THE SUBMICROSCOPIC STRUCTURE OF THE DOLPHIN LUNG

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SUMMARY

This thesis present the first detailed account of the submicroscopic structure of the bronchial tree and lungs of a dolphin, *Tursiops truncatus*. The dolphin respiratory system is the most highly modified from the usual mammalian pattern of all the mammals. I consider, however, that the modifications are within the expressive range exhibited by mammalian lungs. Further, they are particularly suited to the marine existence of the dolphin.

The trachea is short but wide. It is completely surrounded by anastomosing plates of hyaline cartilage embedded in a fibro-elastic membrane. Three types of epithelium line the trachea: (a) proximally there is a transitional type which has an asymmetric unit membrane on its surface cells. This epithelium is similar to the transitional epithelium of the urinary bladder; (b) most of the trachea is lined by a stratified columnar epithelium with micro-villous surface cells, but no cilia. Proximally the asymmetric unit membrane extends into the surface cells, but distally these cells have a clear apical zone, a mitochondrial zone and a supra-nuclear organelle zone. This epithelium looks very like gallbladder epithelium; (c) the distal parts of the trachea and the bronchi are lined by typical respiratory epithelium with cilia and goblet cells. On the ventral aspect of the laryngo-tracheal junction there are tonsil-like lymphatic collections. Throughout the submucosa there are abundant mucous and serous glands with ducts opening onto the surface. A rich vascular plexus and a dense internal elastic lamina complete the submucosa.

The air-conditioning of the inspired air is performed in the trachea because of the modifications in the blowhole and nasal cavities. This means that the epithelium of the proximal trachea is subjected to the particle load carried by the air, resulting in an increase at these levels. Protection of the airway is provided by the transitional epithelium, the flow of secretions from the glands and the tonsil-like lymphatic collections in the most dependent parts. Clearance of the trachea, in the absence of cilia is by gravity and mucous flow towards the larynx. The excess mucus and secretions are removed during the explosive expiratory phase of the "blow". In addition, the trachea must warm and humidify the inspired air and conserve water from the expired air. The venous plexus and the secretions from the glands assist with the former, while the microvillous epithelium performs the latter function.

Cartilage is present in the airways to their termination in the alveolar ducts. A system of myo-elastic sphincters in the terminal airways controls the flow out of the alveoli during the apneustic phase of respiration and diving. In this segment of the airway there is also an extensive capillary plexus covered only by squamous epithelial cells. I have called this the respiratory bronchus.

The alveolar septum has a thick connective tissue core with a capillary plexus on each surface. The alveoli are lined by typical type I and type II cells. Macrophages are frequently seen on the surface. The blood-air barrier is reduced to 200 nm in many areas. During diving the alveoli collapse due to the increasing hydro-static pressure and air is forced into the bronchial tree. The alveolar septum folds in the gaps between the capillaries on

complimentary surfaces, thus maintaining the absorptive surface. It has been shown that dolphins use up to 95% of the inspired oxygen. I consider that the reduction in thickness of the blood-air barrier, and the double capillary plexus are responsible. The main function of the respiratory bronchus is to remove the excess nitrogen which is absorbed by a counter-current flow, thus reducing the risk of the "bends" developing after a dive to shallow depths.

Statement.

This thesis contains no material which has been accepted for the award of any other degree of diploma in any university. To the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except when due reference is made in the text of the thesis.

Joseph C. Fanning.

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" All creatures that have a blowhole respire

and inspire, for they are provided with lungs "

Aristotle.

I. INTRODUCTION

" *All land animals breathe, and even some water animals, such as the whale, the dolphin and all the spouting Cetacea* "

Aristotle

Dolphins are air-breathing mammals which spend their entire life in the water. They are members of the order Cetacea, or whales and belong to the Odotocete, or toothed whale family. Dolphins range in size from the harbour porpoise, less than two metres in length, to the killer whale, up to nine metres in length. They are found in oceans and coastal waters around the world and even far inland in the great rivers of Asia, India and South America.

