%)	20 (13%)

The tables do not include details of pleural fissuring. This was, in fact, 17% in the combined series.

Marginal Lobules.

The incidence of marginal lobules ranges between 15% and 44% in the various groups with an overall incidence of 25%. Inflammatory changes were present in all the lobules and the appearances leave no doubt that these lesions are of inflammatory origin. It would be expected that a batch of rabbits with a low incidence of pulmonary inflammation would have a correspondingly low incidence of marginal lobules, but the present series can give no absolute proof of this.

The figures show that of a total of 38 cases of marginal lobules only 25 showed emphysema in the lobule microscopically. This supports the view that the lobules are first formed by inflammatory changes and subsequently develop emphysema. There is some evidence to suggest that age plays a part in the development of emphysema in marginal lobules. Not one of the three cases in the young rabbits of Group A showed emphysema, whereas 11 out of the 18 cases in the miscellaneous adults of Group B and eight out of the nine cases in the old rabbits of Group C were emphysematous.

The present material provides no direct evidence as to how the emphysema develops but there appear to be two main possibilities. Firstly,

the anatomical connection which remains between the lobule and the parent lung may be such that normal (or abnormal) ventilation of the lung causes abnormal distension of the lobule. Secondly, the inflammatory changes themselves may so alter the nutrition of the lobule that even normal respiratory distension may cause the emphysema. Naturally both these factors could operate together.

Theoretical considerations apart, marginal lobules must be regarded as a common naturally occurring lesion and should be disregarded if they occur in any experimental series. Furthermore, these lobules so commonly become emphysematous in animals with otherwise normal lungs that marginal emphysema of this type can not be considered significant in the experimental production of emphysema.

Vesiculation.

In contrast to marginal lobulation, vesiculation is always a naked eye manifestation of underlying microscopic emphysema. As in the case of marginal lobules, age appears to be an important factor. Microscopically, the incidence ranged from nil, in the young rabbits of Group A, to 33% in the old rabbits of Group C. In the miscellaneous adults of Group B the incidence was 8%.

Histologically, vesiculation presented as the only emphysematous lesion in a few cases but more often it appeared merely to be the local accentuation of a generalised emphysema at the sharp margin of the lung. Foci of interstitial pneumonia were found in the areas of vesiculation in 17 of the 19 cases but in most instances there was not the anatomical deformity of the lung framework as seen in the case of marginal lobules. Nevertheless, in one or two cases there was a suggestion that vesiculation might result from a local deformity of architecture; in addition, vesiculation did occur more frequently in lungs where marginal lobulation was also present. Thus, although no positive proof can be offered, it is thought that, in some cases at least, vesiculation may have a pathogenesis similar to marginal lobulation.

In view of this, care should be taken in interpreting the significance of vesiculation in any attempt to produce emphysema experimentally.

Generalised Emphysema.

The different age incidence in the various groups is striking. No generalised emphysema (or emphysema of any type) was found in the young rabbits of Group A. The incidence was 8% in the miscellaneous adults of Group B and 52% in the old rabbits of Group C.

The present series does not include any animals known definitely to be between the ages of 9 and 18 months; but it is believed that most of the miscellaneous adults were between these ages. Subsequent experience, with a group of 25 rabbits known to be under 18 months old, showed a similarly low incidence of generalised emphysema (Section IV).

Age could operate in two ways in determining the increased incidence of generalised emphysema. Firstly, the emphysema might be a simple senile manifestation; and secondly, the emphysema might be the result of other causative factors which had to be operative over a long period of time before producing emphysema. Obviously the lesions could result from a combination of senile change and other causes.

There was no sign of bronchial disease per se and, further, there were no detectable differences in the elasticae of the young and old rabbits. Thus, it appeared that the only lesion likely to be responsible for the development of generalised emphysema was the interstitial pneumonia.

Therefore, the material was analysed to see if there was any correlation between the two lesions.

The severity of the interstitial pneumonia has been classified on an arbitrary plus basis. Cases showing even a single small focus were recorded as one plus. Those showing several fairly obvious, but still almost wholly interstitial, foci were recorded as two plus. Cases where alveolar consolidation was present in focal lesions, and where there were more diffuse interstitial lesions in many areas, were recorded as three plus.

It must be emphasised that the lesions graded as one or two plus were of very minor severity and that even the most severe lesions, in general, retained their patchy nature. Further, foci of interstitial pneumonia related to marginal emphysema have been ignored. The grading only takes account of what might be called generalised interstitial pneumonia where the lesions, usually trivial, are scattered in all parts of the lung.

The degree of generalised emphysema has also been assessed on a plus basis: details of this are given in Section IV.

Table III shows the total incidence of generalised emphysema and generalised interstitial pneumonia.

Table III

Incidence of Generalised Emphysema and Interstitial Pneumonia

Group	No.in Group	Generalised Emphysema	Interstitial Pneumonia
A	20	0	16 (80%)
B	96	8 (8%)	79 (82%)
C	21	11 (52%)	20 (95%)
D	18	1 (6%)	16 (89%)

These figures show that there is no correlation between the total incidence of generalised emphysema and interstitial pneumonia in the various groups.

Table IV shows the incidence of the various grades of interstitial pneumonia in each group.

Table IV

Incidence of Interstitial Pneumonia

Group	No.in Group	Degree of Interstitial Pneumonia			
		0	+	++	+++
A	20	4 (20%)	11 (55%)	4 (20%)	1 (5%)
B	96	17 (18%)	37 (39%)	29 (30%)	13 (30%)
C	21	1 (5%)	6 (29%)	11 (52%)	3 (14%)
D	18	2 (11%)	6 (33%)	4 (22%)	6 (33%)

These figures show that while there is a lower incidence of the more severe inflammatory changes in the young rabbits of Group A, which had no generalised emphysema, there is no significant difference in the incidence of these lesions in the other groups. Thus the severity of the interstitial pneumonia bears no direct relation to the total incidence of generalised emphysema.

Table V shows the relationship between the degree of generalised emphysema and of interstitial pneumonia in all groups.

Table V

Degree of Generalised Emphysema and Interstitial Pneumonia

Degree of Generalised Emphysema	No. of Cases	Degree of Interstitial Pneumonia			
		0	+	++	+++
0	135	24 (18%)	54 (40%)	38 (28%)	19 (14%)
+	10	0	3	6	1
+ +	6	0	2 (30%)	4 (50%)	0 (20%)
+++	4	0	1	0	3

The figures show that generalised interstitial pneumonia is present in each of the 20 cases of generalised emphysema. The incidence of the

severest grade of pneumonia is the same in the emphysematous and the non-emphysematous and, on the whole, there is little correlation between the degree of inflammation and of emphysema. The results do not suggest a direct relationship.

Histologically, there is no direct relationship between the foci of inflammation, which are essentially of a trivial, patchy nature, and the generalised emphysema. It should be noted that there is no relation between alveolar catarrh and emphysematous fenestration.

It is concluded that the relationship, if any, between interstitial pneumonia and generalised emphysema is indirect; perhaps disturbance in respiratory mechanics is the mechanism involved, e.g. excessive sneezing.

Regardless of whether generalised emphysema is to be regarded purely as a senile lesion or as the indirect result of interstitial pulmonary inflammation or as a combination of both factors, the significant fact is that after the age of 2½ years over 50% of rabbits may be expected to have some degree of it as a naturally occurring lesion.

INFLUENCE OF ACUTE OPERATIVE PROCEDURES.

The animals of Group D, which were miscellaneous adults, died during or within a few hours of operations on the thorax. Nearly all of them had tachypnoea. Table II shows that the incidence of emphysematous lesions in this group did not differ significantly from that in the other adult group. It should be noted that severe tachypnoea, which was frequently present before death, did not cause increased fenestration of the alveolar walls, as is shown by the low incidence (6%) of generalised emphysema in this group.

SUMMARY AND CONCLUSIONS

As a basis for the study of experimental emphysema in rabbits a study was made of the types of spontaneous emphysematous lesions and associated inflammatory changes found in the lungs of 155 rabbits.

The following conclusions have been reached:-

The microscopic appearances of all types of emphysema are essentially the same and are those of

an atrophy of lung tissue which manifests itself in a state of progressive fenestration of the alveolar walls. The elastic tissue shows no changes which ante-date the appearance of fenestration and seems to be the most resistant component of the alveolar framework.

Generalised emphysema can only be diagnosed, with certainty, microscopically. It is not the direct result of inflammatory changes but there may be an indirect relationship. The incidence of generalised emphysema is nil in young rabbits but rises to over 50% in rabbits over 2½ years old. It is thought that the expected natural incidence in adult rabbits under 18 months of age would be of the order of 10%. Various acute operative procedures did not influence the incidence of generalised emphysema.

Marginal lobules are formed by inflammatory deformity of the sharp margins of the lungs. Initially, the lobules are not emphysematous but become so later. They should be disregarded in assessing the results of attempts to produce experimental pulmonary emphysema.

Macroscopic vesiculation along the sharp margins of the lungs is always a manifestation of

underlying microscopic emphysema. Vesiculation may occur as an isolated lesion but it is frequently merely a local accentuation of a generalised emphysema. However, some examples of vesiculation may be the result of local inflammatory changes as in the case of marginal lobules. Therefore care is needed in assessing the significance of vesiculation in the experimental production of pulmonary emphysema.

The inflammatory changes are those of a subacute or chronic interstitial pneumonia which does not involve bronchi except in the presence of alveolar consolidation.

The causative organism of the pneumonia was not identified.

SECTION IV

EXPERIMENTAL PULMONARY EMPHYSEMA

SECTION IV

EXPERIMENTAL PULMONARY EMPHYSEMA

This section specifically excludes the subject of interstitial emphysema and is solely concerned with vesicular emphysema.

In the past, several different methods of producing pulmonary emphysema experimentally have been employed. Nearly all these methods depend on some procedure calculated to produce abnormal distension of all or part of the lungs. It should be noted that many of the authors did not state whether the emphysema they described was acute or chronic.

REVIEW OF THE LITERATURE

PNEUMOTHORAX.

Bayer (1870) was probably the first to claim to have produced experimental emphysema. He induced a pneumothorax in an unstated number of rabbits and reported emphysema at the margins of the contralateral lungs after periods of half an hour or more. Kläsi (1886), also using rabbits,

did not confirm the acute results but stated that marginal emphysema was produced in one rabbit which survived pneumothorax for nine days.

The descriptions of Bayer and Kläsi leave no doubt that they were dealing with chronic emphysema; but it is likely that this was of the spontaneous marginal variety.

VAGAL STIMULATION.

Riegel & Edinger (1882) and Sihle (1903) studied the effect of vagal stimulation in dogs and rabbits but claimed to have produced only a simple over-distension of the lungs - not emphysema. However, Brown-Sequard (1885) stated that even brief stimulation of the vagus or vagal centres produced emphysema in rabbits. He presented no anatomical evidence to substantiate this.

NASAL OBSTRUCTION.

Bullara (1900), in dogs, and Consteau (1900), in rabbits, claimed to have produced emphysema by obstruction of the nares for periods of two weeks to seven months; no details were given and no controls were employed.

SIMPLE STENOSIS OF TRACHEA.

Köhler (1877), investigating the physiological effects of tracheal obstruction produced by means of lead wire bent round between the trachea and oesophagus, reported widespread vesicular and interstitial emphysema in about 20 rabbits which survived for 3-4 weeks. Neither controls nor diagnostic criteria were mentioned.

Following Köhler, Hirtz (1878) narrowed the trachea by ligature in two rabbits which survived four and nine days respectively, and reported generalised and marginal emphysema in the lungs on macroscopic examination. In three other rabbits this effect was abolished by section of the phrenic nerves; no controls were employed.

Sudsuki (1889) ligated the tracheas of nine rabbits. In six no stenosis was produced and no emphysema was found. In three, tracheal stenosis was present at autopsy, 46, 60 and 84 days after ligature. Marked marginal emphysema was present in one but the changes in the others were "less striking". The lesions were confirmed histologically but with some reservation in the least marked cases.

Nissen & Cokkalis (1925) inserted a metal ring into the tracheas of five cats and, after an unstated period, found histological vesic-

ular emphysema in three, the others being unsuitable for examination. No controls were employed. Nissen (1927), in another uncontrolled study, obstructed the trachea by packing the mediastinum with paraffin wax or plaster of paris. Three dogs, four rabbits and three cats survived for periods of 3-6 months. Lung distension was found in eight of the animals. Local traces of true emphysema were present in five but the remaining three showed more marked lesions with bullae. Nissen accepted that these were the result of tracheal obstruction.

Loeb (1930) tried several methods of producing tracheal or bronchial stenosis without long-term success. Finally, he succeeded in keeping eight out of 24 dogs alive, for periods of up to 14 months, after the insertion of a brass tube with a narrow lumen into the trachea. He found no macroscopic emphysema. Microscopically there was, perhaps, some dilatation of alveoli and alveolar ducts, but he did not accept these changes as justifying a diagnosis of true emphysema.

EXTERNAL RESPIRATORY VALVES.

Priese (1909) applied a face mask, made out of a filterfunnel, to seven rabbits for periods of 1-1½ hours daily over three months. The mask was designed to obstruct both phases of respiration

but no emphysema was produced.

Schall (1909) used a mask of celluloid construction which could also obstruct both phases of respiration. This was applied to three dogs for 22 hours daily, for periods of between nine and 11 months, but there was no histological sign of emphysema.

INTERNAL RESPIRATORY VALVES

Friedman & Jackson (1917) obstructed expiration by means of a valve attached to a T-tube inserted into the tracheas of an unstated number of dogs. The experiments lasted only 3-8 hours, but they stated that emphysema was present in the lungs. No details were given. Similar claims were made by Pfanner (1922) in acute experiments in rabbits and dogs. His diagnosis of emphysema was macroscopic and he considered that expiratory obstruction was the important factor.

Harris & Chillingworth (1919), in a deliberate attempt to produce chronic emphysema, obstructed expiration by means of a ball valve in the tracheas of 25 dogs. The animals survived from one day to three weeks. All surviving more than two days were reported to show some degree of emphysema, which was stated to present the usual

features, naked-eye and microscopically, seen in human emphysema. The illustrations do not suggest more than a marked degree of distension of the lungs and Loeb (1930) has refused to accept these changes as emphysematous. Harris & Chillingworth used an unstated number of controls with non-functional valves and discounted the significance of minor changes seen in these lungs.

Kountz et al. (1929) used the method of Harris & Chillingworth in 16 dogs and, although no autopsy details were given, accepted that emphysema was produced on the basis of raised intra-pleural pressure. Sciuto (1945) also used the same method, in dogs, but gave no details of the numbers used or of controls. His illustrations are not convincing. Hinshaw (1938) described an improved version of the ball valve which he used in various animals for periods of up to 18 months. However, no details are given beyond the statement that subpleural emphysematous vesicles were frequently observed.

Paine (1940) devised a flap valve which could be so placed in the trachea as to obstruct expiration or inspiration. Three control dogs, with blank valves, were used. Two of these showed minimal histological emphysema after a period of nine weeks. In ten dogs with expiratory obstruction,

which survived from five to 23 weeks, some evidence of macroscopic emphysema was present in six. Microscopically, acceptable emphysema was present in six out of the ten, three had minimal changes and one was normal. In nine dogs with inspiratory obstruction which survived from four to 30 weeks, definite macroscopic and microscopic emphysema was present in six. Paine assessed his material critically and his illustrations are convincing.

ACTUAL INCREASE IN SIZE OF THORAX.

Nissen (1927) detached the ribs from the sternum in twelve dogs and inserted metal bars which expanded the thorax. Of these only six survived more than a day and, after periods of between two and 12 weeks, no emphysema was seen in the survivors. He also pulled down the diaphragm and sutured it to the anterior abdominal wall and costal margins in four dogs. Two survived for five months. In two other dogs ventral hernia was produced to lower the diaphragm. No emphysema was seen in any of these animals.

Paine (1940), in addition to his work with valves, enlarged the thorax in nine dogs by suturing "reefs" in the diaphragm. Two died shortly after the operation. The remaining seven

survived and were examined at intervals between 16 and 25 weeks. All but one had naked-eye emphysema and in all seven significant microscopic emphysema was present. Paine considered that the emphysema produced by this method was more marked than that in the dogs with valvular tracheal obstruction, noting that, in addition to marginal emphysema, the changes were frequently present in blocks taken from the centre of the lungs.

RELATIVE INCREASE IN SIZE OF THORAX.

While most of the papers in this group are studies of the fate of the remaining lung after pneumonectomy, rather than deliberate experimental studies of emphysema, it seems desirable to include them in this review.

Möllgaard (1909) performed pneumonectomy on three puppies, seven days old, which survived for 2-3½ months and on five adult cats which survived for 14 days. He found the lungs enlarged but did not consider the enlargement a true emphysema as in the human. He regarded it more in the nature of an hypertrophy. He considered that the appearances in one of the cats verged on the pathological. It is interesting to note that the illustration of this lung, his Fig. 7, is more suggestive of emphysema than many other illustrations in the

literature and shows fenestration in all areas although he does not comment on it.

Nissen (1927) ligated selected main bronchi or pulmonary arteries in an unstated number of cats and dogs and claimed that the remaining aerated portions of the lung showed compensatory emphysema after periods of a few days to 16 weeks. The illustrations are convincing.

Adams & Livingstone (1932) were more concerned with surgical technique but claimed that the remaining lung tissue in 28 dogs, 2-12 months after lobectomy or pneumonectomy, showed various degrees of compensatory emphysema. They did not give details and their only histological illustration is not convincing.

Rienhoff *et al.* (1935) in a similar, but more detailed, study of ten dogs for periods of up to six months after pneumonectomy, found no emphysema in the remaining lungs. The previously excised lungs were used as controls. They found dilatation of the terminal respiratory lobules but did not interpret this as emphysematous. It is interesting to note that their Fig. 4 appears more suggestive than many in the literature.

Kountz *et al.* (1936) removed up to 80% of the lungs in 19 dogs by the method of Adams & Livingstone (1932) and implied that emphysema was

present in the remaining lung tissue, although no autopsy details were given.

Longacre & Johansmann (1940) compared the long-term effects of pneumonectomy in adult dogs and puppies. The lungs of two adult dogs were used as controls. Two adult dogs survived two and nearly four years, while three puppies survived 2, 3 and almost four years respectively. No emphysema was found in the lungs of the animals operated on when puppies, but true chronic emphysema was seen in the lungs of the dogs pneumonectomised when adults. However, only one of the illustrations (their Fig. 11A) is suggestive of emphysema.

LOWERING OF OXYGEN TENSION IN THE ATMOSPHERE.

Campbell (1927), in a study of the pathological effects of high altitudes, exposed an unstated number of animals, including rabbits and mice, to atmospheres containing only 10%, or even 7%, of oxygen for prolonged periods. No details were given and he noted, incidentally, that "portions of the lungs of most animals were emphysematous".

Prinzmetal (1934), in a deliberate attempt to produce emphysema by this method, exposed an unstated number of rats to an atmosphere containing

only 8% oxygen for ten weeks. He claimed that true emphysema was produced and, while his illustration is convincing, no details were given and no controls were employed.

OTHER METHODS.

Caradonna (1913) studied the effect of increased respiratory effort on the alveolar pores in an unstated number of young guinea pigs of the same age. One group was kept at rest, undisturbed, as controls. Two other groups were whipped until they were in a state of collapse four times daily for ten minutes before and after food respectively. In the controls, only occasional pores were seen between the ages of 5-15 months. In the whipped animals pores became visible from the age of three months and increased in numbers till, at the age of one year the lungs were definitely emphysematous. The emphysema was most marked in the animals whipped before food.

Kelman (1919) employed various methods using rabbits. In seven the lungs were intermittently inflated through a tracheal canula. In another experiment three rabbits were killed by anaphylactic shock. A further 18 were inoculated intra-tracheally by H. Influenzae cultures or culture filtrates. She claimed that marginal

emphysema was present in all of these animals. No precise criteria were given, though, in places, the emphysema was referred to as being acute. No controls were employed.

Rasmussen & Adams (1942) over-inflated the lungs of seven dogs by a tracheal canula for periods of 15 minutes twice weekly. The dogs survived for periods of one week to eleven months. Vesicular emphysema was noted in only one animal and the appearances were particularly convincing (their Fig. 8). All the other animals showed some degree of over-distension of the lungs but no true emphysema. Rasmussen & Adams made no claim that their single success was, in fact, the result of the over-inflation.

DISCUSSION OF THE LITERATURE

The review of the literature indicates that many of the claims to have produced emphysema experimentally are unacceptable for the following reasons:-

a. Lack of controls.

b. Failure to state precise criteria for the diagnosis of emphysema.

c. Failure of illustrations to substantiate claims made in the text.

The most careful study was that of Paine (1940). He assessed his results very critically and supplemented his anatomical studies by measurements of the intrapleural pressure by the method of Christie & Mackintosh (1934). It is interesting to note that he felt that the anatomical findings in his animals would scarcely have justified the claims he made but for these supplementary studies.

Quite apart from the validity of the experimental work, nearly all the methods employed have been designed to produce abnormal distension of all or part of the lungs or, at least, to increase the amount of functional stress placed upon the lungs.

The claims of the experimental workers, if accepted, lend support to the mechanical theories of pathogenesis of emphysema and show that different remote mechanisms may produce changed conditions of respiration which cause damage to the lung tissues and the production of emphysema.

The methods employed by these workers do not shed any light on the intimate mechanism of pathogenesis and, indeed, few of the authors have commented on this aspect of the problem. Paine (1940), in his preliminary discussion, regards the development

of emphysema as the result of physical stress on the alveolar walls but mentions, in passing, that the possibility of nutritive disturbances, due to capillary occlusion in the course of distension, cannot be excluded.

THE PRESENT STUDY

The method employed in the present study was devised as the result of observations on human lungs in thick sections where it was obvious that the capillary network was a major constituent of the alveolar wall. It seemed possible that the appearances of emphysema might be produced if the capillary bed could be destroyed.

Study of the literature shows that there is ample evidence for regarding chronic emphysema as an atrophy of lung tissue. It has been suggested, from time to time, that ischaemia is at least an ancillary factor involved in the production of emphysematous atrophy.

If chronic emphysema can be regarded as an ischaemic atrophy, it should be possible to produce emphysema by interfering with the blood flow through the pulmonary capillaries without the presence of mechanical over-distension of the lungs.

The present experiments were performed to test this hypothesis. Repeated intravenous injections of a particulate substance were used in an attempt to cause enough vascular obstruction to produce tissue ischaemia.

MATERIALS AND METHODS

HISTOLOGICAL TECHNIQUE

The methods described in Section III were again employed. However, at least one block was taken from each of the major lobes of each pair of lungs. Additional blocks were taken as indicated by the naked eye appearances.

EXPERIMENTAL METHOD.

The method thought most likely to produce an adequate degree of vascular blockage was the oft-repeated intravenous injection of a particulate substance of such a size as to produce blockage of the capillaries and pre-capillaries. The substance should be non-toxic systemically, non-irritant locally and insoluble in body fluids. It should, preferably, be insoluble in histological dehydrating and clearing agents to facilitate subsequent identification of the sites of lodgment.

Caledon Blue R.C. seemed a likely substance. This is an anthraquinone dyestuff, 3',3' dichloro-indanthrone, which is insoluble in water and organic solvents. It is prepared commercially for dyeing leather.

By suitable selection of routine commercial batches it was possible to obtain samples which had a majority particle size of between 10 and 25 microns. The dye was supplied in the form of an aqueous paste containing 10-12% total solids as Caledon Blue. Trials showed that a suitable dilution for intravenous use was an approximate 3% of total solids. The diluent was 0.85% saline with 0.05% of a dispersing agent, Dispersol T, added.

Preliminary trials in mice showed that the material was non-toxic. Tissue reaction was minimal and limited to a slight histiocytic reaction at the site of lodgment of the particles. It appeared that most of the particles which were retained by the lungs became lodged in the precapillaries and capillaries; but aggregation of the particles with extension into vessels of arteriolar or larger dimensions also occurred. This was very marked immediately after an injection, but within a few hours there was a certain amount of re-distribution into finer vessels. It became

obvious that, with Caledon Blue, it would not be possible to block, at will, vessels of a definite calibre by an exact selection of particle size. This was no disadvantage for it was not possible to get batches of exactly the same particle size throughout this work. Rough correspondence could be achieved and this was adequate.

Repeated intravenous injection with Caledon Blue proved to be too difficult and too uncertain in mice and so rabbits were finally employed.

In mice and rabbits there was no immediate tissue reaction to the Caledon Blue but after a day or two there was a very slight histiocytic response at the site of lodgment of the particles. But even this might be absent. After a week occasional histiocytes could be seen transporting engulfed particles of Caledon Blue through the vessel wall into the perivascular lymphatics. Apart from this trivial and inconstant histiocytic response, Caledon Blue provoked no other tissue reaction. This was true even in rabbits which received repeated injections of the dye for periods of a year or more. At no stage was any granulomatous reaction or fibrosis produced.

The removal of Caledon Blue into the lymphatics was a very slow process and considerable quantities remained in the pulmonary vessels in mice for periods of up to 17 months after a single injection. Removal was apparently equally slow in the rabbit and after a few injections the lungs became a uniform deep blue colour.

In rabbits, the majority of the particles lodged in the pre-capillaries and to a lesser extent in the capillary network itself. Even after a single injection the blockage was the result of aggregates of particles rather than of individual particles. With repeated injections aggregation became more marked with the result that the vessels became grossly dilated around the large mass of dye. After prolonged injections aggregates occurred in terminal arterioles but larger vessels did not become involved.

The histological appearances suggest a very marked degree of vascular obstruction; but in spite of this thrombosis has not been observed and infarction has never been produced.

Not all of the particles were retained in the lungs and, even after a single injection, Caledon Blue became lodged in the vessels and taken up by the reticulo-endothelial tissue in other

organs. Some notes on these appearances are included in Appendix B.

DETAILS OF EXPERIMENTS

1st EXPERIMENTAL SERIES (22 Rabbits).

A suitable initial dose was found to be 2.5 ml. of the diluted Caledon Blue, containing approximately 3% of Caledon Blue.

This was injected slowly into an ear vein over a period of 1-2 minutes. On a first injection about one in five rabbits could be expected to die. Death was preceded by tachypnoea, collapse and one or more convulsions in most cases. In about half the rabbits, tachypnoea commenced during or immediately after the injection but lasted only 2-3 minutes, after which the animal remained well.

After two or three injections at weekly intervals the dose was increased to 3.0 ml. of the diluted Caledon Blue and this was maintained till the end of the experiments. After the rabbits had been injected weekly for about two months they rarely had tachypnoea following an injection and the rate of injection could be increased without ill effects.

By this time it became apparent that the rabbits would tolerate the dosage almost indefinitely and it was realised that ageing might interfere with the assessment of results. Consequently, further rabbits added to the series were selected as appearing to be less than one year old.

The weekly injections were continued until the survivors had been receiving them for over a year. These survivors were finally killed by stunning 1-2 weeks after their last injection.

In the meantime, collection of a control series had been in progress and it became evident that spontaneous emphysema occurred in rabbits (Section III). More importantly it was shown that more than 50% of rabbits over the age of 2½ years had some degree of generalised emphysema.

While it was thought that more than half of the 22 animals in the 1st experimental series were under two years of age at the conclusion of the experiments, it is impossible to be certain of this. In spite of the fact that 85% of the experimental rabbits, which had had intravenous Caledon Blue for four weeks or more, had generalised emphysema as compared with just under 10% of a large group of miscellaneous adult rabbits, it was felt that the uncertain ages of the experimental

animals rendered this type of attempted controlling inadequate.

2nd EXPERIMENTAL SERIES (25 "Pairs" of rabbits).

It was therefore decided to repeat the experiment using rabbits of known ages. Most of them were eight or nine months old at the start, the extreme ages being seven and 12 months. The controls in this experiment were the paired litter mates of the animals which received the Caledon Blue.

Encouraged by the results in a rabbit in the first series which had been injected thrice weekly, the animals in the second series were given Caledon Blue intravenously twice weekly, after the second week.

The dosage was maintained at 2.5 ml. of diluted Caledon Blue, containing approximately 3% total solids, as there was a higher death rate initially and in the first few weeks of the second series as compared with the first. The injections were continued twice weekly until the survivors had received Caledon Blue for 24 weeks. These were killed by stunning 1-2 weeks after the last injection so that no rabbit was more than 18 months old at the conclusion of the experiment.

The litter mate controls were treated identically, as far as possible, to the experimental animals. Each control received a bi-weekly intravenous injection of 2.5 ml. of 0.85% saline with 0.05% of the dispersing agent added, but without the Caledon Blue. These injections were given at the same time as the experimental animals received their injections of Caledon Blue. The control rabbits were killed by stunning when the paired experimental animal died or was killed and the control lungs were fixed, embedded, cut and stained in strict parallel to the experimental lungs at all stages.

In this series 25 pairs of rabbits survived for periods of between one and 24 weeks. It should be noted that one of the "pairs" consisted of three litter mates, of which two were given Caledon Blue and one retained as a control for both.

ASSESSMENT OF RESULTS

In view of the experience of spontaneous emphysema in rabbits described in Section III, the assessment of the results of the Caledon Blue experiments will be made solely upon a comparison

Fig. 1 (RSt/17)
Macroscopic vesiculation.
Scale: mm.

of the incidence of microscopic generalised emphysema in the injected rabbits and in the controls.

Emphysema in marginal lobules will be ignored as the lobules have been shown to be spontaneous lesions of inflammatory origin.

While vesiculation is usually a manifestation of marginal accentuation of a generalised emphysema, isolated cases did occur more frequently in the injected animals. However, in spontaneous emphysema in rabbits it is possible that occasional examples of vesiculation may be the result of inflammatory changes and therefore vesiculation will be ignored in the assessment. This type of marginal emphysema is, however, a very useful naked-eye guide as to the success of any individual experiment (Fig. 1).

MICROSCOPIC APPEARANCES.

The generalised or marginal emphysema found in the rabbits injected with Caledon Blue is identical in every respect to the spontaneous emphysema described in Section III. The picture is complicated, however, by the presence of masses of Caledon Blue in the vessels. Apart from rendering assessment of the least severe grade more difficult, the Caledon Blue does not modify the basic histological appearance of the emphysema.

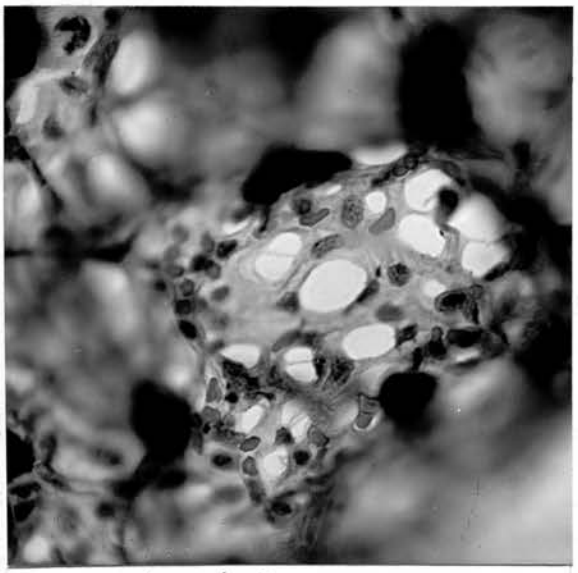


Fig. 2 (RSt/52): Fenestration in experimental emphysema. Black masses are aggregates of Caledon Blue. 100μ. H&E. x 480.

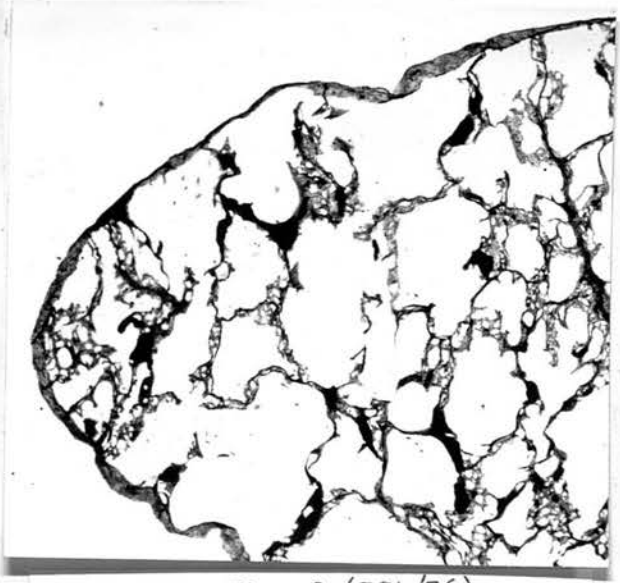


Fig. 3 (RSt/76)
Vesiculation in experimental emphysema showing loss of alveolar septa and Caledon Blue aggregates in vessels.
100μ. H&E. x 30.

As in the spontaneous lesions, there is destruction of the alveolar walls by fenestration (Fig. 2) which, in the marginal accentuation of a generalised emphysema, progresses until there is complete disappearance of the alveolar septa and fusion of neighbouring alveolar sacs and ducts (Fig. 3).

The presence of the Caledon Blue makes the study of the elastic fibres more difficult; but the changes in the elastica are also identical to those seen in spontaneous emphysema.

ASSESSMENT OF DEGREE OF GENERALISED EMPHYSEMA.

In order to assess the results more accurately, an arbitrary system of plus grading has been adopted. This system is based on the extent and severity of the destruction of the lung tissue by fenestration.

Alveolar pores or fenestrations occur in all rabbit lungs and, as the emphysematous process consists of the development of abnormal numbers of fenestrations which enlarge and fuse

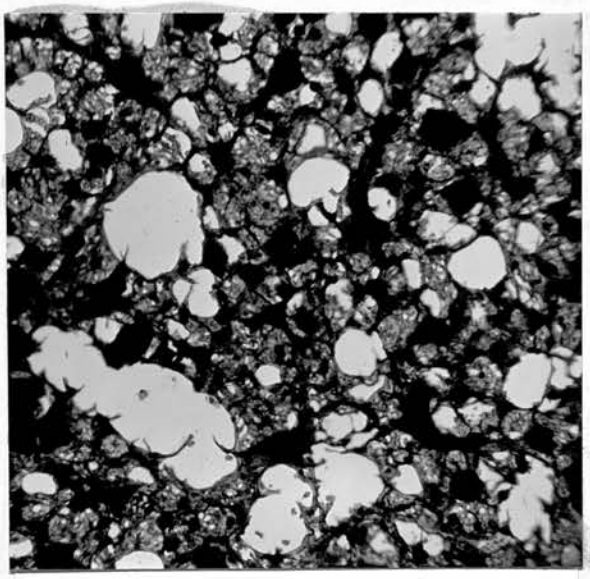


Fig. 4 (RSt/108)
Fenestration in grade + of experimental generalised emphysema.
100μ. H&E. x 60.

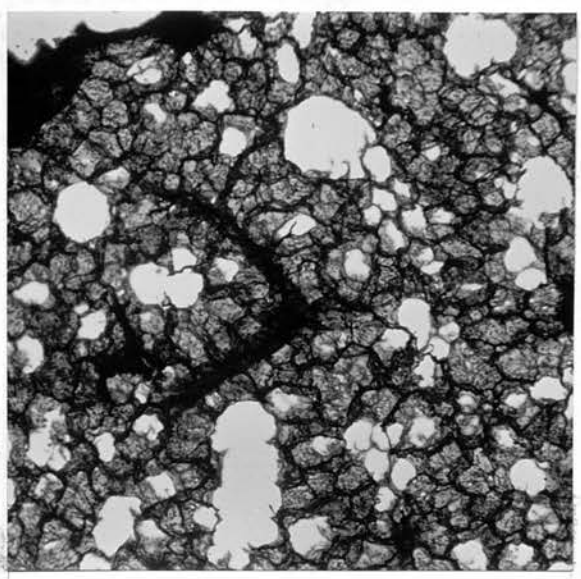


Fig. 5 (RSt/109)
Normal litter mate control for comparison with Fig. 4.
100μ. H&E. x 60.

it will be obvious that there is no hard and fast dividing line between "emphysematous" and "normal". However, in the normal lung, while the number of pores is variable, large pores are rare and there is little or no tendency to fusion.

Grade 0 contains lungs in which the alveolar pores are within normal limits as judged from the experience of the 155 rabbits reported in Section III.

Grade + contains lungs where there is not only a greater amount of fenestration than in the arbitrary normal but where there is also obvious enlargement and fusion of the pores (Figs. 4 & 5). This grade also includes cases where, though individual foci show a degree of fenestration characteristic of the more severe grades, the lesions have a rather patchy distribution throughout the lungs.

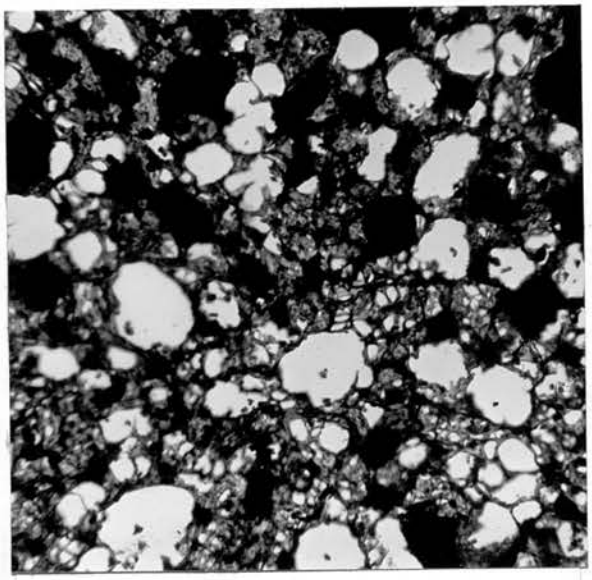


Fig. 6 (RSt/70)
One of the least fenestrated areas in grade +++ experimental generalised emphysema.
100μ. H&E. x 60.

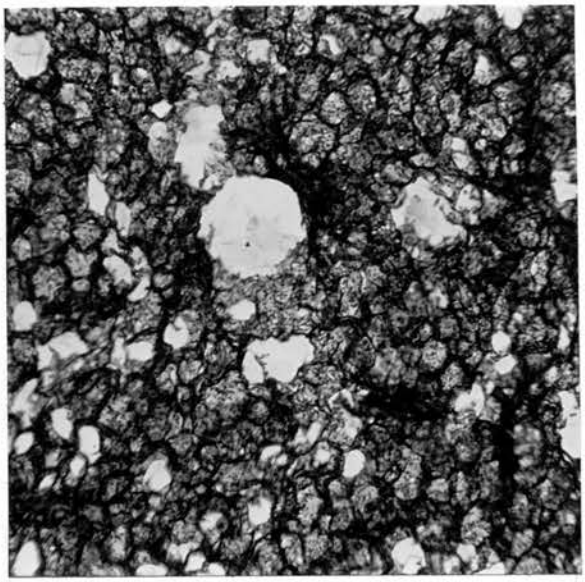


Fig. 7 (RSt/71)
Normal litter mate control for comparison with Fig. 6.
100μ. H&E. x 60.

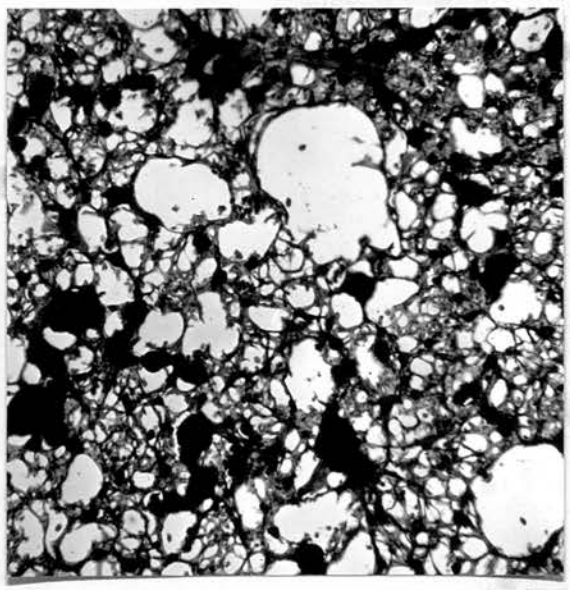


Fig. 8 (RSt/70)
Same case as Fig. 6, showing one of the most fenestrated areas in grade +++ experimental generalised emphysema.
100μ. H&E. x 60.

