STUDIES ON

ACUTE HYPERBARIC PULMONARY OXYGEN TOXICITY

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by

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TABLE OF CONTENTS

SECTION 3

DOG EXPERIMENTS SERIES 1

PATHOPHYSIOLOGICAL STUDY AT 2 ATA

SECTION 4

ELECTRON MICROSCOPY AND STEREOMETRIC ANALYSIS

SECTION 6

INNERVATION OF THE LUNG

SECTION 7

CAT EXPERIMENTS

SYMPATHETICS AND SURFACTANT

SECTION 8

DOG EXPERIMENTS SERIES 3

PATHOPHYSIOLOGICAL STUDY AT 2 ATA

EFFECTS OF ADRENERGIC BLOCKADE

SECTION 9

DOG EXPERIMENTS SERIES 4

PATHOPHYSIOLOGICAL STUDY AT 2 ATA

OXYGEN TOXICITY AND VAGOTOMY

SECTION 10

DISCUSSION AND CONCLUSIONS

LIST OF FIGURES AND ILLUSTRATIONS

1. TABLES

2. LINE DRAWINGS AND GRAPHS

3. PHOTOGRAPHIC ILLUSTRATIONS

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By far the greater part of the work described in this thesis was performed in the Department of Surgery at the Western Infirmary, Glasgow, with which regrettably I had no formal attachment. Dr. Iain Ledingham not only allowed me full access to the laboratories and pressure chambers, but also provided technical assistance from his own staff to cover the experiments. His encyclopaedic knowledge of things hyperbaric was always at my disposal and his enthusiasm for the subject provided a continual impetus. A long-continuing collaboration is happily anticipated.

The majority of the experiments to be described lasted well in excess of 24 hours, and their conduct would have been impossible without the highly skilled assistance of the team of technicians at the Hyperbaric Unit.

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Finally, the last word of thanks must be for my sister, Helen Mackenzie, who has been forced to type this thesis (despite all my good intentions to the contrary) very much at the last moment.

Thank you.

STATEMENT OF COLLABORATION

Dr. Iain Ledingham, Consultant Clinical Physiologist, and Dr. Graham Smith, Senior Lecturer in Anaesthetics, in the Western Infirmary Departments of Surgery and Anaesthesia in the University of Glasgow, introduced me to the dog experimental model.

Graham Smith taught me the necessary surgical techniques and advised and assisted in the setting up of the first few animals. He is also the co-author of the review article reproduced as Appendix 1 to this thesis.

The acknowledgements (pages xii and xiii) indicate the extent to which I have relied on other people. Nevertheless, the ideas presented and developed in this thesis, the conduct of the experiments, the extraction, analysis, interpretation and presentation of results, and the discussions were entirely my own work, and were carried out without collaboration with any other person.

A preliminary report of the findings described in Section 3 was presented at the Anaesthetic Research Society Meeting at Manchester on 21st March, 1975, and is published as an abstract in the British Journal of Anaesthesia:

Shields, T.G., Smith, G. and Ledingham, I.McA. (1975). Mechanisms of oxygen toxicity. Br. J. Anaesth. 47, 904.

STATEMENT OF COPYRIGHT

SUMMARY

A pathophysiological study was made of spontaneously breathing dogs anaesthetised by a neurolept-analgesic technique and exposed to 100% oxygen at 2 ATA to demonstrate the time-course and mechanism of response. The animals remained apparently normal for some 18 hours, following which the majority developed a fulminating intra-alveolar oedema and died of hypoxaemia within a few hours. There was no evidence of systemic nor pulmonary capillary hypertension, and electron microscopy demonstrated complete absence of damage to the endothelial and Type 1 epithelial cells of the alveolar septum. Changes were detected in the Type 2 cells, and oedemagenesis was attributed to an oxygen-induced depression of surfactant activity.

Further experiments on similar animals showed that adrenergic neuronal blockade protected against oedema formation without any effect on survival time. The protection was considered to be due to a pulmonary arterial hypotension which followed blockade, rather than to any modification of surfactant secretion.

Differential intubation of the two main bronchi allowed dogs under similar conditions to breathe oxygen and air at 2 ATA on alternate lungs and showed that, at these pressures, the effect on the lungs was direct, providing further evidence for the lack of involvement of the sympathetic nervous system. A possible synergistic effect of ipsilateral vagotomy on such animals was also demonstrated.

Ancillary experiments demonstrated the presence of fine adrenergic motor nerve fibres deep in the lung parenchyma and terminating close to Type 2 pneumonocytes. Electrical stimulation of the stellate ganglion in closed-chest pithed cats, however, failed to produce any change which could be attributed to disturbances of the

surfactant system.

A general review of pulmonary oxygen toxicity, and of surfactant in relation to pulmonary oedema is included.

UNITS OF PRESSURE AND ABBREVIATIONS USED

The modern practice of using S.I.units was not followed in this thesis: one of the purposes of a thesis is to impart information, and with parts of the diving world still struggling with the conversion of feet to metres of sea water as a unit of pressure, the step to kilopascals seemed just too great.

The units of pressure used are: atmospheres absolute (ATA) for ambient pressures; torr for tensions of dissolved gases in liquids; and mm.Hg. or cms.H_2O as appropriate for hydrostatic pressures.

A list of conversion factors to other commonly used units is given below:

1 ATA* = 33.05 feet sea water
10.33 metres sea water
14.696 lbs/sq. in.
1.033 Kg/sq. cm.
760 mm.Hg. (torr)
1.013 bars
101325 Newtons/sq. metre
101 kilopascals

* defined as a standard atmosphere, equal to the pressure exerted by a column of mercury 760 mm. high at a temperature where the density of mercury is 13.5951 g/cm^3.

The respiratory symbols and abbreviations used are those recommended by the Pappenheimer committee (Pappenheimer, J. (1950). Standardisation of definitions and symbols in respiratory physiology. Fed. Proc. 9, 602-605); they are now universally accepted and will not be detailed here.

Several less well recognised abbreviations have been used, particularly in the tables:

B.G.F.	Blood gas factor
C.	Compliance
C.V.P.	Central venous pressure
Hb.	Haemoglobin
Hct.	Haematocrit
H.R.	Heart rate
O.H.P.	Oxygen at high pressure
P.A.P.	Pulmonary arterial pressure
P.A.P. wedged	Pulmonary arterial wedged pressure
P.V.R.	Pulmonary vascular resistance
Q_S/Q_T	Percentage pulmonary shunt ratio
Q_T	Cardiac output
R.	Respiratory exchange ratio
S.B.P.	Systemic blood pressure
S.D.	Standard deviation
S.E.	Standard error of the mean
W/D ratio	Lung wet/dry weight ratio

Stereometry abbreviations are defined on Page 4:12 et seq.

FOREWORD

'From the greater strength and vivacity of the flame of a candle, in this pure air, it may be conjectured, that it might be peculiarly salutary to the lungs in certain morbid cases, when the common air would not be sufficient to carry off the phlogistic putrid effluvium fast enough. But perhaps, we may also infer from these experiments that though pure dephlogisticated air might be very useful as a medicine, it might not be proper for us in the usual healthy state of the body: for, as a candle burns out much faster in dephlogisticated than in common air, so we might, as may be said, live out too fast, and the animal powers be too soon exhausted in this pure kind of air.'

J. Priestley (1775)

Or, for those who would prefer to attribute the discovery of oxygen to Lavoisier rather than to Priestley:

'..... when there is an excess of vital air (oxygen) the animal only undergoes a severe illness; when it is lacking, death is almost instantaneous.'

A.L. Lavoisier (1782)

SECTION 1

INTRODUCTION

INTRODUCTION

Priestley's original communication to the Royal Society (Foreword) reveals that the awareness of possible toxic effects of oxygen is as old as the discovery of the gas itself.

Historical outline

The most extensive early research on oxygen poisoning was that described by Paul Bert in his monumental work, 'La Pression Barometrique' (1878) which, in the hyperbaric world, has attained the status of a classic. Bert was most interested in the central nervous system manifestations of oxygen toxicity - with which his name has become eponymous - but he also recognised effects on cardiac function and on the lungs.

The first published report referring specifically to pulmonary oxygen toxicity was not by J. Lorrain Smith as is popularly supposed, but by Thompson, who in 1889 described severe pulmonary congestion in a guinea-pig and a dog which had died following exposure to high pressures of oxygen. It is noted that the animals had convulsed and here in the first ever report in the literature was sown the seeds of controversy regarding the possible centri-neurogenic aetiology of pulmonary oxygen toxicity (see Sections 8 and 10).

To Smith, however, (1897, 1899) belongs the first detailed description of pulmonary pathology following oxygen exposure. The original text (Smith 1899) is worth quoting:

'The lungs were deeply congested and sank in the fixing fluid .... On microscopic examination, the tissues of the lungs showed intense congestion in the large and small blood vessels. The alveoli were to a great extent filled with an exudate, which was granular and fibrillated in appearance, but did not give the fibrin stain by Weigert's method, nor with eosin .... There were no leucocytes in the exudate. The pneumonic condition was universal, and could therefore be compared only with the earliest stages of croupous pneumonia. The exudate itself was probably the cause of the embarrassed respiration and the animal's death. It is inconceivable that with inflammation so extensive, the animal could have survived until the process had developed farther.'

These appearances are, of course, now known as the Lorrain Smith effect.

He also clearly demonstrated that the response was dose and time dependent; that the higher the pressure the less time the lungs were able to withstand the consequence of oxygen exposure. Carrying this further, he made a clear statement of the division of oxygen toxicity into its two major manifestations:

'The one consisting in the slowly developing inflammatory effect seen most prominently in the lung tissue. The other a rapidly developing effect on the nervous tissue, which we may in the meantime describe as functional ....'

One of his observations is of direct relevance to the work described in this thesis; that the lung changes in mice exposed to '170 to 180 percent of an atmosphere' (1.8 ATA) were such that

they would interfere with the uptake of oxygen (Smith 1897).

These historical aspects, and the large amount of experimental work carried out during the early decades of this century have been exhaustively reviewed by Bean (1945). Other more concise reviews covering the same period are those of Stadie, Riggs & Haugaard (1944), and Ohlsson (1947).

Present interest

Investigation into the mechanisms of pulmonary oxygen toxicity accelerated with the post-war revival of interest in hyperbaric oxygen therapy, and the 1950's and 60's contributed a large mass of data to the literature (Clark & Lambertsen, 1971).

Unfortunately the high expectations for hyperbaric therapy have not been fulfilled and it is now realised that its indications are limited. Almost concomitant with the decline of interest in hyperbaric oxygenation, however, came the growing use of high concentrations of oxygen at normobaric pressures in the treatment of patients by modern intensive care techniques, and along with this the awareness that oxygen is a dangerous drug (Bendixen, Egbert, Hedley-Whyte, Laver & Pontoppidan, 1965; Nash, Blennerhassett & Pontoppidan, 1967; Thomas & Hall, 1970; Green, 1970; Gould, Tosco, Wheelis, Gould & Kapanci, 1972; Woo & Hedley-Whyte, 1973; Editorials, 1974, 1975).

This interest in the possible dangers of a hyperoxic but normobaric, or even hypobaric environment has received added impetus over the past two decades with the vast amount of effort expended

on satellite and lunar space probes. There are many engineering design advantages in the use of a single gas at low pressure, and the practice has been, in the Western world at any rate, to use 100% oxygen at a pressure of 5 p.s.i. (0.34 ATA) in the capsule and 3.5 p.s.i. (0.24 ATA) in the pressure suit (Lambertsen, 1963).

Much of the continuing interest in oxygen toxicity research lies with the diving world and the recent very great expansion in diving, both commercial and recreational in this country, has provided an added stimulus.

It might be thought that oxygen toxicity is an academic rather than a practical hazard of diving. Compressed air divers (which category ought to include all amateur divers) must descend to 40 metres (5 ATA) before they are breathing the equivalent of pure oxygen on the surface, and duration at that depth is limited by inert gas considerations long before raised oxygen tension becomes a problem. In commercial diving practice using oxygen-helium mixtures, it is relatively simple to adjust the inspired oxygen concentration to ensure an oxygen partial pressure of below 0.5 bars at any depth.

There are, however, three diving situations where dangerously high oxygen tensions may be encountered.

In very deep diving, because the penalty of prolongation of decompression time increases with depth, 'bounce' diving is not cost-effective and it is almost standard practice to use saturation diving techniques where exposure to raised pressure lasts many days, or even weeks. To maintain a normal oxygen tension at great depths,

the fractional concentration of oxygen must be reduced to a very low level (e.g. to obtain a P_IO_2 of 150 torr at 300 metres, F_IO_2=0.677%) and the accurate measurement and control of these extremely small percentages becomes a very real problem (Miller, 1975).

Air saturation diving techniques are also used, mainly by scientific divers engaged on sea-bed surveys (MacInnes, 1975). These divers are of course at much shallower depths, but even so they are exposed to much higher pressures of oxygen than normal for very long periods of time.

The second circumstance is usually confined to the military situation, where the use of pure oxygen closed circuit breathing sets, by virtue of their extended duration and freedom from exhaust bubbles, allows the diver to proceed undetected in the clandestine role. Under normal working or swimming conditions, such divers in the Royal Navy are limited to a maximum depth of 8 metres (1.8 ATA).

The third situation is the increasing use over the past ten years of a minimal-recompression high-oxygen therapeutic regime in the treatment of acute decompression sickness (Goodman & Workman, 1965).

The standard treatment of this condition for very many years has been to return the injured diver to a fixed arbitrary depth (50 metres) on air, the rationale being that the bubble causing the symptoms will be compressed down to such a size as no longer to cause problems. The main objection to this form of treatment is that the patient is being subjected to an extra inert gas load at the very

time when, by definition, his inert gas exchange mechanisms have been damaged.

This disadvantage, together with the recent realisation that bubble formation per se is only a small part of the pathophysiology of decompression sickness has led to the idea of using pure oxygen at 3 ATA instead. In this way, a useful decrease in bubble size is still obtained (theoretically down to approximately one third of its initial volume), a much greater tissue-alveolar gas gradient for the elimination of nitrogen is obtained, and tissue hypoxia can be reversed.

A very great deal of research has been carried out on the effects of breathing 100% oxygen up to 1 ATA, and also at pressures above 3 ATA; the area in between, from 1 ATA to 3 ATA remains relatively poorly investigated, yet it is with this range of pressures that the latter two situations described above are concerned.

Organisation of the thesis

This thesis reports a study of the mechanisms involved in the development of pulmonary oxygen toxicity at a pressure of 2 ATA.

It consists of seven self-contained sections (Sections 3 to 9), each describing one group of experiments, and each with its own literature review and discussion. Section 10 is a general discussion summarising all the findings.

This presentation departs from convention in that there is no general introductory review of the literature. The most

recently published review of pulmonary oxygen toxicity was written by the author in conjunction with Dr. Graham Smith less than a year ago and is presented as Appendix 1 to the thesis.

Section 2 is a short review of two specific topics whose relevance become evident on further reading herein; the physiology of surfactant production, and the influence of surfactant on the formation of pulmonary oedema.

SECTION 2

REVIEW

1) SURFACTANT

2) PULMONARY OEDEMA

REVIEW

SURFACTANT

Discovery

Assuming for the moment that alveolar respiratory mechanics can be analysed in terms of a bubble-shaped model alveolus using the Laplace equation, a simple calculation reveals a marked discrepancy between the forces theoretically necessary to expand the lungs and those actually observed in practice.

Thus, assuming a tissue fluid surface tension of 50 dynes/cm. and an alveolar radius of 50 micrometres, it would require a force of 20,000 dynes/cm. (equivalent to about 20 cms. of water) to overcome surface forces alone.

The contribution of surface tension to the elasticity of the lungs was demonstrated by von Neergaard (1929) in an elegantly simple experiment in which excised lungs were distended either with air or with a 7% gum arabic solution. He found that considerably less pressure was required to inflate the lung preparation with liquid than with air, and from a comparison of the air and liquid pressure volume curves concluded that the difference was due to surface tension effects at the fluid/air interface and that true tissue elasticity contributed only .... 'about $\frac{1}{4}$ to $\frac{1}{3}$ of the total retraction pressure'. He also noted that the separation of the curves became greater (i.e. relative surface tension effect increased) at greater lung volumes and ..... 'that the pressure due to true tissue elasticity regularly reaches the zero point at volumes much larger than would be expected from the pressures in the collapsed

state'.

He also suggested that the lowering of alveolar surface tension might be due to the accumulation of surface active substances at the alveolar interface and ..... 'that this would be useful in the mechanics of breathing, for otherwise the contraction pressures of the lung might become so great as to interfere with adequate expansion'.

Over the years since von Neergaard's original experiment, the presence of such a surface tension lowering agent has repeatedly been demonstrated, in extracts obtained from pulmonary alveoli by such procedures as endobronchial saline lavage, the generation of foam from the alveoli, and the mincing or homogenising of whole lung tissue. Not only did such extracts lower surface tension; they also displayed the properties of surface activity, i.e. the ability to decrease surface tension in inverse proportion to the surface area of the film (Clements, 1957; Avery & Mead, 1959; Bondurant & Miller, 1962; Reiss, 1965). (Note: the difference between a detergent and a surfactant is that the former, being wholly soluble in the liquid phase does not alter surface tension as the surface area changes, whereas the latter by virtue of its unipolar hydrophobic property accumulates on the surface and is thus more active, within limits, when the film is compressed.)

The knowledge that in certain circumstances, the surface tension of a fluid can vary with its surface area is very old (Pockels, 1891). This property explains the widening of the air/liquid pressure-volume curves obtained by von Neergaard (at high lung volumes, 75% of the lung elasticity is due to surface tension

forces, while at low volumes the contribution is only 30%), but the real significance of this was missed at the time and it was left to Clements to stress that, by such a mechanism, the alveoli are stabilised and that many millions of tiny intercommunicating spheres of different sizes can co-exist at different states of inflation in spite of surface tension effects (Clements, Hustead, Johnson & Gribetz, 1961). More recent (Macklem, Proctor & Hogg, 1970) is the demonstration that surfactant is essential also for the maintenance of stability in small bronchioles.

An additional important function of surfactant emphasised by Pattle (1958, 1965) is assisting in the regulation of the intra-alveolar fluid volume; this aspect is discussed later in the section.

Biochemistry

In 1946, an unusual phosphatide, dipalmityl lecithin, was isolated from whole lung homogenate (Thannhauser, Benotti & Boncaddo, 1946) and it soon became apparent that this substance was an important constituent of the surface active film (Salisbury-Murphy, Rubenstein & Beck, 1966; Tierney, Clements & Trahan, 1967; Clements, 1971; Young & Tierney, 1972).

The 'semantic,conceptual and methodologic problems' incurred in the isolation and identification of pulmonary surfactant have been discussed by Clements (1970). The main problem, briefly, is that through common usage, 'surfactant' has come to mean a single compound to most people, while in fact it is made up of complexes of different molecules possibly with different functions within the 'surfactant system' (Allan, Goodman, Besarab & Rasmussen, 1973),

and that as these complexes become separated during the powerful and relatively crude fractionating processes involved, their original interactions become obscured.

It is not surprising therefore that for many years there was disagreement as to the nature of the 'surfactant' molecule.

One group of workers suggested that surfactant is a phospholipopolysaccharide (Clutario, Scarpelli & Taylor, 1966; Scarpelli, Clutario & Taylor, 1967); another group claim that it is the phospholipid itself, unassociated with proteins or polysaccharides which is the active molecule (Steim, Redding, Hauck & Stein, 1969). A very large number of studies have suggested that surfactant is a lipoprotein complex (Pattle & Thomas, 1961; Abrams & Taylor, 1964; Abrams, 1966; Klein & Margolis, 1968; Frosolono, Charms, Pawlowski & Slivka, 1970), and the observation by Spitzer & Norman (1971) that the biological half-life for the ^{14}C-leucine labelled surfactant protein was almost identical to that of the ^{3}H-choline labelled surfactant lecithin, indicating that the surfactant lipoprotein complex appeared to turn over as a single molecule provides strong confirmatory evidence.

All, however, are agreed that dipalmityl lecithin is the most important constituent of surfactant and that although it probably cannot function alone, it contributes most to the lowering of alveolar surface tension.

Site of formation

1) Cellular

Type 2 cells possess the morphological features character-

istic of secretory cells; a large rough endoplasmic reticulum, a well-developed Golgi apparatus, and cytoplasmic inclusion bodies (Sorokin, 1967; Schramm, 1967).

Macklin, in 1954, commented on the similarities between the silver-staining granules of the 'large pulmonary alveolar' (Type 2) cells and granules obtained from pulmonary washings, and observed (by light microscopy) that the granules within these cells appeared to enlarge, coalesce and eventually become extruded onto the alveolar surface, where they disappeared. These findings have been confirmed by electron microscopy and several workers have published micrographs showing inclusion bodies of Type 2 cells discharging their contents onto the alveolar surface (Bensch, Schaeffer & Avery, 1964; Goldenberg, Buckingham & Sommers, 1969; Kikkawa, 1970; Huber, Edmunds & Finley, 1971).

Niden (1967), however, pointed out that in fixed material, it is difficult to distinguish between secretion and phagocytosis and on the basis of histochemical evidence and the knowledge that the Type 2 cell is actively phagocytic (Low & Sampaio, 1957; Ladman & Finley, 1966) suggested that its main function was as an alveolar phagocyte, and that surfactant is in fact produced by the non-ciliated bronchiolar (Clara) cells.

This seems unlikely for several reasons; the osmiophilic inclusions of the Type 2 cells lack a polysaccharide lining which is typical of phagocytic vacuoloes (Kuhn, 1968), labelled lecithin precursors appear in Type 2 cell cytoplasm before appearing in the Clara cells or in the alveolar lumen (Askin & Kuhn, 1971; Darrah & Hedley-Whyte, 1973), and from morphometric analysis (Weibel, 1963a)

of alveolar and bronchiolar cytology applied to known rates of lung lecithin turnover (Tierney, Clements & Trahan, 1967) it becomes obvious that while the Type 2 cells could synthesise sufficient lecithin at a normal metabolic rate, the same production by the known mass of Clara cells would require a 100-fold increase in their oxygen consumption (Clements, 1970).

Nevertheless, the active secretory nature of the Clara cells remains undoubted (Azzopardi & Thurlbeck, 1969), and it has been suggested (Gil & Weibel, 1971b) that they and the Type 2 cells may both secrete surfactant, each to their own areas.

The evidence that the secretory product of the Type 2 cell is surfactant is largely circumstantial.

Pattle (1958) observed that stable bubbles could be expressed from foetal mice lungs at a gestational age of about 17 to 18 days; this correlates well with the appearance of adult-pattern surface activity in mouse-lung extracts at 18 days gestation (Buckingham & Avery, 1962) and the appearance of osmiophilic inclusions in the Type 2 cells at 18 days (Woodside & Dalton, 1958).

In foetal lambs, the temporal relationship between the appearance of inclusion bodies, the rise in the lung's content of dipalmityl lecithin, and the lung's attainment of adult mechanical properties has been demonstrated (Brumley, Chernick, Hodson, Normand, Fenner & Avery, 1967).

In foetal rabbits, injected corticosteroid was found to accelerate the appearance of surfactant (Kotas & Avery, 1971); the same treatment has also been shown to accelerate maturation of

the Type 2 cells and to increase formation of the inclusion bodies (Wang, Kotas, Avery & Thurlbeck, 1971). It is of interest that the treatment had no effect on the Clara cells.

In the human foetus, a surface active lining is present in the lungs from about 5 to 6 months' gestation (Avery & Mead, 1959); osmiophilic inclusions appear at about the same time (Campiche, Gautier, Hernandez & Reymond, 1963).

Adult rats, rendered hyperthyroid, show a progressive enlargement of the Type 2 cells together with an increased size and number of inclusion bodies and an increase in recoverable surfactant (Redding, Douglas & Stein, 1972).

2) Ultrastructural localisation

The most compelling evidence for the Type 2 cell as the origin of surfactant has come from autoradiographic studies of surfactant synthesis.

Following such a study by light microscopy, Buckingham and her co-workers (Buckingham, Heineman, Sommers & McNary, 1966) concluded that as the grains produced on the emulsion by labelled surfactant precursors lay between but not within the inclusion bodies, the inclusions were storage rather than production sites for surfactant. They also stated that more precise localisation would require the then unperfected technique of electron microscopic autoradiography.

This technique was used in 1971 by Askin & Kuhn, who showed that the label (^{3}H-palmitate) was taken up mainly by the Type 2 cells. By electron microscopy it was seen that while the greatest

number of autoradiographic grains were in areas of cytoplasm containing mitochondria, small vesicles and endoplasmic reticulum, there was a 4 to 10-fold concentration of grains over the inclusion bodies compared with the endoplasmic reticulum.

Tombropoulos (1971) demonstrated in vitro that lipid synthesis occurs in both the mitochondrial and microsomal subcellular fractions, but that the microsomal fraction was much more active.

Adamson and Bowden (1973) on the other hand, working with lung tissue cultures, concluded that lecithin synthesis actually took place in the perilamellar membrane of the inclusion bodies, and not remote from them.

Using a multiple-labelling autoradiographic technique, Chevalier & Collet (1972) provided strong evidence for the subcellular site of surfactant synthesis and for its chemical nature. ^{3}H-choline, a specific precursor of lecithin, was initially localised in the endoplasmic reticulum, then was rapidly transported by previously undescribed small lamellar bodies via the Golgi body to the inclusion bodies. The leucine label was also initially confined to the endoplasmic reticulum, but then appeared to be carried by multivesicular bodies, via the Golgi apparatus, to the inclusion bodies. The galactose label was poorly taken up, but appeared also in the inclusion bodies. The labels appeared in the intra-alveolar surfactant at 120 minutes after injection.

These demonstrations of intracellular transport of surfactant precursors are in agreement with the demonstrations by Massaro and his colleagues (Massaro, Weiss & Simon, 1970; Massaro &

Massaro, 1972) of an intracellular transport mechanism for secretory protein within the Type 2 cell.

These studies and others performed since (Darrah & Hedley-Whyte, 1973; Dickie, Massaro, Marshal & Massaro, 1973; Gil & Reiss, 1973; Massaro & Massaro, 1974) have now confirmed that the Type 2 cell actively synthesises a lipoprotein complex and stores it in the inclusion bodies.

Disease states and surfactant

Several pathological conditions and toxic substances are associated with alterations in pulmonary surface activity; they have been reviewed by Avery & Said (1965) and Forrest (1969).

In summary, these are: atelectasis due to pleural effusion, bronchial obstruction or pneumothorax (Tooley, Gardner, Thung & Finley, 1961; Avery & Chernick, 1963; Sutnick & Soloff, 1963; Finley, Tooley, Swenson, Gardner & Clements, 1964; Yeh, Ellison, Manning, Hamlin & Ellison, 1965); impaired pulmonary perfusion (Swenson, Finley & Guzman, 1961; Finley et al., 1964; Chernick, Hodson & Greenfield, 1966; Giammona, Mendelbaum, Foy & Bondurant, 1966; Huber & Edmunds, 1967; Morgan & Edmunds, 1967; Said, Harlan, Burke & Elliot, 1968; Massaro, Weiss & White, 1971); inhalation of amniotic fluid, or in drowning (Johnson, Permutt, Sipple & Salem, 1964; Mantkelow & Hunt, 1967); lung transplantation (Waldhausen, Giammona, Kilman & Daly, 1965; Permutt, 1967); pneumonia (Pattle & Burgess, 1961; Sutnick & Soloff, 1964); radiation pneumonitis (Warren & Gates, 1940; Capers, 1961; Menzel, 1970); carbon dioxide poisoning (Schaeir, Avery & Bensch, 1964); inhalation of mercury vapour

(Matthew, Kirschner, Yow & Brennan, 1958), ozone (Mendenhall & Stockinger, 1962), and cigarette smoke (Bondurant, 1960; Miller & Bondurant,1962); and ingestion of dipyridylium herbicides (Mantkelow, 1967; Fisher & Clements, 1969; Fisher, Clements & Tierney, 1970; Fisher, Clements & Wright, 1973a).

It seems likely, however, that in all of these conditions, disorders of surfactant occur as a consequence rather than a cause of alveolar cellular damage, and in none has a primary role of surfactant been demonstrated.

There appears to be only one condition, namely asphyxia neonatorum, in which the pathological changes may possibly be due to a primary deficiency of surfactant.

Asphyxia neonatorum

In this condition, the newborn, usually premature, infant has great difficulty in expanding its lungs despite maximal inspiratory effort, and eventually dies of asphyxia and exhaustion. The pathological appearances in the lungs are of atelectasis, pulmonary congestion and intrapulmonary haemorrhage, and the formation of eosinophilic hyaline membranes within dilated alveolar ducts.

Gruenwald (1947, 1955) described the uneven expansion pattern of such lungs when inflated with air, but not with kerosene and concluded that the effect was due to an upset in surface tension mechanisms.

Pattle (1958), following his observation that a surface-active material appeared only late in foetal life in guinea-pigs suggested that 'atelectasis neonatorum' may be due to a defect in

this material. Independently, Avery & Mead (1959) performed surface balance studies on infant lungs obtained at autopsy and found that infants under 1.1 Kg. birth weight, and those of any weight, but with atelectasis and/or hyaline membranes, lacked surface active material. These findings were subsequently confirmed (Gruenwald, 1960; Adams, Fujiwara, Emmanoulides & Scudder, 1965) and extended in pressure-volume studies which showed poor distensibility and the tendency to collapse in lungs of infants with hyaline membranes (Gribetz, Frank & Avery, 1959; Gruenwald, Johnson, Hustead & Clements, 1962). The lungs of infants with atelectasis and hyaline membranes were unable to form a normal surface film and retained less air after re-inflation and collapse than normal lungs (Pattle, Claireaux, Davies & Cameron, 1962). The same authors also suggested that the high interfacial surface tension in the alveoli which the infant manages to hold open accounts for the transudation and hyaline membrane formation.

Further evidence for the involvement of surfactant in this condition came from Campiche, Jaccottet & Juillard (1962) who examined the lungs of eight infants with hyaline membranes and found a reduction in the Type 2 cell inclusion bodies.

More recently, a clinical and laboratory study on more than 400 mature and premature babies led to the conclusion that asphyxia neonatorum is a primary disorder of lung development in which there is indeed a failure to produce surfactant (Gluck, Kulovich, Eidelman, Cordero & Khazin, 1972). This causes reduced alveolar distensibility and triggers secondary factors such as alveolar exudation (due to increased negative intrathoracic pressure), a diminished pulmonary

blood flow, hypoxia and acidosis, all of which contribute further to a decrease in any lecithin synthesis.

Hyperoxia and surfactant

The similarities in the pathological changes in pulmonary oxygen toxicity and asphyxia neonatorum were noticed many years ago (Liebegott, 1941; Pichotka, 1941).

With the growing realisation that asphyxia neonatorum could be explained on the basis of surfactant deficiency, attention was directed towards the effects of hyperoxia on surfactant. The results in general have shown the development of defective surfactant mechanisms (as measured by raised surface tension in lung extracts, altered pressure-volume curves, altered uptake of radioactive palmitate, or structural alterations of the Type 2 cell inclusion bodies) following exposure to 100% oxygen at both normobaric (Fujiwara, Adams & Seto, 1964; Caldwell, Giammona, Lee & Bondurant, 1965; Giammona, Kerner & Bondurant, 1965; Morgan, Finley, Huber & Fialkow, 1965; McSherry, Pannossian, Jaeger & Veith, 1968; Morgan, 1968; Beckman & Weiss, 1969) and at hyperbaric (Bondurant & Smith, 1962; Jamieson & van der Brenk, 1964; Kennedy, 1966; Webb, Lanius, Aslami & Reynolds, 1966; McSherry & Gilder, 1970) pressures.

Additional evidence for the implication of surfactant in oxygen toxicity comes from the synergism seen with Paraquat (Fisher, Clements & Wright, 1973).

The biochemical and ultrastructural changes relating to oxygen effects on surfactant described since 1970 are reviewed by Smith and Shields (1975) and are presented on Pages A1:14-16 of this

thesis.

Since the writing of the 1975 review, it has been demonstrated that long-term exposure of mice to 100% oxygen at hypobaric pressures (632 and 315 mm.Hg.) causes lowered surfactant activity and also that there is an adaptive response to gradually increased oxygen tensions (Sheffield, 1975). This appears to indicate that the effect of oxygen is on surfactant synthesis, an active process capable of adaptation, rather than by the passive direct inactivation of pre-formed surfactant.

SURFACTANT AND PULMONARY OEDEMA

Before the advent of the electron microscope, the blood-air barrier was thought to consist of a single layer of tissue, and the development of pulmonary oedema, explained on the basis of a two-compartment model, was considered to be the simple extravasation of a filtrate of plasma into the alveolar space. It is now realised that the structure of the alveolar membrane is considerably more complicated (Weibel, 1969; Ryan, 1969; Fishman, 1972; Szidon, Pietra & Fishman, 1972; Weibel, Burri & Gil, 1972), and that any model of fluid transport must allow for four anatomically distinct compartments, i.e. the vascular, interstitial, alveolar and lymphatic compartments.

Functional anatomy

The ultrastructural cytology of the alveolar membrane is reviewed in Section 4. Here, the structure of the membrane is considered at lesser magnification, and its relation to function is

best appreciated by examining an edge-on view of the alveolar septum.

Almost invariably, the capillaries when seen in either longitudinal (Figure 2:1) or transverse (Figure 2:2) section are found to be eccentrically placed within the septum, and tend to bulge into the alveolar lumen on one side of the septum only. This results in the two sides of a capillary having distinct structural and functional differences.

The side which bulges into the alveolar lumen is extremely thin (less than 0.5 micrometres); it consists only of the finely attenuated processes of the endothelial and Type 1 epithelial cells together with their fused basement membranes. It is ideally adapted to gas exchange. The opposite side is much thicker, and consists of five separate anatomical layers; capillary endothelium, basement membrane, interstitial space, basement membrane, and Type 1 epithelium. Within the interstitial space are found bundles of elastic, collagen and reticulin fibres in a mucopolysaccharide ground substance, fibroblasts and macrophages. This side serves to support the capillary and also plays the major part in fluid exchange (Fishman, 1972).

Fluid leaving the pulmonary capillaries tends to do so on the thick side where the much wider interstitial space can act as a sump leaving the thin gas-exchanging portion of the alveolar septum intact (Cottrell, Levine, Senior, Wiener, Spiro & Fishman, 1967). It is then cleared towards the alveolar ducts where it enters the very extensive pulmonary lymphatic system (Miller, 1937; Ryan, 1969; Lauweryns, 1970; Pump, 1970) for further transport towards the hilum.

The actual mechanisms involved in this transport are still controversial, but it is generally accepted that between the alveolar

and the more centrally-located interstitial spaces there are gradients of sub-atmospheric pressure and that pressures in the interstitial space around the large 'extra-alveolar' (Staub, 1966) vessels and airways are considerably subatmospheric (Howell, Permutt, Proctor & Riley, 1961; Permutt, 1965; Staub, 1970). Respiratory movements probably also assist in pumping the lymph centrally (Fishman, 1972). What is certainly known is that very large volumes of fluid can be moved efficiently from the alveolar areas, and that, if there is a weak point in the system, it is in the dimensions of the final outflow tract, i.e. the thoracic and right lymphatic ducts (Drinker, 1950; Dumont, Clauss, Reed & Tice, 1963). When the flow of lymph overwhelms the exit lymphatics, interstitial oedema accumulates centrally around the large vessels and airways (see Figures 3:25 and 3:26) and only at a late stage is there found any great thickening of the 'sumps' in the alveolar septa (as demonstrated in Figure 2:3) (Staub et al., 1967; Meyrick, Miller & Reid, 1972; Szidon et al., 1972; Staub, 1974).

Surfactant lining layer

The luminal side of the alveolar epithelium is covered by a surface lining layer which consists of two phases, a thin surface film, and an underlying hypophase. It has only recently been visualised by electron microscopy, and then only with difficulty, by means of special techniques such as perfusion-fixation and freeze-etching (Weibel & Gil, 1969; Gil & Weibel, 1968; Kikkawa, 1970; Brooks, 1971; Gil & Weibel, 1971; Untersee, Gil & Weibel, 1971).

The lining layer consists of a very thin osmiophilic surface

film, presumably a monomolecular layer of surface-active lipoprotein, and an underlying hypophase thought to consist mainly of mucopolysaccharides (Roth, 1975) which can attain a thickness of several micrometres, for example in clefts between capillaries. It has been seen to extend to fill and obliterate a pore of Kohn (Gil & Weibel, 1969).

The most striking feature of perfusion-fixed lung tissue is that by virtue of this lining layer, the alveolar lumen presents a perfectly smooth surface. Freeze-etching techniques have demonstrated that the lining is continuous (Untersee et al., 1971).

Fluid exchange across the alveolar septum

Permeability of septal membranes

1) Endothelium

The pulmonary capillary endothelium has similar permeability characteristics to capillaries elsewhere, and does not provide any special barrier to the passage of water and small molecules; it has been likened to the capillaries of cardiac and skeletal muscle (Wangensteen, Wittmers & Johnson, 1969; Taylor & Gaar, 1970). Electron microscopy has revealed clefts approximately 200 Angstroms wide between adjacent endothelial cells, with narrowings along the clefts of down to 50 to 80 Angstroms in places. These allow for continuity of the intravascular and extravascular compartments, and their dimensions and numbers (1 to 2 x 10^9 per square centimetre of capillary wall) are consistent with their representing the 'small pores' of the physiologists' theory of capillary transport (Pappenheimer, 1953; Schneeberger-Keeley & Karnovski, 1968; Szidon et al., 1972). The

dimensions of the 'small pores' are not fixed, however; it has been shown that at normal pulmonary capillary pressures they are sufficiently small to arrest the passage of a marker such as stroma-free haemoglobin (molecular weight 64,000, diameter about 60 Angstroms) whereas when capillary pressure is increased, the pores stretch and allow the marker to pass (Shirley, Wolfram, Wasserman & Mayerson, 1957; Szidon et al., 1972).

Molecules of molecular weight greater than 90,000 must cross the endothelial barrier by other means. On physiological grounds, a system of 'large pores' about 500 Angstroms in diameter has been postulated (Pappenheimer, 1953) and it has been suggested that the pinocytotic vesicles seen in the endothelial membranes (Bruns & Palade, 1968; Bensch & Dominguez, 1971) represent the anatomical equivalent of these large pores (Mayerson, Wolfram, Shirley & Wasserman, 1960).

2) <u>Epithelium</u>

In contrast to the above, physiological studies have shown that the Type 1 epithelial membrane forms a barrier to much smaller molecules; calculations show that, by the pore theory, the pores of the epithelial membrane are only about one tenth the diameter of those of the endothelium (Chinard, 1966; Wangensteen et al., 1969; Taylor & Gaar, 1970). Electron microscopy has revealed that the junctions between adjacent epithelial cells are 'tight' and that the cleft is completely obliterated by fusion of the cell membranes to form zonulae occludentes (Szidon et al., 1972).

Hydrostatic forces across the alveolar membrane

The movement of liquid across an endothelial membrane (Starling 1896) can be expressed by:

$$\dot{Q} = K_f (P_c - P_{i.s.}) - (\pi_{pl} - \pi_{i.s.})$$

where $\dot{Q}$ = rate of liquid movement across a unit surface area of capillary.

K_f = capillary filtration coefficient (a physical constant incorporating capillary permeability and actual surface area units).

P_c and $P_{i.s.}$ = hydrostatic pressures in the capillary and interstitial space.

π_{pl} and $\pi_{i.s.}$ = plasma and interstitial space oncotic pressures.

Drinker (1950), using this approach and allowing 10 mm.Hg. for pulmonary capillary pressure, 30 mm.Hg. for plasma oncotic pressure and a variable 5 to 10 mm.Hg. net intrathoracic negative pressure due to respiratory movements, found a balance of 10 to 15 mm.Hg. in favour of alveolar dryness. This, however, was classical two-compartmental analysis; Drinker did not believe in a continuous alveolar epithelial lining, and thought that any alveolar fluid was immediately removed by the lymphatics. He failed to allow for the oncotic pressure of the interstitial space, and ignored completely the effects of surface tension.

If one again allows for a spherical alveolus 50 micrometres in diameter, an intra-alveolar surface tension of 55 dynes per centimetre would produce an additional 16 mm.Hg. negative pressure in the interstitial space, and under these conditions, transudation would be continuous.

That this is not the case is due to the surface tension lowering effect of the surfactant layer.

Even allowing for this effect, however, it is obvious that there is a very fine balance of hydrostatic forces across the pulmonary capillary membrane. Pulmonary capillary pressure is not uniform throughout the lung, being influenced by gravitational forces (West, 1970), and is pulsatile; it is likely therefore that there are minute to minute fluctuations in interstitial lung water. The tight epithelial cell junctions prevent this from entering the alveolar space, and by collecting initially in the sumps of the interstitium, it does not interfere with gas exchange.

Significance of alveolar geometry

The calculations of the contribution of surfactant to pulmonary mechanics and capillary transudation outlined in this section have assumed that alveoli are spherical.

It is now known that this is an over-simplification. Weibel (1963b) compared the alveoli to a film of soap, where the bubbles formed irregular polyhedra. Staub & Storey (1962) described the walls between adjacent alveoli as being flat, and only where the alveolar wall did not abut directly on another alveolus was the surface curved. Reifenrath & Zimmerman (1973) demonstrated that unfixed sub-pleural alveoli form a dense packing of polyhedra with common boundaries.

The effect of this configuration on the progression of oedema formation has been described by Reifenrath (1975). He suggests that as surface tension is zero along the flat surfaces of

a polyhedron, the balance would be much in favour of dryness, and any movement of liquid would be inwards towards the capillary lumen. Only at the corners, with their sharp radii of curvature would the balance of forces favour transudation. This perhaps explains the phenomenon that intra-alveolar oedema tends to develop first in the corners (Staub et al., 1967; Staub, 1970). Experimental evidence for increase in surface tension effect at the sharp alveolar corners comes from Staub (1966) who raised alveolar pressure slightly above pulmonary capillary pressure in anaesthetised cats and demonstrated by a rapid freezing histological technique that the capillaries of the flat alveolar walls were compressed and occluded, whereas the capillaries at the wall junctions, acted on by surface tension, remained patent.

The alveolar walls are known to be persistently wet (Macklin, 1955); Reifenrath suggests a mechanism whereby the balance of fluid in the alveolar space might be achieved. He postulates that too little fluid will cause a very small radius of curvature, and hence a large surface tension force at the alveolar corners, leading to an influx of fluid. With increasing radius of curvature, the surface tension forces fall and influx decreases. Eventually a balance between influx and efflux dependent on an equilibrium radius (i.e. equilibrium surface tension) is obtained.

This mechanism could also contribute to the 'run-away' phase of progressive pulmonary oedema; the 'oedema begets oedema' phenomenon. Once so much fluid enters the alveolus that it totally fills the corners and in effect makes the fluid/air interface spher-ical, a further increase in influx will, in contrast to the situation

described above, make the radius of curvature smaller rather than greater and will cause an increase rather than a decrease in surface tension forces. The result would be a rapidly increasing (positive feed-back) influx of fluid, further enhanced by the known inactivating effect of fibrinogen on surfactant (Taylor & Abrams, 1966).

Summary

There can be no better summary of the functional morphology of the alveolar membrane in relation to pulmonary oedema than that of Szidon et al. (1972):

'Apparently, the alveolar septum is a supporting structure for an endothelial tube of ordinary permeability. The epithelial lining of alveoli prevents the ready access of water and solutes into the alveolar spaces. Fluid and solutes leaving the capillaries are prevented from interfering with gas-exchanging function of the alveolar-capillary septum by a combination of mechanisms: 'sumps' of connective tissue in the alveolar septum soak up excess fluid, thereby protecting the thin portion; and a system of physical forces promotes its rapid transfer from the connective tissue sump of the septum into a capacious interstitial space in a non-gas-exchanging portion of the lung where it can be stored temporarily until removal by lymphatics. Only when water filtration exceeds the combined storage and reabsorptive ability of the interstitial spaces does alveolar oedema occur.'

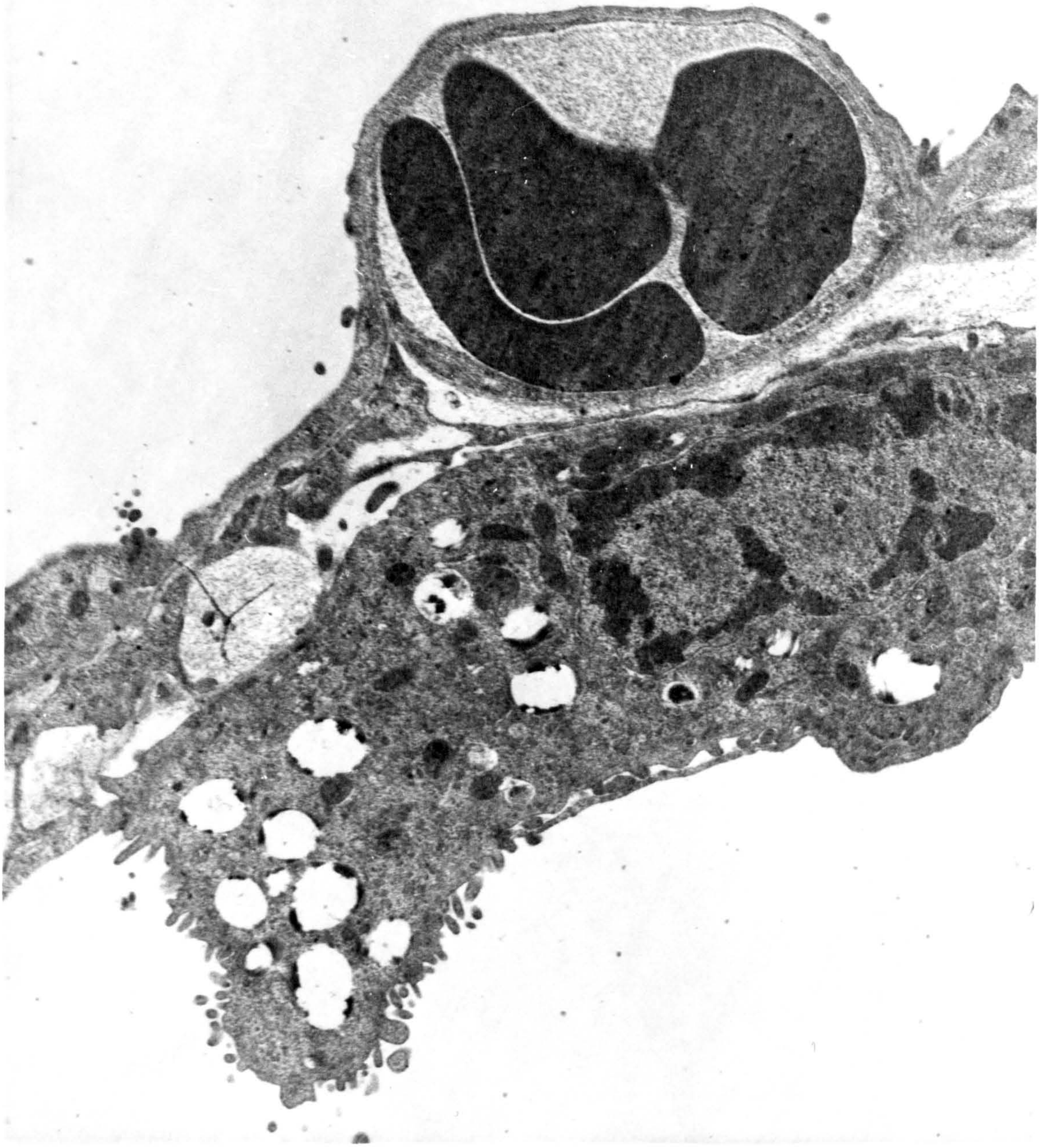


FIGURE 2:1. ALVEOLAR CAPILLARY, TRANSVERSE SECTION.

(x 8,000)

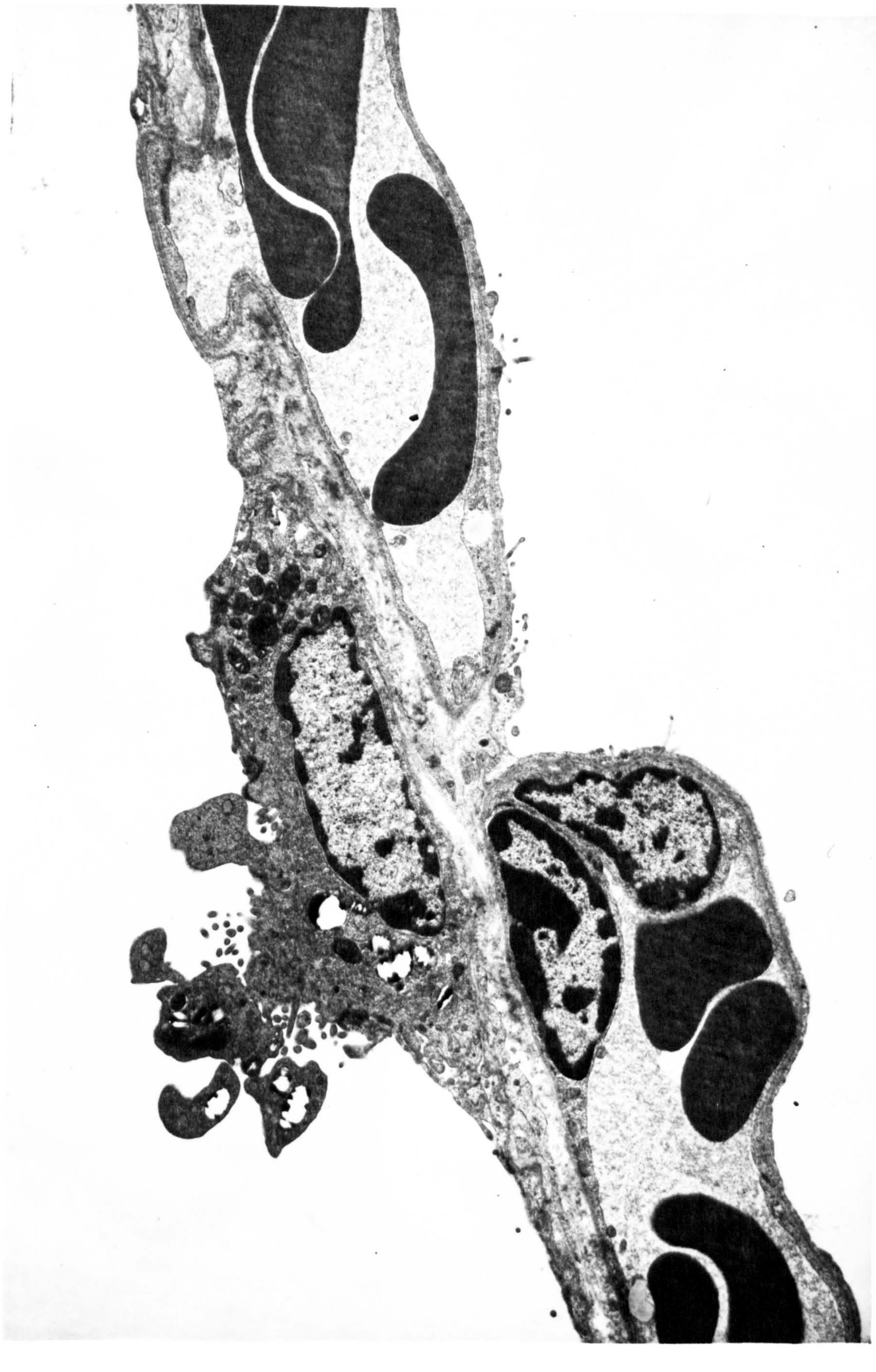


FIGURE 2:2. ALVEOLAR CAPILLARY, LONGITUDINAL SECTION.

(x 7,500)

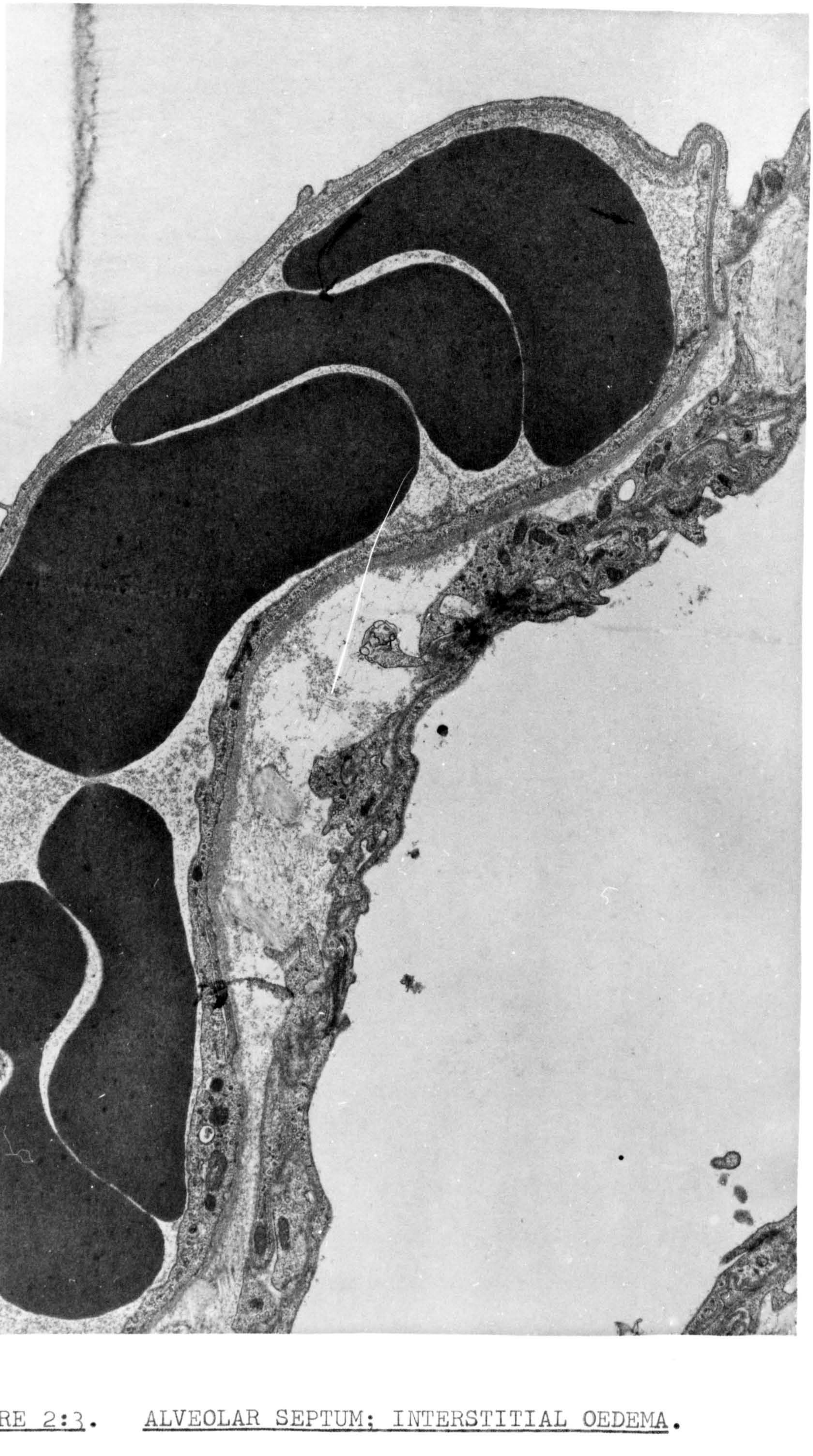


FIGURE 2:3. ALVEOLAR SEPTUM; INTERSTITIAL OEDEMA.

(x 12,000)

SECTION 3

DOG EXPERIMENTS SERIES 1

PATHOPHYSIOLOGICAL STUDY AT 2 ATA

DOG EXPERIMENTS SERIES 1

INTRODUCTION

In 1899 J. Lorrain Smith described the histopathological appearances of acute pulmonary oxygen toxicity as those of atelectasis, consolidation, congestion, inflammation, and intra-alveolar oedema and haemorrhage. These findings have been repeatedly confirmed since (Bean,1945) and seemed to leave little room for doubt as to the cause of death. Very little had been done to determine the time-course of the condition but the tacit assumption appeared to be that the animal went into a steady inexorable decline from the moment it started to breathe high tensions of oxygen and that it finally succumbed from an 'oxygen burn' to the lung.

In 1963 however, Smith, Lehan & Monks, working on conscious dogs breathing 100% oxygen at atmospheric pressure showed that the blood gas and cardiovascular parameters were maintained substantially normal for about 95% of the exposure and that the animals went into respiratory collapse only some two to three hours before death. At autopsy the lungs were dark red, haemorrhagic, oedematous and congested.

Two years later, Durfey (1965) challenged the pathological appearances as possibly being due to post-mortem artifact. He drew attention to the progression of appearances dependent on the time delay between death and histological preparation and demonstrated in mice that any delay in autopsy following exposure to high concentrations of oxygen could produce the gross picture of the classical description of 'oxygen toxicity' before such changes had in fact occurred. He hypothesised that this possible artifact might be due either to the inactivation or alteration of surfactant by high oxygen concentrations

causing an increase in surface tension, or by lack of inert gas 'splinting' of the alveoli.

As animals suffering from acute pulmonary oxygen toxicity die in apnoea and not primarily in asystole, uptake of oxygen will continue across the alveolar membrane as long as the heart continues to pump; if the alveoli contain only a totally absorbable gas such as oxygen, they will collapse. The importance of this was demonstrated, also in 1965, by Pratt who described different histological appearances of the lungs of oxygen poisoned animals which had been allowed a few breaths of air immediately before death compared with those which had died while breathing pure oxygen.

These findings, and those of Smith et al. (above) would appear to have been confirmed in a recent study from Ledingham's laboratory at Glasgow (Clarke, Smith, Sandison & Ledingham, 1973). In this work, six spontaneously breathing anaesthetised dogs breathed 100% oxygen at 2 ATA until death, which occurred at a mean time of 18¾ hours. A wide range of physiological parameters was monitored throughout the experiment and the results showed that there was very little change until within a few hours of death. The animals died in apnoea, and in order to prevent post-mortem artifact the lungs were fixed immediately following this by instillation of buffered formalin. Histological appearances in these lungs were described as being remarkably normal.

The paper can be criticised on several grounds. The first (discussed by the authors themselves) is that by distending the lungs with buffered formalin one might not only reverse any alveolar collapse, but would also tend to mask any intra-alveolar oedema present.

Secondly, the authors chose to present their results as a comparison between 'early' and 'late' findings and did not concentrate

on the time course of the vitally important final three hours. This arose probably because of the large number of parameters being measured and also because of the cumbersome nature of some of the techniques used.

Finally, and this follows largely from the above, no definitive cause of death was established. Of six experimental animals, four appeared to die in apnoea secondary to cardiovascular collapse and in the remaining two, largely due to the lack of any positive findings to the contrary, death was attributed to central nervous system oxygen toxicity causing respiratory failure.

These criticisms, of course, arise easily with hindsight and the paper remains as a valuable pilot study, serving to direct attention to the crucial areas for research.

The 'Dog Series 1' experiments were designed to repeat the work of Clarke et al. with streamlining of techniques to permit intensive investigation of the three hours immediately preceding apnoea and to prepare the lung tissues by a method which would eliminate as far as possible any fixation or post-mortem artifact.

CHOICE AND DEVELOPMENT OF THE ANAESTHETIC TECHNIQUE

Great importance was attached to the choice of anaesthetic agent.

Many early experiments on pulmonary oxygen toxicity (Bean, 1945) were performed on conscious animals and, while this allowed for an accurate description of the mode and time of death, it did not permit access to the animal for detailed study of the mechanisms involved.

General anaesthetic agents are well known to protect against both the central nervous system (C.N.S.) manifestations (Jamieson, 1966a; Behnke, Shaw, Shilling, Thomson & Messer, 1934; Gersh & Wagner, 1945; Harp, Gutsche & Stephen, 1966) and the pulmonary manifestations (Bean & Zee, 1965, 1966; Jamieson, 1966b) of oxygen toxicity. The exact mechanism remains unclear due to the multiple interaction of factors involved. For example, it has been suggested that the pulmonary and C.N.S. manifestations of oxygen toxicity occur completely independently (Brauer, Parrish, Way, Pratt & Pessotti, 1970; McSherry & Veith, 1968); Brauer showed that the exposure of rats to altitude increased their pulmonary tolerance to 1.1 ATA but decreased their central nervous system tolerance to 7.0 ATA of oxygen (Brauer et al., 1970). However, a variety of insults to the central nervous system - e.g. mechanical trauma to the head (Bean & Beckman, 1969; Beckman & Bean, 1970; Beckman, Bean & Baslock, 1971), drug- (and oxygen-) induced convulsions (Bean, Zee & Thom, 1966; Tennekoon, 1954), raised intracranial pressure, both in the clinical (Ducker, 1968) and experimental (Ducker, Simmons & Anderson, 1968; Berman, Ducker & Simmons, 1969) situations, and hypothalamic hypoxia (Moss, Staunton & Stein, 1972)

can lead to a pulmonary pathology apparently identical to that produced by OHP and it is not known to what extent this interaction exists at different tensions of oxygen. It has been conclusively demonstrated both that severe pulmonary damage can occur at high pressures of oxygen when convulsions have been prevented by drugs (Jamieson & Cass, 1967; Thompson & Akers, 1970), and that convulsions due to OHP are not inevitably followed by pulmonary damage (Bean & Zee, 1966; Wood, Stacey & Watson, 1965) (see also page 3:31).

Anaesthetic technique developed by Ledingham

A series of preliminary experiments by Clarke and Ledingham (unpublished) had demonstrated the unsuitability of the more common anaesthetic agents, such as barbiturates, trichlorethylene and phencyclidine in this type of investigation and that at 2 ATA pressure an alteration in the mode of anaesthesia caused a definite alteration in the mode of death. In particular, when halothane was used, the animals would remain remarkably stable over a 24 hour period breathing 40% O_2 at 1 ATA but at 2 ATA of oxygen the period of stability lasted only some 12 hours when the animals developed an acute respiratory failure - a central nervous system death (Smith & Ledingham, 1971; Smith, 1971). They had decided therefore to use a neurolept-analgesic technique with Droperidol (dehydrobenzoperidol) (Janssen Pharmaceuticals) as the neuroleptic combined with a long-acting analgesic RX 320 M (N-(cyclopropylethyl)19-isopentylnororvinal hydrochloride) (Reckitt and Colman).

This had several very important advantages.

In the first place, the animals were extremely lightly anaesthetised - so lightly in fact that local anaesthetic had to be injected

prior to making skin incisions - and the mode of death did not appear to differ to any great extent from that of animals dying at similar pressures of oxygen (Winter, Gupta, Michalski & Lanphier, 1967).

The animals were able to breathe spontaneously throughout the experiment; this allowed for recognition of the possible development of pulmonary oedema and alveolar collapse which could have been masked by positive pressure ventilation and permitted demonstration of the fact that death was preceded by apnoea. The mean time of death in their subsequent experiments (Clarke et al., 1973) was $18\frac{3}{4}$ hrs., whereas Trapp (Trapp, Yoshida & Grant, 1963) maintained mechanically ventilated anaesthetised dogs alive at identical pressures of oxygen far in excess of 36 hours.

The technique preserved the normal sigh mechanism intact throughout; this periodic hyperinflation is thought to be of importance in the reactivation of surfactant (Clements, Tierney & Trahan, 1963).

Development of technique

In each of the four series of dog experiments described in this thesis, this neurolept-analgesic technique was used. In the first series ('Dog Series 1'), the method of administration was identical to that of Clarke et al. - i.e. an initial induction dose of RX 320 M 25 µg/Kg. plus Droperidol 0.75 mg/Kg. administered intravenously, followed by increments of Droperidol (10 mg.) and, less frequently, RX 320 M (100 µg) as necessary throughout the experiment to maintain anaesthesia.

This technique proved adequate but was less than ideal in

that it produced a fluctuating level of anaesthesia. At the end of the first series therefore, the quantities and times of administration of anaesthetic agents were analysed (for 14 dogs). The increments required tended to remain fairly constant for much the greater part of the experiment (Table 3:1), although there appeared to be a marked 'tailing-off' in the terminal stages.

TABLE 3:1

Administration of Anaesthetic Agents in Dog Series 1.

Mean requirements in 14 dogs.

	RX 320 M	DROPERIDOL
INDUCTION	25 ug/Kg	10 mg.
MAINTENANCE Increments required in experimental dogs (N=11)	0.885 µg/Kg/hr.	0.1763 mg/Kg/hr.
MAINTENANCE Increments required in control dogs (N=3)	1.375 µg/Kg/hr.	0.2627 mg/Kg/hr.

In the following three series of experiments (Sections 5, 8 and 9), anaesthesia was maintained by continuous infusion of both Droperidol and RX 320 M in Ringer-lactate delivered via a 'Perpex' infusion pump. The maintenance dose was 'rounded up' somewhat from that shown in Table 3:1 on the grounds that some of the increments given in the Series 1 experiment might not have been noted;

in practice the chosen dosage of Droperidol 0.2 mg/Kg/hr and RX 320 M 1 µg/Kg/hr (which remained considerably less than that required for the control animals) proved entirely satisfactory.

From the Series 1 experiments it was also ascertained that to maintain fluid balance (as monitored by haemoglobin, haematocrit and central venous pressure measurements), 1 litre of fluid had to be infused on average every 10 hours. 500 ml. bottles of Ringer-lactate or Dextrose with the appropriate dosage of anaesthetic were set to infuse via the pump over 5 hours and the rate was finely adjusted to maintain optimum anaesthesia.

In the terminal stages of the experiment, the animals did not seem to require nearly as much anaesthetic as in the early stages, a finding already reported by Trapp, Patrick & Oforsagd (1971). At this point the infusion pump was slowed down or stopped altogether and fluid balance maintained via a separate drip set and cannula.

It was noted also that the test animals (on 2 ATA O_2) required much less anaesthetic throughout than the controls; this fact was also noticed and commented on by Smith (Smith, 1971) and would tend to suggest a central depressant effect of OHP.

<u>Control experiments for effects of anaesthesia</u>

Over the three series of dog experiments, a total of eleven air-breathing control animals were used. Of these, six were 'specialised' controls in that they were either vagotomised or had had some other manoeuvre performed. Of the remaining five, three breathed 10% O_2/90%N_2 at 2ATA (equivalent for inspired oxygen partial pressure to air-breathing at 1ATA) until sacrificed at 24 hours, and

the other two breathed air at ambient pressure until sacrificed at 24 hours. The outstanding feature in these animals was a remarkable degree of cardiopulmonary stability over the 24 hours; the results for the latter two animals are shown in Tables 3:2 a & b,* and the more important values are plotted in Figures 3:1 a & b.

Histological appearances of the lungs, both by light and electron microscopy were normal.

Objections to the use of Droperidol

There are two possible disadvantages in the use of Droperidol.

1) Protective effect

The first lies in a reference to Novelli, Pagni, Pirani, Ariano & Pallini (1967) quoted by Clark & Lambertsen (1971) - which has not been sighted in the original - where Droperidol 'provided considerable pulmonary and less effective central nervous system protection in rats exposed to 5 atm. of O_2 for 30 minutes'. There is no indication of the dosage used, and the different pressures (5 ATA O_2) and species (rats) involved alters the experimental model completely. Certainly some protection is afforded by Droperidol as unanaesthetised animals die more rapidly at similar pressures; whether or not it is more than that afforded by any other general anaesthetic technique is not so certain. A possible answer might be in the second disadvantage, that Droperidol may have a degree of alpha-adrenergic blocking activity.

2) Alpha- blocking properties

Droperidol has long been known to reduce the pressor effects

* presented in Appendix 2

of adrenaline. There remains controversy, however, as to whether or not this is a specific effect mediated by blockage of the alpha-adrenergic receptors.

In a carefully controlled study in dogs, Yelnosky, Katz & Dietrich (1963) concluded that alpha-blockade was probably the case, and quoted a 50% block to adrenaline-induced blood pressure rise following a dose of 0.125 mg/Kg, with a duration of block in excess of 30 minutes. In the same year, however, Schaper, Jageneau & Bogaard (1963) stated that, in dogs, Droperidol produced no myocardial depression and that the other haemodynamic effects were minimal.

In 1970, in a review of the pharmacology of drugs used in neurolept-analgesia, Edmonds-Seal & Prys-Roberts (1970) stated:

> 'We are of the opinion that the alpha-adrenergic blocking activity of the butyrophenones has been overemphasised and would appear to be inconsistent with the cardio-vascular stability which has been well documented.'

This action of Droperidol was appreciated at the outset, but did not assume importance until the belated realisation that the sympathetic nervous system might be intimately involved in the pathological process of pulmonary oxygen toxicity.

The dosage used, 0.2 mg/Kg <u>per hour</u> might be expected to produce a much smaller blocking effect than a single intravenous dose of 0.125 mg/Kg, and there was little evidence of block in any of the experimental traces; it remains, however, a potential source of criticism of the model.

FIGURE 3:1a. Anaesthetic Control Dog 1.

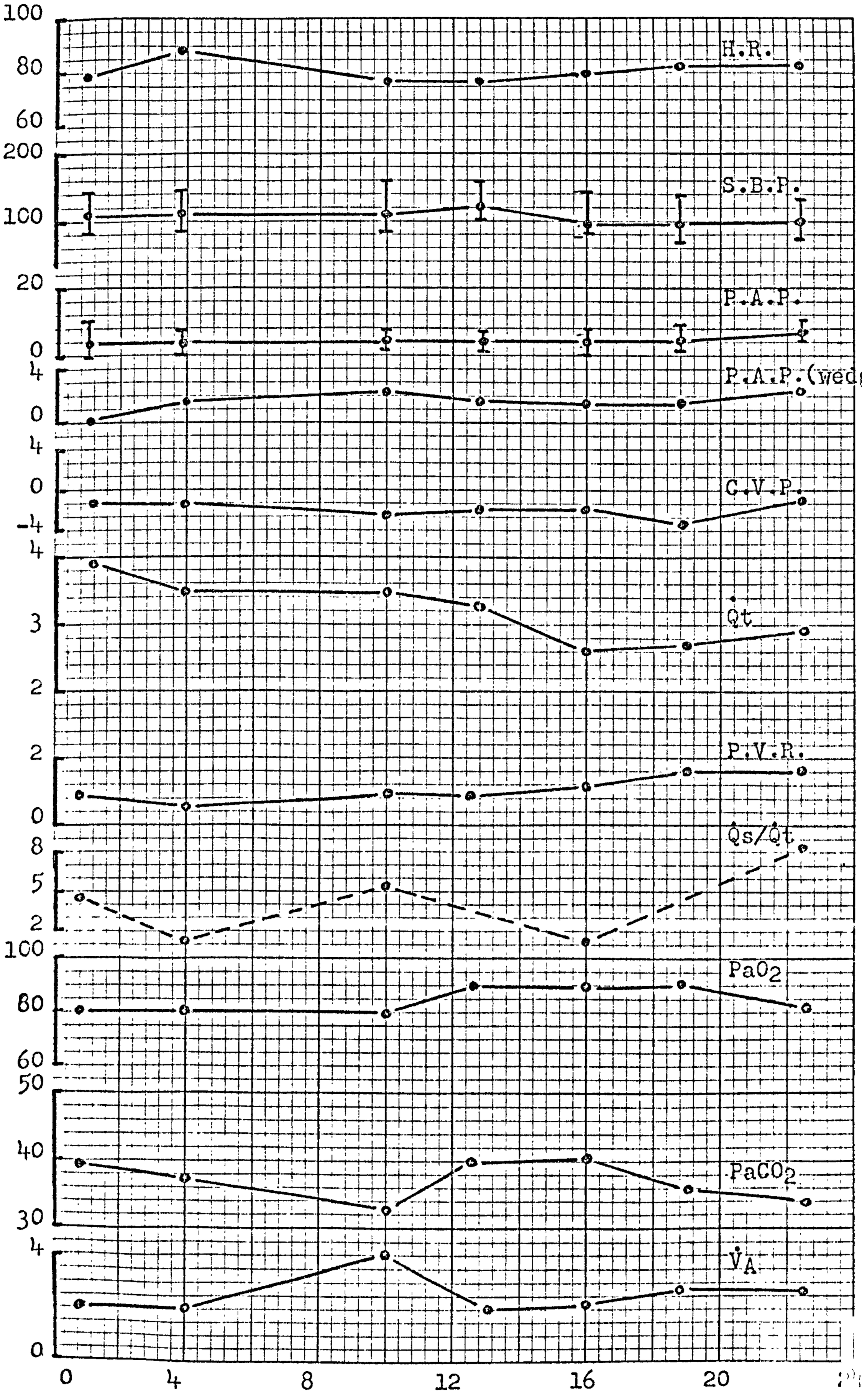
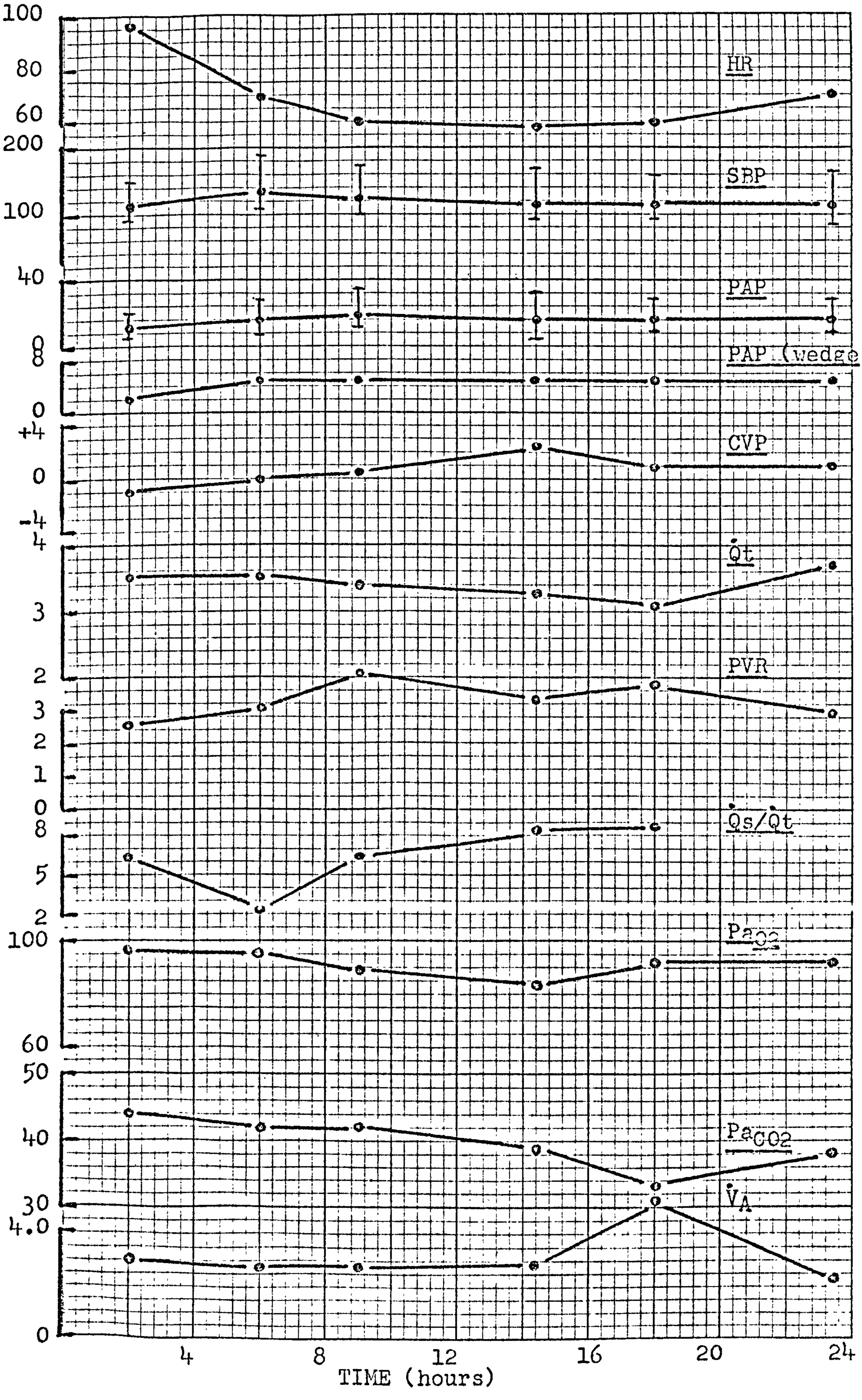


FIGURE 3:1b. Anaesthetic Control Dog 2.



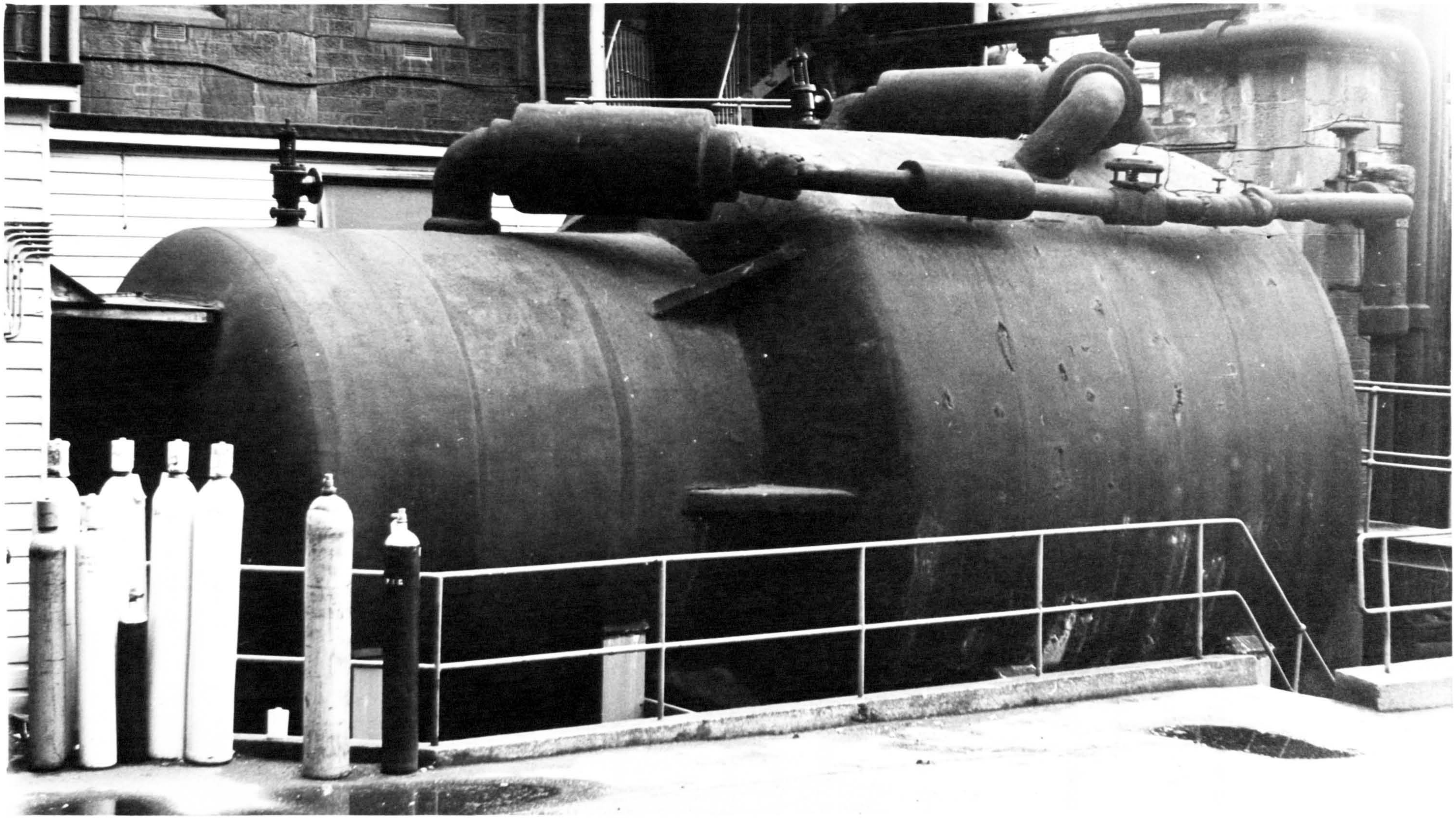


FIGURE 3:2. LARGE PRESSURE CHAMBER. HYPERBARIC UNIT, WESTERN INFIRMARY, GLASGOW.

MATERIALS AND METHODS

EQUIPMENT

Pressure vessel

The chamber used in these experiments was the large compressed air operating theatre of the Hyperbaric Oxygen Unit at the Western Infirmary, Glasgow (Figure 3:2). This chamber, which has a pressure capability of 3 ATA is a walk-in facility with sufficient room for several investigators and a large amount of monitoring equipment. It is equipped with on-line oxygen from a cryogenic source and is provided with ample outlets for special gas mixtures. A built-in gland carries telemetry cables to the laboratory outside. Full air conditioning allows rapid control over environmental temperature and humidity and the total gas content of the chamber is changed every 8 minutes at 2 ATA ensuring against the build-up of expired oxygen and carbon dioxide.

It should perhaps be stressed at this point that the chamber environment consists of compressed air (supplied from its own compressor room, manned on a 24 hour basis by two members of the hospital engineering staff) and that only the experimental animal (or the patient) breathes pure oxygen.

Over the twelve years that this chamber has been in use a considerable record of exposures of patients and staff to pressures up to 3 ATA has been built up. There has been no incidence of any of the decompression disorders and in particular, no case of aseptic bone necrosis for which all members of staff are screened. It seemed reasonable, however, to expose the investigators to pressure for as short a time as possible and therefore, following the setting up of the experi-

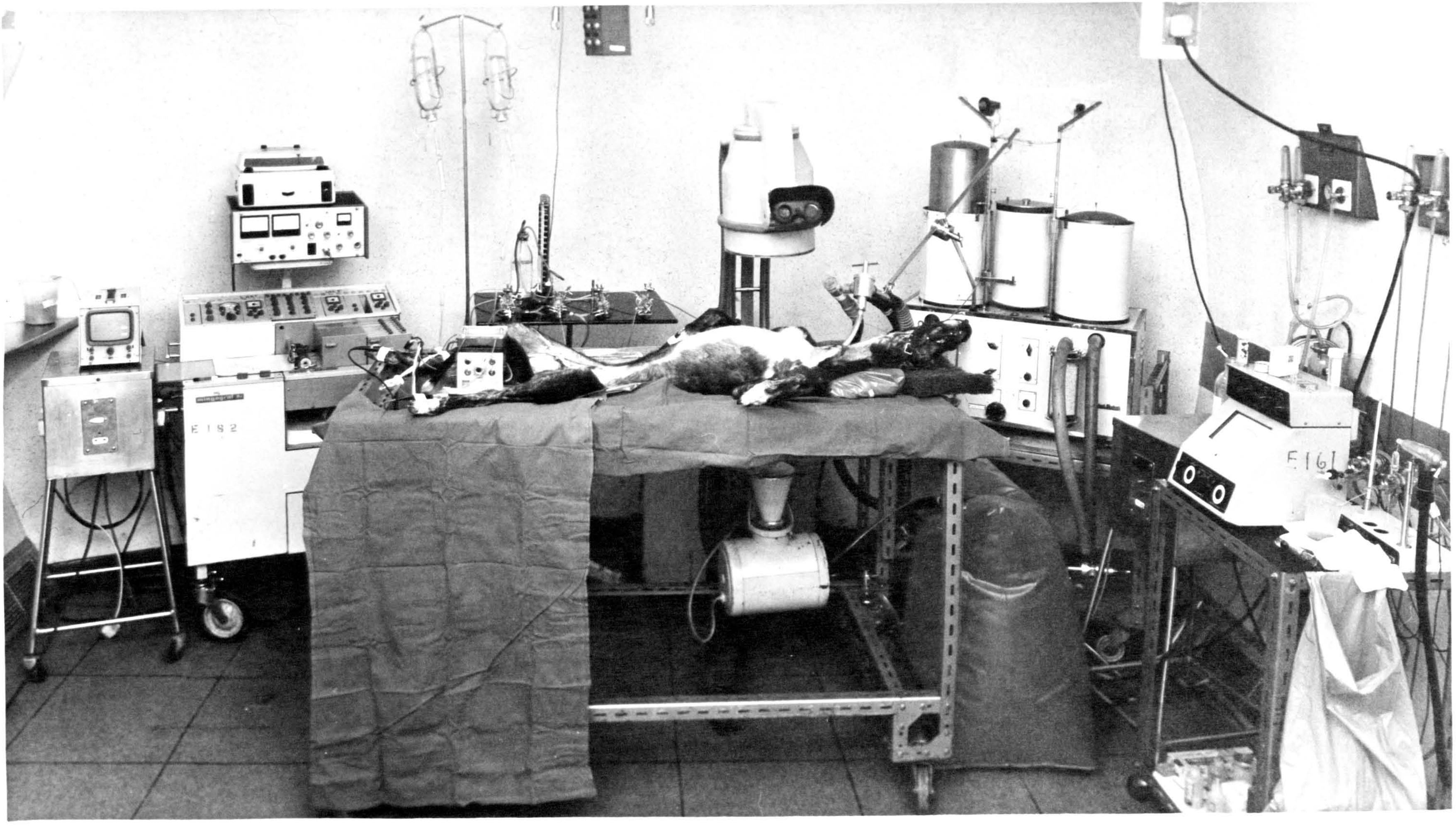


FIGURE 3:3. INTERIOR OF CHAMBER. GENERAL EXPERIMENTAL LAY-OUT.

ment at pressure, the staff decompressed in the air-lock leaving the chamber unmanned except for periods covering the 'runs' of measurements. During the remainder of the time, the experiment was supervised from a laboratory immediately adjacent to the chamber. Vital parameters were monitored on an outside oscilloscope by telemetry and the animal was observed from outside the chamber on closed circuit television. In order to protect the animal against an unexpected lightening of anaesthesia during this time, a syringe containing 10 mg of undiluted Droperidol was connected via a Harvard syringe pump (remotely controlled from the laboratory) to a separate venous cannula.

In this way exposure to pressure was limited to approximately 6 hours per 24 hour experiment. (Over the whole series of experiments described in this thesis, the author was exposed to 2 ATA on 374 separate compressions, totalling 352 hrs., 35 minutes.)

All the experiments are described as being at 2 ATA. In practice, when set at this pressure the chamber maintains its pressure at a constant 770 torr above ambient barometric pressure; the experiments therefore were performed over a range of pressures varying from 1520 to 1541 torr.

Figure 3:3 demonstrates the amount of space available inside the chamber and shows the general experimental lay-out.

Gas delivery and measurement of ventilation

Several gas delivery systems involving inlet demand and expiratory valves of increasing complexity were tried in the early pilot studies but all failed because of the occurrence of inward leakage of air through the expiratory valve(s). They were finally abandoned

in favour of the original (simplest) system. In this, oxygen was supplied via a simple Wolff's bottle bubble humidifier at chamber temperature to a large reservoir (a 100 litre Douglas bag) and from there via a perspex non-breathing valve to the animal. The rate of oxygen flow into the Douglas bag was deliberately set too high so that there was a constant spill out through the expiratory valve; in practice this completely prevented any inward leakage of nitrogen.

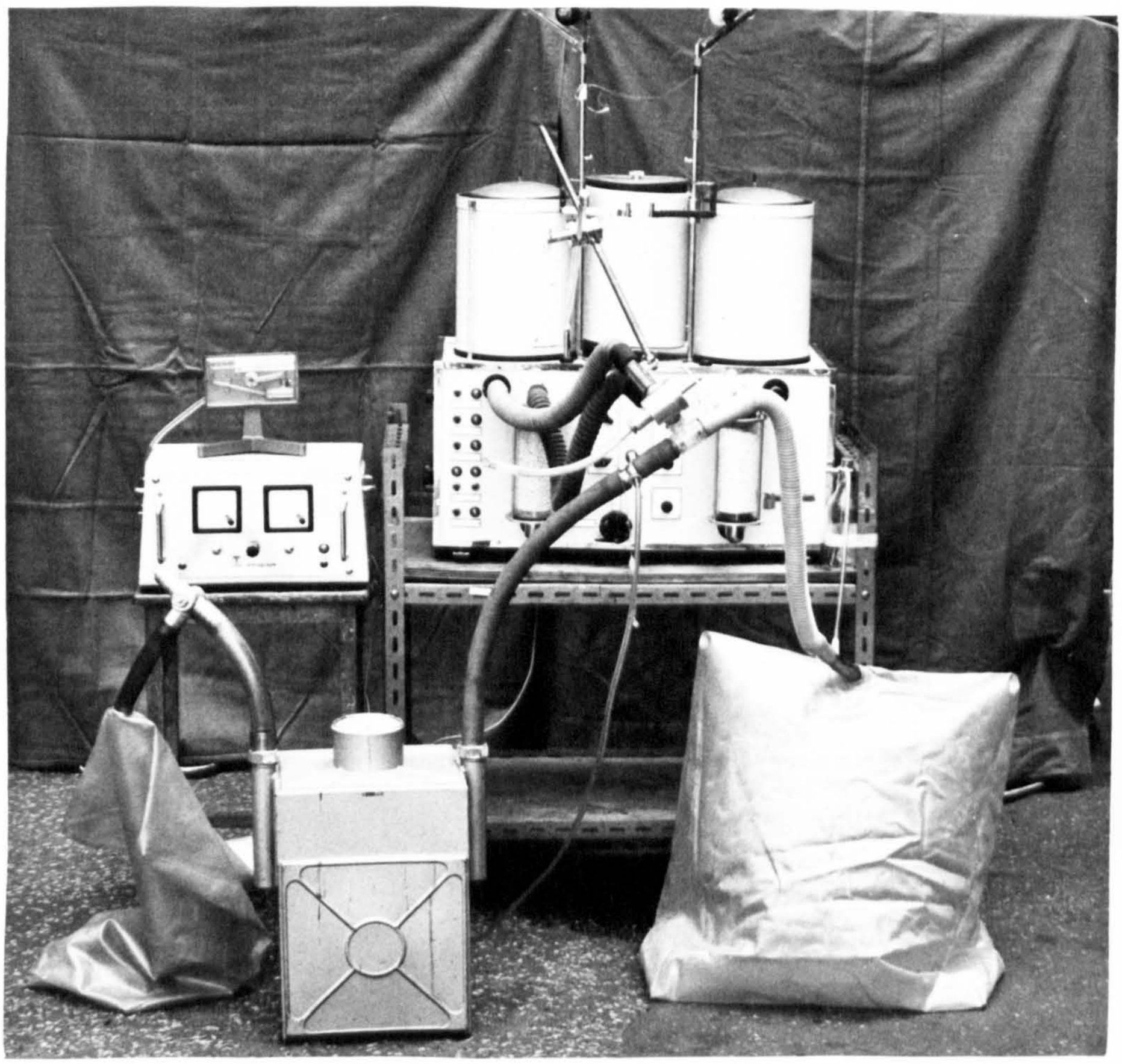


FIGURE 3:4 GAS DELIVERY SYSTEM

The non-rebreathing valve was connected to the animal's endotracheal tube via the two-way tap on the delivery arm of a spirometer (Godart Pulmotest); this allowed the animal to be switched into the spirometer circuit for the recording of respiratory parameters as necessary (Figure 3:4). A tapping in the expiratory line just distal to the non-rebreathing valve was led to a nitrogen analyser (Godart Nitrograph) to provide visual assurance of freedom from contamination.

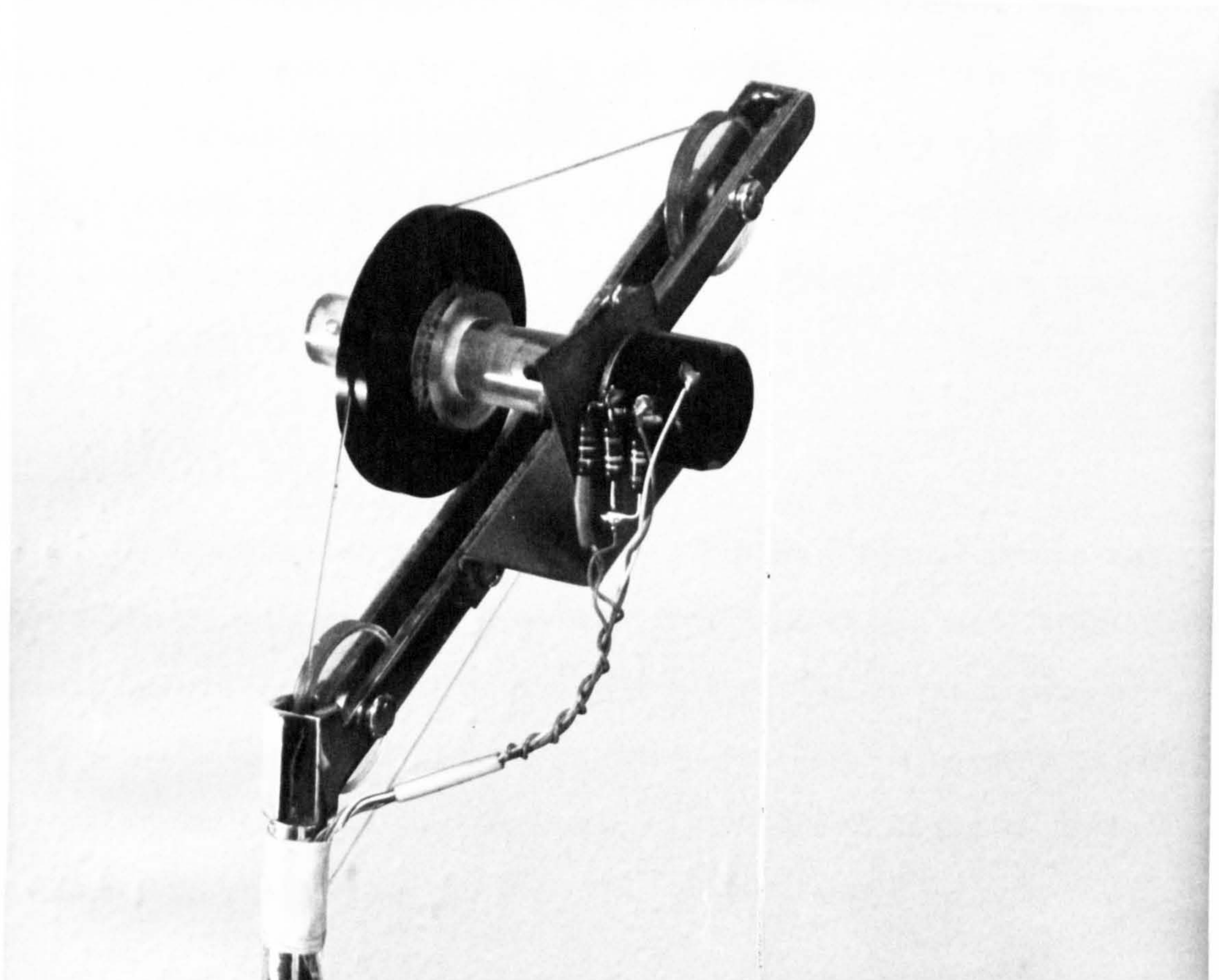


FIGURE 3:5 BRIDGE CIRCUIT MODIFICATION TO PULMOTEST

In the first four experiments in this series ventilation was measured with a pneumotachograph flow head connected to an integrating spirometer (Mercury Electronics CS3). This system proved so unpredictable in its responses and so difficult to calibrate accurately at pressure (a problem still not resolved at the time of writing) that its use was abandoned in favour of a conventional bell spirometer. This was modified to give an electrical output to the pen recorder by leading the operating cord over an extra pulley connected to a ten turn 50 Kohm potentiometer forming one arm of a Wheatstone bridge circuit (Figure 3:5).

Intra-oesophageal pressure changes were recorded from a balloon sensor produced by tying a rubber finger-cote to a Portex cannula. It was found convenient to introduce this by taping it to a thermocouple lead and inserting both into the oesophagus together. Mid-oesophageal temperature was displayed on a direct reading temperature gauge (Ellab, Copenhagen).

Cardiovascular parameters

Systemic arterial, pulmonary arterial, central venous and intra-oesophageal pressures were measured using capacitance transducers (Elema Schonander EMT 35 and 33). Three-way taps on the inputs to each transducer allowed connection direct to either a mercury or water manometer for calibration; this was done before each run of measurements and in practice needed very little adjustment once set.

The output from the transducers together with that from the spirometer, and a Lead 2 electrocardiogram from four limb leads was displayed on an eight-channel ink-jet recorder (Elema Schonander Mingograph 81). The electro-manometers on this piece of equipment have

built-in calibration signals; this was checked and recorded on the paper trace before every measurement.

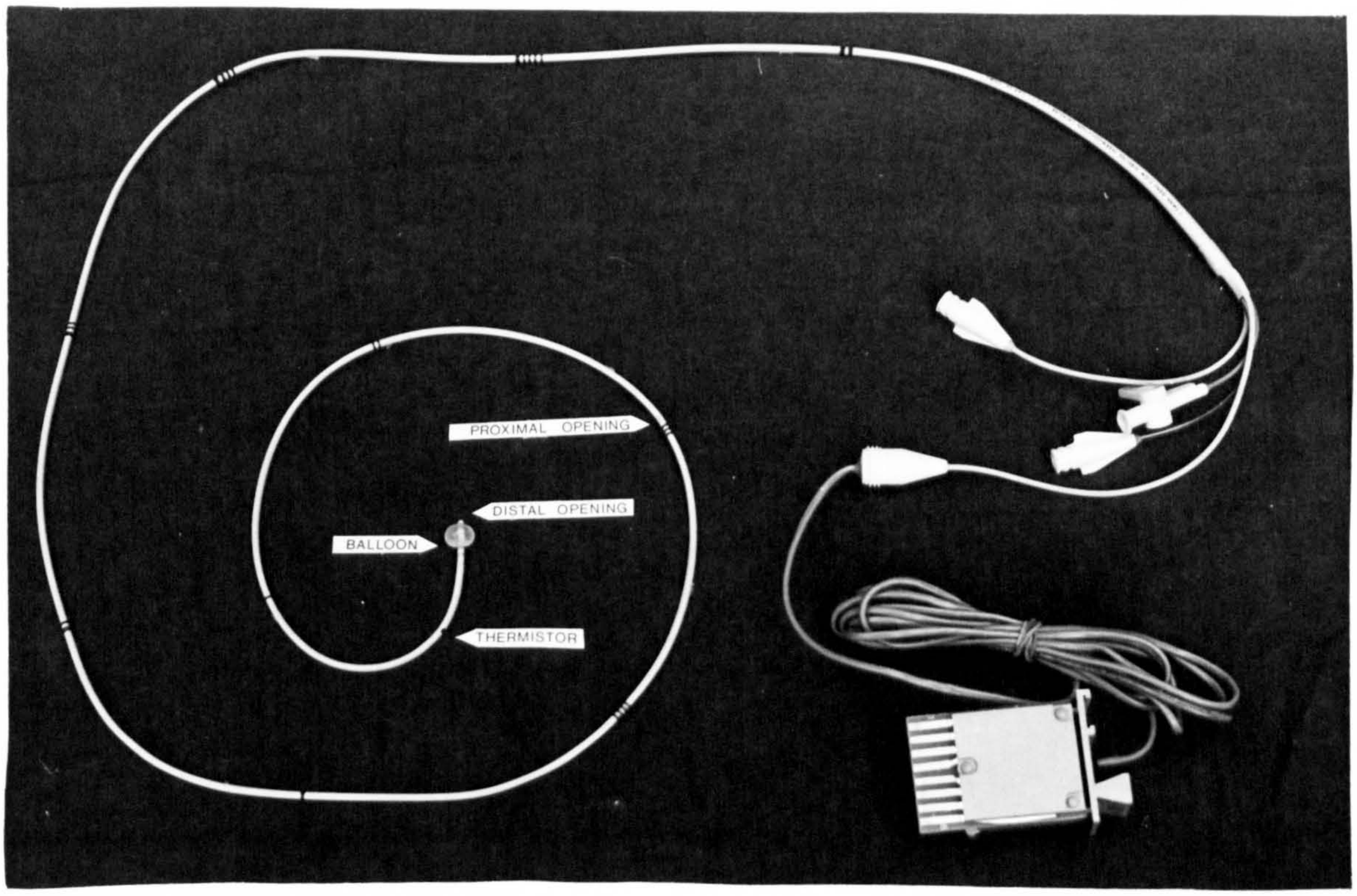


FIGURE 3:6 SWAN GANZ CATHETER

Cardiac output was measured by a thermal dilution technique (Branthwaite & Bradley, 1968) using a Devices Instruments Type 3750 cardiac output computer and a Devices Swan-Ganz Size 7F catheter

(Swan, Ganz & Forrester, 1970) (Figure 3:6). This is a triple lumen catheter with a balloon and thermocouple at the tip. It is inserted via the jugular vein and advanced through the right atrium and ventricle until the tip lies in a fairly small branch of the pulmonary artery, and is so designed that when in this position, a proximal opening lies in the right ventricle. Blood samples can thence be obtained from the right ventricle or pulmonary artery, and pulmonary artery pressure can be recorded. Alternatively, by inflating the balloon and occluding the pulmonary artery, one can obtain the so-called pulmonary artery wedge pressure; since the small pulmonary arteries are end arteries, this reflects the left atrial pressure..

To measure cardiac output a known quantity of cold Dextrose (of known temperature) is injected via the proximal opening and from the changes in temperature recorded at the distal thermocouple an electronically computed measurement of cardiac output can be obtained in less than 30 seconds.

This technique of measuring cardiac output has been shown to have a high degree of correlation with the well-proven dye dilution method (Douglas, McDonald, Milligan, Mellon & Ledingham, 1975).

Before each set of measurements the apparatus was 'zeroed' by triggering the electronic integration process in the absence of any injectate and setting the integrator to give a reading of zero. Care was taken during the actual measurement that the injection (and integration) was commenced at precisely the same phase of the respiratory cycle as the zero-ing run.

The position of the catheter was confirmed by direct fluoroscopic vision using a Siemens X-ray image intensifier.

Respiratory pattern (from the intra-oesophageal pressure

sensor) and electrocardiograph traces were displayed on an Elema Schonander 2-channel oscilloscope and also relayed by telemetry to a similar oscilloscope outside the chamber.

Ringer-lactate and 5% Dextrose (in standard 500 ml bottles) were infused via Baxter administration sets (Travenol Laboratories) by an L.K.B. 'Perpex' perfusion pump set to deliver at approximately 100 ml/hr.

Blood gases (P_{O2}, P_{CO2} and pH) were measured on the appropriate electrodes using Radiometer equipment. The pH electrode was calibrated using standard buffers of known pH and the oxygen and carbon dioxide electrodes using special gas mixtures, the composition of which had previously been checked accurately using the Lloyd-Haldane apparatus. As the oxygen electrode was calibrated in the gas phase, a correction factor had to be applied when measuring P_{O2} in blood (McDowall, Ledingham & Tindal, 1968). This was obtained for each experiment by tonometering a sample of the animals blood against a known tension of oxygen in a rotating syringe (Torres, 1963).

Haemoglobin and haematocrit measurements were performed in the laboratory on blood samples which had been decompressed in the air-lock. Haemoglobin was measured on an Instrumentation Laboratories Model 182 Co-Oximeter, and haematocrit using a microhaematocrit centrifuge (Hawkesley, London).

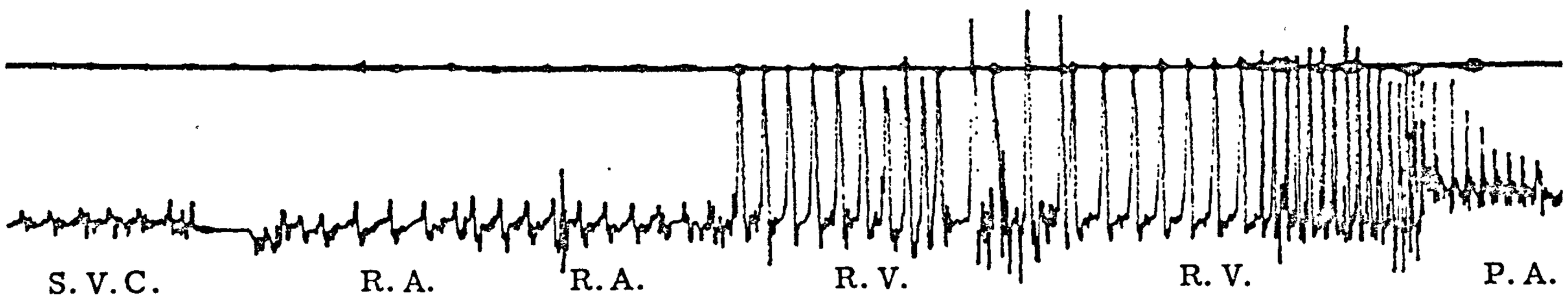
METHODS

Fourteen mongrel dogs of weights ranging from 9 to 16 Kg were used. Of these three breathed 10% O_2/90% N_2 at 2 ATA for 24 hours (normoxic hyperbaric controls) and the remaining eleven breathed 100% O_2 at 2 ATA until death. In addition, two greyhounds, weights 25 and 30 Kg breathed air at barometric pressure for 24 hours (normoxic normobaric controls). These are the two animals referred to on page 3:9; their results are presented in Tables 3:2a & b and Figures 3:1a & b.

The animals were anaesthetised inside the pressure chamber at atmospheric pressure by the technique described on page 3:6 et seq. Atropine (0.3 mg) was given routinely with the anaesthetic agents to lessen the vagotonic effects of RX 320 M. After induction the animals were asleep within ten minutes and were insensitive to pin-prick within fifteen. They remained sensitive to sudden loud noise and vibration, and retained a lash reflex throughout the experiment.

Anaesthesia was too light to permit endotracheal intubation via the larynx and a tracheostomy was performed at the level of the second or third tracheal cartilage. The lines of all skin incisions were infiltrated with 0.5% lignocaine (to a maximum of 5 mls per animal). The cuff of the endotracheal tube (Portex disposable) was inflated with water to prevent its collapse during pressurisation. Following intubation the lungs were inflated manually several times using an anaesthetic balloon of oxygen to reverse any collapse and hypoxaemia.

PRESSURE TRACE: INSERTION OF SWAN GANZ CATHETER



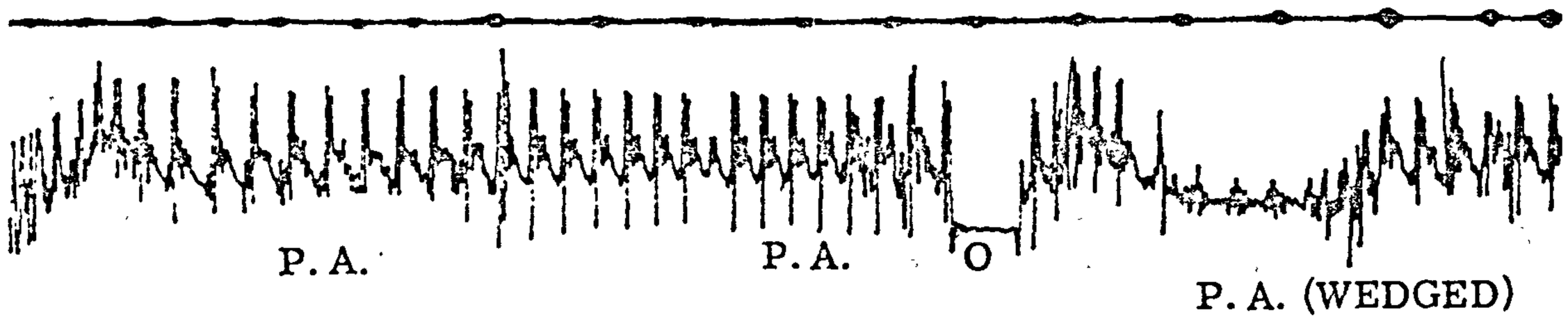


FIGURE 3:7

S.V.C. = Superior vena cava, R.A. = Right atrium
R.V. = Right Ventricle, P.A. = Pulmonary artery.

Long venous and arterial cannulae (Portex polypropylene) were inserted via cut-downs into the femoral artery and vein and advanced proximally until the tips were lying in the thoracic aorta and vena cava; these were used for pressure recording and blood sampling. A separate short femoral venous cannula was inserted into the opposite side for the administration of drugs and for fluid replacement.

The pulmonary artery catheter was inserted via a cut-down on the jugular vein and advanced via the right side of the heart until its tip lay in the pulmonary artery. Its position at any instant was judged from the pressure waveform obtained from the distal opening (Figure 3:7) and its final position was checked by direct fluoroscopic vision. Once in this position a check was made that inflation of the balloon caused the catheter to 'wedge' in the artery.

The animals were not heparinised but all cannulae were flushed with heparinised saline following the aspiration of a blood sample and before any pressure recording.

The combined oesophageal balloon and thermocouple were inserted into the pharynx and advanced down the oesophagus by inducing the animal to swallow by injecting some water from a syringe into the pharynx. The balloon was advanced into the stomach (as shown by a positive pressure deflection on inspiration) and then withdrawn into the oesophagus; this prevented the balloon from becoming kinked. A final position was chosen at a level where the cardiac artifact did not intrude on the pressure trace and the cannula was fixed in this position by taping.

It was found in the course of the experiments that a greater sensitivity was obtained when the system was air-filled rather than liquid-filled. A criticism is that the actual volume of air in the balloon appeared to affect the amplitude of the pressure waveform. To minimise this variation as much as possible the balloon was left vented to chamber atmosphere between recordings and only inflated with a standard 1 ml of air immediately before making a measurement.

Subcutaneous needle electrodes for electrocardiograph leads were inserted in all four limbs.

All the animals in this series lay right side down on the operating table.

During the surgical procedures, the blood gas apparatus, manometers and pen-recorder were calibrated by technical assistants; the chamber was then pressurised, over a period of approximately eight minutes, to 2 ATA. On arrival at pressure the initial calibrations were rechecked and adjusted. The dogs' lungs were again inflated manually to reverse any atelectasis during pressurisation and were then connected to the oxygen supply.

The experiment was timed to start from this point.

'Runs' of measurements were made at approximately four hour intervals initially, increasing to every two hours after twelve hours. When it became evident that the animal was in decline, measurements

were made every 30 minutes and an attempt was made to record date continuously (insofar as was possible within the limitations of the techniques involved) for the last hour or so before death.

The order in which data was obtained was chosen so as to disturb the animal as little as possible. 'Non invasive' measurements such as respiratory parameters (frequency, tidal volume, oxygen uptake and oesophageal pressures) were made first followed by pressure recordings (systemic arterial, central venous, pulmonary arterial, and pulmonary arterial wedged pressures) and a Lead 2 electrocardiogram. Expired gas was then collected over a 5 minute period in a Douglas bag (having previously partially emptied the oxygen reservoir to temporarily avoid spill through the expiratory valve), measured on the Parkinson Cowan gasometer and analysed for mixed-expired P_{CO_2}. During this collection, blood gases were measured (on arterial samples from the aorta and mixed venous samples from the pulmonary artery) and the temperature recorded.

Finally the cardiac output was measured by the injection of 10 mls of cold Dextrose, and a blood sample taken for haemoglobin and haematocrit estimations.

