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SOME ASPECTS OF RESPIRATION AND ITS CONTROL IN THE EASTERN BLUE-TONGUED LIZARD, *Tiliqua scincoides* (1790 *Lacerta scincoides* Shaw)

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TABLE OF CONTENTS

PART A (figures 1A-1C) Page

Abstract..... 6

Respiration in Reptiles..... 7

General Introduction..... 15

PART B RESPIRATORY MORPHOLOGY

Introduction..... 19

SECTION I (figures 1.1-1.28)

Macroscopic and Radiographic Appearances of the Respiratory System of the Blue-tongued Lizard

1.1 Dissection and Macroscopic Respiratory Anatomy

1.1.1 Introduction and Objectives..... 20

1.1.2 Materials and Methods..... 24

1.1.3 The Respiratory Airways..... 24

1.1.4 The Lungs..... 34

1.2 Radiological Examinations of the Respiratory System

1.2.1 Introduction and Objectives..... 40

1.2.2 Materials and Methods..... 41

1.2.3 Results of Radiological Studies..... 43

1.3 Discussion

1.3.1 The Body Wall..... 52

1.3.2 Dead Space..... 53

1.3.3 Intrapulmonary Gas Mixing..... 54

1.3.4 Lung Volume..... 55

1.3.5 Pulmonary Smooth Muscle Contraction..... 57

SECTION 1.3 (continued)

1.3.6 Locomotory Effects on Lungs..... 60
1.3.7 Lung Length Changes..... 60

SECTION II (figs. 2.1 - 2.18)

Histology of the Blue-tongued Lizard Lung

2.1 Introduction and Objectives..... 62
2.2 Materials and Methods..... 62
2.3 Histological Observations
2.3.1 Terminology and Definitions..... 66
2.3.2 Changes Along the Length of the Lung..... 66
2.3.3 Anterior Lung Region..... 67
2.3.4 Mid-lung Region..... 82
2.3.5 Posterior Lung Region..... 86
2.3.6 Histological Lung Measurements..... 86
2.4 Discussion..... 86

SECTION III (figs. 3.1 - 3.56)

Ultrastructure

3.1 Introduction and Objectives..... 90
3.2 Materials and Methods..... 91
3.3 Scanning and Transmission Electronmicroscopic Observations..... 97
3.4 Discussion..... 156

PART C

Some Functional Aspects of Respiration

Introduction and Objectives..... 159

SECTION IV (figs. 4.1 - 4.7)

The Respiratory Cycle and Rate of Respiration

4.1 Introduction..... 161
4.2 Materials and Methods..... 162
4.3 Characteristics of the Respiratory Cycle
4.3.1 Variation of Resting Tidal Volume..... 167
4.3.2 Variation of Respiration Rate at Constant Temperature..... 168
4.3.3 Variation of Respiration Rate with Temperature..... 172
4.3.4 Variation of Respiration Rate with Heart rate..... 172

SECTION IV (continued)

4.4 Discussion..... 176

SECTION V

Intrapulmonary Pressure Changes (figs. 5.1 - 5.7)

5.1 Introduction and Objectives..... 178

5.2 Materials and Methods..... 179

5.3 Results

5.3.1 General Characteristics of Intrapulmonary Pressure Changes..... 181

5.3.2 Gular Respiration and Intrapulmonary Pressure..... 184

5.3.3 Heart Beat and Intrapulmonary Pressure.... 184

5.3.4 Respiratory Movements and Intrapulmonary Pressure..... 188

5.3.5 Intrapulmonary Pressure Relative to Atmospheric Pressure..... 188

5.3.6 Trabecular Smooth Muscle Activity and Intrapulmonary Pressure..... 188

5.4 Discussion..... 189

SECTION VI

Haematological Investigations

6.1 Introduction and Objectives..... 191

6.2 Materials and Methods..... 191

6.3 Results

6.3.1 Microscopy of Blood Cells..... 193

6.3.2 Haematocrit and Haemoglobin..... 194

6.3.3 Haemoglobin Electrophoresis..... 194

6.3.4 Methaemoglobin..... 194

6.3.5 Blood pO_2 and pCO_2..... 194

6.4 Discussion..... 195

SECTION VII

Intrapulmonary pO_2 and pCO_2

7.1 Introduction and Objectives..... 198

7.2 Materials and Methods..... 199

7.3 Results..... 200

7.4 Discussion..... 201

PART D

Summary and Conclusions..... 204

Acknowledgements..... 207

Bibliography and References..... 208

Appendix I, Catalogue of *Tiliqua* Specimens Used in this Study. 230

Appendix II, Lung Surface Area Calculations..... 231

Appendix III, Conservation Postscript..... 234

ABSTRACT

A study of the morphology, macroscopic, light microscopic and electron microscopic appearance of the respiratory system of the Eastern Blue-tongued Lizard, Tiliqua scincoides (reptilia: scincidae). Some basic functional studies of respiratory rate, tidal volume, intrapulmonary pressure and body temperature variations, also some analyses of blood and lung gases.

Key Words: Reptilia, scincidae, Tiliqua, respiratory tract anatomy, histology and ultrastructure, respiratory physiology.

PART A

Respiration in Reptiles

Although William Harvey described the circulatory system in the early seventeenth century and Lavoisier reported the basic principles of gas exchange in respiration in the late eighteenth century it seems (in retrospect at least) that very slow progress was made in the understanding of respiratory physiology, particularly in relation to non-mammalian animals. The mid-nineteenth century physiologist William Carter, in comparing the blood of reptiles with birds stated, "Their blood is very poor in red corpuscles, and its circulation is comparatively languid", (Carter 1859, p. 94), and in describing reptilian respiration he stated, "The lungs of reptiles are for the most part but little divided; so that although they hold a very large quantity of air, this does not act advantageously upon the blood, in consequence of the small surface over which the two are brought together . . . Reptiles have no power of filling their lungs by a process resembling our inspiration, or drawing-in of air; but they are obliged to swallow it, as it were, by the action of the mouth". (ibid. p. 277). These statements serve to illustrate the influence of the Aristotelian principle that function can be deduced from structure. It is only in recent years however that a significant number of physiologists have begun to appreciate some of the many advantages of comparative physiology and of working specifically with poikilothermic animals.

Basically living organisms can be defined as systems of homeostatic mechanisms tending to prevent the natural tendency towards increase in entropy, and it is largely the process of respiration which provides the energy required for this process. Some microorganisms are chemoautotrophic (Levy 1973), many plants are phototropic (Keeton 1980), vertebrates however being aerobic heterotrophs are absolutely dependent on the respiratory processes for the synthesis of adenosine triphosphate (ATP) required to energise virtually all metabolic processes (McCauley 1971).

Because oxygen has a limited solubility in living tissues the maximum thickness of tissue which could be maintained in an active viable state by diffusion alone would be about 1mm, and that would be under ideal conditions of temperature, metabolic rate, environmental pO_2, pCO_2 and tissue permeability (McCauley 1971). However because conditions are seldom ideal and also in order to provide metabolic scope many organisms facilitate the diffusion of gases (the prime function of respiratory systems) by one mechanism or another.

The complexity of the respiratory system in extant vertebrates is highly variable but because soft tissues are rarely demonstrable in fossilized forms the phylogenesis of respiratory systems is largely a matter of conjecture. Among vertebrates there are some fish e.g. lampreys which have no lungs, swim bladders or true gills (although they do possess a simple gill basket and a blood circulatory system). Most fish possess true gills and some have well developed swim bladders even to the extent of becoming obligate air breathers e.g. certain lungfish. Most

amphibians possess both lungs and gills at some stage in their life cycle although a few may possess neither as adults (Gatz and Piiper 1973).

All terrestrial vertebrates possess lungs and although reptiles have the anatomically simplest respiratory systems they are probably less well understood than either mammalian or avian systems. The eastern Blue-tongued lizard (Tiliqua scincoides) like many other reptiles, is a eurythermic poikilotherm and thus requires homeostatic mechanisms which operate effectively over a wide range of temperatures. The eccritic or preferred body temperature for this species is $\sim$ 30-37°, Hammel et al, 1967, but its microhabitat temperature may range anywhere between $\sim$ 0°C and $\sim$ 40°C. The effect of temperature variation on metabolism is not constant although most Q_{10} values lie between 1.5 and 3.1 (Dawson 1967). In the review by Bennett and Dawson 1976 Q_{10} values of $\sim$ 1 and $\sim$ 5.8 are mentioned. Variations of metabolism with thermal acclimation, circadian rhythms, food consumption, age and sex are also reviewed by Bennett and Dawson (1976).

In order to facilitate an understanding of the respiratory physiology it is essential to have a detailed appreciation of the anatomy, histology and ultrastructure of the major components of the reptilian respiratory system. Few studies have covered these aspects in any depth and none has described T. scincoides in detail, hence the devotion of a major part of this study to the subject.

Because reptiles have strongly keratinized (impervious) integuments and relatively large tissue masses lungs are essential for external gas exchange. The lungs of most reptiles are sac-like and possess a relatively small surface area to volume ratio (compared to mammals) although they are frequently quite heterogeneous sometimes having a faveolar (or honeycomb) compartmentalization (Tenney and Tenney 1970). Just why terrestrial reptiles have such a large lung volume to body weight ratio is something of a mystery. Respiratory measurements have shown that reptilian lung surface areas are matched to the general aerobic demands of the species (Bennett, 1973).

The upper respiratory airways in lizards are of little significance to respiratory physiology because of their relatively very small dead space volume compared to lung volume. Although other aspects of physiology such as renal function, acid-base balance and circulation will have a significant effect on respiration it is the control of respiration that forms a major aspect for study in this investigation.

The automatic regulation of respiration in the mammal, which is probably similar to that found in reptiles is brought about by pontine and medullary centres which in turn are influenced by various central and peripheral receptors. A recent outline (Mines 1981) is summarized as follows.

Pontine centres

1) The Pneumotaxic Centre forms the nucleus parabrachialis in the dorsolateral rostral pons. This centre is known to modulate the output of the medullary respiratory centres.

2) The Apneustic Centre found in the lower pons is believed to be the site for the normal control of inspiratory cutoff.

Medullary Centres

1) The Dorsal Respiratory Group (DRG). These bilateral aggregations of specialized neurons are found in the ventrolateral part of the tractus solitarius nucleus. The DRG forms the upper motor neurons of many respiratory muscles and also receives some afferents from pulmonary and airway receptors.
2) The Ventral Respiratory Group. These bilateral neuron groups are found in the nucleus ambiguus and the nucleus retroambigualis. They project contralaterally and ipsilaterally and form the source of the rhythmic drive (both expiratory and inspiratory) to intercostal muscles.

There are three major groups of pulmonary receptors believed to have some influence on the control of respiration.

1) Pulmonary Stretch Receptors (PSR). These are believed to lie in pulmonary airway smooth muscle and are slow adapting. They are largely responsible for the Hering-Breuer reflex where activation by lung inflation causes prolonged expiration and reduced respiratory rate.
2) Pulmonary Irritant Receptors (PIR), are thought to lie between airway epithelial cells and are activated by lung distension, various irritants including histamine and also by pCO_2 changes.

3) Type J (Juxtapulmonary) Receptors, are believed to lie in pulmonary capillary walls. They are rapidly adapting, innervated by vagal fibres and are activated by increases in interstitial fluid.

The chemoreceptors which have an influence on respiratory control mechanims may be divided into central and peripheral. The central chemoreceptors are found bilaterally on the ventral surface of the medulla between the levels of nerve roots 8-12. They respond rapidly to decreases in pH or increases in pCO_2 by stimulating an increase in respiratory rate. These receptors are insensitive to hypoxia.

The peripheral chemoreceptors are divided into two groups, the aortic and carotid receptors. The aortic chemoreceptors situated mainly in the region of the aortic arch are in close association with arterial blood and are innervated by vagal nerve fibres. These receptors are stimulated by a decrease in pH and also by a decrease in pO_2. The carotid chemoreceptors are the most dominant in terms of their effect on respiration. They are sensitive to decrease in pH and pO_2 like the aortic chemoreceptors, they differ however in being stimulated by a decrease in arterial pH.

The interaction and integration of these chemical and neuronal control mechanisms is as yet poorly understood particularly in reptiles, however investigations into general responses e.g. to changes in plasma pCO_2 indicate some similarity with mammals. Reptiles however possess remarkable diversity of behaviour and metabolism so that it is only to be expected

that corresponding differences would be shown by more detailed investigation. One characteristic of many terrestrial reptiles is that respiration tends to be irregular indicating some difference in the mechanism of the respiratory pacemaker of the hindbrain. Another difference is found in the respiratory flow pattern of many reptiles where expiration precedes inspiration, again indicating that different reflex mechanisms must be present. Several investigations of reptile lung innervation have been undertaken e.g. Burnstock and Wood 1967, Berger 1973. Stimulatory vagal (cholinergic) fibres to pulmonary smooth muscle and inhibitory adrenergic sympathetic fibres have also been found. Some pulmonary fibres however appear to be neither cholinergic or adrenergic. These data tend to indicate that autonomic control of pulmonary smooth muscle is important e.g. in the control of lung volume, and possibly in the control of gas mixing within the lungs.

Reptiles tend to be very resistant to hypoxia and large changes in pH (Belkin 1968) and it appears that factors such as change in body temperature have a dominant role in the control of respiration (Howell and Rahn 1976). Temperature and pH are often inversely related in reptiles (Howell and Rahn 1976) and changes in ventilation are used to obtain the appropriate blood pH for a given body temperature. Because of the possibility of right to left central vascular shunting and imbalances in the ventilation/perfusion ratio in the lungs (perhaps at least partly due to the heterogeneous nature of the reptilian lung), there may be large differences in alveolar-arterial pO_2 and pCO_2. This factor must be considered when

testing responses to various concentrations of inspired O_2 or CO_2. Gatz, Fedde and Crawford (1975) demonstrated the presence of CO_2 receptors in the lizard Tupinambis nigropunctatus and although the presence of O_2 sensitive receptors in reptiles remains to be proven unequivocally it does seem that some reptiles are more sensitive to pO_2 changes than to pCO_2 changes (Wood and Lenfant 1976). Some species of terrestrial lizard can store significant quantities of oxygen in the lungs enabling long apnoeic periods.

PART A

GENERAL INTRODUCTION

The Blue-tongued lizards are a group of large, terrestrial, ovoviviparous, short limbed, pentadactyl skinks found throughout most parts of the Australian mainland and Tasmania with one species being found in parts of Indonesia (Cogger 1979, Jenkins and Bartell 1980). The Blue-tongued lizards belong to the Tiliqua genus (an apparently meaningless generic name, Waite 1929), the definition of this genus is still in question, (Storr 1976, Hutchinson 1980). There can be little doubt however that whichever taxonomic position is finally agreed upon it will maintain a close relationship between Tiliqua, Omolepida (Gunther) and Trachydosaurus (Gray). All Australian skinks belong to the subfamily Lygosominae (Greer 1970) which is further subdivided into three distinct groups (Greer 1979), one of which, the Egernia group, contains the two genera Egernia and Tiliqua (Omolepida and Trachydosaurus being included in Tiliqua). The Egernia group can be readily distinguished from all other Lygosominae in having the parietal scales separated from each other by a large interparietal scale as well as by several other commutal characteristics of squamation, osteology and soft tissue anatomy (Greer 1979).

The Eastern Blue-tongued lizard Tiliqua scincoides, is the major subject of the present study. It is one of nine species of Tiliqua recognized (Cogger 1979), all have blue tongues except the Pink-tongued lizard (T. gerrardii, Gray). T. scincoides is one of the largest of extant skinks with a maximum recorded snout-vent length of 335 mm (Greer 1976).

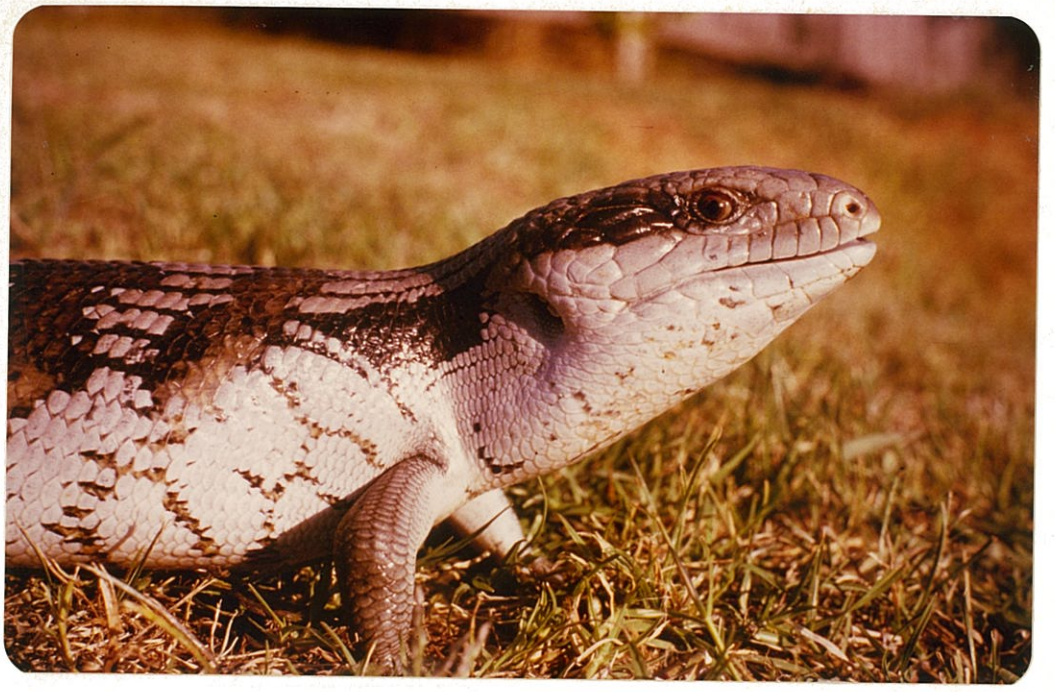


Fig.1A. An adult eastern Blue-tongued lizard *Tiliqua scincoides scincoides*, the buccopharyngeal floor in this instance is in the lowered position.



Fig. 1B. Typical appearance of the lungs of lizards of the Egernia group, *Egernia cunninghami*, note increased vascularity of the anterior lung region (right).

It is a predominantly diurnal skink inhabiting a wide range of habitats in eastern Australia from the mesothermal montane grasslands of New South Wales and Victoria to the humid tropical forests of northern Queensland, (fig. 1C). Like many of the larger members of the family Scincidae the eastern Blue-tongued lizard is omnivorous, consuming a wide range of berries, seeds, flowers, snails, frogs, insects and carrion. There are two recognized subspecies (Cogger 1979) and several colour variations. Availability has limited this study predominantly to the subspecies T. s. scincoides, several other lizards including the following were also used mainly for comparative purposes, T. s. intermedia, T. gerrardii, T. nigrolutea (Quoy and Gaimard), Trachydosaurus rugosus, Egernia cunninghami (Gray), Ctenotus robustus (Storr) and Varanus gouldii (Gray).

The major objectives of this study were,

(1) to analyse the respiratory system of the eastern Blue-tongued lizard in terms of the macroscopic, light microscopic and ultrastructural morphology,

(2) to undertake some basic functional studies related to respiratory rate, tidal volume and body temperature.

(3) to correlate the structural and functional findings, and

(4) to develop some hypotheses related to the control of respiration in this species.

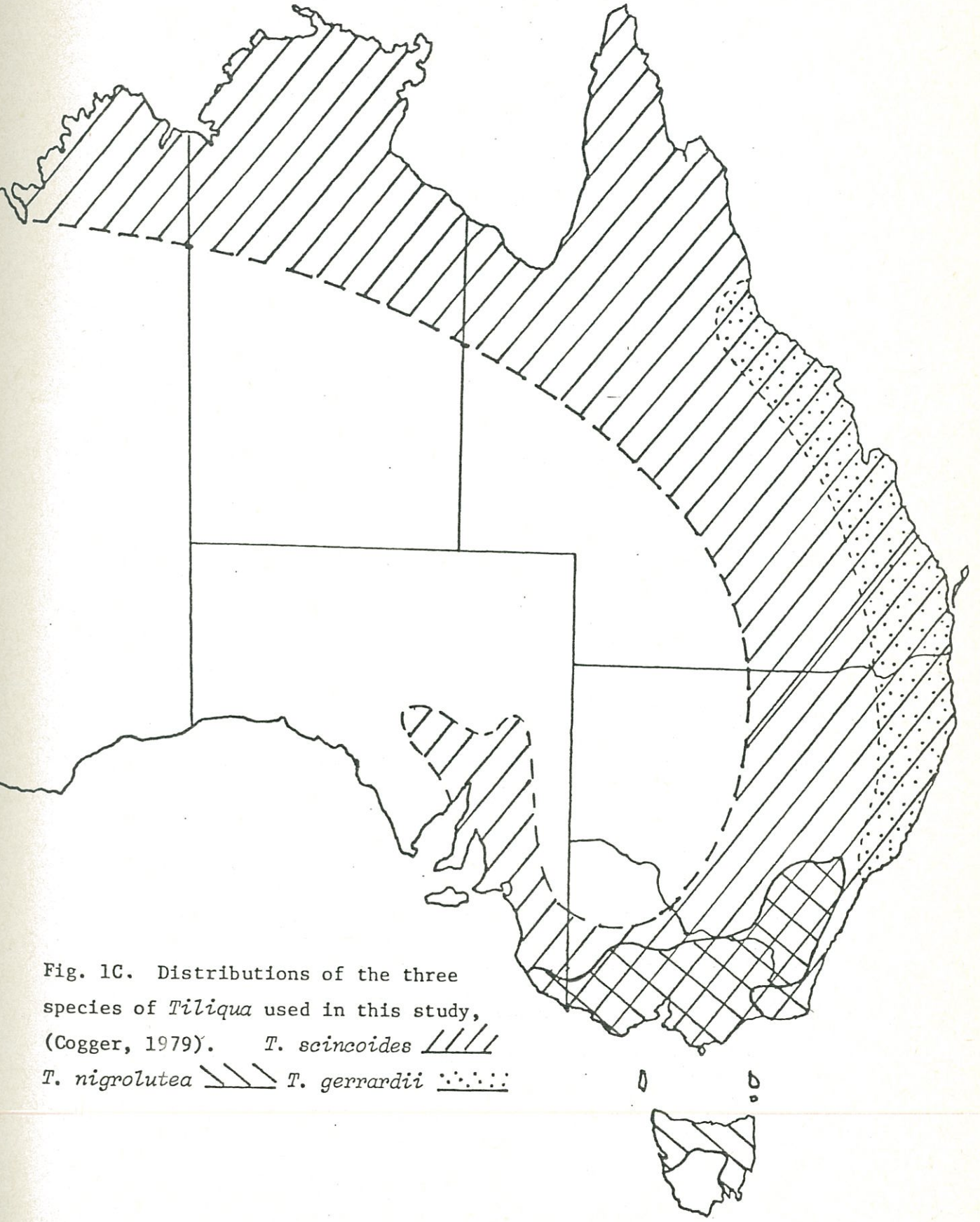


Fig. 1C. Distributions of the three species of *Tiliqua* used in this study, (Cogger, 1979). *T. scincoides* ////
T. nigrolutea \\\\ *T. gerrardii* ∴∴∴

PART B

RESPIRATORY MORPHOLOGY OF THE EASTERN BLUE-TONGUED LIZARD.

INTRODUCTION

Because lizards occupy a great range of habitats it might be expected that they would also exhibit diverse physiological adaptations. Even among terrestrial lizards there is a range of habitats and niches which impose constraints on various body systems including the respiratory system. The eastern Blue-tongued lizard, as already indicated successfully occupies a wide range of environmental conditions so that one might expect to find organ systems or metabolic processes which are less than highly specialized and capable of adaptation to the varied selection pressures operating within the particular biotype concerned.

In order to facilitate understanding of the dynamic physiological processes of respiration in this species group a preliminary morphological study of the respiratory system at the macroscopic, light microscopic and ultrastructural level was undertaken. Additional techniques of investigation included intravascular resin corrosion casting, and radiography (using fluoroscopy and intrapulmonary radiopaque medium injection).

Terminology in this area is something of a problem because there is no recognized system of nomenclature (partly because so little work has been done on comparative reptilian embryology). This study tends to follow the form used by Parsons (1970).

Section I (figs. 1.1-1.28)

Macroscopic and Radiographic Appearance of the Blue-tongued Lizard Lung

1.1 Dissection and Macrosopic Respiratory Anatomy

1.1.1 Introduction and Objectives

Direct observation of the intact living animal, dissection of several specimens and in one case an intravascular resin corrosion cast were carried out in order to help delineate the major characteristics of the respiratory system, (the prime objective of this part of the study). Several comparative observations and dissections were also carried out in order to determine any major variations in respiratory anatomy.

The respiratory system of the blue-tongued skink consists of the following parts -

the two nasal cavities,

the mouth,

the pharynx,

the glottis,

the trachea,

the two bronchi, and

the two lungs.

The anatomy of these organs together with related structures will be considered but with the greatest emphasis being placed on the lungs and the respiratory surfaces. For descriptive purposes the respiratory system is considered in two major sections.

1) The Respiratory Airways

2) The Lungs

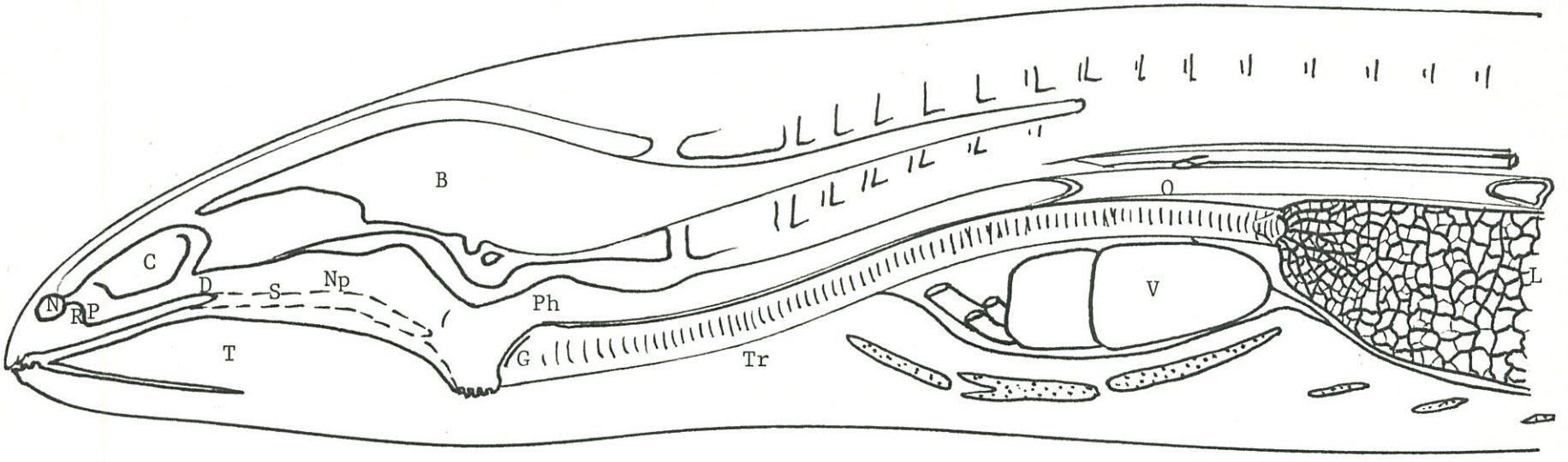


Fig. 1.1, Tiliqua scincoides scincoides, specimen TA50, adult male, longitudinal paramedian section, magnification x 2, snout-vent length 298 mm. N = external naris and vestibulum, R = post-vestibular ridge, P = cavum nasi proprium, C = concha, S = secondary palate, D = ductus nasopharyngeus, N = nasopharynx, T = tongue, B = brain cavity, G = glottis, Tr = trachea, V = ventricle, O = oesophagus, Ph = pharynx, L = luminal surface of left lung.

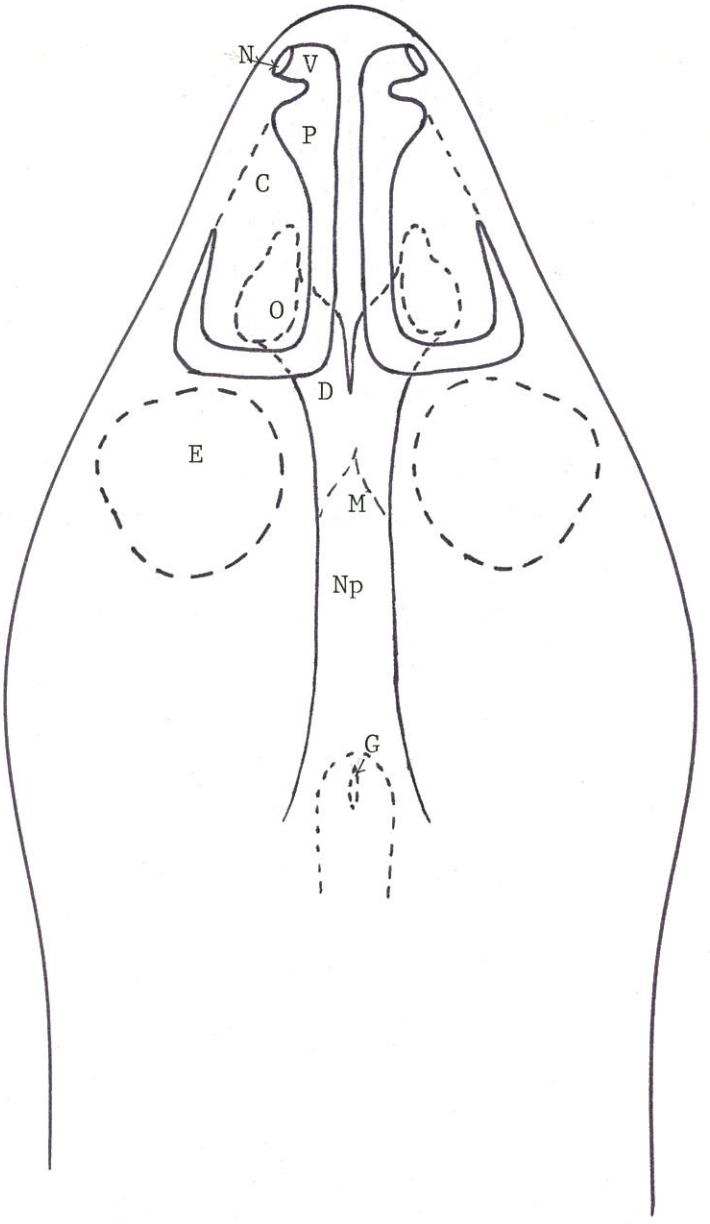


Fig. 1.2, T.s. scincoides, specimen TA54, adult female, frontal section viewed from above, magnification X 2, 265mm snout-vent length. N=external naris, V= vestibulum, P=cavum nasi proprium, C=concha, D=ductus nasopharyngeus, O=opening to nasopharyngeus beneath concha, M=posterior margin of palate, Np=nasopharynx, G=glottis, E=eye.

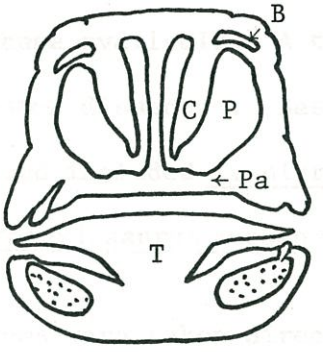


Fig. 1.3, T. s. scincoides, specimen TA53, adult male, vertical section through snout, magnification X 2. P=cavum nasi proprium, C=concha, Pa=palate, B=blood sinus.
(Vertical sections drawn as viewed from posterior aspect.)

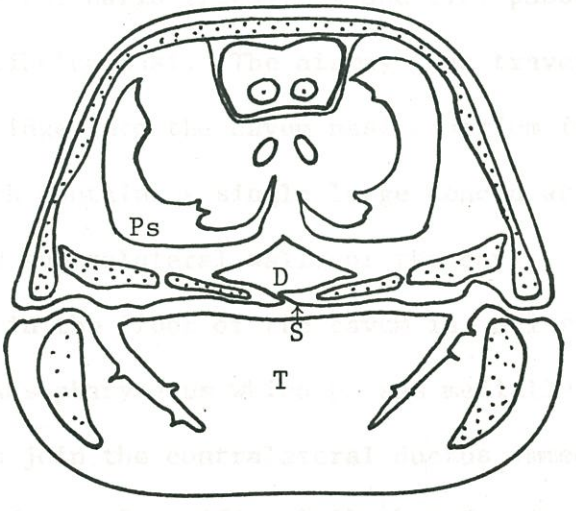


Fig.1.4, Specimen TA53,section through posterior orbital cavity. Ps=periorbital sinus, D=ductus nasopharyngeus, S=secondary palate, T=tongue.

1.1.2 Materials and Methods

Because of the limited availability of Tiliqua specimens there was no specific method of selection and optimum utilization for various parts of this study was made of lizards as and when they became available. A total of twenty specimens of Tiliqua species was dissected. Besides T. s. scincoides other species examined included T. nigrolutea, T. gerrardii, T. s. intermedia, Trachydosaurus rugosus and Egernia cunninghami.

Sectional diagrams were taken directly from preserved specimens which had been deep frozen and then sectioned transversely, longitudinally or coronally, using a thin bladed geological saw.

Dissections were carried out on fresh and preserved specimens generally by use of a mid-ventral incision.

1.1.3 The Respiratory Airways

The external naris (figs. 1.1 and 1.2) passes medially into a short vestibulum nasi. The airway then traverses the post-vestibular ridge into the cavum nasi proprium (the main nasal cavity) which contains a single large concha attached to the superior and superolateral walls of the cavity (figs. 1.2 and 1.3). Posteriorly in the floor of the cavum is a large opening into the ductus nasopharyngeus which passes medially and postero-ventrally to join the contralateral ductus immediately superior to the secondary palate (fig. 1.4) thus forming the posterior choanae which open into the nasopharynx.

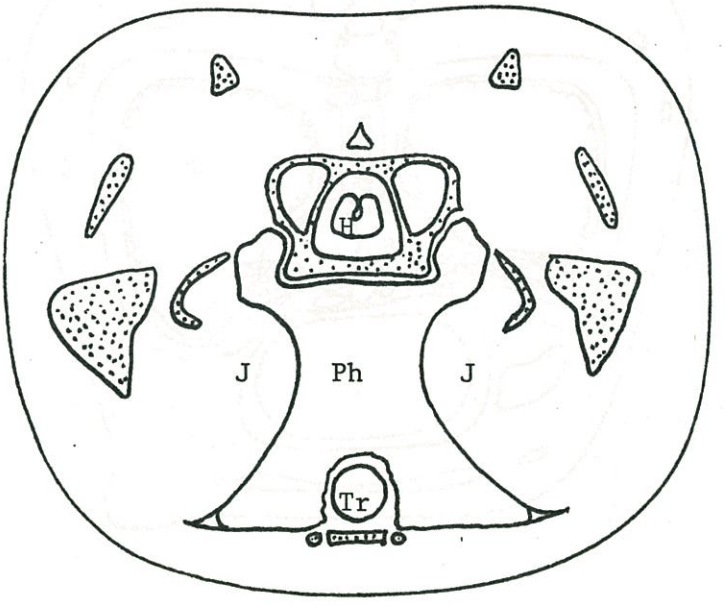


Fig. 1.5, T.s.scincoides, specimen TA53, vertical section through pharyngeal region, magnification X2. Ph=pharynx, J=jaw muscles, Tr=trachea, H=hindbrain.

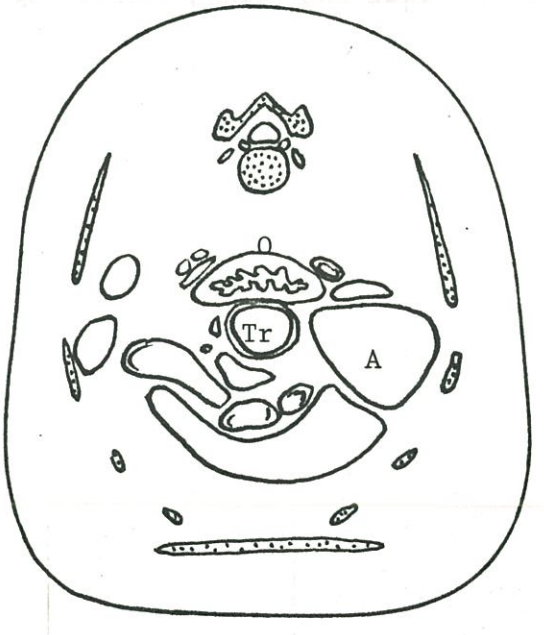


Fig.1.6, Specimen TA53, vertical section through atrial region. O=oesophagus, T=trachea, A=right atrium.

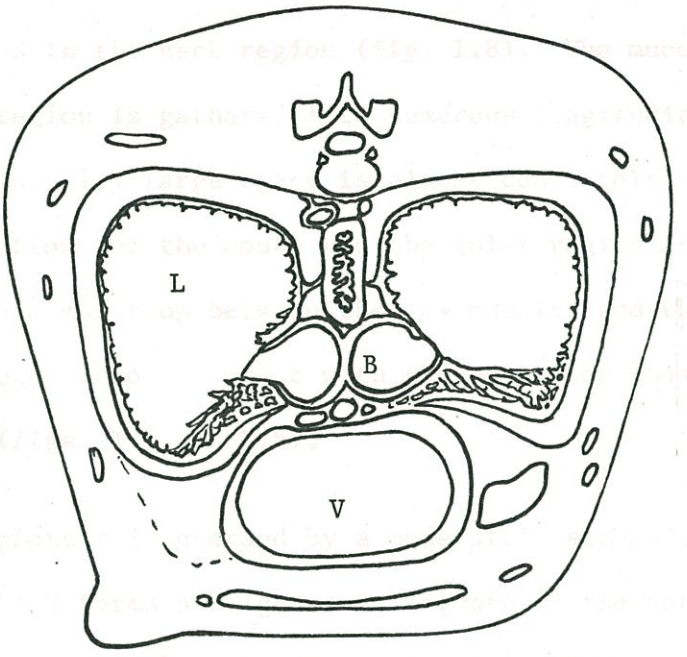


Fig. 1.7, T. s. scincoides, specimen TA53, vertical section through pulmonary hilar region, magnification X 2. L=left lung, B=right bronchus, V=ventricle. The oesophagus with it's convoluted luminal surface is seen between the lungs and below the aorta. The pulmonary vessels pass between the ventricle and the bronchi. Note the increased trabeculation in this area of the lung (Cf. Figs. 1.11 and 1.12). Potential spaces have been drawn as real spaces to facilitate visualization of organ relationships and attachments.

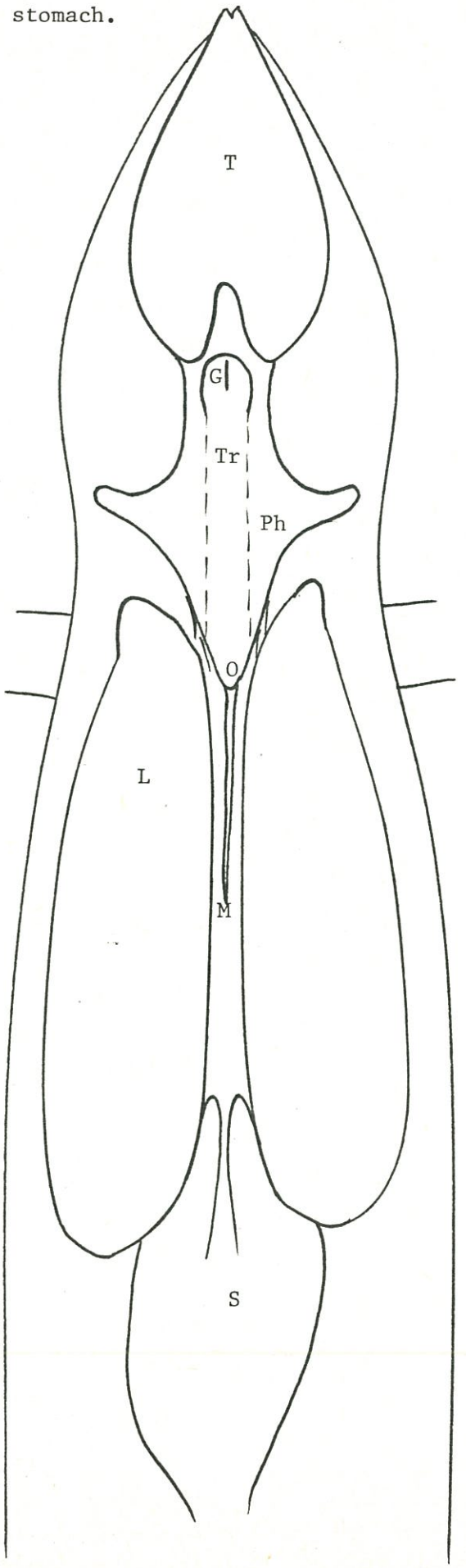
The nasopharynx is continuous below with the buccal cavity which expands to almost the full width of the head just behind the mandibular adductor muscles but tapers rapidly into the oesophagus in the neck region (fig. 1.8). The mucosal surface in this region is gathered into numerous longitudinal folds. This potentially large space is almost completely obliterated when the floor of the mouth and the gular region are raised, the trachea moves up between the jaw muscles and the glottis is then brought into alignment with the posterior choanae and nasopharynx (figs. 1.1 and 1.5).

The glottis is guarded by a moderately strong sphincter muscle which forms a slight swelling around the anterior extremity of the trachea just posterior to the base of the tongue (fig. 1.1). The glottis itself is a longitudinal slit-like opening of variable transverse diameter and inclined at an angle of about 45° to the tracheal axis (fig. 1.1).

The trachea extends in the midline from the glottis to the lungs. Anteriorly it appears to lie on the floor of the pharynx (figs. 1.1 and 1.5), it passes posteriorly immediately inferior to the oesophagus (figs. 1.6 and 1.7), and superior to the heart where it bifurcates near the level of the ventricular apex to form two short bronchi which open directly into the cavities of the lungs. The internal diameter is almost constant throughout except at the glottal constriction and the slight dilation just anterior to the carina (fig. 1.10). The trachea is supported throughout by 70 to 80 cartilaginous rings the majority of which are annular in form although some are partially divided or fused together. At the bifurcation of the trachea there is very little deviation of the axis of each bronchus from that of the trachea,

the carina is sharp so that the inspired air-stream is aligned almost directly in an antero-posterior direction within the lung. Most of the cartilaginous rings supporting the bronchi are incomplete semicircles or arciform.

Fig. 1.8, T.s.scincoides, specimen TA51, adult male, 295mm snout-vent length, frontal section viewed from above, magnification x 1. T = tongue, G = glottis, Tr = trachea, Ph = pharynx, O = oesophagus, L = lung, M = mesopneumonium, S = stomach.



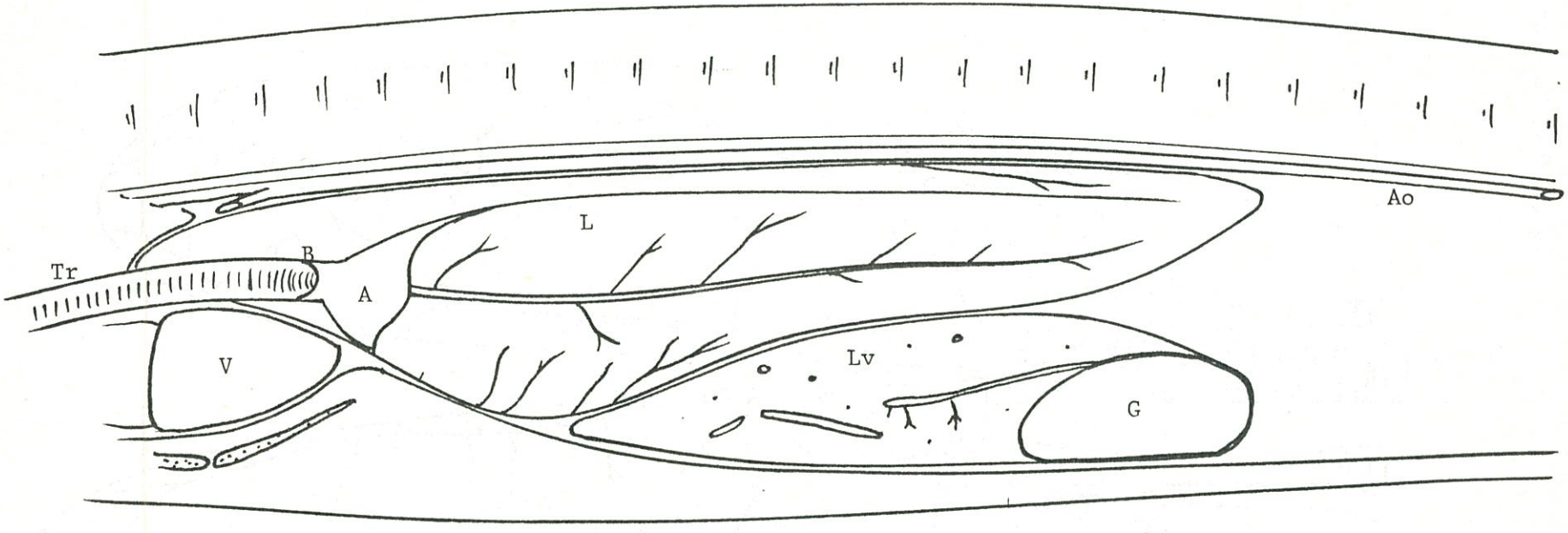


Fig. 1.9, T.s.scincoides, specimen TA50, adult male, 298mm snout-vent length, longitudinal paramedian section, magnification x 2. Tr = trachea, B = bronchus, V = ventricle, A = region of attachment to the contralateral lung, L = line of attachment to the mesopneumonium, Lv = liver, G = gallbladder, Ao = aorta.

Fig. 1.10.T.s.scincoides, specimen TA51, magnification X 2. Ap=pulmonary artery, V=pulmonary vein, Tr=trachea, L=ventral surface of left lung.

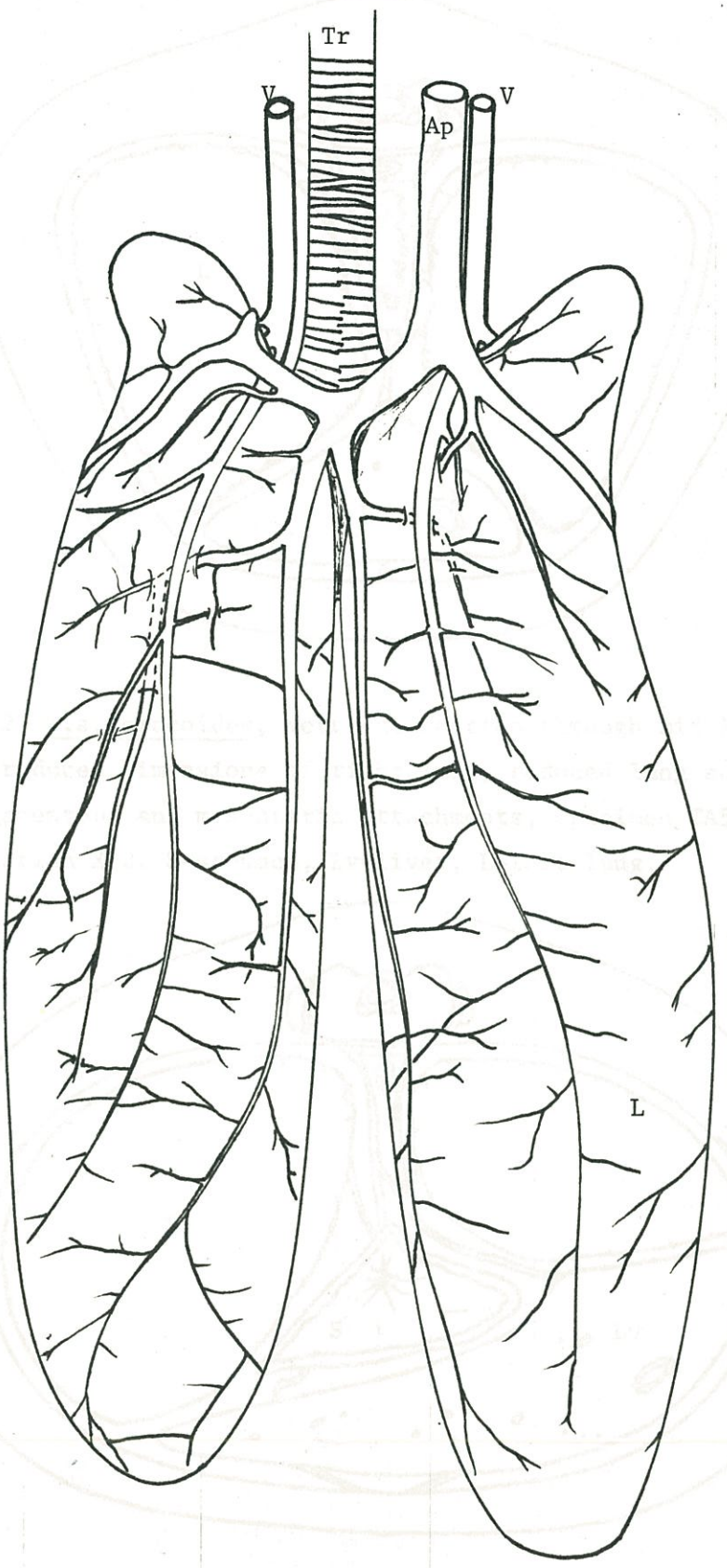


Fig. 1.11, T.s.scincoides, specimen TA53, vertical section through anterior lung region showing flattened lateral body wall and only moderate increase in luminal surface area of lungs, magnification X2. L=left lung, O=oesophagus, Lv=liver, V=posterior vena cava.

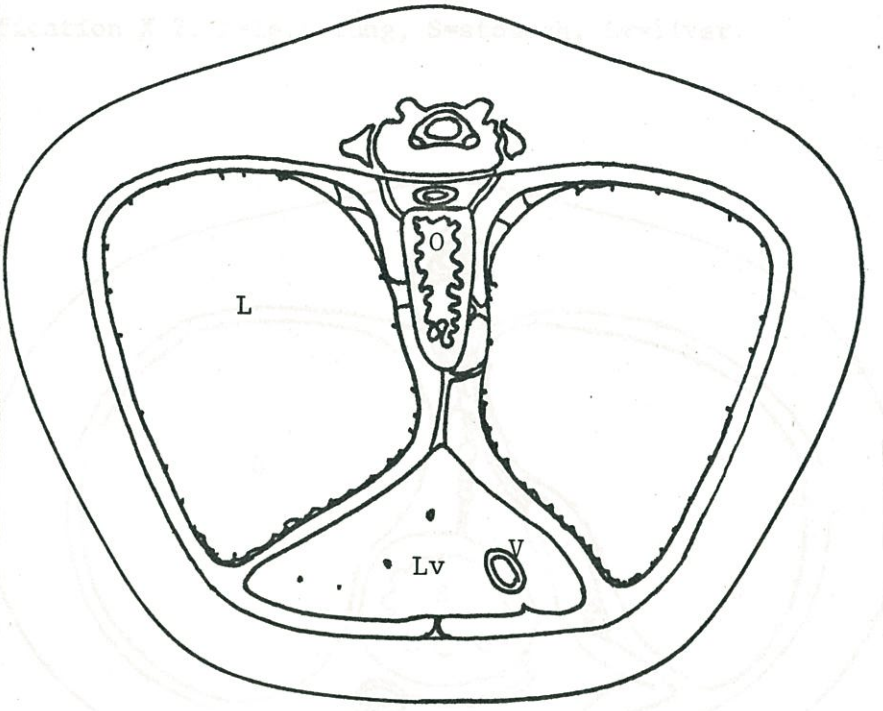


Fig. 1.12, T.s.scincoides, vertical section through mid lung region showing reduced dimensions of right lung, reduced lung surface area also ligamentous and mesenteric attachments, specimen TA53, magnification X 2. S=stomach, Lv=liver, L=left lung.

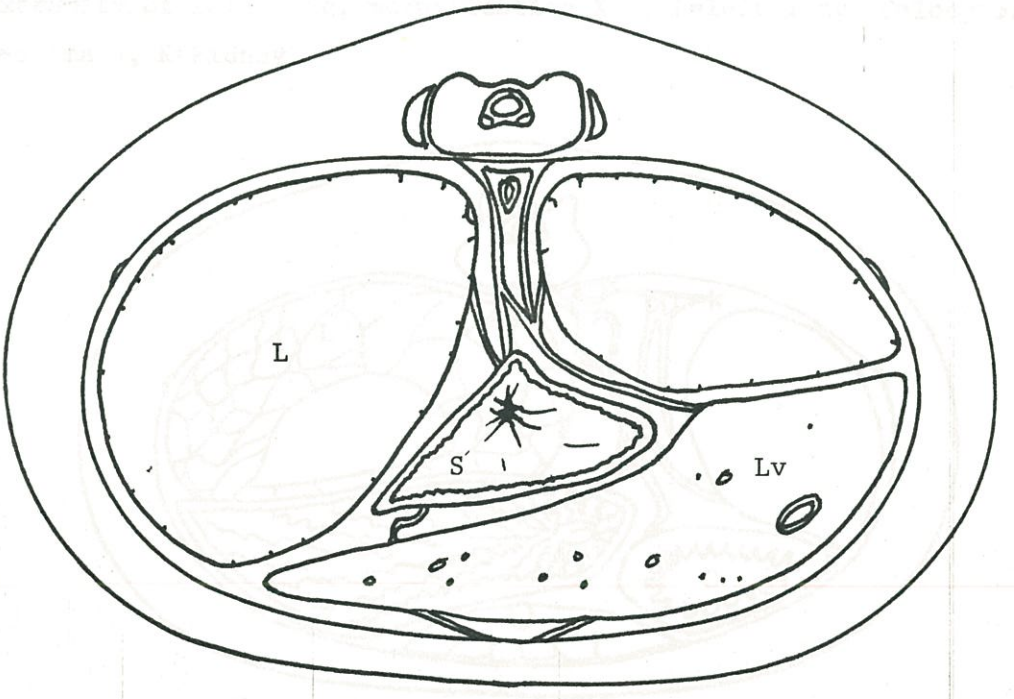


Fig.1.13, T.s.scincoides, specimen TA53, vertical section through posterior lung region showing almost smooth luminal surfaces, magnification X 2. L=left lung, S=stomach, Lv=liver.