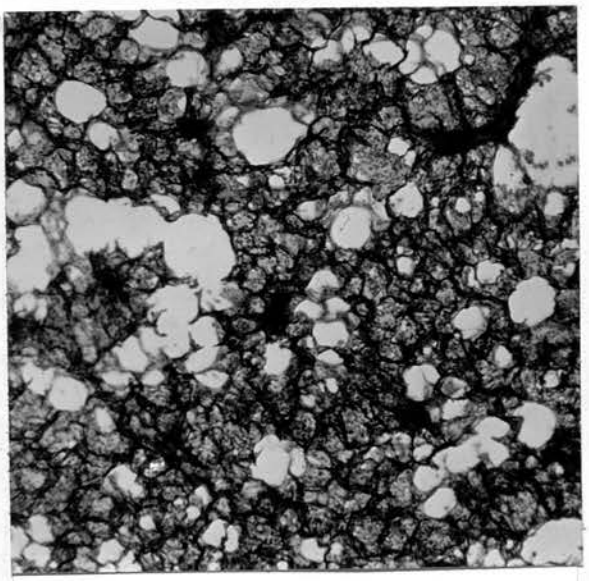


Fig. 9 (RSt/71)
Another field from normal litter mate control for comparison with Fig. 8.
100μ. H&E. x 60.

Grades ++ and +++ include the more severe cases where the fenestration is gross and obvious in all parts of the lungs (Figs. 6, 7, 8, 9 & 10).

In practice, repeated re-examinations showed a remarkable consistency in the grading of normals. Further, in grading the experimental series, any lung which was in any way indeterminate was graded 0. In the case of the litter mate controls, such cases were graded +. In short, an attempt was made to assess the results as critically as possible.

EXPERIMENTAL RESULTS

Details of the incidence of all types of emphysema, duration of injections of Caledon Blue and the amounts of Caledon Blue injected, in both experimental series, are given in Appendix B, Tables 1 and 2.

1st EXPERIMENTAL SERIES.

Table I shows the incidence of the various grades of generalised emphysema found in the rabbits of this series. Although no strict controls are

available, the incidence of spontaneous generalised emphysema found in the 155 "normal" rabbits, described in Section III, is included for comparison. The "normal" rabbits have received no Caledon Blue and have been divided, as in Section III, into three age groups. It is believed that more than half of the rabbits of the 1st experimental series were "miscellaneous adults" i.e. under 18 months of age, but this is not known definitely.

Table I. (Appendix B, Table 1)

Incidence of Generalised Emphysema in 1st Experimental Series.

Degree of Generalised Emphysema	After I.V. Caledon Blue	Normal Rabbits		
		Young	Miscellaneous Adults	Old
+ + +	5	0	1	3
+ +	4	0	2	4
+	8	0	6	4
0	5	20	105	10
Total No. in Group	22	20	114	21

These figures show that the incidence of generalised emphysema, in the rabbits which received Caledon Blue, is very much higher than that in the young and miscellaneous adults. This suggests that

the Caledon Blue has been responsible for the increased incidence.

However, the incidence in the experimental series is not significantly greater than that in the old rabbits. In view of this, no definite conclusion can be drawn as the ages of the experimental animals are uncertain. Nevertheless the results are suggestive.

2nd EXPERIMENTAL SERIES.

Table II shows the incidence of the various grades of generalised emphysema found in the rabbits of the second experimental series and also the incidence of the grades of spontaneous generalised emphysema found in the litter mate controls.

Table II. (Appendix B, Table 2)

Incidence of Generalised Emphysema in 2nd Experimental Series.

Degree of Generalised Emphysema	After I.V. Caledon Blue	Controls (Litter mates)
+ + +	6	0
+ +	4	1
+	2	2
0	14	22
Total No. in Group	26	25

There is a greatly increased incidence of generalised emphysema in the animals which have received intravenous Caledon Blue.

The results were analysed by the exact factorial method of Fisher (1948) in the following manner:-

a. All grades of emphysema were pooled in the injected and control groups, respectively, and the incidence compared with that of the normal grade in the two groups. This gave $P = 0.008$, indicating that the difference in the total incidence of generalised emphysema in the two groups is clearly significant.

b. The ++ and +++ grades of emphysema were pooled in the injected and control groups, respectively, and the incidence compared with that of the pooled grades 0 and + in the two groups. This gave $P = 0.003$, which shows that the difference in the incidence of the more severe grades is even more significant than that of the total incidence of generalised emphysema.

It can, therefore, be concluded that the intravenous Caledon Blue has, in fact, produced experimental emphysema.

INFLUENCE OF DURATION OF CALEDON BLUE INJECTIONS.

Table III shows the relationship between the duration of the Caledon Blue injections and the degree of generalised emphysema in both experimental series.

Table III (Appendix B, Tables 1 & 2)
Duration of Caledon Blue Injections and Degree of Generalised Emphysema.

Degree of Generalised Emphysema	Duration of Caledon Blue Injections (weeks)						
	1st Experimental Series				2nd Experimental Series		
	1-3	4-11	12-23	24-56	1-3	4-11	12-24
+ + +	0	0	0	5	0	1	5
+ +	0	0	1	3	0	0	4
+	0	1	2	5	0	1	1
0	2	3	0	0	5	5	4
Total No. in Group	2	4	3	13	5	7	14

These figures show that the incidence of generalised emphysema increases as the duration of the injections, and hence the amount of Caledon Blue administered, increases. This supports the view that the administration of Caledon Blue has produced the emphysema found in the experimental animals.

MODE OF ACTION OF CALEDON BLUE

The results indicate that the intravenous injection of Caledon Blue has produced generalised emphysema in the rabbits and the mode of action must now be considered.

The experiments were performed on the theoretical basis that chronic emphysema should be regarded as an atrophy of lung tissue and that interference with blood supply might produce such an atrophy.

In so far as emphysema has been produced by the introduction of a particulate substance into the pulmonary vessels, the experimental results appear to substantiate the hypothesis. However, there are other ways in which the Caledon Blue might have acted.

There is no indication that fibrosis is produced and this is definitely not the mechanism involved. Infarction does not occur and there is no evidence that the emphysema is of the complementary type. The Caledon Blue does not produce any acute inflammatory reaction. The slight histiocytic response bears no relation to the anatomical distribution of the emphysema and there is nothing to suggest that the emphysema is the direct result of inflammation or the mere mechanical

presence of the Caledon Blue particles.

It is possible that the particles in the vessels acted as irritants and reflexly altered the mechanics of respiration. However, the only evidence to support this view is the fact that, especially on receipt of the first few injections, about half the rabbits exhibited a transient tachypnoea during or immediately after the injection. This lasted for two or three minutes only. Then the respirations became normal and remained so till the next injection.

This transitory tachypnoea was not a constant feature and was rare once an animal was established on routine injections. In the periods between the injections, the animals were normal, clinically, and auscultation of the chest never gave any hint of bronchial spasm or increased bronchial secretions.

Binger et al. (1924 and 1927), using dogs, made a careful study of the mechanisms involved in the production of transitory tachypnoea following the injection of pulmonary emboli of various types and sizes. They concluded that it was not due to reflex irritation.

Thus, although the possibility cannot be entirely excluded, there is no positive evidence

to support the view that Caledon Blue acts by producing a reflex disturbance of respiration.

The suggestion remains, therefore, that Caledon Blue acts by causing the obstruction of large numbers of small blood vessels in the lungs and thereby produces an ischaemic atrophy of lung tissue - chronic pulmonary emphysema.

SUMMARY AND CONCLUSIONS

An account has been given of the experimental production of generalised chronic vesicular pulmonary emphysema in rabbits by the repeated intravenous injection of a suspension of Caledon Blue R.C.

The experimental lesions are identical to those of spontaneous pulmonary emphysema in rabbits.

The experimental lesions are thought to be the result of an ischaemic atrophy of lung tissue.

SECTION V

RIGHT VENTRICULAR HYPERTROPHY IN SPONTANEOUS AND EXPERIMENTAL PULMONARY EMPHYSEMA IN RABBITS

SECTION V

RIGHT VENTRICULAR HYPERTROPHY IN SPONTANEOUS AND EXPERIMENTAL PULMONARY EMPHYSEMA IN RABBITS

The results presented in this section are based on fractional weighings performed on the hearts of rabbits, the lungs of which have been studied in previous sections with regard to the presence of spontaneous or experimental pulmonary emphysema and associated inflammatory lesions.

MATERIALS AND METHODS

MATERIALS.

With four exceptions* the hearts of the rabbits employed in the study of spontaneous and experimental pulmonary emphysema were available.

---

* Three hearts are missing from the "normal" group: 2 miscellaneous adults and 1 young rabbit. One heart is missing from the subgroup showing spontaneous local emphysema alone.

These animals were classified as follows according to the condition of the lungs with regard to experimental pulmonary embolism and/or emphysema:-

Table I.

Condition of Lungs	No. of Rabbits
Non-emphysematous ("Normal") (Appendix A. Table 1)	133
No pulmonary embolism (Spontaneous emphysema) (Appendix A. Table 1)	43
Experimental pulmonary embolism by Caledon Blue R.C. (With or without experimental emphysema (Appendix B. Tables 1 & 2)	48
Total	224

The three main groups above have been subdivided for comparative purposes as will be seen in the text.

METHODS.

a. Assessment of Degree of Emphysema.

Generalised emphysema has been assessed as detailed in Section IV and the gradings employed in that section are used here, without modification. Where both local and generalised emphysema are present in the same pair of lungs the grading used for analysis is that of the generalised emphysema alone.

b. Method of Fractional Heart Weighing.

The method of Herrmann (1925) was not employed as, in trial dissections, it was found that division of the I.V. septum in the rabbit heart was liable to be very haphazard and, in view of the relatively small weight of the right ventricle, would be liable to give very inaccurate results.

Full details of the method adopted are given in Appendix C. In brief, the procedure was as follows: The hearts were fixed in 10% formol-saline till convenient and then washed. Remaining tags of pericardium etc. were trimmed off and the whole heart weighed after drying with a towel. Next, the auricles, valve cusps, chordae and as much of the sub-epicardial fat as possible were removed. Finally, the right ventricle was separated from the left, cutting as closely as possible, with

fine scissors, to the junction between the right ventricular wall and the septum. The septum was not fractionated but was left intact and weighed as part of the left ventricle. All weighings were performed to the nearest 10.0 mg.

The method gave the following weights:-

Total heart weight.

Right ventricular weight.

Left ventricular weight.

ANALYSIS OF RESULTS.

For assessment of the degree of right ventricular hypertrophy the following expression was used:-

$$LV/RV = \frac{\text{Left ventricular weight}}{\text{Right ventricular weight}}$$

The mean of LV/RV was established for "normal" or control animals of various age groups and these figures were compared with the mean LV/RV ratios of the corresponding age groups with spontaneous or experimental emphysema. A significant decrease in the mean LV/RV ratio was taken to indicate hypertrophy of the right ventricles of the group as a whole.

The mean LV/RV ratios of the various groups were usually compared in pairs by means of the following formula:-

$$X^2 = \frac{(\text{Mean}_1 - \text{Mean}_2)^2}{(\text{S.E.}_1)^2 + (\text{S.E.}_2)^2}$$

In one instance where four groups of "normal" animals were compared the method of James (1951) was employed.

RESULTS.

Full details of the results of fractional heart weighing are given in Appendix C along with the necessary statistical data. The summaries of results given in the text, the graphs and charts have been cross-referenced to the appropriate tables in Appendix C.

NON-EMPHYSEMATOUS ("Normals")

These animals had not been subjected to experimental pulmonary embolism and had no pulmonary emphysema of any type. Many of the lungs, however, had inflammatory lesions as described in Section III. This will be discussed later but for the present it can be stated that inflammation did not cause right ventricular hypertrophy in this or any other group.

The "normal" animals were grouped according to age and the mean LV/RV ratios of the various groups were compared.

Table II. (Appendix C. Table 1)

"Normal" Rabbits

Age of Rabbits	No.in Group	Mean LV/RV	Standard Deviation	Standard Error of Mean
Young (5-11 weeks)	19	4.168	0.236	0.054
Known Adults (7-18 months)	19	4.245	0.305	0.070
Miscellaneous Adults (ages not known)	87	4.096	0.547	0.059
Old (over 2½ years)	8	4.352	0.266	0.094
All ages	133	4.143	0.498	0.043

Comparison of the mean LV/RV by the method of James (1951) gave the following values of X^2, viz: 0.76, 2.69 and 6.29 for 1, 2 and 3 degrees of freedom respectively, showing that there is no significant difference between the mean LV/RV ratios of the four subgroups.

It should be noted that the deviation from the mean is greatest in the case of the miscellaneous adults. This is thought to be due to variation in technique in the earlier stages of the investigation. On re-examining the hearts when taking histology, it was found that less sub-epicardial fat had been

L.V. and R.V. WEIGHTS: NON-EMPHYSEMATOUS ("Normal").

GRAPH 1. (App. C. Table 1.)

All ages = •

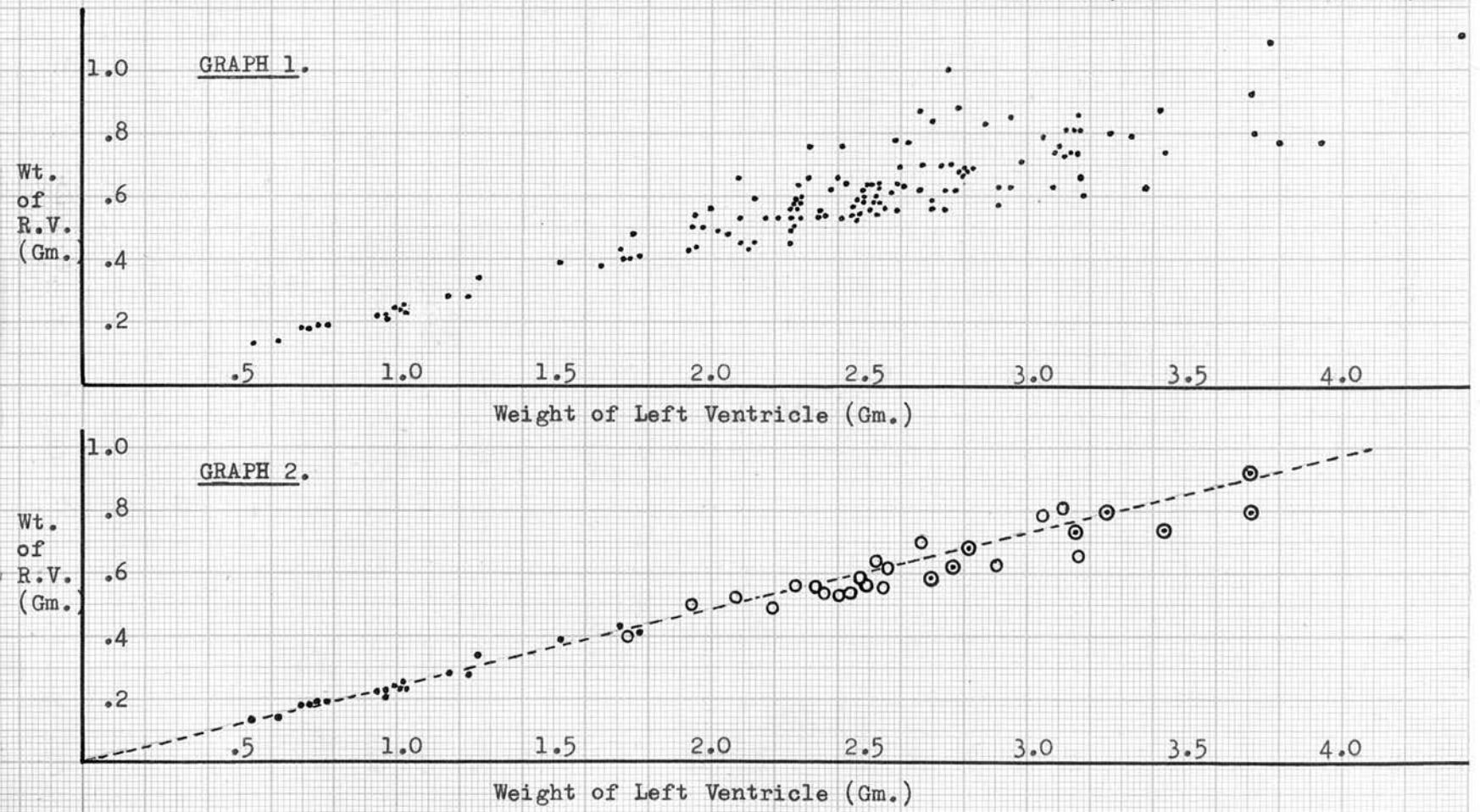
GRAPH 2. (App. C. Table 1.)

Young = •

Known Adults = o

Old = ⊙

Misc. Adults: Mean LV/RV = ------ (4.096)



removed from these hearts, which were the first to be weighed fractionally, than from the others.

Graph 1, where the R.V. and L.V. weights are plotted against each other, shows that the LV/RV ratio maintains a linear relationship within the range of the material studied.

Graph 2, where the R.V. and L.V. weights of the young, known adult and old groups are plotted for comparison with the line of the mean LV/RV ratio of the miscellaneous adults, illustrates the fact that there is no significant difference between the mean LV/RV ratios of the four age groups.

NO PULMONARY EMBOLISM BUT HAVING SPONTANEOUS EMPHYSEMA.

All the rabbits in this group had spontaneous microscopic emphysema: either marginal emphysema alone or generalised with or without marginal emphysema. The rabbits were drawn from all age groups with the exception of the group of young animals; but as there is no significant difference in mean LV/RV ratio between any of the normal groups, the emphysematous animals have been compared with the mean of the entire group of "normals" (Table II).

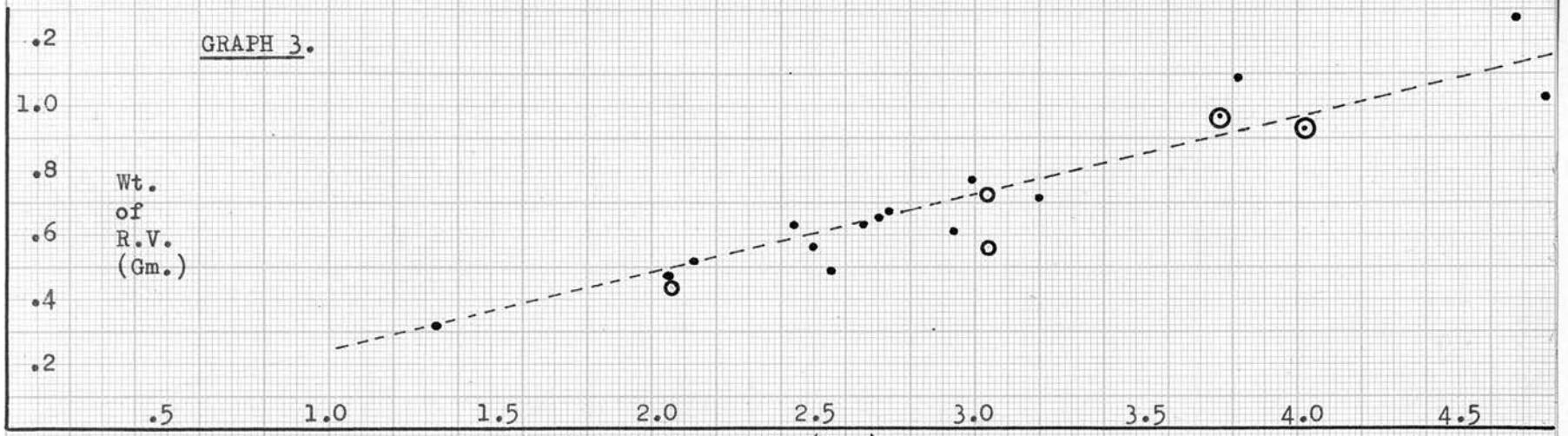
L.V. and R.V. WEIGHTS: SPONTANEOUS EMPHYSEMA.

GRAPH 3. (Table 2.)
Marginal Emphysema.

GRAPH 4. (Table 3.)
Generalised Emphysema (+ or - marginal).

Known Adults = o ; Miscellaneous Adults = • ; Old = ⊙
"Normals": Mean LV/RV = -----(4.143)

GRAPH 3.



GRAPH 4.

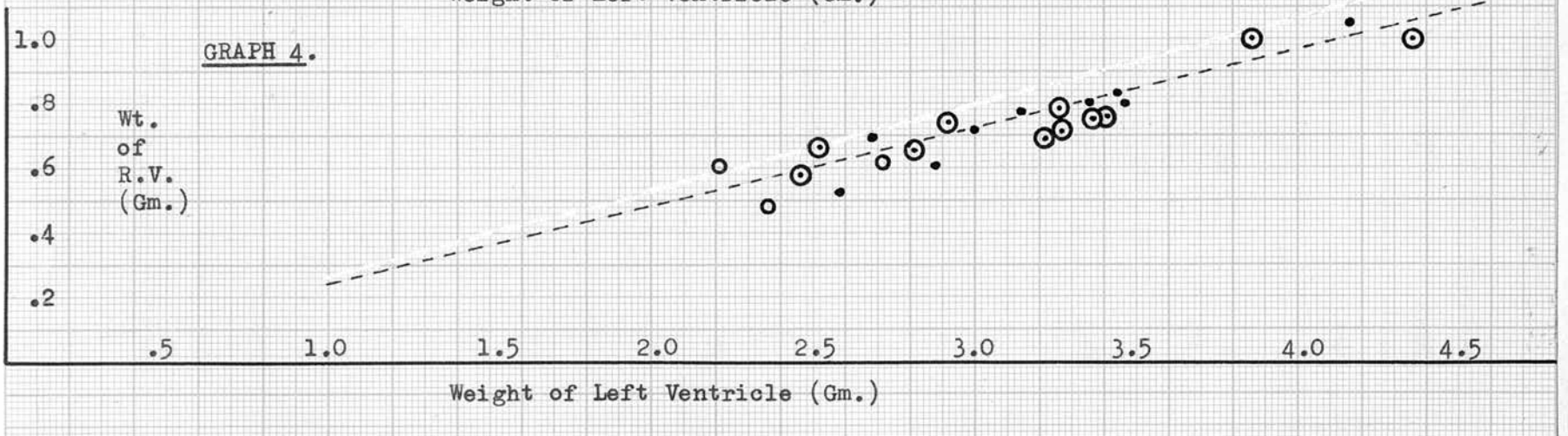


Table III

Condition of Lungs	No. in Group	Mean LV/RV	Standard Deviation	Standard Error of Mean
"Normal" (Appendix C, Table 1)	133	4.143	0.498	0.043
Marginal Emphysema only (Appendix C, Table 2)	20	4.293	0.418	0.093
Generalised Emphysema + or - marginal emphysema (Appendix C, Table 3)	23	4.300	0.347	0.072

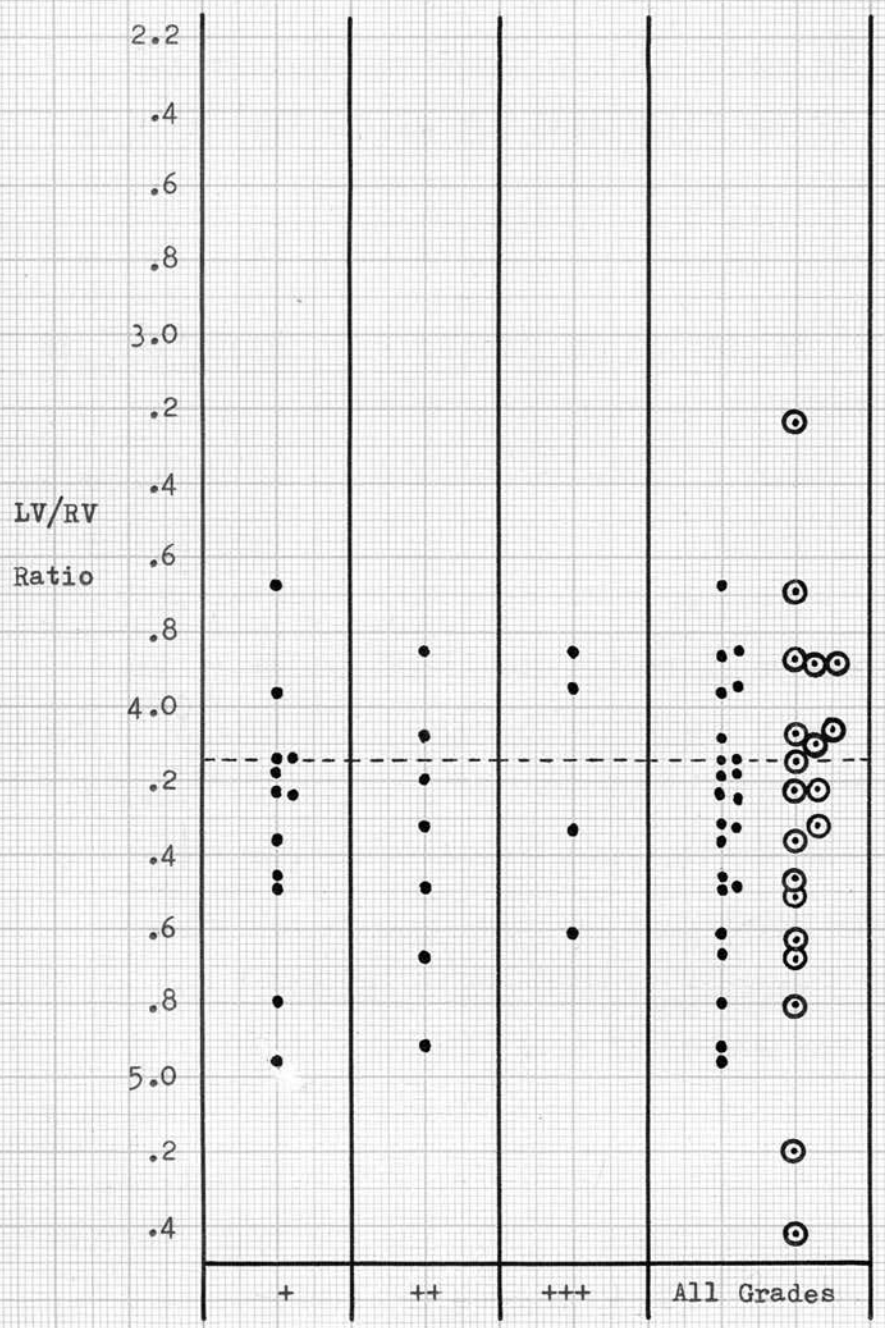
Comparison of the mean LV/RV of the "normals" with that of animals with local emphysema only and generalised with or without local emphysema gave $X^2 = 2.14$ and 3.50 respectively. Comparison of the two emphysematous subgroups gave $X^2 = 0.35$. The differences in the means are not therefore significant and, further, it should be noted that there is not even the suggestion that spontaneous emphysema is causing right ventricular hypertrophy as the mean LV/RV ratios of the emphysematous groups are, if anything, higher than that of the "normals".

Graphs 3 and 4 illustrate these points. The right and left ventricular weights of the emphysematous animals are graphed against each other with

CHART 1. (Tables 2 & 3.)

Influence of Degree of Spontaneous Emphysema on LV/RV Ratio.

Generalised emphysema (+ or - marginal) = ●
Marginal emphysema only = ⊙
"Normals": Mean LV/RV = - - - - - (4.143)



the theoretical line of the mean LV/RV ratio of the "normals" for comparison. The individual emphysematous cases have been symbolised to indicate age and there is no suggestion that age has any effect on the LV/RV ratios in the emphysematous animals, although, in view of the small numbers involved, statistical analysis of the individual age groups has not been performed.

INFLUENCE OF DEGREE OF SPONTANEOUS EMPHYSEMA ON LV/RV RATIO.

Chart 1 shows the results of plotting the degree of spontaneous generalised emphysema against the LV/RV ratios of these animals. It should be noted that the values of the LV/RV ratios on the vertical scale are given in descending order. The mean LV/RV ratio of all groups of "normal" animals is indicated for comparison and it is seen that there is no suggestion that the more severe grades of emphysema decrease or even alter the LV/RV ratios; although the number of cases in the +++ grade is small.

The cases of pure marginal emphysema have been included in Chart 1, but have not been graded.

INFLUENCE OF DEGREE OF INTERSTITIAL PNEUMONIA ON LV/RV RATIO.

Details of the interstitial pneumonia along with an arbitrary grading of the severity of the changes have been given in Section III. The same grading is employed in this section. The mean LV/RV ratio of the cases with the least severe grade of interstitial pneumonia has been compared with that of the most severe grade in the case of "normals" and of rabbits with spontaneous generalised emphysema respectively.

The results may be summarised as follows:-

Table IV (Abstracted from Appendix C, Table 1).

All "normal" rabbits with 0 or +++ grades of interstitial pneumonia

Grade of Pneumonia	No. of Cases	Mean LV/RV	Sum of $(LV/RV)^2$	Standard Deviation	Standard Error of Mean
0	28	3.944	442.916	0.513	0.097
+++	16	4.037	263.290	0.134	0.034

Comparison of the mean LV/RV ratio of the two groups gives $X^2 = 0.08$ (not significant).

Table V (Abstracted from Appendix C, Tables 2 & 3)

All rabbits with Spontaneous Generalised Emphysema (+ or - marginal) having + or +++ grades of Interstitial Pneumonia*.

Grade of Pneumonia	No.of Cases	Mean LV/RV	Sum of $(LV/RV)^2$	Standard Deviation	Standard Error of Mean
+	8	4.141	137.929	0.340	0.120
+++	5	4.522	102.850	0.418	0.187

* No case without some degree of interstitial pneumonia was present in this group.

Comparison of the mean LV/RV ratios of the two groups gives $X^2 = 2.9$ (not significant).

The analysis shows that the mean LV/RV ratio is unaffected by the presence or degree of interstitial pneumonia both in "normal" rabbits and in those with generalised emphysema.

RABBITS SUBJECTED TO PULMONARY EMBOLISM BY CALEDON BLUE R.C. WITH OR WITHOUT MICROSCOPIC GENERALISED EMPHYSEMA.

These animals were divided into two groups as follows:

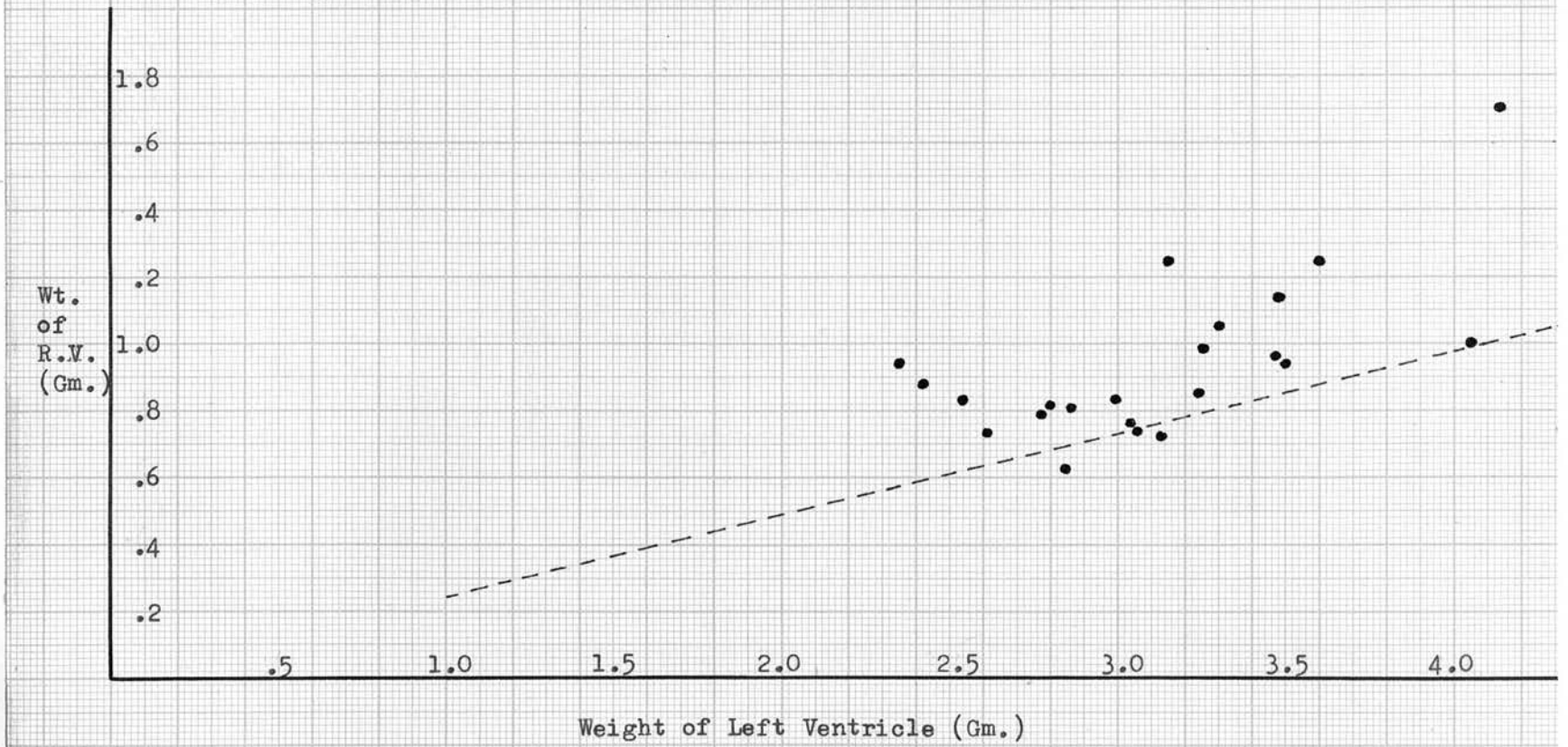
1st. Experimental series: 22 rabbits.

2nd. Experimental series: 26 rabbits.

GRAPH 5. (Table 4.)

L.V. and R.V. WEIGHTS : 1st. EXPERIMENTAL SERIES.

(Miscellaneous Adults: Mean LV/RV = -----(4.096)).



1st Experimental series. This consisted of miscellaneous adults of uncertain ages which were given a weekly intravenous injection of Caledon Blue R.C. suspension as detailed in Section IV, with the exception of one animal (RSt./28) which was injected thrice weekly. The mean LV/RV ratio of this group was compared with that of the "normal" group of miscellaneous adults. The results may be summarised as follows:

Table VI (Appendix C, Table 4)

Group	No.in Group	Mean LV/RV	Standard Deviation	Standard Error of Mean
Miscellaneous adults ("normals")	87	4.096	0.547	0.059
1st Experimental series	22	3.435	0.627	0.134

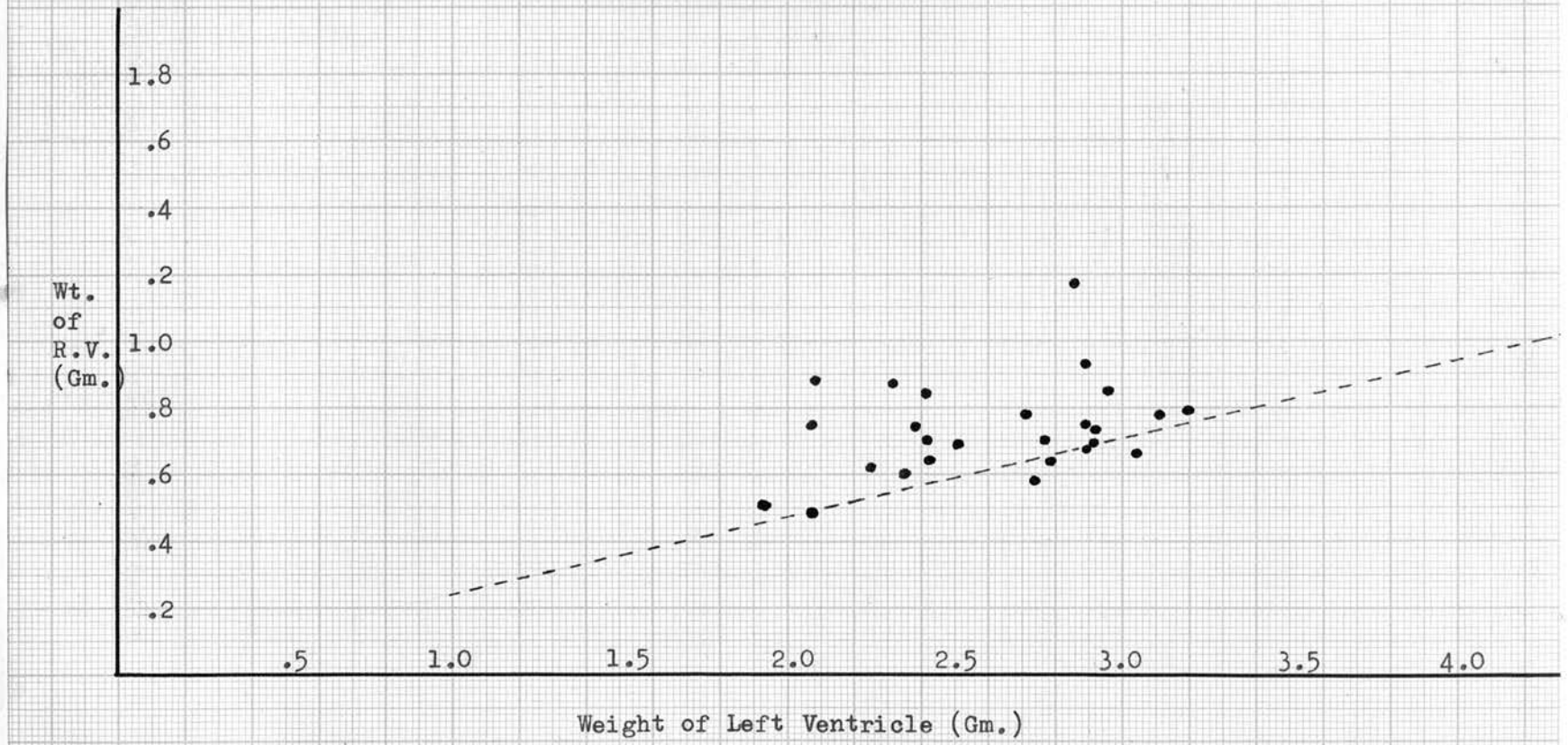
The mean LV/RV ratio of the rabbits which received Caledon Blue intravenously is lower than that of the "normals". Comparison of the two means gives $X^2 = 20.33$, a highly significant difference (P less than 0.001). Graph 5 illustrates this.

2nd Experimental Series. This consisted of adult rabbits of known ages between 7 and 18 months, which were given an intravenous injection of Caledon Blue R.C. twice weekly as detailed in Section IV.

GRAPH 6. (Table 5.)

L.V. and R.V. WEIGHTS : 2nd. EXPERIMENTAL SERIES.

(Known Adults (controls): Mean LV/RV = ----- (4.245)).



The mean LV/RV ratio of this group was compared with that of the specific control group (litter mates) of known adults. The results may be summarised as follows:

Table VII (Appendix C, Table 5)

Group	No.in Group	Mean LV/RV	Standard Deviation	Standard Error of Mean
Known adults ("normal")	19	4.245	0.305	0.070
2nd Experimental series	26	3.647	0.688	0.135

As in the 1st experimental series there is a highly significant reduction in the mean LV/RV ratio of the animals which received Caledon Blue. Comparison of the two mean LV/RV ratios gives $X^2 = 14.17$ (P less than 0.001). Graph 6 illustrates this.

At this stage the results may be summarised as follows:-

a. In "normal" rabbits the LV/RV ratio maintains a linear relationship within the limits of the age groups studied.

b. There is no significant difference in the LV/RV ratios of the various age groups studied.

c. Spontaneous local or generalised emphysema does not influence the LV/RV ratio. No right

ventricular hypertrophy is produced. The degree of spontaneous emphysema has no influence on the LV/RV ratio.

d. Spontaneous interstitial pneumonia does not cause right ventricular hypertrophy in either emphysematous or non-emphysematous rabbits.

e. Pulmonary embolism by repeated intravenous injections of Caledon Blue R.C. produces a significant reduction of the mean LV/RV ratio indicating that the group as a whole exhibits right ventricular hypertrophy.

FACTORS INFLUENCING DEGREE OF R.V. HYPERTROPHY IN EXPERIMENTAL PULMONARY EMBOLISM.