With practice it became possible to complete the whole series of measurements in 15 to 20 minutes and in the final stages, when the more time-consuming techniques such as collection of mixed-expired gas and spirometry were abandoned, the vital pressures, gases and cardiac output could be monitored approximately every 7 to 8 minutes.

During the course of the experiment, fluid balance was maintained (on the basis of haematocrit and central venous pressure

measurements) by the administration of Ringer-lactate and 5% Dextrose. The average rate of infusion was about 1 litre every 10 hours.

The dogs' temperature was kept as close to 37.0° as possible by use of the chamber air conditioning system supplemented by fans or heating lamps as necessary.

Urinary bladder catheterisation and gastric aspiration were performed as indicated for the relief of distension.

CALCULATIONS

The P_{O2} measurements were first corrected for the 'blood-gas' factor (see page 3:18, typical value = 1.06) and then corrected for any difference in temperature between the electrode system (37°) and the animal's mid-oesophageal temperature. It was noted that the usual method of doing this, by use of the dog cursor on the Radiometer blood-gas calculator, was not valid: this instrument is based on 'average' oxy-haemoglobin dissociation curves (Severinghaus, 1966) and is not accurate above 95% saturation. The computer (Hewlett Packard Model 9100B) was programmed to correct for temperature using Severinghaus' data for oxygen tensions below 100 and a combination of special correction factors derived from Severinghaus (1966) and Hedley-Whyte & Laver (1964) for values above 100 mm.Hg.

Temperature correction of P_{CO2} and pH was by use of the Radiometer blood-gas calculator.

Blood oxygen saturation was calculated from P_{O2} and pH taking into account temperature and P_{CO2} (Kelman, 1966).

The oxygen-combining capability of haemoglobin was taken as 1.34 ml/g in all calculations, and blood oxygen contents were calculated as follows:

Blood Oxygen Content (mls(STPD)/100ml = Hb (g) x 1.34 x % saturation + (P_{O2} (torr) x 0.0031) (Bunsen co-efficient)

As the experimental animals were breathing 100% oxygen, alveolar oxygen tension could be calculated thus:

$\underline{\text{Ideal Alveolar } P_{O2}}$ (torr) = Inspired P_{O2} (torr) (measured saturated with water vapour at 37°C.) minus arterial P_{CO2} (mm.Hg.)

In the air-breathing animals, the alveolar gas equation:

$$\underline{P_AO_2} = P_IO_2 - \frac{P_ACO_2}{R} + \left[P_ACO_2 \cdot F_IO_2 \cdot \frac{I-R}{R}\right]$$ was simplified to:

$$\underline{P_AO_2} = P_IO_2 - \frac{P_ACO_2}{R} + 2 \text{ mm.Hg.}$$ (valid where $P_{CO2} = 40$, $F_IO_2 = 0.21$ and $R = 0.8$)

(West, 1974). R was taken to be 0.8 throughout. Equality of arterial and alveolar carbon dioxide tensions was assumed.

Pulmonary end-capillary oxygen content was calculated assuming equilibration with the ideal alveolar oxygen tension, and percentage pulmonary shunt calculated:

$$\underline{\dot{Q}s/\dot{Q}_T} = \frac{C_c'O_2 - C_aO_2}{C_c'O_2 - C_{\bar{v}}O_2} \times 100$$ (all contents at STPD)

Cardiac output was obtained directly from the computer following evaluation of the equation:

$$\underline{\text{C.O. (litre/min)}} = \left[\frac{AV(Ti - Tb)\ Di.Si}{\int \Delta Tb.dt \times Db.Sb} - B\right]$$

where A and B are constants, the suffixes i and b refer to injectate and blood, and:

V = injectate volume (litres)
T = temperature (°C)

ΔT = incremental temperature (°C)

D = density (g/ml)

S = specific heat (cal/g°C)

t = time (mins)

In practice, (Douglas et al., 1975);

Si = 0.964 cal/g°C

Sb = 0.87 cal/g°C

Di = 1.018 g/ml

Db = 1.057 g/ml

A = 0.96 } from the thermal dilution/Fick regression equation

B = 0.20 }

As the relevant data was available, a double check was made on cardiac output using the Fick principle:

$$\text{C.O. (l/min)} = \frac{\dot{V}O_2}{CaO_2 - C\bar{v}O_2} \quad \text{(contents in mls STPD/100ml)}$$

<u>Pulmonary vascular resistance</u> was calculated:

$$PVR = \frac{PAP - PAP\ wedge}{Cardiac\ \ output}$$

and expressed in 'resistance units' - $\frac{mm.Hg.}{l/min}$

Physiological dead space was calculated using the Bohr equation assuming equilibration of arterial with effective alveolar carbon dioxide tensions:

$$V_D = \frac{P_aCO_2 - P_{\bar{E}}CO_2}{P_aCO_2} \times V_T$$

Alveolar ventilation: $\dot{V}_A = f(V_T - V_D)$

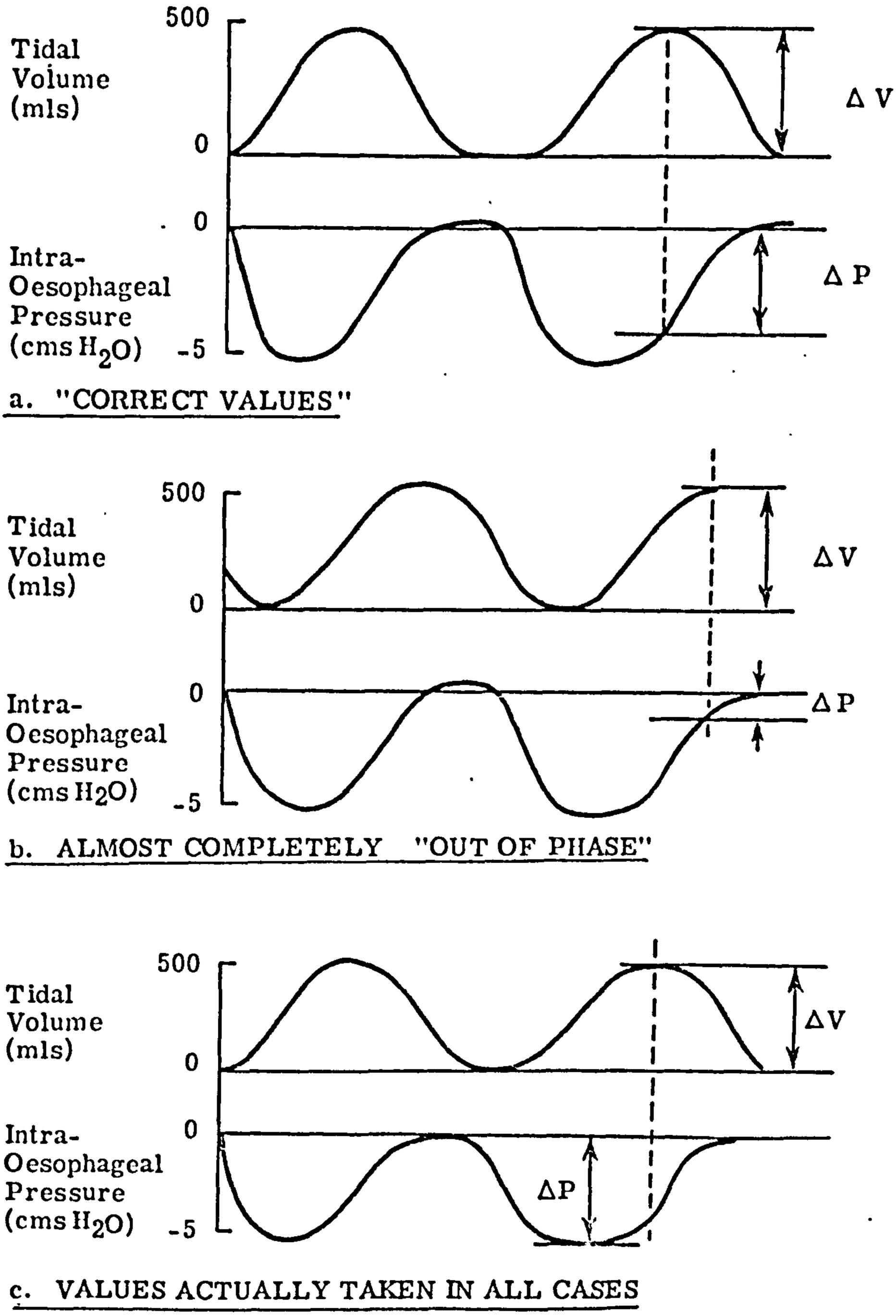
Pulmonary compliance was calculated:

$C = V/P$ where V is lung volume and P is oesophageal pressure.

For various technical reasons the values obtained may be used only as an indication of changes in compliance over the course of the experiment and cannot be presented in absolute terms. The method of obtaining the 'volume' signal on the trace (page 3:14) was crude; also it was found that, due to the inertia in the spirometer bell and to flexibility of the drive cord and pulley mechanism, the recorded volume signal tended to lag behind the pressure signal, and at high breathing rates could get completely out of phase. Figure 3:8b shows this compared with the normal in Figure 3:8a. It was felt that the smallest consistent error in compliance would be obtained by using the peaks of both the volume and pressure waves (Figure 3:8c).

In addition to this approximation, it was found that the magnitude of the pressure swings recorded from the intra-oesophageal balloon varied with the amount of air in the balloon, with the length and diameter of the cannula and also varied (in the same animal) with different balloons.

To allow for mean values of changes to be obtained for the whole series of animals, all the baseline values of compliance obtained for each dog (i.e. all recordings made within the first ten hours of the experiment) were meaned and taken arbitrarily as 100 units. All subsequent values were related to this baseline and expressed as 'compliance units' (Figure 3:11). These values serve to show changes in stiffness of the lungs over the course of the experiments although they do not give quantitative values for compliance.



a. "CORRECT VALUES"

b. ALMOST COMPLETELY "OUT OF PHASE"

c. VALUES ACTUALLY TAKEN IN ALL CASES

Figure 3:8 VALUES USED IN CALCULATION OF COMPLIANCE

Haemoglobin was estimated by measuring the optical density of a haemolysed sample of whole blood.

RESULTS

Controls

Both the two normoxic normobaric controls (described on page 3:9, Tables 3:2a & b and Figures 3:1a & b) and the three normoxic hyperbaric control animals (Dogs 9, 10 & 13) remained essentially normal until sacrifice at 24 hours.

Results eliminated from series

One animal (Dog 7) was discarded completely from the series. At some time between 9 and 13 hours from the start of the experiment the arterial oxygen tension fell from 1305 to 43 mm of mercury and remained at an average value of around 30 mm of mercury for the remaining six hours that the animal survived. This collapse in oxygen tension was accompanied by a rise in pulmonary shunt ratio to 96%. Autopsy confirmed the suspected bilateral pneumothorax. The prolonged survival time at such low arterial oxygen tensions is probably explained by the occasional finding of oxygen tensions of around 115 mm Hg (with shunt falling to 39%) followed immediately by return to very low levels; it is thought that there must have been periodic re-expansion of part of a lung from time to time.

In one other animal (Dog 5) there was an inward leak of air to the inspiratory side of the circuit for an unknown length of time during the earlier part of the experiment. This was corrected and the experiment allowed to continue. As the subsequent events were similar to those in the other animals, the results were included in the final calculations, with the exception of the actual 'time to death', which had been artificially prolonged.

General pattern of results

1) The results confirmed the previous findings of Smith et al. (1963) in normobaric, and Clarke et al. (1973) in hyperbaric conditions, that animals dying of acute pulmonary oxygen toxicity remain essentially normal in most measured parameters until some 3 to 4 hours before death, at which time there is a relatively sudden collapse.

2) The animals formed themselves into two very well-defined groups which died completely differently. The main group of 8 out of 10 dogs succumbed to what might be labelled a 'pulmonary' death while the remaining two animals died a 'cardiac' death.

Time to death

The mean time to death of the 'pulmonary death' animals (less Dog 5 - see above) was 22 hours, 16 minutes with a range of 20 to 25 hours. The two 'cardiac death' animals both died at just over 19 hours.

This agrees well with Clarke's finding, at identical pressures, of a mean time of 18 hours, 45 minutes (range 16:05 to 20:50 hours) as the majority of their animals (4 out of 6) appeared to die a 'cardiac' death.

Convulsions in relation to time to death

There is no reference in Clarke's paper to any of his animals suffering epileptiform convulsions due to CNS oxygen toxicity. In this experiment, five of the ten experimental animals convulsed, four from the 'pulmonary', and one from the 'cardiac' death group.

In view of the conflicting evidence on the effects of brain damage on pulmonary pathology (see page 3:5), the differences between convulsed and non-convulsed animals are potentially of interest and are shown in Table 3:3.

TABLE 3:3

Convulsions/time of death

Dog No.	Mode of Death	Time to Convulse (mins)	Mean P_{O2} until 400 mins before death (mm.Hg.)	P_{O2} time of seizure (mm.Hg.)	Time of Death (hours)
1	Pulmonary	-	1042	-	21:35
4	Pulmonary	-	1049	-	23:10
11	Pulmonary	-	1108	-	19:10
14	Pulmonary	-	1340	-	22:10
MEAN	-	-	1135	-	21:43
3	Pulmonary	25 & 145	1044	1122 & 1185	21:55
5	Pulmonary	1056 *	----	945	----
6	Pulmonary	60	1037	1075	25:00
8	Pulmonary	70	1122	1155	22:05
MEAN	-	----	1068	1096	23:00
2	Cardiac	---	1102	----	19:10
12	Cardiac	100	1249	1277	19:05

* obtained by calculation backward from time of death, on the basis of a mean time of death of 22:16 hours - see page 3:29

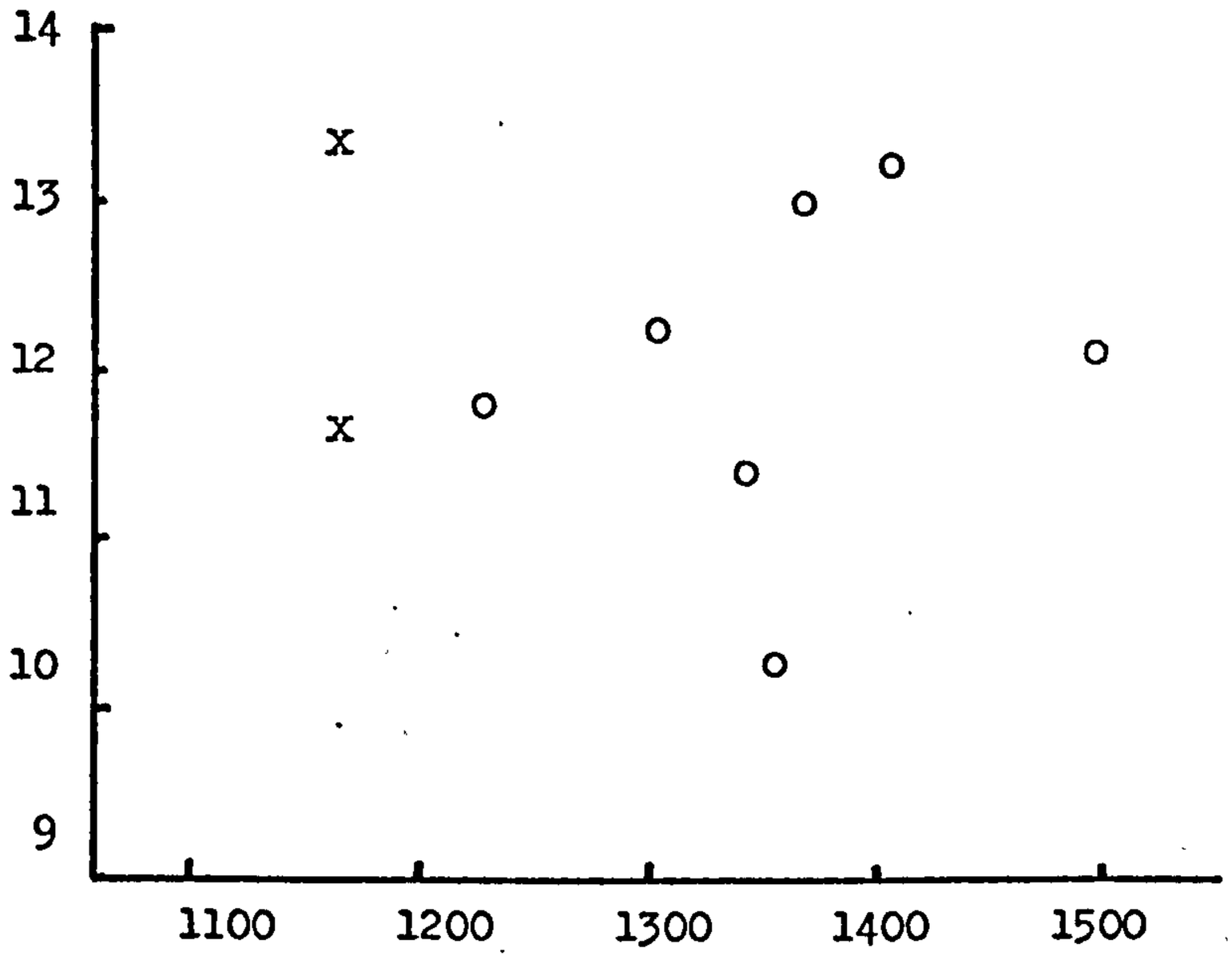
The apparent greater survival time of the animals which had convulsed does not reach the level of significance, but there is no evidence here that an oxygen-induced convulsion shortens the time to death, or has any effect on the mode of death.

Anaemia/Time to death

It has been shown that anaemia is a pre-disposing factor in the aetiology of the 'shock lung' syndrome in dogs (Moss & Stein, 1973). In view of the similarity of pathological appearances in the two conditions, this factor was examined; the haemoglobin level did not appear to have any effect on the final outcome (Figure 3:9).

FIGURE 3:9

Anaemia/time to death



X = 'cardiac death' o = 'pulmonary death'

Cardiorespiratory parameters

All corrected and derived results for each animal were gathered together on a single sheet; Table 3:4 is a typical example.*

In order that the crucial final hours of the experiment could be studied in detail and displayed graphically, the results were meaned and plotted retrospectively against time before death.

This was done for 20 minute increments of time over the last 100 minutes before death, for 50 minute increments for the preceding 100, and for 100 minute increments back to 600 minutes before death.

Figures 3:12 to 3:21 show these mean values plotted together with their standard errors for a variety of parameters; only results over the last ten hours of the experiment are presented, except that the first value plotted at 10 hours before death represents the mean and standard error of all the values recorded before that time.

The results from Dogs 2 and 12, the 'cardiac death' animals, were dealt with separately and are shown where appropriate on the graphs as broken lines.

Ventilation

Clarke et al. (1973) described a consistent sequence of respiratory disturbances in oxygen-poisoned animals, with orderly periods of rapid regular, irregular, and slow regular respiration followed by a decline to apnoea.

This progression of changes was confirmed in both the 'pulmonary' and 'cardiac' death dogs and is illustrated in Figure 3:10. A constant finding was the appearance late in the experiment of a distinctive low-amplitude 'square-wave' respiratory pattern character-

* presented in Appendix 2

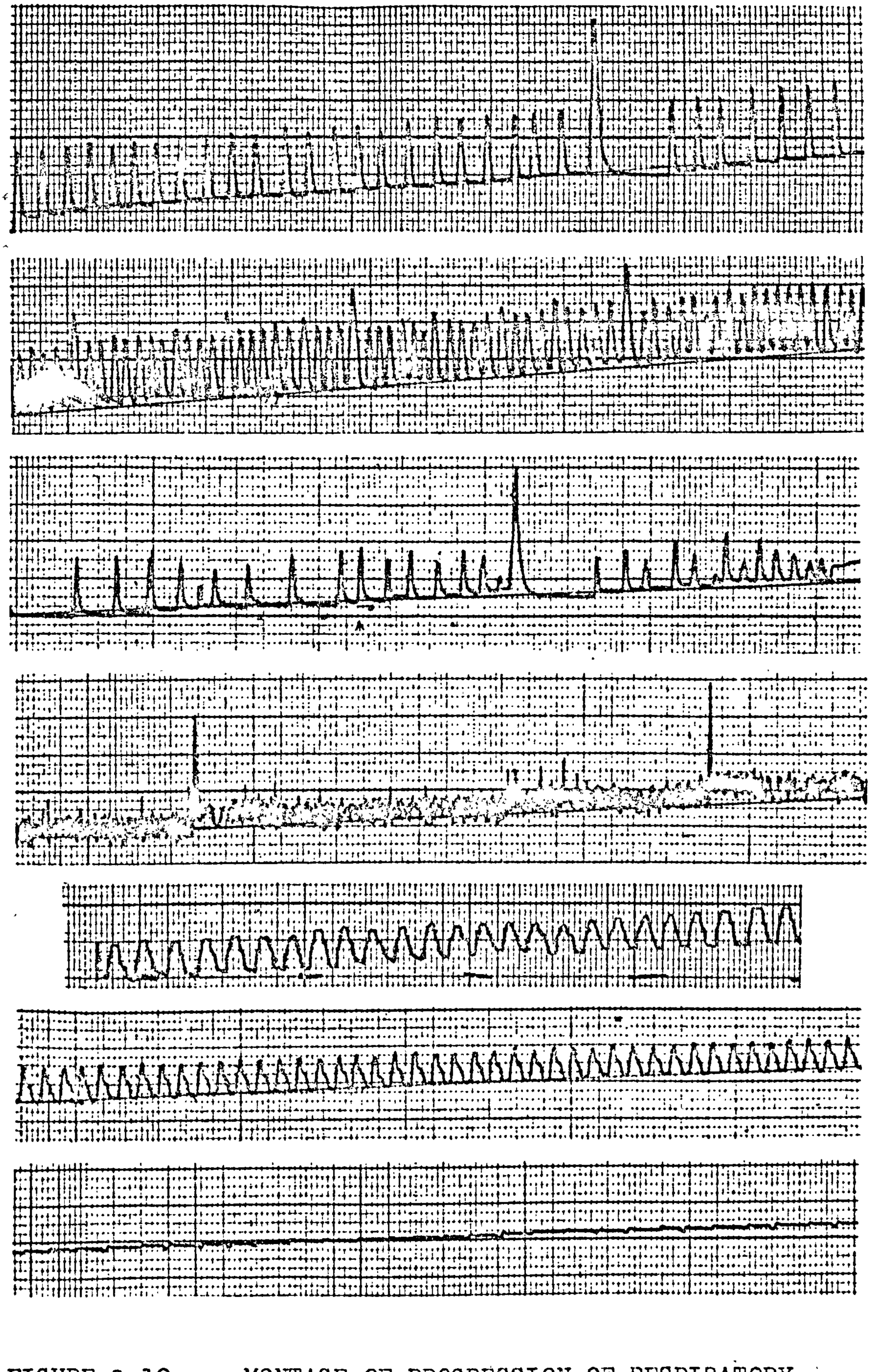


FIGURE 3:10. MONTAGE OF PROGRESSION OF RESPIRATORY PATTERNS.

ised by forceful inspiration causing rib and jaw retraction, a long inspiratory pause, and active use of expiratory muscles. The time of appearance of this pattern was very variable, averaging around some six to eight hours before death, but once established, persisted with a gradually decreasing amplitude to apnoea.

Due to equipment failure, respiratory recordings are available for only one of the 'cardiac death' dogs (Dog 12); in this animal, the appearance of jaw retraction and 'square-wave' breathing did not appear until very late in the study, at 95 minutes before death.

In Clarke's study, during both the regular rapid and irregular phases of respiration there was a marked rise in minute ventilation (simultaneously with a decrease in tidal volume), followed by a return to the initial levels during the final slow regular phase. These changes were not reflected in the alveolar ventilation which remained remarkably constant throughout.

In the present study similar changes in minute ventilation and tidal volume were found, but the level of alveolar ventilation also appeared to fluctuate greatly. No consistent pattern was observed other than that in the terminal stages tidal volume usually fell so far as to encroach on anatomical dead space, reducing alveolar ventilation to zero.

Compliance

It rapidly became obvious in the 'pulmonary death' animals that compliance was the first of the recorded parameters to show any change.

The limitations of the recording technique have been described (page 3:27); Figure 3:11 shows the trend of the changes over the last ten hours. All of the values previous to this time have been meaned and taken as 100%; it can be seen that by ten hours before death, there was already a fall in compliance to about 70% of the base-line value.

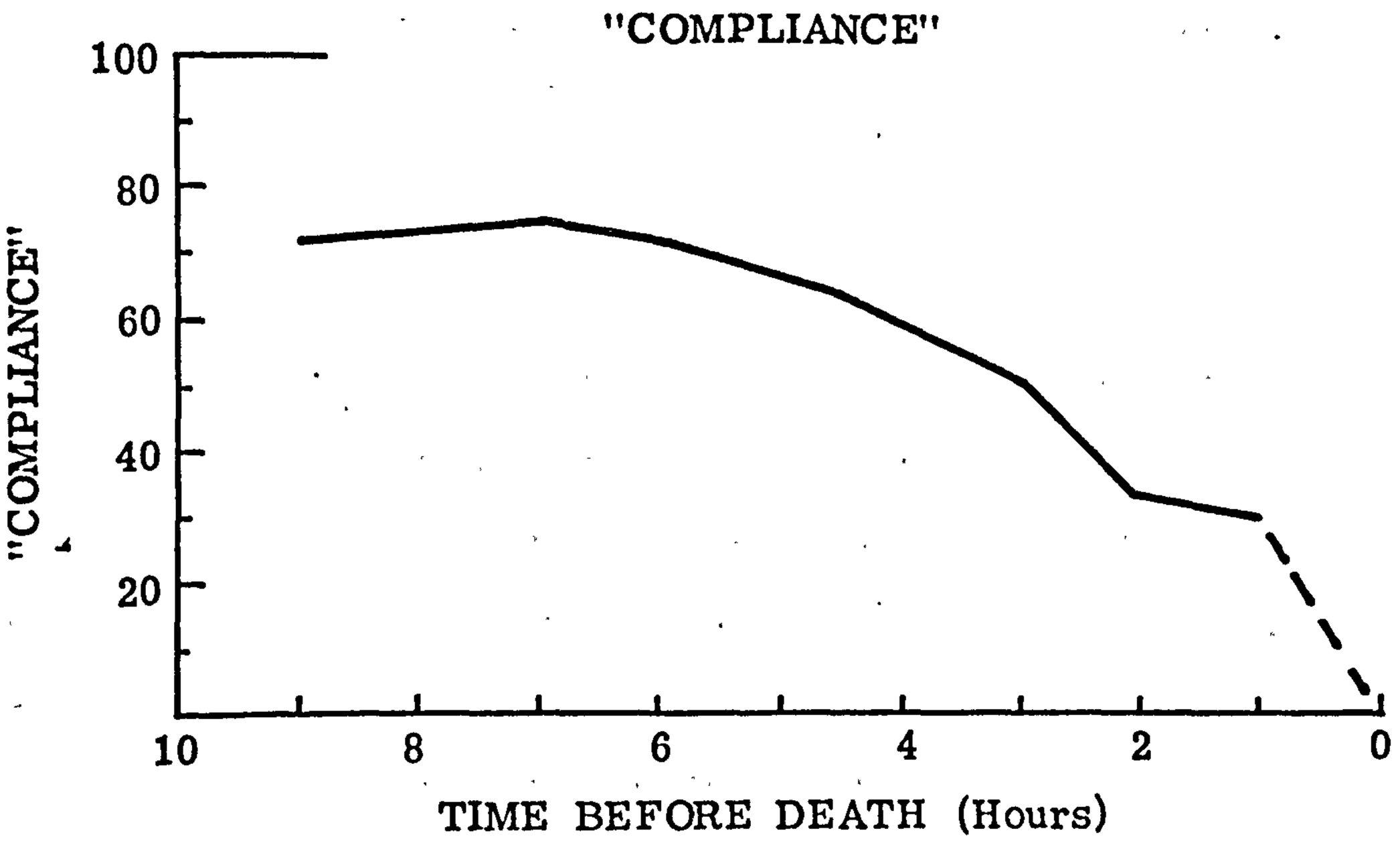


FIGURE 3:11

COMPLIANCE : PULMONARY DEATH ANIMALS

At about one hour before death, the amplitude of the ventilation waveform had usually become so small that a calculation of complaince could not be done with any pretence at accuracy. In the terminal stages, at so-called 'apnoea', very large swings in intra-oesophageal pressure were being recorded with a complete absence of gas movement. Compliance had therefore fallen to zero; this is indicated in Figure 3:11 by the broken line.

Of the 'cardiac death' animals, compliance estimations are not available for Dog 2; in Dog 12 compliance was normal at 10 hours before death and only fell to very low levels at about 90 minutes before death.

Blood gases

In the 'pulmonary death' animals, the arterial oxygen tension (P_aO_2) remained virtually normal until approximately 200 minutes before death, when it suddenly began to fall. The slope of the decline was almost a straight line with a decrement of approximately 300 mm.Hg. per hour (Figure 3:12). The effect of this on the alveolar-arterial oxygen tension difference is shown in Figure 3:13.

The 'cardiac death' dogs differed from this pattern and from each other: in Dog 2, the P_aO_2 had declined from about 1150 at 10 hours before death to 880 at $3\frac{1}{2}$ hours, but then climbed again to reach 974 at the moment of death. In Dog 12, the P_aO_2 remained very high (up to 1376) until 200 minutes before death and then fell rapidly. The slope of the decline was not as steep as that of the 'pulmonary death' dogs, in the initial stages at least, and as the resting baseline was much higher, P_aO_2 decreased only to 760 mm.Hg. at the time of death.

Figure 3:14 shows values of arterial carbon dioxide tensions (P_aCO_2) in the final stages. These remained completely normal until 200 minutes before death and then began to climb, reaching a mean in the 'pulmonary death' animals of 116 mm.Hg. at the moment of apnoea.

The 'cardiac death' animals again differed from each other in that in Dog 12 the climb in P_aCO_2 in the last 200 minutes was steeper than in the 'pulmonary death' animals, and terminated at a P_aCO_2 of 160 while in Dog 2 the rise only occurred in the last 90 minutes and at 15 minutes before death (time of the last blood-gas measurement) had reached only 67 mm.Hg.

Systemic circulatory parameters

The mean heart rate for all the 'pulmonary death' animals up until ten hours before death was 80 (± S.E. of 6) (Figure 3:15). At about ten hours, the rate rose steeply and was maintained at around 135 for about six hours. At about 100 minutes before death there was a gradual slow decline, and the heart rate at the moment of apnoea was 110.

The 'cardiac death' dogs also had baseline values of 75 to 80 up until ten hours before death, and thereafter also displayed a sharp rise. The rise, however, was slowly progressive over the next eight hours, reaching very high values (up to 220 in Dog 2). This was followed about an hour or so before death by a sudden very steep drop in rate.

Mean systemic blood pressure in the 'pulmonary death' dogs remained around 85 mm.Hg. in the early part of the experiments, then

climbed between ten and eight hours before death to 100 mm.Hg. (Figure 3:16). This was followed by a linear decline over five hours back to the baseline, and then by a more rapid decline over the last three hours to reach a mean of just below 40 mm.Hg. at the moment of apnoea.

The 'cardiac death' dogs also had a baseline value of 85 mm.Hg. in the early stages. Dog 2 then showed a progressive rise in mean pressure to 135 at three hours before death followed by a sudden rapid collapse to 25 mm.Hg. at death. Dog 12 remained normal until three hours before death and then also suffered a sudden collapse down to 20 mm.Hg.

Cardiac output was meaned for all the animals up until ten hours before death and plotted as a baseline of 100% (Figure 3:17). Subsequent values were corrected proportionally and plotted as meaned percentages of baseline.

The 'pulmonary death' animals maintained a normal cardiac output until about five hours before death and then showed a fluctuating decline to 63% over the next three hours. This level was maintained until death two hours later. One of the 'pulmonary death' animals (Dog 6) was atypical and was not included in Figure 3:17. In this animal cardiac output began to rise at between five and four hours before death, reaching 290% at just under two hours and falling back to 200% at one hour before death (time of last measurement). It should be noted that, although in all other respects this dog behaved similarly to the other 'pulmonary death' animals, it was the only one of the whole series in which apnoea did not precede asystole.

Both of the 'cardiac death' animals showed a linear decline in cardiac output over the last ten hours reaching very low values at the time of death (44% for Dog 12 and 27% for Dog 2).

Pulmonary circulatory parameters

The meaned value of mean pulmonary artery pressure for all the 'pulmonary death' dogs up until ten hours before death was 11 mm.Hg. (Figure 3:18). This then climbed to 14 at eight hours, returned to and remained at baseline levels from seven to five hours, fell gradually to 6 at just under two hours, suddenly recovered to 8 mm.Hg. and maintained a value of 7 until the moment of apnoea.

The 'cardiac death' dogs showed a similar pattern with a peak at about eight hours before death, followed by a similar decline and levelling off. The absolute values obtained in Dog 2 were approximately double that of the other animals.

Figure 3:19 shows pulmonary artery wedged pressure. The mean value in the early hours was 3.7 mm.Hg. In the 'pulmonary death' animals this had fallen to 2.2 at eight hours and then declined with fluctuations to 1.6 at one and a half hours before death. Then followed a sudden sharp dip to 0.5 mm.Hg. at eighty minutes before death with recovery to approximately 1.6. The mean of the last measured values was 1 mm.Hg.

For technical reasons, wedge pressure is not available from Dog 2. Dog 12 followed the above pattern, with a similar sharp dip at 90 minutes before death.

Pulmonary vascular resistance (Figure 3:20) rose from a

baseline mean of 3.4 'resistance units' to 5.3 at just over six hours before death, fell back to 2.4 at five hours, rose again to 4.8 at around three hours and maintained that level (with large fluctuations and individual variations) until death.

In the 'cardiac death' animals the pattern was similar. The actual values for Dog 2 are artificially high as they are calculated on the basis of pulmonary artery pressure alone, wedge pressure not being available for this animal.

Percentage pulmonary shunt ratio is shown on Figure 3:21. In the 'pulmonary death' animals this remained at the mean baseline level of around 20% until three hours before death when it rose steadily; the mean value at the moment of apnoea was 48%.

In Dogs 2 and 12 percentage shunt showed a net fall over the last ten hours of the experiments, reaching terminal values of 11%.

Autopsy

All the animals with the exception of Dog 6 were apnoeic before death.

The trachea was clamped and the chest opened by shearing through the costal cartilages just to one side of the sternum within thirty seconds of apnoea; in all the animals except Dog 6, the heart was still beating.

The lungs were fully expanded in all cases and there was no evidence of any focal atelectasis.

In the 'pulmonary death' dogs, the lungs were purple in

colour, reflecting the severe terminal arterial desaturation. In the 'cardiac death' dogs, they were bright pink.

A variable (usually very small) quantity of fluid was present in the pleural and pericardial spaces.

In the 'pulmonary death' animals there was copious oedema fluid in the trachea.

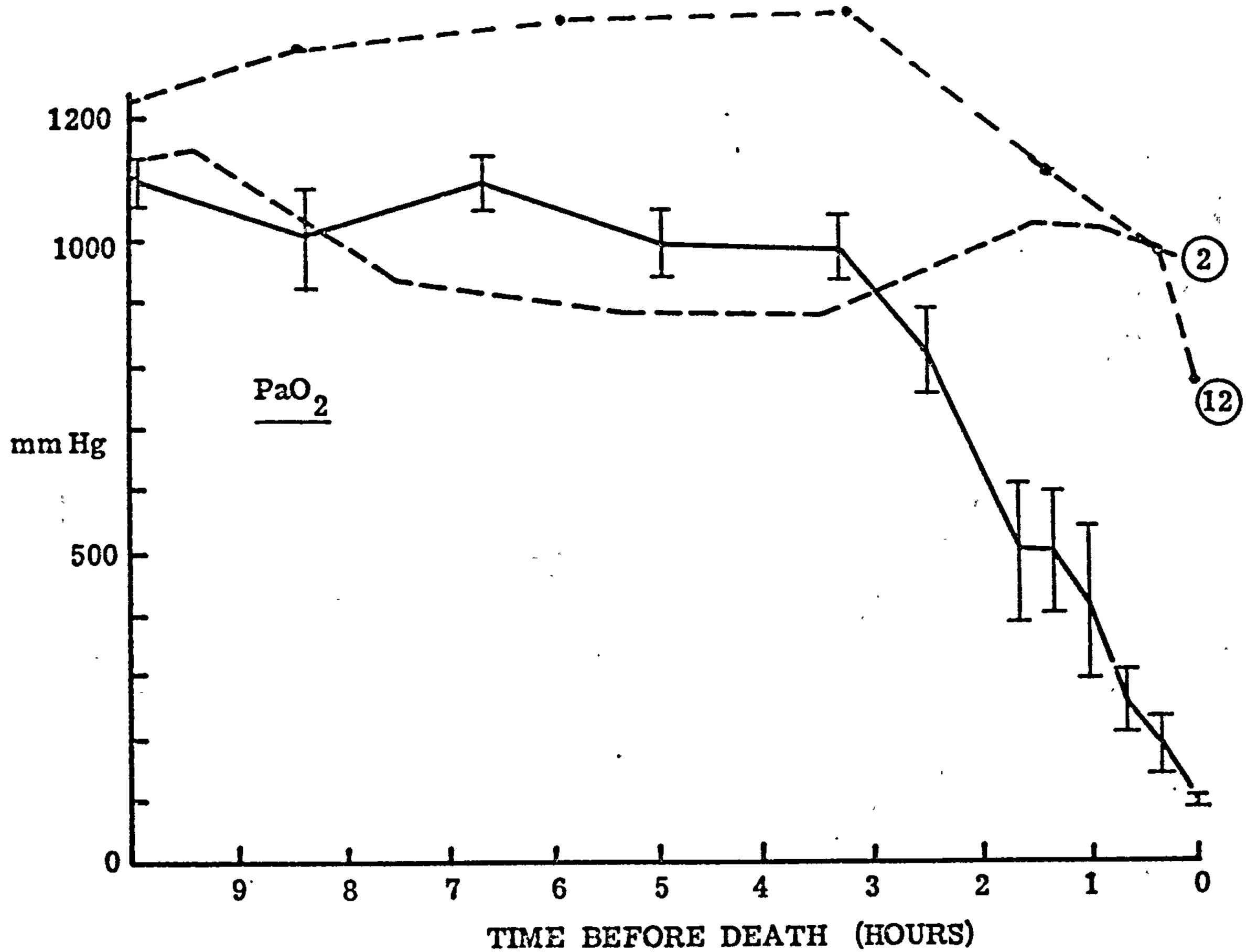

1200
1000
mm Hg
500
0
PaO_2
2
12
9
8
7
6
5
4
3
2
1
0
TIME BEFORE DEATH (HOURS)

FIGURE 3:12.

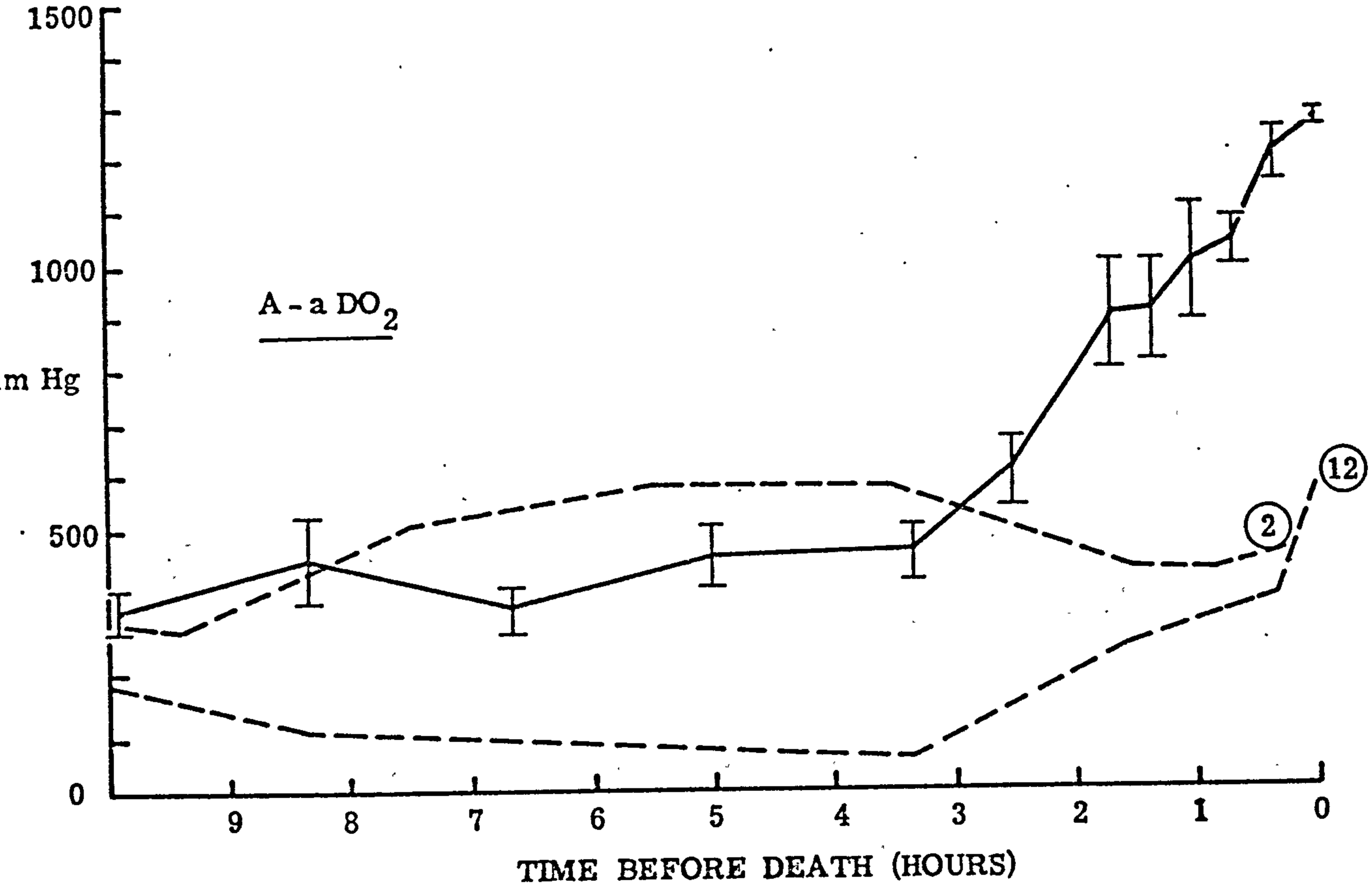

1500
1000
500
0
mm Hg
A - a DO2
9
8
7
6
5
4
3
2
1
0
TIME BEFORE DEATH (HOURS)
2
12

FIGURE 3:13.

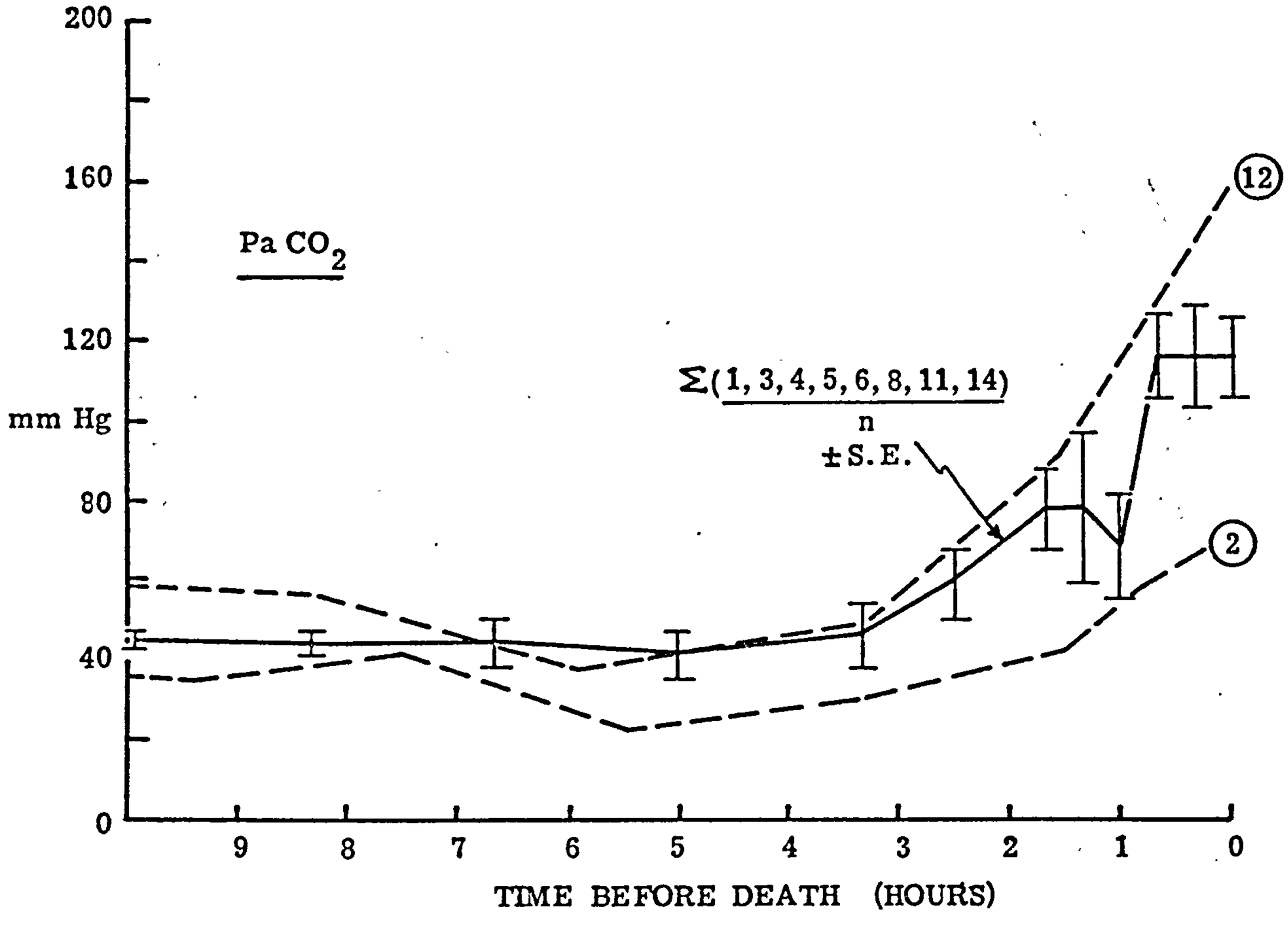

200
160
120
mm Hg
80
40
0
Pa CO2
Σ(1, 3, 4, 5, 6, 8, 11, 14)
n
±S.E.
12
2
9
8
7
6
5
4
3
2
1
0
TIME BEFORE DEATH (HOURS)

FIGURE 3:14.

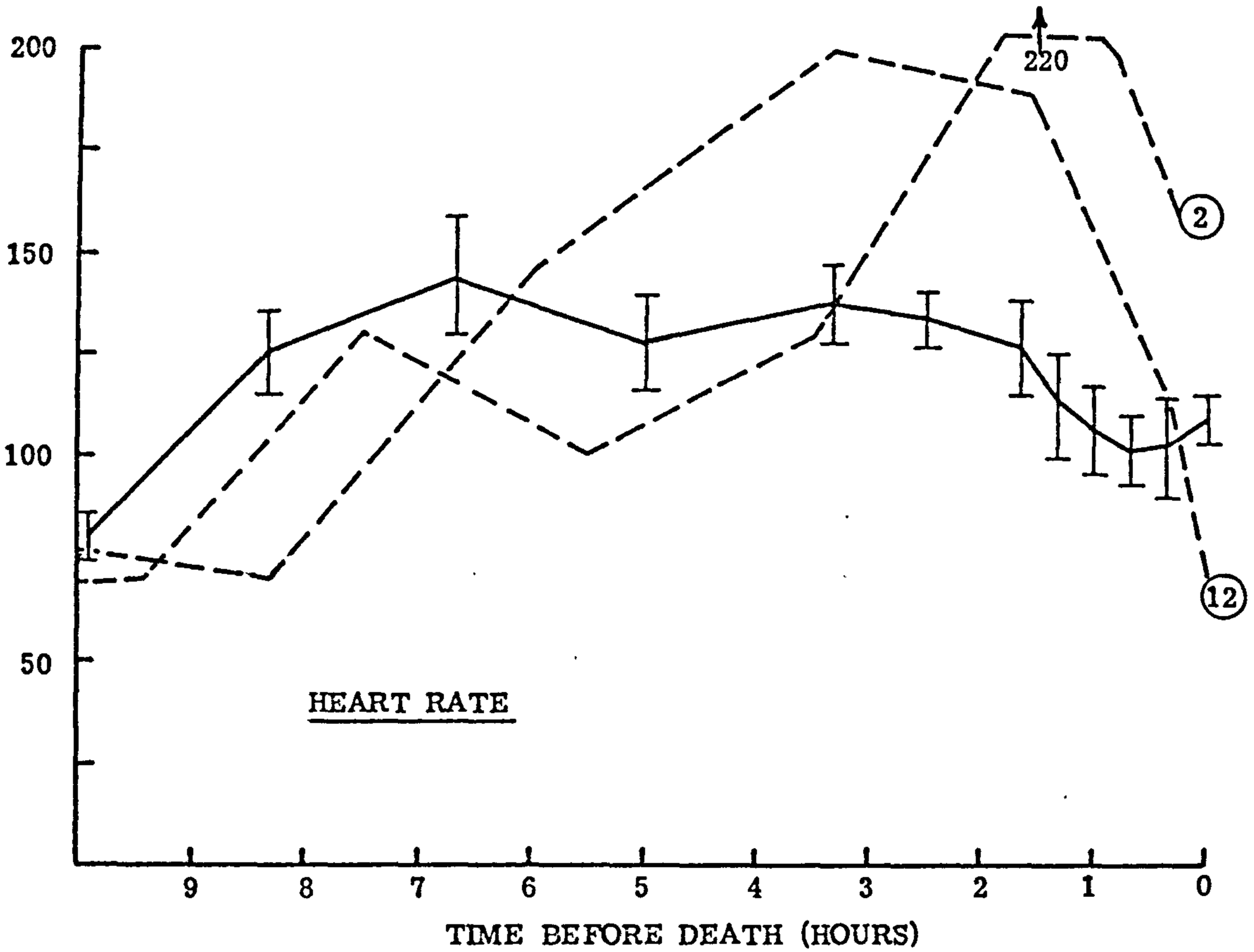

200
150
100
50
220
2
12
HEART RATE
9
8
7
6
5
4
3
2
1
0
TIME BEFORE DEATH (HOURS)

FIGURE 3:15.

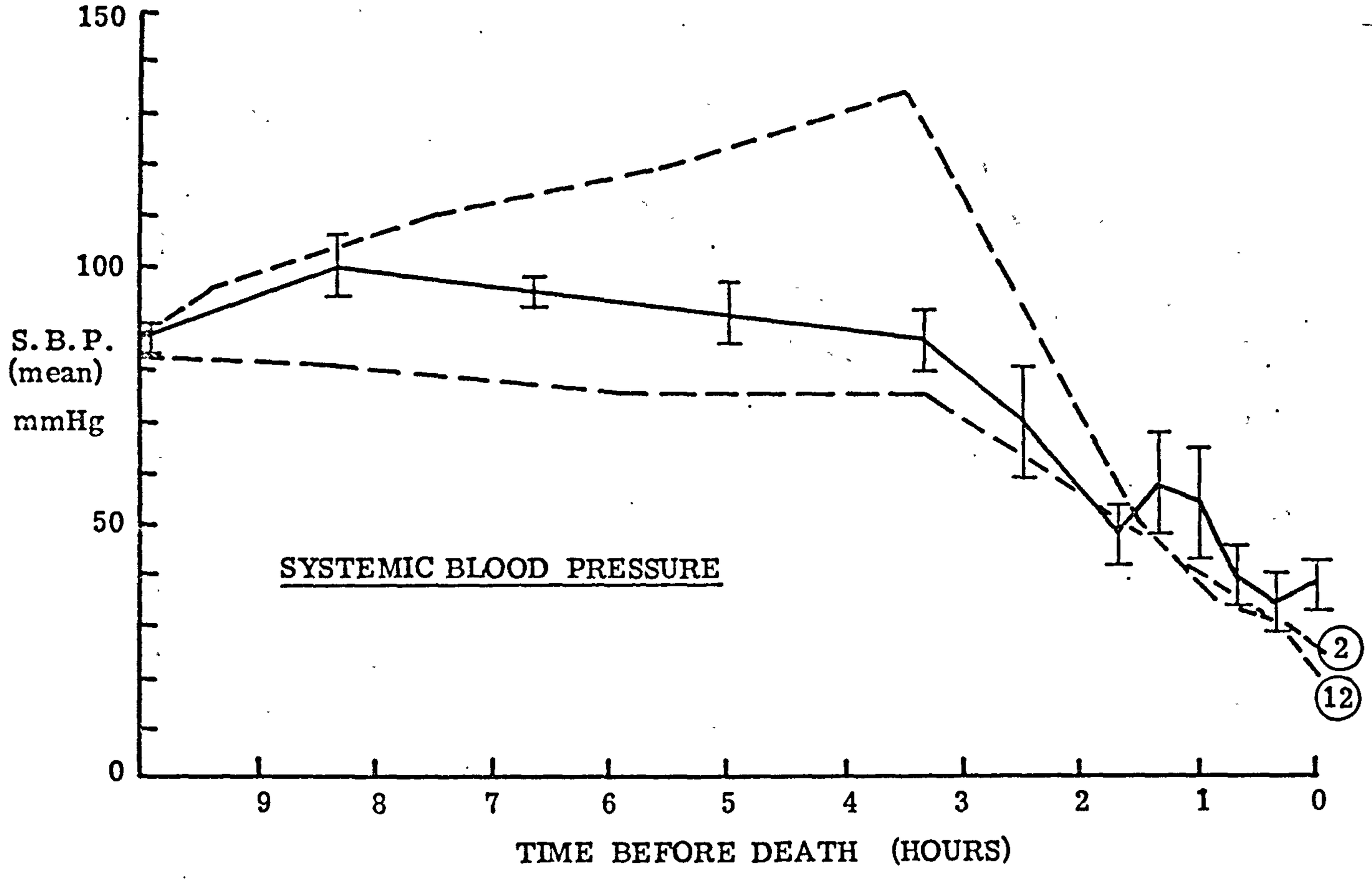

150
100
S.B.P.
(mean)
mmHg
50
0
SYSTEMIC BLOOD PRESSURE
2
12
9
8
7
6
5
4
3
2
1
0
TIME BEFORE DEATH (HOURS)

FIGURE 3:16.

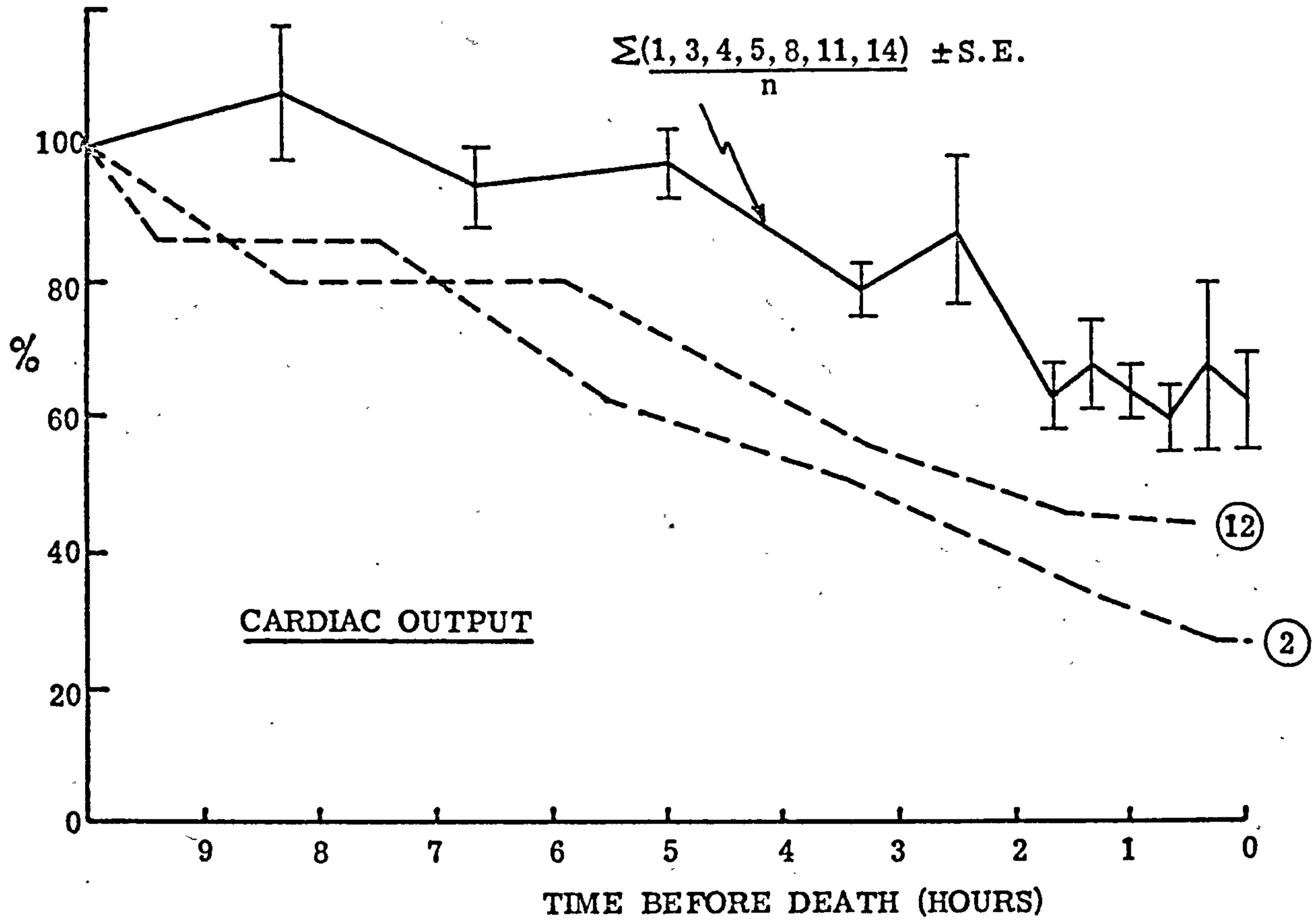

Σ(1, 3, 4, 5, 8, 11, 14) / n ±S.E.
100
80
60
40
20
0
%
CARDIAC OUTPUT
12
2
9
8
7
6
5
4
3
2
1
0
TIME BEFORE DEATH (HOURS)

FIGURE 3:17.

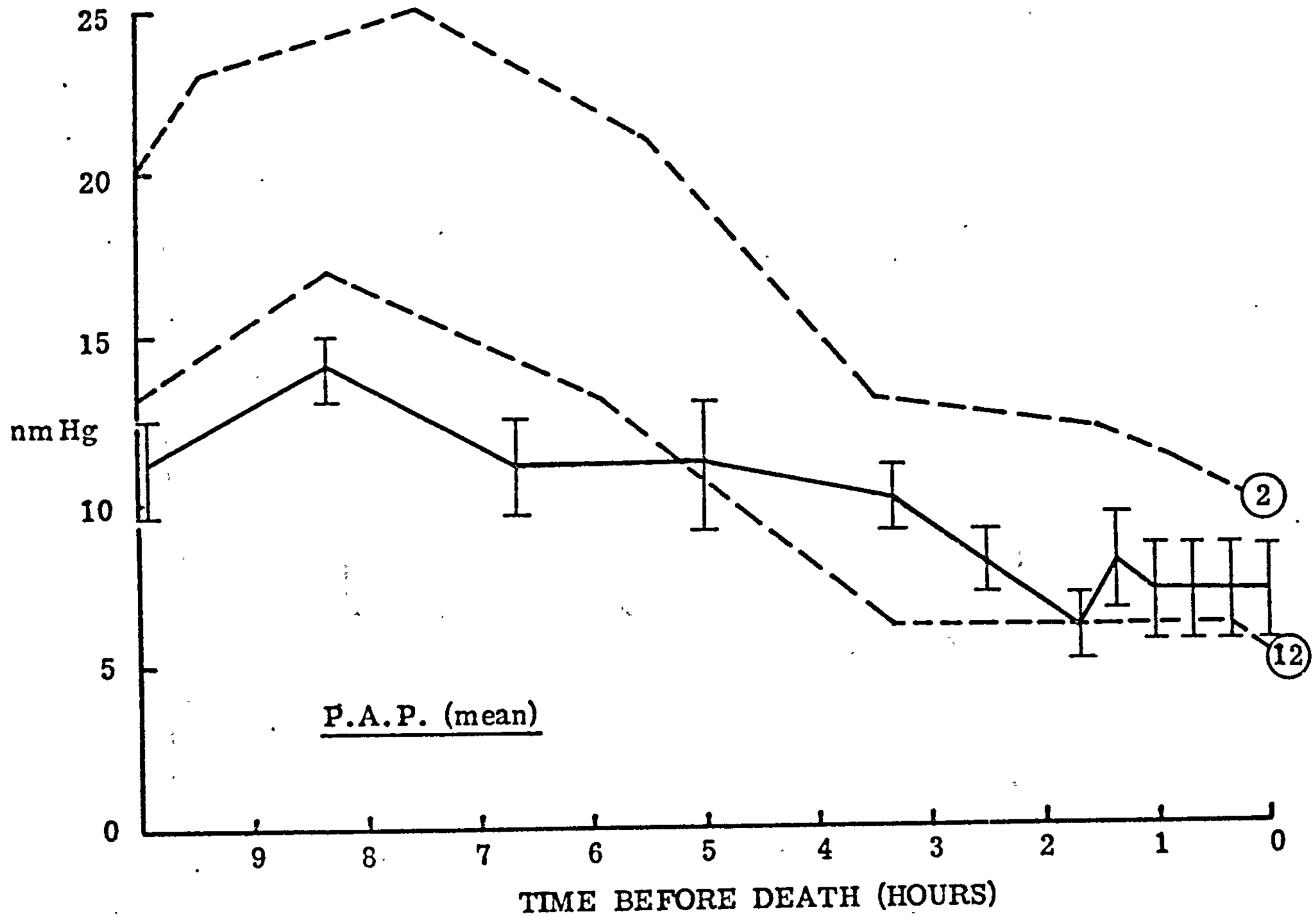

25
20
15
nm Hg
10
5
0
2
12
P.A.P. (mean)
9
8
7
6
5
4
3
2
1
0
TIME BEFORE DEATH (HOURS)

FIGURE 3:18.

PULMONARY ARTERY "WEDGE" PRESSURE (Mean ± S.E.)

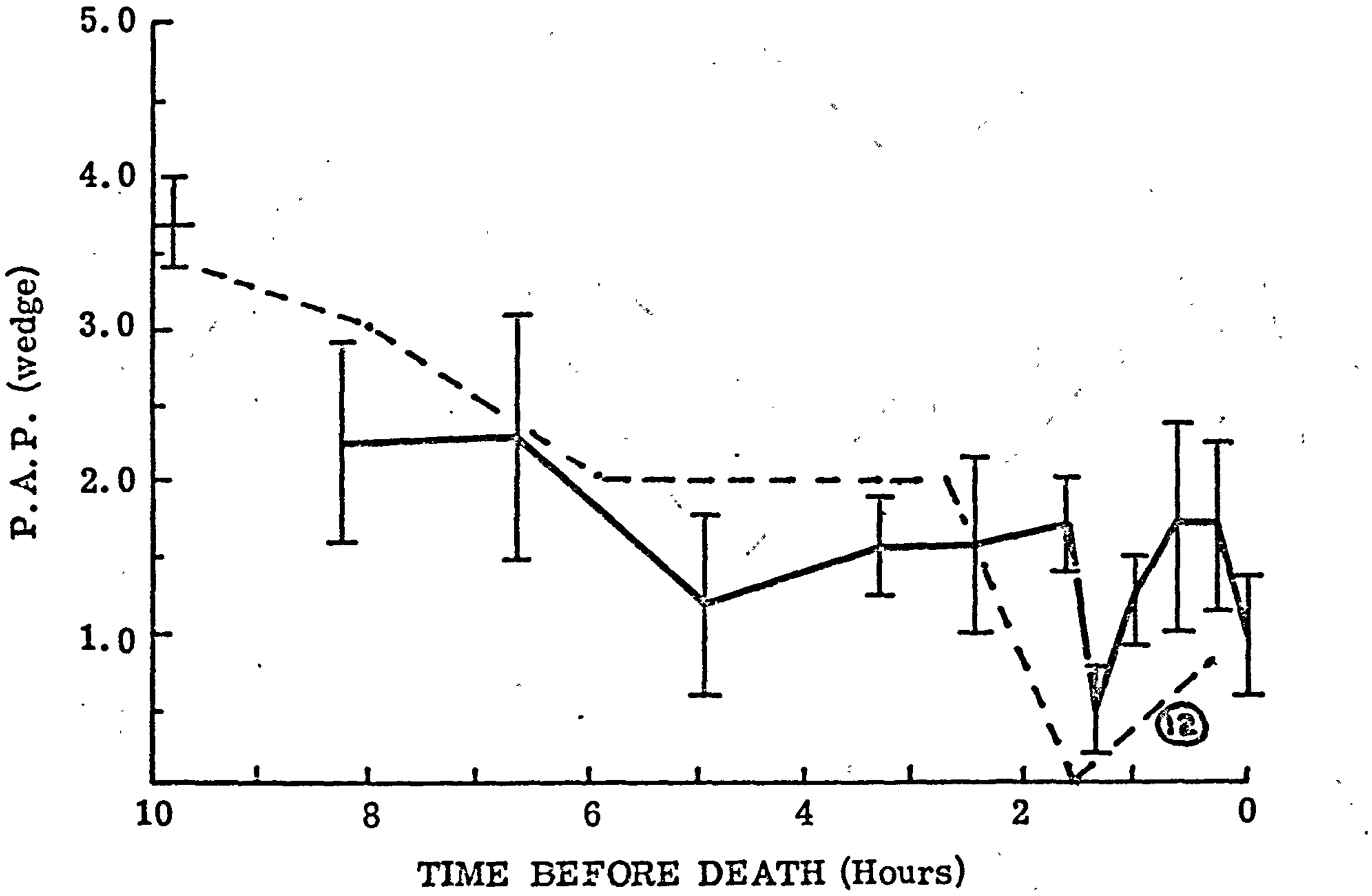


FIGURE 3:19.

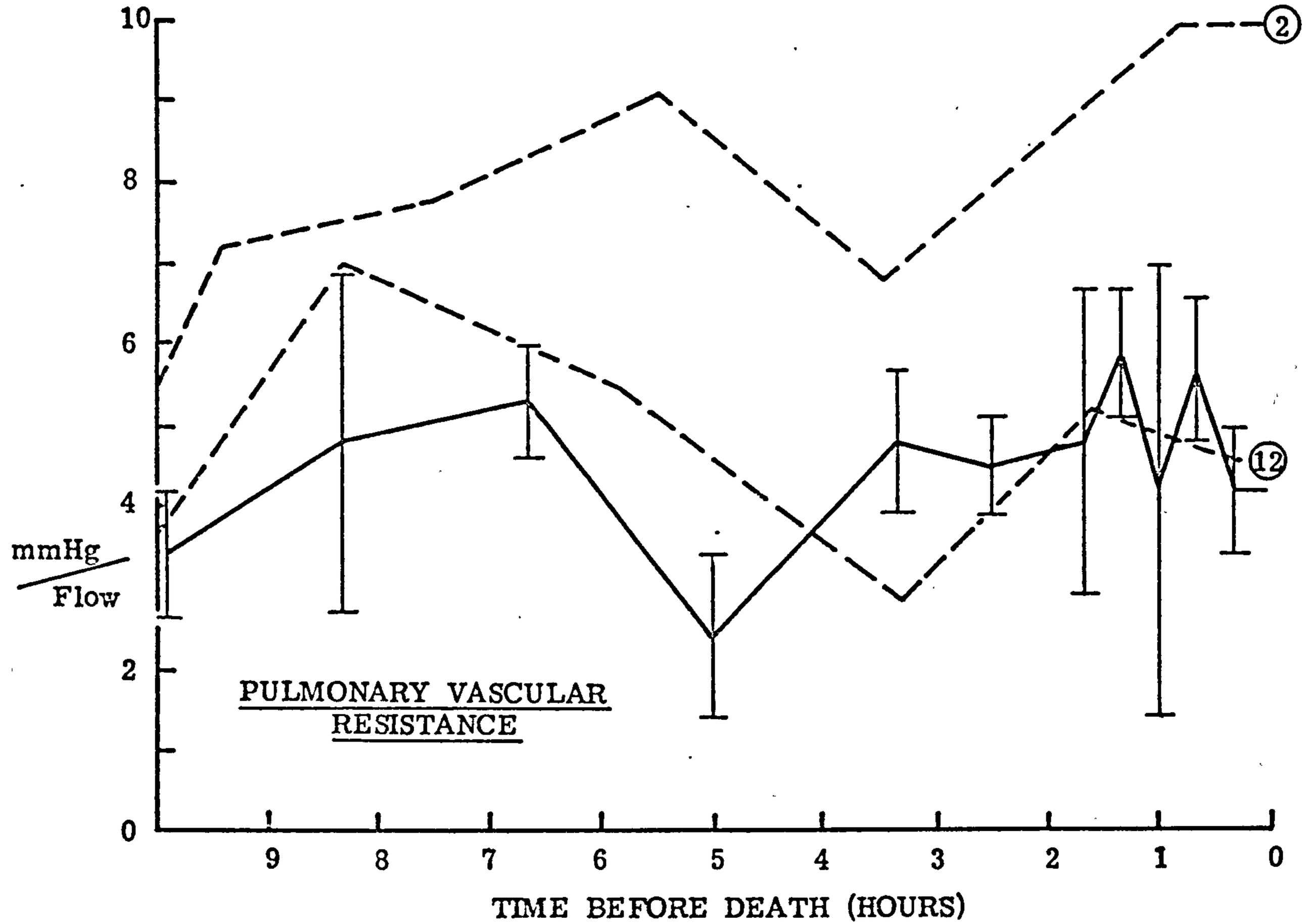

10
8
6
4
2
0
mmHg
Flow
PULMONARY VASCULAR
RESISTANCE
2
12
9
8
7
6
5
4
3
2
1
0
TIME BEFORE DEATH (HOURS)

FIGURE 3:20.

FIGURE 3:21

PERCENTAGE PULMONARY SHUNT

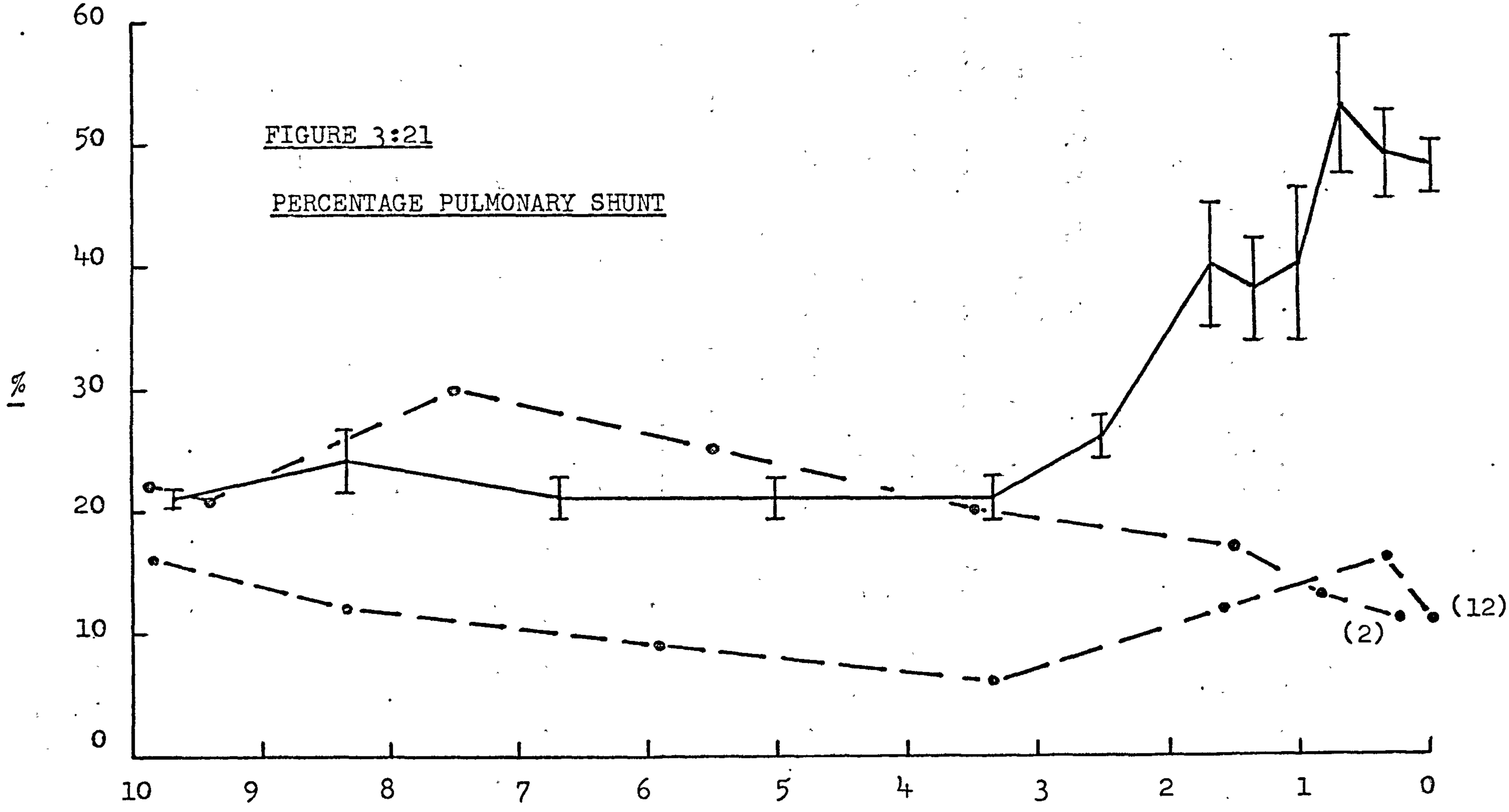


FIGURE 3:21.

TIME BEFORE DEATH (hours)

HISTOLOGY

It had become obvious during the course of the experiments that overt pulmonary oedema was present in at least some of the animals, and the problem then arose as to how to demonstrate this histologically without any masking or artifact.

Development of fixation technique

Experience had already been gained in the special fixation techniques necessary for fluorescence microscopy (see Section 6) in which tissues are quenched in liquid nitrogen immediately following removal from the body. Because of the rapidity of fixation, post-mortem artifact is eliminated. Possible fixation artifacts are shrinkage and distortion of the tissues and cracking due to the very rapid cooling; the first is of no consequence in this context, and the second is easily recognised under the microscope.

The technique had to be modified for use inside the pressure chamber. In the standard technique pieces of tissue are not dropped directly into liquid nitrogen as this causes an insulating layer of vaporised nitrogen to form round the tissue and retard cooling. To overcome this, isopentane or propane/propylene mixtures are first cooled to -190°C. in liquid nitrogen and the tissue quenched in that. The highly inflammable nature of these substances precludes their use in pressure chambers and the little-known technique of powder coating (Moline & Glenner, 1964) was used instead. This works on the principle that if the tissue is first coated with a very fine powder such as lactose or talc, the grains of powder will act as micelles for bubbles and prevent formation of a gaseous envelope around the tissue. This

technique was used successfully on all the tissue samples; on none of the sections of these tissues was there any evidence of ice-crystal formation (caused by slow cooling between 0° and -50° C.).

Method

All the animals lay on the table right side down, i.e. with the right lung dependent. In alternate experiments either the right lung was prepared for electron microscopy and the left for light microscopy, or vice versa.

Within thirty seconds of apnoea in the oxygen poisoned animals and immediately following intravenous injection of potassium chloride in the controls, the trachea was clamped and the chest widely opened. The hilum of the lung destined for light microscopy was clamped and a peripheral portion of the diaphragmatic lobe isolated between curved bowel clamps, severed, coated with talc and quenched in liquid nitrogen. Simultaneously the other (electron microscopy) lung was deflated to a pressure of -5 cms of water, reinflated with cacodylate-buffered gluteraldehyde to 20 cms of water and clamped. Finally the light microscopy lung was deflated to -5 cms of water and reinflated with 10% buffered formalin.

No problems were encountered during decompression of the various tissues in the air-lock.

The nitrogen quenched lung tissue was transferred (in the laboratory) to a shallow tray filled with liquid nitrogen and broken into small portions approximately 5 x 3 x 3 mm with cooled bone forceps. They were transferred rapidly to the freezing head of a Speedivac-Pearse tissue drier which had been previously cooled to -60° C., and

freeze-dried at a vacuum pressure less than 0.001 torr for 24 hours at a temperature of -60° C. At the end of the 24 hours, the freezing head was slowly heated to room temperature to avoid condensation, the vacuum broken and the tissue pieces exposed to gaseous paraformaldehyde at 80° C. for one hour. Following this, they were embedded conventionally in Paraplast in a vacuum oven. Sections were cut at 6 microns on a Leitz rotary microtome and stained routinely with Masson's trichrome, and haematoxylin and eosin.

The formalin-fixed lung tissue was left immersed in a beaker of formalin for at least 48 hours. Blocks, size 2 x 1 x $\frac{1}{2}$ cm, were taken from random areas and dehydrated and impregnated with Paraplast in a Histokinette tissue processor. Embedding and sectioning was as for the freeze-dried tissues.

Sections were stained routinely with haematoxylin and eosin, and Masson's trichrome. In addition, sections from all the animals were stained with Van Gieson's stain for collagen and either Aldehyde fuchsin and Orange G, Resorcein fuchsin and Orange G, or Resorcein fuchsin and azocarmine for elastin. Attempts were made to use Gordon and Sweet's or Foot's silver carbonate technique for the demonstration of reticulin, and Mallory's phosphotungstic acid haematoxylin for the demonstration of fibrin in the hyaline material, but time did not permit acquisition of the necessary technical expertise, and they were abandoned.

All of the freeze-dried tissue was taken from a peripheral portion of either the right or left diaphragmatic lobe. Twelve tissue blocks were taken from each portion for each animal and two sections taken from each block.

The blocks of formalin-fixed tissue were taken at random from the whole of the lung, approximately twenty being taken from each animal. Six sections (for different stains) were taken from each block.

Over the whole experiment approximately two thousand sections were examined.

Results

1) 'Pulmonary death' animals

Examination of the freeze-dried sections from these animals left no doubt whatsoever as to the cause of death.

Every section from every block from all eight animals showed the presence of intra-alveolar oedema, demonstrated by the presence of proteinaceous debris fixed in situ in the alveolar spaces (Figure 3:22).* The amount of this material present varied greatly. In some sections, only a few alveoli were occluded. In others, the material appeared to line the alveolar walls leaving a central clear area. In many of the sections, all the alveoli were totally occluded and a continuous mass of material was present even in the alveolar ducts and respiratory bronchioles.

Intense pulmonary congestion was a constant finding as was also a severe degree of interstitial oedema (made manifest by a widening of the peribronchial spaces). In many sections, erythrocytes were found in the alveolar spaces.

By contrast, freeze-dried sections from the control animals were completely normal (Figure 3:23).*

* presented in Appendix 5

To demonstrate the importance of the fixation technique, sections were taken in several animals from tissues removed from the area of lung immediately adjacent to the isolating bowel clamp (see page 3:43), those from one side of the clamp being fixed by nitrogen quenching and those from the other by formalin instillation. Figures 3:24a & b demonstrate the difference in appearance. It is noted that Figure 3:24b could almost pass as a micrograph of normal lung tissue.

This failure to demonstrate intra-alveolar oedema was common to all the formalin-fixed blocks of tissue taken at random from all over the rest of the lung. Other pathological features, however, were preserved.

The most striking finding on almost all of the formalin-fixed sections was a moderate to severe degree of pulmonary congestion, mainly in the capillaries and venules, but also present in the pulmonary arterioles. A widening of the peri-bronchial and peri-arteriolar spaces by interstitial oedema fluid was also present in almost all of the sections. This varied (in the same lung) from mild (Figure 3:25) to extremely severe (Figure 3:26). Many of the sections showed erythrocytes lying in the alveolar space; in a few sections the degree of intra-alveolar haemorrhage was severe (Figure 3:27). Careful examination of most sections under high power revealed hyaline material lining the alveoli (Figure 3:28). Small areas of focal atelectasis were observed in all the animals, but they were not common and were confined to areas of only a few millimetres or less across (Figure 3:29).

An incidental finding in Dog 6 was a proliferative bronchiolitis in scattered areas affecting the terminal bronchioles. In some

of the lesions, the remains of ascaris larvae could be seen and these were identified as Toxocara canis by a veterinary pathologist who considered the lung lesion to be an immune response to re-infection.

2) 'Cardiac death' animals

In neither of the 'cardiac death' dogs did the freeze-dried sections reveal intra-alveolar oedema. The appearances of the lungs in the two animals were, however, completely different (Figures 3:30 and 3:31).*

This difference was confirmed on formalin-fixed sections taken from over the whole lung field.

The appearance on all the sections from Dog 12 was of intense pulmonary congestion (Figure 3:32). The only other abnormality was an occasional slight widening of the peri-bronchial space, confined to the large bronchioles, due to early interstitial oedema (Figure 3:33).

In Dog 2, the alveolar walls on the freeze-dried sections were greatly thickened (Figure 3:31)*; examination of the formalin-fixed blocks showed, however, that this was a focal lesion and that the bulk of the rest of the lung was completely normal (Figure 3:34). High power examination of the lesion suggested that the appearance was due to Type 2 cell proliferation (Figure 3:35). A mild degree of peri-bronchial interstitial oedema similar to that on Figure 3:33 was present on a few of the sections.

3) Histochemistry

Masson's trichrome staining does not give such a specific colouration to fibrin as does Mallory's phosphotungstic acid haema-

* presented in Appendix 5

toxylin, but from the pale pink colouration of the hyaline 'membrane' at the alveolar margins, it is concluded that fibrin is a constant constituent of the material.

Gupta, Winter & Lanphier (1969) claimed to have demonstrated extensive destruction of elastic tissue around the alveoli and terminal bronchioles in dogs which had been exposed to 2.5 ATA oxygen until convulsions and then to 2 ATA until death. The micrograph presented, however, is from an area showing gross disruption of the normal alveolar architecture.

Examination of material from all of the present animals has revealed scattered areas of atelectasis where the appearance of the elastic tissue is similar to that of Gupta's (Figure 3:36), i.e. fibres are shorter and thicker, appear fragmented, are often curled, and often appear to be displaced from their normal position in the alveolar wall.

Over most of the rest of the lung, however, where the alveolar pattern is normal, there does not appear to be much difference between the oxygen-poisoned animals (Figure 3:37) and the normal controls (Figure 3:38). An interesting finding was the occasional elastic fibre displaced from the alveolar wall lying free in the alveolar space, and the large number of elastic fibres commonly present in the interstitial space in areas of interstitial oedema (Figure 3:39).

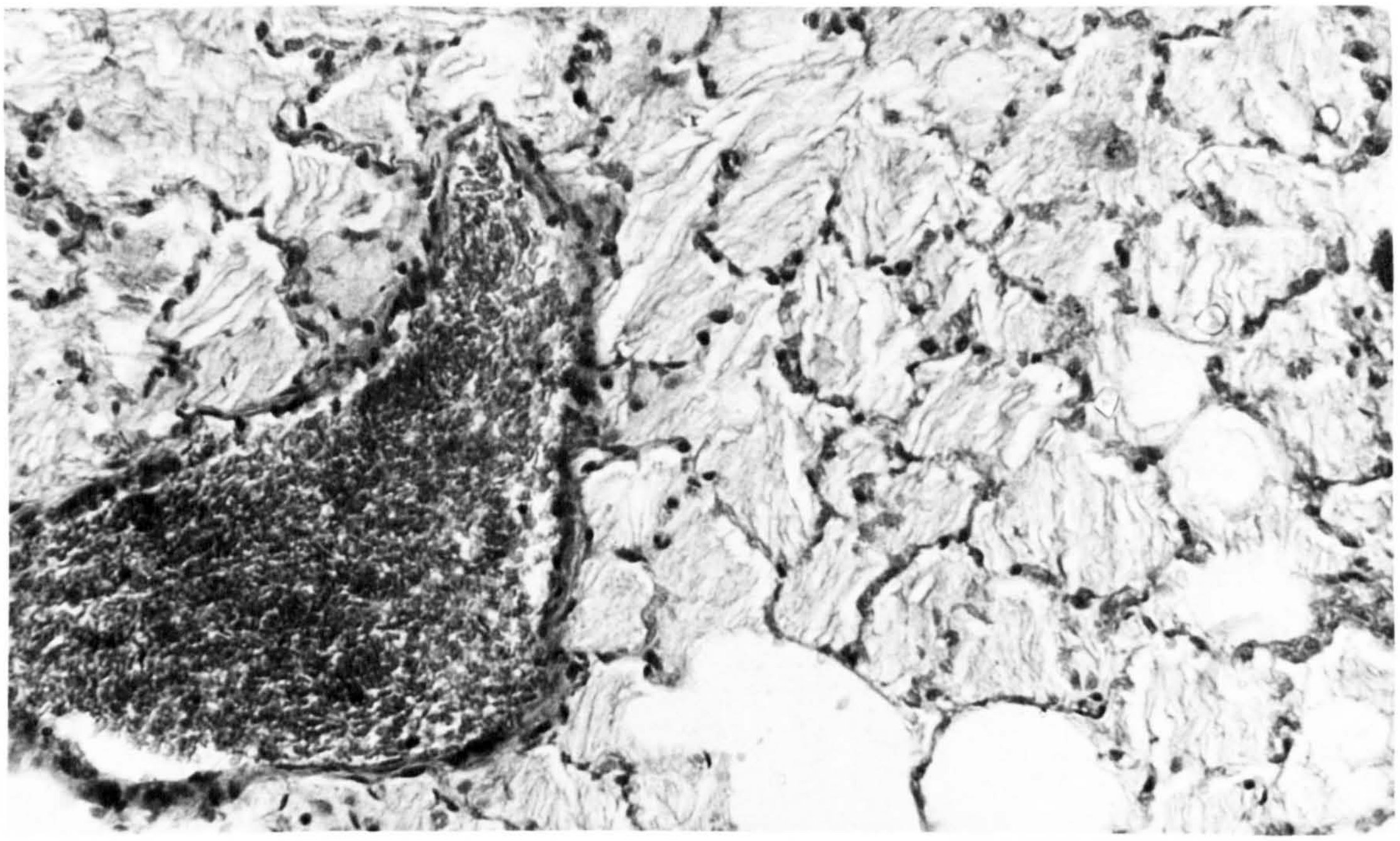


FIGURE 3:24a. 'PULMONARY DEATH' ANIMAL - FREEZE-DRIED TISSUE.

(x 180)

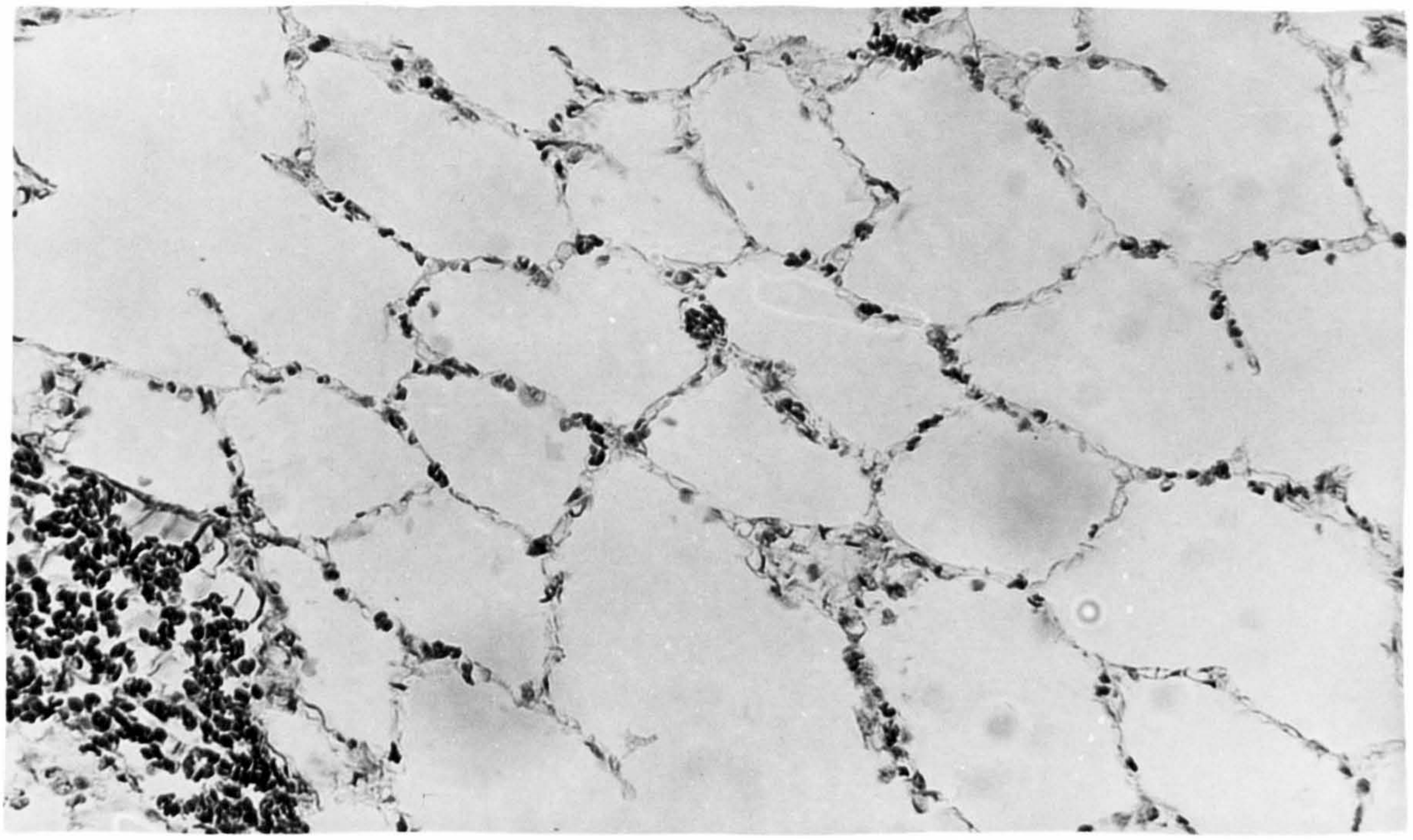


FIGURE 3:24b. 'PULMONARY DEATH' ANIMAL - FORMALIN-FIXED TISSUE.

(x 180)

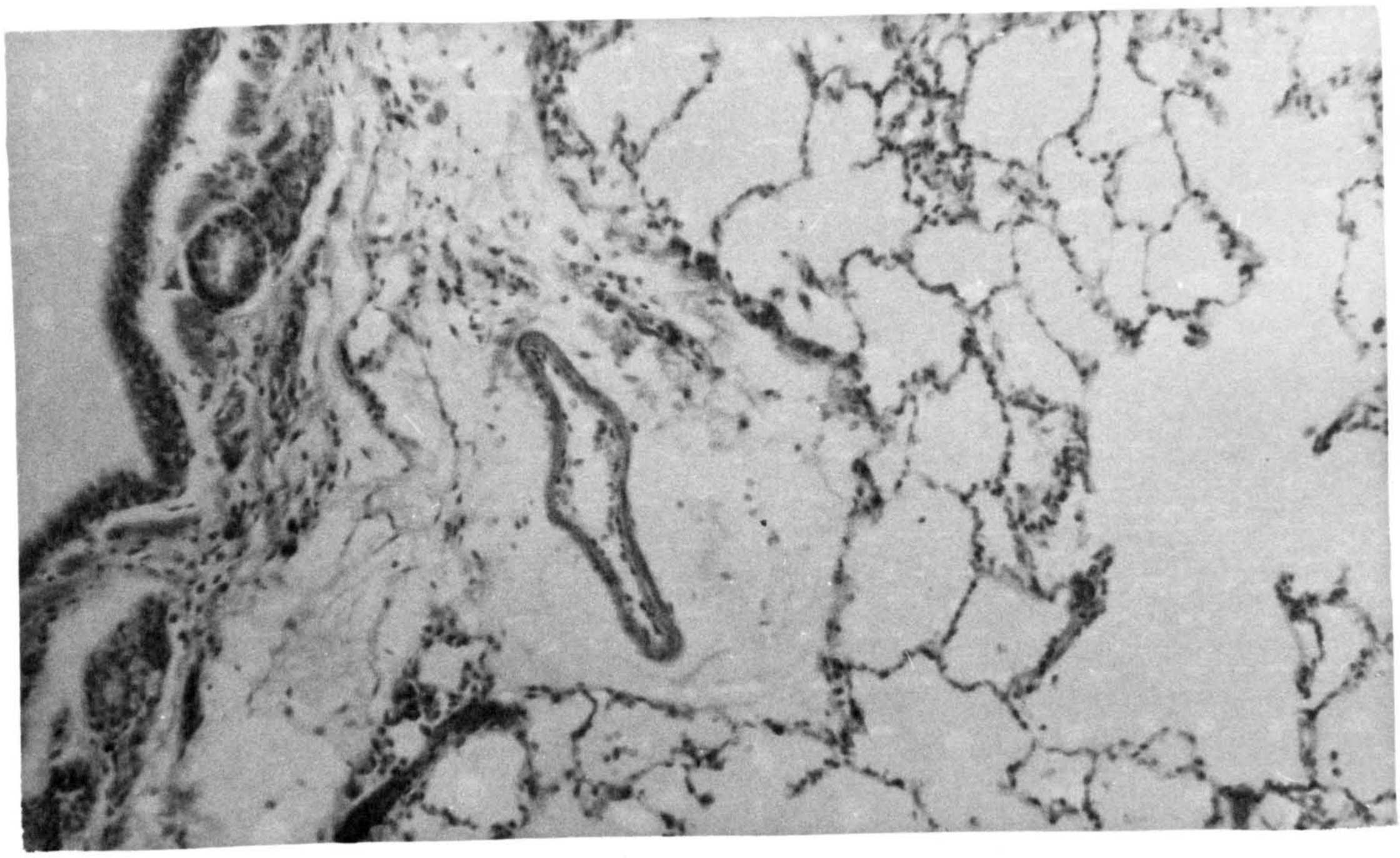


FIGURE 3:25. MILD INTERSTITIAL OEDEMA. (x 75)

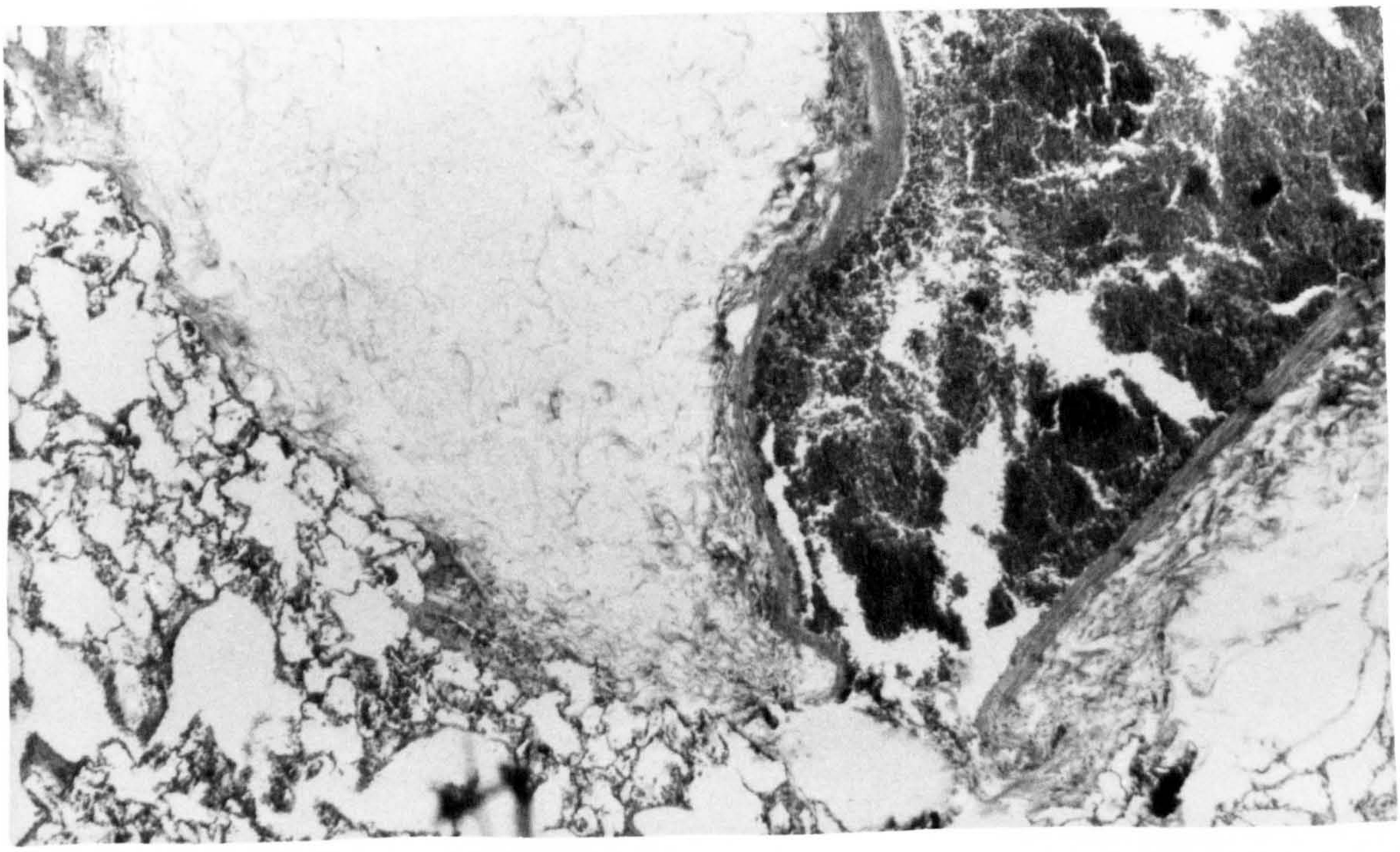


FIGURE 3:26. SEVERE INTERSTITIAL OEDEMA. (x 50)

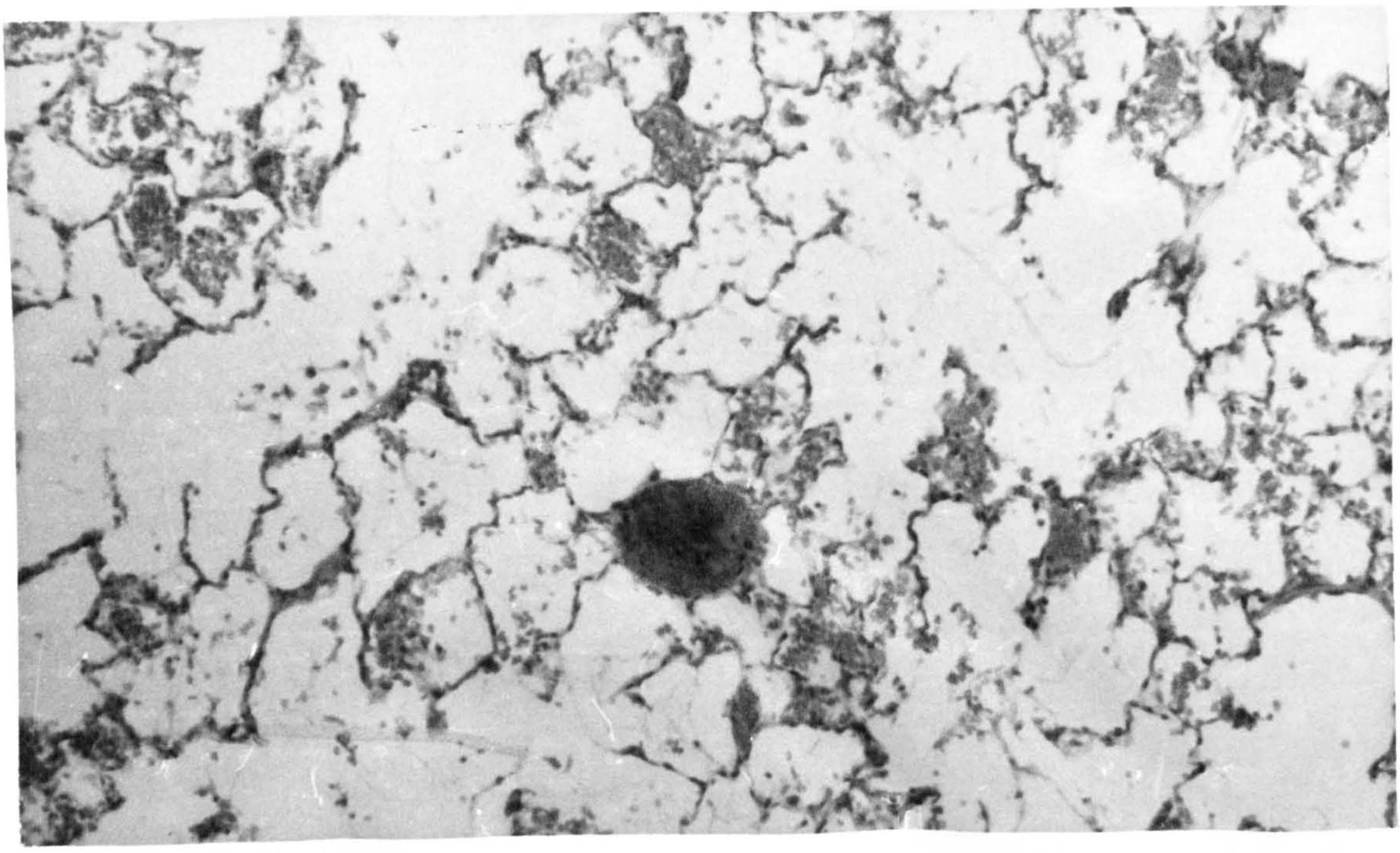


FIGURE 3:27. INTRA-ALVEOLAR HAEMORRHAGE. (x 100)

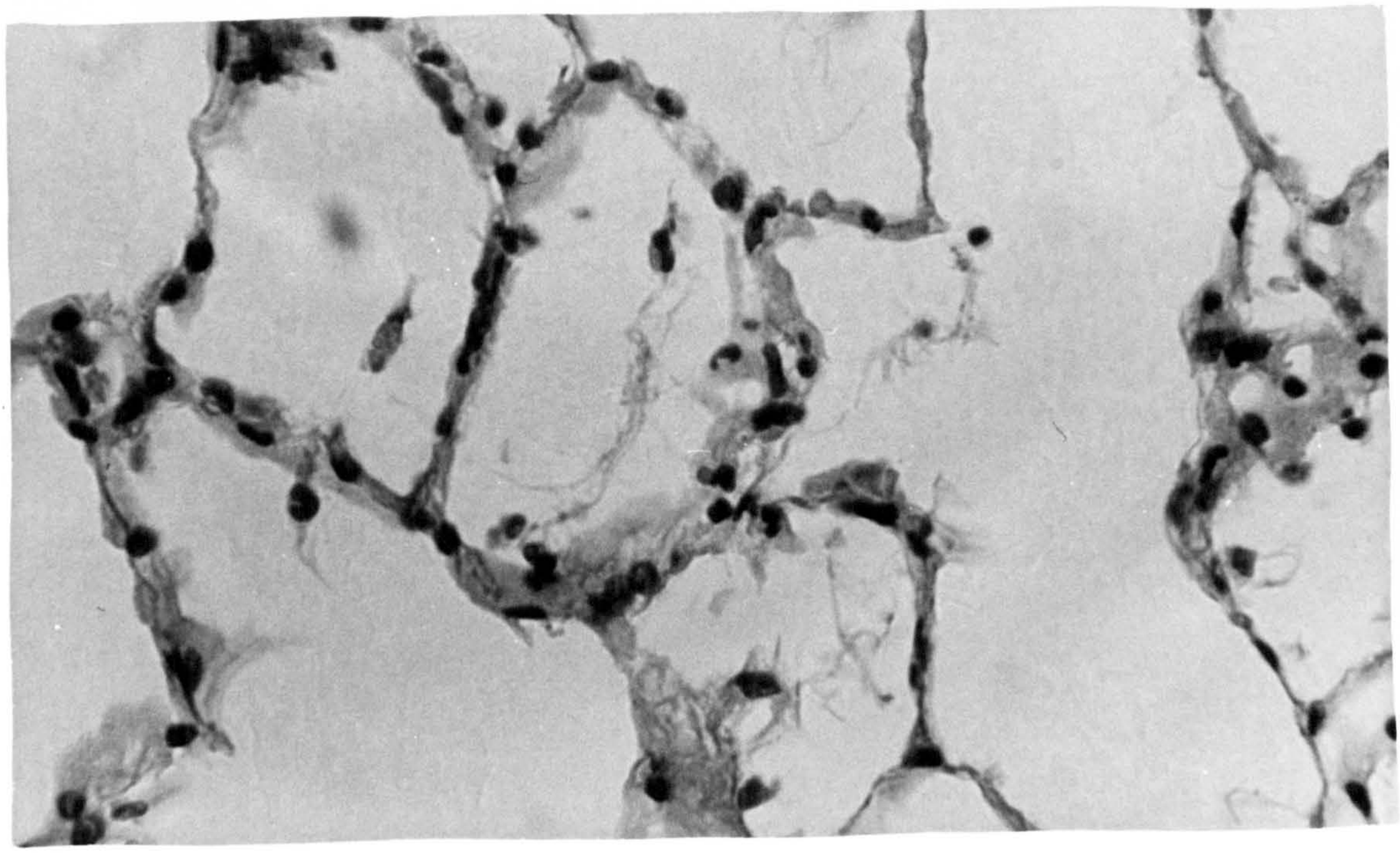


FIGURE 3:28. HYALINE MATERIAL IN ALVEOLI. (x 400)

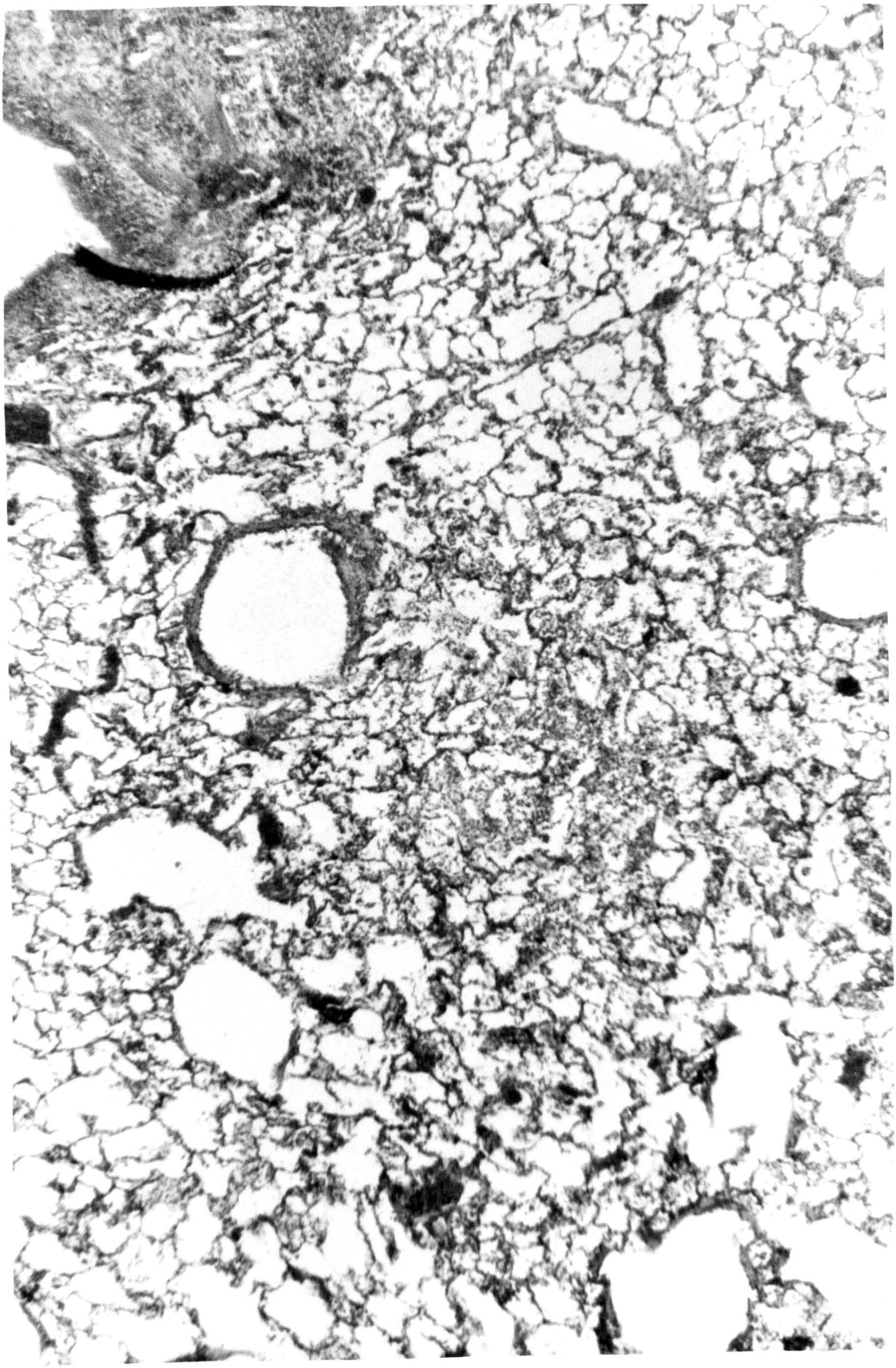


FIGURE 3:29. FOCAL ATELECTASIS. (x 60)