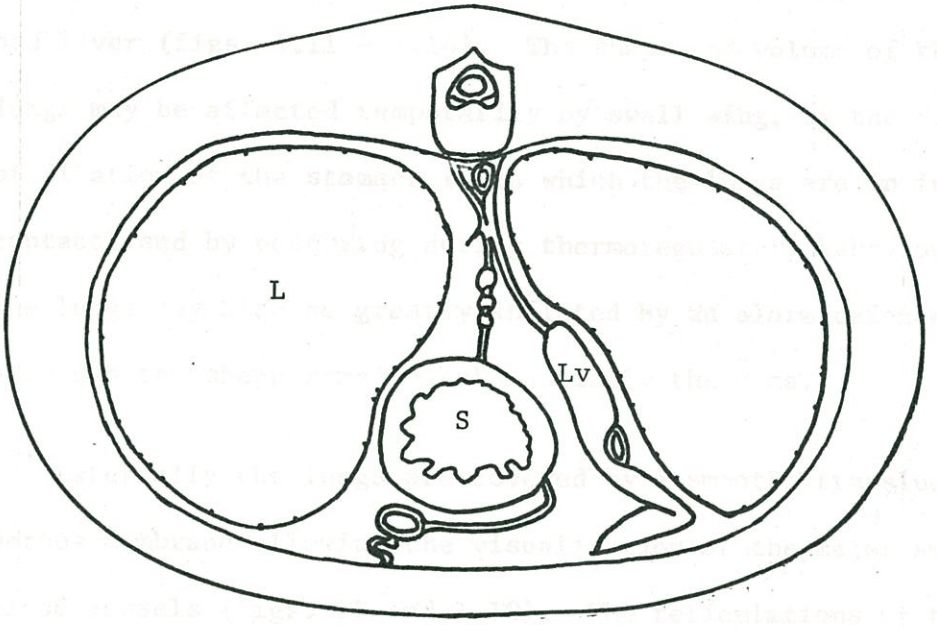
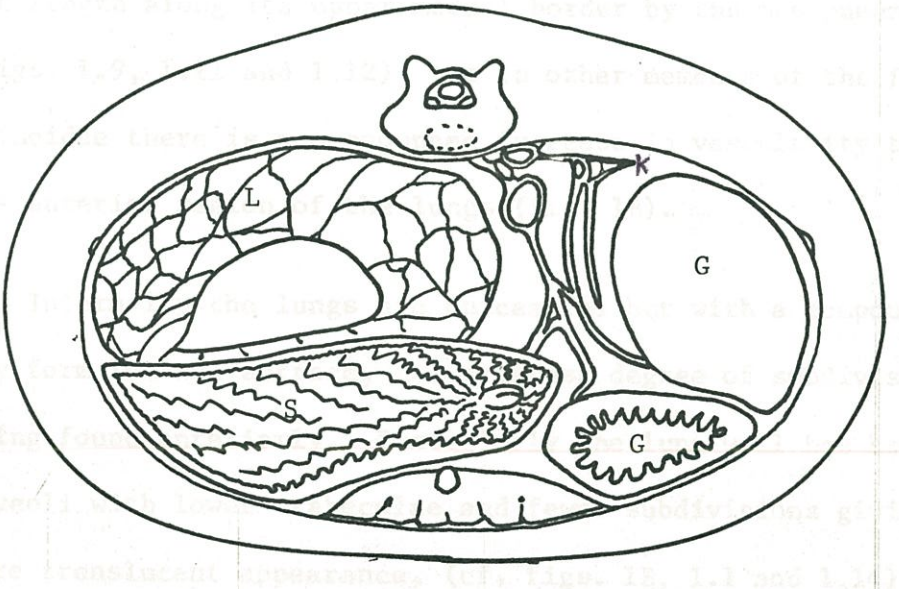


Fig. 1.14, specimen TA53, vertical section through posterior extremity of left lung, magnification X 2. L=left lung, G=loop of gut S=stomach, K=kidney.



1.1.4 The Lungs

The two lungs are large, elongated prolate sacs occupying approximately the anterior two thirds of the body cavity. The left lung is usually slightly longer and of greater volume than the right lung. The anterior extremities are more conical (fig. 1.8). Posteriorly the lungs are impressed by the stomach and liver (figs. 1.11 - 1.14). The shape and volume of the lungs may be affected temporarily by swallowing, by the degree of dilation of the stomach (with which the lungs are in intimate contact) and by posturing during thermoregulatory behaviour. The lungs may also be greatly inflated by an alarm/defence response although the shape remains substantially the same.

Externally the lungs are covered by a smooth, translucent, serous membrane allowing the visualisation of the major subjacent blood vessels (figs. 1B and 1.10). The reticulations of the luminal surface are also visible externally (figs. 1B, 1.1 and 1.14). The lungs are stabilised by their attachement to the bronchi and pulmonary vessels and also by their attachment to each other in the perihilar region. Each lung is also suspended for most of its length along its upper medial border by the mesopneumonium (figs. 1.9, 1.11 and 1.12). As in other members of the family scincidae there is a pronounced increase in vascularity towards the anterior region of the lungs (fig. 1B).

Internally the lungs are unicameral but with a compound faviform luminal surface, the greatest degree of subdivision being found anteriorly. Posteriorly the lung wall has broader faveoli with lower trabeculae and fewer subdivisions giving a more translucent appearance, (cf. figs. 1B, 1.1 and 1.14).

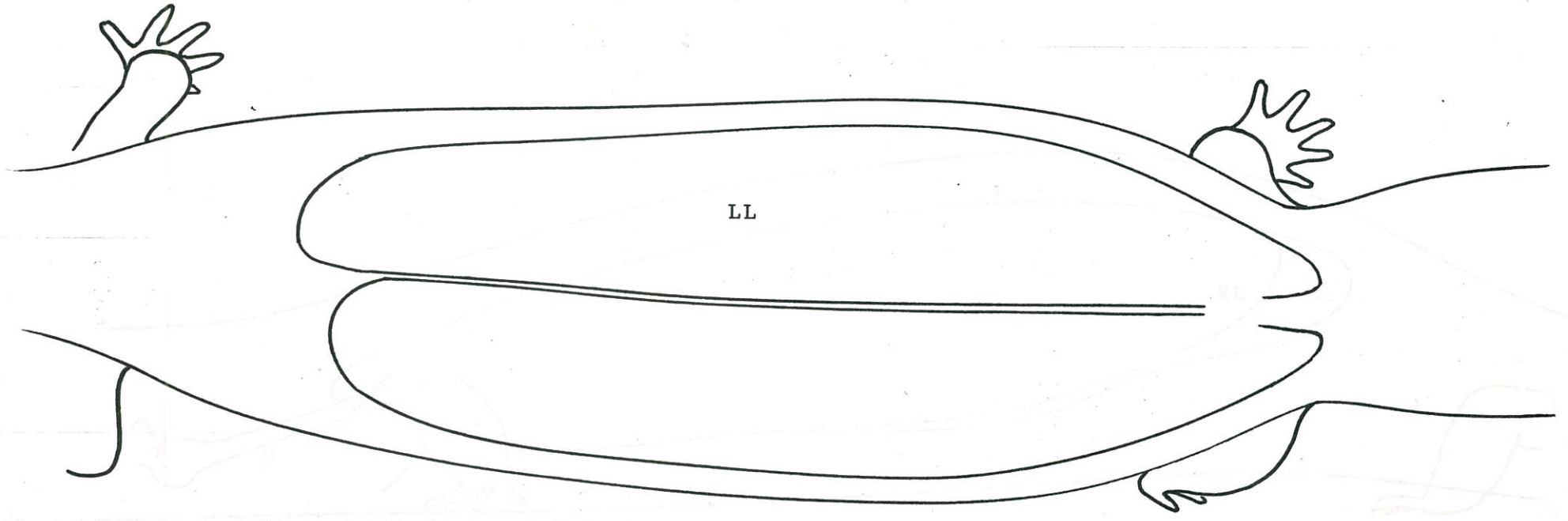


Fig. 1.15, T.s.scincoides, specimen TA07, 315mm snout-vent length, taken directly from a PA radiograph, note proportions of lungs, LL=left lung.

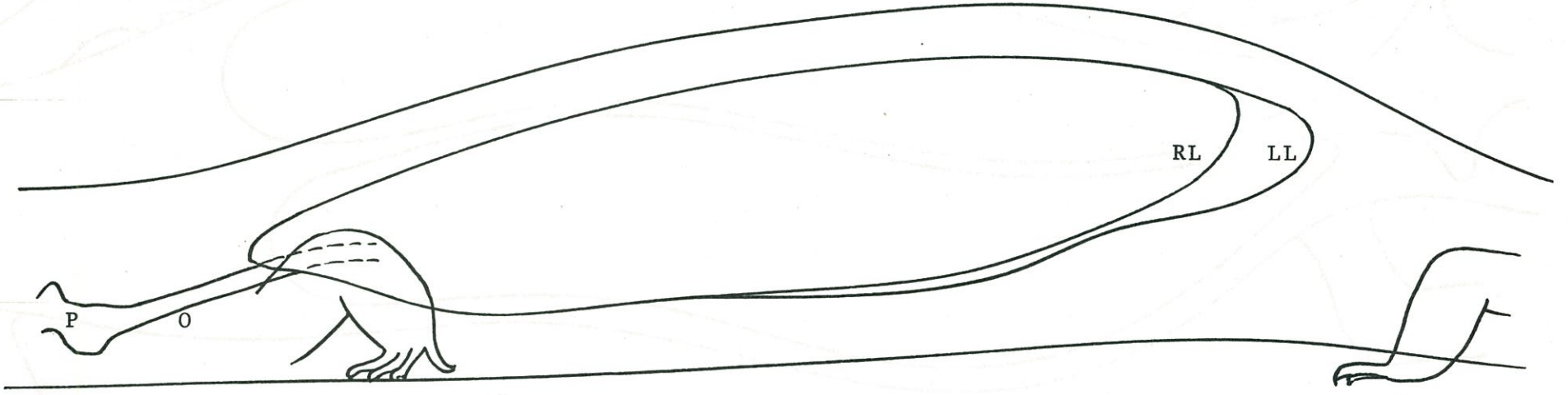


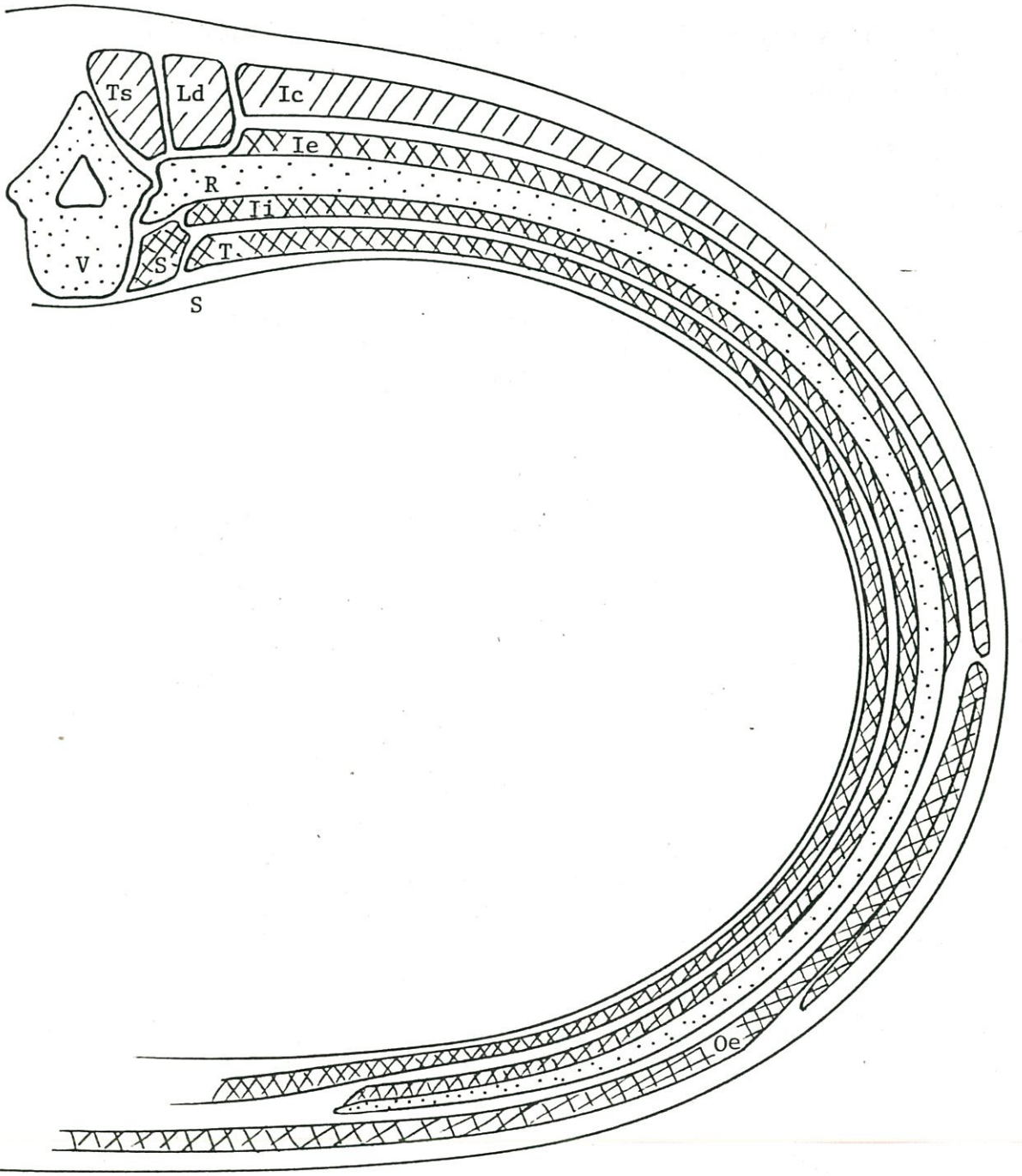
Fig. 1.16, T.s.scincoides, specimen TA07, taken directly from a left lateral radiograph, note positions and proportions of lungs relative to the pleuroperitoneal cavity. LL=left lung, RL=right lung, O=oesophagus, P=pharynx.



Fig. 1.17, T.s.scincoides, specimen TA32, 322mm snout-vent length, taken from a PA radiograph in walking position, note position of lungs also air in pharynx (Ph).

Fig. 1.18 Schematic representation of musculature of reptilian body wall (after Nishi, S. 1919).

V = vertebra, R = rib, Ts = transversospinalis, Ld = longissimus dorsi, Ic = iliocostalis, Ie = intercostalis externus, Oe = obliquus externus, Sv = subvertebralis, Tr = transversus, Ii = intercostalis internus, ///// = epaxial musculature, XXXXX = hypaxial musculature.



The body wall overlying the lungs is tough and muscular (figs. 1.18, and 1.11 - 1.14), being strengthened by ribs, scales and osteoderms. The parietal serous membrane contiguous with the lungs possesses a moderately extensive capillary network although this was not always easy to find macroscopically.

Like other reptiles Tiliqua spp. are occasionally subject to respiratory infections and pulmonary parasites (Ballantyne R. and Pearson J. 1963), during the present study no pulmonary parasites were found and only one specimen was found to have an apparently chronic pulmonary inflammation with a thickened and somewhat shrunken lung on one side only, the specific cause was unknown, no data were used from this animal.

1.2 RADIOLOGICAL EXAMINATIONS OF THE RESPIRATORY SYSTEM

1.2.1 Introduction and Objectives

Being potentially less invasive than many other techniques it was decided to use several radiological procedures to investigate various morphological and functional aspects of respiration. Plain antero-posterior (AP), postero-anterior (PA) and lateral projections were used. Standard high speed condenser discharge techniques, fluoroscopy and intermittent photofluorography with videotaped sequences were also used with some success although problems such as limited radiographic resolution coupled with the small changes that occur in quiet respiration (and some exposure timing problems) resulted in somewhat restricted information gain.

The major objectives of the radiological studies were as follows:

1) to provide accurate determinations of lung volumes,
2) to enable estimates to be made of changes in lung volumes during respiration (tidal volumes),
3) to ascertain whether longer term changes in lung volume occur,
4) to observe external and internal respiratory movements,
5) to visualize faveoli and any changes occurring in them during respiration,
6) to determine whether contralateral gas movement occurs.

1.2.2. Materials and Methods

The general radiographic appearances of four adult T.s. scincoides lizards were compared with several other species including T. nigrolutea, T. gerrardii, Trachydosaurus rugosus, Egernia cunninghami and Varanus gouldii. All the lizards had been in captivity for some time and were used to being handled. Each lizard was left on the X-ray table for about thirty minutes either in a box or under a cloth before any radiographs were taken. This procedure was followed in order to minimize any possible effects of the inflation-alarm response.

For the contrast medium studies lizards were anaesthetized with diethyl ether until the biting reflex was eliminated then one lung was cannulated via the glottis using a 1.5 mm Portex polythene intravenous cannula. Approximately 0.5 ml of contrast medium was slowly injected and the lizard was then upended to allow the contrast medium to run anteriorly and posteriorly in the lung. Each lizard was intermittently observed radiographically and videotaped during and after the period of recovery.

Two different contrast media were used, both containing iodine compounds. Dionosil, suspension (Glaxo, Boronia) was used for one lizard and Lipiodol Ultra, oily solution (May and Baker, Footscray) was used for the others. The x-ray equipment used was a Toshiba system (TVM 18, TVC 18, and a Rotanode tube unit). Videotapes were recorded using a Sony Videocorder (AV 3600 CE).

All radiological studies were carried out in warm weather and at room temperatures of about $25^{o}C$.

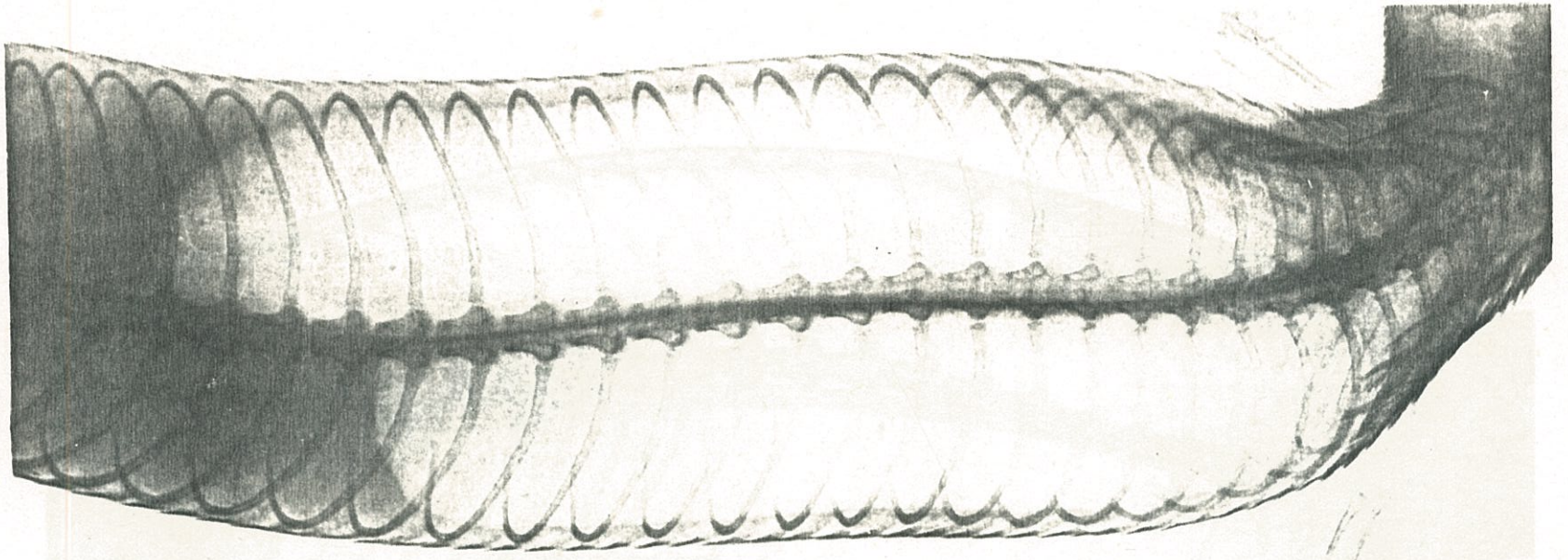


Fig. 1.19. PA radiograph of T. nigrolutea showing lung outlines. The increased radiopacity in the anterior region is only partly due to the presence of extrapulmonary tissues, whereas that in the posterior region is due mainly to the underlying liver and stomach.

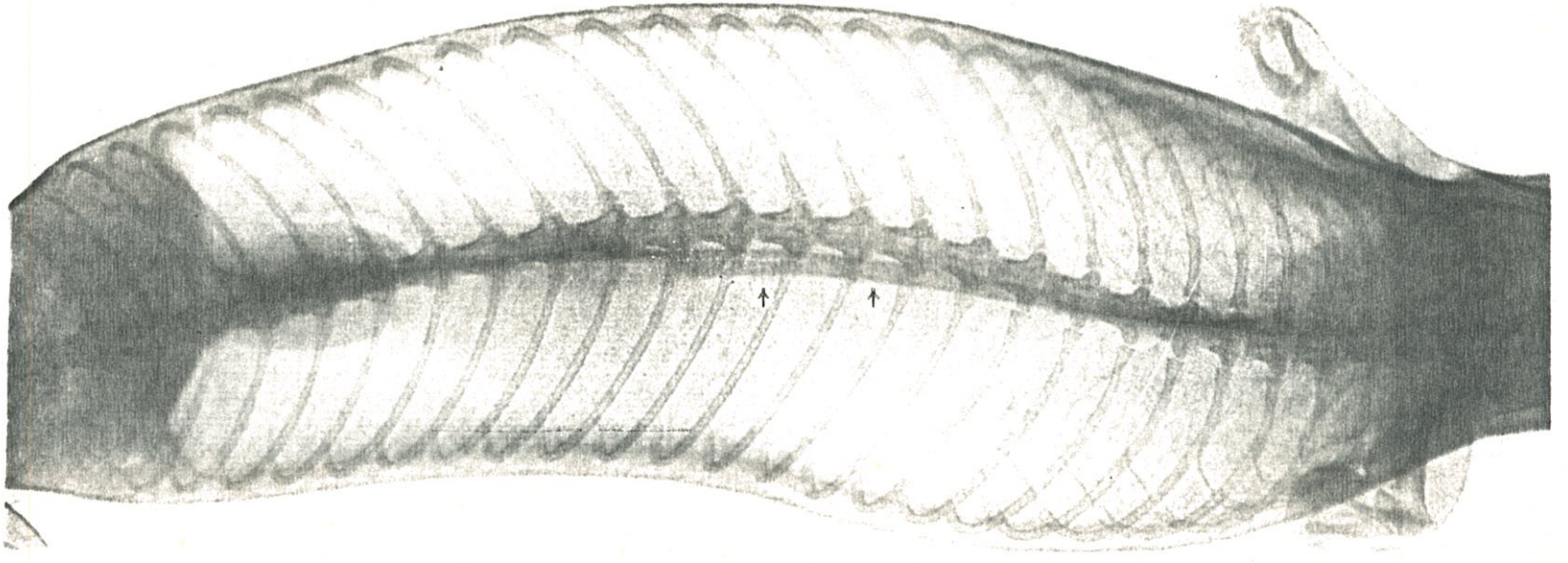


Fig. 1.20. PA radiograph of T. scincoides while walking. The mediastinal shift relative to the vertebral column (↑) may facilitate intrapulmonary gas mixing by increasing interpulmonary gas movement.

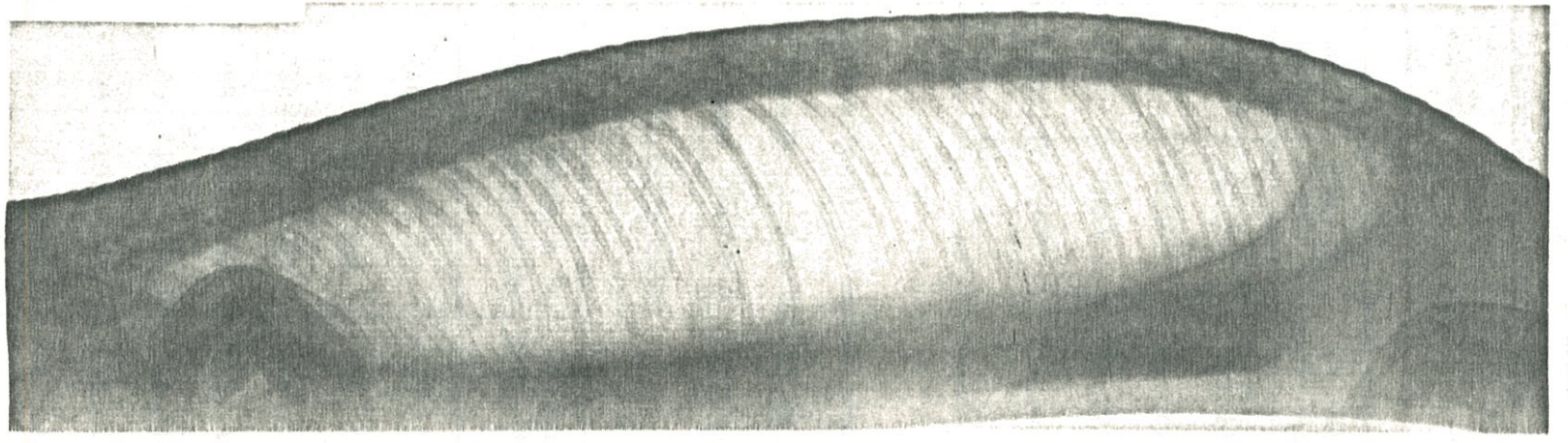


Fig. 1.21. Lateral radiograph of T. scincoides showing lung outlines. The stomach and liver intrude beneath the posterior extremities of the lungs (on the right). The heart, partly obscured by the shadow of the forelimbs, lies beneath the anterior extremities of the lungs (on the left).

Fig. 1.22 Lung volumes of four living adult blue-tongued lizards determined radiographically. TA28, TA38 and TA04 were T. s. scincoides, TA01 was a T. nigrolutea. SVL = Snout-vent length; LL = Lung length; LV = Lung volume.

Specimen Number	SVL (mm)	LL (mm) Left, Right	Mean LL / SVL	LV (ml) Left, Right	SVL / Total LV
TA28	300	109, 101	0.35	59, 27	3.5
TA38	300	125, 127	0.44	62, 47	2.8
TA04	318	172, 172	0.54	76, 74	2.1
TA01	353	175, 158	0.47	137, 92	1.5

Fig. 1.23 Variation in resting lung length over periods of time in the same specimen. A total of 36 observations were made on 8 lizards. TA01 = T. nigrolutea, all other specimens = T. s. scincoides.

Specimen Number	Number of Observations	SVL (mm)	Minimum LL Left, Right	Maximum LL Left, Right	Variation from Median Left, Right (%)
TA42	4	146	60, 53	67, 64	±5.5, ±9.4
TA29	3	276	151, 138	153, 149	±0.7, ±3.8
TA06	3	286	169, 166	174, 168	±1.5, ±0.6
TA41	3	288	166, 161	173, 171	±1.8, ±3.0
TA39	5	300	136, 107	164, 147	±9.3, ±15.7
TA38	5	300	107, 99	125, 127	±7.8, ±12.4
TA30	4	305	145, 136	156, 143	±3.7, ± 2.5
TA01	9	353	143. 125	175, 158	±10.1, ±11.7

1.2.3. Results of Radiological Studies

Lung Length and Volume:

One of the most obvious points of interest was that of lung volume. The PA projections e.g. fig. 1.19, frequently revealed a markedly more cylindrical lung compared to that seen at dissection, e.g. fig. 1B, where the shape is more fusiform as a result of the combined influences of being unconfined by the body wall and the inherent intramural tensions generated by the pulmonary tissues and its surface tensions. The length of the lungs relative to snout-vent length although variable was greater in T. s. scincoides than in all the other species examined, (figs. 1.22 and 1.23), however the relative lung volumes of T. s. scincoides and T. nigrolutea were similar, (fig. 1.22). Volumes were calculated from PA and lateral radiographs, e.g. figs. 1.20 and 1.21 (allowing for the radiographic magnification factor), they varied from 15-23% of body volume.

The variations in lung length and in lung volume, which increase quite dramatically with number of radiographic observations indicated that relatively major but slow changes occur as a normal event possibly unrelated to quiet tidal respiration. These changes may be related to autonomic activity and trabecular smooth muscle activity (discussed later).

During quiet respiration no major breath to breath changes in lung configuration were detected under fluoroscopy, neither were there any detectable changes in length of the lung during the ventilatory cycle. Over a period of time however, varying from about fifteen minutes to several hours, significant changes in lung volume were clearly visible. These major changes in resting lung volume were mainly the result of changes in length of the lung

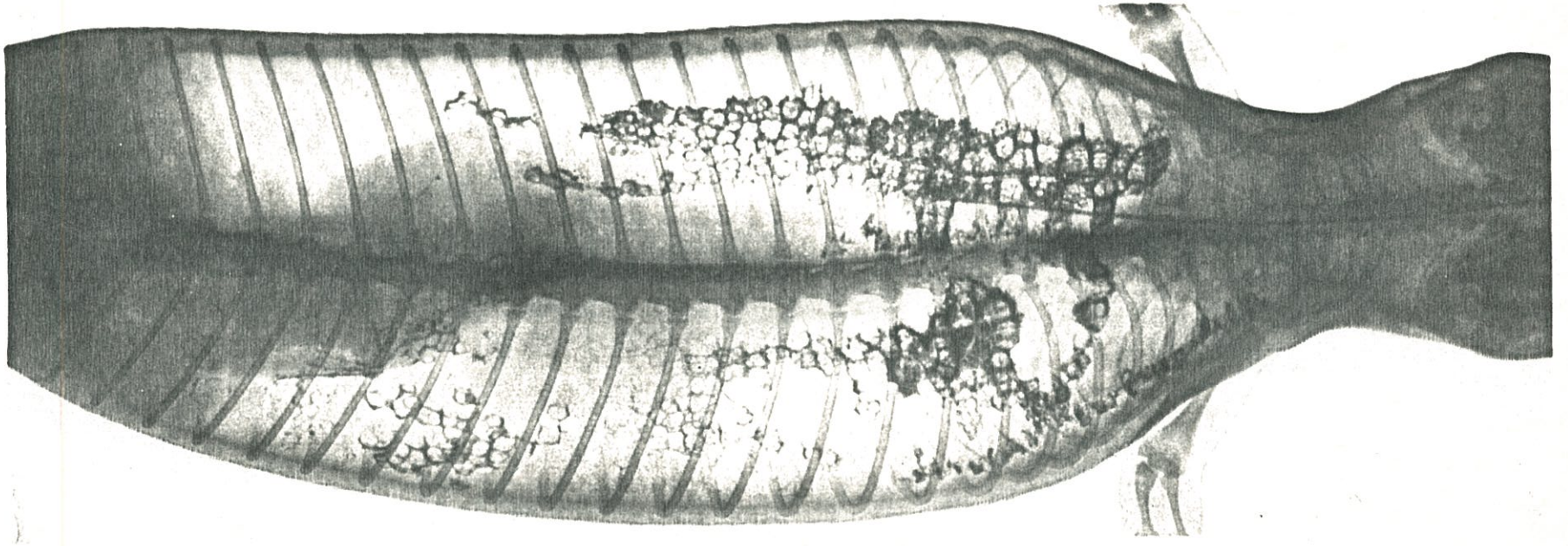


Fig. 1.24. PA radiograph of T. scincoides immediately after injection of radiopaque solution into lungs, catheter still in situ.

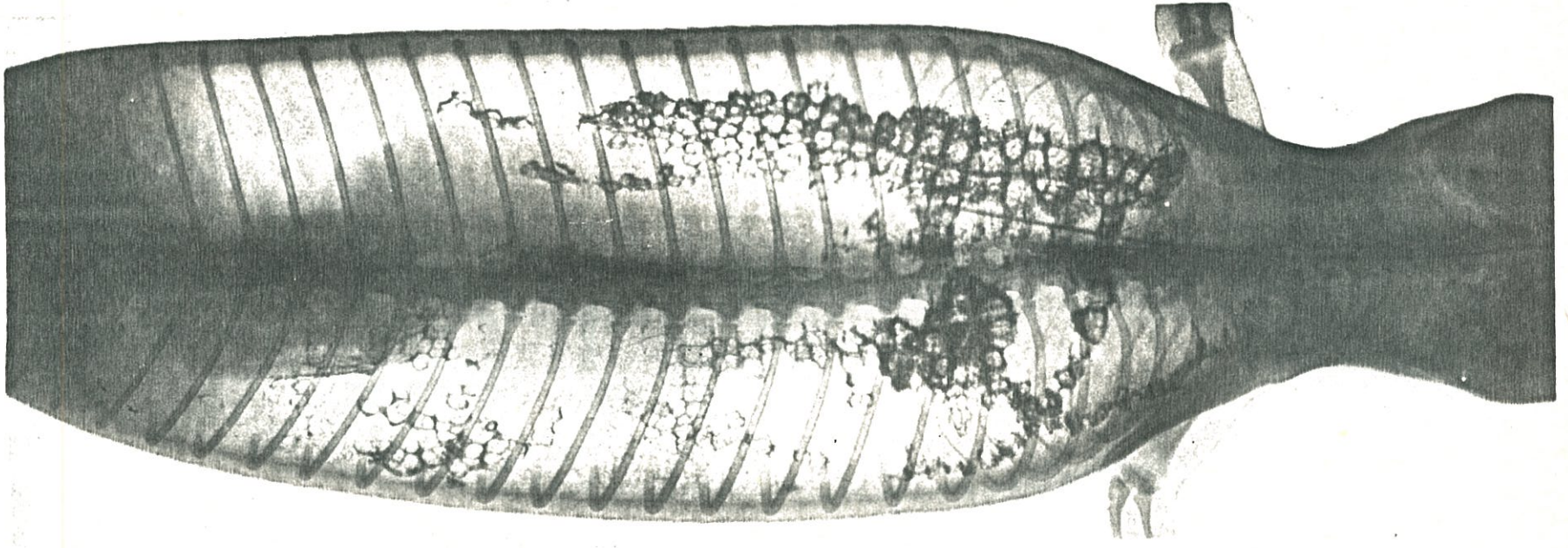


Fig. 1.25. PA radiograph of T. scincoides taken about ten minutes after fig. 2.5. Only minor changes in position and size of faveoli are seen.

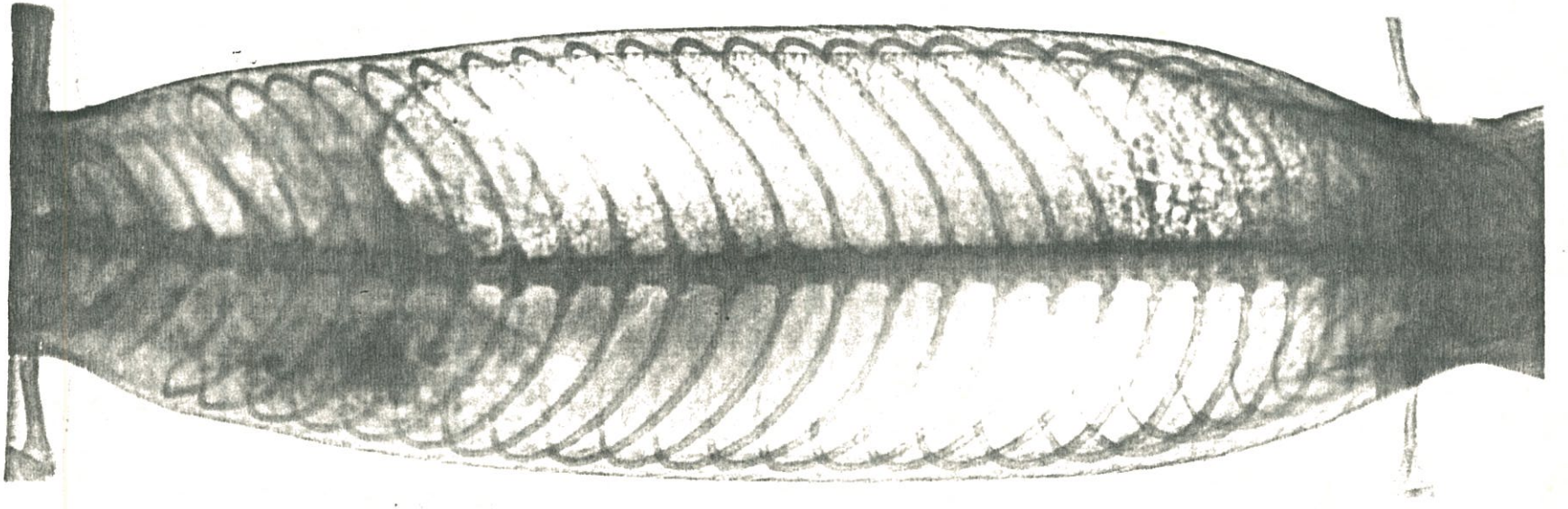


Fig. 1.26. PA radiograph of T. scincoides taken about three hours after injection of Lipiodol Ultra. The contrast medium remaining in the left lung is diffusely spread, however a large proportion is already in the stomach, posterior to the right lung.

although minor changes in the transverse diameters of the lungs accompanied by changes in the angulation of the ribs were seen (figs. 1.24 - 1.26).

Respiratory Movement:

The only fluoroscopically detectable movements in AP projection during quiet respiration were the body wall movements (and concomitant subjacent lung movements) occurring in the post-axillary region and the somewhat less regular buccopharyngeal (or gular) movements. These gular movements were most commonly synchronized with the pulmonary ventilatory movements although occasionally they occurred independently i.e. during the apnoeic or non-ventilatory period, also and quite frequently there were no detectable active gular movements during the ventilatory cycle. Because of the relatively small movements (indicating a small tidal volume) and the poor fluoroscopic resolution no quantitative measurements of tidal volume could be made by this method.

Changes During Walking:

When the animal is walking (fig. 1.20) the pleuroperitoneal organs undergo lateral undulations which appear to cause a mediastinal shift relative to the vertebral column (→), although this does not appear to cause any major change in the volume of one lung relative to the other. All major changes in lung volume which were observed tended to be bilateral so that even with large changes in total lung volume the relative dimensions of the two lungs would tend to remain comparable.

Contrast Medium Studies:

During fluoroscopy using a radiopaque contrast medium the outlines of certain faveoli could be observed. Although there

were no major changes even over periods of about half an hour there were changes in outline of some of the faveoli, (see figs. 1.24 - 1.25). After about three hours much of the contrast medium had been removed from the lung presumably because of ciliary action and the remainder was dispersed throughout the lung (fig. 1.26), with small amounts still remaining in the faveoli. After twenty four hours no contrast medium was visible in the lungs although some was still present in the gut.

1.3 DISCUSSION

1.3.1 The Body Wall

Externally the Blue-tongued lizard's body wall appears extremely tough and giving transcutaneous injections is made particularly difficult by the presence of osteoderms just beneath the scales. However in the post axillary region (as in other areas where increased skin flexibility is required) the scales are smaller than the surrounding scales presumably increasing the compliance of this area. Respiratory work is also reduced by several other factors such as the shape and flexibility of movement of the ribs in the anterior body region. The flattening of the lateral body wall (fig. 1.1) and the complex arrangement of body wall muscles (fig. 1.18) in this area are also no doubt related to an optimum oscillatory mechanism for facilitating gas exchange.

1.3.2 Dead Space

Although the Blue-tongued lizards have a large gape and expansible pharynx it was noted in dissections that the respiratory space could be greatly reduced by raising the floor of the mouth and gular region, this was confirmed as occurring during fluoroscopic observation of respiration, thus increasing respiratory efficiency. Estimates of dead space were difficult to determine but almost certainly it represents less than 2% of the mean living lung volume. This figure would be reduced to less than 1% if gular respiration occurred between normal respirations in which case only

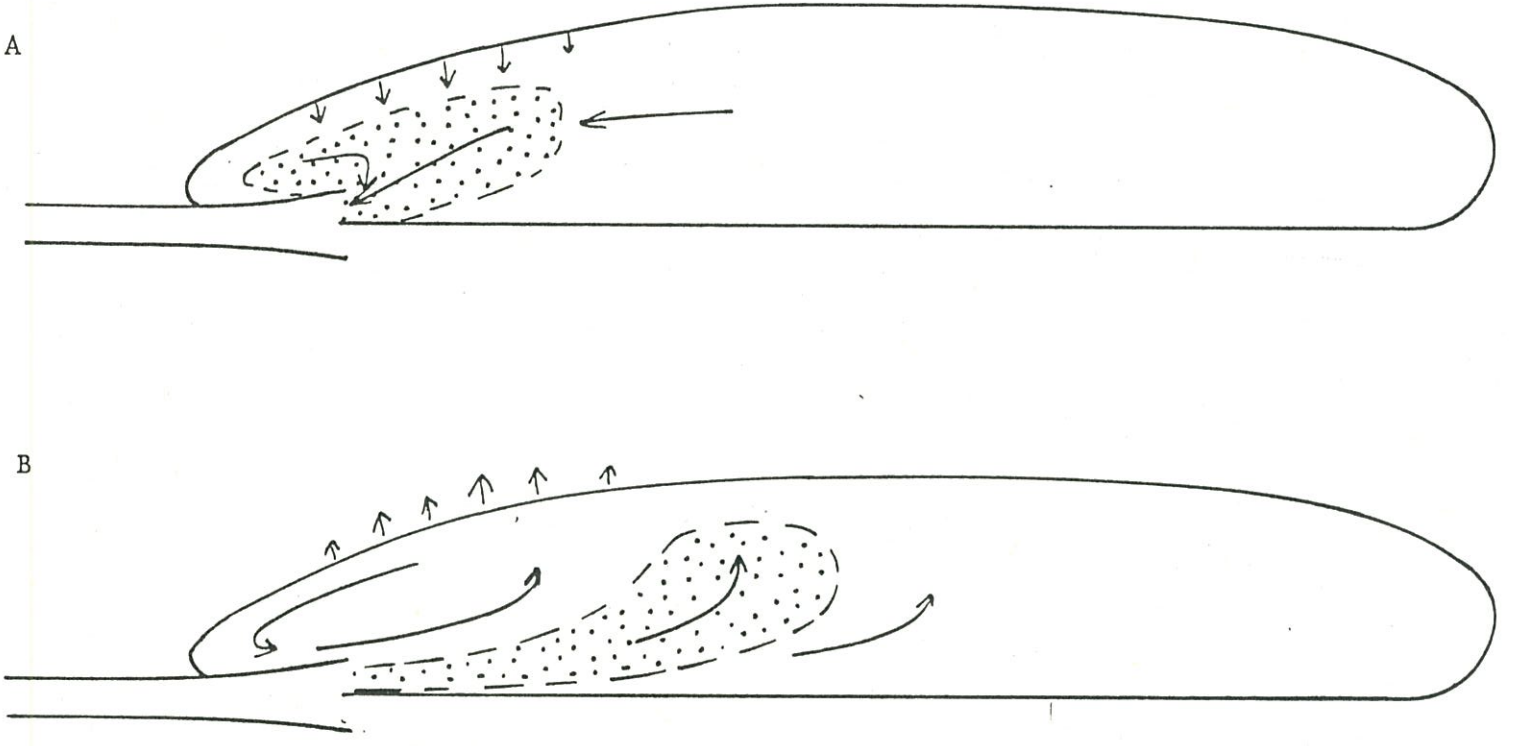


Fig. 1.27. Suggested events occurring during quiet respiration. A. Volume of air (shaded) about to be expired. B. Volume of air (shaded) just inspired. Short arrows show major movements of body wall, long arrows show major air movements. Overlap between the two volumes is minimal.

the trachea could be described as dead space, (tracheal volume is about 0.4 ml in an adult lizard). It is assumed here that air in the lumen of the lung is not defined as dead space air and that mixing in the lung is relatively thorough although this may not actually be the case (this space is not analogous to dead space in the mammalian lung).

1.3.3 Intrapulmonary Gas Mixing

Although tidal volumes are relatively small, the narrow trachea will generate a relatively high velocity for air entering the lung. This will no doubt produce a Venturi effect and cause swirling in a large part of the free space in the lung particularly near the bronchial orifice (fig. 1.27, cf. figs. 1.7 and 1.10 which show respiratory movements as viewed externally and in post-axillary cross section). Along the mid region of the medial lung wall there are numerous low septa and trabeculae which would mitigate against any funnelling or coanda effects along this wall, in fact these irregularities would tend to increase turbulence and the mixing effect. The fact that vascularity of the lungs is markedly reduced in the posterior parts of the lungs would tend to indicate that mixing of fresh air in this region is probably far from complete. If the lungs are thought of in terms of a vascular countercurrent system (perhaps not a very unreasonable idea when considering the small vessels involved and the relatively sluggish circulation), then a variable partial pressure gradient from anterior to posterior of the lung would be a reasonable conclusion.

1.3.4 Lung Volume

The respiratory surfaces although much divided have a low area : lung volume ratio thus supporting the theory that the lungs may have other significant functions besides simple gas exchange. An alternative theory is that the respiratory mechanism is different to that found in multicameral and homogeneous (mammalian) type lungs. A number of alternative (and perhaps concomitant) possibilities are here considered.

Blue-tongued lizards are good swimmers but frequently live in semi-arid areas and seldom take to water spontaneously (personal observation), however the lungs are well situated to give buoyancy and head-up body orientation while swimming. It is unlikely that the large lung volume is in any significant way related to the lizards swimming requirements.

Although Blue-tongued lizards are capable of inflating their lungs moderately in the defence-alarm situation this response is limited (partly perhaps by the presence of the scincid characteristic of osteoderms), and is not nearly as well developed as in the agamids, Amphibolurus spp., for example (personal observation).

As far as predators are concerned there is some doubt whether having large lungs is advantageous or not. At birth there may be an instantaneous increase in body size of about 20% as the lungs expand and although this may make the lizard too large for some predators it no doubt makes it large enough for certain large predators that might otherwise have

considered it too small, however, this would apply to very few extant predators so that there could have been selection pressure in the past towards an increase in lung, and therefor body, volume.

Another possible advantage of having a large reservoir of air in the body is a homeostatic one in that the partial pressures of lung gases would take longer to alter significantly so that frequent, regular respiration is not so vitally essential. This could be of adaptive significance in that Blue-tongued lizards frequently, (but not always), 'freeze ' rather than dash for cover when disturbed and may stop breathing for a short period when disturbed, (personal observation).

A further possibility is related to the defence, nutritional and reproductive functions of the lizard. Having large lungs means that the lizard can swallow large items of food if necessary without having to stretch, and thereby weaken, its body wall. The same reasoning applies during pregnancy, the large number of young (up to 25, Cogger, H. 1979) can be brought to an advanced stage of development before birth without causing undue collapse of the lungs.

Another perhaps less likely advantage of large lungs is the increased thermal insulation they provide between the spinal cord and the viscera (see e.g. fig. 1.7). During insolation when the lizard is below the preferred body temperature (PBT) the dorsum is inclined towards the sun to maximize heat uptake until the PBT is reached. If the

spinal cord is preferentially warmed this will presumably decrease reflex reaction times. Normally the lizard, when emerging from its nocturnal retreat, warms up its head first then increases the degree of exposure until the whole body is exposed (personal observation). On cool days however a lizard may never reach its PBT and the maintenance of quick reactions in such circumstances could be of considerable significance in survival, (T.s. scincoides is sometimes active on days when the maximum air temperatures reach only 12°C, personal observation).

Blue-tongued lizards produce a hissing sound when alarmed or as a warning sign however they do not produce any controlled resonant vocalization and it is doubtful if the large lung volume is of any significance in relation to the hissing activity. Other common respiratory activities include yawning and 'snorting' however neither appears linked to an extra large lung volume.

1.3.5 Pulmonary Smooth Muscle Contraction

Although the radiological studies did not provide any dramatic new information on respiratory function it did reveal several points worth further consideration. The fact that the lungs have a certain tendency to contract before, during or after dissection could be the result of one or more of several factors, e.g.

1) Effect of anaesthesia, direct or indirect.
2) Surfactant level.
3) Elasticity of lung tissue.
4) Contraction of pulmonary smooth muscle.
5) Surgical interference.

1) Diethyl ether is relatively non-toxic and is not likely to be the sole cause of the observed difference between lung size in unanaesthetized and dissected specimens although diethyl ether is known to have an irritant effect on mucosal surfaces, it also stimulates the release of a certain amount of catecholamines and causes relaxation of bronchial smooth muscle (Adam et al. 1973).

2) Surfactant was once thought to be very limited in the simple reptilian lung however it is now known to be present in significant amounts at least in several reptile species (Pattle 1963, Melvan 1980, Stratton, Wetzstein and Hardy 1980, Wetzstein, Stratton and Olson 1980). Although no quantitative measurements were made in the present study the lung washings of blue-tongued lizards were shown to possess considerable detergent activity as shown by the stable foam produced by agitation.

3) Elastic recoil of the lung tissue is a very probable cause of at least part of the observed contraction of the lungs although the tenuity of the tissues involved would probably preclude all but a very small force being developed by this property.

4) Active contraction of smooth muscle in the lung was observed during dissections on more than one occasion, possibly as a result of mechanical irritation, and this could well be the major cause of lung contraction.

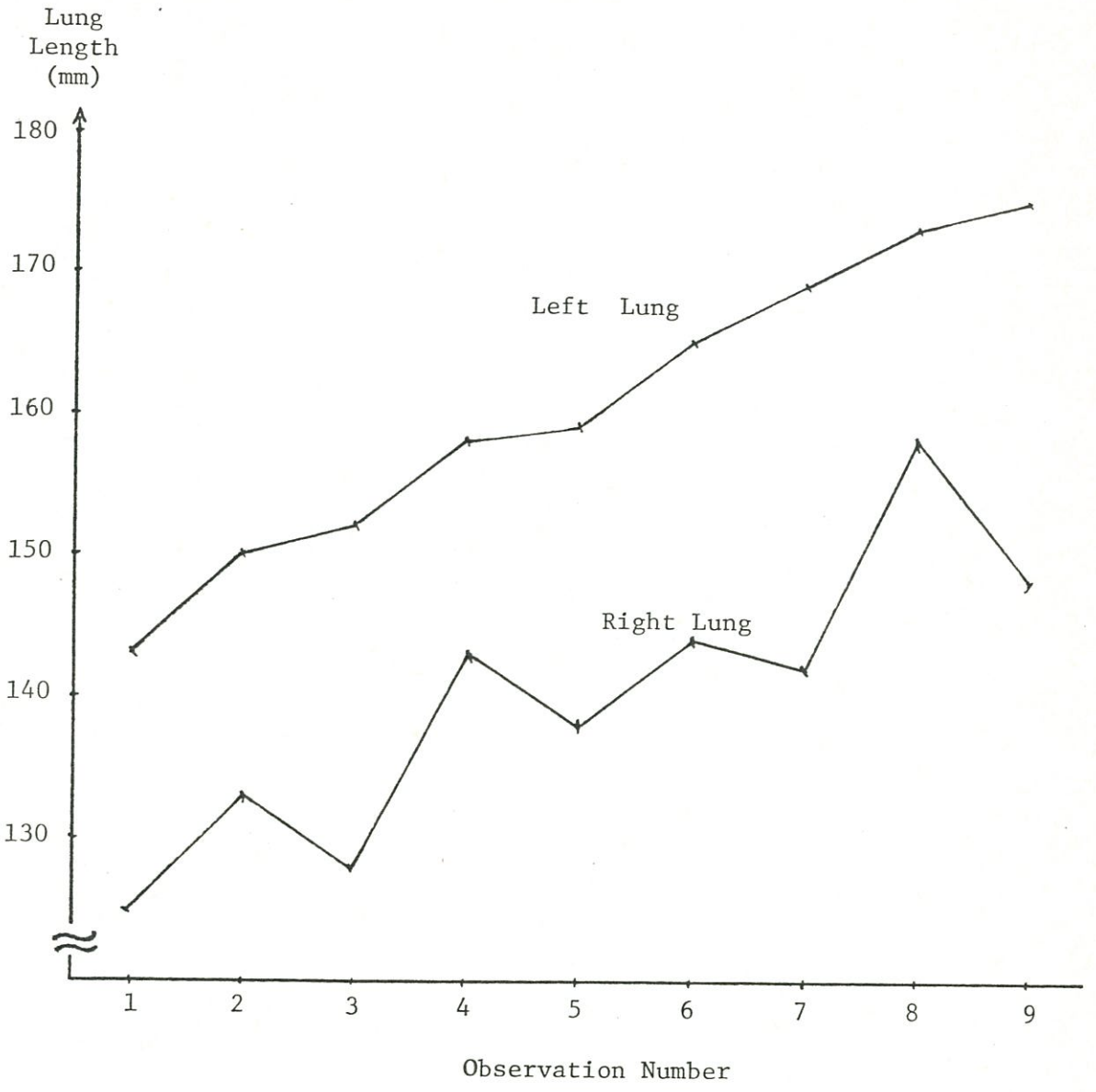


Fig. 1.28. Comparison of changes in lung length over varying periods of time in a single animal determined radiographically. Specimen TA 01, T. nigrolutea, adult female, (observations not in chronological order). Product moment correlation coefficient = 0.90 (significant at 0.05 level).

5) Surgical opening of the pleuroperitoneal cavity may result in immediate partial collapse of the lungs under their own weight, if the glottis is open. If the glottis is closed, as it frequently is then the lungs remain inflated although apparently not always at their normal *in vivo* volume, (fig. 1B).

1.3.6 Locomotory Effects on Lungs

Another question that arose as a result of consideration of the physiological significance of large unicameral lung volumes and small tidal volumes concerned the mechanism by which mixing of gases occurred within the lung and whether this was influenced by voluntary motor activity such as occurs during walking. Simple observation of the walking animal shows that there is usually a certain degree of lateral sinusoidal motion of the body although it is rather variable in amplitude. Blue-tongued lizards have been observed to perform these lateral undulations after feeding, without walking or other movements, (personal observation) however these movements may be related to swallowing rather than to gas mixing. The radiographs, e.g. fig. 1.20) indicate that there are movements of the lungs relative to each other and therefore there must inevitably be some movement of gas from one lung to the other, however this movement does appear to be minimal.

1.3.7 Changes in Lung Length

The fact that the lungs generally contract and expand together (fig.1.28) may indicate that gross changes in lung volume are more under central or autonomic control than local control. However, the fact that there are changes in faveolar dimension and some changes in relative dimensions of the lungs indicates that local factors may also be important in moving

and mixing of gases within the lungs. The radiographic evidence suggests that these mixing processes may be minimal especially in the stationary animal and that other factors must be involved in facilitating gas exchange.

There is the possibility that the endotracheal cannulation and the contrast medium itself could have influenced the findings although observations made before and some hours after the procedure gave no indication that this may have been the case.