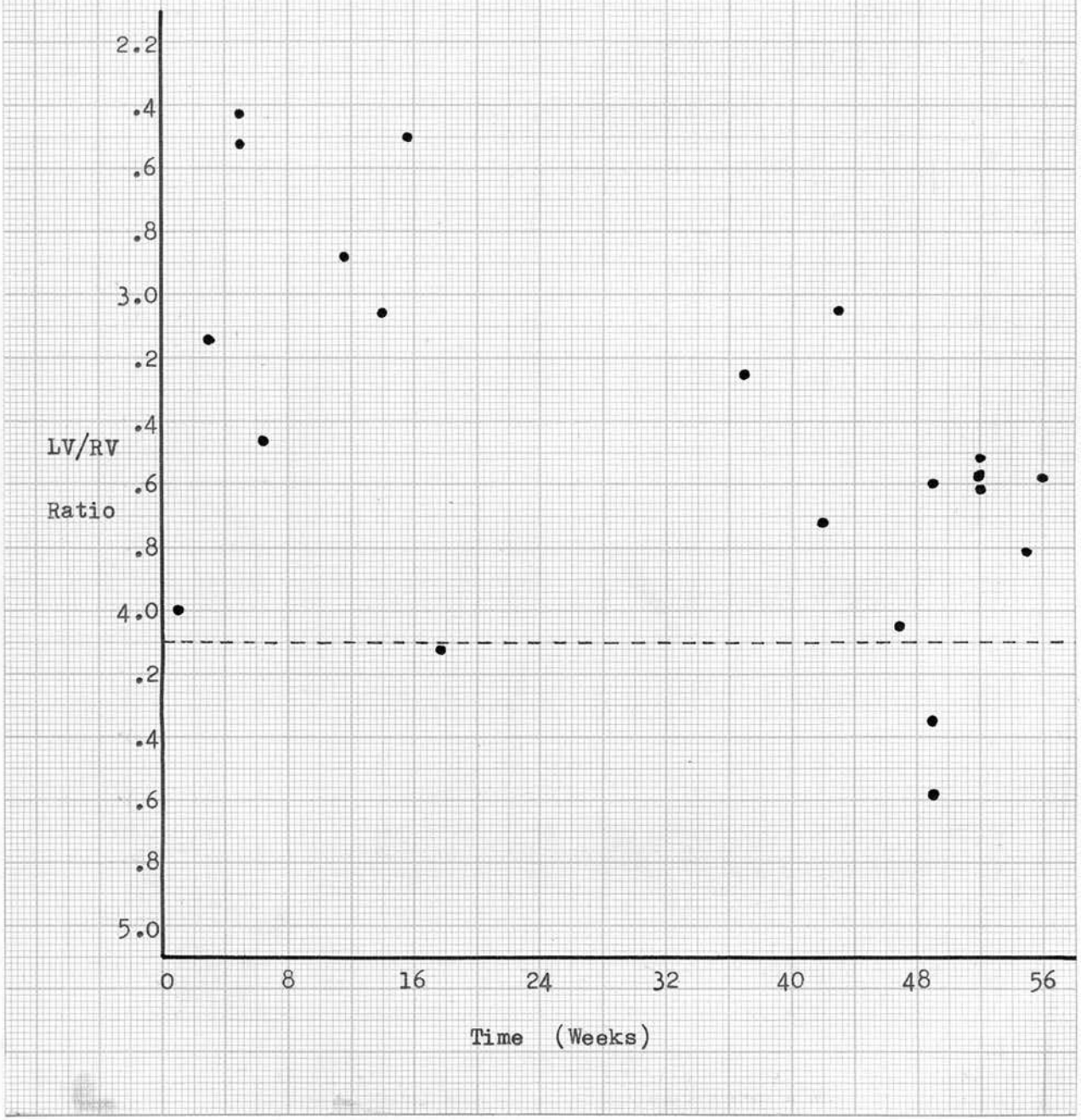
Inspection of the figures and of Graphs 5 and 6 shows that, while both experimental series exhibit right ventricular hypertrophy as a whole, there are many hearts with an LV/RV ratio within normal limits.

Two factors seemed likely to be responsible for this: firstly the duration of the Caledon Blue injections (which reflects the amount of Caledon Blue injected) and secondly the degree of generalised emphysema present.

CHART 2. (Table 4.)

Influence of duration of C.B. injections on LV/RV ratio.
1st. Experimental Series.

(Miscellaneous adults: Mean LV/RV =-----(4.096)).



Analysis of First Experimental Series in regard to Duration of Caledon Blue Injections.

Chart 2 shows the results of plotting the LV/RV ratios of 21 of the 22 rabbits in the first experimental series * against the duration of the injections. The line of the mean LV/RV ratio of the "normal" miscellaneous adult group is included for comparative purposes. The values of LV/RV on the vertical scale have been arranged in descending order.

Chart 2 suggests that there is a greater degree of right ventricular hypertrophy in animals killed or dying in the first half of the experiment.

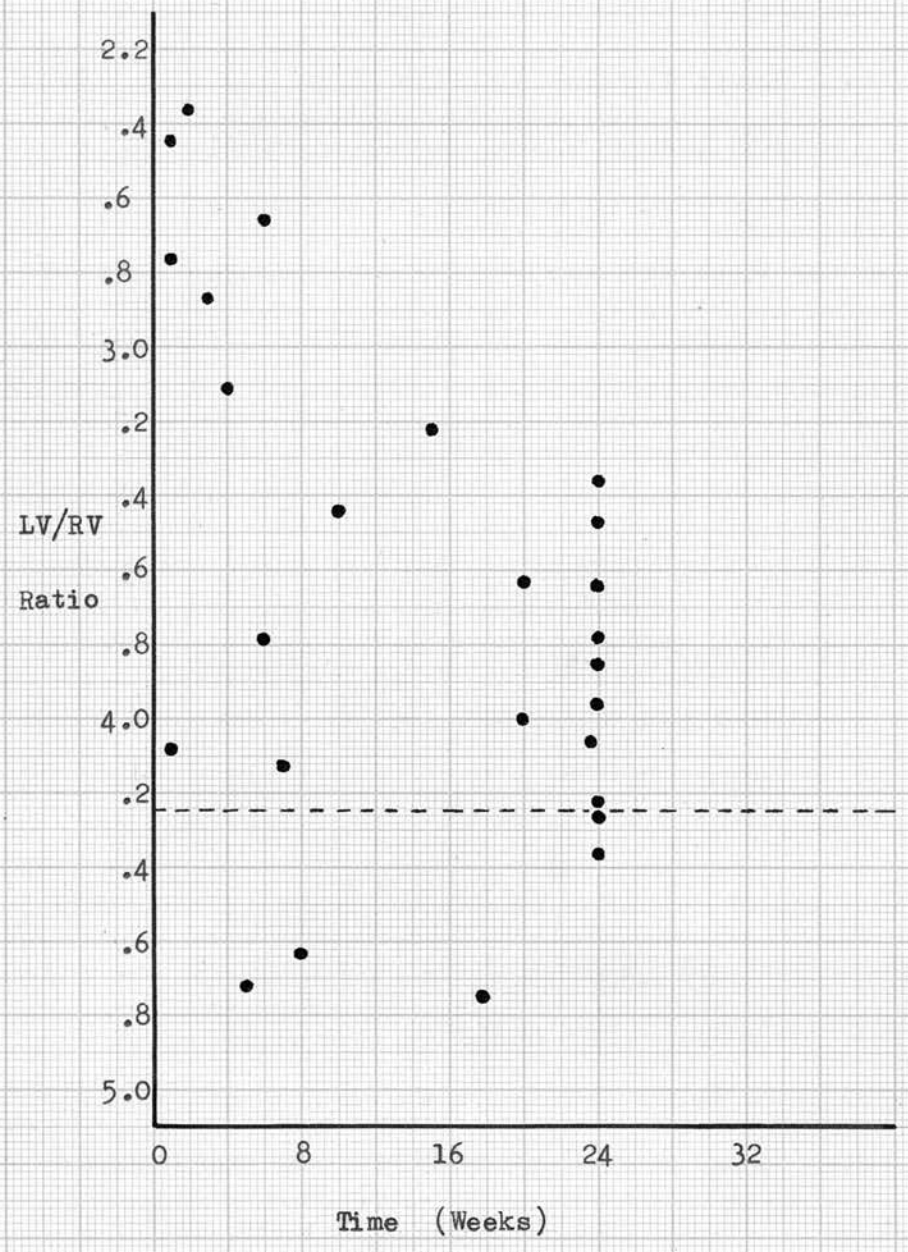
For statistical analysis the cases have been grouped into those receiving Caledon Blue for 1-28 weeks and those receiving it for 29-56 weeks. The following is a summary of the results:

Table VIII (Abstracted from Appendix C, Tables 4 and 1)

Duration of C.B.Injections	No.in Group	Mean LV/RV	Sum of $(LV/RV)^2$	Standard Deviation	Standard Error of Mean
1-28 weeks	9	2.132	90.969	0.689	0.230
29-56 weeks	12	3.725	168.536	0.378	0.109
Misc.Adults (No.C.B.)	87	4.096	1485.334	0.547	0.059

* One rabbit (RSt/28) has been omitted as it received its injections thrice weekly and is therefore not comparable with the others.

CHART 3. (Table 5.)
Influence of duration of C.B. injections on LV/RV ratio. 2nd. Experimental Series.
(Known adults: Mean LV/RV =------(4.245)).



Comparison of the mean LV/RV ratios gave the following results:-

1-28 week group cf. misc. adults:- $X^2 = 16.79$ (P=less than 0.001)

29-56 week group cf. misc.adults:- $X^2 = 8.96$ (P=less than 0.01)

1-28 week group cf. 29-56 week group:- $X^2 = 5.59$ (P=less than 0.02)

These figures confirm that the mean LV/RV ratio in both groups which received Caledon Blue is significantly less than in the "normal" miscellaneous adults and that the ratio is significantly less in those animals receiving Caledon Blue for 1-28 weeks (i.e. in the first half of the experiment) than for 29-56 weeks.

Analysis of Second Experimental Series with regard to the Duration of Caledon Blue Injections.

This was performed in the same way. It should be noted that the animals of this group received the Caledon Blue injections twice weekly. Chart 3 shows the same trend as Chart 2, i.e. the LV/RV ratios are less in the first half of the experimental period. For analysis the cases have been grouped into those receiving Caledon Blue for 1-12 weeks and those for 13-24 weeks. In this case the mean LV/RV ratios have been compared with those of the specific control group, the known adults. The following is a summary of the results:

Table IX (Abstracted from Appendix C, Tables 5 & 1).

Duration of C.B. Injections	No. in Group	Mean LV/RV	Sum of $(LV/RV)^2$	Standard Deviation	Standard Error of Mean
1-12 weeks	12	3.414	147.122	0.827	0.239
12-24 weeks	14	3.897	214.946	0.392	0.105
Known adults (No C.B.)	19	4.245	344.017	0.305	0.070

Comparison of the mean LV/RV ratios gave the following results:-

1-12 week group cf. known adults:- $X^2 = 11.15$ (P=less than 0.001)

13-24 week group cf. known adults:- $X^2 = 7.61$ (P=less than 0.01)

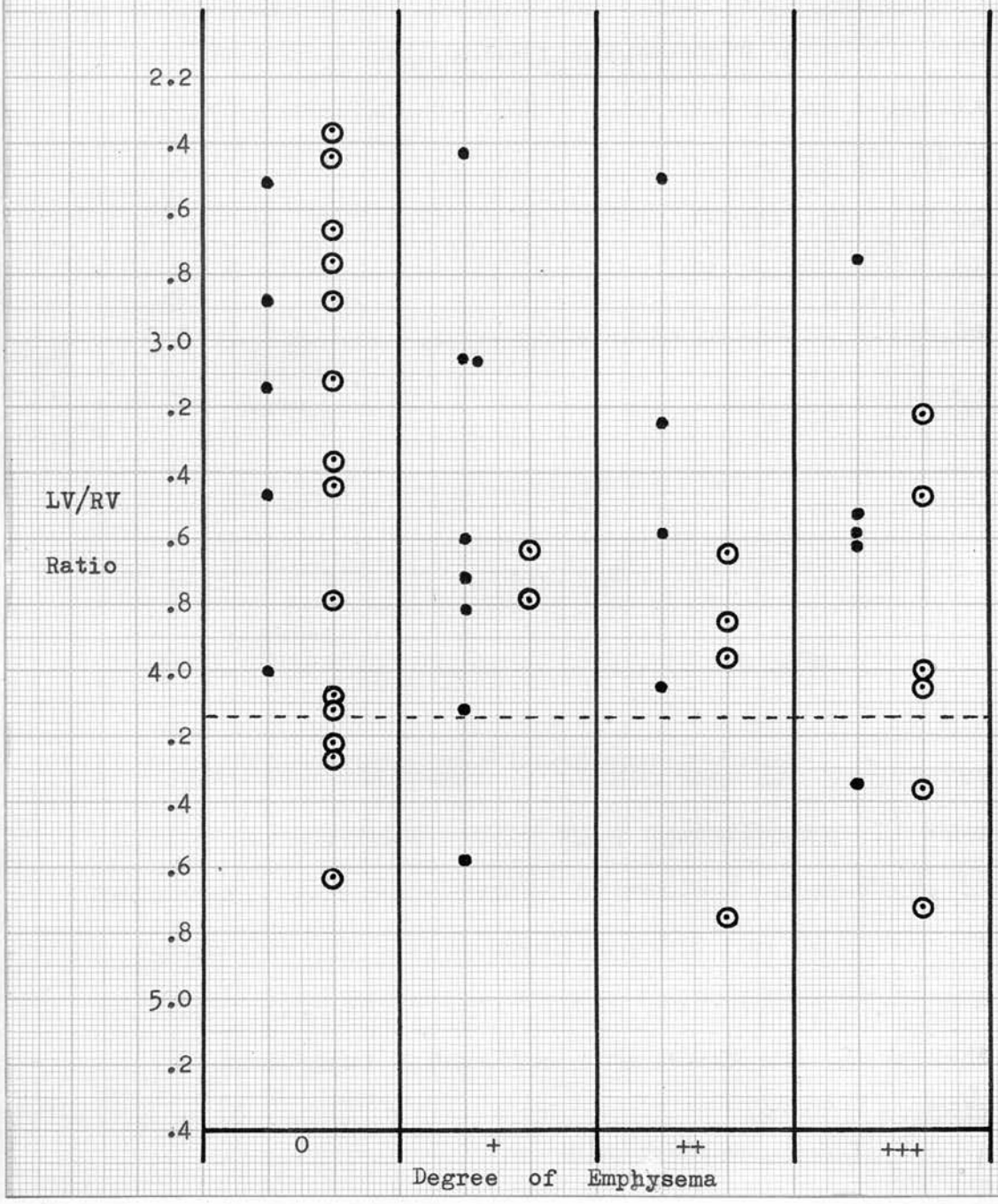
1-12 week group cf. 13-24 week group:- $X^2 = 3.42$ (P=more than 0.05)

These figures confirm that the LV/RV ratio in both groups which received Caledon Blue is significantly less than in the control group (known adults). But the difference in mean LV/RV ratios between those animals receiving Caledon Blue for 1-12 weeks and those for 13-24 weeks falls short of significance. However, the figures are suggestive and lend some support to the evidence of the first experimental series where this difference is significant.

CHART 4. (Tables 4 & 5.)

Influence of Degree of Experimental Generalised Emphysema on LV/RV Ratio.

1st. Experimental series = •
2nd. Experimental series = ⊙
("Normals" : Mean LV/RV = - - - - - (4.143))



Analysis of the Combined Experimental Series with regard to the Degree of Generalised Microscopic Emphysema.

Chart 4 shows the results of plotting the LV/RV ratios of both experimental series against the degree of microscopic generalised emphysema as assessed in Section IV. This chart indicates that there is certainly no decrease in the LV/RV ratio in the more severe grades of experimental generalised emphysema, i.e. there is not an increased incidence of right ventricular hypertrophy.

DISCUSSION

The results of the analysis show that, by the method employed, the mean LV/RV ratio of normal rabbits of all ages between 5 weeks and 2½ years or more is approximately 4.14 with a standard deviation of about 0.5. There is no evidence that spontaneous emphysema of any type or grade causes reduction in the LV/RV ratio, i.e. right ventricular hypertrophy. Similarly, the grade of experimental emphysema is without effect.

However, vascular obstruction by repeated pulmonary embolism with Caledon Blue R.C. produces a significant lowering of the mean LV/RV ratio of both groups of experimental animals. It is concluded that the factor which determines right ventricular hypertrophy is not the emphysema per se but the vascular obstruction.

If obstruction to the pulmonary arterioles and capillaries is responsible for producing right ventricular hypertrophy and if the relationship between the degree of hypertrophy and the vascular obstruction were a simple one, then it would be expected that those animals which had been subjected to a greater degree of embolism would show the greatest degree of right ventricular hypertrophy. However, Charts 2 and 3 and the analysis of the figures suggest that this is not so. The results in the 1st. experimental series show that there is a significantly greater degree of right ventricular hypertrophy in the animals killed or dying in the first half of the experimental period than in those of the second half. The same trend is seen in the 2nd. experimental series, though in this series the difference, while very suggestive, falls just short of significance. Indeed, many of the animals which showed the most marked degree of right ventricular hypertrophy (the lowest LV/RV ratios)

had received considerably less Caledon Blue than those without hypertrophy.

Before discussing this, it should be noted that this apparent anomaly emerged quite unexpectedly and that the experiments were conducted solely with the object of producing experimental emphysema. Consequently, any conclusions drawn must be of a purely tentative nature.

The lungs were required for histological examination and it was impossible to apply injection techniques to study the degree of vascular blockage. The histological appearances indicated that vascular obstruction was, in fact, produced; but histological assessment of the degree of blockage is difficult. A further complication was the fact that many of the animals dying in the first half of the experimental period did so immediately or shortly after an injection of Caledon Blue. In these cases, in addition to Caledon Blue lodged permanently in the lung vessels, considerable quantities of the dye, which experience had shown would normally have cleared from the lungs in the course of the next 24 hours or so, were also present. On the other hand, most of the animals in the second half of the experimental period were killed 7-14 days after the last injection and it is possible that some of the Caledon Blue had become removed in this period. Nevertheless, allowing

for these variables, there is no doubt that the animals of the first half of the experiment had less, and frequently considerably less, Caledon Blue in their pulmonary vessels than those of the second half. Nor did the period of 7-14 days between injection and killing allow of any detectable removal of Caledon Blue.

In short, there is no histological correlation between the degree of hypertrophy and the degree of pulmonary vascular obstruction. Many of the animals with most marked right ventricular hypertrophy had considerably less Caledon Blue, and by inference, less vascular obstruction than those showing relatively little right ventricular hypertrophy.

It could be argued that the histological appearances give no indication of the degree of obstruction and that in fact those rabbits with most marked right ventricular hypertrophy had a greater degree of functional obstruction, in spite of the histological picture. If this were so, the animals which died in the first half of the experimental period did so because of severe functional obstruction with consequent marked right ventricular hypertrophy. This cannot be denied, but the impression gained was that the cause of unexpected

death, early in an experiment, was the presence of a more severe grade of interstitial pneumonia rather than the injections of Caledon Blue per se.

The interstitial pneumonia, itself, might be thought to be responsible for the right ventricular hypertrophy. However, it has been shown already that, in the absence of pulmonary embolism, interstitial pneumonia does not cause right ventricular hypertrophy either in "normals" or in rabbits with spontaneous emphysema. While it cannot be denied that a combination of interstitial pneumonia and experimental pulmonary embolism might cause a greater degree of right ventricular hypertrophy than embolism alone, the present material does not enable this point to be elucidated. On the whole, the evidence suggests that inflammation is not responsible.

It is felt that the most probable explanation lies in the response of the pulmonary vascular bed to embolism. Two mechanisms seem possible:-

a. There may be individual variation with regard to normally occurring anastomotic channels in the pulmonary and/or bronchial circulations. Thus different rabbits would show a different response to the same degree of embolism without the need for further changes.

b. Compensatory changes may occur which would either prevent the development of right ventricular

hypertrophy or alternatively would permit of a return, or near return, to normal after the compensatory mechanism had come into operation. Such compensatory mechanisms might be anatomical changes, e.g. the development of collaterals or anastomoses, or might be functional vasomotor changes.

Naturally a combination of individual variation and any possible compensatory mechanism could occur.

The present material does not justify further speculation; but evidence will be presented in Section VI which suggests that both individual variation in response and the operation of compensatory mechanisms do occur in experimental pulmonary embolism.

SUMMARY AND CONCLUSIONS

There is no significant difference between the mean LV/RV ratios of various age groups of "normal" rabbits without pulmonary emphysema.

Neither the presence nor the degree of spontaneous marginal and/or generalised emphysema influence the mean LV/RV ratio, i.e. there is no right ventricular hypertrophy.

Generalised interstitial pneumonia, also, does not influence the mean LV/RV ratio, either in rabbits with generalised emphysema or in the non-emphysematous.

Experimental pulmonary embolism by Caledon Blue R.C. produces a significant decrease in the mean LV/RV ratio, i.e. right ventricular hypertrophy.

The degree of right ventricular hypertrophy in the rabbits injected with Caledon Blue is not related to the degree of experimental pulmonary emphysema but solely to the experimental pulmonary embolism.

There does not appear to be a direct relationship between the degree of right ventricular hypertrophy and the degree of pulmonary embolism, as inferred from the amount of Caledon Blue injected and the histological appearances. It is suggested that individual variation and/or the operation of compensatory mechanisms in the pulmonary vascular bed may be responsible for this.

SECTION VI

PRESSURE CHANGES IN THE RIGHT VENTRICLE IN EXPERIMENTAL PULMONARY EMBOLISM

SECTION VI

PRESSURE CHANGES IN THE RIGHT VENTRICLE IN EXPERIMENTAL PULMONARY EMBOLISM

While the work reported in previous sections was in progress, but before fractional heart weight figures were available, it was noted that, even on naked eye assessment, the degree of right ventricular hypertrophy, observed in animals subjected to experimental pulmonary embolism, was variable. In view of this, it was decided to determine what pressure changes, if any, were taking place in the right ventricles of rabbits subjected to similar repeated pulmonary embolism.

METHODS

Details of the techniques employed are given in Appendix D. A brief summary of the main points will be given in the text.

BASIC REQUIREMENTS

The object was to study the changes in right ventricular pressure following repeated experimental pulmonary embolism over long periods. The method had to be applicable to the unanaesthetised animal and had to be suitable for repeated use over long periods. Direct needling of the ventricle, as used by Dunn (1919) in goats, was found to be impracticable. Therefore an operative technique for fixing the heart to the sternum was devised.

OPERATIVE TECHNIQUE

The operation consisted of excising the third and fourth costal cartilages on the right. Next the pericardium was opened and stitched to the wound edges. This partly fixed the heart. Finally, the septum and lateral border of the right ventricle were sutured to the wound edges and the skin sutured over the operation site. When the wound had healed (6 weeks was the period usually allowed for this), the heart could be palpated through the gap in the chest wall and a needle inserted directly into the right ventricle. The final relations are shown in Figure 1.

MEASUREMENT OF PRESSURES

The pressure recorded was the mean right ventricular pressure. This was measured on a simple citrate manometer containing 10% potassium citrate. Pressures were recorded to the nearest 0.25 cm. of water, in both long-term and acute experiments. Continuous recording of pressures was not employed. Two assistants were required to hold the rabbit on its back and with firm gentle handling the rabbits remained relaxed without distress.

ESTABLISHMENT OF BASAL NORMAL MEAN R.V. PRESSURES

When the rabbits were ready for pressure readings, but before intravenous injections of particulate matter were commenced, a series of pressure readings was taken from each animal, at weekly intervals, to establish the normal range of pressures recorded by the method. At the time, five weeks seemed an adequate period, although, in retrospect, in relation to the duration of some of the experiments, the control periods on the charts may look rather short.

TYPE OF EXPERIMENTS

The experiments were of two types: long-term and acute.

Long-term Experiments.

a. Two rabbits were kept as controls and weekly pressure readings were taken; but they received no injections.

b. A second group of rabbits, after weekly basal pressures had been taken, was given weekly intravenous injections of an approximate 3% suspension of Caledon Blue R.C. in saline with .05% of dispersing agent (Dispersol T) added. The dosage varied a little but was usually 2.5 or 3.0 ml. Once injections were started, the mean R.V. pressure was read at weekly intervals, usually 2-3 days after the injection.

c. A third group of rabbits was similarly treated but was given, instead of Caledon Blue, weekly injections of 2.0 - 2.5 ml. of a 1.0% suspension of Lycopodium spores in normal saline with 0.05% Dispersol T and a trace of Teepol added.

Acute Experiments.

These were performed to study the immediate effect of injections of Caledon Blue, Lycopodium or the diluting agent on the mean R.V. pressure and were performed partly on animals in the long-term series and partly on animals not previously subjected to experimental pulmonary embolism.

ASSESSMENT OF FINAL LEVEL OF R.V. PRESSURE

This was done at the conclusion of the long-term experiments in order to determine what correlation there was, if any, between the presence of right ventricular hypertrophy or generalised emphysema and the changes in R.V. pressure.

In all cases the mean basal R.V. pressure was taken as the point of reference. In some instances it was obvious that the final pressure was either raised or within normal limits. In all cases the general trend of the last few readings was taken into account in making the assessment. Doubtful changes were recorded as such.

The final level of R.V. pressure has, therefore, been classified as normal (N), doubtful (D) or raised (R).

STATE OF LUNGS AND HEART AT AUTOPSY

The lungs of the rabbits in the long-term experiments were examined at the conclusion of the experiments and the degree of generalised emphysema and generalised interstitial pneumonia was assessed as in previous sections.

In addition, fractional heart weighing was performed and the LV/RV ratios estimated with a view to assessing what degree of right ventricular hypertrophy was present.

Hitherto, in Section V, assessment of right ventricular hypertrophy has been performed on groups and not individuals; but in the present section individual hearts have to be considered. Further, the ages of the rabbits under consideration were not known but certainly varied considerably.

It has been shown, in Section V, that age has no significant effect on the LV/RV ratios of "normal" rabbits and so the data obtained from 133 "normal" rabbits of all ages (Section V, Table II) has been used for comparative purposes. In round figures, the mean LV/RV ratio was 4.14 with a standard deviation of 0.50, in this group. These figures have been used for a somewhat arbitrary assessment of the presence of right ventricular hypertrophy in the animals employed in the pressure experiments, as shown in Table I.

TABLE I

Assessment of Right Ventricular Hypertrophy.

LV/RV Ratio	State of Right Ventricle
4.64 to 3.64	Normal (N)
3.63 to 3.14	Doubtful (D)
3.13 or less	Hypertrophied (H)

PRESENTATION OF RESULTS

a. Long-term Experiments.

Full details of each experiment are given in Appendix D. Charts illustrating the pressure responses are included in the text and are cross-referenced to the appropriate tables in Appendix D.

The following information is also included in the charts:-

i. Mean basal R.V. pressure: interrupted horizontal line.

ii. Points where acute experiments were performed: vertical arrow labelled "A".

iii. Degree of generalised emphysema: Gen. E.

iv. LV/RV ratio to show hypertrophy or otherwise: N, D or H.

v. Degree of generalised interstitial pneumonia: Gen. I.

vi. Final level of R.V. pressure: Final R.V.P. (N, D or R).

b. Acute Experiments.

Examples of these will be given in the text, illustrated by charts cross-referenced to the appropriate tables in Appendix D.

It should be noted that the same scale is used for pressure in the charts illustrating both long-term and acute experiments. However, the time scales are in weeks and minutes respectively.

RESULTS

LONG TERM EXPERIMENTS

Basal mean R.V. pressures were established in 21 rabbits. However, two of these (RO/24 and RO/26) died before the experiments proper could be started and will not be considered, further, here. Details are given in Appendix D, Tables A and B.

The procedures applied to the remaining 19 rabbits may be summarised as follows:-

TABLE II

Long-term Experiments

Procedure	No. of Rabbits
Control: no injections	2
Weekly I.V. Caledon Blue	10
Weekly I.V. Lycopodium	7

The mean R.V. pressure of the control rabbits remained unchanged. The response of the rabbits receiving Caledon Blue or Lycopodium differed only in degree but similar patterns occurred in both groups which may be summarised as follows:-

a. No rise in mean R.V. pressure.

b. Rise in mean R.V. pressure: death while raised.

c. Rise in mean R.V. pressure: followed by return to basal level.

d. Modified response to a second course of injections.

e. Doubtful responses.

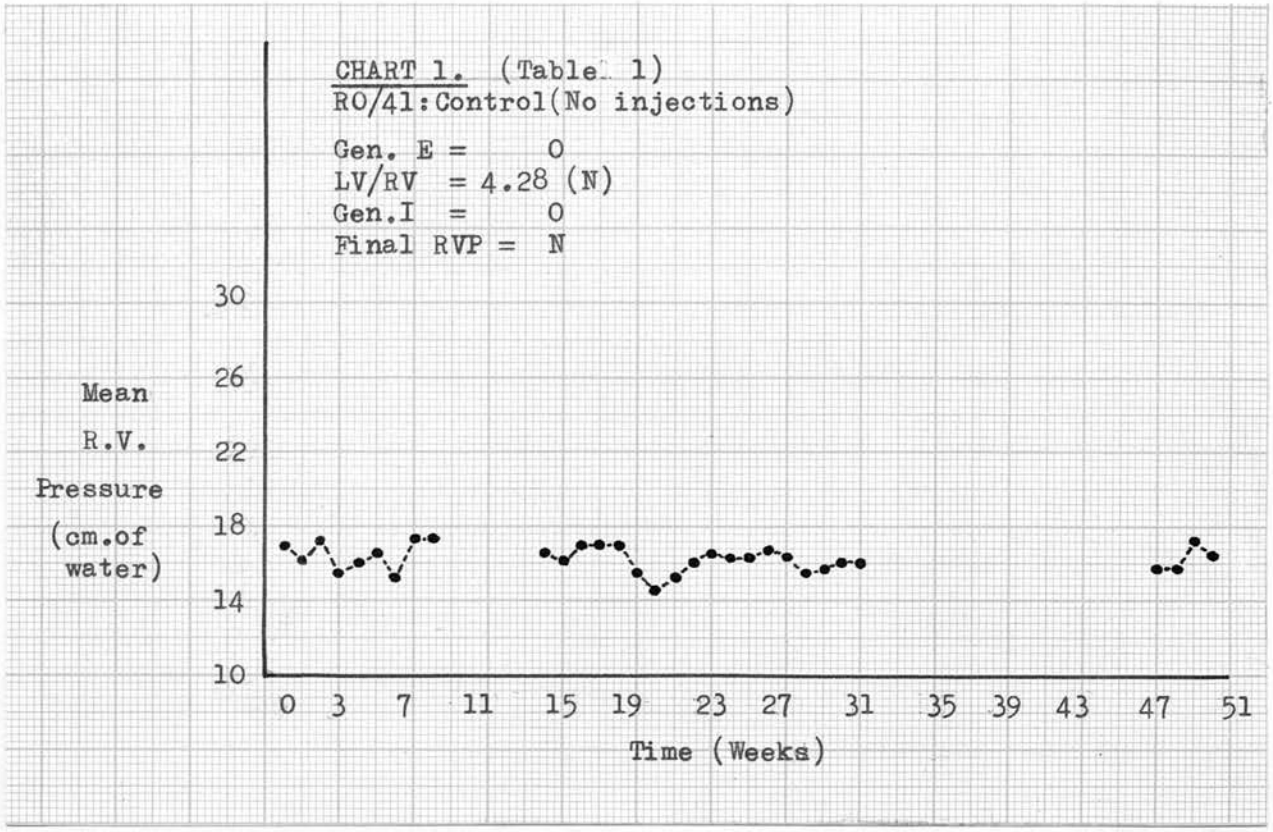


CHART 1. (Table 1)
RO/41: Control(No injections)
Gen. E = 0
LV/RV = 4.28 (N)
Gen.I = 0
Final RVP = N
Mean R.V. Pressure (cm.of water)
30
26
22
18
14
10
0 3 7 11 15 19 23 27 31 35 39 43 47 51
Time (Weeks)

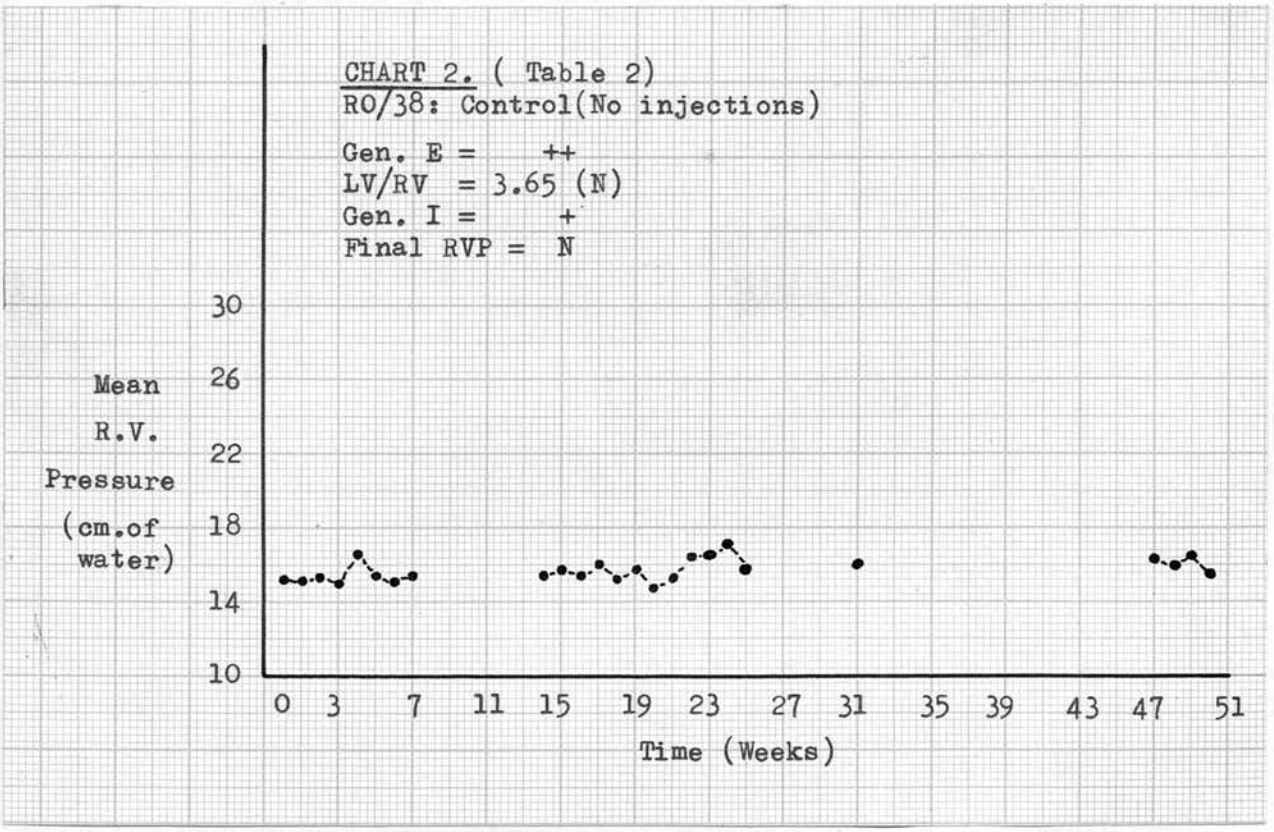


CHART 2. (Table 2)
RO/38: Control(No injections)
Gen. E = ++
LV/RV = 3.65 (N)
Gen. I = +
Final RVP = N
Mean R.V. Pressure (cm.of water)
30
26
22
18
14
10
0 3 7 11 15 19 23 27 31 35 39 43 47 51
Time (Weeks)

CONTROLS

Two rabbits were left without injections and their R.V. pressure recorded over a period of nearly one year. Charts 1 and 2 show the results. The operative procedure did not cause a rise in the mean R.V. pressure. Control by two animals is not perhaps adequate. But it should be noted that the basal pressures in the other rabbits were intended to serve as controls and experience over the whole experimental period gave no suggestion that a persistent rise in the R.V. pressure was due to the experimental technique rather than the injected material.

It will be seen that the level of the mean R.V. pressure in these two animals fluctuated around a level of about 16 cm. of water.

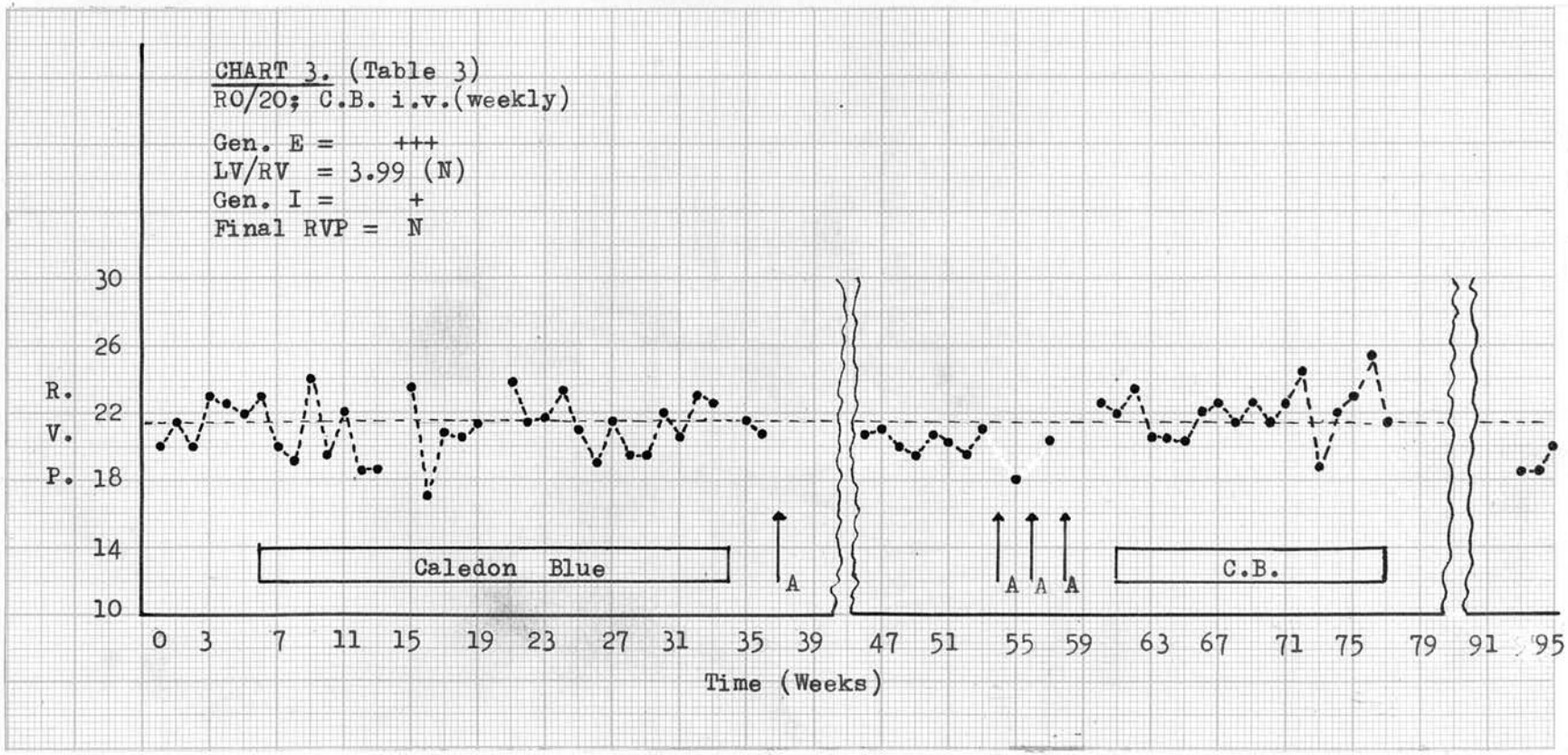


CHART 3. (Table 3)
RO/20; C.B. i.v.(weekly)
Gen. E = +++
LV/RV = 3.99 (N)
Gen. I = +
Final RVP = N
R.
V.
P.
30
26
22
18
14
10
Caledon Blue
A
A A A
C.B.
0 3 7 11 15 19 23 27 31 35 39 47 51 55 59 63 67 71 75 79 91 95
Time (Weeks)

CALEDON BLUE EXPERIMENTS

a. No Rise in Mean R.V. Pressure.

Chart 3 shows the response in the case of one rabbit (RO/20) which was given two successive courses of weekly Caledon Blue injections. There is no sustained rise in mean R.V.P.

The baseline is short in relation to the total duration of the observation period (95 weeks) but, as will be seen in other experiments, there was much more fluctuation in the levels of the weekly pressure readings during the periods when Caledon Blue was being injected.

It should be noted that the mean basal pressure of this rabbit was 21.5 cm. of water: a higher level than in most others. It is impossible to assess the significance of this in relation to the response to Caledon Blue, but it is of interest to note that the LV/RV ratio was 3.99, well within normal limits.