Dolphins and whales have had a special place in the minds of man for thousands of years. They appear in some of the earliest rock carvings; in many myths and legends of coastal dwellers throughout the world; and in numerous passages in the bible and other religious writings (Slijper 1962, 1976; Alpers 1963; Matthews 1968; McIntyre 1974). There has been increasing concern for the biology and conservation of all animals over the last thirty years, and the dolphin has featured prominently in this. This concern has arisen in the case of the dolphin because: the threat of extinction of the great whales by the commercial fishery of some nations; the inadvertent slaughter of large numbers of dolphins in the commercial tuna fishery in the Eastern Pacific Ocean; the rapid increase in the number of marinelands, aquaria and zoos using these animals for display purposes;

and the suggestion that dolphins may be intelligent beings and the subsequent fear that man may exploit this to his own ends (evil or otherwise). Much of this has an emotional bias associated with the primitive beliefs mentioned above. (See McIntyre 1974; Lilly 1975; Greenwood 1976)

The early writers in the field of Cetology appear to have ignored Aristotle's description of the dolphin as a mammal and called them fish. It was only during the last century that dolphins and whales were generally accepted as air-breathing mammals (see Matthews 1968 and Harrison 1972 for a review of the controversy). Dolphins are playful inquisitive creatures in their native environment, and are often seen cavorting in small groups close to the beach. Often all that is seen is a dorsal fin arching out of the water. When one looks at the external features of the dolphin it is not surprising that they were considered for many years to be fish.

Dolphins have a streamlined fusiform body tapering from the thoracic region to the base of the tail. The vertebral column extends distally almost to the base of the tail, but proximally a true neck is not present and there is shortening and fusion of vertebrae. The forelimbs have evolved into flippers with the usual arrangement of bones within, while the hind limbs have disappeared completely externally. The horizontally placed tail flukes have no skeletal support consisting only of fibrous tissue. Some species have a dorsal fin otherwise there are no external appendages to break the streamlined appearance. The ears have been replaced by small orifices between the eyes and the anterior angle of the flippers. The genital organs are

placed in slits anterior to the anus, or retained within the body cavity. The skull is asymmetric and exhibits some degree of telescoping in most species. The maxillary bones are drawn backwards, the occipital bones are pushed forewards and the jaws are elongated and carry teeth. The nostrils have been replaced by a blowhole which is situated at the highest point of the head, just rostral to the frontal bones.

One of the difficulties in accepting dolphins as mammals has been a satisfactory explanation of their diving ability. This ability to dive beneath the surface of the water to search for food and to escape from predators was probably the first and one of the most important of the adaptations which permitted the terrestrial forebears of the modern dolphins to return to the water. Most mammals, man inclusive, are unable to endure submersion for more than a few minutes because of lack of oxygen to the vital tissues of the brain and heart. Man through his ingenuity, has overcome this problem by taking his air supply with him. A great variety of devices have been developed for this purpose in recent years but with them has come an increase in the complications of diving such as "Caisson" disease, or "the bends", and a variety of other problems. The dolphins on the other hand carry only the air in their lungs when they dive - stay submerged for long periods - return to the surface - take a few breathes and dive again. All without apparent ill-effects.

The physiological and anatomical adaptations necessary in diving mammals have been more than adequately reviewed in recent years (Harrison and Tomlinson 1963; Scholander 1964; Andersen 1966, Andersen 1969; Harrison and Kooyman 1971; Ridgway 1972). The problem associated with deep diving have also been extensively reviewed (Kooyman and

Andersen 1969; Kooyman 1972, 1973). I will not attempt to precis these accounts but would like to point to some interesting observations before proceeding with an account of the mammalian lung in general terms and the dolphin lung.