SECTION II HISTOLOGY OF THE BLUE-TONGUED LIZARD LUNG

2.1 Introduction and Objectives

This histological study is concerned mainly with the microanatomy of the internal surfaces of the lungs. The major objectives were as follows:

1) Confirmation of macroscopic findings.
2) To enable the calculation of approximate lung surface areas.
3) To demonstrate at least semi-quantitatively the variation in pulmonary vascularity along the length of the lung.
4) To determine the location of smooth muscle within the pulmonary tissues.
5) To determine qualitatively the amount and distribution of connective tissue fibres.
6) To facilitate the ultrastructural study of the pulmonary tissues.

To a limited extent, at least, all these objectives were achieved.

2.2 Materials and Methods

Two species were used for histological investigation, two specimens of T. nigrolutea (juveniles) and four specimens of T. s. scincoides (adults). All lizards were apparently in good health prior to dissection although the two specimens of T. nigrolutea had suffered transient heat stress which apparently caused the

anaemic appearance of the lungs due to vascular shunting, (see section 2.4).

Both lungs with trachea attached were dissected out of each animal by a mid-ventral incision. They were immediately placed in buffered 10% formalin; this solution was also injected into the lungs to produce varying degrees of inflation. The trachea was then tied off and the lungs were kept in this solution until processed (minimum 24 hours). Pieces of lung tissue up to about 30 mm long x 10 mm wide were cut out from the anterior, middle and posterior regions of the lung. These specimens were then wax embedded and sectioned using standard techniques. Sections were cut in transverse, longitudinal, tangential and oblique planes. Four standard staining techniques were used;

1) Haematoxylin and eosin.
2) Toluidin blue.
3) Gomori reticulin stain with silver impregnation.
4) Picro-mallory.

Drawings were made using a camera lucida device, Olympus 212016. Measurements of perimeters used in calculating lung surface areas were made from the drawings using a Zeiss Digital Image Analyzer (MOP 30).

Photographic prints were made from colour slides taken in a Leitz Orthoplan Photomicroscopy System using Fujichrome ASA 100 film and printed on Ilford Multigrade Photographic paper.

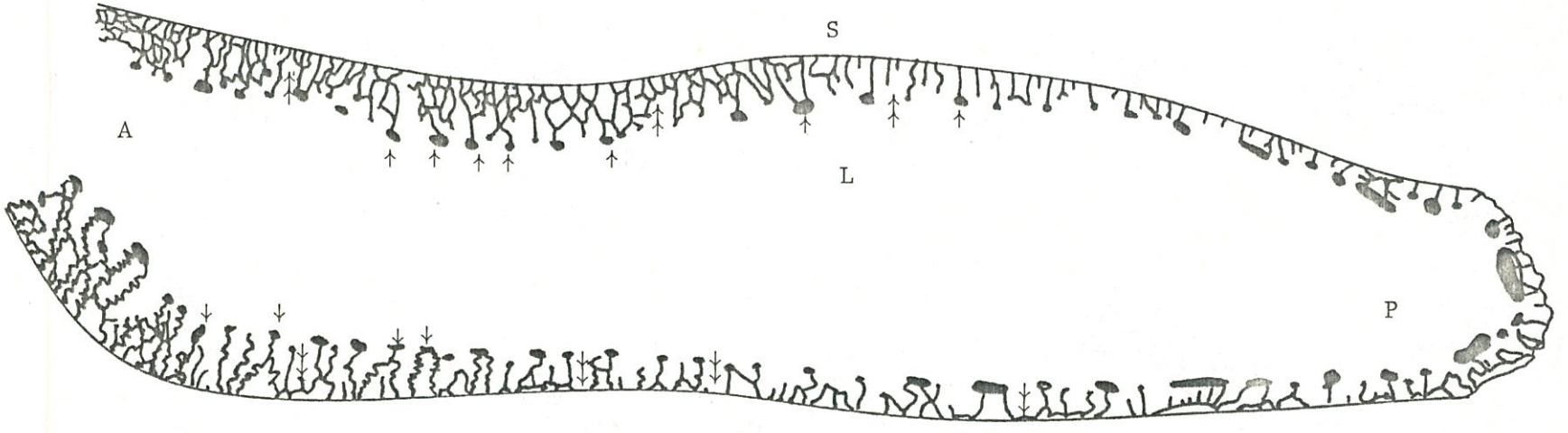


Fig. 2.1, T. nigrolutea, specimen TA40, 135mm snout-vent length, longitudinal section through posterior and middle region of lung, magnification X 11. Anteriorly there is increased depth and subdivision of faveoli, also increased number of trabeculae. A=anterior, P=posterior, S=serosal surface of lung, L=lumen of lung, → = smooth muscle bundles in trabeculae, ↠ =faveolar spaces.

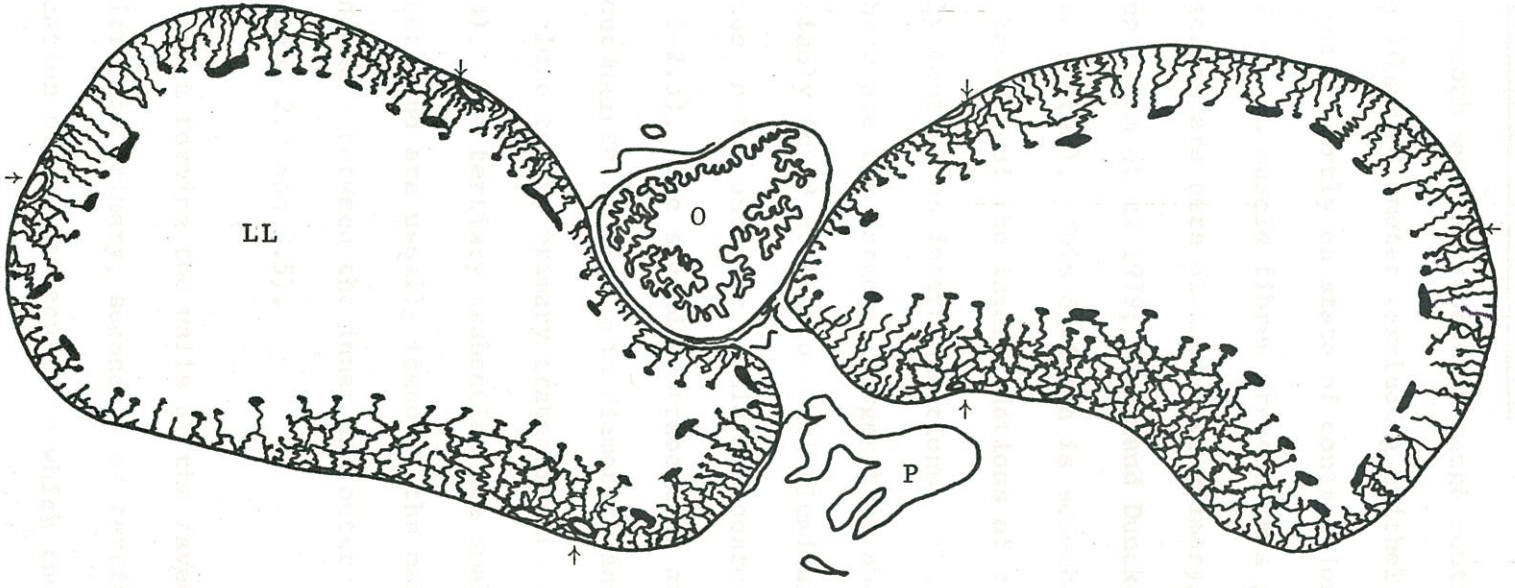


Fig. 2.2, T.nigrolutea, specimen TA40, transverse section through anterior region of both lungs and oesophagus, just posterior to the heart, magnification X 12. LL=left lung, O=oesophagus, P=pericardium, →=major pulmonary blood vessels.

2.3 Histological Observations

2.3.1 Terminology and Definitions

The smooth muscle trabeculae range continuously in size from less than 10μm in diameter (excluding epithelial envelope) to over 150μm depending partly on state of contraction but mainly on the number of smooth muscle fibres present in a given cross section. The trabeculae are here divided into primary, secondary and tertiary (following Klemm et al 1979; Perry and Duncker 1978 also use a tripartite system). This division is somewhat arbitrary however, not only because of the intergradations of size but also the apparently continuous interconnections. The primary trabeculae as defined here are the largest (larger than about 90μm in diameter) and are invariably found at the internal (luminal) boundary of the lung tissue in the anterior, middle and posterior lung regions, (figs. 2.3-2.5). The secondary trabeculae are moderate in size (from about 40μm to about 90μm in diameter) and usually occupy a position close to the primary trabeculae to which they are connected (fig. 2.3). The tertiary trabeculae are small (less than about 40μm in diameter) and are usually found at the margins of small septa almost anywhere between the inner and outer limits of the lung tissue (figs. 2.3 and 2.5).

The septa forming the walls of the faveoli may similarly be classified as primary, secondary or tertiary according to the classification of the trabeculae to which they are attached.

2.3.2 Changes Along the Length of the Lung

Longitudinal sections under low power magnification, e.g. fig. 2.1 show a steadily increasing surface area per unit volume of lung towards the anterior region of the lung resulting from the

increased height of primary septa and the decreasing diameter of individual faveoli.

This tendency is somewhat minimized in fig. 2.1 as shown by the enlarged (contracted) smooth muscle trabeculae and the slightly puckered lung surface at the posterior extremity of the lung.

Concomitant with this tendency is an increase in the amount of smooth muscle towards the anterior lung region, the increase being greatest in the minor trabeculae, there is however little difference in the diameter of the major and minor trabeculae in anterior and posterior regions of the lung, the difference being due almost entirely to the increased numbers of smooth muscle bundles found in the anterior region. The number of smooth muscle fibres found in a single bundle, i.e. trabecula, cross section varied from three or four fibres up to about 700 or more in a major trabecula. The shape of the smooth muscle bundles was frequently circular in cross section and other shapes are believed to be due mainly to the sections being oblique or longitudinal or passing through faveolar-trabecular junctions. The diameters of the trabeculae varied from about 20μm to about 200μm including epithelial covering.

2.3.3 Anterior Lung Region

Fig. 2.2 is a low power cross section of the anterior region of the lungs just posterior to the heart (cf. fig. 1.1). The distribution and gradation of faveoli and trabeculae are asymmetrically distributed around the lung. The ratio of surface area to lung volume is much higher here in the anterior region of the lung but is still much lower than that for the more homogeneous mammalian lung. It is in this region that the lateral aspects of

Fig, 2.3, T.nigrolutea, specimen TA41, 138mm snout-vent length, transverse section of lung, anterior region, magnification x 100. P = primary septum. S = secondary septum, T = tertiary septum, BV = blood vessel, F = faveolus, LC = lymph channel, shaded areas = smooth muscle trabeculae.

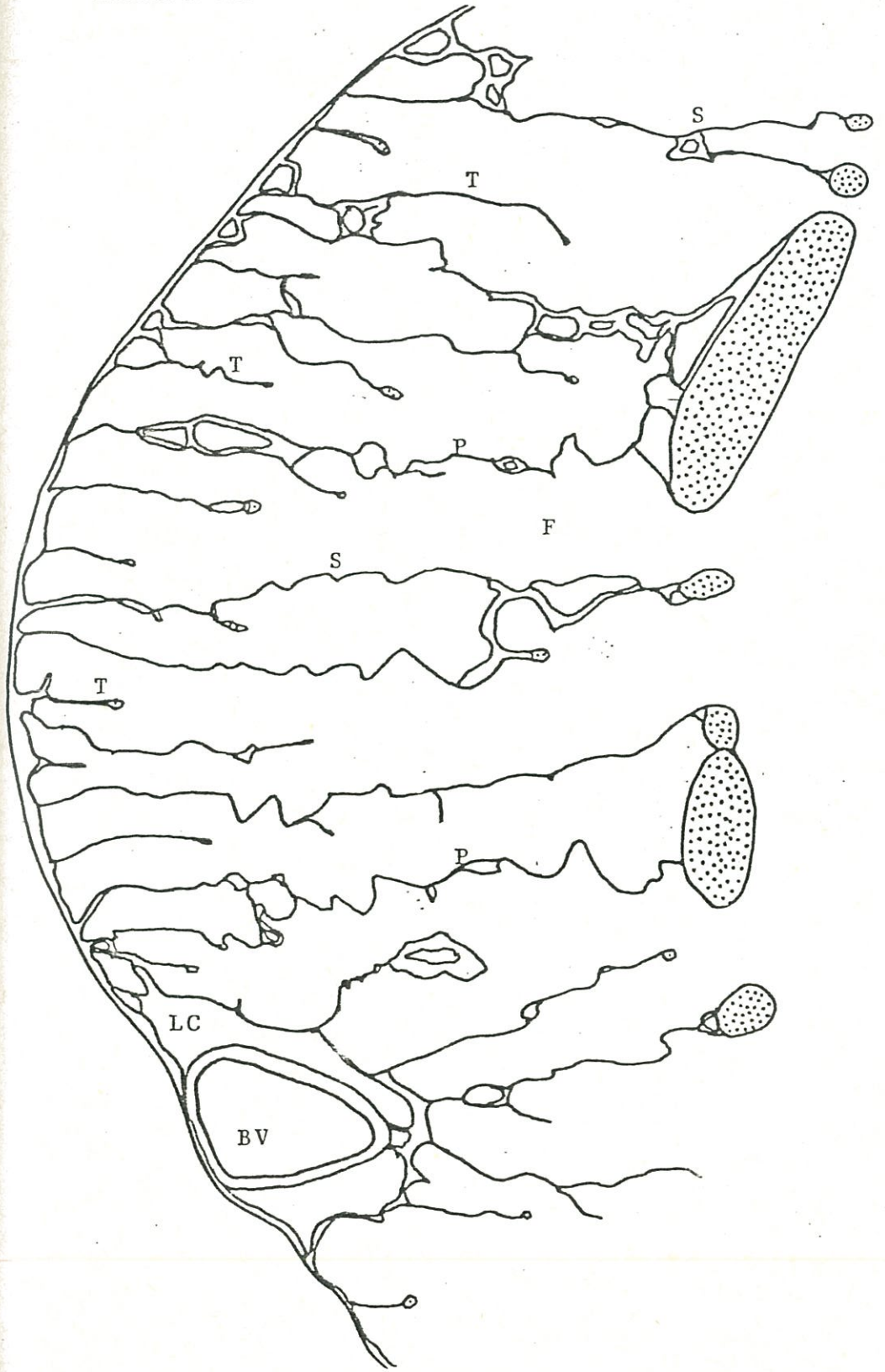
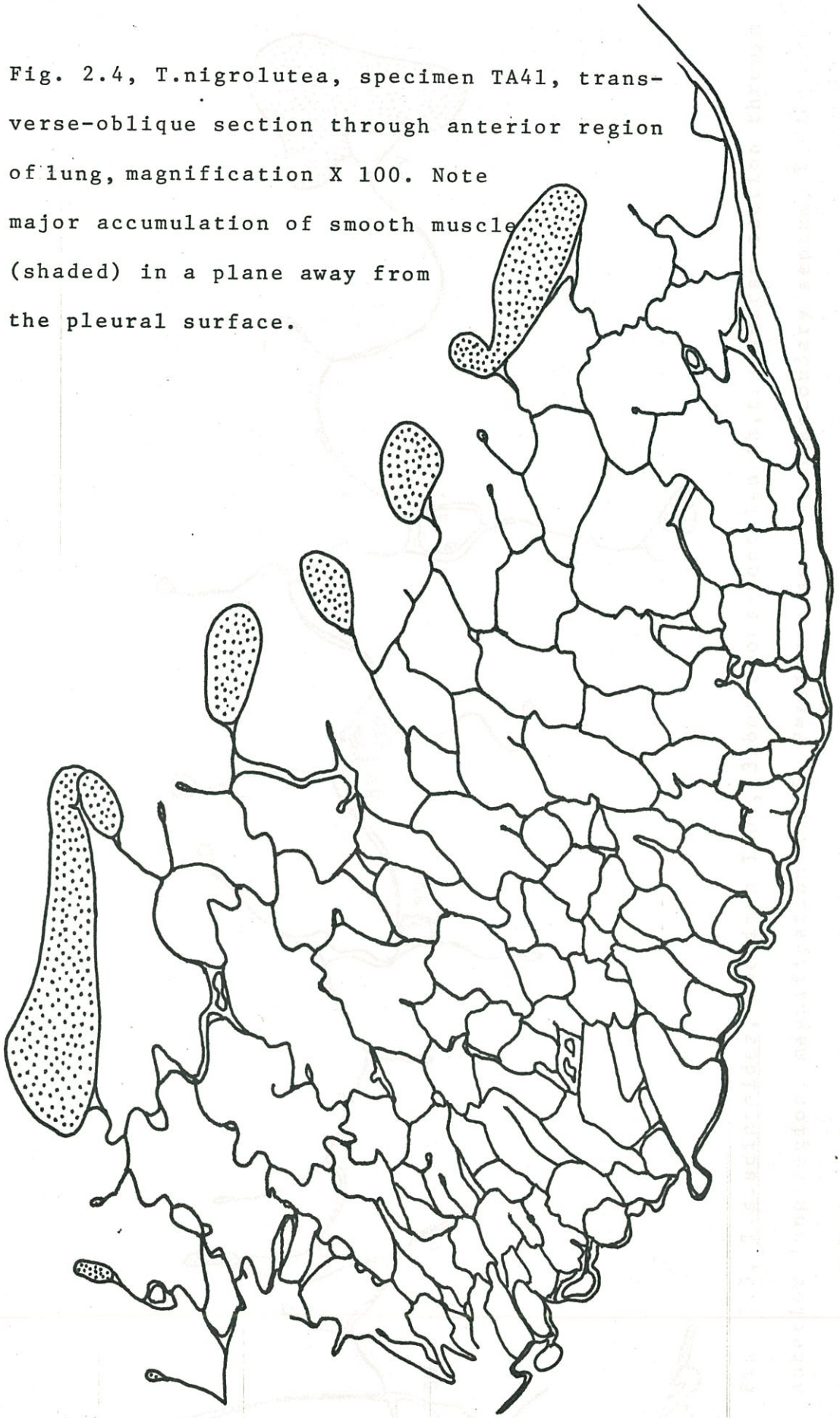


Fig. 2.4, T.nigrolutea, specimen TA41, transverse-oblique section through anterior region of lung, magnification X 100. Note major accumulation of smooth muscle (shaded) in a plane away from the pleural surface.



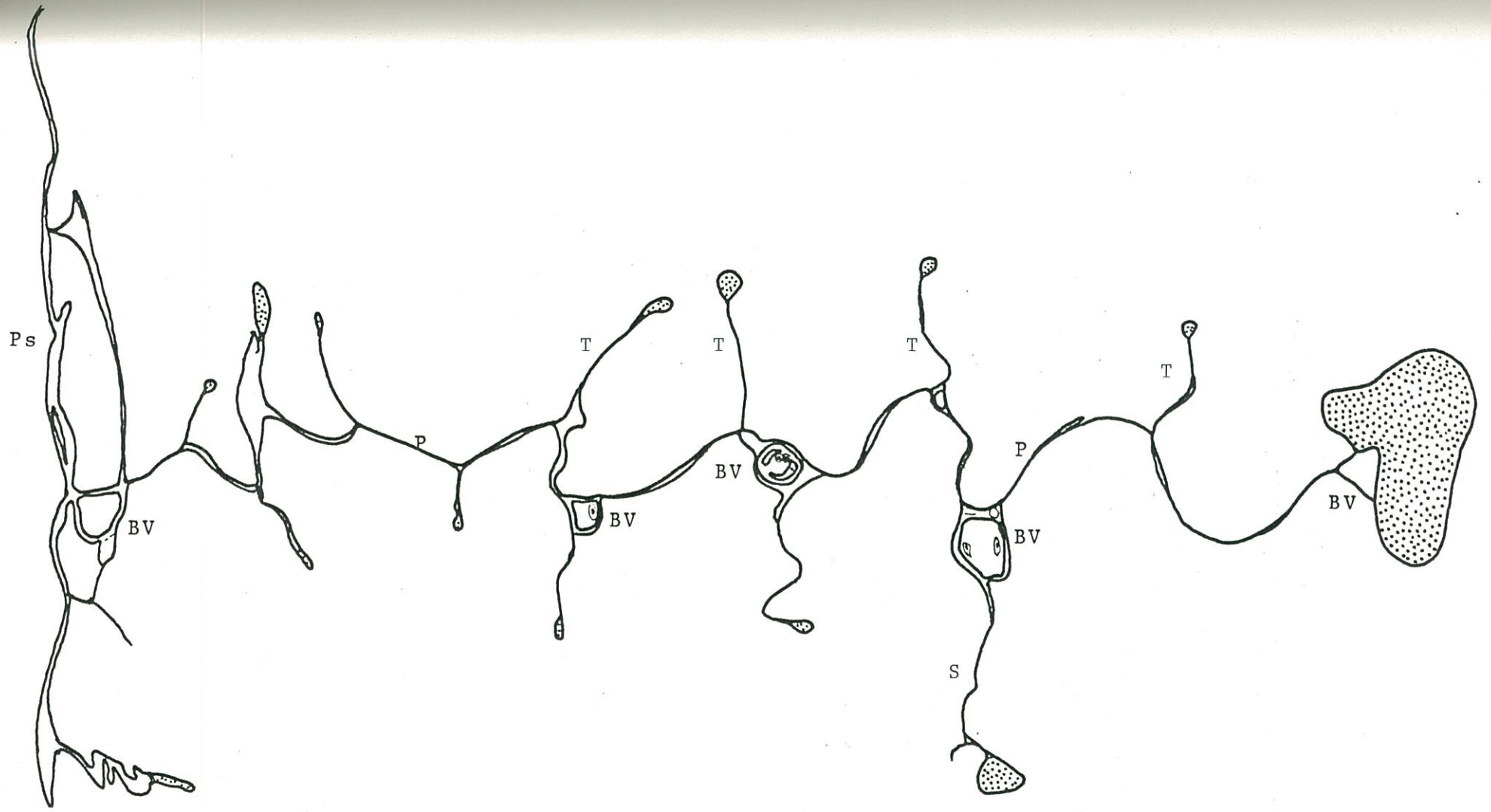


Fig. 2.5, T.s.scincoides, specimen TA04, 318mm snout-vent length, transverse section through anterior lung region, magnification X 125. P=primary septum, S=secondary septum, T = tertiary septum. Ps=pleural surface of lung, shaded areas are smooth muscle bundles in primary, secondary and tertiary trabeculae. Several blood vessels (BV), some containing blood cells are shown.

the lungs will experience the greatest amount of movement during respiratory excursions of the overlying body wall. The outline of the lungs is somewhat distorted by underinflation during fixation and embedding. Most faveoli have radial axes, i.e. they are perpendicular to the external surface of the lung. Major blood vessels can be seen close to the external surface of the lung. There are no major longitudinal muscle strands as found in some other unicameral lungs (Klemm et al. 1979). Fig. 2.3 shows a section running parallel to the long axes of the faveoli. Considerable variation is shown in dimensions of trabeculae (shaded) which consist almost entirely of smooth muscle fibres enveloped in capillaries and cilated epithelium. The large blood vessel (BV) is the lateral branch of the pulmonary artery, it is partly surrounded by large lymphatic channels (Ottaviani and Tazzi 1976) (Cf. fig. 2.6). All the septa appear more or less uniformly thin. Apparently thickened regions are mainly due to the presence of blood vessels or oblique/ tangential sectioning of septa. Scattered smooth muscle fibres and small smooth muscle bundles were occasionally seen in the faveolar parenchyma. Other cells varying in appearance from the types I and II pneumonocytes and ciliated epithelial cells were seen although their significance is unknown, Jeffery and Reid 1975, distinguish ten air passage cell types in the rat and it is quite possible a similar number exist in reptiles.

Fig. 2.4, an oblique section through numerous faveoli, shows an extensive, but unstretched, surface area. The transverse faveolar diameters appear to decrease towards the lung surface due to subdivision mainly by tertiary septa. Fig. 2.5 shows a single unstretched primary septum extending from the external pulmonary membrane to a primary trabecula, it is associated with one secondary and eleven tertiary septa. Blood and lymphatic vessels

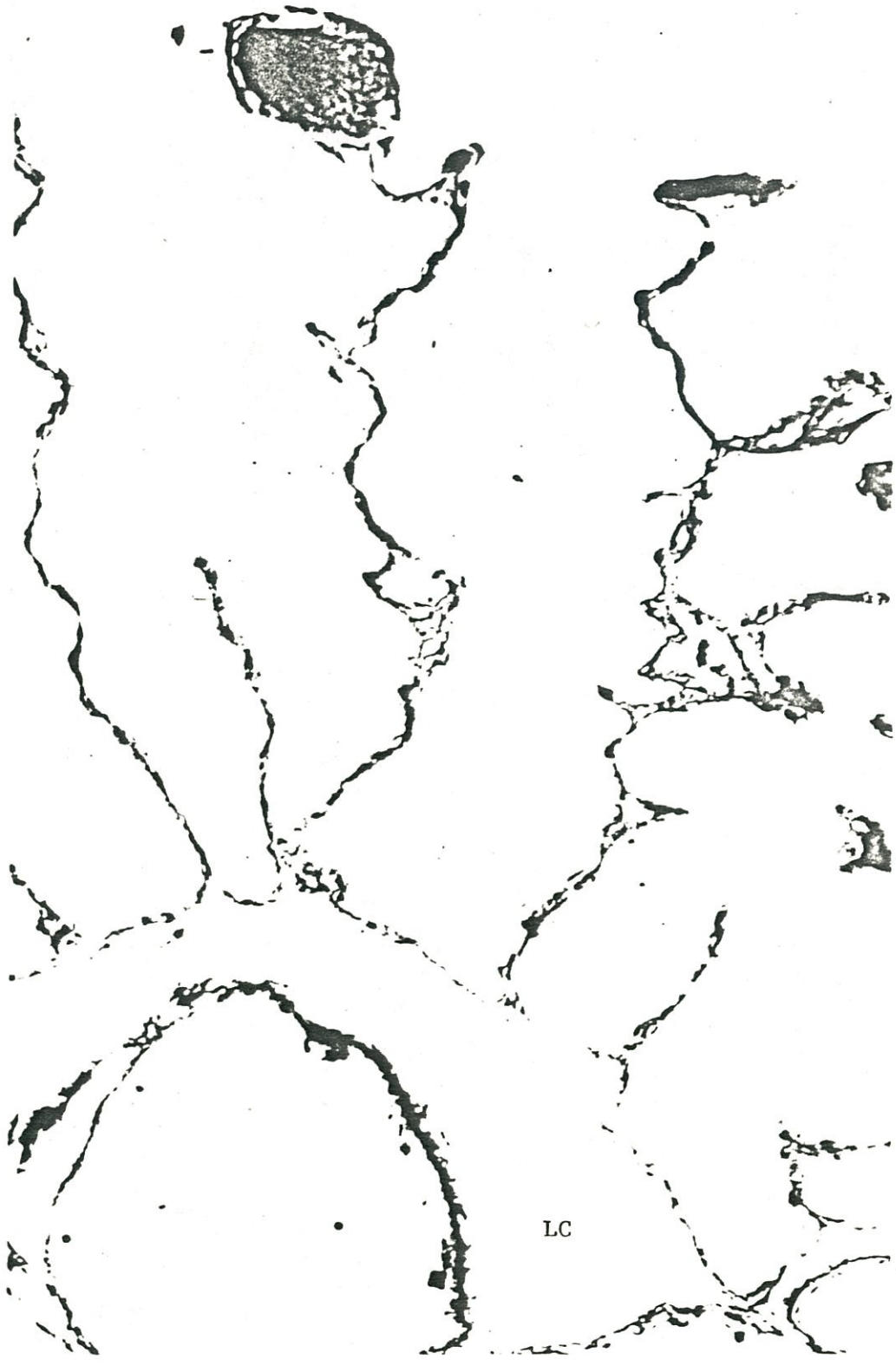


Fig. 2.6. Transverse section from the anterior lung region of a juvenile T. nigrolutea. Magnification x 300, stain picro-mallory. The large dorsolateral branch of the pulmonary vein (below) is partly surrounded by a large lymphatic channel (LC) and several septa (primary, secondary and tertiary) are attached to its wall.

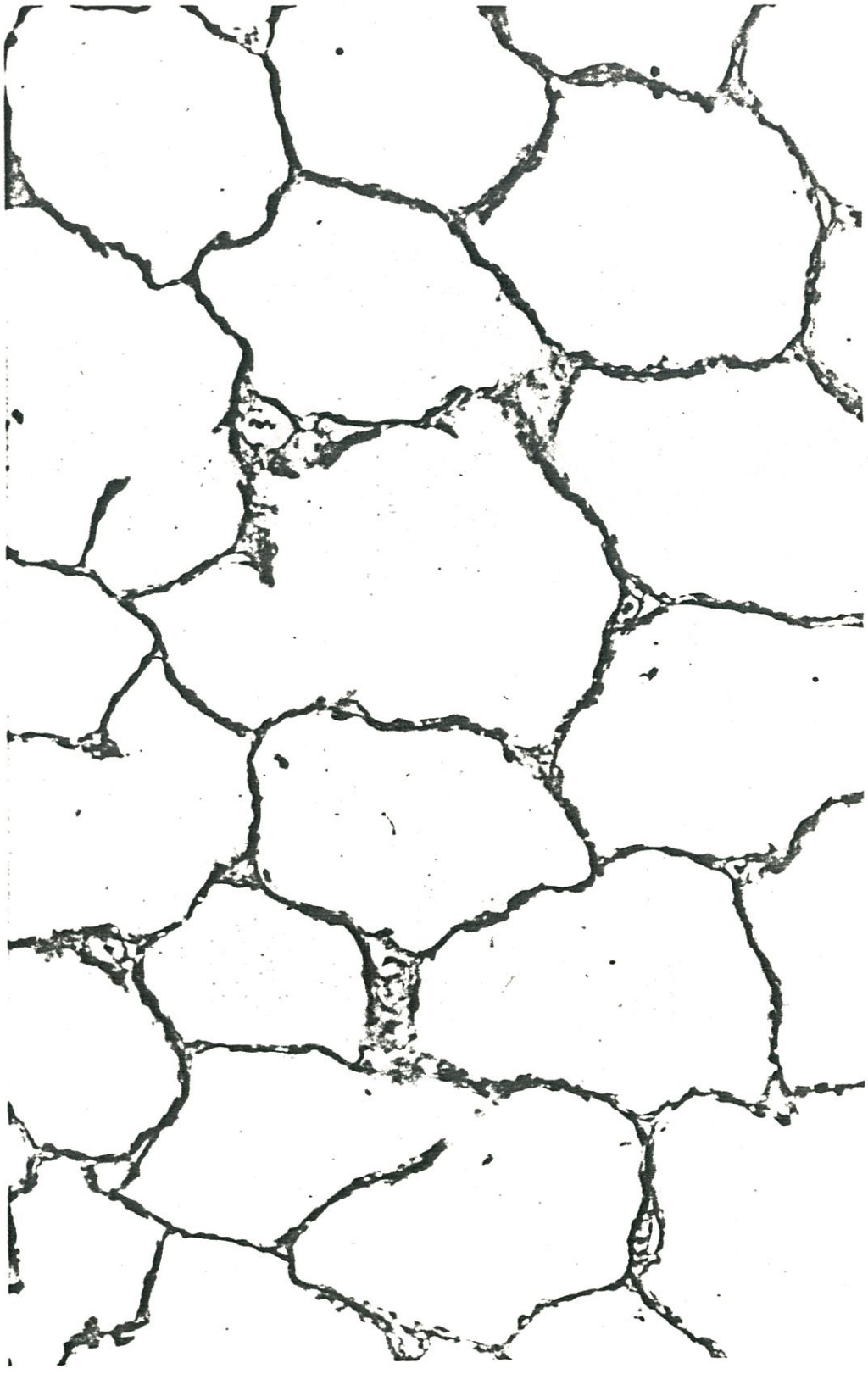


Fig. 2.7. Tangential section from the anterior lung region of a juvenile T. nigrolutea. Magnification x 200, stain gomori reticulin. The irregular shape and size of the faveoli is shown, also the radially directed junctional blood vessels which run between the trabecular networks and the major blood vessels on the periphery of the lung.

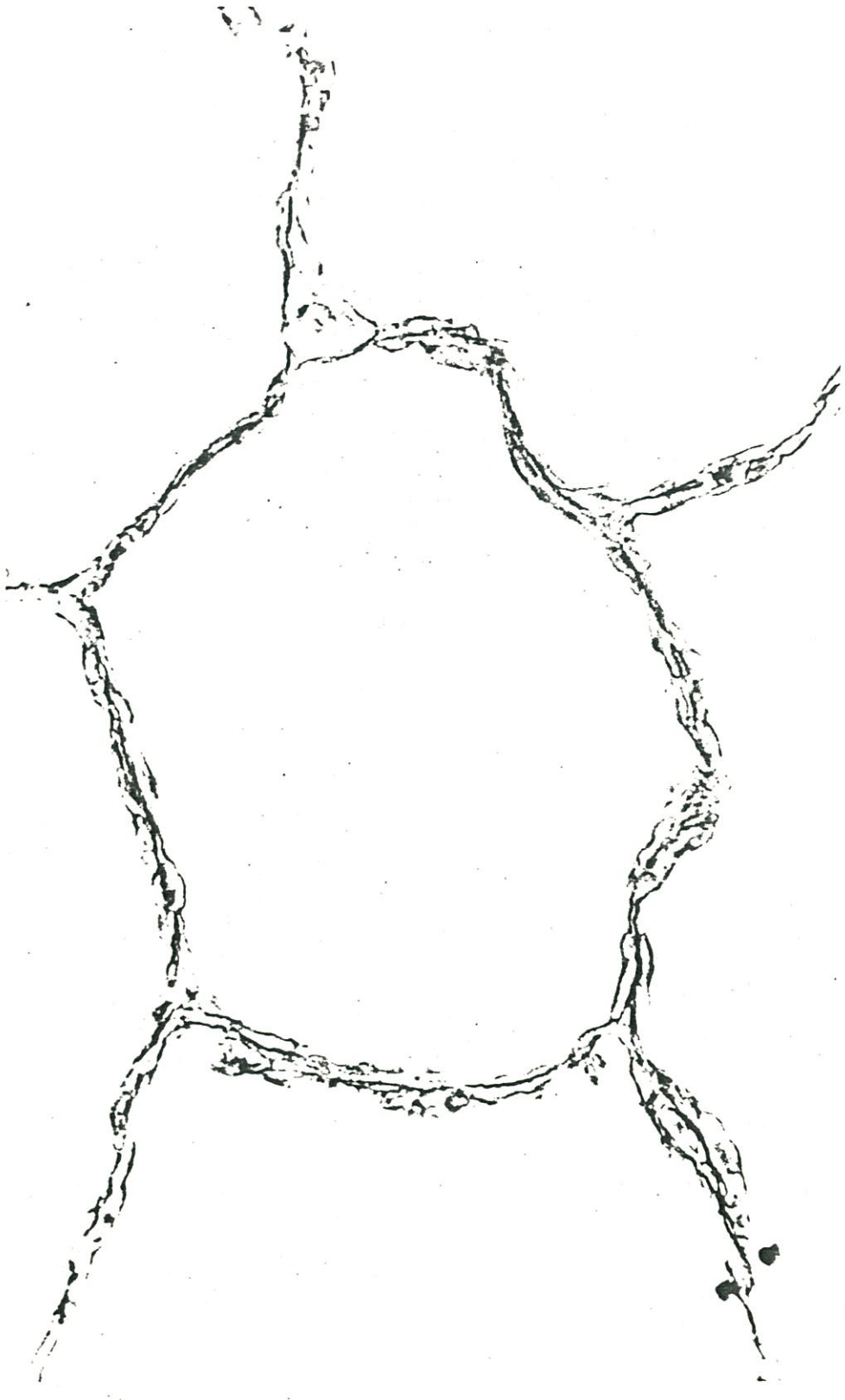


Fig. 2.8. Tangential section through a faveolus from the anterior lung region of a juvenile T. nigrolutea magnification x 530, stain gomori reticulin. The septa between adjacent faveoli consists mainly of two layers of simple squamous epithelium with numerous subjacent capillaries and some connective tissue fibres.

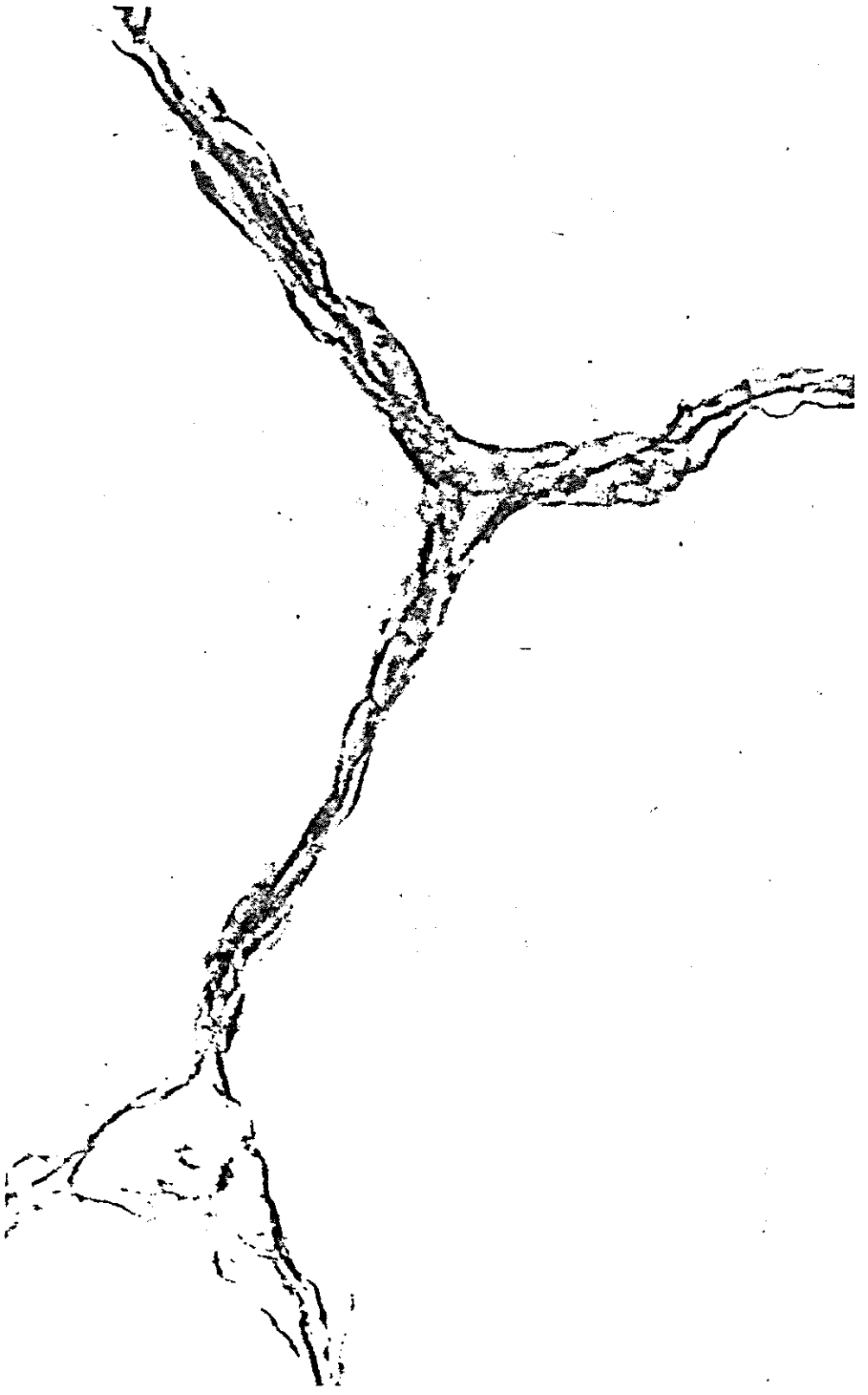


Fig. 2.9. Detail from fig. 2.8. showing a section of a septal wall. Magnification x 1,000. Several capillaries and also a larger junctional blood vessel (lower) containing several blood cells are shown.

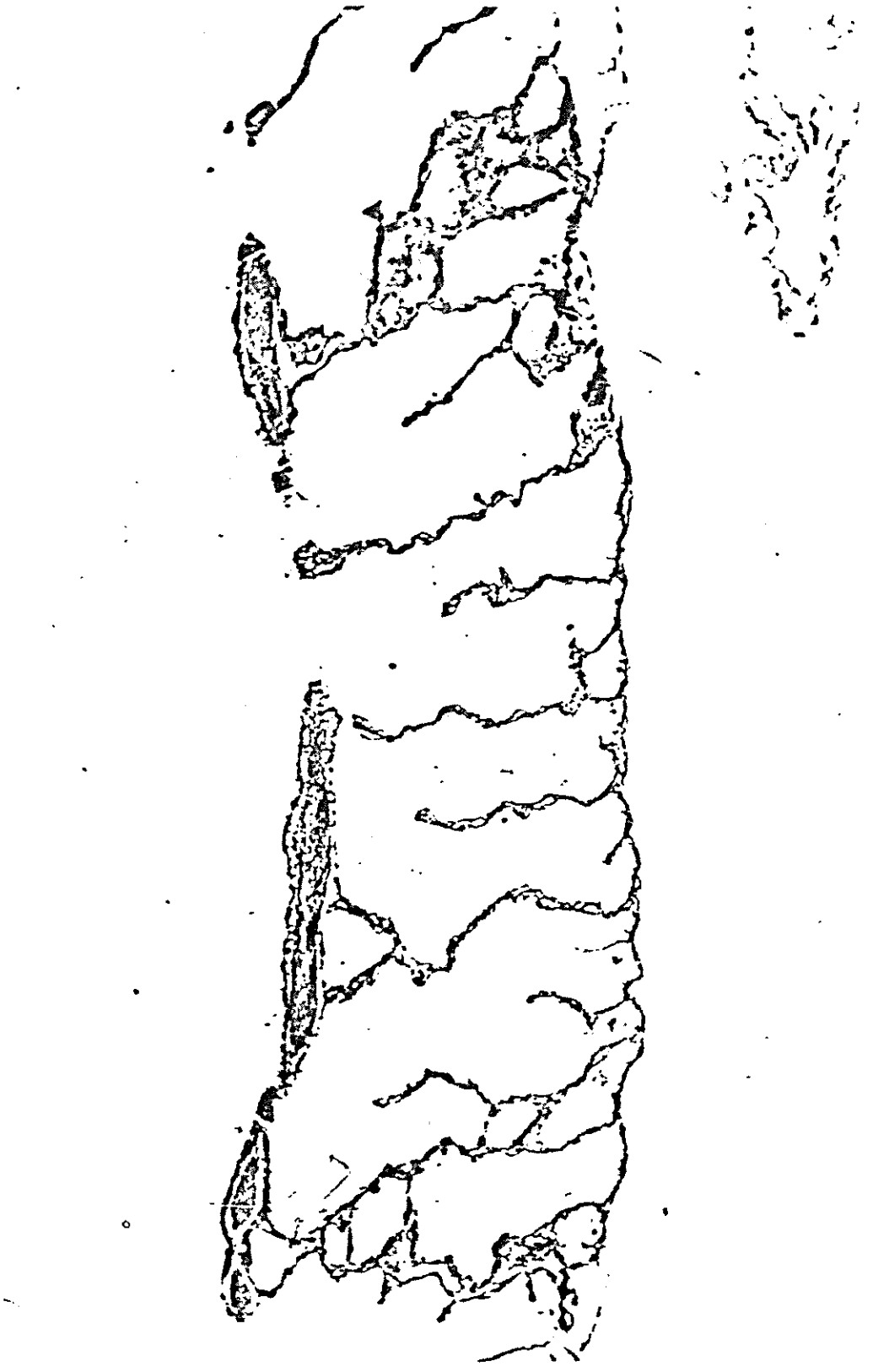


Fig. 2.10. Longitudinal section from the mid-lung region of a juvenile T. nigrolutea. Magnification x 130, stain gomori reticulin. The major trabecular smooth muscle bands (here cut mainly in longitudinal section) are adjacent to the main lumen of the lung. The external pulmonary membrane (towards the right), as elsewhere in the lung, is very thin.

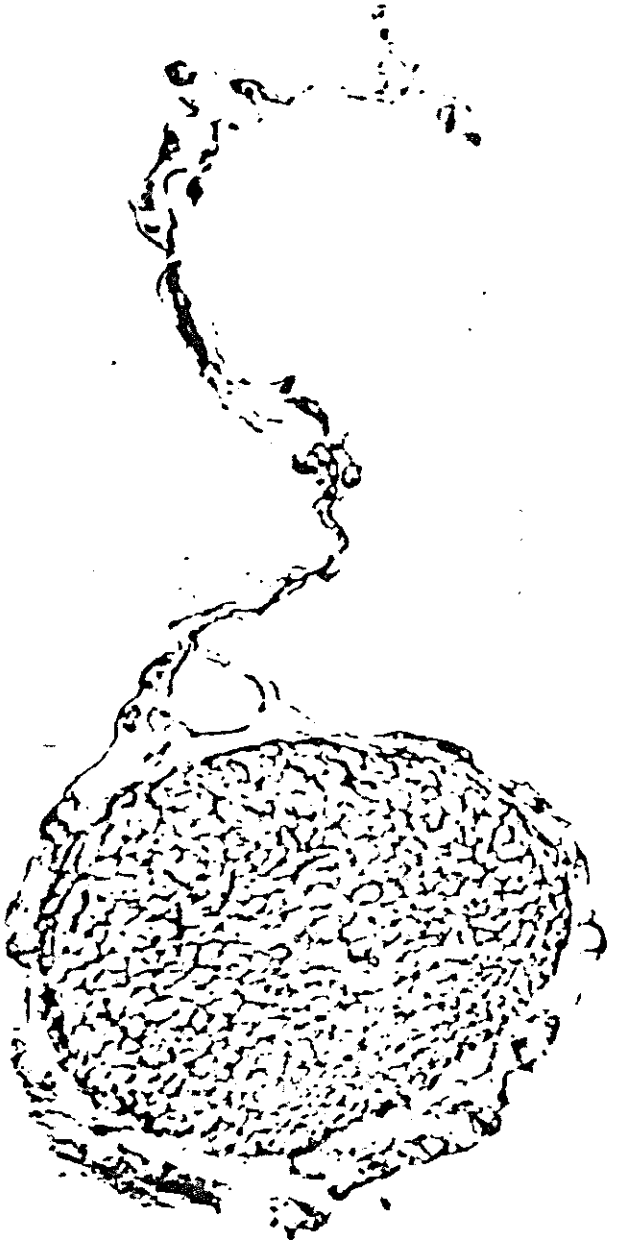


Fig. 2.11. Section through a major trabecula from the mid-lung region of a juvenile T. nigrolutea. Magnification x 600, stain gomori reticulin. The smooth muscle fibres form a discrete bundle surrounded by capillaries and various pneumonocytes. The septum is relatively thin except where other structures are present. The subtrabecular blood vessel is shown just above the smooth muscle bundle.

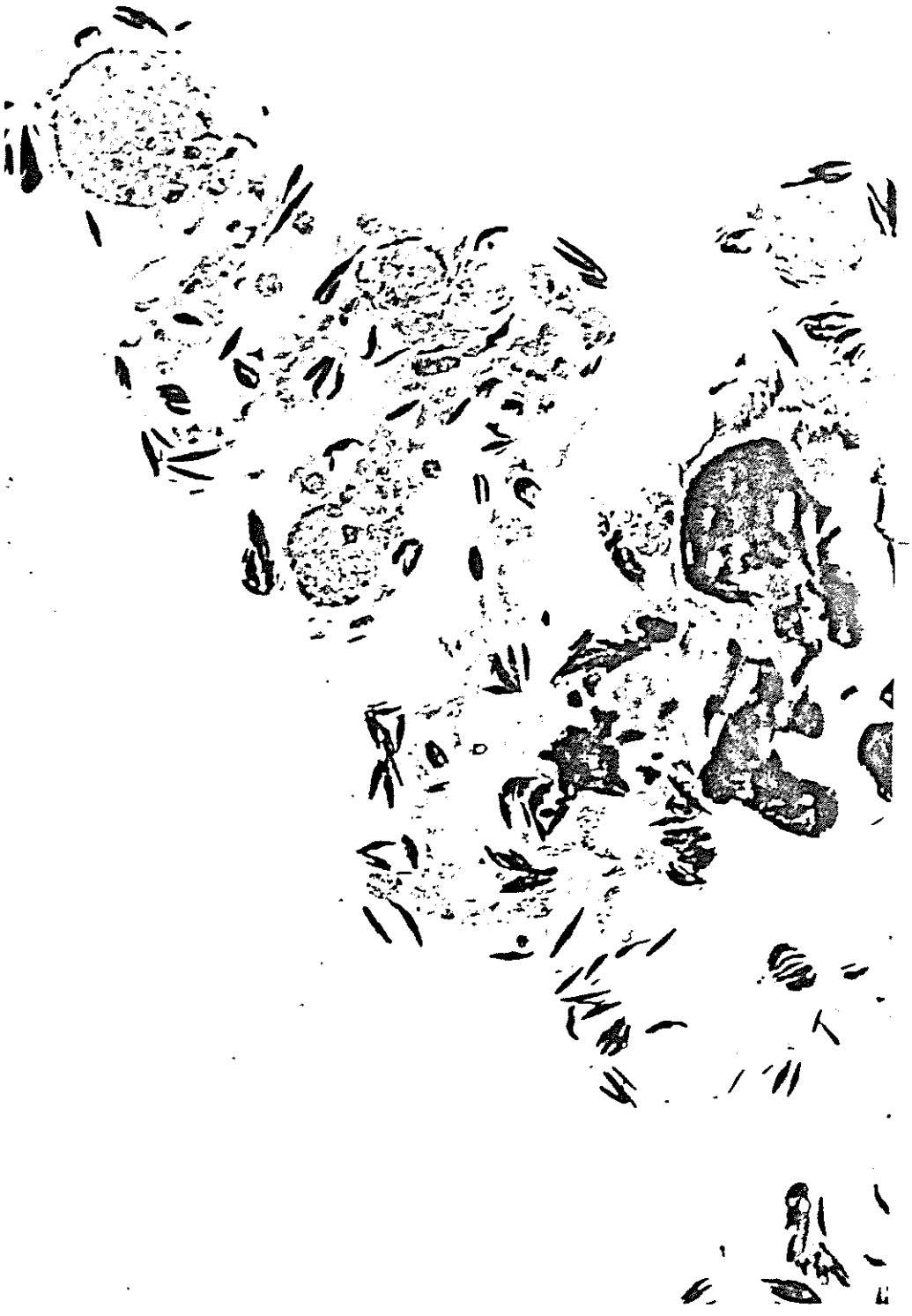


Fig. 2.12. Section from the mid-lung region of an adult T. s. scincoides. Magnification x 540, stain toluidine. A relaxed (folded) septum associated with several smooth muscle trabeculae is shown. Numerous darkly staining erythrocytes are shown in capillaries and larger blood vessels.



Fig. 2.13. Section from the mid-lung region of a juvenile T. nigrolutea. Magnification x 1,000 stain gomori reticulin. This small septum supports few capillaries, scattered ciliated cells and a minor trabecula containing only about twenty smooth muscle fibres in cross section.



Fig. 2.14. Section from the mid-lung region of a juvenile T. nigrolutea. Magnification x 500, stain haematoxylin and eosin. Part of one septum is shown in tangential section. Numerous capillaries containing densely staining, often lanceolate erythrocytes are shown.



Fig. 2.15. Section from the mid-lung region of a juvenile T. nigrolutea. Magnification x 180, stain haematoxylin and eosin. The septa appear partially collapsed and the trabecular smooth muscle relaxed. The large ventral branch of the pulmonary artery shown at the upper right appears to possess a valve-like structure.

are frequently associated with the junctions of the septa with each other, the larger trabeculae and with the external pulmonary membrane.

Fig. 2.6 illustrates the arrangement of a large lymphatic channel around a large blood vessel (adjacent to the serosa below) also primary, secondary and tertiary trabeculae and septa. Figs. 2.7 - 2.9 show faveoli in cross section (tangential to the lung surface) at various magnifications. The high ratio of air space to tissue volume is shown with little connective tissue and irregularly scattered capillaries between the epithelial layers.

2.3.4 Mid-lung Region

Compared to the anterior lung region the faveoli in the mid-lung region tend to be shallower, broader and have less subdivisions with relatively fewer secondary and tertiary septa and trabeculae. Fig. 2.10 is a fairly typical section illustrating these points. Fig. 2.11 is a higher power section of a typical (secondary) trabecula consisting mainly of smooth muscle fibres and surrounded by types I and II pneumonocytes and some ciliated cells. Numerous capillaries in the septum and beneath the epithelial cells are seen. The larger subtrabecular blood vessel is a common occurrence, some of the spaces around this vessel are probably lymphatic. Fig. 2.12, stained to show erythrocytes demonstrates the extensive capillary network in this area. Fig. 2.13 shows a typical tertiary septum and trabecula. Fig. 2.14 illustrates the appearance of part of a septum in tangential section, capillaries, erythrocytes and other cell types are shown. Fig. 2.15 shows lung tissue in a relaxed state with faveoli partially collapsed. It is possible that this is partly an artefact of preparation although the trabeculae do appear to be extended. The possible presence of a vascular valve mechanism

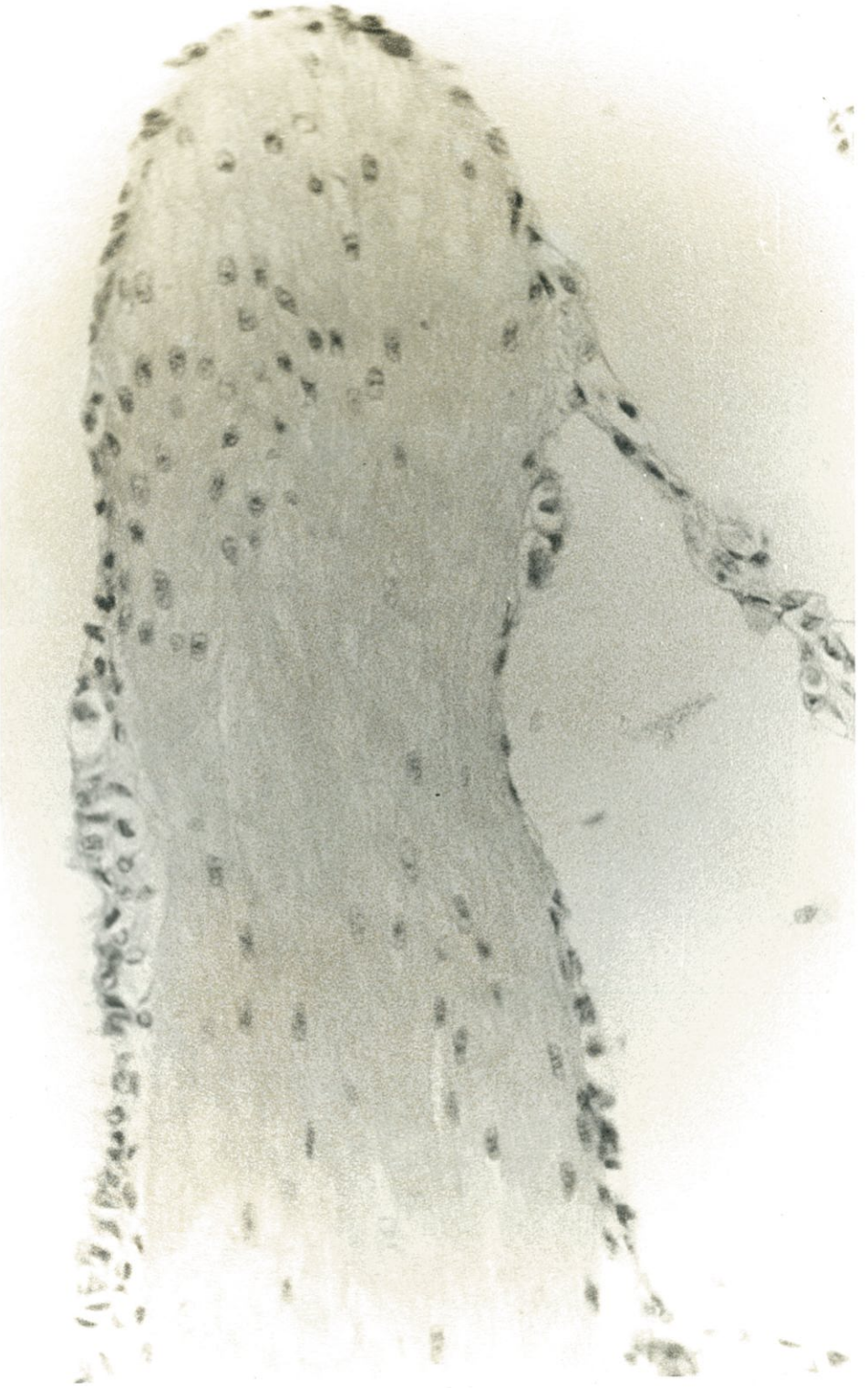


Fig. 2.16. An oblique longitudinal section through a primary trabecula from a juvenile T. nigrolutea. Magnification x 600, stain haematoxylin and eosin. The trabecula consists almost entirely of smooth muscle fibres enveloped by a variety of epithelial cells including types I and II pneumonocytes and ciliated cells. The luminal border is on the left and a septum is attached to the outer border on the right.