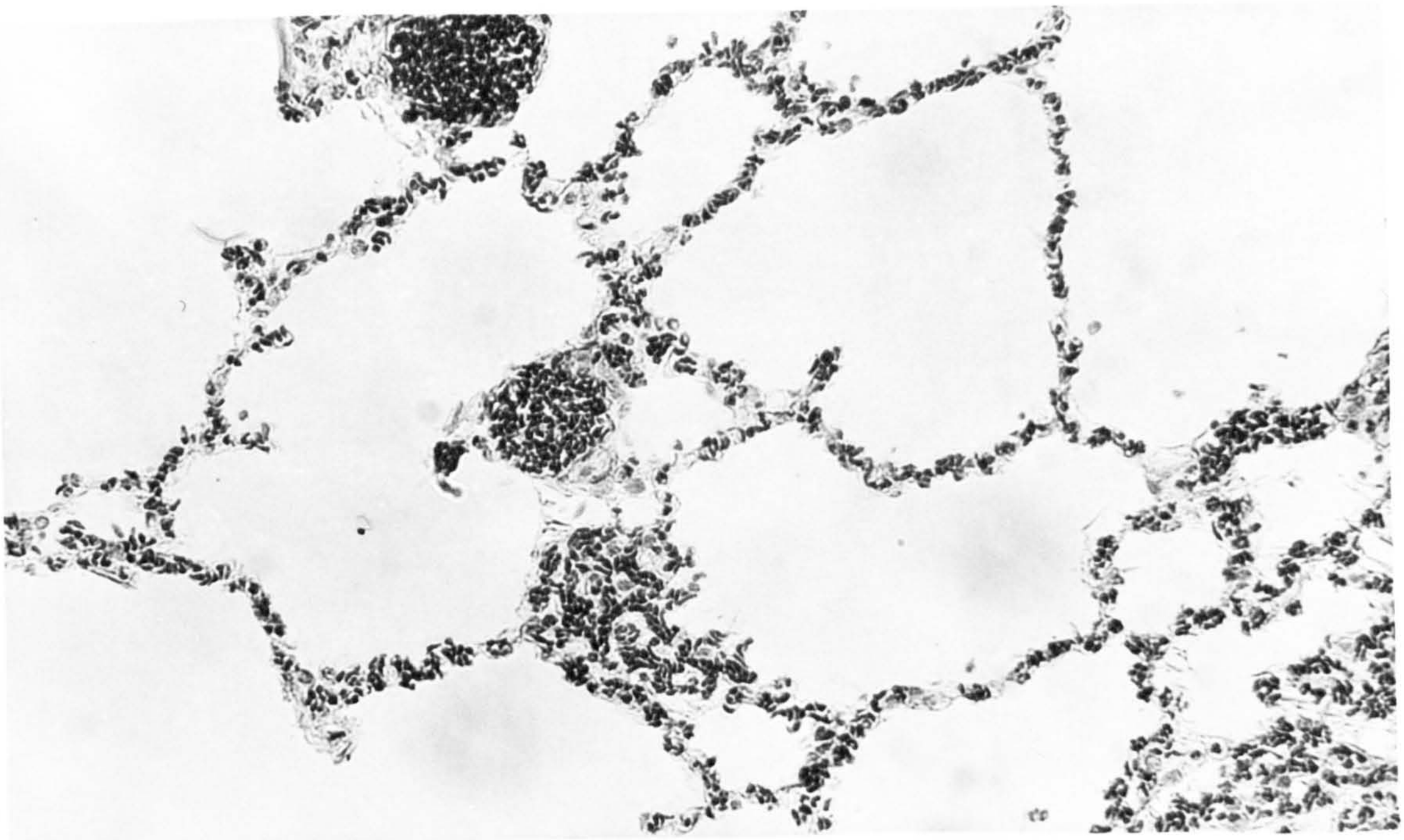


FIGURE 3:32. DOG 12; PULMONARY CONGESTION. (x 300)

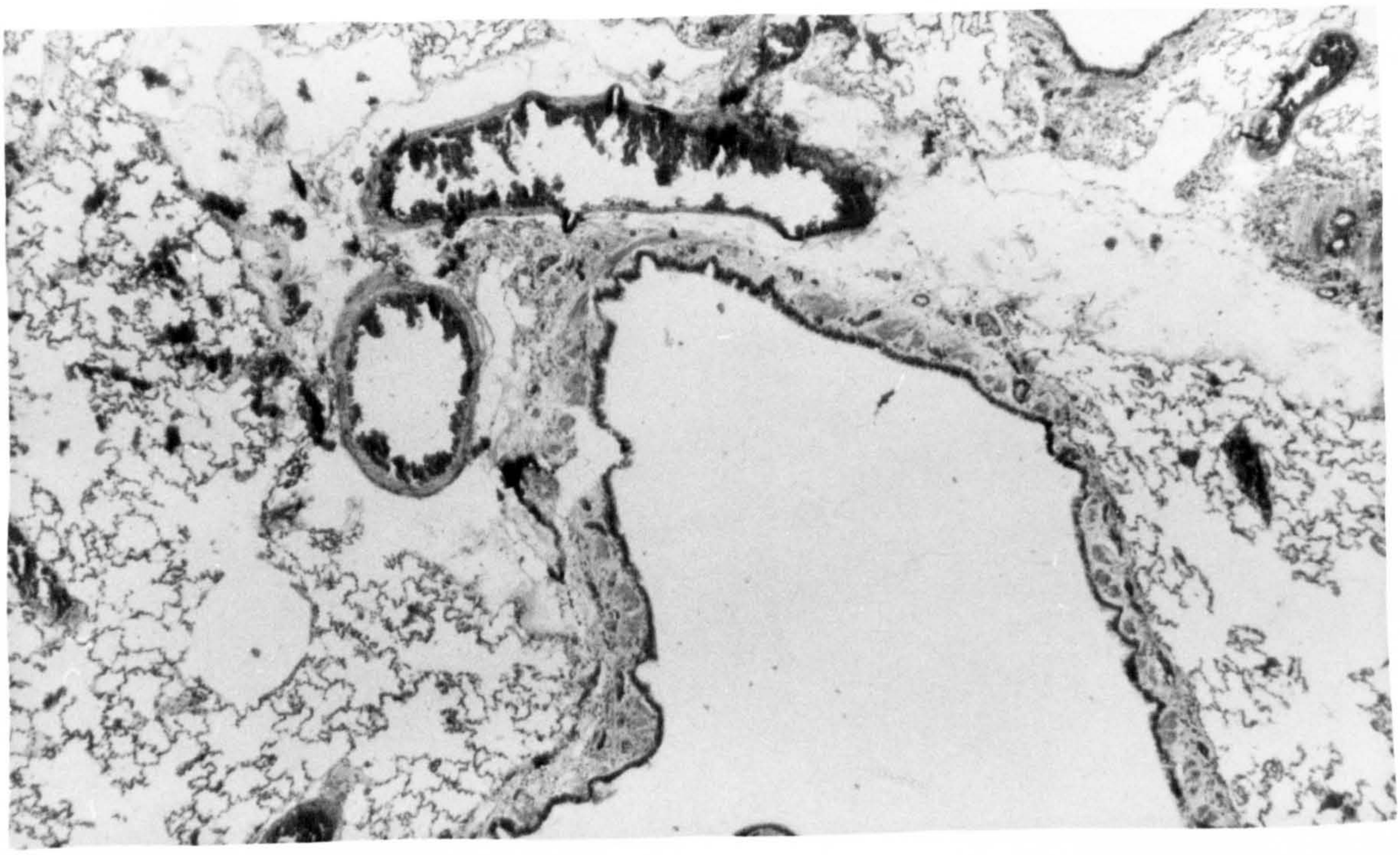


FIGURE 3:33. DOG 12: PERIBRONCHIAL OEDEMA. (x 20)

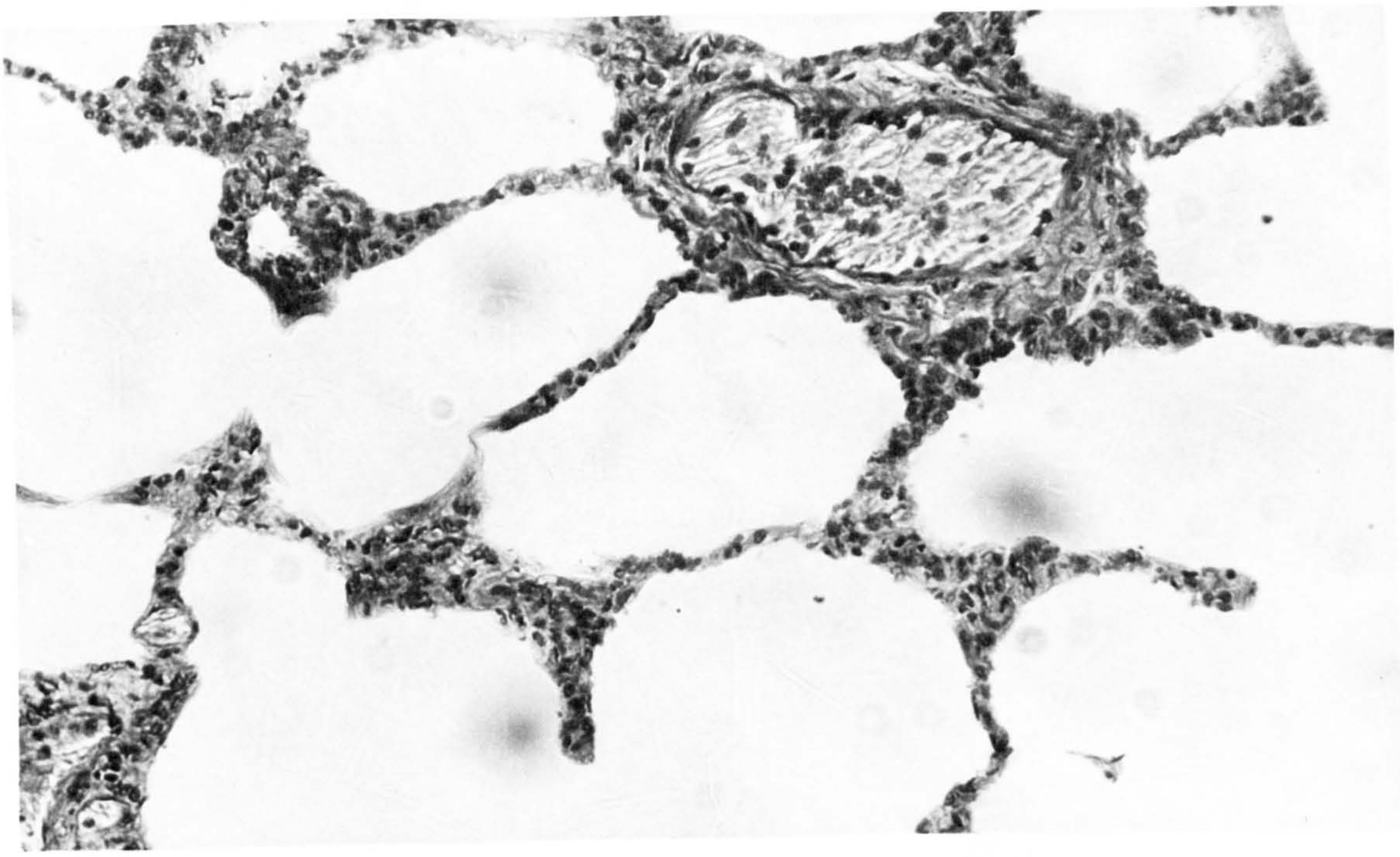


FIGURE 3:35. DOG 2: TYPE 2 CELL PROLIFERATION. (x 250)

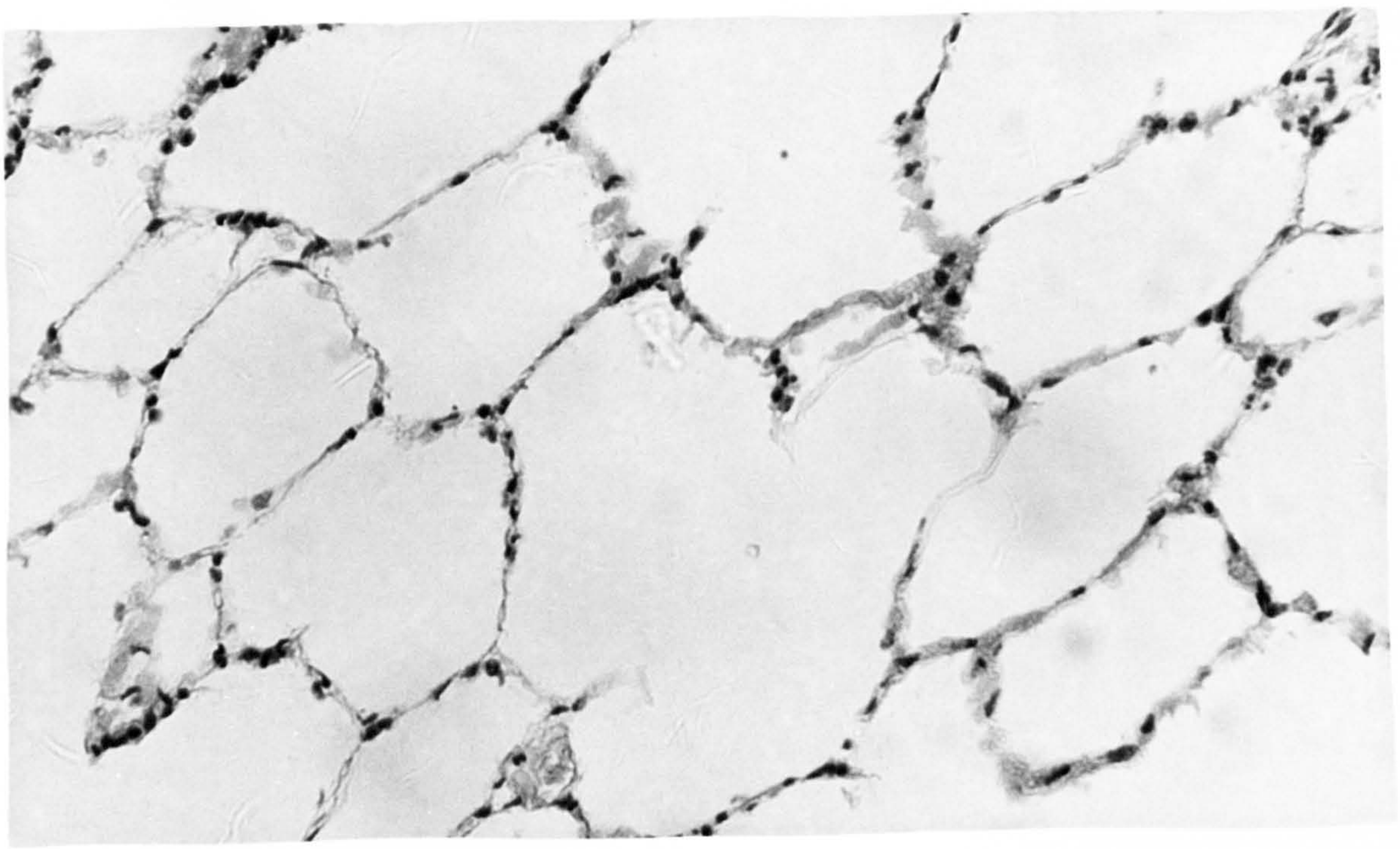


FIGURE 3:34. DOG 2: NORMAL ALVEOLAR WALL. (x 200)

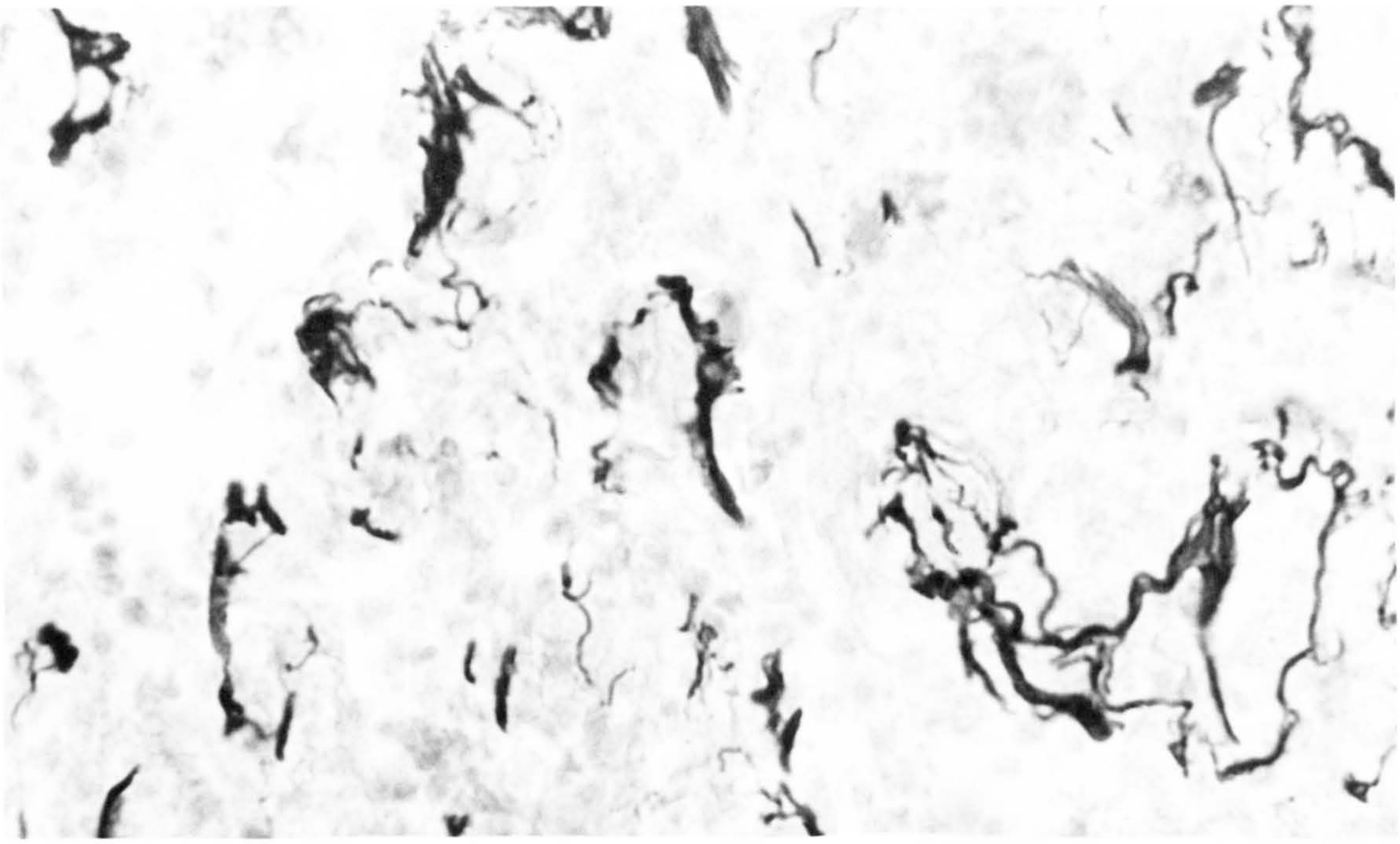


FIGURE 3:36. DISRUPTION OF ELASTIC FIBRES. (x 120)

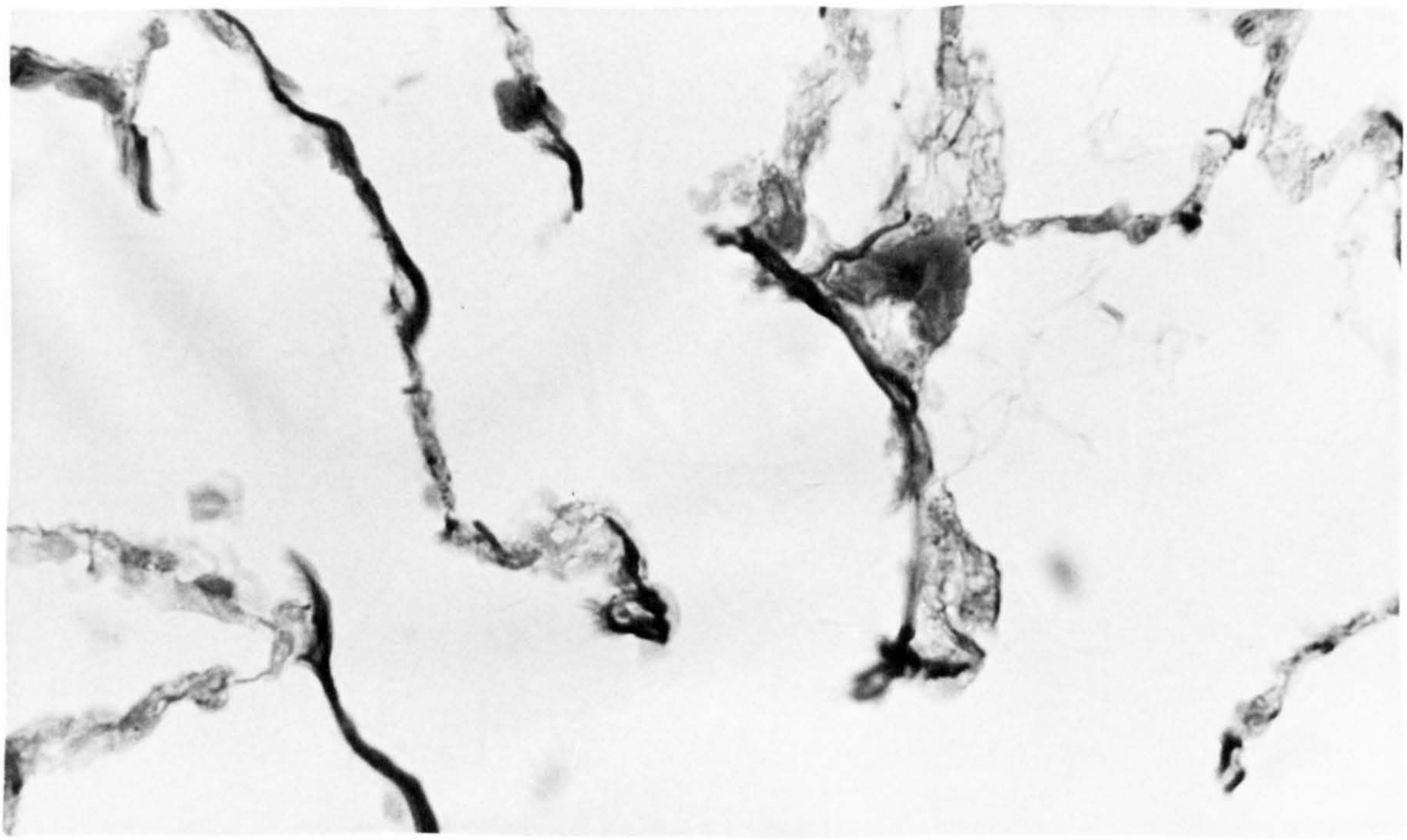


FIGURE 3:37. NORMAL ELASTIC FIBRES FROM EXPERIMENTAL ANIMAL. (x 250)

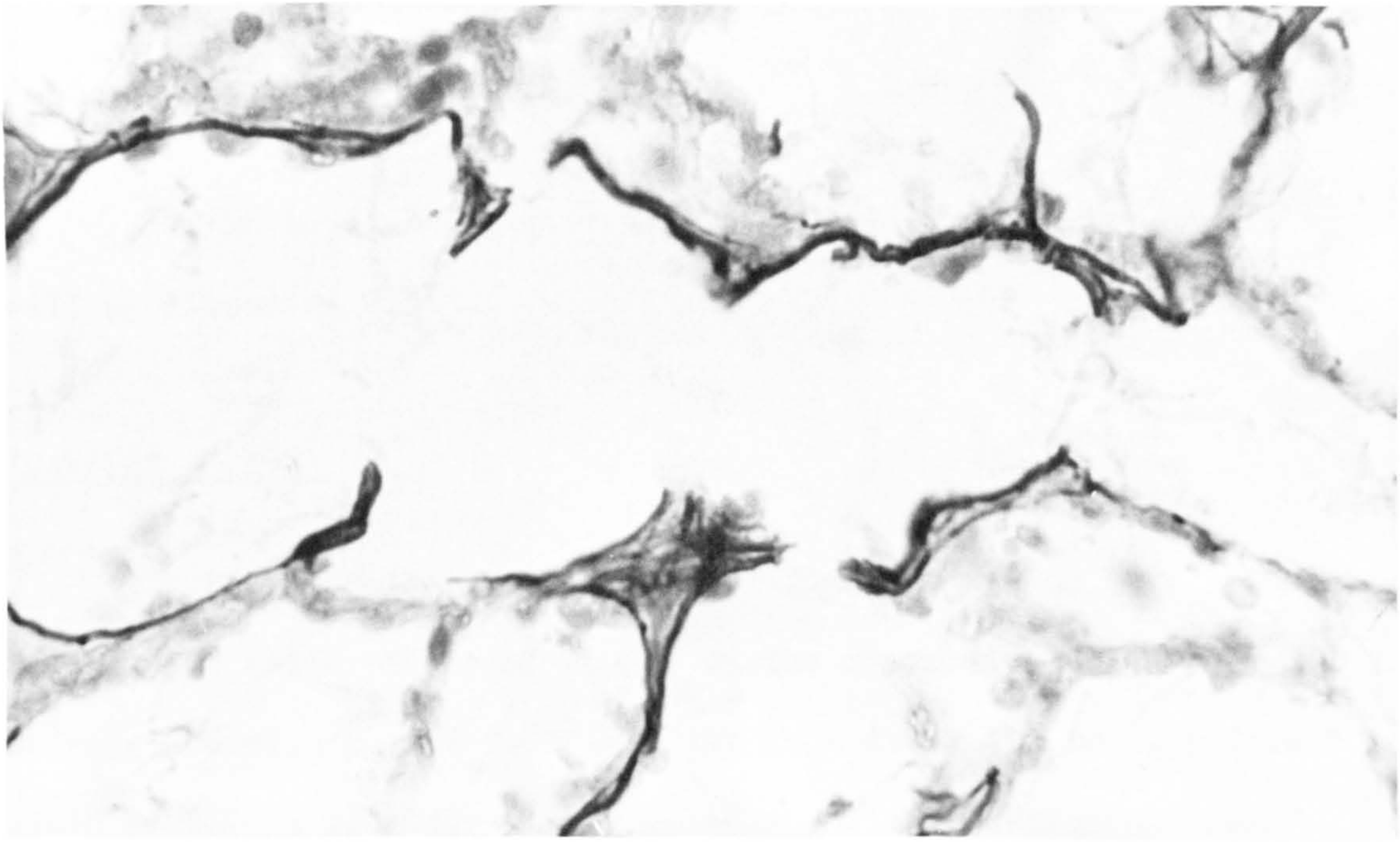


FIGURE 3:38. NORMAL ELASTIC FIBRES - CONTROL (x 250)

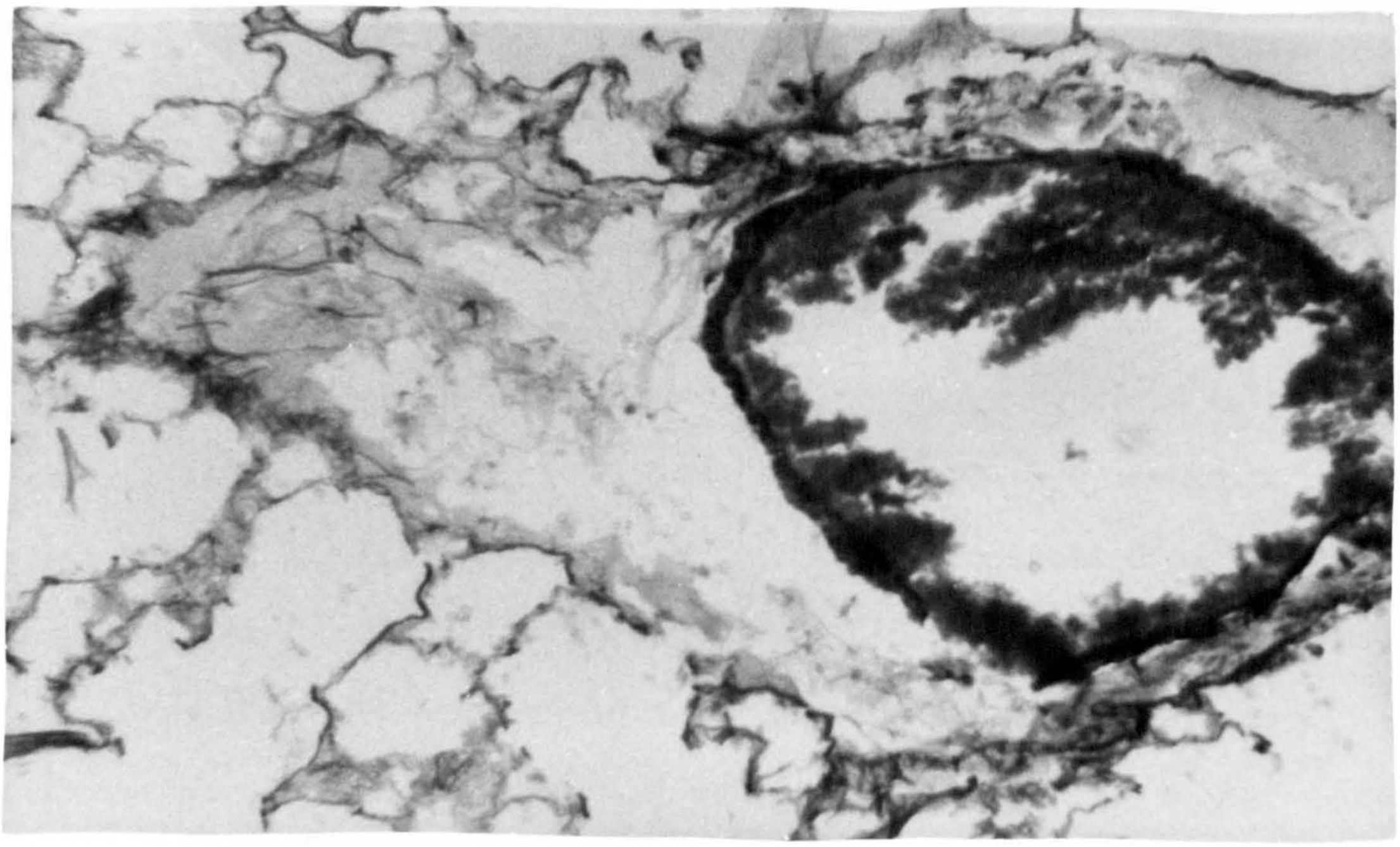


FIGURE 3:39. ELASTIC FIBRES IN INTERSTITIAL SPACE.

(x 60)

DISCUSSION

The smaller group of animals dying from cardiac causes will be discussed first.

'CARDIAC DEATH'

Death in the 'cardiac death' dogs appears to have been due to a direct toxic effect of oxygen on the myocardium. In these animals, cardiac output fell steadily from about ten hours before death despite a steadily rising pulse rate. Blood pressure fell rapidly about three hours before death and at the moment of apnoea had reached very low levels (20 and 25 mm.Hg.). In Dog 2, the fall in blood pressure at three hours was particularly steep and caused an even greater rise in heart rate, up to 220 beats per minute; cardiac output continued to fall at an even steeper rate. Mean pulmonary artery pressure also fell over the last eight hours of the experiment, suggesting that both ventricles were involved.

This toxic effect of oxygen on the myocardium is well known. Myocardial contractility is reduced in dogs breathing oxygen at 1 ATA (Daniell & Bagwell, 1968) while oxygen at 2 ATA has been shown to have a rapid depressant effect on left ventricular function (Kioschos et al., 1969). Smith and Ledingham (1972) showed that, using maximum left ventricular rate of change of pressure as an index of myocardial contractility, oxygen at 2 ATA caused a decline in contractility which was reversible over a course of eight hours. It may be that the changes seen in the present study represent a progression of the above process.

The fall in pulmonary shunt ratio over the last ten hours

can be explained on the basis of a decreasing cardiac output (Smith, Cheney & Winter, 1974).

It is noteworthy that these two animals finally died in apnoea; this is assumed to be due to medullary hypoxia following hypoperfusion.

The two animals differed from each other in several respects.

At the time of death, Dog 12 was in severe respiratory failure; arterial carbon dioxide tension had risen from 48 to 160 mm. Hg. and arterial oxygen had fallen from 1376 to 761 mm.Hg. over the last three hours. The lungs were intensely congested, presumably as a consequence of myocardial failure (although central venous and pulmonary artery wedged pressures remained low). The degree of congestion may explain the terminal fall in compliance.

In Dog 2, cardiovascular changes were similar to the above. Blood gases remained relatively normal, however, with an arterial oxygen tension of almost 1000 mm.Hg. and a carbon dioxide tension rising to only 67 mm.Hg. at the time of death.

Pulmonary changes were unremarkable apart from the focal areas of alveolar wall thickening. This caused a great deal of confusion as the lesions are very similar to those of chronic pulmonary oxygen toxicity produced either by intermittent exposure to high oxygen tensions or by prolonged exposure to relatively low tensions (0.6 to 0.8 ATA) (Vinogradov & Babchinskiy, 1969; Kaplan et al., 1969; Kapanci et al., 1969; Weibel, 1971). It is now thought, however, that they were due to some coincidental pre-existing pathology and were not part of the oxygen toxicity syndrome. They may serve to explain the very high pulmonary artery pressures found in this animal.

The finding that only two out of ten animals died a 'cardiac' death conflicts with Clarke's four out of six. The most likely reason for this is the greater importance attached in the present study to maintaining a correct fluid balance. Despite 'intermittent infusion' of Dextran 110 in 5% Dextrose to 'maintain fluid balance and to replace losses resulting from the withdrawal of blood samples' (Clarke), central venous pressure fell to a mean of -4.5 mm.Hg. at two hours before death and to approximately -8 mm.Hg. at apnoea. No attempt was made to monitor haematocrit and at the moment of apnoea the animals were almost certainly in hypovolaemic shock.

In the present experiment, infusion of Ringer lactate was constantly adjusted to maintain central venous pressure and haematocrit readings within normal limits. It may well be that, had this not been done, the extra load on the heart due to increased blood viscosity might have caused the animals to succumb earlier from cardiac causes. Certainly the appearance of jaw retraction, 'square-wave' respirations and early interstitial oedema in Dogs 2 and 12 suggests that, had the cardiac output been maintained, these animals would eventually have terminated similarly to the 'pulmonary death' group.

In both Clarke's and the present study, one animal (Dog 6) was atypical in that it had sudden terminal ventricular asystole following a prolonged period of greatly increased cardiac output. Dog 6 was also the animal with Toxocara infestation; it is not known if this is significant.

'PULMONARY DEATH'

The 'pulmonary death' dogs died from hypoxaemia caused by a fulminating intra-alveolar oedema. The oedema fluid produced both a diffusion block and such a fall in compliance that ventilation decreased to zero.

The alterations in the respiratory pattern (Figure 10) could arise either as a direct effect of oxygen on the respiratory centres or from peripheral receptors in the lung. Smith (1971) investigated the importance of vagal afferent fibres by performing bilateral cervical vagotomy on three dogs, and reported that this made no change in the pattern. He concluded that the mechanism of action was central. This conflicts with experiments described elsewhere in this thesis (see Sections 5 and 9), where vagotomy completely abolished the response and respiration remained regular throughout, suggesting a peripheral action.

The time of onset of jaw retraction and 'square-wave' respirations (six to eight hours) tended to coincide with a rise in pulmonary artery pressure and an increase in pulmonary vascular restance. The cause of these changes is not known, but it is tempting to suggest that they may have the same cause as the decrease in compliance which was becoming apparent at this time. Compliance change is likely to signal the development of interstitial oedema and it is interesting to note that in the 'cardiac death' dogs, where jaw retraction and 'square-wave' respirations became established only a very short time before death, early interstitial oedema was in fact present.

Compliance continued to fall over the next five hours, possibly due to the continuing decrease in cardiac output causing congestion, but perhaps also due to an increase in the degree of

interstitial oedema.

Then, at about three hours before death, there was a sudden catastrophic fall in arterial oxygen tension together with a sharp fall in compliance and a rise in pulmonary shunt ratio. It is at this point that one must postulate the appearance of intra-alveolar oedema which once established tends to be rapidly progressive.

The consistent 'dip' in pulmonary artery wedged pressure seen in both groups of dogs at about ninety minutes before death remains unexplained.

Cause of oedema

In general, pulmonary oedema may be considered to be due to changes either in the balance of hydrostatic forces, or in alveolar membrane permeability.

Increased hydrostatic pressures as a cause of oedema has been accepted for very many years, since the demonstration by Welch (1878) that acute left ventricular failure caused waterlogging of the lungs. The ultimate condition, pulmonary capillary hypertension may be due either to a raised pulmonary venous pressure, as above, or to an increase in pulmonary arterial pressure (Fishman, 1972).

More recent is the recognition that the alveolar-capillary barriers can become exceptionally permeable following injury (Crone & Lassen, 1970).

Increased pulmonary artery pressure in response to hyperoxia has been reported previously (Bennett & Smith, 1934; Brooksby, Datnow & Menzel, 1967) and histological changes suggestive of pulmonary hypertension demonstrated (Kydd, 1967). Clarke et al. (1973) described a rise in mean pulmonary artery pressure of 6 mm.Hg.; in the present experiment the rise appeared to be in the order of only 3 mm.Hg. These rises are modest in physiological terms, and occur only transiently some five hours before the suggested onset of intra-alveolar oedema; their significance is uncertain.

In a series of experiments on rats and dogs at pressures of oxygen of 1, 3 and 5 ATA, Wood has shown (Wood, Seager & Perkins, 1967; Wood & Perkins, 1970; Wood, Perkins, Smith & Reaux, 1972; Wood & Perkins, 1974) that pulmonary oedema appeared secondary to a severe sustained systemic hypertension which caused left ventricular failure and a rise in pulmonary venous pressure. In the present experiment mean systemic arterial pressure remained below 100 mm.Hg. and over the last nine hours fell progressively to 40 mm.Hg. Pulmonary artery wedged pressure fell from an initial level of around 4 mm.Hg. to a mean of about 2 mm.Hg. at apnoea.

It must be pointed out, however, that in the lung, a very large number of parallel vessels are being perfused, and that the distribution of pulmonary vascular resistance may be non-uniform (Lehr, Tuller, Fisher, Ellis & Fishman, 1963). It follows from this that small areas of localised pulmonary venous constriction may cause a local capillary hypertension leading to oedema even although the left atrial pressure remains normal. This has been suggested as a possible mechanism for the pulmonary oedema of high altitude (Grover, Reeves, Will & Blount, 1963; Viswanathan, Jain & Subramanian, 1969)

and as the mechanism for the pulmonary oedema associated with pulmonary emboli (Visscher, 1962).

Thus, if the Swan-Ganz catheter were wedged in a 'patent channel', the recorded pressure would appear to be normal. However, in all of the experiments covered in this thesis involving over fifty animals, there was no single instance of a high pulmonary artery wedged pressure recorded at any time.

In the absence of haemodynamic factors, therefore, one must look either for changes in alveolar membrane permeability, or for other factors altering the transmural capillary pressure gradient, for example changes in alveolar surface tension. Section 4 describes an electron microscopic investigation of tissues from these animals.

SECTION 4

DOG EXPERIMENTS SERIES 1

EIECTRON MICROSCOPY AND STEREOMETRIC ANALYSIS

ELECTRON MICROSCOPY AND STEREOMETRIC ANALYSIS

Cell types of the alveolar wall

The alveolar blood-gas barrier consists of the endothelial cells forming the walls of the pulmonary capillaries and an epithelial layer lining the alveoli. This epithelial layer was demonstrated by Low & Daniels (1952) to be continuous and was first stated to consist of two different cell types ('septal' and 'alveolar') by Policard, Collet, Ralyte & Renet in 1954. (It is noted that Sorokin (1967) quotes Reinhardt (1847) as 'one of the first to note that the alveolar epithelium of the mammalian lung contains cuboidal alveolar cells as well as squamous lining cells and to suggest that the squamous cells may be capable of conversion into cuboidal cells.' Just how this was achieved by primitive light microscopy is not clear.)

Various different names have been applied to these cell types, but there is now a general acceptance of the term 'pneumonocyte' (Macklin, 1953), subdivided into the Type 1 and Type 2 of Campiche (1960).

Type 1 pneumonocyte

This cell covers by far the greater part of the alveolar surface. The cytoplasmic extensions are extremely attenuated and may be as thin as 0.2 micrometres. The nucleus (the only part visible by light microscopy) is also elongated. An average total diameter (human) is 54 micrometres, and in each human alveolus there are approximately 110 Type 1 cells covering an area of 259,000 square micrometres (Meyrick & Reid, 1970). A Golgi system and endoplasmic reticulum are

found close to the nucleus, but elsewhere in the cell, organelles are sparsely distributed. Numerous pinocytotic vesicles may be found in the attenuated parts. Adjacent cell membranes fuse together to form zonulae occludentes (see Section 2). The extremely thin portions are ideally adapted to gas exchange.

Type 2 pneumonocyte

In the Type 2 cell the cytoplasm is concentrated around the nucleus and does not extend beyond the perikaryon. This confers on it a roughly cuboidal shape, but for an epithelial derivative, it is remarkably pleomorphic. The Type 2 cell forms junctional complexes with the Type 1 cell, and rests directly on the basement membrane. It is most commonly seen with a broad base applied to the basement membrane, partially buried by the extensions of neighbouring Type 1 cells; but forms which are less extensively attached, pedunculated forms,and even isolated forms lying free in the alveolar lumen are occasionally found. It has been suggested by Forrest (1969) that this might represent not so much a static spectrum of cell position in the alveolar wall as an actual turnover route for the cells, with the effete cells being sloughed off and discarded. He noted in this context that the number of inclusion bodies (q.v.) appeared to decrease with the relative degree of detachment from the alveolar wall, suggesting that the number of inclusion bodies diminished with cell ageing.

The free borders of the Type 2 cells have extensive microvilli.

The internal organisation of the Type 2 cell attests to its

active secretory nature (Sorokin, 1967). There is an extremely well-developed endoplasmic reticulum extending throughout the whole of the cytoplasm. Ribosomes extend along the whole of the cisternal membranes and are often closely associated with the numerous large mitochondria. The Golgi apparatus is so extensive that portions of it appear in almost every plane of section. Multivesicular bodies are present to an extent found in few other cell types. They are most conspicuous close to the Golgi regions where they appear to originate. Multivesicular bodies are also found in intimate association with another structure which is the most conspicuous morphological feature of the Type 2 cell, the lamellated inclusion body. Various intermediary forms between the two structures can be recognised, and although there is still some disagreement (Pattle, Schock, Dirnhuber & Creasey, 1972; Schock, Pattle & Creasey, 1973), it is now fairly generally accepted that the inclusion bodies are derived from the multivesicular bodies.

The inclusion bodies are ovoid structures 0.2 to 1.0 micrometres in diameter which by conventional light microscopy fixation present a vacuolated appearance. By electron microscopy they are seen to be bounded by a double-layer plasma membrane and to contain osmiophilic material usually arranged in concentric lamellae with a repeat interval of about 50 to 100 Angstroms.

The evidence for the inclusion bodies as being storage sites for pre-formed surfactant is presented in Section 2.

There are about 170 Type 2 cells per human alveolus, covering an area of 11,000 square micrometres (Meyrick & Reid, 1970). They are commonest at the junctions of the alveolar walls ('corner

cells') and are usually single (Bertalanffy, 1964). Occasionally, several Type 2 cells may be found side by side, particularly where the epithelium abuts against the adventitia of small vessels and airways, and rarely, a clump of Type 2 cells may be found just below the pleural surface (Sorokin, 1967).

Capillary endothelium

The capillary endothelium consists of cells which are morphologically almost indistinguishable from Type 1 epithelial cells. Occasional multivesicular bodies and small mitochondria may be found, mainly confined to the perinuclear cytoplasm; numerous pinocytotic vesicles are present in the extensions. It has been stated that Weibel-Palade bodies (Weibel & Palade, 1964) - organelles peculiar to endothelium and thought to have some function in blood clotting - are more numerous in the pulmonary circulation (Meyrick & Reid, 1970). The junctions between adjacent endothelial cells have 'pores' approximately 50 Angstroms in diameter (see Section 2).

Other cell types

Other cell types found in the alveolar walls include macrophages, pericytes, Type 3 epithelial cells, and fibroblasts.

Macrophages

The alveolar macrophages are large cells (20 micrometres) with a large eccentric nucleus and a prominent nucleolus. The cell surface is irregular, but they do not have cytoplasmic processes comparable with the Type 2 cells. The endoplasmic reticulum is sparse.

The most prominent feature of the alveolar macrophages is the large number of densely osmiophilic inclusions (0.18 to 2.2 micrometres in diameter) representing phagocytosed alveolar material. The comparative histochemistry of the macrophage and the Type 2 cell has been studied by Karrer (1958), Buckingham, McNary & Sommers (1964), Sorokin (1967), Goldfischer, Kikkawa & Hoffman (1968), and Vatter, Reiss, Newman, Lindquist & Groeneboer (1968).

Pericytes

The electron microscopical appearance of the pericyte in rabbit skin has been described by Rhodin (1968) who thought it was an immature muscle cell. It is also present in the alveolar septum. Its main significance in the context of this thesis is that the fine pericyte cytoplasmic extensions, complete with tubules approximately 200 Angstroms in diameter (Meyrick & Reid, 1971) may be mistaken for nerve fibres (see Section 6). The pericytes are absent from the 'thin' portions of the alveolar septum where the basement membranes are fused (see Section 2) and it has been suggested that their function is to monitor the passage of liquid across the capillary wall (Robin, Cross & Zelis, 1973).

Type 3 epithelial cell

A third type of alveolar epithelial cell has been described, peculiar to the rat lung (Meyrick & Reid, 1968). It is a low cuboidal cell with squat blunt microvilli on its free edge, similar to the 'brush cell' present in the large airways of the rat and in man. It is said to comprise 5 to 10% of the alveolar pneumonocytes and it is

suggested that it may be a stretch or chemoreceptor. An alveolar wall nerve fibre has been demonstrated adjacent to (Meyrick & Reid, 1971), and in a basal invagination of (Weibel, 1973) a Type 3 cell.

Response of the alveolar septum to hyperoxia

1) Endothelial and Type 1 epithelial cells

A series of experiments reported between 1967 and 1969, utilising the stereometric techniques of Weibel (Weibel, 1963, Weibel & Elias, 1967) has greatly increased the understanding of the response of the endothelial and Type 1 epithelial cells to hyperoxia.

In a study on rats exposed to 100% oxygen at 1 ATA (Kistler, Caldwell & Weibel, 1967) it was found that following exposure for 48 hours, the animals became dyspnoeic on return to room air. By electron microscopy it was shown that, whereas both the endothelial and epithelial cells appeared normal, there was a doubling of the average thickness of the interstitial space causing an estimated 20% decrease in diffusing capacity. Following 72 hours exposure, dyspnoea and cyanosis on return to room air was severe and several animals died. The gross pathological appearances of pulmonary oxygen toxicity were obvious and electron microscopy revealed the interstitial space to be invaded by leucocytes, thrombocytes, cell fragments and fibrin. The most remarkable finding was that while the Type 1 epithelial cells appeared normal or showed some hyperplasia, the capillary endothelium had suffered extensive damage and was completely destroyed over large areas. Some 65% of the alveolar lumen was obliterated by oedema fluid.

Morphometric analysis showed that the average thickness of

the epithelial layer had increased by 50% (due to cellular hyperplasia), while that of the endothelial layer had decreased, due to cell destruction, to 60% of the control value. Total barrier thickness was doubled; this caused a decrease in estimated diffusing capacity to 25% of the control, with a further fall to about 9% when corrected for the presence of alveolar oedema.

The unexpected finding of capillary endothelial rather than alveolar epithelial destruction has been confirmed repeatedly in several different species (Schaffner, Felig & Trachtenberg, 1967; Bowden, Adamson & Wyatt, 1968; Vinogradov & Babchinskiy, 1969; Adamson, Bowden & Wyatt, 1970; Yamamoto, Wittner & Rosenbaum, 1970; Coalson, Beller & Greenfield, 1971; Nash, Bowden & Langlinais, 1971; Weibel, 1971; Gould, Tosco, Wheelis, Gould & Kapanci, 1972; Valimaki, Kivisaari & Niinikoski, 1974; Bonikos, Bensch, Ludwin & Northway, 1975).

When the same type of experiment was performed on monkeys (Robinson, Harper, Thomas & Kaplan, 1967; Kaplan, Robinson, Kapanci & Weibel, 1969; Kapanci, Weibel, Kaplan & Robinson, 1969; Robinson, Sopher, Witchett & Carter, 1969), a marked species difference in response was observed. Monkeys survived much longer, about two weeks as opposed to 72 hours, and displayed two distinct phases of response; an early acute exudative phase and a subsequent subacute proliferative phase.

Exposure was to 90 to 100% oxygen at 750 mm.Hg. for up to 12 days. Approximately 50% of the animals died of acute oxygen toxicity by the seventh day, whereas the remainder survived longer and entered the subacute phase.

Stereometric examination revealed that after two days the

only abnormality was slight swelling of the endothelial cells. By the fourth day this swelling had increased (a 35% increase over control values), but there was widespread destruction (up to 90%) of the Type 1 epithelial cells together with a 2.5 times increase in the relative volume of the Type 2 cells. At seven days exposure, the Type 1 cells were almost completely destroyed and were replaced by a massive hyperplasia of the Type 2 cells, resulting in a focal but extensive increase in membrane thickness (average epithelial thickness increased by 300%).

By the twelfth day of exposure, the Type 1 cells had been completely replaced by Type 2 cells (a 22 times increase in Type 2 cell volume) resulting in a seven-fold increase in mean epithelial cell thickness. There was a decrease in endothelial cell volume to one third of the control value, but capillary surface area was unchanged.

It has been suggested by Kapanci et al. (1969) that the difference in the pathological response to hyperoxia between the rat (Kistler et al. 1967) and the monkey lies in a species difference in susceptibility to oxygen; that the rats, which all died within about three to four days, exhibited only the acute exudative response and did not survive sufficiently long to develop proliferative lesions (see also Figure 10:1).

2) <u>Type 2 epithelial cells</u>

a) <u>Mitochondria</u>

Degenerative changes in the mitochondria of the Type 2 cells

in response to 100% oxygen at 1 ATA were described by Rosenbaum, Wittner & Lenger (1969), and have been confirmed repeatedly (Yamamoto et al., 1970; Adamson et al., 1970; Massaro & Massaro, 1973a). Massaro & Massaro (1973a) have reported a decrease in the number of mitochondrial granules following exposure to oxygen and suggest that this might represent the loss of intramitochondrial cations. Calcium ions have been shown to be involved in the activation of alpha-glycerophosphate oxidation (Fisher, Basset, Scarpa & Williamson,1972) and as this substrate plays an important role in the synthesis of phospholipid in the lung (Scholz, Woodward & Rhoades, 1972), an alteration of mitochondrial granules might signify an alteration in surfactant synthesis.

In animals which were 'adapted' to high oxygen tensions by prolonged exposure to sub-toxic levels, a large number of cup-shaped mitochondria - a form thought to be associated particularly with lipid metabolism (Seljelid & Ericsson, 1965) - were found (Rosenbaum et al., 1969). These changes did not appear in response to higher toxic levels of oxygen and were thus considered to be adaptive rather than degenerative. Yamamoto et al. (1970) showed that adaptation by prior exposure to non-toxic levels of oxygen was associated with elongation of the mitochondria and with an increase in mitochondrial volume. With exposure to 100% oxygen at 1 ATA following adaptation, the mitochondria developed cup shapes. Massaro & Massaro (1973b), on the other hand, found no difference in the percentage cytoplasmic volume, surface area to volume ratio, nor absolute volume of mitochondria following 48 hours exposure to 100% oxygen at 1 ATA. Bonikos et al. (1975), working on newborn mice whose tolerance to

oxygen resembles that of monkeys (see page 4:8) found no change in the mitochondria before the sixth day of exposure, but the development of elongation, pleomorphism and cup shaping thereafter.

b) Inclusion bodies

In an early study of the effects of oxygen (100% O_2 at 1 ATA for 38 hours, or at 3 ATA for 8 hours) on rats, Treciokas (1959) described increased vacuolation in the inclusion bodies compared with the control animals.

Similar degenerative changes in response to normobaric hyperoxia have been described since (Morgan, Finlay, Huber & Fialkow, 1965; Motlagh, Kaufman, Guisti, Cramer, Garzon & Karlson, 1969; Yamamoto et al., 1970). Massaro & Massaro (1973b) showed that, following exposure of rats to 98% oxygen for 48 hours, there was no change in the numbers of inclusion bodies (thus confirming an earlier observation by Rosenbaum et al., 1969) but that the percentage organelle cytoplasmic area and absolute organelle volume were significantly reduced. Further studies (Massaro & Massaro,, 1974) on adapted rats showed that at 96 hours this decrease in size of the inclusion bodies had been reversed and that there was no longer any difference from the control values. In newborn mice exposed to similar pressures (Bonikos et al., 1975), there was no difference in the size or numbers of inclusion bodies up until the fifth day of exposure, whereupon there was a large and progressive increase in numbers per cell; this coincided with the marked hyperplasia of Type 2 cells which signified the onset of the subacute proliferative phase of response.

3) Macrophages

Reports on the effects of hyperoxia on macrophage activity are conflicting. Morgan et al. (1965) (in dogs), and Schaffner et al. (1967) (in rats) reported an increase in numbers in response to 100% oxygen, whereas Meyrick, Miller & Reid (1972) found a significant reduction in numbers after 24 hours exposure in rats. In a study of cell turnover in mice by tritiated thymidine labelling, Bowden et al. (1968) found the total number of macrophages to be unaltered under hyperoxic conditions (90% O_2 at 1 ATA).

Circumstantial evidence of impairment of macrophage function comes from the demonstration of increased mortality and a decrease in survival time in animals with viral or bacterial pneumonia combined with exposure to 100% oxygen at 1 ATA (Finder, LaForce & Huber, 1972; Ayers, Tierney & Imagawa, 1973; Angrick, Somerson & Weiss, 1974). It has been suggested that this possibly results from a reduced ability of the macrophages to ingest bacteria (Thurlbeck, 1974). In a study of radiolabeled Staphylococcus aureus pulmonary infection (Huber, LaForce & Mason, 1970), a progressive impairment of bacterial inactivation in response to 100% oxygen at 0.87 ATA was found to correlate with progressive vacuolisation of the macrophages. Once the biochemical and structural changes of oxygen toxicity became manifest, bacterial replication exceeded inactivation by the macrophages.

A different situation results under hyperbaric conditions. It has been found that following exposure to 100% oxygen at 2 to 3 ATA, the toxic effect of oxygen was greater on the bacteria than on the host's defence mechanisms, and that such exposure prolonged the sur-

vival of mice infected with pneumococci (Ross & McAllister, 1965).

Inhalation of ozone (5 ppm for 3 hours) by rabbits has also been shown to impair antibacterial defence mechanisms and is associated with intracellular vacuolisation, swelling of mitochondria and cell lysis of the alveolar macrophages (Huber, Mason, LaForce, Spencer, Gardner & Coffin, 1971).

4) Type 3 cells

Following exposure of rats to 100% oxygen at 1 ATA for 24 hours, a significant reduction in the number of Type 3 epithelial cells was found (Meyrick, Miller & Reid, 1972). It was noted, however, that this might have been more apparent than real as certain structural alterations, for example regression of microvilli and encroachment of a cytoplasmic covering from adjacent Type 1 epithelial cells, might have disguised the Type 3 cells sufficiently to prevent their being counted.

Principles of stereometric analysis

Stereology allows estimates of numbers, volumes and surface areas of three-dimensional objects to be made from counting operations on two-dimensional sections of these objects. The principles involved are simple (Elias, Hennig & Schwartz, 1971).

1) Volumetric analysis

The Principle of Delesse (Delesse, 1846) states that the volume fraction V_V of a given component in a tissue can be estimated

by measuring the area fraction A_A covered by transections of the component on a very thin random section of the tissue (i.e. one where the volume of an individual component greatly exceeds the thickness of the slice through it).

$$\text{i.e. } V_V = A_A$$

Glagoleff (1933) showed that if a lattice of regularly spaced points were superimposed on the tissue section, the fraction of points lying over the component was equal to its area fraction, i.e.

$$V_{Va} = A_{Aa} = P_{Pa} = \frac{Pa}{P_T}$$

where V_{Va} = volume fraction of component a.
P_a = number of points lying on a.
P_T = total number of points on the section.

2) <u>Surface area</u>

On a section of tissue, the surface membrane of a component appears as a line surrounding the sectioned component; the length of this line is proportional to the surface area (Sv) of the membrane. It has been shown by Hennig (1956) that the number of intersections (N) that this line makes with a test line of total length L_T is proportional to the surface area of the membrane. If N_L is the number of intersections between the surface and the unit length of the test line, then:

$$Sv = \frac{2N}{L_T} = 2.N_L$$

3) <u>Surface area to volume ratios</u>

The surface area to volume ratio may be derived from a

combination of the data inserted into the above equations (Chalkley, Cornfield & Park, 1949).

$$\text{Surface area/volume} = \frac{4.Nz}{Z.P}$$

where Nz = number of intercepts with the surface membrane.

Z = length of each line.

P = number of points lying on the component.

4) Number of particulate structures

The number of approximately spherical structures N_V contained in a unit volume of tissue is proportional to the number of particle trans-sections N_A found in a unit area of section.

$N_A = N_V.\overline{D}$ where $\overline{D}$ = mean diameter.

Further corrections must be made, however, where the particle shape varies.

The three required test systems, a lattice of test points for volumetric estimation, test lines for surface measurements and a test area for particle counting, can be combined in a single grid (Weibel, Kistler & Scherle, 1966) as described below.

MATERIALS AND METHODS

Tissue sampling

Alternate right and left (dependant and superior) lungs were removed from the eight 'pulmonary death' animals and the three normoxic hyperbaric control animals described in the previous section. Twelve tissue samples approximately 1 centimetre square were taken at

random from widely separated areas covering the whole lung, and were diced under gluteraldehyde into 1 millimetre square tissue blocks.

Fixation, embedding and sectioning

The initial fixation by tracheal instillation of 2% gluteraldehyde buffered to a pH of 7.3 with 0.1 M sodium cacodylate was as described on Page 3:43. The diced tissue blocks were washed in cacodylate buffer solution for a minimum of 4 hours to remove any free aldehydes and were post-fixed in 1% osmium tetroxide in 0.1 M sodium cacodylate at pH 7.3 for 1 hour. They were then dehydrated through graded alcohols, cleared in propylene oxide and embedded in Araldite.

This fixation schedule was chosen as being optimal for preservation of the alveolar membrane (Gil & Weibel, 1968); it is not ideal for the preservation of the saturated phospholipids in the inclusion bodies (Schock, Pattle & Creasey, 1973), but unfortunately, as the study was not specifically of inclusion bodies, this was not realised to be a disadvantage at the time.

Ultrathin sections for electron microscopy were cut at 500 Angstroms with a glass knife on an L.K.B. ultramicrotome and mounted on uncoated copper grids. One micrometre sections for cellular stereometry were mounted on glass slides.

The ultrathin sections were stained in alcoholic uranyl acetate followed by lead citrate, and examined on either an A.E.I. AM6B or a J.E.O.L. 100C electron microscope. The one micrometre sections were stained in 1% para-phenylenediamine, cover-slipped and examined by phase contrast microscopy on a Zeiss Ultraphot microscope.

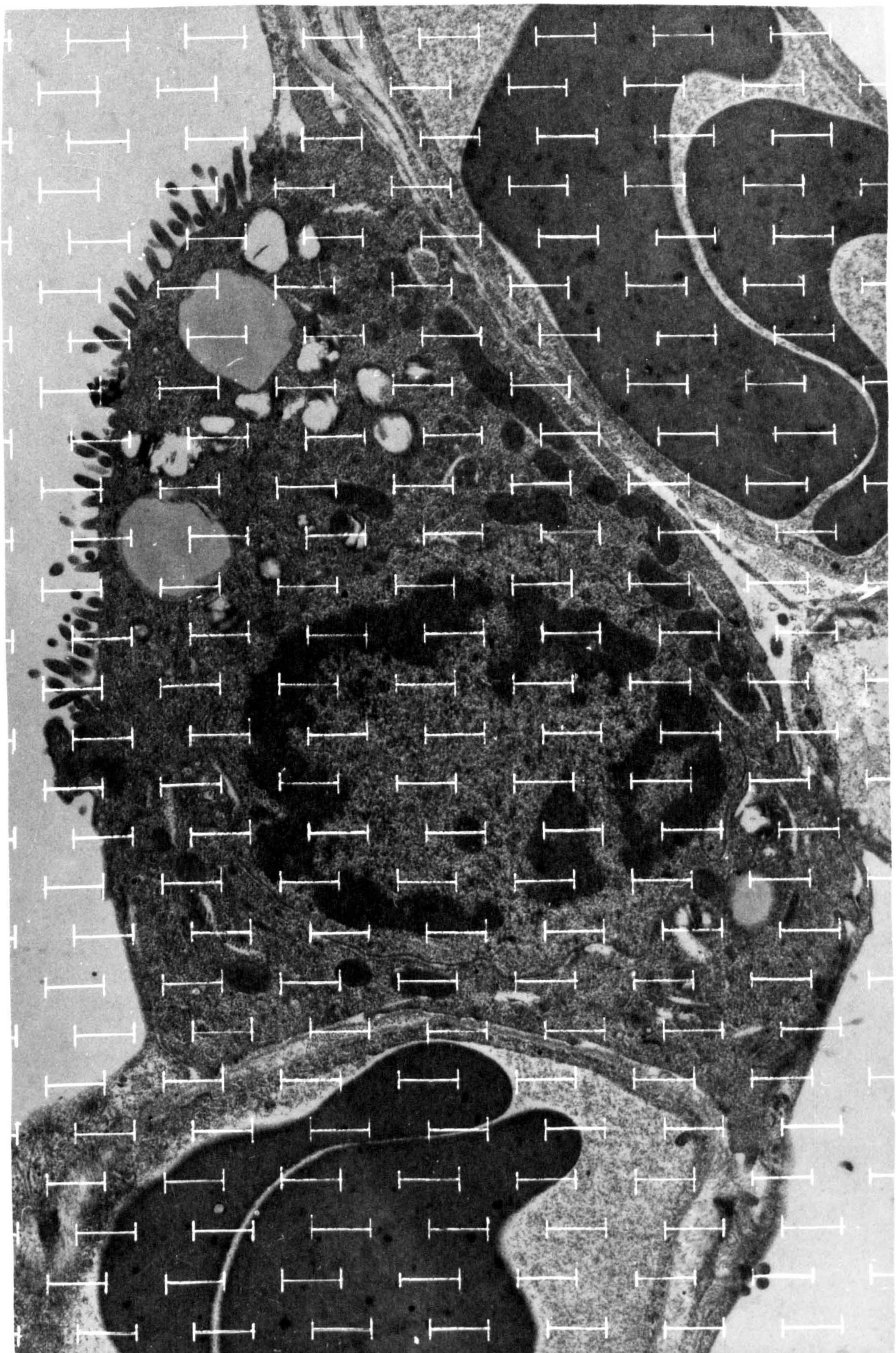


FIGURE 4:1. WEIBEL GRID ON TYPE 2 CELL. (x 20,000)

Electron micrographs were taken of random fields throughout each section. Particular attention was paid to the integrity of the alveolar membrane and to the Type 2 cells and macrophages.

Stereometry

Stereometric analysis was performed on the Type 2 epithelial cells.

Micrographs of 44 cells from the oxygen poisoned animals and 31 from the control animals were selected at random and enlarged to 10" x 8" prints. A multipurpose test grid was made consisting of 300 lines of length 1 centimetre arranged in equidistant ($\frac{1}{2}.\sqrt{3}$) parallel rows in a lattice of equilateral triangles (side length = 1 centimetre). The lines on this system are used for counting surface intersections (N_L) while their two end-points (a total of 600 per grid) provide a lattice of test points (P_P) for the estimation of relative volume density (V_V). Figure 4:1 shows a portion of the grid at actual size superimposed on a Type 2 cell and Table 4:1 lists the actual observations made.

For the cell counts, nine sections approximately one millimetre square were taken from each animal and some ten to fifteen alveoli were examined in each section. The total number of alveoli counted was 840 in the experimental and 315 in the control animals.

In the estimation of the numbers and position of the Type 2 cells and macrophages (from the one micrometre sections), the test area of the grid was not used as the lung has a convenient grid system 'built in'. Cell numbers were expressed simply as so many per alveolar section. Variations in alveolar size due to differences in planes of

TABLE 4:1

ULTRASTRUCTURAL STEREOMETRY : TYPE 2 CELLS

TOTAL OBSERVATIONS

a) EXPERIMENTAL — Number of fields = 44

	POINT COUNTING				INTERCEPT COUNTING			
	Vv nuc	Vv cyt	Vv incl	Vv total	Sv free	Sv basal	Sv nuc	Sv incl
TOTAL	3121	7563	774	10684	934	414	488	615
n	44	44	44	44	44	44	44	44
M	71	172	18	243	21	9	11	14
S.D.	51	56	13	72	9	7	7	8
S.E.	5.19	5.69	1.35	7.35	0.9	0.75	0.66	0.86

b) CONTROL — Number of fields = 31

	POINT COUNTING				INTERCEPT COUNTING			
	Vv nuc	Vv cyt	Vv incl	Vv total	Sv free	Sv basal	Sv nuc	Sv incl
TOTAL	2230	4538	476	6768	528	381	420	379
n	31	31	31	31	31	31	31	31
M	72	146	15	218	17	12	14	12
S.D.	58	45	9	60	7	7	9	6
S.E.	6.98	5.48	1.06	7.32	0.86	0.81	1.08	0.77

section could be expected to be compensated for by the numbers of alveolar sections (1155) examined.

RESULTS

1) Capillary endothelium

The electron microscopical examination was undertaken in the expectation of demonstrating capillary endothelial degeneration causing gross permeability changes in the alveolar membrane. The most surprising result of the experiment therefore was an almost complete lack of damage to the endothelial cells. There was no evidence whatsoever of the extensive endothelial degeneration found following exposure to normobaric hyperoxia (see Page 4:6).

In addition, the junctions between adjacent endothelial cells remained 'tight' (Figures 4:2, 4:3 and 6:31) and there was no evidence of pore stretching or disruption which might be expected to follow capillary hypertension.

In two fields only out of the many hundreds examined, there was blebbing of the endothelial cells (Figures 4:4 and 4:5); they are reproduced here as a demonstration of exceptions to normal findings.

Figure 4:3 also demonstrates the extent of pinocytosis seen on many micrographs from the experimental animals. Work is in progress to quantitate this stereometrically, but a preliminary simple count of number of vesicles per unit length of transversely sectioned endothelial cell indicates an approximately two-fold increase in pinocytosis over the control values.

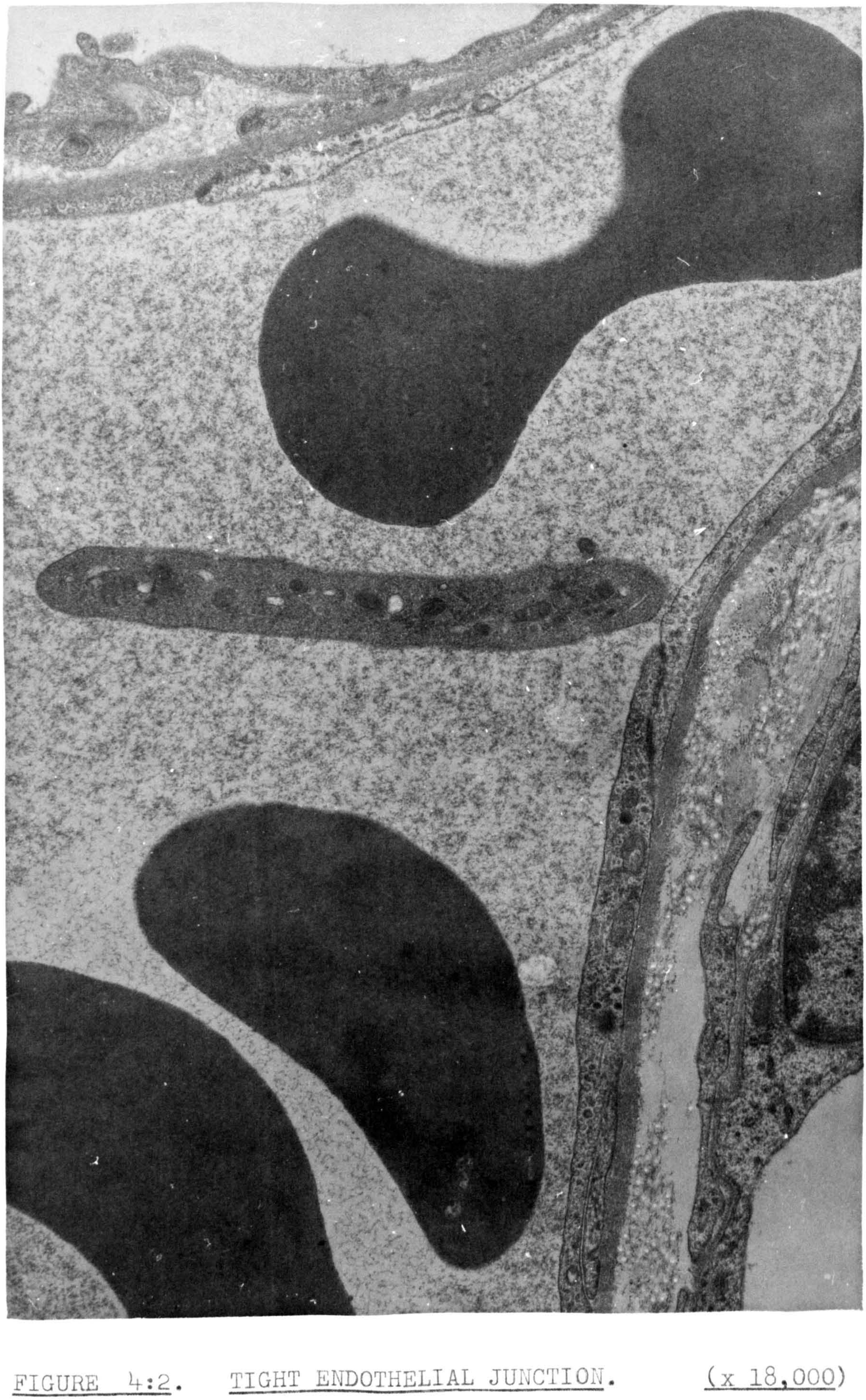


FIGURE 4:2. TIGHT ENDOTHELIAL JUNCTION. (x 18,000)

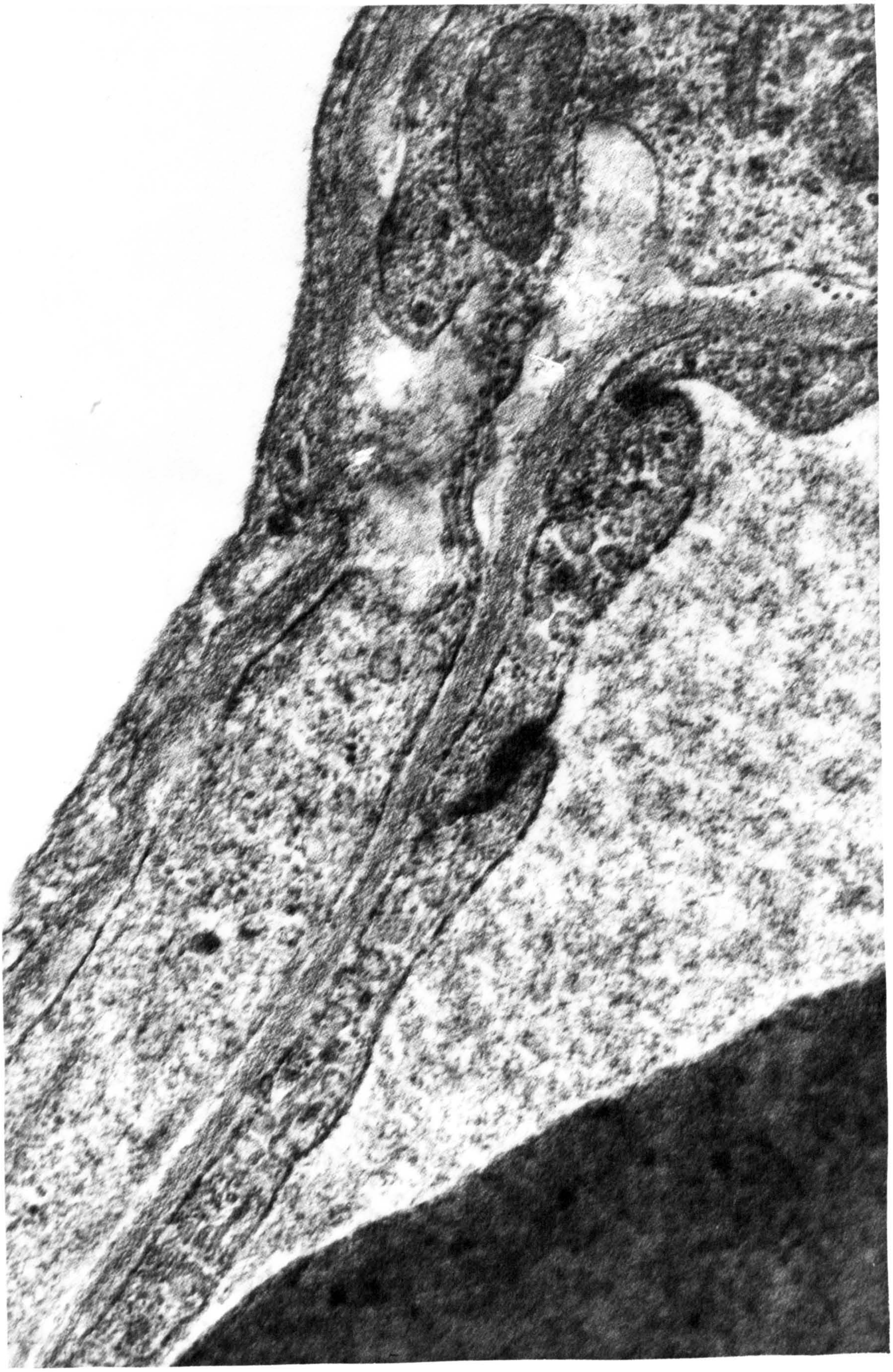


FIGURE 4:3. TIGHT ENDOTHELIAL JUNCTION. (x 60,000)

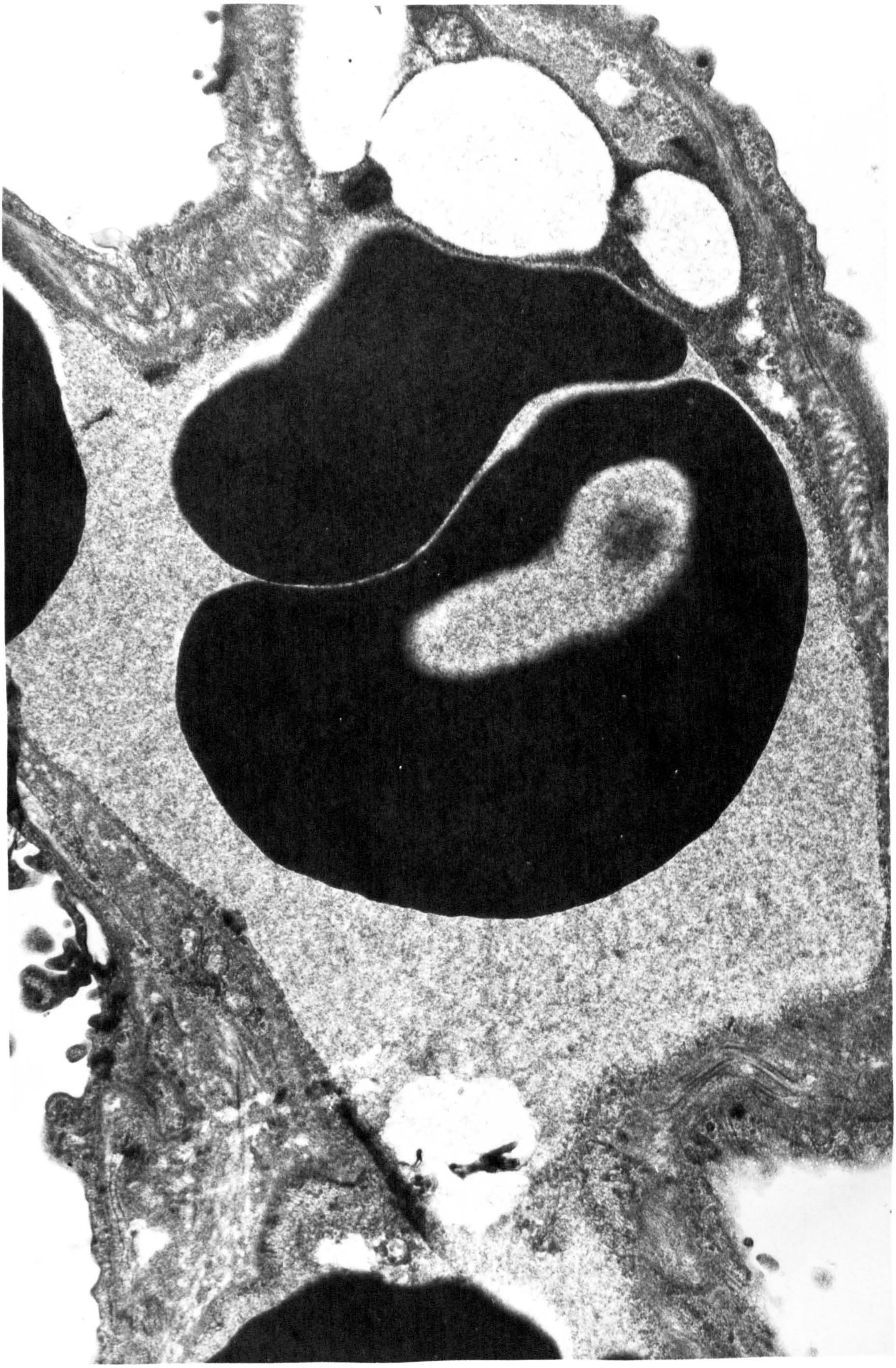


FIGURE 4:4. ENDOTHELIAL 'BLEBBING'. (x 20,000)

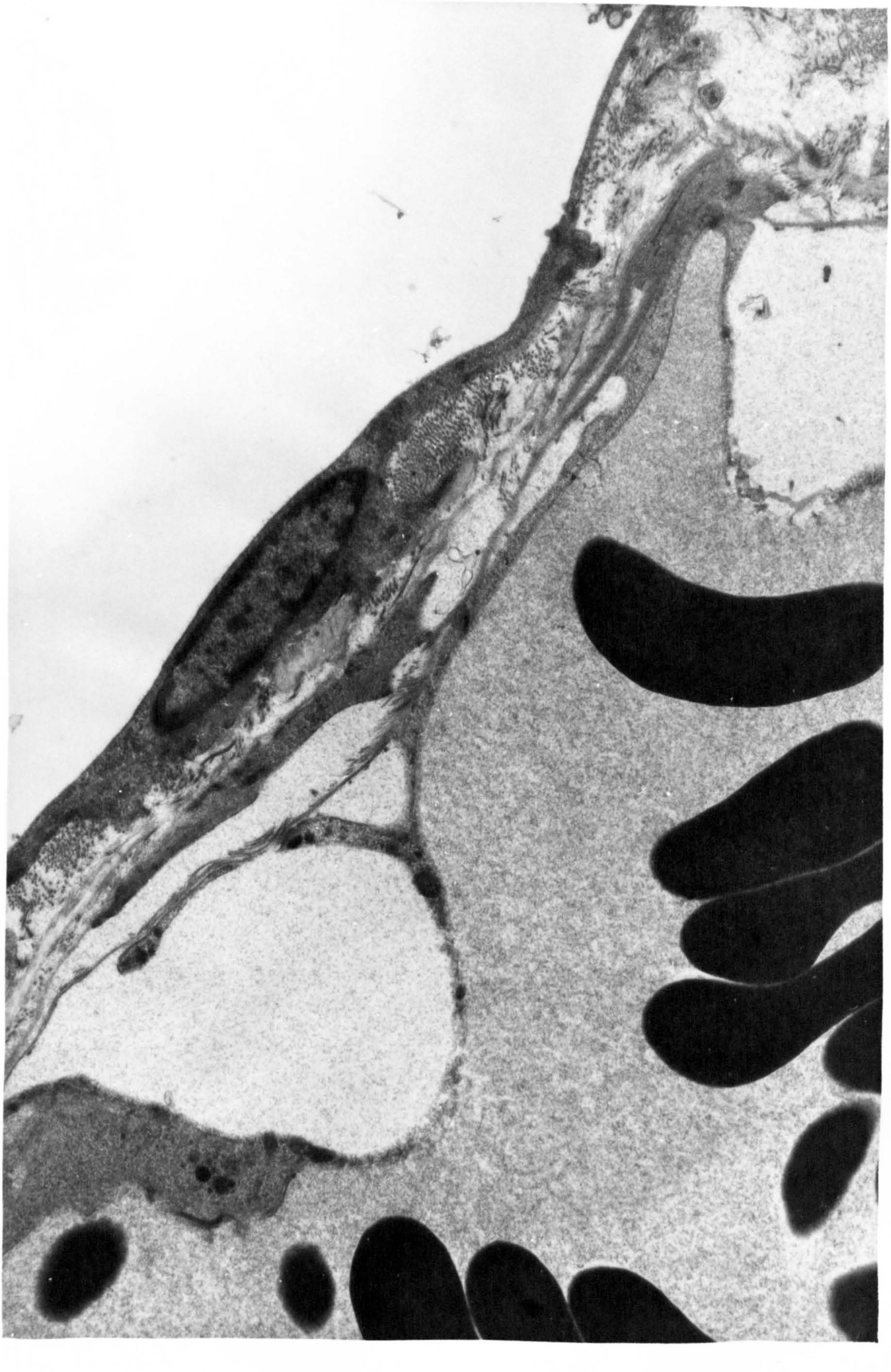


FIGURE 4:5. ENDOTHELIAL 'BLEBBING'. (x 10,000)

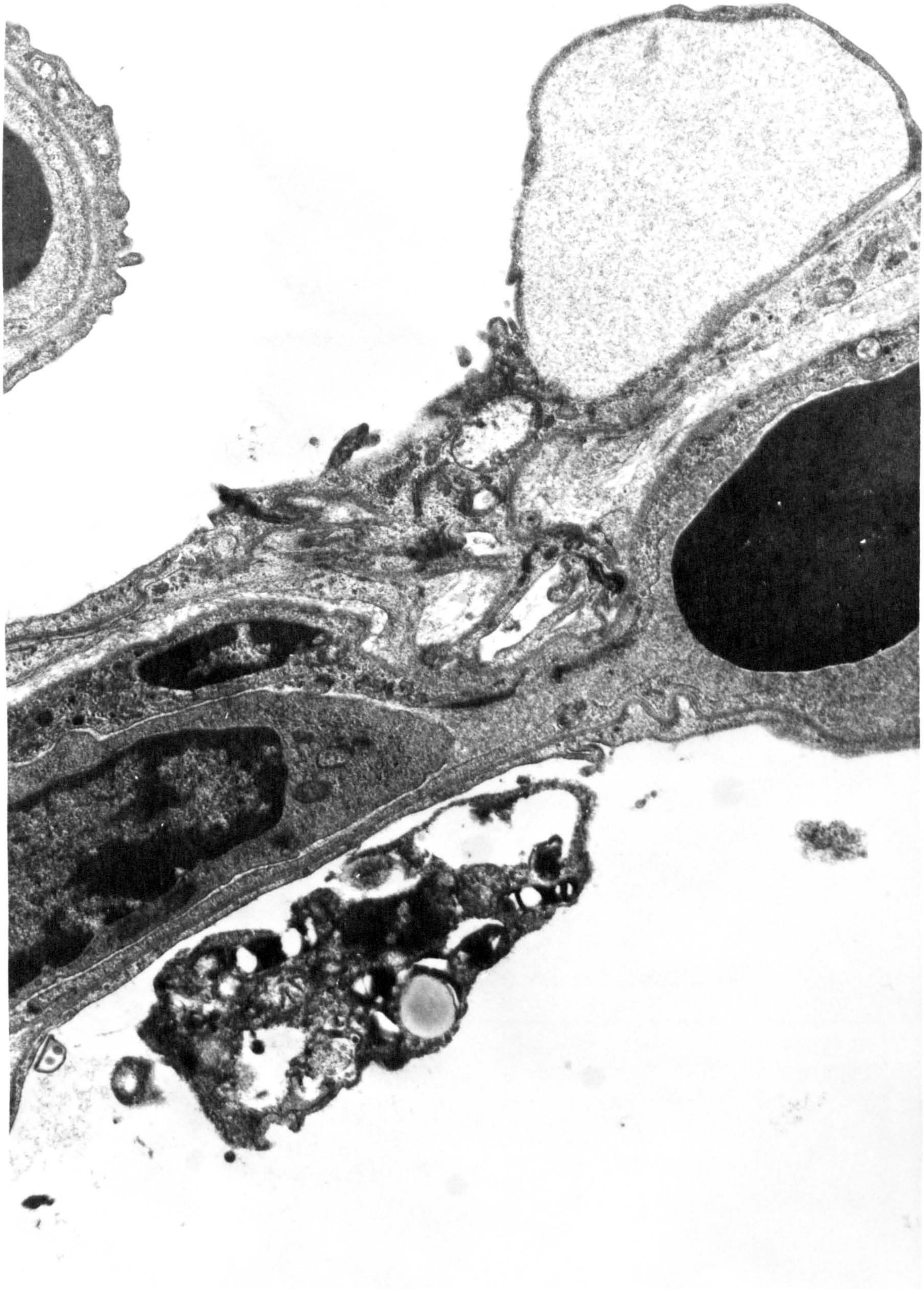


FIGURE 4:6. EPITHELIAL 'BLEBBING'. (x 14,000)

2) Type 1 epithelial cells

Almost no damage was found in the Type 1 epithelial cells. The zonulae occludentes remained tight and the basement membranes intact.

An epithelial bleb (Figure 4:6) was found in one field only out of the total number examined.

3) Type 2 epithelial cells

Examination of large numbers of alveoli on the one micrometre sections by phase contrast microscopy revealed that there was a large increase in the numbers of Type 2 cells, from a mean of 2.9048 per alveolus in the control animals to 4.574 per alveolus in the experimental animals ($P = <0.005$). (Note that in the presentation of these results, 'per alveolus' is used to represent 'per average alveolar section'.)

TABLE 4:2

CELLULAR STEREOMETRY : TYPE 2 CELLS AND MACROPHAGES

PARAMETER	EXPERIMENTAL	CONTROL	SIGNIFICANCE
Total no. of alveolar sections counted	840	315	-
Total no. of Type 2 cells counted	3842	915	-
Mean no. of Type 2 cells per alveolus	4.574	2.9048	$P=<0.005$
S.E.	0.5359	0.946	-
% of Type 2 cells free in lumen	18.01%	1.86%	$P=<0.001$
% of Type 2 cells pedunculated	32.17%	13.99%	$P=<0.005$
Total no. of macrophages counted	1445	119	-
Mean no. of macrophages per alveolus	1.3631	0.3778	$P=<0.001$
S.E.	0.915	0.0392	

In the experimental animals, Type 2 cells were found in sheets lining the alveolar walls (Figure 4:7) and occasionally in clumps, usually at the mouth of the alveolar sac (Figure 4:9). Where such clumps existed, the individual cells were connected by desmosomes (Figure 4:10). The greatest number of Type 2 cells found in any single alveolar section was 18 in the experimental animals, and 7 in the controls.

Table 4:2 also quantitates the greatly increased numbers of pedunculated (Figure 4:11) and completely isolated (Figure 4:12) Type 2 cells found in the experimental animals. It should be noted here that there was the occasional difficulty in distinguishing between Type 2 cells (particularly the more atypical isolated forms - see Figure 4:14) and macrophages by light microscopy and there may have been some transposition of numbers between the two groups. In view of the extremely high significance values for the increase in both groups, however, (see Table 4:2) this is not considered to be important.

From the electron microscopical examination of Type 2 cells in the experimental animals, a strong subjective impression was obtained that as the cells became more and more pedunculated, until they eventually became free in the alveolar lumen (Figures 4:11, 4:12, 4:13, 4:14, 4:16) they became more spherical, lost their cytoplasmic processes and developed a greater cytoplasmic to nuclear volume ratio than the controls, This, however, was not confirmed by stereometry (Table 4:3) and no significant difference was found in percentage nuclear, cytoplasmic nor inclusion body volumes between the experimental animals and the controls.

TABLE 4:3

ULTRASTRUCTURAL STEREOMETRY : TYPE 2 CELLS

PERCENTAGE VOLUME AND SURFACE AREA/VOLUME RATIOS

PARAMETER	EXPERI-MENTAL	CONTROL	SIGNI-FICANCE
Cytoplasmic volume / Total cell volume	70.79%	67.05%	N.S.
Nuclear volume / Total cell volume	29.21%	32.95%	N.S.
Inclusion body volume / Total cell volume	7.24%	7.03%	N.S.
Free cell surface area / Cell volume	0.3497	0.3121	N.S.
Basal cell surface area / Cell volume	0.1550	0.2252	P=<0.02
Nucleus surface area / Nuclear volume	0.6254	0.7534	N.S. (P=0.3)
Inclusion body surface area / Inclusion body volume	3.1783	3.1849	N.S.

Neither was there any increase in actual inclusion body number, nor inclusion body surface area to volume ratio (an index of inclusion body shape) between the experimental and the control animals.

Indeed the only ultrastructural morphometric difference to reach any level of significance was the cell basal surface area (i.e. the area of the cell resting on a basement membrane) to volume ratio, which is a reflection of the increased numbers of cells which are either pedunculated or lying free in the alveolar lumen (Table 4:2).

Many of the Type 2 cells from the experimental animals appeared to have increased numbers of multivesicular bodies in their cytoplasm (Figure 4:15), but this has not yet been quantitated by stereometry. In addition, an analysis has not yet been made of the effects of the oxygen exposure on mitochondria or on the endoplasmic reticulum.

In the experimental animals, a large number of Type 2 cells (Figure 4:16) and macrophages (Figure 4:17) were found trapped in the pores of Kohn.

4) Macrophages

A greatly increased number of macrophages was found in the experimental animals (1.3631 per alveolus compared with 0.3778 per alveolus in the controls). It was immediately obvious, however, that the presence of macrophages was focal, as 362 of the 840 'experimental' alveoli had no macrophages present at all, whereas in some other alveoli there were large groups (Figure 4:18). The greatest number observed in a single alveolus was 16 in the experimental animals, and 3 (on only one occasion) in the controls.

Many macrophages were observed and studied by electron microscopy. They were seen frequently to contain whole lamellated inclusion bodies (Figures 4:19, 4:20 and 4:21) in addition to other osmiophilic material from the alveolar lumen. Figure 4:21 shows a macrophage possibly in the process of engulfing an inclusion body.

In the material from Dog 6 (the animal with the proliferative bronchiolitis), there were large numbers of macrophages with extensive fine vacuolisation of the cytoplasm (Figure 4:22). It is

possible that these correspond to Bertalanffy's (1964) 'foamy macrophages' which have occasionally been found in animals with a mild lung infection (Meyrick & Reid, 1970).

5) Alveolar space

In the oxygen poisoned animals, large quantities of dense osmiophilic material was found to accumulate on the alveolar walls or to lie free in the alveolar space either in clumps or in structures reminiscent of tubular myelin figures (Weibel, Kistler & Tondury, 1966; Gil & Reiss, 1973) (Figure 4:23). On higher magnification (Figure 4:24), the substance lining the alveolar walls is seen to consist of a large number of parallel lamellations, and Figure 4:25 shows the possible contribution of two osmiophilic inclusion bodies to the formation of this structure.

Similar material was regularly observed inside the alveolar macrophages (Figures 4:20 and 4:21) and Figures 4:26 and 4:27 show, at different magnifications, the possible phagocytosis of such material by a macrophage.

A not uncommon finding in the experimental, but not the control animals was the presence of red blood cells in the alveolar lumen (Figure 4:28). There was no indication on any of the micrographs as to how they arrived there; this point is discussed below.

FIGURE 4:7. PHASE CONTRAST MICROGRAPH: SHEET OF TYPE 2 CELLS. (x 2,000)

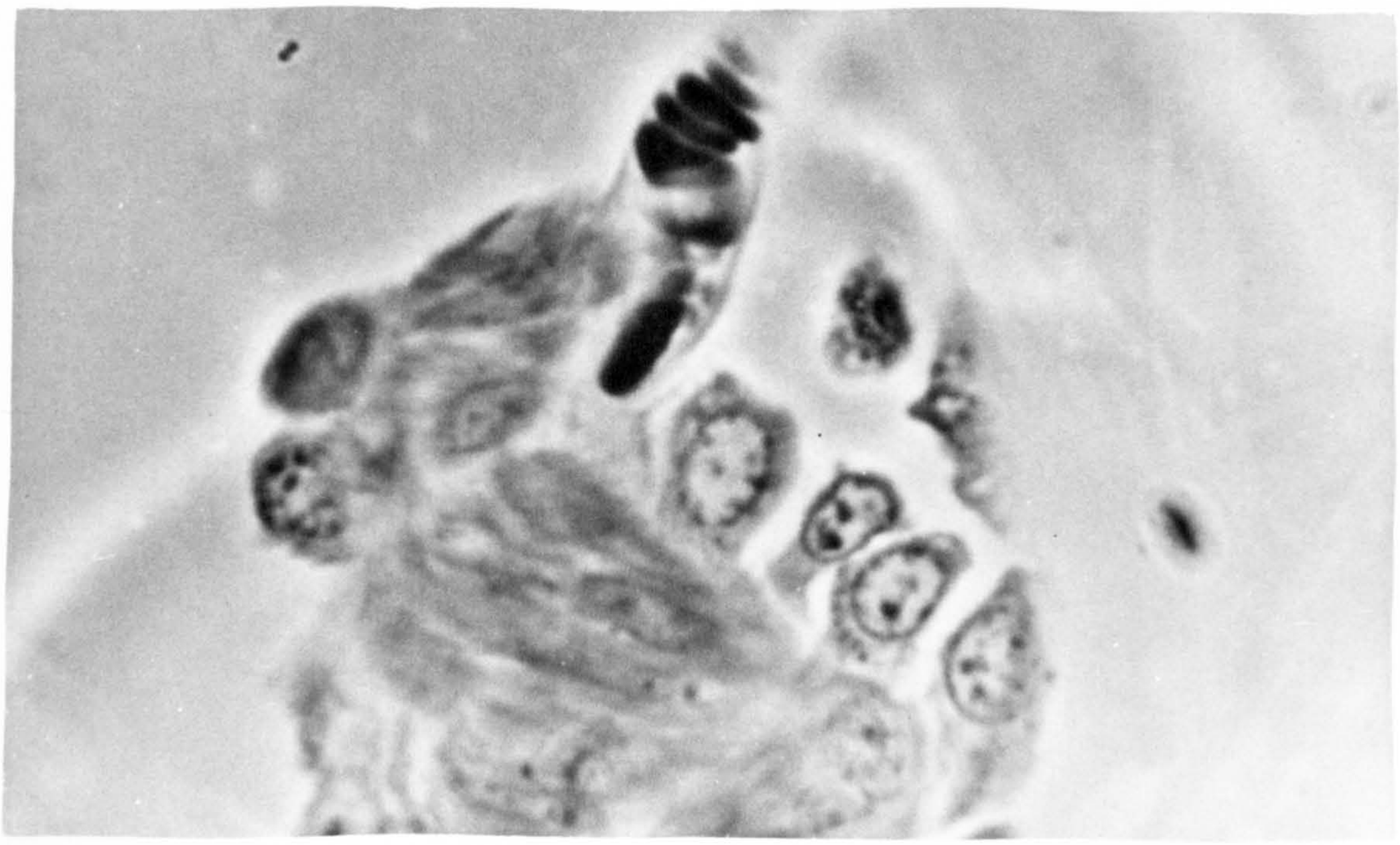


FIGURE 4:8. PHASE CONTRAST MICROGRAPH: CLUMP OF TYPE 2 CELLS. (x 2,000)

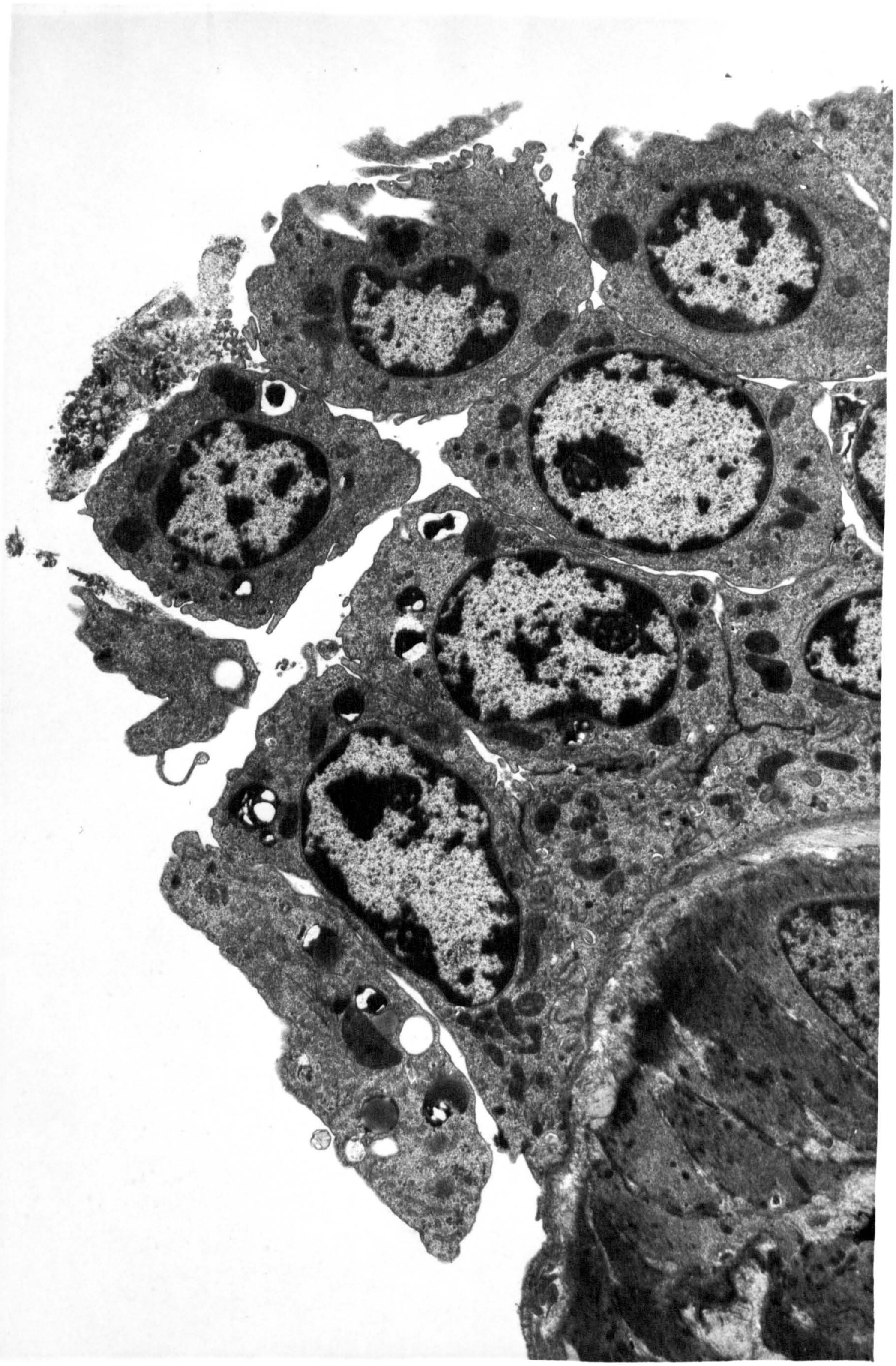


FIGURE 4:9. CLUMP OF TYPE 2 CELLS. (x 8,000)

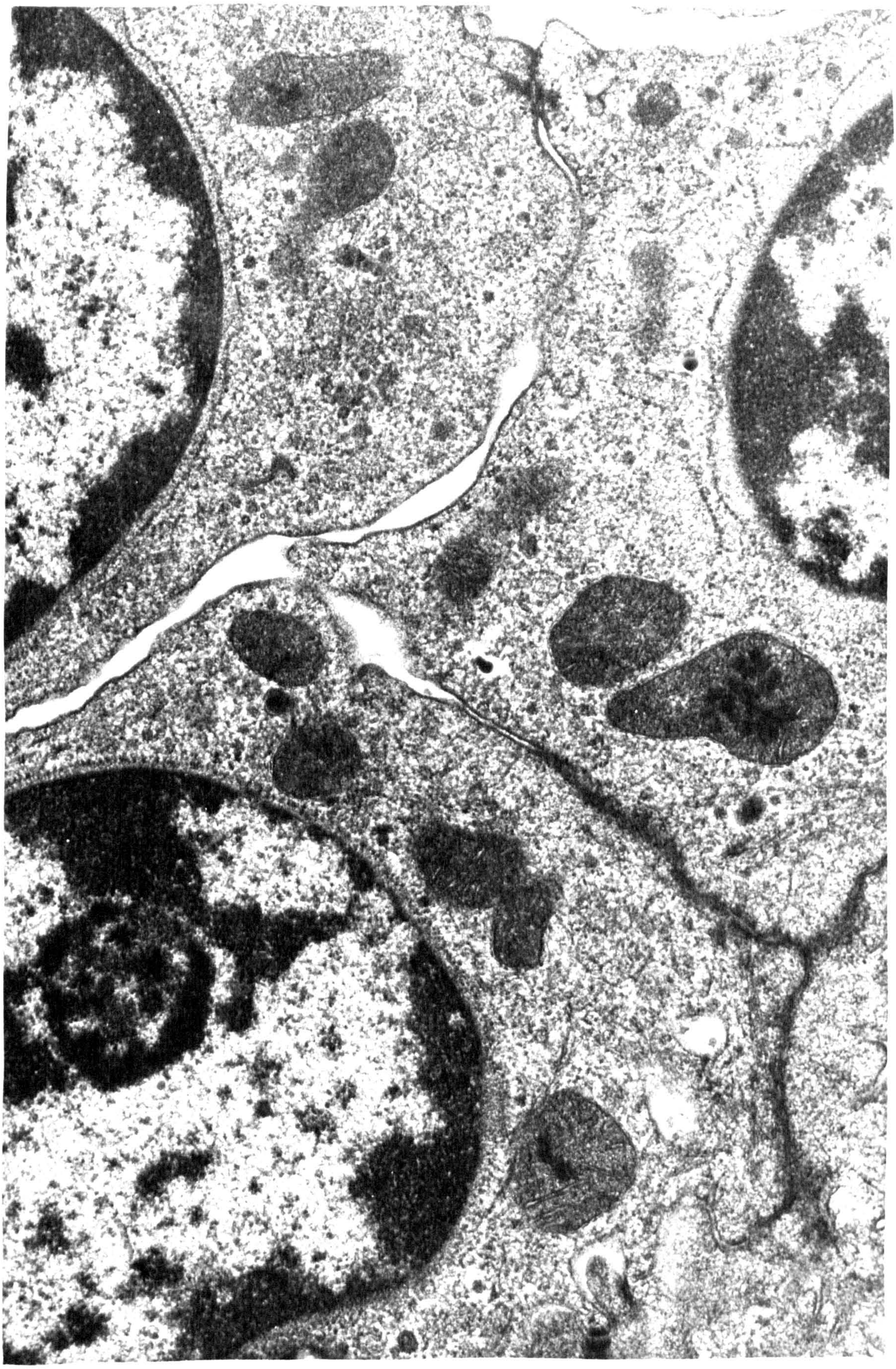


FIGURE 4:10. DESMOSOMES BETWEEN TYPE 2 CELLS. (x 36,000)

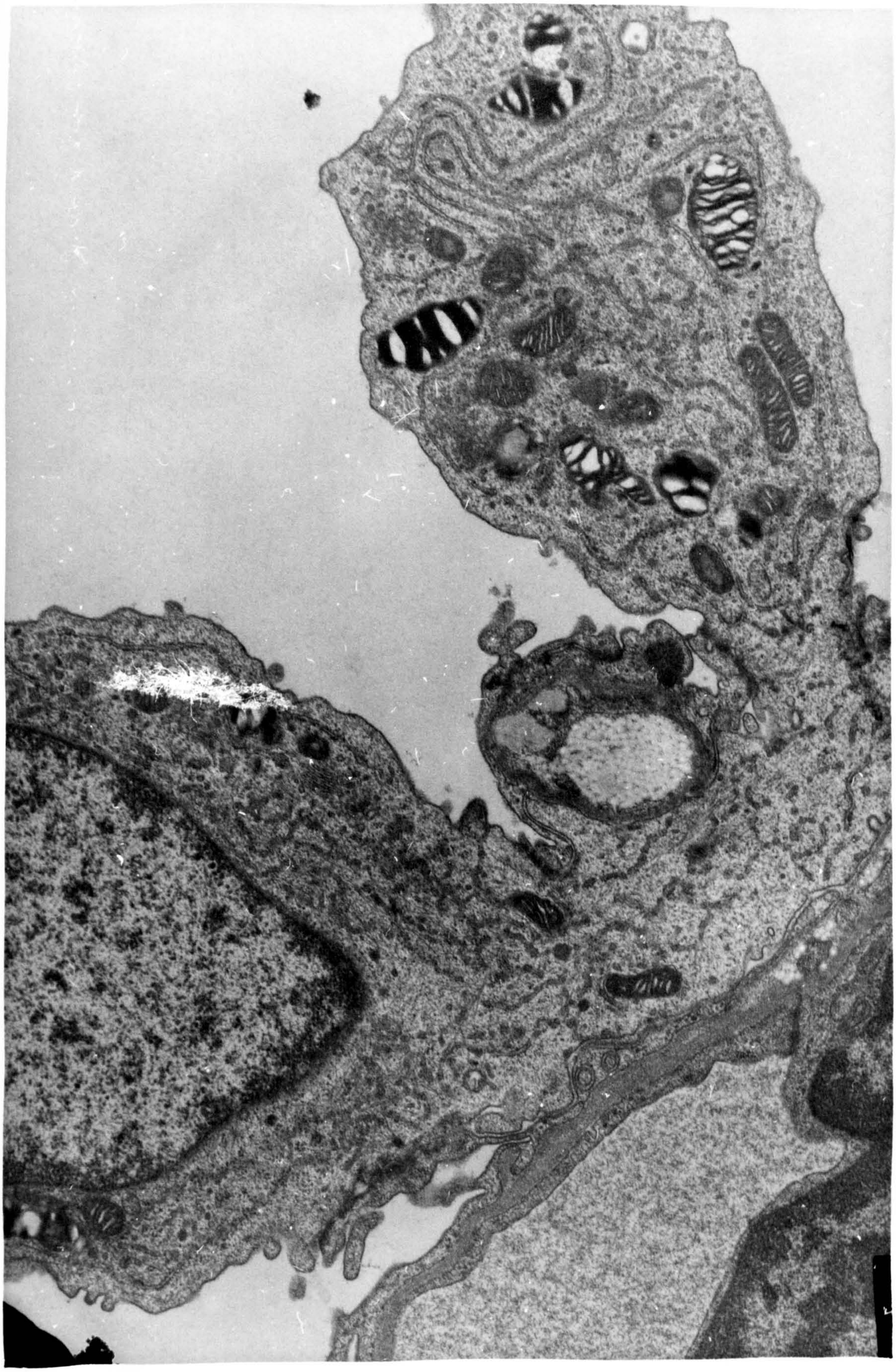


FIGURE 4:11. PEDUNCULATED TYPE 2 CELL. (x 30,000)

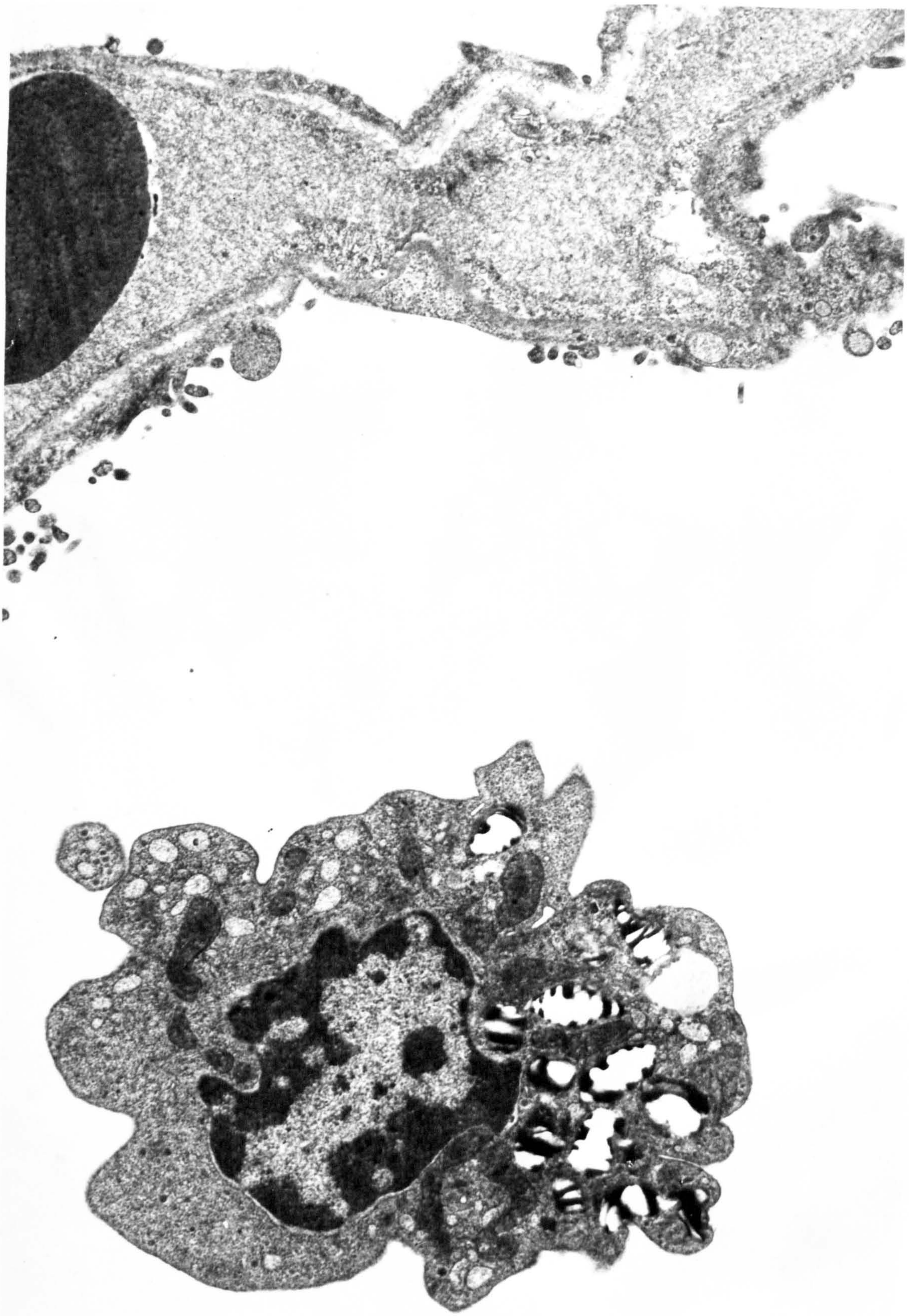


FIGURE 4:12. DETACHED TYPE 2 CELL. (x 15,000)