Prolonged breathe holding is essential for successful diving in any air-breathing animal. Cessation of breathing results very quickly in a deficiency in the amount of oxygen available to the vital areas of the brain and the heart. This is accentuated by the muscular activity which goes with diving. It has been shown that there is a marked reduction in aerobic metabolism in all marine mammals during diving, which is a marked distinction from their usual metabolic rates, which are generally higher than terrestrial mammals of the same size (Scholander 1940, 1964; Andersen 1966; Ridgway 1972). The reduction in aerobic metabolism is due to a change in the distribution of the blood supply which is caused by the diving reflexes with bradycardia and shunting of the blood to vital areas (Scholander 1940, 1964; Andersen 1966; Elsner 1969); there are insufficient oxygen stores in the body to sustain aerobic metabolism for the whole duration of the dive, and there is good evidence that the oxygen stores in the lungs are not available to the blood during the dive due to collapse of the alveoli and sequestration of the air in the tracheo-bronchial tree (Irving 1939; Scholander 1940, 1964; Andersen 1966; Kooyman and Andersen 1969; Kooyman 1973; Ridgway 1972). It is true however, that there are additional stores of oxygen available to the muscles in the form of myoglobin, but these are soon exhausted and are not capable of replenishment until the circulation returns to normal on completion of the dive.

In this thesis I have used the term dolphin as a general term to include all of the members of this group, including Killer whales, *Orcinus orca*; Pilot whales, *Globicephala melaena, G. scamoni*; and members of the following species: *Lagenorhynchus, Tursiops, Stenella.* Where I have intended only a particular species be referred to, I have included its generic name. There is still some debate concerning the classification of the Cetaceans (see Gaskin1972; Ridgway 1972; Mitchell 1975 for details), and I have chosen this method to refer to a group of animals which have similar structural and functional characteristics with respect to their respiratory systems.

CHAPTER II.

THE MAMMALIAN RESPIRATORY SYSTEM

THE MAMMALIAN RESPIRATORY SYSTEM

2-1 Introduction

The mammalian respiratory system has traditionally been divided into three zones: (1) the conducting zone; (2) the intermediate zone; (3) the respiratory zone.

The components of the three zones are:

(1) The conducting zone: the nose and nasopharynx; the larynx; the trachea; the right and left main bronchi; the secondary, or lobar bronchi; the tertiary, or segmental, bronchi and their branches.

(2) The intermediate zone: the terminal bronchi; the bronchioles; the respiratory bronchioles; the alveolar ducts; and the alveolar sacs.

(3) The respiratory zone: the alveoli.

Not all the components are present or well developed in every species, and even within species there may be considerable variation. Tyler et al (1971) have attempted a classification based on the functional unit of the lung, but at this time it is still very general. My main aim in this work is to discuss the microscopic structure so I will not consider the sub-divisions of the respiratory system at the macroscopic level any further.

2-2 The microscopic structure.

The microscopic structure of the respiratory system is now well known and is described in many textbooks and reviews (see Krahl 1964; Nagaishi 1971; Sorokin 1973; Rhodin 1974; Murray 1976; to mention only a few). I will discuss the structure of the nose,

nasopharynx and the larynx in the section on functional aspects. I will therefore commence a review of the current concepts of the microscopic structure of the respiratory system with the trachea.

2-2 A The Trachea.

The wall of the trachea is composed of: mucous membrane; submucosa; tunica fibro-cartilaginosa; and the adventitia (Krahl 1964).

2-2 A-1 The mucous membrane.

The mucous membrane consists of: the epithelium; a prominent basement membrane; and the lamina propria.

(a) the epithelium.