Fig. 2.17. Longitudinal section from the posterior lung region of a juvenile T. nigrolutea. Magnification x 160, stain picro-mallory. Primary, secondary and tertiary trabeculae and septa are seen, also the junction between a primary and tertiary trabecula (centre).

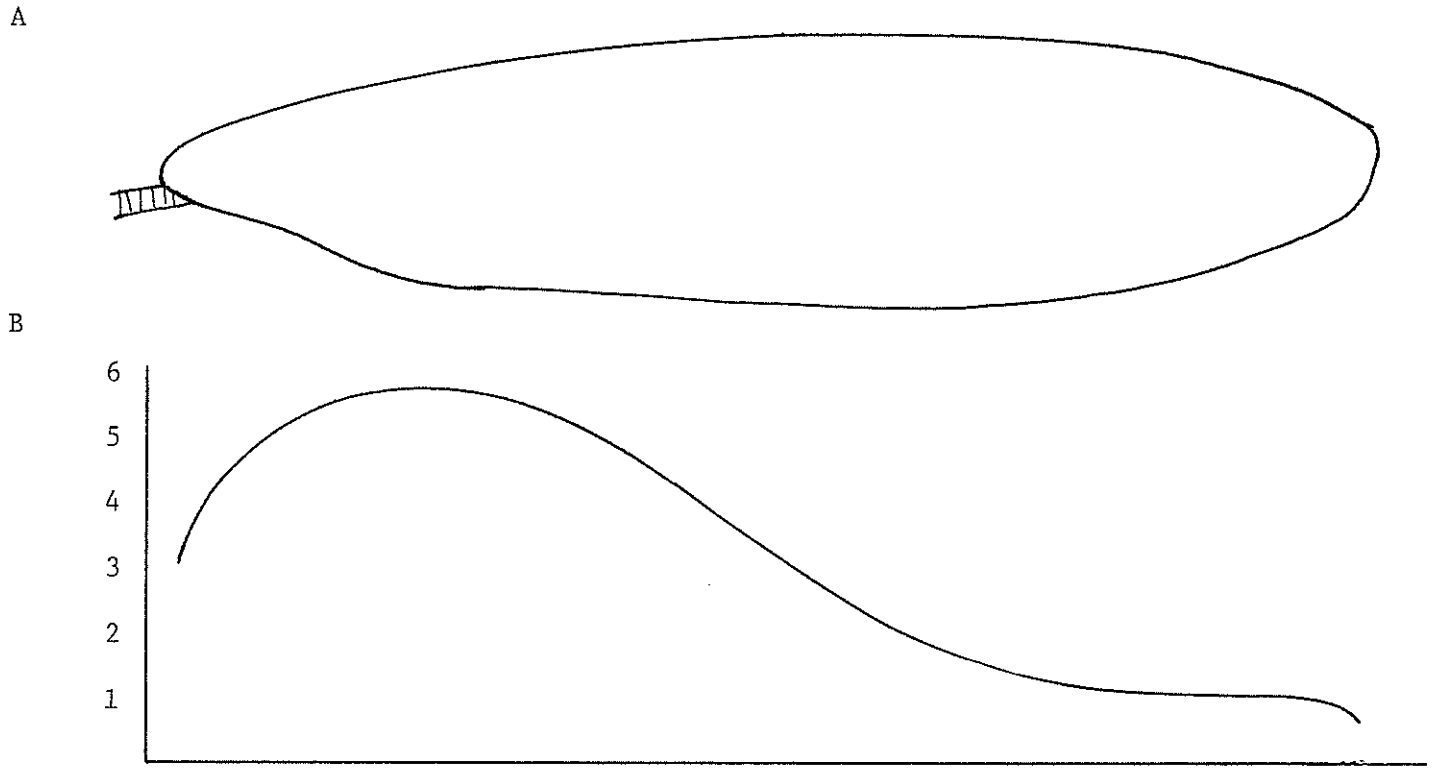


Fig. 2.18. A. Lateral view of lizard lung.

B. Schematic representation of relative amounts of surface area available along the length of the lung, (arbitrary units).

is also shown. Fig. 2.16 shows several epithelial cell types covering a primary trabecula shown partly in longitudinal section. Numerous capillaries are seen beneath type II pneumonocytes and ciliated cells on the left.

2.3.5 Posterior Lung Region

In this region the faveoli tend to be verybroad and shallow, the low septa have few branches (fig. 2.17). The trabeculae are similar to those found elsewhere in the lung although fewer blood vessels of all sizes are seen.

2.3.6 Histological Lung Measurements

Measurements taken from histological sections showed that the faveolar area exposed to the pulmonary air space was approximately six times higher in the anterior lung region than in the posterior lung region, fig. 2.18. This factor could be doubled if the capillary surface area rather than entire faveolar surface area was considered.

2.4 Discussion

Because of the temporal variations in lung volumes and septal configurations it is difficult to make estimates of lung surface area with any degree of certainty. The lungs of two juvenile T. nigrolutea were fixed and wax embedded in a relaxed position then sectioned transversely or longitudinally, mounted, stained and projected to facilitate measurement of lung surface area.

A transverse section in the anterior lung region showed 256 septa (primary, secondary and tertiary). These were measured to the nearest 0.04 mm and summed giving a total internal perimeter of 218 mm including surfaces of trabeculae and bases of faveoli. The septal surface available in the posterior lung region was found to be only one sixth of that found in the anterior lung region. From these measurements the total area of two, lengths 62 mm and 47 mm, were calculated to be 12,400 mm^2 and 9,200 mm^2 respectively, (See Appendix II, p. 231 for method of calculation). The total area of 21,600 mm^2 for a 52 g lizard is considerably above the reptile lung surface area predicted by Tenney and Tenney 1979, who found that the lung surface area in a range of adult reptiles (no scincids) was proportional to body weight (BW) in the ratio of $BW^{0.75}$, this gives an expected surface area for a 52 g lizard of about 350 mm^2. This discrepancy is difficult to explain wholly on the basis of differences in technique or possible factors such as age of lizard.

The histological appearances of the lungs of the two species examined were (as expected) very similar. Slight variations such as the increased ratio of secondary to primary septa in T. s. scincoides could not be held to be significant because of the low number of specimens examined, (n = 6).

The method of preparation of the lungs for histological examination is very important if meaningful morphometric data are to be obtained. Future studies using more closely controlled inflation fixing techniques, e.g. formalin/steam method, (Weibel

and Vidone 1961) also control of contraction/relaxation state of pulmonary smooth muscle during fixation would facilitate the determination of more precise morphometric data.

In summary it would seem that the anterior part of the lung is functionally efficient in terms of perfusion, as judged by the high capillary density, and ventilation as judged by body wall movements which are greatest in the anterior region of the lung. The fact that slight body wall movement does occur along the whole length of the lung during quiet breathing and the fact that an appreciable amount of blood capillaries, although at a reduced density, are found in the posterior part of the lungs would indicate that this region is much less significant as a region of gas exchange, particularly in quiet respiration. The mechanism by which gas is moved around in the lung and particularly in the deeper faveoli is uncertain although the relatively large volume of smooth muscle present forming a complex reticulation is almost certainly the major key to this problem.

The valve like arrangement seen in fig. 2.17 is interesting in that it may indicate an intrapulmonary mechanism of local perfusion control for ventilation/perfusion matching. For example, when the tidal volume is increased the gas exchange to the posterior part of the lung would be increased and it would then become advantageous to increase the blood supply to this area in order to obtain optimum gas exchange for work expended in respiratory effort. The reverse situation where the tidal volume is decreased, e.g. during brumation, is probably just as significant in that respiratory effort, tidal volume and circulation are all greatly

attenuated so that the perfusion of less vital tissues can be controlled giving preference to vital organs.

The manner in which the primary septa are attached to the major lymphatic channels (fig. 2.6) suggests a method of facilitating the flow of lymph. Contraction of major trabecula (fig. 2.5) could well cause compression of the small septal lymphatic vessels thus increasing rate of flow into the major vessels. Tension on the septa generated by contraction of the major circularly arranged trabeculae (which are perpendicular to the longitudinal orientation of most of the major blood and lymph vessels) would decrease pressure in the major lymphatic channels thus assisting lymph flow. The shape and loose attachments of the inner walls (i.e. towards the lung lumen) would appear to allow considerable distenstion to accommodate large variations in volume and flow. Similarly these effects may also influence blood flow in the capillaries and perhaps to a lesser extent, in the larger blood vessels. The fact that these small vessels almost invariably occur at septal junctions is evidence to support this hypothesis.

Section III Ultrastructure of the Blue-Tongued Lizard Lung

3.1 Introduction and Objectives

In contrast to the rapidly increasing volume of literature relating to the ultrastructure of mammalian respiratory systems there remains a considerable paucity of such material in the area of non-mammalian and particularly of reptilian vertebrates. Wetzstein, Stratton and Olson (1980) have given a brief introduction to the subject although this is a comparative study related primarily to surfactant systems. Several other papers, e.g. Perry 1976, Duncker 1978, Perry and Duncker 1978, Meban 1980, also make useful contributions to the subject.

The major objectives of the present study were initially as follows:

1) To confirm the macroscopic and histological findings.
2) To determine the general ultrastructural characteristics of the blue-tongued lizard lung.
3) To determine the major cell types present.
4) To determine the nature and effective thickness of the air-blood barrier.
5) To estimate total respiratory area.
6) To observe variations of ultrastructure in relaxed and contracted lungs.
7) To improve understanding of the structure-function relationships of scincid respiration.
8) To facilitate the development of hypotheses regarding the mechanisms of scincid respiration.

The first seven objectives at least were achieved to some extent.

Both scanning (SEM) and transmission (TEM) electron microscopy were used to observe the ultrastructure here described, although the emphasis was on the surface features obtained by SEM.

3.2 Materials and Methods

Four animals, all T. scincoides, were used. Samples of tissue for SEM and TEM were prepared as follows. The animal was euthanized with chloroform or diethyl ether, weighed, measured and the lungs and trachea removed via a midventral incision. Small sections about 3 mm in diameter were taken from the anterior, middle and posterior regions of one lung and placed in aldehyde fixative for later TEM. The rest of the lungs was placed in 3% gluteraldehyde in 0.1M Sorenson's phosphate buffer, pH 7.4. The intact lung was injected with the same solution to produce varying degrees of inflation and the bronchus was tied to maintain shape and volume during the fixation period of about sixteen hours. Small sections about 6 mm in diameter were then removed from the anterior middle and posterior lung regions, washed in cold 0.1 M Sorenson's phosphate buffer in 0.25 M sucrose, pH 7.4, for about eighteen hours. The lung samples were then washed in distilled water for ten minutes followed by dehydration to 100% ethanol over about 8 hours then replacement of the ethanol in stages to 100% molecular sieved acetone. The tissue samples were then dried in a Polaron critical point drier, mounted on brass stubs using cyanoacrylate adhesive and gold sputtered ready for SEM and photography.

The tissues for TEM were prepared using standard techniques of epoxyresin embedding and double staining with uranyl acetate and lead citrate.



Fig. 3.1, magnification bar = 1.0 mm, luminal surface of posterior region of lung. The smooth muscle is relaxed and the low septa collapsed, very few blood vessels are present here.



Fig. 3.2, magnification bar = 10 μm, from posterior region of lung. Scattered effated and polygonal microvillous cells are shown.

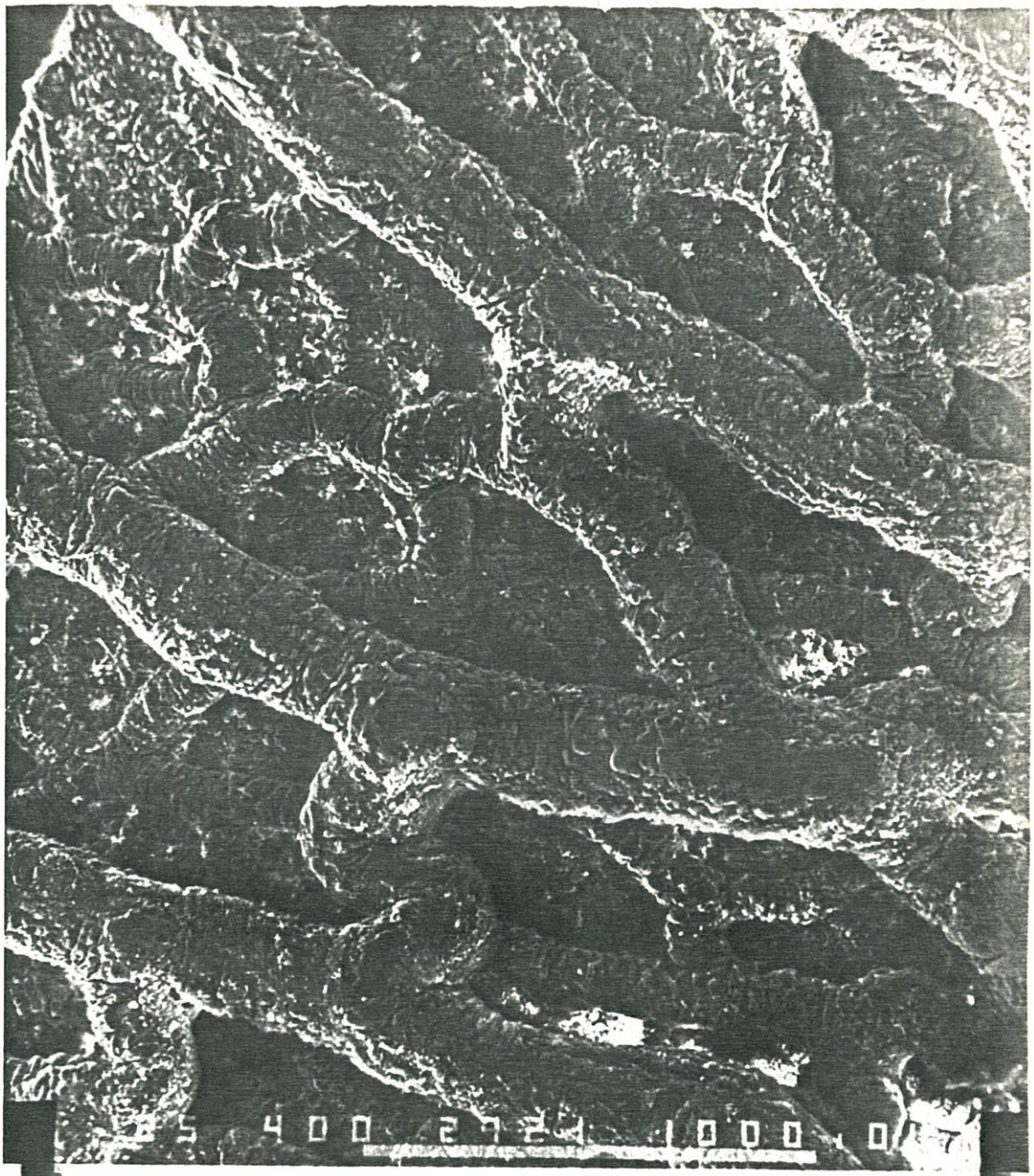


Fig. 3.3, magnification bar = 1.0 mm, from the posterior region of the lung. Here the smooth muscle is contracted giving thick, slightly puckered trabeculae.

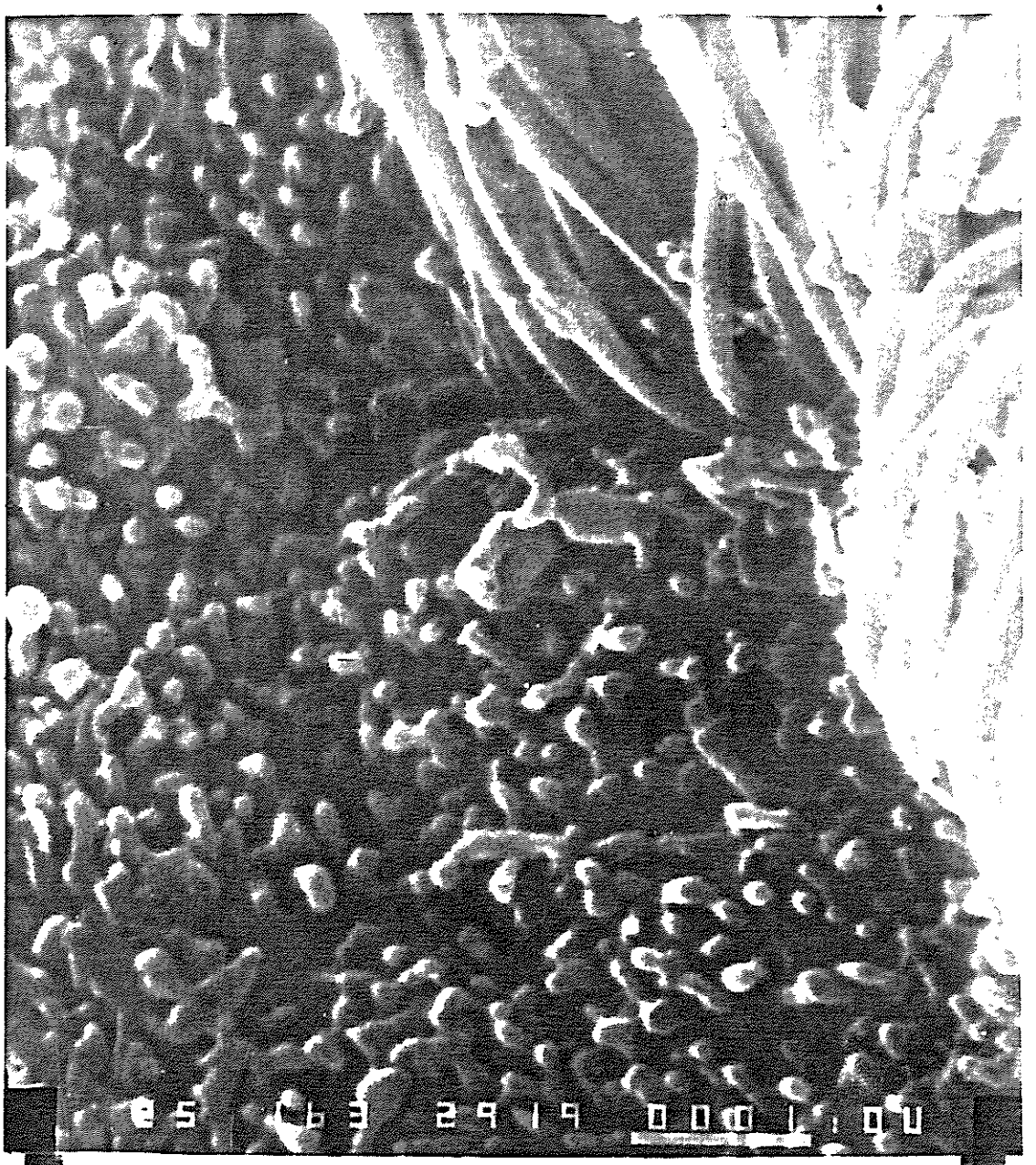


Fig. 3.4, magnification bar = 1.0 μm, from the posterior region of the lung. The margin between ciliated and non-ciliated areas is shown, the microvilli here are long and in some cases form complex structures.



Fig. 3.5, magnification bar = 1.0 μm, from the posterior lung region. This appears to be a secretory cell having several secretory pores surrounded by secretory material.

The electron microscopes used were the JEOL JSM-35 for SEM and the AEI Electronmicroscope 801 for TEM.

3.3. Observations and Results

The posterior region of the lung being of a relatively simple structure will be considered first. The open trabecular network and low septal walls are well shown in fig. 3.1. The few blood vessels that are present tend to follow the septa and are not visible here. The septa are collapsed because the tissue is so tenuous in this area and also because the smooth muscle in the trabeculae is relaxed. At higher magnification, e.g. fig. 3.2 the bases of the faveoli, i.e. the flat areas in fig. 3.1. are shown to consist of mainly squamous (type I) pneumonocytes which usually have numerous low microvilli and ridged intercellular borders. Interspersed among these cells are patches of ciliated cells and frequently isolated ciliated cells most of which also possess numerous microvilli. When the smooth muscle of the trabeculae is contracted quite a different appearance is presented with major and minor trabeculae apparently interconnecting freely and deeper faveoli being formed, (the depth has been minimized somewhat in the preparation and mounting of this specimen). The trabeculae in the posterior lung region are largely covered on their luminal surfaces at least by ciliated epithelium. Fig. 3.4 at high magnification shows the border between a ciliated cell to the right and a type II pneumonocyte showing complex surface features including small openings (probably pinocytotic vesicles) at lower left. These features probably indicate increased metabolic activity although there is no evidence of secretion in this cell. Fig. 3.5 again at high magnification shows what appears to be the opening to a secretory cell on a

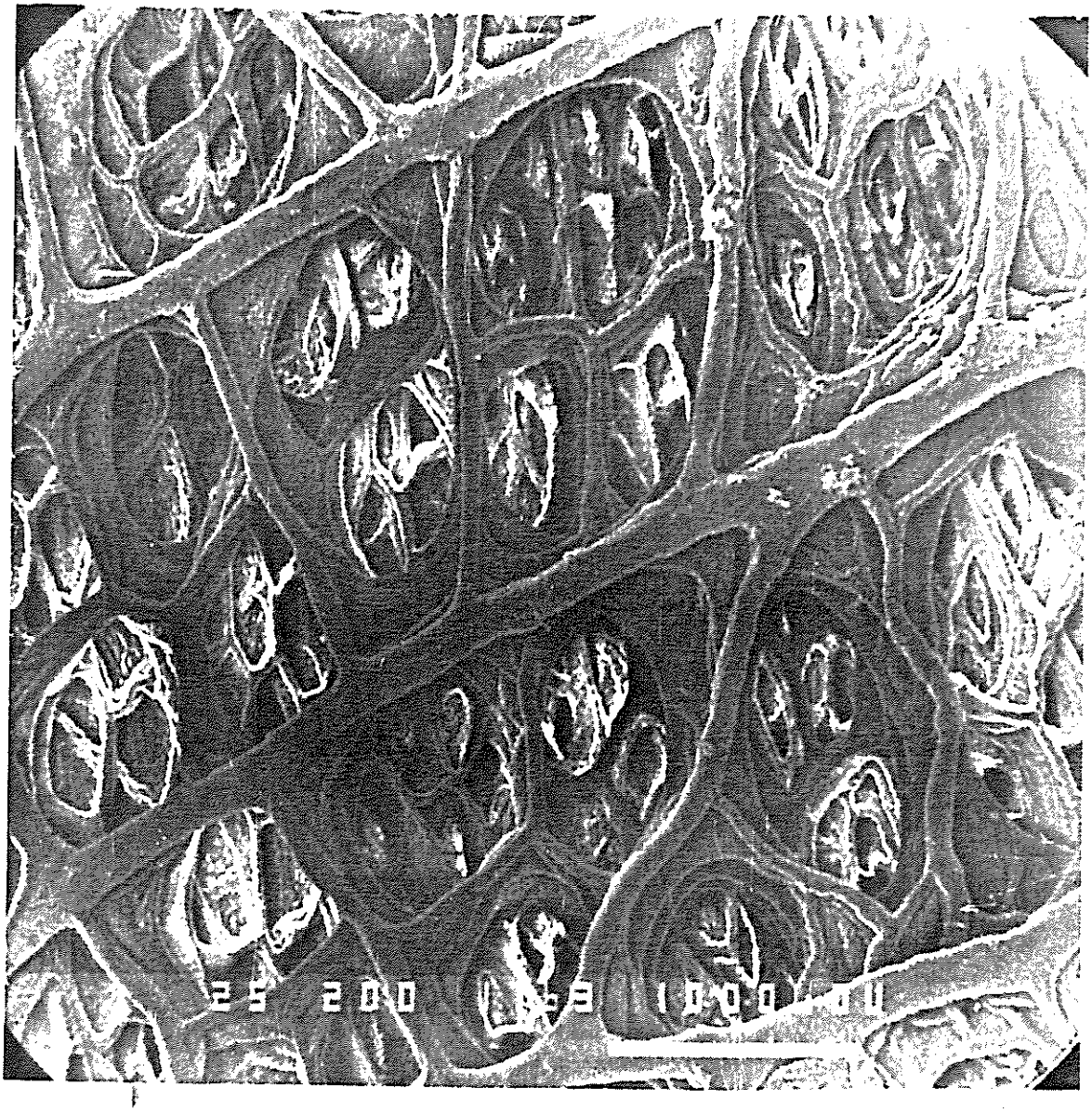


Fig. 3.6, magnification bar = 1.0 mm, from the mid-lung region. All trabeculae appear almost equally relaxed, several orders of subdivision of the faveoli are seen.

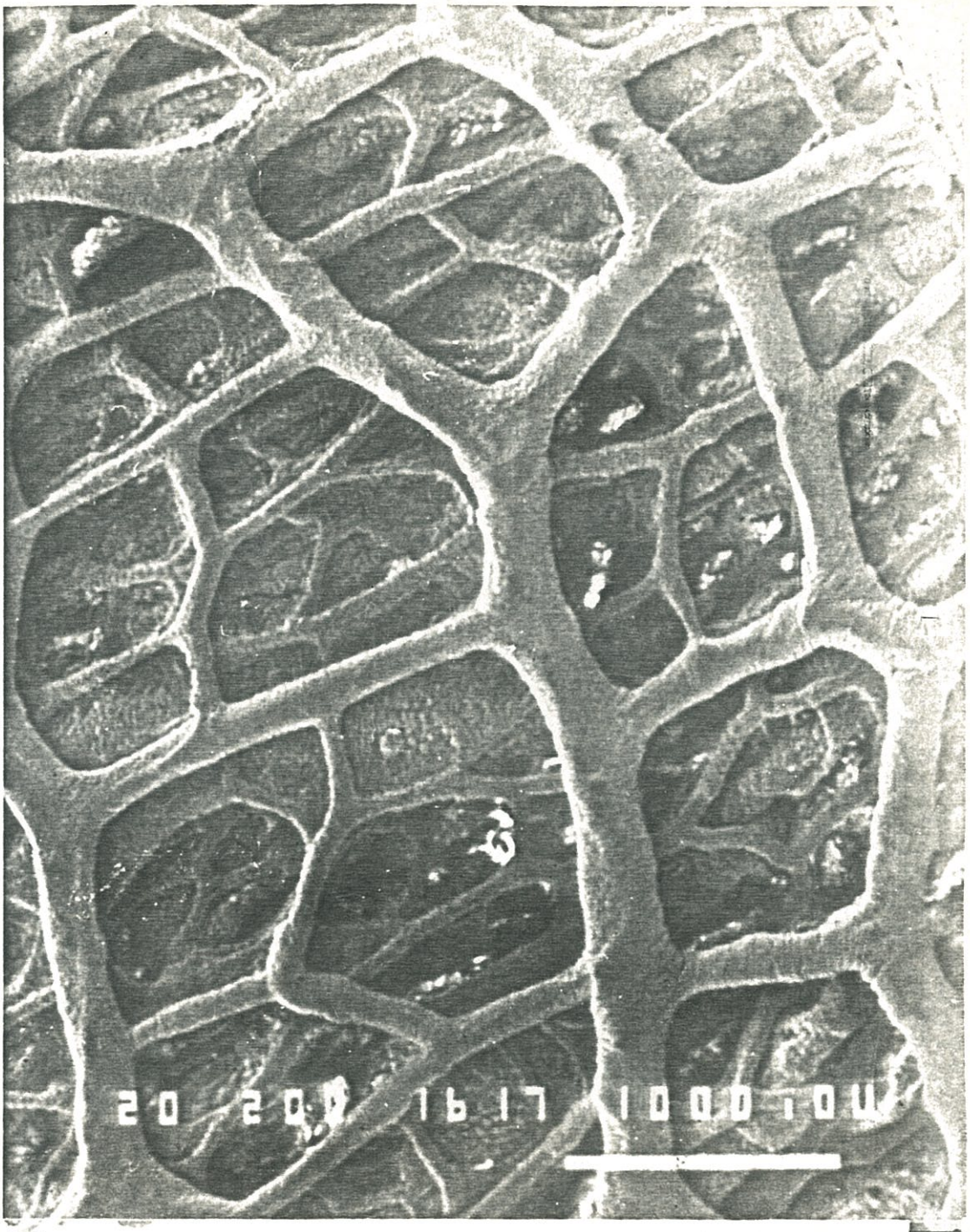


Fig. 3.7, magnification bar = 1.0 mm, from the mid-lung region. All trabeculae appear at least partially contracted.

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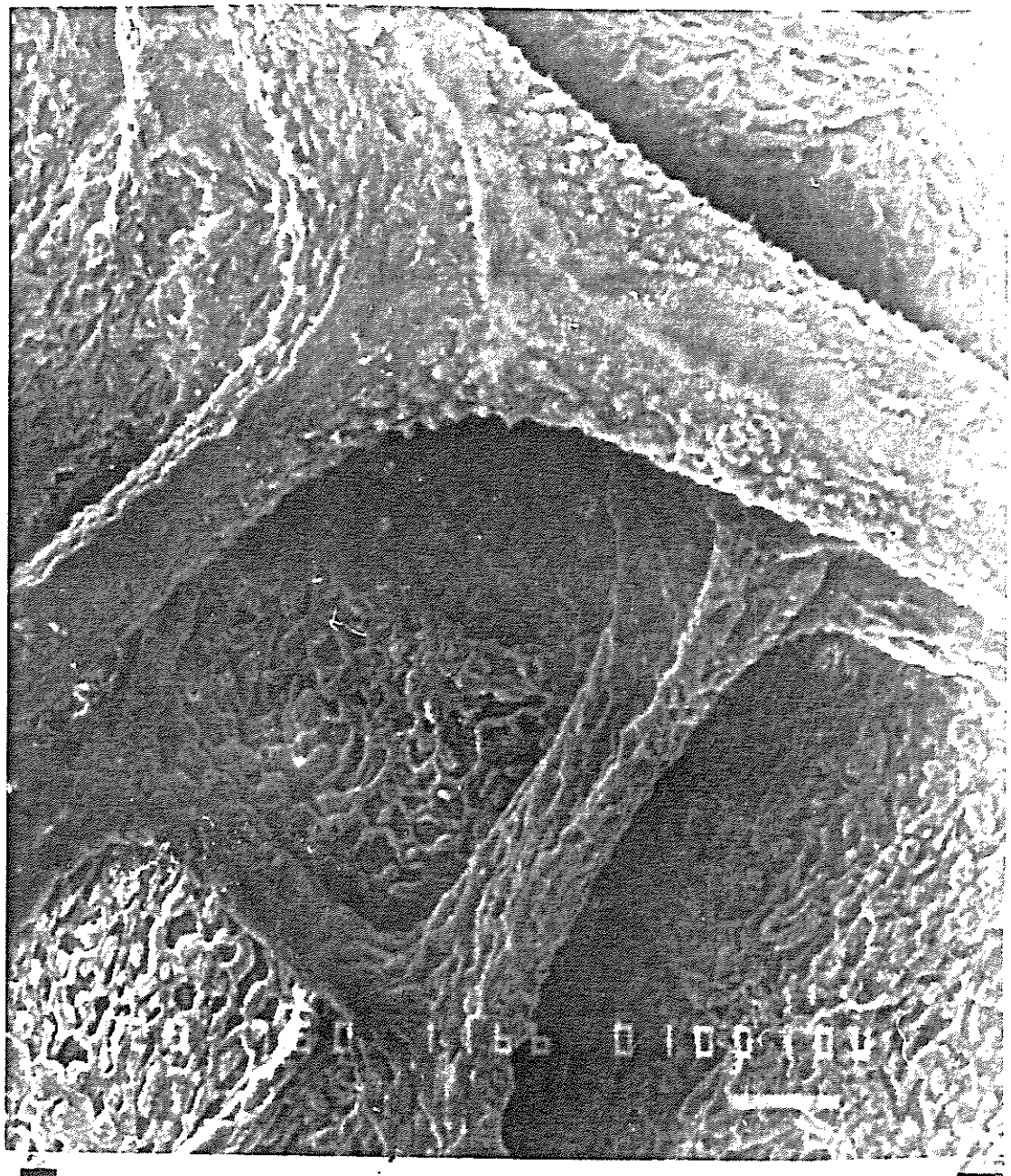


Fig. 3.8, magnification bar = 0.1 mm, from the mid-lung region. Extensive capillary networks cover the surfaces of the faveoli, bands of ciliated epithelium are seen on the major trabeculae.



Fig. 3.9, magnification bar = 0.1 mm, from the mid-lung region. A contracted major trabecula showing ciliated and non-ciliated areas is shown, no capillaries are visible here.

trabecula in the posterior lung region, possibly a surfactant secreting greater alveolar cell.

In the mid-lung region the trabeculae are arranged in a more complex manner forming deep compound faveoli. This is shown in fig. 3.6 under low power magnification although there has been some collapse of the septa during preparation and mounting. The large amount of smooth muscle present in the trabeculae is well shown here. The parallel arrangement of the major trabeculae is not always followed in this or other regions of the lung although they are sometimes seen running around the lung with also a few major trabeculae running lengthwise in the lung for varying distances. From this and other figures, e.g. fig. 3.7 it can be envisaged that differential contraction of minor trabeculae could significantly alter the volumes of individual faveoli and thus produce mixing and exchange of faveolar air with inspired air in the major luminal cavity of the lung. For effective gas exchange there must of course be an extensive capillary network. This network is better developed here in the mid lung region than in the posterior lung region as shown here and also at higher magnification in fig. 3.9 which also shows a major trabecula in a contracted state but with no capillaries visible. It is suggested as a distinct possibility that numerous superficial capillaries are present but that trabecular contraction may alter blood flow by increasing capillary resistance and simultaneously increasing the thickness of the air-blood barrier thus producing a ventilation-perfusion matching effect. The transverse ridges seen here may partly represent the subepithelial capillary network, further evidence for this was seen in light microscopy (e.g. fig. 2.11) also in SEM (e.g. fig. 3.43). This mechanism together with vascular shunting suggested earlier could provide a

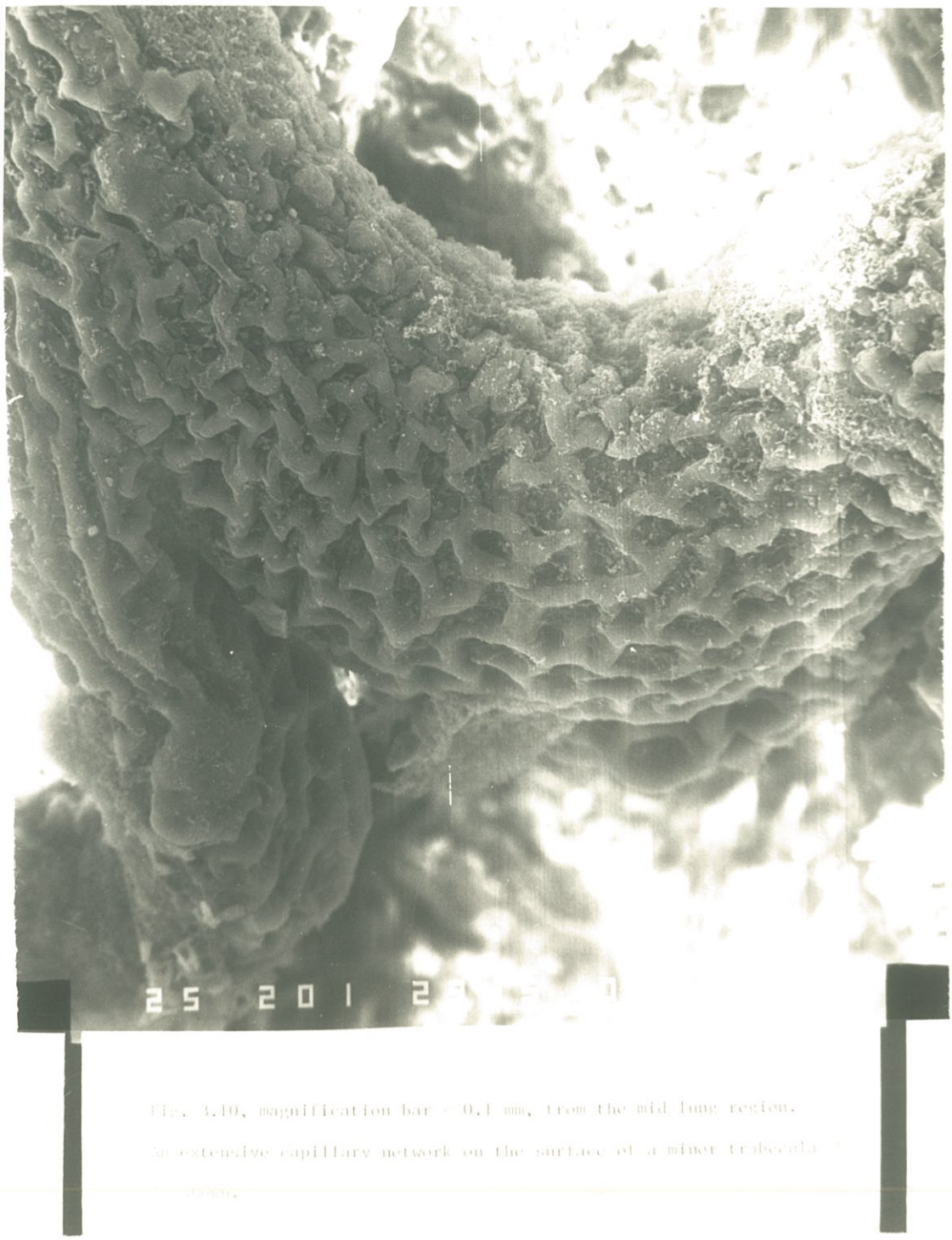


Fig. 3.10, magnification bar = 0.1 mm, from the mid lung region. An extensive capillary network on the surface of a minor trabecula [illegible] septum.

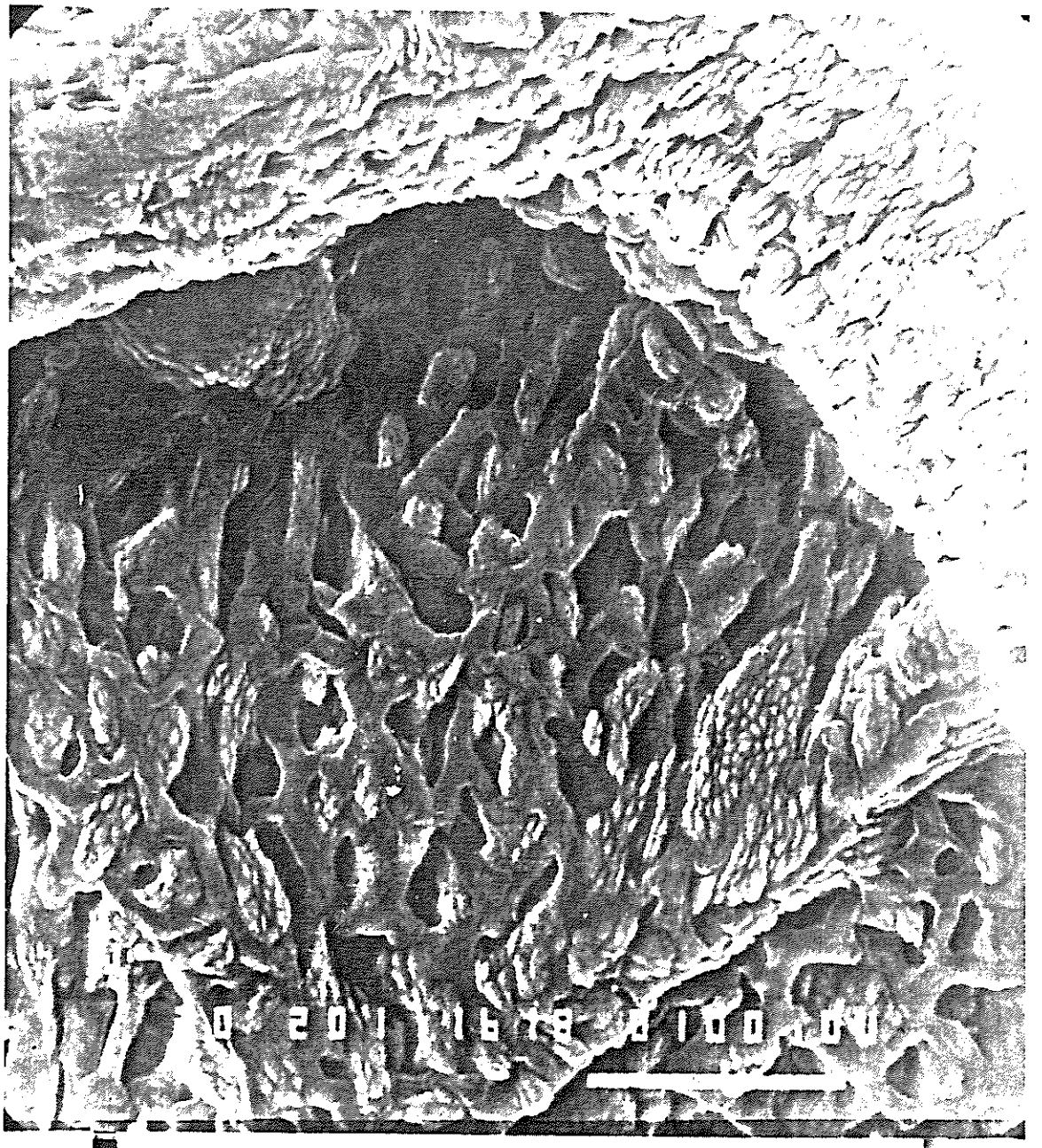


Fig. 3.11, magnification bar = 0.1 mm, from the mid-lung region. An extensive capillary network is interspersed with patches of type II pneumonocytes across the base of a faveolus.



Fig. 3.12, magnification bar = 10 μm, from the mid-lung region. Strands and sheets of dried secretory material are superimposed on areas of the capillaries. The junctions between some of the type I pneumonocytes are also shown.



Fig. 3.13, magnification bar = 10 μm, from the mid-lung region. Microvillous, type II pneumonocytes are scattered between the capillaries.

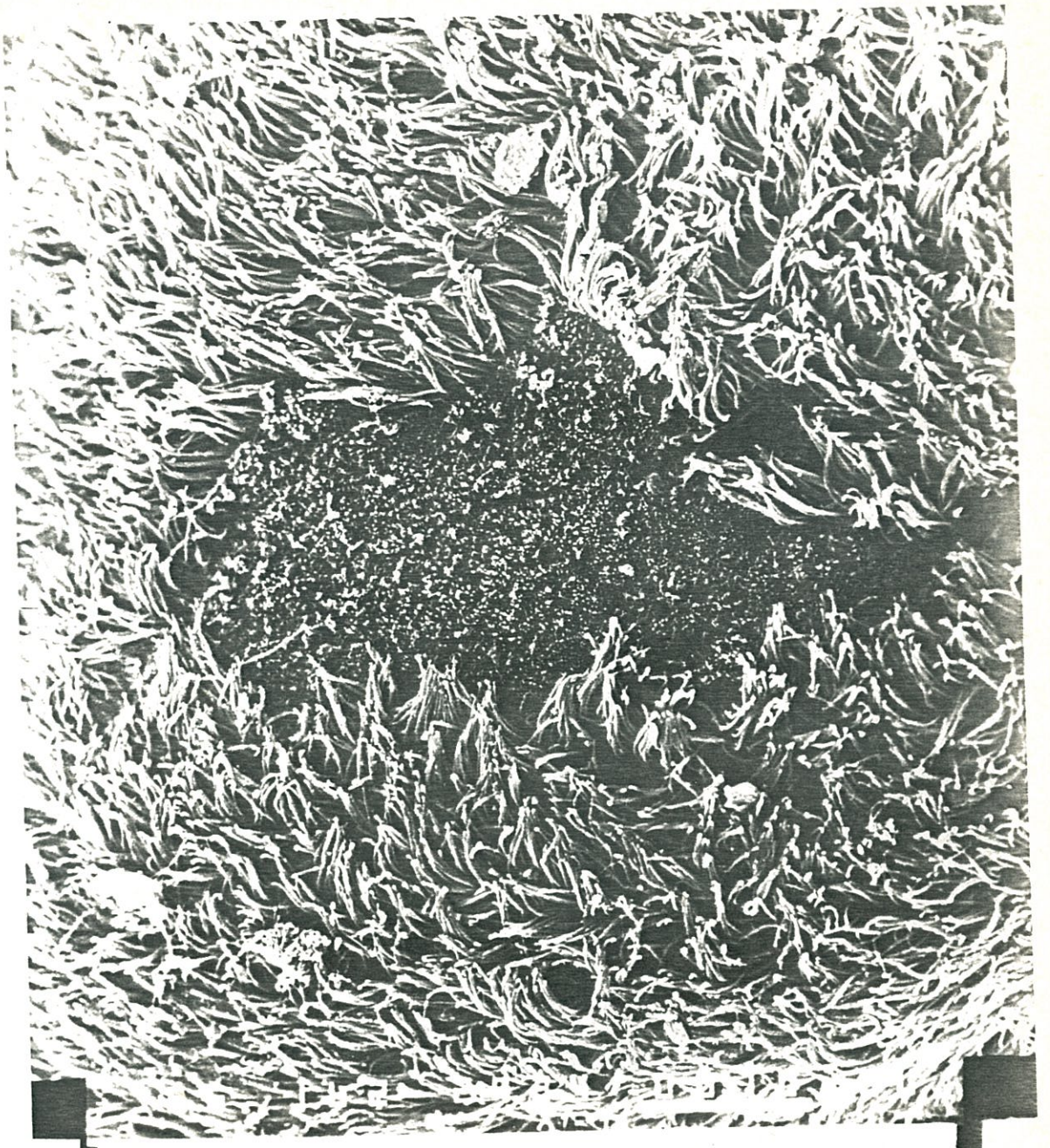


Fig. 3.14, magnification bar = 10 μm, from the mid-lung region. In the ciliated epithelial areas there may be scattered patches of one or more microvillous, type II pneumonocytes as shown here.

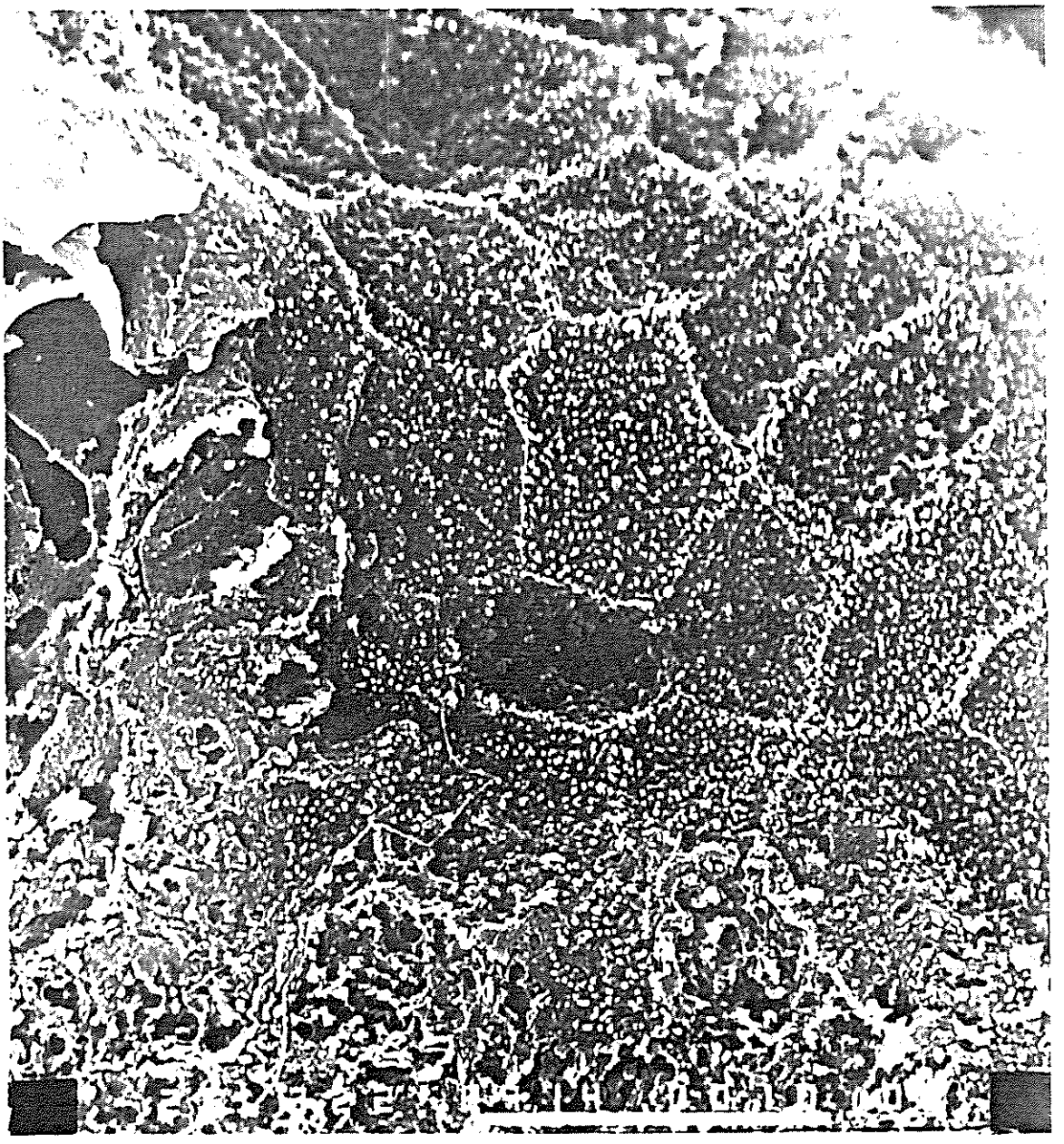


Fig. 3.15, magnification bar = 10 µm, from the mid-lung region. In addition to the usual type I and type II pneumonocytes there are several other cell types or variants including this cell with numerous superficial cavities.



Fig. 3.16, magnification bar = 10 µm, from the mid-lung region. A large (20 µ), smooth, clearly delineated type II pneumonocyte with a few low microvilli around the margin.

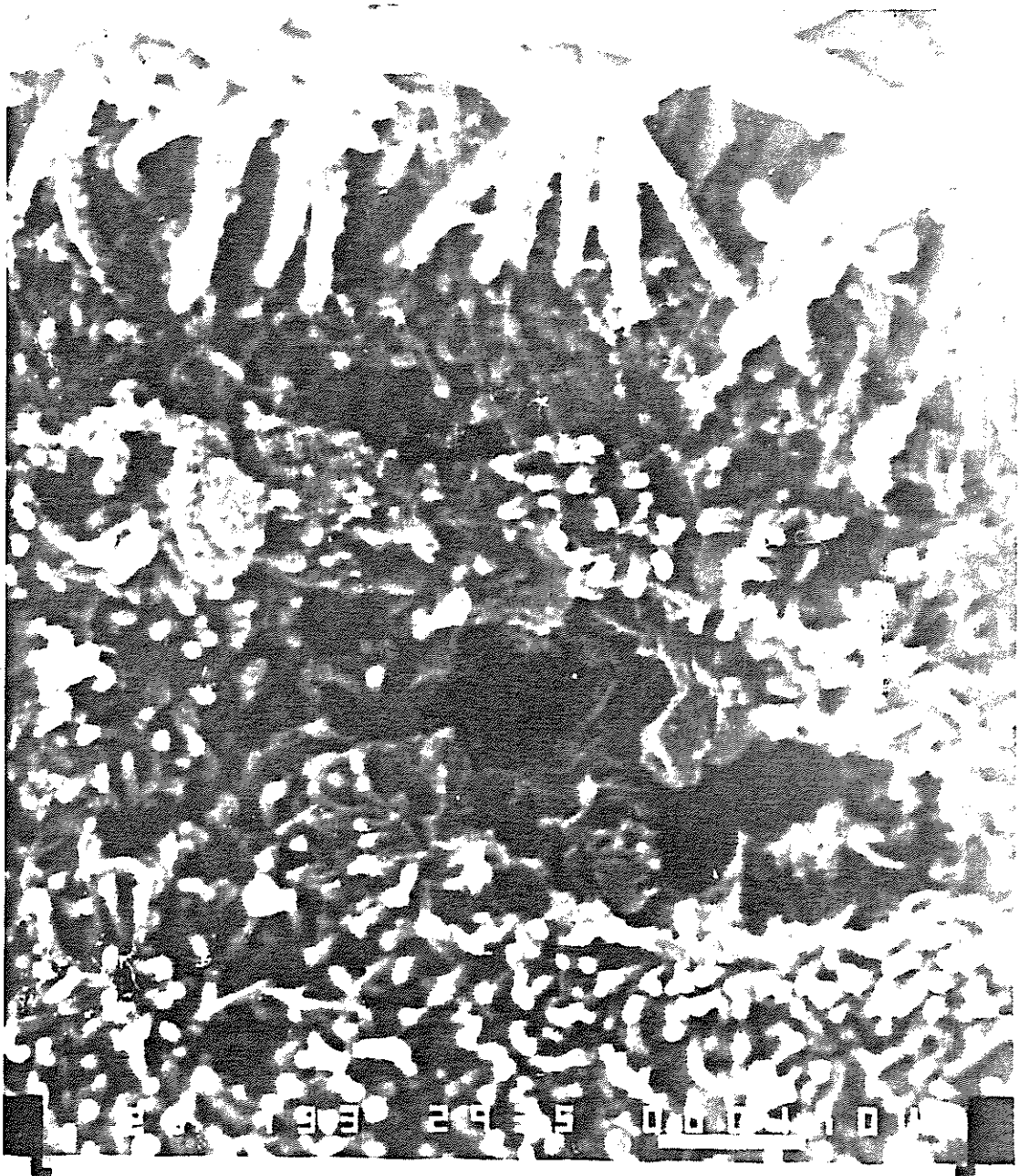


Fig. 3.17, magnification bar = 1.0 μm, from the mid-lung region. This microvillous cell appears to have both secretory and perhaps pinocytotic pores.