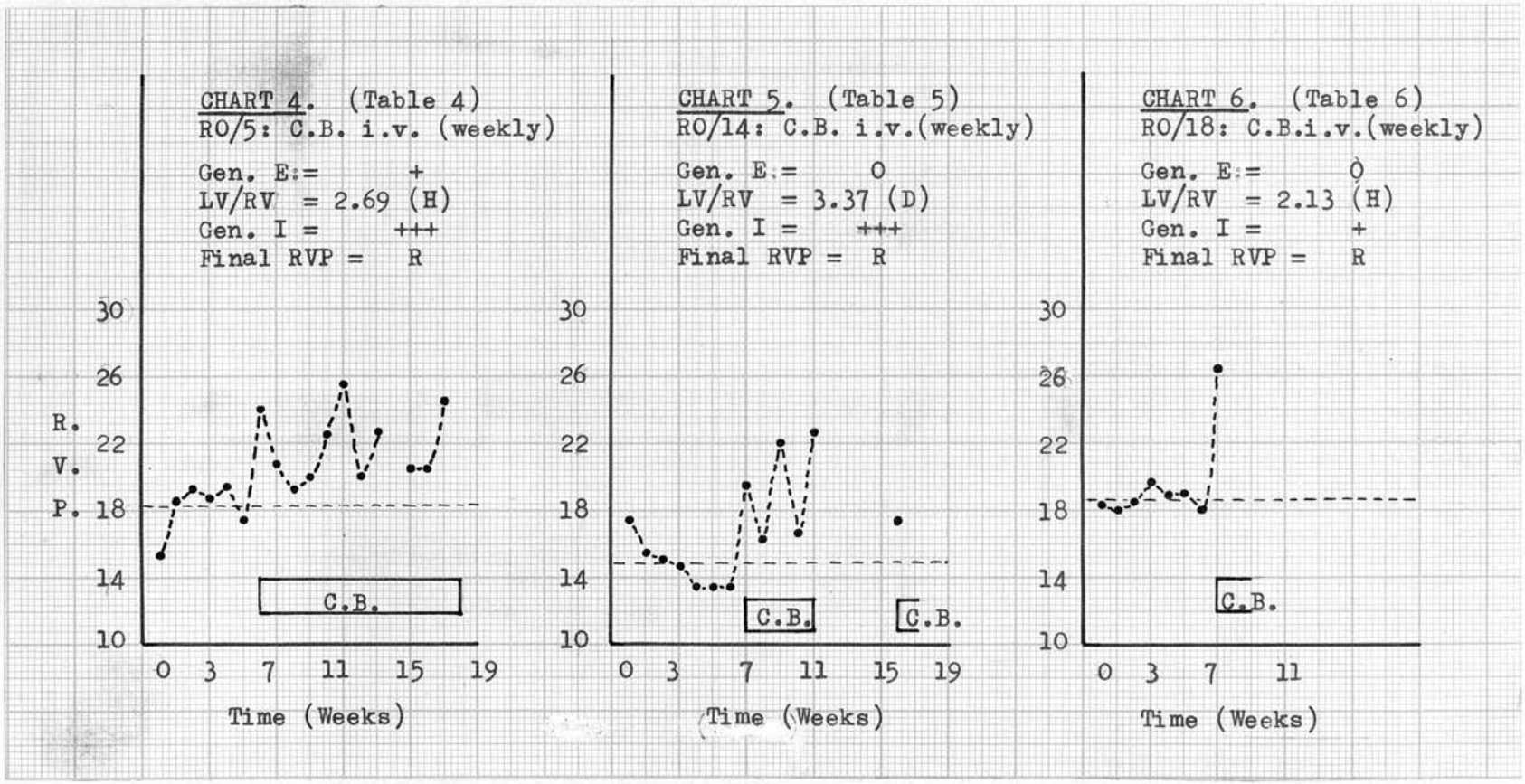


CHART 4. (Table 4)
RO/5: C.B. i.v. (weekly)
Gen. E:= +
LV/RV = 2.69 (H)
Gen. I = +++
Final RVP = R
30
26
22
18
14
10
R.
V.
P.
C.B.
0 3 7 11 15 19
Time (Weeks)
CHART 5. (Table 5)
RO/14: C.B. i.v.(weekly)
Gen. E.= O
LV/RV = 3.37 (D)
Gen. I = +++
Final RVP = R
30
26
22
18
14
10
C.B.
C.B.
0 3 7 11 15 19
Time (Weeks)
CHART 6. (Table 6)
RO/18: C.B.i.v.(weekly)
Gen. E:= O
LV/RV = 2.13 (H)
Gen. I = +
Final RVP = R
30
26
22
18
14
10
C.B.
0 3 7 11
Time (Weeks)

b. Rise in Mean R.V. Pressure: Death while raised.

Charts 4, 5 and 6 (Rabbits RO/5, RO/14 and RO/18 respectively) show this response. All three rabbits died immediately after their last injection of Caledon Blue but it should be noted that one of them (RO/14) was ill for a few weeks which caused the injections to be suspended.

The response in these rabbits was immediate and quite striking. Two of them showed marked fluctuations in the level of the weekly pressures during the period of injection, but the pressure did not fall to the mean basal level.

Two of the animals in this group showed definite right ventricular hypertrophy while the third showed suggestive hypertrophy.

CHART 7. (Table 7)
RO/16: C.B. i.v. (weekly)

Gen. E = ++
LV/RV = 3.34 (D)
Gen. I = +
Final RVP = N

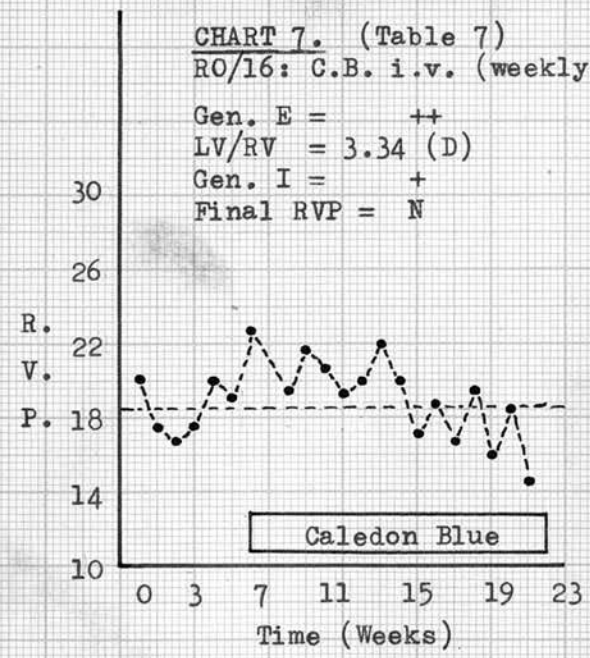


CHART 8. (Table 8)
RO/40: C.B. i.v. (weekly)

Gen. E = ++
LV/RV = 3.95 (N)
Gen. I = +
Final RVP = N

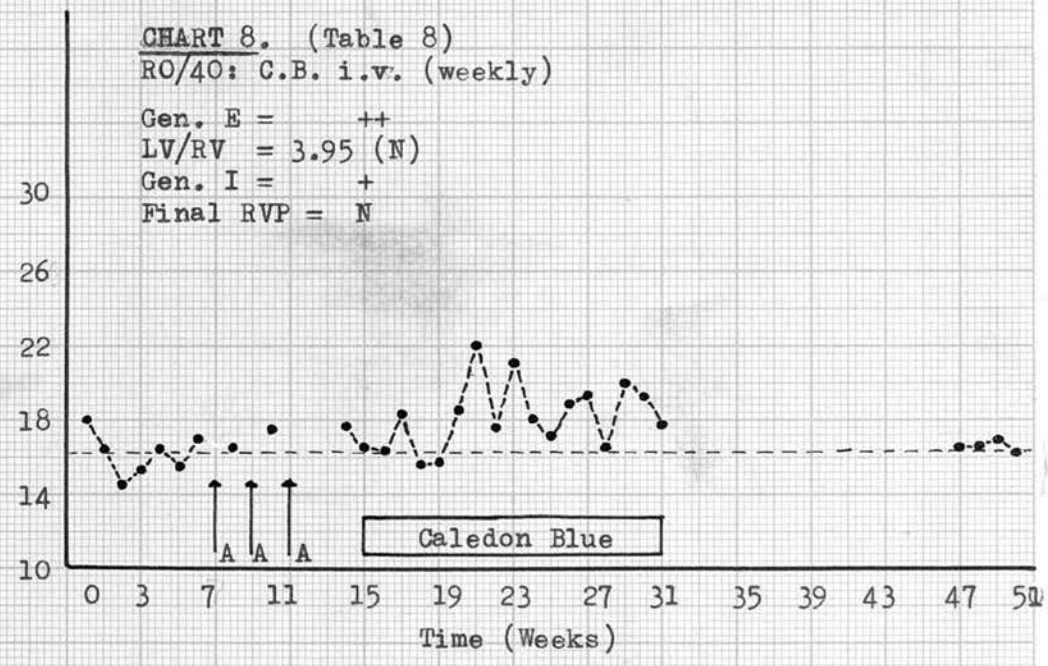
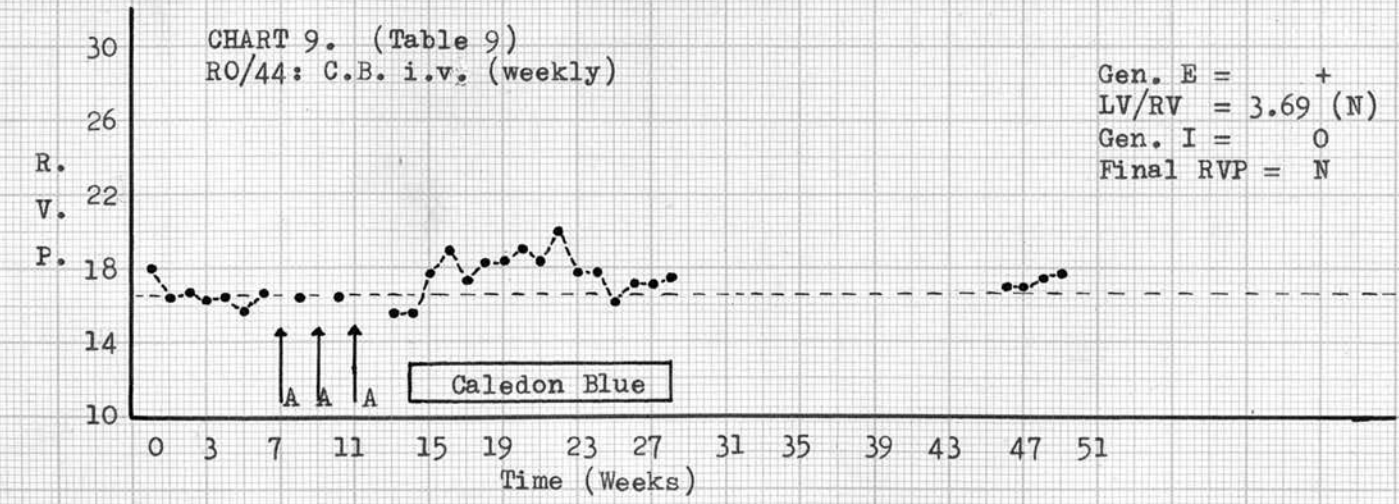


CHART 9. (Table 9)
RO/44: C.B. i.v. (weekly)

Gen. E = +
LV/RV = 3.69 (N)
Gen. I = 0
Final RVP = N



c. Rise in Mean R.V. Pressure: followed by return to Basal Level.

This response is illustrated in Charts 7, 8 and 9 (Rabbits RO/16, RO/40 and RO/44 respectively).

RO/16 died immediately after an injection of Caledon Blue but had been normal, clinically, during the preceding weeks and showed no sign of cardiac failure either during life or at autopsy. The other two rabbits survived and were killed later.

Chart 8 (RO/40) is not such a clear-cut example of this type of response as the other two but it is felt that the similarity is enough to justify its inclusion in this group.

It should be noted that only one animal (RO/16) in this group shows even suggestive hypertrophy of the left ventricle.

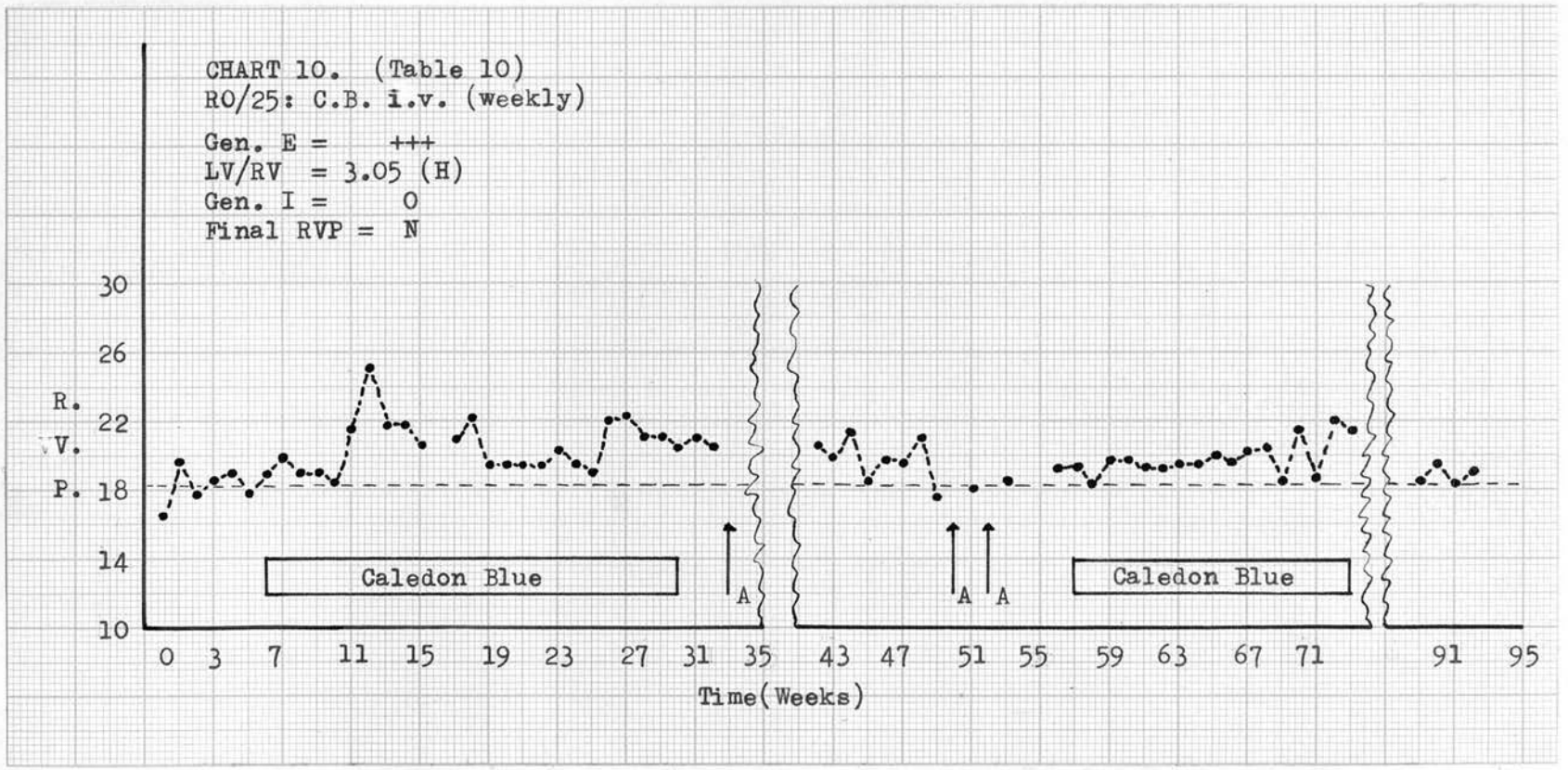


CHART 10. (Table 10)
RO/25: C.B. i.v. (weekly)
Gen. E = +++
LV/RV = 3.05 (H)
Gen. I = 0
Final RVP = N
R. V. P.
30
26
22
18
14
10
Caledon Blue
A
A
A
Caledon Blue
0 3 7 11 15 19 23 27 31 35 43 47 51 55 59 63 67 71 91 95
Time(Weeks)

d. Modified Response to a Second Course of Caledon Blue Injections.

Chart 10 (RO/25) illustrates this.

The response to Caledon Blue was delayed for four weeks, but then the initial rise, as seen in other rabbits, took place. While injections were being continued at weekly intervals the mean R.V. pressure fell somewhat, in the manner of the response in the previous sub-group.

Caledon Blue injections were stopped and then recommenced about 25 weeks later. However, no rise in pressure occurred for a period of about 10 weeks following the commencement of the second course of injections, but, after that, there was a short period of fluctuation with a tendency to rise.

The pressure response during the second period of injections was less dramatic than during the first.

At the end of the observation period the pressures had returned to basal levels but the right ventricle was definitely hypertrophied.

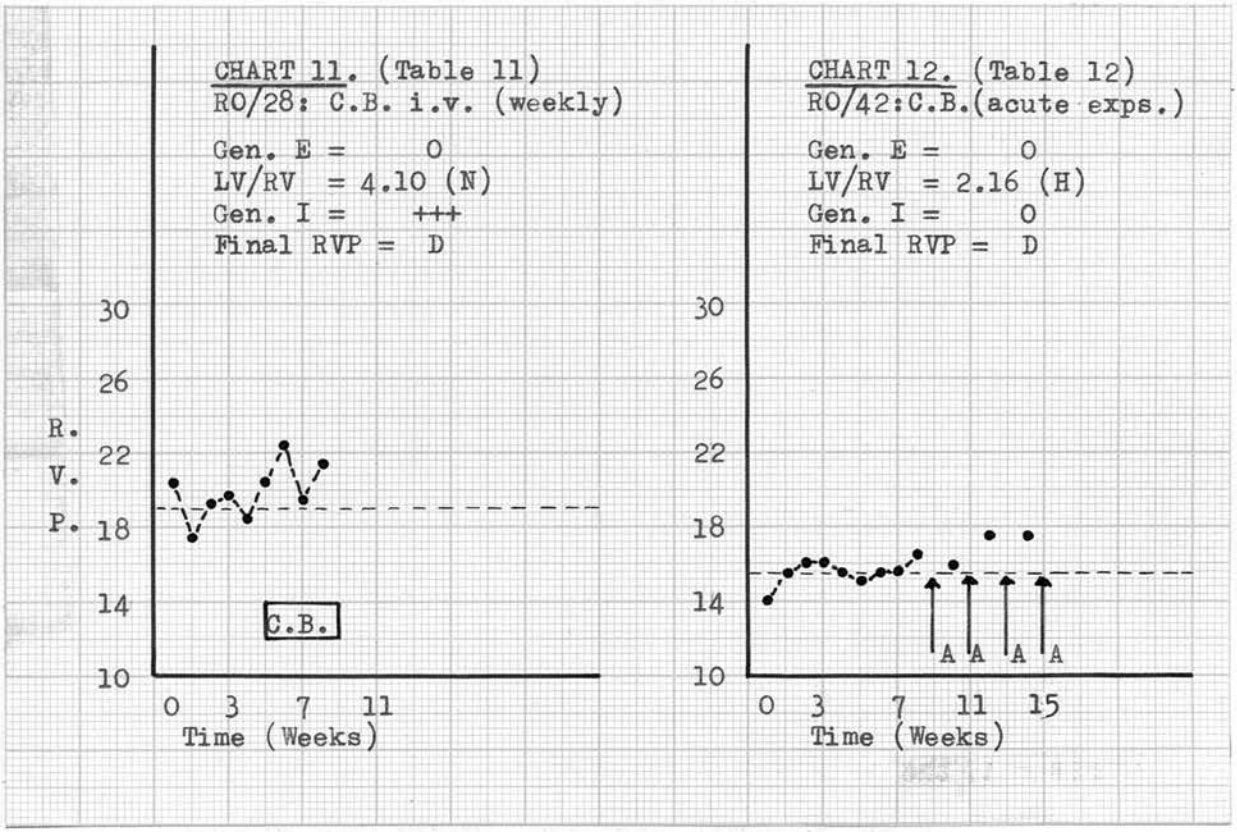


CHART 11. (Table 11)
RO/28: C.B. i.v. (weekly)
Gen. E = 0
LV/RV = 4.10 (N)
Gen. I = +++
Final RVP = D
30
26
22
18
14
10
R.
V.
P.
C.B.
0 3 7 11
Time (Weeks)
CHART 12. (Table 12)
RO/42:C.B.(acute exps.)
Gen. E = 0
LV/RV = 2.16 (H)
Gen. I = 0
Final RVP = D
30
26
22
18
14
10
A A A A
0 3 7 11 15
Time (Weeks)

e. Doubtful Responses.

Charts 11 and 12 (RO/28 and RO/42 respectively) show doubtful responses to the injection of Caledon Blue.

RO/28 died immediately after an injection and was found to have a marked degree of interstitial pneumonia. The pressure readings in Chart 11 suggest a slight rise, but the survival time was not long enough to enable a definite conclusion to be drawn.

RO/42 was used for a series of four acute experiments at intervals of two weeks. Caledon Blue was injected at each acute experiment and the animal survived for six weeks after the first injection. Chart 12 shows a doubtful rise in the mean R.V. pressure.

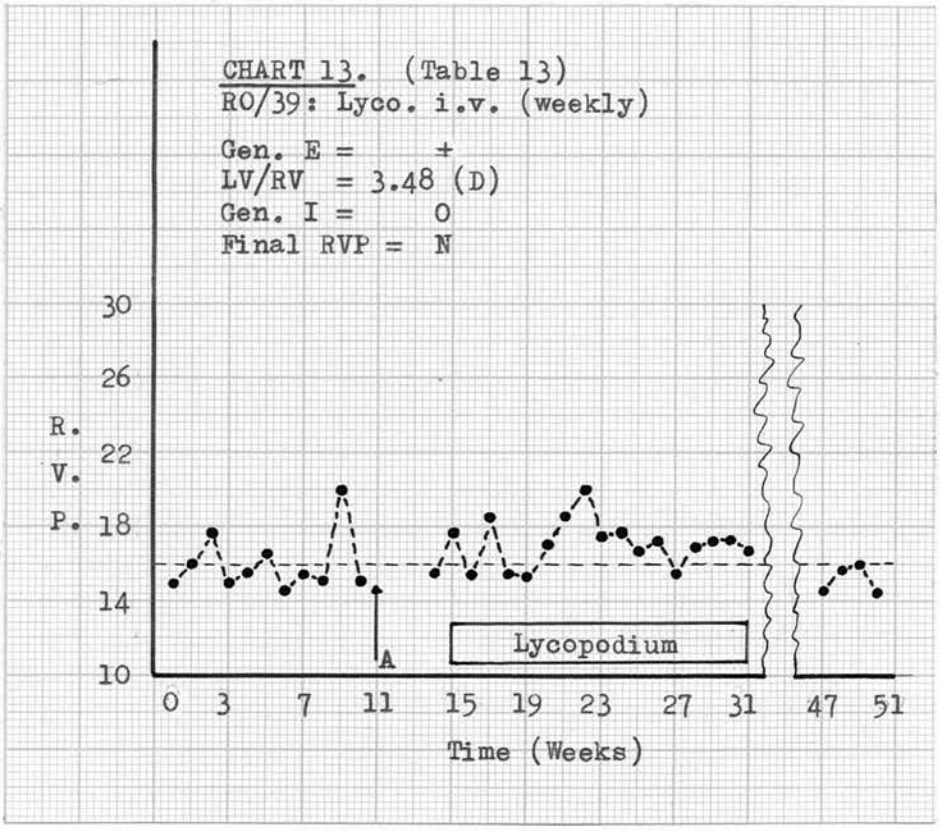


CHART 13. (Table 13)
RO/39: Lyco. i.v. (weekly)
Gen. E = +
LV/RV = 3.48 (D)
Gen. I = O
Final RVP = N
30
26
R.
V.
P.
22
18
14
10
A
Lycopodium
0 3 7 11 15 19 23 27 31 47 51
Time (Weeks)

LYCOPODIUM SPORE EXPERIMENTS

The responses in these fell into the same groups as those in the Caledon Blue experiments.

a. No Rise in Mean R.V. Pressure.

Chart 13 (RO/39) shows this type of response.

There is considerable fluctuation in the basal pressures and, while there is a suggestion of a slight overall rise in mean R.V. pressure in the middle of the injection period, the general appearance of the chart indicates lack of response to I.V. Lycopodium.

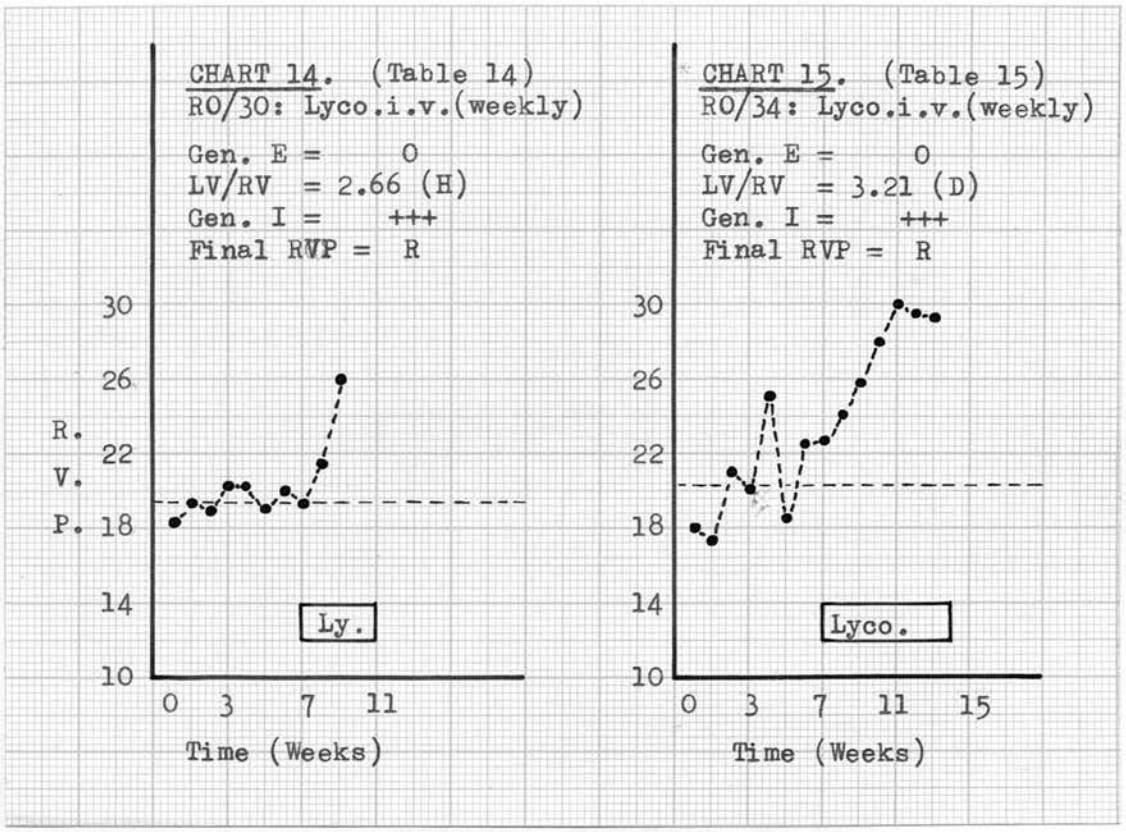


CHART 14. (Table 14)
RO/30: Lyco.i.v.(weekly)
Gen. E = 0
LV/RV = 2.66 (H)
Gen. I = +++
Final RVP = R
30
26
22
18
14
10
R.
V.
P.
Ly.
0 3 7 11
Time (Weeks)
CHART 15. (Table 15)
RO/34: Lyco.i.v.(weekly)
Gen. E = 0
LV/RV = 3.21 (D)
Gen. I = +++
Final RVP = R
30
26
22
18
14
10
Lyco.
0 3 7 11 15
Time (Weeks)

b. Rise in Mean R.V. Pressure: Death while raised.

Charts 14 and 15 (RO/30 and RO/34 respectively) show this type of response which is striking and quite clear-cut in spite of the marked fluctuations in the basal pressures in the case of RO/34.

The response is similar, but perhaps a little more dramatic than in the corresponding groups in the Caledon Blue experiments.

It should be noted that RO/30 shows definite right ventricular hypertrophy and the other rabbit shows suggestive, but not definite, hypertrophy.

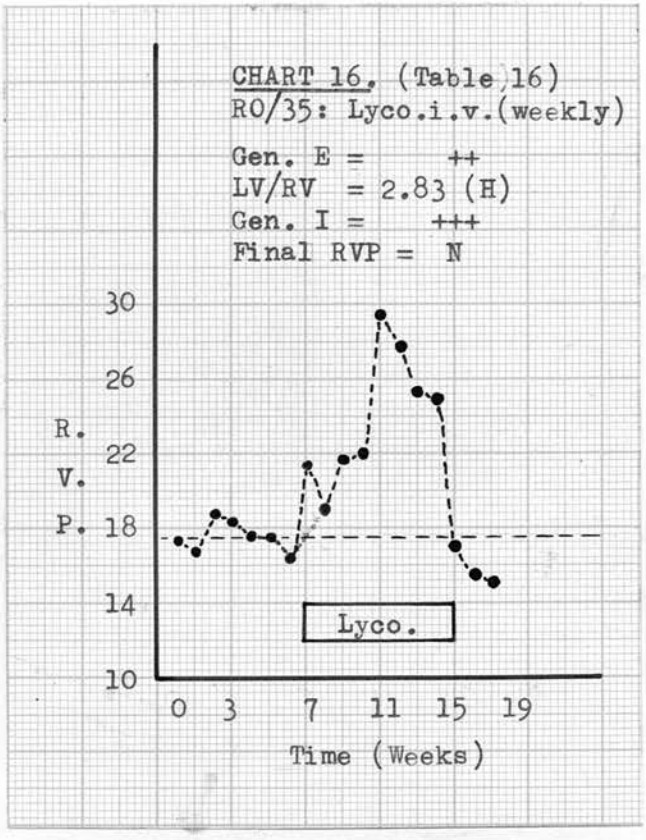


CHART 16. (Table 16)
RO/35: Lyco.i.v.(weekly)
Gen. E = ++
LV/RV = 2.83 (H)
Gen. I = +++
Final RVP = N
30
26
22
18
14
10
R.
V.
P.
Lyco.
0 3 7 11 15 19
Time (Weeks)

c. Rise in Mean R.V. Pressure: followed by Return to Basal Level.

Chart 16 (RO/35) illustrates this response.

The pressure fell during the course of the Lycopodium injections and the rabbit died two weeks later apparently due to acute respiratory infection.

At no time, either during the injections, subsequently or at autopsy, was there any sign of heart failure.

Definite right ventricular hypertrophy was present.

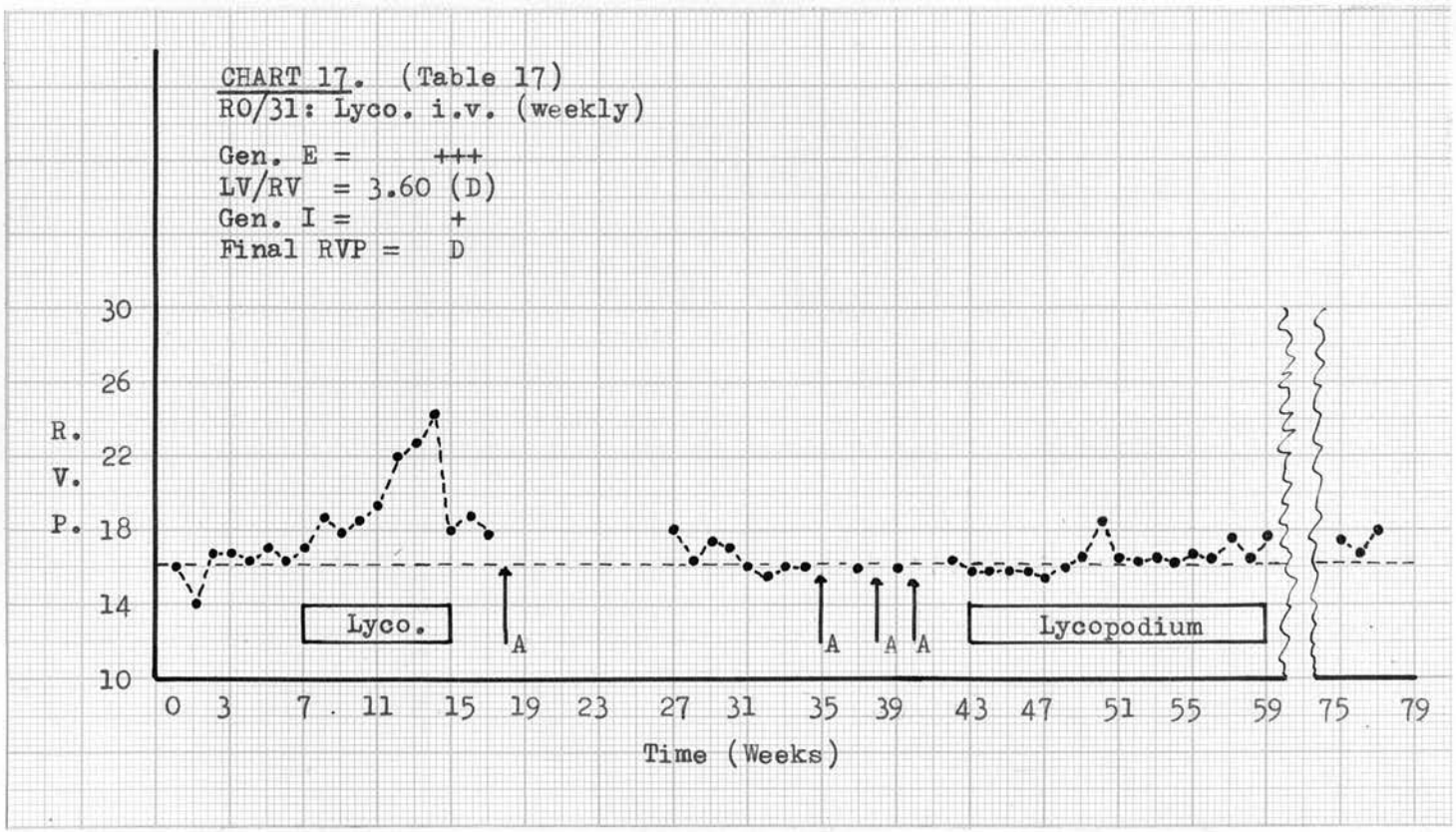


CHART 17. (Table 17)
RO/31: Lyco. i.v. (weekly)
Gen. E = +++
LV/RV = 3.60 (D)
Gen. I = +
Final RVP = D
R. V. P.
30
26
22
18
14
10
Lyco.
A
A
A
A
Lycopodium
0 3 7 11 15 19 23 27 31 35 39 43 47 51 55 59 75 79
Time (Weeks)

d. Modified Response to a Second Course of Lycopodium Injections.

Chart 17 (RO/31) illustrates this very clearly.

There was an initial sharp rise in mean R.V. pressure during the first course of injections but the pressure fell to near basal level while the injections were still in progress.

After a rest period the pressure fell to basal level and when systematic weekly injections of Lycopodium were recommenced there was virtually no response at all except at the end of the course of injections when there was a slight, doubtful rise.

This modified response was all the more striking in view of the fact that, in the second course of injections, a weekly dose of 2.5 ml. of 1% Lycopodium suspension was used as compared with 2.0 ml. in the first course.

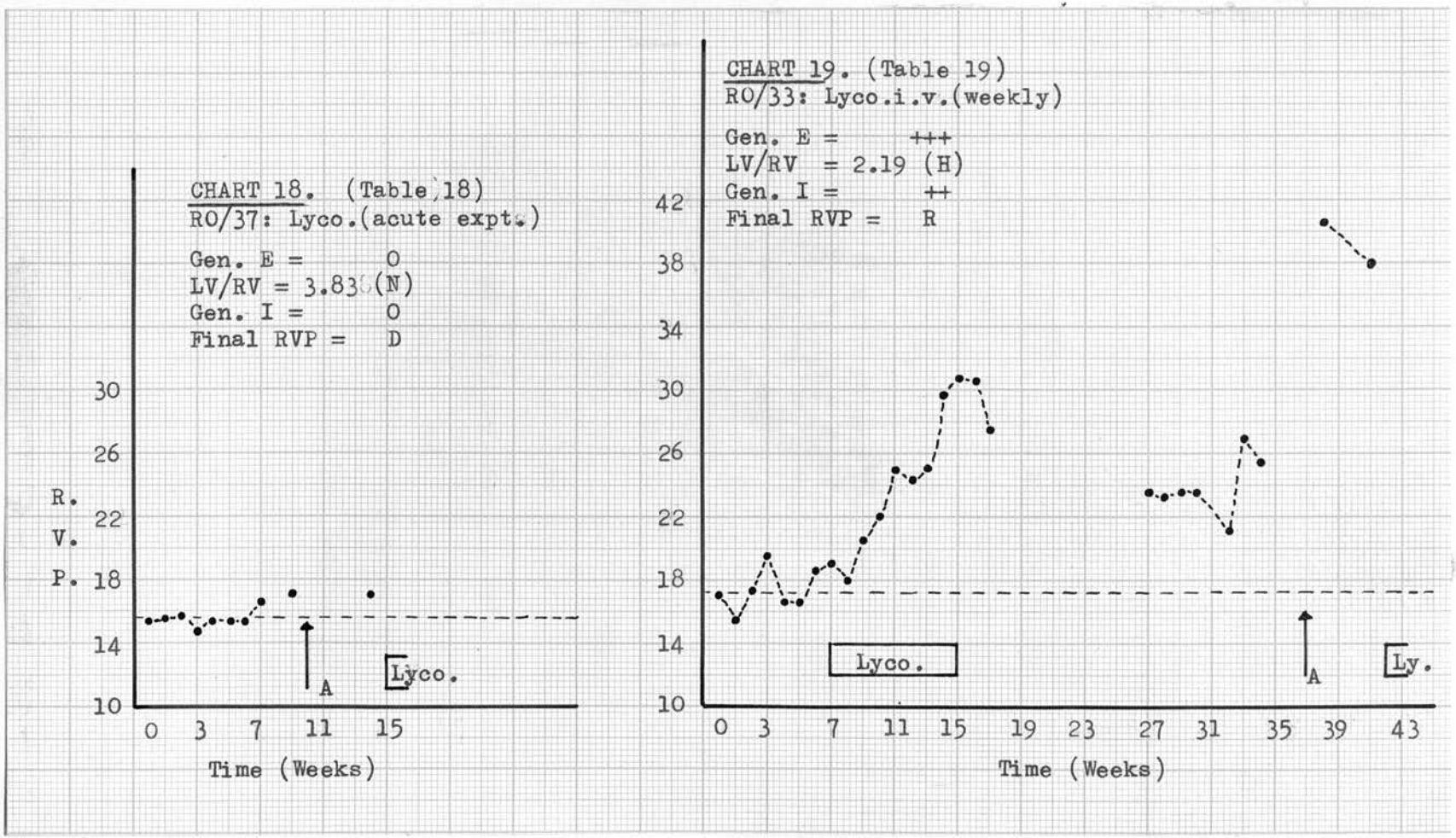


CHART 18. (Table 18)
RO/37: Lyco.(acute expt.)
Gen. E = O
LV/RV = 3.83 (N)
Gen. I = O
Final RVP = D
R.
V.
P.
30
26
22
18
14
10
0 3 7 11 15
Time (Weeks)
A
Lyco.
CHART 19. (Table 19)
RO/33: Lyco.i.v.(weekly)
Gen. E = +++
LV/RV = 2.19 (H)
Gen. I = ++
Final RVP = R
42
38
34
30
26
22
18
14
10
0 3 7 11 15 19 23 27 31 35 39 43
Time (Weeks)
Lyco.
A
Ly.

e. Doubtful Responses.

Chart 18 (RO/37) shows the response in a rabbit which survived 6-7 weeks after a single injection of Lycopodium. There was a doubtful rise of the mean R.V.P.

Chart 19 (RO/33) shows a very dramatic response but is included in the "doubtful" group as in every sense this rabbit reacted atypically to the injections of Lycopodium. Its tolerance was very low and, clinically, it was felt that, even with small doses, death was liable to occur at any moment. The curve is presented as it shows the heights to which the pressure can rise without causing immediate death. Although the injections had to be stopped before the pressure reached its peak in the initial period of the experiment, the subsequent fall resembles that seen in other animals. But the pressure never came back to normal and a single additional injection at week 37 was sufficient to nearly double the pressure.

ACUTE EXPERIMENTS

These experiments were instituted to investigate one of the points which arose during the long-term studies, viz. the fall in R.V. pressure which occurred in some rabbits at a time when the Caledon Blue or Lycopodium injections were being continued and when the animals showed no sign of cardiac failure or other clinical upset.

It happened that this phenomenon was noted in a rabbit where the weekly pressure reading was taken three days after the Lycopodium injection instead of one day after.

This prompted two questions: (a) Was this fall due merely to the alteration in timing of the pressure reading? (b) Was the apparently more dramatic response to Lycopodium due to the fact that less time elapsed between the injections and the pressure readings?