The epithelium was first described in detail by Kolliker (1853 and again in 1881) and by Miller (1932) as a pseudostratified, ciliated, columnar epithelium with goblet cells, Four cell types are described by light microscopy: basal cells; ciliated cells and goblet cells. The first transmission electron microscopic (TEM) studies of mammalian trachea were by Rhodin and Dalhmann (1956) using the rat. The first scanning electron microscopic (SEM) studies were on the rabbit by Holma (1969). Since these early descriptions most laboratory animals, many of the larger animals, many of the primates, and man have been described. The following is a list of many of the descriptions: the Rat (Rhodin 1959 TEM; Andrews 1974 TEM and SEM; Alexander et al 1975 TEM and SEM); the Mouse

(Hansell and Moretti 1969 TEM; Greenwood and Holland 1972 SEM); the Guinea pig (Dahlgren et al 1972 TEM and SEM); the Hamster (Harris et al 1971 TEM; Port et al 1973 SEM); the Rabbit (Hilding and Hilding 1966 TEM; Konradova 1966 TEM; Wang et al 1972 TEM and SEM); the Dog (Frasca et al 1968 a TEM; Groniowski et al 1972 SEM); the Cat (Rhodin 1974 TEM); the Bat (Sorokin 1973 TEM); the Cow (Mariassy et al 1975 SEM); the Horse (Tyler et al 1971 TEM and SEM); Non-human primates (Greenwood and Holland 1972 SEM; Rhodin 1974 TEM); and Human (Rhodin 1966 TEM).

It is now generally agreed that at least six cell types may be present in the epithelium of the mammalian trachea (Krahl 1964; Sorokin 1973; Rhodin 1974). The cells are: ciliated cells; goblet cells; basal cells; intermediate cells; brush cells; and small granule cells.

The ciliated cell is the most numerous cell seen in the epithelium. In LM preparations and low power TEM the ovoid nuclei of these cells occupy varying heights within the cells, giving the appearances of a stratified epithelium. They are columnar in shape tapering below the nucleus to extend thin processes between the basal cells to reach the basement membrane. A feature of this epithelium is that all cells, with the exception of the occasional wandering leucocyte, have attachments to the basement membrane. Numerous mitochondria, a prominent Golgi complex, some profiles of endoplasmic reticulum, many free ribosomes

and some lysosomes occupy the supra-nuclear region of the cell. Slender microvilli 0.8 to 1.0 µm long and cilia up to 5.0 µm in length project from the luminal surface of the cell. The basal bodies of the cilia are situated immediately beneath the plasma membrane in close proximity to the mitochondria. Each cilium has the typical 9+2 microtubular structure (Satir 1974). It has been estimated that each cell has about 250 cilia (Krahl 1974), however, recent SEM studies suggest that the actual number is very variable (Andrews 1974). Near the luminal surface the cells are attached by tight junctions, but deep to the surface the lateral plasma membrane has numerous plications and interdigitating processes joined only by occasional desmosomes.

The goblet cells are typical of those found elsewhere in mammalian tissues (Rhodin 1974). They are flask-shaped cells with the nucleus towards the base of the cells. The luminal portion of the cell is usually packed with mucus droplets which sometimes coalesce. Microvilli 0.4 x 0.12 µm with a prominent filamentous coat are aggregated around the periphery of the cell. These cells are the most variable of the epithelial cells in their appearance, often being empty, or in one or other of their secretory phases.

The basal cells are small polygonal cells resting on the basement membrane. The nucleus is rounded, fairly densely staining and surrounded by scanty cytoplasm containing few organelles. The cells are attached by prominent half desmosomes and associated bundles of tonofilaments to the

basement membrane.

The intermediate cells are pyramidal cells with a broad base, which may rest on the basement membrane, or send processes between the basal cells, and an apex wedged between the goblet cells and the ciliated cells. The round to ovoid nucleus is usually situated above the level of the basal cell nuclei and is paler staining. The cytoplasm generally contains many free ribosomes and tonofilaments. Transitional stages are observed, where the cells take on some of the characteristics of the goblet or ciliated cells.

The brush cells are most frequently seen in rodents (Sorokin 1973; Rhodin 1974) but are occasionally seen in other mammals. They are columnar cells with numerous microvilli up to 2.0 x 0.2 µm, containing prominent central filaments. Other cells, which have been called brush cells, probably represent developing ciliated or goblet cells.