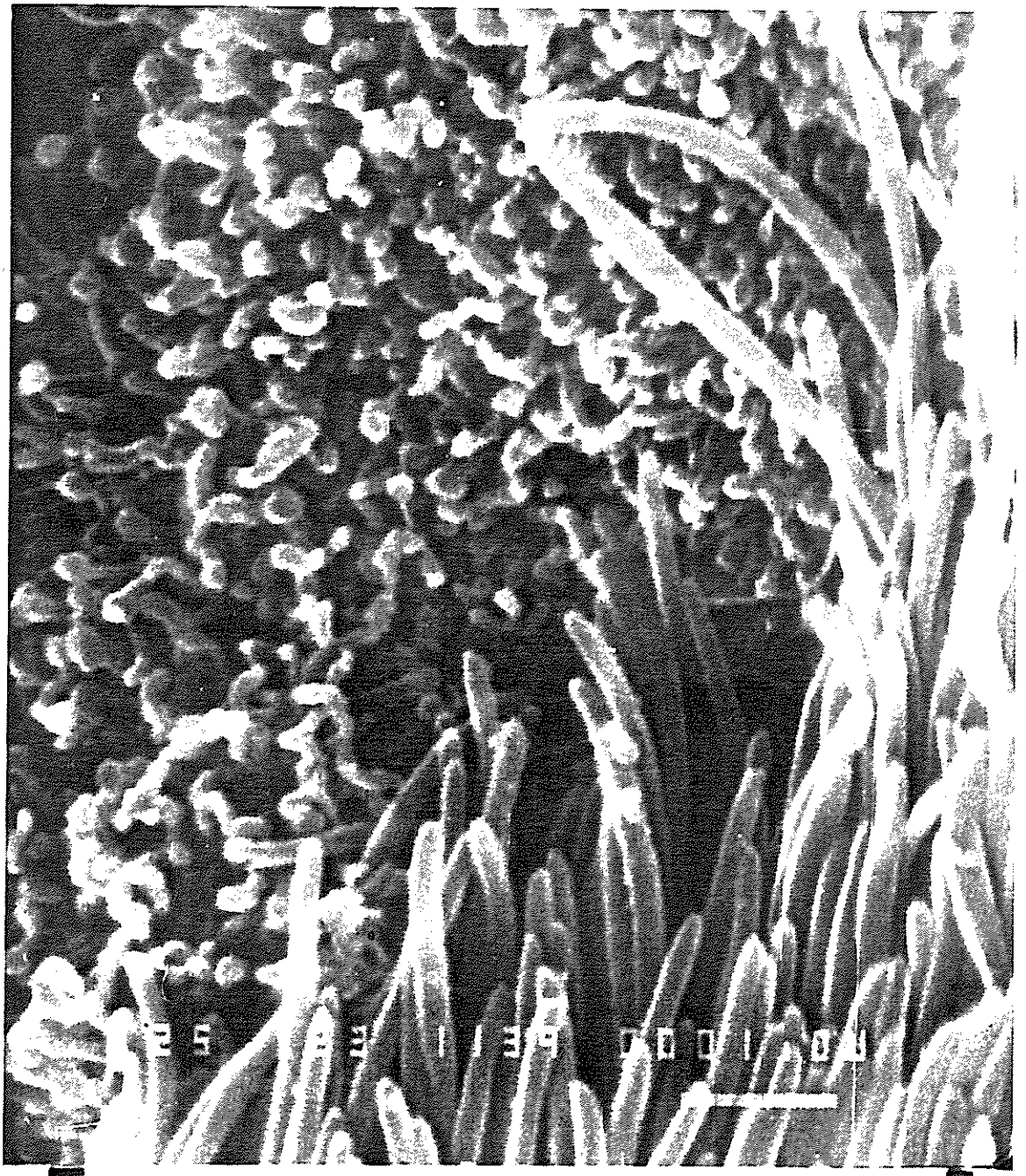


Fig. 3.18, magnification bar = 1.0 μm, from the mid-lung region. The type II pneumonocyte on the left shows several forms of microvilli, cylindrical, fused and even club shaped examples.

very effective means of controlling blood flow through the respiratory surfaces. Fig. 3.10 shows an area of a trabecula in the midlung region where the extensive capillary network is somewhat distended, although the cause cannot be determined it does appear that the trabecula is relaxed. In some areas of the mid lung region the septa and faveolar base are also well covered with a capillary network as shown in fig. 3.11. Sometime the capillaries appear to lift free (or almost free) from the underlying surface thus presenting a maximum surface area for gas exchange. The epithelium-endothelium layer appears quite thin in some views of the capillaries at high magnification, fig. 3.12 and 3.13. where the outlines of contained erythrocytes may be seen as bulges in the capillary surface. Although the percentage capillary cover of the respiratory surface is very variable, in the areas shown it is well over 50%. Among the capillaries and also in the ciliated areas there are scattered microvillous cells, (fig. 3.14) forming a significant proportion of the total epithelial cells. Fig. 3.15 shows a patch of such cells in the mid-lung region together with other possibly secretory cells. Some of the type II pneumonocytes are relatively large and smooth, (fig.3.16) although the significance of these cells is not certain. Fig. 3.17 shows an active, microvillous, type II pneumonocyte adjacent to a ciliated cell. What appear to be cilia rootlets are seen near the bases of some of the cilia. Just why certain cells in the respiratory epithelium require numerous microvilli is not clear but fig. 3.18. showing a range of forms of microvilli, indicates a considerable increase in surface area in these cells (the area is increased by a factor of 10 for cylindrical microvilli covering half the surface area of the cell and having a height of five times their diameters). Surfactant is almost certainly secreted by cells scattered throughout the respiratory epithelium. Although few and

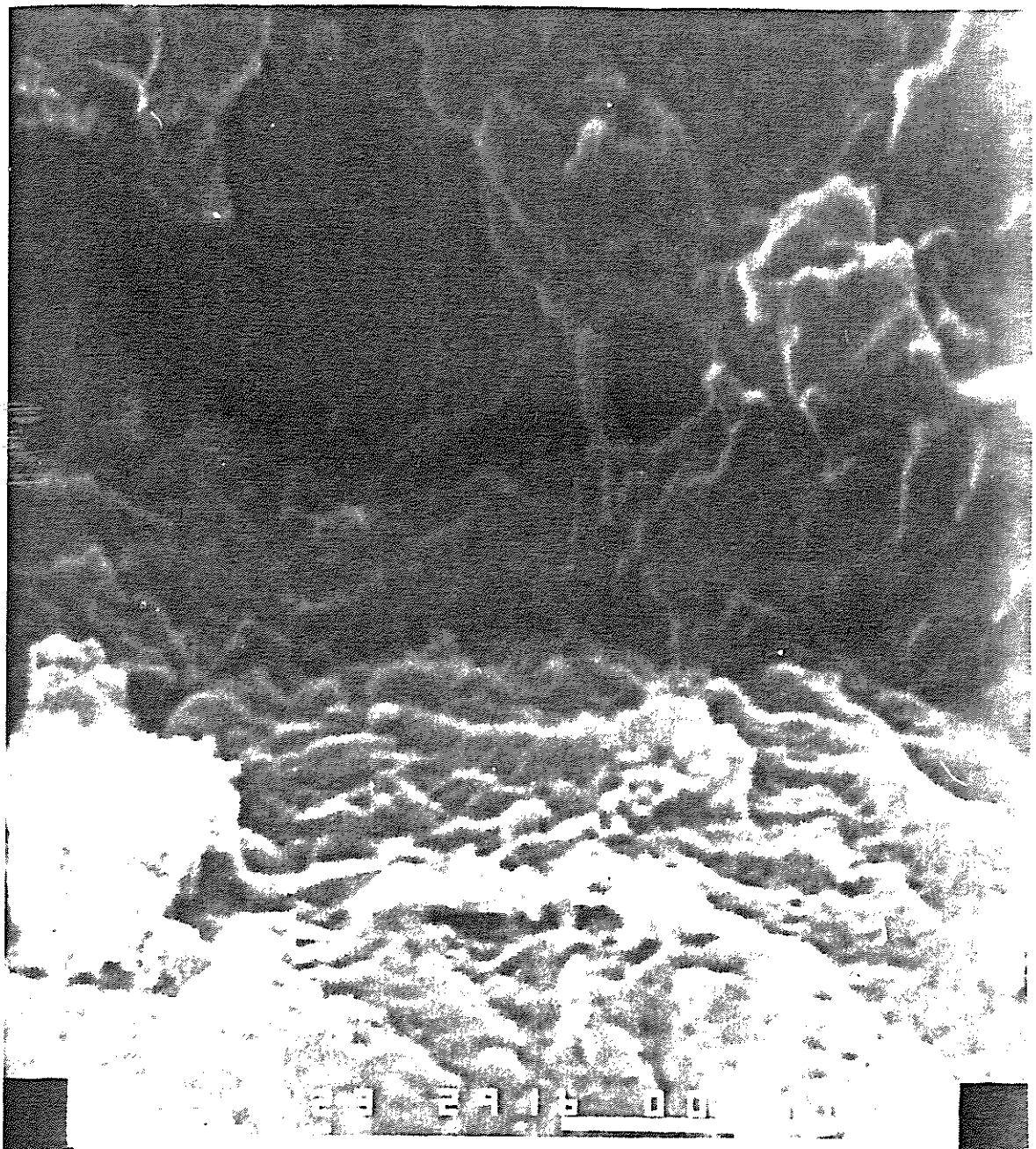


Fig. 3.19, magnification bar = 1.0 µm, from the mid-lung region. Detail of a secretory pore surrounded by a layer of secretory material, the material in the lower area is probably also secretory.

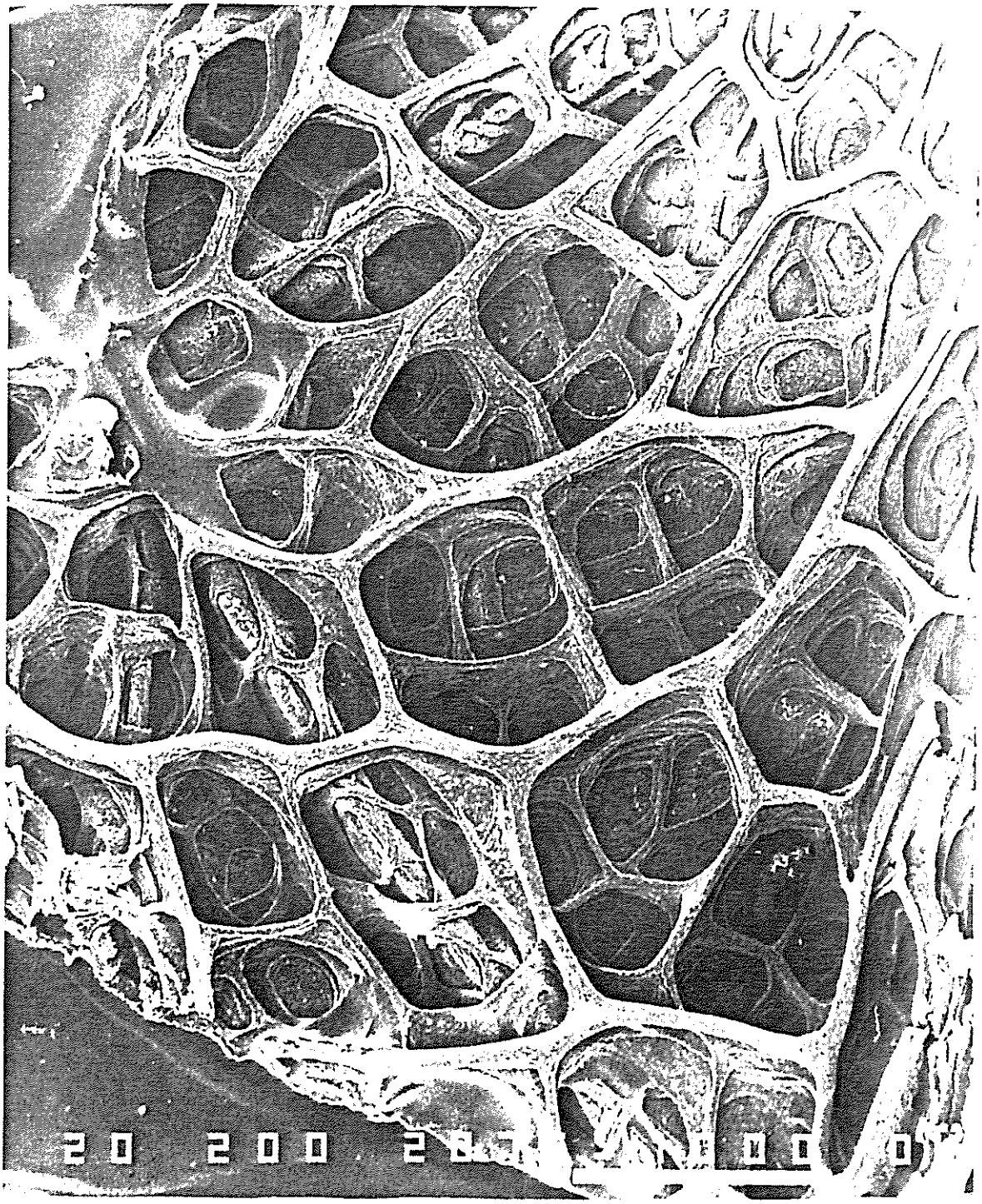


Fig. 3.20, magnification bar = 1.0mm, from the anterior lung region. The arrangement of the deep faveoli is shown, (some flattening around the periphery has occurred during tissue preparation)

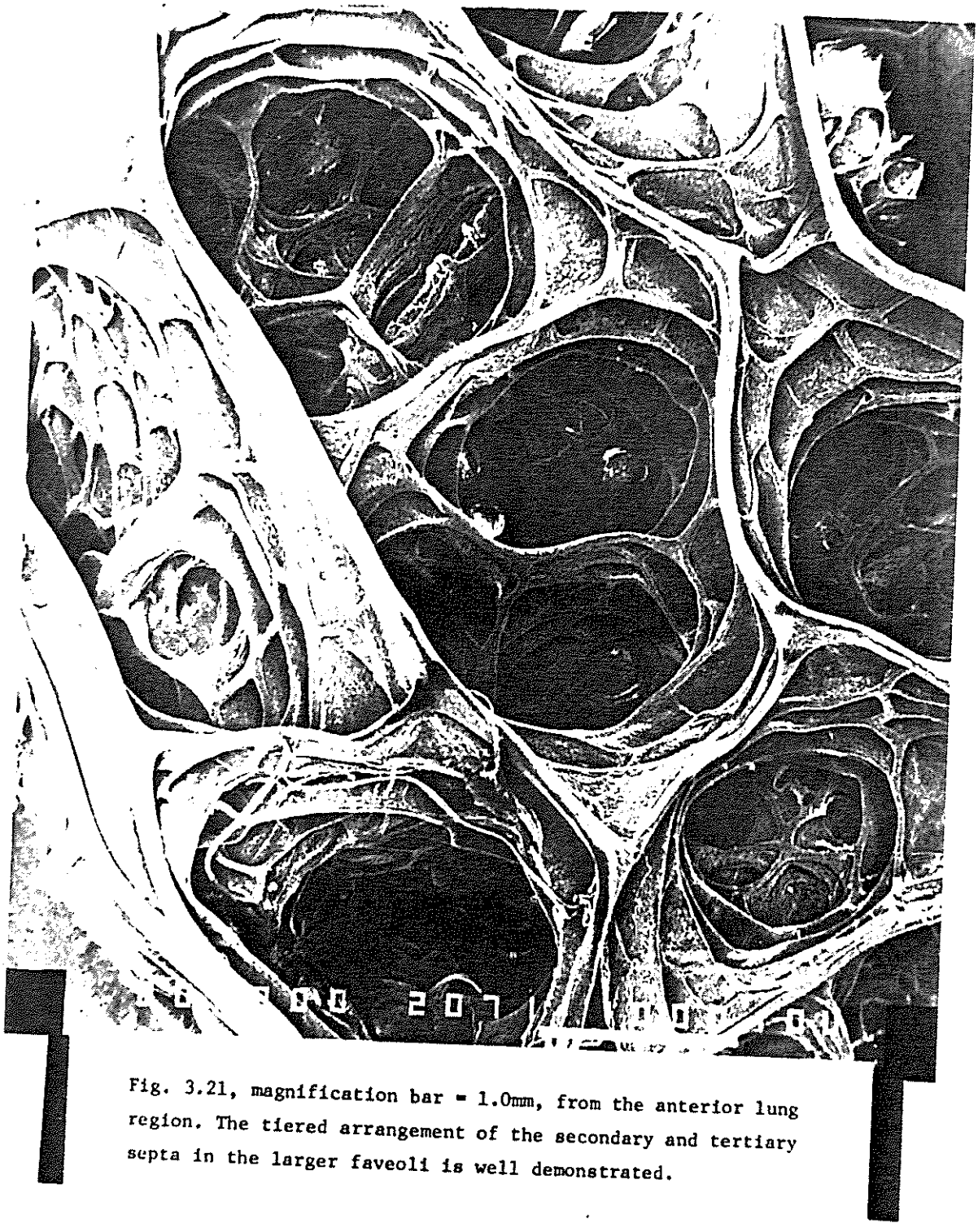


Fig. 3.21, magnification bar = 1.0mm, from the anterior lung region. The tiered arrangement of the secondary and tertiary septa in the larger faveoli is well demonstrated.

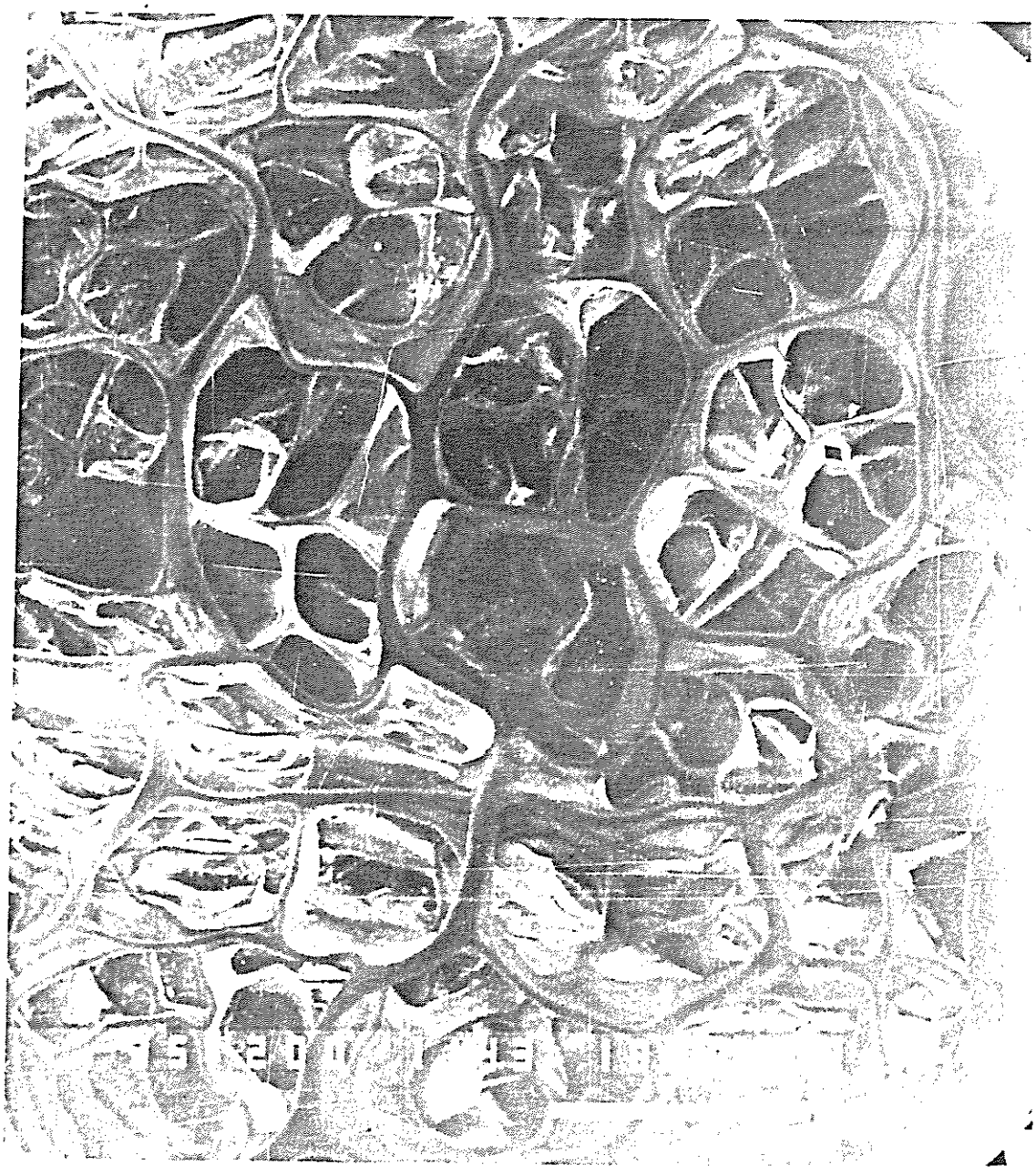


Fig. 3.22, magnification bar = 1.0mm, from the anterior lung region. These faveoli are smaller and shallower than those in Figs. 3.20 and 3.21.

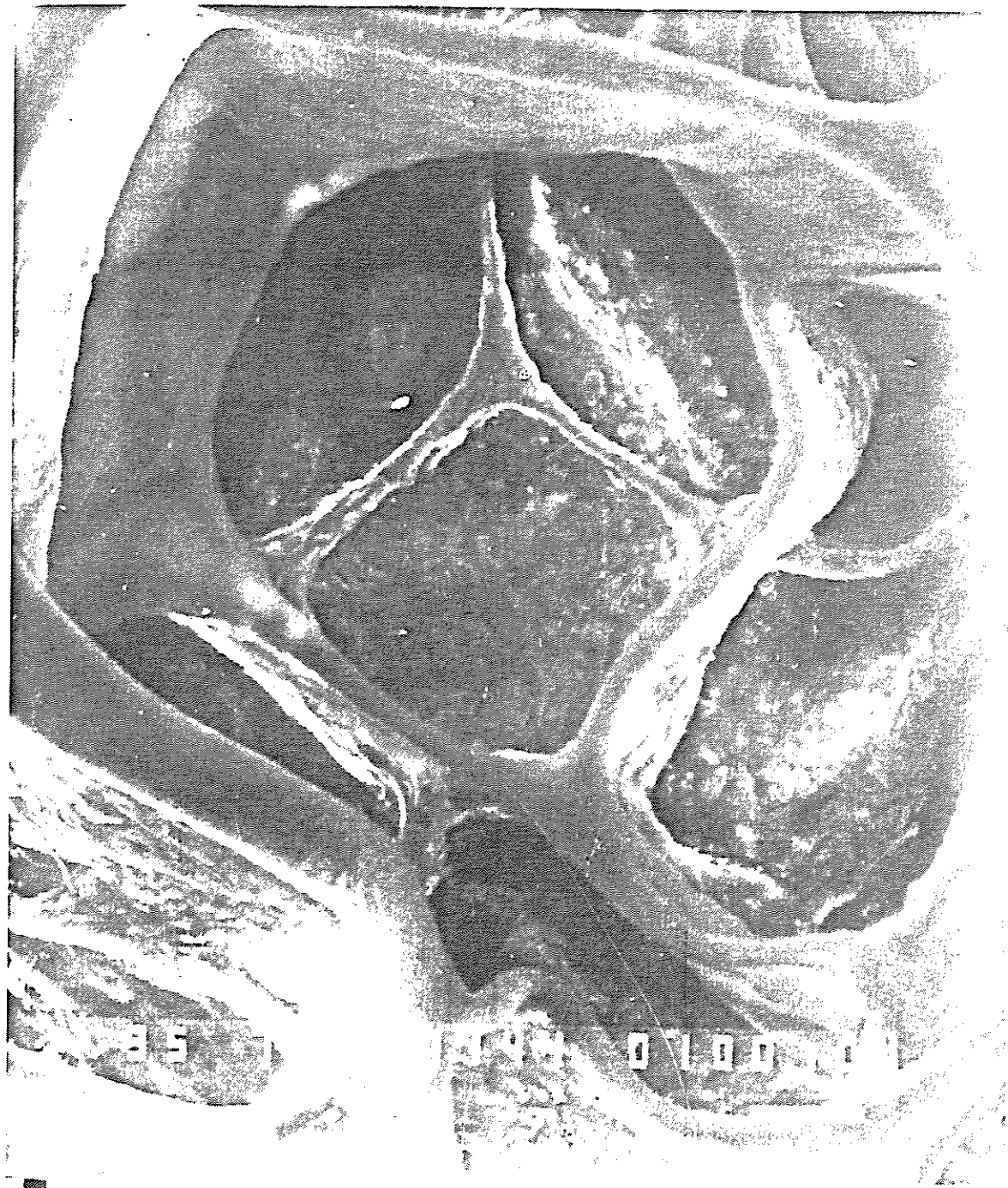


Fig. 3.23, magnification bar = 0.1mm, from the anterior lung region. The faveolar subdivisions and septa have a less complex arrangement here with minimal tiering.



Fig. 4.2, magnification bar = 0.1mm, from the anterior lateral region. The interconnexions of primary, secondary and tertiary septa are shown.



Fig. 3.25, magnification bar = 1.0mm, from the anterior lung region. The major trabeculae are contracted while the minor trabeculae appear relaxed.

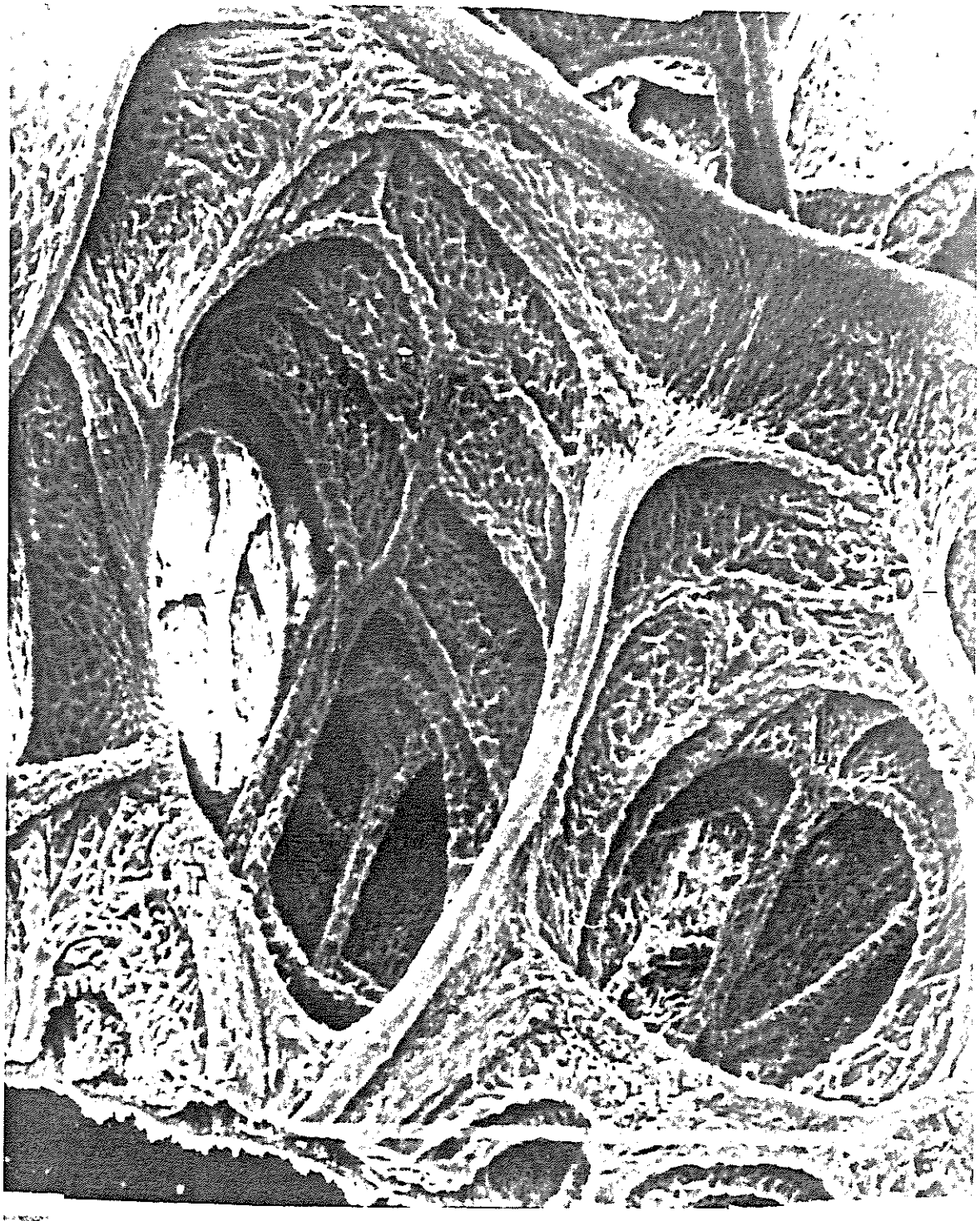


Fig. 3.26, magnification bar = 1.0mm, from the anterior lung region. Details of the interconnexions of the minor smooth muscle trabeculae between tiers are shown.

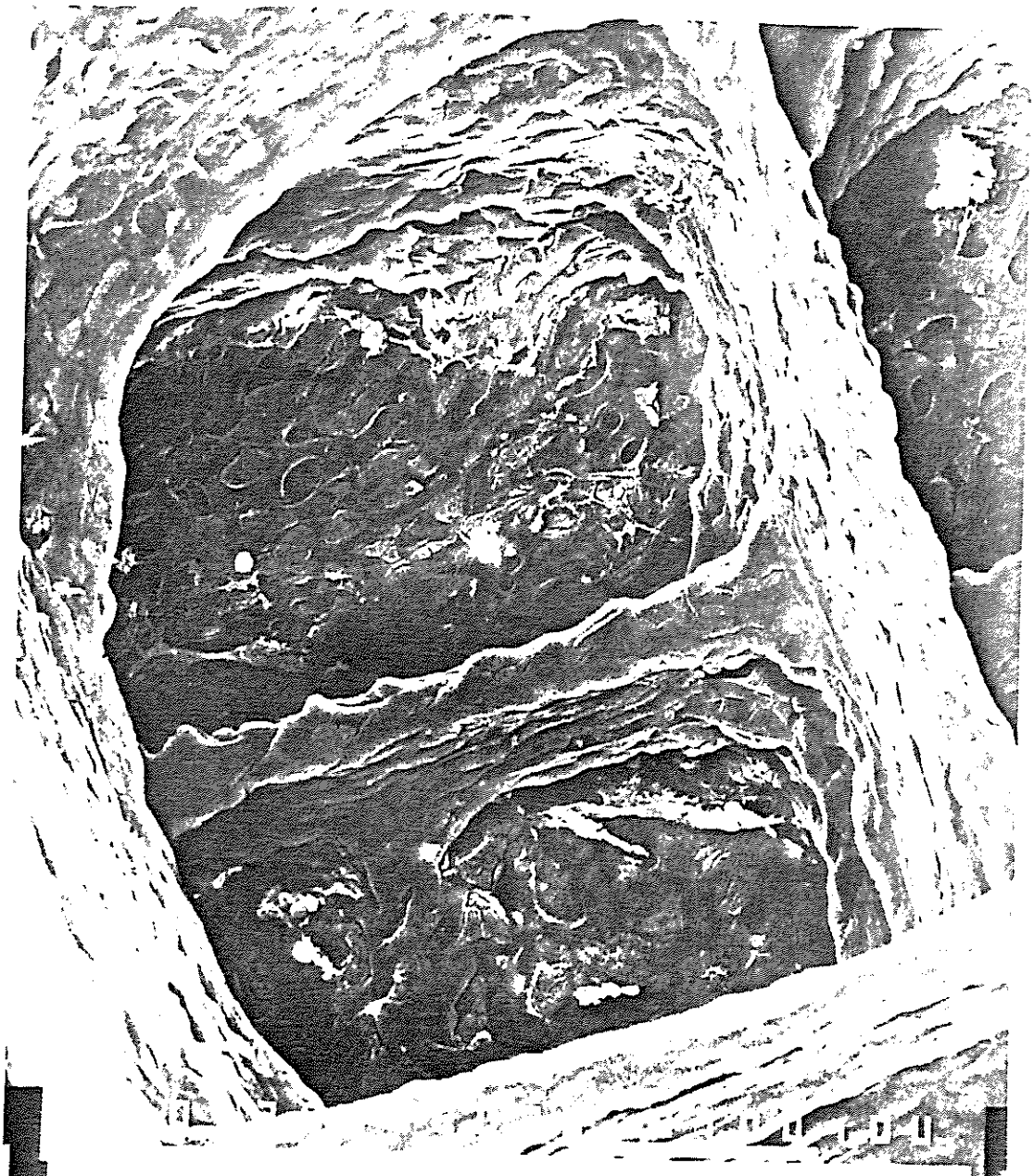


Fig. 3.27, magnification bar = 0.1mm, from the anterior lung region. The extensive capillary coverage of minor trabeculae, septa and faveolar base are shown.

far between, fig. 3.19 appears to be one of these showing secretory material similar to that in fig. 3.5.

In the anterior lung region the faveolar network is even more complex than in the midlung region with the deepest and most complex compound faveoli being found in this region. The trabecular network is just as complex although there is a tendency for some of the major trabeculae to radiate out from the hilar region of the lung, (fig. 3.20) top left hand corner. The complexity of the faveoli is well shown in fig. 3.21 where the multiple tiers of the lateral faveolar walls are shown with only minimal evidence of smooth muscle trabeculae in the borders of the tertiary septa. Interconnections between tiers are also shown possibly providing a functional syncytium of smooth muscle throughout the trabecular network. Fig. 3.22 taken from an adjacent area of the anterior lung region demonstrates shallower and less complex faveoli. The large ratio of air volume to lung tissue volume is well shown here. Fig. 3.23 shows a detail of fig. 3.22 at higher magnification demonstrating the interconnections of septa and trabeculae. These interconnections are also shown in figs. 3.24 and 3.25, some of the relaxed and contracted trabeculae are directly connected perhaps indicating a slow progression of contractions through the trabecular network or possibly an alternation between contracting and relaxing trabeculae. The capillary network is most prominent on the relaxed trabeculae. The capillary network and also the interconnections of the minor smooth muscle trabeculae in the complex compound faveoli of the anterior lung region is well demonstrated in fig. 3.26 although some stretching has occurred in the preparation of this specimen. The extensive coverage of the capillary network on septa and faveolar bases in the anterior lung region (fig 3.27), produces the highest ratio of respiratory surface to external lung surface area of any part of the lung. The

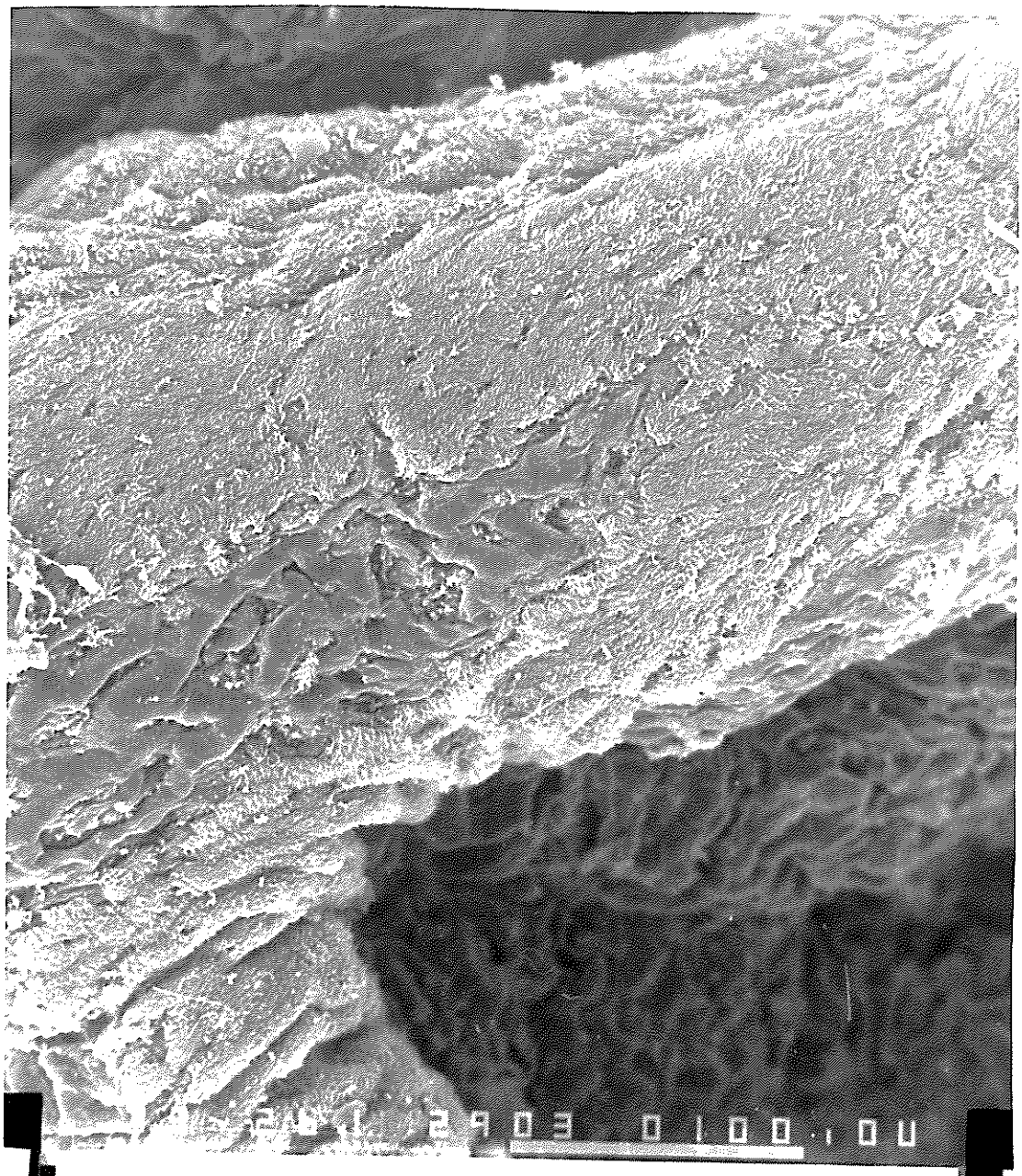


Fig. 3.28, magnification bar = 0.1mm, from the anterior lung region. This major trabecula demonstrates a patch of exposed capillaries within a region of ciliated epithelium.

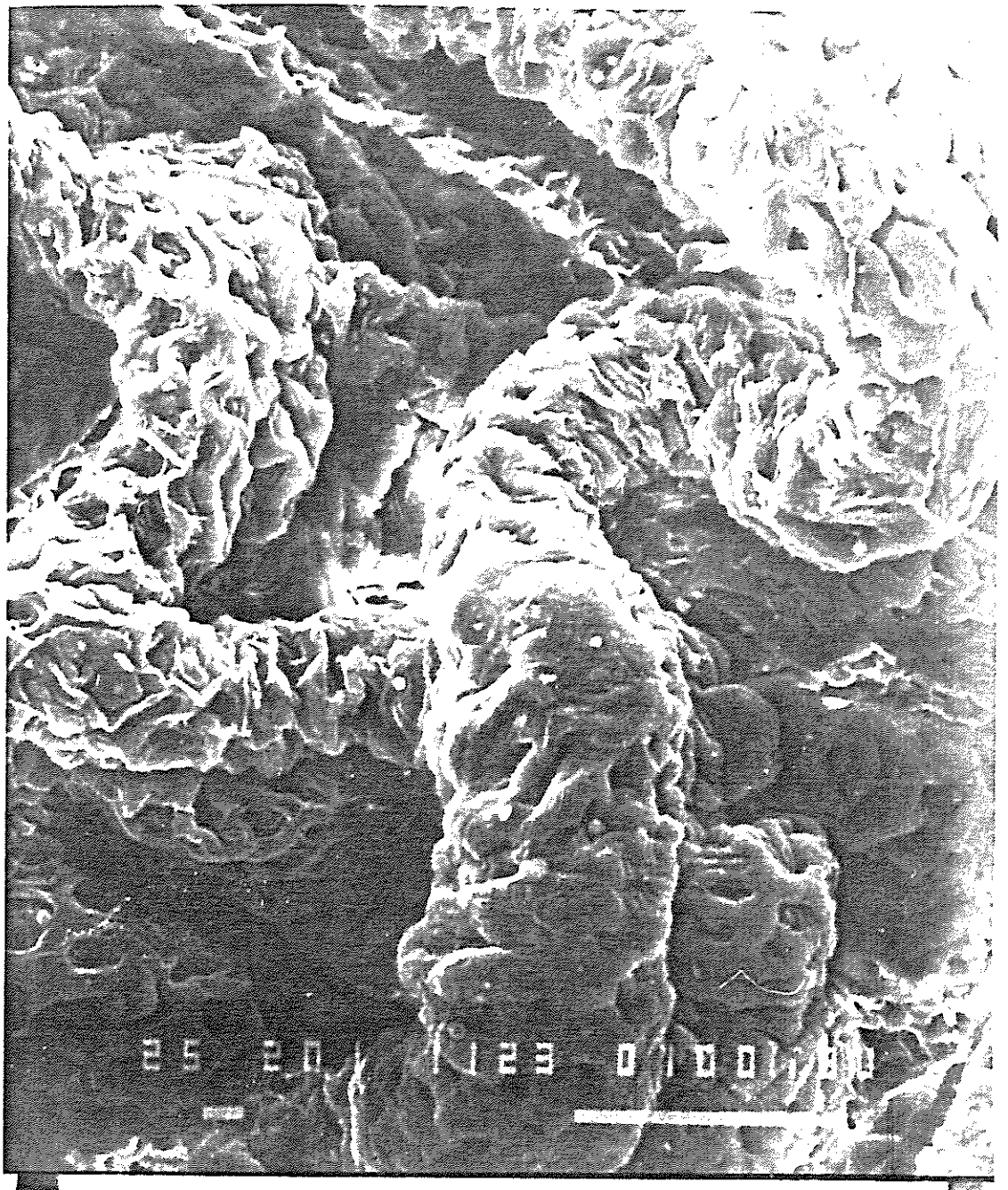


Fig. 3.29, magnification bar = 0.1mm, from the anterior lung region. This view illustrates the extensive capillary coverage of (relaxed) minor trabeculae in this area.



Fig. 3.30, magnification bar = 0.1mm, from the anterior lung region. There is an extensive capillary coverage even at the base of this deep,tiered faveolus.



Fig. 3.31, magnification bar = 10μm, from the anterior lung region. Views similar to this were used to help calculate values for capillary coverage.

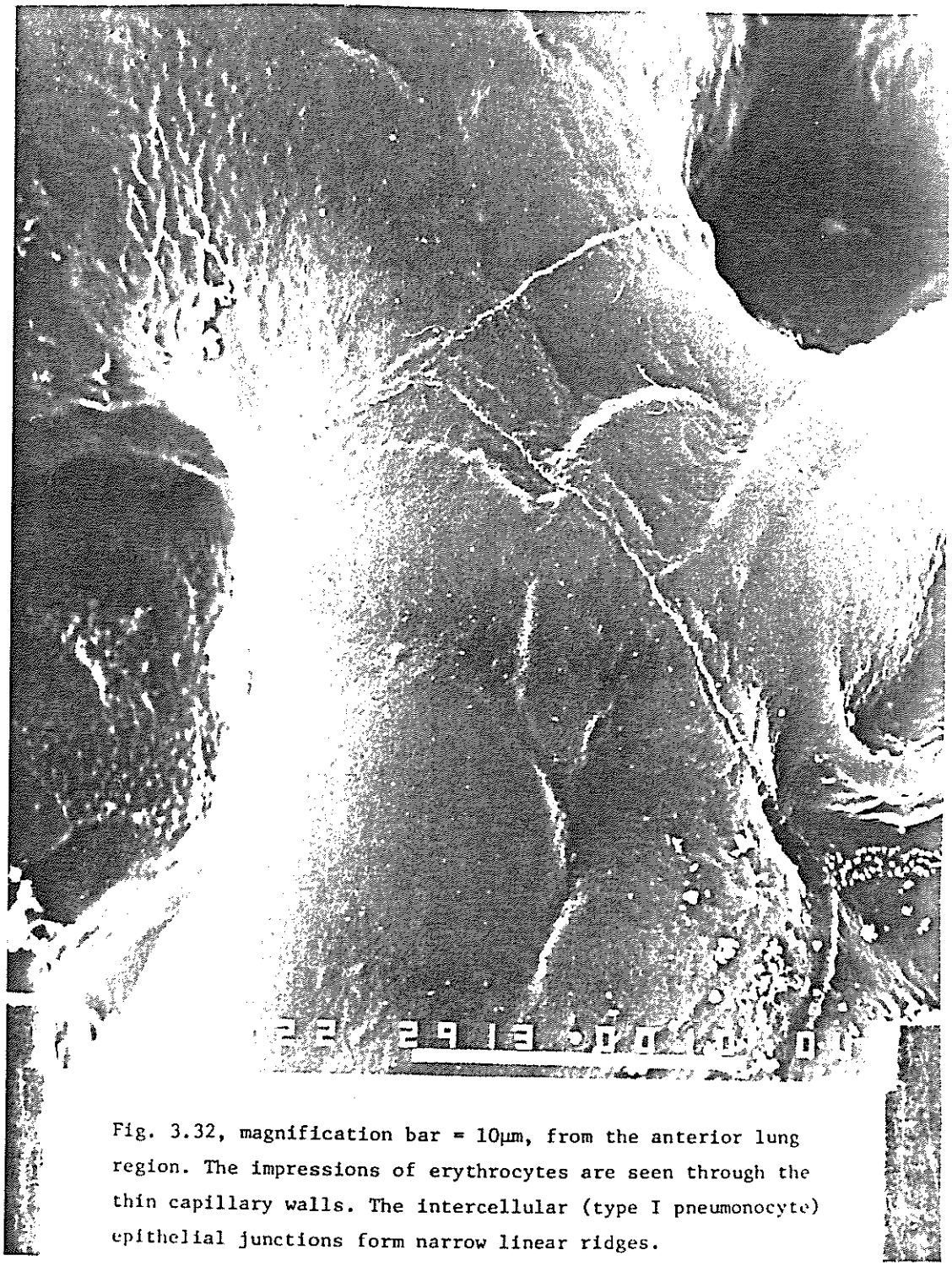


Fig. 3.32, magnification bar = 10μm, from the anterior lung region. The impressions of erythrocytes are seen through the thin capillary walls. The intercellular (type I pneumonocyte) epithelial junctions form narrow linear ridges.

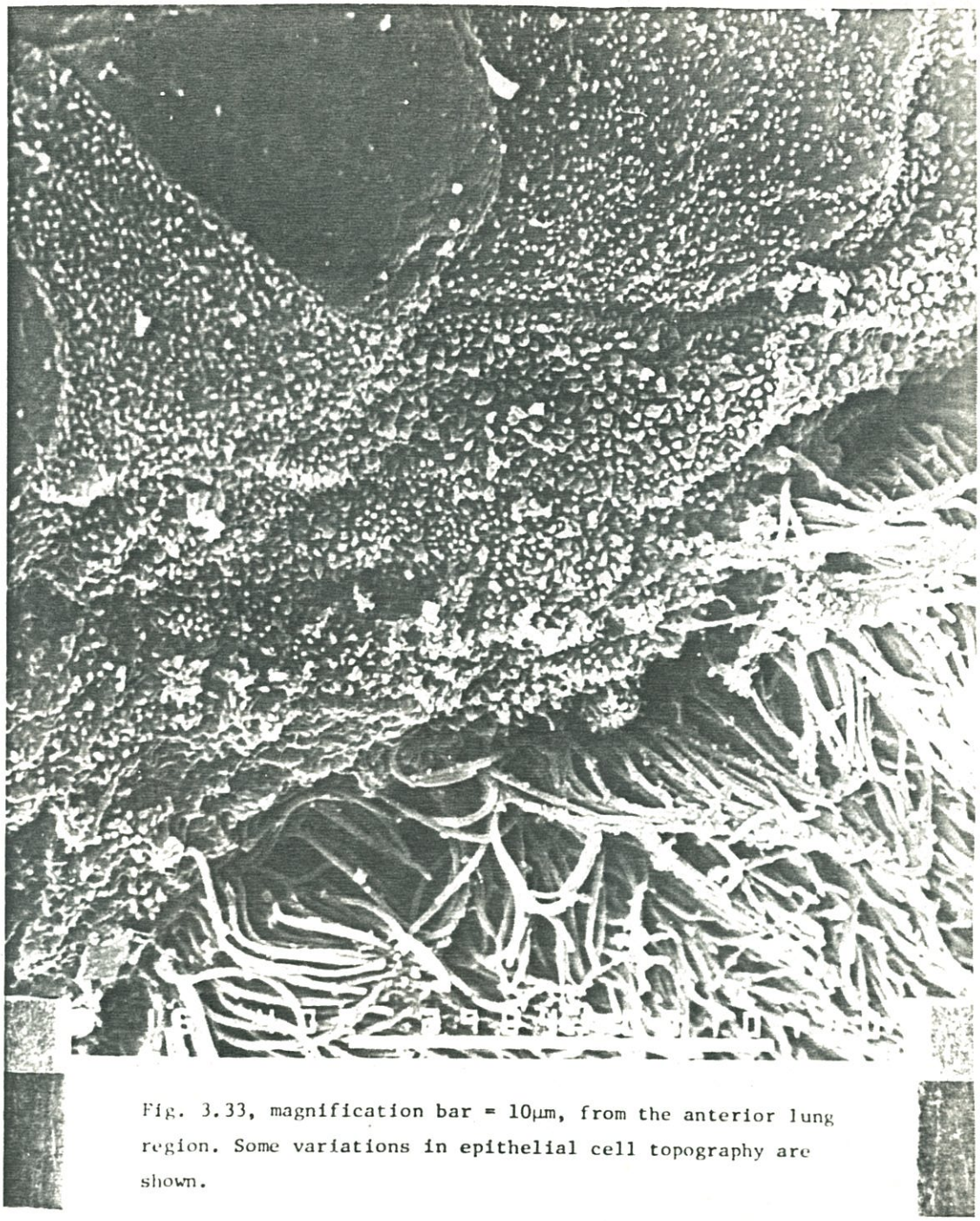


Fig. 3.33, magnification bar = 10μm, from the anterior lung region. Some variations in epithelial cell topography are shown.

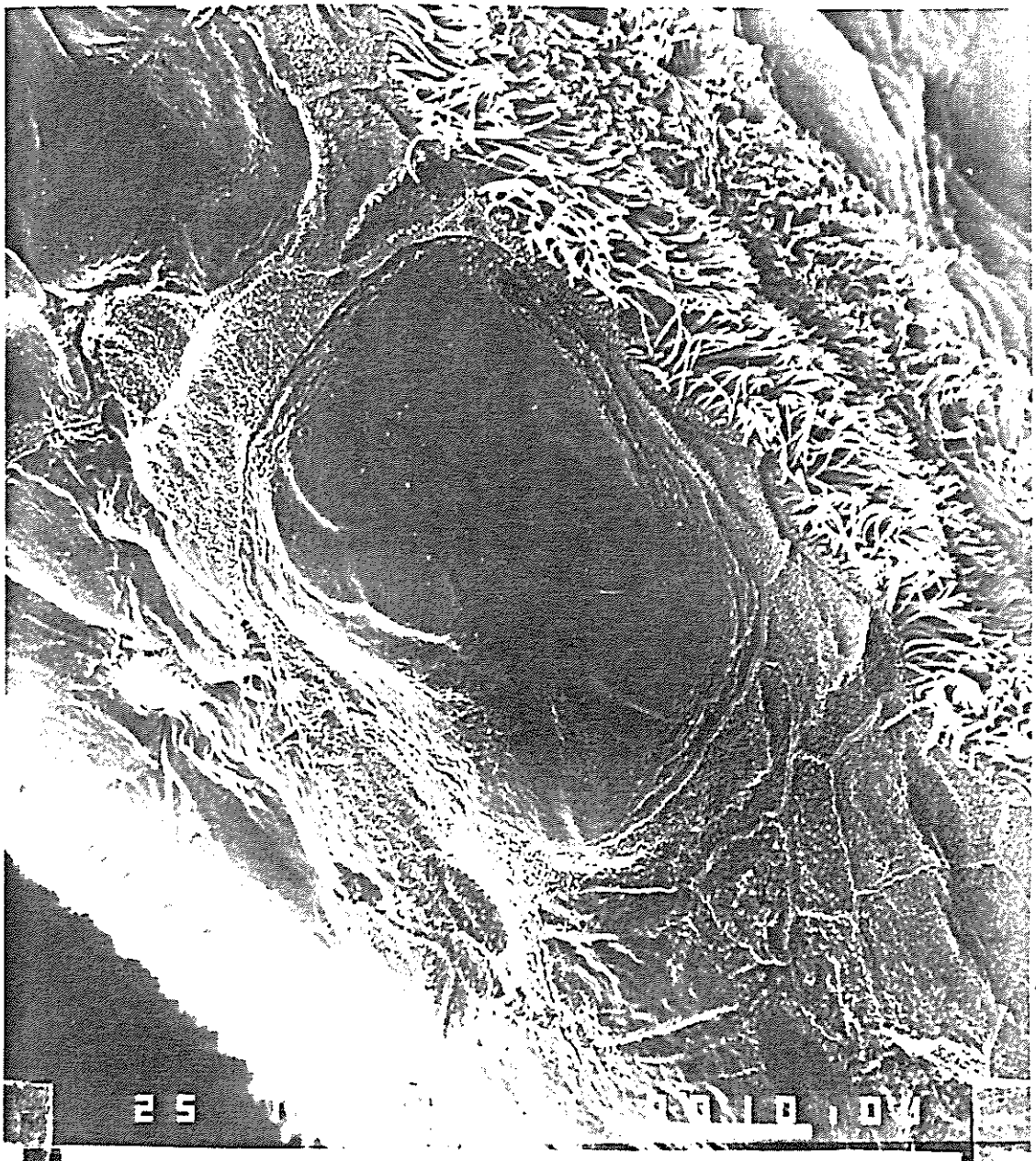


Fig. 3.34, magnification bar = 10μm, from the anterior lung region. A large, type I pneumonocyte is shown also a variety of type II pneumonocytes.