In anticipation, it may be said that the acute experiments showed that it was most unlikely that the time of the pressure reading influenced the results of the long-term experiments. Consequently, although the results of the acute experiments are not without interest in themselves, they will not be given in detail in the text.

Where/

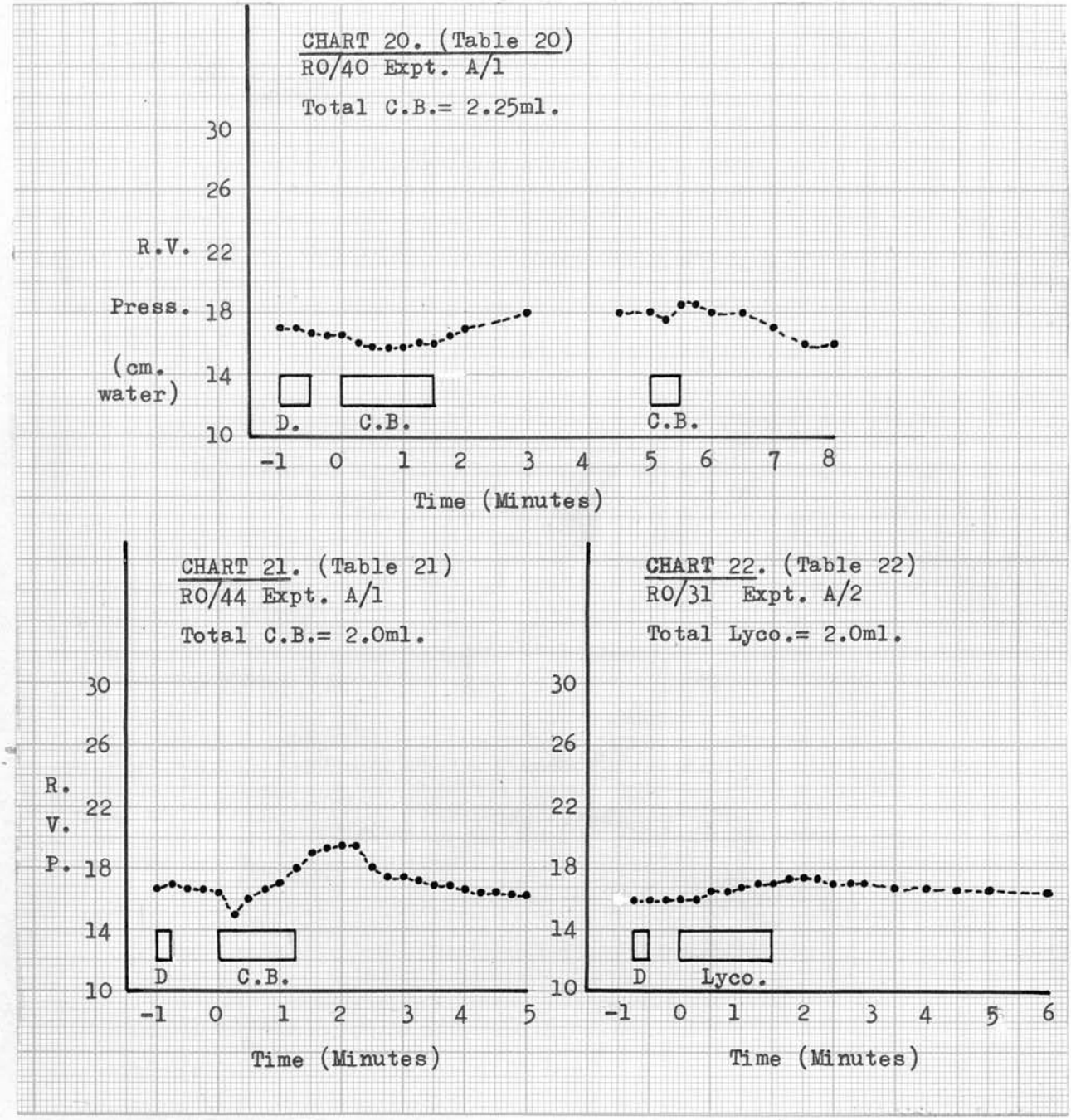


CHART 20. (Table 20)
RO/40 Expt. A/1
Total C.B.= 2.25ml.
R.V. Press. (cm. water)
30
26
22
18
14
10
D.
C.B.
C.B.
-1 0 1 2 3 4 5 6 7 8
Time (Minutes)
CHART 21. (Table 21)
RO/44 Expt. A/1
Total C.B.= 2.0ml.
R. V. P.
30
26
22
18
14
10
D
C.B.
-1 0 1 2 3 4 5
Time (Minutes)
CHART 22. (Table 22)
RO/31 Expt. A/2
Total Lyco.= 2.0ml.
30
26
22
18
14
10
D
Lyco.
-1 0 1 2 3 4 5 6
Time (Minutes)

Where acute experiments involving injection of Caledon Blue or Lycopodium have been performed this has been indicated in the long-term charts.

CALEDON BLUE AND LYCOPODIUM EXPERIMENTS

a. Pressure Readings taken One Hour after Injection.

This was done in three animals only, viz. experiments A/1 in RO/20, RO/25 and RO/31 (Charts 3, 10 and 17 respectively). Each received what had been their usual dose of Caledon Blue (RO/20 and RO/25) or Lycopodium (RO/31) and the pressure was read one hour later. The levels recorded were not above the levels for the last few readings. Thus it appeared likely that the time factor in reading the pressures probably did not account for the fall in pressures which had been noted.

b. Pressure Readings taken during the actual Injection Period.

In all these experiments a preliminary injection of diluting agent was given: usually 3.0 ml. in 30 seconds. The injection of Caledon Blue or Lycopodium was given 30-45 seconds later, in doses of 2-3 ml. as indicated by the response.

1. Injections given slowly over more than 1.0 mins.

The pressure changes seen in three out of four experiments are shown in Charts 20, 21 & 22.

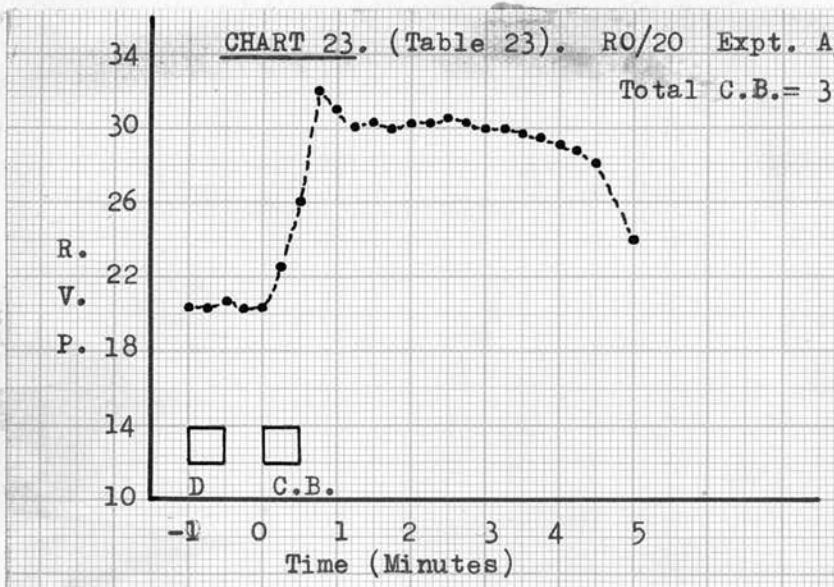


CHART 23. (Table 23). RO/20 Expt. A/4
Total C.B.= 3.0ml.
R.
V.
P.
34
30
26
22
18
14
10
D
C.B.
-1
0
1
2
3
4
5
Time (Minutes)

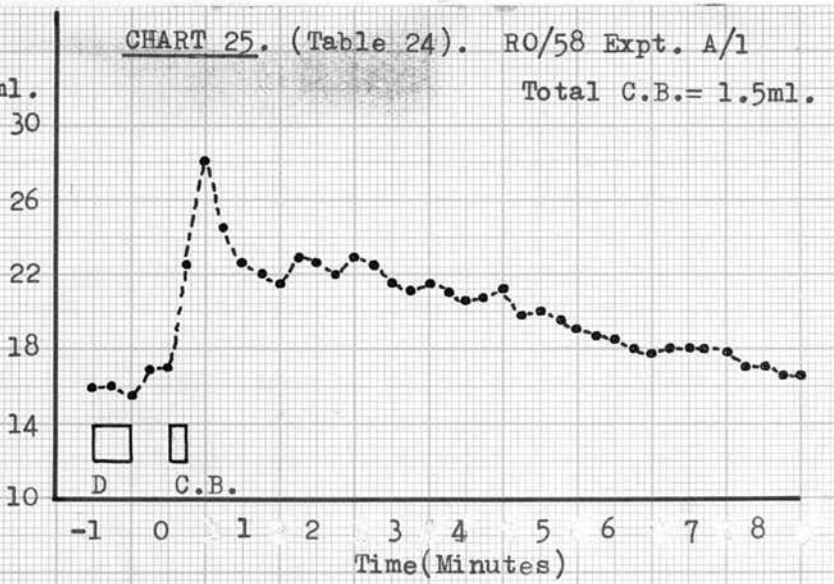


CHART 25. (Table 24). RO/58 Expt. A/1
Total C.B.= 1.5ml.
30
26
22
18
14
10
D
C.B.
-1
0
1
2
3
4
5
6
7
8
Time(Minutes)

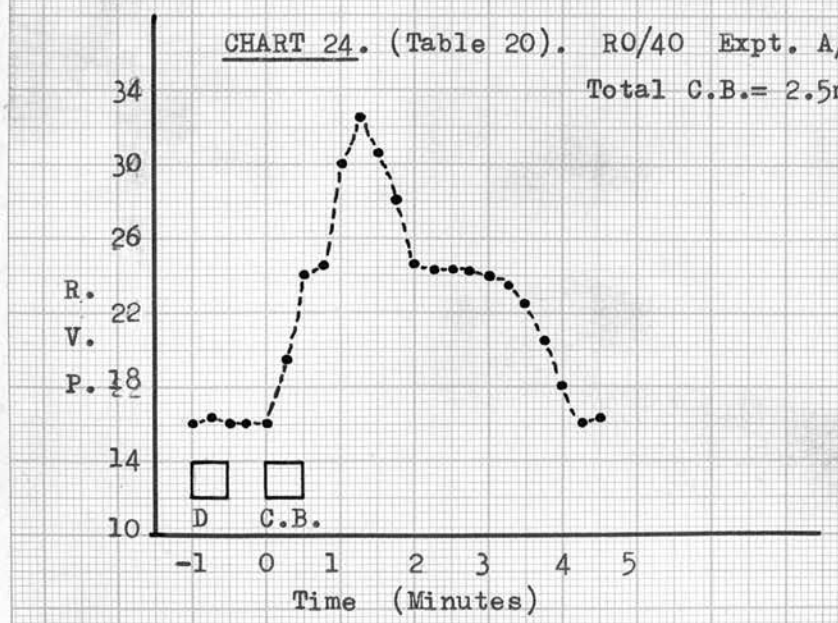


CHART 24. (Table 20). RO/40 Expt. A/2
Total C.B.= 2.5ml.
R.
V.
P.
34
30
26
22
18
14
10
D
C.B.
-1
0
1
2
3
4
5
Time (Minutes)

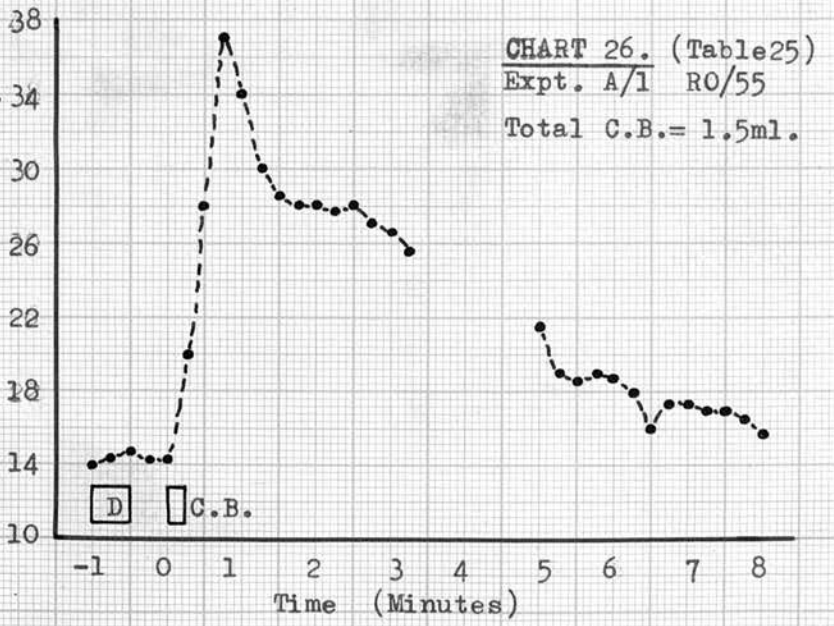


CHART 26. (Table25)
Expt. A/1 RO/55
Total C.B.= 1.5ml.
38
34
30
26
22
18
14
10
D
C.B.
-1
0
1
2
3
4
5
6
7
8
Time (Minutes)

RO/40 and RO/44 were receiving their first injections of Caledon Blue while RO/31 had already had numerous injections of Lycopodium. The doses were those usually given in the long-term experiments, but the rate of injection was somewhat slower. The very slight rise of pressure was surprising in view of the fact that similar doses have been known to kill rabbits, especially on a first injection.

2. In view of the apparently slight pressure rise, subsequent injections were given more quickly, usually in about half a minute.

The responses fell roughly into three groups:-

A. Spiked: In this type there was an immediate more or less dramatic pressure rise to a peak followed by a sudden drop to an intermediate level at which the pressure sustained itself for a few minutes, giving a plateau-like appearance to this portion of the curve. Then, finally, there was a fairly rapid drop to basal or near basal level.

This type of response was the common one with Caledon Blue irrespective of whether or not the rabbit had received previous injections.

Examples of this type of response are shown in Charts 23, 24, 25 and 26.

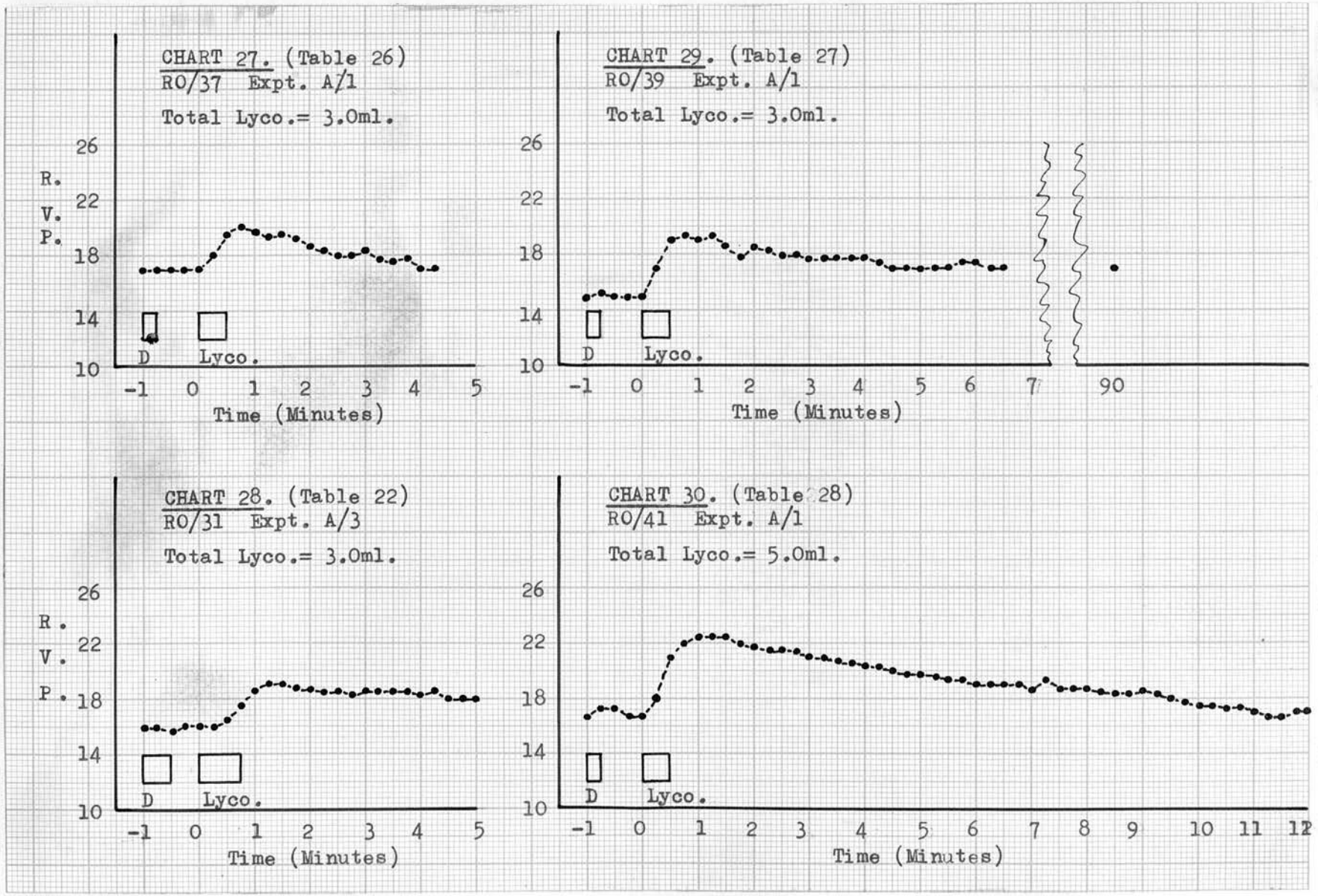


CHART 27. (Table 26)
RO/37 Expt. A/1
Total Lyco.= 3.0ml.
R.
V.
P.
26
22
18
14
10
D
Lyco.
-1 0 1 2 3 4 5
Time (Minutes)
CHART 29. (Table 27)
RO/39 Expt. A/1
Total Lyco.= 3.0ml.
26
22
18
14
10
D
Lyco.
-1 0 1 2 3 4 5 6 7 90
Time (Minutes)
CHART 28. (Table 22)
RO/31 Expt. A/3
Total Lyco.= 3.0ml.
R.
V.
P.
26
22
18
14
10
D
Lyco.
-1 0 1 2 3 4 5
Time (Minutes)
CHART 30. (Table 28)
RO/41 Expt. A/1
Total Lyco.= 5.0ml.
26
22
18
14
10
D
Lyco.
-1 0 1 2 3 4 5 6 7 8 9 10 11 12
Time (Minutes)

B. Flat: Here the pressure rose fairly sharply but not to the same height as in the previous type. No peak or spike was produced and the return to normal was even and gradual. On the whole, especially in relation to the initial pressure rise, the return to basal or near basal level took longer than in the case of the spiked type.

This type of curve was the common one in acute experiments using Lycopodium, irrespective of whether or not the animal had had previous injections of Lycopodium.

Charts 27, 28, 29 and 30 illustrate this type of response.

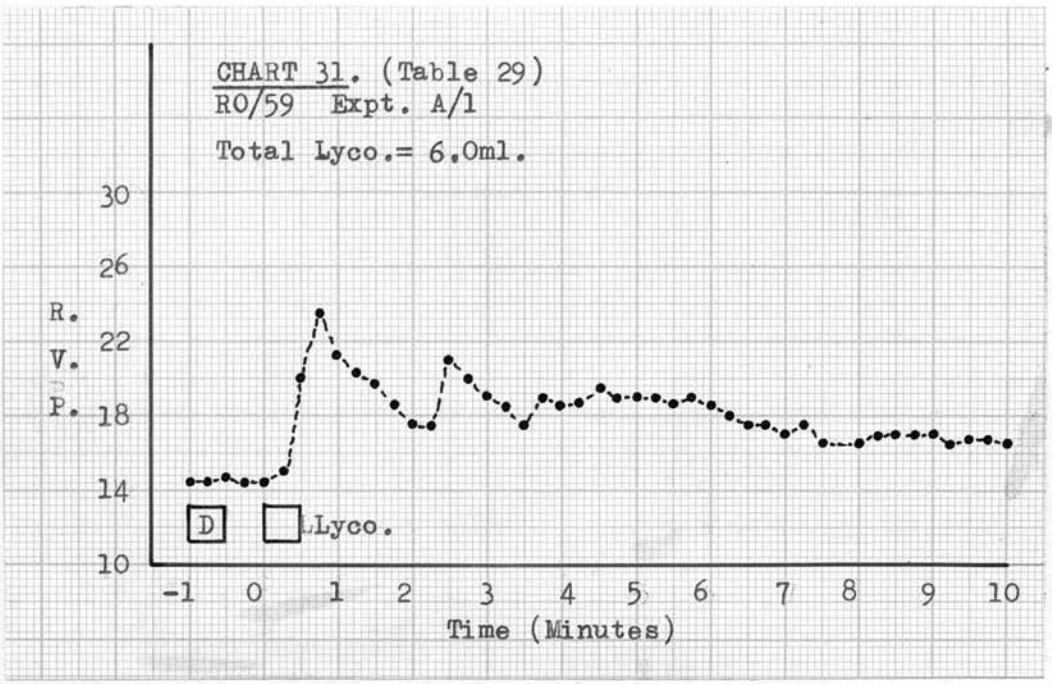


CHART 31. (Table 29)
RO/59 Expt. A/1
Total Lyco.= 6.0ml.
30
26
R.
V.
P.
22
18
14
10
D
Lyco.
-1
0
1
2
3
4
5
6
7
8
9
10
Time (Minutes)

C. Doubtful Responses: Anything which did not conform to the spiked or flat variety of response was classified as doubtful. It should be noted that, in general, the doubtful curves resulting from the Caledon Blue experiments were much closer in form to the spiked variety.

Similarly, the doubtful curves resulting from Lycopodium injections more closely resembled the flat type of curve.

This is true even of the response shown in Chart 31 (RO/59) in which a first injection of 6.0 ml. of 1% Lycopodium suspension was given rapidly in an attempt to produce a spiked response. It is true that spiking is present but the general character of the response remains that of the flat type.

CHART 32. (Table 30)
RO/38 (No previous injections)

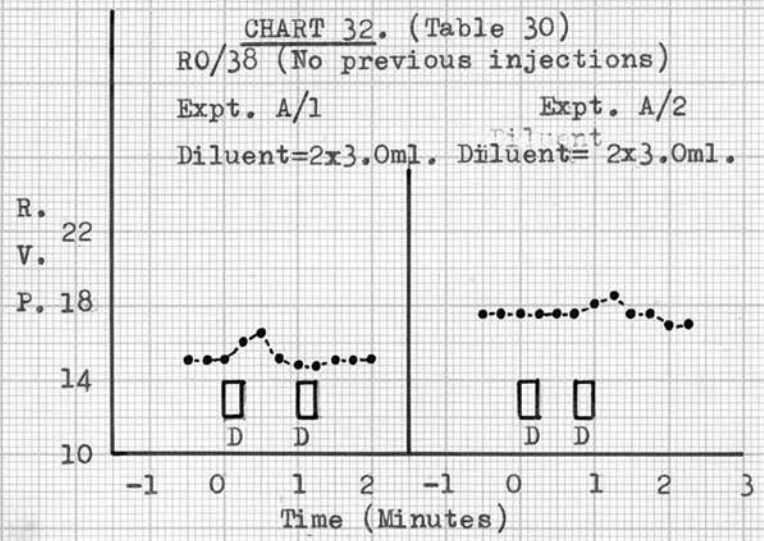


CHART 33. (Table 31)
RO/25 (Previous C.B.)

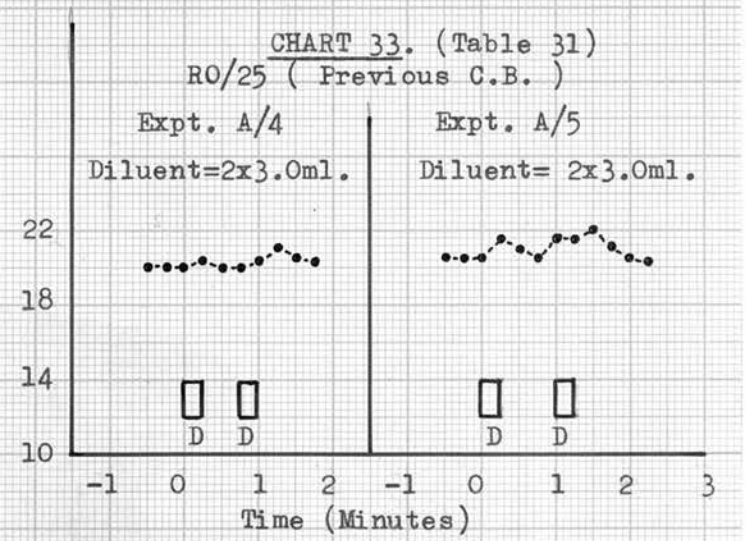
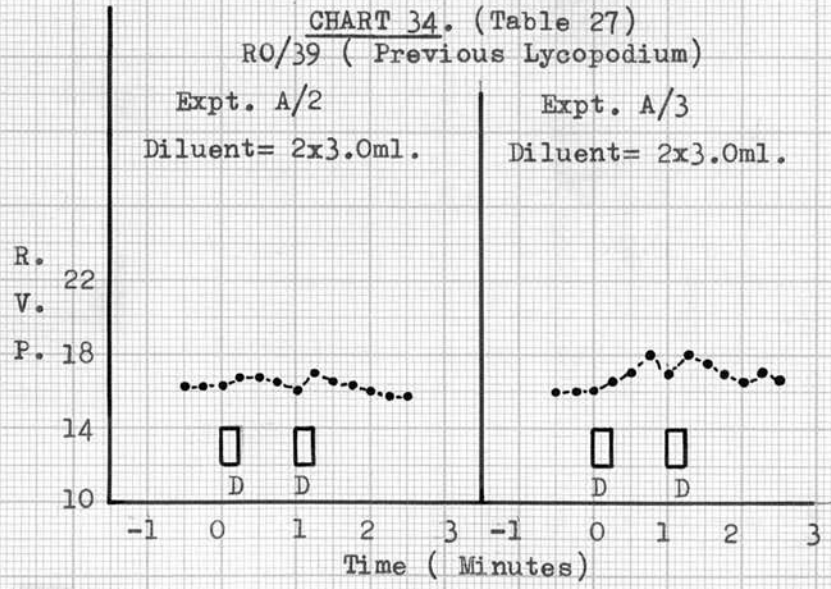


CHART 34. (Table 27)
RO/39 (Previous Lycopodium)



TWO SUCCESSIVE INJECTIONS OF DILUENT.

Charts 32, 33 and 34 show the greatest responses recorded by this procedure from three rabbits, RO/38 (no previous injections), RO/25 (previous Caledon Blue injections) and RO/39 (previous Lycopodium injections). It is evident that mere volume of the second injection is not responsible for the effects noted in the other acute experiments, especially as the injections of 3.0 ml. diluent were given quicker than in most of the experiments with Caledon Blue and Lycopodium.

DISCUSSION AND ANALYSIS OF RESULTS

GENERAL

The results have been presented in detail as it is believed that the method is new.

There was no clinical indication that the operative procedure interfered with cardiac function or upset the rabbits in any way. The appearance of the position of the heart at autopsy did not suggest that cardiac embarrassment had been produced.

In the circumstances, rigid control was impossible but the results gave no indication that the method itself produced a rise in mean R.V. pressure, nor did they suggest that the operation or the repeated cardiac punctures were responsible for producing right ventricular hypertrophy.

PRESSURE RESPONSES

LONG-TERM EXPERIMENTS

1. Basal Pressures.

In the 21 rabbits, where basal R.V. pressures were established over a period of five weeks, the average basal value was 17.1 cm. of water.

The extreme range of mean basal pressure was between 14.8 and 21.5 cm. However, in 17 out of the 21 rabbits, the range of the mean basal pressure was between 15.5 and 18.5 cm. (Appendix D, Table 35)

It should be remembered that the pressures are expressed in cm. of water and are referred to table level and not to the level of the ventricle. Consequently, variations in the size of the rabbits may account for some of the differences in the basal pressures.

Considerable fluctuation occurred in the individual pressure readings from the same animal, even in the period before pulmonary embolism was commenced. But, on the whole, reasonable basal readings were obtained. Some of this fluctuation may have been due to positioning of the rabbits although every effort was made to standardise this. The state of relaxation of the rabbit was another important factor. This depended, to a great extent, on the technique of the operators which improved with experience. Another possible factor was the size of the needle used for puncturing the ventricle. It is thought that a finer needle would have been an advantage but, with the crude manometer used, it was found desirable to employ a No.3 serum needle throughout.

2. Pressure fluctuations during the period of Embolism.

It was noted that there was a greater variation in individual readings, in many rabbits, during the period of administration of Caledon Blue or Lycopodium than in the basal periods or in the intervals between courses of injections. While the experiments gave no explanation of this finding, it is suggested that, because of this variation, pressure readings should be taken more frequently than once weekly in any more detailed study of the long-term effects of experimental pulmonary embolism.

3. Varied Responses to repeated Pulmonary Embolism.

The injection of repeated doses of Caledon Blue or Lycopodium produced a marked degree of pulmonary embolism, as assessed histologically. In view of this, the variability in the response was somewhat unexpected and emphasised the complexity of the problem.

In general it may be said that, in the long-term experiments, the responses to Caledon Blue and Lycopodium were similar but, with the doses employed, the response to Lycopodium was the more dramatic. Apart from this difference the responses may be grouped as follows:-

a. No Rise in Mean R.V. Pressure.

Though this was observed in only two rabbits, each received large amounts of Caledon Blue and Lycopodium, respectively, over long periods and the histological degree of pulmonary embolism did not differ from that in other rabbits which showed a marked response.

b. Rise in Mean R.V. Pressure; death while raised.

This is what might have been expected with repeated pulmonary embolism; but it should be noted that other rabbits survived much longer under similar circumstances. Death followed immediately on the injection in these cases but though embolism was obviously the immediate cause of death, it should be noted that a marked degree of spontaneous interstitial pneumonia was also present. It is thought that death resulted from the combined effects of embolism and pre-existing pneumonia rather than embolism per se.

c. Rise in Mean R.V. Pressure; followed by return to Basal Level.

In these rabbits the fall in pressure occurred while the injections were still in progress and, at no time, was there any clinical or autopsy

evidence that cardiac failure was responsible for the fall. In the circumstances, however, this possibility cannot be excluded.

d. Modified Response to a second period of Pulmonary Embolism.

This response was seen in two rabbits which had shown an initial rise in pressure followed by a return to normal during a first course of injections of Caledon Blue and Lycopodium, respectively. There was virtually no response to a second course of injections.

It must be emphasised that these experiments were of a purely exploratory nature and that firm conclusions are unjustifiable.

It is apparent that, in the rabbit, the response to experimental pulmonary embolism is variable. Even prolonged and repeated embolism cannot be guaranteed to produce pulmonary hypertension. This suggests that certain rabbits have differences in the arrangement of the lesser circulation which modify the response to embolism. It is impossible to say whether these differences are purely functional or have an anatomic basis.

Certainly no histological differences could be detected.

Further, if the possibility of cardiac failure can be excluded, it appears that any pulmonary hypertension produced is not necessarily sustained, even although embolism is being continued. This could be due to the development of compensatory mechanisms in the lesser circulation. The experiments give no hint as to the nature of the compensatory mechanism. This might be a purely functional vasomotor effect or might involve the development of collateral channels, either by enlargement of pre-existing vascular connections or by the development of new ones. Naturally, both pulmonary and bronchial vascular systems could be involved in such a process.

The modified response to a second period of pulmonary embolism supports the view that some alteration has occurred in the structure or behaviour of the lesser circulation.

It will be remembered that, in Section V, when considering the effects of pulmonary embolism on the right ventricle, a greater degree of right ventricular hypertrophy was found to be present in the first half of the experimental period. It is felt that the results of the pressure studies support the view that the reduction in the degree of R.V.

hypertrophy was the result of a compensatory mechanism.

ACUTE EXPERIMENTS

These were originally performed to determine whether the fall in R.V. pressures noted in the long-term experimenta was the result of reading certain of the pressures three days after the weekly injection instead of one day.

The acute experiments, with either Caledon Blue or Lycopodium, showed that the material had to be injected fairly quickly to produce a marked rise in pressure, and that the pressure usually fell back to the starting level within a matter of minutes after the injection.

It is concluded that the fall in pressure in the long-term experiments was genuine and not dependent on the time of the pressure reading.

No experiments were conducted to determine at what time a sustained rise in R.V. pressure became established.

The difference between the immediate response to Caledon Blue and Lycopodium has not been investigated. But it is interesting to note that, although the immediate response to Caledon Blue was more dramatic than in the case of Lycopodium,

the reverse was generally the case in the long-term experiments.

MEAN R.V. PRESSURE AND R.V. HYPERTROPHY

An attempt has been made to determine what relationship exists between R.V. pressure and the state of the right ventricle. However, quite apart from the small number of rabbits in the long-term experiments, there are other difficulties which prevent any but the most tentative conclusions from being drawn, viz:

a. It is not known whether the rabbits with higher basal mean R.V. pressure have a lower LV/RV ratio than the others, although this does not, in fact, appear to be the case.

b. It was impossible to determine whether or not any of the animals had right ventricular hypertrophy before the start of the experiments.

c. In the case of rabbits killed at the end of the experiments the period between death and the last pressure reading was variable.

The analysis is based on the 17 rabbits which received intravenous Caledon Blue or Lycopodium and which survived for at least one week after the first injection. (Appendix D, Table 35). The results are summarised in Table III.

Table III

Relation between Final R.V. Pressure and R.V. Hypertrophy.

Final R.V. Pressure	State of Right Ventricle		
	Normal	Doubtful	Hypertrophied
Normal	3	2	2
Doubtful	2	1	1
Raised	0	2	4

In all cases where the final pressure was definitely raised there was either definite or doubtful hypertrophy of the right ventricle.

Where the pressure remained unchanged, the results were less definite. With one exception (RO/20, Chart 3) all the rabbits in this group had shown a raised R.V. pressure at some time during the experiment and the right ventricular hypertrophy could be regarded as residual in nature.

A tentative interpretation of the results is that when R.V. pressure can be shown to be raised, there is usually evidence of right ventricular hypertrophy. If the R.V. pressure remains, or returns to, normal the ventricle may also remain, or return to, normal. However, there is a suggestion that hypertrophy of the right ventricle may

be present when the R.V. pressure is normal. In other words, right ventricular hypertrophy may not always reflect a raised R.V. pressure.

MEAN R.V. PRESSURE AND GENERALISED EMPHYSEMA

The results of the analysis on the same 17 rabbits (Appendix D, Table 35) are summarised in Table IV.

Table IV.

Relation between Final R.V. Pressure and Generalised Emphysema

Final R.V. Pressure	Degree of Generalised Emphysema			
	0	+	++	+++
Normal	0	2	3	2
Doubtful	3	0	0	1
Raised	4	1	0	1

In view of the fact that the ages of these rabbits were not known, it is impossible to assess how much of the emphysema developed during the experimental period; but there is certainly no suggestion that raised R.V. pressure is associated with emphysema per se.

SUMMARY AND CONCLUSIONS

An account has been given of a method of fixation of the heart, in rabbits, which enables direct readings of the right ventricular pressure to be taken repeatedly, over prolonged periods, without the use of an anaesthetic.

The procedure itself does not appear to cause either a rise in mean R.V. pressure or right ventricular hypertrophy.

In long-term experiments involving repeated pulmonary embolism by the intravenous injection of clinically comparable doses of either Caledon Blue R.C. or Lycopodium spores, the changes in mean R.V. pressure were similar in type but, in general Lycopodium produced the more marked rise in pressure. The responses may be classified as follows:-

a. No rise in mean R.V. pressure.

b. Rise in mean R.V. pressure: death while raised.

c. Rise in mean R.V. pressure followed by a return to basal level while injections were being continued.

d. Modifications of response to a second course of injections.

e. Doubtful responses.

It is suggested that responses of types c. and d. may be the result of the development of compensatory mechanisms, either anatomical or vasomotor, in the lesser circulation.

In acute experiments, the responses to i.v. Caledon Blue or Lycopodium differed from each other. The rise in mean R.V. pressure was greater, but less sustained, after Caledon Blue than after Lycopodium. It was necessary to inject the material fairly quickly to produce an appreciable acute response, which was always transitory.

The long-term experiments suggest that, when mean R.V. pressure is raised, right ventricular hypertrophy is usually present. On the other hand, a normal R.V. pressure may be associated with an hypertrophied right ventricle.

There is no suggestion that raised R.V. pressure is related to generalised emphysema.

SECTION VII

GENERAL DISCUSSION

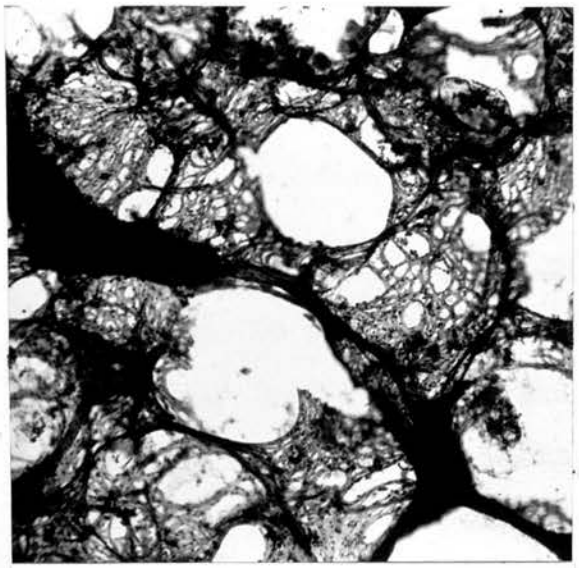


Fig. 1
Fenestration in human hypertrophic emphysema.
200μ. H&E. x 60.

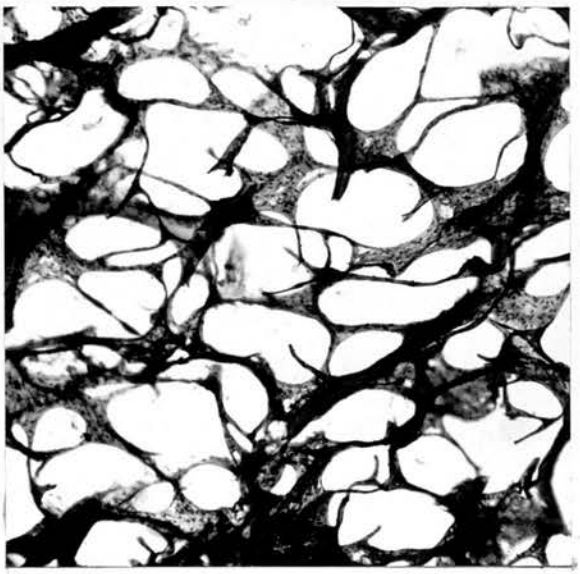


Fig. 2
Complete destruction of alveolar walls in human hypertrophic emphysema.
Same case as Fig. 1.
200μ. H&E x 60.

SECTION VII

GENERAL DISCUSSION

PATHOGENESIS OF CHRONIC VESICULAR EMPHYSEMA

CHRONIC HYPERTROPHIC EMPHYSEMA.

In Section I it was concluded that the published anatomical accounts of the histo-pathology of chronic vesicular emphysema provided ample evidence in support of the view that all forms of chronic emphysema should be regarded as examples of an atrophic process affecting the lung tissues. Further, the majority of workers have been unable to detect changes in the elastic tissue which precede the appearance of fenestration which is accepted by all as being the essential histological manifestation of chronic emphysema. There was no anatomical evidence that tearing of the lung tissues played a significant part in the pathogenesis of the condition.

In Section III, the anatomical features of spontaneous chronic pulmonary emphysema in rabbits have been described. The lesions in the rabbit are identical, in all essentials, to those described in the human disease. My own experience of human emphysema agrees with the views expressed in the literature. If thick sections are employed, the basic lesion, destruction of the alveolar walls by fenestration, can be seen (Figs. 1 & 2).

The experimental lesions in rabbits, described in Section IV, are identical to those of spontaneous rabbit emphysema and are also strictly comparable with the lesions of the human disease.

These experimental lesions were produced by the repeated intravenous injection of Caledon Blue R.C. on the theoretical basis that, if tissue ischaemia could be produced by this means, the appearances of emphysema should result. The possible modes of action of Caledon Blue have been discussed in Section IV and it was suggested that the most likely mode of action was, in fact, ischaemia due to vascular obstruction.