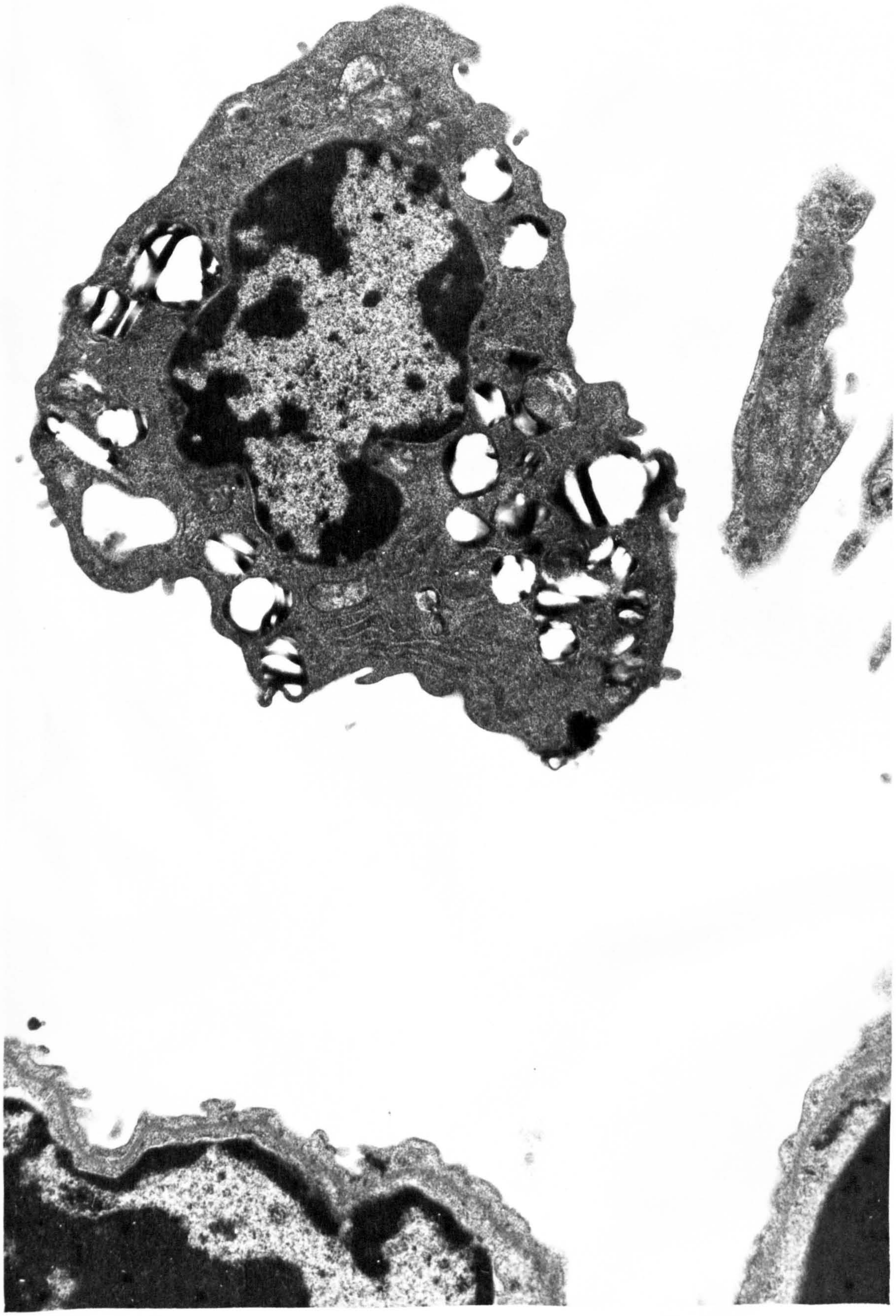


FIGURE 4:13. DETACHED TYPE 2 CELL. (x 18,000)

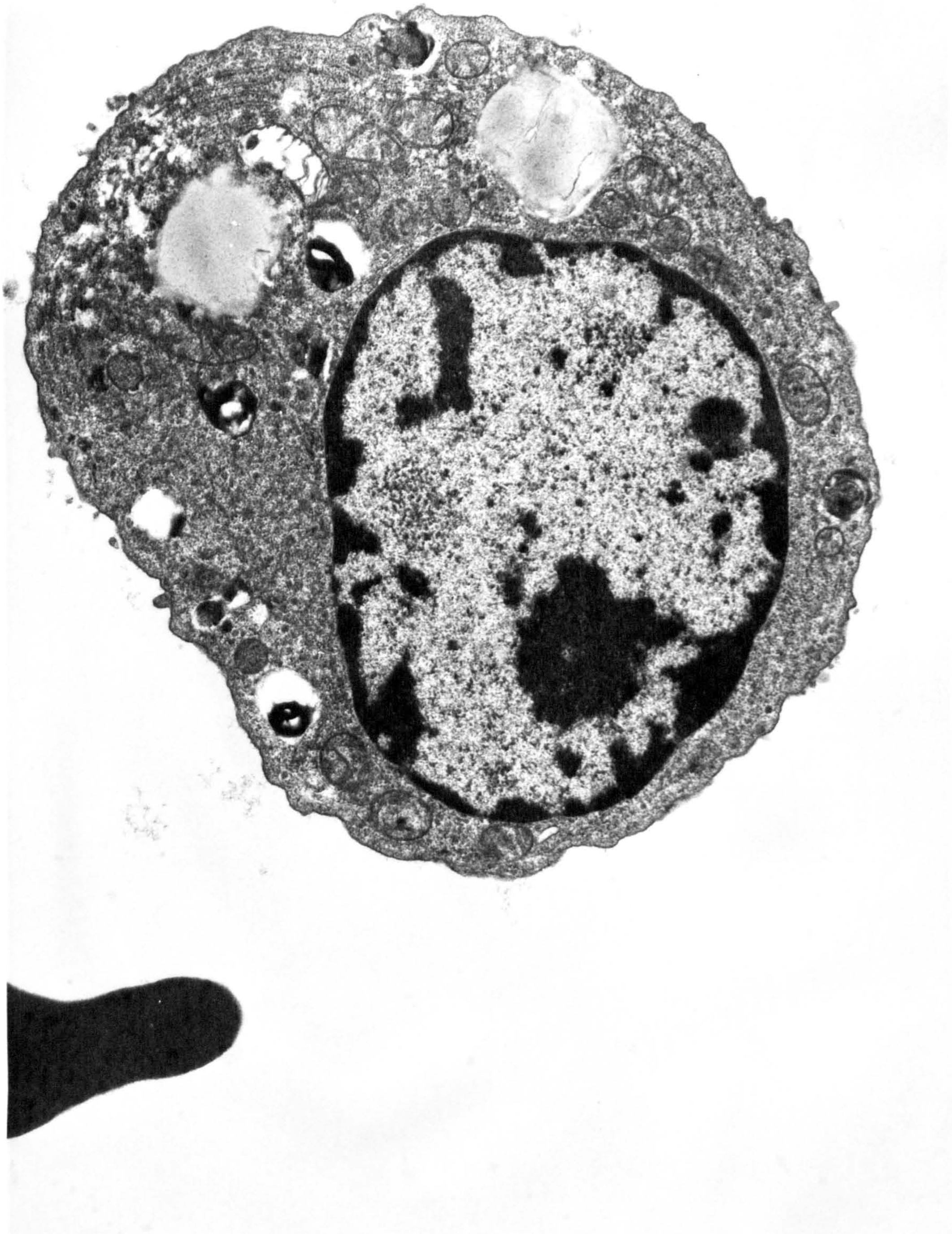


FIGURE 4:14. SPHERICAL TYPE 2 CELL. (x 20,000)

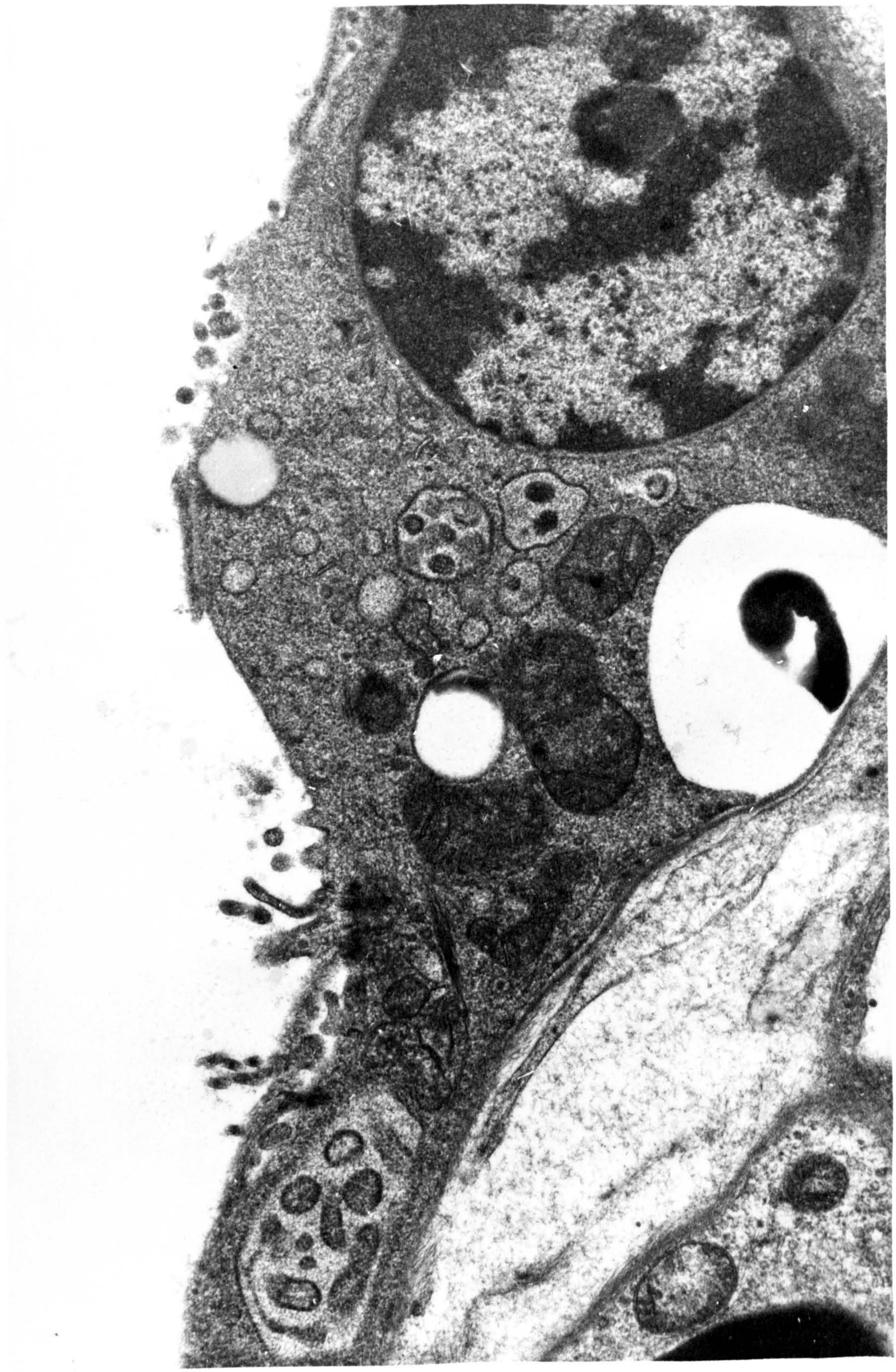


FIGURE 4:15. MULTIVESICULAR BODIES. (x 25,000)

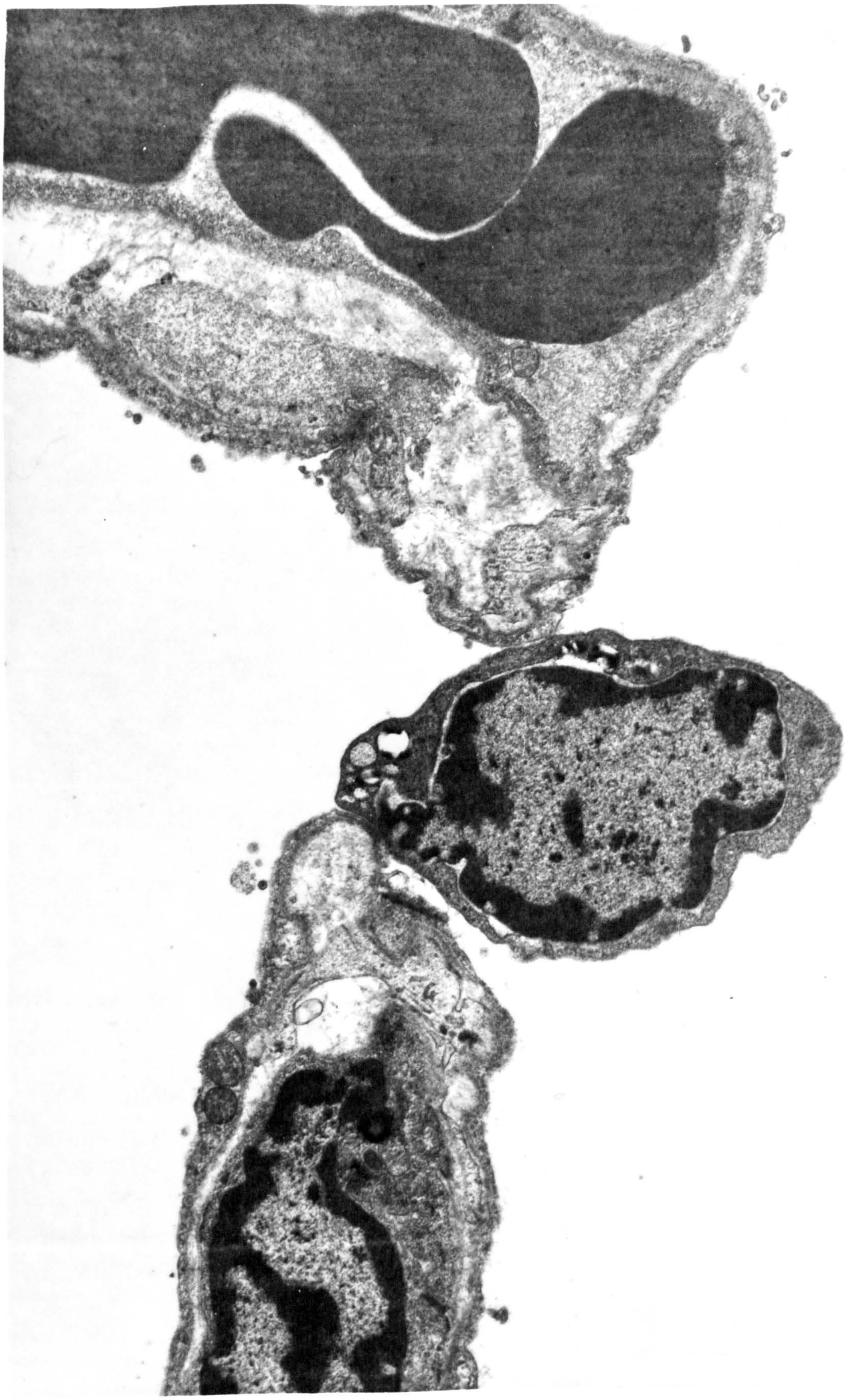


FIGURE 4:16. TYPE 2 CELL IN PORE OF KOHN. (x 14,000)

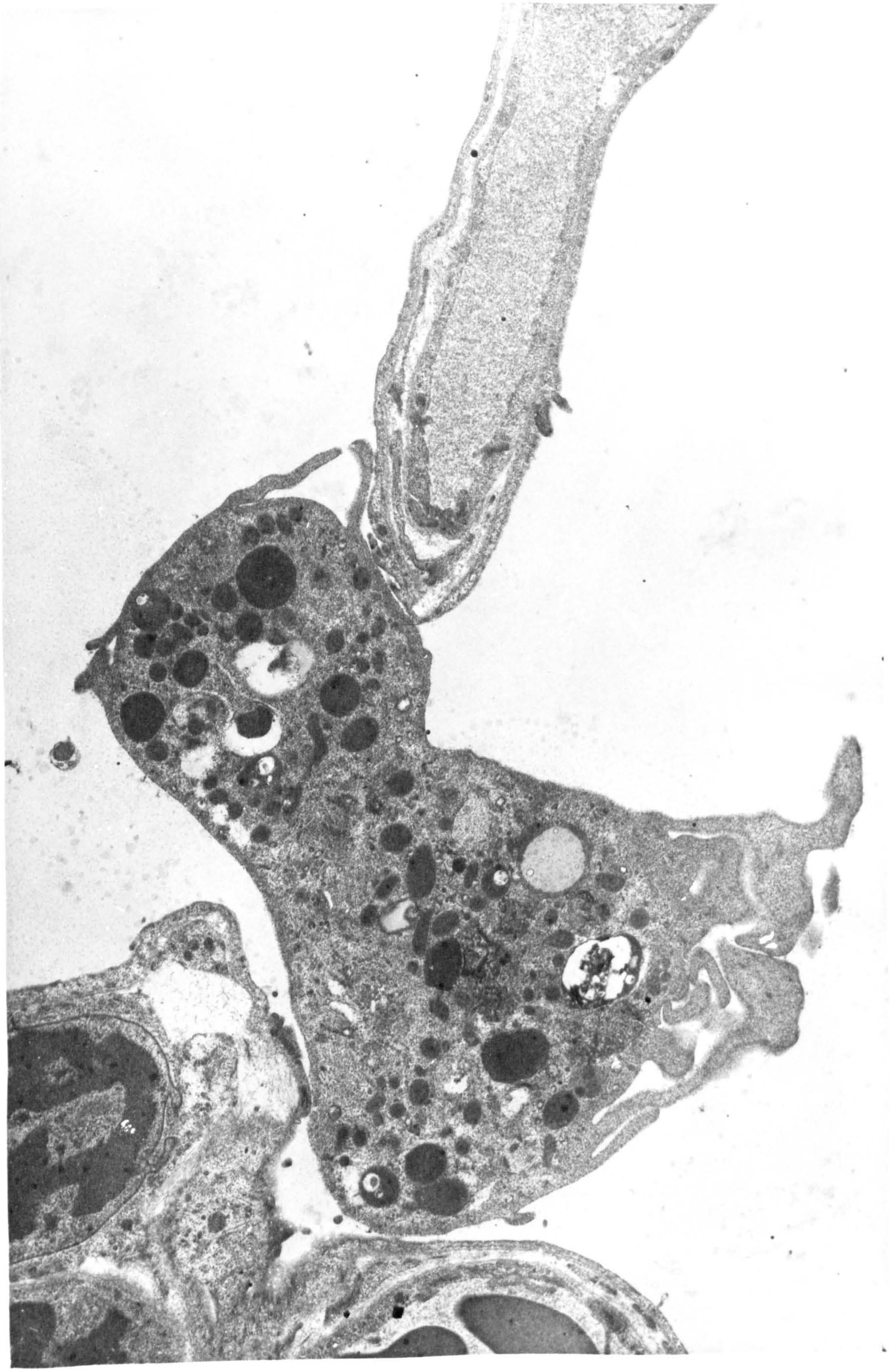


FIGURE 4:17. MACROPHAGE IN PORE OF KOHN. (x 1,000)

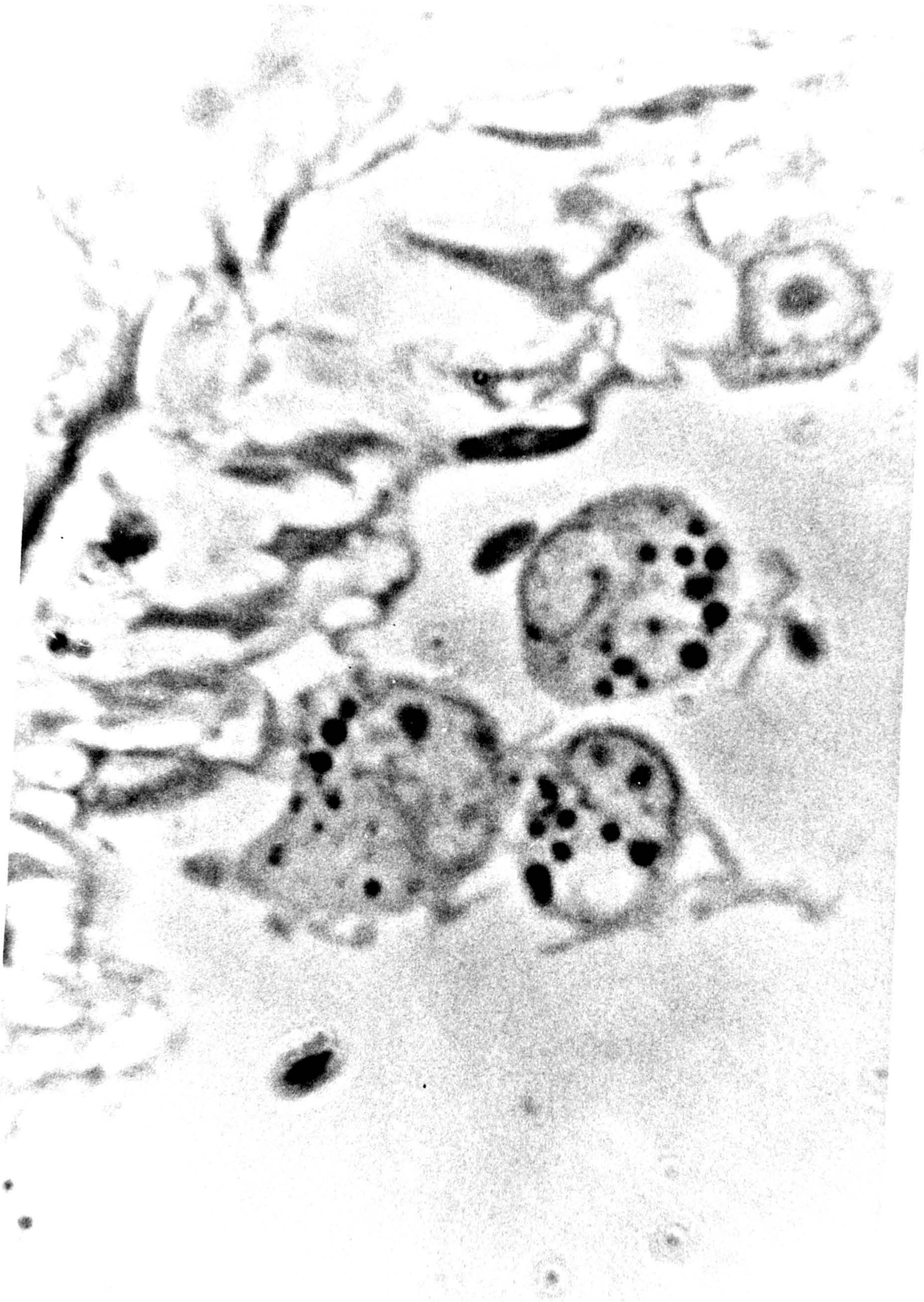


FIGURE 4:18. PHASE CONTRAST MICROGRAPH: MACROPHAGES.

(x 3,000)

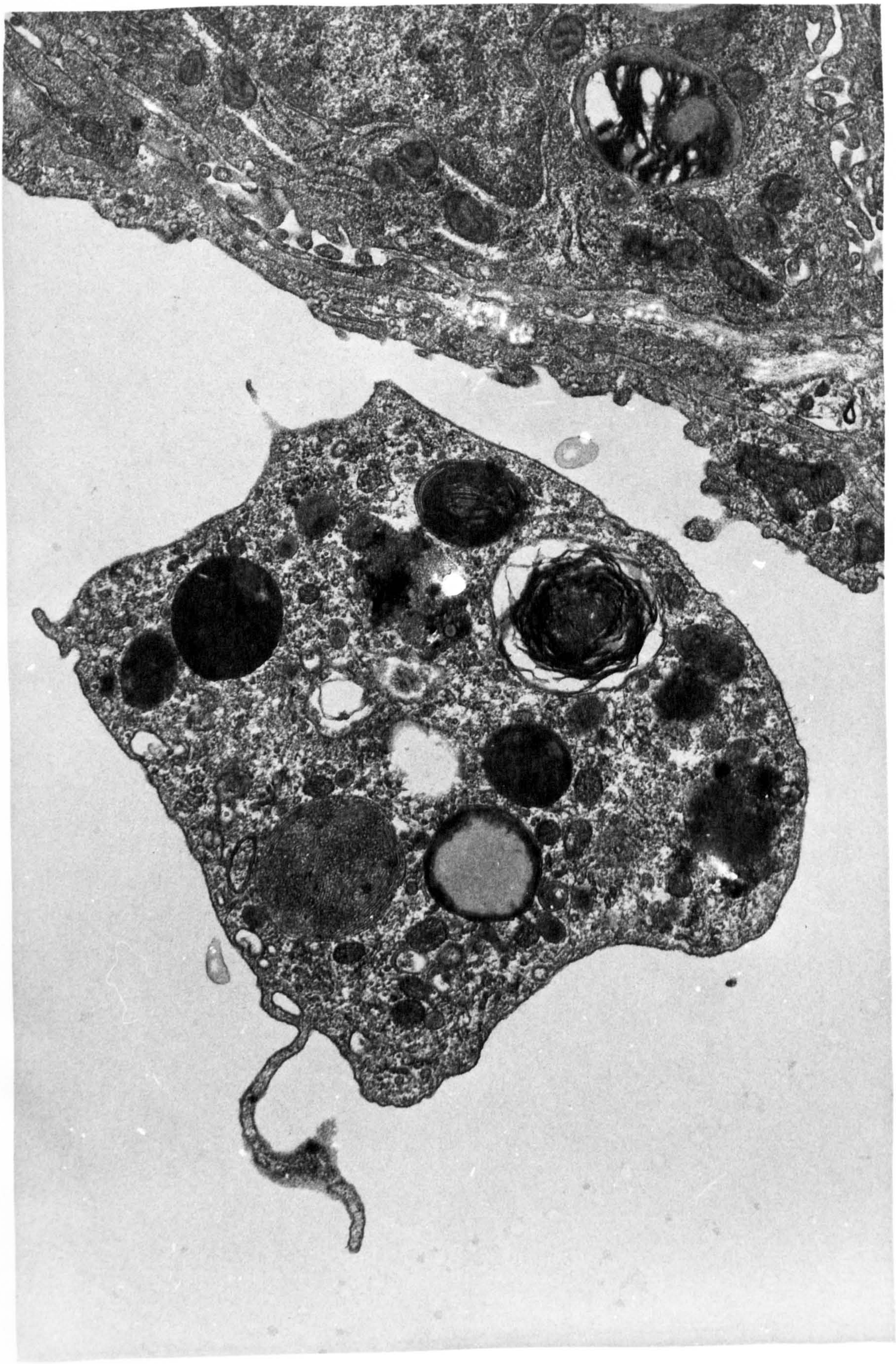


FIGURE 4:19. MACROPHAGE CONTAINING LAMELLATED BODY.

(x 30,000)

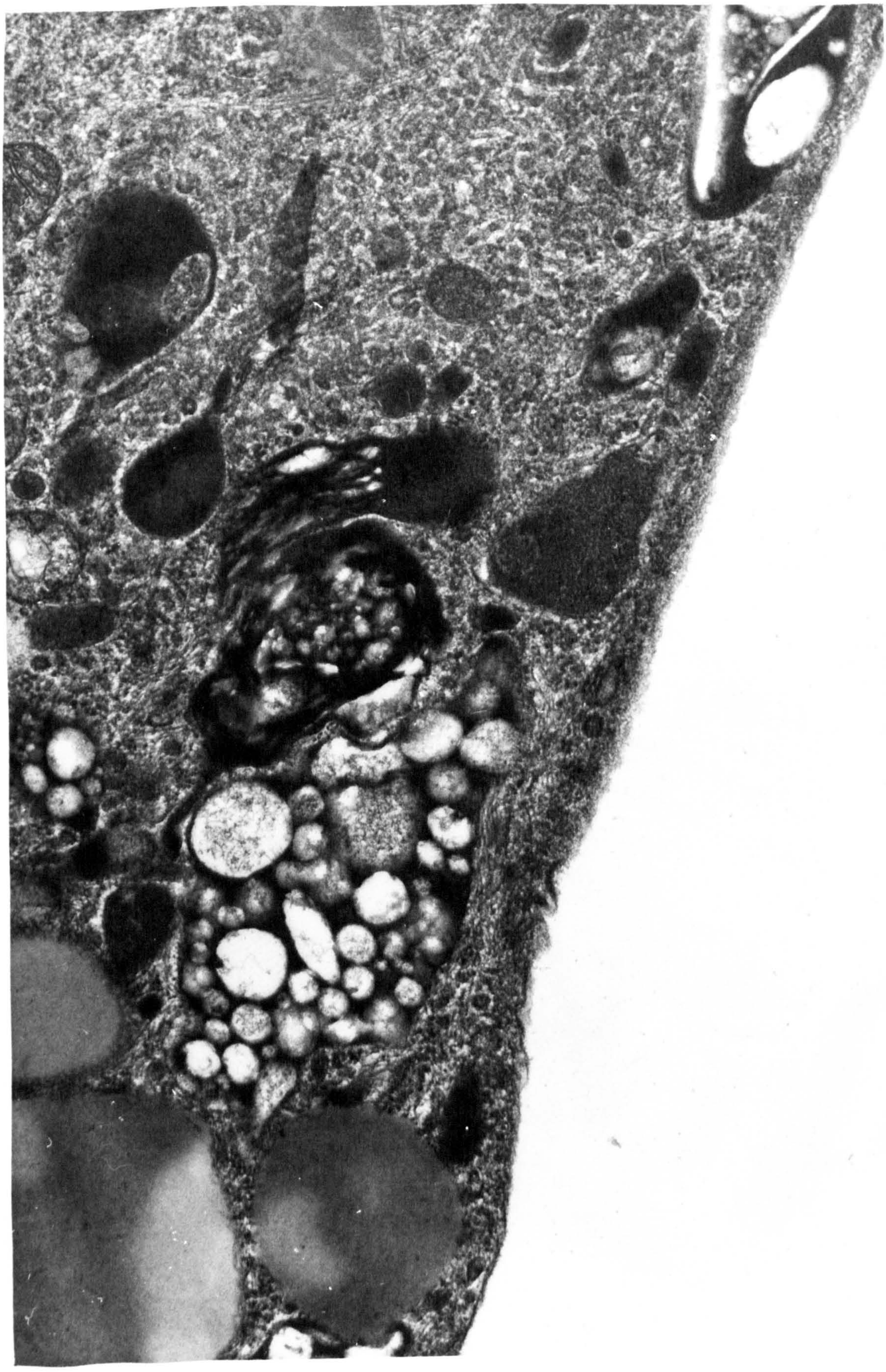


FIGURE 4:20. OSMIOPHILIC MATERIAL IN MACROPHAGE.

(x 65,000)

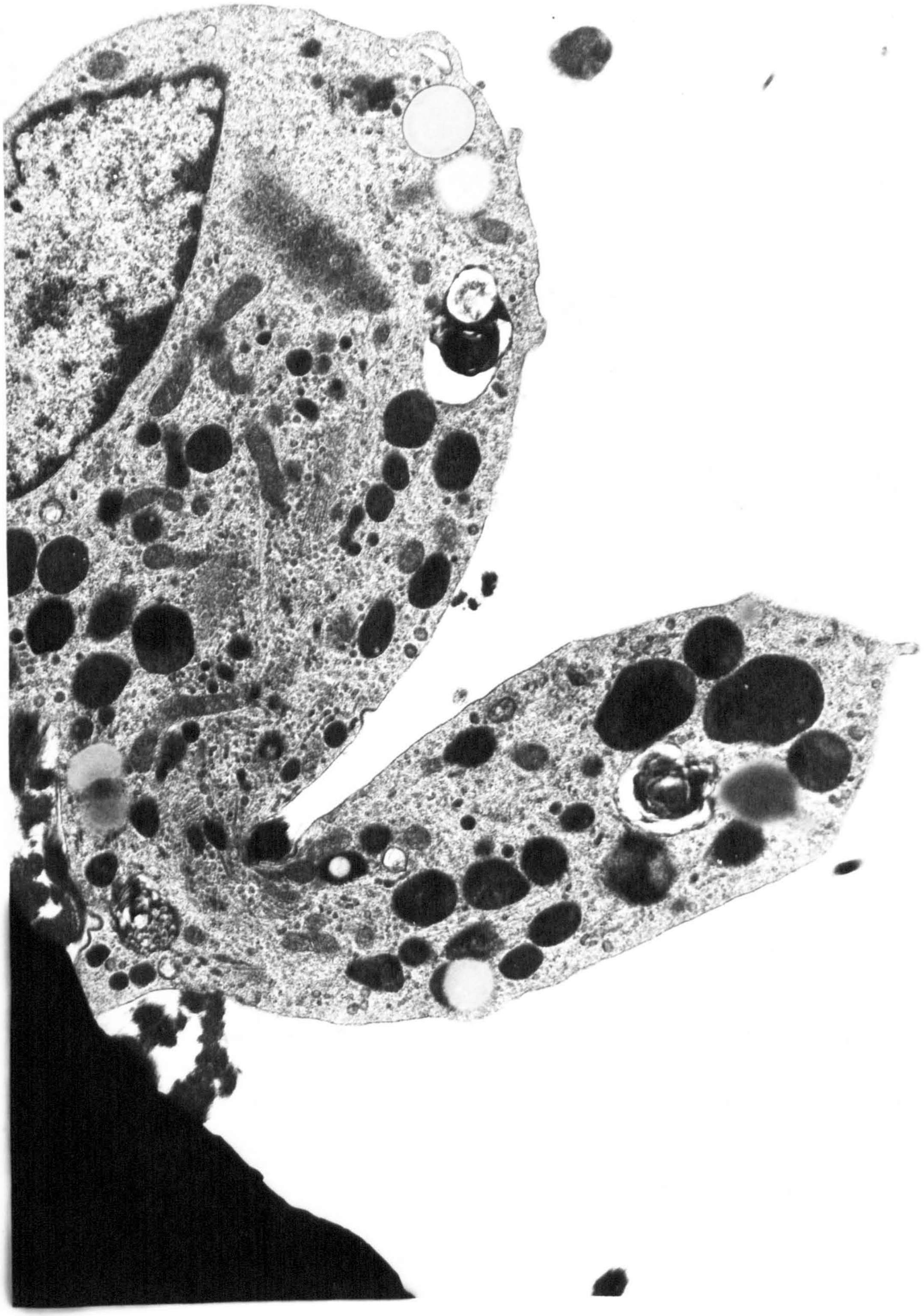


FIGURE 4:21. MACROPHAGE INGESTING OSMIOPHILIC MATERIAL.

(x 14,000)

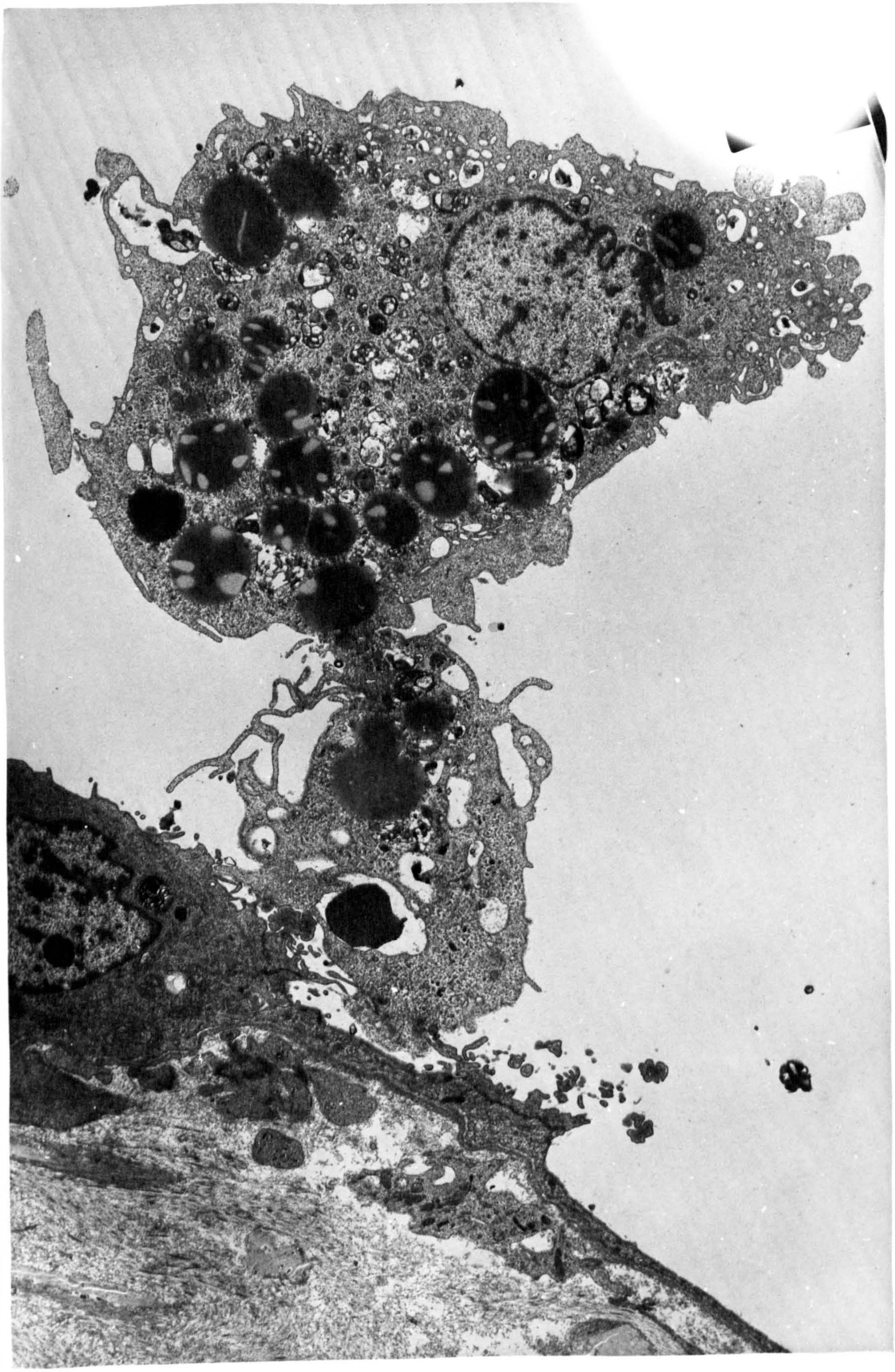


FIGURE 4:22. 'FOAMY' MACROPHAGE. (x 15,000)

FIGURE 4:23. OSMIOPHILIC ALVEOLAR DEBRIS. (x 800)

FIGURE 4:24. ACCUMULATION OF MATERIAL ON ALVEOLAR WALL.

(x 60,000)

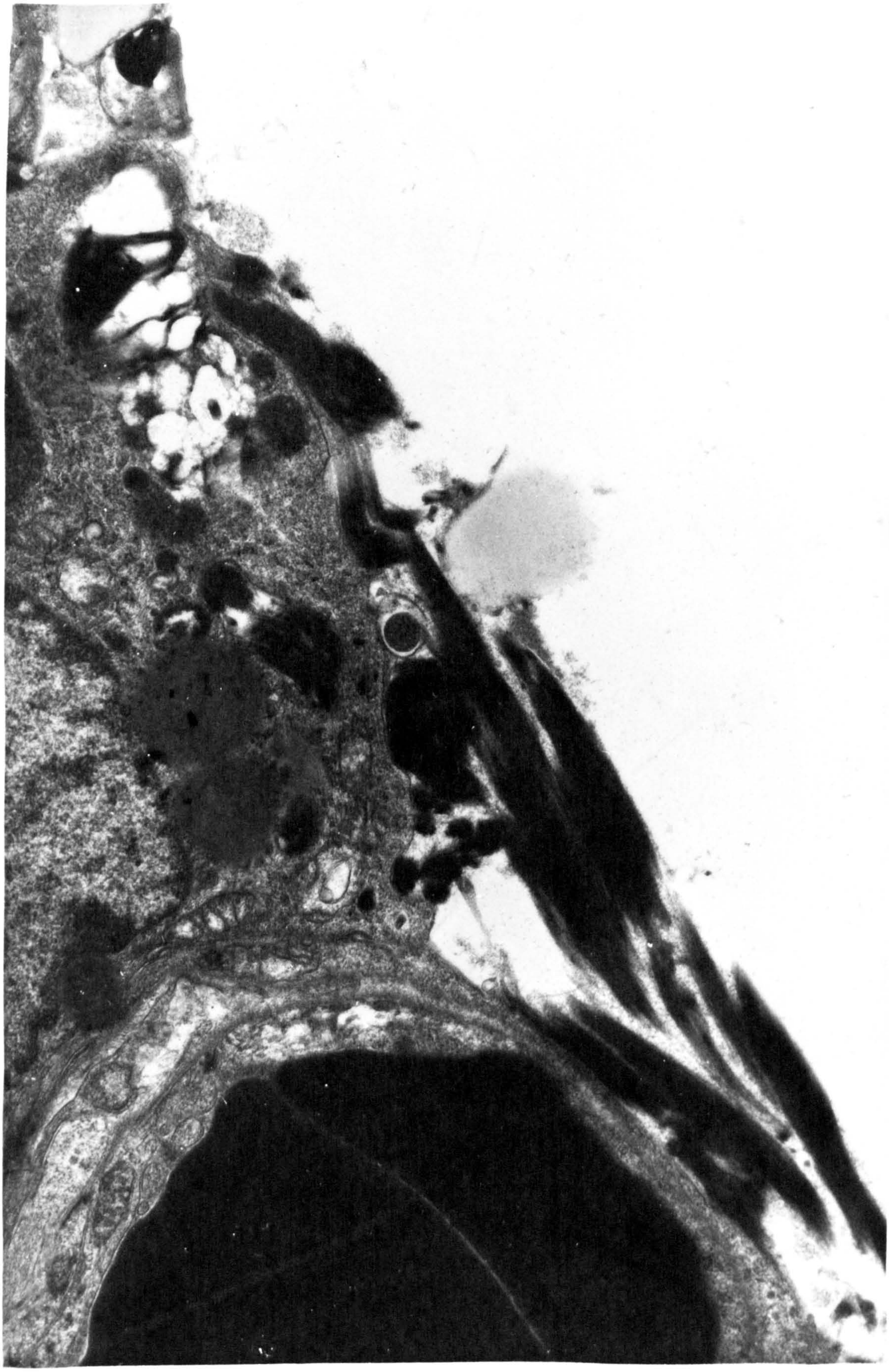


FIGURE 4:25. CONTRIBUTION OF INCLUSION BODIES TO ALVEOLAR DEBRIS (x 20,000)

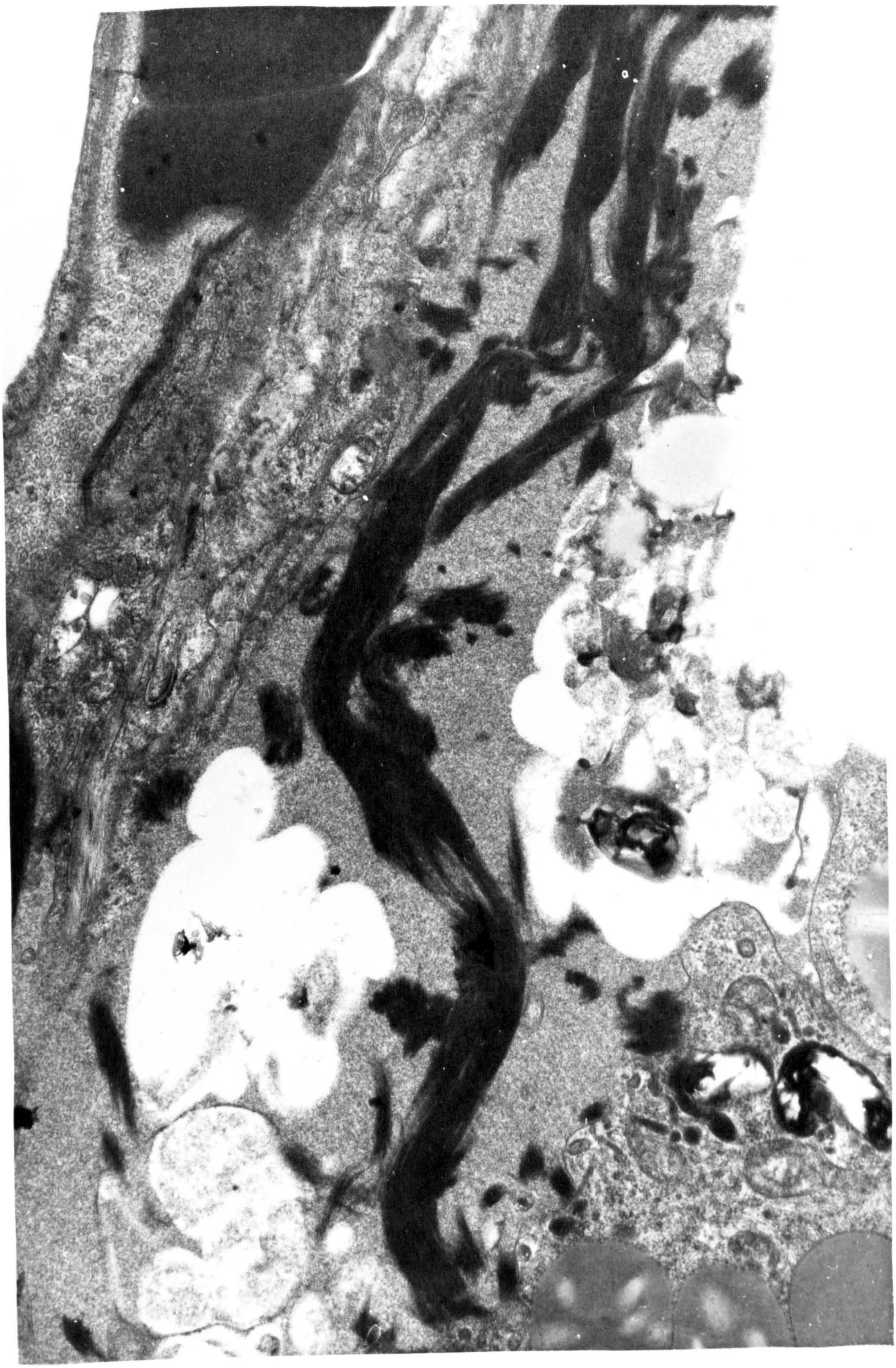


FIGURE 4:26. PHAGOCYTOSIS OF OSMIOPHILIC MATERIAL.

(x 20,000)

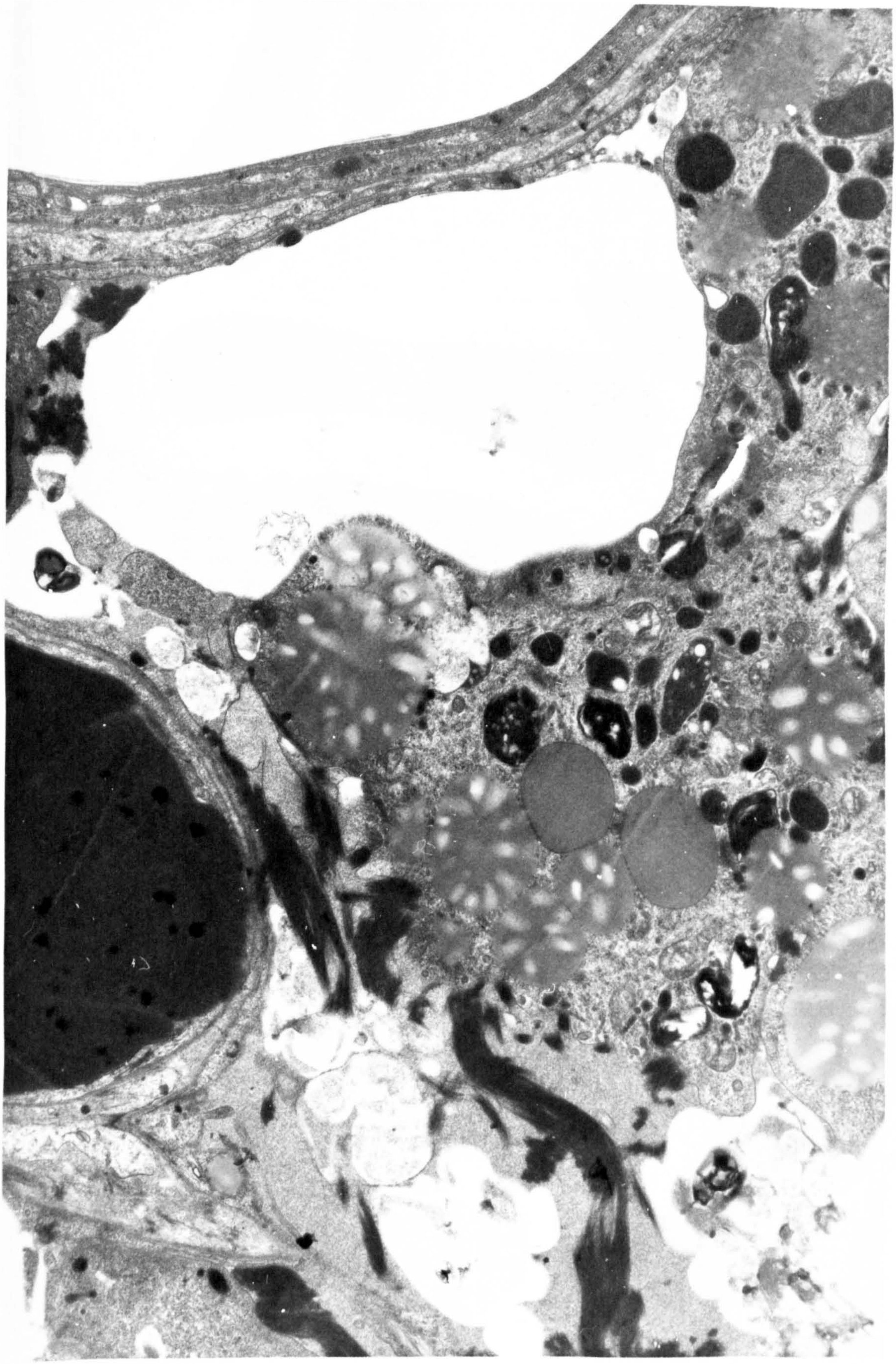


FIGURE 4:27. PHAGOCYTOSIS OF OSMIOPHILIC MATERIAL.

(x 25,000)

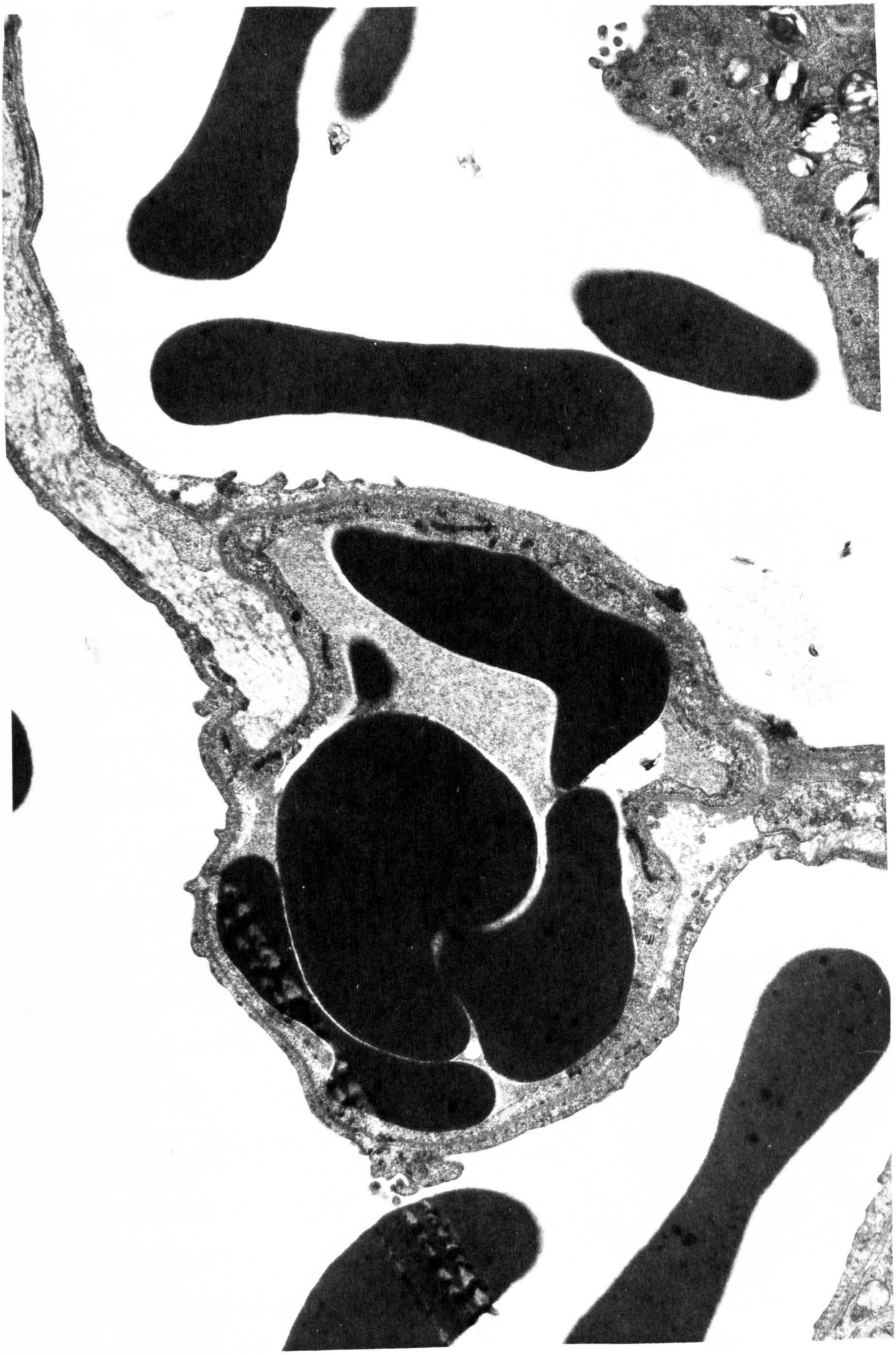


FIGURE 4:28. INTRA-ALVEOLAR ERYTHROCYTES. (x 13,000)

DISCUSSION

In view of the absence of any physiological evidence of a pulmonary capillary hypertensive aetiology for the presence of intra-alveolar oedema, and in face of consistent confirmation of endothelial and/or Type 1 epithelial cell destruction in response to normobaric hyperoxia (see Page 4:7), the absolute normality of the alveolar membrane came as a complete surprise.

The first suspicion must be that, although many hundreds of fields were scanned by electron microscopy and several hundred photomicrographs produced, the actual lesion was overlooked. It was well-demonstrated by the phase contrast microscopy that the cellular response was focal, and it can be suggested that the searching for a focal lesion by electron microscopy in a structure as large as a dog's lung makes the needle in the haystack appear simple by comparison.

Bright field light microscopy, however, (see Section 3) showed almost complete obliteration of alveoli by oedema fluid over very large areas. In addition, electron microscopical examination was performed on alveoli which were definitely abnormal in terms of macrophage and Type 2 cell count and the presence of intra-alveolar debris, and yet in almost no instance was there seen any damage to the alveolar membrane.

A second possible explanation of the normality of the membrane is that the oxygen toxic response is both dose x time dependent and species dependent: had the dog at 2 ATA been able to survive longer, membrane damage might have become apparent. In the rat, Kistler et al. (1967) found no endothelial response to 100% oxygen

at 1 ATA before 48 hours, while Meyrick et al. (1972) reported damage at 24 hours. Bowden et al. (1968) found no endothelial damage in mice exposed to 90% oxygen at 1 ATA before 48 hours, and Kapanci et al. (1969) reported no response in monkeys before 4 days exposure to similar tensions. On the other hand, Coalson et al. (1971) reported both endothelial and epithelial damage in dogs after only one hour of exposure to 100% oxygen at 1 ATA.

As far as is known, there has been no previous study of the effect of oxygen at 2 ATA on the alveolar membrane ultrastructure in the dog or in any other species.

The appearance of sheets of Type 2 cells lining parts of the alveoli on the one micrometre sections (Figure 4:7) would suggest an early proliferative response possibly similar to that seen (although at a much later stage) in primates (Kapanci et al., 1969) and newborn mice (Bonikos et al., 1975). It has been shown recently that the Type 2 cell acts as a 'stem cell' in the reparative processes of epithelial regeneration following exposure to oxygen (Adamson & Bowden, 1974) and it may be that the increase in Type 2 cells seen in the present experiment represents the response to a still latent damage to the epithelium. The endothelial integrity remains unexplained.

The material piled up in the alveoli (Figures 4:23 and 4:24) is presumed to be surfactant; it is osmiophilic, forms parallel lamellations, and appears to incorporate the inclusion body material in its substance. In addition it forms figures in the alveolar space which while not identical to, are very similar

to the tubular myelin figures thought to be a crystalline form - and possibly a breakdown product - of surfactant which are found in the hypophase and occasionally in the alveolar lumen in normal lungs (Gil & Weibel, 1969). An increase in tubular myelin has previously been clearly demonstrated after oxygen poisoning (Kistler et al., 1967; Harrison & Weibel, 1968).

As the efficacy of surfactant is thought to be due to its polar arrangement in a monomolecular film (see Section 2) it seems unlikely that such an accumulation of material can remain functional; it is more likely to represent surfactant damaged either by the presence of fibrinogen or blood in the alveolar space (Taylor & Abrams, 1966; Reifenrath & Zimmerman, 1973) or even more probably by the direct destructive effect of oxygen itself (Klaus et al., 1961).

It is not clear whether the accumulation represents an increased production of surfactant or a decreased removal. There is a large increase in numbers of Type 2 cells in the experimental animals, but a very large proportion of these cells lie free in the alveolar lumen and must be effete (and presumably non-surfactant-producing) varieties. No change was found in the inclusion body numbers or percentage volumes between the experimental and control animals, but it has been suggested that decreased surfactant production may be indicated by a decrease in electron density rather than in size or number of the inclusion bodies (Goldenberg et al., 1967). Unfortunately, because of the fixation techniques used in the present study, an estimate of inclusion body electron density could not be made.

Investigations into the ability of the Type 2 cell to continue producing surfactant following exposure to hyperbaric oxygen have produced conflicting results which are confounded by more than usual problems of cause and effect, but the bulk of the evidence suggests a diminished surfactant activity (see Appendix 1, Pages A1: 14 to 16). Trapp et al. (1971) demonstrated a decrease in surfactant activity in dogs at 3 ATA of oxygen, and McSherry & Gilder (1970) showed a progressive decline in surfactant production in rabbits exposed to oxygen at 2 and 3 ATA.

There is also a large increase in the numbers of alveolar macrophages in the experimental animals, but judging by the amount of debris remaining in the alveoli, it may be that there was little increase in total phagocytic activity. There seems little doubt that macrophage activity is inhibited at 1 ATA and this effect might be expected to be more pronounced at 2 ATA.

It would appear from the results described in this and in the previous section that the mechanism of oedema formation falls into neither the 'haemodynamic' nor 'permeability' categories, but operates by an imbalance of hydrostatic forces across the alveolo-capillary membrane caused by a severe impairment of surfactant activity; i.e. the mechanism described in Section 2.

Such a mechanism could account for the movement of large quantities of water and small ions across the capillary walls and would presumably lead to flooding of the interstitial space with the eventual overwhelming of the lymphatic transport capacity. At this stage, which from the time-course of events described in Section 3 would appear to occur at around 18 to 20 hours, the interstitial

pressure would rise sufficiently to force water through the epithelium into the alveolar space. As soon as this happened, the condition would become rapidly self-perpetuating, as described in Section 2. That this is so is suggested from the absence of trapped gas or bubbles within alveoli (which suggests orderly filling) and the presence side by side of normal and completely obliterated alveoli, with little evidence of intermediate forms; individual alveoli fill rapidly and independently of their neighbours. Previous examples of this mechanism have been reported (Cook, Mead, Schreiner, Frank & Craig, 1959; Staub et al., 1967).

This leaves several observations unexplained. Fluid movement due to hydrostatic imbalance can be regarded as an exaggeration of normal water exchange across the capillary wall, and there is no need to postulate alterations in 'pore sizes' or cell permeability. Yet the material present in the alveoli of the animals with fulminating oedema consists of more than just water which, of course, cannot be stained for by any histological technique (see Figures 3:23, 4:23, 4:27).

The origin of this material, assumed to be fibrin, is not known. It has been suggested (Staub et al., 1967) that in some types of oedema there may be a leakage of fluid from vessels larger than capillaries, with a centrifugal spread of fluid towards the periphery of the lungs, and vascular leakage from the peri-bronchial venules has been demonstrated in haemodynamic oedema and histamine-induced oedema (Gabianni, Badonnel, Gervasoni, Portman & Majno, 1972; Pietra, Szidon, Leventhal & Fishman, 1971). These vessels were not examined by electron microscopy in the present study and they may well have

contributed to the leakage, but it would seem highly unlikely that they were selectively damaged. Bowden and Adamson (1974) recently reported a study of the specific site of endothelial damage following normobaric hyperoxic exposure; using ^{3}H-thymidine autoradiographic-labelled mitotic figures in regenerating endothelium as a marker, they found that the bulk of the damage occurred in the capillary endothelium and that there was also some damage to pre- and post-capillary thin-walled vessels up to 200 micrometres in diameter; there was no evidence whatsoever of damage to larger vessels, either pulmonary or bronchial.

A further possibility is that the material is not in fact fibrin, but consists of some smaller molecule. It was noted in Smith's original description (1899) that the alveolar exudate 'did not give the usual fibrin stain by Weigart's method, nor with eosin', while in the present study, although a faint pink colouration (the normal reaction for fibrin) was consistently found in the hyaline membranes of the conventionally fixed tissue stained by the Masson trichrome technique, in the freeze-dried sections, the alveolar debris more commonly stained green (Figure 3:23).

A much more difficult problem lies in the presence of red blood cells in the alveolar lumen. This was a common finding and must surely be expected to indicate the presence of fairly large 'holes' in the blood-air barrier.

The problem, however, is not new. Staub and his colleagues (1967) in a study comparing 'haemodynamic' with 'permeability' (alloxan-induced) oedema found that paradoxically, red cells appeared in the alveoli in very large numbers in the haemodynamic form but not

at all when endothelial permeability was increased. This enigma has been confirmed and underlined in an electron microscopical comparison of the same two models of oedema (Cottrell et al., 1967). In the permeability form, although both the endothelial and epithelial membranes showed extensive degenerative changes, no red cells were found in the alveolar space. There was no fibrin visible in the interstitial space, but large amounts of fibrin and other unidentified granular electron-dense material were present in the alveoli. It is perhaps significant from the point of view of the lack of red cells that although the epithelial and endothelial cells were seriously damaged, numerous tight inter-cellular junctions were found, and that both basement membranes remained intact. In the haemodynamic form on the other hand, the endothelial and epithelial cells, together with their inter-cellular junctions and basement membranes remained normal, yet there were numerous red cells, both intact and in various stages of disintegration in the alveoli. Just as puzzling was the fact that there was no fibrin present in the alveoli, and when vascular markers (carbon suspension with particles 250 Angstroms in diameter and thorium dioxide, diameter 70 Angstroms) were injected into these animals to detect the site of leakage, they were found both free in the capillary lumen and within intra-vascular macrophages, but nowhere within the interstitial or alveolar space.

Meyrick et al. (1972) studied two different forms of permeability oedema produced either by injection of alpha-naphthylthiourea or by exposure to normobaric hyperoxia, and found no intra-alveolar red cells in either.

Yet in asphyxia neonatorum, where the oedema is almost certainly of hydrostatic origin, intra-alveolar red cells are a constant feature.

It is just possible to imagine that, as parts of the lung become either atelectatic due to surfactant inactivity or fill up with fluid, shear forces may be set up between the focal areas of reduced or zero compliance and those parts which are still moving with ventilation. This may through time cause sufficient minor damage to allow leakage of small quantities of blood into the airways where it could be dispersed to the still-ventilating alveoli.

The anomalous presence of intra-alveolar blood cells in haemodynamic, but not permeability, oedema may be associated with differences in the time-course of development of the two forms (Staub et al., 1967); in the haemodynamic form fluid begins to collect slowly, but virtually immediately, and continues as long as the capillary pressure remains raised, while in the permeability form there is a latent period of 20 to 40 minutes while the animal remains apparently normal followed by an extremely rapid fulminating extravasation of fluid. Staub et al. (1967) have suggested a possible hypothesis on the interrelation of atelectasis and the availability of fluid to 'splint open' the alveoli:

> 'If the capillaries are not leaky and surface tension is relatively high, then alveoli collapse to the atelectatic state. If the capillaries are very leaky and surface tension is relatively low, then the alveoli will fill with fluid at normal volume. If the capillaries are moderately leaky and surface tension is intermediate, then the alveoli will be fluid

filled but at a volume less than in the air-filled state.'

It is put forward here, although without much conviction, that in the haemodynamic (i.e. capillaries not leaky and surface tension normal) and surfactant-deficiency forms (capillaries not leaky and surface tension high) that, although any individual alveolus fills rapidly, the condition is only slowly progressive, creating many areas of differing compliance possibly sufficient to cause micro-extravasation of blood, whereas in the permeability forms (capillaries very leaky and surface tension possibly normal), alveoli are much more rapidly, uniformly and suddenly splinted open.

Notwithstanding the anomalies discussed above, it is concluded that the oedema observed following exposure to dogs to 2 ATA of oxygen is due primarily to an impairment of surfactant activity.

SECTION 5

DOG EXPERIMENTS SERIES 2

DISTINCTION BETWEEN DIRECT AND INDIRECT MECHANISMS OF TOXICITY

DISTINCTION BETWEEN DIRECT AND INDIRECT MECHANISMS OF TOXICITY

INTRODUCTION

The findings described in the previous section (Section 4) suggest that the most important lesion in animals dying at 2 ATA of oxygen is a direct toxic effect of the oxygen on the alveolar Type 2 cells. That this is demonstrably not the case under normobaric conditions (Kistler et al., 1967) gives rise to the idea that indirect mechanisms (either in the form of circulating co-factors or of a neurohumoral response to toxic effects on some distant organ) may be involved. A review of the literature reveals many contradictory reports, and it seems likely that both direct and indirect mechanisms exist and that their contributions vary according to the partial pressure of inspired oxygen and the species under investigation.

Effect of arterial oxygen tension

The tolerance limits of normal man for oxygen are well known (Winter & Smith, 1972). It is, however, common clinical experience that some patients with hypoxaemia due to impairment of pulmonary gas exchange are able to tolerate high inspired tensions of oxygen for much longer than might be predicted (Bendixen, Egbert, Hedley-Whyte, Laver & Pontopiddan, 1965; Northway, Rosan & Porter, 1967; Nash, Blennerhassett & Pontoppidan, 1967). This protective effect of a large alveolar-arterial oxygen tension difference has been investigated in dogs at both hyperbaric and normobaric pressures.

1. Effect of experimental venous admixture

Hyperbaric

A veno-arterial shunt was produced surgically in a group of 10 dogs by anastomosing the inferior vena cava to the right inferior pulmonary vein (Winter et al., 1967). A control group breathed oxygen at 2.5 ATA until convulsion (at a mean of 5.1 hours) and at 2 ATA from then until death (12.3 hours). The operated dogs breathed oxygen at 2.5 ATA (mean P_AO_2 = 1816 mm.Hg., P_aO_2 = 127 mm.Hg.) for 5.1 hours and then at 2 ATA until death, which occurred at a mean time of 21.1 hours. The lungs of both groups showed both the gross and microscopic changes of oxygen poisoning while the lungs of an additional group of operated animals exposed on the same regimen, but sacrificed at the mean time of death of the control group (12.3 hours) showed minimal changes. Electron microscopy was not performed.

Experimental venous admixture therefore delayed the development of pathological changes and almost doubled the survival time.

The importance in the development of lung pathology of the oxygen tension of the blood perfusing the lung was demonstrated in a series of experiments involving intermittent exposure (1 hour, 4 times a day) to oxygen at 3 ATA for 30 days (Thomas, Ketchum, Hall & Zubrin, 1969). In the first group of dogs, the right pulmonary artery was ligated and the bronchial artery collateral circulation allowed to develop. In the second group, the animals were first rendered cyanotic by anastomosing the left pulmonary artery to the left atrial appendage and then several weeks later had their right middle lobe pulmonary artery ligated.

Both groups tolerated the exposures to oxygen well. Follow-

in sacrifice, the most severe damage was found in the right lung of the first group - i.e. in those lungs which had been perfused solely by hyperoxic arterial blood via the greatly enlarged bronchial arterial circulation. The left lung showed moderate changes. In the cyanotic group, the right middle lobe (perfused by the bronchial artery) showed minimal changes while the left lung was normal. Pulmonary venous blood oxygen tension was taken as the best indicator of oxygen dosage to any particular lung or lobe, and in all cases there was a good correlation between degree of lung damage and that oxygen tension. As the inspired oxygen tension remained constant throughout, an indirect mechanism of action is indicated.

Normobaric

A group of dogs were made cyanotic by the surgical creation of a large intracardiac veno-arterial shunt, ($\dot{Q}_S/\dot{Q}_T$ of 40 - 60% causing a P_aO_2 of 29 to 58 mm.Hg. while breathing air) (Miller, Waldhausen & Rashkind, 1970). They were then exposed with a group of controls to 100% oxygen at 1 ATA for two days. Pulmonary damage was assessed on the basis of respiratory distress, increased minimum surface tension of lung extracts, gross atelectasis, and alveolar oedema and haemorrhage. There were no observable differences between the two groups.

This finding was confirmed by Ashbaugh (1971) in a careful study in which cardiorespiratory parameters were monitored over many days. Chronic arterial hypoxaemia was created by anastomosing the inferior vena cava to the left atrium. Following a recovery period, these animals and a control group were exposed to 540 to 580 mm.Hg. O_2 at 1 ATA (equivalent to 100% oxygen as the work was carried out at

Denver, Colorado, altitude 5280 feet) until death. There were no differences between the groups in either survival time (8.2 days for hypoxaemic group, 8.25 days for controls) or in measured parameters. Pathological changes, such as congestion, atelectasis and interstitial and intra-alveolar oedema were the same for both groups.

The conclusion from these experiments must be that while large alveolar-arterial oxygen tension differences can protect against pulmonary oxygen toxicity under hyperbaric conditions, there is no evidence for protection at normal pressures.

In contrast to the above are the findings in an experiment where rabbits with artificial lung damage due to the intravenous administration of oleic acid were exposed to 100% oxygen at 1 ATA until death (Smith, Winter & Wheelis, 1973). These animals were moderately hypoxaemic compared with the control group (P_aO_2 = 320 mm. Hg., hypoxaemic; 463 mm.Hg., control) and survived about twice as long. On histological examination, however, all of the injected animals demonstrated a marked Type 2 cell proliferation, and as this reaction is also a feature of the recovery phase of chronic oxygen toxicity (Yamamoto, Wittner & Rosenbaum, 1970) where it is known that there is an increased tolerance to oxygen, the extent to which this change per se influenced survival time is not certain.

It may, however, suggest a mechanism for the, as yet unexplained, increased oxygen tolerance of lung-damaged patients.

2. Differential intubation of the lungs

A different approach to the problem of distinguishing between direct and indirect effects of oxygen is to supply the two lungs with

different gas mixtures, one with high and the other with low oxygen, and observe the effect. Any appearance of pathological changes in the 'low oxygen' lung would be strong evidence in favour of an indirect mechanism.

Several such experiments have been reported, with conflicting results.

Normobaric conditions

In 1954, Chapin and Hohl (cited as a personal communication in Bruns & Shields, 1954, and apparently unpublished elsewhere) aerated the right lung of a dog with 100% oxygen at 1 ATA and the left lung of the same animal with air over a period of seven days. At post-mortem the right lung showed a liver-like consolidation while the left lung appeared normal. No mention is made of histological examination or of precautions taken against post-mortem artifact.

This evidence in favour of a direct toxic effect of oxygen is augmented by a report of dogs with lungs ventilated respectively with air and oxygen at 1 ATA for 72 hours (Yhap, Zeller, Levin & Solis, 1971). Examination showed that, although both lungs showed a patchy atelectasis, only the lung receiving high oxygen had gross signs of oxygen toxicity, i.e. epithelial and endothelial cell damage, and interstitial oedema.

In contrast, Motlagh et al. (1969) demonstrated an indirect effect in a group of dogs ventilated with both air and oxygen at 1 ATA for twelve hours. Both the oxygen-ventilated and the air-ventilated lungs showed similar lesions.

This was confirmed in a similar group of dogs (Coalson,

Beller & Greenfield, 1971) similarly ventilated in which both the air and the oxygen-ventilated lungs were damaged. This particular experiment is interesting for the time-course demonstrated; evidence of capillary endothelial cell and Type 1 epithelial cell damage being present after only one hour.

Conversely, when dogs were ventilated with oxygen to only a single lobe, and arterial tension remained relatively low, no lung lesions were found elsewhere (Suga, Tait & Reich, 1970).

Hyperbaric conditions

Only one reference has been found to such experiments being carried out under hyperbaric conditions. Penrod (1958),using cats, exposed one lung to 100% oxygen at 5 ATA and the other to nitrogen. At seven hours the inert gas lung was normal, whereas the high oxygen lung showed both macroscopic and microscopic signs of damage. 'Many combinations of gases' (no further details given) were used and the conclusion reached was that oxygen exerted its toxic effect on the alveolar membrane directly rather than by any blood-borne or neurological route.

EXPERIMENT

It was decided to perform a similar experiment at 2 ATA in spontaneously breathing dogs.

The anaesthetic techniques, apparatus, materials, methods and calculations were similar to those set forth in Section 3, and only the differences will be described.

EQUIPMENT

The main difference was in the gas delivery system.

Development of the dual-lumen tube

In the experiments described above little or no indication was given of the techniques and equipment used to functionally separate the animals' lungs and ensure differential ventilation without leakage. The development of a reliable catheter for canine bronchospirometry has been difficult (Benfield, Coon & Cree, 1966; Garzon, Cheng, Pangan & Karlson, 1968) and the problem is not yet fully resolved.

The problem lies in the anatomy of the dog's tracheal bifurcation (Miller, 1974); the right apical lobe bronchus arises very high up the right principal bronchus, almost directly from the trachea. The left apical lobe bronchus, while arising more distally along the left principal bronchus still leaves very little space between its origin and the tracheal bifurcation. Attempts to lodge a catheter cuff in this space often results in the balloon herniating across the carina and occluding the right principal bronchus.

A series of preliminary experiments were performed on small mongrels using Robertshaw bronchospirometry tubes of different sizes. Position of the tube cuffs was observed radiologically using an opaque medium (Conray 280, May and Baker) to fill the cuffs, and was confirmed directly at post-mortem. They were unsatisfactory; if the tube was pushed sufficiently far home to ensure against herniation of the cuff across the carina, the cuff then occluded both the left apical and left cardiac lobe bronchi.

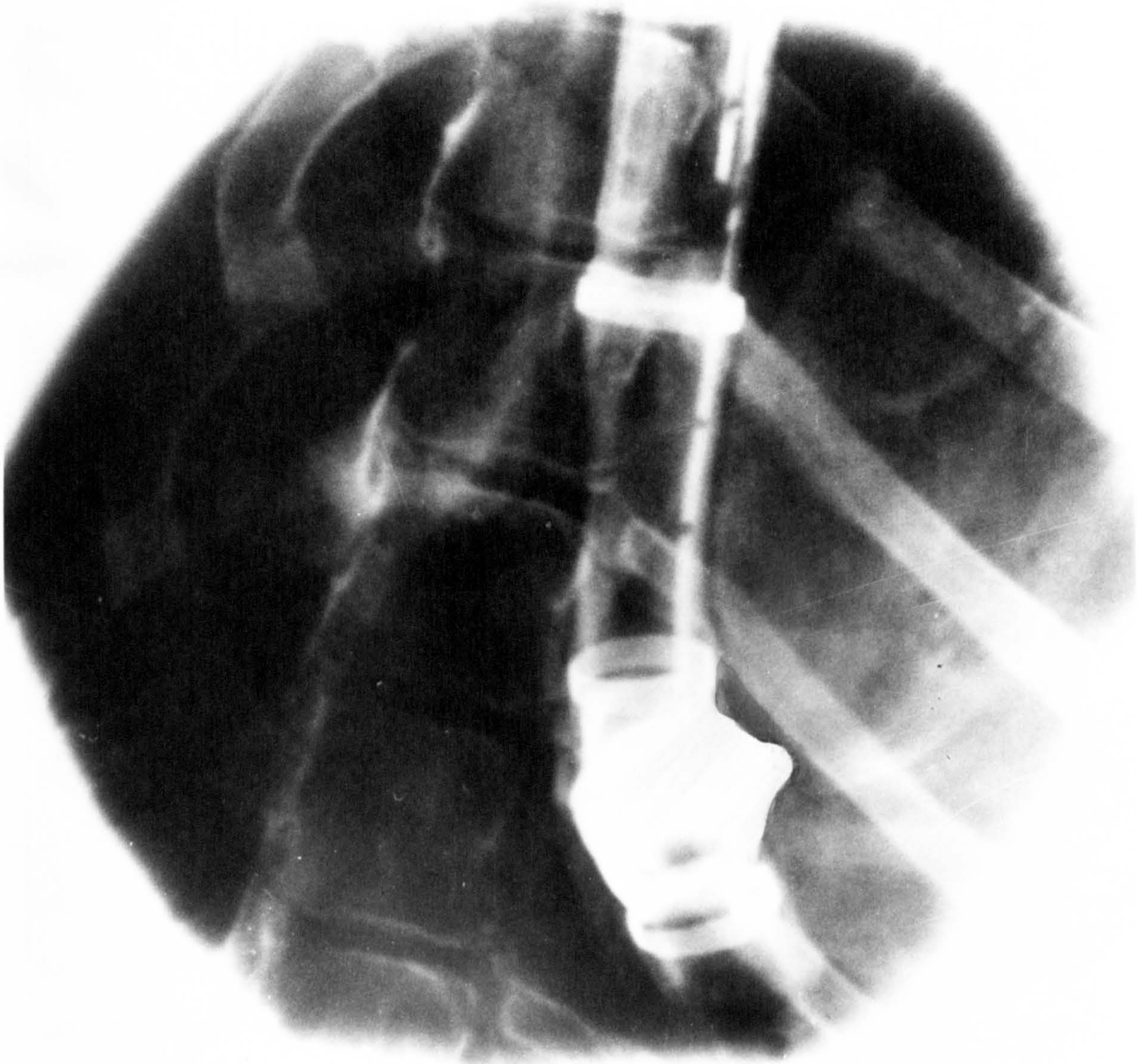


FIGURE 5:1. RADIOGRAPH: CARLENS TUBE IN SITU.

A solution to the problem was found in the use of larger animals (greyhounds) and a modified Carlens tube (Carlens, 1949). This catheter proved to be much more satisfactory; the left hand branch is shorter and the small hook at the carina allows the tube to be pushed right home and ensures positive location. The balloon on the left branch continued to block one or other of the left lobar bronchi and it was discovered that, if the balloon were modified by spiral taping to give a shorter 'squatter' shape it could consistently be persuaded to herniate into the left cardiac lobe bronchus (Figure 5:1). Once in this position, the catheter was very difficult to dislodge.

This produced a model different from that intended in that the left cardiac lobe became collapsed and the left apical lobe became part of the right lung as far as ventilation was concerned. It was decided to accept this compromise, mainly because of its reproduceability, but also because in the dog the left cardiac lobe contributes to only 5.8% (S.E. = ± 0.13) of the total weight of both lungs and the left apical lobe 9.6% (S.E. = ± 0.20) (Rahn & Ross, 1957).

Henceforth in this section the term 'right lung' refers anatomically to right lung plus left apical lobe, and 'left lung' to the left diaphragmatic lobe only, representing approximately 60% and 30% respectively of the total lung weight.

Confirmation of position of tube

The position of the tube in each experiment was ascertained by several different methods.

The cuffs were inflated with Conray and a check made under

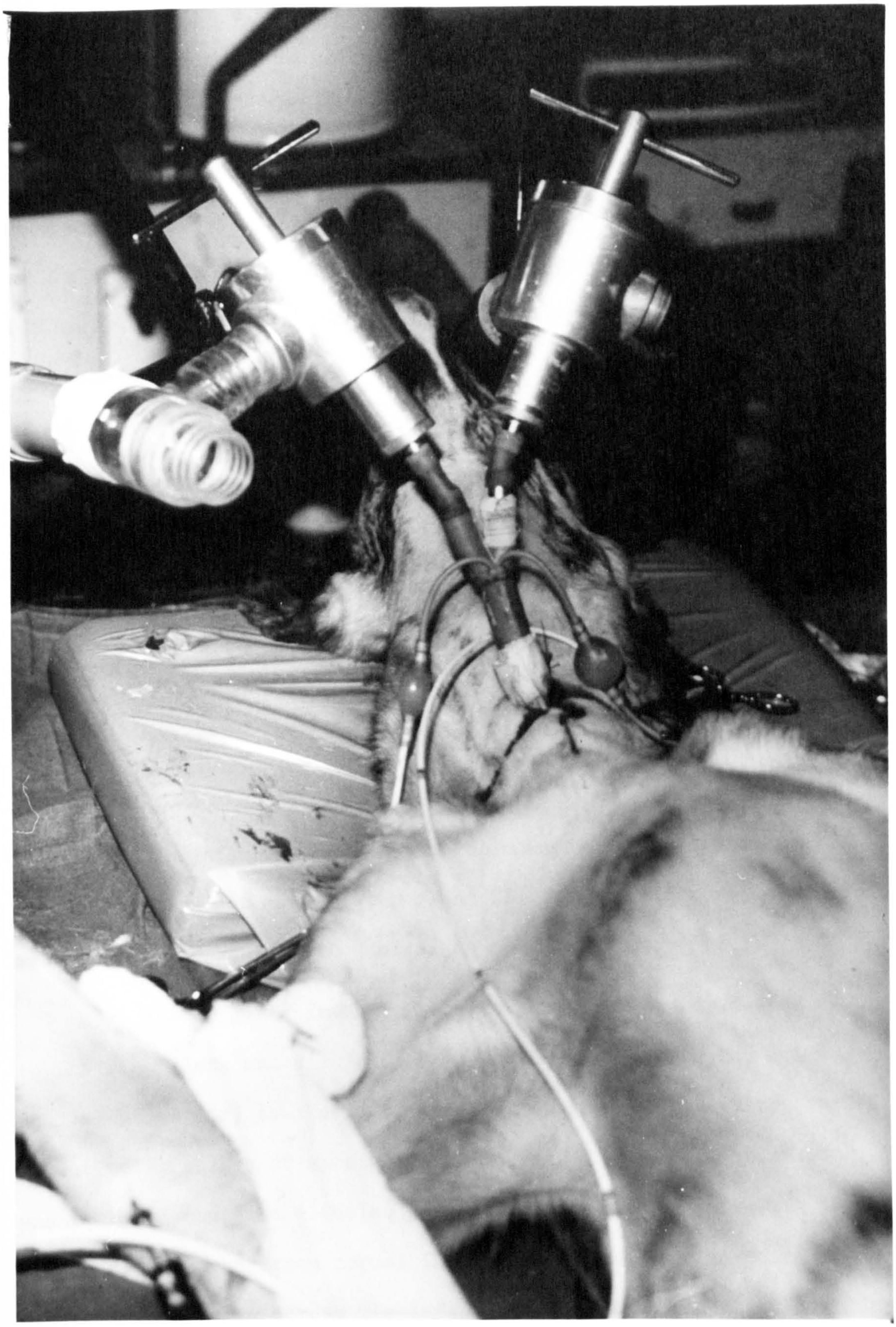


FIGURE 5:2. CARLENS TUBE: GAS DELIVERY SYSTEM.

fluoroscopic vision that the left branch balloon had herniated into the left cardiac lobe bronchus.

The lungs were separately inflated by means of an anaesthetic balloon and chest movements observed.

The right and left lungs were separately ventilated with 100% oxygen and the effects on blood gases observed. (For example, at 2 ATA with the right lung on oxygen, a typical P_aO_2 would be 600 mm.Hg., whereas with the very much smaller left lung on oxygen, P_aO_2 was around 250 mm.Hg.)

Leaks past the tracheal cuff could be detected by a fall in both bells of the spirometer whereas a leak past the left branch cuff (i.e. a leak between lungs) showed as an apparent emptying of one bell into the other.

The exact position of the tube was finally confirmed at autopsy.

Gas delivery system

The left hand side of the spirometer was modified in a manner identical to that of the right (Page 3:14, Figures 3:4, 3:5); gases were delivered to the animal as shown in Figure 5:2.

The original intention was to have 100% oxygen delivered to the right lung and a 10%/90% O_2/N_2 mixture (i.e. P_IO_2 at 2 ATA equivalent to air) to the left, as shown in Figure 5:3. This created logistical problems of special gas mixtures, and on reflection it was realised that the alveolar oxygen tensions in the left lung were liable to be higher than normal in any case due to the reversed gradient for oxygen across the left lung. (By the same token alveolar

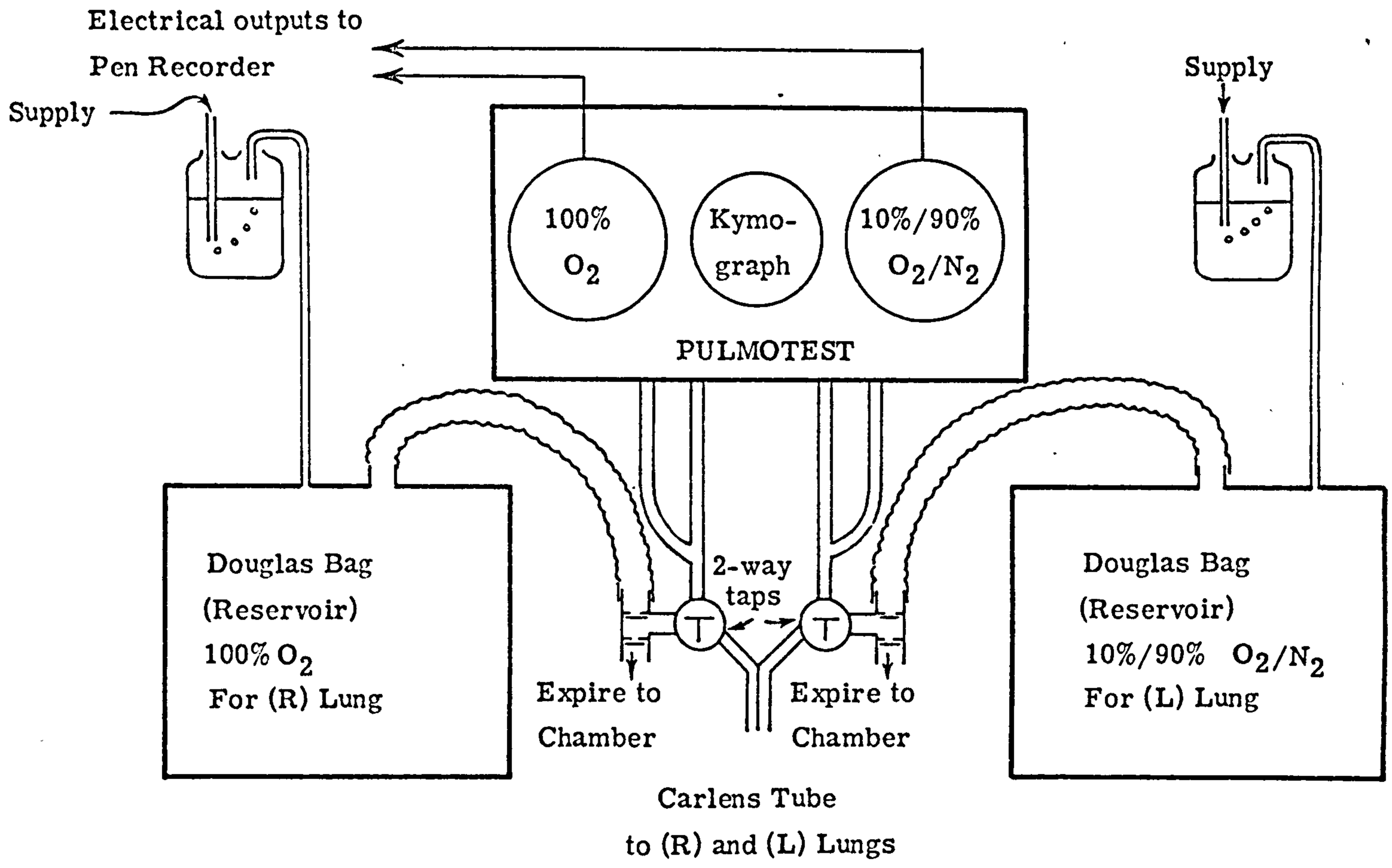


Figure 5:3 ORIGINAL GAS DELIVERY SYSTEM

oxygen tension in the right lung would be rather smaller than the theoretical 'ideal' alveolar P_{O_2} because of the uptake of nitrogen by the left lung and its excretion by the right.)

It was decided therefore to allow the animals to breath oxygen with the right lung, and chamber air (P_IO_2 = 310 mm.Hg.) with the left. This was predicted to give alveolar values of around 1425 mm.Hg. and 250 mm.Hg. respectively.

MATERIALS AND METHODS

Fifteen greyhounds, of weights ranging from 22 to 30 Kg were used. They lay supine in a harness in a specially constructed operating table (i.e. neither the right nor the left lung was dependent).

The physiological measurements made are listed in Table 5:1.* These were made as described in Section 3 other than for the exceptions set forth below.

Electrical output from each bell of the spirometer was displayed on the pen-recorder along with the intra-oesophageal pressure trace.

Calculation of percentage pulmonary shunt ratio was not possible as there were no means in a closed-chested animal of determining the pulmonary venous oxygen content for each lung.

In the calculation of compliance, allowance had to be made for the different volumes of each 'lung'. This was done by a method similar to that described in Section 3; compliance was assumed to be equal in both lungs in the early stages of the experiment, and the early values obtained for each lung were taken to represent 100%.

* presented in Appendix 2

Subsequent values were corrected in proportion and displayed as a percentage change.

Pathological investigation

Immediately following death, the endotracheal tubes were clamped and the chest opened. The condition of the lungs and particularly the state of collapse of the left cardiac lobe and of expansion of the left apical lobe was assessed.

Portions of both diaphragmatic lobes were isolated between clamps, excised, powder-coated, and quenched in liquid nitrogen.

An adjacent slice of tissue about 1 to 2 mm thick was removed from each diaphragmatic lobe, placed under the surface of some freshly prepared cacodylate-buffered gluteraldehyde, diced into 1 mm cubes and placed in fresh cold gluteraldehyde.

The hilum of each lung was divided between clamps and the lungs removed. The right lung together with the left apical lobe was placed in a beaker, and the left cardiac and left diaphragmatic lobes placed in separate beakers. They were weighed and then placed in a drying oven (Gallenkamp Vacuum Oven) at 60°C. and maximum vacuum (about 1 - 2 torr) until two identical consecutive dry weight readings were obtained.

No attempt was made to correct for the amount of blood in the lung tissue. This was considered to be justified as the results are presented as lung wet weight/dry weight ratios rather than as an absolute value for lung water, and the water fraction of normal blood (0.83) is so close to that of normal lung (0.75 to 0.80) that large differences in residual blood content would have only modest effects on

the overall wet/dry weight ratio (Staub, 1974). As the animals tended towards haemoconcentration in the terminal stages, the effect on the ratio would be even less.

RESULTS

Distribution of animals

Of the fifteen dogs, two were anaesthetic controls, one was sacrificed to obtain an additional wet/dry lung weight ratio, and the remaining twelve were experimental animals.

Of these twelve, three were discarded from the series for reasons discussed below and the remaining nine formed themselves into two groups; a main group of six dogs whose results are presented below and a subsidiary group whose results are not plotted, but are discussed subsequent to the main group.

Animals eliminated from the series

During one experiment a power failure caused the chamber to partially decompress. It was eventually repressurised, but the animal died soon after, with both lungs collapsed. It is not known whether this was due to a pneumothorax secondary to pulmonary barotrauma during the decompression, or was a consequence of atelectasis during the recompression.

One animal suddenly died at 11½ hours for no obvious reason. At autopsy the right lung was completely collapsed.

A third animal appeared to be well during a normal run of measurements, but was noticed to be dead by the time the investigators

had decompressed in the air-lock. There was an unavoidable delay of at least thirty minutes between death and autopsy, and the findings were those of a classical Lorrain Smith type lesion - almost certainly a post-mortem artifact.

Control animals

Two greyhounds, having undergone full surgery and with the Carlens tube in place, breathed air at 2 ATA for 24 hours until sacrificed. They remained essentially normal in all recorded parameters. Pulmonary histology was normal and their wet/dry lung weight ratios were similar to those of the normal control animal.

MAIN GROUP

Time to death

The main group of animals died at a mean time of 23:47 hours (range 21:00 to 26:15 hours).

Respiratory pattern

A variable pattern of respiration similar to that shown in Figure 3:10 was observed.

Physiological measurements

Corrected and derived data were tabulated and plotted in a manner similar to that described in Section 3. Table 5:1 (presented in Appendix 2) shows typical values for one animal. Meaned values from which the graphs were constructed are reproduced in Appendix 3.

Compliance

Changes in compliance of the right and left lungs are demonstrated in Figure 5:4. Compliance fell steadily in the right lung over the last ten hours of the experiment, but remained essentially unchanged in the left lung until immediately before death.

Tidal volume (right and left)

Figure 5:5 demonstrates both the much smaller actual size of the left 'lung' initially, and the change in relative contribution to ventilation over the course of the experiment. By about five hours before death ventilation was about equal in the two lungs. It continued to fall in the right lung due to a continuing decrease in compliance, and during the last hour of the experiment much the greater part of ventilation was performed by the left lung.

Blood gases

The mean arterial oxygen tension over the first twelve hours in the main group of dogs was 665 mm.Hg. (S.E. = ± 13). This tended to rise slightly later in the experiment and was still normal (for this preparation) at two hours before death. It then showed a sudden collapse down to 78 mm.Hg. at one hour before death and fell slightly (down to 50 mm.Hg.) over the final hour (Figure 5:6).

Arterial carbon dioxide tension remained completely normal until the last few minutes before death when it showed a sudden climb (mean value at death = 70 mm.Hg., S.E. = ± 18) (Figure 5:6).

Cardiovascular parameters

Mean systemic arterial blood pressure remained remarkably constant throughout the experiment, the value at fifteen minutes

before death being identical to the mean of values for the first twelve hours (96 mm.Hg.). It fell over the last fifteen minutes to a mean value of 75 mm.Hg. (S.E. = ± 15) at the moment of apnoea (Figure 5:7).

Cardiac output also remained steady over much the greater part of the experiment being the same at one hour before death as at the start (4.1 l/min., S.E. = ± 0.1) (Figure 5:7). It then fell gradually to 3 l/min. (S.E. = ± 0.4) at the moment of apnoea.

Mean pulmonary artery pressure rose from its baseline value of 9 mm.Hg. to a terminal value of 14 (S.E. = ± 2) at apnoea ($p < 0.025$) (Figure 5:8). It is appreciated that this method of displaying meaned results tends to disguise transients in individual animals, and as may be inferred by the large standard errors, actual values considerably higher than those displayed in the graph were obtained. In addition, these were values of mean arterial pressure; systolic peaks of 25 to 30 mm.Hg. were not uncommon.

Care had to be taken also that the method of display did not produce artifacts; for example, the 'dip' in pressure at 8½ hours before death is largely artificial due to sampling error - a very small number of recordings being available for this particular moment of time.

Pulmonary artery wedge pressure tended to mirror pulmonary artery pressure, rising from 2 mm.Hg. initially to 3.6 mm.Hg. at 45 minutes before death (Figure 5:8). It did not, however, display the steep terminal rise seen in pulmonary artery pressure, and fell to 2.75 mm.Hg. at the moment of apnoea. Once again the peculiar 'dip' in wedge pressure (not artifactual) was seen around one hour before

death (cf Figure 3:19).

Pulmonary vascular resistance (Figure 5:9) was remarkably consistent in the early parts of the experiments (1.78 units, S.E. = ± 0.01). It rose to 2.33 (S.E. = ± 0.18) at seven hours before death, returned to approximately baseline values for four hours, then climbed steeply to 3.28 units at one hour before death. The spread of values in the latter stages became very wide (S.E. at 30 minutes before death was 0.58).

Pathological appearances

In all of the animals the heart was still beating strongly at thoracotomy.

In all cases there was copious oedema fluid in the right limb of the endotracheal tube. This was clear, or only very faintly pink-tinged.

The right lung was fully expanded and pink in colour in five of the six dogs. In the sixth animal, the right lung had areas of partial haemorrhagic collapse, and only the diaphragmatic lobe was fully expanded.

Findings in the left lung were variable.

In all animals the left diaphragmatic lobe was fully expanded, but was a dull greyish-purple colour, in striking contrast to the right lung.

In only one animal had the proposed pattern of ventilation (i.e. cardiac lobe collapsed, apical lobe ventilated with oxygen) been achieved.

In three animals, both the apical and cardiac lobes were totally collapsed and consolidated. These lobes were not included in the wet/dry ratio results presented below.

One animal had obviously been ventilating both the apical and cardiac lobes with oxygen as they were pink, expanded and oedematous. These were included for weighing purposes with the right lung.

In the last animal the catheter cuff had lodged in the apical lobe bronchus; this lobe was completely collapsed, and the cardiac and diaphragmatic lobes were expanded and markedly cyanosed.

Lung wet/dry weight ratio

Four controls were used; two anaesthetised dogs which had breathed air at 2 ATA for 24 hours, one animal which was sacrificed to provide a completely normal control value, and an animal from a different experiment (Section 8) which had died unexpectedly shortly after arriving at pressure. Results were highly consistent (Figure 5:10), the actual values being: right lung, 4.65 (S.E. = ± 0.11), left lung, 4.54 (S.E. = ± 0.09).

In the experimental animals the values were: right lung (oxygen breathing) 9.37 (S.E. = ± 0.53), left lung (air breathing) 4.42 (S.E. = ± 0.22) (Figure 5:10).

This difference is highly significant ($p < 0.001$).

Histology

Typical appearances are shown in Figure 5:11a and b.

Sections taken from the oxygen breathing lungs were indistinguishable from those from the 'pulmonary death' animals

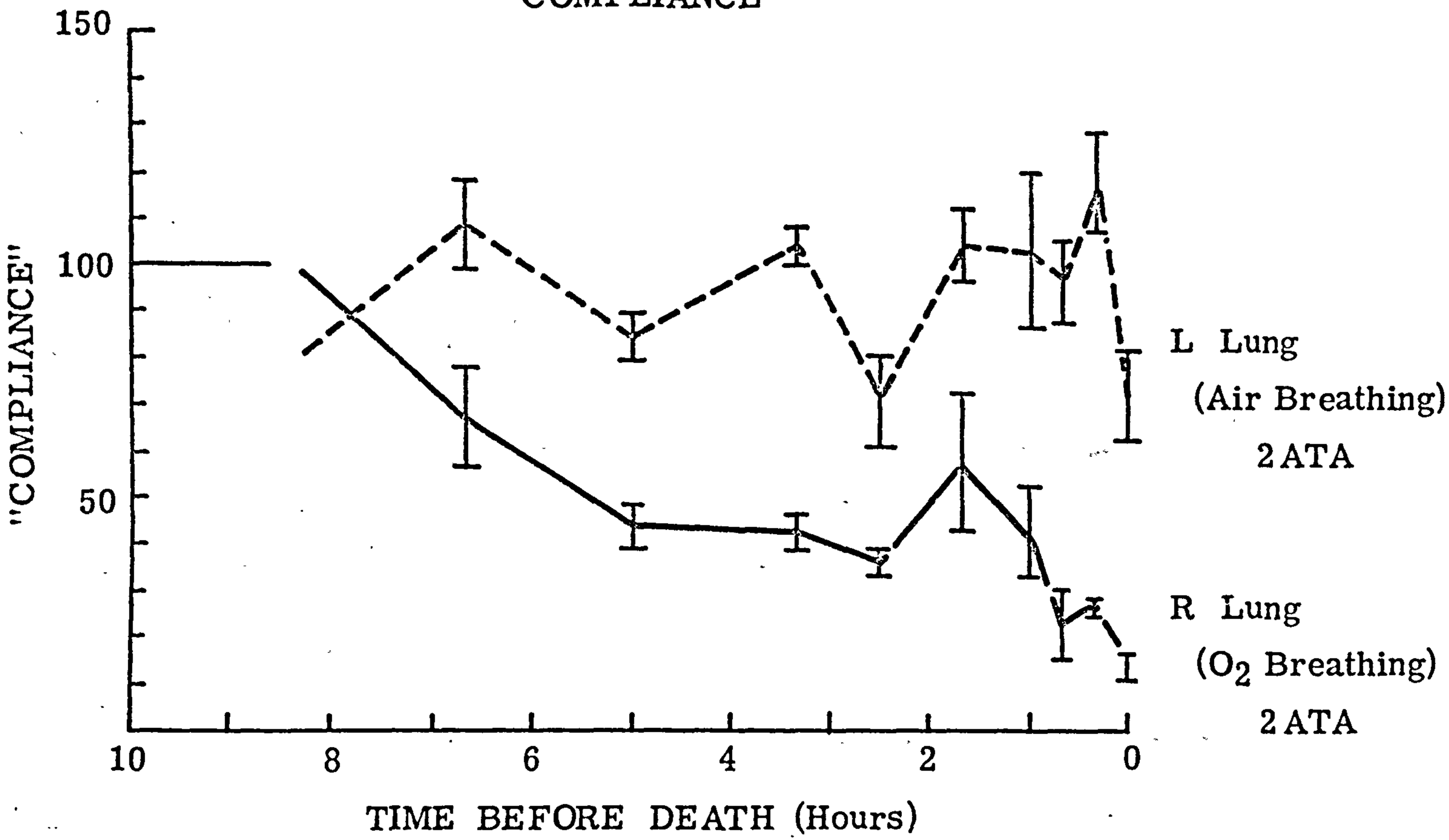
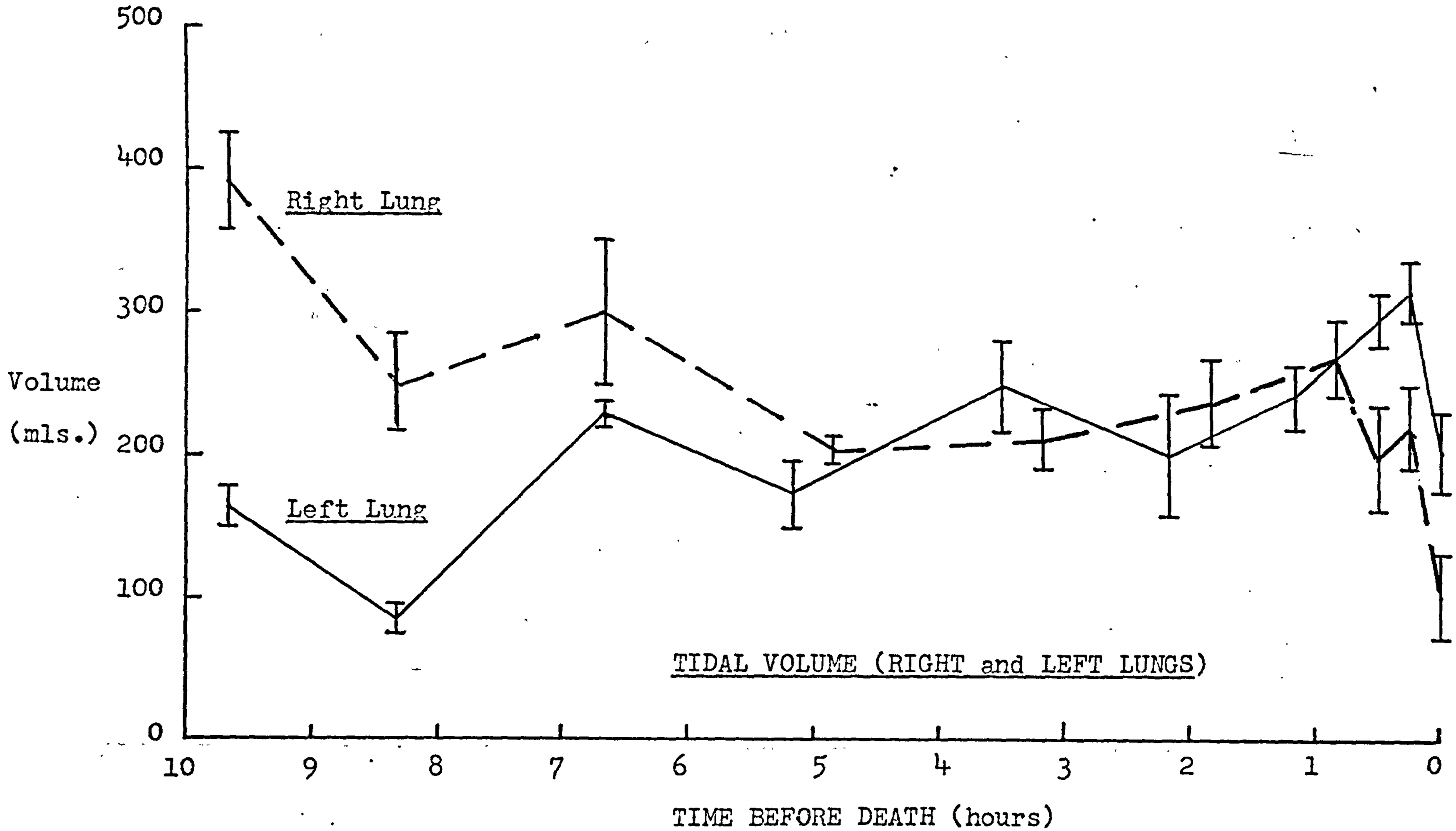

"COMPLIANCE"
150
100
50
"COMPLIANCE"
L Lung
(Air Breathing)
2 ATA
R Lung
(O_2 Breathing)
2 ATA
10
8
6
4
2
0
TIME BEFORE DEATH (Hours)

FIGURE 5:4.