The small granule cells occupy a basal position and are so named because they contain many small, dense-core, granules 100 to 200 µm in diameter. These cells resemble enterochromaffin cells and have been described in the upper part of the mouse trachea (Ericson et al 1971; Sorokin 1973) and also in the bronchi of many species (Lauweryns and Peuskens 1972; Lauweryns et al 1972, 1973, 1974; Tersakis et al 1972).

As well as these six resident cells, wandering laucocytes have also been described (Kent 1966). Lymphocytes are the most frequently seen but plasma cells and mast cells are often seen.

There is still some disagreement between the SEM and TEM descriptions because of the difficulty of recognising cells which are not typical ciliated, goblet or brush cells. The ciliated cells are easily recognised by the clearly visible cilia protruding from the surface. Andrews (1974) has described three types of microvillous cells; goblet cells with smaller peripheral microvilli interconnected by fine strands a second type with a dense population of microvilli 1 x 0.06 µm, without a prominent filamentous coat, but with occasional cilia emerging from the surface; and brush cells with a dense population of longer and wider microvilli 1.5 x 0.17 µ Alexander et al (1975) described only four types of cells in the rat: ciliated; microvillous; brush; and goblet cells in that order of frequency. Greenwood and Holland in the mouse (1972) and non-human primate (1973) described only ciliated and microvillous cells. These differences will no doubt be resolved during the next few years. Several species differences have been described within each cell type, but they are generally of degree rather than substance.

Neuronal processes are also seen within the epithelium but no definite nerve endings have been identified (Rhodin and Dalhmann 1956; Rhodin 1966; Sorokin 1973; Castleman et al 1975).

(b) The basement membrane.

The basement membrane is prominent in all species which have so far been examined. It consists of a basal lamina and a thick, dense network of collagenous and reticular fibres which is continous with the lamina propria.

(c) The lamina propria.

The lamina propria consists of two layers; an inner layer which is relatively narrow contains a plexus of thin-walled bloodvessels and wandering leucocytes; the outer layer is thicker and consists of longitudinally running elastic fibres. This layer of elastic tissue extends from the larynx to the termination of the bronchi and is often called the internal elastic lamina.

2-2 A-2 The submucosa.

The submucosa lies between the internal elastic lamina and the tunica fibro-cartilaginea. It varies in thickness and is thinner where it is applied to the cartilages and thicker between the cartilages and dorsally. This layer consists of loosely organised connective tissue containing plexuses of blood vessels, nerves, lymphatics and the bodies of the tracheal glands.

(a) Tracheal glands.

The most important component of the submucosa in most mammals is the tracheal glands, which are located principally in intervals between the cartilages. The glands open onto the surface through ducts which are

arranged in grooves between the cartilages (Wang et al 1972). Dorsally however, the bodies of the glands lie external to the trachealis muscle and their ducts open into longitudinal furrows in the mucous membrane. The glands vary in number and complexity in different species but there is general agreement that the glands are compound tubulo-acinar with mixed mucous and serous elements (Goco et al 1963; Krahl 1964; Sorokin 1973; Rhodin 1974).

The mucus secreting cells are typical pale-staining cells containing membrane-bound droplets which tend to coalesce near the luminal surface (Bensch et al 1965; Sorokin 1965; Meyrich and Reid 1970).

The serous secreting cells are more pleomorphic and darker staining than the mucous cells. They contain secretion granules of varying electron-density and size. Typical myoepithelial basket cells surround the acini between the secretory cells and the basement membrane.

Another cell type, the Kultchitsky cell or argentaffin cell, which contains small dense granules has also been described (Bensch et al 1965; Meyrich and Reid 1970).

The ducts of the glands are lined by cuboidal to columnar epithelial cells which in some animals (Sorokin 1965; Meyrick et al 1969) appear to have a secretory role. Near the openings, the ducts are often lined by typical ciliated epithelium with goblet cells.

Nerve plexuses are frequently seen surrounding the acini

and numerous lymphocytes and plasma cells are also found.

2-2 A-3 The tunica fibro-cartilaginosa, or fibrocartilaginous membrane. This layer is made up of two parts; the paries annulatus or ventral three-fifths and the paries membranosa or dorsal two-fifths.