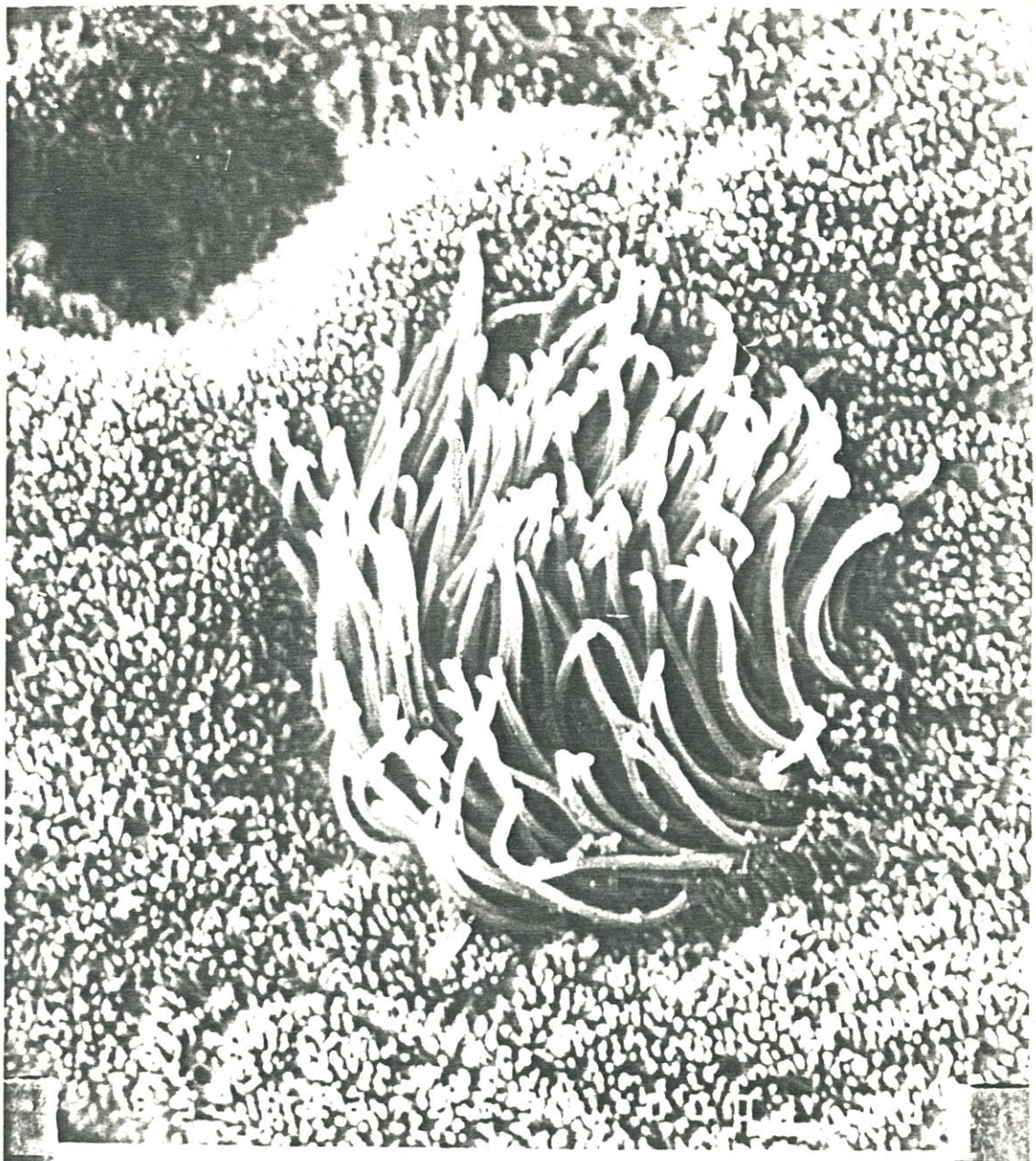


Fig. 3.35, magnification bar = 1.0μm, from the anterior lung region. An isolated ciliated cell with microvilli is shown surrounded by microvillous cells.

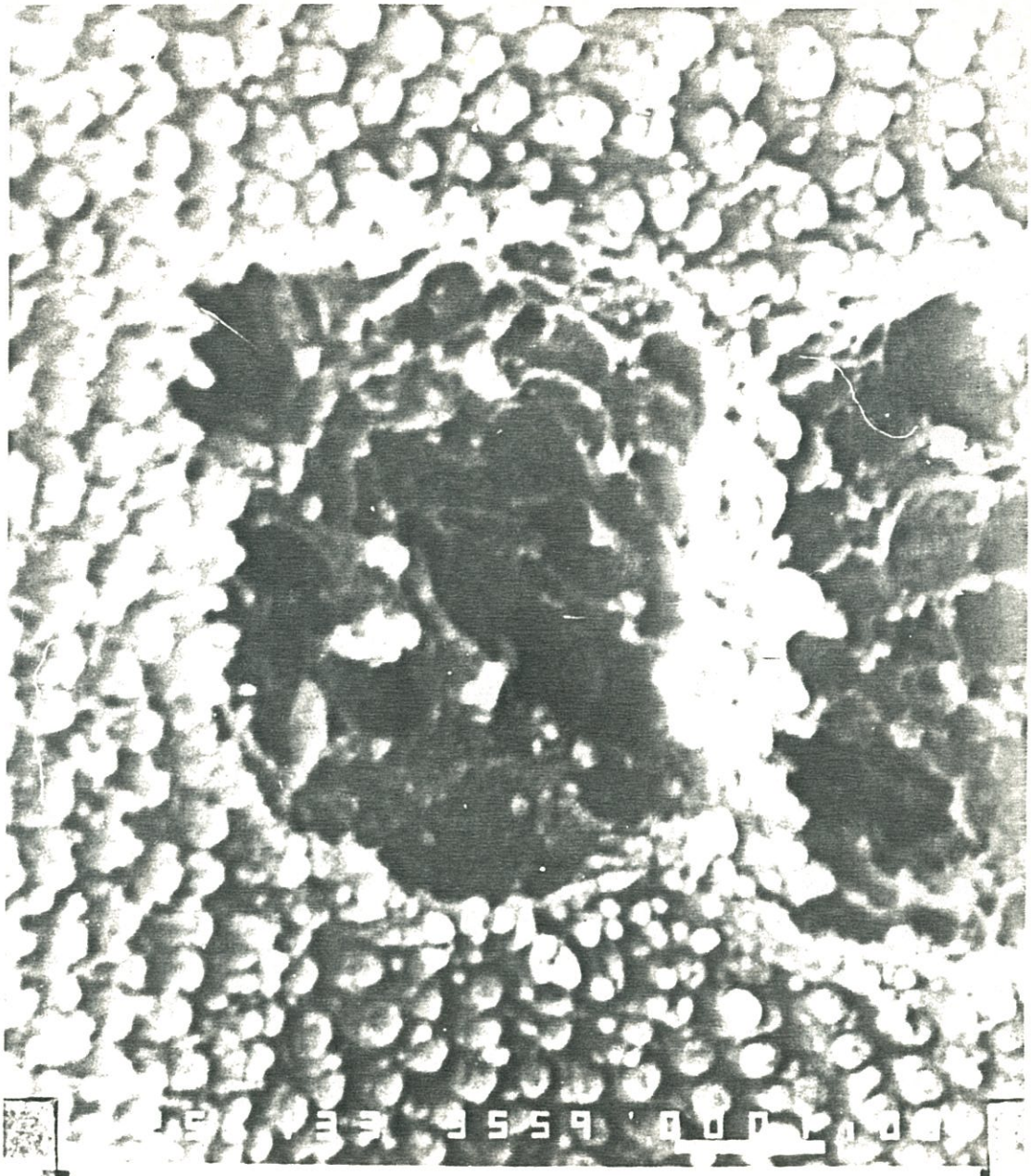


Fig. 3.36, magnification bar = 1.0μm, from the anterior lung region. These small cells have deeply scalloped free surfaces, the microvilli of surrounding type II pneumonocytes also show interesting variations of form.



Fig. 3.37, magnification bar = 10 μm, from the anterior lung region. This collection of cells on a trabecula show considerable variation in size and form.

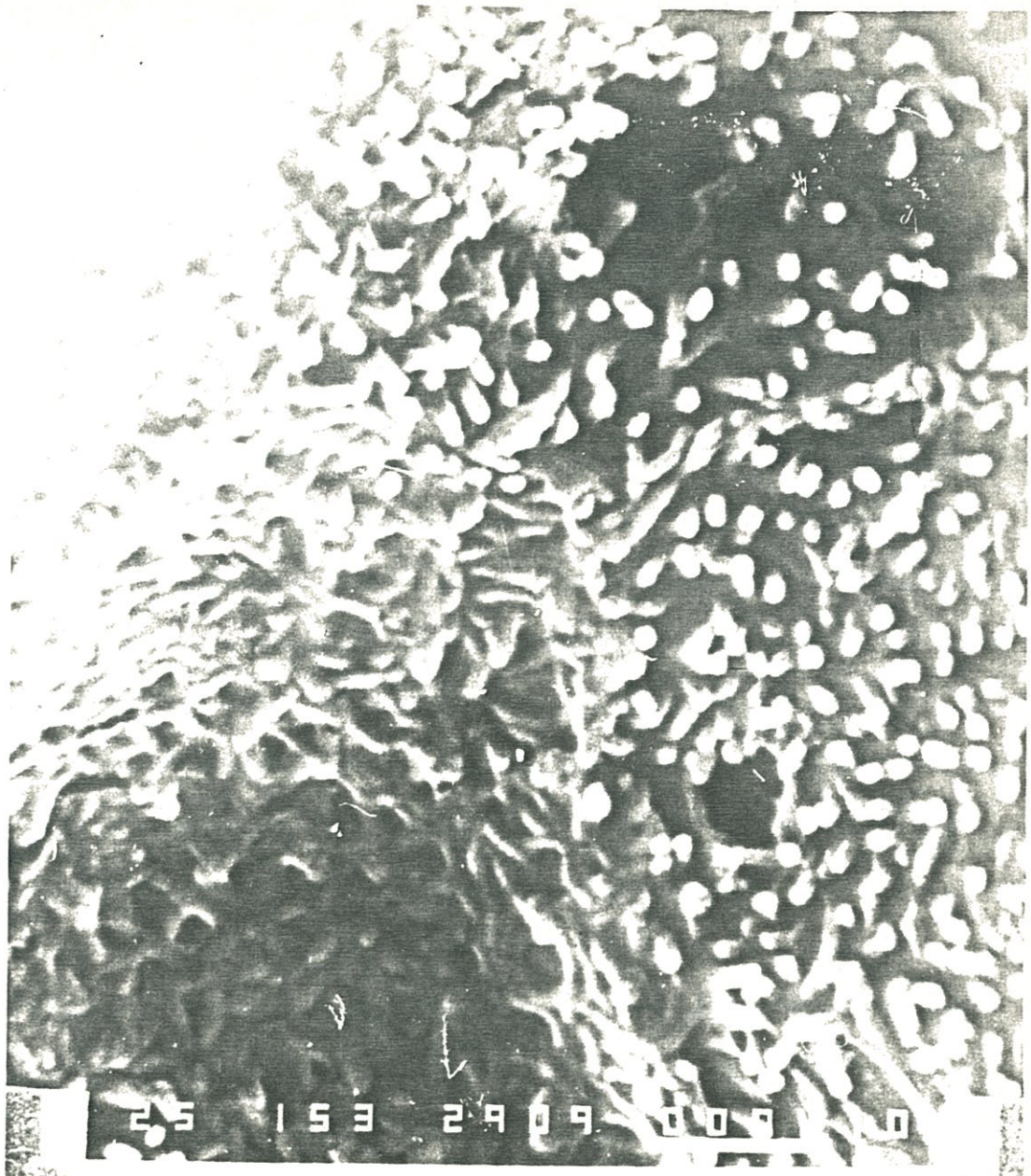


Fig. 3.38, magnification bar = 1.0μm, from the anterior lung region. Detail of apertures in free surfaces of type II pneumonocytes.

major trabeculae in the anterior lung region are very similar to those found elsewhere in the lung, (fig. 3.28) with bands of ciliated epithelium and numerous capillaries shown here in the relaxed trabecula. Fig 3.29 shows the extensive capillary network found again on relaxed secondary trabeculae. Some of the capillaries appear collapsed probably due to loss of blood during preparation however the respiratory surface of the capillaries probably represents well over 100% of the available trabecular surface on which the capillaries rest. The faveolar base is a region where it might be expected that little fresh air could penetrate in quiet respiration however as fig. 3.30 indicates this region is also covered by an extensive capillary network which almost certainly indicates that air must be efficiently exchanged even down to the faveolar base. Fig 3.31 shows an area of clearly defined capillaries, although somewhat collapsed they give a measurable indication of capillary coverage. Fig. 3.32 is a high power view from an area similar to fig. 3.31. Fig. 3.33 illustrates several of the cell types found in the anterior lung region. Some cells have few if any microvilli others have large irregular microvilli. The presence of cells of intermediate description may indicate different stages of celluar activity as well as different cell types. Fig. 3.34 illustrates a large type I pneumonocyte which appears to overly some capillaries as indicated by the large projections outlined in its surface which are of erythrocyte shapes and dimensions. Although no positive identification of any sensory receptor cell was made there were a few instances such as the specialized cells of figs. 3.34 and 3.35 which could conceivably have been sensory receptors. Fig. 3.36 is also another possibility for some kind of chemoreceptor. Fig. 3.37 is a patch of small cells, possibly goblet cells adjacent to a capillary (upper right) to which a pale erythrocyte is attached. Fig. 3.38 shows several apertures or depressions in the free surfaces of epithelial

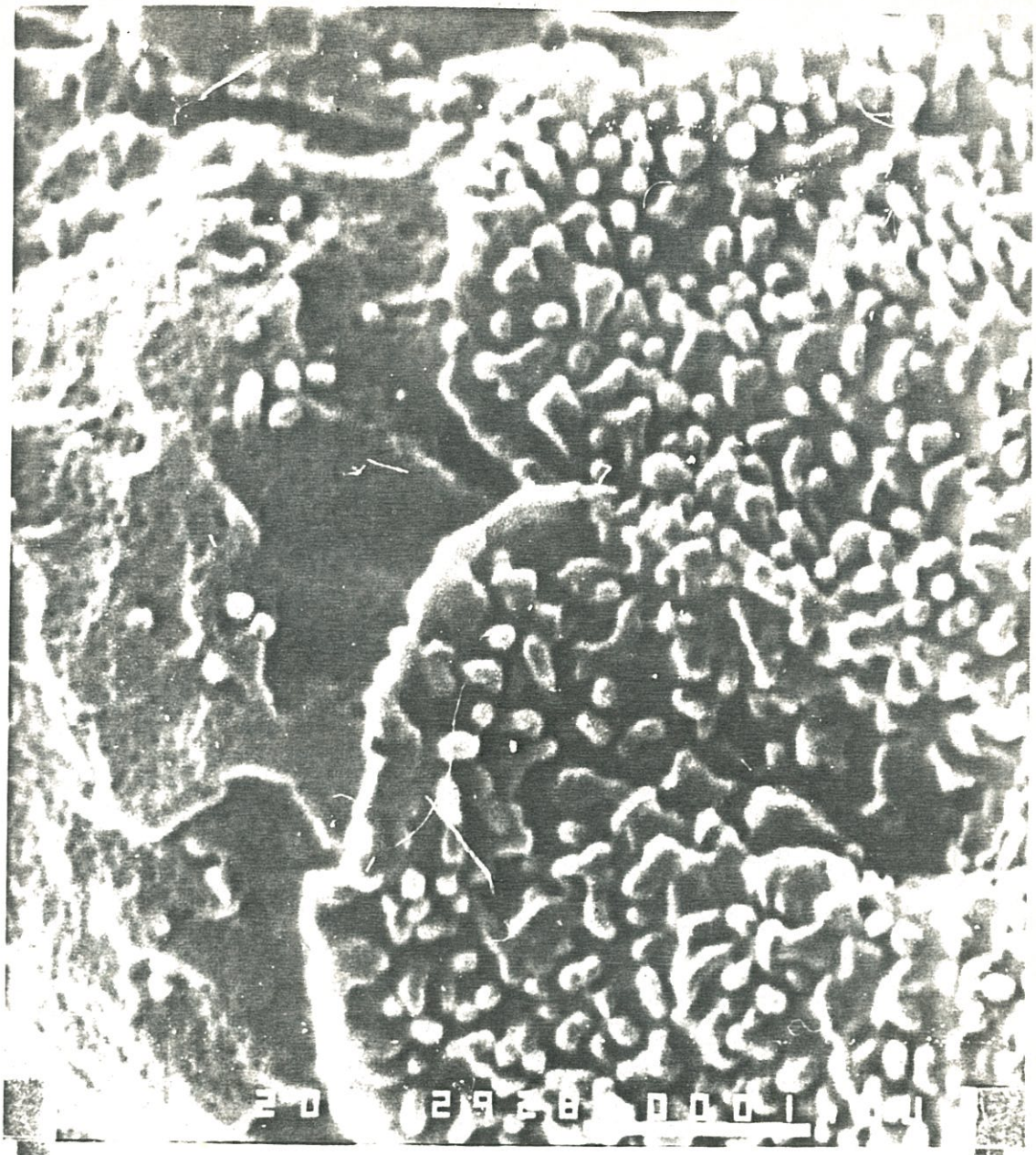


Fig. 3.39, magnification bar = 1.0μm, from the anterior lung region. Cells with pleomorphic microvilli and pitted surfaces are shown.



Fig. 3.40, magnification bar = 1.0μm, from the anterior lung region. Ciliary 'roots' appear to bridge the intercellular junction.

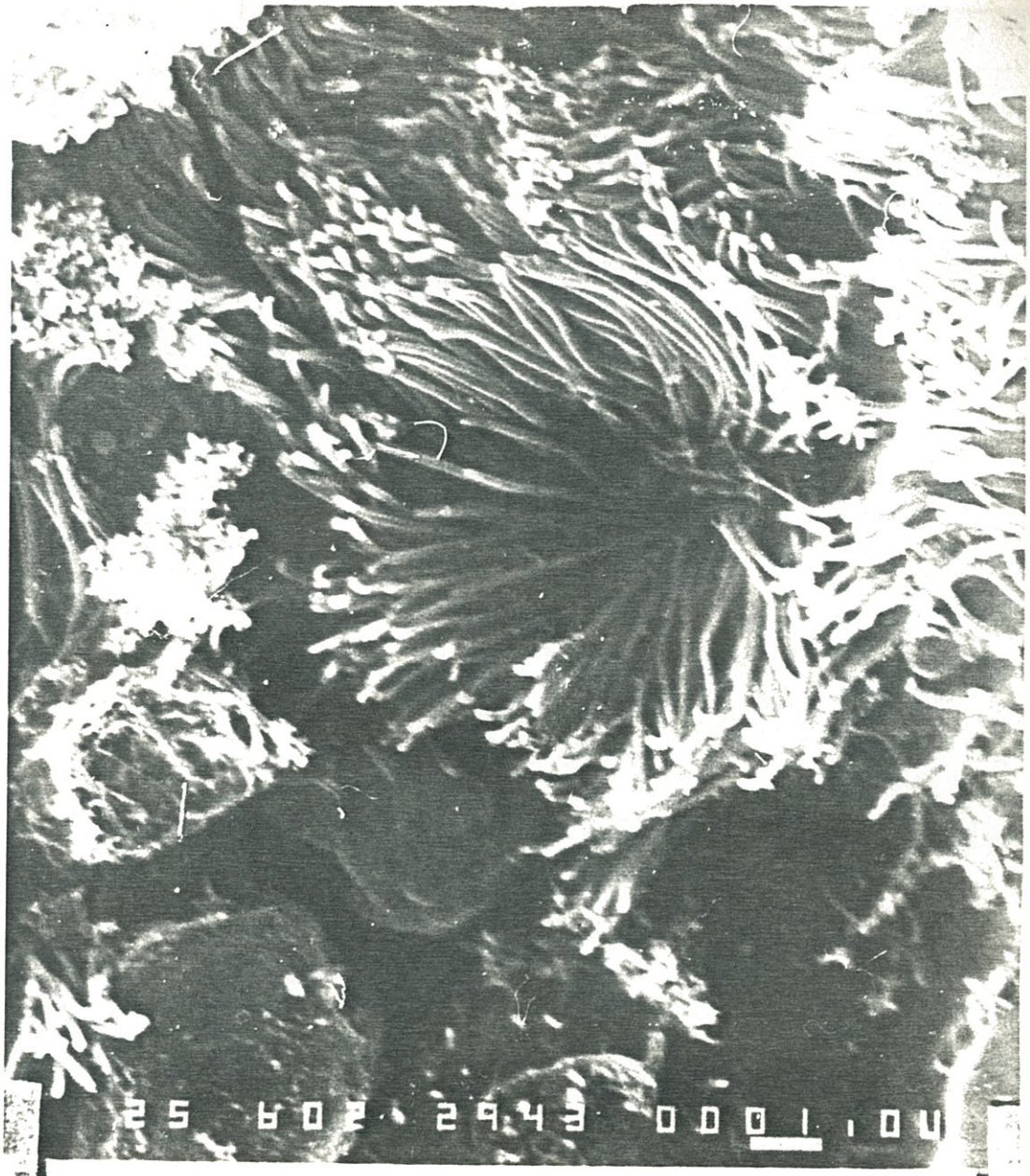


Fig. 3.41, magnification bar = 1.0μm, from the anterior lung region. A 'ciliary floret' adjacent to other ciliated cells.

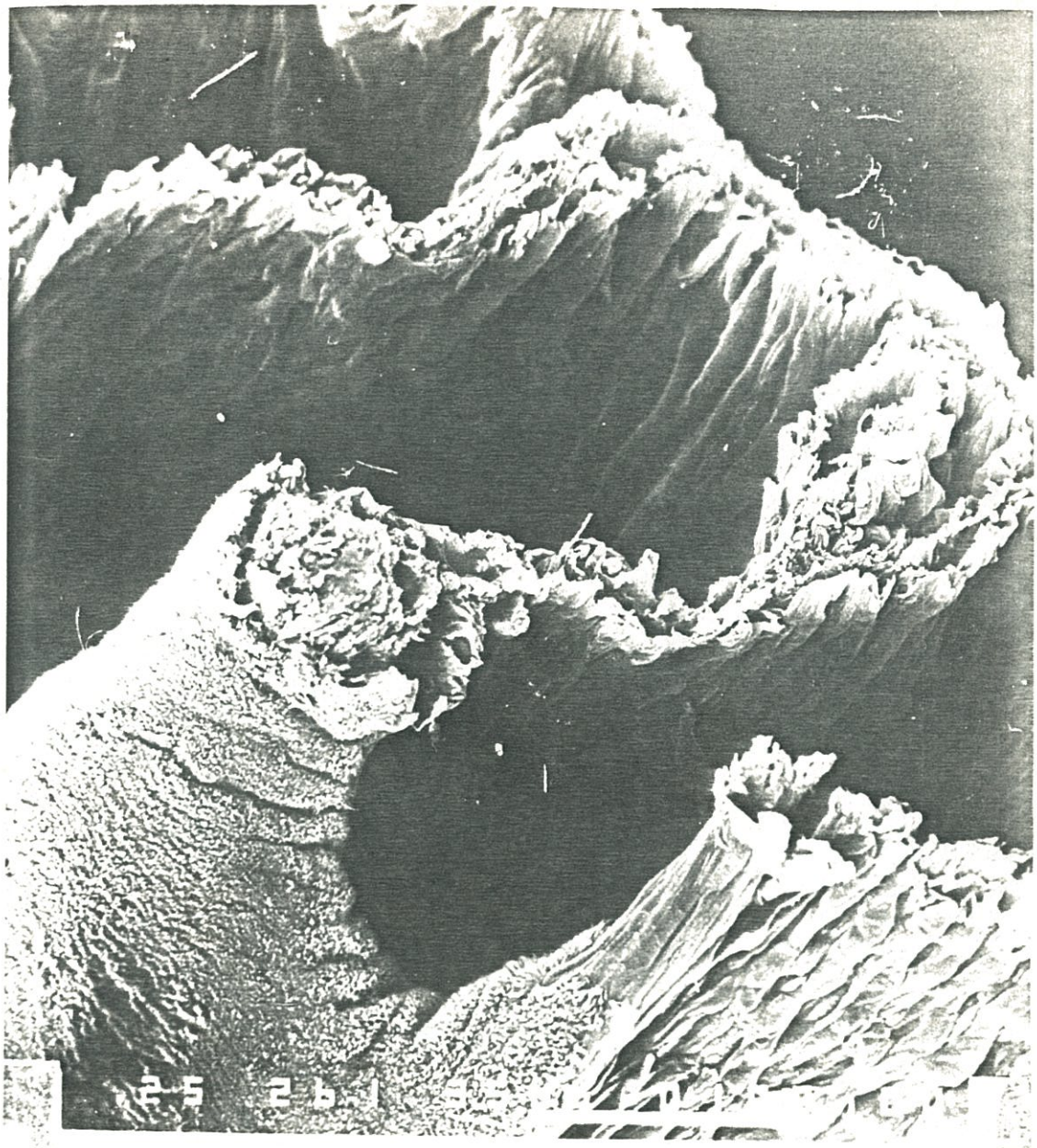


Fig. 3.42, magnification bar = 0.1mm, from the mid-lung region. Trabeculae and septa have been cut through to reveal some of the internal structure.

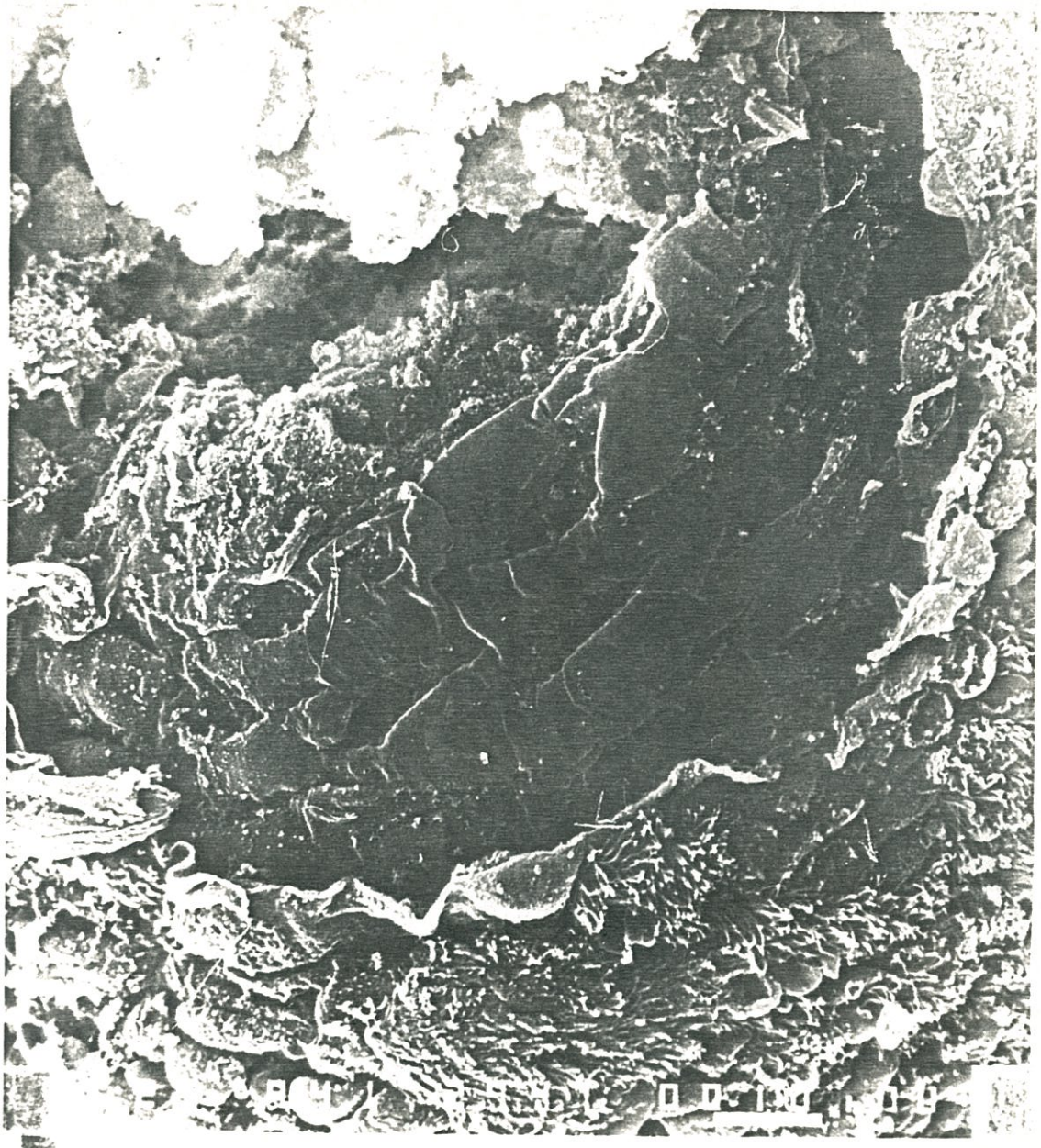


Fig. 3.43, magnification bar = 10μm, from the anterior lung region. The epithelial layer has been removed to show the capillary endothelium.



Fig. 3.44, magnification bar = 10µm, from the anterior lung region. A tangled mass of branched connective tissue fibres also several erythrocytes are shown within a trabecula.



Fig. 3.45, magnification bar = 10μm, from the anterior lung region. Several cell types are shown including capillary endothelium and possibly small macrophages in a trabecula.

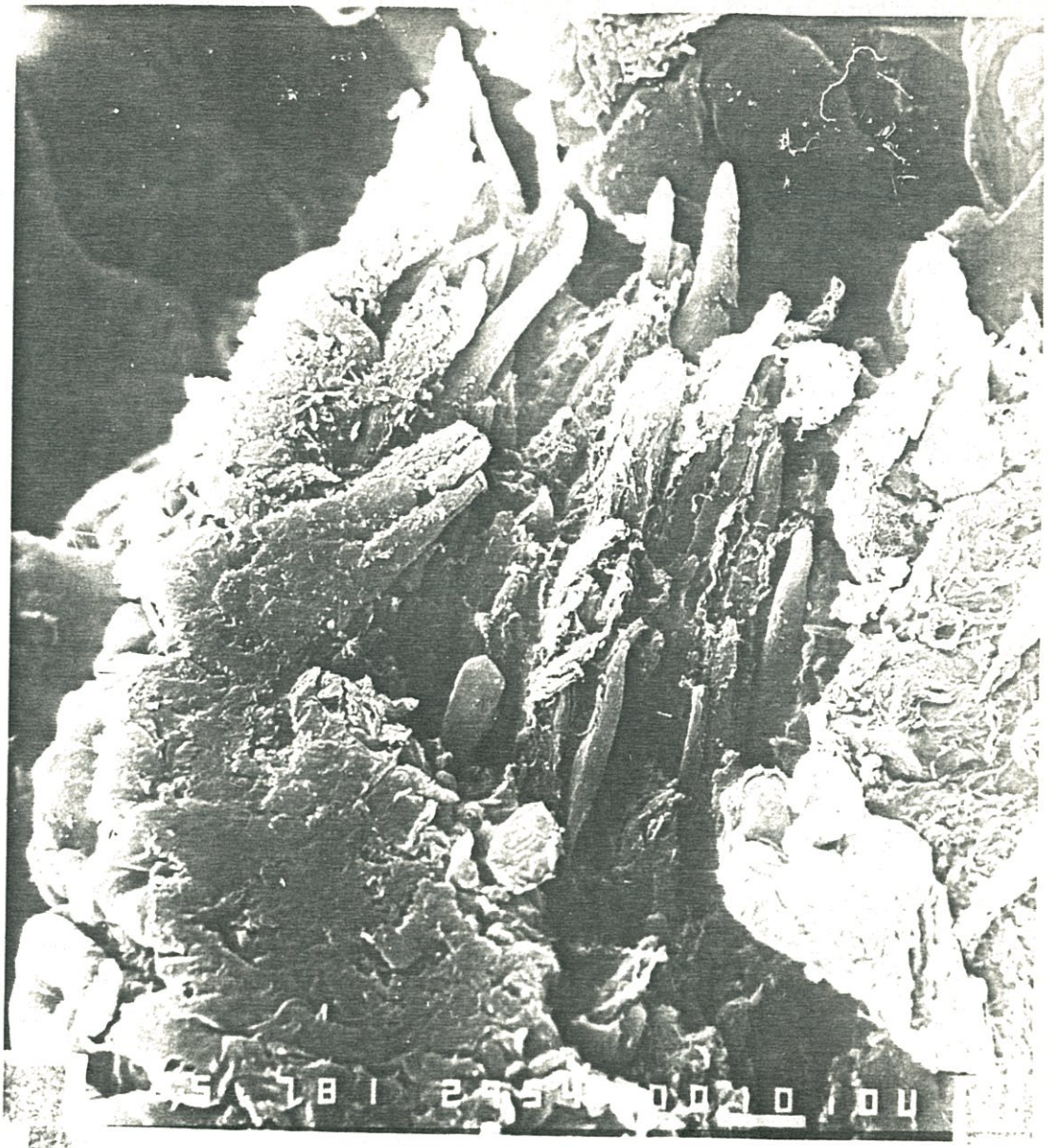


Fig. 3.46, magnification bar = 10μm, from the anterior lung region. This oblique'rough cut' section of a minor trabecula shows the ends of several smooth muscle fibres.

cells in the anterior lung region. The presence of anything equivalent to the interalveolar pores of mammals could not be shown in the septa. Various topographic features are also seen in fig. 3.39, the significance of the numerous micropores or pits shown on the left is not known. Although the junctions between many epithelial cells were marked by definite ridges other intercellular appearances were seen, in fig. 3.40, for example two such junctions are marked by depressions apparently bridged in some places by microvilli or ciliary rootlets.

Generally the appearance of the ciliated epithelium was quite normal indicating nothing but a co-ordinated 'sweeping' activity. However occasionally a rosette arrangement of cilia (or ciliary floret) was observed. This could have been just coincidence, on the other hand it could possibly throw light on the mechanism of ciliary action in that it may indicate a spontaneous unicellular origin of ciliary activity unco-ordinated with adjacent ciliary activity, (fig. 3.41).

In addition to examining the topographic features of the lung SEM was also used to observe several cut surfaces of lung tissue. Fig. 3.42 shows several septa and trabeculae cut through to show something of their internal structure. At higher magnification, (fig.3.43), a cut trabecula shows an extensive capillary network immediately beneath the ciliated epithelium which has been removed in the central area. Just beneath the capillary layer surrounding the trabecula, fig. 3.44, is a tangled layer of collagen fibres which give some tensile strength to the lung tissue. Fig. 3.45, shows several cell types within a trabecula probably including fibroblasts, lymphocytes, macrophages and endothelial cells of perfusing capillaries. In fig. 3.46, a bundle of smooth muscle fibres in the

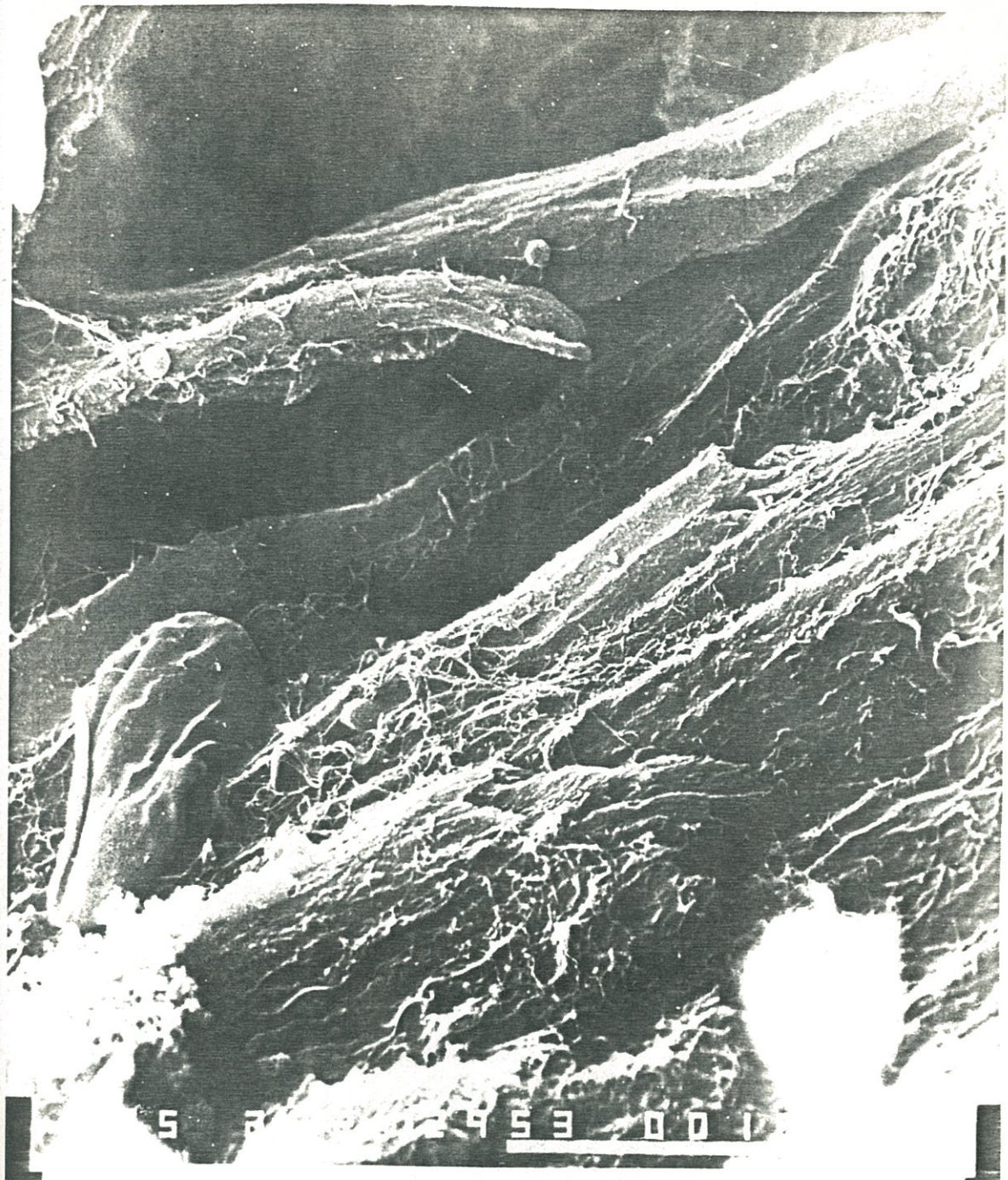
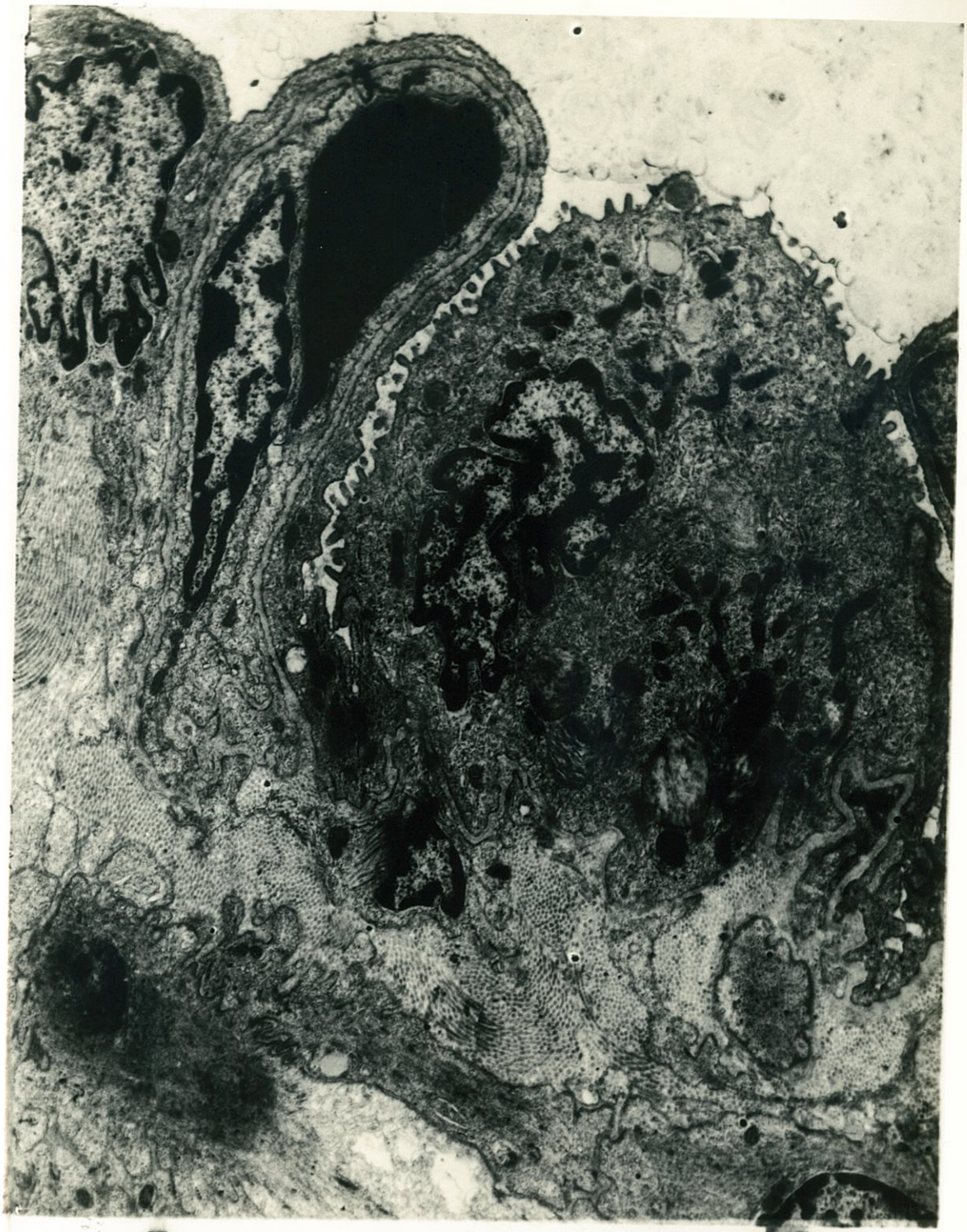


Fig. 3.47, magnification bar = 10μm, from the anterior lung region. There are several smooth muscle fibres present with an erythrocyte on the left, only a small amount of connective tissue fibres is present between the muscle fibres.



1.0μm

Fig. 3.48, from the posterior lung region. A nucleated erythrocyte fills a capillary projecting into the faveolar air space. Both the epithelial and endothelial cell layers appear pinocytotically very active.



1.0μm

Fig. 3.49, from the anterior lung region. Adjacent to the capillary is a large, type II pneumonocyte containing golgi bodies, mitochondria, ribosomes, vacuoles, multilamellar bodies and possibly secretion granules. Immediately beneath this cell is the nucleus of a fibroblast-like cell and numerous connective tissue fibres.

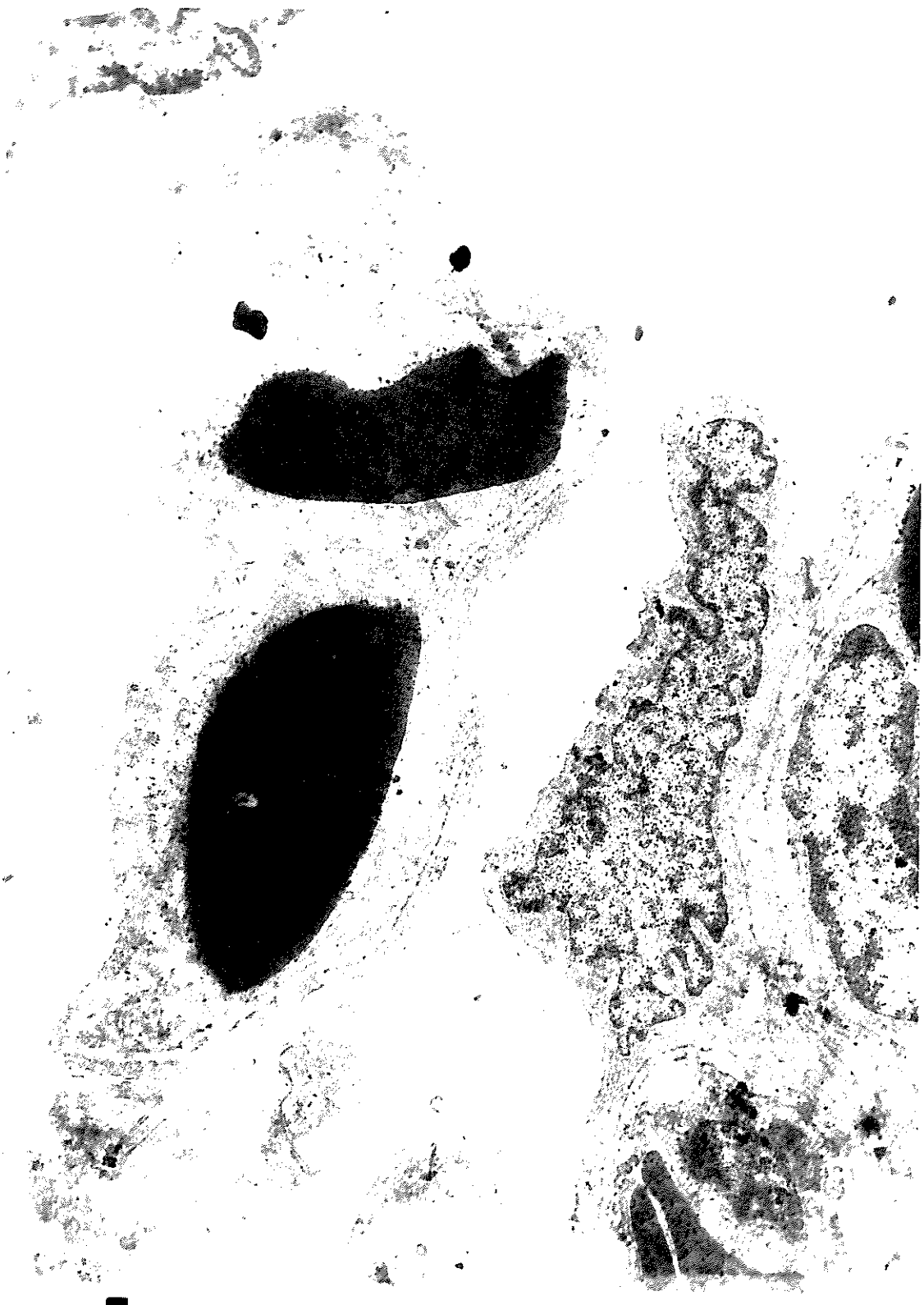


Fig. 3.50. A section through the base of a faveolus in the anterior region of the lung, showing an isolated capillary entirely surrounded by air space.



Fig. 3.51. Section of a minor trabecula in the anterior lung region. Numerous smooth muscle fibres (M) are sectioned, note junctional complexes between muscle fibres (arrows) also nerve

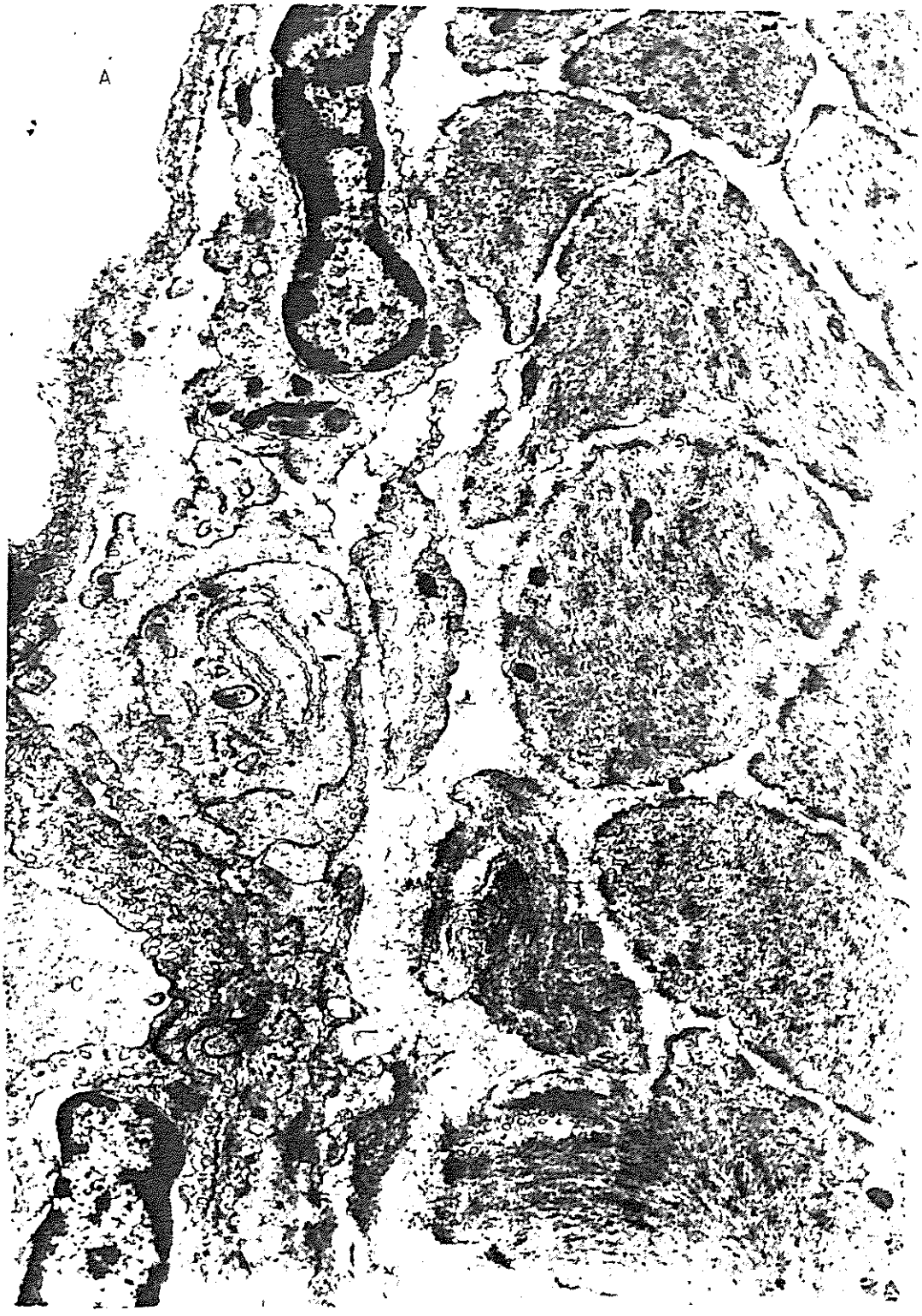
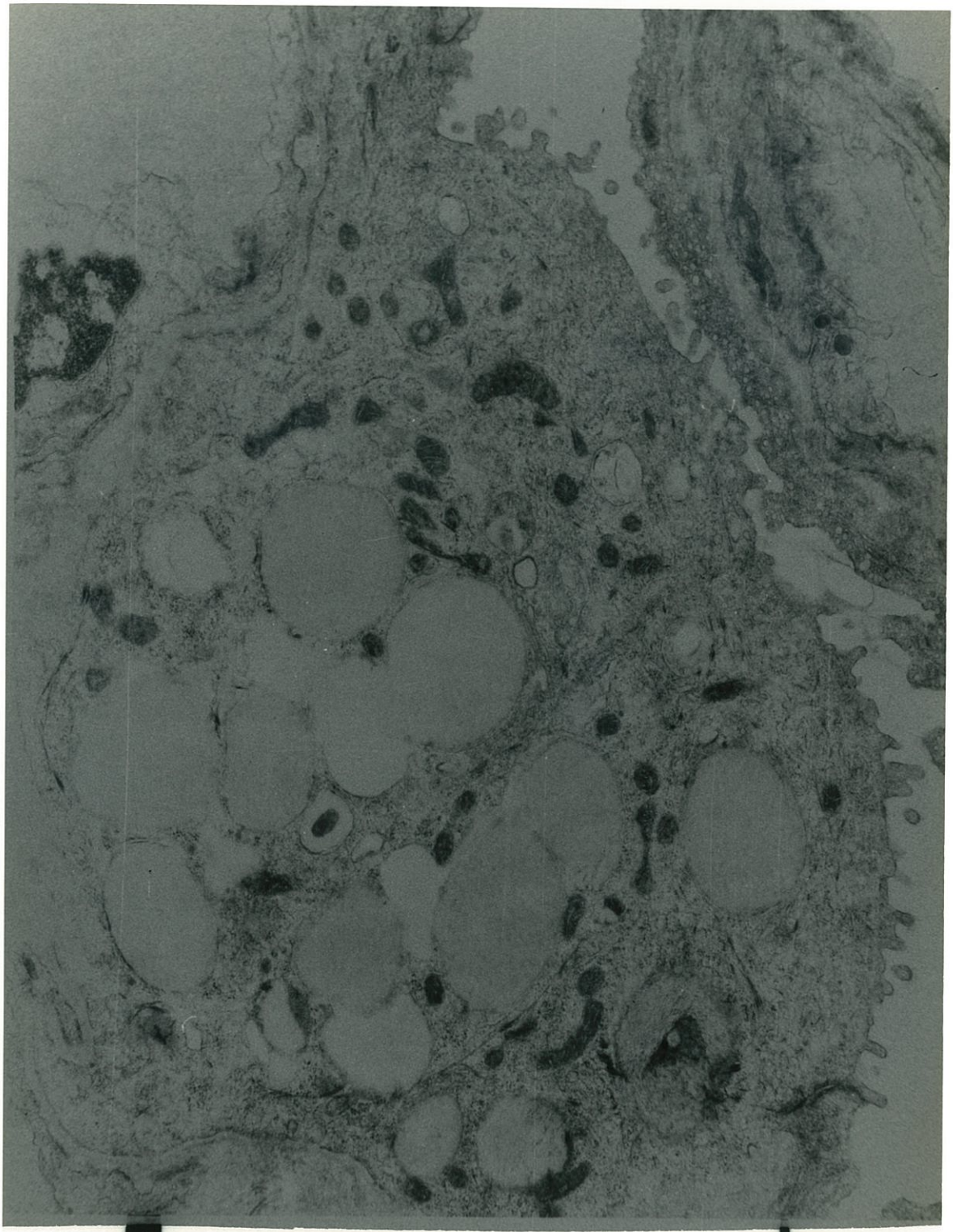


Fig. 3.52. Section of smooth muscle fibres from the anterior lung region. Although there are many junctional complexes (arrows) between the muscle cells no tight junctions were seen. A = air space, C = capillary.