TIDAL VOLUME (RIGHT and LEFT LUNGS)

FIGURE 5:5

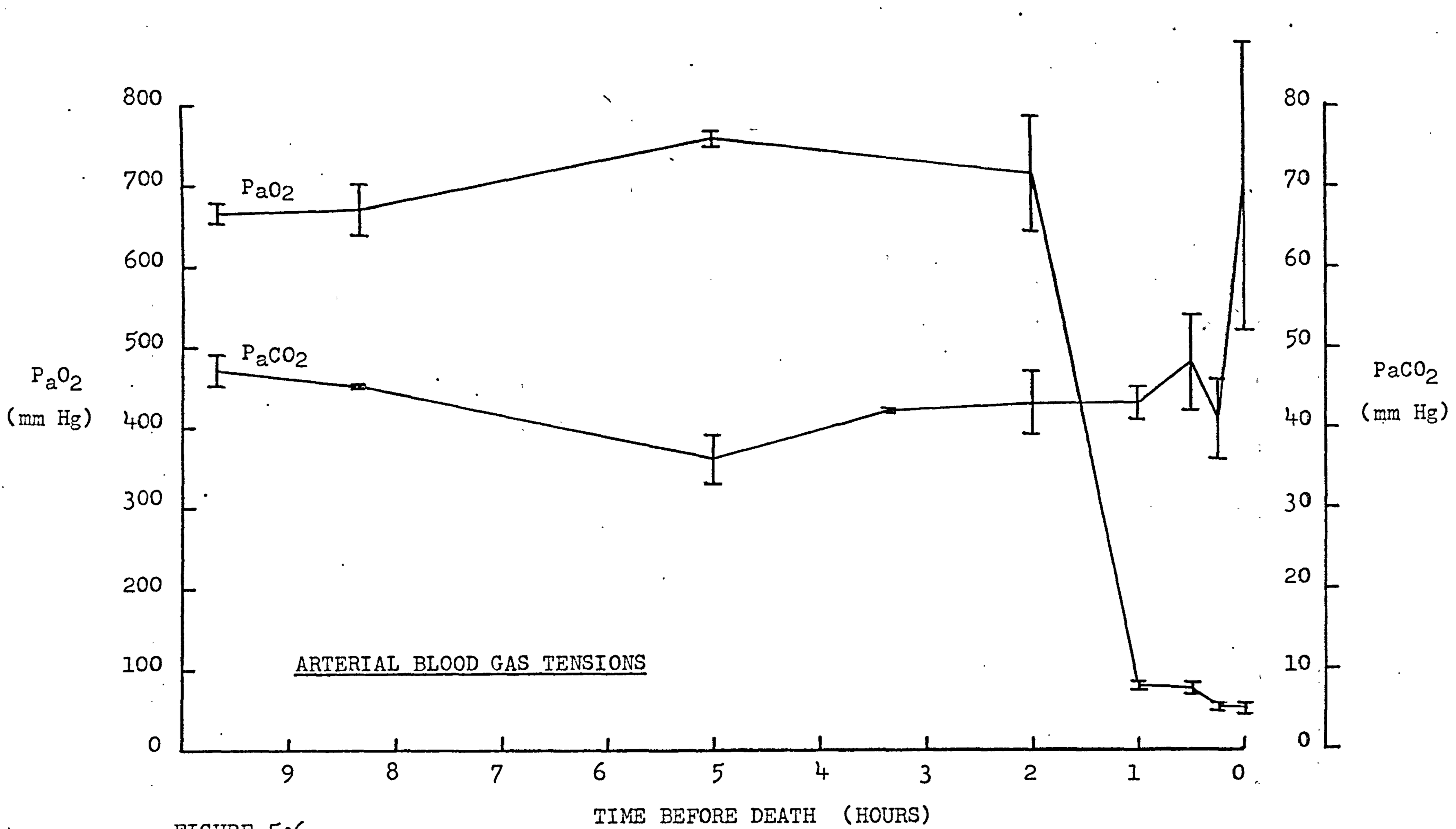

800
700
600
500
400
300
200
100
0
Pa_{O_2}
(mm Hg)
Pa_{O_2}
Pa_{CO_2}
80
70
60
50
40
30
20
10
0
Pa_{CO_2}
(mm Hg)
ARTERIAL BLOOD GAS TENSIONS
9
8
7
6
5
4
3
2
1
0
TIME BEFORE DEATH (HOURS)

FIGURE 5:6

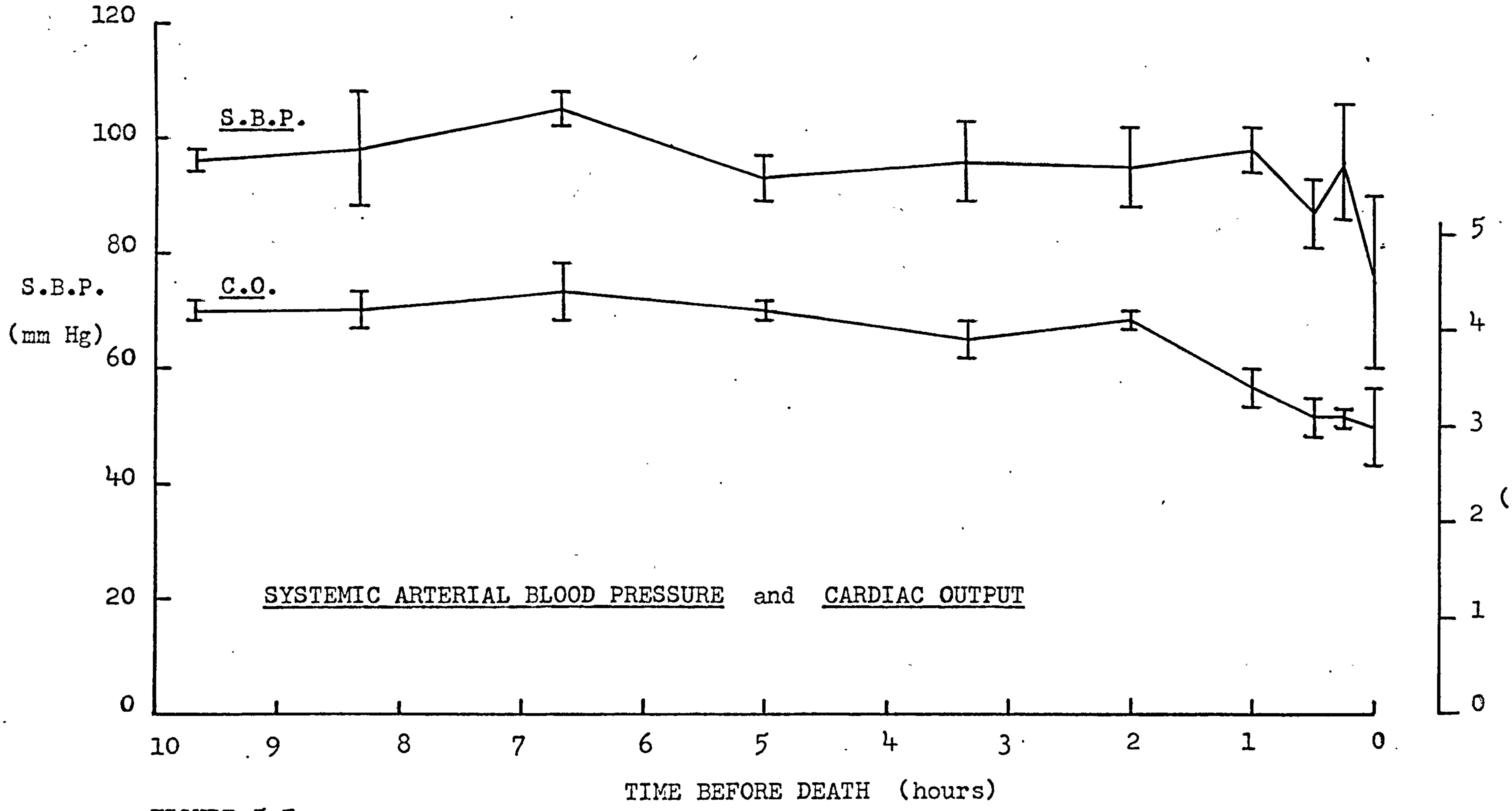

120
100
80
60
40
20
0
S.B.P.
C.O.
S.B.P.
(mm Hg)
SYSTEMIC ARTERIAL BLOOD PRESSURE and CARDIAC OUTPUT
10
9
8
7
6
5
4
3
2
1
0
TIME BEFORE DEATH (hours)
5
4
3
2
1
0
C.O.
(l/min

FIGURE 5:7

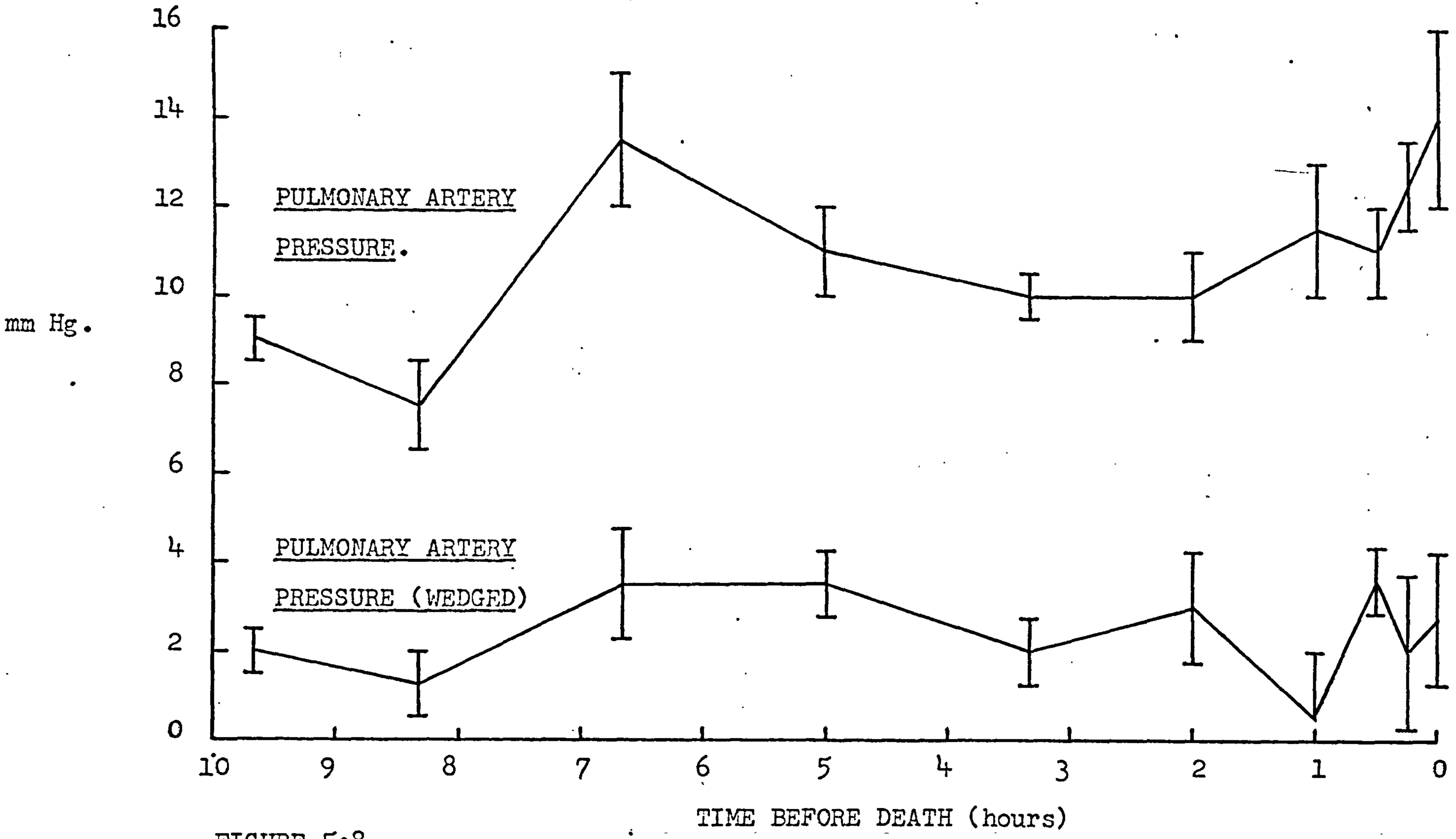

16
14
12
10
8
6
4
2
0
mm Hg.
PULMONARY ARTERY
PRESSURE.
PULMONARY ARTERY
PRESSURE (WEDGED)
10
9
8
7
6
5
4
3
2
1
0
TIME BEFORE DEATH (hours)

FIGURE 5:8

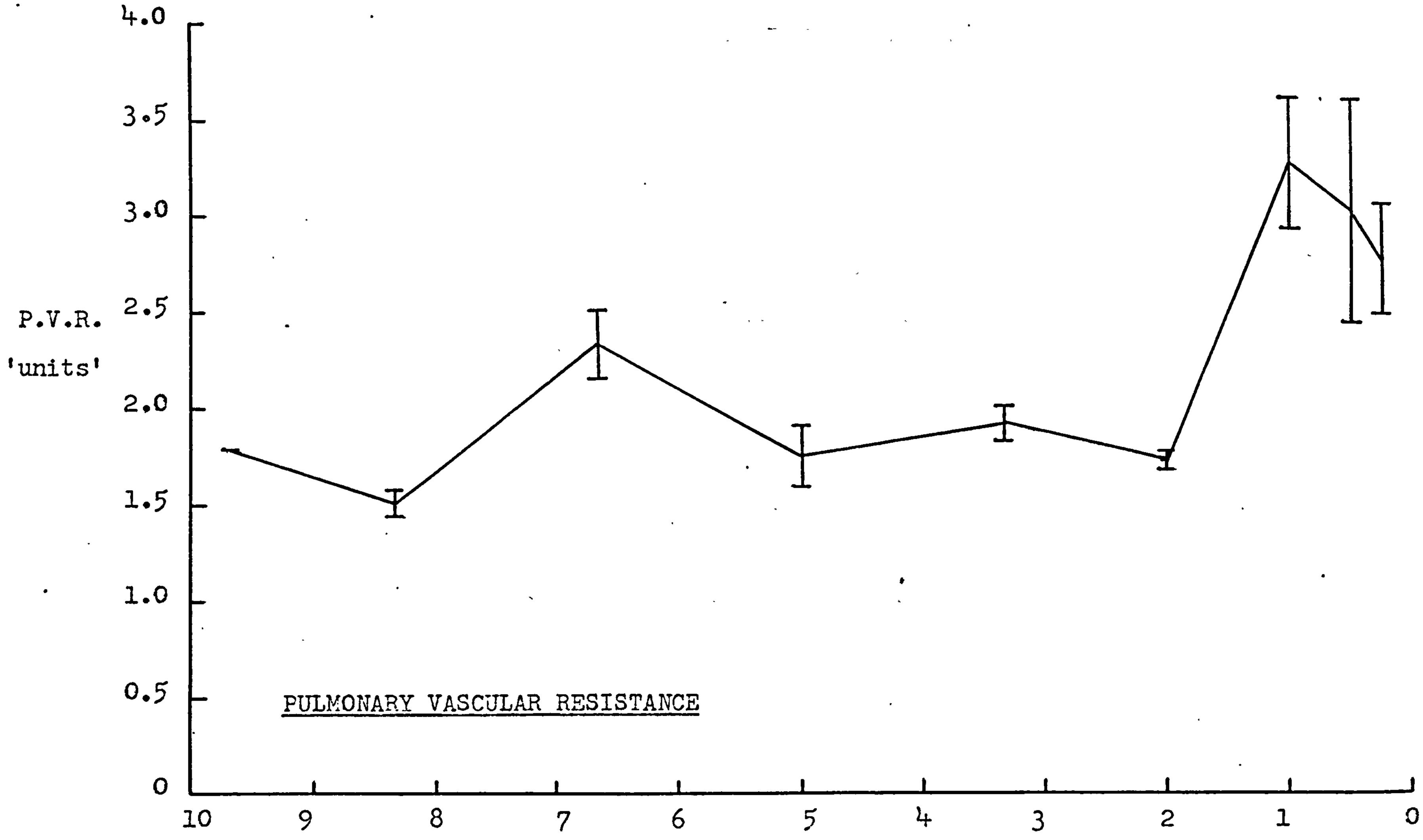

4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
P.V.R.
'units'
PULMONARY VASCULAR RESISTANCE
10
9
8
7
6
5
4
3
2
1
0
TIME BEFORE DEATH (hours)

FIGURE 5:9

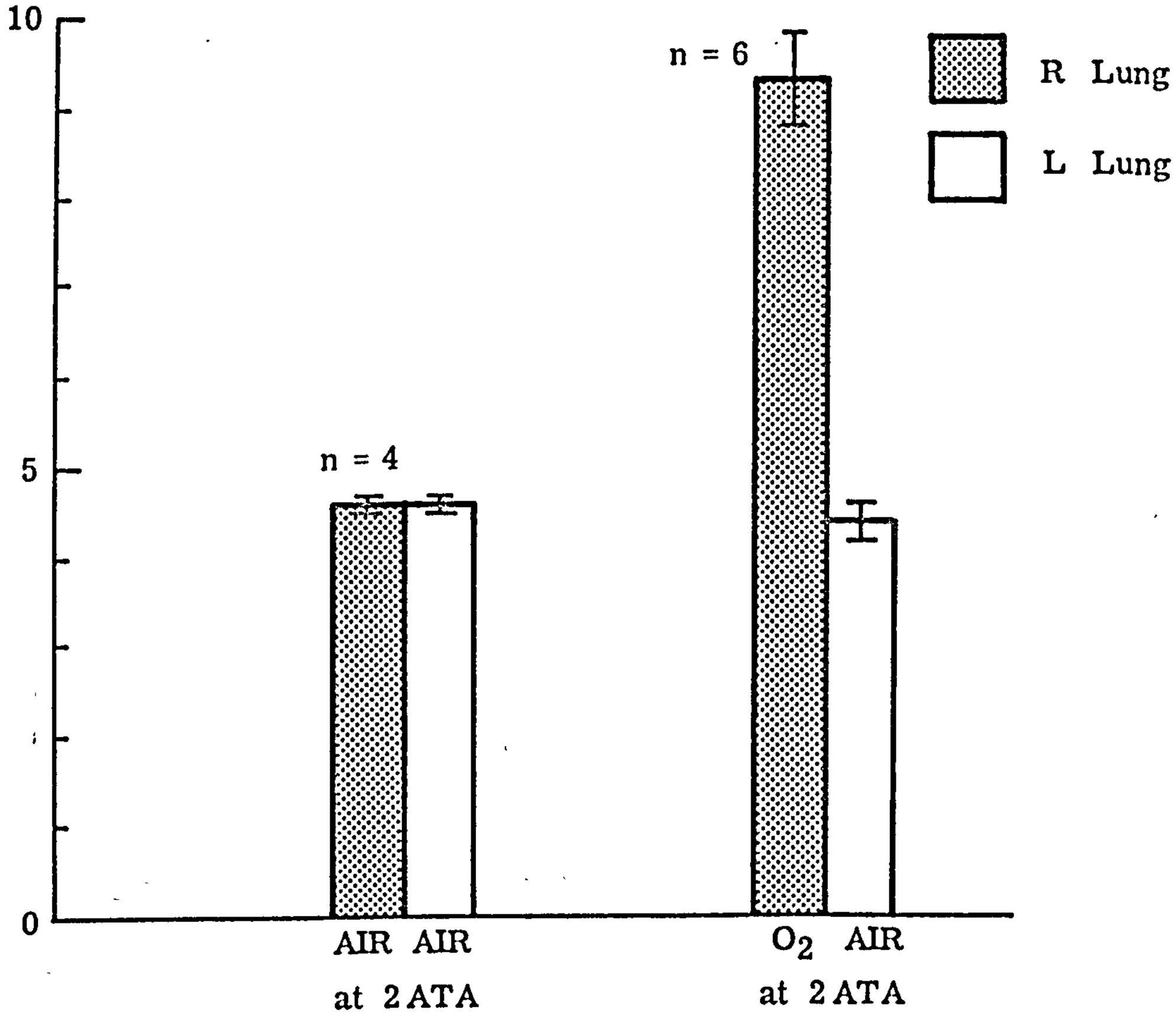


FIGURE 5:10

described in Section 3; i.e. there was gross intra-alveolar oedema, occasional intra-alveolar haemorrhage, intense vascular congestion (involving arterioles, capillaries and venules) and appearances suggestive (at light microscope level) of severe interstitial oedema.

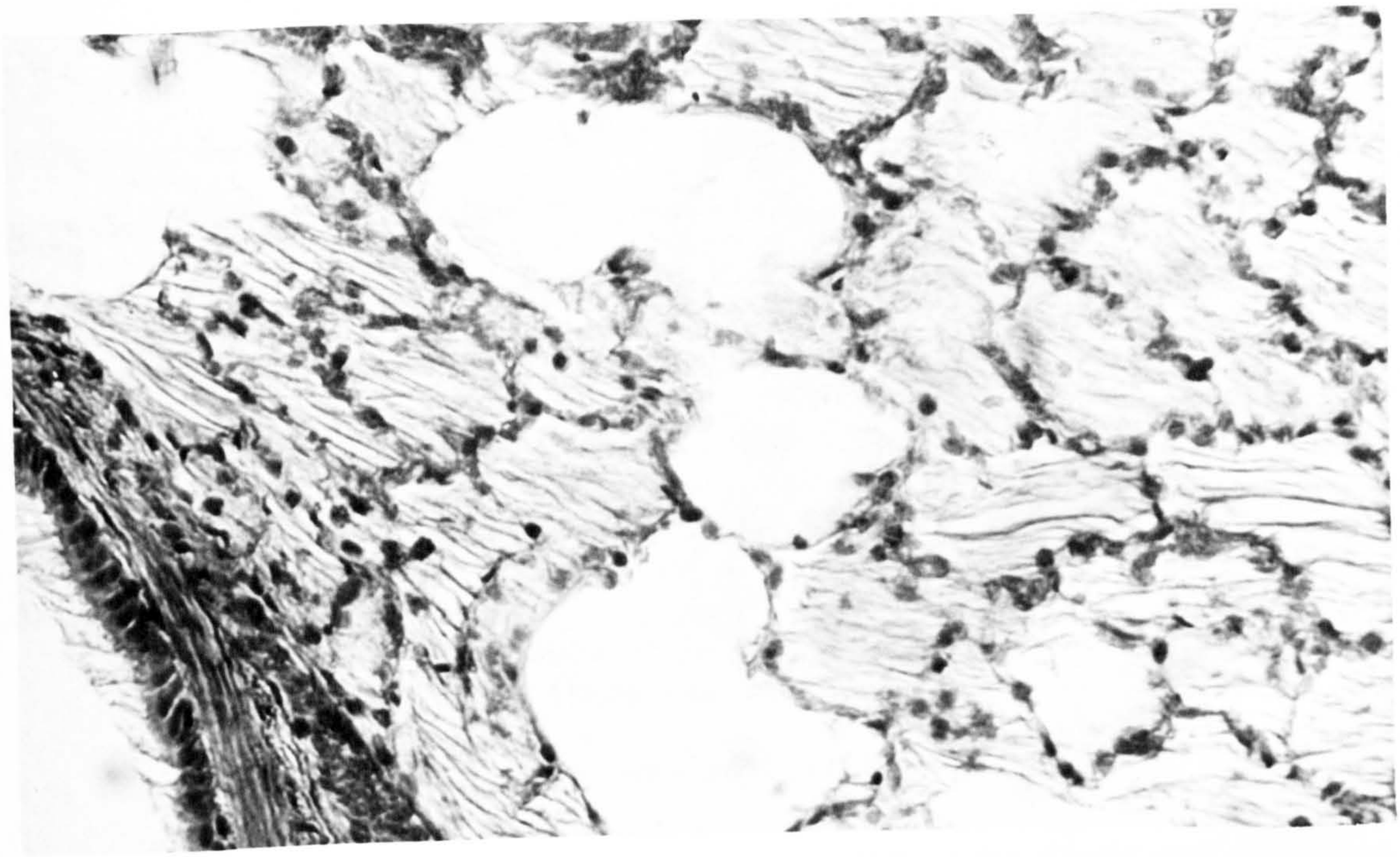


FIGURE 5:11a. HISTOLOGY: RIGHT LUNG. (x 180)

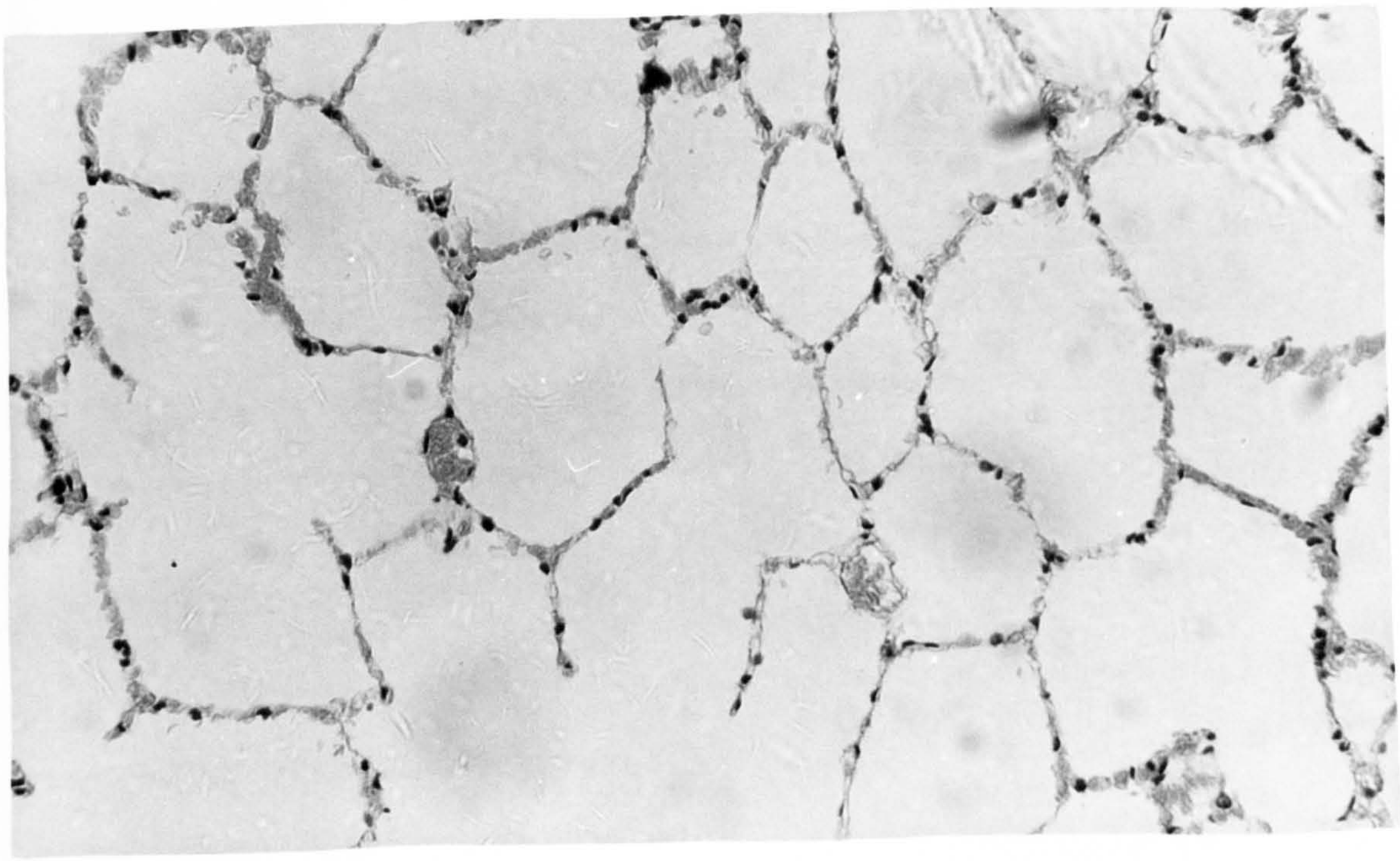


FIGURE 5:11b. HISTOLOGY: LEFT LUNG. (x 180)

Sections taken from the air-breathing lungs were relatively normal. There was no sign of intra-alveolar oedema, although in some sections thickening of the alveolar membrane suggested interstitial oedema. Peri-bronchiolar and peri-venular interstitial oedema was minimal, although it is noted that all the sections were taken from a peripheral portion of lung which did not include any large vessels or airways.

In most sections there was marked arteriolar, capillary and venular congestion. There was no fibrin in the alveolar space.

Tissues for electron microscopy were post-fixed and embedded as described in Section 4, but time did not permit of their sectioning.

SUBSIDIARY GROUP

Over the course of this series of experiments, which lasted about four months, a total of six dogs were originally rejected. It was only after completion that it was realised that three of these had been rejected for the same reason, namely that at autopsy they had large bilateral pleural effusions. Retrospective analysis of their results revealed some interesting similarities.

Time to death

They died more rapidly than the main group (mean = 19.17 hours, range 18 to 20:05 hours) ($p<0.005$).

Mode of death

All three animals ceased to ventilate with their left

diaphragmatic lobe some time before death (the interval varying between 30 minutes and two hours). Following this, two animals suddenly became apnoeic with no gradual fall in the tidal volume of the right lung. In the third animal, ventricular asystole preceded apnoea.

Cardiorespiratory parameters

A striking difference from the main group is that in these three animals arterial oxygen tension was much greater throughout the experiment (mean value over the first 12 hours = 959 mm.Hg. compared with 665 mm.Hg. in the main group) and remained high terminally.

Arterial carbon dioxide tension on the other hand began to rise several hours before death and by two hours before death had already reached 65 mm.Hg. in all three dogs.

Systemic blood pressure and cardiac output fell over the last two hours, reaching approximately 60% of baseline terminally.

There was a small (4 mm.Hg.) rise in pulmonary artery pressure at about 7 hours before death (cf Figures 3:18 and 5:8) with a decline back to normal values at apnoea. Pulmonary artery wedged pressure remained steady in one animal and rose terminally (by about 3 mm.Hg.) in the other two.

In these two animals this was reflected (in the face of a falling cardiac output) in a stable pulmonary vascular resistance. In the third animal, pulmonary vascular resistance rose in the late stages.

Compliance of the right lung decreased to 65% of baseline in two dogs and to 85% in the third. Compliance of the left lung had fallen to about 50% at 6 hours before death and collapsed to zero when the lung ceased to ventilate.

Autopsy

All three animals had very large bilateral pleural effusions.

In all three, the left diaphragmatic lobe was partially collapsed; in two there was extensive haemorrhagic consolidation in the dependent part.

The right lung differed in all three animals. In one it was fully aerated and pink; in another it was also aerated and pink, but at a level well below functional residual capacity presumably due to the copious amount of fluid in the intrapleural space; while in the third it was expanded superiorly, but with extensive haemorrhagic consolidation in the dependent part.

In two of the animals both the left apical lobe and the left cardiac lobe were well expanded and pink, which helps to explain the high P_aO_2's; in the third animal the apical lobe was aerated and pink, but the cardiac lobe was collapsed.

An interesting incidental finding in two of the animals (Dogs 20 and 21) was scattered 'tubercles' similar to those seen in Dog 6 of Section 3 (see Page 3:46). These were confirmed histologically in Dog 21 to be of the same aetiology, i.e. an immune response to toxocara canis re-infection. It is interesting also that one of these animals (21) was, like Dog 6 of Section 3, the only animal in

this series in which asystole preceded apnoea.

Autopsy findings are tabulated in Table 5:2.

Lung weights

Fortunately, although the animals were discarded from the series, the lungs had been retained and processed routinely for wet/dry weights.

Lung weights are shown in Table 5:2; in two animals the left lung contained more water than the right.

Histology

The tissues of only one animal (Dog 21) could be salvaged for histology.

Material had been sampled from the aerated parts of the lobes (see Table 5:2) and the sections were remarkable for their relative normality.

The right lung was intensely congested with areas of intra-alveolar haemorrhage and focal emphysematous change, but there was no evidence whatsoever of intra-alveolar oedema. Mild interstitial oedema was evident round the small venules only.

The left diaphragmatic lobe was almost completely normal. The venules were packed with red cells, but the septae were not congested. There was no evidence of septal thickening and peri-arteriolar and peri-venular interstitial oedema was minimal. There was no intra-alveolar oedema.

The left apical lobe showed mild venular congestion and

TABLE 5:2

Autopsy Findings and Lung Weights

DOG NO.		(R) LUNG	LEFT APICAL LOBE	LEFT CARDIAC LOBE	LEFT DIAPHRAG. LOBE	HISTOLOGY	DEATH
18	Condition	Expanded, pink.	Expanded, pink.	Totally consolidated	Partially consolidated	No	Apnoea
	Wet/Dry	6.0	5.0	5.83	5.125		
20	Condition	Partially collapsed but pink.	Expanded, pink.	Expanded, pink.	Partial haemorrhagic consolidation in dependent part.	No	Apnoea
	Wet/Dry	5.2	5.125	5.0	8.84		
21	Condition	Partial haemorrhagic consolidation in dependent part.	Expanded, pink.	Expanded, pink.	Partial haemorrhagic consolidation in dependent part.	Yes	Asystole
	Wet/Dry	5.69	9.42	6.71	7.22		

mild peri-venular interstitial oedema. Intra-alveolar oedema was present, but was confined to a few alveoli only.

DISCUSSION

SUBSIDIARY GROUP

The subsidiary group will be dealt with first.

It is questionable whether in fact these three animals represent a distinct entity or whether the similarities are coincidental.

From the cardiorespiratory point of view the pattern which they most resembled was that of the two 'cardiac death' dogs in Section 3. They showed a progressive fall in systemic blood pressure and cardiac output and died with a high arterial oxygen tension and a rising carbon dioxide tension. The terminal rise in pulmonary artery wedge pressure, however, was atypical.

Compliance of the right lung had fallen to levels where one might expect interstitial oedema only and this was confirmed by the lung weights and by histology.

A high wet/dry weight ratio of the left diaphragmatic lobes was expected as compliance had fallen to zero and the lobes had ceased to ventilate, but histology (admittedly performed in only one animal) did not show any sign of the expected intra-alveolar oedema.

It is tempting to suggest that the functional failure of the left diaphragmatic lobes reveals an indirect humoral mechanism

of oxygen toxicity, but there could be other reasons. Leakage of oxygen past the left bronchial cuff is a possibility and would help to explain the high overall oxygen tensions. There was, however, no indication during the experiments that such leakage was occurring, and the lobes at autopsy were cyanosed. It seems more likely that the findings in the 'air-breathing' lung are irrelevant to oxygen toxicity; that the collapse of the lobes was possibly due to the large volume of fluid accumulating in the pleural cavity. In two of the animals at least, this pleural effusion could have been a consequence of Toxocara infection.

MAIN GROUP

Direct/Indirect mechanism of action

The absence of pathological changes in the air-breathing lungs of the main group of animals confirms Penrod's claim (1958) that under hyperbaric conditions oxygen exerts a direct toxic action on the lung.

Protection by relative arterial hypoxaemia

Although in the present study the degree of relative arterial hypoxaemia was much less than in that described by Winter et al. (1967), when survival time is used as a criterion of protection, the present experiments tend not to corroborate Winter's findings that a lowering of arterial oxygen tension protects against oxygen toxicity. The difference in the mean time of death in this group of animals ($\bar{m}$ = 2?:47 hours) and those described in Section 3 ($\bar{m}$ = 22:16 hours) does not reach the level of significance ($p < 0.2$).

There are, however, several important differences in the two experiments. In Winter's study, the control animals had all convulsed whereas none of the 'shunted' animals convulsed. The pressure profile was different (see Page 5:2) and the animals were unanaesthetised. Arterial oxygen tensions in the 'shunted' dogs were much lower than in the present study (127 mm.Hg. compared with 600 mm.Hg.); it follows that the bronchial arteries were perfused with virtually normoxic blood in Winter's study and that pulmonary arterial (mixed venous) oxygen tensions were at a normal low value (compared with 80 to 100 mm.Hg. in the present experiments).

Nor did the findings agree with those of Thomas et al. (1969), although once again the pressure profiles were very different. In the present experiment, bronchial arterial and mixed venous oxygen tensions were much higher than normal to both lungs, and yet the air-breathing lung suffered little detectable damage.

Pattern of effect

The pattern of respiratory changes over the whole experiment, similar to those shown in Figure 3:10, suggests, in the face of a very much lower P_aO_2, that the site of stimulation is peripheral rather than central. This is substantiated by the finding in a later experiment (Section 9) where animals prepared in a manner identical to that described in this section were vagotomised; whereupon the respiratory pattern changes were abolished and respiration remained regular throughout.

The mechanism of death was very similar to that already demonstrated in the main group described in Section 3.

Compliance fell steadily in the right lung, presumably due to an increasing interstitial oedema. Gas exchange was not compromised until about two hours before death, when the accumulation of oedema fluid must be assumed to have overwhelmed the drainage mechanisms and to have flooded into the alveoli. Unlike the animals in Section 3, arterial oxygen tension did not plummet to zero, but levelled off at around 50 to 80 mm.Hg. for the final hour. At this stage, gas exchange was being performed almost wholly via the undamaged left diaphragmatic lobe.

Pulmonary vascular resistance

A striking difference between this group of animals and those described previously is the rise in pulmonary arterial pressure over the later stages. In both groups of animals, there was a largely fluctuating, but progressive increase in pulmonary vascular resistance, certainly over the last few hours, but in the Section 3 experiments this was offset by a fall in cardiac output which caused the pulmonary arterial pressure actually to decrease from about five hours before death (Figure 3:18). In the present experiment, cardiac output was maintained at normal levels until very late and pulmonary arterial pressures rose significantly ($p = < 0.025$).

The actual values of pulmonary vascular resistance are calculated from pressure drops over the whole of both lungs and without knowing the relative distribution of cardiac output between the two lungs, it is not possible to state whether the contribution to vascular resistance is greater in one lung or the other. The intense congestion seen on sections of both lungs might suggest that they were both affected to some extent.

The mechanism of the rise in pulmonary vascular resistance is not known. Small pulmonary veins are known to be the site of active vasoconstriction in response to many stimuli (Gilbert, Hinshaw, Kuida & Visscher, 1958; Keller, Schramel, Hyman & Creech, 1963; Sugg, Craver, Webb & Ecker, 1969; Murakami, Wax & Webb, 1970; Moss et al., 1972); the appearance of severe congestion of the whole of the pulmonary vascular bed as far as the venules, together with a normal or low pulmonary venous pressure would suggest that this is certainly so in the present case.

The controversy regarding the mechanism of action of vasoconstriction in response to alveolar hypoxia is not yet finally resolved (Kazemi, Bruecke & Parsons, 1972), but recent work (Malik & Langford Kidd, 1973) favours a local control, thus confirming a large body of earlier investigations (Duke, 1957; Barer, 1966). Very recently Forrest (1976), in an elegant preparation involving multiple simultaneous bio-assay techniques, showed that alveolar hypoxia caused the release of prostaglandin F2a and serotonin, presumably from somewhere upstream of the venules.

It is beyond the scope of the present experiment to provide evidence for a similar mechanism in response to hyperoxia, but such a mechanism would be a reasonable suggestion. It is of interest that the hypoxic vasoconstrictor action operates in response to alveolar, but not to arterial hypoxia. In both the present experiment and that described in Section 3, although arterial oxygen tensions differed, alveolar tensions in the 'high oxygen' lungs were identical, and a similar overall effect on vascular resistance was observed.

The increased pulmonary arterial pressure was, of course, transmitted equally to both lungs and one might therefore expect a

similar degree of damage in each. If, however, the venules are the site of increased resistance in this condition, and if the two lungs reacted differently, the pressure drop in the 'low resistance lung' (assumed to be the air-breathing lung) would be over a much greater segment of the vascular bed and capillary pressures would be correspondingly lower.

It is therefore impossible to say, from the evidence available so far, to what extent the rise in pulmonary arterial pressure contributed to the development of oedema. Perhaps the most interesting part of this study will be the part which remains to be done, that is the examination by electron microscopy of tissues from the pairs of lungs.

SECTION 6

INNERVATION OF THE MAMMALIAN LUNG

INNERVATION OF THE MAMMALIAN LUNG

INTRODUCTION

The findings described in Sections 3 and 4, and possibly also those in Section 5 suggest that the pathological changes in the lungs in acute hyperbaric oxygen toxicity are caused by alterations in alveolar surfactant.

There is in the literature a large body of evidence that pulmonary oxygen toxicity may be modified by sympathomimetics and sympatholytics (see review in Section 8). In addition, work by Goldenberg and his colleagues (Goldenberg, Buckingham & Sommers, 1967, 1969) on alterations in the Type 2 alveolar epithelial cell following vagotomy or cholinergic secretory stimulation by pilocarpine injection suggests parasympathetic interaction. There is, however, little evidence elsewhere for a direct autonomic control mechanism for the production or release of surfactant (Clements, 1970).

The investigation of a possible mechanism was tackled in two ways: in an experimental physiological approach by stimulating the sympathetic nerves to the lungs and looking for effects which could be attributed to alterations in surfactant (Section 7), and by a search for the micro-anatomical basis of such a control.

PULMONARY INNERVATION: REVIEW

Light microscopy

The earliest attempts at describing the innervation of the mammalian lung seem to have been by Reisseisen & von Sommering in 1808

followed by Remak (quoted by Gaylor, 1934) who in 1844 dissected out the nerve supply to an ox lung. At a microscopical level, Berkley (1894), using the newly described Golgi silver impregnation technique, demonstrated peribronchial and sub-epithelial nerve plexuses in the large bronchi, and fine nerve fibre ramifications around blood vessels.

In the early part of this century Larsell published a series of papers on pulmonary innervation in the rabbit, dog and human which, despite subsequent advances in staining techniques, remain largely unchallenged (Larsell, 1921, 1922; Larsell & Mason, 1921; Larsell & Dow, 1933).

He described the lung as having a joint innervation, both from the vagus and the upper thoracic sympathetic ganglia. The nerves divide after entering the lung at the hilum and become arranged as peribronchial and periarterial plexuses. The former, which sub-divides into extrachondrial and subchondrial plexuses, has numerous ganglion cells scattered throughout its substance; the latter, thought to be sympathetic in origin, has no ganglion cells.

Several different groups of sensory nerve endings were described. One group which was present in the epithelium of all orders of bronchi as far distal as the respiratory bronchioles was considered to respond to tactile stimuli, a suggestion later supported by experimental evidence (Larsell & Burget, 1924). Another group found in the bronchial muscle at the bifurcation of the large bronchi and described by Larsell as 'smooth muscle spindles' (a term more recently arrogated to refer to specialised receptors in skeletal muscle) may be involved in the Hering-Breuer reflex. A third type of ending is described as

a specialised structure lying in the walls of the atrium of Miller, i.e. in the proximal part of the alveolar sacs. Larsell suggests that, as their position virtually precludes mechanical stimulation of any sort, they must be 'regarded as chemical receptors which are probably stimulated by increase of carbon dioxide tension beyond a certain point', and points out that their position at the confluence of some 4 to 10 air sacs is ideally suited to the sampling of the 'respiratory product'. The interest in an alveolar chemoreceptor declined with the discovery of the peripheral arterial chemoreceptors (Heymans & Heymans, 1927; Biscoe, 1971), but has been revived very recently following a study which provides strong experimental evidence for its existence (Bartoli, Cross, Guz, Jain, Noble & Trenchard, 1974).

The atrium was the most distal point in the lung at which afferent fibres were found.

Sensory endings were also described in the pulmonary artery and on the luminal perichondrium of the bronchial cartilage plates.

Motor endings of fibres from the nerve cells of the bronchial ganglia (presumed on this basis to be parasympathetic in origin - an assumption since confirmed experimentally in denervation experiments by Elftman in 1943) were described in the bronchial muscles and bronchial mucous glands.

Of great interest in the present context is the description of motor nerve fibres from the peri-arterial plexus - thought to be formed of post-ganglionic fibres from the upper thoracic sympathetic ganglia - which end not only on the muscularis of the pulmonary and bronchial arteries, but also pass out into the parenchyma. The text is worth quoting (Larsell & Dow, 1933):

'Some of these fibres take their course away from the arterial wall into the lung parenchyma. In the lung parenchyma more slender filaments follow the course of the capillaries about alveolar ducts and air sacs, at intervals giving off short terminal twigs .... These twigs end in relation to the capillary walls, but whether on endothelial cells or on some other type of cell in the capillary wall could not be ascertained in the methylene blue material. Patches of such fibres on capillaries were found in favorably stained portions of the lung, where the epithelium was not stained with the methylene blue. Without doubt such nerve fibres have a wide distribution throughout the lung. So far as can be determined in our material, they are continuations of the nerve fibres surrounding the arteries.'

It should be remembered that a description of the structure of the alveolar membrane awaited the development of the electron microscope (Low & Daniels, 1952) and in the light of present knowledge the statement above may be taken to represent a description of the innervation of the alveolar epithelium.

This early work has been repeated and largely confirmed in other species over the years (Elftman, 1943; Honjin, 1956; Spencer & Leof, 1964). Neither Honjin nor Spencer & Leof were able, however, to demonstrate nerve fibres in the alveolar wall and concluded that bronchial motor innervation extended as far as the respiratory bronchioles and vascular innervation as far as the terminal arterioles and fine intrapulmonary venous branches.

In contrast, Hirsch and his associates (Hirsch, Kaiser, Barner, Cooper & Rams, 1968; Nigro, Hirsch, Rams, Hamouda & Adams,

1968; Hirsch, Kaiser, Barner, Nigro, Hamouda, Cooper & Adams, 1968) using a combined Bielschowski-Gros and Gomori trichrome staining technique, claim to have demonstrated an extensive network of fine nerve fibres in the alveolar walls.

A recurrent problem in pulmonary histology, however, is the lack of specificity of the staining techniques employed; both elastic and reticulin fibres are agyrophylic and also show an affinity for methylene blue (Fillenz & Woods, 1970), and it is not obvious from Hirsch's published micrographs to what extent these 'nerve' fibres might be some other structure.

Electron microscopy

The first demonstration by electron microscopy of nerve fibres in the alveolar walls was by Meyrick & Reid as recently as 1971. They were rare, being seen in only two of a total of eighty blocks, from forty animals (rats). The axons were seen on several sections from each block, were deep in the acinus and remote from any large structure, and were positively identified by the presence of a Schwann cell sheath. Morphological features of these axons implied a sensory function, and in one section a terminal varicosity surrounded by a seemingly specialised group of cells ('guard cells') suggested a receptor.

The comment was made that these intra-alveolar nerves were so fine that they failed to show on a toluidine blue stained section; this may be the cause of the conflicting results in the classical light-microscopical studies described above.

These findings have since been confirmed and extended by Hung and his colleagues (Hung, Hertweck & Loosli, 1972; Hung, Hertweck, Hardy & Loosli, 1972, 1973a) who have not only found similar fibres in the alveolar walls of mouse lungs, but have found them in abundance, at all section levels of all blocks examined (again from 40 animals).

In the alveolar ducts, groups of unmyelinated nerves containing up to nine axons from 0.12 to 1.03 micrometres in diameter were found. They were surrounded completely or partly by a Schwann cell. Neurotubules and mitochondria were demonstrated in the axoplasm. Bundles of up to five axons were present in the alveolar walls, in the interstitium between the epithelial and endothelial cells. They also contained numerous neurotubules and were surrounded by a thin Schwann cell sheath.

Meyrick and Reid (1971) stress the extreme difficulty in distinguishing between fine nerve fibres and the long cytoplasmic processes of alveolar pericytes which were frequently seen. In Hung's micrographs, however, not only were the nerves identified by their Schwann cell sheaths, but in some sections the axons lay in close proximity to pericyte processes and could be distinguished from them.

Two different types of nerve endings were described by Hung et al.

Endings of the first type, which were the most numerous, were associated with the Type 1 alveolar epithelial cell. They lay under the epithelial cell body or its processes and the enlarged nerve terminals were separated from the cell basal surface membrane only by a layer of basal lamina. The opposite surface of the nerve fibres was capped by a Schwann cell covering, the basal lamina of

which became continuous with that under the Type 1 cell. These nerve terminals had numerous mitochondria and occasional small dense-cored vesicles and were considered on morphological grounds to be sensory.

In the second type of ending the nerve formed a bell-shaped swelling with its wide base applied to the lateral surface of the Type 2 epithelial cell. The area of contact between the bare surface of the nerve terminal and the surface of the Type 2 cell was given as approximately 1.4 micrometres and the synaptic cleft as 100 to 200 Angstroms wide. Again a Schwann cell capped the ending and its basal lamina became continuous with the basal lamina under the Type 2 cell. The pre-terminal varicosities were crowded with numerous dense-cored vesicles (1200 Å) and a few mitochondria, and the fibres were considered to be motor in function.

Nerve fibres have been demonstrated closely associated with specialised bronchiolar cells (Hung, Hertweck, Hardy & Loosli, 1973b). These cells are characterised by having numerous dense-cored granules in the basal cytoplasm, a portion of the apical cytoplasm exposed to the bronchiolar lumen, and a single cilium on the surface adjacent to the lumen. The nerve-epithelial cell complex is thought to be a sensory receptor. It is tempting to suggest that they correspond to Larsell's 'carbon dioxide receptor' (see Page 6:3), but they appear to lie rather more proximally in the airways, at the junctions of the bronchioles.

Finally, Weibel (1973) in a recent review of lung structure and function produced a photomicrograph which shows the possible presence of a nerve ending in a basal invagination of a Type 3 alveolar epithelial cell.

Histochemical studies

To demonstrate that a tissue is actually innervated, one must show the presence of terminal fibres; the presence of non-terminal fibres has no functional significance (Fillenz & Woods, 1970). The classical histological appearances attributed to terminal fibres (e.g. 'beading' with methylene blue, 'boutons' and fibre thickenings with silvers and golds) have no anatomical counterpart in electron microscopical preparations and must be regarded to a certain extent as artifactual, or at least unreliable.

Where terminal fibres are demonstrated by electron microscopy, it is usually possible to suggest on morphological grounds whether a terminal fibre is motor or sensory, but it is often not possible to classify a motor fibre as definitely being either sympathetic or parasympathetic in origin.

In classical histology, as described above, this was done, with immense effort, by multiple serial sectioning in an attempt to trace the nerve back to its ganglion cell, on the assumption that parasympathetic ganglia lie in the substance of the organ innervated, while sympathetic ganglia are remote. Alternatively, working on the same assumption, denervation could be performed and the pattern of terminal fibre degeneration observed.

Modern histochemical methods can, however, distinguish between adrenergic (sympathetic) and cholinergic (probably parasympathetic) nerve endings. The presence of monoamines in sympathetic post-ganglionic fibres can be demonstrated by fluorescence microscopy (Falck & Owman, 1965), and although there is as yet no specific stain

for acetylcholine, the cholinergic nature of a nerve fibre can be inferred from the presence within it of acetylcholinesterase (Koelle, 1951).

a) Cholinergic innervation

In the techniques used to demonstrate acetylcholinesterase there are two unavoidable complications which tend to limit their accuracy. The first is the presence in almost all peripheral nerves of butyrylcholinesterase (non-specific, or pseudo-cholinesterase) which must be selectively inactivated, and the second is the degree of diffusion of acetylcholinesterase which inevitably occurs as the tissue sections pass through the various substrates and solutions used in processing. These techniques therefore are more suited to the demonstration of parasympathetic ganglion cells and pre-ganglionic fibres where there is presumably a higher concentration of acetylcholinesterase and a larger number of wholly cholinergic fibres. They are much less effective in demonstrating single terminal fibres.

Notwithstanding these limitations, it is generally agreed that by far the larger proportion of the cholinergic nerve fibres in the lung are associated with the bronchial tree. This was originally described in cat lung (Koelle, 1950) where fibres were found to be distributed both to the extrachondrial and subchondrial plexuses, to the smooth muscle of all orders of bronchioles, to the bronchial arteries, and to the bronchial mucous glands. These observations were confirmed by Honjin (1956) who described cholinergic innervation in the mouse lung extending to the level of the respiratory bronchiole, and has been repeatedly confirmed since in all other species examined

(Daly & Hebb, 1966; Hebb, 1969; El-Bermani, McNary & Bradley, 1970; El-Bermani, 1973).

There is no report in the literature of acetylcholinesterase activity in the alveolar walls, nor indeed distal to the respiratory bronchioles, although in view of the limitations described above this must not be taken to mean that such activity does not exist.

There is less agreement on the extent of the cholinergic innervation of the pulmonary vasculature and a large species variation has been described. Daly and Hebb (1966) found a sparse innervation of both the medium-sized pulmonary arteries and veins in the dog and kitten lung and possibly also in the pulmonary artery of the pig. Fisher (1965) also described extensive innervation to parts of the vascular bed, the particular parts varying with the species examined. Thus in the monkey the media of the pulmonary artery to the level of arterioles was richly innervated, while in the cat, guinea-pig and rat, the arterial vasculature was devoid of cholinergic activity, but the veins were well innervated, either from fibres running parallel to the smooth muscle, or from a well-defined plexus beneath the intima.

This variation was further emphasised by Hebb (1969) who tabulated the results for all orders of arteries and veins for six different species. Of particular interest is the fact that in only the sheep and the cat were cholinergic fibres found in pulmonary arterioles smaller than 70 micrometres in diameter, and none were found in pulmonary veins as small as this in any of the species.

b) <u>Adrenergic innervation</u>

Hebb (1969) examined the adrenergic innervation of the lungs

in six species (rat, rabbit, cat, sheep, pig and calf) and found a remarkable variation.

The bronchial arteries in all the species were well supplied with adrenergic fibres.

The bronchial muscle was well supplied in the cat, calf, sheep and pig, but not in the rabbit nor in the rat. This last finding (i.e. in the rat) was confirmed by El-Bermani et al. (1970), but is in disagreement with the work of Zussman (1966) who reported an extensive network of fine fluorescent fibres in the mucosa of the entire bronchial tree. In the cat bronchial muscle, adrenergic fibres have been described as building up a high-density plexus of single nerve terminals approaching both the inner and outer surface of the muscle cells and extending as far distal as the respiratory bronchioles (Dahlstrom, Fuxe, Hokfelt & Norberg, 1966).

On the vascular side, the extra-pulmonary arteries and intra-pulmonary veins, both deep within the lung substance and on the surface of the lung received an adrenergic nerve supply in all the species examined (Hebb, 1969). There was marked variation in the innervation of the intra-pulmonary arteries and smaller intra-pulmonary veins, and it is of interest that, of the more commonly used laboratory animals, only the rabbit and cat, but not the rat, have an adrenergic innervation to pulmonary arterioles smaller than 70 micrometres. These findings confirm an earlier report on the same animals (Cech & Dolezal, 1967). Pulmonary veins of this size were reported to be devoid of innervation.

A search of the literature has failed to discover a morphological description of the autonomic innervation of the dog

lung, but several physiological studies point to its existence.

Bronchial dilation or constriction was demonstrated in dogs following stimulation of either the thoracic sympathetic nerves (stellate ganglion) or the cervical vagus (Cabezas, Graf & Nadel, 1971). From the same laboratory, a study of the re-innervation of the auto-transplanted dog lung revealed that cholinergic re-innervation was functionally complete within three to six months after operation (Edmunds, Graf & Nadel, 1971). A similar experiment demonstrated the functional re-innervation by adrenergic fibres over the course of forty-five months, but in addition demonstrated directly by fluorescence microscopy that adrenergic fibres were present in the large and small bronchi (Lall, Graf, Nadel & Edmunds, 1973).

Adrenergic innervation of the alveolar wall

In none of the above work was there any report of adrenergic nerve fibres being present in the alveolar walls.

In a short abstract in 1972, Bean and Nakamoto claimed to have demonstrated the presence of fluorescent sympathetic nerve cell bodies in the lungs of rats, a finding at variance with the concept of sympathetic ganglia being far removed from the organ innervated. They also stated that there was 'adrenergic beaded fluorescence, related and unrelated to blood vessels .... (suggesting) .... functions other than vascular and bronchial control', but no micrographs were published and an exact location of the fibres was not given (but cf 6:36).

At the time at which the work to be described in this section was performed (late 1973), there had been no positive demonstration that adrenergic nerve fibres were present in the alveolar wall.

DEMONSTRATION OF INNERVATION

Portions of lung tissue were taken from either the rabbit or the rat and were processed by three different methods.

1) METHYLENE BLUE

The general pattern of innervation of the rat lung was determined by a supravital perfusion technique.

Materials and methods

A series of preliminary experiments was performed to determine the optimal concentrations and pH of the staining solutions and the best method of application. The technique finally evolved was that of continuous supravital perfusion simultaneously with positive pressure oxygen ventilation.

Adult rats were anaesthetised with Nembutal (approximately 35 mg/Kg intraperitoneally). A tracheal cannula was inserted, the abdomen was opened, and the abdominal vena cava cannulated. The renal arteries were cut and the vasculature perfused (from a simple Wolff's bottle perfusion apparatus set at a pressure of 10 cms H_2O) with a solution of 0.01% sodium nitrite plus Heparin (25,000 i.u./ litre) in normal saline at 37°C until the effluent returned clear.

Methylene blue (Gurr C-1-52015 Batch No. 23553) 0.01% in normal saline, phosphate buffered to a pH of 6.5 was then perfused at body temperature continuously for 30 minutes while the lungs were ventilated with 100% oxygen via a Palmer ventilation pump.

On completion of the perfusion the lungs were deflated to

-5cms H_2O and the stain was fixed by re-expanding with cold (4°C) 8% ammonium molybdate. They were then excised and stored overnight immersed in molybdate at 4°C.

Tracheal irrigation with many changes of saline was performed to get rid of the molybdate and the lungs were then re-expanded with and left immersed in 10% formal saline for 48 hours. Blocks were taken from all parts of the lobes and were dehydrated and embedded conventionally in Paraplast in a Histokinette tissue processor.

Pairs of serial sections were cut at 6 micrometres; one of each pair was left unstained other than by the methylene blue, while the other was stained with Resorcein fuchsin to demonstrate elastic fibres.

Results

In the hilar sections, large nerve trunks containing both coarse and fine nerve fibres ran with the bronchi. Deeper in the lung, along the course of these fibres, clusters of ganglion cells could be seen in the peribronchial tissues. Fine nerve fibres appeared to run from the ganglia towards the bronchial muscle bands.

Fine nerve fibres were positively demonstrated in the wall of the pulmonary arteries, but not in the pulmonary veins. Ganglion cells were not found associated with these fibres.

Alveolar wall

With the technique described, there was very little background staining of the alveolar wall. Stain was taken up by the

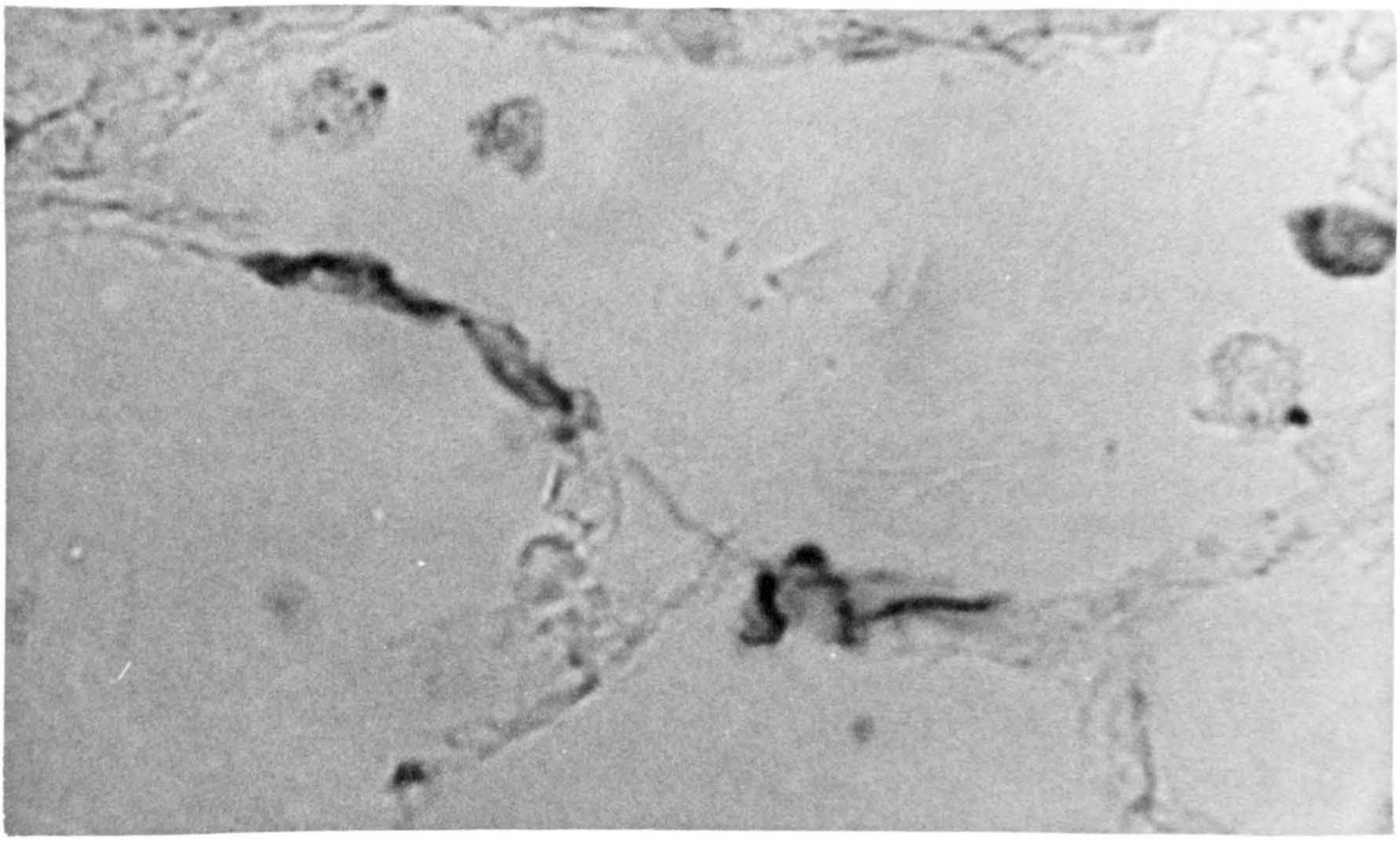


FIGURE 6:1. COARSE ALVEOLAR FIBRE: METHYLENE BLUE.

(x 1,000)

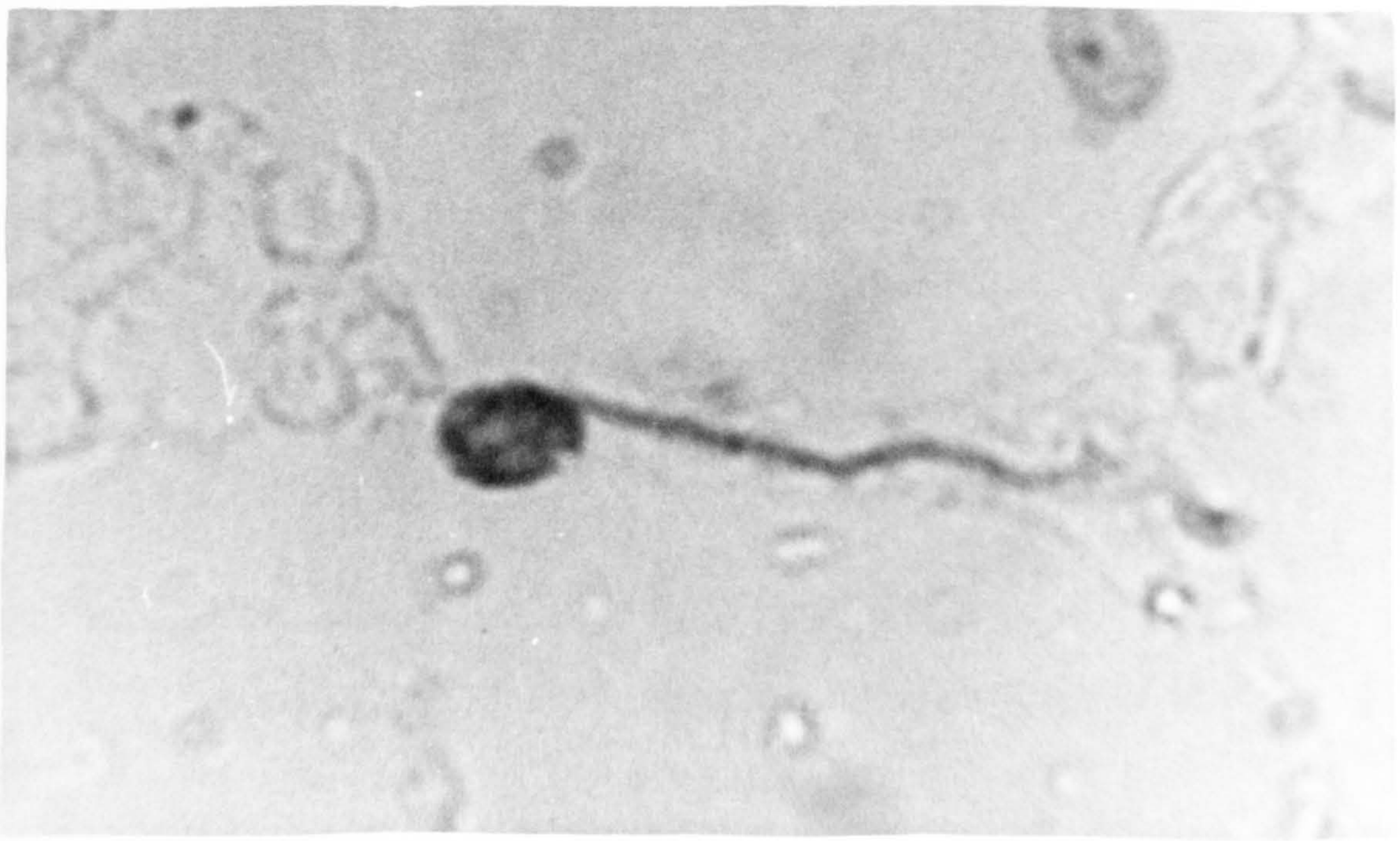


FIGURE 6:2. FINE ALVEOLAR FIBRE: METHYLENE BLUE.

(x 1,400)

epithelial and endothelial cell nuclei, and by two distinctly different types of fibre.

One type, by far the more common, was very coarse and appeared to be made up of several strands frayed at the ends (Figure 6:1). Their appearance was very similar to that of elastic fibres and on the Resorcein-fuchsin stained serial sections were seen to lie (despite difficulties of accurate registration due to the 6 micrometre difference in section level) in much the same areas.

The second type was much finer and definitely single (Figure 6:2). Many of these fibres were very similar to Larsell's drawings (Larsell & Dow, 1933) of fibres ending on alveolar capillaries, but there was no evidence of the 'beading' commonly described in methylene blue stained terminal fibres.

Serial sections stained for reticulin were not performed, mainly because of the impossibility of accurate registration of such very fine fibres on relatively thick sections, and it is therefore not possible to state categorically that the structures taking up stain are in fact nerve fibres. Their general size, appearance and distribution, however, bear a striking resemblance to the alveolar wall fibres unequivocably demonstrated by fluorescent microscopy (see Page 6:29).

2) ACETYLCHOLINESTERASE

An attempt was made to demonstrate the extent of the cholinergic innervation of the rat lung.

Materials and methods

20 micrometre sections were cut either from blocks of fresh lung tissue in the cryostat, or from blocks fixed for 24 hours in cold (4°C) Baker's formal calcium and sectioned on a Leitz freezing microtome. The latter procedure was the more satisfactory as there was less distortion of the tissues than with fresh frozen cryostat sections, and also because the lung could be inflated with formal calcium and sectioned in the expanded state.

Better consistency for frozen sectioning was obtained by prior immersion of the block for several hours in gum-sucrose solution at 4°C.

The sections were stained for cholinesterases by Gomori's (1952) modification of Koelle's (1951) method.

Results

In general the results were disappointing because of severe diffusion of the enzymes through the tissues and because of the masking effects of esterases in the red cells.

There was definite cholinesterase activity at all levels of the bronchial tree down to the respiratory bronchioles. Staining was also present in the large intrapulmonary veins, but not in the arteries.

There was no definite evidence of cholinesterase activity in the alveolar walls, but the appearances were difficult to interpret because of staining of red cells in the pulmonary capillaries and because of poor localisation of the enzyme.

The conclusion must not be that such activity definitely

does not exist, but that the technique may not be sufficiently sensitive to demonstrate it (see Page 6:9).

3) NORADRENALINE

Portions of rat, rabbit and cat lung were processed to demonstrate catecholamines by fluorescent microscopy (Falck & Owman, 1965).

Rationale

Direct histochemical methods for the demonstration of the biogenic monoamines are largely empirical, insensitive, and of low specificity.

The demonstration in 1961 (Carlsson, Falck, Hillarp, Thieme & Torp, 1961) that certain monoamines could be converted into highly fluorescent tri-hydroxyindoles led to the development of a highly specific histochemical technique (Falck, 1962).

Exposure to formaldehyde gas causes catecholamines to condense to form fluorescent hydroisoquinolines. The reaction takes place in two stages, the first readily under almost any conditions, but the second (which yields the actual fluorophore) only in the presence of a dry protein layer which acts as a catalyst.

By careful attention to details of technique, intracellular localisation can be close to perfect. Tissues are taken live and quenched immediately in liquid nitrogen to prevent enzyme diffusion. If processing and examination thereafter takes place under conditions of complete dryness, diffusion remains insignificant. A crucial factor is the relative humidity of the formaldehyde vapour (Corrodi &

Jonsson, 1967); some moisture must be present for the reaction to proceed at all, but a fine balance must be obtained between intensity (i.e. conditions of high humidity) and localisation (i.e. conditions of low humidity) of the fluorescent products.

Some of the different monoamines may be distinguished by their fluorescent emission spectra; for example, to the naked eye on fluorescence microscopy, the catecholamines (adrenaline and noradrenaline) and dihydroxyphenylalanine fluorophores show a green to greenish-yellow fluorescence while those of 5-hydroxytryptamine are unmistakably yellow.

The primary and secondary catecholamines may be separated by differential processing; a longer condensation reaction time is required by secondary catecholamines (adrenaline), and, once formed, the fluorophore of adrenaline can be extracted completely by mounting in a medium containing organic solvents.

Materials and methods

Tissues were taken from live anaesthetised cats, rats and rabbits. Small (less than 1 centimetre cubed) portions of lung lobes were isolated in the inflated state with artery forceps, excised and quenched immediately in iso-pentane cooled to the temperature of liquid nitrogen (-196°C). Portions of frozen tissue were removed with cooled bone forceps under the surface of liquid nitrogen and transferred to the tissue tray of a Speedivac-Pearse freeze-drier which had previously been running at a temperature of -60°C for at least one hour. They were freeze-dried over phosphorus pentoxide at -60°C and a vacuum greater than 0.001 torr for 18 hours. The phosphorus pentoxide was replaced after one hour, and again about ten

hours later.

Paraformaldehyde stock was kept in a dessicator over sulphuric acid with a specific gravity of 1.250 (i.e. in an atmosphere of relative humidity 70%) (Hamberger, 1967). About one hour before use, 5 grams were put in a sealed beaker in an oven at 80°C.

Following the 18 hours of freeze-drying, the temperature of the drier head was raised to +30°C to prevent condensation on breaking the vacuum, the tissues were removed as rapidly as possible and suspended in gauze bags in the sealed beaker of formaldehyde vapour. They remained there for exactly one hour at a temperature of exactly 80°C.

During this period, the tissue tray in the freeze-drier was filled with degassed wax which was further degassed under high vacuum and was then allowed to cool and solidify. Following the combined fixation and condensation reactions in the formaldehyde vapour, the tissues were returned under as dry conditions as possible to the tissue tray, and a vacuum was drawn, at room temperature, for one hour to ensure complete dryness. The temperature of the drier head was then raised to 60°C to melt the wax and allow the tissues to sink and embed. They were then transferred to conventional copper embedding pans in a vacuum oven, and blocked out in the normal manner.

Control tissues, freeze-dried as described above and incubated in a separate oven under identical conditions, but in the absence of paraformaldehyde, were processed routinely.

Sections were cut at 10 micrometres under strictly dry conditions (mere breathing on the sections was found to be sufficient

to destroy the fluorescence), mounted by heat on scrupulously clean glass slides and cleared with several changes of liquid paraffin. They were examined immediately on a Zeiss 'Ultraphot' fluorescence microscope with a BG 12/4 mm exciter filter over the ultra-violet light source, and either a 470 or 500 nanometre barrier filter in the microscope tube.

With experience it was usually possible to distinguish visually between the specific fluorescence of monoamines and the duller greyish-green autofluorescence of, say, elastic fibres, but to confirm the differentiation sections were routinely exposed following examination to either water vapour or sodium borohydride (Corrodi, Hillarp & Jonsson, 1964), both of which quenched specific fluorescence while leaving autofluorescence unchanged.

Photomicrographs were taken on Ilford HP4 film (exposure times around 2 to 4 minutes) with a Zeiss camera attachment, processed in Microphen, and printed on extra-hard paper.

There were two problems encountered in the production of photomicrographs suitable for publication. The first was due to the enormous difference in contrast between the highly fluorescent nerve fibres and the almost completely opaque (by ultraviolet light) alveolar wall. Any attempt to expose for the alveolar wall caused complete 'burning out' and loss of definition of the nerve fibre and in the micrographs presented in this thesis exposure has been made for the fibre, leaving the alveolar wall barely visible.

The second problem was that of depth of field of focus. The sections were fairly thick (unavoidable because of the brittle nature of freeze-dried lung tissue) and the nerve fibres were

tortuous. On observation these fibres could be followed over considerable distances by 'focussing down' through the tissues, but micrographs could be taken on only one plane of focus at a time. Attempts at 'image enhancement' by making multiple exposures on different planes allowed a much greater length of fibre to be demonstrated, but caused almost complete loss of definition due to flare.

This multiple exposure technique was not used in any of the micrographs presented here and consequently they must be taken to represent only a portion of the innervation which could be observed on microscopy by direct vision.

An additional problem unconnected with the photography was that of identification of individual cells and structures in the sections, as the alveolar wall was, of course, unstained. For example, all of the sections contained large numbers of highly fluorescent cells whose presence was completely unexpected, and in addition, with the positive demonstration of nerve fibres in the alveolar walls, it became highly desirable to show on what structure such fibres terminated.

This was attempted on many of the micrographs reproduced here by counterstaining the sections conventionally following fluorescence microscopy. The sections were prepared as described above, were examined and photographed on the ultraviolet microscope, were then washed clear of liquid paraffin, rehydrated and stained with haematoxylin and eosin. They were then re-photographed and the two resulting micrographs were superimposed.

This technique met with only partial success. For example, it was sufficiently accurate in its localisation to demonstrate that

the fluorescent cells in the lungs were probably mast cells (see below). It was of little use in determining the exact termination of the nerve fibres as the rehydration and staining processes caused expansion and distortion of the section, and a sufficiently accurate registration of the micrographs was not possible.

Very belatedly (long after the work described in this section was completed) came the realisation that it is possible to examine a section simultaneously by fluorescence and phase-contrast microscopy. Knowledge of this technique would have solved all the problems of registration, and a very recent repeat of some of the work described has demonstrated its usefulness (see Page 6:28).

Results

1) Background fluorescence

In the lungs of all three species examined, the alveolar walls showed a faint greenish autofluorescence (Figure 6:3) which persisted following exposure to water vapour or sodium borohydride (Figure 6:4).

Three types of cell contributed to the background fluorescence. The most prominent were numerous spherical cells showing an intense greenish-yellow specific fluorescence indistinguishable from that of noradrenaline in the nerve endings (Figure 6:3). These were identified by the counterstaining technique described as probably being mast cells containing dopamine. These are known to be present in abundance in the lungs of ruminants (Falck, Nystedt, Rosengren & Stenflo, 1964; Hebb, 1969), but were not anticipated in such numbers in the species examined.

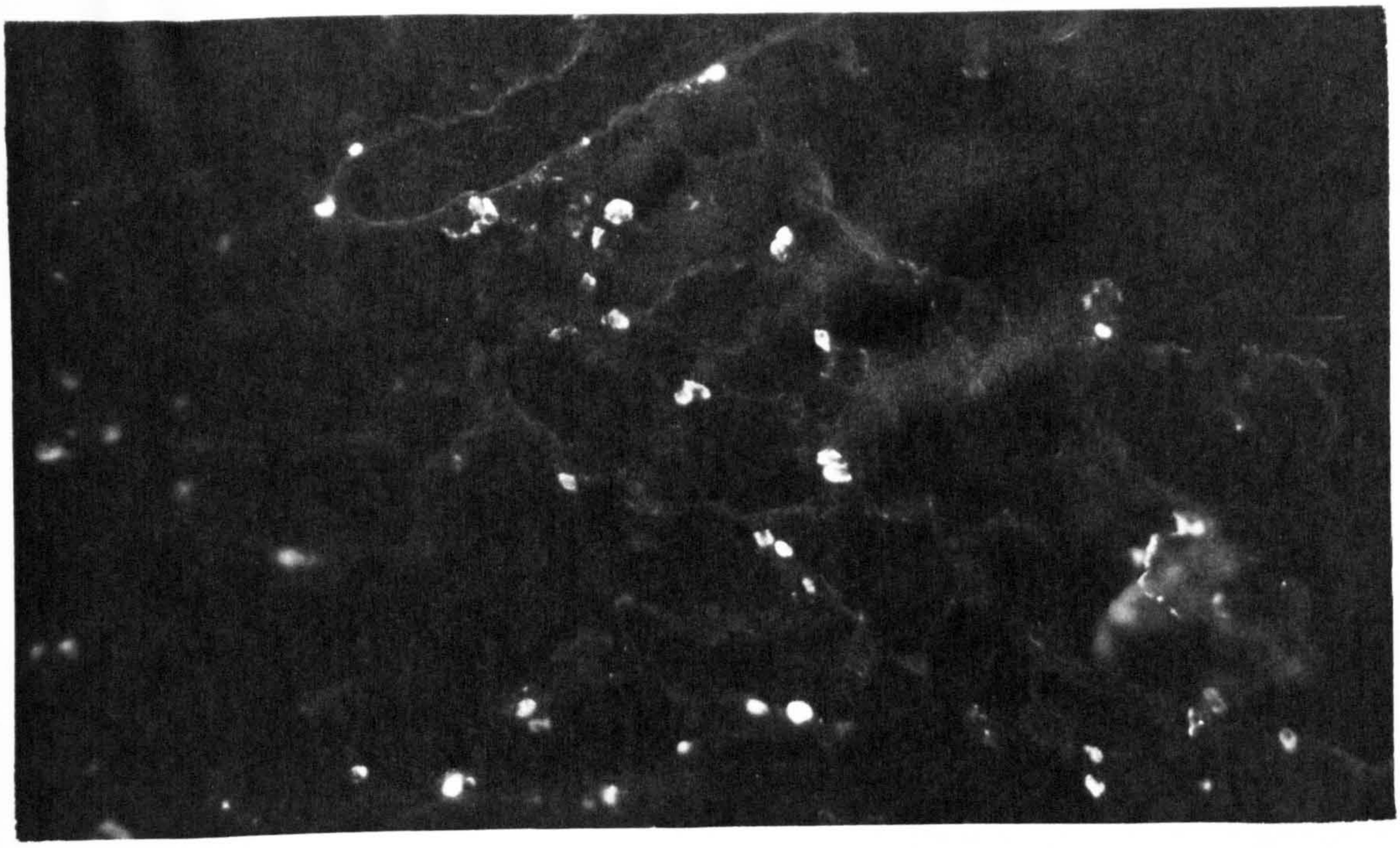


FIGURE 6:3. BACKGROUND FLUORESCENCE. (x 150)

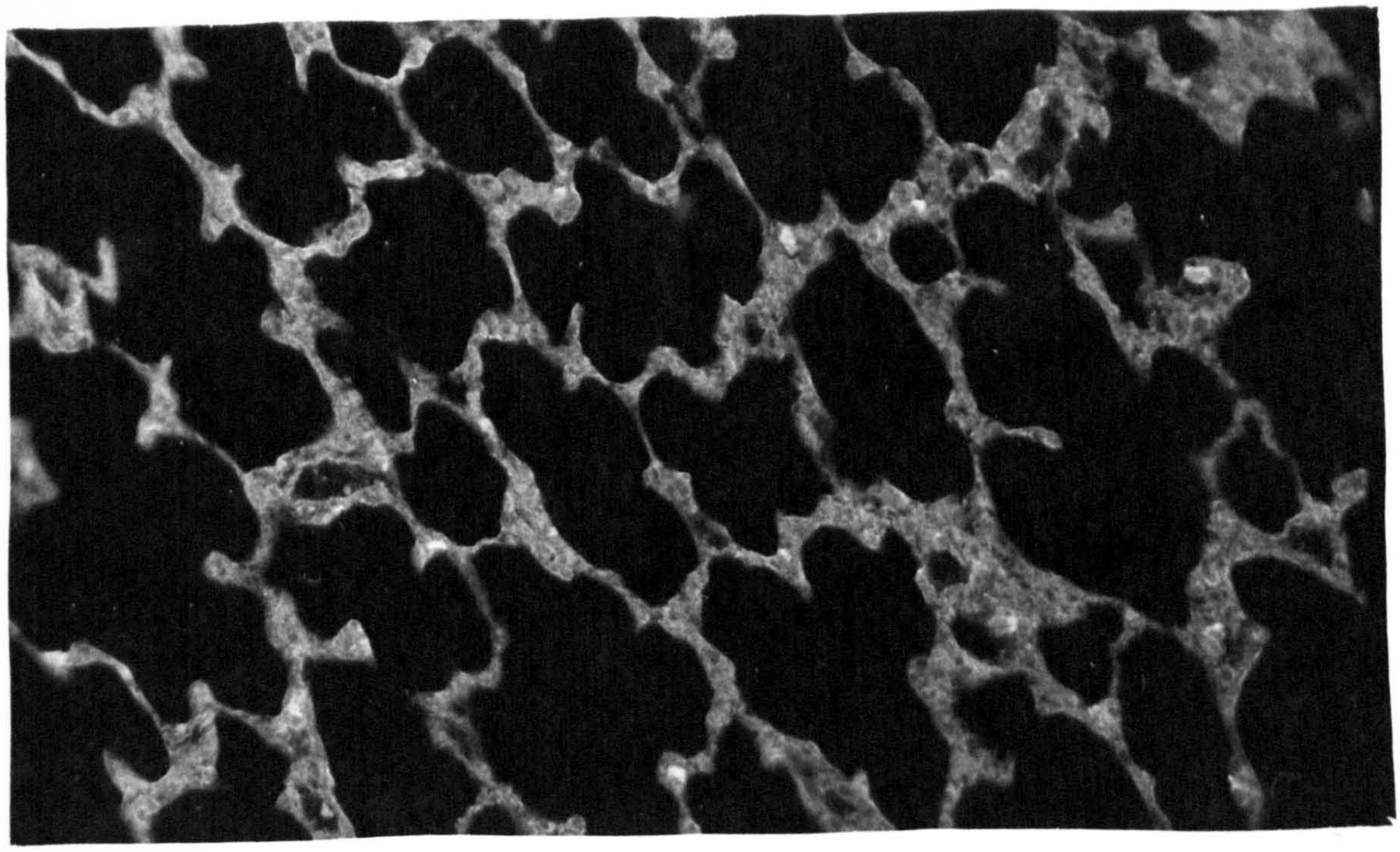


FIGURE 6:4. BACKGROUND AUTOFLUORESCENCE (CONTROL).

(x 200)

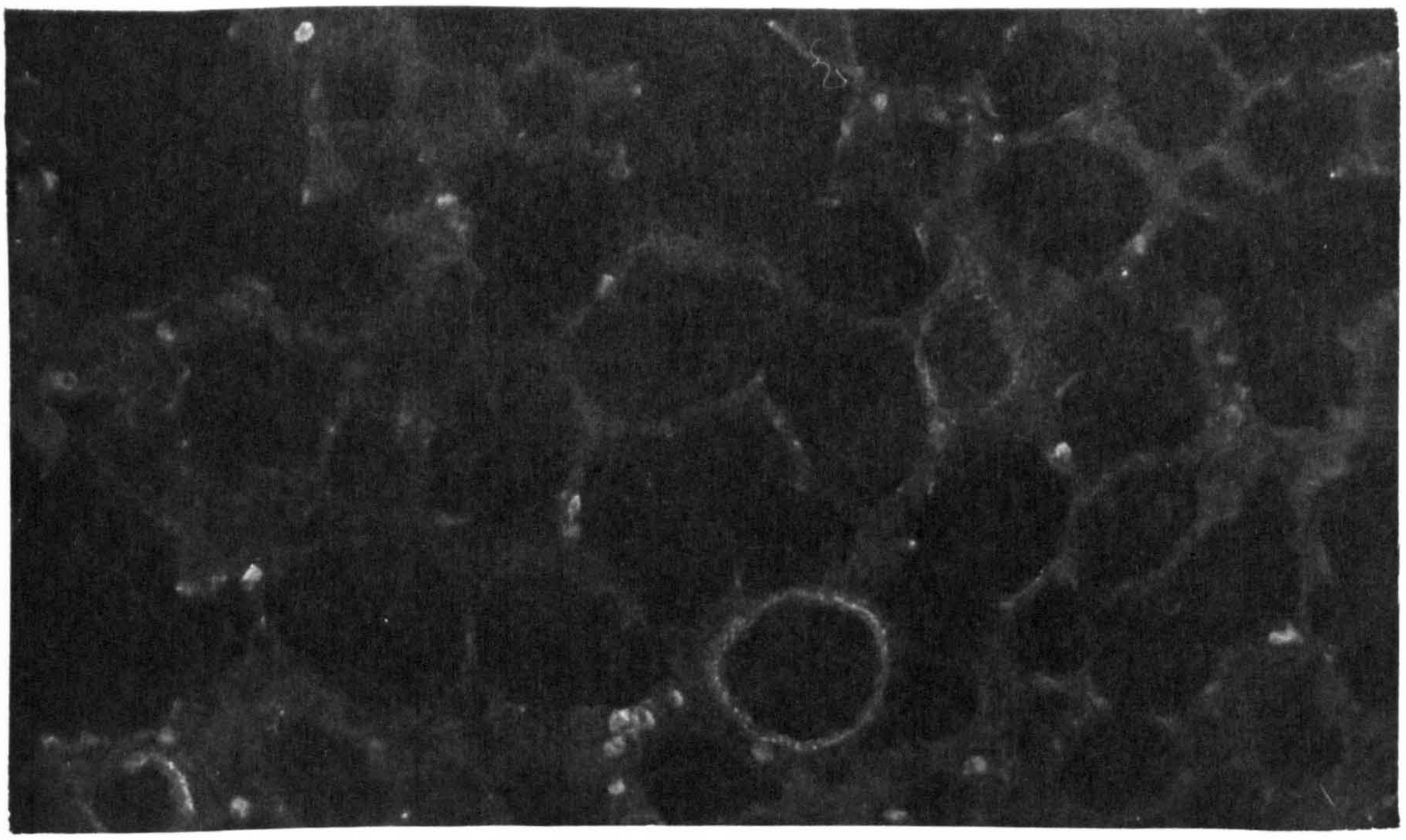


FIGURE 6:5. AUTOFLUORESCENCE: TYPE 2 CELLS. (x 200)

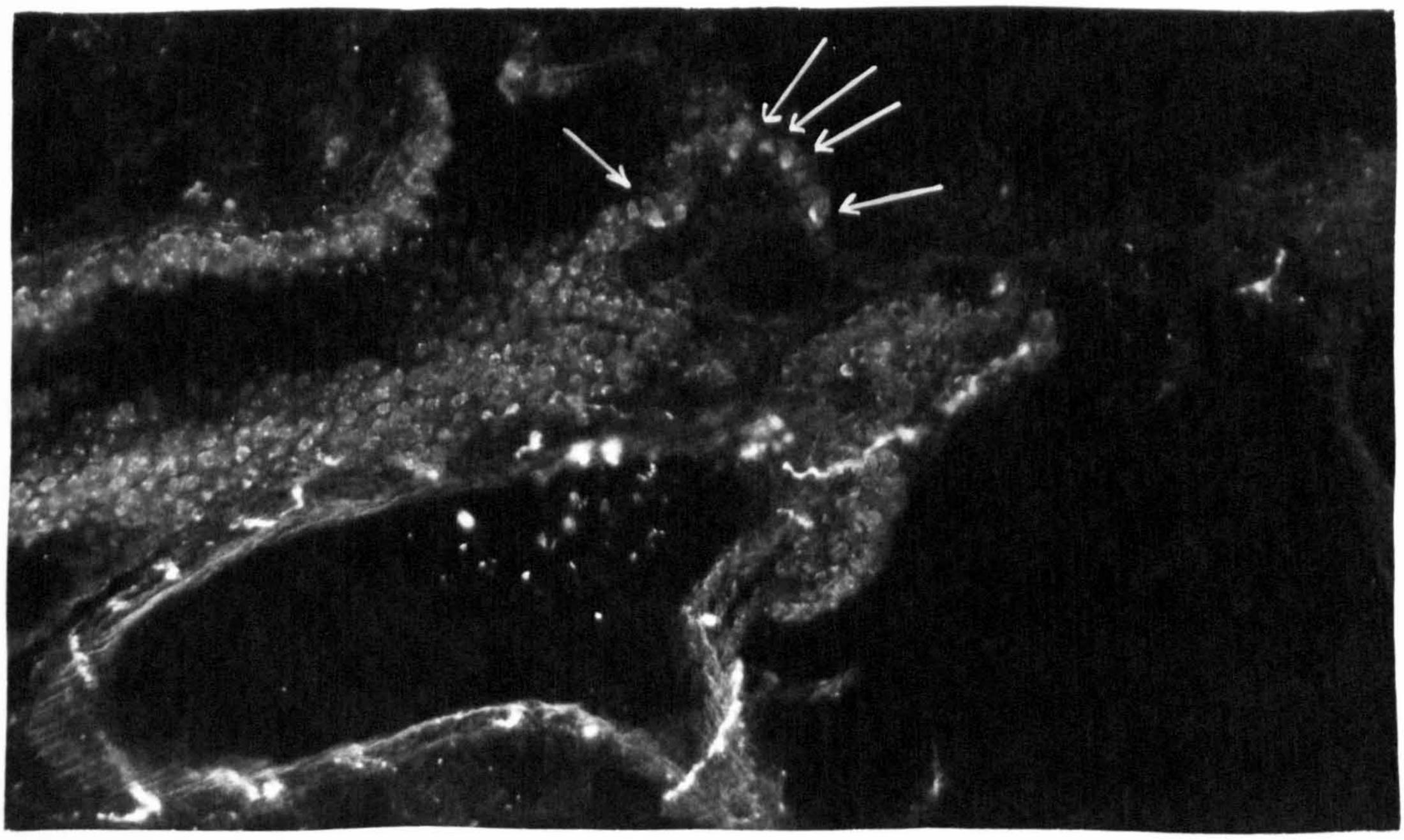


FIGURE 6:6. SPECIFIC ORANGE FLUORESCENCE (arrowed)

(x 150)

The second type of cell did not display specific fluorescence, but autofluoresced with a pale yellowish glow. They are demonstrated in Figure 6:5 which is of a section similar to that shown in Figure 6:3, but which has been treated with sodium borohydride to quench specific fluorescence and exposed photographically to show the autofluorescent cells to advantage. From their general size and position mainly at the corners of the alveoli it was thought that they might be Type 2 alveolar epithelial cells, and this was confirmed on subsequent counterstaining.

The third type of cell was extremely rare, and was seen only in the rat lung. The cells were spherical and contained coarse bright orange fluorescent granules which disappeared on exposure to water vapour and borohydride. They lay in the peribronchial connective tissue, but have not been positively identified (Figure 6:6). Such specific orange fluorescence has been described previously, in the rat acoustic nerve (Ross, 1969), but its significance is not known.

Bronchial tree

In the cat, the bronchial muscle was found to be densely innervated down to the level of the respiratory bronchioles, confirming the findings of Hebb (1969) and Dahlstrom et al. (1966). In the other two species, bronchial innervation was found to be sparse, but not absent as stated by Hebb (1969).

Figures 6:7 and 6:8 show sections from a rat lung containing both a bronchiole and a branch of the pulmonary artery. The artery shows many fine fluorescent dots representing nerve fibres

in transverse section both internal and external to the media. In contrast, apart from the fibre in longitudinal section arrowed in Figure 6:8, there is little evidence of adrenergic innervation, the other structures visible being autofluorescent.

Bronchial arteries

The bronchial arteries in all three species received an abundant adrenergic nerve supply. This formed a coarse plexus in the adventitia, but occasional fine fibres were seen to penetrate to the media.

Figure 6:9, which is almost a true transverse section of a large bronchial artery of a rabbit shows the extent of the plexus. The difference in appearance between the nerve fibres and the autofluorescent elastic fibres of the internal lamina is obvious.

Figure 6:10, a tangential section of a similar artery, shows a beaded branching terminal fibre supplying the vessel wall.

Pulmonary arteries

Innervation of the pulmonary arteries was found in all three species, although much more profusely in the cat and rabbit than in the rat.

Figures 6:11 and 6:12 show (at different magnifications) the pattern of innervation at both poles of a medium-sized pulmonary artery from the rabbit, cut in tangential section. The extent of the innervation is obvious and the fibres can be clearly distinguished from other fluorescent structures. Fibres were not seen actually to penetrate the media.

Figure 6:13 is technically a poor micrograph, but was the closest obtained to a true transverse section. It shows circular and longitudinal nerve fibres confined to the adventitia, with no evidence whatsoever of fibres penetrating the media. The internal elastic lamina appears particularly bright in this micrograph, but was readily distinguished by its colour under the microscope. Also shown is a surprisingly large vasa vasorum which also appears to receive a sympathetic innervation.

Figure 6:14 shows in longitudinal section the point of branching of a pulmonary arteriole and precapillary, again in the rabbit. Nerve fibres form a plexus in the adventitia encircling the vessels, and appear to be more concentrated around the junction; this may represent a sphincter mechanism controlling regional blood flow.

Figure 6:15 shows two branches of a rat pulmonary arteriole just distal to their point of bifurcation; the accompanying conducting bronchiole is also seen. The nerve fibres supplying the vessels have presumably just branched in the section immediately above that shown and are seen entering the section in the centre between the two vessel branches and running towards their specific vessel. The bronchiole is devoid of innervation.

In Figure 6:16, the same nerve fibre is shown under much higher power to demonstrate the 'beading'.

Pulmonary pre-capillary vessels

Figure 6:17, which is a serial section to Figure 6:14, shows adrenergic innervation extending along a small pulmonary arterial

vessel about 25 micrometres in diameter (rabbit lung). In Figure 6:18 innervation is shown further distal down the vascular bed in cat lung, at the level of the pre-capillaries, about 10 to 15 micrometres in diameter. The gaps arrowed appeared to be the sites of origin of a capillary leash, but no nerve fibres were seen leaving the pre-capillary arteriole to pass to the parenchyma.

Pulmonary veins

The pulmonary veins of all three species were innervated at all levels, but the fibres were much finer than those supplying the arteries, and in the rat and the rabbit were much more sparsely distributed.

Figure 6:19 shows a vein from a rat lung, apparently devoid of innervation other than for a few small portions of nerve fibre in the adventitia. A sequential serial section from only 20 micrometres away, however, is shown under much higher power in Figure 6:20 (site of field is outlined on Figure 6:19) and shows a length of beaded nerve fibre.

A similar but much longer fibre, again in a rat lung, is shown in the wall of a vein in Figure 6:21 and under high power in Figure 6:22.

In the cat, the pulmonary veins seemed to be much more extensively innervated, a finding in agreement with that of Hebb (1969). Figure 6:23 shows a large number of fine nerve fibres in transverse section in the longitudinal section of a very small (20 to 40 micrometres) pulmonary venule.

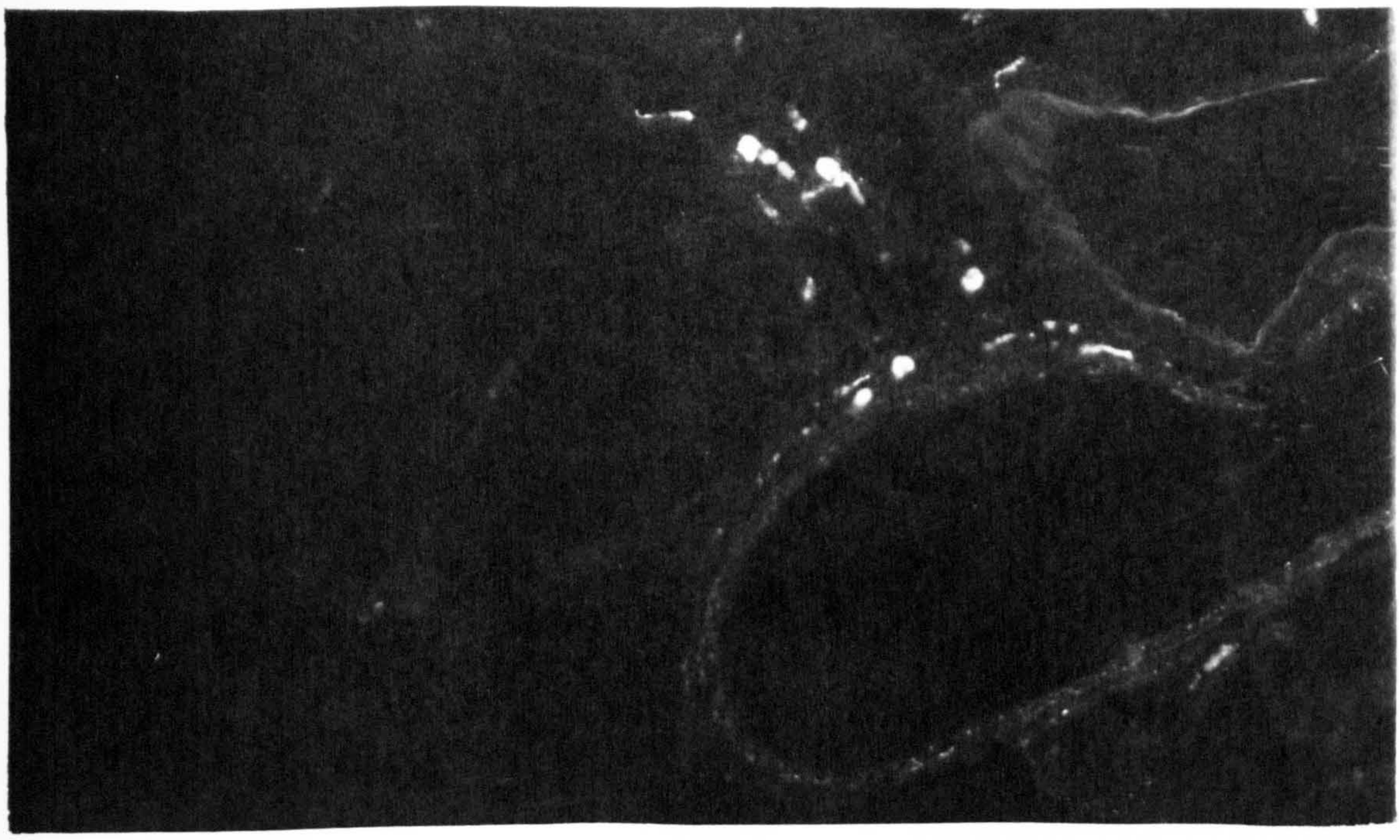


FIGURE 6:7. BRONCHIOLE AND PULMONARY ARTERIOLE.

(x 100)

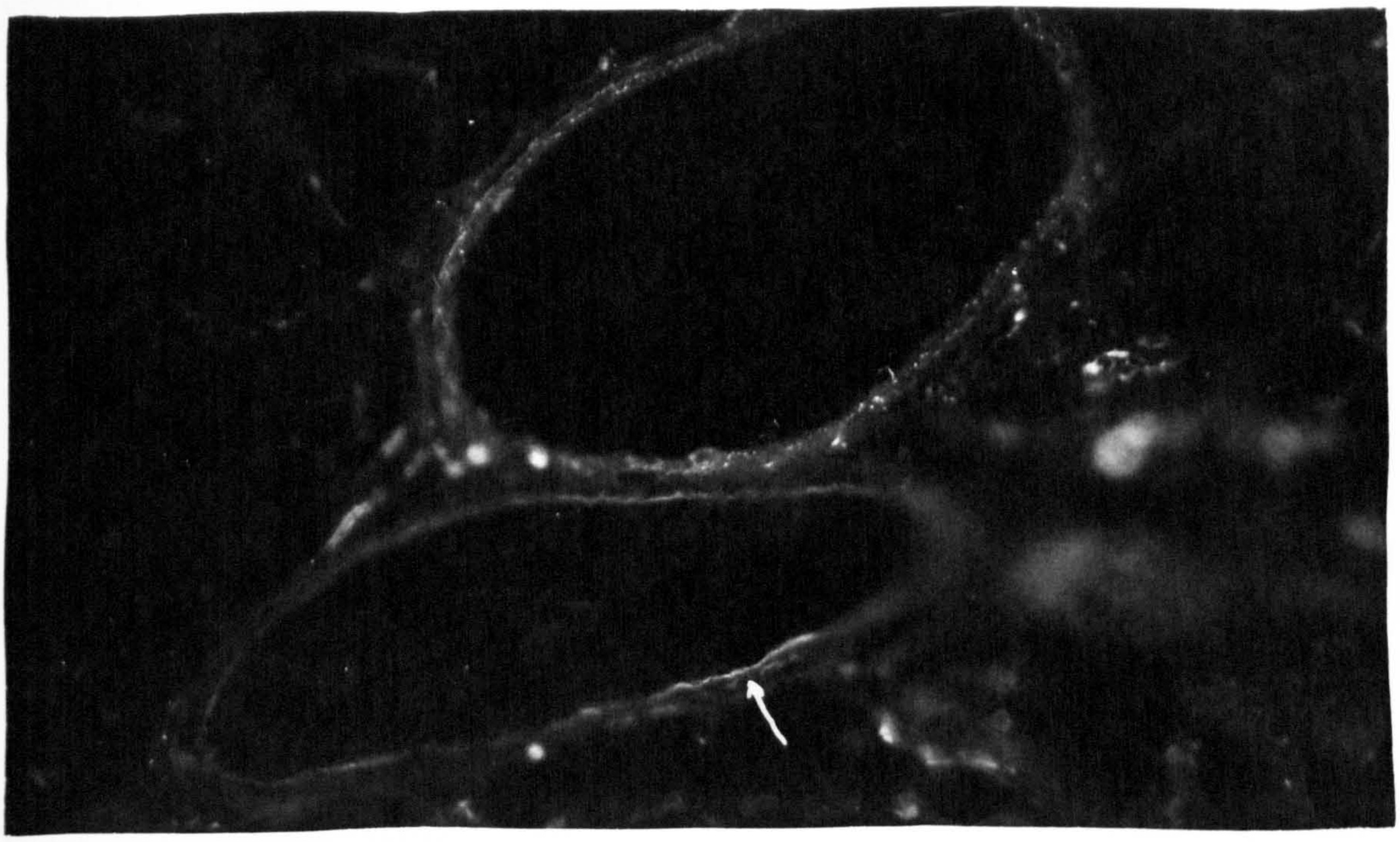


FIGURE 6:8. BRONCHIOLE AND PULMONARY ARTERIOLE.

(x 100)

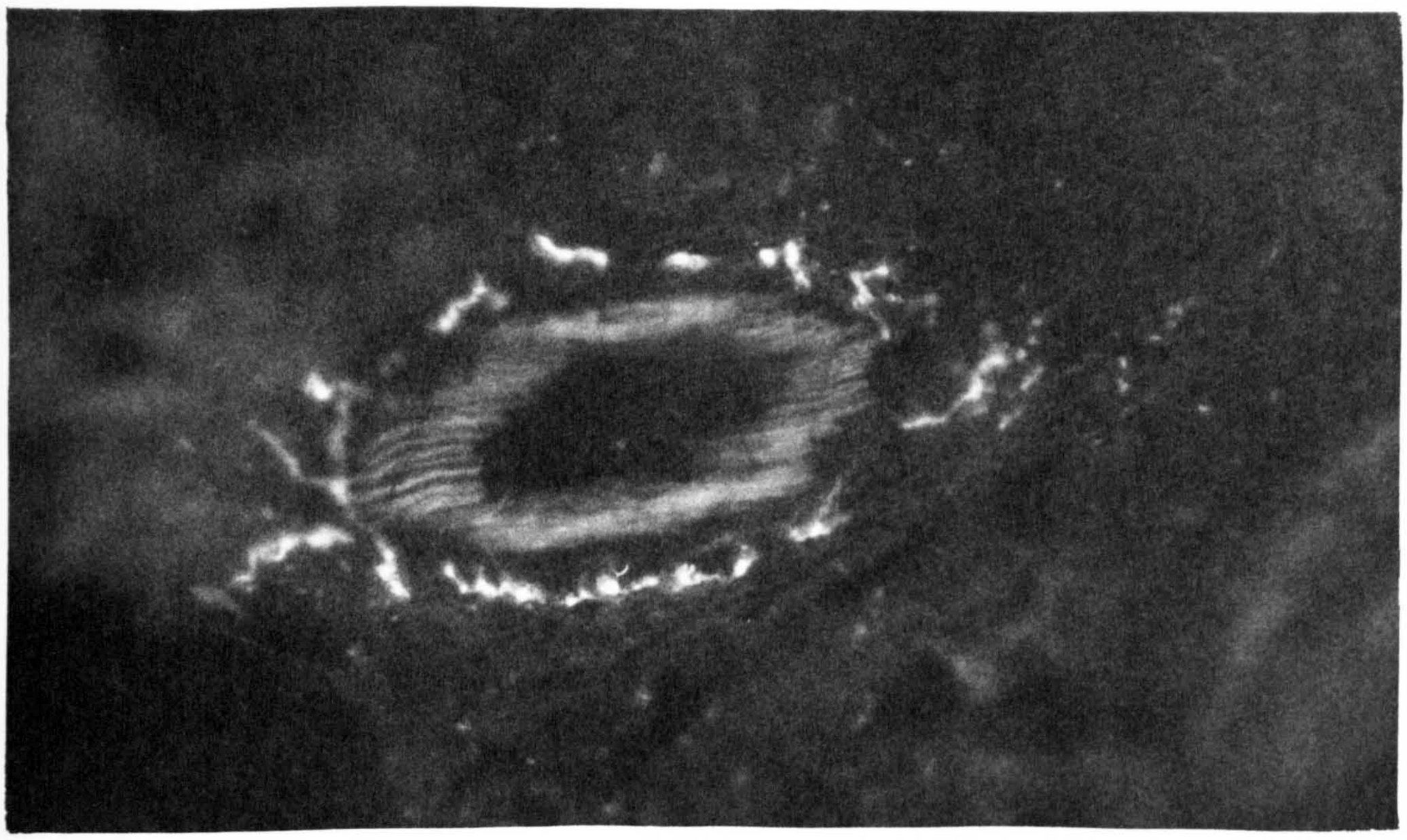


FIGURE 6:9. BRONCHIAL ARTERY: TRANSVERSE SECTION.

(x 300)

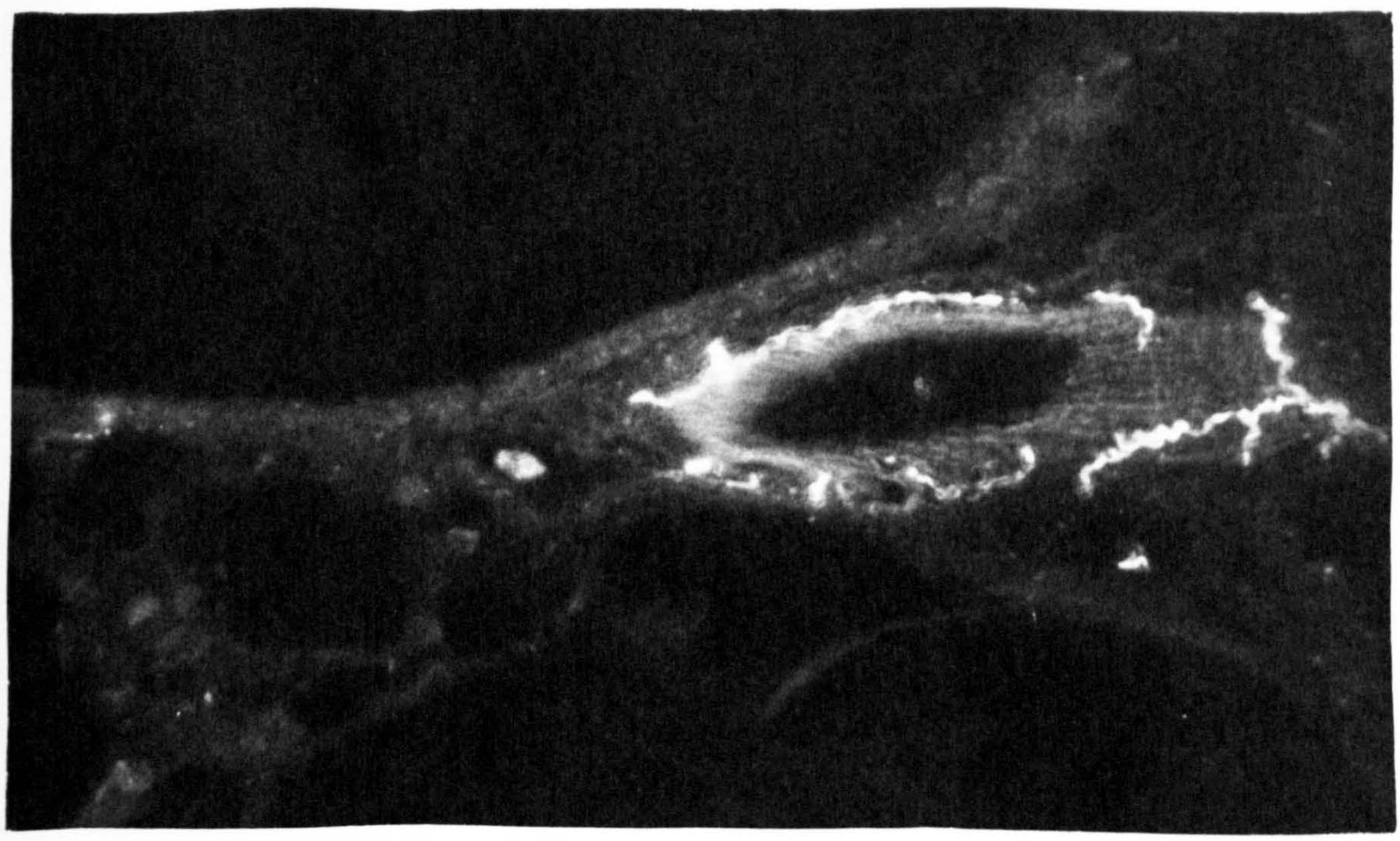


FIGURE 6:10. BRONCHIAL ARTERY: TANGENTIAL SECTION.

(x 300)

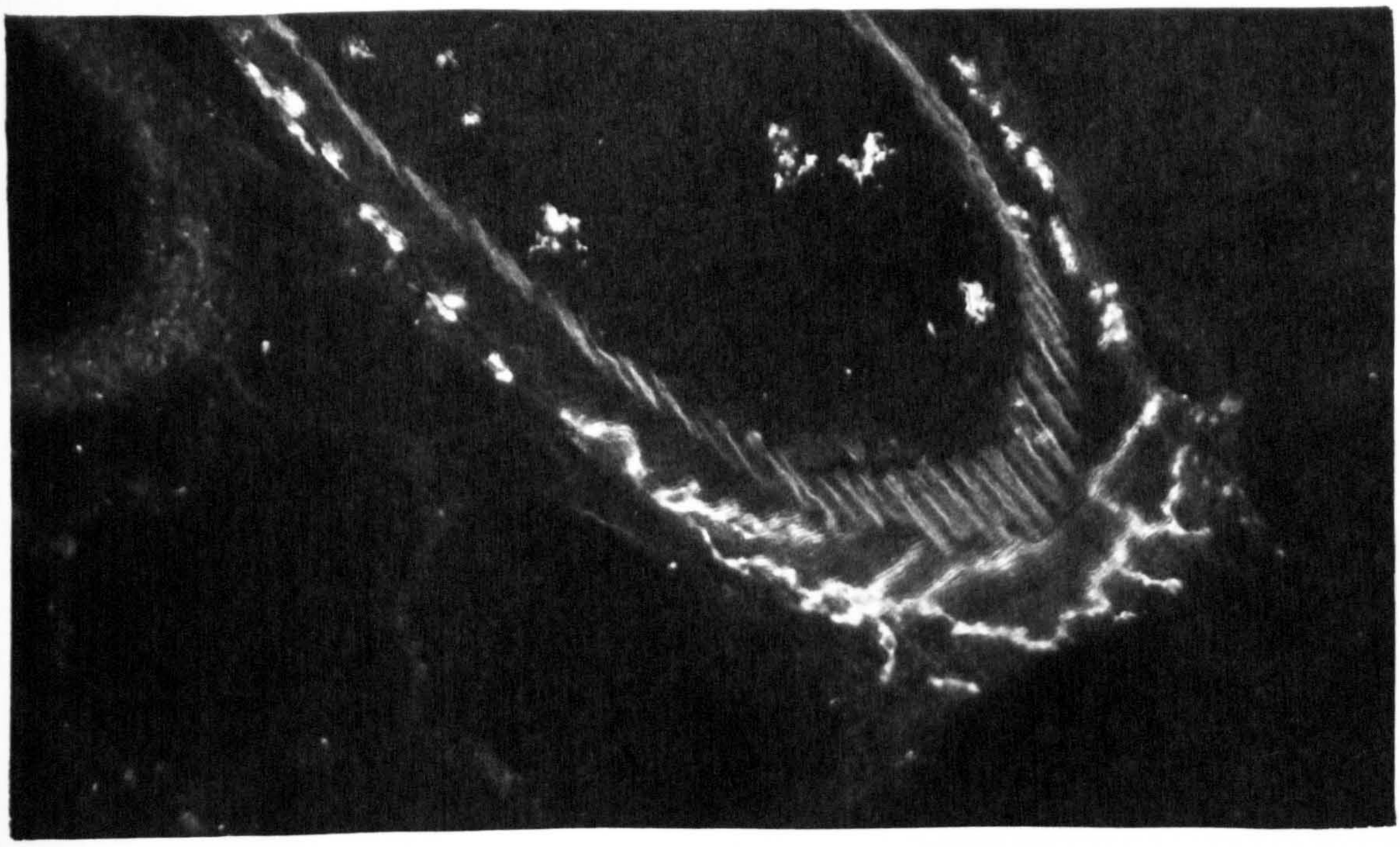


FIGURE 6:11. PULMONARY ARTERY: TANGENTIAL SECTION.

(x 100)

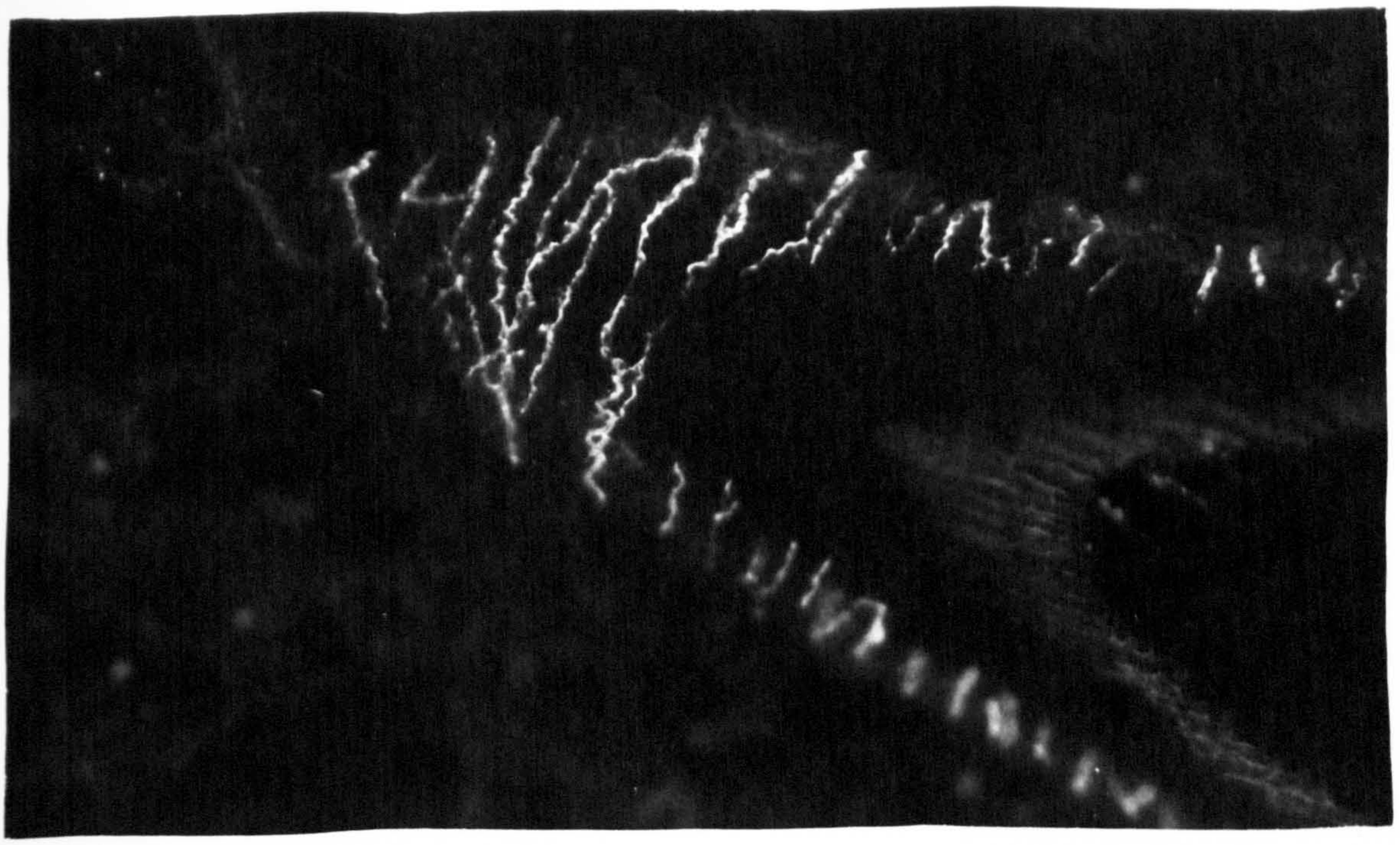


FIGURE 6:12. AS ABOVE: OPPOSITE POLE.