If this is accepted, the experiments provide direct evidence in support of the view that chronic emphysema is an ischaemic atrophy of lung tissue, in spite of the fact that the method by which the ischaemia has been produced is quite different from mechanisms known to be associated with the development of the human condition.

Previous experimental work, reviewed in Section IV, lends some support to the view that chronic emphysema is the result of distensive forces or abnormal stresses acting on the alveolar tissues but provides no direct evidence as to how these forces injure the lung.

As has been shown in Section II, most writers who have discussed the pathogenesis of emphysema from other points of view have come to similar conclusions. However, it will be remembered that various authors have considered that capillary occlusion, the result of distension, plays a part in the development of the lesions. This view has been most convincingly expressed by Loeschcke (1922, 1928a and 1928b) who had no doubt that this was the actual mechanism by which the tissue atrophy was produced. Others have put the case less convincingly or merely mentioned it in passing.

It should be remembered that those who have considered emphysema to be the direct result of "mechanical damage" to the lung tissues have based their conclusion on inference from other observed facts. Direct evidence as to how the damage was produced has not been available. The same is true of the alternative view that pressure changes lead to capillary occlusion.

The present experimental evidence does not provide an explanation of how tissue ischaemia is produced in human cases but it appears that there are two main ways in which this might occur:-

1. If it is accepted that distension of part of the lung occurs due to any of the recognised remote mechanisms, there is no theoretical objection to considering that capillaries may become occluded by linear stretching or distortion in the course of the distension.

On the other hand, Christie (1944) argued that there is no evidence that the forces involved are capable of producing distension initially and he considered that the tissue damage resulted from the sudden changes of pressure during coughing.

It is suggested that such pressure changes are just as likely to operate by the mechanism of capillary occlusion as by the effect of "wear and tear", of unspecified nature, on the alveolar tissues.

It has never been shown, directly, that capillary occlusion by either of these mechanisms does occur. Physiological work on the relation of pulmonary blood flow to lung distension has produced conflicting results, e.g. Cloetta (1912 and 1913) and Chillingworth & Hopkins (1920) concluded that distension did hinder the passage of blood through the capillaries while Daly (1930) failed to demonstrate this effect. However, these experiments were concerned with the total blood flow through the lungs and this problem is irrelevant to the conditions pertaining in the genesis of emphysema

in which the anatomical distribution of the lesions indicates that all parts of the lung are not affected at the same time nor to the same degree.

On the other hand, the upper limit of normal systolic pulmonary arterial pressure is given as 30 mm. Hg. by Cournand (1947) and McMichael (1948) while the upper limit of normal pulmonary "capillary" pressure is considered to be 15 mm. Hg. by Dexter et al. (1950). Estimates of intrathoracic pressures attained during coughing considerably exceed these levels. Rasmussen & Adams (1942) recorded intra-bronchial pressures of +50 mm. Hg. in their experimental dogs and Sharpey-Schafer (1953) showed that the intrathoracic pressure, in humans, reached levels of +250 mm. Hg. during coughing.

These figures suggest that mechanical occlusion of capillaries might reasonably be expected to occur in the course of respiratory disturbances known to be associated with the development of emphysema.

Thus, it appears that acceptance of the view that chronic emphysema is an ischaemic atrophy of lung tissue, due to pressure changes within the lungs, would reconcile the mechanical views of pathogenesis with the apparently conflicting nutritional theories. This point of view was first tentatively suggested by Rindfleisch (1871).

2. While it is accepted that cough and bronchitis lead to hypertrophic emphysema, Christie (1944) and Whitfield (1952), among others, have pointed out that the apparent severity of the emphysema cannot always be correlated with the severity of the cough or bronchitis and that the association is not invariable.

It is suggested that mechanical effects may not be the only factors operative but that direct interference with the blood supply to any given part of the lungs (or the lack of it), in the course of the bronchial inflammation, might afford an explanation for such apparent anomalies. In this connection it should be remembered that the anatomical studies of Orsos (1907 and 1936), Letulle (1928), Antoniazzi (1934a and 1934b) and Bezançon & Delarue (1947) led them to consider that inflammatory changes were an integral part of the emphysematous process.

CHRONIC COMPLEMENTARY (COMPENSATORY) EMPHYSEMA.

The above arguments can be applied to the pathogenesis of complementary emphysema. However, in view of the close association between complementary emphysema and inflammatory scarring, there is a stronger case for considering that inflammatory vascular sclerosis may be more important than mechanical

distensive effects. This has been suggested by Korol (1938) in connection with emphysema in tuberculosis.

SENILE EMPHYSEMA.

Whether senile emphysema or senile atrophy is the better term for this condition is a matter which could give rise to much speculation. It does not appear to be a condition strictly separable from hypertrophic emphysema. In my own experience, the factor which determines whether the lung, at autopsy, is large or small is the presence or absence of bronchial obstruction.

It is suggested that there is little need for regarding senile emphysema as an entity apart from hypertrophic emphysema. In considering the development of simple senile atrophy, i.e. the "normal" senile lung, it is tempting to ascribe this to vascular factors but this would, perhaps, be an over-simplification of the process of senescence.

OTHER FORMS OF CHRONIC EMPHYSEMA.

While emphysema in association with the pneumoconioses has been specifically excluded from the scope of this thesis, it is suggested that vascular factors should be considered in relation to the pathogenesis of emphysema in these conditions.

RIGHT VENTRICULAR HYPERTROPHY AND EMPHYSEMA

The results of fractional heart weighing, in rabbits, reported in Section V showed that the presence of spontaneous chronic emphysema did not cause right ventricular hypertrophy and there was no suggestion that the degree of emphysema, either spontaneous or experimental, influenced the LV/RV ratio.

It might appear that these results do not agree with the findings in human emphysema where it is generally accepted that emphysema is associated with right ventricular hypertrophy. It would be ridiculous to deny this association.

However, there is no published evidence which shows that right ventricular hypertrophy is the direct result of emphysema, per se. It is thought that, if this were the case, it ought to be possible to correlate the degree of emphysema and the degree of right ventricular hypertrophy. Published work fails to establish this correlation.

There are few published anatomical investigations of the problem.

Müller (1883), in his study of the fractional weights of 1,481 human hearts, included 49 cases where emphysema was considered to be the

main cause of death. Analysis of his figures, employing his own standards, shows that right ventricular hypertrophy was present in 32 of the emphysematous cases. Unfortunately, no details of the type or degree of emphysema were given.

Einhorn (1910) was impressed by the apparent lack of correlation between the degree of emphysema and right ventricular hypertrophy and made a specific investigation of the problem. He excluded cases with minimal emphysema or hypertension from his series. In 160 cases of hypertrophic emphysema, he found no correlation between the degree of emphysema and the degree of right ventricular hypertrophy which was present in only 45% of his cases.

White and Brenner (1933) and Brenner (1935) studied the pathology of the pulmonary vessels in 100 unselected autopsy cases dying from various causes and, while not primarily concerned with emphysema, they considered that even marked degrees of emphysema did not "ordinarily" produce right ventricular hypertrophy.

Griggs et al. (1939) found right ventricular hypertrophy present in just over 30% of 129 cases of emphysema diagnosed at autopsy, but they did not attempt to correlate the degree of emphysema and right ventricular hypertrophy.

On the other hand, Scott and Garvin (1941), in their study of 50 cases of cor pulmonale at autopsy, found chronic emphysema, "together with varying grades of bronchitis, bronchiectasis and fibrosis", present in 48 of these cases. They concluded that the traditional view on the causative connection between emphysema and cor pulmonale was correct.

Fulton (1953) also studied 50 cases of cor pulmonale at autopsy and was unable to correlate the degree of right ventricular hypertrophy with the degree of capillary destruction as estimated in large lung sections prepared by the Cardiff technique (Gough et al., 1949).

Quite apart from the merits of the techniques employed and the possibility of differences in criteria for the assessment of the degree of emphysema, there is nothing in the above work which provides evidence establishing a causal connection between emphysema and cor pulmonale; yet, the association between the two conditions cannot be denied.

In Section V, it was shown that, although right ventricular hypertrophy in rabbits was not related to emphysema, experimental pulmonary embolism

by Caledon Blue R.C. did produce right ventricular hypertrophy.

This is what might have been expected. However, the relationship between the degree of right ventricular hypertrophy and the duration of the Caledon Blue injections (which reflects the amount of Caledon Blue injected) was not a simple quantitative one. Various degrees of hypertrophy, or no hypertrophy at all, were found in rabbits which had sustained the same histological degree of pulmonary embolism. It was suggested that this was the result of the operation of compensatory mechanisms, either functional or anatomical, e.g. the development of anastomoses, in the pulmonary circulation.

The pressure studies in Section VI, also, showed a varied response pattern to similar degrees of pulmonary embolism and provided some supportive evidence for the existence of compensatory mechanisms as mentioned above. Further, these studies suggested that, while there is usually a correlation between a raised right ventricular pressure and right ventricular hypertrophy, there was no relation between right ventricular pressure and the degree of generalised emphysema in rabbits.

The experimental work suggests that, in the rabbit, vascular factors other than capillary destruction, as reflected by the degree of generalised emphysema are responsible for the development of right ventricular hypertrophy.

It is possible that similar considerations may govern the occurrence of right ventricular hypertrophy in association with emphysema in man.

This was first suggested by Eppinger (1876) who noted that not all cases of hypertrophic emphysema developed cor pulmonale. He observed the development of anastomoses between the pulmonary and bronchial circulations in certain cases and thought that this explained the absence of right ventricular hypertrophy. Similar anatomical changes have been investigated, more recently, by a few groups of workers.

Liebow et al. (1949), employing injection techniques, demonstrated the enlargement of anastomoses between the bronchial and pulmonary arteries in bronchiectasis. Bloomer et al. (1949) showed that the blood flow in the bronchial arteries increased, after a period, following the ligation of one of the main pulmonary arteries in dogs. The anatomical changes in these animals were described by Liebow et al. (1950) who saw increased collaterals between the pulmonary and bronchial circulations nine weeks

after ligation of the pulmonary artery. Cockett and Vass (1950) demonstrated similar changes in their animals. Marchand et al. (1950) studied the pulmonary and bronchial vessels in normal and diseased human lungs and concluded that anastomoses normally existed between the bronchial and pulmonary arteries. In disease, including bronchiectasis and emphysema, these were more prominent while, at the same time, there was an increase in the size and numbers of anastomoses between the pulmonary and the pleuro-hilar veins. Liebow (1953) reported similar changes in, what he called, the bronchopulmonary veins.

The discussions in the last two articles indicate the complexity of the problem and show that, in diseased lungs including the emphysematous, attempts to explain the alterations in the circulatory arrangements must consider factors other than mere obstruction of vessels and capillary destruction.

The results reported in Section V and VI and the failure of published work to establish a causal relationship between chronic emphysema and right ventricular hypertrophy suggest that it might be more profitable to ignore emphysema per se and concentrate on the vascular changes when investigating cor pulmonale. Injection techniques, as employed by Liebow and the other workers mentioned

above, or angiography, which has been used by Miscall and Duffy (1953) and by Hornykiewytsch and St.Stender (1955), seem to be hopeful lines of approach to the problem.

One point remains for consideration. It might be asked, why do the "vascular factors" lead to cor pulmonale in human emphysema and not in rabbit emphysema?

It has been noted in Section III that bronchitis, as seen in humans, does not occur in rabbits. Involvement of bronchi was only an occasional, incidental, feature in a few of the most severe foci of interstitial pneumonia. On the other hand, in humans, chronic bronchitis is accepted as the commonest remote cause of hypertrophic emphysema. The absence of this type of bronchial inflammation might explain the absence of cor pulmonale in rabbit emphysema.

In humans, the one common pathological feature in pulmonary diseases, known to result in right ventricular hypertrophy, is inflammation, usually chronic and associated with fibrosis. Bronchiectasis is an accepted cause of cor pulmonale and the dividing line between bronchiectasis and bronchitis is a fine one. It may be that bronchial inflammation is the connecting link between human emphysema and cor pulmonale, the development of

cor pulmonale not being determined by the emphysema, per se, but by vascular changes occurring in the course of the bronchial inflammation.

GENERAL CONSIDERATIONS

This thesis has been devoted to advancing the view that chronic vesicular emphysema can be regarded as an atrophy of lung tissue and evidence has been presented which suggests that ischaemia plays, at least, a part in the intimate pathogenesis of the condition.

It is felt that this outlook provides a means of reconciling apparently conflicting views on the aetiology and pathogenesis of the condition. Further, a case could be made for ceasing to regard "emphysema" as a disease in its own right. Pathologically, emphysema could be considered to be a non-specific atrophic degeneration of lung tissue which could be produced by many remote aetiological factors operating through the immediate agency of tissue ischaemia. In this respect, emphysema would be no more an entity than nephrosclerosis or myocardial fibrosis.

In the main, the functional aspects of emphysema have been excluded from consideration, but it has been suggested that emphysema is no more than an incidental finding in cases of cor pulmonale. There is some evidence that bronchial infection is the factor which produces cor pulmonale and emphysema, but independently of each other. In other words, the acceptance that emphysema causes cor pulmonale has merely confused the issue.

While it would be unwise to indulge in further speculation, it seems that the tendency to regard emphysema as a disease, per se, can lead to confusion. It is suggested that concentration on the functional effects of diseases known to upset respiratory and/or cardiac function, without undue preoccupation with the presence or absence of emphysema, might clarify the position with regard to the functional effects of "emphysema".

SUMMARY AND MAIN CONCLUSIONS

Spontaneous chronic vesicular emphysema in rabbits was studied to provide a basis for the assessment of results in the experimental production of emphysema.

Some degree of generalised emphysema is present in over 50% of rabbits over 2½ years old.

Bronchitis, as seen in humans, is not found in rabbits.

Experimental generalised emphysema has been produced in rabbits by the repeated intravenous injection of Caledon Blue R.C.

It is thought that the experimental emphysema is the result of ischaemic atrophy of lung tissue due to widespread vascular blockage caused by Caledon Blue.

A study of the fractional heart weights of "normal" rabbits, rabbits with spontaneous chronic emphysema and rabbits subjected to pulmonary embolism by Caledon Blue (in the course of the experimental production of emphysema) gave the following results:-

Spontaneous emphysema in rabbits is not associated with right ventricular hypertrophy.

The degree of spontaneous or experimental emphysema has no influence on the LV/RV ratio.

Pulmonary embolism by Caledon Blue produces right ventricular hypertrophy, but there is not a direct relationship between the degree of embolism and the degree of right ventricular hypertrophy.

A method was described which enabled repeated, direct, readings of the right ventricular pressure to be made in unanaesthetised rabbits.

A study of the mean right ventricular pressure during repeated pulmonary embolism by Caledon Blue or Lycopodium spores gave the following results:-

The response to similar degrees of pulmonary embolism is varied.

Raised right ventricular pressure is usually associated with right ventricular hypertrophy.

There is no suggestion that generalised emphysema is associated with raised right ventricular pressure.

The studies of fractional heart weights and of right ventricular pressure suggest that vascular factors, other than destruction of capillaries or simple occlusion, influence the degree of right ventricular hypertrophy and the pressure changes in the right ventricle.

The experimental results were discussed in relation to the literature on the anatomy and pathogenesis of human chronic vesicular emphysema and its association with cor pulmonale.

It is suggested that chronic emphysema, in humans, should be regarded as an ischaemic atrophy of lung tissue and that acceptance of

this view would provide a means of reconciling the apparently conflicting opinions as to the significance, and mode of action, of the various aetiological factors involved.

There is no published evidence that cor pulmonale is caused by emphysema, per se, and it is suggested that chronic inflammation may be the factor which is responsible for the independent development of both the cor pulmonale and the emphysema.

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APPENDIX A

APPENDIX A

HISTOLOGICAL TECHNIQUES

The use of thick paraffin sections required only minor modifications in standard histological methods.

EMBEDDING.

It was advisable, but not imperative, to use a slightly softer paraffin wax than would normally be used for routine thin sections. This minimised minor splits in the sections which were liable to occur during cutting. In this laboratory, wax of a melting point of 52° C. was found suitable.

CUTTING.

The important point was that the sections should be prevented from curling up as they came off the knife. This was done most conveniently by lightly holding down the leading edge of the section with the tips of the fingers.

MOUNTING AND DRYING.

The sections were mounted in the usual way. Albuminised slides were used. The sections had to be dried in an oven hot enough to melt the wax in order to prevent tham from coming off the slide during staining.

STAINING.

Removal of wax and dehydration were slower in thick sections but staining times did not need to be altered.

Haemalum and Eosin Stain.

No special modification of any of the standard methods was required. It was advisable to avoid over-staining with haemalum but, on the other hand, it was an advantage to employ a fairly heavy eosin stain for the study of the alveolar pores and the fenestrations in emphysema.

Elastic Tissue Stain.

Hart's modification of Weigert's resorcin-fuchsin elastic tissue stain was found to be the most suitable.

The method detailed by Mallory (1938) was used with the following slight modifications:-

a. Preparation of stock solution: dry commercial (Revector) resorcin-fuchsin was used as follows:-

	Weigert's resorcin-fuchsin		1.5 gm.
Dissolve in	:	95% Ethyl alcohol	100.0 ml.
Add	:	Conc. HCl.	2.0 ml.
Add	:	10% Ferric chloride(fresh)	1.0 ml.

b. After the potassium permanganate and oxalic acid stages, the sections were left in 70% alcohol for 3 minutes.

c. A minimum of 24 hours was allowed for staining but some sections required even longer.

d. Counter-staining presented a problem. Nuclear or any intense stains obscured the pattern of the elastic fibres.

Picric acid (Sat. aqueous soln. : 15-30 secs.) was found to be the most satisfactory counter-stain. This, however, had the disadvantage of making visualisation of alveolar pores and fenestrations difficult.

However, by omitting the final rinse in alcohol which normally removed excess elastic tissue stain from the background and placing the sections directly into the picric acid, this difficulty was overcome. A brownish counter-stain was produced but even this obscured the general pattern a little.

TABLE 1

Details of Spontaneous Emphysema and Interstitial Pneumonia in 155 Rabbits.

Rabbit No.	Body Weight (kg.)	MACROSCOPIC			MICROSCOPIC						
						Degree & Type of Emphysema			Degree & Distribution of Interstitial Pneumonia		
		Normal	Marginal Lobule	Vesiculation	Non-Emphysematous	Marginal Lobule	Vesiculation	Generalised	In Marginal Lobule	In Vesiculation	Generalised
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)
RC/					GROUP A (Young Rabbits)						
117	-	-	+	-	+	0	0	0	++	0	+
118	1.14	+	-	-	+	0	0	0	0	0	+
119	1.03	+	-	-	+	0	0	0	0	0	0
120	1.10	+	-	-	+	0	0	0	0	0	0
121	1.12	+	-	-	+	0	0	0	0	0	+
122	1.07	+	-	-	+	0	0	0	0	0	+
123	1.15	+	-	-	+	0	0	0	0	0	+
124	1.56	+	-	-	+	0	0	0	0	0	+
125	1.33	+	-	-	+	0	0	0	0	0	0
126	1.51	+	-	-	+	0	0	0	0	0	0
137	0.73	+	-	-	+	0	0	0	0	0	++
138	0.98	+	-	-	+	0	0	0	0	0	++
139	1.26	+	-	-	+	0	0	0	0	0	+
140	0.89	+	-	-	+	0	0	0	0	0	+
141	0.55	-	+	-	+	0	0	0	++	0	+
142	0.52	+	-	-	+	0	0	0	0	0	+
143	0.48	+	-	-	+	0	0	0	0	0	++
144	0.51	+	-	-	+	0	0	0	0	0	++
145	0.46	+	-	-	+	0	0	0	0	0	+
146	0.65	-	+	-	+	0	0	0	+++	0	+++
Total											
20		17	3	0	20	0	0	0	3	0	16
RC/					GROUP B (Miscellaneous Adults)						
10	2.79	+	-	-	+	0	0	0	0	0	0
11	1.96	+	-	-	+	0	0	0	0	0	0
12	1.86	+	-	-	+	0	0	0	0	0	+
13	2.05	+	-	-	+	0	0	0	0	0	++
14	2.69	+	-	-	+	0	0	0	0	0	+++
15	1.99	+	-	-	+	0	0	0	0	0	+++
16	2.54	+	-	-	+	0	0	0	0	0	+
17	3.4	+	-	-	+	0	0	0	0	0	++

TABLE 1 - contd.

Column No. (1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)
18	2.27	+	-	-	+	0	0	0	0	0	0
19	1.67	+	-	-	+	0	0	0	0	0	0
20	1.47	+	-	-	+	0	0	0	0	0	0
21	1.87	-	+	-	-	+	0	0	+++	0	+
22	2.09	+	-	-	-	0	+	0	0	+	0
23	2.14	+	-	-	-	0	0	++	0	0	++
24	1.69	-	+	-	+	0	0	0	++	0	0
25	1.67	-	+	-	-	+	0	0	+	0	+
26	1.80	+	-	-	+	0	0	0	0	0	+
27	2.00	-	-	+	-	0	++	0	0	0	+
28	2.05	+	-	-	+	0	0	0	0	0	++
29	1.96	+	-	-	+	0	0	0	0	0	++
30	1.89	+	-	-	+	0	0	0	0	0	+
31	2.22	-	-	+	-	0	++	++	0	0	++
32	1.77	+	-	-	+	0	0	0	0	0	++
33	1.92	+	-	-	+	0	0	0	0	0	+
34	1.99	+	-	-	+	0	0	0	0	0	+
35	1.89	+	-	-	+	0	0	0	0	0	+
36	2.02	-	+	-	+	0	0	0	+	0	0
37	1.15	+	-	-	+	0	0	0	0	0	++
38	2.35	+	-	-	+	0	0	0	0	0	+
39	2.97	-	+	-	+	0	0	0	+++	0	++
40	2.15	+	-	-	+	0	0	0	0	0	+
41	2.22	+	-	-	+	0	0	0	0	0	+
42	2.06	+	-	-	+	0	0	0	0	0	++
43	1.84	+	-	-	+	0	0	0	0	0	+++
44	2.51	+	-	-	+	0	0	0	0	0	0
45	1.80	+	-	-	+	0	0	0	0	0	0
46	2.49	+	-	-	+	0	0	0	0	0	0
47	1.98	+	-	-	+	0	0	0	0	0	++
48	2.40	+	-	-	+	0	0	0	0	0	+
49	2.04	+	-	-	+	0	0	0	0	0	+
50	2.47	+	-	-	+	0	0	0	0	0	+
51	3.00	-	+	+	-	+++	+	0	++	+	+++
52	2.98	+	-	-	+	0	0	0	0	0	+++
53	2.89	+	-	-	+	0	0	0	0	0	+
54	2.58	+	-	-	+	0	0	0	0	0	+
55	2.11	-	+	-	+	0	0	0	+++	0	++
56	3.08	+	-	-	+	0	0	0	0	0	+
57	2.63	+	-	-	+	0	0	0	0	0	++
58	2.40	+	-	-	+	0	0	0	0	0	++
59	-	+	-	-	+	0	0	0	0	0	++
60	2.67	+	-	-	-	0	0	+++	0	0	+++
61	2.94	-	+	+	-	++	++	+	+++	+	+
62	2.62	+	-	-	+	0	0	0	0	0	+++
63	2.16	+	-	-	+	0	0	0	0	0	++
65	3.40	+	-	-	+	0	0	0	0	0	+++
66	2.49	+	-	-	+	0	0	0	0	0	++
67	2.24	+	-	-	+	0	0	0	0	0	++
68	1.87	+	-	-	+	0	0	0	0	0	+
69	1.81	+	-	-	+	0	0	0	0	0	++
70	2.02	+	-	-	+	0	0	0	0	0	0
71	1.84	+	-	-	-	0	+	0	0	++	0

TABLE 1 - contd.

Column No. (1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)
72	1.31	-	+	-	-	+	0	+	+	0	++
73	1.37	+	-	-	+	0	0	0	0	0	+
74	-	-	+	-	-	+	0	0	+++	0	++
75	1.92	+	-	-	+	0	0	0	0	0	+
76	1.68	+	-	-	+	0	0	0	0	0	++
77	-	+	-	-	+	0	0	0	0	0	+++
78	2.68	-	+	-	-	++	0	0	++	0	++
79	2.00	+	-	-	+	0	0	0	0	0	+
80	2.12	+	-	-	+	0	0	0	0	0	+
81	2.22	+	-	-	+	0	0	0	0	0	+
82	2.89	+	-	-	+	0	0	0	0	0	++
94	1.40	+	-	-	+	0	0	0	0	0	+
95	2.13	+	-	-	+	0	0	0	0	0	0
96	2.25	+	-	-	+	0	0	0	0	0	++
97	2.25	+	-	-	+	0	0	0	0	0	+
98	2.16	-	+	-	+	0	0	0	++	0	+
99	2.53	+	-	-	+	0	0	0	0	0	+
100	2.47	+	-	-	-	0	0	+	0	0	+
101	2.61	-	+	-	-	+	0	0	++	0	+
102	-	+	-	-	+	0	0	0	0	0	0
103	2.47	+	-	-	+	0	0	0	0	0	0
104	2.35	+	-	-	+	0	0	0	0	0	+++
105	2.10	+	-	-	+	0	0	0	0	0	0
106	2.45	+	-	-	+	0	0	0	0	0	++
107	2.65	+	-	-	+	0	0	0	0	0	++
108	2.08	-	+	-	+	0	0	0	++	0	+
109	2.30	+	-	-	+	0	0	0	0	0	+
110	2.60	-	+	+	-	++	+++	+	+++	++	++
111	1.76	+	-	-	+	0	0	0	0	0	++
112	2.16	+	-	-	+	0	0	0	0	0	+
113	2.34	+	-	-	+	0	0	0	0	0	+++
114	2.90	-	+	+	-	++	++	+	++	++	+
115	2.02	-	+	-	-	+	-	-	+	0	+
116	2.49	+	-	-	+	0	0	0	0	0	+++
147	-	-	+	-	+	0	0	0	+++	0	+++
Total 96		76	18	6	78	11	8	8	18	6	79
RC/			GROUP C (Old Rabbits)								
83	3.06	+	-	-	+	0	0	0	0	0	0
84	2.45	-	+	-	+	0	0	0	+++	0	++
85	2.41	-	+	+	-	+	+	+	++	+	++
86	2.39	+	-	-	-	0	0	++	0	0	+
87	2.99	+	-	-	+	0	0	0	0	0	+
88	2.20	+	-	-	+	0	0	0	0	0	++
89	2.23	+	-	-	-	0	0	+	0	0	++
90	3.32	+	-	-	-	0	0	+++	0	0	+
91	2.51	-	+	-	-	++	0	++	+	0	+
92	2.68	-	+	+	-	+	+	+++	+	+	+++
93	3.52	+	-	-	+	0	0	0	0	0	++
127	3.00	-	+	+	-	++	+++	0	+	++	+
128	2.42	+	-	-	-	0	0	++	0	0	++
129	2.99	-	+	+	-	+	+	+	+	+++	++

TABLE 1 - contd.

Column No. (1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)
130	2.47	-	+	+	-	+	+++	+++	++	+++	+++
131	2.82	-	+	+	-	++	++	0	+++	+++	++
132	2.27	-	+	+	-	+	++	+	++	++	++
133	2.33	+	-	-	-	0	0	++	0	0	++
134	2.69	+	-	-	+	0	0	0	0	0	+
135	1.91	+	-	-	+	0	0	0	0	0	+++
136	2.16	+	-	-	+	0	0	0	0	0	++
Total											
21		12	9	7	8	8	7	11	9	7	20
					GROUP D (Miscl. Adults: Acute Operations)						
RO/											
4	-	-	+	+	-	+++	++	0	+++	++	+
6	1.99	-	+	-	-	+++	0	+	+++	0	+++
7	1.32	-	-	+	-	0	+	0	0	+	++
8	2.14	+	-	-	+	0	0	0	0	0	++
9	2.43	+	-	-	+	0	0	0	0	0	+
10	1.76	-	+	-	-	+	0	0	++	0	+++
11	1.66	-	+	-	+	0	0	0	+	0	+
12	1.58	+	-	-	+	0	0	0	0	0	+
17	2.17	+	-	-	+	0	0	0	0	0	+++
19	2.03	+	-	-	+	0	0	0	0	0	+
22	3.08	-	+	+	-	+	++	0	+	+	+++
23	2.00	+	-	-	+	0	0	0	0	0	++
26	2.45	+	-	-	+	0	0	0	0	0	+++
27	3.27	-	+	-	-	+	0	0	+++	0	+++
29	2.08	+	-	-	+	0	0	0	0	0	0
32	2.68	-	+	-	+	0	0	0	++	0	+
36	2.55	-	+	+	-	+	+	0	+++	++	++
43	2.25	+	-	-	+	0	0	0	0	0	0
Total											
18		9	8	4	11	6	4	1	8	4	16

APPENDIX B

APPENDIX B

CALEDON BLUE R.C. IN TISSUES OTHER THAN THE LUNGS

Particles of Caledon Blue which are not retained in the lungs pass into the other tissues of the body. After repeated injections no organ is free from the dye. The particles either lie free in the vessels or are taken up by the reticulo-endothelial cells. Aggregation of the particles, as noted in the lungs, occurs. Consequently, although most of the particles that pass through the lungs are presumed to be the smaller ones, aggregates in other tissues may be large.

In spite of its widespread deposition in the tissues, Caledon Blue is inert and granulomatous or other reactions are not seen even after periods of many months.

The main sites of deposition of Caledon Blue are the liver and spleen which, after weeks of injections, become a deep blue colour. Other organs also show a faint blue staining by this time. Dye particles are transported from the lungs to the

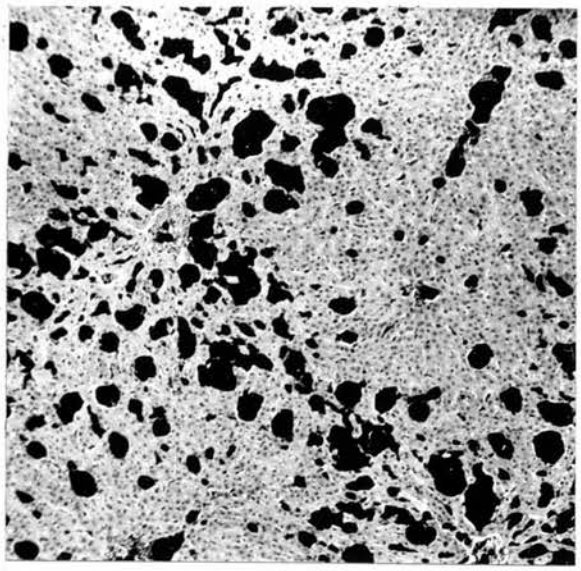

Fig. 1
Periportal arrangement of Caledon Blue aggregates in liver.
4μ. H&E. x 60.

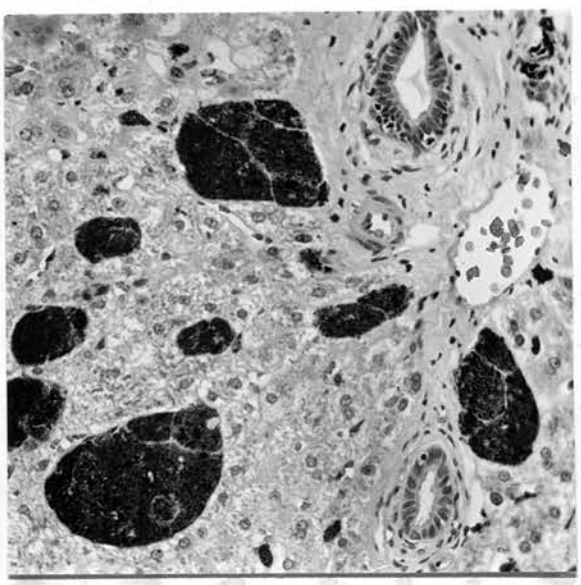

Fig. 2
Caledon Blue aggregates in Kupffer cells. Thin rims of cytoplasm subdivide the masses of dye.
4μ. H&E. x 240.

hilar and mediastinal nodes. The coeliac nodes are also involved due to transportation from liver and spleen.

By comparison with the litter mates there is a great increase in the weight of the liver and spleen. In general terms, after some weeks of injections of Caledon Blue, the livers showed a 50% to 80% increase in weight while the spleens were from twice to four times as heavy as those in the normals.

MICROSCOPIC APPEARANCES.

Liver.

The dye is taken up by the Kupffer cells. Most of the dye aggregates appear to be intracellular and nuclei or a thin rim of cytoplasm may be seen around some of the aggregates, although the Caledon Blue obscures much of the detail. Dye is found in all parts of the lobule but there is a tendency to increased deposition in the periportal zones. There is no cellular reaction to the Caledon Blue and fibrosis does not occur (Figs. 1 & 2).

Spleen.

The Caledon Blue is found in large aggregates in the reticulo-endothelial cells in the pulp although

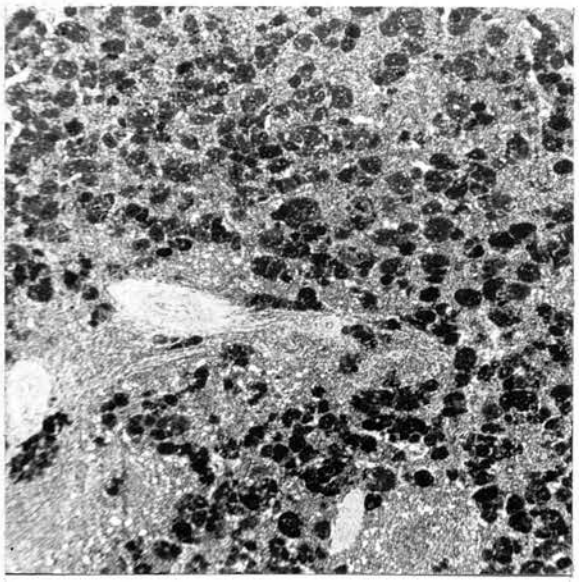

Fig. 3
Caledon Blue aggregates in spleen.
4μ. H&E. x 60.

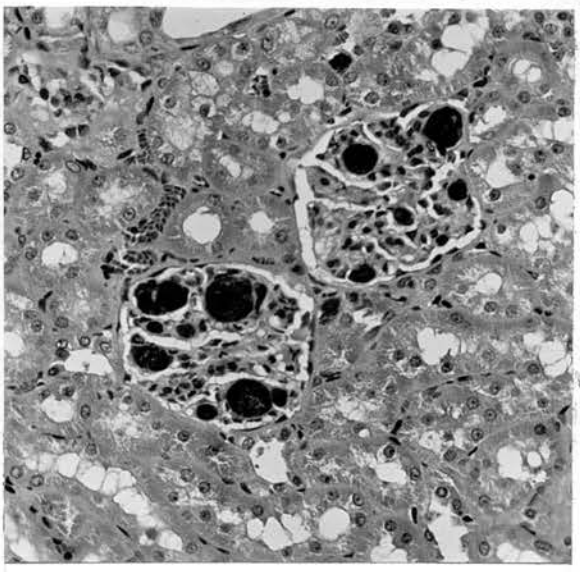

Fig. 4
Glomerular capillaries distended by Caledon Blue aggregates.
4μ. H&E. x 240.

occasional deposits are seen in the Malphigian corpuscles. Relatively, there is more dye in the spleen than in the liver (Fig. 3).

Lymph Nodes.

The appearances are similar to those in the spleen and masses of dye can be seen in macrophages in the sinuses.

Kidney.

Caledon Blue aggregates are seen in the glomerular and, to a lesser extent, in the intertubular capillaries. The dye, in the kidney, is almost wholly free in the vessels and not intracellular like that in the liver and spleen. Relatively large masses occur in the glomerular capillaries (Fig. 4) and the appearances closely resemble those in the lungs. No evidence of glomerular or tubular necrosis is seen.

Other Tissues.

Caledon Blue aggregates are seen in the myocardium, thyroid, adrenal and brain. These are usually small and scanty as compared with those in the liver and spleen and, as in the kidney, are usually free in the vessels.

Table 1.

Details of 1st Experimental Series

Rabbit No.	Body Wt. at Death (Kg.)	Duration of C.B. Injections (wks.)	Total C.B.(ml.of approx. 3.0% suspension)	Macroscopic			Microscopic				Degree of Generalised Interstitial Pneumonia
				Normal	Marginal Lobule	Vesiculation	Non-emphysematous	Degree & Type of Emphysema: Marginal Lobule	Vesiculation	Generalised	
R.St/											
13	2.41	1	5.5	+	-	-	+	0	0	0	+++
4	2.41	3	8.5	+	-	-	+	0	0	0	+
6	3.95	5 1/7	15.0	+	-	-	-	0	0	+	+++
9	2.13	5 1/7	15.0	+	-	-	-	0	0	0	+++
17	2.23	6 5/7	17.5	-	-	+	-	0	+++	0	0
20	2.64	11 5/7	30.0	-	-	+	-	+++	+++	0	+++
26	2.43	14 2/7	39.0	-	-	+	-	+	++	+	0
24	2.03	15 5/7	42.0	-	-	+	-	0	++	++	+++
16	2.33	17 6/7	48.0	-	-	+	-	0	+	+	+
28*	2.34	31 1/7	169.5	-	-	+	-	0	+	+++	+
25	2.68	37	87.0	-	-	+	-	++	+++	++	++
18	2.87	42	92.0	-	-	+	-	0	+	+	+++
11	2.73	43	91.5	-	-	+	-	0	+++	+	+++
7	3.73	47	103.0	-	+	+	-	++	0	++	+
21	2.80	49	107.5	-	-	+	-	0	+	+	++
22	2.36	49	108.0	+	-	+	-	0	0	++	+
23	2.74	49	108.0	-	-	+	-	0	0	+	+
10	2.84	52	110.5	-	+	+	-	0	++	++	++
14	3.12	52	111.5	-	+	+	-	++	++	+++	++
15	3.11	52	114.0	-	+	+	-	0	+	+++	+
8	2.74	55	122.0	+	-	-	-	0	0	+	+
5	3.26	56	126.0	+	-	-	-	0	0	+++	+
Total Incidence of Lesions					5	13		5	13	17	20

Total No. in Series:- 22.

* R.St/28: Injected thrice weekly.

Table 2.

Details of 2nd Experimental Series and Controls

Pair No.	Rabbit No. (Odd Nos.: controls)	Body wt. at death (Kg.)	Age at start (months)	Duration of C.B. Injections (weeks)	Total C.B. (ml. of approx. 3% suspension)	Macroscopic			Microscopic				Degree of Generalised Interstitial Pneumonia
						Normal	Marginal Lobule	Vesiculation	Non-emphysematous	Degree and Type of Emphysema: Marginal Lobule	Vesiculation	Generalised	
1	2	3	4	5	6	7	8	9	10	11	12	13	14
	R.St/												
1	64	2.53	9	1	5.0	+	-	-	+	0	0	0	++
	65	2.11	9	-	0	-	+	-	+	0	0	0	+
2	90	2.06	9	1	4.5	+	-	-	+	0	0	0	+++
	91	2.72	9	-	0	-	+	-	+	0	0	0	0
3	94	2.25	10	1	4.5	+	-	-	-	0	+	0	++
	95	2.26	10	-	0	+	-	-	+	0	0	0	0
4	80	1.93	12	2 2/7	9.5	+	-	-	+	0	0	0	+++
	81	1.75	12	-	0	-	+	-	-	++	0	++	++
5	86	2.02	9	3	15.0	+	-	-	+	0	0	0	+++
	87	2.36	9	-	0	+	-	-	+	0	0	0	0
6	56	2.56	9	4 2/7	22.5	-	-	+	-	0	++	0	+++
	57	2.89	9	-	0	+	-	-	+	0	0	0	+
7	110	2.66	10	5 3/7	26.5	-	+	+	-	++	+	+++	++
	111	2.29	10	-	0	-	-	+	-	0	+	+	++
8	44	3.01	10	6	31.5	+	-	-	+	0	0	0	++
	45	3.04	10	-	0	+	-	-	+	0	0	0	0
9	46	1.59	11	6	31.5	+	-	-	-	0	0	+	++
	47	1.62	11	-	0	+	-	-	+	0	0	0	+
10	78	2.65	12	7	35.5	-	+	-	+	0	++	0	+++
	79	2.57	12	-	0	-	+	-	+	0	0	0	++
11	104	2.06	7	8	40.0	-	-	+	-	0	+	0	++
	105	2.32	7	-	0	+	-	-	+	0	0	0	++
12	48	2.33	10	10	50.0	+	-	-	+	0	0	0	+
	49	2.01	10	-	0	+	-	-	+	0	0	0	+

Table 2 - contd.

Column No.