(a) Paries annulatus.

The paries annulatus consists of generally U-shaped hyaline cartilages embedded in a dense outer fibro-elastic membrane. This membrane consists of mainly longitudinally running elastic fibres which are particularly prominent at the dorsal tips of the cartilages (Krahl 1964).

(b) Paries membranacea.

The paries membranacea consists of transverse and obliquely oriented smooth muscle fibres connecting the cartilages. In man the muscle is inserted into the perichondrium at the tip of the cartilages; in carnivora the attachment is onto the external aspect; and in ungulates and insectivores the attachment is to the internal aspect (Miller 1947).

2-2 A-4 The adventitia.

The adventitia is a layer of loose connective tissue rich in blood vessels, nerves and lymphatics which is external to the fibro-cartilage layer and continuous with the fasciae of the neck.

2-2 B The Bronchi.

The bronchi are generally divided into extra-pulmonary and intra-pulmonary bronchi. Proximally the extra-pulmonary bronchi have the same structure as the trachea, while distally they

show a transition to the intra-pulmonary pattern.

The intra-pulmonary bronchi are usually subdivided into three groups: secondary or lobar bronchi; tertiary or segmental bronchi; and branches of tertiary bronchi (Rhodin 1974); or, large; medium; and small bronchi (Krahl 1964). The principal differences between the groups are of degree and size so that for the purposes of ease of description I have combined the three groups together and will consider their structure under the same headings as the trachea.

2-2 B-1 The mucosa.

(a) The epithelium.

The epithelium lining the larger bronchi is very similar to that in the trachea, namely pseudostratified ciliated columnar epithelium with goblet cells. The goblet cells are relatively more numerous in the smaller bronchi. The epithelium becomes thinner, the basal cells less numerous and eventually there is a simple columnar ciliated epithelium with numerous goblet cells (Rhodin 1974; Castleman et al 1975). Small dense-core vesicle cells are relatively more frequently seen. Neuronal processes are also more frequently found.

(b) The lamina propria.

The lamina propria becomes relatively more prominent in the smaller bronchi, consisting of a rich capillary network and loose connective tissue.

The internal elastic lamina is thicker and produces prominent longitudinal folds in the relaxed state.

2-2 B-2 The submucosa.

In the bronchi the submucosa contains the muscularis which in the trachea is confined to the dorsal wall external to the submucosa.

(a) The muscularis.

The muscularis is arranged in a geodesic network completely encircling the bronchi (Macklin 1929). Intertwining with the muscle fibres and also forming a geodesic network, is the connective tissue framework of the lung (Krahl 1964). Radially running elastic fibres can be found connecting the perichondrium, or the outer elastic membrane, to the inner elastic membrane in the lamina propria.

(b) The glands.

The glands become more numerous in the medium sized bronchi but gradually decrease in number after this point. Glands are not found in the bronchioles.

2-2 B-3 The fibro-cartilaginous membrane.

The hyaline cartilages which are U-shaped in the larger bronchi become irregular plates in the smaller bronchi. Around the bifurcations they form saddle shapes with longitudinal connections. Sections of the smallest bronchi often shows only small portions of cartilage in their walls.

2-2 B-4 The adventitia.

When the bronchi enter the hilum of the lung the adventitia and the outer elastic membrane become continuous with the fibrous framework of the lung. This layer is now called the peri-

bronchial tissue. It contains the bronchial vessels and is continuous with the layer surrounding the pulmonary vessels.

2-2 C The bronchioles.

The bronchioles are the smallest division of the conducting zone. The definition of "when is a bronchiole a bronchiole" is still somewhat confused but it is generally accepted that a bronchiole has NO cartilage in its walls, no glands, and is less than 1 mm in diameter. It is the size of a bronchiole about which there is much debate and I shall return to this later. A bronchiole generally also has a well developed circular muscle layer.

2-2 C-1 The mucosa.