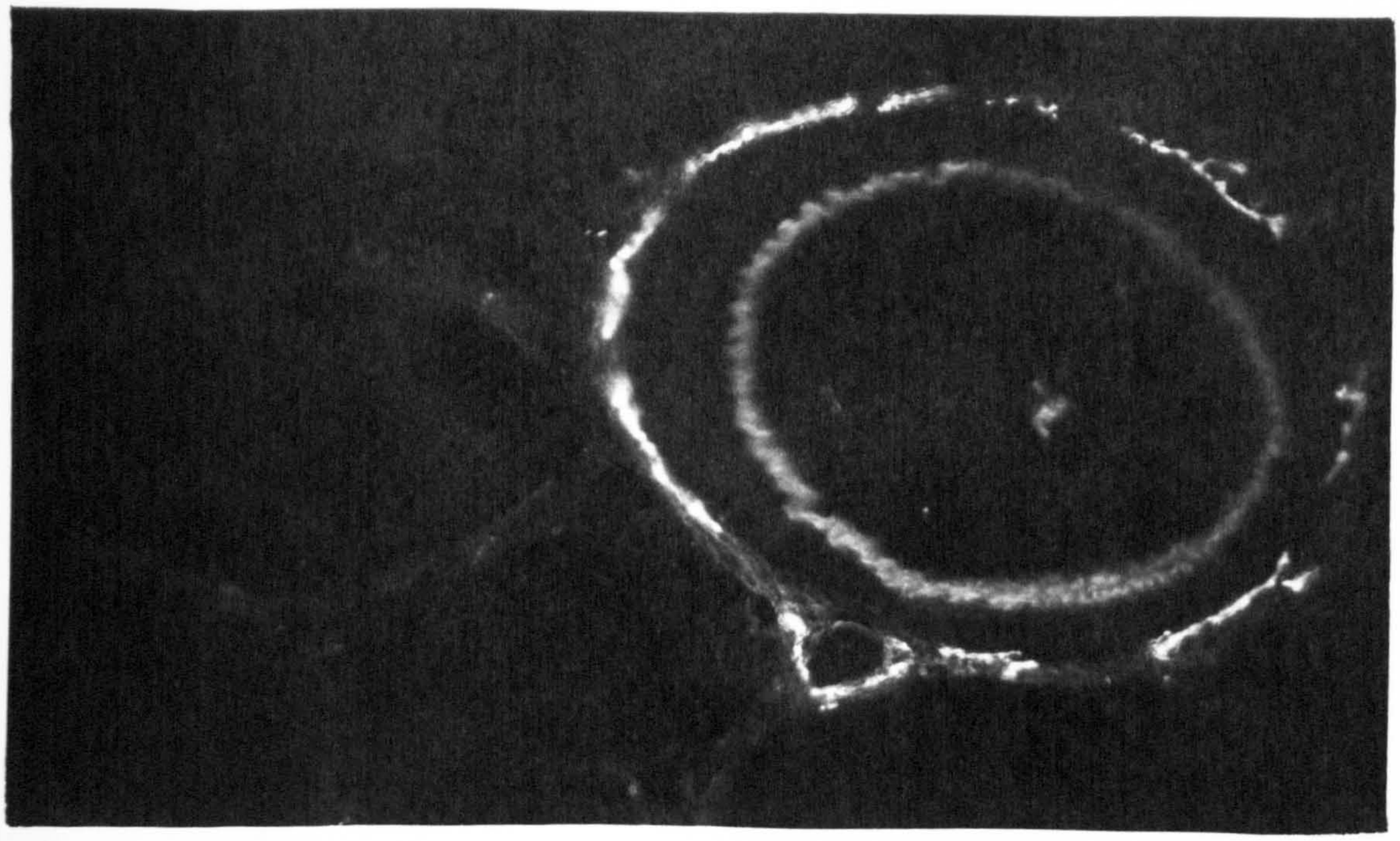


FIGURE 6:13. PULMONARY ARTERY: TRANSVERSE SECTION.

(x 100)

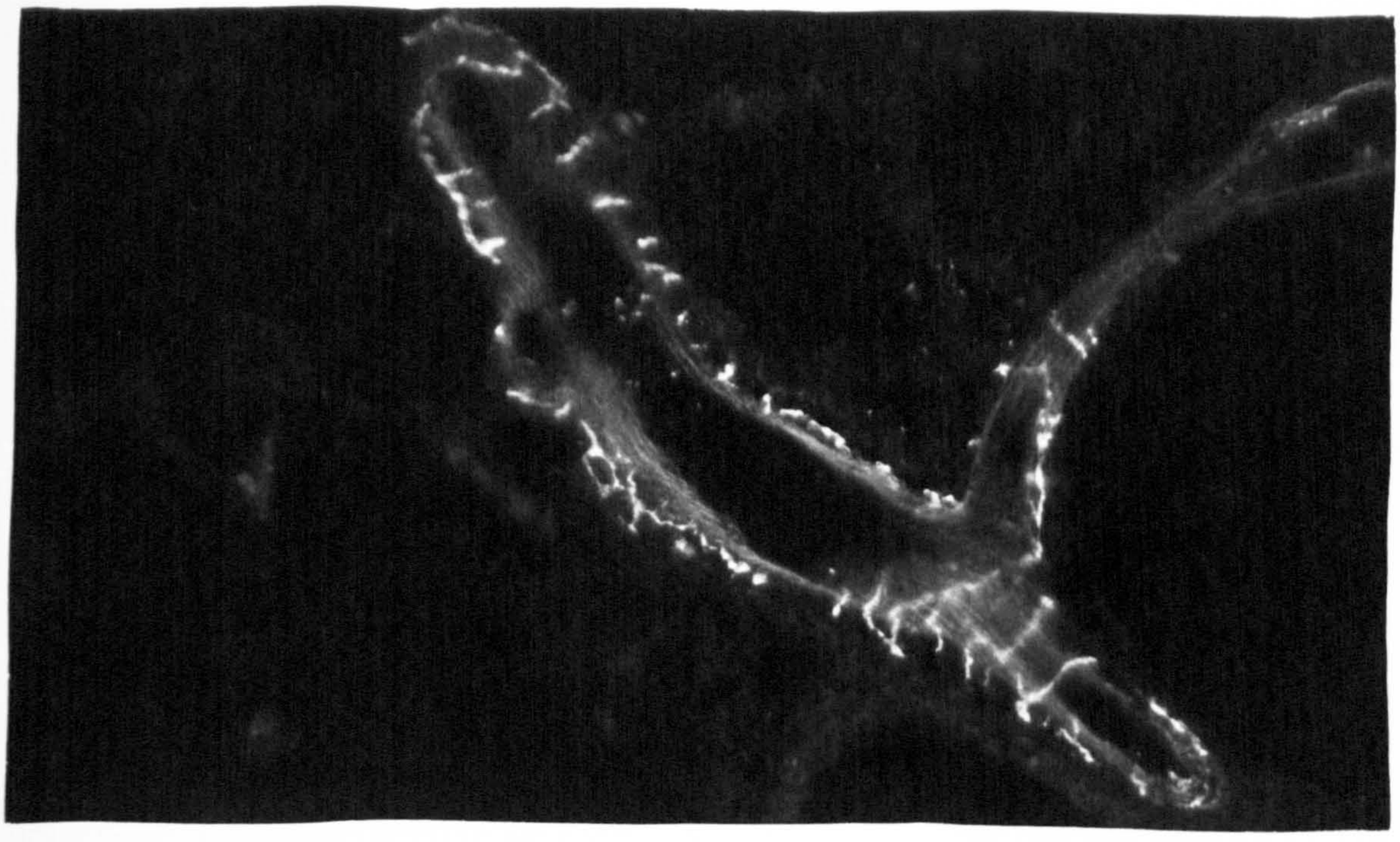


FIGURE 6:14. BRANCHING OF ARTERIOLE. (x 500)

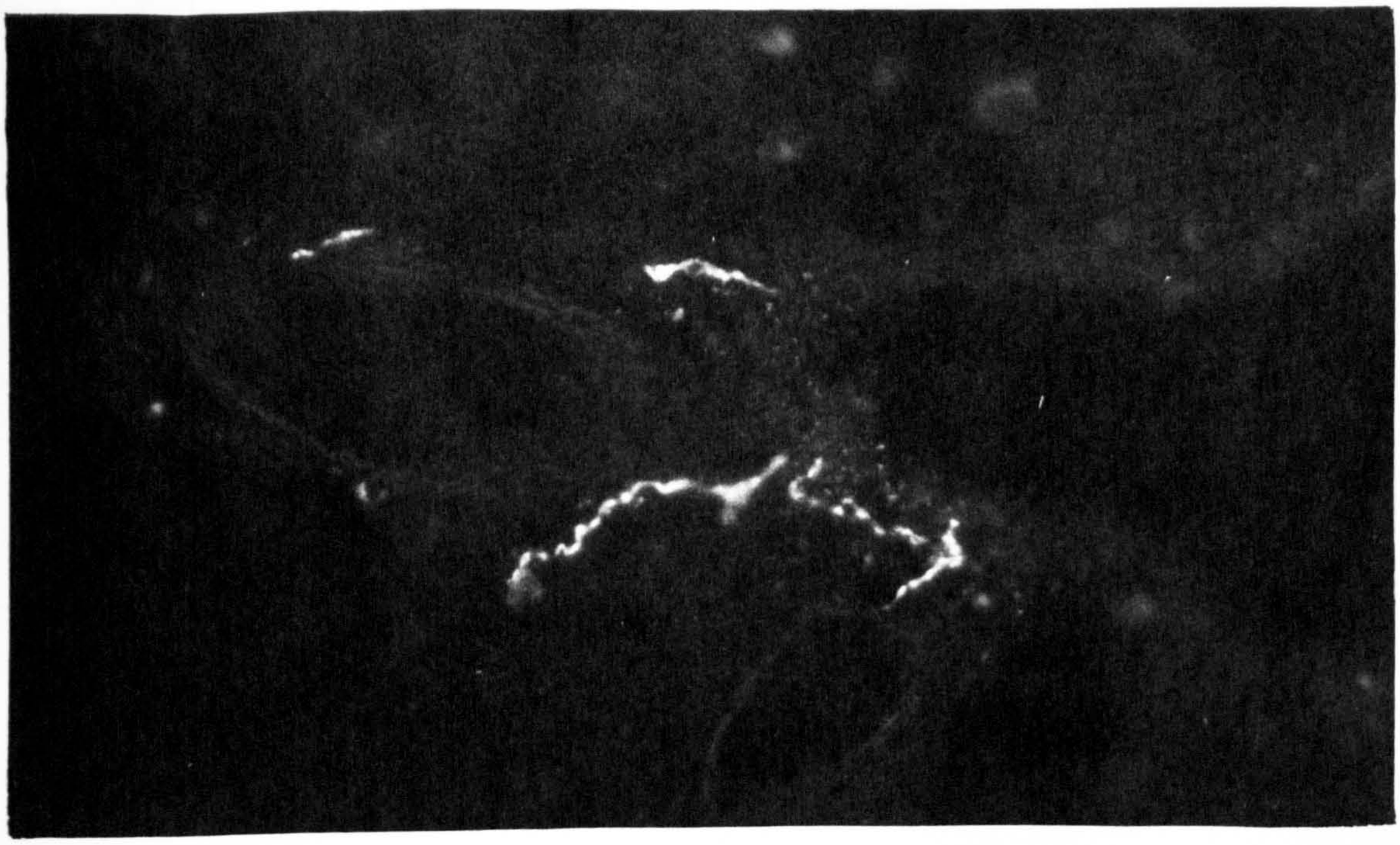


FIGURE 6:15. BRANCHING OF NERVE FIBRE. (x 700)

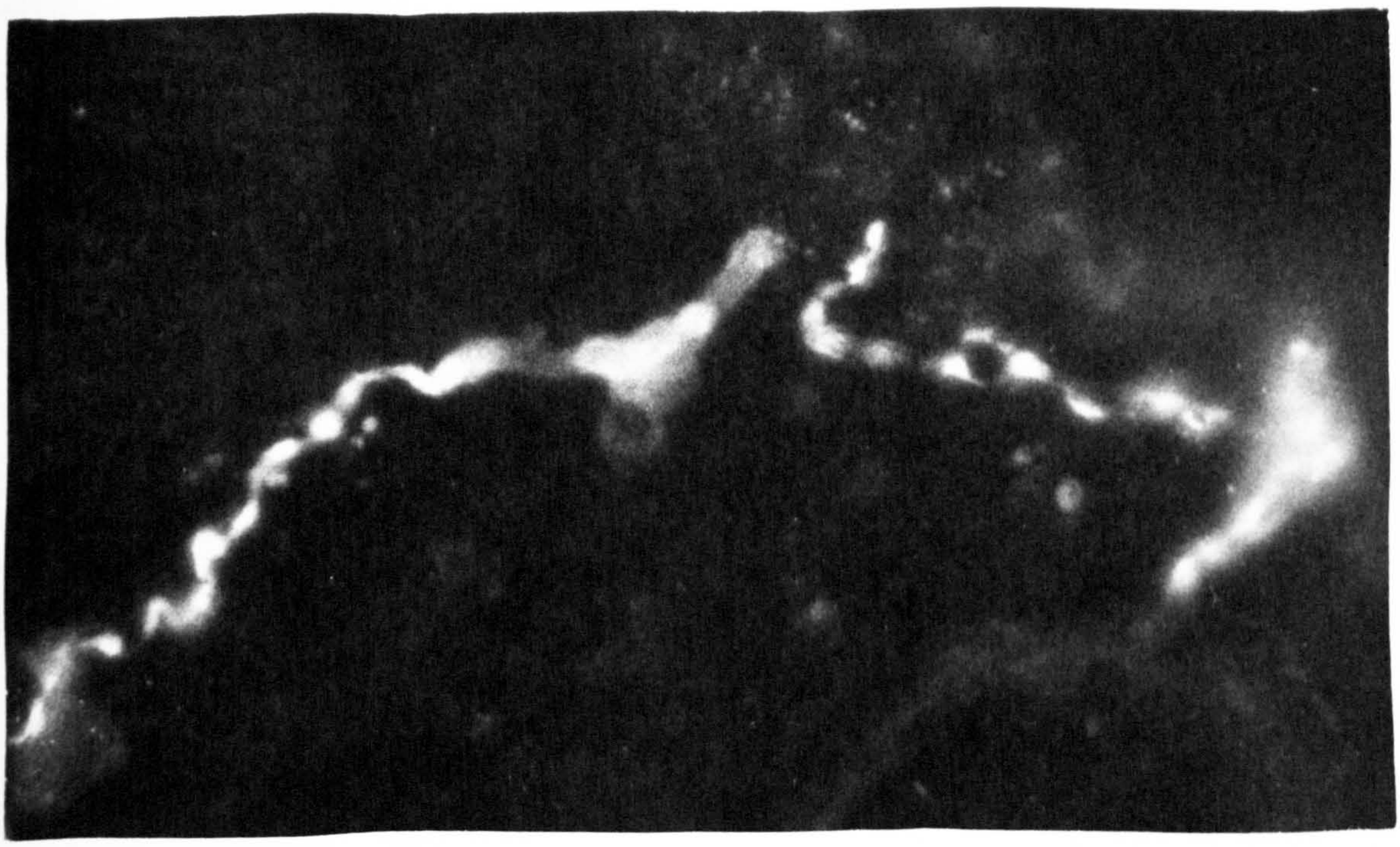


FIGURE 6:16. AS ABOVE. (x 2,100)

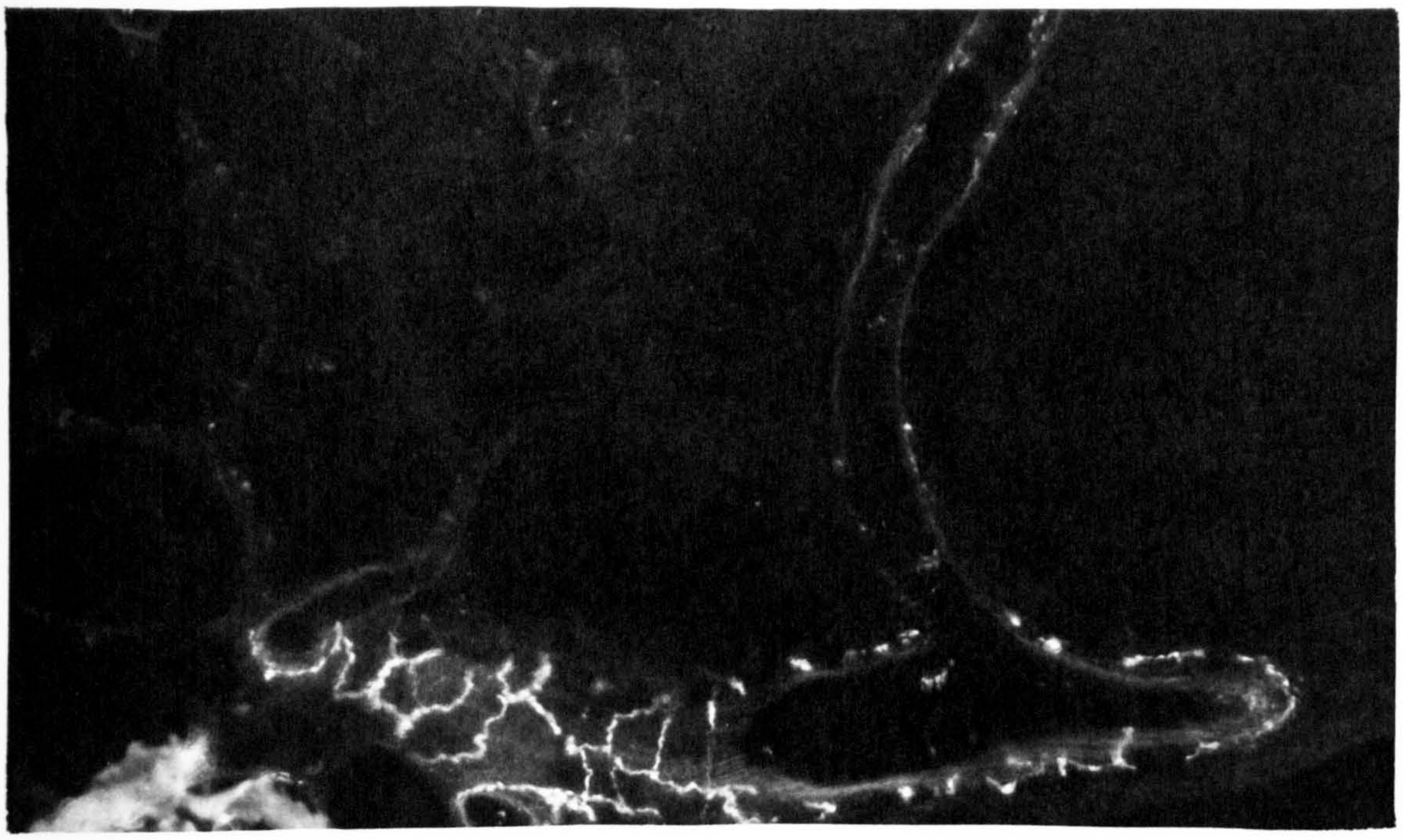


FIGURE 6:17. ORIGIN OF PRE-CAPILLARY. (x 500)

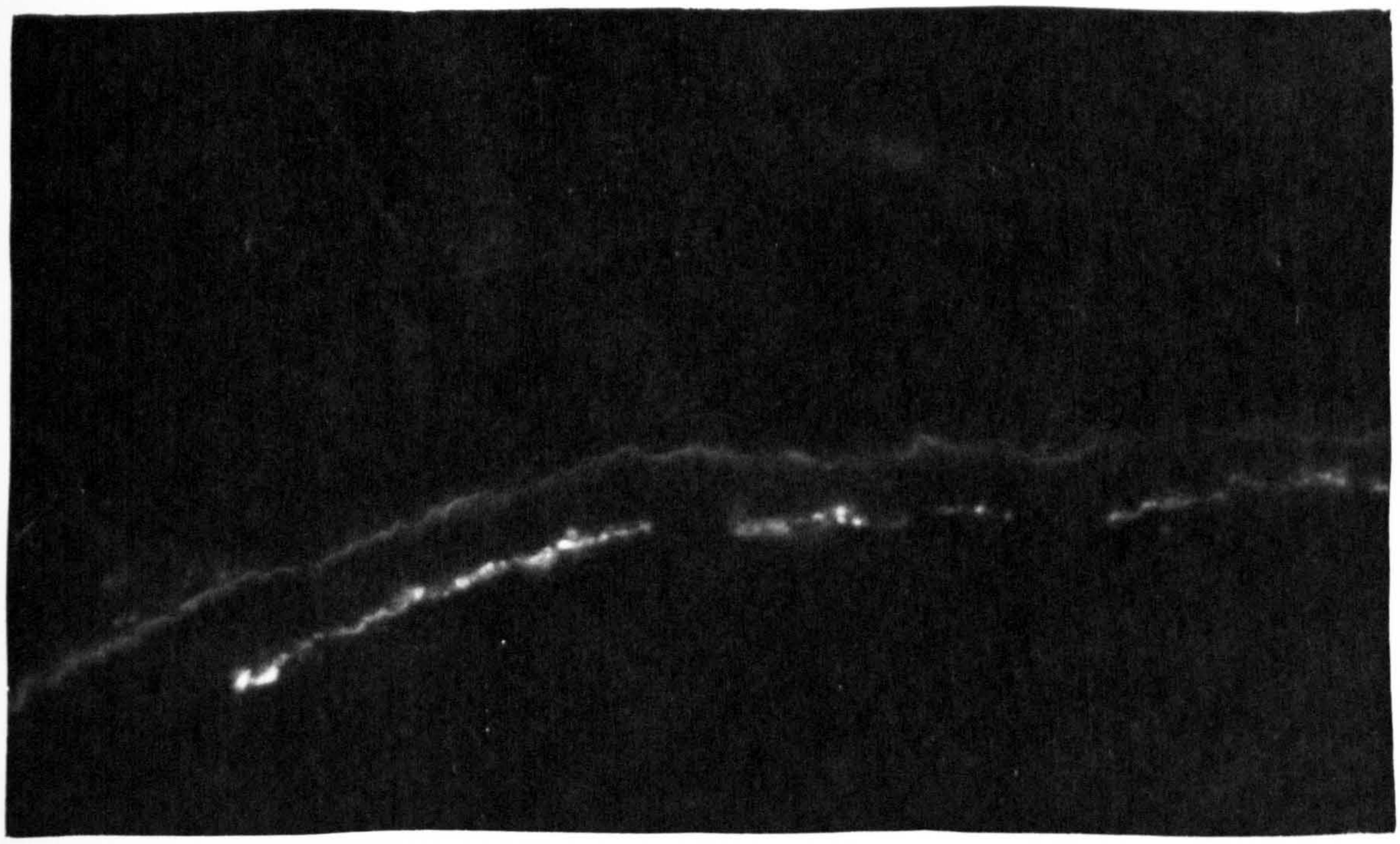


FIGURE 6:18. FINE NERVE FIBRES IN PRE-CAPILLARY.

(x 500)

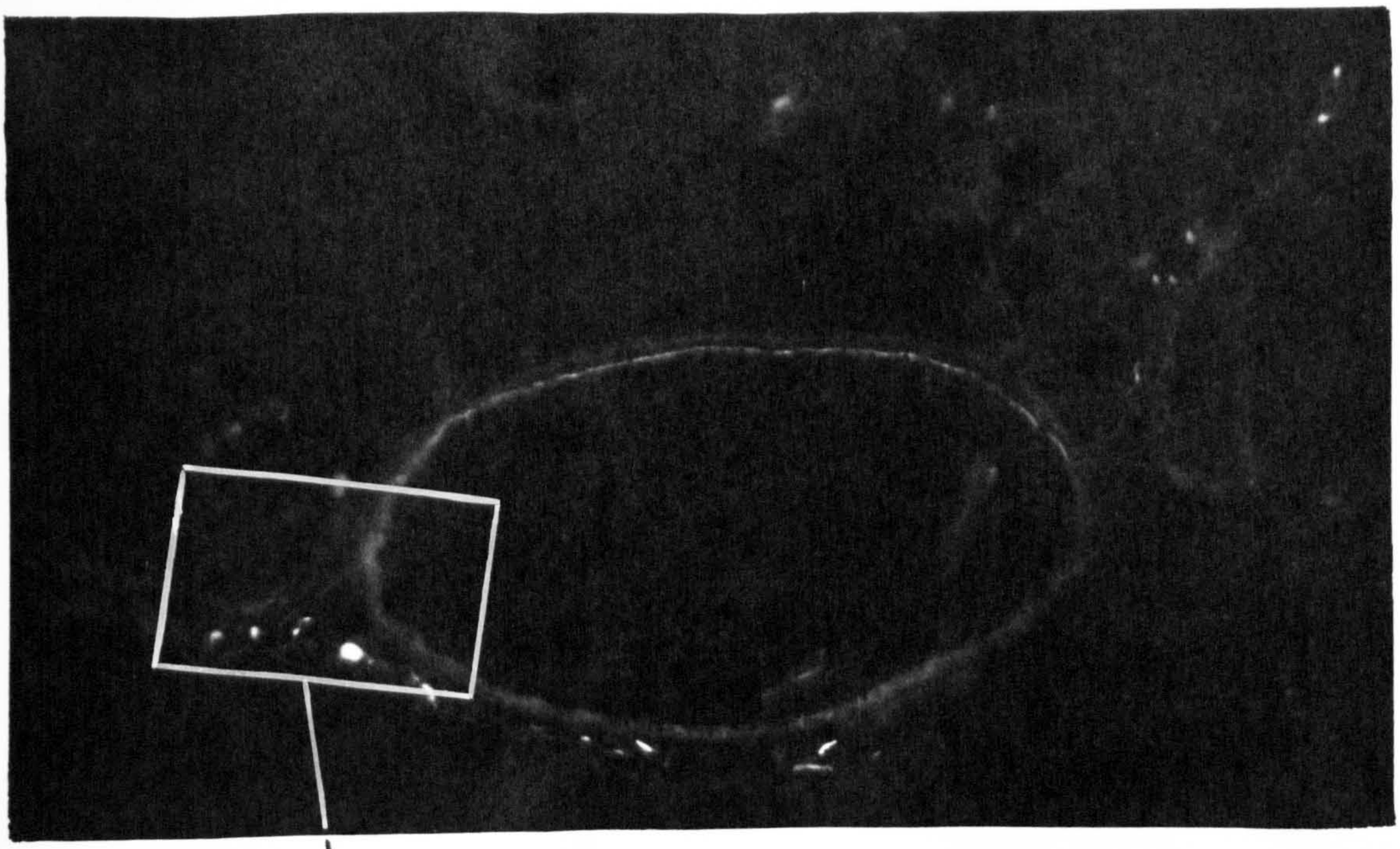


FIGURE 6:19. SMALL PULMONARY VEIN. (x 500)

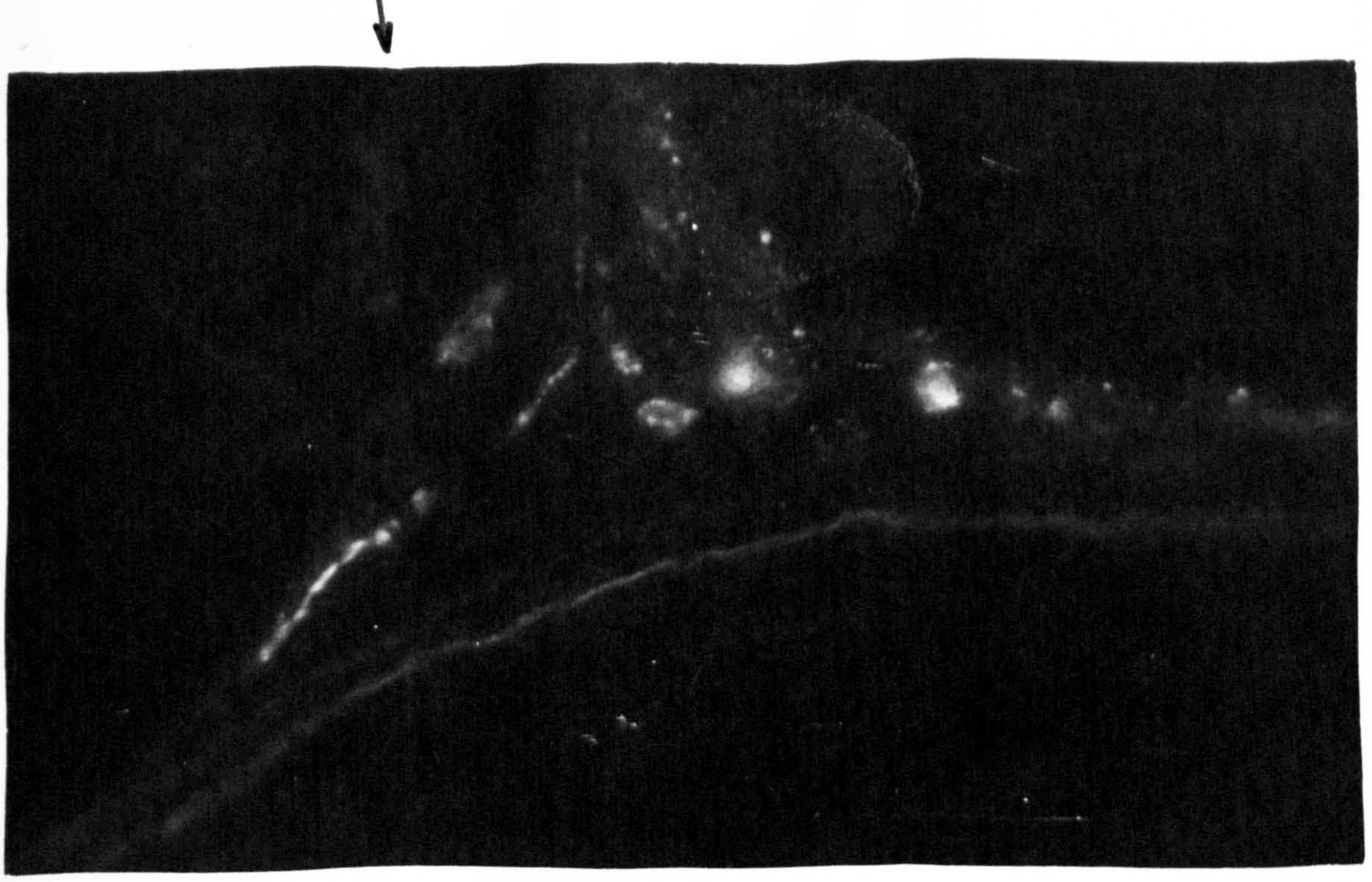


FIGURE 6:20. NERVE FIBRE IN SERIAL SECTION TO ABOVE.

(x 2,500)

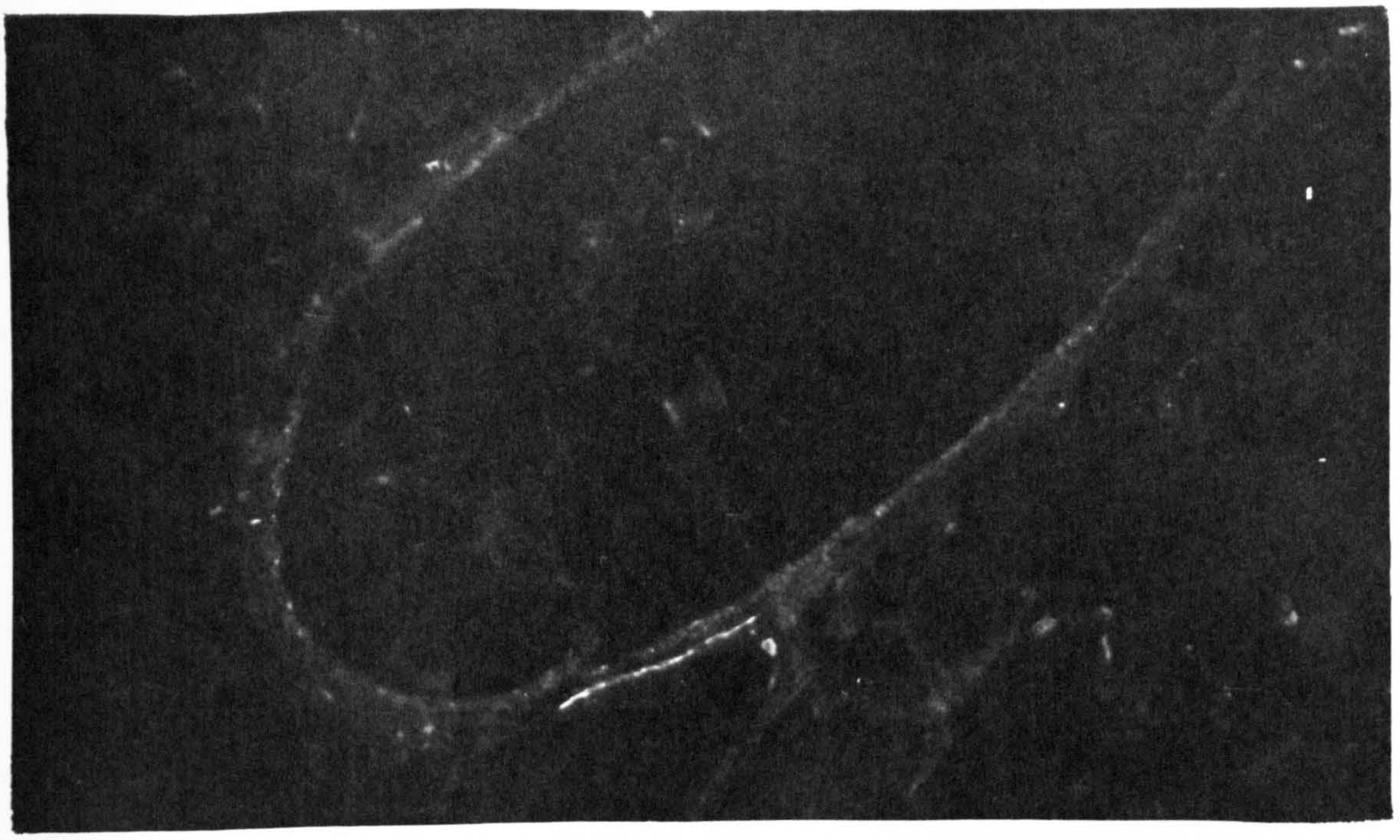


FIGURE 6:21. NERVE FIBRE IN PULMONARY VEIN. (x 200)

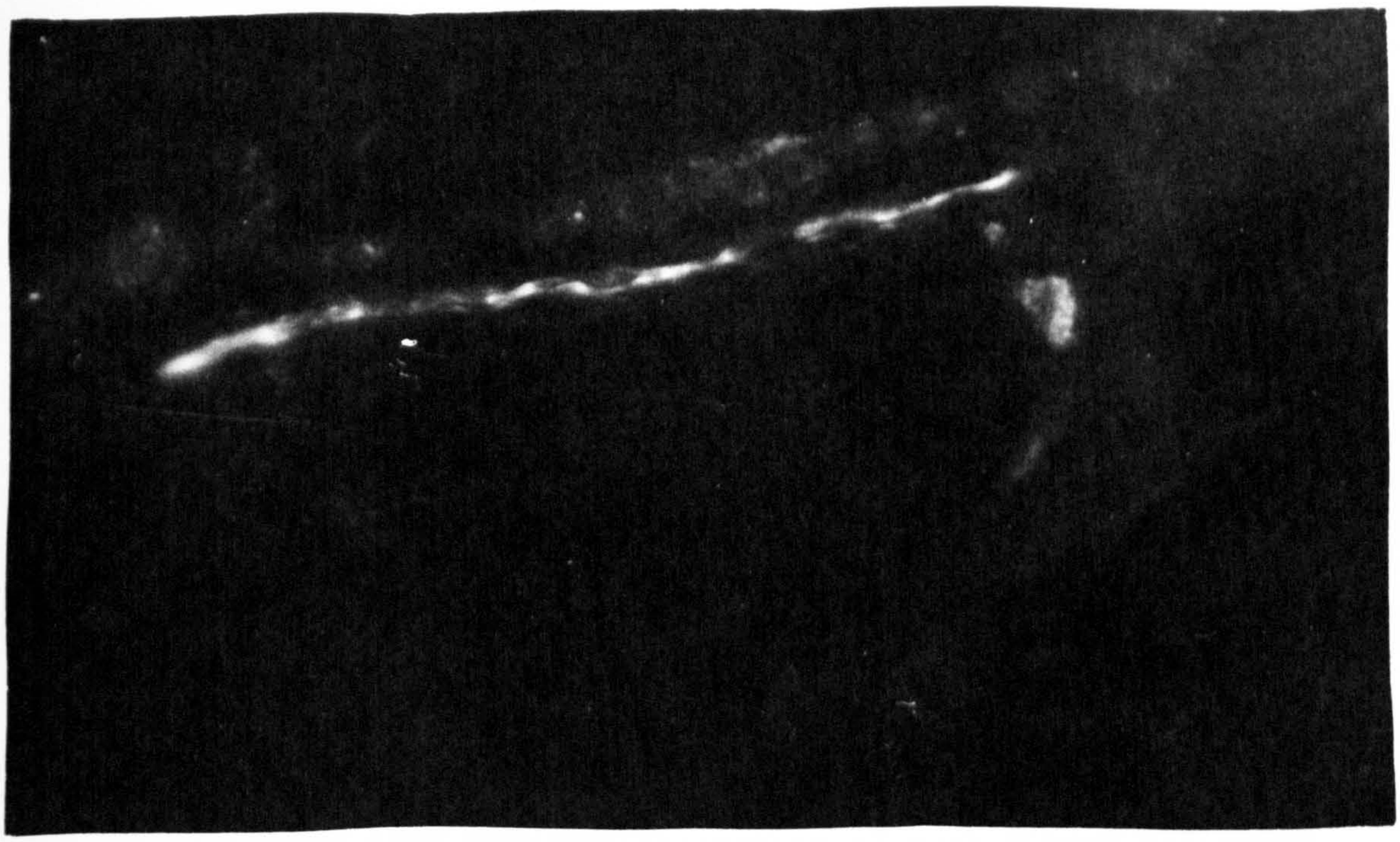


FIGURE 6:22. AS ABOVE. (x 800)

Alveolar wall

The main object of this study was to look for nerve fibres in the alveolar walls and if possible to demonstrate their relationship to Type 2 alveolar cells.

There is no doubting that such fibres have been positively demonstrated (Figures 6:24 to 6:29), but unfortunately these were the most difficult to reproduce photographically as the fibres were extremely fine and therefore only weakly fluorescent. Even with the fastest film (HP4 rated at 1200 ASA and 'forced' in Microphen) exposure times lasted many minutes and general vibration in the building added to the general loss of definition.

From time to time a large non-beaded brightly fluorescent fibre could be seen leaving the vicinity of a pulmonary arteriole or bronchiole and heading towards the parenchyma (Figure 6:24). It would then disappear from the plane of section, but if serial sections were examined, several much smaller beaded fibres could be found in the alveolar walls roughly along the predicted distribution path of the large fibre. There is no direct evidence here that these small fibres did arise from the large one, but the above relationship of fibres was seen on several occasions.

The small beaded fibres were extremely fine and very faintly fluorescent; in addition, such fluorescence as was present faded rapidly on exposure to ultra-violet light. A typical appearance is shown (arrowed) in Figure 6:25.

This appearance could be enhanced photographically and Figures 6:26 and 6:27 show the appearance of such fibres following photographic enlargement and 'burning out' of the alveolar walls.

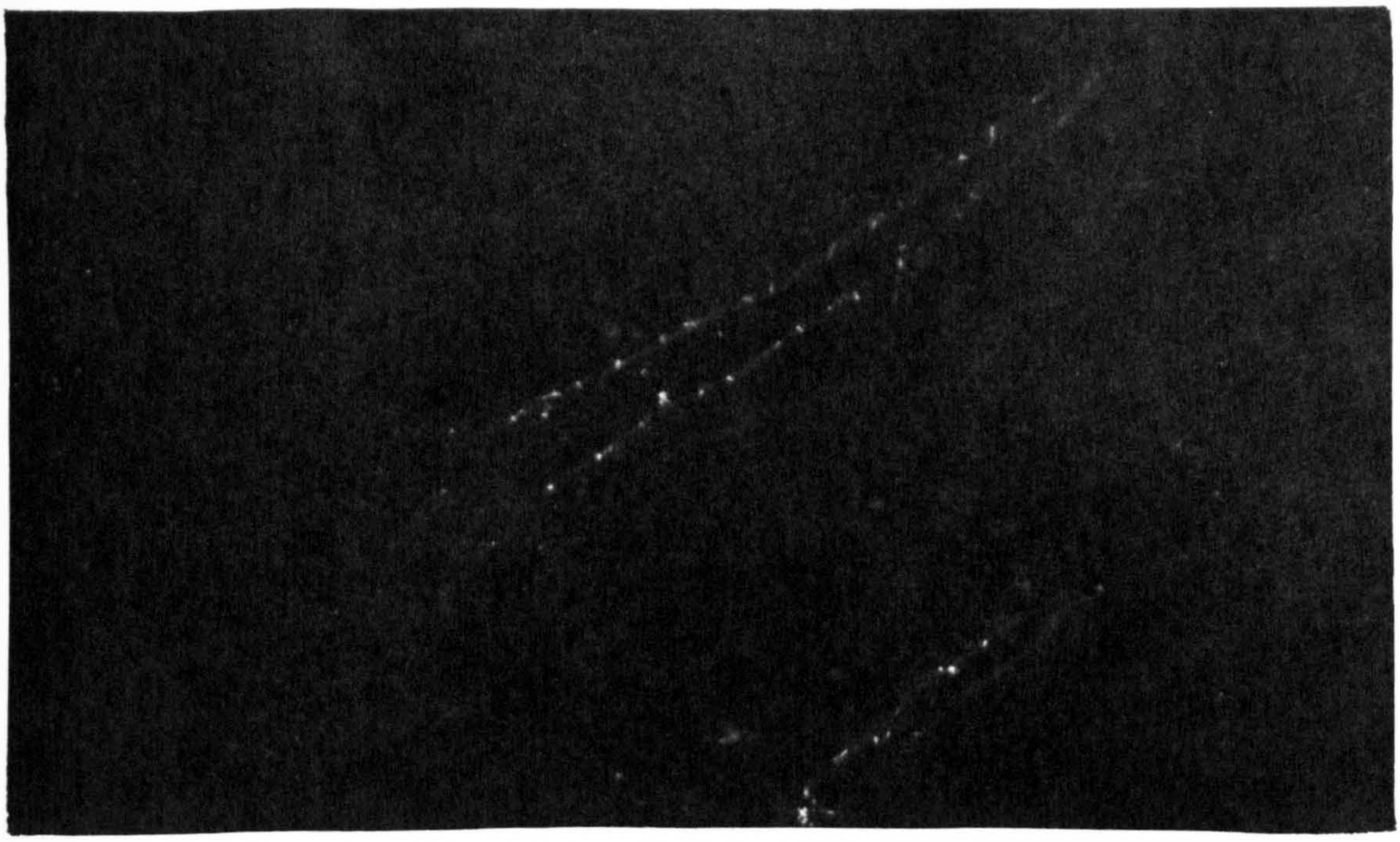


FIGURE 6:23. FINE FIBRES IN PULMONARY VENULE. (x 200)

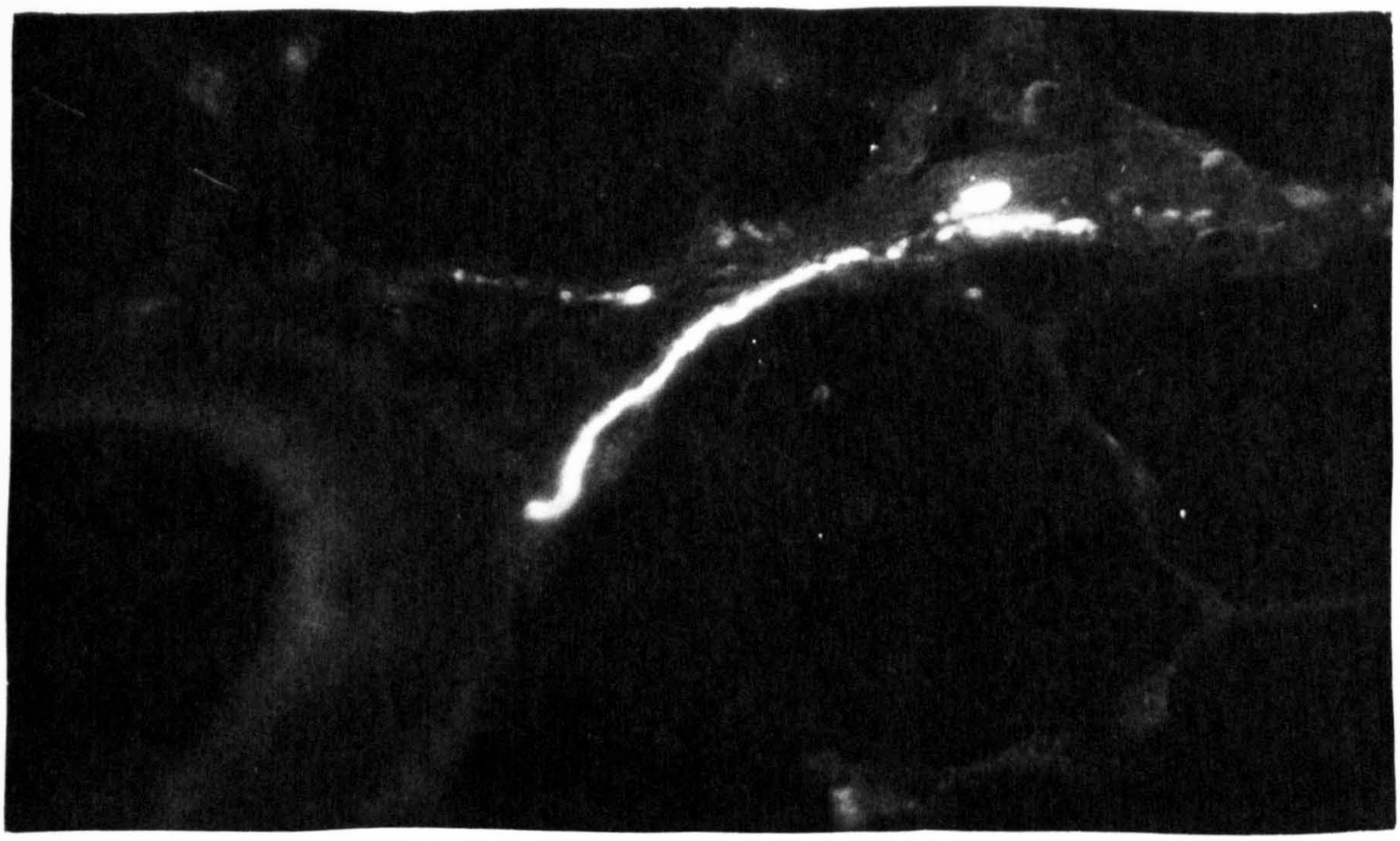


FIGURE 6:24. NERVE FIBRE ENTERING PARENCHYMA. (x 700)

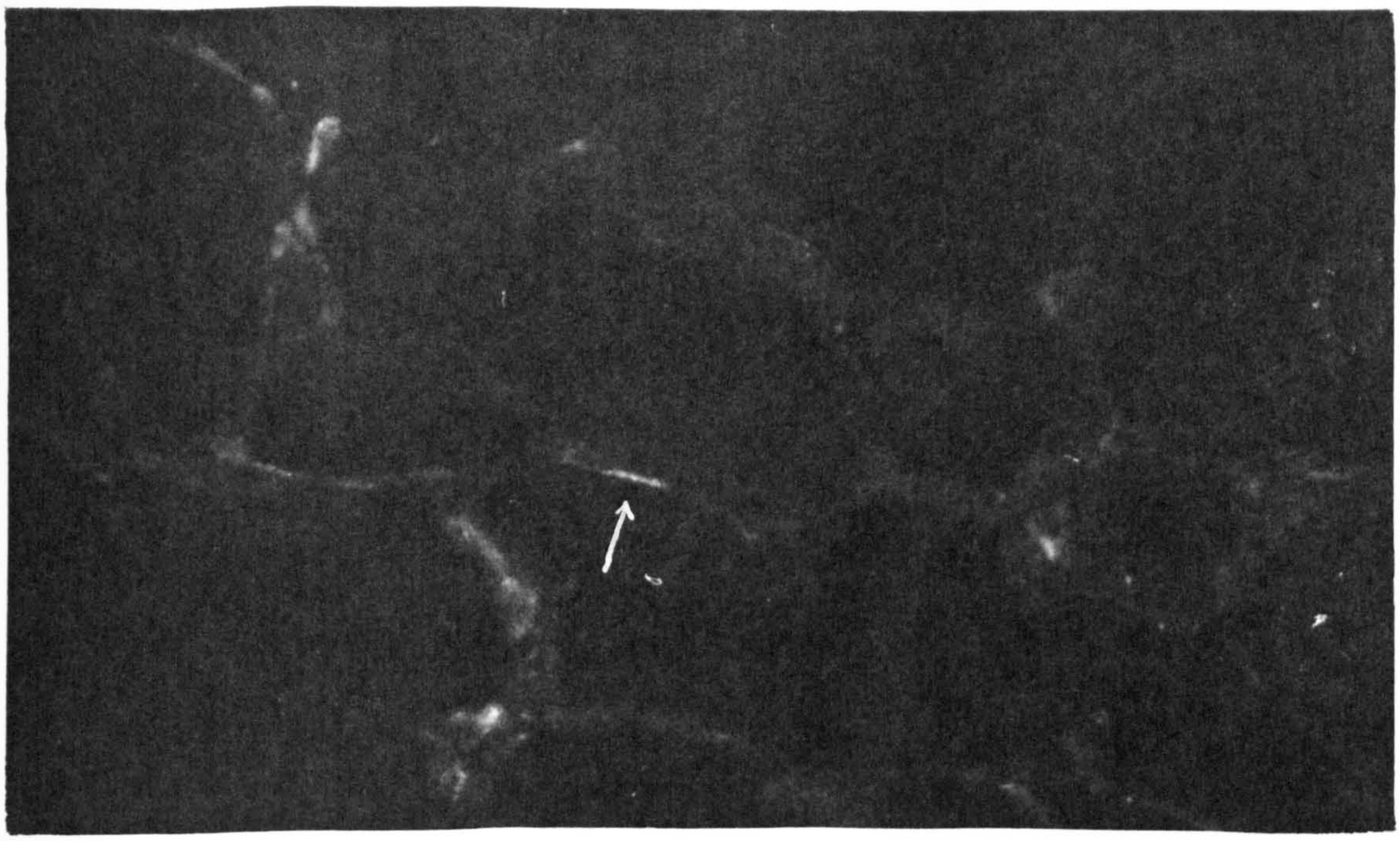


FIGURE 6:25. FINE FIBRE IN ALVEOLAR WALL. (x 400)

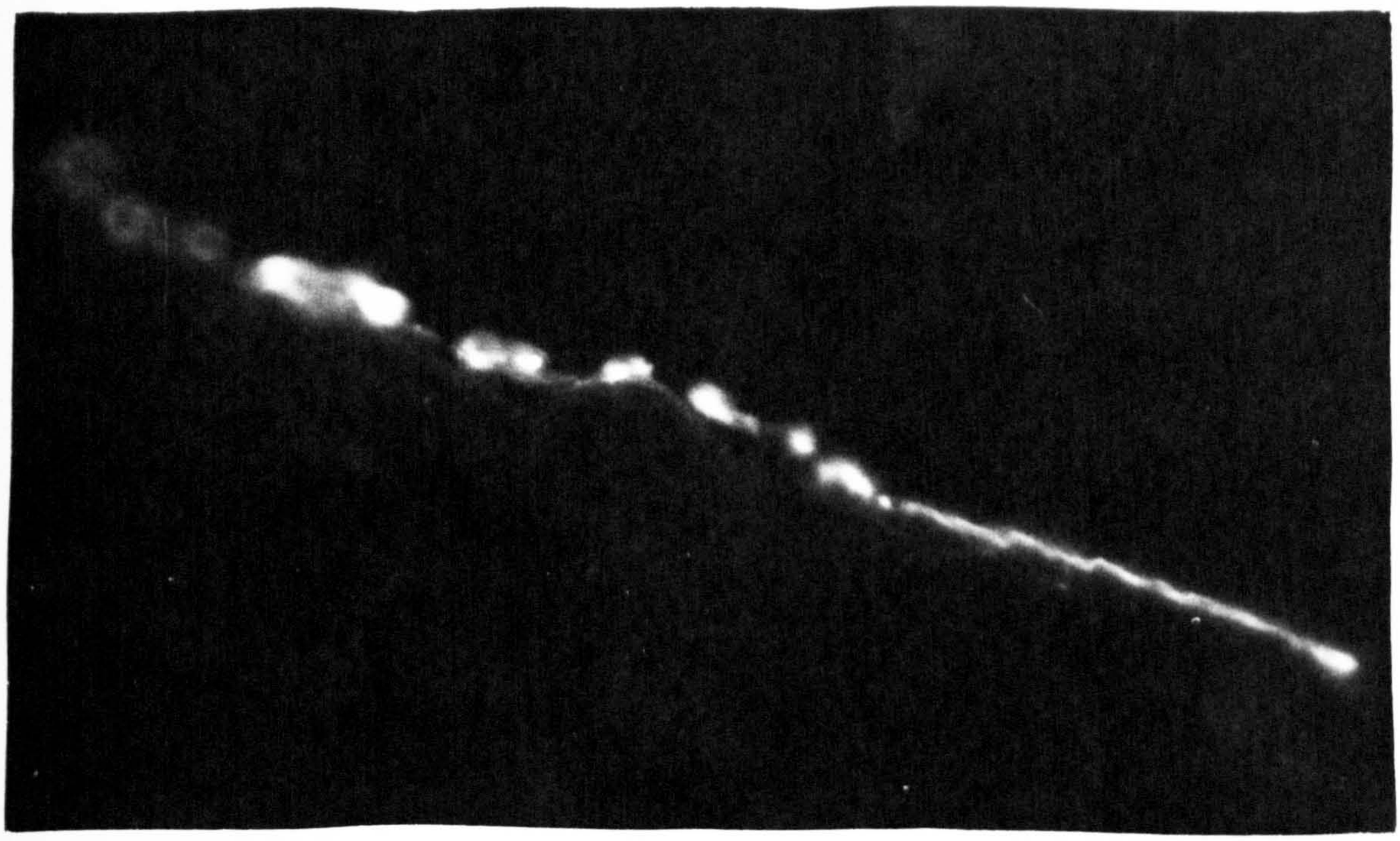


FIGURE 6:26. FIBRE IN ALVEOLAR WALL. (x 5,000)

Relationship to Type 2 cells

It is impossible to be certain that nerve fibres actually terminate on the Type 2 cells.

In many instances, fibres were seen passing close to or terminating near faintly autofluorescent cells which were later shown to be Type 2 cells, but in no case did the fluorescent fibre actually touch the cell.

The two micrographs demonstrating the closest relationship are presented as Figures 6:28 and 6:29 (rabbit lung). They are both of single exposures, but in both cases the nerve fibre had to be masked and 'held back' during photographic exposure to allow 'burning in' of the faintly autofluorescent Type 2 cell.

The fibre in Figure 6:28 appears much coarser and brighter than it was in reality, due to flair and inadequate masking. That in Figure 6:29 is a good representation of the actual appearance and shows the close proximity of the fibre to the cell. It could be argued from this figure that a nerve termination is present against the cell body, but a break in the continuity of the fibre and an apparent continuation of the fibre beyond the cell boundary probably invalidates this.

The problems of multiple exposure registration have been mentioned (see Page 6:21) as has the technique of combined phase contrast/fluorescence microscopy. This latter was used in a confirmatory experiment on rat lung during the actual writing of this Section. The specific fluorescence in the tissues had been enhanced by prior administration of a monoamine-oxidase inhibitor ('Pargyline' 50 mg/Kg intraperitoneally daily for three days before sacrifice)

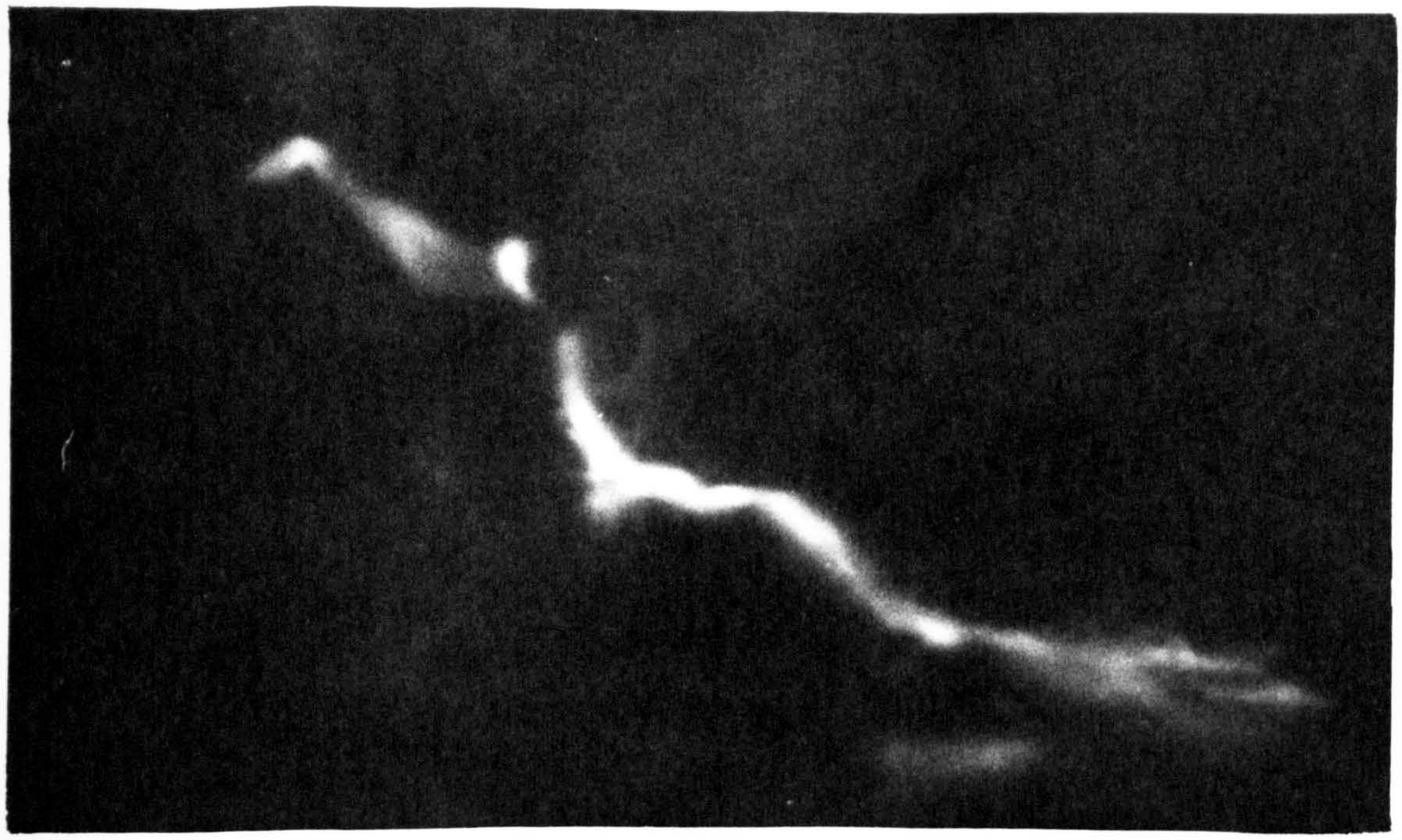


FIGURE 6:27. FIBRE IN ALVEOLAR WALL. (x 7,500)

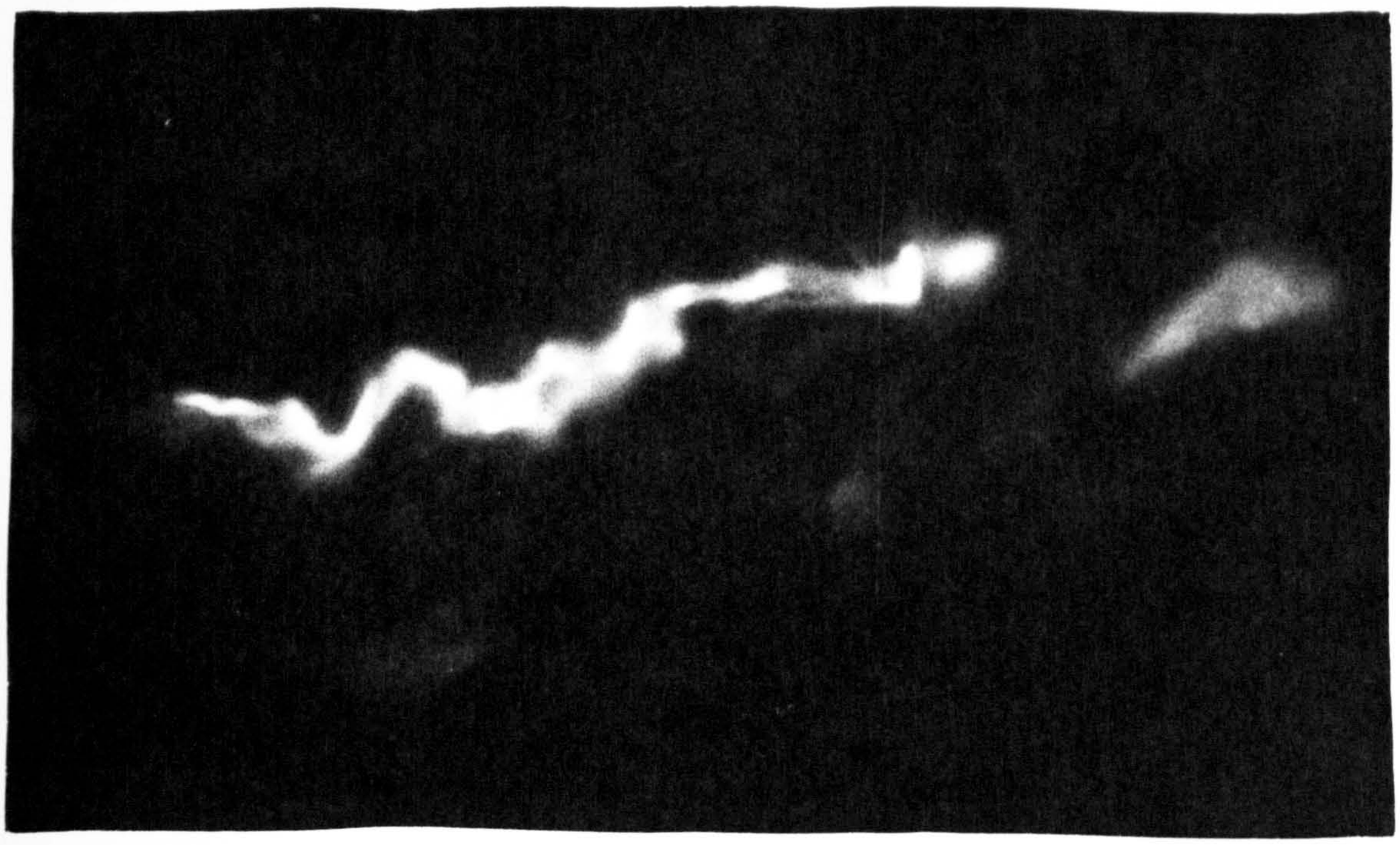


FIGURE 6:28. ALVEOLAR WALL FIBRE CLOSE TO AUTOFLUORESCENT CELL. (x 7,500)

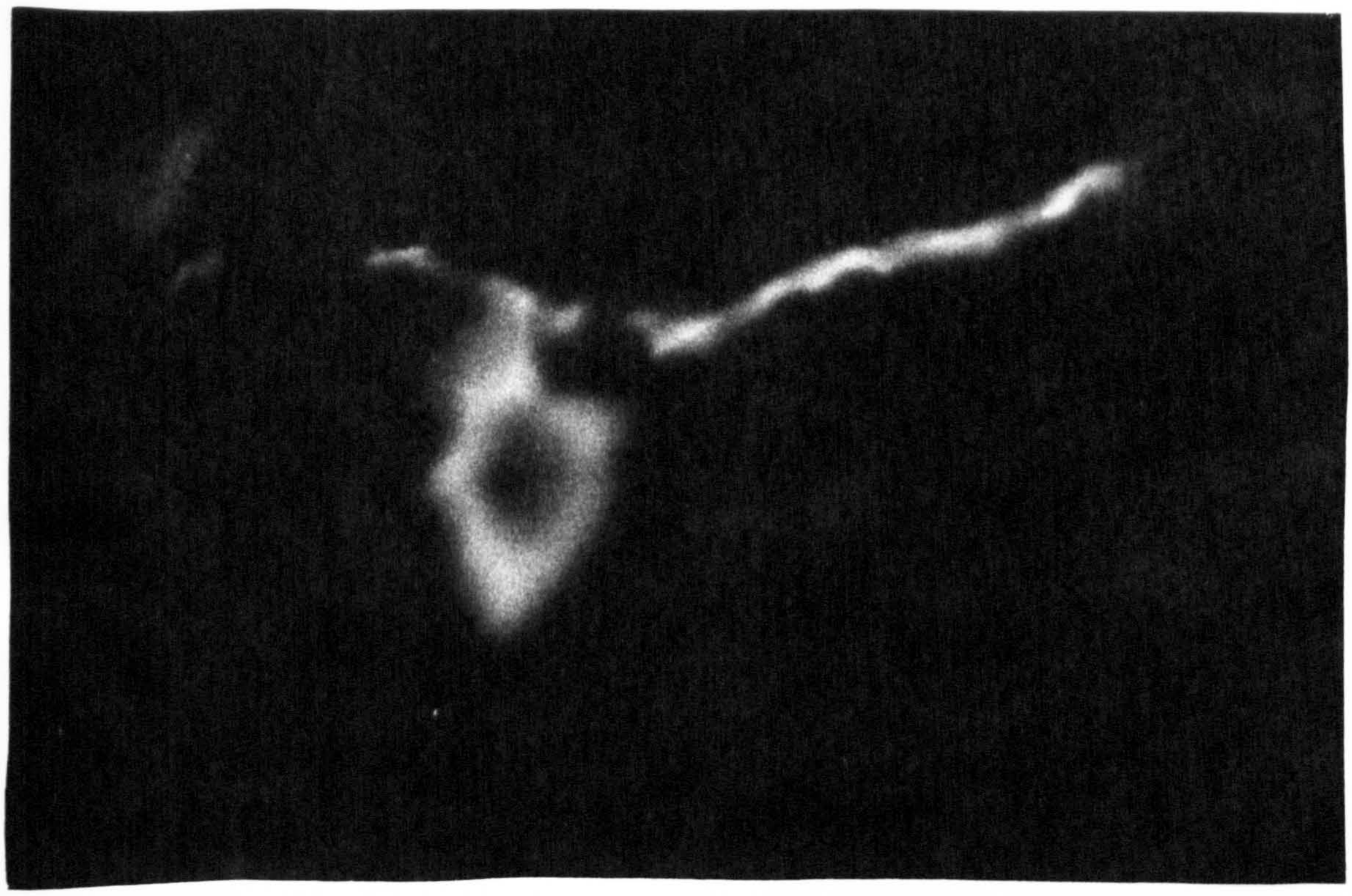


FIGURE 6:29. ALVEOLAR WALL NERVE FIBRE AND TYPE 2 CELL.

(x 3,500)

and this together with the vastly superior microscopic technique gave a very much brighter and more sharply defined image. Nevertheless, although adrenergic nerve fibres could be seen passing close to and even behind positively identified Type 2 cells, there was no single instance of a fibre actually terminating on a cell.

ELECTRON MICROSCOPY

Finally, all the sections prepared for electron microscopy from the lungs of dogs described in Sections 3 and 4 were scrutinised for the presence of nerve fibres.

Out of the several hundred fields examined, one single nerve fibre was found.

It lay in a crevice in the interstitial space between the nucleus of a capillary endothelial cell and the alveolar membrane (Figure 6:30). It was deep within the acinus at the junction of four alveoli and was not associated with any large structure. It was separated from the alveolar epithelium by the processes of an unidentified cell, probably a fibrocyte whose nucleus could be seen alongside. In the interstitium surrounding the fibre were numerous fine collagen and elastic fibres. There was no Type 2 cell anywhere in the field.

The enlarged micrograph of the fibre itself (Figure 6:31) shows that it consisted of a single axon surrounded by a Schwann cell sheath; portions of (presumably) the same Schwann cell lay alongside in the same plane of section. In the cytoplasm of the Schwann cell were many mitochondria and ribosomes.

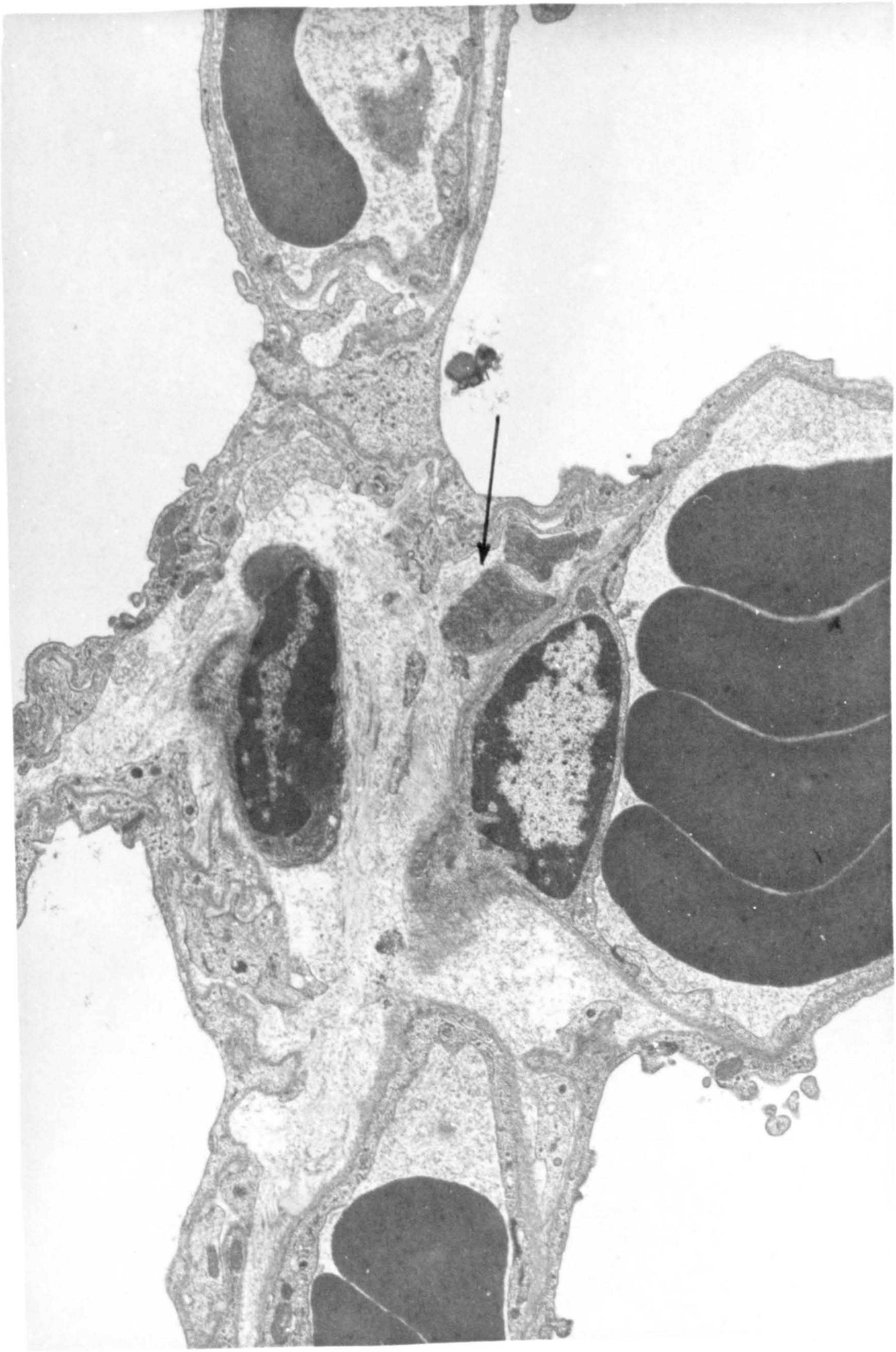


FIGURE 6:30. NERVE FIBRE (arrowed) IN ALVEOLAR WALL.

(x 10,000)

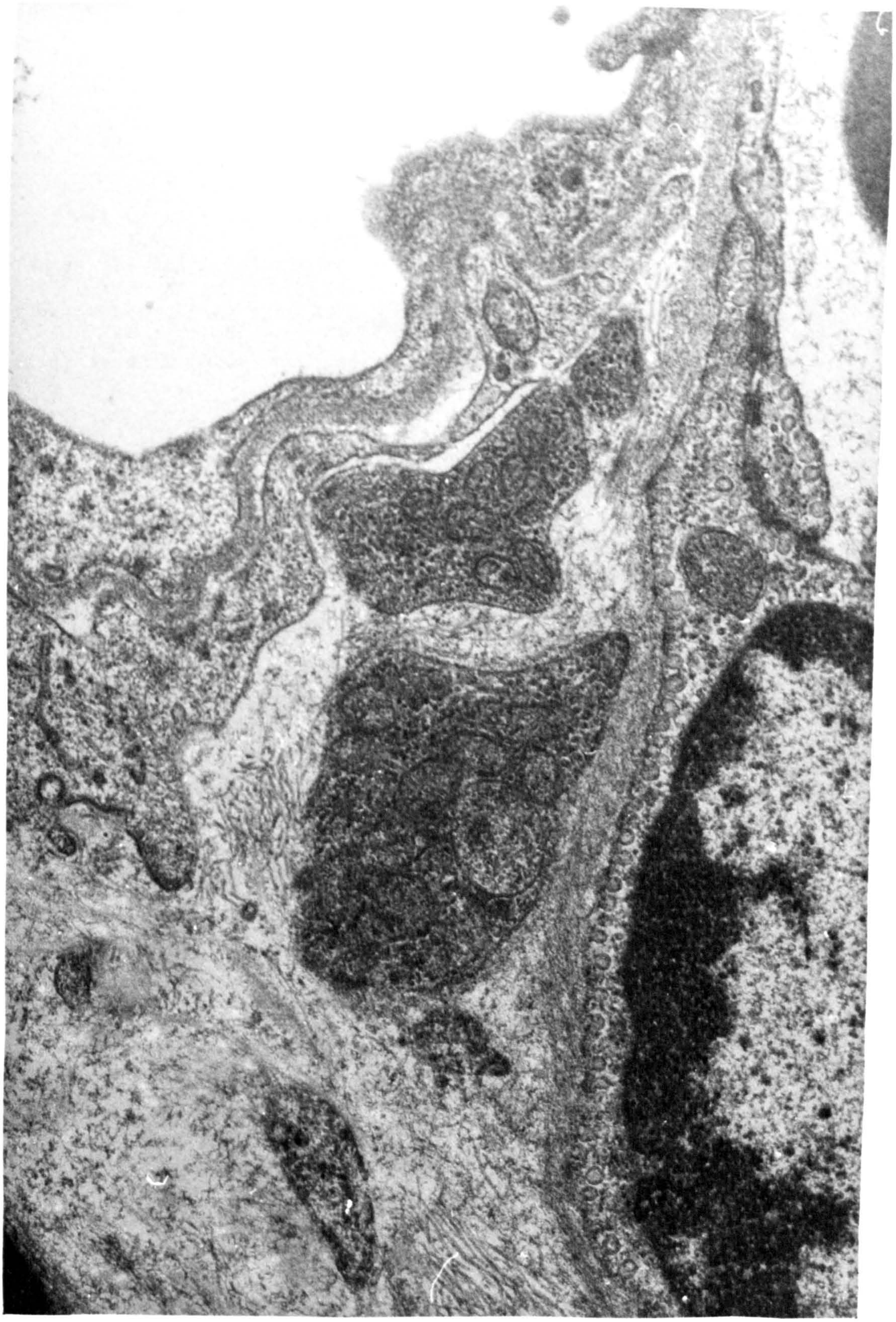


FIGURE 6:31. ALVEOLAR NERVE FIBRE. (x 40,000)

DISCUSSION

The methylene blue sections described confirm the bulk of previous work that pulmonary innervation extends at least as far as the small arterioles and respiratory bronchioles. The fluorescent micrographs have demonstrated unequivocably that there is at least a motor innervation extending to the alveolar walls.

The discrepancy between the two sets of findings can readily be explained by the fact that the Falck fluorescence technique is much more sensitive than methylene blue impregnation, and that any fibres present in the alveolar walls must be extremely fine.

Unfortunately there is no correspondingly sensitive technique for demonstrating sensory fibres. The recent demonstration, however, that stretch receptors ('J-receptors') must exist in the interstitial space adjacent to pulmonary capillaries (Paintal, 1969) provides strong experimental evidence for their existence.

Assuming that Paintal's prediction is valid, then to make physiological sense even as a coarsely local monitor of raised interstitial pressure, there must be many millions of terminal nerve fibres; in the adult human lung there are an estimated 300 million alveoli, each surrounded by a network of 1800 to 2000 capillary segments (Weibel, Burri & Gil, 1972). If the hypothesis to be advanced in this thesis is accepted, i.e. that the production and/or release of surfactant is under local sympathetic control, then again a very large number of fibres would be involved.

In exocrine glands under neural control it is not necessary to postulate that each secretory cell is individually innervated;

presumably local current spread can activate groups of cells situated around the motor fibre. Type 2 cells, however, do not fit into this pattern as they are usually widely separated from each other. Nevertheless it hardly seems feasible that each Type 2 cell has its own innervation as it is estimated that there are approximately 170 Type 2 cells in the average human alveolus (Meyrick & Reid, 1970). In addition, the same authors, in an analysis of several previous reports suggest a cell turnover time (i.e. the time taken to replace a given cell population, and representative of the mean life span of a single cell) for the Type 2 cell of 3 to 4 weeks. This implies 2,000 million new Type 2 cells being produced daily, and it is difficult to understand how this vast number of growing cells could be supplied continually with new nerve fibres.

A possible answer to this problem has been suggested by Hung and his co-workers (Hung et al., 1972), who in their report on mouse lung commented that the fibres which they had observed ending on Type 2 cells were typical neither of normal adrenergic nor cholinergic motor endings, but resembled more a special type of motor axon with many large dense-cored vesicles which had previously been described in the parathyroid gland and in the pars tuberalis of the pituitary. The suggested that:

> 'it is possible that the substance in the dense cores diffuses out of the axon into the interstitium of the alveolar septum and affects not only the Type 2 pneumocyte close by, but also other pneumocytes at some distance away from the axon.'

Nevertheless, even if one allows for only one motor nerve fibre per alveolus, and accepts that many J-receptor terminals may

come together in a single sensory fibre and that a single motor fibre may branch distally (as suggested on Page 6:27) there must still be an extremely large number of nerve fibres running through parts of the alveolar wall.

Probably the most puzzling feature therefore of alveolar wall innervation is the almost universal failure to demonstrate these nerves by electron microscopy.

Since the original investigation of pulmonary ultrastructure by Low and Daniels in 1952 there have been numerous electron microscopical investigations of the alveolar wall (reviewed in Section 4). Over the last decade, the most extensive investigations must be those of Meyrick and Reid (e.g. 1970) and Weibel (e.g. 1973), yet out of the vast mass of material which has passed through all their laboratories there has been only the extremely rare demonstration of an alveolar nerve.

The only group who have claimed consistently to have demonstrated alveolar nerves is Hung and his colleagues (see Page 6:6).

It is fairly easy to reconcile the discrepancy between the fluorescence microscopical and electron microscopical demonstration of fibres on any single section; the fluorescence microscopy sections were cut at 10 micrometres and therefore included a sizeable proportion of the alveolar wall (the average diameter of a rat alveolus being 60 to 70 micrometres - Meyrick & Reid, 1970) whereas ultrathin sections for electron microscopy were cut at 300 Angstroms, and it would therefore require some 2000 serial sections to cover a whole rat alveolus. In the conventional histological demonstration of Type 2 cells, a whole cell (average diameter 9 micrometres) can be accommodated in

the thickness of a single section, whereas more than 300 serial ultrathin sections would be required for electron microscopy.

What is much more difficult to reconcile is the failure of many workers over many years to find even a single nerve fibre, followed by the seeming facility of Hung and his colleagues.

The only suggestion that can be advanced, perhaps with some naivety, is that they were not looking for them.

Up until very few years ago there was no need to postulate autonomic innervation of the alveolar walls; motor nerve fibres 'required to be distributed' to bronchial and vascular muscle only and the idea of receptors and hence sensory nerve fibres at an alveolar capillary level had long been abandoned. In the context of finding only what one is looking for, it is interesting to note that, while Meyrick and Reid in 1968 described a new type of pneumonocyte, the alveolar brush cell, as comprising 5 to 10% of pneumonocytes, Weibel as late as 1973 stated:

> 'it seems to be rather rare; we have since found only about a dozen of these cells in very extensive material.'

The single example of a nerve fibre found in lung tissues taken from dogs described in Section 3 and presented in this thesis (Figure 6:30) was completely overlooked even during a painstaking morphometric study of the micrograph, and was only found on retrospective scrutiny when looking specifically for nerve fibres.

By similar reasoning it is noted that while there have been several studies which have produced extremely clear fluorescent

micrographs of fine adrenergic fibres in the airways and pulmonary vessels (reviewed on Page 6:11 et seq.), in none of them is there any mention of fluorescent fibres in the alveolar walls. Yet they must have been there to see, had they been looked for.

Some time after the work described herein had been completed, Bean and Nakamoto (1974) published a single micrograph which was presumably the one referred to in their abstract in 1972 (see Page 6:12). There were no details given of techniques and controls, and the fluorescent cells described as sympathetic ganglion cells look very similar to those identified as mast cells in this thesis. There are, however fluorescent fibres albeit of a rather odd appearance, visible in the alveolar walls, which, assuming that they exhibited specific rather than autofluorescence, must represent nerve fibres.

SECTION 7

SYMPATHETICS AND SURFACTANT

SYMPATHETICS AND SURFACTANT

REVIEW

There is in the literature a large body of evidence that a variety of insults to the central nervous system can lead to pulmonary damage. This 'centrineurogenic pulmonary oedema' has been demonstrated following mechanical trauma to the head (Bean & Beckman, 1969; Beckman & Bean, 1970; Beckman, Bean & Baslock, 1971), idiopathic, drug and oxygen induced convulsions (Bean, 1945; Bonbrest, 1965; Bean, Zee & Thom, 1966), cerebral hypotension (Kusajima, Wax & Webb, 1974), hypothalamic hypoxia (Moss, Staunton & Stein, 1972), and raised intracranial pressure, both in the clinical (Ducker, 1968) and experimental (Campbell & Visscher, 1949; Ducker, Simmons & Anderson, 1968; Berman, Ducker & Simmons, 1969) situation.

Anaesthetic agents are known to depress the response (Buckingham, Sommers & McNary, 1968; Bean & Beckman, 1969). A causal involvement of the sympathetic nervous system is indicated by the fact that sympatholytic agents, particularly alpha-adrenergic blockers (Johnson & Bean, 1957; Bean, Zee & Thom, 1966; Bean & Beckman, 1969), and denervation (Staunton, Stein & Moss, 1973) protect against the response, while administration of sympathomimetics augments it (Bean & Johnson, 1955).

The exact site of action is not known, but a hypothalamic origin is suggested by experiments demonstrating the development of pulmonary oedema and haemorrhage in rats with pre-optic hypothalamic lesions and its prevention by cervical cord section and bilateral splanchnicectomy (Gamble & Patton, 1953; Maire & Patton, 1956 a & b).

Stereotactic stimulation of the same area in rats has been shown to produce similar pulmonary lesions, preventable by administration of sympatholytic drugs (Wood, Seager & Ferrell, 1964).

A further pointer towards a neurogenic mechanism is the extremely rapid progression of response. Gamble and Patton (1953) describe the 'explosive' onset of acute respiratory distress leading to death within a few seconds. In a study of Vietnam soldiers killed almost instantly with clean bullet wounds of the head, pulmonary oedema was often found (Simmons, Martin, Heisterkamp & Ducker, 1969)

Possible mechanisms arising from the different aetiologies have been suggested. Moss, Staunton and Stein (1973) postulate that derangement of hypothalamic function leads to a sympathetically-mediated pulmonary venular constriction causing a transient, but large, increase in pulmonary capillary pressure. Theodore and Robin (1975) accept this, but suggest also that the massive centrally-mediated alpha-adrenergic discharge results in an intense generalised systemic vasoconstriction which produces a large shift of blood from the systemic to the pulmonary circulation, further raising the pulmonary capillary pressure.

This leads to an intra-alveolar oedema both by massive upset to the balance of hydrostatic forces across the alveolar membrane and by permeability changes following direct damage to the pulmonary capillary walls, The latter effect persists following return of the cardiovascular response to normal.

That this is not the only mechanism involved is evident from a recent series of experiments by Beckman and his colleagues.

Lethal mechanical head injury in the rat induced by a captive bolt mechanism had been shown to produce severe epileptiform convulsions and pulmonary congestion, haemorrhage, atelectasis, and oedema (Bean & Beckman, 1969; Beckman & Bean, 1969); the lung changes could be prevented by prior administration of the sympatholytic Dibenzyline (Bean & Beckman, 1969). Further work with the same experimental model demonstrated a decrease in pulmonary compliance following the head injury (Beckman & Bean, 1970) which, from the change in the air pressure/volume, but not the saline pressure/volume curve, appeared to be due to an alteration in surface tension. In view of the gross pulmonary damage, however, it was impossible to state whether the surface tension change was a primary event.

When these experiments were extended to primates (squirrel monkeys), however, it was found that mechanical head injury could induce changes in compliance (again, from air and saline pressure/volume studies, apparently due to surface tension changes) even in the absence of gross lung damage as judged by macroscopic appearances and lung weight/body weight ratios (Beckman, Bean & Baslock, 1971). An increase in the minimum surface tension of lung washings was also reported.

Again, the effect was prevented by alpha-adrenergic blocking agents.

This strong evidence for the primary involvement of surfactant changes in centrineurogenic lung damage and of the influence of the sympathetic nervous system was further investigated by direct electrical stimulation of the sympathetic nerve supply to the lung. In both the cat (Beckman & Mason, 1973) and the squirrel monkey

(Beckman, Bean & Baslock, 1974) stimulation of the stellate ganglion caused a significant decrease in pulmonary compliance in the absence of any gross lung damage.

Multiple controls were set up. The effect of airway constriction was eliminated in one group by pre-treatment with isoproterenol, and a haemodynamic effect would appear to have been obviated by the demonstration of a typical response in two exsanguinated cats. Inadvertent vagal stimulation was eliminated by demonstrating a continuing response after atropine administration.

In surface balance estimations of lung washings from the monkeys, minimum surface tension measurements rose following stellate ganglion stimulation from 4.8 ± 1.0 dynes in sham operated controls, to 21.2 ± 3.8 dynes in the ipsilateral, and 18.0 ± 5.1 dynes in the contralateral lungs.

There appears from these experiments to be little doubt that sympathetic stimulation of the lungs can in some way cause an alteration in the surfactant system; it was therefore decided to repeat the latter experiment on cats, and following the anticipated demonstration of a change in compliance to fix the lungs for an investigation by electron microscopy in the hope that ultrastructural changes in the Type 2 cells, possibly similar to those described in Section 4, might be demonstrated.

MATERIALS AND METHODS

Preliminary experiments

Various different routes of exposure were attempted in order to place stimulating electrodes on the stellate ganglion; in all instances the location and identification of the ganglion proved difficult.

The most reliable method was to open the chest widely by a sternum-splitting incision and to remove surgically one half of the rib cage. The operation, however, was performed without access to diathermy equipment and in each case by the time that the electrodes had been implanted, the animal was moribund. In addition, the unavoidable handling of the lung could have proved a possible source of damage.

The ideal preparation appeared to be a closed-chest animal, and a method of stimulating the stellate ganglion in such a preparation was found in the work of Gillespie, Maclaren and Pollock (1970).

In this technique the animal is pithed by a steel pithing rod which then can be used as a stimulating electrode. A movable insulating Teflon shield around the steel electrode allows for a variable number of spinal segments to be stimulated, and the whole structure can be moved up and down inside the vertebral canal in order to stimulate different levels of the cord outflow.

Pithed cat experiments

The experiments were performed on adult cats of approximately 2 to 2.5 Kg. in weight.

Anaesthesia was induced by passing a stream of 4% Halothane in a nitrous oxide/oxygen mixture (4 l/min and 2 l/min respectively) through an animal box, following which they were transferred to the operating table. Tracheostomy was performed immediately and a cannula inserted, after which the animal was immediately pithed.

Operative procedures

A cork borer with an internal diameter slightly larger than that of the Teflon-shielded pithing rod was used as a trocar. It was introduced in an upwards direction through the upper table of the orbit to avoid bleeding from the retinal artery. The head was then rotated and tilted until the orbit was in line with the spinal column and the trocar was advanced through the foramen magnum; it was usually left in situ where it acted as a splint for the head. The pithing rod was introduced into the spinal canal via the trocar and advanced as far as the sacrum. It was then withdrawn to its approximate stimulating position.

To aid localisation of the electrode, a standard length of rod was used, and comparison of the length left protruding from the orbit with a similar length inserted in a specially mounted cats' vertebral column allowed for accurate positioning. The final position was confirmed under direct vision by fluoroscopy on completion of each experiment (Figure 7:1).

An indifferent electrode was inserted all the way down the back of the animal, between the spinal column and the skin.

When properly performed, the operation was bloodless.

Immediately the animal was pithed, it was connected to a ventilator.

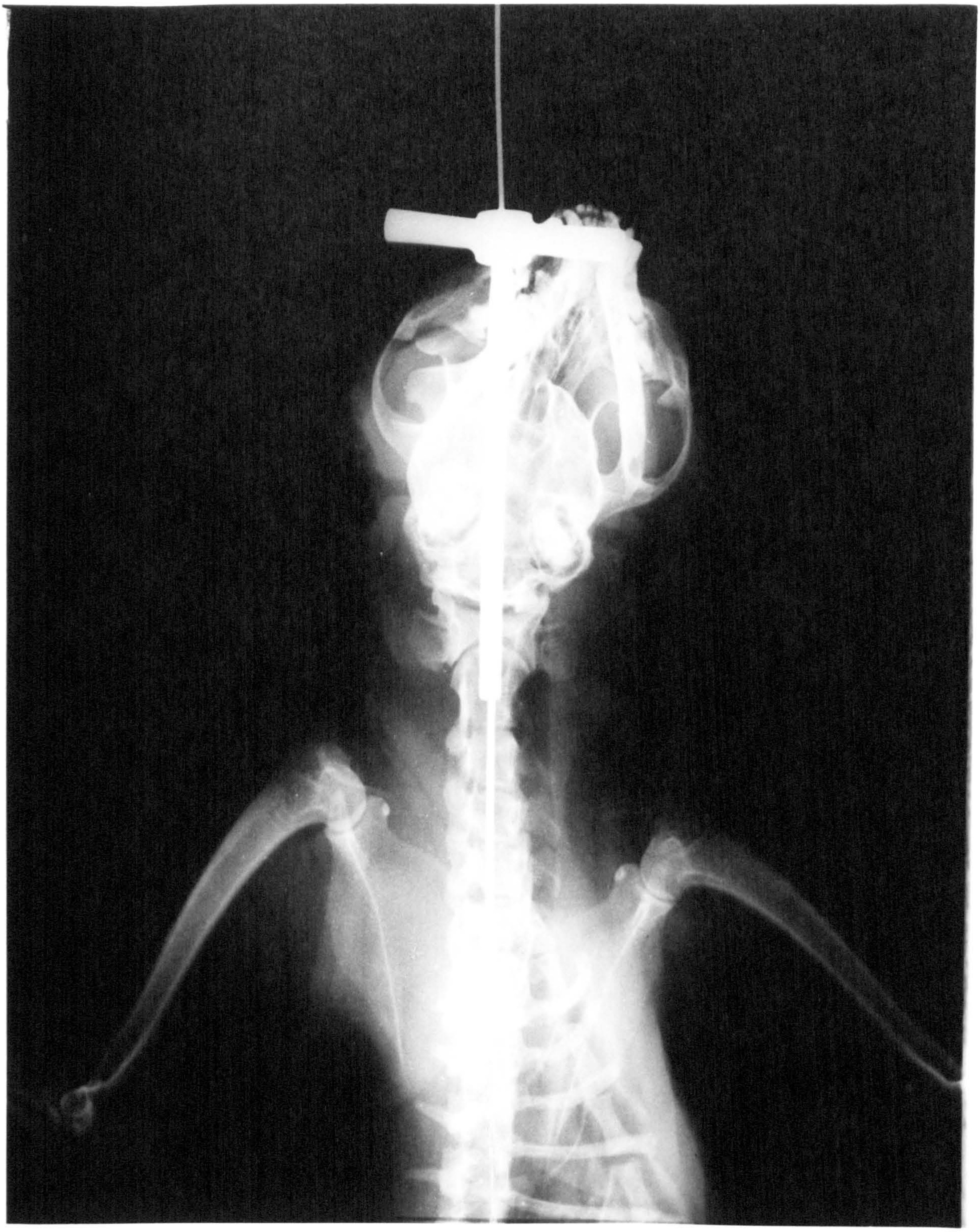


FIGURE 7:1. RADIOGRAPH: PITHED CAT.

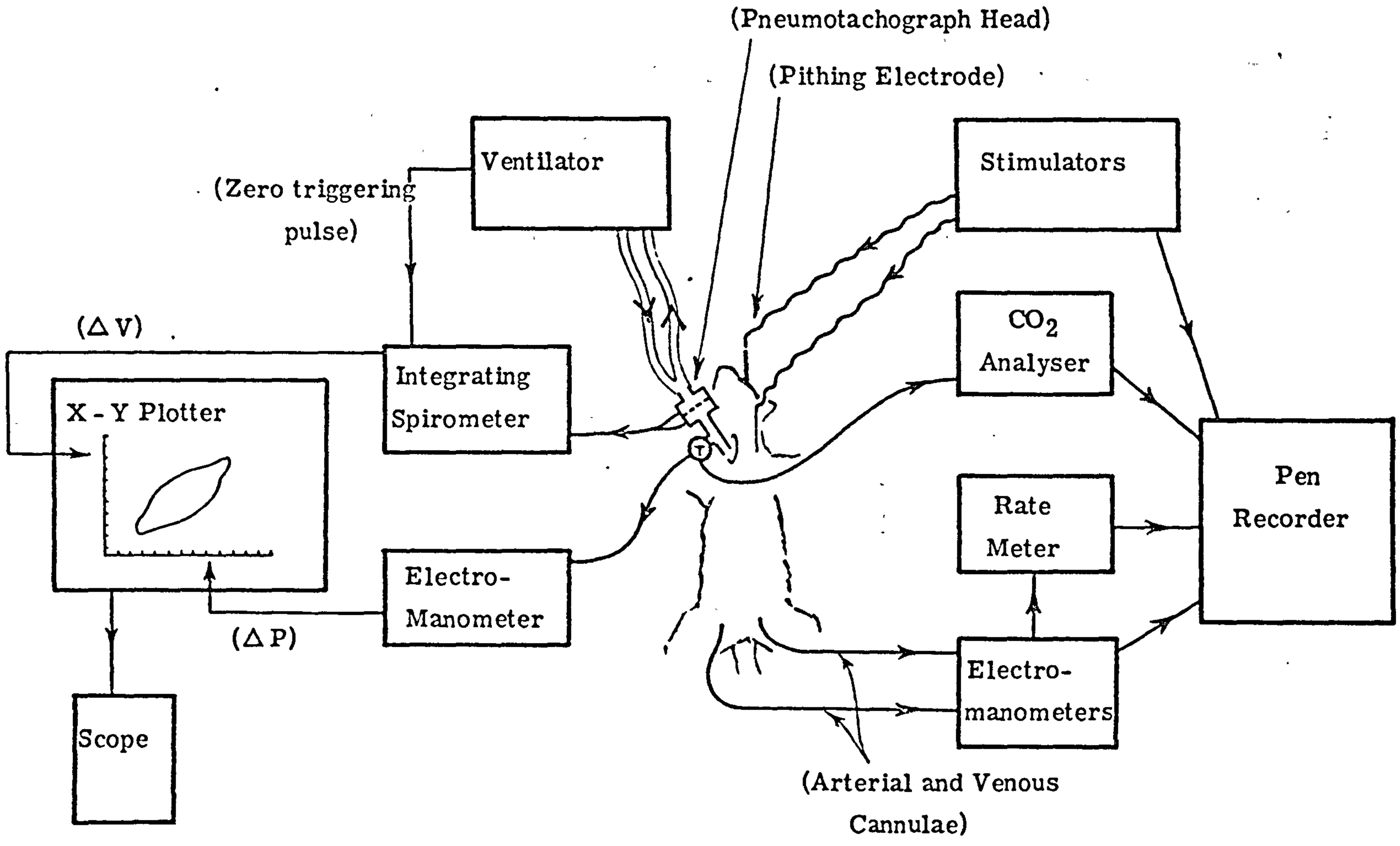


Figure 7:2 PITHED CAT : GENERAL LAY-OUT

A rectal thermometer was inserted and the temperature was kept at 38 ± 1°C.

Femoral arterial and venous cannulae were inserted and advanced proximally.

Instrumentation

The general lay-out is shown in Figure 7:2.

a) Pressure/volume trace

A pneumotachograph head was connected to the tracheal cannula and the differential pressure outputs taken to an integrating spirometer (Mercury Electronics CS1). From there, a continuously-integrated volume signal was taken to the Y axis of a Yokogawa Electric Works X-Y recorder.

A cannula inserted just proximal to the pneumotachograph head conveyed airway pressure information to an Elcomatic EM 750 pressure transducer. The signal, after passing through the appropriate amplifiers was inserted into the X axis of the recorder. The dual input to the X-Y recorder was split so that an additional continuous trace of pressure/volume could be displayed on an oscilloscope (Tektronix Type 502A).

b) Ventilation

A Palmer oscillating pump supplied room air during the inspiratory phase and allowed passive expiration to atmosphere. The rate and stroke were adjusted initially (within physiological limits) to maintain a normal end-tidal P_{CO_2} (circa 35 torr); thereafter the

stroke volume was not altered. Gas sampling for P_{CO_2} was via a three-way tap from the same cannula used to measure airway pressure; it was analysed on a Hartmann & Braun infra-red CO_2 analyser (Model URAS4).

A specially designed cam mounted on the crankshaft spindle of the ventilation pump operated a microswitch which acted as a trigger to zero the CS1 integrator at the start of each respiratory cycle.

c) Cardiovascular parameters

Systemic arterial and central venous cannulae were connected to Elcomatic pressure transducers and the signal after amplification was displayed on an S.E. Laboratories U-V recorder (Model SE 2005). Also displayed on the recorder were the outputs from a heart-rate meter (Devices Model 2750) triggered from the arterial pressure waveform, from the CO_2 analyser, and an 'incident signal' from the stimulator.

d) Stimulator

The distance between the stimulating electrode inside the spinal column and the stellate ganglion called for high voltage stimulation, and this was obtained by using two stimulators in series; i.e. with a Mercury Electronics Model S1 (low voltage) triggering a Mercury Electronics 'Isolated Stimulator' which acted as a slave unit.

A consistently supramaximal value was found to be 50 volts, and this was used as standard. Frequency was set at 50 Hz, and the waveform, being a very highly chopped sine wave, was almost square.

METHODS AND RESULTS

On completion of the surgical procedures and calibration of the equipment, the stimulating electrode was inserted as far as the sacrum. Gallamine triethiodide (5 mg/Kg) was injected intravenously and single low-voltage pulses were delivered approximately every 2 seconds until neuromuscular block was complete.

The stimulating electrode was then withdrawn to the upper thoracic region (approximately T2-4) with about one centimetre of electrode exposed.

A baseline pressure/volume trace was obtained, and a 50 volt, 50 Hz stimulus applied for 30 seconds.

The pressure/volume trace was observed continuously on the oscilloscope and either recorded continuously on slowly moving paper or recorded every fifth or so cycle on stationary paper on the X-Y recorder. Recording continued for 10 minutes after each stimulus.

<u>On no single instance was there any deviation on the trace to indicate a change in compliance</u>.

Many different combinations of electrode length, electrode level and stimulator voltage and frequency were tried, but none of them made the slightest difference to the pressure/volume trace.

It was consistently possible to separate the cardio-accelerator and cardio-pressor responses, as described by Gillespie, Maclaren and Pollock (1970). With one centimetre of electrode exposed at about C7 - T1, the standard stimulation produced an immediate marked rise in heart rate with a minimal or absent pressor effect. On further insertion of the electrode to about T5-8, the

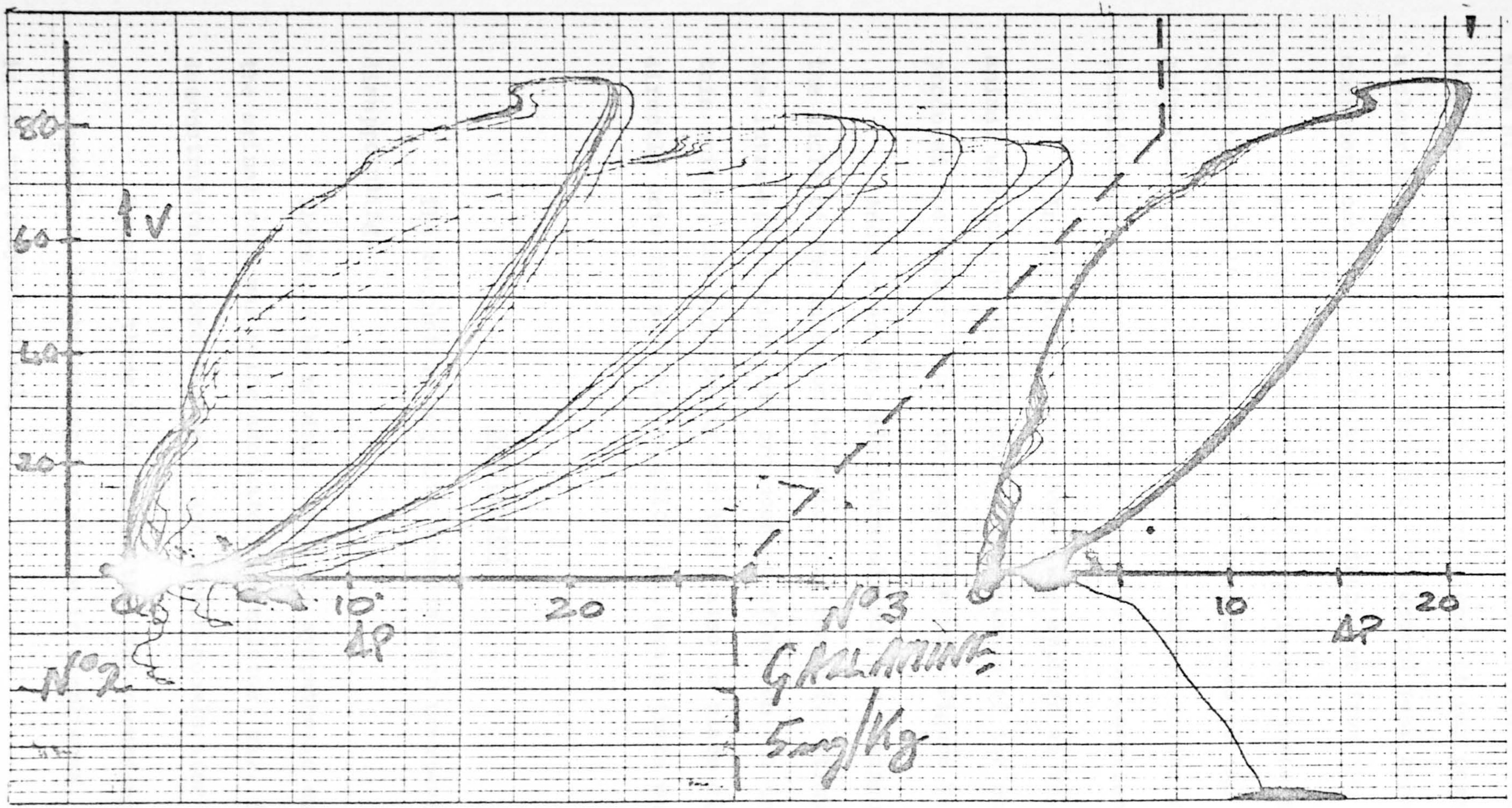


FIGURE 7:3. MONTAGE (UNRETOUCHED) OF COMPLIANCE CHANGE ON STIMULATION, BEFORE AND AFTER ADMINTSTRATION OF GALLAMINE.

same stimulus produced a much smaller rise in heart rate, but a large rise in blood pressure. On many occasions, it was possible to see a clear biphasic pressor response, the second peak representing a component caused by the release of adrenaline from the adrenal glands.

At least once during each experiment, these two positions were 'straddled' by exposing 6 to 8 centimetres of the electrode and stimulating for 30 seconds; immediate cardiovascular responses were consistently observed, but never any effect on compliance.

In one animal, stimulation was applied at T1-4 before the animal was paralysed; the consequent tetanus of the chest muscles produced the expected fall in compliance (see Figure 7:3).

Another animal, also unparalysed, was exsanguinated and the whole of the anterior thoracic wall removed. Stimulation via the intra-spinal electrode produced no effect on the trace. In this animal, the opportunity was taken also to place electrodes directly onto the stellate ganglion, but again there was no effect.

DISCUSSION

The completely negative results were unexpected and disappointing.

There can be no doubt that the sympathetic outflow from the spinal cord was being adequately stimulated, viz. the classical demonstration of cardio-pressor and cardio-accelerator responses.

Equally, there is no doubt that the recording equipment was capable of demonstrating any change in compliance, as, for example, in the unparalysed animal.

Beckman refers to the varying effect of different anaesthetic agents on the response to stellate ganglion stimulation (Beckman, Bean & Baslock, 1974). Thus, pentobarbital anaesthesia 'prevented most compliance and surface tension changes from .... stellate ganglion stimulation', and phencyclidine 'blocked three fourths of the usual compliance decrease after head injury'. Even with his anaesthetic agent of choice, ketamine, he was careful to stress that only 'light anaesthesia' was used.

It is difficult, however, to reconcile this presumably central effect with blockade in what is virtually an isolated lung/nerve preparation - particularly so in the case of his exsanguinated cats.

Following repeated stellate ganglion stimulation, the lungs of two animals from this series were prepared by freeze-drying and conventional formalin fixation for histology as described in Section 3. They were completely normal, and it was not considered justified in terms of time or money to look at them further by electron microscopy.

After consistently negative results on eight animals, the experiment was abandoned.

SECTION 8

DOG EXPERIMENTS SERIES 3

PATHOPHYSIOLOGICAL STUDY AT 2 ATA

EFFECTS OF ADRENERGIC BLOCKADE

PATHOPHYSIOLOGICAL STUDY AT 2 ATA

EFFECTS OF ADRENERGIC BLOCKADE

REVIEW

It has been known for many years that the sympathetic nervous system is involved in the development of pulmonary oxygen toxicity (Bean, 1945; Bennett, 1972).

Bilateral excision of the adrenal medulla, or total bilateral adrenalectomy has been shown to decrease mortality and protect against pulmonary damage in rats (Gerschman, Gilbert, Nye, Nadig & Fenn, 1954; Bean & Johnson, 1955; Taylor, 1958; Rucci, Satta & Campodinico, 1965), and mice (Gerschman, Gilbert, Nye, Price & Fenn, 1955) exposed to 3.0 to 6.0 ATA of oxygen.

Administration of adrenaline augments the oxygen-produced pulmonary damage (Bean, Johnson & Smith, 1954; Bean & Johnson, 1955; Gerschman et al., 1955; Taylor, 1958) and also reverses the protection afforded by adrenalectomy (Bean & Johnson, 1955; Gerschman et al., 1954, 1955; Taylor, 1958). Noradrenaline also has been shown to enhance pulmonary oxygen toxicity (Gerschman et al., 1955; Maritano, Cabrai, Pattono & Marchiaro, 1966).

Pretreatment with tyramine, a catecholamine-releasing agent, caused a greater increase in lung water content following exposure of rats to 5 ATA of oxygen than exposure to oxygen alone (Hammond & Akers, 1974).

The sympathomimetic agents amphetamine and L-Dopa both enhance the development of pulmonary oedema in rats at 3.7 ATA (Wood

& Perkins, 1970).

Reserpine, a catecholamine depleting agent, protects against oxygen-induced pulmonary pathology in rats (Bean, 1956a, 1956b; Hammond & Akers, 1974).

The ganglion-blocking agents, hexamethonium and tetraethylammonium have both been shown to have a beneficial effect on the mortality and pulmonary pathology in rats exposed to oxygen at 80 p.s.i.g. (6.44 ATA) (Johnson & Bean, 1957).

Peripheral alpha-adrenergic blockade protects against oxygen poisoning.

Phenoxybenzamine (Johnson & Bean, 1957; Pagni, Zampolini & Frullani, 1967), dibenamine and SKF 501 (Johnson & Bean, 1957) and phentolamine (Hammond & Akers, 1974) prevented pathological changes in rat lungs following oxygen exposures at pressures of 3.0 to 6.44 ATA.