1	2	3	4	5	6	7	8	9	10	11	12	13	14
13	76	1.92	8	15	72.0	-	+	+	-	++	++	+++	0
	77	2.05	8	-	0	-	-	+	-	0	+	0	++
14	102	2.88	7	17 4/7	83.5	+	-	-	-	0	0	++	++
	103	3.32	7	-	0	+	-	-	+	0	0	0	+
15	106	3.04	9	20	90.5	+	-	-	-	0	0	+++	+
	107	2.89	9	-	0	+	-	-	+	0	0	0	+++
16	108	2.16	7	20	90.0	+	-	-	-	0	0	+	++
	109	2.16	7	-	0	+	-	-	+	0	0	0	+
17	52	2.80	12	23 4/7	104.0	-	+	+	-	+++	++	+++	0
	53	2.84	12	-	0	-	+	-	-	+	0	0	+
18	54	2.70	12	24	116.5	+	-	-	-	0	0	++	0
	55	2.82	12	-	0	+	-	-	+	0	0	0	+
19	62	2.82	9	24	116.5	-	+	+	-	+++	+++	+++	+
	63	2.53	9	-	0	-	+	-	-	+	0	+	+
	70	2.08	9	24	113.5	-	-	+	-	0	+	+++	0
20	72	2.32	9	24	114.5	+	-	-	-	0	+	0	0
	71	2.00	9	-	0	+	-	-	+	0	0	0	+
21	82	2.13	11	24	115.5	+	-	-	+	0	0	0	0
	83	2.15	11	-	0	+	-	-	+	0	0	0	+++
22	88	2.33	9	24	115.5	+	-	-	-	0	+	++	+
	89	2.75	9	-	0	-	+	-	-	++	0	0	+
23	92	2.87	7	24	115.5	-	-	+	-	0	+	++	+
	93	3.04	7	-	0	+	-	-	+	0	0	0	0
24	96	2.41	8	24	115.5	+	-	-	+	0	0	0	0
	97	2.67	8	-	0	+	-	-	+	0	0	0	++
25	100	2.73	7	24	116.0	-	-	+	-	0	+	0	+
	101	2.91	7	-	0	+	-	-	+	0	0	0	0
Total Incidence of Lesions					After i.v.C.B.		4	10		4	13	12	19
					Controls		7	2		4	2	3	19

Total Nos. in Series: Experimental - 26.
Control - - - 25.

Discrepancies between the duration of injections and the total dosage of Caledon Blue were the result of unavoidable interruptions of the weekly or bi-weekly injections.

APPENDIX C

APPENDIX C

DISSECTION OF HEART FOR FRACTIONAL WEIGHING

At autopsy the heart was detached from the lungs. The right heart was opened by a scissors cut passing through the lateral wall of the atrium, the tricuspid valve and the antero-lateral wall of the right ventricle, but stopping a few millimetres short of the apex. The left heart was opened similarly, the cut extending down through the anterior wall of the atrium, mitral valve and the anterior wall of the left ventricle.

Next, all blood and clot was washed out of the heart which was then fixed in 10% formol-saline till dissection and weighing were performed.

Before dissection all hearts were washed in running water for a few minutes, purely to get rid of the formalin. Remaining tags of parietal pericardium or mediastinal fat were removed. The stumps of the aorta and pulmonary artery were cut

off as close to the valve rings as possible. Similarly the remains of the veins were excised from the atria.

Then the heart was dried, using a hand towel and what can be best described as "good firm pressure" between thumb and forefinger. This drying was necessary in view of the small weight of the right ventricle but had the disadvantage of disrupting the planes between the muscle bundles, to the detriment of subsequent histological examination.

The whole heart was then weighed to the nearest 10.0 mg.

Next, the atria, valve cusps, chordae and valve rings were removed. Finally, as much as possible of the sub-epicardial fat was cut off. This step, and all others, was performed with fine dissecting scissors.

This left the right and left ventricles. The right was then cut off from the left, with the scissors. The cut started at the junction of the right ventricle and the septum, posteriorly, and was continued downwards towards the apex, keeping as close to the septum as possible. It was continued up from the apex following close to the septum, anteriorly, to finish in the region of

the pulmonary valve. Thus the entire right ventricle was severed from the left.

The papillary muscles of the right ventricular side of the septum were removed and included with the right ventricle for weighing.

No attempt was made to fractionate the septum which was left intact and weighed as part of the left ventricle.

Both ventricles were then weighed, to the nearest 10.0 mg. The left ventricle was always weighed before the right.

TABLE 1

Fractional Heart Weights of Non-Emphysematous Rabbits

Rabbit No.	Body Weight (kg.)	Heart Weights (Gm.)			LV/RV	$(LV/RV)^2$	Degree of Generalised Interstitial Pneumonia
		Total	L.V.	R.V.			
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
RC/		Young Rabbits					
118	1.14	1.56	1.02	0.25	4.08	16.646	+
119	1.03	1.55	1.03	0.23	4.48	20.070	0
120	1.10	1.25	0.97	0.22	4.41	19.448	0
121	1.12	1.47	0.97	0.21	4.62	21.344	+
122	1.07	1.44	1.00	0.24	4.13	12.057	+
123	1.15	1.59	1.01	0.24	4.21	17.724	+
124	1.56	2.60	1.77	0.41	4.32	18.662	+
125	1.33	1.78	1.23	0.28	4.39	19.272	0
126	1.51	2.35	1.52	0.39	3.89	15.132	0
137	0.73	1.19	0.78	0.19	4.11	16.892	++
138	0.98	1.76	1.17	0.28	4.18	17.472	++
139	1.26	2.55	1.71	0.43	3.98	15.840	+
140	0.89	2.03	1.26	0.34	3.71	13.764	+
141	0.55	1.37	0.94	0.22	4.27	18.233	+
142	0.52	1.10	0.70	0.18	3.89	15.132	+
143	0.48	0.90	0.62	0.14	4.43	19.625	++
144	0.51	1.09	0.75	0.19	3.95	15.603	++
145	0.46	0.80	0.54	0.13	4.15	17.223	+
146	0.65	1.09	0.72	0.18	4.00	16.000	+++

No. in sub-group = 19.
Mean LV/RV = 4.168. Sum of $(LV/RV)^2$ = 331.139.
S.D. = 0.236. S.E. of Mean = 0.054.

R.St/		Known Adults					
45	3.04	4.82	2.91	0.63	4.62	21.344	0
47	1.62	2.64	1.74	0.40	4.35	18.923	+
49	2.01	3.28	2.20	0.49	4.49	20.160	+
55	2.82	3.92	2.45	0.54	4.54	20.612	+
57	2.89	3.73	2.41	0.53	4.55	20.703	+
65	2.11	3.51	2.34	0.55	4.25	18.063	+
71	2.00	3.77	2.67	0.70	3.81	14.516	+
79	2.57	3.72	2.55	0.56	4.57	20.885	++
83	2.15	3.67	2.50	0.56	4.29	18.404	+++
87	2.36	3.44	2.27	0.56	4.05	16.403	0
91	2.72	2.90	1.94	0.50	3.88	15.054	0
93	3.04	3.96	2.48	0.58	4.28	18.318	0
95	2.26	4.56	3.05	0.79	3.86	14.900	0
97	2.67	3.87	2.57	0.61	4.21	17.724	++
101	2.91	4.69	3.17	0.66	4.80	23.040	0
103	3.32	5.15	3.12	0.81	3.85	14.823	+
105	2.32	3.16	2.09	0.53	3.94	15.524	++
107	2.89	4.04	2.53	0.64	3.94	15.524	+++

TABLE 1 - contd.

Column No. (1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
109	2.16	3.53	2.36	0.54	4.37	19.097	+

No. in sub-group = 19.
Mean LV/RV = 4.245. Sum of $(LV/RV)^2$ = 344.017.
S.D. = 0.305. S.E. of Mean = 0.070.

RC/							
		Miscellaneous Adults					
10	2.70	6.65	4.38	1.11	3.94	15.524	0
11	1.96	3.93	2.41	0.76	3.17	10.049	0
12	1.86	4.11	2.62	0.77	3.40	11.560	+
13	2.05	4.08	2.58	0.78	3.31	10.956	++
14	2.69	4.86	3.15	0.81	3.88	15.054	+++
15	1.99	4.71	3.10	0.76	4.08	16.646	+++
16	2.54	4.35	2.80	0.67	4.17	17.389	+
17	3.40	4.71	3.14	0.74	4.24	17.978	++
18	2.27	4.63	2.75	1.00	2.75	7.563	0
19	1.67	3.72	2.50	0.64	3.91	15.288	0
20	1.47	3.76	2.51	0.64	3.92	15.366	0
24	1.69	3.59	2.48	0.62	4.00	16.000	0
26	1.80	3.26	2.28	0.58	3.93	15.445	+
28	2.05	4.18	2.79	0.68	4.10	16.810	++
29	1.96	2.88	1.93	0.43	4.49	20.160	++
30	1.89	3.63	2.46	0.53	4.64	21.530	+
32	1.77	3.29	2.21	0.53	4.17	17.389	++
33	1.92	2.93	2.05	0.48	4.27	18.233	+
34	1.99	4.26	2.95	0.63	4.68	21.902	+
35	1.89	2.97	2.13	0.45	4.73	22.373	+
36	2.02	3.96	2.66	0.62	4.29	18.404	0
37	1.15	3.37	2.27	0.57	3.98	15.840	++
38	2.35	3.10	2.25	0.45	5.00	25.000	+
39	2.97	5.57	3.93	0.77	5.10	26.010	++
40	2.15	3.31	2.26	0.50	4.52	20.430	+
41	2.22	3.28	2.28	0.53	4.30	18.490	+
42	2.06	3.89	2.74	0.56	4.89	23.912	++
43	1.84	3.28	2.53	0.54	4.69	21.996	+++
44	2.51	4.35	2.66	0.87	3.06	9.364	0
45	1.80	2.68	1.73	0.41	4.22	17.808	0
46	2.49	4.81	2.78	0.88	3.16	9.986	0
47	1.98	3.69	2.31	0.66	3.50	12.250	++
48	2.40	4.32	2.70	0.84	3.21	10.304	+
49	2.04	3.73	2.43	0.64	3.80	14.440	+
50	2.47	3.28	2.17	0.53	4.09	16.728	+
52	2.98	5.44	3.77	1.09	3.46	11.972	+++
53	2.89	3.79	2.53	0.69	3.74	13.988	+
54	2.53	3.42	2.99	0.71	4.21	17.724	+
55	2.11	3.19	2.09	0.45	4.64	21.530	++
56	3.08	5.05	3.33	0.79	4.22	17.808	+
57	2.63	4.03	2.53	0.64	3.95	15.603	++
58	2.40	4.35	2.59	0.64	4.05	16.403	++
62	2.62	3.85	2.40	0.66	3.64	13.250	+++
63	2.16	3.52	2.34	0.55	4.25	18.063	++
65	3.40	4.66	3.08	0.63	4.86	23.620	+++
66	2.49	4.14	2.60	0.69	3.77	14.213	++

TABLE I - contd.

Column No. (1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
RC/							
67	2.24	3.77	2.47	0.54	4.58	20.976	++
68	1.87	3.34	2.25	0.53	4.25	18.063	+
69	1.81	3.14	2.00	0.56	3.57	12.745	++
70	2.02	3.65	2.28	0.68	3.35	11.223	0
73	1.37	3.96	2.70	0.56	4.82	23.232	+
75	1.92	4.28	2.76	0.70	3.94	15.524	+
76	1.68	3.10	1.95	0.54	3.43	11.765	++
79	2.00	3.88	2.59	0.55	4.71	22.184	+
80	2.12	4.53	3.18	0.60	5.30	28.090	+
81	2.22	3.57	2.26	0.57	3.96	15.682	+
82	2.89	5.89	3.80	0.77	4.94	24.404	++
94	1.40	5.10	3.38	0.63	5.37	28.837	+
95	2.13	4.02	2.53	0.58	4.36	19.010	0
96	2.25	3.71	2.45	0.56	4.38	19.184	++
97	2.25	3.72	2.38	0.62	3.84	14.746	+
98	2.16	4.68	2.87	0.83	3.46	11.972	+
99	2.47	4.25	2.74	0.62	4.42	19.536	+
102	-	5.51	3.42	0.87	3.93	18.445	0
103	2.47	3.97	2.46	0.59	4.17	17.389	0
104	2.35	4.72	3.09	0.74	4.18	17.472	+++
105	2.10	3.67	2.52	0.58	4.52	20.430	0
106	2.45	4.85	3.11	0.73	4.26	18.148	++
107	2.65	4.50	2.73	0.70	3.90	15.210	++
108	2.08	3.72	2.48	0.59	4.20	17.640	+
109	2.30	3.01	1.97	0.50	3.94	15.524	+
111	1.76	2.88	1.95	0.44	4.43	19.625	++
112	2.16	3.05	2.12	0.43	4.93	24.305	+
113	2.34	4.24	2.95	0.85	3.47	12.041	+++
116	2.49	3.59	2.61	0.63	4.14	17.140	+++
147	-	4.57	2.80	0.69	4.06	16.403	+++
RO/8	2.14	4.26	2.91	0.57	5.11	26.112	++
9	2.43	3.82	2.52	0.60	4.20	17.640	+
11	1.66	3.38	2.26	0.50	4.52	20.430	+
12	1.58	2.35	1.65	0.38	4.34	18.836	+
17	2.17	5.19	3.16	0.86	3.67	13.469	+++
19	2.03	3.40	2.08	0.66	3.15	9.923	+
23	2.00	3.66	2.14	0.59	3.64	13.250	++
26	2.45	2.93	1.75	0.43	3.65	13.323	+++
29	2.08	3.51	2.28	0.59	3.95	15.603	0
32	2.63	5.17	3.16	0.81	3.90	15.210	+
43	2.25	4.34	2.31	0.76	3.04	9.242	0

No. in sub-group = 87.
Mean LV/RV = 4.096. Sum of $(LV/RV)^2$ = 1485.334
S.D. = 0.547. S.E. of mean = 0.059.

TABLE 1 - contd.

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
RC/			Old Rabbits				
83	3.06	5.95	3.71	0.92	4.03	16.241	0
84	2.45	4.88	3.26	0.80	4.08	16.646	++
87	2.99	5.62	3.72	0.80	4.65	21.623	+
88	2.20	4.88	3.16	0.74	4.27	18.233	++
93	3.52	5.67	3.44	0.74	4.65	21.623	++
134	2.69	4.43	2.77	0.62	4.47	19.981	+
135	1.91	3.97	2.70	0.59	4.58	20.976	+++
136	2.16	4.38	2.82	0.69	4.09	16.728	++

No. in sub-group = 8.
Mean LV/RV = 4.352. Sum of $(LV/RV)^2$ = 152.051.
S.D. = 0.266. S.E. of mean = 0.094.

Total No. in Group = 133.
Mean LV/RV = 4.143. Sum of $(LV/RV)^2$ = 2312.541
S.D. = 0.498. S.E. of mean = 0.043.

TABLE 2

Fractional Heart Weights of Rabbits with Marginal Emphysema only.

Rabbit No.	Body Weight (kg.)	Heart Weights (Gm.)			LV/RV	$(LV/RV)^2$
		Total	L.V.	R.V.		
	Young Rabbits - nil.					
R.St/	Known Adults					
53	2.84	4.32	3.040	0.56	5.42	29.376
77	2.05	3.04	2.06	0.44	4.68	21.902
89	2.75	4.48	3.04	0.72	4.22	17.808
R.C/	Miscellaneous Adults					
21	1.87	4.07	2.73	0.67	4.07	16.565
22	2.09	4.56	2.99	0.77	3.88	15.054
25	1.67	3.88	2.70	0.65	4.15	17.223
27	2.00	3.22	2.13	0.52	4.10	16.810
51	3.00	5.25	3.82	1.18	3.24	10.498
71	1.84	3.72	2.44	0.63	3.87	14.977
78	2.68	4.52	2.93	0.61	4.80	23.040
101	2.61	3.82	2.50	0.56	4.46	19.892
115	2.02	3.82	2.66	0.63	4.22	17.808
R.O/4	2.03	3.04	2.05	0.47	4.36	19.010
7	1.32	2.06	1.33	0.32	4.16	17.306
10	1.76	3.55	2.55	0.49	5.20	27.040
22	3.08	7.03	4.77	1.03	4.63	21.437
27	3.27	8.2	4.68	1.27	3.69	13.616
36	2.55	4.90	3.20	0.71	4.51	20.340
R.C/	Old Rabbits					
127	3.00	6.07	4.02	0.93	4.32	18.662
131	2.82	5.75	3.76	0.97	3.88	15.054

No. in Group = 20. Sum of $(LV/RV)^2$ = 373.418

Mean LV/RV = 4.293

S.D. = 0.418 S.E. of Mean = 0.093

TABLE 3

Fractional Heart Weights of Rabbits with Generalised Emphysema + or - Marginal Emphysema.

Rabbit No.	Body Weight (Kg.)	Heart Weights (gm.) Total	L.V.	R.V.	LV/RV	$(LV/RV)^2$	Degree of Generalised Interstit. Pneumonia	Degree of Generalised Emphysema
Young Rabbits - nil.								
R.St/	Known Adults							
63	2.53	3.54	2.21	0.60	3.68	13.542	+	+
111	2.29	4.08	2.72	0.61	4.46	19.892	++	+
81	1.75	3.20	2.36	0.48	4.92	24.206	+++	++
RC/	Miscellaneous Adults							
23	2.14	4.89	3.35	0.80	4.19	17.556	+	++
31	2.22	3.62	3.14	0.77	4.08	16.646	++	++
60	2.67	5.26	3.46	0.80	4.33	18.749	+++	+++
61	2.94	6.13	4.16	1.05	3.96	15.682	+	+
72	1.31	3.78	2.58	0.52	4.96	24.602	++	+
100	2.53	5.34	3.44	0.83	4.14	17.140	+	+
110	2.60	4.53	3.00	0.71	4.23	17.893	++	+
114	2.90	4.22	2.69	0.69	4.14	17.140	+	+
RO/6	1.99	4.08	2.88	0.60	4.8	23.040	+++	+
RC/	Old Rabbits							
85	2.41	6.84	4.36	1.00	4.36	19.010	++	+
86	2.39	4.98	3.41	0.76	4.49	20.160	+	++
89	2.23	5.00	3.26	0.78	4.18	17.472	++	+
90	3.32	6.24	3.86	1.00	3.86	14.900	+	+++
91	2.51	4.83	3.22	0.69	4.67	21.809	+	++
92	2.68	4.92	3.27	0.71	4.61	21.252	+++	+++
128	2.42	4.00	2.52	0.66	3.85	14.823	++	++
129	2.99	3.76	2.46	0.58	4.24	17.978	++	+
130	2.47	4.44	2.92	0.74	3.95	15.603	+++	+++
132	2.27	5.02	3.37	0.75	4.49	20.160	++	+
133	2.33	4.24	2.81	0.65	4.32	18.662	++	++

No. in Group = 23.

Mean LV/RV = 4.300. Sum of $(LV/RV)^2$ = 427.917.

S.D. = 0.347. S.E. of Mean = 0.072.

TABLE 4

Fractional Heart Weights of Rabbits of 1st Experimental Series.

Rabbit No.	Body Weight (Kg.)	Heart Weights (gm.) Total	L.V.	R.V.	LV/RV	$(LV/RV)^2$	Degree of Gen. Interst. Pneumonia	Deg. of Gen. Emphysema	Durat'n of w'kly C.B.inj'ns
R.St/									
4	2.41	5.58	3.30	1.05	3.14	09.860	+	0	3
9	2.13	5.68	3.15	1.25	2.52	06.350	+++	0	5 1/7
13	2.41	4.85	3.04	0.76	4.00	16.000	+++	0	1
17	2.23	4.67	2.80	0.81	3.46	11.972	0	0	6 5/7
20	2.64	6.66	3.60	1.25	2.88	08.294	+++	0	11 5/7
6	3.95	8.11	4.14	1.70	2.43	05.905	+++	+	5 1/7
8	2.74	5.24	3.24	0.85	3.81	14.516	+	+	55
11	2.73	6.65	3.48	1.14	3.05	09.303	+++	+	43
16	2.33	4.90	3.05	0.74	4.12	16.974	+	+	17 6/7
18	2.87	5.38	3.50	0.94	3.72	13.838	+++	+	42
21	2.80	4.74	2.99	0.83	3.60	12.960	++	+	49
23	2.74	4.42	2.84	0.62	4.58	20.976	+	+	49
26	2.43	4.55	2.54	0.83	3.06	09.364	0	+	14 2/7
7	3.73	7.09	4.05	1.00	4.05	16.403	+	++	47
10	2.84	4.26	2.61	0.73	3.58	12.816	++	++	52
24	2.03	4.35	2.35	0.94	2.50	06.250	+++	++	15 5/7
25	2.68	6.08	3.25	0.98	3.25	10.563	++	++	37
5	3.26	5.07	2.86	0.80	3.58	12.816	+	+++	56
14	3.12	6.37	3.47	0.96	3.61	13.032	++	+++	52
15	3.11	4.84	2.78	0.79	3.52	12.390	+	+++	52
22	2.36	4.65	3.13	0.72	4.35	18.923	+	+++	49
28*	2.34	4.10	2.42	0.88	2.75	07.563	+	+++	31 1/7

No. in Group = 22.
Mean LV/RV = 3.435. Sum of $(LV/RV)^2$ = 267.068
S.D. = 0.627. S.E. of Mean = 0.134.

* Omitted from Chart 2.

TABLE 5

Fractional Heart Weights of Rabbits of 2nd Experimental Series.

Rabbit No.	Body Weight (Kg.)	Heart Weights (gms.) Total	L.V.	R.V.	LV/RV	$(LV/RV)^2$	Degree of Gen. Interst. Pneumonia	Deg. of Gen. Emphysema	Durat'n of w'kly C.B.inj'ns
R.St/									
44	3.01	4.52	2.31	0.87	2.66	07.076	++	0	6
48	2.33	3.79	2.41	0.70	3.44	11.834	+	0	10
56	2.56	4.38	2.89	0.93	3.11	09.672	+++	0	4 2/7
64	2.53	4.90	2.86	1.17	2.44	05.954	++	0	1
72	2.32	4.16	2.91	0.69	4.22	17.808	0	0	24
78	2.65	4.90	3.11	0.78	4.12	16.974	+++	0	7
80	1.93	3.92	2.08	0.88	2.36	05.570	+++	0	2 2/7
82	2.13	3.72	2.42	0.64	3.78	14.288	0	0	24
86	2.02	4.07	2.41	0.84	2.87	08.237	+++	0	3
90	2.06	3.65	2.07	0.75	2.76	07.618	+++	0	1
94	2.25	3.80	2.45	0.60	4.08	16.646	++	0	1
96	2.41	4.34	2.90	0.68	4.26	18.148	0	0	24
100	2.73	4.67	2.96	0.85	3.36	11.290	+	0	24
104	2.06	3.61	2.07	0.49	4.63	21.437	++	0	8
R.St/									
46	1.59	3.02	1.93	0.51	3.78	14.288	++	+	6
108	2.16	3.37	2.25	0.62	3.63	13.177	0	+	20
R.St/									
54	2.70	4.62	2.77	0.70	3.96	15.682	0	++	24
88	2.33	4.08	2.51	0.69	3.64	13.250	+	++	24
92	2.87	4.63	2.89	0.75	3.85	14.823	+	++	24
102	2.88	4.88	3.14	0.66	4.76	22.658	++	++	17 4/7
R.St/									
52	2.80	4.77	3.20	0.79	4.05	16.403	0	+++	23 4/7
62	2.82	4.54	2.71	0.78	3.47	12.041	+	+++	24
70	2.08	4.20	2.79	0.64	4.36	19.010	0	+++	24
76	1.92	3.72	2.38	0.74	3.22	10.368	0	+++	15
106	3.04	4.42	2.92	0.73	4.00	16.000	+	+++	20
110	2.66	4.14	2.74	0.58	4.72	22.278	++	+++	5 3/7

No. in Group = 26.

Mean LV/RV = 3.674. Sum of $(LV/RV)^2$ = 362.530

S.D. = 0.688. S.E. of Mean = 0.135.

APPENDIX D

OPERATIVE TECHNIQUE

In order to fix the heart to the sternum, so that repeated cardiac puncture could be performed without anaesthesia, the following procedure was adopted.

The rabbit was anaesthetised by Nembutal and open ether. The chest was shaved and thoroughly cleaned with spirit.

First Stage.

An incision was made in the skin which started about 2.0 cm. to the left of the midline at the level of the xiphisternum and was carried almost horizontally to 2-3 cm. to the right of the midline and curved upwards to complete a rough semi-circle which ended at the level of the first rib 1-2 cm. to the left of the midline. This gave good exposure and ensured that the scar was well clear of the region of the 3rd and 4th right inter-spaces near the sternal margin.

The pectoral muscles were then detached, with scissors, from their sternal insertion and a strip, 2-3 cm. wide, was cut off the end of the

muscles to increase the exposure of the underlying costal cartilages and intercostal muscles.

Second Stage.

The right internal mammary artery and vein were located and ligatured in the 2nd interspace. These vessels lie deep to the intercostal muscles about 0.75 cm. lateral to the right border of the sternum. Cautious separation of the fibres of the intercostal muscle in their natural plane revealed the vessels which were then cleared and ligated. Care must be taken lest the pleura is perforated at this stage. A similar dissection was performed in the 4th right interspace and the internal mammary vessels were ligated in this space also.

The ligation of these vessels was the most important step in the operation as it ensured freedom from major bleeding in the later stages. Any slight oozing could be ignored or easily controlled by light packing with Alginate wool.

Third Stage.

The intercostal muscles were cleared from the 3rd and 4th costal cartilages which were ultimately resected. The muscles were removed partly by blunt dissection and partly by scissors. They were cleared, laterally, right out to the ribs; but medially it was advisable to leave a little

muscle attached to the border of the sternum so that sutures could be passed through it later.

Ligatures were passed round each cartilage and tied firmly, one at the rib end and one at the sternal end. The cartilages were then cut between the ligatures and the entire length removed to give maximum exposure. The ligatures were a great convenience as retractors in the later stages.

There was risk of opening the pleural sac during this atage. Unilateral pneumothorax made the remainder of the operation more difficult but was of no importance otherwise.

Fourth Stage.

The mediastinal fat was pushed gently aside and the pericardium grasped at its central point by fine-toothed forceps. Care was taken at this stage to avoid perforating the pleural cavity on the left, especially if the right pleural sac had already been opened accidentally. The pericardium and heart were then drawn forward into the aperture of the wound. Then the pericardium was opened by scissors by making a vertical slit about 0.5 cm. long on the sternal side relative to the gripping forceps. Next, the costal margin of the opened pericardium was sutured to the corresponding margin of the wound aperture in as natural a position as possible. The slit in the pericardium was then

enlarged and the edges sutured to the corresponding edges of the wound aperture. The object was to make the aperture in the pericardium as large as possible without actually splitting the sac posteriorly. Usually, two sutures on either side gave good apposition of the pericardium to the wound edges. It was better to be content with relatively poor apposition than to risk splitting the pericardium posteriorly which deprived the heart of support and made the next stage difficult.

Fifth Stage.

This consisted of suturing the heart to the edges of the wound aperture. The heart was grasped with the forceps near the apex, just to the left of the coronary artery, and pulled forward into the wound. Then sero-muscular sutures were used to stitch the heart to the wound edges. It was best to start with one suture near the apex of the heart, in the region of the septum just to the right of the coronary vessels, and stitch this to the soft tissue remaining on the right border of the sternum. Thereafter, another similar suture near the base of the ventricle on the sternal side, and one, or at the most two, sutures between the right border of the right ventricle and the right edge of the wound completed the fixation.

Every attempt was made to keep the heart in as natural a position as possible. However,

even if the position did not seem ideal at operation, this did not appear to make any difference for, at autopsy, in every case, the heart was found to be in good position.

Sixth Stage.

All sutures and ligatures were cut off as closely as possible and the skin incision closed with interrupted sutures. The pectoral muscles were not re-attached to the sternum but this did not incapacitate the rabbit on recovery. If a right-sided pneumothorax had occurred, aspiration of this was advisable.

The wound was kept covered for ten days, till the stitches were removed, to prevent infection and interference by the rabbit. This was done by bandages kept in place with a "figure-of-eight" of adhesive strapping round the chest. This was renewed anteriorly as the rabbit chewed it away. The rabbits were left for six weeks before being subjected to pressure readings.

MEASUREMENT OF PRESSURES

The pressure recorded was the mean right ventricular pressure.

A simple manometer filled with 10% potassium citrate was used for all readings. It was found that ordinary No.3 serum needles were required to give a reasonable degree of sensitivity to this apparatus and these were employed throughout. The bevels of the needles were ground down to a half or two-thirds of their original length.

The needle was connected, by an adaptor, to about nine inches of polythene tubing which in turn was connected to the manometer by a short length of rubber tubing which could be clamped easily. The manometer was fixed to the table at a convenient position, level with the throat of the rabbit, and the pressures were read directly in cm. of water. Zero pressure was taken as the level of the table and no attempt was made to correct the individual pressures to the level of the ventricle.

The level of the citrate was adjusted to approximately 5.0 cm. above the expected pressure

and the rubber connecting tube clamped. The operator then palpated the exposed right ventricle through the skin and inserted the needle into the ventricle. The clamp on the connecting tube was then opened and successful entry of the ventricle could be gauged from the fluctuations of the manometer fluid.

Experience showed that the pressure took a variable time to settle down after entry of the ventricle. This varied between a few seconds and a minute or more. When a steady reading was attained the value was noted and the needle withdrawn. Continuous recording was not employed. The pressures could be read to the nearest 0.25 cm. of water with this apparatus.

Skilled assistance in holding the rabbits was the most essential part of taking successful pressure readings. Two assistants were required, the person at the head end being the more important of the two. It was found advisable to adhere to a strict ritual, even if certain steps of this were not strictly necessary at the time of any particular pressure reading.

The rabbit was turned on its back and one assistant held the hind legs while the ears and fore-legs were held by the other. With firm, gentle handling most rabbits relaxed in this position

provided even tension was maintained on the legs. The chest was then shaved with electric clippers and the area cleaned with spirit. When the rabbit was relaxed the pressure was read as above . No anaesthetic, local or otherwise, was employed.

If any difficulty was experienced in getting the needle into the ventricle, it was better to stop immediately and leave the rabbit till later in the session.

This routine was observed in the long-term experiments where the pressure was taken in each animal at weekly intervals.

In the acute experiments, where the pressures were read while the injection was being given, the procedure was identical in most respects. However, before shaving the chest, a hypodermic needle barrel, fitted directly into fine polythene tubing to which a syringe could be attached by an adaptor, was inserted into an ear vein. The ventricle was needled as before and, when the pressure had settled, the starting level was recorded. The control injection of diluent was given followed by the test injection of Caledon Blue or Lycopodium. The pressure changes were read by the operator, as before, and recorded every 15 seconds by a third assistant.

EFFECT OF REPEATED PUNCTURE ON THE HEART

At autopsy there was no evidence of mechanical embarrassment of the heart by the operation. At the site of puncture, however, when frequent punctures had been performed, there was, invariably, a small area of fibrosis in the myocardium. Frequently there was slight thickening of the endocardium of the septum of the right ventricle at a point opposite the site of entry of the needle. This was due to trauma by the point of the needle and it was for this reason that the bevels of the needles were shortened.

Both these effects were trivial, especially the latter, and did not appear likely to have seriously affected the results of the experiments. The control animals certainly showed no sign of any change in the mean R.V. pressure over periods of nearly a year. Naturally, both these effects could be minimised if a more sensitive manometer and a finer needle were used.

APPENDIX D

PROTOCOLS OF LONG TERM PRESSURE EXPERIMENTS

TABLE A - Rabbit No. RO/24.

Time (weeks)	I.V. Caledon Blue (mls.)	Interval between Injection and Pressure Reading (days)	Mean R.V. Pressure (cm. of water)
0	-	-	17.0
1	-	-	17.0
2	-	-	16.25
3	-	-	14.75
4	-	-	14.0
5	-	-	13.5
6	-	-	14.0
7	2.0	-	-

Died after injection.

Total C.B. = 2.0 ml.
Basal mean R.V.P. = 15.2 cm.
Gen. E. = 0
LV/RV = 3.90 (N)
Gen. I. = +++

TABLE B - Rabbit No. RO/26.

Time (weeks)	I.V. Caledon Blue (mls.)	Interval between Injection and Pressure reading (days)	Mean R.V. Pressure (cm. of water)
0	-	-	19.25
1	-	-	15.0
2	-	-	15.75
3	-	-	13.25
4	-	-	14.50

Died: ? Respiratory Infection

Total C.B. = nil.
Basal mean R.V.P. = 15.6
Gen. E. = 0
LV/RV = 3.65 (N)
Gen. I. = +++

TABLE 1.
Rabbit No. RO/41

Control: No Injection	
Time (weeks)	Mean R.V.Pressure (cms. of water)
0	17.00
1	16.25
2	17.25
3	15.50
4	16.00
5	16.75
6	15.25
7	17.50
8	17.50
9	)
10	) no
11	) press.
12	)
13	)
14	16.50
15	16.25
16	17.00
17	17.00
18	17.00
19	15.50
20	14.50
21	15.25
22	16.00
23	16.50
24	16.25
25	16.25
26	16.75
27	16.25
28	15.5
29	15.75
30	16.0
31	16.0
32 to 46	) no press.
47	15.75
48	15.75
49	17.25
50	16.50
Killed: week 59	

Mean Basal R.V.P. = 16.3cm.
Gen. E. = ++
LV/RV = 3.65 (N)
Gen. I. = +
Final R.V.P. = N.

TABLE 2.
Rabbit No. RO/38

Control: No Injection	
Time (weeks)	Mean R.V.Pressure (cms. of Water)
0	15.25
1	15.25
2	15.50
3	15.00
4	16.50
5	15.50
6	15.25
7	15.50
8	)
9	) no
10	) press.
11	)
12	)
13	)
14	15.50
15	15.75
16	15.50
17	16.0
18	15.25
19	15.75
20	14.50
21	15.25
22	16.50
23	16.50
24	17.25
25	15.75
26	)
27	) no
28	) press.
29	)
30	)
31	16.00
32 to 46	) no press.
47	16.25
48	16.00
49	16.50
50	15.50
Killed: week 59	

Mean Basal R.V.P. = 15.7cm.
Gen. E. = 0
LV/RV = 4.28 (N)
Gen. I. = 0
Final R.V.P. = N.

TABLE 3 - Rabbit No. RO/20

Time (wks.)	I.V.Caledon Blue (ml.)	Interval between Injection and Pressure Reading (days)	Mean R.V. Pressure (cm. of water)
0	-	-	20.0
1	-	-	21.5
2	-	-	20.0
3	-	-	23.0
4	-	-	22.5
5	-	-	22.0
6	2.5	3	23.0
7	3.0	3	20.0
8	3.0	3	19.25
9	3.0	3	24.0
10	3.0	3	19.5
11	3.0	3	22.0
12	3.0	2	18.5
13	3.0	2	18.5
14	3.0	-	-
15	3.0	3	21.5
16	3.0	1	17.0
17	3.0	3	20.75
18	3.0	3	20.5
19	3.0	1	21.25
20	3.0	-	-
21	3.0	2	23.75
22	3.0	2	21.5
23	3.0	5	21.75
24	3.0	2	23.25
25	3.0	2	21.0
26	3.0	1	19.0
27	3.0	1	21.5
28	3.0	2	19.5
29	3.0	1	19.5
30	3.0	1	22.0
31	3.0	1	20.5
32	3.0	1	23.0
33	3.0	1	22.5
34	3.0	-	-
35	-	7	21.5
36	-	14	20.75
37	3.0	acute	-
38) to 45)	-	-	-
46	-	-	20.75
47	-	-	21.0
48	-	-	20.0
49	-	-	19.5
50	-	-	20.75
51	-	-	20.25
52	-	-	19.5
53	-	-	21.0
54	2.0	acute	-
55	-	-	18.0
56	3.0	acute	-
57	-	-	20.25
58	3.0	acute	-
59	-	-	-
60	-	-	22.5
61	2.5	4	22.0
62	2.5	3	23.5
63	2.5	3	20.5
64	2.5	3	20.5
65	2.5	2	20.25
66	2.5	2	22.0
67	2.5	2	22.5
68	2.5	1	21.5
69	2.5	2	22.5
70	2.5	1	21.5
71	2.5	1	22.5
72	2.5	1	24.5
73	2.5	1	18.75
74	2.5	1	22.0
75	2.5	1	23.0
76	2.5	1	25.5
77	2.5	1	21.5
78) to 92)	-	-	-
93	-	-	18.5
94	-	-	18.5
95	-	-	20.0

Killed: week 103

Total C.B. = 137.0 ml.
Mean Basal R.V.P. = 21.5 cm.
Gen.E. = +++
LV/RV = 3.99(N)
Gen.I. = +
Final R.V.P. = N.

TABLE 4 - Rabbit No.RO/5

Time (wks)	I.V.Caledon Blue (ml.)	Interval between Injection & Pressure Reading (days)	Mean R.V. Pressure (cm. of water)
0	-	-	15.5
1	-	-	18.5
2	-	-	19.25
3	-	-	18.75
4	-	-	19.5
5	-	-	17.5
6	2.5	3	24.0
7	3.0	3	20.75
8	3.0	3	19.25
9	3.0	3	20.0
10	3.0	3	22.5
11	3.0	1	25.5
12	3.0	2	20.0
13	3.0	1	22.75
14	3.0	-	-
15	3.0	1	20.5
16	3.0	3	20.5
17	3.0	3	24.5
18	3.0	-	-
Died after Inject.			

Total C.B. = 38.5 ml.
Mean Basal R.V.P. = 18.2 cm.
Gen. E. = +
LV/RV = 2.69 (H)
Gen. I. = +++
Final R.V.P. = R.

TABLE 5 - Rabbit No. RO/14

Time (Wks.)	I.V.Caledon Blue (ml.)	Interval between Injection & Pressure Reading (days)	Mean R.V. Pressure (cm. of water)
0	-	-	17.5
1	-	-	15.5
2	-	-	15.25
3	-	-	14.75
4	-	-	13.5
5	-	-	13.5
6	-	-	13.5
7	2.5	3	19.5
8	3.0	3	16.25
9	3.0	3	22.0
10	3.0	3	15.5
11	3.0	3	22.5
12)	Ill with "snuffles".		
13)	Well by wk. 14.		
14)	No infect. or press.		
15)			
16	3.0	3	17.5
17	3.0	-	-
Died after Inject.			

Total C.B. = 22.5 ml.
Mean Basal R.V.P. = 14.8 cm.
Gen. E. = 0
LV/RV = 3.37 (D)
Gen. I. = +++
Final R.V.P. = R.

TABLE 6 - Rabbit No. RO/18

Time (wks.)	IV.Caledon Blue (ml.)	Interval between Injection & Pressure Reading (days)	Mean R.V. Pressure (cm. of water)
0	-	-	18.25
1	-	-	18.0
2	-	-	18.5
3	-	-	19.75
4	-	-	19.0
5	-	-	19.0
6	-	-	18.0
7	2.5	3	26.25
8	3.0	-	-
Died after Inject.			

Total C.B. = 5.5 ml.
Basal Mean R.V.P. = 18.6 cm.
Gen.E. = 0
LV/RV = 2.13 (H)
Gen.I. = +
Final R.V.P. = R.

TABLE 7 - Rabbit No. RO/16

Time (wks.)	I.V.Caledon Blue (ml.)	Interval between Injection & Pressure Reading (days)	Mean R.V. Pressure (cm. of water)
0	-	-	20.0
1	-	-	17.5
2	-	-	16.75
3	-	-	17.5
4	-	-	20.0
5	-	-	19.0
6	2.5	3	22.75
7	3.0	-	-
8	3.0	3	19.5
9	3.0	3	21.75
10	3.0	3	20.5
11	3.0	3	19.75
12	3.0	2	20.0
13	3.0	2	22.0
14	3.0	3	20.0
15	3.0	1	17.25
16	3.0	3	18.75
17	3.0	3	16.75
18	3.0	1	19.5
19	3.0	2	16.0
20	3.0	2	18.5
21	3.0	3	14.5
22	2.5	-	-
Died after Inject.			

Total C.B. = 50.0 ml.
Mean Basal R.V.P. = 18.5 cm.
Gen.E. = ++
LV/RV = 3.34 (D)
Gen.I. = +
Final R.V.P. = N.