(a) The epithelium.

The epithelium found in the bronchi gradually changes from a simple columnar ciliated type with some goblet cells to a simple cuboidal ciliated with "non-ciliated" cells. The bronchiolar epithelium was first described by Kolliker (1881) who found ciliated cuboidal and non-ciliated cuboidal cells. Clara (1937) repeated these observations and described granules in the non-ciliated cells. These cells are now generally referred to as Clara cells (Krahl 1964). The Clara cell has been the subject of numerous TEM investigations, most of which have been on rodents. This has led to much debate, not only about the structure of these cells but also about their function. In the mouse, the Clara cell has a bulbous apex protuding well above the ciliated cells (Karrer 1956; Niden and Yamada 1966; Niden 1967; Okada 1969;

Petrik and Collet 1970 a; 1970 b; Wang et al 1971; Etherton et al 1973; Parkinson and Stephens 1973). This bulbous portion of the cell contains whorls of concentric lamellae of smooth endoplasmic reticulum, usually surrounding mitochondria, and some secretory granules containing amorphous material. One or sometimes two prominent Golgi zones are found in the supranuclear region. The surface of the cell has no microvilli. In the rat the apex of the Clara cell is bulbous or dome shaped, with a few microvilli projecting above the ciliated cells. The cytoplasm contains well developed secretory granules, whorls of smooth endoplasmic reticulum and ovoid mitochondria (Askin and Kuhn 1971; Gil and Weibel 1971; Kuhn et al 1974; Smith et al 1974; Kuhn and Callaway 1975; Jeffrey and Reid 1975).

Similar appearances have been described for Clara cells in the following species: pigs (Baskerville 1970a, b); rabbits (Cutz and Conen 1971); and bats (Sorokin 1973). In horses (Tyler et al 1971); non-human primates (Rhodin 1974; Castleman et al 1975); and humans (Watson and Brinkman 1964; Jarkovska 1970; Basset et al 1971; Cutz and Conen 1971; Rosan and Lauweryns 1972) the Clara cells do not have such pronounced bulbous projections, but they do contain very densely staining secretory granules and have microvilli on their apical surface.

The SEM studies have confirmed the TEM appearances in the species so far examined: mouse (Okada 1969; Parkinson and

Stephens 1973); horse (Tyler et al 1971); cow (Mariassy et al 1975); non-human primates Castleman et al 1975); human (Ebert and Terracio 1975).

The ciliated cells are similar to those in the trachea and need no further description.

At least three other cell types have been described in the bronchial epithelium:- wandering white blood cells, also called globule leucocytes (Kent 1966); brush cells (Sorokin 1973; Rhodin 1974); and small granule cells or Kulchitsky cells (see above). These cells do not differ significantly from those in the trachea and will not be described again.

Some authors have described neuro-epithelial bodies in the bronchiolar epithelium (Bensch et al 1965; Lauweryns and Peuskens 1972; Lauweryns et al 1972, 1973, 1974; Cutz and Conen 1972; Hung et al 1973 a; Castleman et al 1975). These bodies are thought to be receptors, possibly chemo- or pressor- receptors (Hirsch and Kaiser 1969; Comroe 1974).

(b) Basement membrane.

The epithelium rests on a thin basement membrane.

(c) Lamina propria.

The lamina propria consists of a narrow band of fine elasti fibres and collagen fibres containing few cells.

2-2 C-2 The submucosa.

(a) Muscularis.

The muscularis is a prominent bundle of predominantly spirally running smooth muscle fibres proximally,

but becomes broken up into an incomplete spiral in the smallest bronchioles.

2-2 C-3 The adventitia.

The adventitia is thin and continous with the framework of the structures in the intermediate and respiratory zones.

THE INTERMEDIATE ZONE.

This zone derives its name from its position between the conducting and respiratory zones and because its structures show a transition between the two zones. It is composed of: terminal bronchioles; respiratory bronchioles; alveolar ducts; and alveolar sacs.