Beta-adrenergic blockade (propranolol), however, appeared to afford no protection against the formation of pulmonary oedema (Hammond & Akers, 1974).

The cation lithium which is known to decrease the sensitivity of man to infused noradrenaline (Fann, Davis, Janowski, Cavanaugh, Kaufmann, Griffith & Oates, 1972) possibly by reducing the sensitivity of receptor sites, protects against the development of pulmonary oedema in rats exposed to 100% oxygen at 5.5 ATA (Radomski & Watson, 1973).

In all of the experiments reviewed above, extremely high pressures of oxygen were used (i.e. greater than 3 ATA) and all the

animals had convulsed. It is impossible to say to what extent the effects on the lungs were directly due to oxygen or whether there is a contribution from a non-specific centri-neurogenic oedema mechanism as discussed in the previous section. It is probable that both mechanisms are involved, and with this in mind, the role of the circulating biogenic amines in the pathogenesis of pulmonary oxygen toxicity was recently investigated in dogs exposed to various high pressures of oxygen (Demeny, Manger, Naftchi & Reich, 1973).

Animals which had convulsed following exposure to high pressures of oxygen showed a significant elevation of both adrenaline and noradrenaline both during and for up to 30 minutes following convulsion, whereas animals which were exposed for 8 to 10 hours to sub-ictal pressures (2.0 to 2.3 ATA) showed no such rise. The lungs of these animals showed the early changes of oxygen toxicity, however, suggesting that, while adrenaline and noradrenaline may contribute to pulmonary oxygen toxicity, their role is secondary to the onset of convulsions.

There is much less information available concerning the involvement of the sympathetic nervous system at normobaric pressures.

Administration of exogenous adrenaline has been shown to potentiate oxygen toxicity in rats breathing 100% oxygen at 1 ATA (Bean & Smith, 1953; Smith & Bean, 1955), but these findings must be interpreted with caution as it is now recognised that administration of adrenaline per se may result in pulmonary oedema (Ersoz & Finestone, 1971). Following exposure of rabbits to 100% oxygen at 1 ATA for 72 hours, Smith, Winter and Wheelis (1973) found a large (sevenfold) increase in circulating total catecholamines, with

noradrenaline being affected slightly more than adrenaline.

There appears to be nothing in the literature concerning a protective effect of sympatholytics at a pressure of 2 ATA, and certainly nothing regarding the time-course and mechanisms of such an effect.

It was decided therefore to repeat the pathophysiological study described in Section 3, following prior administration of an adrenergic blocking agent.

PHARMACOLOGY

Two pharmacological substances were used: the alpha-adrenergic blocking agent phentolamine mesylate B.P. ('Rogitine' CIBA) and the adrenergic neurone blocking agent guanethidine monosulphate B.P. ('Ismelin' CIBA).

Phentolamine

Phentolamine is a competitive antagonist at alpha-adrenoreceptors.

It suffers from the disadvantage that it is relatively short-acting, and hence the persistence of alpha-adrenergic blockade had to be monitored at regular intervals.

In a preliminary experiment on a 25 Kg. greyhound anaesthetised and prepared as described in Section 3, it was found that 50 nanograms/Kg. of noradrenaline ('Levophed' WINTHROP) produced a barely discernable rise in blood pressure whereas 100 nanograms/Kg. consistently produced an obvious, but transient, rise. This was used

as the 'test dose' thereafter.

Phentolamine was given intravenously in 2 mg. doses at two minute intervals until the test dose of noradrenaline failed to produce a response; the total dose of phentolamine required was found to be 6 mg.

The animal was then pressurised to 2 ATA and allowed to breathe 100% oxygen. The noradrenaline response was tested at 15 minute intervals over the course of six hours, with increments of phentolamine given as necessary to maintain a block; it was determined that the drug was being metabolised at the rate of 5 mg/hour.

During the experiments proper, on three greyhounds, the test dose of noradrenaline was determined for each animal prior to pressurisation. A standard 5 mg. of phentolamine given intravenously produced a block in all three dogs and thereafter it was administered by continuous intravenous infusion at the rate of 5 mg/hour. Noradrenaline responses were tested every four hours; on several occasions minimal responses, indicating recovery from blockade, were obtained, necessitating additional increments of 5 mg. of phentolamine.

Guanethidine

The pharmacological actions of guanethidine are fully described in a review by Boura & Green (1965) from which most of the information quoted here is taken.

Whereas phentolamine blocks alpha-adrenergic receptor sites

to both locally released and circulating catecholamines, guanethidine blocks only the release of transmitter from adrenergic nerve fibres, leaving the receptor sites fully functional.

There is good evidence that following guanethidine administration, adrenal medullary function is left largely intact. For example, the pressor response to splanchnic nerve stimulation or the intravenous administration of dimethylphenylpiperazinium in anaesthetised cats or dogs is normally biphasic; the initial rapid component attributable to post-ganglionic vasomotor fibres is suppressed by guanethidine whereas the second delayed component, caused by catecholamines released from the adrenal medulla may be unaffected or even enhanced. Following adrenalectomy, both responses are abolished.

This 'adrenal sparing' action of guanethidine has very recently been confirmed (Clarke & Romanyshyn, 1976).

The effect produced by guanethidine therefore is in many ways similar to a post-ganglionic sympathectomy, i.e. local neurally-mediated responses are abolished, whereas any centrally mediated release of catecholamines from the adrenal glands can still exert a full or exaggerated effect on peripheral adrenergic receptors.

In the context of this experiment it can thus be proposed that any toxic effects on the lungs produced by oxygen and protected against by guanethidine can to a large extent be attributed to a local sympathetic nerve discharge.

Two problems arise in the use of guanethidine.

The first is that there is a very marked pressor response

immediately following the intravenous injection of the drug. This is thought to be due not to a direct sympathomimetic effect, but to the local release of catecholamines from nerve endings, as the pressor response following guanethidine is abolished by prior administration of an alpha-adrenergic receptor antagonist. The hypertensive effect on the animal can be minimised by giving the drug in divided doses some 15 minutes apart, but the demonstration that another catecholamine releasing agent, tyramine, can cause an increase in lung water (Hammond & Akers, 1974) suggests a more serious complication, that guanethidine itself may contribute to the oedema process.

Two normobaric air-breathing, guanethidine-blocked controls were therefore set up; they showed that this effect was minimal (see Table 8:3).

The second problem was that of demonstration of effective neuronal block. There is no simple method of doing this analogous to the noradrenaline response test for competitive receptor blockers and it was decided to rely on a dose which according to a review of the relevant literature was fairly certain of producing a block. It is felt that this approach was justified in retrospect by the degree of protection demonstrated.

Additional evidence for the effectiveness of the dosage came from fluorescence microscopy of lung tissue. Inflated peripheral portions of lung were taken routinely for liquid nitrogen quenching and fixation by freeze-drying to demonstrate histologically any intra-alveolar oedema (see Section 3); in six of the guanethidine-blocked dogs the opportunity was taken to process these tissues by the Falck technique and to look at the distribution of catecholamines.

There is controversy as to the extent to which tissue depletion of catecholamines occurs following administration of guanethidine (Boura & Green, 1965), but in all of the sections examined there was an almost complete absence of specific fluorescence even in structures such as arterioles.

MATERIALS AND METHODS

Distribution of the animals

Eighteen greyhounds of weights ranging from 23 to 30 Kg. were used.

One animal died suddenly for no obvious reason during pressurisation. At autopsy the lungs appeared normal; they were retained and processed for wet/dry weight ratio and used as a part of the control (see Section 5:17).

Controls

Three animals, unblocked, breathed 100% oxygen at 2 ATA until death, exactly as described in Section 3. These provided control wet/dry lung weight ratio values following oxygen exposure and in addition served as controls to demonstrate any possible species difference in response to oxygen between greyhounds and the mongrels which had been used previously.

Two guanethidine-blocked dogs breathed air at normobaric pressures to serve as a drug control. One was sacrificed at 25¼ hours (i.e. at a convenient time which was outwith the longest survival time of the experimental animals), and the other at 21¾ hours (mean time of death of the guanethidine-blocked experimental

animals).

Experimental

Of the twelve 'experimental' animals, one was used to determine a typical dose schedule for phentolamine (see Page 8:4), three were blocked with phentolamine and exposed to 100% oxygen at 2 ATA until death, and eight were blocked with guanethidine and were similarly exposed.

Equipment and techniques

The equipment, gas delivery system, measurement techniques and calculations were exactly as described in Section 3.

Particular emphasis was placed on the measurement of terminal blood gas values, cardiovascular parameters, pulmonary compliance, and percentage pulmonary shunt ratio.

Anaesthesia

Anaesthesia was by intravenous induction followed by continuous intravenous infusion, as described in Section 5.

Preparation of tissues

At autopsy, the macroscopic appearances of the lungs were noted; they were then excised and processed for wet/dry weight ratios as described in Section 5. Peripheral portions of lung tissue were taken routinely and fixed by freeze-drying to demonstrate any intra-alveolar oedema (see Section 3). In addition, in the guanethidine-blocked dogs, a small portion of lung tissue was diced under

gluteraldehyde and fixed for examination by electron microscopy; these tissues have not yet been sectioned.

RESULTS

Control animals

1) Physiological parameters

a) Anaesthetic controls

These animals have already been described (see Page 3:9); they remained normal.

b) Normobaric air-breathing, guanethidine-blocked controls

The animal which was sacrificed at 25¼ hours had shown a fall in P_aO_2 from 93 torr at 14 hours to 56 torr (S_aO_2 = 89%) at 22 hours, and to 51 torr (S_aO_2 = 85%) immediately before death. Cardiac output had fallen slightly (5.7 to 4.7 l/minute) over the last three hours, but all other parameters, in particular P_aCO_2, systemic and pulmonary arterial pressures, compliance, and shunt ratio remained normal.

The other animal (sacrificed at 21¾ hours) also showed a late fall in P_aO_2 (84 to 67 torr - S_aO_2 = 90%) and a fall in systemic arterial pressure from 100 to 75 mm.Hg. All other parameters were normal at the time of sacrifice.

c) Unblocked oxygen-poisoned controls

These three animals behaved exactly as the main group described in Section 3; i.e. they demonstrated a late fall in P_aO_2, compliance and shunt ratio, and died from a fulminating pulmonary oedema.

2) Lung wet/dry weight ratios

a) Anaesthetic controls

The mean weight ratio for four animals was 4.60 (S.E. = ± 0.09) (see Figure 8:8 and Appendix 4).

b) Normobaric air-breathing, guanethidine-blocked controls

The weight ratio for the animal sacrificed at 21¾ hours was 4.75 and for that at 25¼ hours 5.41 (see Table 8:3 and Appendix 4).

c) Unblocked oxygen-poisoned animals

The mean weight ratio for these three animals was 9.65 (S.E. = ± 0.175) (see Figure 8:8). This is in good agreement with the ratio obtained in the oxygen-exposed lung of animals described in Section 5, where a value of 9.37 (S.E. = ± 0.53, n = 6) was obtained (see Figure 5:10).

Experimental animals (block + oxygen)

General pattern of results

There was little difference between the phentolamine and the guanethidine blocked animals, and in general they are not separated in the graphs of results. Where differences were observed, they are mentioned in the text.

Time of death

There was large variation in the survival times of both groups of animals.

The three phentolamine-blocked animals died at 17, 18 and

24¼ hours.

The mean time of death for the guanethidine-blocked animals was 21¾ hours, with a range from 18 to 25¼ hours.

There is no significant difference between this mean and that of the unblocked oxygen-poisoned dogs described in Section 3 (mean of 22¼ hours).

The individual times of death are listed in Table 8:1.

Mode of death

Ventricular asystole or fibrillation preceded apnoea in six of the eleven blocked dogs; this is in marked contrast with those described in Section 3.

The mode of death relative to the type of block is demonstrated in Table 8:1; the number of animals involved is insufficient to allow any conclusions to be drawn.

Physiological parameters

The corrected and derived data for one animal (Dog 5) are presented in Table 8:2 (see Appendix 2). The meaned values from which the graphs were constructed are reproduced in Appendix 3.

1) Blood gases

Arterial oxygen tension fell gradually over the last ten hours of the experiment, but was still at very high levels terminally (mean of 838 torr at one hour and 628 torr at 15 minutes before death) (Figure 8:1).

Arterial carbon dioxide tensions were consistently higher than normal throughout the experiment (overall mean of 55 torr (S.E.= 1.7) for the first twelve hours). A steep terminal rise in P_aCO_2 occurred at about two hours before death; i.e. it preceded the sharp fall in P_aO_2 (Figure 8:2).

2) Cardiovascular parameters

Heart rate, mean systemic arterial pressure and cardiac output are shown as a combined graph in Figure 8:3.

There is a gradual, but progressive, fall in all three parameters over the last eight hours before death. Arterial pressure fell from a mean of 82 mm.Hg. at four hours before death to 28 mm.Hg. at 10 minutes before death. Cardiac output recorded a few minutes before death showed a fall to less than 40% of the baseline value, i.e. from 4.52 l/min (S.E. = ± 0.13) to 1.77 l/min (S.E. = ± 0.17).

Mean pulmonary arterial pressure over the first twelve hours of the experiment was 9.0 ± 1.5 torr. There was an apparent rise at eight hours before death which did not attain significance, after which it fell steadily to a mean value of 4.6 mm.Hg. a few minutes before death (Figure 8:4).

Mean pulmonary arterial wedged pressure (Figure 8:4) remained steady at around 1 to 2 mm.Hg. throughout the experiment. Yet again the unexplained dip in pulmonary arterial and pulmonary arterial wedged pressures was seen at about one hour before death (cf Figures 3:18, 3:19 and 5:8).

Pulmonary vascular resistance remained much more steady

throughout this experiment than in the two previous series (cf Figures 3:20 and 5:9). The terminal rise was much less marked and failed to reach the level of significance.

Percentage pulmonary shunt ratio also remained at normal low levels throughout the experiment. There was a slight rise (from 19 to 22.5%) terminally (Figure 8:6).

3) Compliance

For the graphical demonstration of compliance, the animals were divided into two groups: those whose lungs were completely normal at autopsy and those with any focal atelectasis or consolidation.

By doing this, the sample size at each increment of time was much reduced, and some of the meaned values plotted on these two curves do not contain contributions from all of the animals; this is reflected in the large standard error bars.

The graphs are plotted as before, i.e. by calculating compliance as a percentage drop from a baseline value which incorporates all the results obtained prior to 10 hours before death. In the case of the 'atelectatic group', this has tended to mask the actual time of onset of the fall, but in most cases it was in fact around 10 to 12 hours before death.

The 'normal' group showed a fall in compliance over the last six hours or so to about 70% of the baseline value; the fall in the 'atelectatic' group commenced much earlier, at around 10 to 12 hours before death, and reached a value of 17% of baseline immediately before death.

Autopsy

The macroscopic appearances of the lungs are summarised in Table 8:1.

In only four animals did the lungs appear to be completely normal.

In two animals there were patchy areas of atelectasis not extending to whole lobes, in one or both lungs.

In the remaining five animals there was a total haemorrhagic consolidation of one or more lobes - appearances very reminiscent of the classical description of lung changes in pulmonary oxygen toxicity, and in marked contrast to those seen in Section 3.

In no case was there an overt intra-alveolar oedema.

Lung wet/dry weight ratios

These are shown in Figure 8:8.

The actual mean values obtained, including those from the air-breathing guanethidine-blocked controls are shown together with their significance limits in Table 8:3.

An interesting correlation is that between the mode of death (i.e. primarily in apnoea or asystole) and lung weight ratios (see Table 8:1).

The animals dying primarily in apnoea had larger wet/dry lung weight ratios (mean of 5.78, S.E. = $\pm$ 0.147, n = 5) than those dying in ventricular asystole or fibrillation (mean of 5.35, S.E. = $\pm$ 0.120, n = 6). This difference just failed to reach significance at the 5% level (t at 0.05 = 2.2622; t obtained = 2.2521).

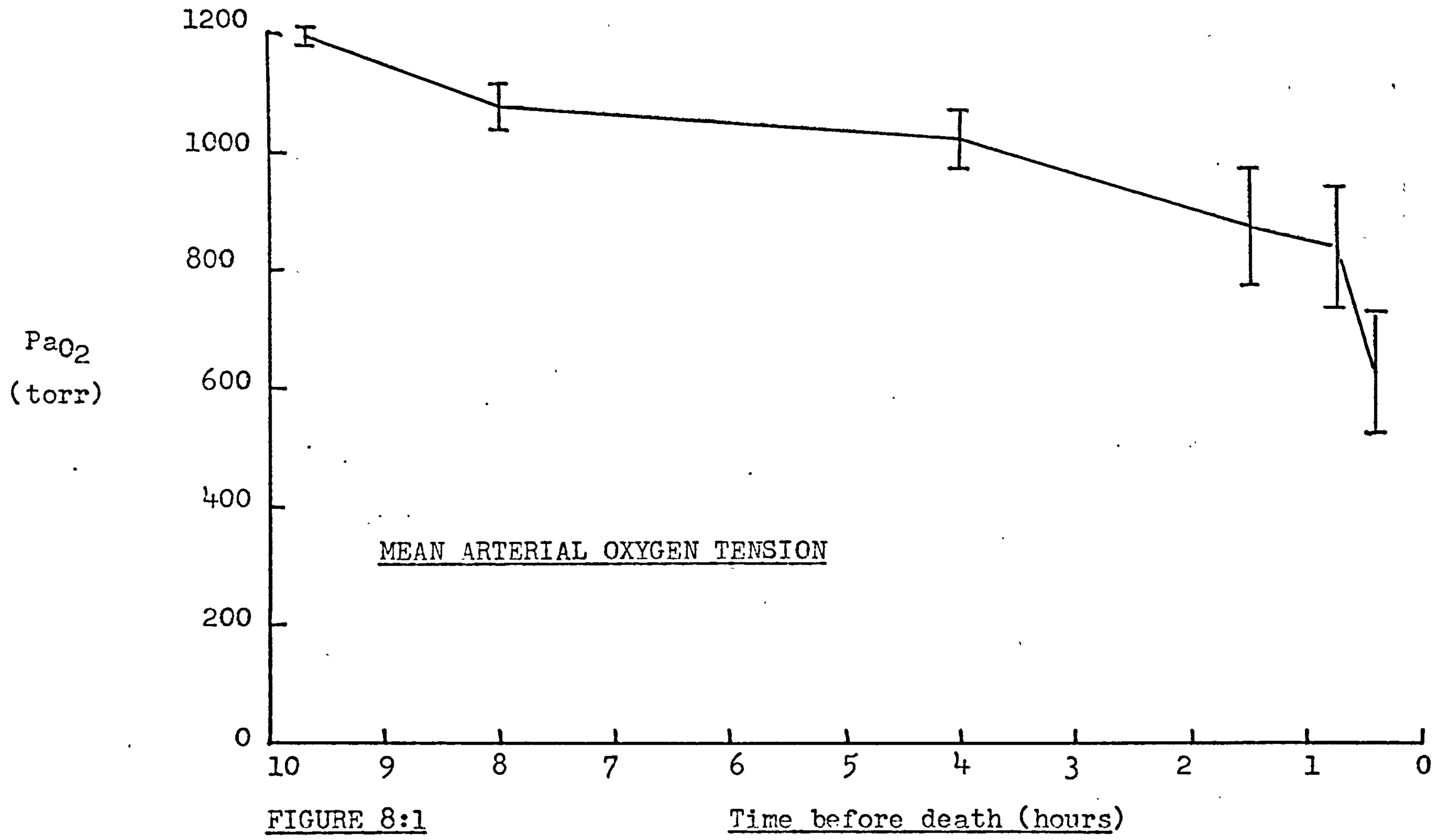

1200
1000
800
600
400
200
0
Pa_{O_2}
(torr)
MEAN ARTERIAL OXYGEN TENSION
10
9
8
7
6
5
4
3
2
1
0
Time before death (hours)

FIGURE 8:1

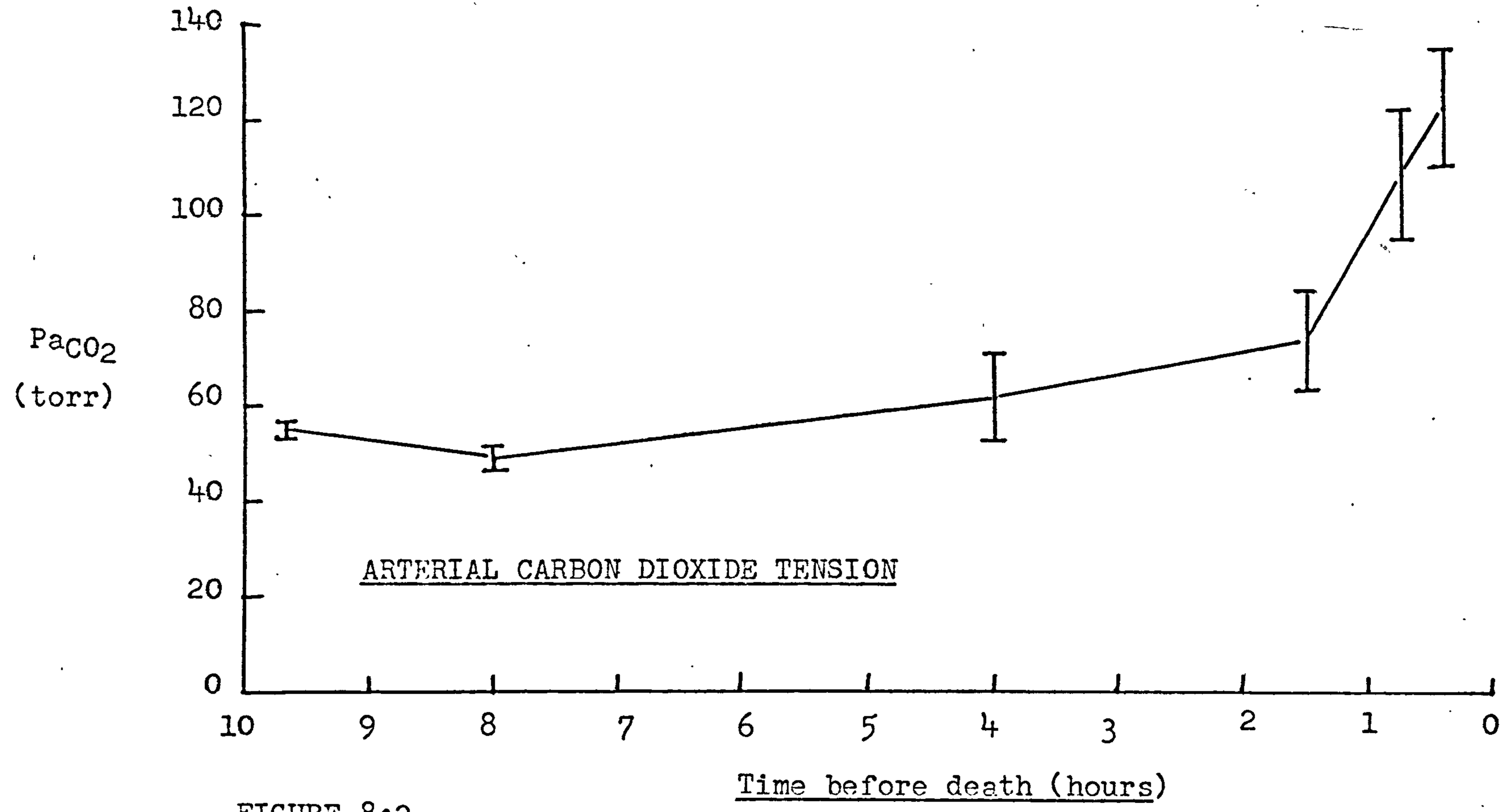

140
120
100
80
60
40
20
0
Pa_{CO_2}
(torr)
ARTERIAL CARBON DIOXIDE TENSION
10
9
8
7
6
5
4
3
2
1
0
Time before death (hours)

FIGURE 8:2

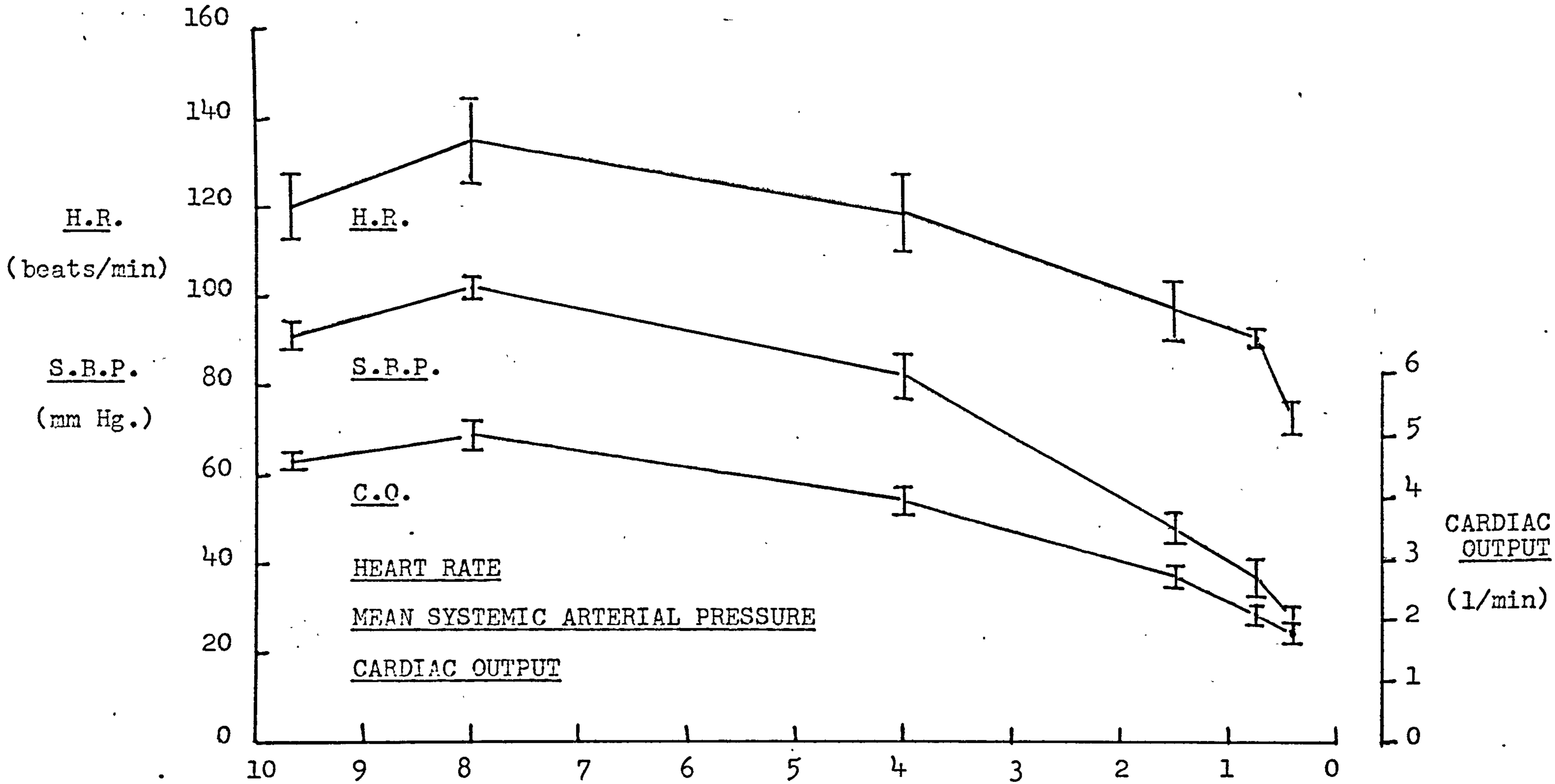

160
140
120
100
80
60
40
20
0
H.R.
(beats/min)
S.B.P.
(mm Hg.)
H.R.
S.B.P.
C.O.
HEART RATE
MEAN SYSTEMIC ARTERIAL PRESSURE
CARDIAC OUTPUT
6
5
4
3
2
1
0
CARDIAC OUTPUT
(l/min)
10
9
8
7
6
5
4
3
2
1
0
TIME BEFORE DEATH (hours)

FIGURE 8:3

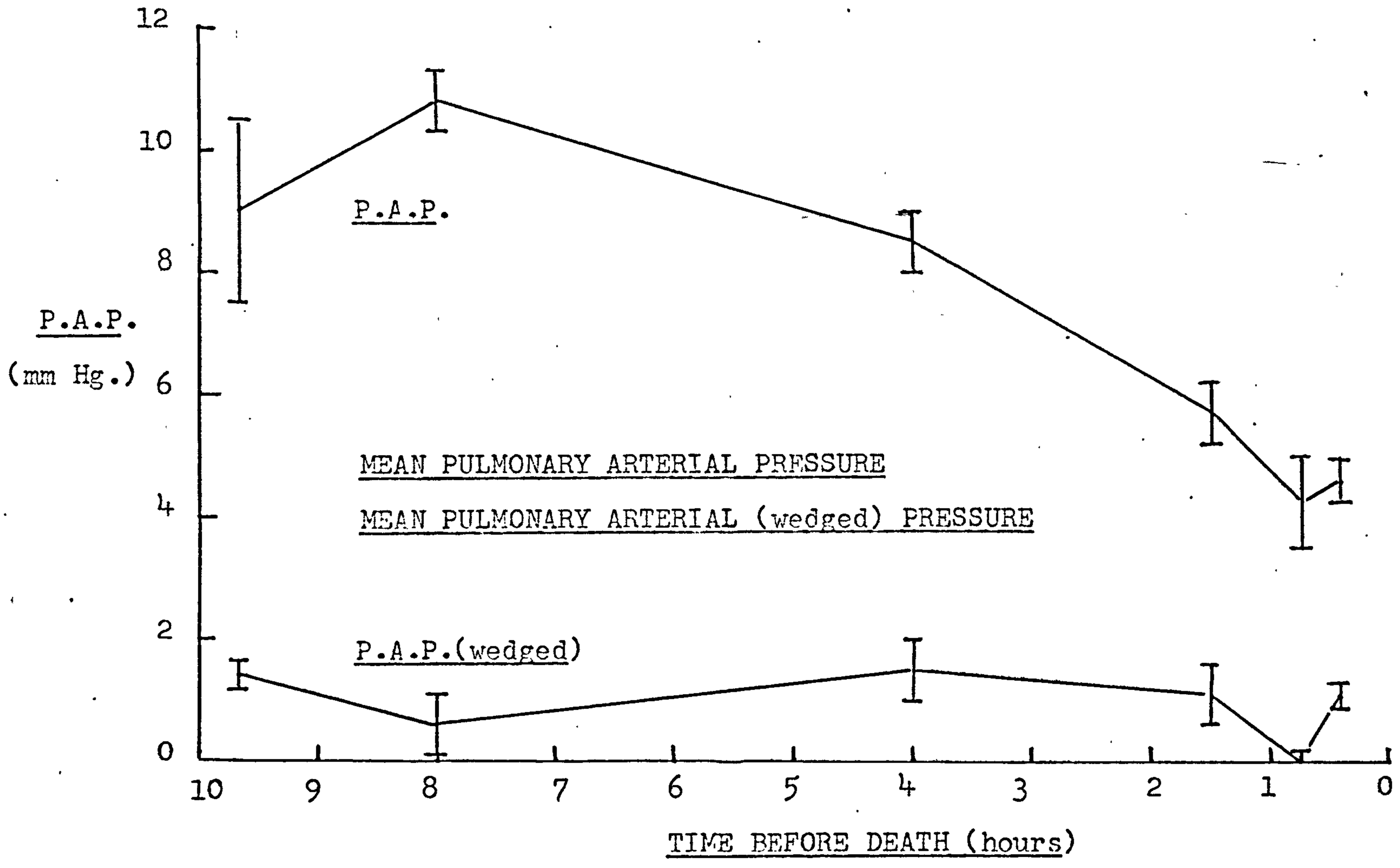

12
10
8
6
4
2
0
P.A.P.
(mm Hg.)
P.A.P.
MEAN PULMONARY ARTERIAL PRESSURE
MEAN PULMONARY ARTERIAL (wedged) PRESSURE
P.A.P.(wedged)
10
9
8
7
6
5
4
3
2
1
0
TIME BEFORE DEATH (hours)

FIGURE 8:4

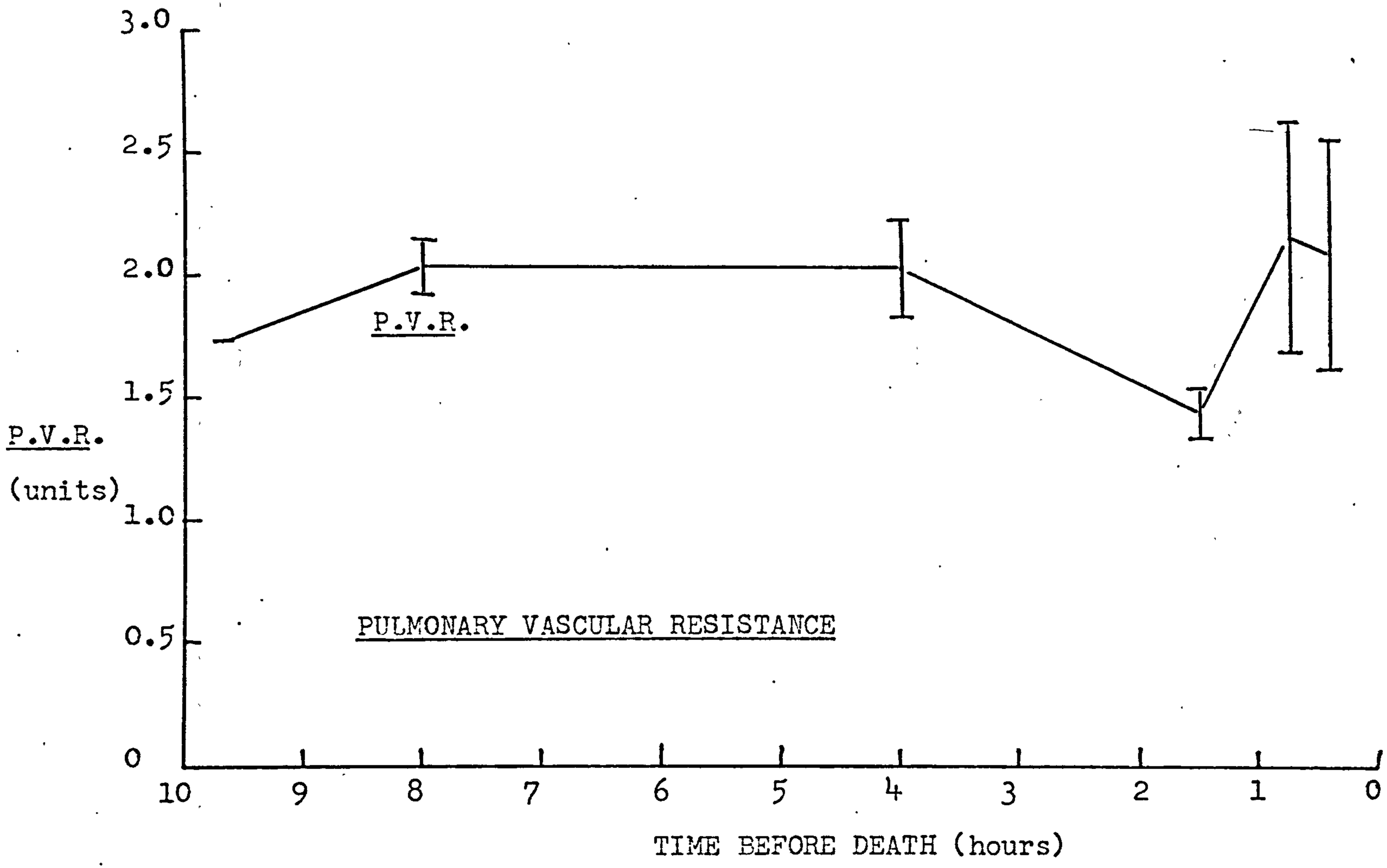

3.0
2.5
2.0
1.5
1.0
0.5
0
P.V.R.
(units)
P.V.R.
PULMONARY VASCULAR RESISTANCE
10
9
8
7
6
5
4
3
2
1
0
TIME BEFORE DEATH (hours)

FIGURE 8:5

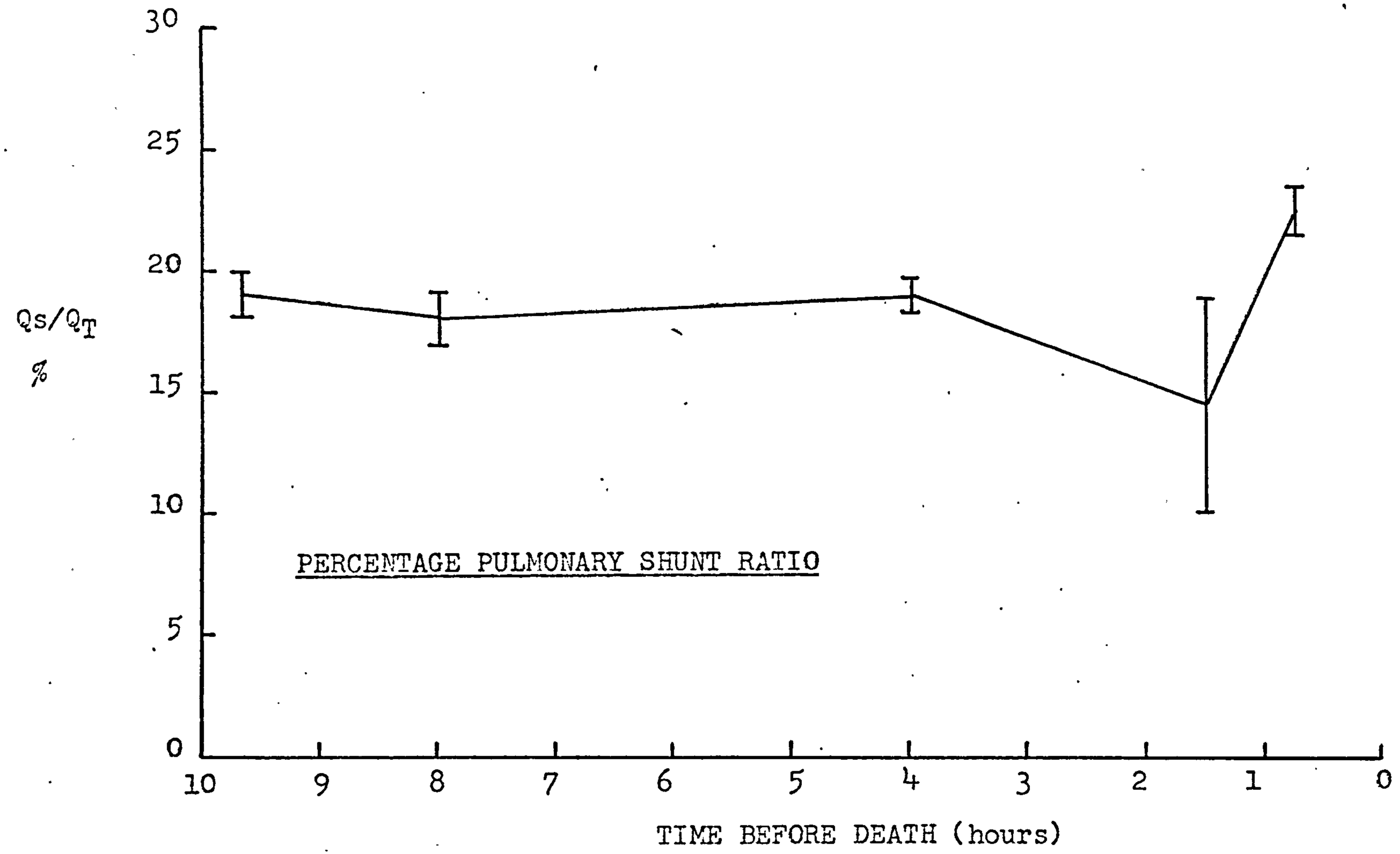

30
25
20
15
10
5
0
Qs/QT
%
PERCENTAGE PULMONARY SHUNT RATIO
10
9
8
7
6
5
4
3
2
1
0
TIME BEFORE DEATH (hours)

FIGURE 8:6

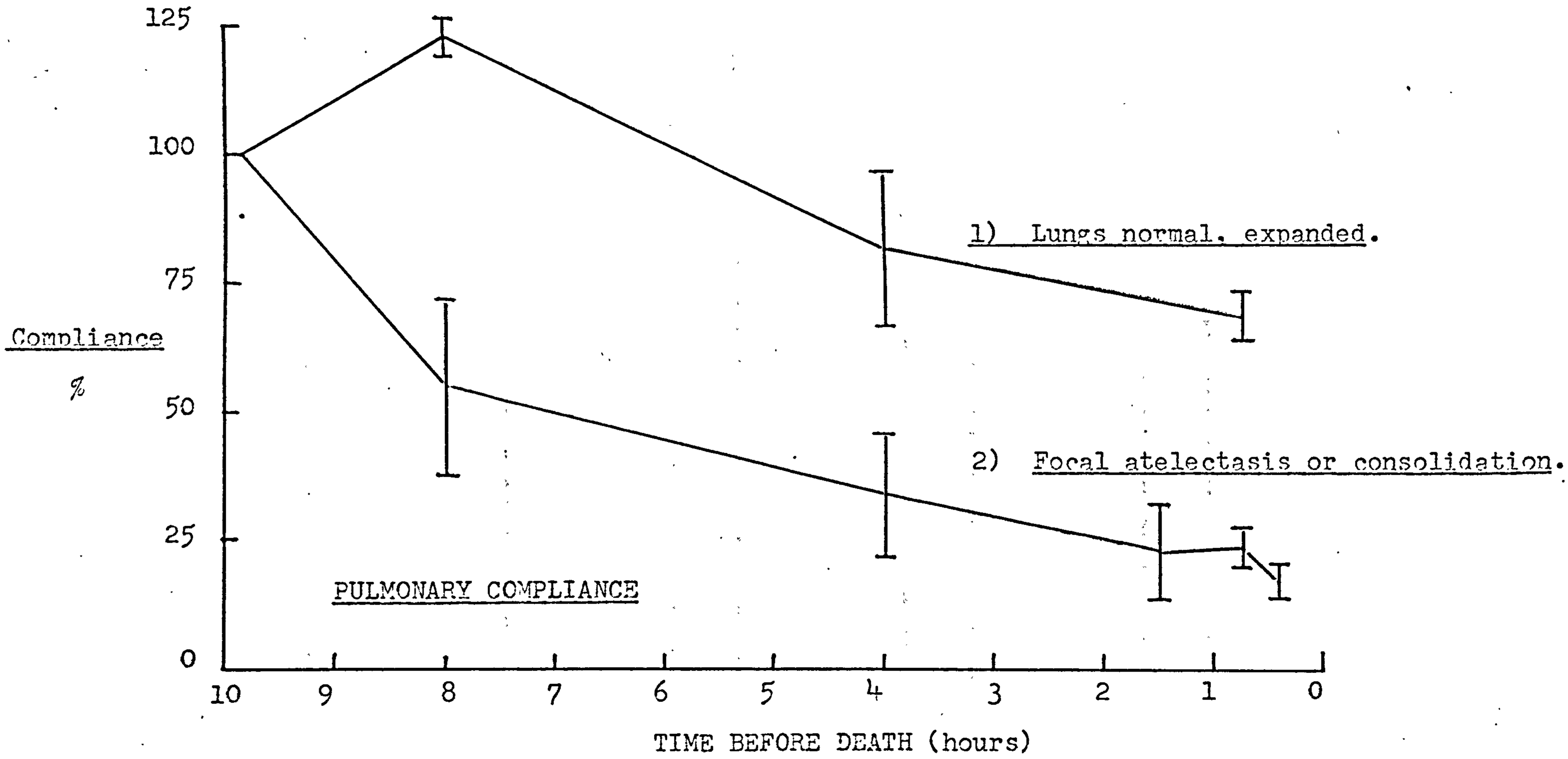

125
100
75
50
25
0
Compliance
%
1) Lungs normal, expanded.
2) Focal atelectasis or consolidation.
PULMONARY COMPLIANCE
10
9
8
7
6
5
4
3
2
1
0
TIME BEFORE DEATH (hours)

FIGURE 8:7

LUNG WET/DRY WEIGHT RATIOS (± S.E.)

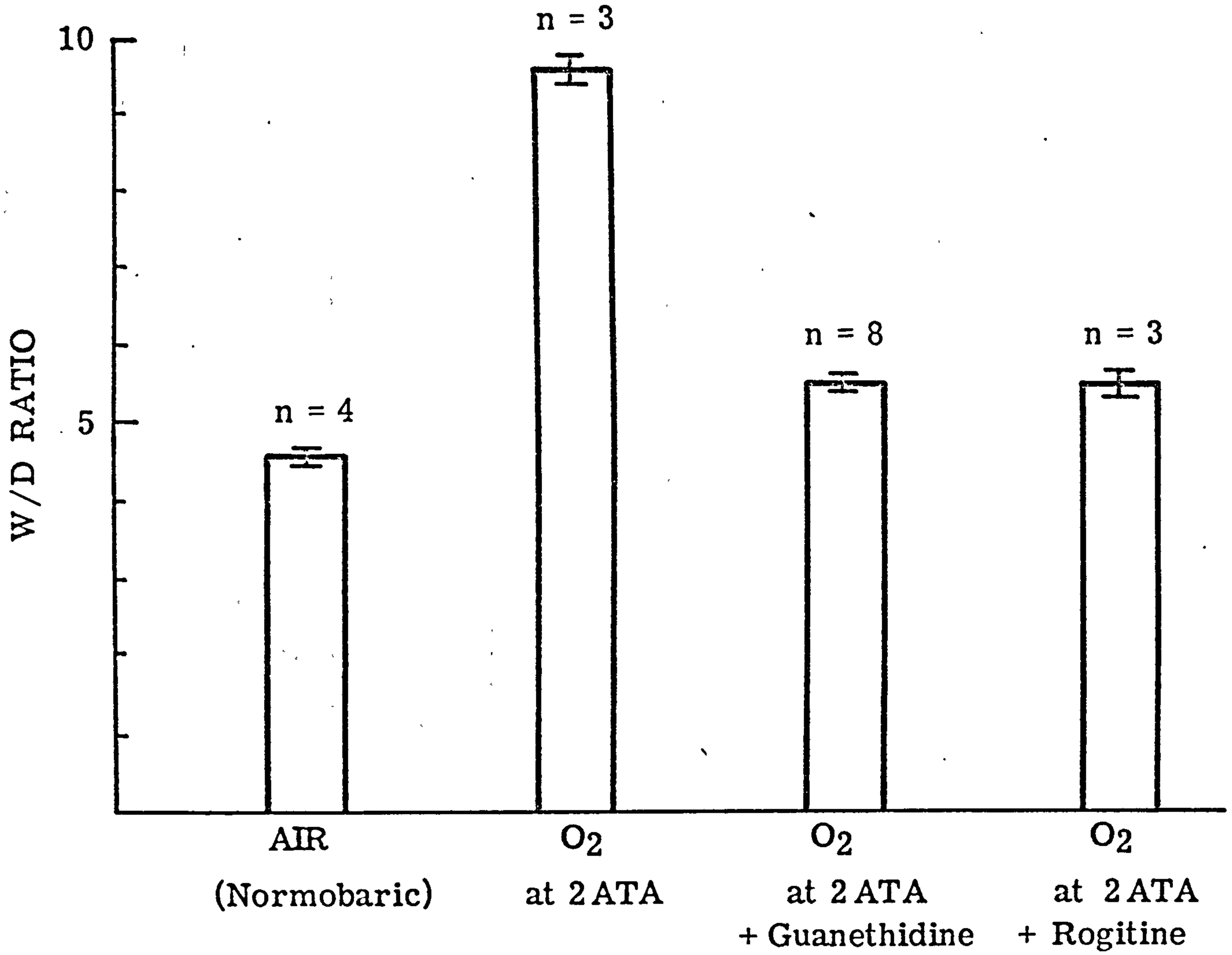


FIGURE 8:8.

TABLE 8:1 LUNG WEIGHTS, TIMES OF DEATH AND PATHOLOGICAL APPEARANCES

DOG NO.	DRUG	CONVULSION	TIME OF DEATH (hours)	MODE OF DEATH	PATHOLOGICAL APPEARANCES	WET/DRY WEIGHT RATIOS
3	P	Nil	17	V.F.	Normal, expanded.	5.39
2	P	Nil	$24\frac{1}{4}$	Apnoea	Focal atelectasis. Both lungs.	5.22
4	P	Nil	18	Apnoea	Collapse and consolidation: (L) lung.	6.01
6	G	Nil	18	V.F.	Normal, expanded.	4.95
7	G	Nil	20	Apnoea	" "	5.76
11	G	Nil	$23\frac{1}{2}$	Asystole	" "	5.32
8	G	at $3\frac{1}{4}$ hrs.	$21\frac{1}{2}$	Asystole	Focal atelectasis: (L) lung.	5.19
5	G	at $18\frac{1}{2}$ hrs.	$25\frac{1}{4}$	Apnoea	Collapse and consolidation: RCL	5.88
10	G	Nil	$23\frac{3}{4}$	Asystole	" " " : LCL LDL	5.84
12	G	at 14 hrs.	$22\frac{1}{4}$	Apnoea	" " " : RCL	6.02
13	G	Nil	$19\frac{1}{2}$	Asystole	" " " : RDL RCL RAL	5.41

Notes: 1) where a lobe or part of a lobe was totally consolidated, this part was not included in the wet/dry weight ratio estimations.

2) Abbreviations: P = phentolamine G = guanethidine V.F. = ventricular fibrillation
Lung lobes: RCL, LCL = (R) & (L) cardiac lobes, RAL = (R) apical lobe,
RDL, LDL = (R) & (L) diaphragmatic lobes.

TABLE 8:3

LUNG WET/DRY WEIGHT RATIOS : SIGNIFICANCE LIMITS

	CONDITION	n	W/D (mean)	W/D (S.E.)	SIGNIFICANCE LIMITS
1	Air-breathing, unblocked.	4	4.60	0.09	————
2	Air-breathing, guanethidine-blocked.	2	5.08	0.023	1:2, P = < 0.2 N.S.
3	O_2 at 2 ATA, unblocked.	3	9.65	0.175	1:3, P = < 0.001
4	O_2 at 2 ATA, guanethidine-blocked.	8	5.55	0.09	3:4, P = < 0.001 2:4, P = < 0.001 1:4, P = < 0.001
5	O_2 at 2 ATA, phentolamine-blocked.	3	5.54	0.16	3:5, P = < 0.001 2:5, P = < 0.6 N.S. 1:5, P = < 0.005

N.S. = not significant (but note very small number of degrees of freedom in each case)

Histology

Light microscopic examination of freeze-dried sections showed that there was no evidence whatsoever of intra-alveolar oedema in any of the animals, and that interstitial oedema was either completely absent or minimal.

DISCUSSION

Mode of death

The unblocked dogs died very differently from the unblocked dogs described in Section 3.

The high incidence of ventricular fibrillation and asystole points towards a much greater degree of myocardial involvement, and the continuing fall in blood pressure and cardiac output over the last eight hours followed by a secondary central respiratory failure was very similar to that observed in Dog 12 of Section 3 - one of the so-called 'cardiac death' dogs.

In particular, there was no evidence either from the pulmonary shunt ratios, macroscopical appearances of the lungs, wet/dry weight ratios, or histology to suggest the development of intra-alveolar oedema.

There was no significant difference in the survival times of blocked and unblocked dogs exposed to the same pressures of oxygen. This pattern of effect (protection against the development of pulmonary oedema without any prolongation of survival time) has previously been reported in phentolamine-blocked rats exposed to 4 ATA of oxygen (Maritano et al., 1966).

The prolonged survival time and relative normality of the air-breathing guanethidine-blocked control animals serve to show that death was not simply a drug-induced phenomenon and that oxygen at least contributed to death; protection against oxygen poisoning was not complete.

Type of blockade

The results from the three phentolamine-blocked animals confirm and extend the widely-reported observation that alpha-adrenergic blocking agents protect an oxygen-poisoned animal against the development of pulmonary oedema and endorse the concept of a causal involvement of the sympathetic nervous system.

The protection afforded by an adrenergic neurone blocking agent would appear to suggest that the oxygen-induced damage is mediated by local adrenergic neurone discharge. This theory is, however, probably untenable; evidence is presented later (see Page 8:21) which suggests that the integrity of the adrenoreceptors to circulatory catecholamines is not sufficient to allow normal function.

Mechanism of protection

1) Prevention of hypertension

A possible means by which adrenergic blockade could protect against the development of pulmonary oedema would be by lowering arterial pressures and by preventing any sympathetically-induced hypertension.

Systemic hypertension, however, has already been discarded as a possible aetiological factor in pulmonary oxygen toxicity, at

least in dogs at 2 ATA (see discussion in Section 3:54).

In the pulmonary circulation, blockade appeared to prevent the fluctuations in pulmonary vascular resistance seen in the unblocked animals (Figures 3:20, 5:9), in that pulmonary arterial pressures were more uniform throughout the experiment. As discussed previously, however, (see Page 3:54) the rises in pulmonary arterial pressure in the unblocked dogs were modest in physiological terms and while they would have added to the net outflow of fluid to the interstitial space, their contribution was not thought to be of great importance.

It should be noted in this context that over the first twelve hours or so of the experiment, the fall in systemic and pulmonary arterial pressures observed following adrenergic blockade were minimal. Over the last eight hours, however, at the time when the events leading to oedema might be anticipated, arterial pressures showed a gradual and sustained fall; systemic arterial down to 30% and pulmonary arterial to 50% of baseline values. The significance of this low terminal pulmonary arterial pressure (mean of 4.6 mm.Hg.) is discussed later.

2) <u>Effects on surfactant</u>

The results presented in Sections 3, 4 and 5 in this thesis provide circumstantial evidence for the role of surfactant in the development of pulmonary oxygen toxicity. Despite the negative findings reported in Section 7, it is further suggested here that the protective effect of adrenergic blockade is mediated by interfering with the sympathetic control of surfactant release.

This is discussed more fully later in the Section, but, as a working hypothesis, it is postulated for the moment that exposure to high pressures of oxygen causes an increased sympathetic tone, and that this results in an increased release of surfactant, leading to depletion. This depletion is compounded with the known oxygen-lability of surfactant (Klaus, Clements & Havel, 1961), and by the direct depressant effect of oxygen on surfactant synthesis (Gacad & Massaro, 1973; Gilder & McSherry, 1974).

If this is the case, however, pharmacological blockade ought not only to prevent the excessive secretion of surfactant leading to depletion, but might also be expected to interfere with the normal secretion process, resulting in effects similar or identical to those caused by depletion.

In fact, an effect which could possibly be due to diminished or defective surfactant is demonstrated in this section; a large proportion of the animals had either a focal atelectasis or complete collapse and consolidation of parts of the lungs.

As this was not a feature of the animals described in Section 3, it can reasonably be attributed to adrenergic blockade. Possible mechanisms are discussed below, but whatever the cause, one might expect the effect to be more pronounced in the phentolamine-blocked than in the guanethidine-blocked animals, as in the latter the sympathetic receptor sensitivity to circulating catecholamines is actually enhanced (Boura & Green, 1965).

The small sample size involved does not allow any valid comparison to be made, but the results listed in Table 8:1 suggest that this was not the case; the pathological appearances of the lungs

of the three phentolamine-blocked animals ranged neatly, but completely unhelpfully, over the full spectrum, from completely normal to totally consolidated.

Compliance change

There are several possible explanations for the fall in compliance.

The first is that there is a defect in the maintenance of the alveolar surfactant layer causing a progressive increase in alveolar surface tension forces and leading eventually in some cases to atelectasis.

This could be due to a direct effect of high levels of alveolar oxygen damaging the surfactant already formed and upsetting synthesis in the Type 2 cells, although if this is the case it is difficult to explain the continuing relatively normal compliance in the 'normal' lung animals in this Section or in the 'cardiac death' animals described in Section 3.

Alternatively, as suggested previously, it could be due to adrenergic secretomotor blockade.

It is not easy to reconcile this hypothesis with the long delay before a fall in compliance becomes obvious. However, when it is remembered that, by the techniques used, 'compliance' as calculated cannot be translated into absolute values for comparison with controls, and that as the first measurement of compliance was made several hours after adrenergic blockade, it may be that all the early values of compliance, meaned and assigned a nominal value of 100%, were already very low. If this were the case, the fall in compliance

over the last 10 hours would appear artificially large in percentage terms, and the real drop might have occurred before any measurements were made.

Whichever of the above alternatives is accepted, one is forced by the length of the survival time and relative normality of some of the animals to postulate some continuing degree of surfactant secretion, which must be independent of sympathetic control.

Another potential mechanism, possibly with a decrease in surfactant as a contributing factor, is the accumulation of water in the lungs.

There is no evidence from P_aO_2 or shunt measurements, nor from histology to suggest the development of intra-alveolar oedema. These parameters, however, do not rule out the possibility of a fairly severe degree of interstitial oedema; this is known to have a negligible effect on pulmonary gas exchange (Szidon, Pietra & Fishman, 1972) and in the early stages is confined to the interstitium around the large vessels and airways (Staub, Nagano & Pearce, 1967), thus explaining the normal appearances on the peripheral freeze-dried sections. The lung wet/dry weight ratio in the 'atelectatic' animals (mean = 5.56) was increased by 23% over the control value (4.60); it is not known to what extent such an increase would contribute to the reduction in compliance.

A third mechanism which might be in operation is a parasympathetically-mediated bronchoconstriction unleashed by adrenergic blockade (note that, although all the animals were given atropine 0.3mg. routinely to mitigate against the vagotonic effect of the anaesthetic RX 320 M, this was given intravenously at the time of induction and

that its duration of action might be expected to be no more than one hour).

There is unfortunately no means whereby, from the data available, the contribution of increased airway resistance can be separated from the overall decrease in compliance, and it may be that this factor alone could account for the total observed fall. Bronchiolar constriction and increased bronchial mucus secretion could also, by promoting absorption collapse, account for the atelectasis. In addition, it could help explain another perplexing observation: the consistently high P_aCO_2 values obtained throughout the experiments.

Carbon dioxide tensions were observed to be high (55 torr ± 1.7) right from the time of the first measurement, and if this is taken as a reflection of increased airway resistance, by a similar argument to that advanced on Page 8:20, it may be that again the early baseline measurements of compliance were much lower than normal.

The problem will never be resolved, as baseline values of blood gases and compliance were not recorded before adrenergic blockade.

When the conclusions arrived at in Sections 3 and 4 are taken into consideration, the main objection to the theory of a sympathetically mediated surfactant deficiency as the major contributing factor to the fall in compliance must lie in the absence of any intra-alveolar oedema. It might be argued that the fall in pulmonary arterial pressure over the last eight hours of the experiment was quite possibly sufficiently large to avoid overwhelming the inter-

stitial space drainage mechanisms even in the face of a continuing rise in alveolar surface tension. However, for the idea of a sympathetic effect to be credible, a reduction in alveolar surfactant sufficient to cause a compliance change must have occurred within an hour or so following blockade (see Page 8:20) and at that time, and for some twelve hours after that, pulmonary arterial pressures were at their normal high levels.

CONCLUSIONS

It is not possible from the evidence available in this Section to come to a firm conclusion regarding the mechanism of protection afforded by sympathetic blockade.

The fall in compliance seems most likely to be due to a rise in airway resistance and a focal atelectasis due to a parasympathetically-mediated bronchoconstriction.

There is little here to suggest that the normal resting secretion of surfactant is abolished by adrenergic blockade. It may be that it is decreased, but this is an imponderable.

From the results presented in Sections 3, 4 and 5, it seems likely that the damage inflicted by oxygen at 2 ATA (in the dog) is mediated mainly by a direct toxic effect on the Type 2 cell which after some 18 to 20 hours or so is unable to continue secreting surfactant (synthesis possibly having stopped immediately following exposure to oxygen).

It is probable that the same type and degree of damage was inflicted on the animals described in this Section, but that they were protected against the development of intra-alveolar oedema in

the late stages by the large fall in pulmonary arterial pressure.

It is concluded therefore, that at pressures of 2 ATA, the sympathetic nervous system has little part to play in the oedema of pulmonary oxygen toxicity.

SECTION 9

DOG EXPERIMENTS SERIES 4

PATHOPHYSIOLOGICAL STUDY AT 2 ATA

OXYGEN TOXICITY AND VAGOTOMY

OXYGEN TOXICITY AND VAGOTOMY

INTRODUCTION

This Section reports in outline form a short experiment which was set up in an attempt to demonstrate the interaction between the parasympathetic nervous system and oxygen at high pressure in the development of pulmonary oedema.

REVIEW

Parasympathetic nervous system and oedema

The early history of experimentation into vagotomy-induced pulmonary oedema has been reviewed by Visscher, Haddy and Stephens (1956) who quote an experiment performed by Vieussens in 1716 on post-vagotomy lung changes and discuss vagotomy experiments by such famous names as Valsalva (1740) and Claude Bernard (1858).

Among the several mechanisms suggested as a cause of post-vagotomy lung oedema were the aspiration of food, 'laryngeal paralysis with inspiratory leakage', and 'pulmonary vasomotor paralysis'.

Lorber (1939 a & b) attributed post-vagotomy pulmonary oedema to airway obstruction due to laryngeal paralysis, a view to which Visscher et al. (1956) subscribed.

Farber (1937 a & b) came very close to modern thinking by suggesting that interrupted vasomotor impulses to the pulmonary capillaries caused an increase in permeability, and in 1940 demonstrated a 20% decrease in total blood volume and varying changes in haematocrit following cervical vagotomy in rabbits.

Bilateral cervical vagotomy in guinea-pigs was shown to produce an increase in the surface tension of lung extracts (Tooley, Gardner, Thung & Finlay, 1961) and a reduction in the number of osmiophilic inclusion bodies in the Type 2 cell (Klaus, Reiss, Tooley, Piel & Clements, 1962; Bolande & Klaus, 1964).

24 hours after unilateral vagotomy the surface tension of lung extracts was reported to be higher on the vagotomised side, the effect being more pronounced when a right vagotomy was performed (Tooley et al., 1961).

These surface tension effects were substantiated by Goldenberg, Buckingham and Sommers (1967) who in a careful electron microscopical study in rat lungs at 1 to 6 hours post-vagotomy demonstrated a progressive decrease in osmiophilia in the Type 2 cell inclusion bodies and an increase in the number of endothelial pinocytotic vesicles. Atelectasis and abnormal surface activity were also demonstrated.

The same workers provided additional compelling evidence for the involvement of the parasympathetic nervous system in surfactant secretion by the demonstration of massive extrusion of pre-formed osmiophilic inclusions from the Type 2 cell and increased synthesis of new inclusions following a 'cholinergic' dose of pilocarpine (Goldenberg, Buckingham & Sommers, 1969).

Parasympathetics and pulmonary oxygen toxicity

The implication from the findings reviewed above is that normal cholinergic traffic is essential for the continuing normal

secretion of surfactant. This received support in the hyperbaric oxygen situation where carbachol and methacholine have been shown to prolong survival time and decrease pulmonary pathology in mice exposed to oxygen at 6 ATA; furthermore, this protection is reversed by prior administration of atropine (Gerschman, Gilbert, Nye, Price & Fenn, 1955). The same workers showed a decreased survival time in the same model when atropine was given alone.

Under normobaric hyperoxic conditions, it has been shown that atropine augments hyaline membrane formation in mice breathing 100% oxygen (Buckingham & Sommers, 1960).

Despite all this evidence, the influence of vagotomy on the development of pulmonary oxygen toxicity appears not to have attracted much attention.

The most extensive investigations must be those of Shanklin who originally tackled the problem in reverse. He demonstrated that bilateral cervical vagotomy in newborn rabbits had a 100% mortality rate, the animals dying with a gross pulmonary oedema and atelectasis leading to total consolidation, then showed an increasing synergistic effect (in terms of time course of response) of increasing oxygen tensions in the inspired gas mixture up to 100% at 1 ATA (Shanklin & Cunningham, 1965). On extending such studies to the hyperbaric situation, he showed that, at pressures up to 3 ATA, the extent of the lung damage in vagotomised animals was proportional to the inspired percentage and not to the partial pressure of oxygen, implying that much of the effect was due to absorption collapse (Shanklin, 1967, 1969).

In an ancillary study to investigate the mechanism of the respiratory pattern changes in spontaneously breathing anaesthetised dogs exposed to 100% oxygen at 2 ATA (see Figure 3:10), Smith (1971) performed a bilateral cervical vagotomy on three dogs at different times in the course of the experiment. Unfortunately, there is no record of cardiorespiratory parameters in these animals, but it was reported that 'the mode of death appeared to be a gross hypotension'.

Penrod (1958) in his report on differentially intubated cats (reviewed in Section 5) reported that vagotomy appeared 'to enhance oxygen damage', but no details are given and it is not stated whether or not vagotomy was also unilateral.

INVESTIGATION

It was decided to set up a series of differentially intubated dogs identical to those described in Section 5, all receiving 100% oxygen at 2 ATA to their right lung and air at 2 ATA to their left, and then to perform either right or left unilateral vagotomies and observe any degree of protection or synergism.

The magnitude of the complexities of the model were only belatedly realised and it is perhaps as well that the results were for the most part negative and did not require detailed interpretation.

MATERIALS AND METHODS

Equipment, anaesthesia, gas delivery, measurement techniques, calculations and post-mortem investigations were identical

to those described in Section 5 except that, following tracheostomy, one or other of the cervical vagi was exposed and divided.

Nine greyhounds, weights 24 to 29 Kg., were used. All were intubated with a modified Carlens tube (see Section 5).

Four of the animals were set up as controls; two had a right and two a left vagotomy, and all breathed air at 2 ATA with both lungs.

A hyperbaric rather than a normobaric air-breathing control situation was chosen a) because it was more directly comparable with the experimental group (the original intention of supplying the left lung of the experimental animals with a 10%/90% O_2/N_2 mixture having been abandoned - see Page 5:9), and b) it prevented any hypoxaemia in the event of collapse of the left apical and cardiac lobes following intubation.

The five experimental animals all breathed 100% oxygen at 2 ATA with the right lung and air at 2 ATA with the left; in three animals a right (ipsilateral) vagotomy was performed, and in the other two, a left (contralateral) vagotomy (see Table 9:1).

RESULTS

The main findings are presented in Table 9:1.

1) CONTROL GROUP

Time and mode of death

All of the control animals survived beyond 24 hours and were sacrificed at a convenient time ($24\frac{1}{2}$ to $26\frac{3}{4}$ hours) by intra-

TABLE 9:1

EXPERIMENTAL MODEL, TIME OF DEATH, PATHOLOGICAL APPEARANCES, AND LUNG WET/DRY RATIOS

DOG NO.	GAS (at 2 ATA)		VAGOT.	TIME OF DEATH (hours)	MODE OF DEATH	PATHOLOGICAL APPEARANCES					W/D RATIO	
	(R)	(L)				Right Lung		LAL/LCL	LDL		RL	LDL
12	Air	Air	(R)	$24\frac{1}{2}$	KCl	Expanded,	purple	Collapsed	Expanded,	purple	4.85	3.56
16	Air	Air	(R)	$24\frac{3}{4}$	KCl	"	"	"	"	"	5.03	7.45
13	Air	Air	(L)	26	KCl	"	"	"	"	"	5.04	5.35
15	Air	Air	(L)	$26\frac{3}{4}$	KCl	"	"	"	"	"	4.37	4.34
7	O_2	Air	(R)	$22\frac{1}{4}$	Apnoea	"	pink	"	"	"	10.98	4.18
8	O_2	Air	(R)	25	Apnoea	"	"	"	"	"	6.3	4.33
9	O_2	Air	(R)	$15\frac{1}{2}$	Apnoea	"	"	"	"	"	10.23	6.18
11	O_2	Air	(L)	20	Apnoea	"	"	"	"	"	9.19	7.14
10	O_2	Air	(L)	13	Apnoea	"	"	"	"	pink	11.5	9.8

KCl = potassium chloride. RL = right lung. LAL, LCL, LDL = left apical, cardiac and diaphragmatic lobes.

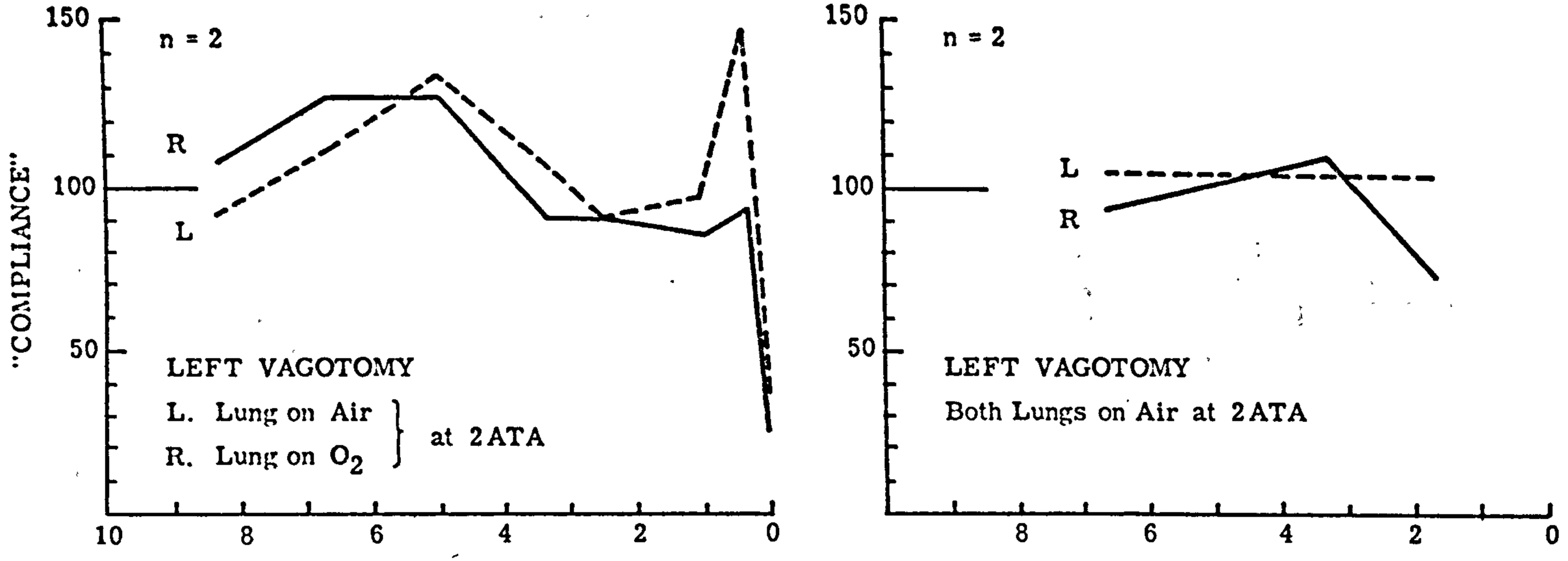

"COMPLIANCE"
150
100
50
"COMPLIANCE"
n = 2
R
L
LEFT VAGOTOMY
L. Lung on Air
R. Lung on O_2
at 2ATA
10
8
6
4
2
0
150
100
50
n = 2
L
R
LEFT VAGOTOMY
Both Lungs on Air at 2ATA
8
6
4
2
0
150
100
50
"COMPLIANCE"
n - 3
L
R
RIGHT VAGOTOMY
L. Lung on Air
R. Lung on O_2
at 2ATA
10
8
6
4
2
0
TIME BEFORE DEATH (Hours)
150
100
50
n = 2
R
L
RIGHT VAGOTOMY
L. Lung on Air
R. Lung on O_2
at 2ATA
10
8
6
4
2
0
TIME BEFORE DEATH (Hours)

FIGURE 9:1.

LUNG WET/DRY WEIGHT RATIOS (± S.E.)

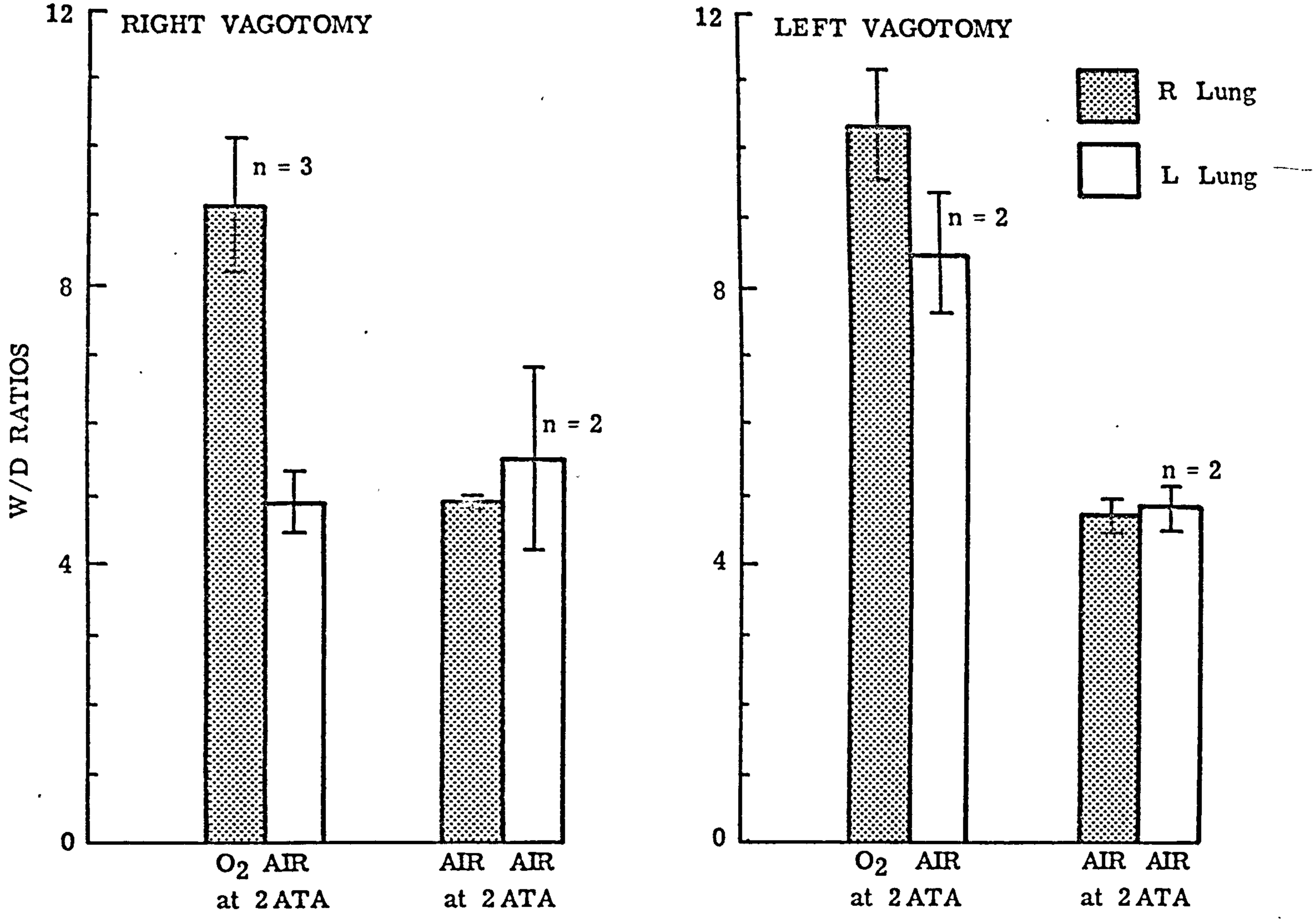


FIGURE 9:2.

venous injection of pentothal followed by potassium chloride.

Cardiorespiratory parameters

One animal (Dog 12) was almost moribund at the time of sacrifice, systemic arterial pressure and cardiac output having fallen steadily for three hours before death.

The other three animals remained remarkably normal.

In one there was a slight fall in systemic arterial pressure possibly indicating incipient cardiovascular failure, but pulmonary arterial wedge pressure was also falling, and cardiac output remained unchanged. In another animal there was a slight fall in P_aO_2 and a simultaneous rise in haematocrit over the last five hours.

Compliance

Neither a right nor a left vagotomy appeared to have the slightest effect on pulmonary compliance in the air-breathing animals (Figure 9:1).

Lung wet/dry weight ratios

These are shown in Figure 9:2.

There was no significant difference between the ipsilateral and contralateral lungs of the vagotomised group, nor was there any significant difference between these lungs and corresponding lungs from non-vagotomised air-breathing control animals (Section 5).

In one animal with a right vagotomy there was an unexpected

and completely unexplained increase in weight ratio in the left lung. It is this value which accounts for the large standard error bar on the relevant histiogram in Figure 9:2.

2) EXPERIMENTAL ANIMALS

Time and mode of death

Apnoea preceded asystole in all of the animals.

There was a large range of survival times, from 13 to 25 hours; the small sample size does not permit any analysis.

Cardiorespiratory parameters

With one exception (Dog 10) discussed below, the pattern of cardiorespiratory responses was identical to that described in the non-vagotomised dogs described in Section 5, and the results of the vagotomised animals could have been compounded with those of Section 5 without affecting the shape of the curves.

An interesting incidental finding is that the typical progression of respiratory pattern changes (see Figure 3:10) was abolished by vagotomy. This is discussed on Pages 3:33 and 5:25.

Dog 10 (vagotomised on the left side) died earlier than any other animal, at 13 hours. The most prominent difference observed during the experiment was a fall in P_aO_2 commencing only 4 hours from the start of the experiment, and some 4 hours later, at $4\frac{1}{2}$ hours before death reaching a value of 77 torr (baseline value = 542 torr).

Pulmonary arterial pressure began to rise within four hours

of the start of the experiment and had reached 19 mm.Hg. (baseline = 7 mm.Hg.) at one hour before death. Cardiac output rose in the later stages, and despite attempts at maintaining fluid balance, there was a large rise in haematocrit over the last four hours.

Compliance

The general pattern followed that seen in the animals described in Section 5 (Figure 5:4), i.e. a fall in compliance in the oxygen-exposed right lung and a relatively unaffected left lung.

Unilateral vagotomy, however, seemed to impose a synergystic effect, although it must be stated emphatically that the small sample size does not allow of any statement regarding significance, and Figure 9:1 must be taken to indicate a possible trend only. The fall in compliance in the right lung appeared to be more steep following ipsilateral than contralateral vagotomy.

Compliance in the left lung was completely unaffected by contralateral vagotomy. Ipsilateral vagotomy may have effected a fall in compliance, but unfortunately the results are possibly invalidated by incorporating those of Dog 10, which is suspect.

Lung wet/dry weight ratios

The changes seen in compliance are reflected in the weight ratios.

For the oxygen-exposed right lungs, there is no significant difference between the weight ratios of the ipsilateral and contralateral vagotomised groups, nor between all these animals and the non-vagotomised animals described in Section 5.

Similarly, for the air-exposed left lungs, there is no significant difference between those with contralateral vagotomy and the equivalent non-vagotomised control. On the other hand, with ipsilateral vagotomy, the increase in weight ratio becomes highly significant ($P = < 0.005$). Yet again, however, it must be remembered that values taken from the possibly abnormal Dog 10 have influenced this result.

DISCUSSION

Within the limits imposed by the small numbers involved, it would appear that unilateral vagotomy in air-breathing animals has no effect on pulmonary compliance or wet/dry weight ratios, i.e. it does not cause oedema (at least within the time-scale of this experiment).

In view of the universally accepted oedemagenic action of bilateral vagotomy, this observation is surprising.

It is possible that laryngeal paralysis is avoided following unilateral nerve section, but a more probable explanation is that there is a large degree of cross-innervation in the lungs. This has been demonstrated in rabbits (Larsell & Mason, 1921; Honjin, 1956) in experiments designed to investigate the pattern of degeneration of nerve fibres around intra-pulmonary ganglion cells following cervical vagotomy; both observers commented that some fibres remained intact, having come from the opposite vagus through communicating branches below the level of section.

The present findings are in conflict with those of Tooley

et al. (1966) who, 24 hours after unilateral vagotomy in guinea-pigs found an increased surface tension in the lung extract from the vagotomised side.

It is impossible to be dogmatic about the interaction between vagotomy and hyperbaric oxygen.

There may have been an increased loss of compliance in the oxygen-exposed right lung following ipsilateral, but not contralateral vagotomy, but the effect, if any, is minimal; neither is there any observed change in wet/dry weight ratios.

The air-breathing left lung presents a very definite lack of response to contralateral vagotomy, but shows a possible fall in compliance and a highly significant increase in wet/dry weight ratio following ipsilateral vagotomy.

If the possibly confounding effects of Dog 10 are ignored, it would appear that the oedemagenic effects of oxygen toxicity and vagotomy are at least synergistic.

Although the left lung in these animals was breathing air, it is possible to postulate that it was under stress; for example, the 'air' was at 2 ATA (P_IO_2 = 310 torr), pulmonary arterial (mixed venous) oxygen tensions were much higher than normal (ranging from 50 to 100 torr), bronchial arterial oxygen tension was very high (over 700 torr up until about 2 hours before death), and there may have been a sympathetically-mediated component present due to hypothalamic hyperoxia.

By the end of the experiment, the right lung was so flooded

with oedema fluid due to the oxygen-induced damage that any additional effect of vagotomy would be masked. Such an effect, however, might possibly have become apparent in the 'normal', but pre-stressed left lung.

SECTION 10

DISCUSSION AND CONCLUSIONS

DISCUSSION AND CONCLUSIONS

This short section serves to summarise the points raised in the individual sections.

What has been learned from this study?

It has been demonstrated yet again that in all work on oxygen toxicity, the exact pressures and duration of exposure must be stated as there is likely to be a spectrum of biological response which is dose and time dependent. Experimental conditions must be accurately recorded because of the possibility, for example, of hyperoxia/drug interactions. The species and maturity of the animal must be declared; all the work herein was performed on adult greyhounds, but there is ample evidence quoted in the review paragraphs to demonstrate the marked inter- and intra-species differences in response.

In common with most other whole animal work, one must be content with probabilities rather than dogmatic statements of fact. From the original experiments, however, (Sections 3 and 4) it seems fairly certain that exposure to hyperbaric oxygen at 2 ATA does not cause a slowly progressive decline, but that the animal remains apparently well until a few hours before death. It is also likely under these conditions that spontaneously breathing dogs anaesthetised by a neuroleptanalgesic technique and kept in good fluid balance will die of pulmonary rather than cardiac causes. Death is due to a fulminating intra-alveolar oedema (as originally described by Lorain Smith in 1899), but to demonstrate this, special fixation techniques are required. The other pathological appearances described

by Smith - the totally consolidated red beefy lung - are considered to be due to post-mortem artifact.

The mechanism of oedema formation would appear to fall into neither of the neat conventional divisions, 'permeability' nor 'haemodynamic', but rather into that miscellaneous ragbag which contains the less well understood forms such as follow sudden exposure to high altitude, or heroin overdosage. The work described in Section 4 strongly suggests a disturbance of Type 2 cell and possibly also of macrophage activity, and the implication is that oxygen at 2 ATA depresses Type 2 cell metabolism sufficiently either to diminish seriously or to arrest completely surfactant production. The eighteen or so hours of apparent normality are taken to represent the reservoir of pre-formed surfactant existing within the Type 2 cells.

The conclusion that the primary aetiology of pulmonary oedema in oxygen poisoning is a defect of surfactant production was arrived at only hesitantly as disease states due to a primary lack of surfactant are extremely rare (possibly only asphyxia neonatorum) and to primary overproduction unknown.

Alpha-adrenergic blockade (Section 8) protects against the oedema formation without any prolongation of survival time; it seems likely that this is due to its pulmonary hypotensive effect. There is little here to suggest that sympathetic hyperactivity is involved in the aetiology of the hyperoxic oedemagenic response, nor indeed that the autonomic nervous system is greatly involved at all (Section 9). This conclusion supports the work of Reich and Demeny (1974) who studied the effects of identical tensions of oxygen (70%/30% O_2/N_2) at 2.9 ATA) on dogs' lungs denervated by autotransplantation and

found that, although noradrenaline levels were reduced significantly on the denervated side, there were no differences between the two lungs in either gross appearance, light microscopical findings nor lung wet/dry weight ratios.

This, however, only holds for the 2 ATA situation and is manifestly not the case where higher pressures are involved. In a study of the sympathoadrenomedullary response of rats exposed to increasing pressures of oxygen, Cross & Houlihan (1969) showed that while at low, non-lethal levels (0.6 ATA) there is a depression of circulating catecholamines, as the pressures rise to toxic levels (through 0.9 ATA) there is a sustained sympathetic outflow and concommitant excessive release of adrenaline. At higher pressures (up to 6 ATA), following convulsion there is a hypothalamic depletion of noradrenaline. Faiman & Heble (1966) had previously demonstrated in mice a progressive decrease in brain noradrenaline with an increase in oxygen tensions from 1 to 6 ATA. In dogs which had convulsed following exposure to high pressures of oxygen there was a marked increase in circulating catecholamines, whereas animals exposed to non-convulsive levels, in whose lungs were the changes of oxygen toxicity, showed no such rise (Demeny, Manger, Naftchi & Reich, 1974).

What happens thereafter is less clear; i.e. whether the centrally-mediated effect operates by a direct action on the Type 2 cells as suggested by Beckman and his colleagues (Beckman et al., 1971, 1973, 1974), or is 'neurohaemodynamic' (Sarnoff, 1952) in nature as suggested by Moss et al. (1972) and Theodore and Robin (1975).

In either case it results in a centrineurogenic pulmonary

oedema and if a rigid classification of oxygen toxicity were being attempted, this would more correctly be labelled a pulmonary complication of C.N.S. toxicity rather than pulmonary oxygen toxicity per se.

It is probable that once again there is a spectrum of different effects dependent on the pressures involved.

In normobaric hyperoxia, the animal survives for a relatively long time and the main mechanism of toxicity is a wholly direct effect on the epithelial and endothelial cell membranes. As the endothelium appears to suffer greater damage than the epithelium, it may be that circulating co-factors - possibly even adrenaline itself - are also involved.

Under moderately hyperbaric conditions there is not sufficient time for membrane damage to occur and the major effect appears to be directly on the Type 2 cell, whereas following exposure to pressures higher than 2.5 ATA where the animals convulse, the time course is even shorter and the centrineurogenic mechanism predominates.

Figure 10:1 is a modification of the diagram of mechanisms of pulmonary oxygen toxicity which appeared in the Smith & Shields (1975) review (see Page A1:9) which takes these mechanisms into account.

The normal day to day control of surfactant production and release remains a mystery. Turnover is extremely rapid, thought to be around 14 hours for the lecithin moiety (Tierney et al., 1967) and radiolabelled precursors appear concentrated within the Type 2 cells within 5 minutes of injection (Askin & Kuhn, 1971) and free in the alveolar surface layer within 2 hours (Chevalier & Collet, 1972).

It performs a vital function and the consequences of a disturbance of normal production are catastrophic; it would seem unlikely that some sort of control mechanism does not exist.

Pulmonary compliance and the surface active properties of lung extracts were reported to be normal in homografted lungs of dogs up to 14 days following transplantation, suggesting that denervation had no effect on surfactant production. Notwithstanding this and the negative results reported in Section 7, however, the work of Beckman et al. (1973, 1974) and Goldenberg et al. (1967, 1969) provide strong experimental evidence for some influence of the autonomic nervous system.

Fine nerve fibres have been shown unequivocably to exist in the alveolar walls (Section 6). Furthermore these contain noradrenaline, i.e. they are sympathetic motor fibres. Why are they there? The only structures which they can be innervating are the capillary walls (although it is hard to imagine why), the pericytes, which are thought to possess some contractile properties, or the Type 2 cells as Hung and his colleagues (1972) claim to have demonstrated. If they supply the Type 2 cells, then they must be secretomotor.

It is tempting to suggest that while a resting normal secretion of surfactant can and probably does continue in the absence of a neural contribution, the autonomic nervous system can cause an increased production or release in the face of any emergency liable to result in alveolar instability or oedema. Possible stimuli could include a greatly increased interstitial space pressure with the risk of impending intra-alveolar oedema (perhaps to be considered as part

of Paintal's J-reflex), or the presence of alveolar hyperoxia. The teleological objection to the latter, that hyperoxia does not occur in nature, can be dismissed by the fact that other responses to hyperoxia do exist. The mechanism might also form part of the deflation reflex.

If considered at possibly an acinar level, a localised alteration in surfactant production in response to localised hyperoxia might contribute to the optimisation of ventilation/perfusion ratios. There are now several indications in the literature that chemoreceptors at an alveolar level might exist (Meyrick & Reid, 1968; Kazemi et al., 1972; Bartoli et al., 1974).

The main objection to all these theories, however, is that it is difficult to imagine how, if the surface lining film must exist as a monomolecular layer, an increased production could enhance surfactant activity.

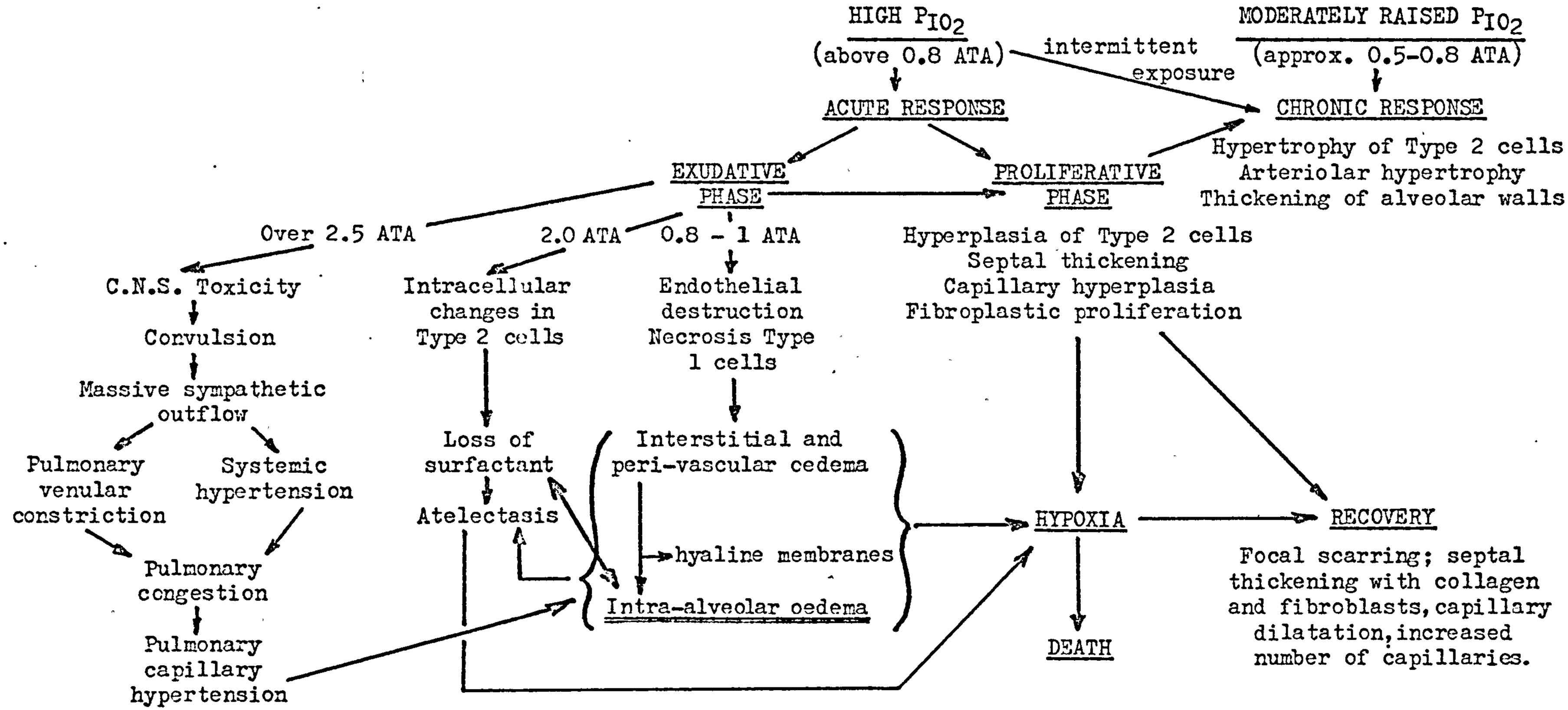


FIGURE 10:1 POSSIBLE MECHANISMS OF PULMONARY OXYGEN TOXICITY

Work to be done

1) To complete the thesis

The stereometric analysis described in Section 4 is incomplete. Numerical values are required for the increase in pinocytosis across the endothelial membrane following exposure to hyperoxia, and ultrastructural stereological analysis of the response of the Type 2 cell mitochondria and endoplasmic reticulum has yet to be made.

Lung tissues from the animals described in Sections 5, 8 and 9 were fixed and embedded for electron microscopy, but have yet to be sectioned and examined.

2) As an extension to the thesis

Although the oxygen poisoned animals appear to be normal until very late in the experiment, it would be interesting to know from what stage recovery is possible. If the condition is due to functional impairment of surfactant, it ought to be possible to return the animal to room air, possibly with the assistance of mechanical ventilation to permit surfactant recovery, at any time up until the time of appearance of the fulminating intra-alveolar oedema.

Serial sacrifice at different times around 16 to 22 hours might permit demonstration of a progression of interstitial oedema and possibly allow correlation with clinical signs, e.g. compliance change, jaw retraction or onset of 'square-wave respiration', of predictive value.

The facilities for assaying circulating catecholamines were not available during the performance of these experiments, but such

measurements would assist in deciding whether or not the sympathetic nervous system is involved in the oedemagenic process.

It was only realised on completion of the experiments that the haemodynamic and permeability forms of pulmonary oedema may be distinguished by the osmolality of the oedema fluid (Robin, Carey, Grenvik, Glauser & Gaudio, 1972; Brigham, Woolverton, Blake & Staub, 1974). In 'hydrostatic' oedema due to surfactant deficiency, the oedema fluid would be expected to be of low osmolality, and such a demonstration would provide additional evidence for this mechanism.

3) Other points raised

A better demonstration of alveolar nerve fibres, by use of more sophisticated techniques such as combined phase contrast and fluorescence microscopy and perhaps by a more highly skilled histologist than the author, should be made. There should also be an intensive search for further evidence of Type 2 cell innervation.

The lack of knowledge of surfactant control mechanisms has been discussed above.

Much work remains to be done to elucidate the mechanisms of pulmonary oedema and the movement of interstitial and lymphatic fluid within the lung. In particular, values are required of differences in interstitial space pressures from the alveoli to the more proximal regions of the lung.

The fascinating paradox of the presence of intra-alveolar blood cells in haemodynamic but not in permeability oedema remains to be investigated.

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APPENDIX 1

REVIEW

'OXYGEN TOXICITY'

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OXYGEN TOXICITY

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1. INTRODUCTION

The knowledge that oxygen possesses noxious as well as beneficial properties dates almost from the discovery of the gas. Shortly after its isolation in 1775, Priestley theorized on the possible dangers of the inhalation of high concentrations of oxygen, and a few years later Lavoisier (1783) noted the 'incendiary' effects of oxygen on the lungs of animals and described congestion and consolidation as the main pathological features. In 1878 Paul Bert described in considerable detail the toxic effects of hyperoxia both on the lungs and on the central nervous system. He recognized three important factors influencing the time to onset of convulsions, namely, the pressure of oxygen, the duration of exposure to oxygen, and the considerable interspecies variation.

The description of pulmonary oxygen toxicity is credited generally to J. Lorrain Smith (1899) who worked extensively with both normobaric and hyperbaric pressures of oxygen. The term Lorrain Smith effect is now given to the pathological features of acute pulmonary oxygen toxicity (see below).

The findings of these early workers were confirmed and amplified by many other investigators and the reader is referred to an excellent review by Bean (1945) for information on studies performed during the first four decades of this century.

In the last two decades there has been a considerable resurgence of interest in the toxic effects of oxygen as a result of the increasing use of high concentrations of this gas in the commercial (diving and compressed air work), military (aerospace and submarine activities) and medical fields. In the clinical situation, the initial flush of enthusiasm for the use of hyperbaric oxygen has waned as a result of the realization that the vasoconstrictive effects of hyperbaric oxygen largely offset the increase in arterial oxygen content, producing relatively little change in tissue oxygen availability. The clinical uses of hyperbaric oxygen are regarded now as fairly limited and include the treatment of:

(1) carbon monoxide poisoning;
(2) anaerobic infections; and
(3) decompression sickness

Of greater importance then these indications for hyperoxia, however, is the use of high concentrations of oxygen at normobaric pressures in the treatment of patients with modern intensive care techniques. The extent of the oxygen toxicity hazard in intensive therapy units is not known, but the subject is of sufficient importance to require the physician to have knowledge of human oxygen tolerance in treating any patients who require prolonged oxygen therapy (Editorial, 1974).

As much of the research in the field of oxygen toxicity has utilized exposure to hyperbaric conditions, familiarity with the terminology of hyperbaric pressures is necessary. In a normobaric environment the total pressure exerted by the atmosphere at sea-level is equal to 760 mmHg [standard barometric pressure or 1 atmosphere absolute (1 atm)]. If a hyperbaric chamber at sea-level is compressed so that the total pressure is 1520 mmHg, the pressure is said to be 2 atmospheres absolute (2 atm) or 1 atm gauge pressure.

The purpose of the present review is to cover the significant advances in the field of oxygen toxicity in the last two decades with particular emphasis on the pulmonary aspects. As central nervous oxygen toxicity in the form of convulsions is manifest only at pressures higher than 2.5 atm, where it overshadows the effects of oxygen on all other systems, this article will deal predominantly with oxygen toxicity in the range 0.5–2.5 atm.

2. PHYSIOLOGICAL EFFECTS OF HYPEROXIA

Ventilation with increased partial pressures of oxygen produces various physiological, as opposed to toxic effects. These effects are characterized by a fast onset and by a rapid and complete reversibility. They do not endanger life.

2.1. RESPIRATORY SYSTEM

The immediate effect of an abrupt increase in the inspired oxygen partial pressure (P_{O_2}) is a transient decrease in ventilation, as a result of the removal of any residual 'hypoxic' drive from the arterial chemoreceptors (DeJours *et al.*, 1959). This decrease causes an initial increase in arterial carbon dioxide tension (P_{CO_2}).

As the inspired, and hence arterial oxygen partial pressures, increase more oxygen is carried in physical solution in the plasma and, in theory at least, at pressures of oxygen above 3 atm, total tissue oxygen requirements may be supplied from oxygen in physical solution, leaving the mixed venous hemoglobin completely saturated. This oxygenated hemoglobin is less able to form carbamino compounds, and in addition is a less effective buffer for the hydrogen ions from carbonic acid. This causes a generalized increase in tissue P_{CO_2} and [H^+] which in the brain leads to stimulation of ventilation (Behnke *et al.*, 1934). The end result is therefore a state of increased ventilation with resulting hypocapnia and a co-existing central nervous acidosis and arterial alkalosis.

Studies of the effects of high inspired oxygen partial pressures on pulmonary gas exchange have revealed conflicting results. In a careful study of different groups of human breathing 100% oxygen at 1, 2 and 3 atm, McDowall *et al.* (1968) obtained mean values for alveolar-arterial difference (A-aP_{O_2}) of 17 mmHg at 1 atm, 10 mmHg at 2 atm and 36 mmHg at 3 atm. However several of the subjects had hegative values which suggests over-estimation of the arterial P_{O_2}. Many earlier studies in which large increases in A-aP_{O_2} were obtained with hyperoxic ventilation are open to criticism, e.g. failure to obtain a 'blood-gas' calibration factor for the P_{O_2} electrode (Rosenberg *et al.*, 1966), leakages of room air around face masks (Whalen *et al.*, 1964) and the use of the methods necessary before the practical availability of oxygen electrodes (Lambertsen *et al.*, 1953). However, a most recent study by Clark and Lambersten (1971a) indicated that the A-aP_{O_2} did increase with elevations in alveolar P_{O_2} but the values were of a relatively small order—9.4, 46, 74, and 121 mmHg at inspired oxygen partial pressures of 0.2, 1.0, 2.0 and 3.5 atm respectively. These values closely approximated to theoretically calculated values for A-aP_{O_2} at these inspired oxygen partial pressures (Clark and Lambertsen, 1971a). It was suggested that probable causes of this effect included an initial alveolar collapse, increased arteriovenous oxygen content difference and increased intrapulmonary shunting. Recent work confirms the suggestion that an increased Pa_{O_2} produces effects on blood gas exchange by 'active' alterations of ventilation/perfusion relationships as well as by a 'passive' effect by a simple increase of oxygen transfer across the alveolar-capillary membrane. Kerr (1975) has observed that an increased inspired

oxygen concentration leads to an increased pulmonary capillary-arterial oxygen content difference in patients at normobaric pressures and has excluded changes in cardiac output and absorption collapse as major factors. It is also of interest that Smith *et al.* (1974) suggested that, in the physiological range, elevation of the oxygen tension in mixed venous blood may increase the extent of intrapulmonary shunting.

2.2. Cardiovascular System

The immediate response of the cardiovascular system to an elevated arterial P_{O_2} is an increase in the systemic vascular resistance with a concomitant fall in cardiac output, the mean systemic pressure remaining unchanged. (Eggers *et al.*, 1962; Murray *et al.*, 1964; Murray, 1964). At a regional level, vascular resistance has been observed to increase, with reduction in blood flow, in the limbs (Bergofsky and Bertun, 1966; Bird and Telfer, 1966), the retina (Dollery *et al.*, 1964), the bowel (Bergofsky and Bertun, 1966), the liver (Hahnloser *et al.*, 1966), the heart (McBride and Ledingham, 1968; Ledingham *et al.*, 1970; Winter *et al.*, 1970), the kidney (Kioschos *et al.*, 1967; Rennie *et al.*, 1964; Norman *et al.*, 1965) and the retina (Sieker and Hickam, 1953). The net result is that, despite the high arterial oxygen tensions, the oxygen tension in most tissues is not elevated substantially.

Two vascular beds merit particular mention, namely the cerebral and pulmonary circulations.

2.2.1 *Cerebral Circulation*

It has been known for many years that oxygen at high pressure causes a decrease in cerebral blood flow (Dautrebande and Haldane, 1921; Kety and Schmidt, 1948; Lambertsen *et al.*, 1953; Betz, 1972). In the study of Lambertsen *et al.* (1953) a reduction in cerebral blood flow of 25 per cent and a 55 per cent increase in cerebral vascular resistance were found in subjects breathing oxygen at 3.5 atm. However it was noticed that the subjects hyperventilated, reducing the arterial P_{CO_2} by 5 mmHg, and it was concluded that the oxygen effect on cerebral vascular resistance was indirect, and was mediated via arterial hypocapnia. In an attempt to distinguish between the direct and indirect effects of oxygen, experiments were performed on anesthetized dogs breathing oxygen at 2 atm when the arterial carbon dioxide tension was maintained constant by controlled ventilation (Jacobson *et al.*, 1963; Harper *et al.*, 1965). A decrease in cerebral blood flow of the order of 21 per cent was demonstrated. However in a similar study at 3 atm where the arterial carbon dioxide tension was again kept constant, a significant increase in flow was observed (Ledingham *et al.*, 1966). Ledingham (1969) suggests that these conflicting results may be resolved by assuming that oxygen has two opposing actions on cerebral perfusion: a direct constrictive effect on arterial smooth muscle and an indirect vasodilator effect resulting from an increase in the carbon dioxide tension of cerebral tissues and the cerebral venous blood (which was a constant finding in his study). The net effect on cerebral perfusion would depend on the balance of these two opposing effects. Thus at 2 atm, the direct vasoconstrictive effect would be predominant, while at 3 atm vasodilatation induced by increased tissue carbon dioxide tensions would be evident. In *spontaneously* breathing man at 3 atm, oxygen causes vasoconstriction as a result of the hypocapnia from hyperventilation induced by raised cerebral tissue P_{CO_2}.

2.2.2. *Pulmonary Circulation.*

In contrast to the well-documented pressor responses of the pulmonary circulation to hypoxia, data on the effects of hyperoxia are sparse and conflicting. In general, in the intact organism, small systemic blood vessels dilate in response to hypoxia (Duke, 1957; Fishman *et al.*, 1951; Kety and Schmidt, 1948) and constrict in response to hyperoxia. The response of the pulmonary vasculature to hypoxia is the opposite, a

marked vasoconstriction being demonstrated consistently (Fishman, 1961). Conversely one might expect vasodilatation to occur in response to hyperoxia, but in fact most studies of the effects of breathing 100% oxygen at normobaric pressures fail to show any appreciable response (Storstein, 1952; Westcott *et al.*, 1951). This is consistent with the concept that the resistance vessels in the normal pulmonary circulation have little 'tone' (despite the low oxygen tensions of mixed venous blood). One study (Bain *et al.*, 1965) demonstrated that perfusion of the pulmonary vascular bed with hyperoxic blood with a P_{O_2} near to 600 mmHg resulted in an increase rather than a decrease in vascular resistance. Other studies have demonstrated a reduction in pulmonary artery pressure in response to inhalation of 100% oxygen (Logoras, 1947; Euler and Liljestrand, 1946; Duke, 1951), and a rise in pulmonary vascular resistance in the isolated perfused cat lung (Nisell, 1951).

In patients with pre-existing pulmonary hypertension, hyperoxia appears to reverse, at least in part, the vasoconstrictive element. Thus, in a study of nine patients breathing 100% oxygen, Westcott *et al.* (1951) found no effect on the pulmonary artery pressure in four who had normal control values, but a significant decrease in pressure in five (chronic emphysematous patients) with long-standing pulmonary hypertension. This was confirmed by Krongrad *et al.* (1973), Wilson *et al.* (1955), and Holt and Branscomb (1965) who found a 'highly significant' reduction in pulmonary artery pressure and pulmonary vascular resistance in thirteen patients with moderate to severe chronic obstructive emphysema. In altitude-acclimatized subjects, however, the administration of a 35% oxygen mixture does not relieve pulmonary hypertension (Rotta *et al.*, 1956).

When inspiration of high concentrations of oxygen is prolonged sufficiently for toxicity to develop, it appears that pulmonary artery pressure increases but that the magnitude of the response is quite small (see below).

2.3. ERYTHROPOESIS

One of the physiological manifestations of adaptation to long-term exposure to high altitude is a hyperactivity in the formation of red cells and hemoglobin. Plasma volume remains normal, or slightly reduced, and the resulting polycythemia permits the adapted individual to carry more oxygen per given quantity of blood (Viault, 1891; Hurtado, 1960). The reverse also occurs. A progressive decrease in hemoglobin concentration during the course of prolonged exposure to high partial pressures of oxygen (380 mmHg over 30 days) has been shown in the experiments leading up to the recent manned space flights (Helvey *et al.*, 1962). This, however, may not represent a suppression of erythrocyte formation but rather an early manifestation of hematological oxygen toxicity (see below).

3. TYPES OF OXYGEN TOXICITY

It is paradoxical that a substance so essential to life as oxygen should prove toxic at high concentrations. When considered in evolutionary terms, however, there is good evidence that the atmospheric concentration has been gradually increasing to its present level (Gilbert, 1972) and that the fight for survival of the organism has centered around the development of biological antioxidant defence systems. These antioxidant defences are at their most spectacular in the chloroplast-containing cells of green plants and in the cells lining the swim-bladders of fishes, where the oxygen tension may be concentrated to 100 atm (Fange, 1966; Steen, 1971).

The supply of oxygen at pressures greater than normal is therefore likely to affect all cell and tissue types, and toxic effects have been described for example in striated muscle (Bhakthan *et al.*, 1974), membrane transport in brain (Kaplan and Stein, 1957), frog skin (Falsetti, 1959) and striated muscle (Gilbert and Lowenberg, 1963), connective tissue (Richmond and D'Aoust, 1974), peripheral nerve (Perot and Stein, 1956), renal tissue (Mautner, 1966), the eye (Margolis and Brown, 1966; Yanoff *et al.*, 1970), the testis (Gershman, 1962), the blood (Mengel and Kann, 1966; Larkin *et al.*, 1973;

Blenkarn *et al.*, 1974; Senior *et al.*, 1974), and the endocrine (Hale *et al.*, 1973) and cardiovascular systems (Wood *et al.*, 1972; Hardenberg *et al.*, 1973).

The most florid effects of oxygen toxicity are those involving the pulmonary and central nervous systems. Although an animal exposed to an overdose of oxygen will probably succumb to the resulting derangement of lung or brain function it is important to realize that probably all tissues and systems of the body are involved in the toxic process.

4. TOXIC EFFECTS ON RESPIRATION OF PROLONGED OXYGEN BREATHING

4.1. Studies on Human Volunteers

The prolonged inspiration of oxygen leads to the development of symptoms, the rate of onset of which are related exponentially to the partial pressure of inspired oxygen (Fig. 1). With 100% oxygen at 1 atm, symptoms start to occur in the volunteer at between 8–10 hr (Dolezal, 1962). The symptoms commence with a mild irritation in the retrosternal region—this becomes progressively worse with the development of uncontrollable coughing until there is extremely severe pain on inspiration. The dyspnea starts to diminish rapidly several hours after changing the inspired gas from oxygen to air.

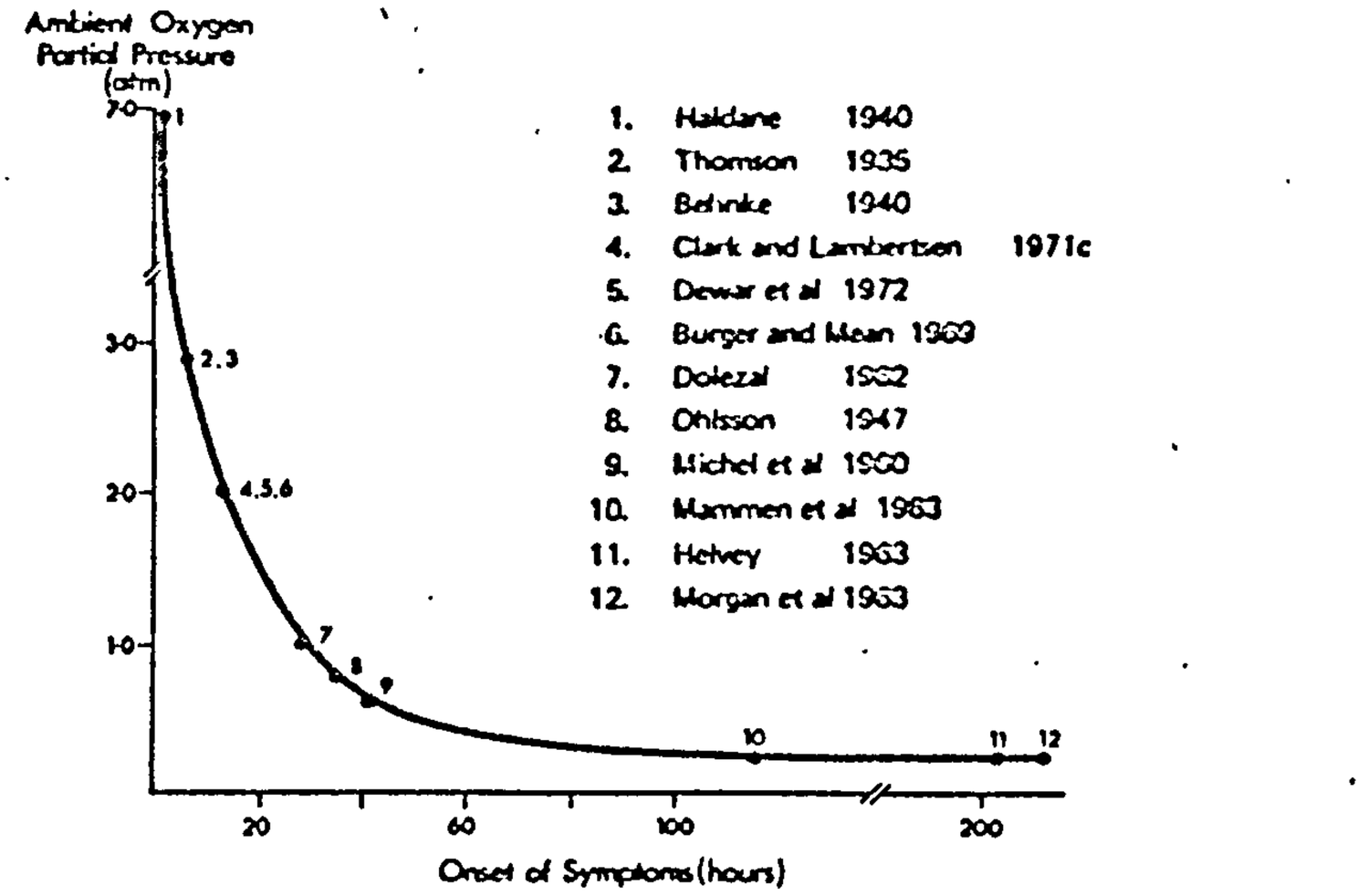

FIG. 1. Rate of development of early oxygen toxicity (abscissa, time to onset of symptoms) as a function of the inspired partial pressure of oxygen (ordinate, atm).