TABLE 8 - Rabbit No. RO/40

Time (wks.)	I.V. Caledon Blue (mls.)	Interval between Injection & Pressure Reading (days)	Mean R.V.Pressure (cm. of water)
0	-	-	18.0
1	-	-	16.5
2	-	-	14.5
3	-	-	15.25
4	-	-	16.5
5	-	-	15.5
6	-	-	17.0
7	2.0	ac.fail.	-
8	-	-	16.5
9	2.5	acute	-
10	-	-	17.5
11	2.0	acute	-
12	-	-	-
13	-	-	-
14	-	-	17.75
15	2.5	4	16.5
16	2.5	3	16.25
17	2.5	3	18.25
18	2.5	4	15.5
19	2.5	2	15.75
20	2.5	2	18.5
21	2.5	2	22.0
22	2.5	1	17.5
23	2.5	2	21.0
24	2.5	1	18.0
25	2.5	1	17.25
26	2.5	1	19.0
27	2.5	1	19.25
28	2.5	1	16.5
29	2.5	1	20.0
30	2.5	1	19.25

Time (wks.)	I.V. Caledon Blue (mls.)	Interval between Injection & Pressure Reading (days)	Mean R.V.Pressure (cm. of water)
31	2.5	1	17.75
32 to 46	Rest		
47	-	-	16.5
48	-	-	16.5
49	-	-	17.0
50	-	-	16.25

Killed: week 58

Total C.B. = 49.0 ml.
Mean Basal R.V.P. = 16.2 cm.
Gen. E. = ++
LV/RV = 3.95 (N)
Gen. I. = +
Final R.V.P. = N

TABLE 9 - Rabbit No. RO/44

Time (wks.)	I.V.Caledon Blue (ml.)	Interval between Injection & Pressure Reading (days)	Mean R.V.Pressure (cm. of water)
0	-	-	18.00
1	-	-	16.50
2	-	-	16.75
3	-	-	16.25
4	-	-	16.50
5	-	-	15.75
6	-	-	16.75
7	2.0	acute	-
8	-		16.50
9	2.5	acute	-
10	-		16.50
11	3.0	acute	-
12	-		-
13	-		15.50
14	2.5	4	15.50
15	2.5	4	17.75
16	2.5	3	19.00
17	2.5	3	17.25
18	2.5	2	18.25
19	2.5	2	18.25
20	2.5	2	19.00
21	2.5	1	18.25
22	2.5	2	20.00
23	2.5	1	17.75
24	2.5	1	17.75
25	2.5	1	16.00
26	2.5	1	17.25
27	2.5	1	17.25
28	2.5	1	17.50

Time (wks.)	I.V.Caledon Blue (ml.)	Interval between Injection & Pressure Reading (days)	Mean R.V.Pressure (cm. of water)
29 to 45	Rest. No inj. No press.		
46	-	-	17.00
47	-	-	17.00
48	-	-	17.5
49	-	-	17.75

Killed: week 58

Total C.B. = 45.0 ml.
Mean Basal R.V.P. = 16.6 cm.
Gen. E. = +
LV/RV = 3.69 (N)
Gen. I. = 0
Final R.V.P. = N.

TABLE 10 - Rabbit No. RO/25.

Time (wks.)	I.V.Caledon Blue (ml.)	Interval between Injection & Pressure Reading (days)	Mean R.V.Pressure (cm. of water)
0	-	-	16.5
1	-	-	19.75
2	-	-	17.75
3	-	-	18.5
4	-	-	19.0
5	-	-	17.75
6	2	1	19.0
7	3	1	20.0
8	3	3	19.0
9	3	2	19.0
10	3	2	18.5
11	3	3	21.5
12	3	1	25.0
13	3	3	21.75
14	3	3	21.75
15	3	1	20.5
16	3	1	-
17	3	2	21.0
18	3	2	22.25
19	3	5	19.5
20	3	2	19.5
21	3	2	19.5
22	3	1	19.5
23	3	1	20.25
24	3	2	19.5
25	3	1	19.0
26	3	1	22.0
27	3	1	22.25
28	3	1	21.0
29	3	1	21.0
30	3	3	20.5
31	-	-	21.0
32	-	-	20.5
33	3.0	acute	-
34 to 41	-	-	-
42	-	-	20.5
43	-	-	20.0
44	-	-	21.5
45	-	-	18.5
46	-	-	19.75
47	-	-	19.5
48	-	-	21.0
49	-	-	17.5
50	-	-	-
51	-	-	18.0
52	3.0	acute	-
53	-	-	18.5
54	3.0	acute	-
55	-	-	-
56	-	-	19.25
57	2.5	4	19.25
58	2.5	4	18.25
59	2.5	3	19.75
60	2.5	3	19.75
61	2.5	2	19.25
62	2.5	2	19.25
63	2.5	2	19.5
64	2.5	1	19.5
65	2.5	2	20.0
66	2.5	1	19.75
67	2.5	1	20.25
68	2.5	1	20.5
69	2.5	1	18.5
70	2.5	1	21.5
71	2.5	1	18.75
72	2.5	1	22.0
73	2.5	1	21.5
74 to 88	Rest		
89	-	-	18.5
90	-	-	19.5
91	-	-	18.25
92	-	-	19.0

Killed: week 100

Total C.B. = 125.5 ml.
Mean Basal R.V.P. = 18.2 cm.
Gen. E. = +++
LV/RV = 3.05 (H)
Gen. I. = 0
Final R.V.P. = N

TABLE 11

Rabbit No. RO/28

Time (wks.)	I.V.Caledon Blue (ml.)	Interval between Injection & Pressure Reading (days)	Mean R.V.Pressure (cm. of water)
0	-	-	20.5
1	-	-	17.5
2	-	-	19.25
3	-	-	19.75
4	-	-	18.5
5	2	1	20.5
6	2	1	22.5
7	3	3	19.5
8	3	2	21.25
9	3	-	-
Died of resp. infect.			

Total C.B. = 13.0 ml.
Mean Basal R.V.P. = 19.1 cm.
Gen. E. = 0
LV/RV = 4.10 (N)
Gen. I. = +++
Final R.V.P. = D.

TABLE 12

Rabbit No. RO/42

Time (wks.)	I.V.Caledon Blue (ml.)	Interval between Injection & Pressure Reading (days)	Mean R.V.Pressure (cm. of water)
0	-	-	14.0
1	-	-	15.5
2	-	-	16.0
3	-	-	16.0
4	-	-	15.5
5	-	-	15.0
6	-	-	15.5
7	-	-	15.5
8	-	-	16.5
9	2.0	acute	-
10	-	14	16.0
11	2.0	acute	-
12	-	14	17.5
13	3.0	acute	-
14	-	14	17.5
15	3.0	acute	
Died in acute experiment: week 15			

Total C.B. = 10 ml.
Mean Basal R.V.P. = 15.5 cm.
Gen. E. = 0
LV/RV = 2.16 (H)
Gen. I. = 0
Final R.V.P. = D.

TABLE 13

Rabbit No. RO/39

Time (wks.)	I.V.Lycopodium (ml. 1% w/v suspension)	Interval between Injection & Pressure Reading (days)	Mean R.V.Pressure (cm. of water)
0	-	-	15.00
1	-	-	16.00
2	-	-	17.75
3	-	-	15.00
4	-	-	15.50
5	-	-	16.50
6	-	-	14.50
7	-	-	15.50
8	-	-	15.00
9	-	-	20.00
10	-	-	15.00
11	3.0	acute	-
12	-	-	-
13	-	-	-
14	-	-	15.50
15	2.0	4	17.75
16	2.5	4.	15.50
17	2.5	3	18.75
18	2.5	3	15.50
19	2.5	2	15.25
20	2.5	2	17.00
21	2.5	2	18.50
22	2.5	1	20.00
23	2.5	2	17.50
24	2.5	1	17.75
25	2.5	1	16.75
26	2.5	1	17.25
27	2.5	1	15.50
28	2.5	1	17.00
29	2.5	1	17.25
30	2.5	1	17.25
31	2.5	1	16.75
32 to 46	Rest. No injects. No. press.		
47	-	-	14.50
48	-	-	15.75
49	-	-	16.00
50	-	-	14.25

Killed: week 59

TABLE 14

Rabbit No. RO/30

Time (wks.)	I.V.Lycopodium (ml.) 1% w/v suspension)	Interval between Injection & Pressure Reading (days)	Mean R.V. Pressure (cm. of water)
0	-	-	18.25
1	-	-	19.25
2	-	-	19.00
3	-	-	20.25
4	-	-	20.25
5	-	-	19.00
6	-	-	20.00
7	1.5	1	19.25
8	3.0	1	21.5
9	3.0	2	26.0
10	3.0	-	-

Died after injection

Total Lyco. = 10.5 ml.
Mean Basal R.V.P. = 19.4 cm.
Gen. E = 0.
LV/RV = 2.66 (H)
Gen. I = +++
Final R.V.P = R.

TABLE 13 - continued.

Total Lyco. = 45.0 ml.
Mean Basal R.V.P. = 16.0 cm.
Gen. E. = +
LV/RV = 3.48 (D)
Gen. I. = 0
Final R.V.P. = N.

TABLE 15

Rabbit No. RO/34

Time (wks.)	I.V.Lycopodium (ml. 1% w/v suspension)	Interval between Injection & Pressure Reading (days)	Mean R.V. Pressure (cms.of water)
0	-	-	18.00
1	-	-	17.25
2	-	-	21.00
3	-	-	20.00
4	-	-	25.00
5	-	-	18.50
6	-	-	22.50
7	2.0	1	22.75
8	3.0	1	24.00
9	3.0	2	25.75
10	2.0	1	28.00
11	2.0	1	30.00
12	2.0	1	29.50
13	2.0	1	29.25
14	3.0	-	-
Died after injection			

Total Lyco. = 19.0 ml.
Mean Basal R.V.P. = 20.3 cm.
Gen. E. = 0
LV/RV = 3.21 (D)
Gen. I. = +++
Final R.V.P. = R.

TABLE 16

Rabbit No. RO/35

Time (wks.)	I.V.Lycopodium (ml. 1% w/v suspension)	Interval between Injection & Pressure Reading (days)	Mean R.V. Pressure (cm. of water)
0	-	-	17.25
1	-	-	16.75
2	-	-	18.75
3	-	-	18.25
4	-	-	17.50
5	-	-	17.50
6	-	-	16.25
7	2.0	1	21.25
8	3.0	1	19.00
9	3.0	2	21.75
10	2.0	1	22.00
11	2.0	1	29.50
12	2.0	1	27.75
13	2.0	1	25.25
14	2.0	1	25.00
15	2.0	3	17.00
16	-	-	15.50
17	-	-	15.00
Died resp. infect.			

Total Lyco. = 20.0 ml.
Mean Basal R.V.P. = 17.5 cm.
Gen. E. = ++
LV/RV = 2.83 (H)
Gen. I. = +++
Final R.V.P. = N.

TABLE 17. - Rabbit No. RO/31

Time (wks.)	IV. Lycopodium (ml. 1% w/v suspension)	Interval between Injection & Pressure Reading (days)	Mean R.V. Pressure (cm. of water)
0	-	-	16.00
1	-	-	14.00
2	-	-	16.75
3	-	-	16.75
4	-	-	16.25
5	-	-	17.00
6	-	-	16.25
7	2.0	1	17.00
8	3.0	1	18.75
9	3.0	1	17.75
10	2.0	1	18.50
11	2.0	1	19.25
12	2.0	1	22.00
13	2.0	1	22.75
14	2.0	1	24.25
15	2.0	3	18.00
16	-	-	18.75
17	-	-	17.75
18	2.0	acute	-
19 to 26	Rest		
27	-	-	18.00
28	-	-	16.25
29	-	-	17.25
30	-	-	17.00
31	-	-	16.00
32	-	-	15.50
33	-	-	16.00
34	-	-	16.00
35	2.0	acute	-
36	-	-	-
37	-	-	16.00
38	3.0	acute	-
39	-	-	16.00
40	3.0	acute	-
41	-	-	-
42	-	-	16.25
43	2.0	4	15.75
44	2.5	3	15.75
45	2.5	3	15.75
46	2.5	3	15.75
47	2.5	2	15.50
48	2.5	2	16.00
49	2.5	2	16.50
50	2.5	1	18.50
51	2.5	2	16.50
52	2.5	1	16.25
53	2.5	1	16.50
54	2.5	1	16.25
55	2.5	1	16.75
56	2.5	1	16.50
57	2.5	1	17.50
58	2.5	1	16.50
59	2.5	1	17.75
60 to 74	Rest		
75	-	-	17.50
76	-	-	16.75
77	-	-	18.00

Killed: week 84

Total Lyco. = 72.0 ml.
Mean Basal R.V.P. = 16.1 cm.
Gen. E. = +++
LV/RV = 3.60 (D)
Gen. I. = +
Final R.V.P. = D.

TABLE 18

Rabbit No. RO/37

Time (wks.)	IV. Lycopodium (ml. 1% w/v suspension)	Interval between Injection & Pressure Reading (days)	Mean R.V. Pressure (cm. of water)
0	-	-	15.25
1	-	-	15.50
2	-	-	15.75
3	-	-	14.75
4	-	-	15.50
5	-	-	15.50
6	-	-	15.00
7	-	-	16.50
8	-	-	-
9	-	-	17.00
10	3.0	acute	-
11)			
12)	No press.		
13)			
14	-	-	17.00
15	1.0	-	-
Died after injection			

Total Lyco. = 4.0 ml.
Mean Basal R.V.P. = 15.6 cm.
Gen. E. = 0
LV/RV = 3.83 (N)
Gen. I. = 0
Final R.V.P. = D.

TABLE 19 - continued

Total Lyco. = 22.0 ml.
Mean Basal R.V.P. = 17.3 cm.
Gen. E. = +++
LV/RV = 2.19 (H)
Gen. I. = ++
Final R.V.P. = R.

TABLE 19

Rabbit No. RO/33

Time (wks.)	I.V. Lycopodium (ml. 1% w/v suspension)	Interval between Injection & Pressure Reading (days)	Mean R.V. Pressure (cm. of water)
0	-	-	17.0
1	-	-	15.5
2	-	-	17.25
3	-	-	19.5
4	-	-	16.5
5	-	-	16.5
6	-	-	18.5
7	2.0	1	19.0
8	3.0	1	18.0
9	3.0	2	21.5
10	3.0	1	22.0
11	3.0	1	25.0
12	2.0	1	24.25
13	2.0	1	25.0
14	1.5	1	29.75
15	1.0	3	30.75
16	-	-	30.5
17	-	-	27.5
18)			
to)	-	-	-
26)			
27	-	-	23.5
28	-	-	23.25
29	-	-	23.5
30	-	-	23.5
31	-	-	-
32	-	-	21.0
33	-	-	27.0
34	-	-	25.5
35	-	-	-
36	-	-	-
37	1.5	acute	-
38	-	7	40.5
39	-	-	-
40	-	-	-
41	-	-	38.0
42	1.5	-	-
Died after injection			

APPENDIX D

PROTOCOLS OF ACUTE EXPERIMENTS

TABLE 20 - Rabbit No. RO/40

Acute Experiments A/1,2,3 & 4

Time (Mins.)	Mean R.V. Pressure (cm.water)	Detail
Expt. A/1. Performed in week 7.		
-1.0	17.0	) 3 ml.
-0.75	17.0	) diluent
-0.50	16.75	) I.V.
-0.25	16.5	
0	16.5	) 1.5 ml.
.25	16.0	) C.B.
.5	15.75	) I.V.
.75	15.75	)
1.0	15.75	)
1.25	16.00	)
1.5	16.00	)
1.75	16.5	
2.0	17.0	
3.0	18.0	
-	-	Manometer
-	-	needle clotted
4.5	18.0	
5.0	18.0	) 0.75 ml.
5.25	17.5	) C.B.
5.5	18.5	) I.V.
5.75	18.5	
6.0	18.0	
6.5	18.0	
7.0	17.0	
7.5	16.0	
8.0	16.0	
68.0	15.5	
Expt. A/2. Performed in week 9.		
-1.0	16.5	) 3.0 ml.
-0.75	16.75	) dil.I.V.
-0.5	16.5	
-0.25	16.5	
0	16.5	) 2.5 ml.
.25	19.5	) C.B.
.5	24.0	) I.V.
.75	24.5	)
1.0	30.0	
1.25	32.5	
1.5	30.5	
1.75	28.0	
2.0	24.5	
2.25	24.25	
2.5	24.25	
2.75	24.25	
3.0	24.0	
3.25	23.5	
3.5	22.5	
3.75	21.5	
4.0	18.0	
4.25	16.0	
4.5	16.25	
Expt. A/3. Performed in week 11		
-1.0	17.5	) 3.0 ml.
-0.75	17.5	) diluent
-0.5	18.0	) I.V.
-0.25	17.5	
0	17.5	) 2.0 ml.
.25	20.0	) C.B.
.5	30.0	) I.V.
.75	35.0	
1.0	32.0	
1.25	30.0	
1.5	28.0	
1.75	27.0	
2.0	27.0	
2.25	27.0	
2.5	27.0	
2.75	26.5	
3.0	26.0	
3.25	25.5	
3.5	25.0	
3.75	24.5	
4.0	24.0	
4.25	23.25	
4.5	22.75	
4.75	22.25	
5.0	21.75	
5.25	21.50	
5.5	21.50	
5.75	21.0	
6.0	21.0	
6.25	20.5	
6.5	needle clotted.	
Expt. A/4. Performed in week 58.		
-0.5	18.5	
-0.25	18.5	
0	18.5	) 3.0 ml.
.25	19.5	) dil. I.V.
.5	18.75	
.75	18.25	) 3ml.
1.0	19.5	) dil.I.V.
1.25	19.25	
1.5	18.5	
1.75	18.25	
2.0	18.25	
2.25	18.5	
Killed		

TABLE 21 - Rabbit No. RO/44

Acute Experiments A/1, 2, 3, 4 & 5.

Time (mins.)	Mean R.V. Pressure (cm.water)	Detail
Expt. A/1. Performed in week 7.		
-1.0	16.75	2.0 ml.
-0.75	17.0	diluent
-0.5	16.75	
-0.25	16.75	
0	16.5	
.25	15.0	2.0 ml.
.5	16.0	C.B.
.75	16.75	I.V.
1.0	17.0	
1.25	18.0	
1.5	19.0	
1.75	19.25	
2.0	19.5	
2.25	19.5	
2.5	18.0	
2.75	17.5	
3.0	17.5	
3.25	17.25	
3.5	17.0	
3.75	17.0	
4.0	16.75	
4.25	16.5	
4.5	16.5	
4.75	16.25	
5.0	16.25	
65.0	16.75	
Expt. A/2. Performed in week 9.		
-1.0	16.5	3.0 ml.
-0.75	16.5	diluent
-0.5	16.5	I.V.
-0.25	16.5	
0	16.5	
.25	16.5	2.5 ml.
.5	18.0	C.B.
.75	20.0	I.V.
1.0	24.0	
1.25	24.0	
1.5	21.75	
1.75	20.5	
2.0	20.0	
2.25	19.0	
2.5	18.5	
2.75	18.25	
3.0	18.0	
3.25	17.5	
3.5	17.5	
3.75	17.0	
4.0	17.0	
4.25	16.75	
4.5	16.75	
4.75	16.5	
Expt. A/3. Performed in week 11.		
-1.0	16.5	3.0 ml.
-0.75	16.5	diluent
-0.5	16.5	I.V.
-0.25	16.5	
0	16.5	3.0 ml.
.25	18.0	C.B.
.5	23.0	I.V.
.75	22.0	
1.0	20.75	
1.25	20.25	
1.5	19.5	
1.75	18.75	
2.0	18.75	
2.25	18.25	
2.5	17.5	
2.75	17.25	
3.0	17.25	
3.25	17.25	
3.5	17.0	
3.75	16.5	
4.0	16.5	
4.25	16.25	
Expt. A/4. Performed in week 57		
-0.5	17.0	
-0.25	17.0	
0	17.0	3.0ml.
.75	18.25	dil.I.V.
.5	18.0	
.75	17.5	
1.0	17.5	3.0 ml.
1.25	18.25	dil. I.V.
1.5	18.25	
1.75	18.0	
2.0	17.5	
2.25	17.25	
2.5	17.0	
2.75	17.0	
Expt. A/5. Performed in week 58		
-0.5	17.0	
-0.25	17.0	
0	17.0	3.0 ml.
.25	17.75	dil. I.V.
.5	17.5	
.75	17.5	
1.0	17.0	3.0 ml.
1.25	17.5	dil.I.V.
1.5	17.25	
1.75	17.0	
2.0	17.0	
2.25	16.75	
2.5	16.75	
Killed		

TABLE 22 - Rabbit No. RO/31

Acute Experiments A/1, 2, 3, 4, 5 & 6

Time (mins.)	Mean R.V. Pressure (cm.water)	Detail
Expt. A/1. Performed in week 18		
0	-	)2 ml.
1	-	)1%Lyco
60	19.0	
Expt. A/2. Performed in week 35		
-0.75	16.0	)2 ml.
-0.5	16.0	) dil.I.V.
-0.25	16.0	
0	16.0	)
.25	16.0	)
.5	16.5	) 2 ml.
.75	16.5	) Lyco.
1.0	16.75	) I.V.
1.25	17.0	)
1.5	17.0	)
1.75	17.25	
2.0	17.25	
2.25	17.25	
2.5	17.0	
2.75	17.0	
3.0	17.0	
3.5	16.75	
4.0	16.75	
4.5	16.5	
5.0	16.5	
6.0	16.25	
Expt. A/3. Performed in week 38		
-1.0	16.0	)
-0.75	16.0	) 3 ml.
-0.5	15.75	) Dil.I.V.
-0.25	16.0	
0	16.0	)
.25	16.0	)3 ml.
.5	16.5	)Lyco.
.75	17.5	)I.V.
1.0	18.5	
1.25	19.0	
1.5	19.0	
1.75	18.75	
2.0	18.75	
2.25	18.5	
2.5	18.5	
2.75	18.25	
3.0	18.5	
3.25	18.5	
3.5	18.5	
3.75	18.5	
4.0	18.25	
4.25	18.5	
4.5	18.0	
4.75	18.0	
5.0	18.0	
5.25	Manometer needle clotted	
Expt. A/4. Performed in week 40		
-1.0	16.0	)3 ml.
-0.75	16.0	)dil.
-0.5	16.0	)I.V.
-0.25	16.0	
0	16.0	)3 ml.
.25	19.5	)Lyco.
.5	20.5	)I.V.
.75	22.5	
1.0	24.5	
1.25	25.0	
1.5	25.5	
1.75	25.0	
2.0	25.0	
2.25	24.5	
2.5	24.5	
2.75	24.5	
3.0	24.25	
3.25	24.0	
3.5	24.0	
3.75	24.0	
4.0	24.0	
4.25	24.0	
4.5	23.75	
4.75	24.0	
5.0	24.0	
5.5	24.0	
60.0	17.5	
Expt. A/5. Performed in week 83		
-0.5	18.0	
-0.25	18.0	
0	18.0	)3 ml.dil.
.25	18.25	) I.V.
.5	17.75	
.75	17.25	)3 ml. dil.
1.0	17.25	) I.V.
1.25	17.25	
1.5	17.5	
1.75	17.0	
Expt. A/6. Performed in week 84		
-0.5	18.75	
-0.25	18.75	
0	18.75	) 3 ml.dil.
.25	18.75	) I.V.
.5	18.75	
.75	18.75	) 3 ml.dil.
1.0	18.75	) I.V.
1.25	18.75	
1.5	18.75	
1.75	18.75	
Killed		

TABLE 23 - Rabbit No. RO/20

Acute Experiments A/1, 2, 3, 4 and 5

Time (mins.)	Mean R.V. Pressure (cm.water)	Detail
Expt. A/1. Performed in week 37.		
0	-	)3 ml.
1.0	-	)C.B.,I.V.
60	21.5	
Expt. A/2. Performed in week 54		
-1.0	21.0	)2 ml.dil.
-0.75	21.25	) I.V.
-0.5	21.5	
-0.25	21.0	
0	21.0	)
.25	22.0	) 3 ml.
.5		) C.B.
.75		) I.V.
1.0		)
Animal struggled Needle dislodged		
Expt. A/3. Performed in week 56.		
-1.0	18.0	)3 ml.
-0.75	18.0	) dil.
-0.5	17.5	) I.V.
-0.25	17.5	
0	17.5	)
.25	17.5	)3 ml.
.5	18.5	) C.B.
.75	18.5	) I.V.
1.0	20.0	)
Needle blocked during injection. Discontinued		
Expt. A/4. Performed in week 58.		
-1.0	20.25)	3 ml.
-0.75	20.25)	dil.
-0.5	20.75)	I.V.
-0.25	20.25	
0	20.25)	3 ml.
.25	22.5)	C.B.
.5	26.0)	I.V.
.75	32.0	
1.0	31.0	
1.25	30.0	
1.5	30.25	
1.75	30.0	
2.0	30.25	
2.25	30.25	
2.5	30.5	
2.75	30.25	
3.0	30.0	
3.25	30.0	
3.5	29.75	
3.75	29.5	
4.0	29.0	
4.25	28.75	
4.5	28.0	
4.75	-	Struggle
5.0	23.0	
5.25	-	Struggle
60.0	20.0	
Expt. A/5. Performed in week 103.		
-0.5	23.5	
-0.25	23.5	
0	23.5	) 3 ml. dil.
.25	23.75)	I.V.
.5	24.0)	
.75	24.0	
1.0	23.5	) 3 ml. dil
1.25	23.5	) I.V.
1.5	23.5	
1.75	23.5	
2.0	23.5	
Killed		

TABLE 24 - Rabbit No. RO/58

Acute Experiment No. A/1.

Time (mins.)	Mean R.V. Pressure (cm. of water)	Detail
-1.0	16.0	3.0 ml. diluent I.V.
-0.75	16.0	
-0.5	15.5	
-0.25	17.0	
0	17.0	1.5 ml. C.B. I.V.
.25	22.5	
.5	28.0	
.75	24.5	
1.0	22.5	
1.25	22.0	
1.5	21.5	
1.75	23.0	
2.0	22.75	
2.25	22.0	
2.5	23.0	
2.75	22.5	
3.0	21.5	
3.25	21.0	
3.5	21.5	
3.75	21.0	
4.0	20.5	
4.25	20.75	
4.5	21.0	
4.75	19.75	
5.0	20.0	
5.25	19.5	
5.5	19.0	
5.75	18.75	
6.0	18.5	
6.25	18.0	
6.5	17.75	
6.75	18.0	
7.0	18.0	
7.25	18.0	
7.5	17.75	
7.75	17.0	
8.0	17.0	
8.25	16.5	
8.5	16.5	

TABLE 25 - Rabbit No. RO/55

Acute Experiment A/1

Time (mins.)	Mean R.V. Pressure (cm. of water)	Detail
-1.0	14.0	) 3.0 ml.
-0.75	14.25	) diluent
-0.5	14.75	) I.V.
-0.25	14.25	
0	14.25	) 1.5 ml. C.B.
.25	20.0	) I.V.
.5	28.0	
.75	27.0	
1.0	34.0	
1.25	30.0	
1.5	28.5	
1.75	28.0	
2.0	28.0	
2.25	27.5	
2.5	28.0	
2.75	27.0	
3.0	26.5	
3.25	25.5	
3.5	Manometer needle clotted	
5.0	21.5	
5.25	19.0	
5.5	18.5	
5.75	19.0	
6.0	18.75	
6.25	18.0	
6.5	16.0	
6.75	17.25	
7.0	17.25	
7.25	17.0	
7.5	17.0	
7.75	16.5	
8.0	15.75	

TABLE 26 - Rabbit No. RO/37

Acute Experiment A/1
Performed in Wk.10

Time (mins.)	Mean R.V. Pressure (cm. of water)	Detail
-1.0	17.0	3 ml. diluent I.V.
-0.75	17.0	
-0.5	17.0	
-0.25	17.0	
0	17.0	3.0 ml. Lyco. I.V.
.25	18.0	
.5	19.5	
.75	20.0	
1.0	19.75	
1.25	19.25	
1.5	19.5	
1.75	19.0	
2.0	18.75	
2.25	18.25	
2.5	18.0	
2.75	18.0	
3.0	18.25	
3.25	17.75	
3.5	17.5	
3.75	17.75	
4.0	17.0	
4.25	17.0	

TABLE 27 - Rabbit No. RO/39

Acute Experiments A/1,2, & 3

Time (mins.)	Mean R.V. Pressure (cm.water)	Detail
Expt.No.A/1. Performed in week 11.		
-1.0	15.0	) 3.0 ml.
-0.75	15.25	) dil. I.V.
-0.5	15.0	
-0.25	15.0	
0	15.0	) 3.0 ml.
.25	17.0	) Lyco.
.5	19.0	) I.V.
.75	19.25	
1.0	19.0	
1.25	19.25	
1.5	18.5	
1.75	17.75	
2.0	18.5	
2.25	18.25	
2.5	18.0	
2.75	18.0	
3.0	17.75	
3.25	17.75	
3.5	17.75	
3.75	17.75	
4.0	17.75	
4.25	17.25	
4.5	17.0	
4.75	17.0	
5.0	17.0	
5.25	17.0	
5.5	17.0	
5.75	17.25	
6.0	17.25	
6.25	17.0	
6.5	17.0	
90.0	17.0	
Expt. No.A/2. Performed in week 58		
-0.5	16.25	
-0.25	16.25	
0	16.25	) 3.0 ml.
.25	16.75	) dil. I.V.
.5	16.75	
.75	16.5	
1.0	16.0	) 3.0 ml.
1.25	17.0	) dil. I.V.
1.5	16.5	
1.75	16.25	
2.0	16.0	
2.25	15.75	
2.5	15.75	
Expt. No. A/3. Performed in week 59		
-0.5	16.0	
-0.25	16.0	
0	16.0	) 3.0 ml.
.25	16.5	) dil. I.V.
.5	17.0	
.75	18.0	
1.0	17.0	) 3.0 ml.
1.25	18.0	) dil. I.V.
1.5	17.5	
1.75	17.0	
2.0	16.75	
2.25	17.0	
2.5	16.75	
Killed		

TABLE 28 - Rabbit No. RO/41

Acute Experiment A/1

Performed in week 59

Time (mins.)	Mean R.V. Pressure (cm.water)	Detail
-1.0	16.75	) 3.0ml.
-0.75	17.25	) dil.I.V.
-0.5	17.25	
-0.25	16.75	
0	16.75	) 5.0 ml.
.25	18.0	) Lyco.
.5	21.0	) I.V.
.75	22.0	
1.0	22.5	
1.25	22.5	
1.5	22.5	
1.75	22.0	
2.0	21.75	
2.25	21.5	
2.5	21.5	
2.75	21.25	
3.0	21.0	
3.25	21.0	
3.5	20.75	
3.75	20.5	
4.0	20.25	
4.25	20.25	
4.5	20.0	
4.75	19.75	
5.0	19.75	
5.25	19.5	
5.5	19.25	
5.75	19.25	
6.0	19.0	
6.25	19.0	
6.5	19.0	
6.75	19.0	
7.0	18.75	
7.25	19.25	
7.5	18.75	
7.75	18.75	
8.0	18.75	
8.25	18.5	
8.5	18.25	
8.75	18.25	
9.0	18.5	
9.25	18.25	
9.5	18.0	
9.75	17.75	
10.0	17.5	
10.25	17.5	
10.5	17.25	
10.75	17.25	
11.0	17	
11.25	16.75	
11.5	16.75	
11.75	17.0	
12.0	17.0	
	Killed	

TABLE 29 - Rabbit No. RO/59

Acute Experiment A/1

Time (mins.)	Mean R.V.Pressure (cm. of water)	Detail
-1.0	14.5	) 3.0 ml.
-0.75	14.5	) diluent
-0.5	14.75	) I.V.
-0.25	14.5	
0	14.5	) 6.0 ml.
.25	15	) Lyco.
.5	20	) I.V.
.75	23.5	
1.0	21.25	
1.25	20.25	
1.5	19.75	
1.75	18.5	
2.0	17.5	
2.25	17.5	
2.5	21.0	
2.75	20.0	
3.0	19.0	
3.25	18.5	
3.5	17.5	
3.75	19.0	
4.0	18.5	
4.25	18.75	
4.5	19.5	
4.75	19.0	
5.0	19.0	
5.25	19.0	
5.5	18.75	
5.75	19.0	
6.0	18.5	
6.25	18.0	
6.5	17.5	
6.75	17.5	
7.0	17.0	
7.25	17.5	
7.5	16.5	
8.0	16.5	
8.25	17.0	
8.5	17.0	
8.75	17.0	
9.0	17.0	
9.25	16.5	
9.5	16.75	
9.75	16.75	
10.0	16.5	

TABLE 39 - Rabbit No. RC/38

Acute Experiments No.A/1.2 & 3

Time (mins.)	Mean R.V. Pressure (cm.water)	Detail
Expt. A/1. Performed in week 57		
-0.5	15.0	
-0.25	15.0	
0	15.0	) 3 ml.
.25	16.0	) dil.I.V.
.5	16.5	
.75	15.0	
1.0	14.5	)3 ml.
1.25	14.5	)dil.I.V.
1.5	15.0	
1.75	15.5	
2.0	15.5	
Expt. A/2. Performed in week 58		
-0.5	17.5	
-0.25	17.5	
0	17.5	) 3ml.
.25	17.5)	) dil.I.V.
.5	17.5	
.75	17.5	) 3ml.
1.0	18.0	)dil.I.V.
1.25	18.5	
1.5	17.5	
1.75	17.5	
2.0	17.0	
2.25	17.0	

Time (mins.)	Mean R.V. Pressure (cm.water)	Detail
Expt. A/3. Performed in week 59		
-1.0	15.0	) 3 ml.
-0.75	15.5	) dil.I.V.
-0.5	15.25	
-0.25	15.25	
0	15.25	) 3 ml.
.25	15.5	)Lyco.I.V.
.5	23.5	
.75	16.75	
1.0	17.25	
1.25	17.25	
1.5	17.25	
1.75	16.5	
2.0	16.5	
2.25	16.5	
2.5	16.5	
2.75	16.25	
3.0	16.5	
3.25	16.75	
3.5	17.0	
3.75	17.0	
4.0	16.5	
	Killed	

TABLE 31 - Rabbit No. RO/25

Acute Experiments A/1,2,3,4 & 5

Time (mins.)	Mean R.V. Pressure (cm.water)	Detail
Expt. A/1. Performed in week 33		
0	-	)3ml.
1.0	-	)C.B.I.V.
60.0	19.25	
Expt. A/2. Performed in week 52		
-1.5	18.0	)
-1.25	18.0	) 3 ml.
-1.0	18.0	) dil.
-0.75	18.0	) I.V.
-0.5	18.0	)
-0.25	18.0	
0	18.0	)
.25	18.0	) 3 ml.
.5	18.5	) C.B.
.75	20.0	) I.V.
1.0	23.5	)
1.25	26.0	
1.5	26.5	
1.75	27.5	
2.0	28.0	
2.25	27.75	
2.5	27.75	
2.75	26.5	
3.0	21.5	
3.25	19.5	
Expt. A/3. Performed in week 54		
-1.0	18.5	) 3 ml.
-0.75	18.5	) dil.
-0.5	18.5	) I.V.
-0.25	18.5	
0	18.5	) 3 ml.
.25	19.0	) C.B.
.5	20.5	) I.V.
.75	21.5	
1.0	21.0	
1.25	21.0	
1.5	21.25	
1.75	21.25	
2.0	20.5	
2.25	20.5	
2.5	20.25	
2.75	20.25	
3.0	20.0	
3.25	20.0	
3.5	20.0	
3.75	19.75	
4.0	19.75	
4.25	19.5	
4.5	19.5	
4.75	19.25	
5.0	19.0	
Expt. A/4. Performed in week 98		
-0.5	20.0	
-0.25	20.0	
0	20.0	) 3 ml.
.25	20.25	)dil.I.V.
.5	20.0	
.75	20.0	) 3 ml.
1.0	20.25	)Dil.I.V.
1.25	21.0	
1.5	20.5	
1.75	20.25	
Expt. A/5. Performed in week 100		
-0.5	20.5	
-0.25	20.5	
0	20.5	) 3 ml.
.25	21.5	)dil.I.V.
.5	21.0	
.75	20.5	
1.0	20.5	)3 ml.
1.25	21.5	)dil.I.V.
1.5	21.5	
1.75	22.0	
2.0	21.0	
2.25	20.5	
2.5	20.25	

TABLE 32 - Rabbit No. RO/33

Acute Experiment A/1.

Performed in week 37.

Time (mins.)	Mean R.V. Pressure (cm. of water)	Detail
-1.0	22.0	3 ml. dil. I.V.
-0.75	22.0	
-0.5	19.5	
-0.25	22.0	
0	22.0	
.25	25.0	1.5 ml. Lyco. I.V.
.5	27.0	
.75	28.0	
1.0	31.0	
1.25	36.5	
1.5	33.5	
1.75	30.5	
2.0	30.0	
2.25	31.0	
2.5	31.0	
2.75	29.0	
3.0	25.0	
3.25	24.0	
3.5	23.5	
3.75	23.0	
4.0	Manometer needle clotted	

TABLE 33 - Rabbit No. RO/42

Acute Experiments A/1, 2, 3 & 4.

Time (mins.)	Mean R.V. Pressure (cm.water)	Detail
Expt. A/1. Performed in week 9.		
-1.0	16.5	) 2 ml.
-0.75	17.0	) dil.I.V.
-0.5	17.25	
-0.25	17.0	
0	17.0	)
.25	17.5	)
.5	18.0	) 2 ml.
.75	18.0	) C.B.
1.0	18.0	) I.V.
1.25	19.0	)
1.5	Manometer needle clotted during injection	
Expt. A/2. Performed in week 11.		
-1.0	16.0	)
-0.75	16.0	) 3 ml.
-0.5	16.0	) dil.I.V.
-0.25	16.0	
0	16.0	) 2 ml.
.25	18.5	) C.B.
.5	24.0	) I.V.
.75	30.0	)
1.0	33.0	
1.25	29.0	
1.5	28.0	
1.75	27.5	
2.0	27.75	
2.25	28.25	
2.5	28.0	
2.75	28.25	
3.0	28.0	
3.25	27.25	
3.5	26.5	
3.75	25.25	
4.0	24.0	
4.25	24.25	
	Manometer needle clotted	
60.0	16.5	

Time (mins.)	Mean R.V. Pressure (cm.water)	Detail
Expt. A/3. Performed in week 13.		
-1.0	17.5	) 3 ml.
-0.75	18.0	) dil.
-0.5	17.5	) I.V.
-0.25	17.5	
0	17.5	) 3 ml.
.25	21.5	) C.B.
.5	27.0	) I.V.
.75	32.5	
1.0	31.5	
1.25	Manometer needle clotted	
Expt. A/4. Performed in week 15.		
-1.0	17.5	) 3 ml.
-0.75	18.25	) dil.
-0.5	18.25	) I.V.
-0.25	17.5	
0	17.5	) 3 ml.
.25	19.5	) C.B.
.5	30.0	) I.V.
.75	38.0	
1.0	31.5	
1.25	31.5	
1.5	28.0	
1.75	27.5	
2.0	27.75	Cardiac arrest

TABLE 34 - Rabbit No. RO/53

Acute Experiment No. A/1

Time (mins.)	Mean R.V.Pressure (cm. of water)	Detail
-1.0	16.0	) 3 ml. dil.
-0.75	17.0	) I.V.
-0.5	17.0	
-0.25	16.0	
0	15.5	) 3 ml.
.25	16.0	) C.B. I.V.
.5	25.0	
.75	50.0	
1.0	27.0	
1.25	22.0	
1.5	20.25	
1.75	Cardiac arrest	

TABLE 35

LONG TERM EXPERIMENTS

(Details of Basal and Final R.V. Pressures, LV/RV Ratios and Generalised Emphysema)

Serial No.	Substance Injected	Mean Basal R.V. Pressure (cm. water)	Final Level of R.V. Pressure (N, D or R)	LV/RV Ratio	State of R.V. (N, D or H)	Degree of General Emphysema
RO/24)	nil	15.2	-	3.90	N	0
26)		15.6	-	3.65	N	0
38)	nil	15.7	N	3.65	N	+ +
41)		16.3	N	4.28	N	0
20)	C.B.	21.5	N	3.99	N	+ + +
5)		18.1	R	2.69	H	+
14)		14.8	R	3.37	D	0
18)		18.6	R	2.13	H	0
16)		18.5	N	3.34	D	+ +
40)		16.2	N	3.95	N	+ +
44)		16.6	N	3.69	N	+
25)		18.2	N	3.05	H	+ + +
28)		19.1	D	4.10	N	0
42)		15.5	D	2.16	H	0
39)	Lyco.	16.0	N	3.48	D	+
30)		19.4	R	2.66	H	0
34)		20.3	R	3.21	D	0
35)		17.5	N	2.83	H	+ +
31)		16.1	D	3.60	D	+ + +
37)		15.6	D	3.83	N	0
33)		17.3	R	2.19	H	+ + +

Average of Mean Basal R.V. Pressure = 17.1 cm.water.