1.0μm

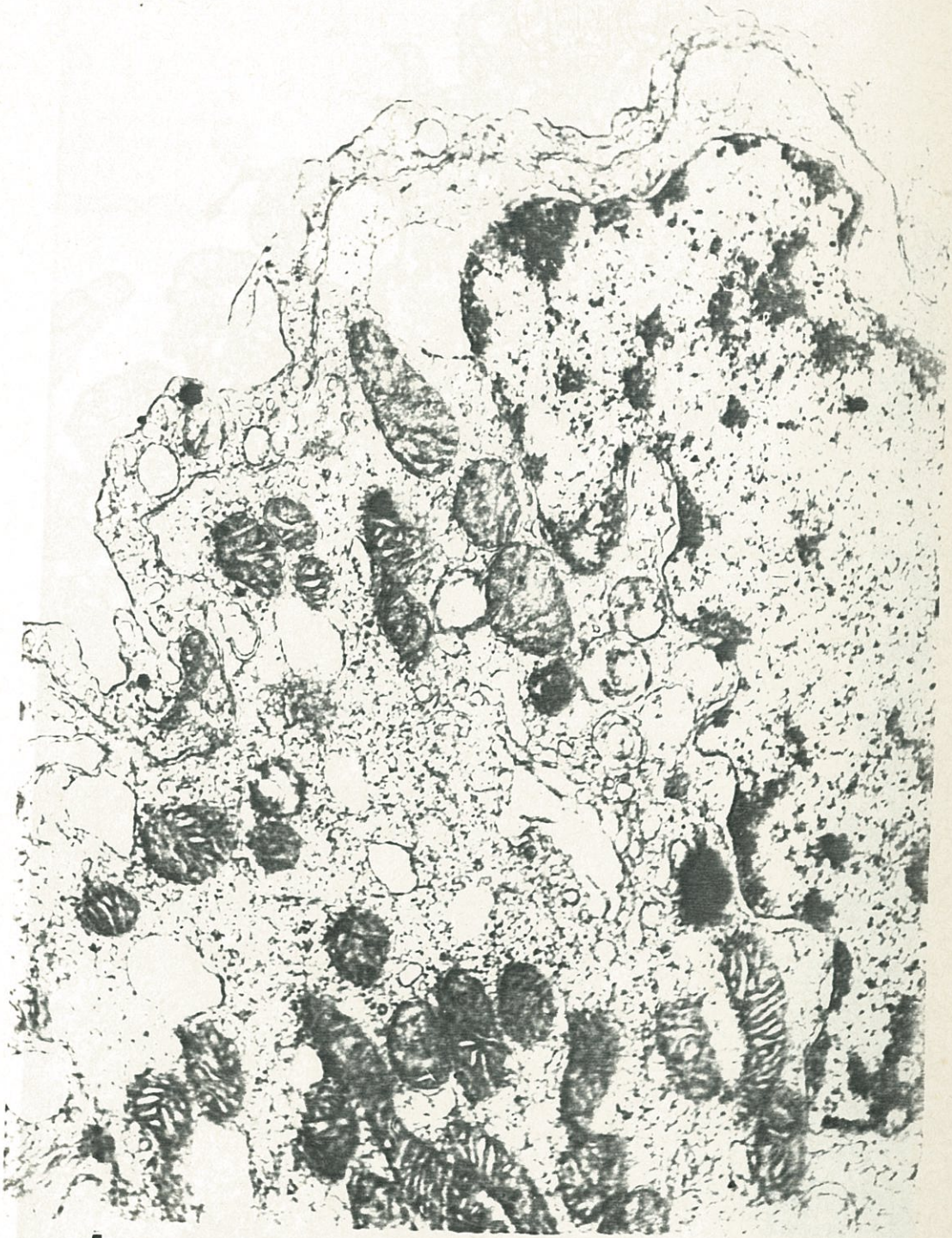
Fig. 3.53. Section through a septum in the posterior lung region. This large microvillous cell contains numerous secretory vesicles and signs of increased metabolic activity. C = capillary, A = air space.



Fig. 3.54. Section from the anterior lung region, showing the junctional complex between a microvillous cell and a smooth surfaced cell.

centre of a minor trabecula are shown projecting from the cut surface. A few collagen fibres are interspersed among the muscle fibres. Fig. 3.47, is a higher power magnification of smooth muscle fibres with a collagen fibre net also shown. An erythrocyte is seen on the left.

Although TEM is often more difficult to explain it was found to be useful in helping to determine the three dimensional structure of the respiratory tissues. Fig. 3.48, shows a cross section of a respiratory capillary with a nucleated erythrocyte (top right) and a leucocyte (centre left) filling the entire lumen. The minimum thickness of the air-blood barrier is about 0.4 μm. In this particular section the nuclei of both the epithelial and endothelial cells are sectioned but it can be seen that a large proportion of the capillary surface is exposed to faveolar air, (top). Fig. 3.49, also shows a similar respiratory capillary from the anterior lung region but also a large, active, type II pneumonocyte with numerous microvilli. Fig. 3.50 shows a capillary which appears to be free from the underlying surface and containing two erythrocytes. This could be just a capillary loop cut in tangential section although two other 'free' capillaries were observed in other sections. The minimum air-blood barrier thickness is about 0.4 μm. Figs. 3.51, and 3.52, are cross sections of minor trabeculae showing numerous smooth muscle fibres in various cross sections, numerous junctional complexes between fibres are seen, also several nerve fibres undoubtedly related to nervous control of trabecular contraction. Fig. 3.53 shows a large secretory cell with signs of increased metabolic activity. Numerous vesicles, mitochondria, golgi bodies, ribosomes and endoplasmic reticulum are visible. Fig. 3.54 shows a junctional complex between two epithelial cells. The function is probably mainly mechanical although there may also be some control



1.0 μm

Fig. 3.55. Section through the pleural surface of the anterior lung region. The mesothelium is very thin but metabolically active as is the type II pneumonocyte which demonstrates numerous mitochondria, various vesicles and a golgi body.

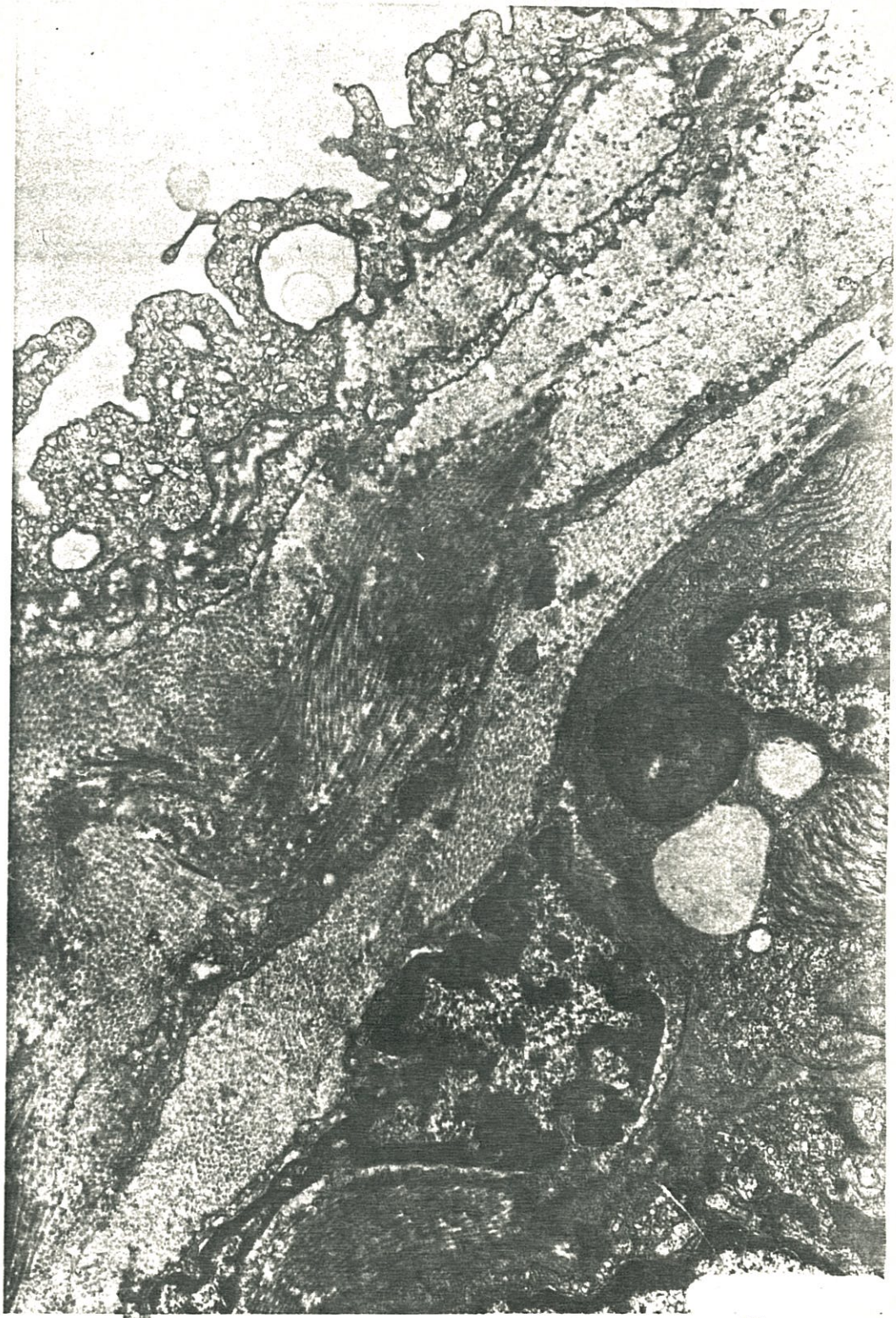


Fig. 3.56. Section through the pleural surface of the anterior lung region. In some areas as here the mesothelial layer overlays a layer of connective tissue fibres. A = air space, F = connective tissue fibres, N = nucleus of fibroblast-like cell.

mechanism related to the passage of material between cells or between the free surface and the subepithelial region.

Although SEM was used only briefly to observe the pleural (or serosal) surface of the lung there were a few features of interest. The surface was mainly smooth with few microvilli the mesothelial cells were shown to cover a network of collagen fibres with numerous spaces (probably lymph spaces) around the blood vessels. In fig. 3.55 there appears to be little more than a basement membrane between the mesothelium and the type II pneumonocyte below. Fig. 3.56, on the other hand shows several large bundles of collagen fibres passing obliquely across the field of view between the serosal mesothelium in the upper left hand corner and the epithelial and fibroblast cells in the lower right hand corners.

3.4 DISCUSSION

The general requirements for a lung include the possession of moderate mechanical strength to maintain the integrity of the lung as a functional organ and to maintain the continuity of the pulmonary circulation. An efficient gaseous exchange mechanism with a low diffusion resistance must also be provided. These opposing requirements result in an anatomical situation in which the number of pulmonary capillaries are greatly increased and are very thin walled thus reducing diffusion resistance. The large number of capillaries in parallel also results in a reduced pulmonary arterial blood pressure requirement.

According to the law of Laplace the equation linking circumferential tension (T_c) and transmural pressure (P_t) in a cylindrical vessel is given by;

$$T_c = kR.P_t$$

where R is the radius of the vessel and k is a constant. From this equation it can be seen that a reduction in diameter effects a proportional reduction in tension for a given transmural pressure. The mean external capillary diameter (excluding collapsed capillaries) was 14.1 μm. This value is about twice the value for many mammals. Measurements indicate that the size of the Blue-tongued lizard's erythrocyte is over twice that of most (non-nucleated) mammalian erythrocytes thus setting a proportionately greater lower limit to capillary diameter. This increased capillary diameter may limit the optimum and/or maximum pulmonary systolic blood pressure.

No rigorous stereological methods for morphometric analysis were undertaken, however a selection of measurements were made

indicating that the minimum observed thickness of the air/blood barrier was about 0.25 μm and the mean thickness was 0.6 μm. This mean is considerably less than in human lung (2.2 μm) and most other mammals (range 0.6 - 1.5 μm), (Gehr, Bachofen and Weibel 1978). From the point of view of mechanical strength requirements a large diameter capillary would require a stronger and therefore thicker wall. The fact that the capillary wall is not thicker than in many mammals may indicate that the Blue-tongued lizard has a low systolic blood pressure.

The capillary beds seen beneath the ciliated trabecular epithelium, e.g. fig. 3.44, may be related to the gas exchange requirements of the trabecular smooth muscle fibres. Although some gas exchange may occur through a thin ciliated epithelium the fact that patches of capillaries were exposed among areas of ciliated epithelium, e.g. fig. 3.28 could indicate that some oxygenated blood is carried directly from exposed pulmonary capillaries to the deeper trabecular smooth muscle capillaries.

Because the faveoli are large (compared to mammalian alveoli) and because they are often columnar in shape and attached directly to the external pulmonary membrane it might be suspected that surfactant would have little place in stabilizing such a system even if there are large relative changes in faveolar dimensions as a result of trabecular smooth muscle activity. The fact that surfactant is present in moderate amounts suggests there may be other reasons for its presence, e.g. it could well be a significant factor in optimizing the compliance of the lung and therefore respiratory muscle work loads. How such a system is controlled is open to speculation.

In relation to respiratory surface area it is estimated that in the anterior lung region approximately two thirds of the available septal surface area was occupied by superficial capillaries or capillaries that were covered by only a thin layer of e.g. ciliated epithelial cells, which may allow a certain amount of gaseous diffusion. This factor plus the increased surface area generated by the capillaries being frequently raised from the septal surfaces would give a respiratory surface area approaching 100% of available septal surface. In the posterior lung region the value probably lies closer to 50% but this value may not be very meaningful because of 1) probably reduced gaseous exchange in this area and 2) possible shunting of blood around this part of the lung.

Whether or not there is a direct mechanism for coupling ventilation and perfusion remains to be seen however the evidence from electronmicroscopy particularly SEM indicates a strong possiblity for a mechanism related to trabecular smooth muscle contraction patterns. This mechanism could be influenced by either intrapulmonary chemoreceptors or intravascular chemo-receptors (or both). The longitudinal arrangement of major pulmonary blood vessels would also allow a simple shunting of blood to facilitate matching of ventilation and perfusion.

PART C

SOME FUNCTIONAL ASPECTS OF RESPIRATION

Introduction and General Objectives

The first step in oxygen transport is tidal ventilation. This is followed by passive diffusion of gas molecules through the respiratory epithelium, then convective movement or circulation of the blood followed by a second passive diffusion phase where the oxygen passes from the capillaries to the mitochondria and other sites in the tissues where it is used. This part of the study is concerned mainly with the first two stages and is of particular interest not only because of the taxonomic position of reptiles but also because of the wide range of fluctuations in the parameters affecting metabolism and the physiological plasticity which enables the organism to cope so well with these variations.

The major objectives were:

1) to characterise the respiratory cycle

2) to analyse the variations of respiration

3) to analyse variation of respiratory rate with temperature

4) to analyse variations of respiratory rate and heart rate

5) to help clarify the mechanisms of respiratory control.

SECTION IV THE RESPIRATORY CYCLE AND RATE OF RESPIRATION

4.1 Introduction

Observation of respiration in a Blue-tongued lizard, even for a brief period, demonstrates that the respiratory movements are quite different from those generally associated with terrestrial mammals. The respiratory cycle usually consists of a brief ventilatory period followed by an apnoeic period of irregular duration. Tidal volumes and flow patterns may also be rather irregular even when the animal is at rest. Respiration is also complicated by rhythmic gular movements, (figs 4.1 and 4.10), which are usually (but not invariably) synchronized with the ventilatory movements. According to Krogh (1941), Naifeh, Huggins and Hoff (1970) and Gans (1976), gular movements or gular pumping (in some reptiles at least) is associated with olfactory or social behavioural functions rather than with respiration. Reptiles have no diaphragm muscle however they are generally regarded as aspiration breathers, Gans (1976, p. 229). Gentle lateral compression of the post axillary region, thus preventing aspiration breathing, soon results in the institution of positive pressure breathing resulting from gular pumping. This occurs in numerous species of skinks (pers. obs.) and implies that the lizard is able to block the nasal expiratory channels presumably by using the tongue. Some lizards produce defensive inflation of the lungs at least partially by gular pumping (pers. obs) although it is not known whether this is the only situation where gular pumping is normally used.

4.2 Materials and Methods

The majority of recordings were made on adult Blue-tongued lizards of the species T. s. scincoides and T. nigrolutea. Some comparative observations were also made on T. gerrardii, T. rugosus and Ctenotus robustus Storr.

Most recordings were made using a mercury in silastic tubing strain gauge (after Whitney, 1953). Changes in electrical resistance were recorded on a Physiograph or Hewlett Packard recorder. An attempt was also made to use a phototransducer attached directly to the body wall to record respiratory movements. Both methods are relatively non-invasive and gave reproducible qualitative and semi-quantitative results.

Electrocardiographs (ECGs) were recorded using three needle electrodes (centre one earthed) placed in the skin over the heart and recordings were made on a Physiograph recorder.

Temperatures were monitored continuously using a copper-constantan thermocouple in the cloaca and recorded on the Physiograph recorder or taken manually from a digital read-out thermometer (custom made and calibrated to $\pm$ 0.1°C). The animals were kept in the laboratory at least overnight before recordings were made to stabilize body temperature and minimize sympathetic activity. During recording the animal was either placed in a darkened box or had its head covered by a cloth to reduce possible effects of visual disturbance.

In order to obtain recordings of respiration outside the normal range of laboratory temperatures lizards were placed in

large insulated containers and either cooled to a minimum temperature of about 5°C or warmed to a maximum of about 35°C and then allowed to return slowly (over a period of several hours) to room temperature while recordings were being made as described above.

Quantitative respiratory flow patterns and tidal volumes were determined using a body plethysmograph and a Greer differential pressure spirometer and recorded using a Hewlett-Packard two channel recorder. The plethysmograph consisted of a rigid polymethylmethacrylate tube approximately 600 mm long and 100 mm in diameter, one end of which was sealed and the other covered by a rubber diaphragm with a hole cut in the middle which provided a snug fit around the posterior part of the lizards head. The seal was improved by using a little high vacuum grease (some other sealants are more volatile and liable to affect respiration). A small nozzle attached to the side of the tube provided for connection to the spirometer and a second port with sealing ring allowed passage of electric leads. The flow was calibrated using individually calibrated G.P. Meer (U.K.) rotameters. Volumes were also cross checked using 1 mL, 2 mL and 5 mL disposable syringes.

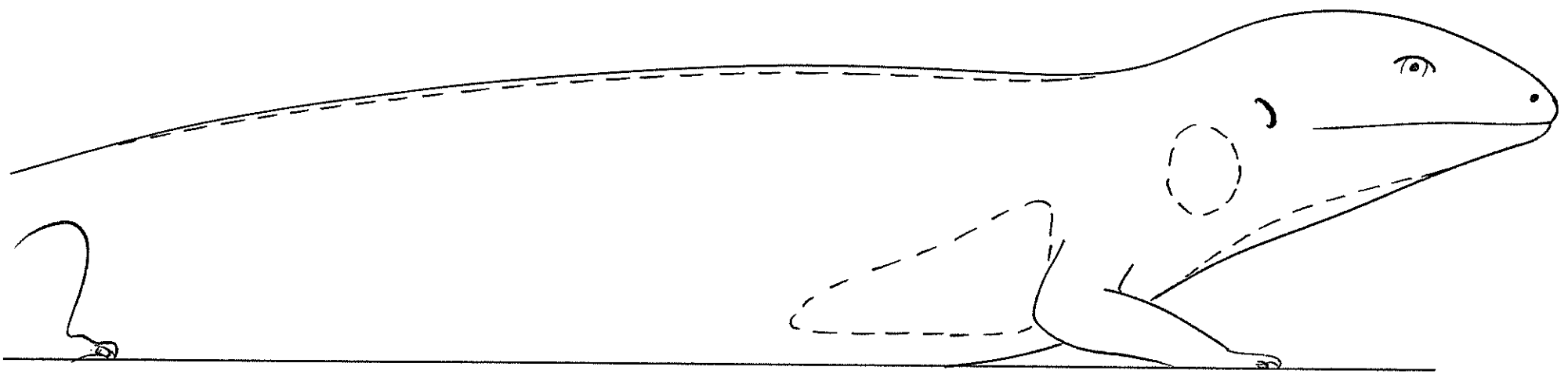


Fig. 4.1. Showing areas of the lizard body where significant movement occurs during quiet respiration. If the body is held off the ground then the venter may be seen to move up and down, quite commonly however the lizard just slides along the ground so that general expansion/contraction movements of the body are seen as slight vertical movements of the dorsum.

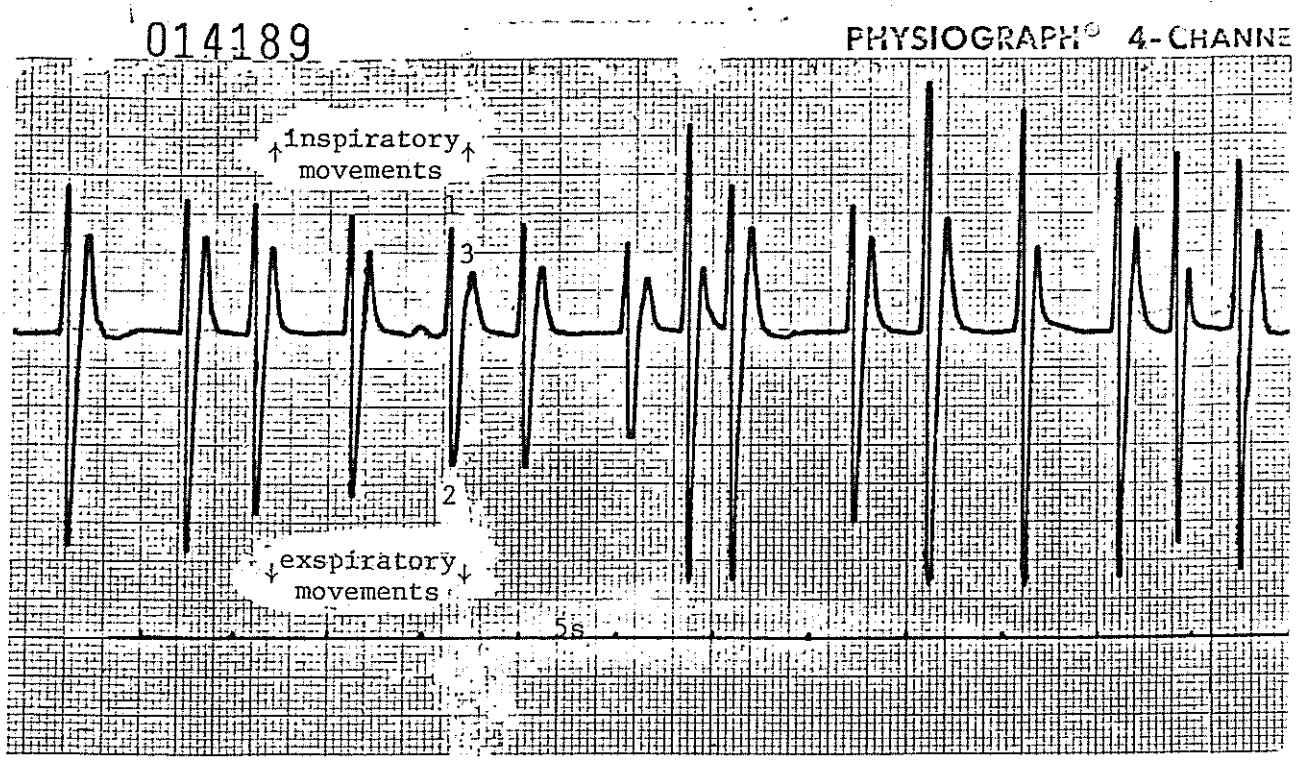


Fig. 4.2. Showing movements usually accompanying quiet respiration. T. nigrolutea, adult at about 22°C. The initial expiratory movement (1) lasts about 0.6s (± 0.3s). The inspiratory movement (2) lasts about 1.2s (± 0.4s). The second expiratory movement (3) may last about 2s but is very variable. The animal's head was not covered during this recording so that variations in amplitude could be partly due to visual stimulation. The recording was made using a phototransducer in the mid-lateral post axillary region.

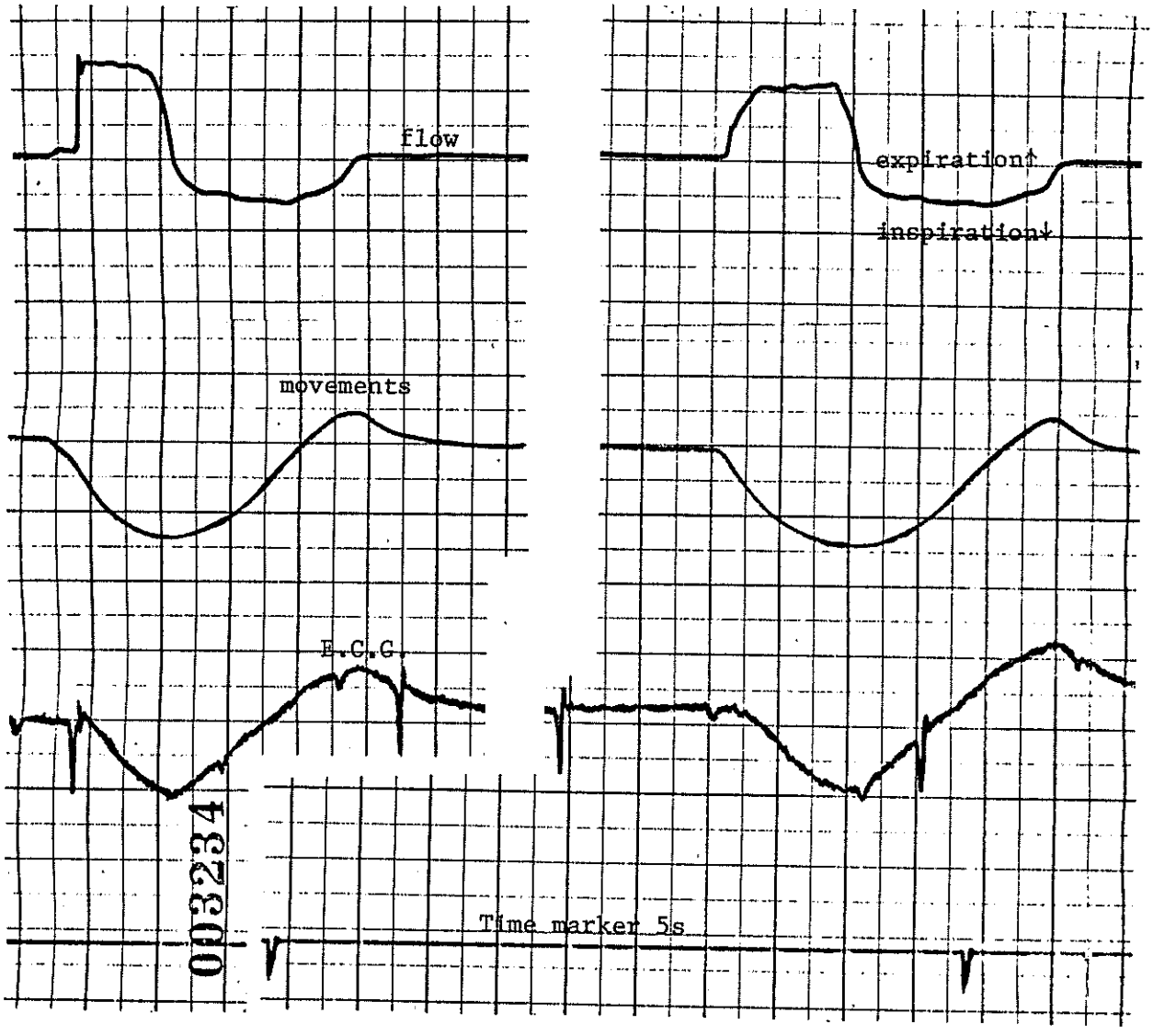


Fig. 4.3. Respiratory air flow, respiratory movements and electrocardiograph of adult T. nigrolutea, showing two consecutive respirations. In the first there is a sudden onset to expiration probably indicating that the glottis did not open until after the onset of expiratory effort. The rise of expiratory flow in the second respiration is smoother indicating that the glottis opening was synchronised with expiratory effort. (Respiratory movements here were recorded using a mercury strain gauge). Respiration rate is 12/minute, heart rate is 24/minute.

4.3 Characteristics of the Respiratory Cycle

Observation and recording of respiratory movements indicate that the post-axillary region is most active in producing pulmonary ventilation (fig. 4.1). The gular region movements although active are probably only of minor significance in quiet respiration. The general changes in body girth are also quite small (usually less than 1 mm) and are probably only of major significance when large changes in lung volume occur, e.g. in threat display hissing and defensive lung inflation. The post-aural movements are quite minor and are probably passive, resulting from changes in intrapharyngeal air pressure (there is only a thin layer of skin overlying the pharynx in this region, see fig. 1.8).

In the Blue-tongued lizards as in many other reptiles the ventilatory period commonly begins with a short exhalation followed by a slightly more prolonged inhalation, this in turn is followed by an apnoeic period (figs. 4.2 and 4.3). This basic pattern is however subject to considerable variation, some aspects of these variations are considered here.

4.3.1 Variation of Resting Tidal Volume

Although it might be expected *a priori* that there would be some correlation between respiratory rate and metabolic rate, it might also be suspected in view of the irregularity of resting respiration that the lizard could conceivably compensate for irregularities in rate of respiration by reciprocal changes in tidal volume. Therefore several experiments measuring respiratory rate and tidal volume were undertaken to investigate this possibility.

In one experiment an adult lizard, TA31, at a body temperature of 32°C had a mean resting respiratory rate of 17.7 min^{-1} and a mean heart rate of about 90 min^{-1}.

In this series of recordings the tidal volume and the respiratory interval were measured for each of 142 consecutive respirations. The mean tidal volume was 5.3 ± 1.8 mL (± one standard deviation). The correlations of the tidal volume to the preceding respiratory interval and to the following respiratory interval were both low (0.34 and 0.39 respectively). In two other series of recordings the correlations were closer to zero although both were positive.

In several experiments resting minute volume was measured at intervals. At a body temperature of 32.5 ± 0.5°C the mean minute volume was found to be 83.8 $mL.min^{-1}$ with a 95% confidence interval of ± 20.7 $mL.min^{-1}$.

4.3.2. Variation of Respiration Rate at Constant Temperature

An analysis of respiratory frequency and its variations was carried out over several thousand respiratory cycles in an attempt to throw light on the respiratory triggering and control mechanisms.

Because respiration is so irregular it was first decided to check statistically the variation in rate both on a breath to breath basis and also rate changes measured over set periods of time.

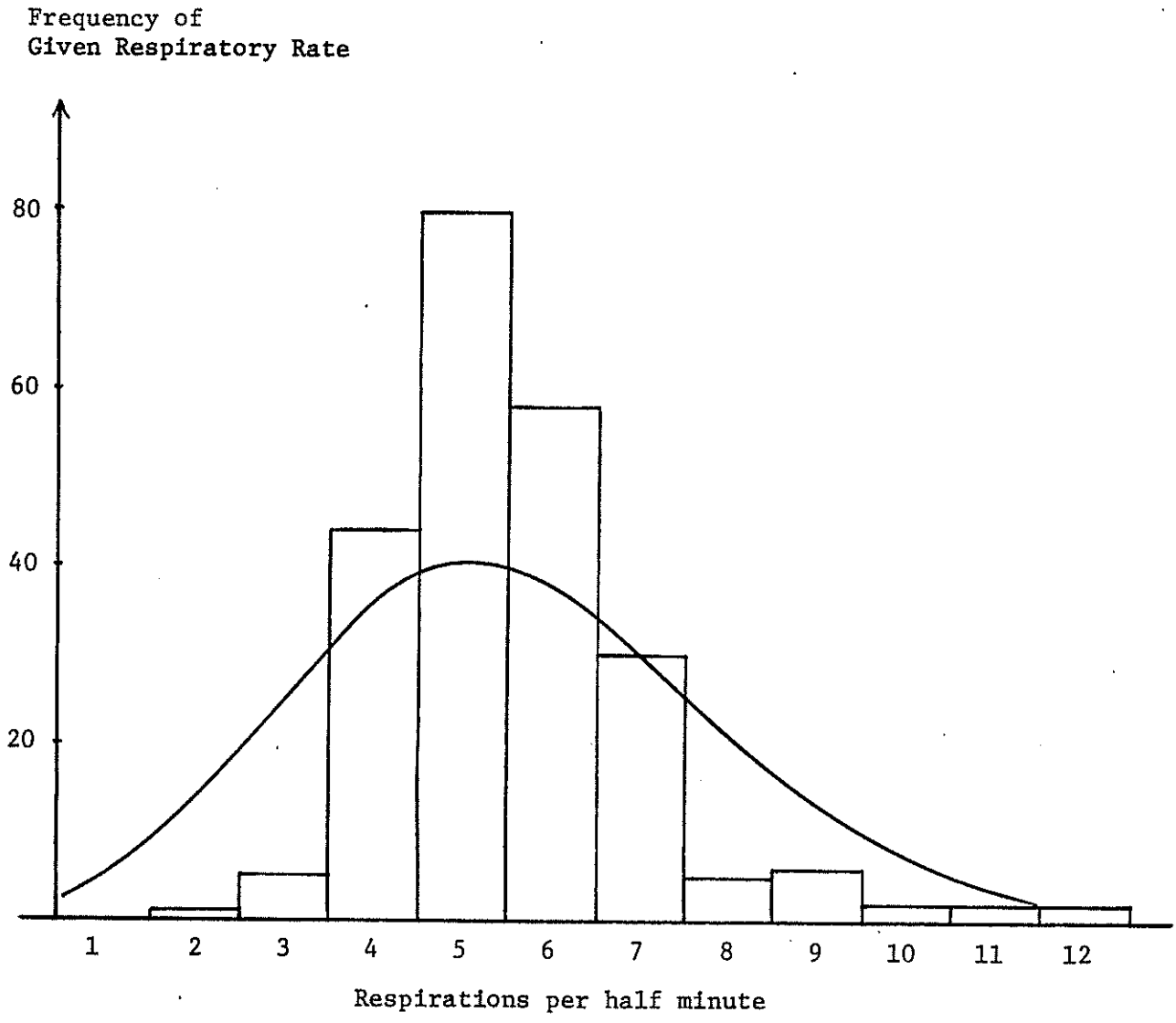


Fig. 4.4. Analysis of resting respiration rate of an adult T.s.scincoides at a constant body temperature ($23.5^{\circ}C \pm 0.5^{\circ}C$). The number of respirations occurring in consecutive half minute intervals was recorded giving the frequency histogram shown. The equivalent Poisson distribution is superimposed. There is marked leptokurtosis.

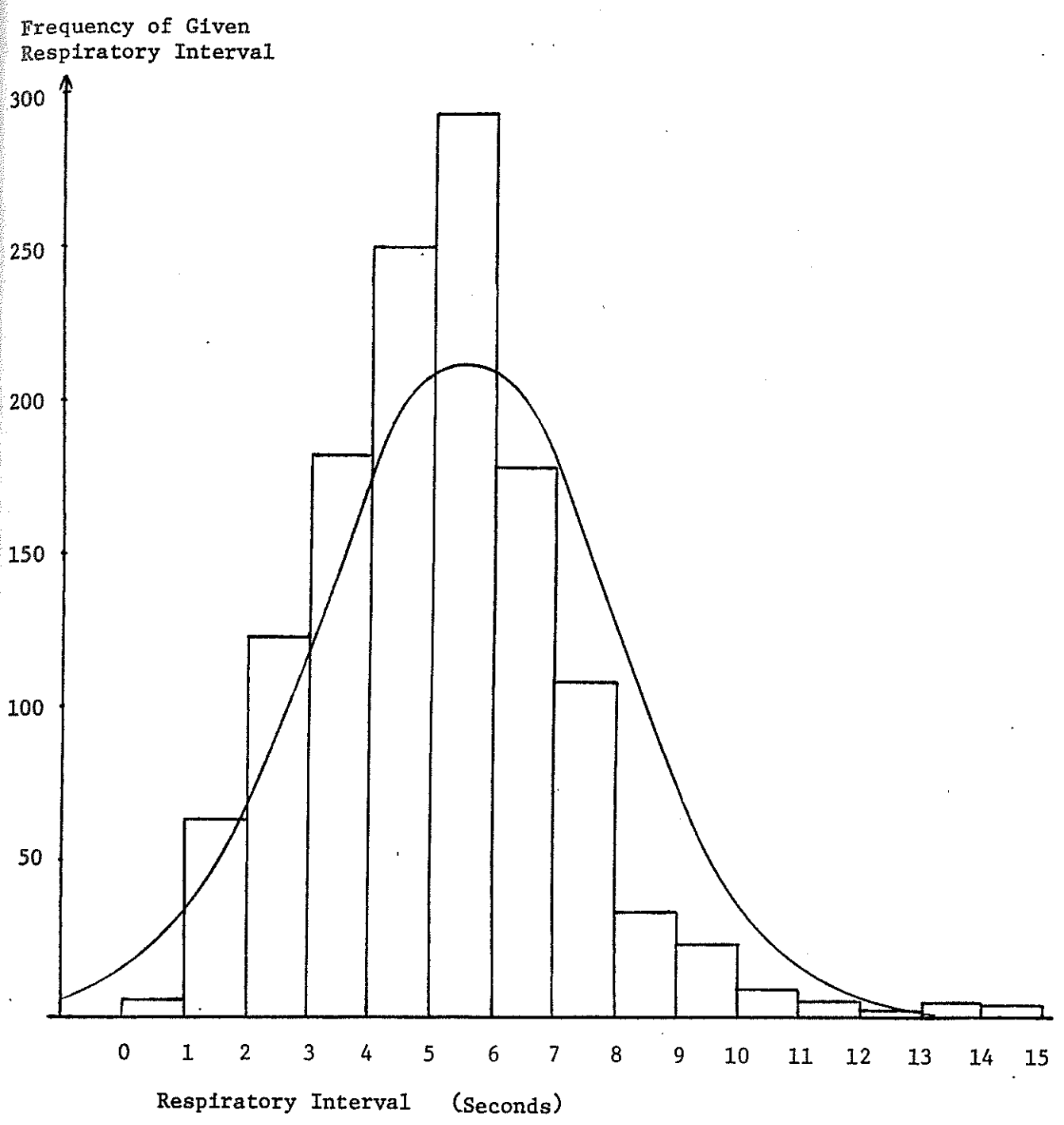


Fig. 4.5. Analysis of the respiratory interval at a given temperature ($23.5^{\circ}C \pm 0.5^{\circ}C$). Superimposed on the frequency histogram is the standard curve having the same sample size and standard deviation. The respiratory interval distribution has a positive skew (with the tail to the right), it is also moderately leptokurtic.

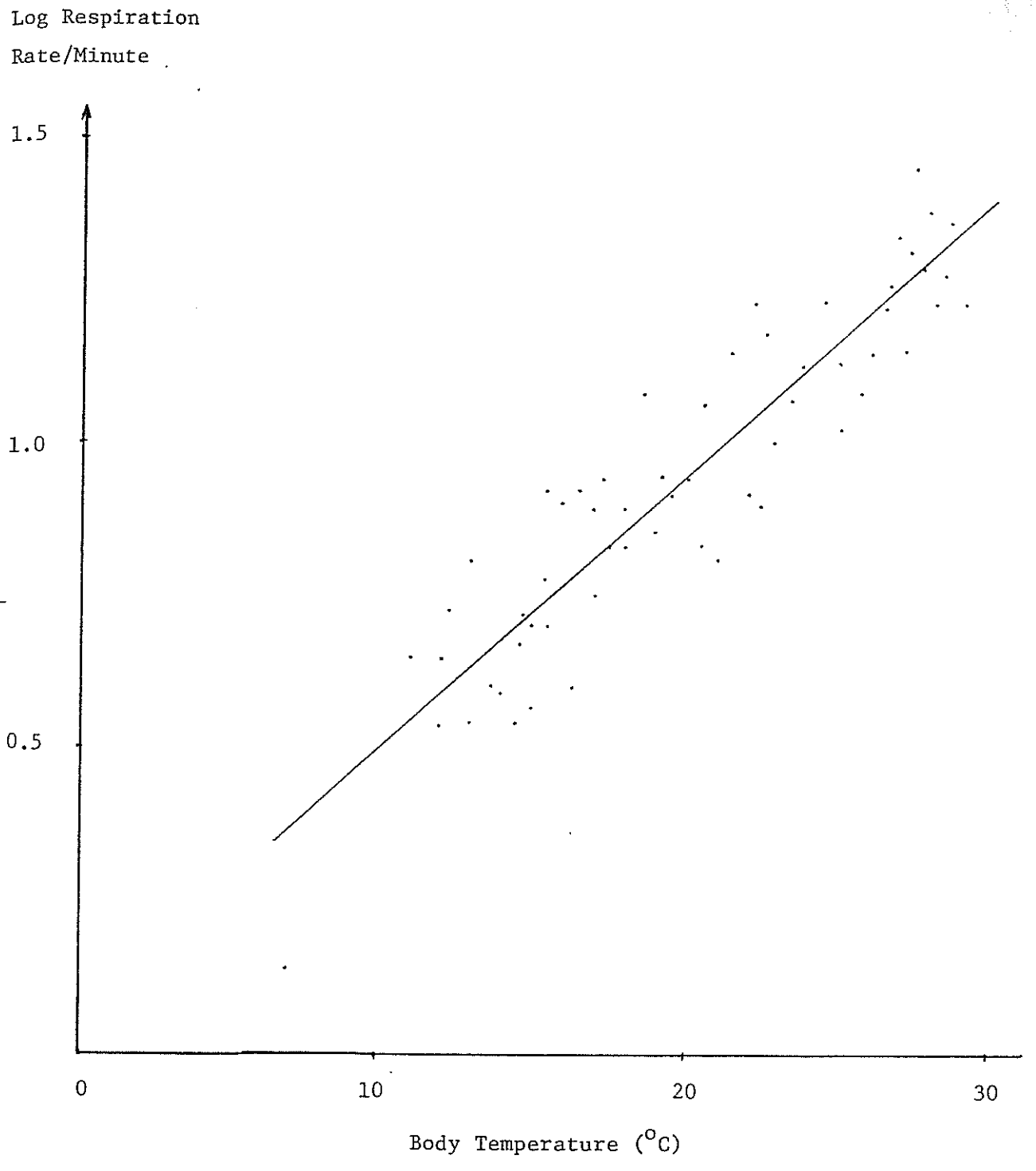


Fig. 4.6. Regression analysis of log respiration rate on body temperature. There is a close correlation between the two parameters (r = 0.91).

The respiratory rate was measured over several hundred half minute intervals and a frequency histogram was generated, (fig. 4.4). This distribution was then tested against a Poisson distribution using the chi-square test. This distribution was rejected at well below the 0.005 level of significance indicating that individual breaths are not random events, independent of each other.

Over one thousand individual respiratory intervals (timed from the start of one ventilatory cycle to the start of the next) were recorded and measured to the nearest 0.1 s and a frequency histogram was plotted (fig. 4.5) and tested against a normal distribution. The hypothesis that the sample was taken from a normally distributed population had to be rejected at well below the 0.005 level of significance using the chi-square test.

4.3.3 Variations of Respiration Rate with Temperature

The results of analysis of the variation in resting respiration rate with body temperature are shown in fig. 4.6. As expected the correlation of log respiration rate against body temperature was quite high 0.91 although the Q_{10} of 2.7 was higher than the value of around 2.0 which is usually predicted (Gordon, 1977).

4.3.4 Variation of Respiration Rate with Heart Rate

During one experiment at a body temperature of 32°C the heart rate was 115 per minute near the start but soon settled down to about 82 to 86 per minute. This rate would fluctuate

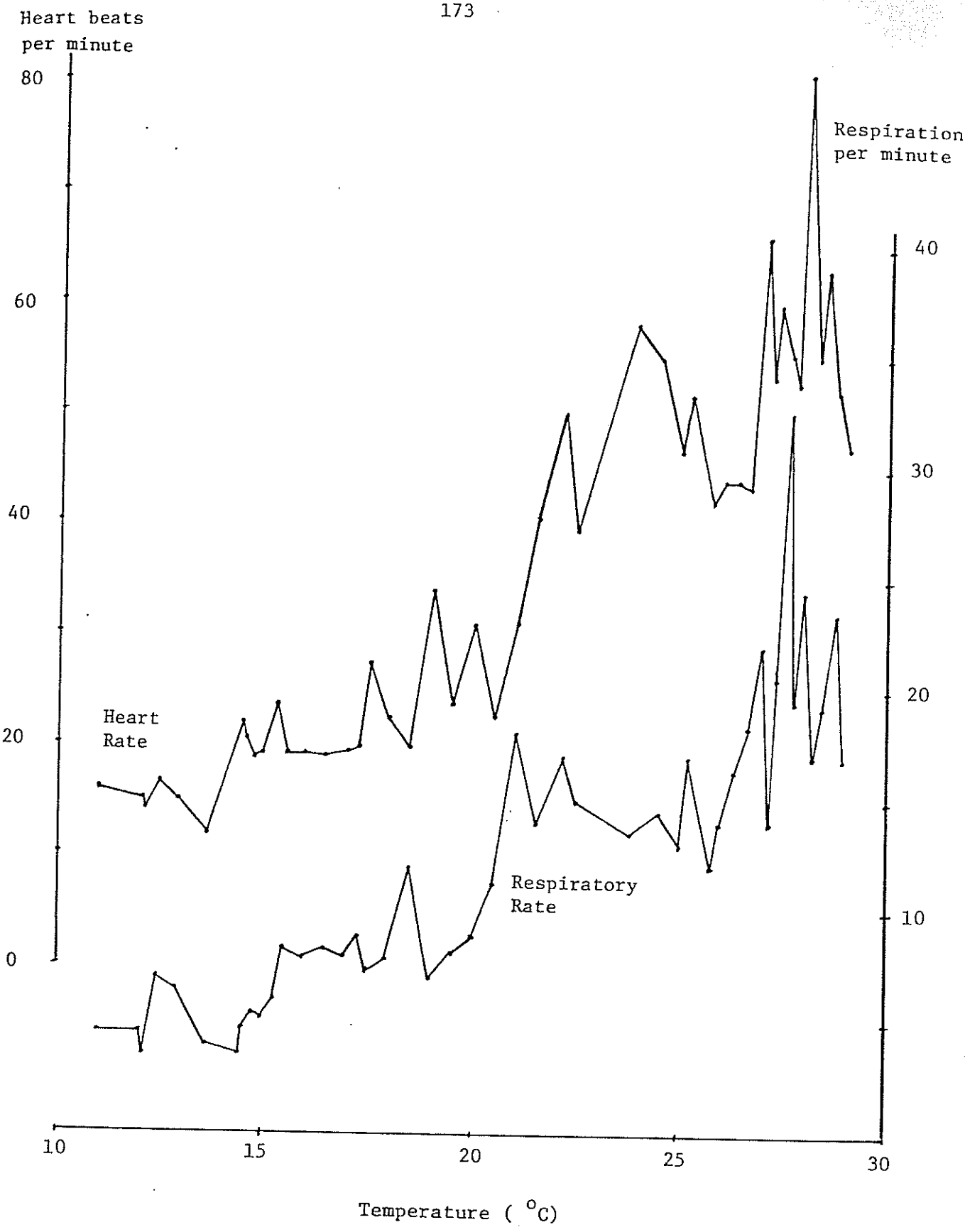


Fig. 4.7 Correlation of resting heart rate and respiration rate during gradual warming of adult lizard, Specimen TA01. r = 0.90.

only slowly although slight visual or tactile stimuli would increase the heart rate up to 100 per minute.

The results of another experiment in which body temperature was varied are shown in fig. 4.7, this shows a close correlation between heart rate and respiratory rate.

4.4 Discussion

Because of the wide range of variations in the characteristics of the respiratory cycle it was decided that a mathematical model was not only complex but probably of little value especially in view of the as yet poor understanding of the mechanical and control factors involved.

The fact that the major respiratory body wall movements in the post-axillary region (fig. 4.1) only cover a small area near the anterior region of the underlying lung tends to support the idea that the posterior lung may have a significant reservoir function. The post-aural movements are not thought to be significant in terms of gas exchange although they may be of use in observations of pressure changes occurring in the bucco-pharyngeal region, e.g. during positive pressure ventilation. The gular movements are thought to be mainly related to olfaction. Usually these movements were synchronized with pulmonary respiration but occasionally they occurred alone and sometimes pulmonary respirations were not accompanied by significant gular movements.

The body wall movements (C in Fig. 4.1) although very small in amplitude could be significant in terms of mixing gas in the posterior lung region during quiet respiration. These movements are probably caused mainly by active intercostal activity along the length of the body.

The fact that the tidal volume was so variable is another indication of the plasticity of respiratory physiology in this species especially considering the poor correlation between tidal volume and the previous or following respiratory interval. This

however may be interpreted as indicating that inspired air may not be fully available for exchange until some time later when a certain amount of mixing with the rest of the lung gases has occurred.

The relatively high minute tidal volume of 83.8 mL min^{-1} is almost certainly related mainly to the maintenance of a high intrapulmonary pO_2 and low intrapulmonary pCO_2 (see Section V).

The analysis of respiratory rate (fig. 4.4) at a constant body temperature shows statistically that individual breaths are not random events. There is no clue to the form of the link between breaths however this analysis is compatible with a central respiratory centre having a major input from peripheral chemoreceptors (vascular and/or pulmonary). The analysis of variation in individual respiratory intervals (fig. 4.5) also confirms that individual respirations are not random events and also demonstrates the wide range of respiratory intervals (up to a maximum of 34.2 s at 23.5°C) indicating a large respiratory reserve capacity.

The results of the analysis of changes in respiration rate with temperature (fig. 4.6) show that the wide variation in respiratory rate is present at least between body temperatures of 12°C and 30°C. The high Q_{10} value of 2.7 is interesting in that it may reflect the sensitivity of respiratory chemoreceptors to blood pH and/or pCO_2 changes. As temperature increases the blood pH decreases and pCO_2 increases even at constant CO_2 content (Reeves 1972) thus an increase in temperature (especially in a non-acclimated animal may be expected to stimulate respiration more than would be expected from merely increasing metabolic rate.

The analysis of variation in respiratory rate with temperature (fig. 4.6) was performed in order to determine if there was a close connection between respiratory and cardiac control mechanisms. Although the correlation between the two is high the fact that on occasions the heart rate increased when the respiratory rate decreased, and vice versa indicates that their control mechanisms are not so closely linked as to prevent this. In several instances, e.g. between 19°C and 22°C there is a large rise in respiratory rate which precedes a large rise in heart rate possibly indicating a response of the respiratory centre to decreased pH and increased pCO_2 which then indirectly influences the heart rate as a result of increasing metabolic rate and perhaps the increased neuronal input from proprioceptors in the respiratory system or body wall.

SECTION V

Intrapulmonary Pressure Changes

5.1 Introduction and Objectives

In order to facilitate the understanding of the basic mechanics of respiration and also the related aspects of respiratory control mechanisms several functional measurements and recordings were made. Intrapulmonary pressure measurements correlated with respiratory movements, air flow and volume, electrocardiograph and body temperature. Blood and respiratory gas analyses were also carried out (see sections V1 and V11).

The major objectives were to find answers to the following questions:

1) Is the resting intrapulmonary pressure significantly different from atmospheric pressure?

2) Does the glottis close during the second expiratory movement?

3) What kind of variations occur in intrapulmonary pressure?

5.2 Materials and Methods

Adult lizards of T. s. scincoides were used and recordings of respiratory movements, heart rate and cloacal temperature were made as previously described. In addition recordings of intrapulmonary pressure were made as follows. Local anaesthetic (Lignocaine hydrochloride 2%, Astra), about 0.2 ml was injected beneath the scales in the dorsolateral midbody region. A large bore stainless steel hypodermic needle, 1.20 x 38 mm was then plunged through the body wall and into the lung cavity. A specially prepared multiorifice polythene cannula (1.0 mm outside diameter, Portex) was then passed about five centimetres into the lung. The hypodermic needle was withdrawn and the polythene tubing was taped to the skin surface using flexible elastoplast. The animal was then left to recover for at least an hour. The tubing was connected to a Greer spirometer and recordings made on the physiograph recorder and also, in a second experiment, on a Grass polygraph recorder. Respiratory flow was recorded in this second experiment by placing the animal in a tubular body plethysmograph apparatus sealed around the back of the head with a rubber diaphragm and with only the head exposed. Pressure changes were recorded using a Gould-Statham pressure transducer number 15424 connected via a low level D.C. preamplifier, model 7 PIA, and a D.C. driver amplifier, model 7DAC, to the recorder.