Owing to the wide variability in the reporting of symptoms by volunteers, attention has turned to other, hopefully more sensitive, indices of the rate of development of pulmonary oxygen toxicity. Clark and Lambertsen (1971*b*, *c*) have used the reduction in vital capacity which occurs to construct oxygen tolerance curves. Thus, in 50 per cent of subjects, a 10 per cent reduction in vital capacity occured at about 26 hr with 100% oxygen at 1 atm., 10 hr with 100% oxygen at 2 atm and about 7 hr with 100% oxygen at 3 atm. A decrease in vital capacity frequently commenced before the development of symptoms and the decline progressed for some time after removal from the oxygen environment (Caldwell *et al.*, 1966; Clark and Lambertsen, 1971*c*), unlike symptoms which tended to diminish more rapidly. Complete restoration of vital capacity usually occurred within 1–3 days following exposure to oxygen at 2 atm (Clark and Lambertsen, 1971*c*). A large number of other studies testify to the reduction in vital capacity provided that oxygen exposure is prolonged sufficiently (Clark and Lambertsen, 1971*b*).

The mechanism of this reduction in vital capacity, which occurred mainly at the expense of the inspiratory reserve volume, is unclear. The major causes of the decline in vital capacity might include chest pain, absorption collapse, pulmonary edema, and diminished pulmonary compliance. Chest pain is an unlikely mechanism as changes in vital capacity may occur in the absence of symptoms. In several studies vital capacity changes have occurred in the presence of normal chest X-rays and before any increase in A-aP_{O_2} developed, which renders absorption collapse and pulmonary edema unlikely (Clark and Lambertsen, 1971c, Caldwell *et al.*, 1966) as mechanisms in the early stages of toxicity, although these would obviously be important in the late stages. Clark and Lambertsen (1971*c*) postulated that the measured decrease in pulmonary compliance was of little importance but that the diminished force of inspiration, resulting possibly from pain, reflex inhibitions and muscle weakness, was the main factor causing a reduction in vital capacity.

Changes in arterial oxygenation attributable to oxygen toxicity during oxygen breathing in man occur only after prolonged periods of exposure. Thus with 100% oxygen at 2 atm, no progressive changes in A-aP_{O_2} were found after 5 hr exposure by Dewar *et al.* (1972) or after 8–10 hr by Clark and Lambertsen (1971*c*). With four volunteers exposed to 100% oxygen at 1 atm, there was no evidence of a progressive increase in A-aP_{O_2} over 30–74 hr (Caldwell *et al.*, 1966). Two well-controlled prospective clinical studies also suggest that changes in arterial oxygenation attributable to oxygen toxicity occur only relatively late during exposure. Studying two groups of patients following cardiopulmonary bypass, Singer *et al.* (1970) found no difference after 24 hr in dead space-tidal volume ratio (V_D/V_T), compliance or A-aP_{O_2} in those ventilated with 100% oxygen or in those ventilated with an inspired oxygen content of 40%. Also, no changes were recorded in two patients ventilated with 100% oxygen for 4 and 7 days respectively. Barber *et al.* (1970), examining patients with irreversible brain damage, found in a group of five ventilated with 100% oxygen a significant increase in V_D/V_T at 30 hr and a significant increase in venous admixture ($\dot{Q}_s/\dot{Q}_t$) at 40 hr, compared with five patients ventilated with air. It should be noted that these patients were receiving steroids which are known to diminish oxygen tolerance in animals and this factor may account for the difference between these findings and those of the study of Caldwell *et al.* (1966). That this deterioration in arterial oxygenation occurs only in the later stages of oxygen toxicity correlates with similar findings in animal studies (Smith, 1971).

Specific airway conductance was found to decrease in ten subjects exposed to 100% oxygen at 2 atm for 5 hr in the absence of any change in lung volumes or arterial oxygenation (Dewar *et al.*, 1972). In contrast, Fisher *et al.* (1968) had found no change 5 hr after exposure of six subjects to 100% oxygen at 2 atm for 6–11 hr. Dewar and her colleagues explained this apparent discrepancy by implicating constriction of the smooth muscle of the bronchioles induced by hyperoxia and postulating relaxation during the 5 hr recovery period.

Changes in the elastic properties of the human lung have been demonstrated in the early stages of oxygen toxicity before there is evidence of collapse, edema or gross pulmonary congestion (Fisher *et al.*, 1968; Clark and Lambertsen, 1971c). In view of the considerable body of evidence accumulating on the effect of oxygen on the alveolar type 2 cells (see below) it is tempting to speculate that a reduction in pulmonary surfactant is the most important mechanism of a reduction in lung compliance in the early stages of toxicity.

Changes in pulmonary capillary blood volume and diffusing capacity also occur, although data from a study with oxygen at 0.98 atm revealed a decrease in the latter and no change in the former (Caldwell *et al.*, 1966) whilst data from a study at 2.0 atm indicated a decrease in pulmonary capillary volume but not diffusing capacity (Clark and Lambertsen, 1971c). It has been suggested that the higher toxic levels of oxygen may be accompanied predominantly by vasoconstriction and capillary destruction, whilst interstitial edema is more prominent than capillary destruction during prolonged exposure to lower toxic levels of oxygen (Clark and Lambertsen, 1971b). In view of the

many mechanisms responsible for pulmonary damage and the suggestion that the contribution of the indirect mechanisms varies according to the inspired P_{O_2} this explanation seems feasible.

4.2. Animal Studies

There are many descriptions in the older literature of the visible events occurring in animals during the development of acute pulmonary oxygen toxicity. More recent studies have verified these descriptions of variation in ventilation followed by coughing, the development of irregularity of ventilation, gasping ventilation and finally death in apnea (Smith, 1973). The rate of development of these events depends on the inspired P_{O_2} and on the species of animal under investigation. In general, conscious dogs die in oxygen at 1 atm at about 55 hr (Ashbaugh, 1971) and at about 12 hr in oxygen at 2 atm, whilst anesthetic agents prolong survival time. Thus anesthetized dogs die in 100% oxygen at 2 atm, at about 18 hr under neuroleptanalgesia (Clarke *et al.*, 1973) and at around 14 hr under halothane anesthesia (Smith and Ledingham, 1971). Rats and mice perish in a slightly shorter time than dogs—around 48 hr at 1 atm. Unanesthetized rabbits survive for a mean time of 82 hr at 1 atm (Smith *et al.*, 1973). The survival time of primates is much greater; monkeys survive as long as 9–12 days in 100% oxygen at 1 atm. The variations in the reported survival times of animals reputedly inspiring the same P_{O_2}, described in a voluminous older literature on the subject reviewed by Clark and Lambertsen (1971b), can undoubtedly be accounted for by minor variations in inspired P_{O_2} and in the temperature, humidity, carbon dioxide, ammonia, and nitrogen contents of the oxygen exposure chamber together with differences in types of species, and age, nutritional and endocrine state of animals.

Recently, attention has been paid to the physiological changes occurring in animals during the development of toxicity. A consistent finding in several studies has been that of a reduction in pulmonary compliance (Pautler *et al.*, 1966; Smith *et al.*, 1963). Until recently, it has been possible to account for this change in terms of the development of interstitial edema, absorption collapse or frank intra-alveolar edema. Recent evidence, however, suggests that the change in compliance may also result from decreased synthesis of surfactant. Gacad and Massaro (1973) have examined pressure/volume curves of excised rat lungs exposed to oxygen at 1 atm and correlated these measurements with protein synthesis. After 24 hr of hyperoxia, at which time there was no change in static compliance, there was diminished incorporation of radioactive-labeled leucine into total protein and surface-active protein. At 48 hr of hyperoxia, the incorporation into surface active protein was much less than that into total protein. Although the interpretation of these biosynthetic changes is open to dispute, a growing body of evidence points to diminished surfactant activity occurring as a primary as well as a secondary event during oxygen exposure (see below).

There is good general agreement that changes in arterial oxygenation occur only in the late stages of toxicity (Smith *et al.*, 1963; Clarke *et al.*, 1973), and studies by the present authors, currently in progress, indicate that changes in lung compliance may occur long before any changes in arterial oxygenation. It seems reasonable, therefore, to postulate that in the early stage of oxygen toxicity the decreased lung compliance is produced by diminished surfactant activity. With the gradual development of interstitial and alveolar edema, as toxicity progresses, the magnitude of reduction in compliance increases.

Changes in the cardiovascular system do not appear to be the major cause of terminal hypoxemia in as much as systemic arterial pressure is well maintained. The evidence tentatively suggesting that left ventricular failure may occur is reviewed below. Pulmonary hypertension has been reported but the order of magnitude is quite small in physiological terms—increases of between 4 and 5 mmHg being observed between the early and late stages of toxicity (Clarke *et al.*, 1973; Smith *et al.*, 1963; Smith and Ledingham, 1971, 1972).

5. PATHOLOGICAL FEATURES OF PULMONARY OXYGEN TOXICITY

5.1. EXPERIMENTAL ANIMAL STUDIES

J. Lorrain Smith (1899) described the general pneumonic changes of pulmonary oxygen toxicity comprising congestion, consolidation and intra-alveolar exudation. These early findings have been confirmed repeatedly and, together with descriptions of atelectasis, alveolar thickening, and intra-alveolar edema and hemorrhage, are reviewed by Bean (1945).

In 1965 however, it was suggested (Durfey, 1965) that some at least of these changes might be artifactual and result from *post mortem* absorption collapse. Using mice exposed to 100% oxygen at 1 atm, he demonstrated a progression of changes in the lungs dependent on the delay between time of death and actual *post mortem* examination. In the same year, Pratt (1965) demonstrated a different pathological appearance in the lungs of mice that had been permitted to breathe air for short periods of time before death compared with those that had died while breathing oxygen. It is generally found in the terminal stages of oxygen toxicity that apnea precedes cardiac arrest by several minutes. Most earlier investigators failed to recognize this and made no attempt to fix the lungs at the moment of apnea. Consequently, only work published since 1965 will be reviewed.

The most comprehensive recent review of pulmonary pathology in oxygen toxicity is that of Clark and Lambertsen (1971*b*). The authors reviewed the results of experiments on oxygen breathing in thirteen different species citing some fifty references and commented on the marked species variation in susceptibility to oxygen. In particular, there is a species difference in the type of pathological response to oxygen. Exposure of rats to 100% oxygen at 1 atm caused death at approximately 70 hr (Kistler *et al.*, 1967) and electron microscopy revealed extensive damage to the pulmonary capillary endothelial cell with consequent interstitial and intra-alveolar edema. On the other hand, monkeys survived exposure to similar partial pressures of oxygen for approximately 2 weeks (Kapanci *et al.*, 1969) and the most obvious pathological change was a disappearance of type 1 alveolar epithelial cells and a marked proliferation of type 2 cells.

It is this species difference in susceptibility, together with a totally unpredictable strain susceptibility (Robinson *et al.*, 1967a) and even individual susceptibility to oxygen toxicity, that has produced much of the difficulty in correlating different experiments and interpreting results. In general, however, it may be stated that there are two distinct patterns of reaction to increased oxygen partial pressures; an acute exudative response characterized by capillary endothelial destruction and necrosis of type 1 cells and a chronic proliferative response showing mainly a proliferation of type 2 cells (Robinson *et al.*, 1967b; Kistler *et al.*, 1967; Schaffner *et al.*, 1967; Bowden *et al.*, 1968; Kaplan *et al.*, 1969; Kapanci *et al.*, 1969; Weibel, 1971). By and large, the acute response is a feature of exposure to high pressures of oxygen (in excess of 0.8 atm) and the chronic response is the result of prolonged exposure to relatively low pressures (0.5–0.8 atm) but it should be emphasized that, as a result of species variation, a spectrum of response is seen, and the two types of lesion may co-exist in one animal (Robinson *et al.*, 1967b; Kydd, 1967; Schwinger *et al.*, 1967; Robinson *et al.*, 1969; Harrison, 1974). Figure 2 illustrates these changes.

That the damage is not confined to the cellular elements of lung tissue is revealed in a histochemical study (Gupta *et al.*, 1969) which demonstrates damage to elastic fibers and reticulin in dogs exposed to pressures of 2.5 atm oxygen for 5.1 hr and then to 2 atm oxygen until death.

By using the stereologic and morphometric techniques of Weibel (1963a, b, 1969, 1970, 1973), Kistler has demonstrated the time course of events leading to death (Kistler *et al.*, 1967). Groups of rats were exposed to 100% oxygen at 1 atm and examined at 6, 24, 48 and 72 hr. There were few changes before 48 hr exposure but thereafter a return to room air caused dyspnea, and electron microscopy revealed interstitial edema and

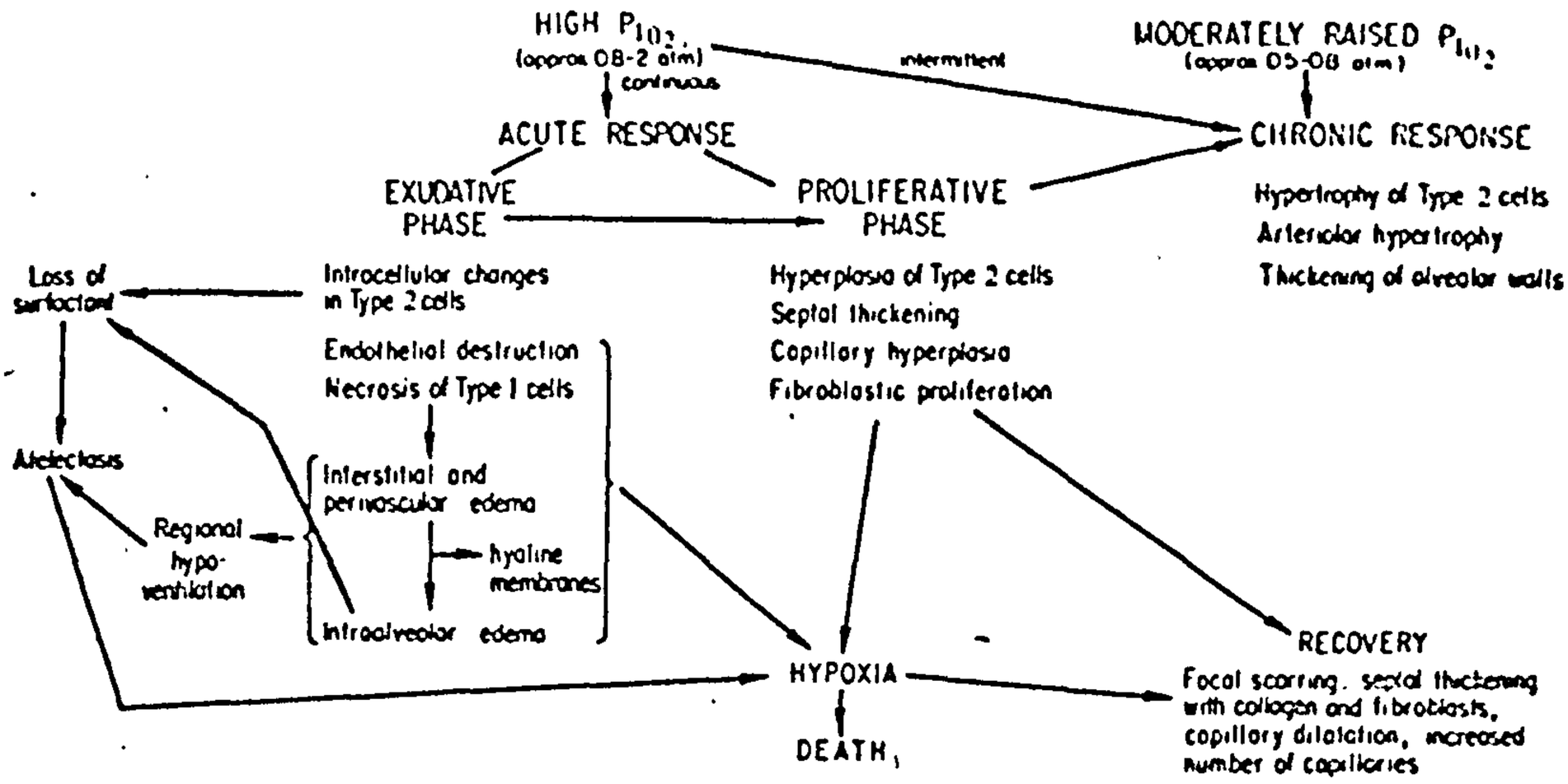

FIG. 2. Schematic diagram of the features of pulmonary oxygen toxicity. Reproduced by permission from Winter (1972).

early capillary endothelial changes. Restoration of rats to air after 72 hr of oxygen exposure caused severe dyspnea and cyanosis. Electron microscopy revealed interstitial infiltration and gross damage to capillary endothelium with complete destruction in some areas. There was little evidence of damage to alveolar epithelial cells but the alveolar membrane had thickened as a result of widening of the interstitial space and a hyperplasia of the epithelium. Morphometric analysis (Weibel, 1970) suggests that the diffusion capacity of such lungs has decreased to less than 20 percent of the normal value. Furthermore, by using the vascular perfusion fixation techniques developed by Gil and Weibel (1969, 1971), one may demonstrate intra-alveolar edema.

Monkeys survive for much longer periods of time in 100% oxygen at 1 atm. In experiments similar to the above (Kaplan *et al.*, 1969; Kapanci *et al.*, 1969) monkeys were examined after 2, 4, 7 and 12 days. Again the first lesion observed, at 2 days, was slight swelling and vacuolization of the endothelium. At 4 days, there was gross destruction of 90 per cent of the type 1 alveolar cells and early proliferation of type 2 cells. By the 7th day the alveolar membrane consisted almost entirely of type 2 cells and there was considerable variation in endothelial thickness. By the 12th day the proliferative changes were more marked and morphometric analysis revealed that the mean epithelial thickness was approximately seven times that of the control, and that the absolute increase in volume of the type 2 cells was approximately twenty-two fold. The endothelium had suffered massive destruction, the relative volume of endothelium per unit volume of tissue being reduced to some 30 per cent of the control value. The interstitium was more than twice as wide as in the controls, 50 per cent of its volume comprising erythrocytes, fibroblasts and inflammatory cells, and the harmonic mean of the total blood-gas barrier thickness was three times greater than in normal lung.

In this same investigation, (Kapanci *et al.*, 1969) the opportunity was taken to study the degree of regression of the lesions produced. Two monkeys, one exposed to pure oxygen for 8 days and the other for 13 days, were weaned back gradually to room air by slowly reducing the oxygen content of the inspired gas over 7–10 days. These animals made a complete clinical and functional recovery, and they were killed after 56 and 84 days recovery. In both animals, considerable restoration of normal lung architecture occurred. In the animal exposed for 8 days (i.e. after 56 days recovery) the endothelium was entirely normal, the alveoli were again lined with normal type 1 epithelium, and the type 2 cells were much less numerous although still greater than in normal lungs. There was a focal increase in the number of fibroblasts and collagen fibers and some residual edema fluid accumulation but the harmonic mean thickness of the barrier was in the normal range.

After 84 days of recovery (following 13 days exposure) the most obvious feature was septal scarring occupying 7 per cent of the total alveolar tissue. There was some thickening of the 'thin barrier portions' of the alveolar wall causing an increase in the harmonic mean thickness of the barrier of 28 per cent and an increase of 30 per cent in the capillary volume.

A recent study, using radioisotopes on rats exposed to 100% oxygen at 1 atm, showed progressive accumulation of fluid in the pulmonary interstitial space, intra-alveolar space and pleural cavity (Valimaki *et al.*, 1974). In recovery experiments, the pleural effusion and intra-alveolar edema had disappeared 24 hr after resumption of air breathing and interstitial fluid accumulation was diminishing. After 5 days recovery, interstitial edema had disappeared, indicating full recovery of the endothelium.

It is thought that the different effects of oxygen on the two epithelial cell types in the lung may be accounted for by their different ultracellular differentiation (Weibel, 1971). The type 2 cell is rich in mitochondria and has a well-developed endoplasmic reticulum. It is probably capable of rapid repair following damage by high oxygen tensions. There is, in fact, evidence of ultrastructural modifications in response to high levels of oxygen which would enhance anabolic function (Rosenbaum *et al.*, 1969). The type 1 cell (and endothelial cell) on the other hand is thin and attenuated, consisting predominantly of cell membrane and little else; it is poorly adapted to regenerate. It has not yet been explained why two apparently similar cell types, the capillary endothelial cell and the alveolar type 1 cell show such a different response to oxygen. In addition, the endothelium, which can be exposed only to oxygen after it has passed through the type 1 cell, suffers considerable damage while the type 1 cell remains comparatively unscathed. This has produced concepts of circulating 'humoral' factors which combine with oxygen to activate it, rendering the endothelium the 'target' tissue.

5.2. Clinical Studies

There are a large number of retrospective studies of the lung histology of patients who have been exposed to high concentrations of oxygen, and most of the important reports prior to 1971 are tabulated in the review by Winter and Smith (1972). These authors reviewed the literature and in most instances elicited other possible etiologies which could account for the histological findings in the lungs of patients alleged to have encountered oxygen toxicity. It is important to appreciate that there are neither histological features which by themselves are diagnostic of oxygen toxicity, nor any diagnostic radiological features. A diagnosis of oxygen toxicity in a patient depends on obtaining a history of exposure to a sufficiently large dose of oxygen known to produce changes in human volunteers or in patients in the two prospective clinical studies which exist. Thus, from the latter it is suggested that exposure of patients to 100% oxygen might be expected to cause sufficient lung damage to augment the degree of any existing intrapulmonary shunt (Barber *et al.*, 1970) after 40 hr of continuous exposure. It is of interest that Barber and his colleagues could not detect any histological differences in the lungs of patients ventilated with 100% oxygen for 40 hr from those ventilated with air for the same period and this is further testimony to the nonspecificity of oxygen-induced lung damage. Unfortunately electron microscopy was not performed and this might have been useful in elucidating any possible differences.

An important earlier study and one typical of many investigations was that of Nash *et al.* (1967). These authors retrospectively reviewed the pathological records of seventy patients who had been receiving prolonged intermittent positive pressure ventilation. They allocated the patients to four groups; those ventilated for less than 10 days with oxygen concentrations below 90% and those above 90%, and those ventilated for more than 10 days with oxygen concentrations below 90% and those above 90%. They found in the patients receiving higher oxygen concentrations a higher incidence of hyaline membranes, thickening of the interlobular and alveolar septa by edema and fibroplastic proliferation. These findings did not correlate with the clinical etiologies or with the duration of ventilation.

More recently, studies of human oxygen toxicity have been extended by the use of electron microscopy. In 1973, Anderson and his associates described the features in the lungs of seventy-four infants with respiratory distress syndrome ventilated with 40–60% oxygen for periods varying from 3 hr to 135 days. The observations were of an early exudative phase minimal at about 12 days progressing to a fibroproliferative phase which became more obvious in the lungs of infants who survived for longer periods. The study suggested that initially there was an alteration of the alveolar lining cells followed by capillary endothelial damage and hyperplasia of type 2 cells. Hyaline membranes were incorporated by extension of the type 2 cells and later there was a proliferation of septal cells.

Similar changes were described in a study by Gould *et al.* (1972) of the lungs of fifteen patients without pre-existing pulmonary disease who had succumbed after periods of 14 hr to 30 days following ventilation with oxygen concentrations varying from 40–100% at 1 atm. As the dose of oxygen increased, there was a change in the ultrastructural lesions. Initially cytoplasmic changes were seen in the type 2 cells. Later, endothelial damage occurred parallel with sloughing of the type 1 cells. After 2 days exposure, interstitial edema was invariably present. After 6 days exposure the most striking features were proliferation of the type 2 cells, followed by hyperplasia of the septal cells (corresponding to 'septal thickening' as seen by light microscopy). The increases in numbers of septal cells and collagen fibers progressed in the patients with the longest oxygen exposures. However, many of the patients in this study suffered from irreversible brain damage and this may have potentiated the rate of development of lung damage.

These changes, although non-specific, are identical to those occurring in primate lungs exposed to oxygen and the rates of development are similar. This provides strong circumstantial evidence of the existence of pulmonary oxygen toxicity as a clinical entity.

6. MECHANISMS OF PULMONARY OXYGEN TOXICITY

The cellular mechanisms and biochemical aspects of oxygen toxicity are beyond the scope of this article and the reader's attention is drawn to reviews of this subject by Haugaard (1968) and Davies and Davies (1965). Although there is a very large literature on the subject, it should be noted that many studies have used very high pressures of oxygen, and many are observations of changes in tissue culture, the relevance of which to the intact organism exposed to oxygen at 0.5–2.5 atm is not clear.

High pressure oxygen inactivates many enzyme systems leading to depression of cellular metabolism. The most important ways in which this occurs and which are most likely to be relevant to the *in vivo* situation include:

(1) oxidation of SH groups on essential enzymes such as Co enzyme A;
(2) peroxidation of lipids and the resulting lipid peroxides inhibit the function of the cell; and
(3) inhibition of the pathway of reversed electron transport possibly by inhibition of iron and SH containing flavoproteins.

Additionally, high pressure oxygen may oxidize other components of the tissues which are amenable to oxidation including glutathione and ascorbic acid.

Recent evidence suggests that the site of action of oxygen may be on the membrane rather than on intracellular locations (Allan *et al.*, 1973).

The physiological pathways whereby oxygen may exert toxic effects leading to lung damage are shown in Fig. 3. A high inspired P_{O_2} has been shown to diminish ciliary activity possibly by direct diffusion into the cells of the tracheobronchial mucosa (Laurenzi *et al.*, 1968). This is not a mechanism of any great importance as retention of secretions is not a significant feature of pulmonary oxygen toxicity.

The elevated alveolar P_{O_2} may damage alveolar cells of all types and also capillary endothelial cells by a direct effect but it is of great interest that, in all species so far

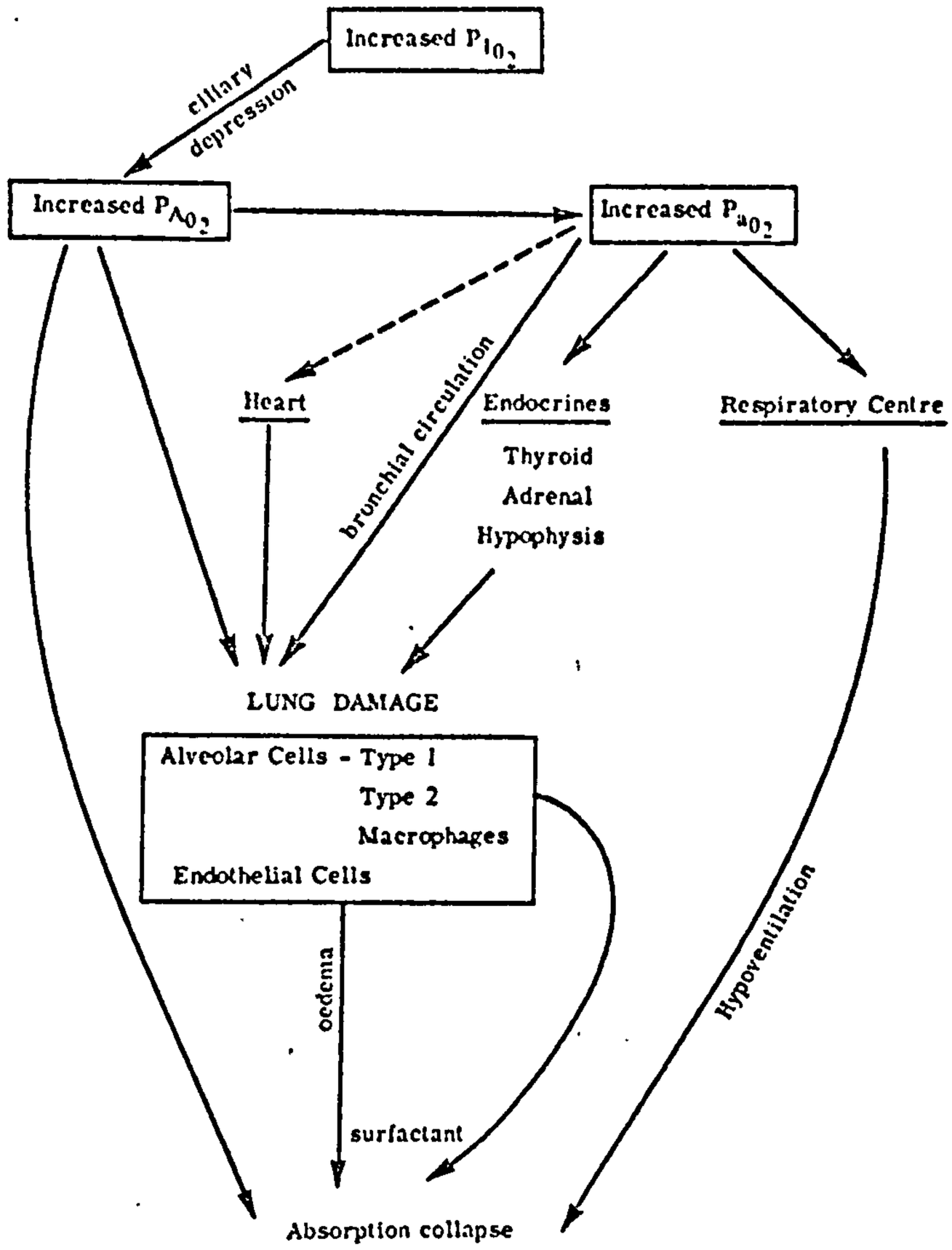

FIG. 3. Diagram of the mechanisms by which toxic partial pressures of oxygen may produce lung damage.

investigated, the endothelial cell demonstrates changes before pathological features occur in the alveolar cells. Elevation of the alveolar P_{O_2} leads to an increase of arterial P_{O_2} which potentiates the toxic effects either via the bronchial circulation or by eliciting contributions from several indirect mechanisms; the heart, the neuroendocrine system and the respiratory center. Absorption collapse occurs more readily in the presence of a high alveolar P_{O_2} as a result of inhibition of surfactant, the presence of edema and by hypoventilation. These mechanisms will now be reviewed in greater detail.

6.1. DIRECT EFFECT

There is little doubt that oxygen has a direct toxic effect on the lung. Experiments on cats using a double lumen catheter to ventilate one lung with air and the other with oxygen have revealed that the oxygen-ventilated lung suffered gross damage with oxygen at 5 atm whilst the lung ventilated with inert gas suffered little effect (Penrod, 1958). Work designed to separate the effect of a high alveolar P_{O_2} from those of a high arterial P_{O_2} (discussed at length in the following section) have revealed that at normobaric pressures the rate of development of toxicity is not reduced by lowering the arterial P_{O_2} (Ashbaugh, 1971; Miller *et al.*, 1970) although at hyperbaric pressures (Winter *et al.*, 1967; Thomas and Hall, 1970) an increased A-aP_{O_2} does improve oxygen tolerance. One concludes that both direct and indirect mechanisms exist and that their contributions vary according to the partial pressure of inspired oxygen and the species under investigation.

6.2. Central Respiratory Failure

There is some evidence to show that, at a pressure of 5 atm, oxygen may cause apnea in the anesthetized rat as a result of central respiratory failure, if the inspired carbon dioxide concentration is high (Jamieson and Cass, 1967). There is further tenuous and circumstantial evidence that oxygen at 2 atm may have an effect on the respiratory center in anesthetized dogs (Clarke *et al.*, 1973). Furthermore, dogs anesthetized with halothane and inspiring 100% oxygen at 2 atm appear to die as a result of central respiratory failure (Smith and Ledingham, 1971). In both these studies, apnea occurred in spontaneously breathing dogs when the arterial oxygen saturation was still of a high order and compatible with life, although there had been a progressive increase in the A-aP_{O_2} commencing shortly before death. Although it is likely that central respiratory failure may occur at hyperbaric pressures, there is no evidence that this mechanism may play a role in the development of apnea at normobaric pressures.

6.3. Absorption Collapse

Collapse, congestion and consolidation are the classical pathological features of acute pulmonary toxicity and it was thought for many years that absorption collapse was important in the etiology of this condition. This view has been strengthened recently by work on the physiological effects of breathing 100% oxygen in humans. Collapse of alveoli has been demonstrated during oxygen breathing at reduced lung volumes (Burger and Macklem, 1968; Nunn *et al.*, 1965), at low lung volume in the presence of airway closure (DuBois *et al.*, 1966), and at normal lung volumes in the absence of coughing or sighing (Burger and Mead, 1969). However, there is good evidence that absorption collapse is not important as an initiating event in the development of toxicity but occurs at a later stage subsequent to the development of lung damage (Winter and Smith, 1972). Factors which would encourage the development of lung collapse include narrowing of the airways by interstitial and intra-alveolar edema, reduction in pulmonary surfactant either as a primary effect of oxygen or subsequent to the development of edema, and hypoventilation. It is our contention however, that less absorption collapse than previously suggested is seen in the pre-terminal stages of pulmonary oxygen toxicity (Smith and Ledingham, 1971; Smith, 1971; Clarke *et al.*, 1973) and that the red, beefy, congested lung of the 'Lorrain Smith effect' is predominantly a terminal event.

6.4. Myocardial Failure

Oxygen at normal atmospheric pressure reduces myocardial contractility in the open-chested dog (Daniell and Bagwell, 1968) whilst oxygen at 2 atm has a rapid depressant effect on left ventricular function (Kioschos *et al.*, 1969). Recently, following the observation of persistent hypertension in rats exposed to 100% oxygen at 3 atm (Wood *et al.*, 1972), it has been suggested that left ventricular failure may be a feature of oxygen toxicity. By processes of elimination and elucidation, Clarke *et al.* (1973) also postulated that left ventricular failure may have occurred in four of six anesthetized dogs exposed to 100% oxygen at 2 atm. These observations were the subject of another study by Smith and Ledingham (1972) who ventilated six dogs, anesthetized with trichloroethylene, with 100% oxygen at 2 atm and measured maximum left ventricular rate of change of pressure [dp/dt(max)] as an index of myocardial contractility. They found that contractility declined over a 4 hr period, was relatively stable for a subsequent 4 hr, and recovered following ventilation with 15% oxygen and 85% nitrogen at 2 atm. These results are consistent with the view that oxygen produces a reversible reduction in myocardial contractility, but do not conclusively substantiate the concept of left ventricular failure contributing to the development of pulmonary edema. In contrast, in a study of conscious dogs exposed to 100% oxygen at 1 atm, there was no tendency for the left atrial pressure to increase in

the terminal stages (Smith *et al.*, 1963). One may conclude that, although there is some tentative evidence to implicate left ventricular failure at hyperbaric pressures of oxygen, there is no evidence to suggest that this process contributes to the production of lung damage at normobaric pressures.

6.5. NEUROENDOCRINE EFFECTS

There is little doubt that the general level of metabolic activity affects the rate of development of oxygen toxicity (Popovic *et al.*, 1964) and, interestingly, poikilotherms are known to be particularly resistant to oxygen toxicity (Faulkner and Binger, 1927). Depression of metabolism by anesthesia is known to improve both pulmonary and central nervous oxygen tolerance (Bean and Zee, 1965).

Thyroid activity influences oxygen tolerance (Bean and Bauer, 1952). Thus thyroxine potentiates the rate of development of pulmonary oxygen toxicity whilst thyroidectomy improves oxygen tolerance. Hypophysectomy produces an increase in oxygen tolerance either by reducing the secretion of TSH (Bean and Bauer, 1952; Smith *et al.*, 1960) or by reducing the secretion of ACTH, as it is known that the adrenal cortical hormones potentiate the development of oxygen toxicity (Bean and Smith, 1953).

There is a large amount of experimental data on the protective effects against oxygen toxicity produced by ablation of these endocrine glands and of the potentiating effect produced by administration of the secretions of these glands. The reader is referred to the review by Clark and Lambertsen (1971b) for a fuller discussion. It should be noted though that the majority of studies have used very high pressures of oxygen (in excess of 3 atm) and these results should be interpreted with caution for toxicity in the range 0.5–2.5 atm. However, effects of thyroxine, hypophysectomy, cortisone and adrenalectomy are all demonstrable with normobaric pressures of oxygen.

There is a considerable body of experimental data which substantiates the concept that the sympatho-adrenal system is associated with the development of pulmonary oxygen toxicity. Thus sympatho-adrenal stimulation and sympathomimetic drugs potentiate the development of oxygen toxicity whilst adrenalectomy and sympatholytic drugs delay the rate of development of oxygen toxicity (Bean, 1964, 1965; Hammond and Akers, 1974; Taylor, 1958; Bean and Johnson, 1955; Johnson and Bean, 1957; Drysdale, 1971; Beckman, 1974; Demeny *et al.*, 1974). Although most of these data have been obtained using hyperbaric pressures of oxygen it is known that oxygen at 1 atm is associated with augmented secretion of catecholamines (Smith *et al.*, 1973) and that the administration of exogenous epinephrine potentiates oxygen toxicity in rats breathing 100% oxygen at 1 atm (Smith and Bean, 1955).

Current concepts suggest that sympatho-adrenal stimulation may augment oxygen toxicity at low pressures by an effect on pulmonary surfactant and the evidence for this is reviewed below.

6.6. PULMONARY SURFACTANT

Research on the effects of oxygen and pulmonary surfactant is one of the areas of significant advance in the last few years in the field of oxygen toxicity. Pulmonary surfactant stabilizes the alveolus by virtue of its unique surface tension properties (Clements *et al.*, 1961) and also prevents transudation of fluid into the capillary lumen (Pattle, 1965). Impairment of the surfactant system by oxygen would explain many of the morphological features of pulmonary oxygen toxicity. Unfortunately there is no standard method of measuring surface activity. The use of many different methods for assessing the effect of oxygen on surfactant, such as measuring the surface tension of pulmonary extracts obtained either by endobronchial lavage (Giammona *et al.*, 1965) or from minced lung (McSherry *et al.*, 1968), observing bubble stability (Newman and Naimark, 1968), comparison of air and saline pressure/volume curves (Beckman and Weiss, 1969) and estimating the uptake of labeled palmitate into surfactant lecithin (Gilder and McSherry, 1974), has made it difficult to validate results and to correlate data from different centers. In addition to this, other mechanisms unrelated to oxygen

toxicity, or induced by other etiologies, such as contamination by fibrinogen (Taylor and Abrams, 1966) and blood (Reifenrath and Zimmerman, 1973), cholinergic stimulation (Bolande and Klaus, 1964), atelectasis (Levine and Johnson, 1965), edema (Said *et al.*, 1965) mechanical overinflation of the lung (Forrest, 1972) and rapid decompression (McSherry *et al.*, 1968), are known to diminish surfactant activity.

Despite many conflicting results (Clark and Lambertsen, 1971b) the bulk of the evidence suggests that there is diminished surfactant activity following exposure to high partial pressures of oxygen.

It is not certain if the surfactant changes in pulmonary oxygen toxicity are a primary event or if they are consequent upon mechanical derangement induced by oxygen. To resolve this, the ultrastructural changes occurring in the lung during exposure to high pressures of oxygen have been investigated with particular reference to those cytoplasmic organelles—such as the lamellated inclusion bodies and the rough endoplasmic reticulum—known to be involved in surfactant synthesis (Askin and Kuhn, 1971). In addition alterations in the biochemical events leading to the production of surfactant have been studied (Spitzer and Norman, 1971). The results are conflicting. Thus, in studies on the lipid component of surfactant, Trapp *et al.* (1971) (using dogs exposed to 3 atm oxygen for 5 and 10 hr) demonstrated that the diminished surface activity of lung extracts, which occurred in the absence of light or electron microscopic evidence of damage, was associated with the appearance of fatty acids not normally found in the lining layer. These fatty acids have a potentially destructive effect on surface activity. Also, McSherry and Gilder (1970) showed that acute exposure of rabbits to 100% oxygen at 2 and 3 atm for 1, 3, and 5 hr was associated with a progressive decrease in surfactant production. Chronic exposures also decreased the rate of incorporation of labeled palmitate into lung phospholipids. Conversely, Newman and Naimark (1968) found that in rats oxygen at 3 atm for 3.5 hr appeared to *enhance* the uptake of labeled palmitate into lung phospholipid and was not associated with impairment of surface activity. In a more recent study, Gilder and McSherry (1974) demonstrated two stages of lecithin synthesis which were inhibited by high pressures of oxygen, and an inexplicable increase in mitochondrial lecithin.

Partial explanation of these conflicting findings may be obtained from the observation of Young and Tierney (1972) that dipalmitoyl lecithin may exist in the lung in the form of several 'pools' and that it is not confined to the surface active component.

In studies on the protein constituents of surfactant, Gacad and Massaro (1973) exposed rats to oxygen at 1 atm for 12, 24 and 48 hr. After 24 hr there was a significant decrease in protein synthesis before any compliance changes occurred. After 48 hr, when there was a decrease in compliance, the extent of incorporation of labeled leucine was decreased more in the surface active fraction than in the total protein. More recent work by Massaro and Massaro (1974) confirmed these observations and demonstrated that during adaptation to hyperoxia the lungs recovered the ability to synthesize protein. These biochemical findings are associated with consistent ultrastructural alterations (see below).

The degenerative changes described by Rosenbaum *et al.* (1969) in mitochrondria of the type 2 cells in response to high pressures of oxygen have been confirmed consistently (Yamamoto *et al.*, 1970); Adamson *et al.*, 1970; Massaro and Massaro, 1973a). It has been suggested that a marked decrease in the number of mitochondrial granules may represent a loss of intramitochondrial cations which could affect surfactant synthesis by interfering with α-glycerophosphate oxidation (Massaro and Massaro, 1973a). In addition, exposure to oxygen causes degenerative changes in the lamellated inclusion bodies (Chevalier and Collet, 1972; Kuhn, 1968) thought to be the intracellular storage sites for preformed surfactant (Morgan *et al.*, 1965; Motlagh *et al.*, 1969; Yamamoto *et al.*, 1970). Recent work by Massaro and Massaro (1973b) confirms that there is a decrease in size but not in number of lamellated bodies, yet failed to detect any change in the surface density of the rough endoplasmic reticulum.

Circumstantial evidence for the involvement of the surfactant system in pulmonary oxygen toxicity may be found in the ultrastructural response of the type 2 cell during

adaptation to and recovery from high pressures of oxygen. Rosenbaum and his colleagues (1969) described the appearance of cup-shaped mitochondria, [an adaptation thought to be associated particularly with lipid metabolism (Engers and Lyons, 1964; Seljelid and Ericsson, 1965)], an increase in free ribosomes and a dilatation of the endoplasmic reticulum. That these changes were compensatory rather than degenerative was demonstrated by the absence of such features during exposure to higher, more toxic pressures of oxygen. Yamamoto *et al.* (1970) increased the tolerance of rats to pure oxygen by pre-exposure to 0.85 atm oxygen and showed that this was associated with an elongation of mitochondria and a significant increase in mitochondrial volume. With exposure to pure oxygen following adaptation, the mitochondria developed cup shapes, the endoplasmic reticulum became dilated, and there was an increase in the number of lamellated bodies. Massaro and Massaro (1974) revealed that adaptation reverses the reduction in size of the inclusion bodies caused by exposure to toxic pressures of oxygen so that, after 96 hr, their surface area has increased in size to normal values. Furthermore, at 96 hr the surface density of the rough endoplasmic reticulum was increased significantly, indicating an increased synthesis of secretory proteins (Ganoza and Williams, 1969).

6.6.1. *Pulmonary surfactant and the sympathetic system*

That pulmonary edema is associated with a variety of insults to the central nervous system has been recognized for many years (Weisman, 1939; McKay, 1950) and recent work by Bean and his colleagues (Bean and Beckman, 1969; Beckman and Bean, 1970; Beckman *et al.*, 1971) has demonstrated the involvement of the sympathetic system in the etiology of 'centri-neurogenic' pulmonary edema.

Evidence is growing that these sympathetic effects on the lung are mediated via the surfactant system.

In a series of experiments exposing cats and rats to very high oxygen pressures, Beckman and Houlihan (1973) showed that, while rats have both altered surfactants and gross lung damage, cats have only altered surfactants and no gross lung damage. In other experiments not involving exposure to hyperoxia, it has been demonstrated in cats (Beckman and Mason, 1973) and monkeys (Beckman *et al.*, 1974) that electrical stimulation of the stellate ganglion caused a significant decrease in compliance and altered surfactants in the absence of any gross lung damage.

There is a little evidence elsewhere for the existence of a control mechanism for surfactant at organ level (Clements, 1970). It is of related interest that Bean and Nakamoto (1974) have demonstrated adrenergic nerve fibres in the alveolar wall, a finding confirmed by one of the present authors (T. G. Shields, unpublished) who has found very fine adrenergic nerve fibers terminating adjacent to a type 2 cell. This, together with the recent demonstration by electron microscopy (Hung *et al.*, 1972) of motor nerve fibers forming a synapse with a type 2 cell provides strong circumstantial evidence for a central nervous control of surfactant production or release.

However, it should be noted that almost all of the experiments demonstrating a 'centri-neurogenic' effect in pulmonary oxygen toxicity were conducted at relatively high pressures of oxygen, at a level where central nervous oxygen toxicity might be expected to become evident. At these levels, the observed pulmonary pathology might be one of the manifestations of central nervous toxicity mediated via the sympathetic system. There is very little evidence for the involvement of the sympathetic system at normobaric pressures, and this remains a potentially fruitful field of study.

7. MODIFICATION OF PULMONARY OXYGEN TOXICITY

In recent years there has been considerable interest in the relationship between pre-existing lung damage and oxygen tolerance, partly by virtue of its clinical importance, and partly as a means of exploring the mechanisms underlying the development of pulmonary oxygen toxicity. Thus if the direct mechanism of oxygen

toxicity is the predominant one, pre-existing lung damage may reduce oxygen tolerance whilst, if the indirect mechanisms are predominant, pre-existing lung damage may reduce the magnitude of their effects by reducing the level of P_{v} in the systemic or bronchial circulations or by inhibiting reflexes from the lung stimulated by hyperoxia, and prolong oxygen tolerance. Unfortunately studies designed to investigate this question provide conflicting data.

In experiments designed to expose lung tissue to a toxic level of oxygen whilst maintaining physiological systemic oxygen tensions, Winter *et al.* (1967) found that the oxygen tolerance of dogs with veno-arterial shunts exposed to oxygen at a pressure of 2.0–2.5 atm was increased in comparison with that of control, unanesthetized dogs. The conclusion that pulmonary shunting and an increased A-aP_{O_2} improved oxygen tolerance is partly confirmed by data obtained by Thomas and Hall (1970) using a slightly different model. In dogs which underwent either pulmonary artery ligation, or ligation following anastomosis between the opposite pulmonary artery and the left atrial appendage, a positive correlation was found between increased surface tension of lung extracts and the P_{O_2} of venous blood draining from the lung from which the extract was obtained. It should be stressed however that the study of Winter *et al.* (1967) was of acute pulmonary oxygen toxicity whilst that of Thomas and Hall (1970) was of chronic oxygen toxicity as the animals were exposed intermittently to oxygen at 3 atm pressure in the study of the latter group.

Although these two studies appear to indicate that chronic pulmonary oxygen toxicity and acute pulmonary oxygen toxicity induced by hyperbaric pressures of oxygen are influenced by the level of systemic oxygen tension, studies with normobaric oxygen have demonstrated the opposite results. Ashbaugh (1971) found that the survival times of dogs with experimentally created veno-arterial shunts were not altered during exposure to an inspired P_{O_2} of 540–580 mmHg in comparison with control animals whilst Miller *et al.* (1970) found no difference in pulmonary surfactant measurements or pulmonary histology between shunted and non-shunted dogs after 48 hr exposure to 98–100% oxygen at 1 atm.

These four studies suggest that in the absence of pre-existing pulmonary damage, the direct toxic effect of oxygen may be dominant at normobaric pressures but that, at hyperbaric pressures of oxygen, the indirect mechanisms play a greater role.

Unfortunately, it is clear that this hypothesis is not applicable in the situation where there is pre-existing pulmonary damage, where one might expect oxygen tolerance to be diminished at normobaric pressures. In contrast it is known that occasionally hypoxic patients with lung damage may survive for very prolonged periods (Nash *et al.*, 1967; Bendixen *et al.*, 1965; Northway *et al.*, 1967) and in addition exposure to phosgene gas (Ohlson, 1947) and prior intermittent exposure to hyperoxia (Wright *et al.*, 1966; Kydd, 1968) are known to be associated with the production of lung damage and an increase in oxygen tolerance. Smith *et al.* (1973) have concluded that the structural changes present in lung damage induced by oleic acid comprise elements which are more resistant to oxygen toxicity and constitute the major mechanisms of increased oxygen tolerance. In studies of rabbits with oleic acid-induced lung damage, it was found that the survival times of treated animals exposed to 100% oxygen at 1 atm were double those of control animals. In particular, it was found that fibroproliferative changes, particularly hyperplasia of type 2 cells, appeared to be associated with an increase in oxygen tolerance although other possible explanations for the findings include the creation of an increased A-aP_{O_2}. Pre-treatment with oleic acid induced type 2 cell hyperplasia which was associated with increased tolerance to oxygen following immediate exposure or where exposure of rabbits to oxygen had been delayed for 24 hr. Delay for 1 week was associated with neither type 2 cell hyperplasia nor an increase in oxygen tolerance (Winter *et al.*, 1974).

Another situation in which prior parenchymal change is related to improved oxygen tolerance is that of altitude acclimitization (Brauer *et al.*, 1970). These workers found that rats exposed to altitude survived three times as long as control rats whilst inspiring oxygen at a pressure of 825 mmHg. The protective effect of acclimatization persisted for as long as 30 days. The absence of protection against the convulsive effects of oxygen

at 7 atm indicated that the protection was exerted at the level of the pulmonary parenchyma and not by any reduction in systemic level of oxygen. The mechanism of this tolerance was interpreted in terms of increased oxygen carrying capacity of the blood, increased density of capillary networks and changes in oxidative enzyme chains. The mechanisms which persist long enough in the partially deacclimatized rats to protect against toxicity were thought to include biochemical adaptations and microcirculatory changes. Support for this concept that prior exposure to hypoxia produces increased oxygen tolerance at 1 atm by mechanisms other than increased shunting comes from the work of Brashear *et al.* (1973). These workers exposed rats to hypoxia for 5 days, at which time their systemic oxygen tensions whilst breathing 100% oxygen at 1 atm were not significantly different from those of control rats breathing oxygen. Hypoxia-stressed animals survived for considerably longer than control rats in 100% oxygen at 1 atm. Unfortunately pulmonary histological examination and electron microscopy of animals treated by hypoxia alone were not performed, so it is not possible to speculate on the mechanisms of increased oxygen tolerance in this study other than to exclude increased intrapulmonary shunting.

There is some evidence that infections may be potentiated in the presence of high inspired oxygen concentrations and, although this does not constitute a primary mechanism of pulmonary oxygen toxicity, this process may augment the development of lung damage especially in clinical situations. It has been shown that mice with influenzal viral pneumonia have an increased mortality and a shortened survival time with exposure to 100% oxygen at 1 atm (Ayers *et al.*, 1973). The mechanism of this process is uncertain. Impairment of pulmonary *antibacterial* activity in rats exposed to 100% oxygen at 1 atm (Finder *et al.*, 1972) possibly results from the reduced ability of alveolar macrophages to ingest bacteria (Thurlbeck, 1974). In a study of mice infected with diplococci pneumoniae, Angrik *et al.* (1974) found that exposure of infected animals to 75% oxygen at 1 atm was associated with prolongation of survival.* However, it should be noted that, with *hyperbaric* pressures of oxygen, the toxic effects of oxygen on bacteria are greater than the toxic effects of oxygen on the host's defence mechanisms. Thus Ross and McAllister (1965) found that the resistance of mice to infections with the pneumonococcus was increased by exposure to 100% oxygen at 2–3 atm.

Although there are many chemicals which delay or potentiate the rate of development of pulmonary oxygen toxicity, few, by themselves, produce structural changes in the lungs. Thus adrenal cortical hormones, thyroid hormones and catecholamines or sympathomimetic agents all augment oxygen toxicity whilst agents with converse effects, and many antioxidants and reducing agents delay the rate of development of pulmonary oxygen toxicity without affecting pulmonary structure. A notable exception to this statement is the substance Paraquat® (dimethylbipyridinium dichloride) which has been shown to enhance the development of toxicity in rats exposed to an inspired P_{O_2} of 630 to 700 mmHg (Fisher *et al.*, 1973). Interestingly the pathological lesions induced by both Paraquat® and oxygen were virtually identical. On a biochemical basis, it would appear that both oxygen (Haugaard, 1968) and Paraquat® (Gage, 1968) produce an increased net oxidation of pyridine nucleotides and an additive effect or potentiation may be postulated. Dimethylbipyridinium dichloride may indirectly accept electrons from NADPH, bypass the normal electron transfer system and pass on electrons to molecular oxygen. It is known that in the reduction of oxygen, the first radical produced is O_2^- or superoxide, a substrate which is catalyzed to hydrogen peroxide by the enzyme superoxide dismutase. Cells which contain a higher concentration of superoxide dismutase are more resistant to oxygen toxicity than comparable cells which contain a lower concentration of this enzyme (Gregory and Fridovich, 1973). Other pieces of evidence suggest that the radical superoxide may be a basic agent in the causation of oxygen toxicity and that the enzyme superoxide dismutase may be an important defence mechanism against the lethal effects of the free radical (Editorial, 1973). Confirmatory data have been provided recently in rats rendered oxygen-tolerant by prior exposure to 85% oxygen at 1 atm for 7 days, and by

* ERRATUM: should read: 'associated with a decrease in survival time.'

the demonstration of an increase in pulmonary superoxide dismutase activity which parallels the rate of development of oxygen tolerance. Under similar circumstances hamsters, mice and guinea pigs did not develop oxygen tolerance and they did not have as large a change in pulmonary superoxide dismutase as did the rat (Crapo and Tierney, 1974).

7.1. Inert Gas and Carbon Dioxide

At relatively low pressures, oxygen toxicity depends on the partial pressure of inspired oxygen rather than on its concentration, but the presence of inert gas may modify the rate of development of toxicity (neglecting absorption collapse which has been discussed above). When the partial pressure of inert gas is sufficient to exert a narcotic action, delay in the onset of toxicity may be anticipated (Almquist *et al.*, 1969; Clarke *et al.*, 1973), whilst increase in the rate of development of hyperbaric oxygen toxicity at a given pressure of oxygen by the addition of high partial pressures of helium or nitrogen has been interpreted in the light of increases in inspired gas density (Thomson *et al.*, 1970). The latter mode of action may be attributed to hypoventilation and an increase in arterial P_{CO_2} which has been known for many years to reduce oxygen tolerance at pressures in excess of 3 atm. Although an increased alveolar P_{CO_2} of a small order causes enhancement of oxygen toxicity, a high alveolar P_{CO_2} increases oxygen tolerance presumably by exerting an anesthetic effect (Jamieson, 1966).

7.2. Pharmacological Modifications of Oxygen Toxicity

It is known that agents which diminish metabolic, thyroid, adrenal cortical or sympatho-adrenal activity all delay the rate of development of oxygen toxicity in experimental animals. In addition it has been known for many years that various substances including alanine, antihistamines, arginine, cysteine, antioxidants, glutathione and vitamin E all offer partial protection against pulmonary oxygen toxicity in the experimental animal exposed to very high pressures of oxygen (Wood, 1969; Bennett, 1972; Willis and Kratzing, 1972; Clark and Lambertsen, 1971b). Clinically no agents have yet been demonstrated to affect oxygen tolerance in patients. As in many areas of research in the field of oxygen toxicity, the amount of data available on the effect of drugs at pressures of 0.5–1.5 atm is sparse.

8. CONCLUSIONS AND CLINICAL RECOMMENDATIONS

It is clear that oxygen has very widespread effects upon the animal organism, attacking many systems simultaneously. The system which is affected overtly depends upon many factors including the partial pressure of oxygen, pressure of inert gas, species and age of the animal, neuroendocrine status, etc. In one system alone, notably the lung, there appear to be many pathways whereby oxygen might lead to pulmonary damage, including inhibition of ciliary activity, impaired bacterial phagocytosis, a tendency to develop absorption collapse, a direct toxic effect upon endothelium to give rise to 'leaky capillaries', inhibition of surfactant activity and a tendency for left ventricular failure to occur. Superimposed on these direct effects and potentiating them are effects related to toxicity at distant sites including the adrenal and thyroid glands and central nervous system.

Despite the enormous volume of research in this area in the last few decades, there are many gaps in our knowledge. Although the physiological events occurring during the development of pulmonary oxygen toxicity are well described, it is necessary to correlate these changes with sequential histological changes occurring in the lung. The role of the sympatho-adrenal system at normobaric pressures of oxygen is not clear and this is of considerable clinical relevance as many patients undergoing treatment in intensive care units receive medication affecting sympatho-adrenal activity. Also it is not clear if the reduction in surfactant activity is a primary effect of oxygen on type 2 cells, as may be suspected from biochemical measurements, or if the major mechanism

whereby surfactant activity declines in pulmonary oxygen toxicity is one secondary to alterations in lung mechanics.

All these events are based upon the cellular toxic effects of oxygen and the inactivation of essential enzyme systems giving rise to cellular disruption. In view of the differing susceptibilities of different types of lung cell to oxygen toxicity, it seems likely that the biochemical effects of oxygen on intracellular processes are not uniform but many and varied. These biochemical events and their connection with physiological events and the importance of the enzyme superoxide dismutase are important areas of research. Of considerable clinical importance is the further elucidation of the way in which oxygen tolerance is prolonged by interruptions of continuous oxygen exposure by brief periods of air administration. This prolongation of oxygen tolerance is possibly the result of resynthesis, during the air-breathing period, of critical enzymes which are depressed by hyperoxia. It is known that the process can occur very quickly and in a period as short as 5 min in humans exposed to 100% oxygen at 2 atm for 20 min periods (Widell *et al.*, 1974), and to 100% oxygen at 3 atm for 20 min periods (Workman, 1968). It is obviously necessary to construct oxygen tolerance curves for humans exposed to hyperoxia at normobaric pressures and to study the biochemical mechanisms underlying the rapid reversibility of oxygen toxicity.

In the clinical management of hypoxic patients, it is important to appreciate that an increase in the inspired oxygen concentration above 0.4–0.5 atm is potentially toxic (Winter and Smith, 1972). For patients in chronic ventilatory failure with prolonged hypoxemia and chronic carbon dioxide retention, a small increase in the inspired oxygen concentration may precipitate carbon dioxide narcosis (Campbell, 1960).

Treatment of patients with high concentrations of oxygen depends on our knowledge of the oxygen tolerance of human volunteers with the assumption that patients with high degrees of intrapulmonary shunting are not more susceptible to oxygen; indeed, the evidence appears to be to the contrary. Oxygen tolerance of humans has been discussed in detail above but, in summary, there is no evidence that changes occur over 24 hr of breathing 100% oxygen at 1 atm, whilst changes in arterial oxygenation start to occur after approximately 40 hr continuous exposure (Winter and Smith, 1972). From this, one may conclude that patients may be exposed to 100% oxygen at 1 atm continuously for approximately 40 hr if the inspired oxygen is reduced to 40% thereafter. If it is obvious that patients will require an inspired oxygen of more than 50% for several days then steps must be taken at the outset of treatment to reduce the inspired oxygen content to a minimum compatible with viable arterial oxygen availability to the tissues. It should also be appreciated that tissue availability may be improved by measures designed to improve cardiac output, increase the hemoglobin concentration and perhaps alter the position of the oxyhemoglobin dissociation curve (Woo and Hedley-White, 1973), whilst arterial oxygen saturation may be improved, without an increase in inspired oxygen content, by 'large tidal volumes, bronchial toilet and chest physiotherapy and, perhaps, the use of a positive end-expiratory pressure' (Editorial, 1974).

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APPENDIX 2

DOGS, SERIES 1, 2, & 3;

TYPICAL CORRECTED VALUES

Tables 3:2 a) and b)

3:4

5:1

8:2

TABLE 3:2a Anaesthetic Control Dog 1

Cardiorespiratory Data

Time from start (hrs)	1:20	4:00	10:15	13:30	16:00	19:45	22:30
P_aO_2	81	79	77	90	90	92	82
P_aCO_2	39	37	33	40	41	36	34
pHa	7.363	7.371	7.437	7.377	7.373	7.381	7.376
Sa %	94	94	96	96	96	96	95
C_aO_2	15.93	15.59	16.11	18.58	16.97	16.91	16.42
$P_{\bar{v}}O_2$	84	90	78	78	72	84	84
$P_{\bar{v}}CO_2$	43	42	40	43	44	39	42
$pH\bar{v}$	7.343	7.391	7.407	7.357	7.403	7.451	7.356
$S\bar{v}$%	64	75	77	81	83	81	82
$C_{\bar{v}}O_2$	10.59	12.46	12.89	15.64	14.53	14.13	14.03
H.R.	84	90	78	78	73	84	84
S.B.P.	155/90	150/90	165/90	170/105	145/80	140/75	135/75
S.B.P. (mean)	120	120	120	130	100	95	100
C.V.P.	-1	-1	-2	-1.5	-1.5	-2.5	-1
P.A.P.	12/2	8/3	8/4	7.5/3	7.5/3	9/3	10/5
P.A.P. (mean)	5	5	6	5	4.5	6	7
P.A.P. (wedge)	0.5	2	2.5	2	1.5	1.5	2.5
$\dot{Q}_T$	3.9	3.5	3.5	3.3	2.6	2.7	2.9
P.V.R.	1.15	0.85	1.00	0.90	1.15	1.66	1.55
S'_cO_2	97.16	97.36	97.82	97.13	97.06	97.48	97.55
C'_cO_2	16.18	15.95	16.29	18.53	17.01	16.88	16.64
$\dot{Q}_s/\dot{Q}_T$	4.5	1.05	5.3	-	1.7	-	8.3
P_AO_2	102.9	105.4	110.4	101.7	100.4	106.7	109.2
$(a-\bar{v})D_{O_2}$	5.243	3.128	3.226	2.937	2.448	2.782	2.393
$(A-a)P_{O_2}$	21.9	26.4	33.4	11.65	10.4	14.65	27.15
$\dot{V}O_2$ (Fick)	204	109	113	97	64	75	69
f	10	8	12	8	10	9	10
V_T	400	400	550	450	400	500	400
$\dot{V}_E$	4.0	3.2	6.6	3.6	4.0	4.5	4.0
$P_{\bar{E}}CO_2$	21	22	21	24	24	23	22
V_D	185	162	200	180	166	181	141
$\dot{V}_A$	2.15	1.90	4.2	2.16	2.34	2.871	2.59
$\dot{V}_{O_2}$(direct)	140	120	120	120	100	100	100
Hb.	12.4	12.2	12.4	14.2	13.0	12.9	12.7
Hct.	38	38	42	44	41.5	43	43
Temp.	37.5	37.6	36.5	37.2	37.5	37.6	37.3

Note list of abbreviations on Page xviii.

TABLE 3:2b

Anaesthetic Control Dog 2

Cardiorespiratory Data

Time from start (hrs)	2:00	6:00	9:00	14:30	18:30	23:30
P_aO_2	96	95	89	84	92	92
P_aCO_2	44	42	42	39	33	38
pHa	7.361	7.337	7.363	7.400	7.390	7.414
Sa%	95.5	95.95	95.72	95.29	96.07	96.32
C_aO_2	15.86	18.53	18.35	18.76	19.31	20.13
$P_{\bar{v}}O_2$	50	49	51	45	50	52
$P_{\bar{v}}CO_2$	53	45	45	41	34	48
$pH\bar{v}$	7.302	7.317	7.343	7.37	7.37	7.404
$S\bar{v}$ %	71.4	74.53	78.99	73.35	78.48	80.93
$C_{\bar{v}}O_2$	11.8	14.32	15.07	14.38	15.7	16.85
H.R.	96	70	60	56	60	70
S.B.P.	150/95	190/110	175/100	170/95	160/95	165/85
S.B.P. (mean)	115	135	125	115	115	115
C.V.P.	-1	0	0.5	2.5	1.0	1.0
P.A.P.	20/7	30/8	35/11	33/5	30/10	30/10
P.A.P. (mean)	11	16	19	16	17	16
P.A.P. (wedge)	2	5	5	5	5	5
$\dot{Q}_T$	3.5	3.5	3.4	3.3	3.1	3.7
P.V.R.	2.57	3.14	4.12	3.33	3.87	2.97
$S_c'O_2$	96.872	96.402	96.907	97.352	97.753	97.523
$C_c'O_2$	16.125	18.628	18.577	19.167	19.662	20.125
$\dot{Q}_S/\dot{Q}_T$	6.1	2.3	6.5	8.5	8.9	-
P_AO_2	96.44	98.94	98.95	102.69	110.19	103.94
$(a-v)D_{O_2}$	4.06	4.21	3.28	4.38	3.60	3.29
$(A-a)P_{O_2}$	0.44	3.94	9.94	18.69	18.19	11.94
$\dot{V}_{O_2}$ (Fick)	142	147	111	144	112	122
f	8	9	12	9	14	10
V_T	540	500	390	450	600	400
$\dot{V}_{\bar{E}}$	4.32	4.50	4.68	4.05	8.40	4.00
$P_{\bar{E}}CO_2$	31	26	26	28	21	23
V_D	160	190	150	127	218	158
$\dot{V}_A$	3.04	2.79	2.88	2.90	5.35	2.42
$\dot{V}_{O_2}$ (direct)	150	150	120	130	120	120
Hb.	12.2	14.2	14.1	14.5	14.8	15.4
Hct.	45	44	43	46	44	48
Temp.	39	37.9	37.5	37.7	38.2	38.1

Note list of abbreviations on Page xviii.

TABLE 3:4

Typical Values; Dog Series 1

Time from start (mins)	60	300	420	540	660
Time before death (mins)	1260	1020	900	780	660
P_aO_2 (corrected t° and BGF)	1155	1134	1179	1026	1155
P_aCO_2 (corrected t°)	64	25	47	49	31
pHa (" ")	7.229	7.452	7.375	7.38	7.44
S_aO_2	99.97	99.98	99.98	99.99	99.98
C_aO_2	16.7	18.85	16.9	20.14	21.73
$P_{\bar{v}}O_2$ (corrected t° and BGF)	45	86	118	116	99
$P_{\bar{v}}CO_2$ (corrected t°)	87	34	49	51	43
$pH_{\bar{v}}$ (" ")	7.15	7.373	7.296	7.32	7.38
$S_{\bar{v}}O_2$	58.89	93.89	97.15	96.55	95.82
$C_{\bar{v}}O_2$	7.89	14.81	13.35	16.85	17.84
P_AO_2	1414	1454	1431	1433	1448
$(A-a)DO_2$	259	320	252	407	293
$(a-\bar{v})DO_2$	8.8	4.04	3.55	3.29	3.89
H.R.	68	108	80	130	120
S.B.P.	120/55	130/80	105/70	130/70	135/90
S.B.P. (mean)	80	105	80	110	110
C.V.P.	2	1	5	3	3
P.A.P.	14/6	15/4	11/5	15/5	18/4
P.A.P. (mean)	9	7	7	9	12
P.A.P. (wedged)	0	2	1	3	4
$\dot{Q}_T$ (l/min)	2.4	1.6	1.4	1.4	1.6
P.V.R. ('units')	3.75	3.12	4.3	4.3	5.0
$\dot{Q}_S/\dot{Q}_T$ (%)	8	20	18	28	19
f	11	13	17	19	21
V_T (mls)	-	327	135	120	150
$\dot{V}_E$ (mls/min)	-	4250	2295	2280	3150
$P_{\bar{E}}CO_2$	19	10	14	11	14
V_D (mls)	-	178	92	89	75
$\dot{V}_A$ (mls/min)	-	1937	731	590	1575
$\dot{V}_{O_2}$ (mls/min)	55	121	74	110	129
C (%)	←		100		→
Temp.	37.8	39.5	38	39.7	39.5
Hb.	9.9	11.6	10	12.8	13.7
Hct.	31	37	33	42	45

Series 1 Dog 8 Mongrel bitch, 9 Kg. Start time 1030, 13 May 1974.
Chamber pressure 1523 mm.Hg. Blood gas factor 1.06.

(continued overleaf)

TABLE 3:4 (contd)

Typical Values: Dog Series 1

Time from start (mins)	780	900	990	1095	1170
Time before death (mins)	540	420	330	225	150
P_aO_2 (corrected t° and BGF)	1033	1172	937	825	584
P_aCO_2 (corrected t°)	45	33	45	47	53
pHa (" ")	7.404	7.298	7.231	7.173	7.27
S_aO_2	99.97	99.98	99.96	99.94	99.89
C_aO_2	19.16	19.57	17.06	19.79	18.38
$P_{\bar{v}}O_2$ (corrected t° and BGF)	86	99	82	88	63
$P_{\bar{v}}CO_2$ (corrected t°)	75	41	84	66	72
$pH\bar{v}$ (" ")	7.304	7.248	7.13	7.108	7.128
$S_{\bar{v}}O_2$	93.95	95.67	92.0	93.09	83.72
$C_{\bar{v}}O_2$	15.36	15.67	13.35	16.39	14.12
P_AO_2	1432	1444	1428	1426	1420
$(A-a)D_{O_2}$	399	272	491	601	836
$(a-v)D_{O_2}$	3.80	3.90	3.72	3.40	4.26
H.R.	120	150	130	130	120
S.B.P.	130/95	100/65	105/70	80/50	70/40
S.B.P. (mean)	105	80	85	60	50
C.V.P.	2	1	1	2	0
P.A.P.	11/5	18/8	17/6	15/7	13/5
P.A.P. (mean)	8	11	11	10	8
P.A.P. (wedged)	2	4	4	2	1
$\dot{Q}_T$ (l/min)	1.4	1.8	2.1	-	1.4
P.V.R. ('units')	4.3	3.9	3.3	-	5.0
$\dot{Q}_S/\dot{Q}_T$ (%)	24	17	28	34	37
f	16	13	6	8	6
V_T (mls)	215	216	192	107	85
$\dot{V}_E$ (mls/min)	3440	2808	1150	856	510
$F_{\bar{E}}CO_2$	18	18	-	15	19
V_D (mls)	127	94	-	75	56
$\dot{V}_A$ (mls/min)	1408	1586	-	256	174
$\dot{V}_{O_2}$ (mls/min)	121	129	92	51	51
C (%)	53	47	-	73	60
Temp.	37.4	37.5	35.4	35.6	35.6
Hb.	13	13	10.6	12.9	12.4
Hct.	42	42	37	41	42

Series 1 Dog 8 Mongrel bitch, 9 Kg. Start time 1030, 13 May 1974.
Chamber pressure 1523 mm.Hg. Blood gas factor 1.06.

(continued overleaf)

TABLE 3:4 (contd)

Typical Values: Dog Series 1

Time from start (mins)	1215	1240	1255	1270	1290
Time before death (mins)	105	80	65	50	30
P_aO_2 (corrected t° and BGF)	345	234	104	134	100
P_aCO_2 (corrected t°)	94	104	104	106	151
pHa (" ")	7.045	7.016	6.985	6.955	6.965
S_aO_2	99.49	98.84	93.24	96.27	91.57
C_aO_2	18.11	16.35	15.44	15.65	14.79
$P_{\bar{v}}O_2$ (corrected t° and BGF)	78	68	64	63	53
$P_{\bar{v}}CO_2$ (corrected t°)	112	113	127	123	160
$pH\bar{v}$ (" ")	7.024	7.006	6.975	6.945	6.955
$S_{\bar{v}}O_2$	86.56	80.24	75.38	73.04	63.07
$C_{\bar{v}}O_2$	15.10	12.91	12.43	11.76	10.15
P_AO_2	1377	1366	1366	1364	1316
$(A-a)D_{O2}$	1032	1132	1262	1230	1216
$(a-\bar{v})D_{O2}$	3.01	3.44	3.01	3.89	4.64
H.R.	120	115	88	72	80
S.B.P.	60/30	55/25	50/25	40/15	30/15
S.B.P. (mean)	40	35	30	20	20
C.V.P.	1	1	0.5	0	1
P.A.P.	13/5	11/3	12/4	-	-
P.A.P. (mean)	9	6	8	-	-
P.A.P. (wedged)	2	0	2	-	-
$\dot{Q}_T$ (l/min)	1.2	1.0	1.0	-	-
P.V.R. ('units')	5.8	6.0	6.0	-	-
$\dot{Q}_S/\dot{Q}_T$ (%)	51	51	62	53	52
f	6	6	6	-	-
V_T (mls)	53	64	43	-	-
$\dot{V}_E$ (mls/min)	318	384	258	-	-
$F_{\bar{E}}CO_2$	-	-	-	-	-
V_D (mls)	-	-	-	-	-
$\dot{V}_A$ (mls/min)	-	-	-	-	-
$\dot{V}_{O2}$ (mls/min)	37	46	46	-	-
C (%)	32	27	27	-	-
Temp.	35.8	35.7	35.7	-	-
Hb.	12.8	11.8	12.1	11.8	11.8
Hct.	43	43	42	42	42

Series 1 Dog 8 Mongrel bitch, 9 Kg. Start time 1030, 13 May 1974.
Chamber pressure 1523 mm.Hg. Blood gas factor 1.06.

(continued overleaf)

TABLE 3:4 (contd)

Typical Values: Dog Series 1

Time from start (mins)	1300	1310	1320
Time before death (mins)	20	10	0
P_aO_2 (corrected t° and BGF)	85	74	67
P_aCO_2 (corrected t°)	141	141	156
pHa (" ")	6.945	6.905	6.935
S_aO_2	86.94	79.93	75.34
C_aO_2	14.01	12.86	12.1
$P_{\bar{v}}O_2$ (corrected t° and BGF)	51	42	37
$P_{\bar{v}}CO_2$ (corrected t°)	151	160	160
$pH_{\bar{v}}$ (" ")	6.925	6.895	6.925
$S_{\bar{v}}O_2$	59.32	43.91	37.91
$C_{\bar{v}}O_2$	9.55	7.08	6.12
P_AO_2	1326	1326	1311
$(A-a)D_{O2}$	1241	1252	1244
$(a-v)D_{O2}$	4.46	5.78	5.99
H.R.	66	78	-
S.B.P.	35/20	40/15	-
S.B.P. (mean)	22	21	-
C.V.P.	1	1	-
P.A.P.	-	-	-
P.A.P. (mean)	-	-	-
P.A.P. (wedged)	-	-	-
$\dot{Q}_T$ (l/min)	-	-	-
P.V.R. ('units')	-	-	-
$\dot{Q}_S/\dot{Q}_T$ (%)	57	55	56
f	-	-	-
V_T (mls)	-	-	-
$\dot{V}_E$ (mls/min)	-	-	-
$P_{\bar{E}}CO_2$	-	-	-
V_D (mls)	-	-	-
$\dot{V}_A$ (mls/min)	-	-	-
$\dot{V}_{O2}$ (mls/min)	-	-	-
C (%)	-	-	-
Temp.	-	-	-
Hb.	11.8	12.6	13.0
Hct.	44	49	51

Series 1 Dog 8 Mongrel bitch, 9 Kg. Start time 1030, 13 May 1974.
Chamber pressure 1523 mm.Hg. Blood gas factor 1.06.

TABLE 5:1

Typical Values: Dog Series 2

Time from start (mins)	90	285	540	720	1020
Time before death (mins)	1455	1260	1005	825	525
P_aO_2 (corrected t° and BGF)	627	602	563	627	623
P_aCO_2 (corrected t°)	48	43	49	40	45
pHa (" ")	7.32	7.29	7.38	7.39	7.33
S_aO_2	99.9	99.9	99.9	99.9	99.9
C_aO_2	19.6	19.6	19.3	19.4	18.6
$P_{\bar{v}}O_2$ (corrected t° and BGF)	78	76	162	210	201
$P_{\bar{v}}CO_2$ (corrected t°)	60	50	67	53	55
$pH\bar{v}$ (" ")	7.29	7.26	7.28	7.32	7.27
$S_{\bar{v}}O_2$	91.4	89.9	98.5	99.2	98.9
$C_{\bar{v}}O_2$	16.4	16.4	17.9	18.0	17.3
$(a-v)D_{O2}$	2.9	3.3	1.4	1.3	1.4
H.R.	115	110	160	130	90
S.B.P.	100/65	125/80	135/65	130/65	160/90
S.B.P. (mean)	80	100	80	90	115
C.V.P.	2	2.5	2	2	1.5
P.A.P.	10/5	9/4	9/3	10/2	11/3
P.A.P. (mean)	6	6	5	6	6
P.A.P. (wedged)	0	1	-1	0.5	-2
$\dot{Q}_T$ (l/min)	3.6	2.8	3.4	3.35	3.8
P.V.R. ('units')	1.66	1.78	1.76	1.64	2.1
f	7	-	-	-	11
V_T (L) (mls)	190	50	-	-	70
V_T (R) (mls)	400	120	-	-	200
$\dot{V}_{O2}$(L) (mls/min)	13	25	15	-	14
$\dot{V}_{O2}$(R) (mls/min)	32	91	73	-	100
C (L) (%)	← 100 →				80
C (R) (%)	← 100 →				99
Temp.	39.1	38.1	37.3	37.4	37.8
Hb.	13.2	13.4	13.2	13.1	12.6
Hct.	40	-	39	40	41

Series 2 Dog 3 Greyhound bitch, 26 Kg. Start time 1130, 13 Nov. 1974
Chamber pressure 1511 mm.Hg. Blood gas factor 1.05

(continued overleaf)

TABLE 5:1 (contd)

Typical Values: Dog Series 2

Time from start (mins)	1320	1440	1470	1480	1500
Time before death (mins)	225	105	75	65	45
P_aO_2 (corrected t° and BGF)	656	104	70	60	55
P_aCO_2 (corrected t°)	41	51	40	38	-
pHa (" ")	7.39	7.31	7.40	7.40	-
S_aO_2	99.9	96.3	90.9	86.9	-
C_aO_2	19.1	19.8	19.1	18.6	-
$P_{\bar{v}}O_2$ (corrected t° and BGF)	78	41	-	-	-
$P_{\bar{v}}CO_2$ (corrected t°)	48	64	-	-	-
$pH\bar{v}$ (" ")	7.3	7.34	-	-	-
$S_{\bar{v}}O_2$	91.9	62.3	-	-	-
$C_{\bar{v}}O_2$	16.1	12.7	-	-	-
$(a-v)D_{O2}$	3.0	7.0	-	-	-
H.R.	80	140	170	-	70
S.B.P.	170/100	150/105	120/80	135/85	180/80
S.B.P. (mean)	120	120	95	105	110
C.V.P.	1	2	2	1	2
P.A.P.	9/4	12/6	12/9	11/8	16/8
P.A.P. (mean)	6	8	10	9	10
P.A.P. (wedged)	1	2	3	0	1
$\dot{Q}_T$ (l/min)	3.0	3.05	2.9	3.0	2.6
P.V.R. ('units')	2.33	1.96	2.41	3.0	3.46
f	13	13	16	16	16
V_T (L) (mls)	90	130	250	300	400
V_T (R) (mls)	150	200	370	300	320
$\dot{V}_{O2}$(L) (mls/min)	10				25
$\dot{V}_{O2}$(R) (mls/min)	75				72
C (L) (%)	89	116	124	107	138
C (R) (%)	65	79	81	48	50
Temp.	37.8	38.4	38.4	38.4	38.5
Hb.	12.9	15.1	15.5	15.8	16.2
Hct.	41	47	48	50	50

Series 2 Dog 3 Greyhound bitch, 26 Kg. Start time 1130, 13 Nov. 1974
Chamber pressure 1511 mm.Hg. Blood gas factor 1.05

(continued overleaf)

TABLE 5:1 (contd)

Typical Values: Dog Series 2

Time from start (mins)	1515	1530	1545
Time before death (mins)	30	15	0
P_aO_2 (corrected t° and BGF)	43	44	29
P_aCO_2 (corrected t°)	43	48	64
pHa (" ")	7.34	7.29	7.17
S_aO_2	65.5	62.9	30.0
C_aO_2	4.9	14.7	7.1
$P_{\bar{v}}O_2$ (corrected t° and BGF)	-	-	-
$P_{\bar{v}}CO_2$ (corrected t°)	-	-	-
$pH\bar{v}$ (" ")	-	-	-
$S_{\bar{v}}O_2$	-	-	-
$C_{\bar{v}}O_2$	-	-	-
$(a-v)D_{O_2}$	-	-	-
H.R.	60	70	-
S.B.P.	200/120	175/80	-
S.B.P. (mean)	135	100	-
C.V.P.	3	2	-
P.A.P.	16/12	16/12	-
P.A.P. (mean)	13	13	-
P.A.P. (wedged)	2	2	-
$\dot{Q}_T$ (l/min)	2.9	3.15	2.4
P.V.R. ('units')	3.79	3.49	-
f	20	20	15
V_T (L) (mls)	400	300	250
V_T (R) (mls)	280	180	30
$\dot{V}_{O_2}$(L) (mls/min)	50	75	73
$\dot{V}_{O_2}$(R) (mls/min)	66	66	15
C (L) (%)	107	111	93
C (R) (%)	32	30	5
Temp.	38.7	39	39.1
Hb.	16.8	17.3	17.6
Hct.	53	55	58

Series 2 Dog 3 Greyhound bitch, 26 Kg. Start time 1130, 13 Nov. 1974

Chamber pressure 1511 mm.Hg. Blood gas factor 1.05

TABLE 8:2

Typical Values: Dog Series 3

Time from start (mins)	180	480	750	1080	1260
Time before death (mins)	1340	1040	770	440	260
P_aO_2 (corrected t° and BGF)	1162	1037	1241	1187	1064
P_aCO_2 (corrected t°)	41	42	32	36	52
pHa (" ")	7.33	7.33	7.45	7.39	7.28
S_aO_2	99.98	99.97	99.98	99.98	99.97
C_aO_2	25.58	24.64	24.9	25.53	24.31
$P_{\bar{v}}O_2$ (corrected t° and BGF)	131	125	102	97	76
$P_{\bar{v}}CO_2$ (corrected t°)	55	55	41	45	63
$pH\bar{v}$ (" ")	7.29	7.3	7.39	7.34	7.34
$S_{\bar{v}}O_2$	97.7	97.5	96.85	96.58	93.66
$C_{\bar{v}}O_2$	22.11	21.26	21.05	21.52	19.84
P_AO_2	1435	1434	1445	1439	1423
$(A-a)D_{O2}$	273	397	204	252	359
$(a-\bar{v})D_{O2}$	3.47	3.42	3.85	4.01	4.47
H.R.	100	100	150	140	120
S.B.P.	155/95	130/80	130/85	120/80	90/55
S.B.P. (mean)	120	100	105	90	70
C.V.P.	-0.5	0	0.5	2	2.5
P.A.P.	9/4	14/4	12/6	8/4	9/3
P.A.P. (mean)	7	8	8	6	5
P.A.P. (wedged)	-1	0	-1	0	2
$\dot{Q}_T$ (l/min)	5.0	4.5	4.5	4.2	3.6
P.V.R. ('units')	1.6	1.8	2.0	1.4	0.8
$\dot{Q}_S/\dot{Q}_T$ (%)	21	23	18	15	20
f	12	8	11	11	8
V_T (mls)	500-900	950	750	500	400-450
$\dot{V}_E$ (mls/min)	8400	7600	8250	5500	3360
$\dot{V}_{O2}$ (mls/min)	143	115	83	45	112
C (%)	←	100	→	28	26
Temp.	37.6	37.7	38	37	37.2
Hb.	16.6	16.0	16.0	16.4	15.8
Hct.	50	50	54	55	50

Series 3 Dog 5 Greyhound bitch, 27 Kg. Start time 1100, 30 Sept. 1975
Chamber pressure 1522 mm.Hg. Blood gas factor 1.065

(continued overleaf)

TABLE 8:2 (contd)

Typical Values; Dog Series 3

Time from start (mins)	1350	1470	1485	1500	1515
Time before death (mins)	170	50	35	20	5
P_aO_2 (corrected t° and BGF)	1272	877	835	724	467
P_aCO_2 (corrected t°)	54	96	118	155	199
pHa (" ")	7.29	7.18	7.11	7.01	6.83
S_aO_2	99.98	99.94	99.92	99.86	99.49
C_aO_2	22.25	24.00	23.84	23.48	22.63
$P_{\bar{v}}O_2$ (corrected t° and BGF)	88	73	-	-	-
$P_{\bar{v}}CO_2$ (corrected t°)	66	127	-	-	-
pH$\bar{v}$ (" ")	7.25	7.13	-	-	-
$S_{\bar{v}}O_2$	93.97	83.9	-	-	-
$C_{\bar{v}}O_2$	20.41	18.2	-	-	-
P_AO_2	1421	1380	1358	1321	1277
$(A-a)D_{O2}$	149	503	523	597	810
$(a-v)D_{O2}$	4.84	5.8	-	-	-
H.R.	105	100	90	65	145
S.B.P.	80/45	42/15	35/15	35/15	35/20
S.B.P. (mean)	55	25	20	20	25
C.V.P.	2	2	1	0.5	0.5
P.A.P.	8/2	4/1	4/1.5	6/1	8/1
P.A.P. (mean)	4	1.5	2	2	2
P.A.P. (wedged)	0	-1	0	1	1
$\dot{Q}_T$ (l/min)	3.65	1.9	1.55	1.5	1.45
P.V.R. ('units')	1.1	1.3	1.3	0.7	0.7
$\dot{Q}_S/\dot{Q}_T$ (%)	8	21	-	-	-
f	8	6	4	5	-
V_T (mls)	300-400	350-400	400	200	-
$\dot{V}_E$ (mls/min)	2800	2220	1600	1000	-
$\dot{V}_{O2}$ (mls/min)	112	83	75	75	-
C (%)	19	22	22	14	-
Temp.	37	37.3	37.5	37.7	38
Hb.	16.0	16.0	16.0	16.0	16.0
Hct.	50	50	50	50	50

Series 3 Dog 5 Greyhound bitch, 27 Kg. Start time 1100, 30 Sept. 1975

Chamber pressure 1522 mm.Hg. Blood gas factor 1.065

APPENDIX 3

DOGS, SERIES 1, 2, & 3;

CORRECTED MEANED VALUES

(Data from which all graphs of cardiorespiratory parameters were plotted)

APPENDIX 3

MEANED VALUES : DOGS, SECTION 3

Parameter	Time before death (minutes)											
	up to 600	500-600	400-500	300-400	200-300	150-200	100-150	80-100	60-80	40-60	20-40	0-20
P_aO_2	1097	1006	1097	999	990	822	499	494	416	254	183	87
S.E.	40	80	44	56	52	72	110	101	125	52	47	7
P_aCO_2	45	44	44	41	46	59	78	78	68	116	116	116
S.E.	2	3	6	6	8	9	10	19	14	11	13	10
A-a D_{O2}	340	435	342	444	449	603	903	909	1000	1041	1204	1280
S.E.	41	82	44	59	53	67	106	101	114	48	54	15
H.R.	80	125	144	128	138	134	127	113	107	102	103	110
S.E.	6	10	15	12	10	7	12	13	11	9	13	6
S.B.P.(mean)	86	100	95	91	86	70	48	58	54	39	34	38
S.E.	3	6	3	6	6	11	6	10	11	6	6	5
P.A.P.(mean)	11	14	11	11	10	8	6	8	7	7	7	7
S.E.	1.5	1	1.5	2	1	1	1	1.5	1.5	1.5	1.5	1.5
P.A.P.(wedged)	3.7	2.25	2.3	1.2	1.6	1.6	1.75	0.5	1.25	1.75	1.75	1.0
S.E.	0.32	0.70	0.8	0.6	0.3	0.6	0.3	0.3	0.3	0.7	0.6	0.4
$\dot{Q}_T$	100	108	94	98	79	88	63	68	64	60	68	63
S.E.	-	10	6	5	4	11	5	7	4	5	13	7
P.V.R.	3.4	4.8	5.3	2.4	4.8	4.5	4.8	5.9	4.2	5.7	4.2	4.2
S.E.	0.8	2.1	0.7	1.0	0.9	0.6	1.9	0.8	2.8	0.9	0.8	-
$\dot{Q}_S/\dot{Q}_T$	21	24	21	21	21	26	40	38	40	53	49	48
S.E.	0.8	2.6	1.7	1.7	1.8	1.7	5.1	4.2	6.2	5.6	3.6	2.2
C	100	-	75	-	64	51	33	-	33	-	-	-

APPENDIX 3

MEANED VALUES : DOGS, SECTION 5

Parameter		Time before death (minutes)									
		up to 600	500-600	400-500	300-400	200-300	120-200	60-120	30-60	15-30	0-15
P_aO_2		665	670	-	758	729	715	78	75	52	50
	S.E.	13	32		10	49	71	6	8	5	7
P_aCO_2		47	45	-	36	42	43	43	48	41	70
	S.E.	2	0.3		3	0.3	4	2	6	5	18
H.R.		127	85	158	157	139	133	160	144	93	119
	S.E.	6	3	5	11	10	16	6	14	11	15
S.B.P.(mean)		96	98	105	93	96	95	98	87	96	75
	S.E.	2	10	3	4	7	7	4	6	10	15
P.A.P.(mean)		9	7.5	13.5	11	10	10	11.5	11.0	12.5	14.0
	S.E.	0.5	1.0	1.5	1.0	0.5	1.0	1.5	1.0	1.0	2.0
P.A.P.(wedged)		2.0	1.25	3.5	3.5	2.0	3.0	0.5	3.6	2.0	2.75
	S.E.	0.5	0.75	1.25	0.75	0.75	1.25	1.5	0.75	1.75	1.5
$\dot{Q}_T$		4.2	4.2	4.4	4.2	3.9	4.1	3.4	3.1	3.1	3.0
	S.E.	0.1	0.2	0.3	0.1	0.2	0.1	0.2	0.2	0.1	0.4
P.V.R.		1.78	1.5	2.33	1.75	1.92	1.73	3.28	3.03	2.78	
	S.E.	0.01	0.07	0.18	0.16	0.09	0.05	0.34	0.58	0.29	
V_T (L)		163	85	228	172	248	200	240	295	315	202
	S.E.	14	10	9	24	32	43	23	19	21	28
V_T (R)		391	250	300	203	211	237	268	198	220	101
	S.E.	34	34	51	10	21	30	27	37	29	30
C (L)		100	80	109	85	105	71	105 | 104	97	119	73
	S.E.	-	-	10	5	4	10	8 | 17	9	11	10
C (R)		100	99	67	44	43	36	58 | 43	23	27	14
	S.E.	-	-	11	5	4	3	15 | 10	8	2	3

APPENDIX 3

MEANED VALUES : DOGS, SECTION 8

Parameter		Time before death (minutes)					
		more than 600	360–600	120–360	60–120	30–60	0–30
P_aO_2		1193	1074	1020	872	838	628
	S.E.	15	38	49	99	103	102
P_aCO_2		55	49	62	74	109	123
	S.E.	1.7	2.5	9.1	10.4	13.7	12.3
H.R.		120	135	119	97	91	73
	S.E.	7.4	9.5	8.75	6.6	2.0	3.75
S.B.P.		91	102	82	48	37	28
	S.E.	3.3	2.5	5.0	3.4	4.3	2.7
P.A.P.		9.0	10.8	8.5	5.7	4.25	4.6
	S.E.	1.5	0.5	0.5	0.5	0.75	0.35
P.A.P.(wedged)		1.4	0.6	1.5	1.1	0	1.1
	S.E.	0.25	0.5	0.5	0.5	0.2	0.2
$\dot{Q}_T$		4.52	4.94	3.89	2.68	2.06	1.77
	S.E.	0.13	0.24	0.23	0.17	0.16	0.17
P.V.R.		1.73	2.04	2.03	1.44	2.16	2.09
	S.E.	0.06	0.11	0.20	0.10	0.47	0.47
$\dot{Q}_S/\dot{Q}_T$		19	18	19	14.5	22.5	-
	S.E.	0.9	1.1	0.7	4.4	1.0	-
C ('normal')		100	123	82	-	69	-
	S.E.	-	3.68	15.12	-	4.77	-
C ('atelectatic')		100	55	34	23	24	17.5
	S.E.	-	17.15	11.96	9.27	3.84	3.34

APPENDIX 4

LUNG WET/DRY WEIGHT RATIOS

(OVERALL MEANED VALUES)

APPENDIX 4

LUNG WET/DRY WEIGHT RATIOS. OVERALL MEANED VALUES

CONDITION		M	n	S.D.	S.E.
1) Air breathing anaesthetic controls		4.60	4	0.27	0.09
2) Sect. 8 Oxygen breathing at 2 ATA		9.65	3	0.37	0.175
3) Sect. 8 Air breathing/guanethidine		5.08	2	0.467	0.223
4) Sect. 8 Oxygen at 2 ATA/guanethidine		5.55	8	0.38	0.09
5) Sect. 8 Oxygen at 2 ATA/phentolamine		5.54	3	0.42	0.16
6) Sect. 8 Oxygen at 2 ATA/{guanethidine, phentolamine} (grouped together)		5.56	11	0.40	0.08
7) Sect. 5 (R) lung O_2/(L) lung air at 2 ATA	(R lung)	9.37	6	1.91	0.53
	(L lung)	4.42	6	0.8	0.22
8) Sect. 9 (R) lung O_2/(L) lung air at 2 ATA (R vagotomy)	(R lung)	9.17	3	2.51	0.98
	(L lung)	4.9	3	1.11	0.43
9) Sect. 9 (R) lung O_2/(L) lung air at 2 ATA (L vagotomy)	(R lung)	10.35	2	1.6	0.78
	(L lung)	8.47	2	1.88	0.90
10) Sect. 9 (R) vagotomy, air-breathing control	(R lung)	4.94	2	1.24	0.06
	(L lung)	5.51	2	2.75	1.31
11) Sect. 9 (L) vagotomy, air-breathing control	(R lung)	4.71	2	0.47	0.23
	(L lung)	4.85	2	0.71	0.34
12) Sects. 5 & 9 (R) lung on O_2/(L) lung on air (vagotomies & non-vagotomies combined)	(R lung)	9.49	11	1.88	0.38
	(L lung)	5.29	11	1.86	0.38

APPENDIX 5

COLOUR PHOTOMICROGRAPHS

Figures: 3:22

3:23

3:30

3:31

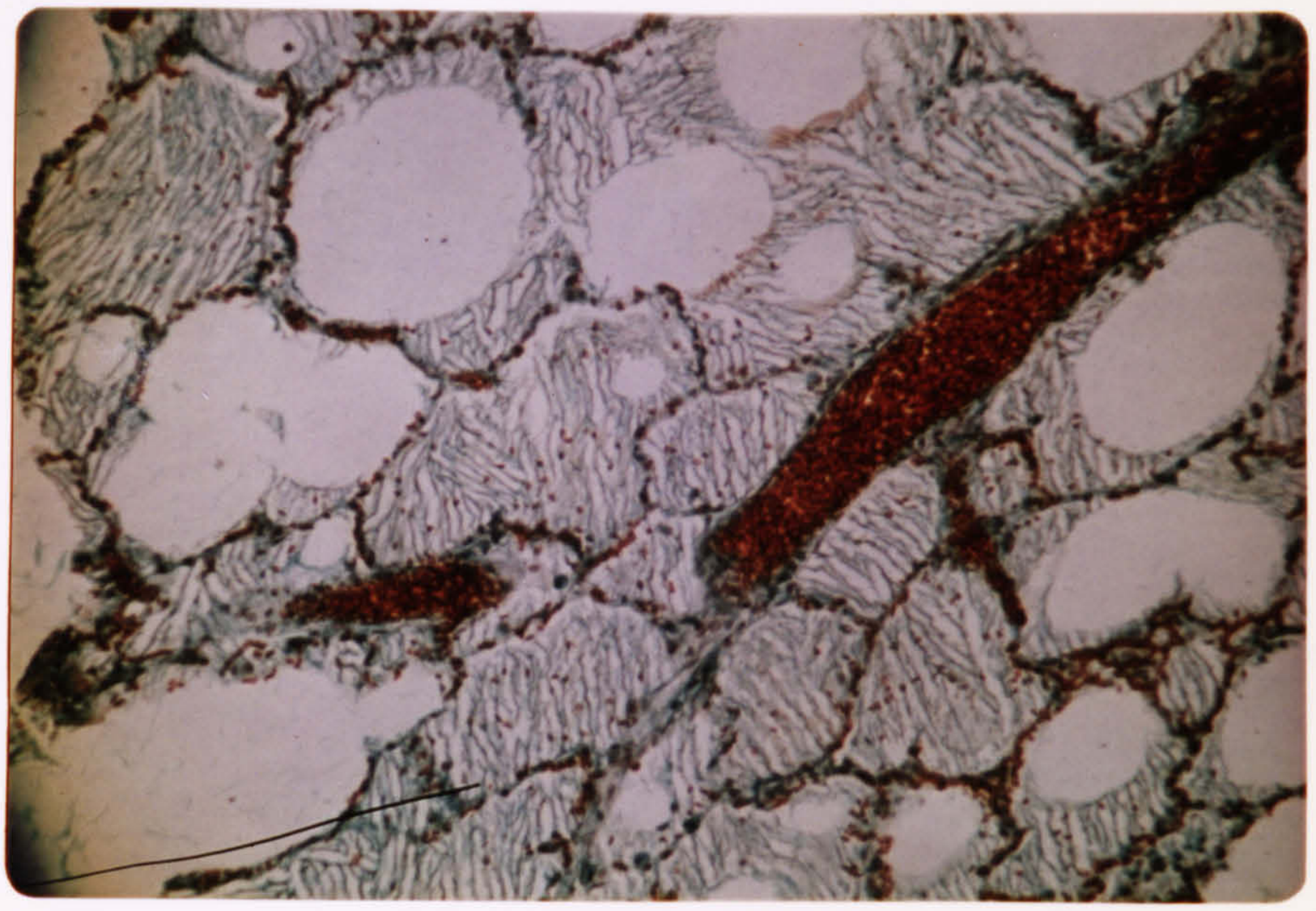

FIGURE 3:22. EXPERIMENTAL ANIMAL: INTRA-ALVEOLAR OEDEMA.

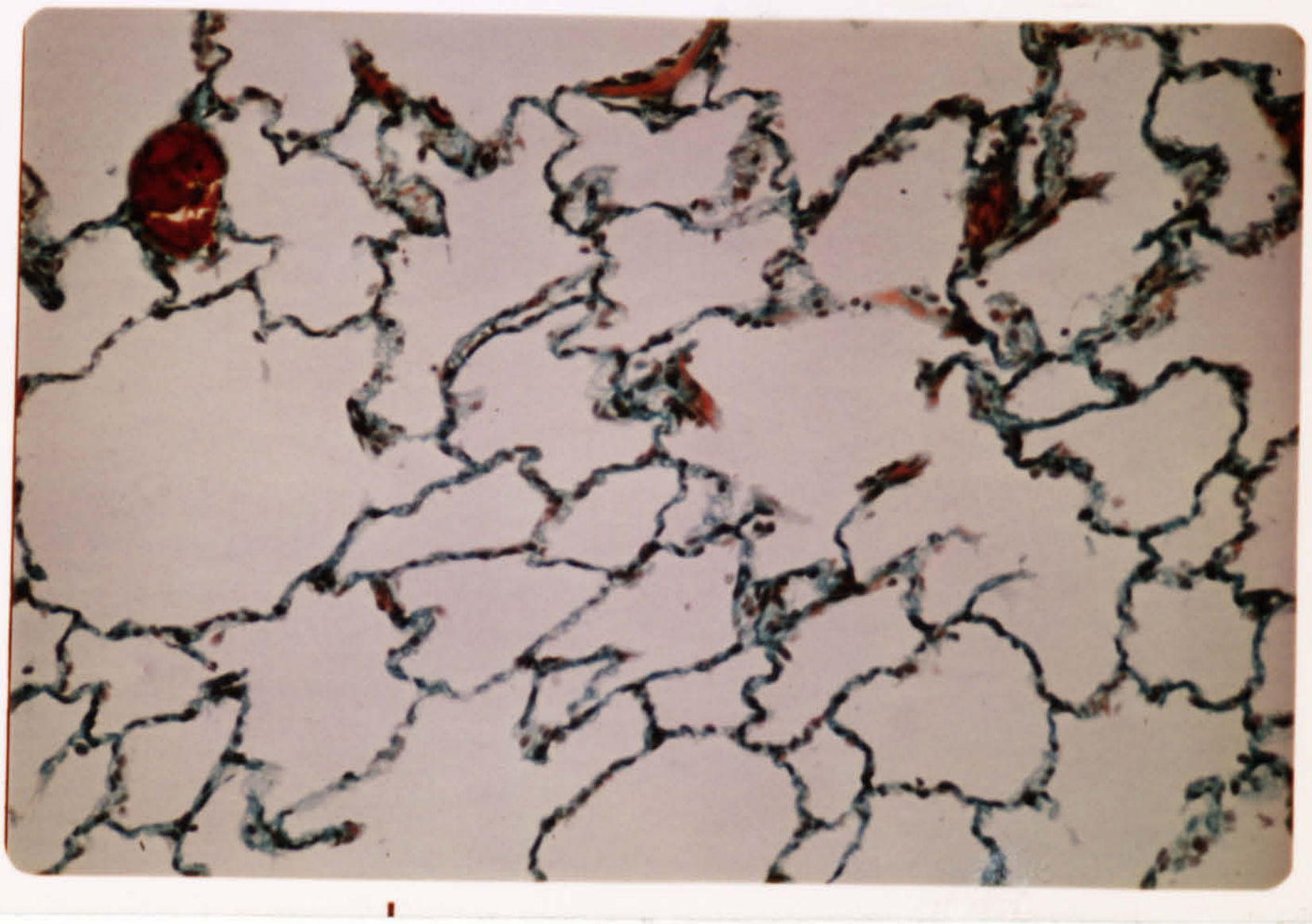

FIGURE 3:23. CONTROL ANIMAL.

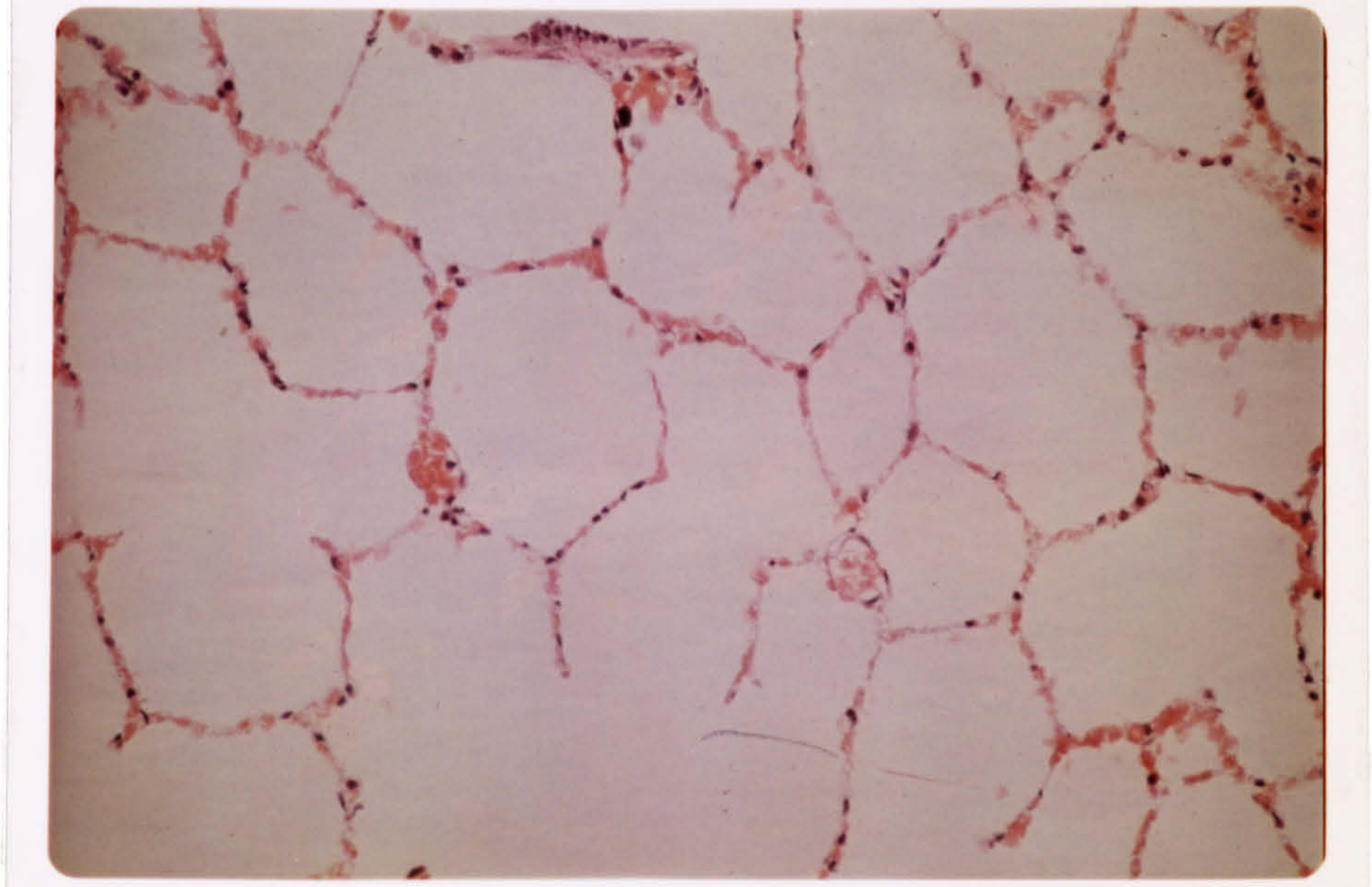

FIGURE 3:30. 'CARDIAC DEATH', DOG 12.

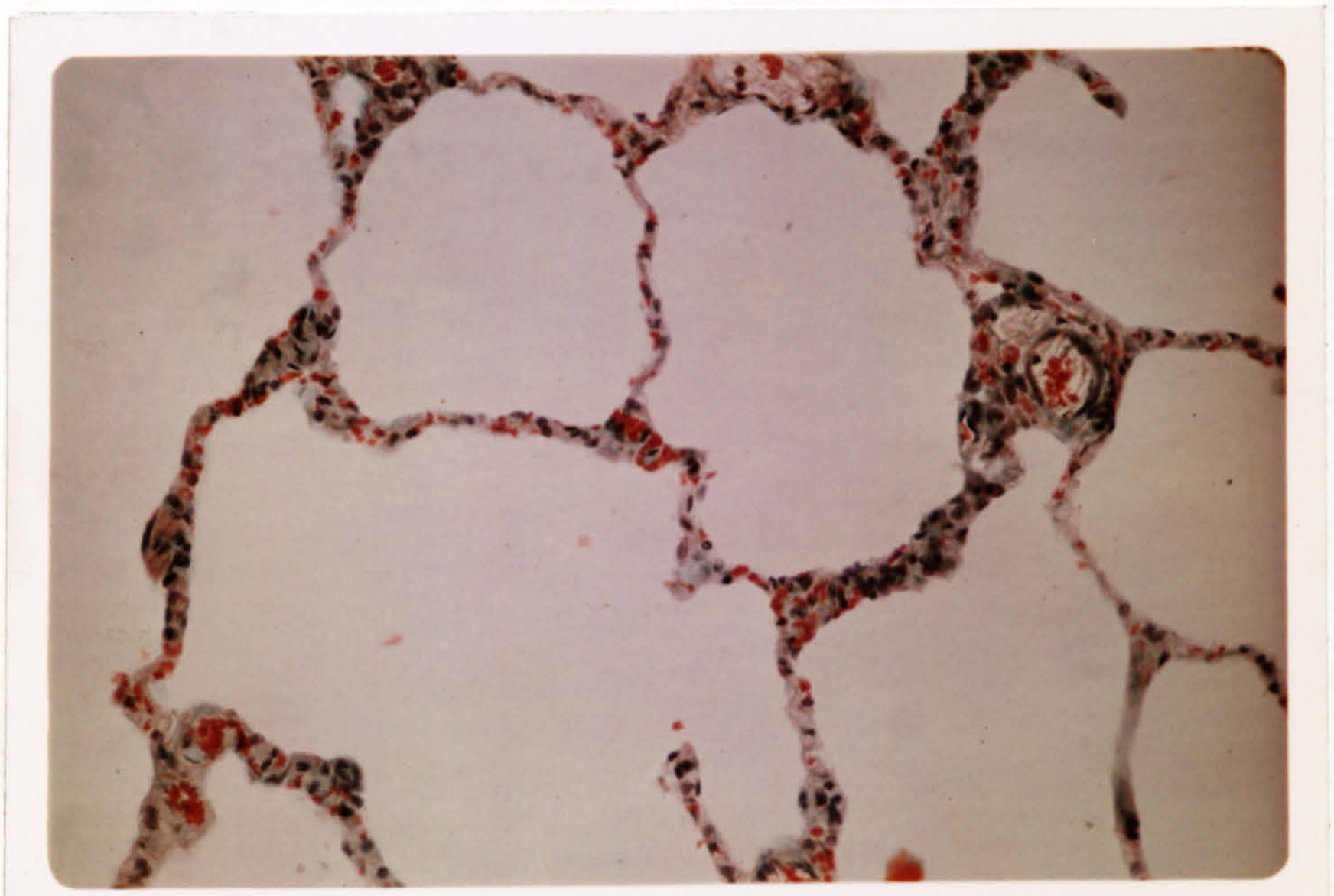

FIGURE 3:31. 'CARDIAC DEATH', DOG 2.

POSTSCRIPT

Je n'ai fait celle-ci plus longue que parceque je n'ai pas eu le loisir de la faire plus courte.

Blaise PASCAL

(Lettres Provinciales, 14 Dec. 1656)