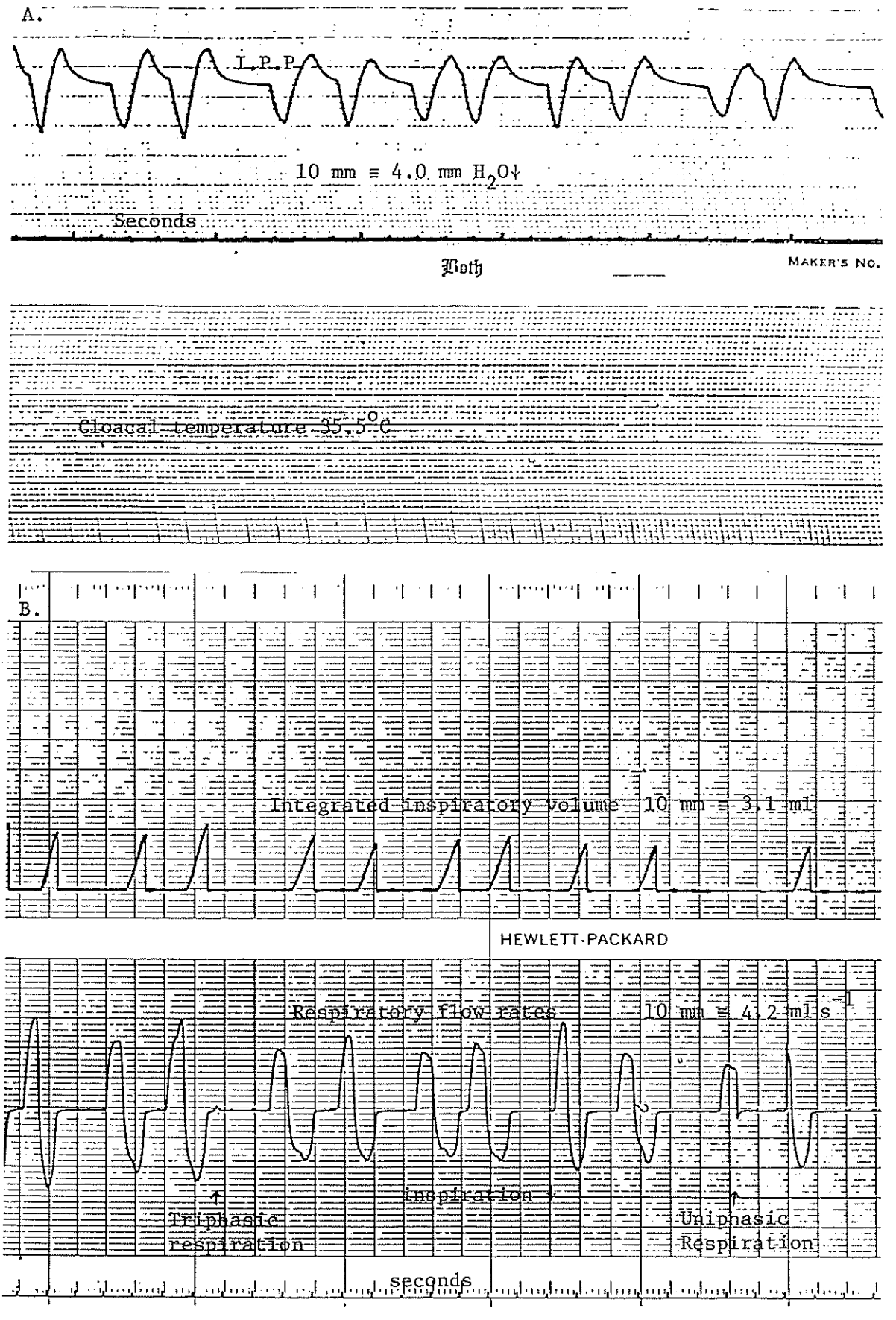


Fig. 5.1 A. Intrapulmonary pressure (I.P.P.) changes during quiet respiration in an adult T. s. scincoides. B. Inspiratory volume and respiratory flow patterns recorded using a body plethysmograph.

5.3 Results

5.3.1 General Characteristics of Intrapulmonary Pressure Changes

As indicated in Fig. 5.1A intrapulmonary pressure varies irregularly in amplitude from breath to breath. The rate of change also varies but less markedly. The ratio of duration of the positive pressure phase to the negative pressure phase is also very variable. Each cycle is biphasic, the initial positive pressure phase commences abruptly, comes to a sharp peak and is followed by a return towards the resting pressure level. The second negative pressure phase (relative to resting pressure) follows smoothly and continuously usually without sign of interruption. In this particular run (at a body temperature of 35.5^{o}C) the positive pressure (expiratory) phase lasted an average of 0.54 seconds whereas the negative pressure (inspiratory) phase lasted an average of 0.76 seconds. Examination of the simultaneous recordings of respiratory flow and volume, fig. 5.1B shows that peak expiratory flow is usually slightly greater than peak expiratory flow. The flow pattern was almost invariably biphasic, rarely triphasic or uniphasic. A small third phase of respiration is shown in fig. 5.1B, this was almost certainly due to failure of the glottis to close completely at the end of the inspiratory phase and before the second 'expiratory' movement occurred. A uniphasic respiration, expiration only, is also shown in fig. 5.1B indicating apparently that the glottis closed prematurely. The accompanying intra-pulmonary pressure trace was slightly extended but otherwise

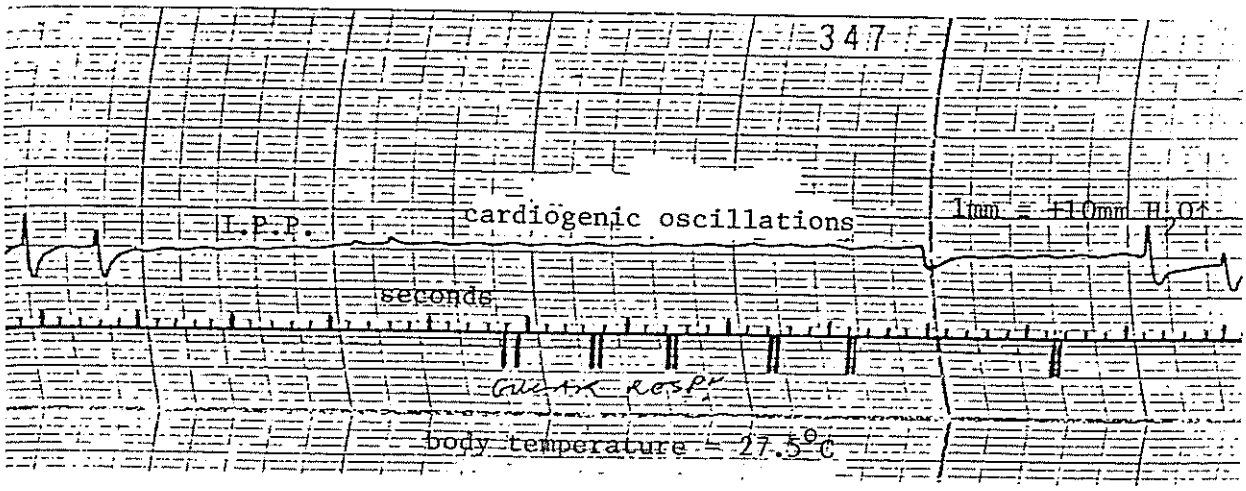


Fig. 5.2. Gular respiration and intrapulmonary pressure (I.P.P.). No detectable changes in pressure were seen to result from gular pumping movements.

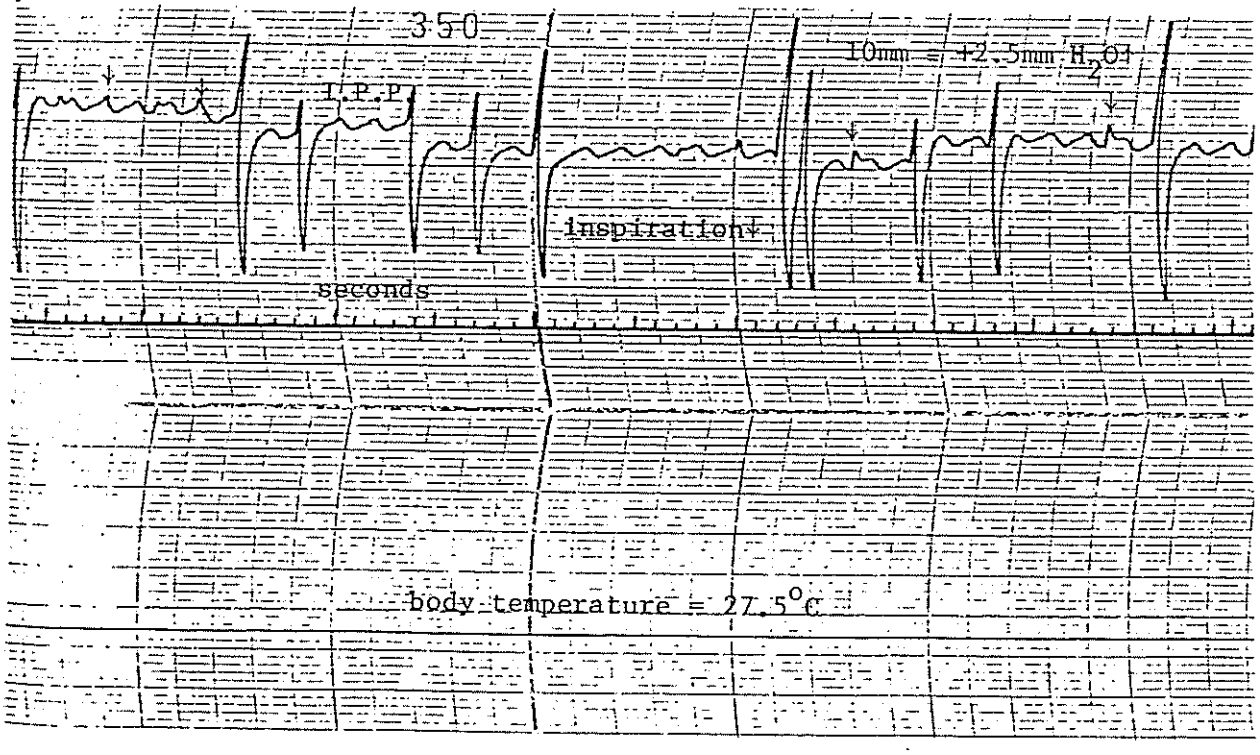


Fig. 5.3. Anomalous variations (↓) to intrapulmonary pressure (I.P.P.) superimposed on normal quiet respiration with cardiogenic oscillations also shown.

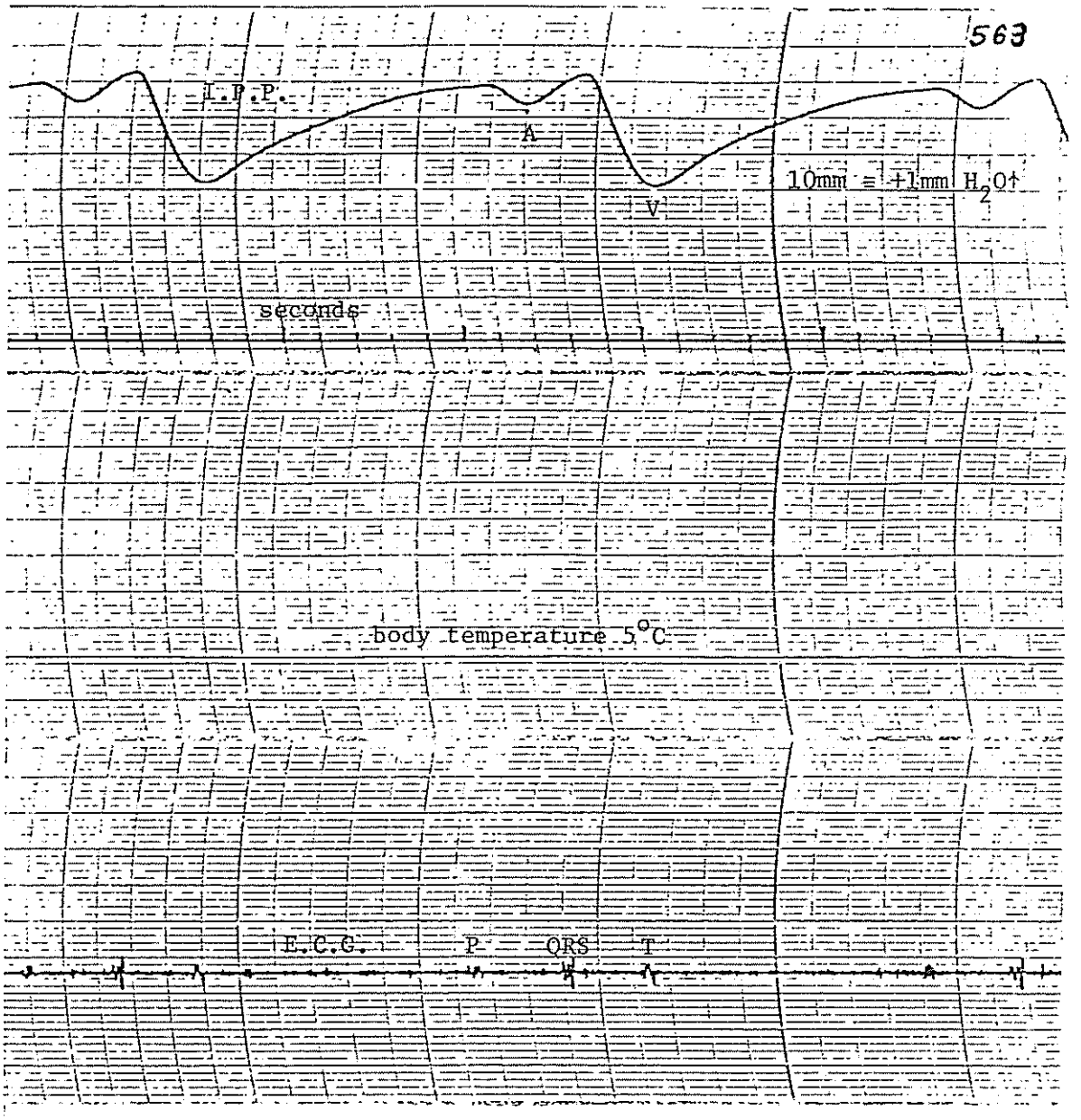


Fig. 5.4 Cardiogenic oscillations in intrapulmonary pressure (I.P.P.). The responses to atrial contraction A, and ventricular contraction V are well shown in relation to the electrocardiograph (E.C.G.) below.

normal. The glottis was late in opening for the subsequent breath as shown by the very rapid onset of peak expiratory flow and reduced duration of expiratory period. Neither of these phenomena show on the volume trace which was integrating only the inspiratory volumes. The variations in tidal volumes and flow rates are also shown on the volume trace. The plateau that occurs between each breath indicates that the glottis is closed at this stage (as the intrapulmonary pressure may still be changing). This plateau occasionally disappeared in very rapid breathing in which case the glottis probably remained open continously. During this recording there were no significant changes to the resting intrapulmonary pressure level.

5.3.2
Gular Respiration and Intrapulmonary Pressure

Gular respirations recorded during several apnoeic periods showed no changes in intrapulmonary pressure indicating that the glottis is kept closed at these times, fig. 5.2.

5.3.3
Heart Beat, and Intrapulmonary Pressure

In another experiment using the physiograph recorder and at higher sensitivity cardiogenic oscillations of intrapulmonary pressure were easily detected, (figs. 5.3 and 5.4). Superimposed on one of these traces, (fig. 5.3), were other small deviations possibly due to small trabecular smooth muscle contractions. The atrial contraction produced a reduction in intrapulmonary pressure of about 0.2 mm H_2O whereas the ventricular contraction produced a reduction of about 1.5 mm H_2O.

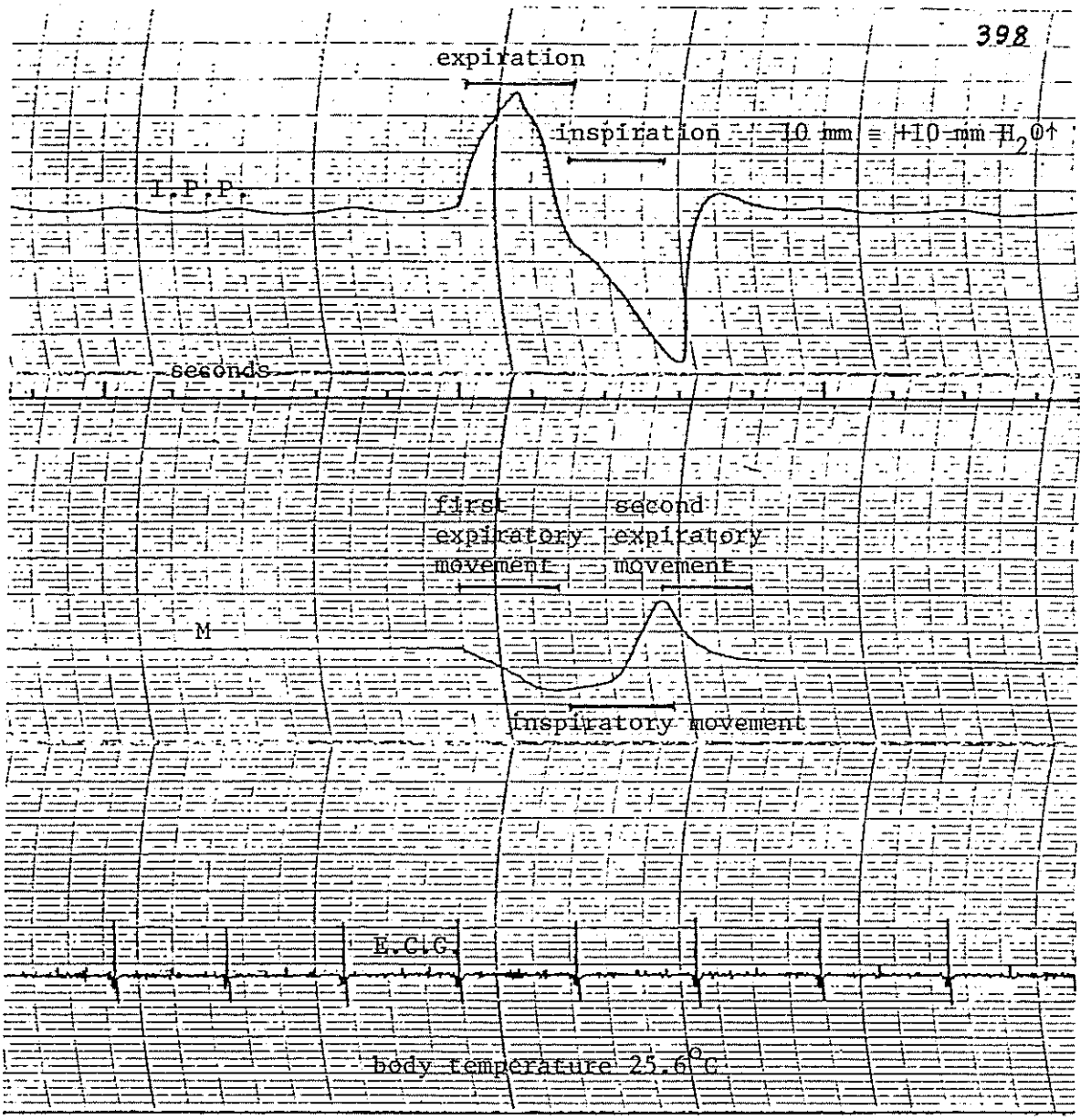


Fig. 5.5. Intrapulmonary pressure (I.P.P.) and post-axillary body movements (M) measured using a mercury in silastic tubing strain gauge. The electrocardiograph (E.C.G.) is also shown.

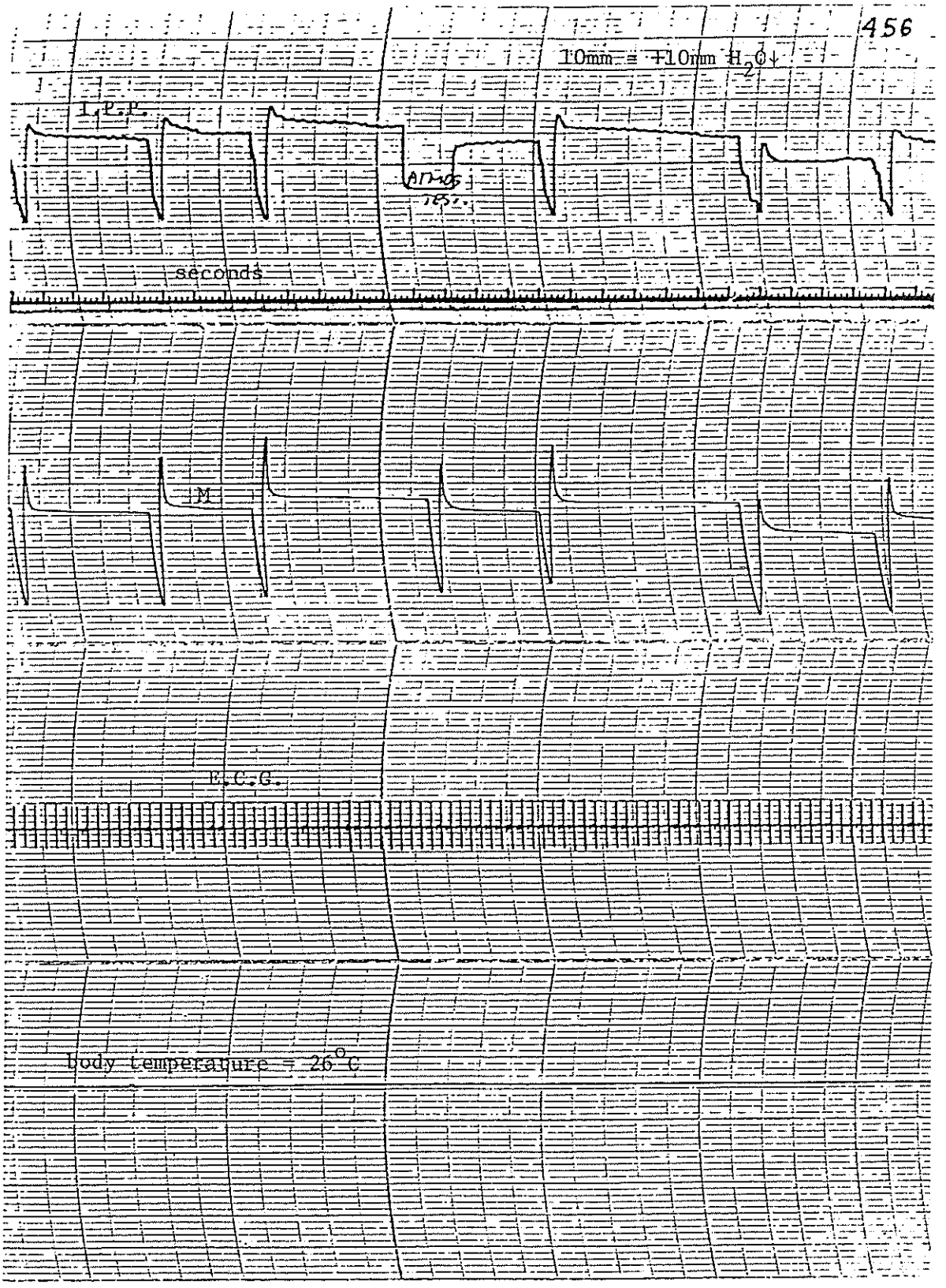


Fig. 5.6. In this recording there appears to be little evidence of active expiration as the intrapulmonary pressure (I.P.P.) falls towards atmospheric pressure during this phase. The respiratory movements (M) are however quite normal.

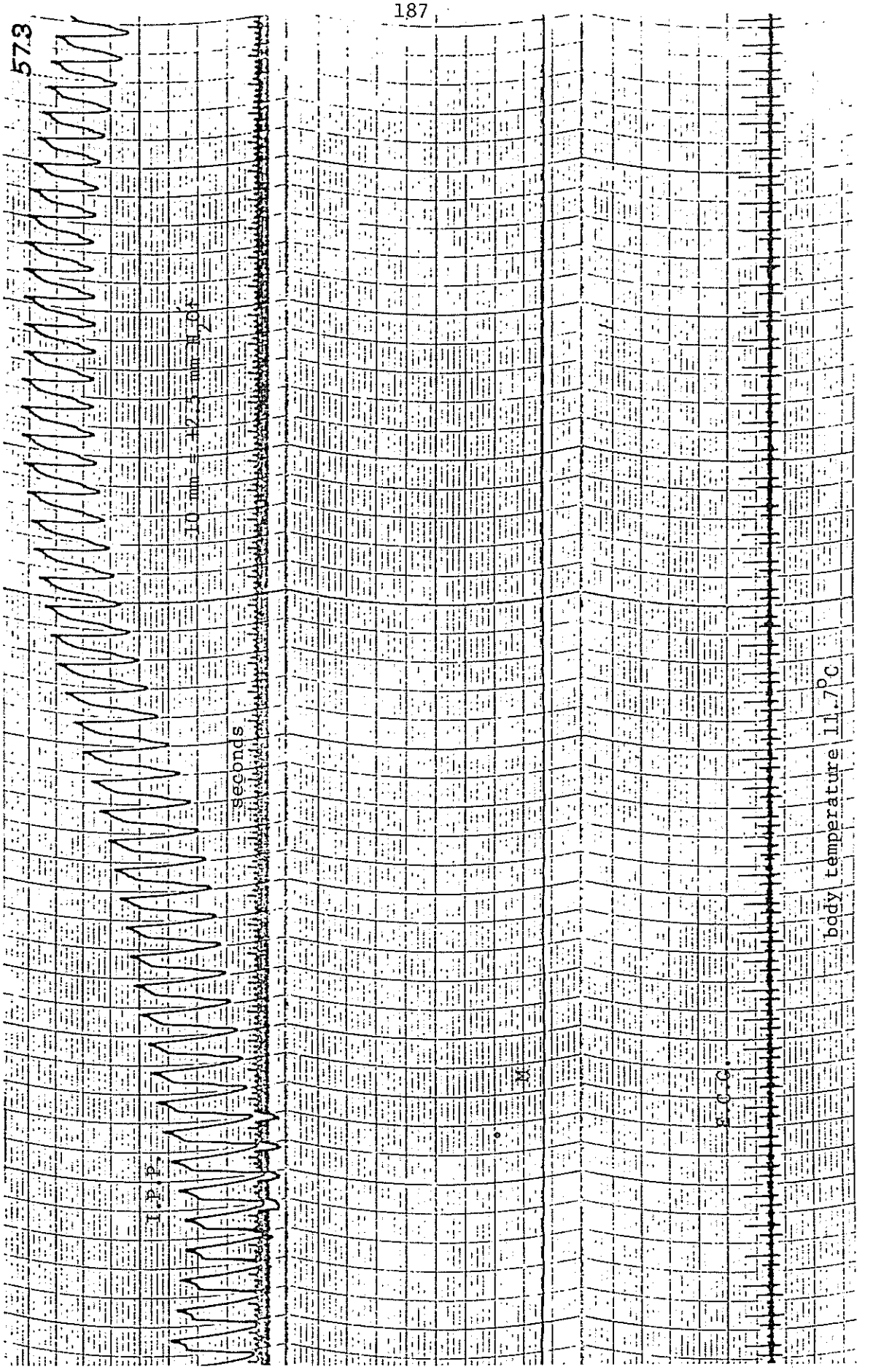


Fig. 5.7. Cardiogenic oscillations of intrapulmonary pressure (I.P.P.) during an apnoeic period in a cooled adult T. s. scincoides. There are no significant post-axillary body movements (M) and the electrocardiograph (E.C.G.) appears normal.

5.3.4

Respiratory Movements and Intrapulmonary Pressure

Fig. 5.5 shows intrapulmonary pressure changes related to respiratory movements (also the electrocardiograph). The respiratory movement trace shows three phases, the initial expiratory movement phase (coincides with increased intrapulmonary pressure), the inspiratory movement phase (coincides with decreased intrapulmonary pressure), the second expiratory movement phase, which is normally preceded by closure of the glottis and coincides with the rapid return of the intrapulmonary pressure to the resting level.

5.3.5

Intrapulmonary Pressure Relative to Atmospheric Pressure

Of 22 random checks made of intrapulmonary pressure during three days of recording 18 were above atmospheric and 4 were subatmospheric. The mean positive pressure was 6.0 mm H_2O and range -30 to +12 mm H_2O. Fig. 5.6 shows one of these atmospheric pressure tests, it also demonstrates the stepwise changes in intrapulmonary pressure which sometimes occur on a breath to breath basis.

5.3.6

Trabecular Smooth Muscle Activity and Intrapulmonary Pressure Changes

On several occasions (as in fig. 5.3) 'extraneous' changes to intrapulmonary pressure were noted. In addition to these short term changes there were also longer term changes which were particularly noticeable in cooled animals. Fig. 5.7 shows one recording made at a body temperature of about 11.7°C with no body movements. This high sensitivity recording shows the cardiogenic oscillations in pressure as well as the longer term cyclic changes (period 4½ minutes, amplitude 3 mm H_2O).

5.4 Discussion

These results tend to confirm what was thought to happen in terms of the respiratory cycle and closure of glottis from the end of the inspiratory phase to the beginning of the next expiratory phase. Just why there is a sharp peak to the expiratory pressure wave instead of a sinusoidal wave is difficult to interpret. It seems to indicate that the inspiratory muscles are already active before the expiratory phase is complete and probably accounts for the smooth transition to the inspiratory phase. It could be related to the fact that the resting intrapulmonary pressure is frequently positive relative to atmospheric pressure so that expiration is partly achieved simply by opening the glottis (although expiration is definitely an active process). The contraction of inspiratory muscles may serve to halt expiration as well as initiate inspiratory movements. A rapid respiratory phase followed by a prolonged apnoeic period could also be of useful survival significance in relation to detection by potential predators.

The stepwise changes that sometimes occur in intrapulmonary pressure probably indicate that the expiratory volume of a given respiration is not equal to the inspiratory volume of the same respiratory cycle. On several occasions when such changes occurred it was noted that there was a close correlation between girth in the post axillary region (as indicated by the mercury strain gauge) and the intrapulmonary pressure.

The maintenance of a positive intrapulmonary pressure may be related to the mechanics of respiration but it could also

be advantageous in terms of increasing the intrafaveolar pO_2 particularly if the pCO_2 can be kept at a low level.

The considerable variation in the various aspects of intrapulmonary pressure serve to underline the plasticity of the system and the mechanisms used to maintain adequate gaseous exchange.

Section VI Haematological Investigations

6.1 Introduction and Objectives

As part of the general investigation into respiration in the blue-tongued lizard it was decided to make a few general investigations of the properties of the blood which might be expected to have some bearing on respiratory function. The tests carried out included:

1) microscopic examination and measurement of blood cells
2) blood cell counts
3) haematocrit determinations
4) haemoglobin concentrations
5) haemoglobin electrophoresis
6) methaemoglobin levels
7) pO_2 and pCO_2 levels
8) $[HCO_3^-]$ and pH levels

The major objectives of this part of the study were:

1) to determine the major characteristics of the blood which might influence respiration
2) to facilitate the development of hypotheses related to respiratory function and its control.

6.2 Materials and Methods

Five adult blue-tongued lizards were used and venous blood was taken from the retroorbital blood sinus using the technique described by Maclean, Lee and Wilson (1973).

Blood smears were stained with Romanowski prior to microscopic examination. Blood cell counts were made using the

improved Neubauer hemacytometer. Haematocrits were determined using microhaematocrit capillary tubes and centrifugation at 10,000 g for five minutes. Haemoglobin was obtained by haemolysis of erythrocytes using one or two crystals of saponin per ml. (The erythrocytes were found to be quite resistant to osmotic stress, e.g. induced by 1% Triton X-100 solution). Electrophoresis was carried out using standard techniques on cellulose acetate in a Helena Zip Zone electrophoresis chamber (Beaumont, Texas) at 320 volts for 25 minutes.

Haemoglobin concentrations were determined using an EEL 197 Spectra photometer (Evans Electroselenium, Halstead, Essex) at 540 nm with cyanmethaemoglobin standards.

Methaemoglobin concentrations were measured on small samples (0.4 mL) of haemolysate obtained as above. Methaemoglobin has a characteristic absorption band at 630 nm which is abolished by the addition of sodium cyanide. The haemolysate was analysed photometrically as above at 630 nm both before and after the addition of about 0.5 mg of sodium cyanide. The ratio of methaemoglobin to total haemoglobin is given by the ratio of these readings and the absolute value of the total haemoglobin is determined by using standard solutions as described above.

Blood pO_2, pCO_2, bicarbonate and pH were determined as follows. 1 mL heparinized disposable syringes were used to collect small samples (about 0.5 mL) of venous blood from the retro-orbital sinus. Some samples were also collected using 270 µL heparinized capillary tubes. Blood was collected as quickly as

possible to reduce any possible effects of sympathetic stimulation and was then analyzed, usually within one or two minutes before metabolic processes could significantly alter the factors being analyzed. Gas analyses were carried out using a Corning 168 pH Blood Gas Analyzer, (Medfields, Massachusetts). Calibrations were carried out using standard Corning solutions and gas mixtures. Corrections for variations to atmospheric pressure, haemoglobin concentration and lizard body temperature were made. The Corning 168 automatically calculates $[HCO_3^-]$ and total blood CO_2 from the pH and pCO_2 determinations using the following equations.

$$[HCO_3^-] = 0.031\ pCO_2.10^{(pH-6.1)}$$

$$\text{and total } CO_2 = 0.031\ pCO_2 + [HCO_3^-]$$

where partial pressures are measured in torr and concentrations in mML^{-1}.

6.3 Results of Blood Tests

6.3.1 Microscopic Examination of Blood Cells

The erythrocytes are large flattened ovoid cells, 16-19 μm in largest diameter by 9-11 μm in the lesser diameter and about 2-3 μm thick. All circulating erythrocytes are nucleated. The nuclei have a flattened ovoid shape and measure about 6-7 x 4-5 x 1.5-2 μm, and occupy about 15-25% of the cell volume. The cytoplasm of the erythrocytes is similar to that of mammals being very dense and homogeneous with little stroma. The erythrocyte count ranged from 824,000 μL^{-1} to 956,000 μL^{-1} ($\bar{n}$ = 3).

A range of white blood cells including lymphocytes (large, medium and small), eosinophils, basophils, neutrophils and monocytes also thrombocytes were seen. The total white cell count ranged from 17,900 to 18,500 μL^{-1} (n = 3). No differential count was made.

6.3.2 Haematocrit and Haemoglobin Determinations

Haematocrit values varied between 23% and 34% with a mean of 26.4% (n = 5). The haemoglobin concentration ranged from 5.4 to 10.9 g/100 ml, mean = 7.4 (n = 15).

6.3.3 Haemoglobin Electrophoresis

The vast majority of the haemoglobin was found to be of only one electrophoretic variety presumably the usual vertebrate tetramer. There was also a very small amount of a second slow haemoglobin.

6.3.4 Methaemoglobin Concentration

One specimen of blood was found to have 0.94% methaemoglobin in it representing 17.2% of the total haeme protein present. A specimen of blood from a second lizard showed only 0.12% methaemoglobin or 2.2% of the total haeme protein.

6.3.5 Blood pO_2 and pCO_2

Fifteen samples of venous blood taken from five lizards gave an average pO_2 of 52.1 mm Hg, range 31.8-97.3. The mean pCO_2 was 13.0 mm Hg, range 8.6-18.9. The mean bicarbonate concentration was 13.7 M/L, range 9.9-18.3.

6.4 Discussion

As already indicated (section 3) blue-tongued lizards have large diameter capillaries and these are matched to the large erythrocytes. Larger erythrocytes, particularly thicker, erythrocytes will require increased gaseous diffusion times and therefore the erythrocytes may require a longer time period in the capillaries for saturation of haemoglobin to occur. Although the erythrocytes are biconvex in shape the cytoplasmic, haemoglobin containing region is effectively biconcave because the centre of the cell is occupied by a nucleus, thus producing a more diffusion-efficient morphology, albeit less efficient than the typical biconcave mammalian erythrocyte.

The considerable range in haemoglobin concentrations 5.4-10.9 g/100ml could be the result of numerous factors, e.g. age, sex, season, nutrition, all of which are known to have an effect (Saint Giron, 1970), temporary sequestering of erythrocytes by the spleen or liver is another possibility. However this variation could well be another reflection of the metabolic plasticity of the Blue-tongued lizards rather than an indication of the degree of haemoglobinization or anaemia. A mean haemoglobin concentration of 7.4 g/100 ml indicates a mean oxygen capacity of 9.9 ml/100 ml assuming the normal haemoglobin capacity of 1.34 g O_2/g (Adams and Hahn, 1979), and also assuming that the normal haemoglobin is 100% efficient in its oxygen carrying capacity. This estimate of oxygen capacity is about half of that found in humans and many other mammals, and indicates a low oxygen requirement for general body activities and/or a low reserve capacity.

The haematocrit is also very variable and when allowance is made for nuclear volume (the high white cell count has less than

1% effect on haematocrit) then the mean corpuscular haemoglobin concentration is very similar to that found in mammalian erythrocytes. This indicates that there is probably very little difference in the physical aspects of oxygen absorption by the erythrocytes in the two classes of animals. The electrophoretic analysis of haemoglobin showing only one major type and also the determination of a low methaemoglobin level provide evidence that there is probably little fundamental difference between the mechanisms of haemoglobin action in Blue-tongued lizards and mammals, (the small amount of the second, 'slow' haemoglobin could well have been a 'double haemoglobin' which is sometimes found in reptiles (Dessauer, 1970).

The wide range of values for mixed venous pO_2 is influenced by factors such as variation in temperature, blood pH and pCO_2 which produce shifts in the oxyhaemoglobin dissociation curve (Adams and Hahn 1979). However even allowing for these factors there is a wider range than expected for resting animals. The high pO_2 levels found on occasion (up to a maximum of 97.3 mm Hg) may be partly explained by the possibility of sympathetic stimulation producing a rapid increase in cardiac output unmatched by a corresponding increase in tissue metabolism. Whatever the cause however, it does indicate an adequate oxygenation mechanism with reserve capacity. It is also evidence for at least temporary mismatching of ventilation and perfusion.

The mean blood pCO_2 level (when temperature corrected), of 13.0 mm Hg was just below the minimum value (14.1) given in the review of blood chemistry of reptiles by Dessauer (1970) (determined by back calculation from values given for $[HCO_3^-]$

and pH, at 'room temperature'). Crawford (1974) indicates physiological pCO_2 values for carotid blood of 11 and 20 mm Hg at 16°C and 27°C respectively in the lizard Sauromalus obesus when acclimatized for '3-5 days' to the experimental temperature. The low pCO_2 values obtained in the present study match the high pO_2 values discussed above and similar arguments relating to the cause may apply.

The mean blood bicarbonate concentration of 13.7 mML^{-1} and range of 9.9-18.3 mML^{-1} are all within the lower range of values given by Dessauer (1970) for mainly terrestrial snakes and lizards. The low values for $[HCO_3^-]$ could be related to the lizard's relatively sedentary life style with only very occasional, and short, bursts of more vigorous activity, and thus little requirement for a large buffering reservoir. The pH range recorded matched the pCO_2 and $[HCO_3^-]$ values given above.

Section VII. Intrapulmonary pO_2 and pCO_2 Measurements

7.1 Introduction and Objectives

The relatively large volume of the Blue-tongued Lizards lungs suggests some kind of reservoir function. A large lung volume could allow a large tidal volume and a corresponding decrease in respiratory rate with the possibility of extended periods of apnoea and concomitant increases in the fluctuations of partial pressures of gases in the lungs and blood vessels. Conversely a large lung volume could also have several homeostatic effects mainly in relation to stabilization of blood pO_2 and pCO_2. These effects of a large lung volume will depend largely on the mean partial pressures of the intrapulmonary gases.

The major objectives of this section were:

1) to determine intrapulmonary partial pressures of oxygen and carbon dioxide
2) to help develop hypotheses related to the control of respiratory function.

The abbreviations pAO_2 and $pACO_2$ are used to indicate the partial pressures of intrapulmonary oxygen and carbon dioxide respectively although strictly speaking these are not directly comparable to mammalian alveolar gas partial pressures, paO_2 and $paCO_2$ are used to indicate the partial pressures found in oxygenated (or arterial) blood.

7.2 Materials and Methods

Three adult Blue-tongued lizards each had an intrapulmonary catheter introduced as described for intrapulmonary pressure measurements (see section 5.2). The distal end of the catheter was estimated to lie just inside the anterior third of the lung. The lizard was then left overnight or at least for several hours to recover. 3 mL and 6 mL samples of lung air were taken at not less than about fifteen minute intervals using plastic disposable syringes. Each 6 mL sample was analysed as two, 3 mL samples within two or three minutes using a Corning 168 pH Blood Gas Analyzer (Medfields, Massachusetts), calibrated using Corning calibration gases. Attempts were made to obtain samples quickly and in the latter part of the interrespiratory apnoeic period to reduce the possibility of sampling unmixed incoming air. A continuous record of respiratory activity during the periods of sampling was made using a Hewlett Packard recorder type 740 2A (Palo Alto, California). Body temperature was monitored using an intracloacal thermocouple, room temperature and air pressure were also recorded.

7.3 Results

The mean $pACO_2$ was 8.2 $\pm$ 2.8 mm Hg (n = 23) and the mean lung pAO_2 was 68.5 $\pm$ 15.9 mm Hg (n = 18). Body (cloacal) temperatures ranged from 21.5 → 28°C and barometric pressures ranged from 671 → 681 mm Hg.

7.4 Discussion

When the lizard is resting and breathing quietly at a given body temperature, it would be expected that the partial pressures of the lung gases would oscillate on a breath to breath basis around some mean value which itself may vary slightly over a period of time. Age, sex, season, body temperature and other factors could also influence the lizards metabolism and the mean partial pressures of oxygen and carbon dioxide found in the lungs at any one time. The objects of this part of the study however were only to determine these partial pressures and relate them to possible mechanisms of respiration and its control. If there is a steady tissue output of carbon dioxide into the lungs and a steady uptake of oxygen then one might expect an approximate inverse proportionality of the partial pressures of these two gases related to ventilation and provided other factors such as temperature and barometric pressure remain reasonably constant.

The variations in $pACO_2$ and pAO_2 found in the lung could be a reflection of several factors in addition to those mentioned above. The heterogeneous lizard lung results in uneven gas distribution, this variation is compounded by the uneven distribution of the pulmonary capillaries within the lung. The fact that the respiration pattern is arhythmic will also tend to produce variations in partial pressures of lung air samples. Pulmonary gas exchange could also be influenced by the degree of central (right to left)

vascular shunting (Wood and Lenfant 1976). Such potential for variation makes it difficult to make meaningful analyses and predictions relating to respiratory function and its control. There is a large difference between alveolar and arterial pCO_2 and pO_2 values in some reptiles (Wood and Lenfant 1976) although according to Pough (1969) the $pACO_2$ and $paCO_2$ are closely related in Uma inornata, the pAO_2 and paO_2 appear closely correlated but with the paO_2 lagging by about 5-30 mm Hg.

The fact that considerable variations of pAO_2 and $pACO_2$ occurred in resting animals without producing major changes in ventilation indicates that any intrapulmonary chemoreceptors are not significantly active at these partial pressures.

During this experiment it was noted that aspiration of small samples (3-6 mL) of lung air would almost invariably trigger a respiratory cycle, this response occurred even with an aspiration volume of as little as 0.5 mL especially if it was removed rapidly. The respiratory cycle thus triggered was usually the normal triphasic movement-biphasic ventilation type with a slightly decreased expiratory volume and a correspondingly increased inspiratory volume. The rapidity of triggering, increased probability of triggering with rapid aspiration even at small aspiration volumes and the slight changes in the triggered respiratory cycle all support the theory that stretch receptors are involved in normal quiet respiration and in the maintenance of appropriate lung volumes. This concept was also supported by the results of experiments in which air was injected into the lungs which frequently resulted in delay before the next respiratory

cycle occurred. In view of the fact that these responses were initiated with volume changes approximating the normal tidal volumes it is suggested that they are involved in the control of normal quiet respiration. Guyton (1981), p. 517 suggests that the Hering-Breuer reflex in humans (and presumably in other mammals) is mainly an overinflation protective mechanism, however in view of the above responses and the very different respiratory cycle found in many reptiles it may be safer to regard these responses as analogous rather than homologous to the mammalian Hering-Breuer reflex.

The fact that the Blue-tongued lizard is able to regulate its intrapulmonary pressure by muscular action may be of physiological value. Because the pAO_2 is always much greater than the $pACO_2$ the effect of any change in intrapulmonary pressure will affect the partial pressure gradients for oxygen much more than for carbon dioxide. This differential effect will also be increased by the presence of plasma and tissue fluid buffers.

SUMMARY

A study of the respiratory system of the large terrestrial skink Tiliqua scincoides was undertaken to provide basic information on morphological and physiological characteristics which could help determine respiratory control mechanims.

This study covers morphological aspects at the macroscopic, light microscopic and ultrastructural levels and includes radiological investigations. The functional aspects include observations of respiratory rate, over a period of time as well as on a breath to breath basis, tidal volume changes, variations of respiration with temperature, lung gas composition, intra-pulmonary pressure variations and also some basic haematological investigations.

Macroscopically the lungs were found to be large and unicameral but variable in dimensions, occupying from 16-30% of total body volume. The internal surface area of the lungs is increased by the presence of numerous faveoli of variable dimensions. Histologically these were found to be usually rimmed by smooth muscle trabeculae. The faveoli were concentrated in the anterior lung region where the surface area was as much as six times the corresponding value in the posterior region. The posterior lung region is however not just a gas reservoir, it contains a moderate number of low faveoli and a relatively large amount of smooth muscle in the trabeculae. The pulmonary capillary beds although found largely in the anterior lung region are also found to a lesser extent in the posterior lung region. The mechanism of exchange of gases between the

faveoli and the main cavity of the lungs is conjectural although several mechanisms including the contraction of the trabecular smooth muscle are almost certainly involved.

A moderate amount of surfactant was demonstrated in the lungs although its significance is somewhat obscure.

The observations of blood distribution in the lungs, also the histological observation of a valve-like strucutre in a pulmonary blood vessel indicate that vascular shunting almost certainly does occur and may be important in ventilation/perfusion matching. The arrangement of tissues within the lung wall and the contraction of the trabecular smooth muscle is suggested as being conducive to the flow of lymph.

The measurements of thickness of the air/blood barrier showed considerable variation and a minimum thickness of about 0.25 μm was observed (somewhat greater than that found in humans) and the mean value was estimated at about 0.6 μm.

The total internal lung surface area in two juvenile specimens was estimated to be approximately 21,600 mm^2, considerably greater than published values for reptiles.

Morphological evidence supporting the notion of ventilation/perfusion matching included increased surface area and increased vascularity of lung tissues close to the bronchial orifice and also the probable presence of a blood flow controlling mechanism in the pulmonary arteries. The arrangement and possible mechanism of action of the trabecular smooth muscle could also be interpreted

as having a major controlling influence on both intrapulmonary capillary blood flow and faveolar air movements.

The pattern of the respiratory cycle was found to be similar to that of other terrestrial skinks. Most commonly the respiratory cycle consists of a biphasic flow pattern (expiration followed by inspiration) but with a triphasic movement pattern in which a second expiratory type movement occurs with the glottis closed. This active phase is followed by an apnoeic period of very variable duration. Observations and recordings of variations to this basic pattern and analyses of certain variations were made including the following: variations in resting respiratory rate at constant temperature, variation in respiration rate with temperature, variation of respiration rate with heart rate, variation of tidal volume and minute volume. Some discussion of the possible significance of these factors is included. An analysis of intrapulmonary pressure changes during and between the active respiratory phases was made including a demonstration of cardiogenic and possibly pulmonary smooth muscle activity.

Several samples of mixed venous blood from the retro-orbital sinus were analysed for pH, pO_2, pCO_2 and HCO_3^- levels. Tests were also made for haemoglobin, methaemoglobin, haemoglobin electrophoresis, haematocrit, blood cell counts and microscopic appearances of blood cells. There is a brief discussion of some of these results and their significance in relation to respiratory function. Of particular interest was the considerable range of blood haemoglobin concentration and therefore of oxygen capacity.

The mean values for $pACO_2$ and pAO_2 were 8.2 mm Hg and 68.5 mm Hg respectively at room temperature, but considerable variation was found.

ACKNOWLEDGEMENTS

The work reported in this thesis was performed predominantly in the Department of Physiology at the University of New England, Armidale, New South Wales and in the School of Applied Science at the Riverina College of Advanced Education, Wagga Wagga, New South Wales, under the supervision of Associate Professor M.E.D. Webster, Head, Department of Physiology, University of New England, whose unfailing encouragement and assistance are gratefully acknowledged.

Experimental work including electronmicroscopy and photography reported in this thesis is original but would have been impossible were it not for the considerable technical support, academic advice and secretarial assistance received from staff at the two institutions, all such assistance is also very gratefully acknowledged.

Signed: T. Annable

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Date: 15-2-'83

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APPENDIX II

Catalogue of *Tiliqua* specimens used in this Study.

No.	Species	Origin	Snout/Vent Length (mm)
TA02	*T. scincoides*	Albury	245
TA03	*T. scincoides*	Wagga Wagga	294
TA04	*T. scincoides*	Armidale	318
TA05	*T. scincoides*	Dubbo	298
TA06	*T. scincoides*	Adelong	285
TA07	*T. scincoides*	Temora	315
TA08	*T. scincoides*	Armidale	123
TA09	*T. scincoides*	Armidale	103
TA10	*T. scincoides*	Yaven Creek	313
TA11	*T. scincoides*	Coff's Harbour	293
TA12	*T. scincoides*	Point Cook	315
TA13	*T. scincoides*	Armidale	167
TA14	*T. scincoides*	Armidale	112
TA15	*T. scincoides*	Armidale	160
TA16	*T. scincoides*	Armidale	295
TA17	*T. scincoides*	Armidale	123
TA18	*T. scincoides*	Armidale	115
TA19	*T. scincoides*	Arnhem Land	247
TA28	*T. scincoides*	Armidale	300
TA29	*T. scincoides*	Armidale	262
TA30	*T. scincoides*	Glen Innes	305
TA31	*T. scincoides*	Armidale	280
TA32	*T. scincoides*	Armidale	322
TA36	*T. scincoides*	Armidale	296
TA40	*T. scincoides*	Wagga Wagga	138
TA41	*T. scincoides*	Wagga Wagga	146
TA42	*T. scincoides*	Armidale	288
TA01	*T. nigrolutea*	Wee Jasper	353
TA23	*T. nigrolutea*	(Born in captivity)	140
TA24	*T. nigrolutea*	(Born in captivity)	131
TA37	*T. nigrolutea*	(Unknown)	295
TA25	*T. gerrardii*	Glen Innes	220
TA34	*T. rugosus*	Balranald	305
TA35	*T. rugosus*	Balranald	315

APPENDIX III

LUNG SURFACE AREA CALCULATIONS

Estimations of internal lung surface area were made using measurements taken from histological transaxial and longitudinal sections of lung tissue.

If it is assumed that faveoli are shaped as square sectioned prisms then the internal surface area (A_s) of each prism is given by:

$$A_s = w^2 + 4wH$$

where w = side of square

H = height of prism

If the faveoli are all cuboidal then the internal surface area of the lung can be calculated as follows. The area of the longitudinally arranged faveolar walls can be estimated by calculating the product of the mean of the sum of septal perimeters ($\overline{C}_p$) seen in transaxial sections, (measured histologically) and lung length (L), (the ends of the lungs are approximately hemispherical and since the area of a hemisphere, radius r, is equal to the area of a cylinder height r, there is probably no need to make corrections). Similarly the area of the circumferentially arranged faveolar walls can be estimated by calculating the product of the internal perimeter (L_p), (measured histologically) and the mean external circumference ($\overline{C}$) (measured on the transaxial sections). The faveolar base areas are added to the sum of these two products to give the total internal surface area. The area of the faveolar bases (A_b) is estimated to be 95% of the external lung area and is given approximately by:

$$A_b = 0.95\overline{C}L$$

The total internal surface area (A) is now given approximately by:

$$A = \bar{C}_p L + L_p \bar{C} + 0.95\bar{C}L$$

This formula applies equally well to rectangular faveoli of any dimensions. Most faveoli are somewhat irregular polygons in cross section with a tendency to form hexagonal shapes, however, as shown below the calculations based on rectanuglar faveoli do not require correction assuming that the faveoli are of equal depth and base area. In order to simplify calculations it is assumed that faveolar sections are either squares or regular hexagons. The effects of the two major parameters expected to alter surface area calculations are determined as follows.

1. The internal surface area (A_c) of a cuboidal faveolus, of edge H and open at one end is given by:

$$A_c = 5H^2$$

The internal surface area (A_h) of a hexagonal prism (faveolus) open at one end, of height H and the same volume as the cuboidal faveolus is given by

$$A_h = \frac{6y}{2}.\sqrt{(y^2 - (\frac{y}{2})^2)} + 6yH$$

(where H = height of faveolus, and
y = side of hexagon)

$$= \frac{3y^2}{2}.\sqrt{3} + 6yH$$

If the base areas and heights for cubes and hexagonal prisms are equal then the volumes will be equal and:

$$\therefore \quad H^3 = \frac{3y^2}{2}. \sqrt{3}\, H$$

$$\therefore \quad H^2 = \frac{3y^2}{2}. \sqrt{3}$$

$$\therefore \quad H = \sqrt{\frac{3y^2}{2}. \sqrt{3}}$$

or $H = 1.6119$ when $y = 1$

The ratio of the internal surface areas of cuboidal to hexagonal faveoli of equal base area and height is then given by:

$$4H^2 : 6yH$$

$$= 2H : 3y$$

$$= 2 \times 1.6119 : 3 \text{ (when } y = 1)$$

$$= 3.224 : 3$$

$$= 1.07457 : 1$$

Thus the calculation of faveolar wall surface areas based on square sections requires correcting by multiplying by 1.075.

2. If randomly oriented sections are made through regular arrays of squares or hexagons of equal area one might expect a priori a difference in the number of walls sectioned per unit of surface area. This ratio was determined as follows. The internal surface of the lung is considered continuous (only the small bronchial orifice breaks this continuity) in which case each additional square section added requires two sides only whereas each additional hexagon requires

three sides for its formation. Thus if the base areas of hexagons and squares are equal then the ratio of the wall densities present will be that of the two sides of the square to three sides of the hexagon

$$2H : 3y$$
$$= 1 : 0.9306$$

This factor is however the reciprocal of that derived in 1. and they cancel each other out so that the equation for finding the total internal surface area of the lung remains

$$A = \overline{C}_p L + L_p \overline{C} + 0.95\overline{C}L$$

N.B. No allowance has been made for the irregular variations due to; 1. compound subdivision of the faveoli, 2. tiering of certain large faveoli in the anterior lung region, 3. temporal variations in faveolar dimensions due to muscular action. Factors 2. and 3. could well lead to increased values for lung surface areas.

APPENDIX III

Conservation Postscript

During the period of this study a number of the Blue-tongued lizards were maintained in a large ($\sim 30m^2$) outdoor holding and observation area where breeding has occurred in *T.s.scincoides* and *T.nigrolutea* enabling over twenty juvenile and also several adult lizards to be released into the wild.