2-2 D The terminal bronchiole.

The structure of the terminal bronchiole has been described in the previous section. As its name suggests, it is the last of the true bronchioles and gives rise to from one to three or four respiratory bronchioles. The area of the lung supplied by a terminal bronchiole is generally called the primary lobule or acinus (Green 1973; Kilburn 1975).

2-2 E The respiratory bronchiole.

The respiratory bronchiole is so named because alveoli arise directly from its walls as outpouchings, as well as from its termination. It is thus the first area in which gas exchange is possible. The wall of the respiratory bronchiole consists of pillars of smooth muscle and connective tissue, between the openings of the alveolar ducts, alveolar sacs and alveoli, covered by an epithelium of ciliated cells and Clara cells.

Despite its seemingly insubstantial wall, the respiratory bronchiole is one of the most important sites of airway resistance (See Comroe 1974 for a review). The respiratory bronchiole may divide several times before terminating by giving rise to up to four alveolar ducts.

2-2 F Alveolar ducts and alveolar sacs.

These two components of the intermediate zone have a very similar structure consisting of a framework of connective tissue pillars, with a few smooth muscle fibres, covered by an epithelium of cuboidal cells or squamous cells. The SEM has demonstrated the arrangement of the intermediate zone in a way that was only possible previously by reconstruction techniques (Kuhn and Finke 1972; Wang and Thurlbeck 1970; Nowell and Tyler 1971; Castelman et al 1975). All of the structures are important in the control of air flow patterns.

THE RESPIRATORY ZONE.

2-2 G The alveoli.

The alveoli vary considerably in size and shape between and within species. Macklin and Hartcroft (1943) reported a range of diameters of:- 38.7 μm in the mouse to 166 μm in humans. Tenney and Remmers (1963) and more recently Weibel (1973), have related alveolar size, and in particular alveolar surface area, to oxygen consumption in a series of logarithmic equations or allometric relationships. It has also been shown that the shape of alveoli varies according to the following:- (a) the degree of inflation (Kuhn and Finke 1972; Weibel et al 1973; Weibel 1973) (b) the method of fixation (Klingele and Staub 1970;

Kuhn and Finke 1972; Weibel 1973); (c) the region of the lung sampled (Siegwart et al 1971; Weibel 1972).

The walls of the alveoli consist essentially of a capillary network supported by a fibrous tissue framework. In 1953 Low demonstrated for the first time that epithelium lined the alveolar septum. Bertalanffy (1964) and Weibel (1971) have reviewed the early literature and I will not attempt a precis. The alveolar septum consists therefore of:- the alveolar epithelium; the capillaries; and the interstitial tissues.

2-2 G-1 The alveolar epithelium.

This consists of three cell types resting on a basement membrane.

(a) Type I cells (cellula respiratoria)

This cell has also been called variously: the small alveolar cell; the squamous epithelium cell; type 1 pneumonocyte (pneumocyte); type A cell; type 1 cell; membranous pneumonocyte; and many others. I will use the term type I cell. This cell has the largest surface area of any of the epithelial cellsin the lung. Its thin cytoplasmic processes often extend for 50 µm or more from the nucleus and may appear on the surface of two or more alveoli (Weibel 1971; Fanning unpublished data).

The nucleus of the cell is often difficult to locate but is usually found in a niche between capillaries. The perinuclear cytoplasm contains a few mitochondria, a Golgi complex and some profiles of endoplasmic reticulum. The thin lateral extensions of the cell may be only 0.2 µm and contain some ribosomes and pinocytotic vesicles. Adjacent cells are

joined by tight junctions.

The cuboidal epithelium (cellula magna).

Two types of cuboidal cell have been described; the type II and type III cell. These have been variously called:- the great alveolar cell; septal cells; alveolar cells; type B cells; specific cells; and other names.

(b) Type II cell.

This is by far the most numerous of the cuboidal cells and has been found in all mammalian lungs (Sorokin 1967 and 1973; Kikkawa and Spitzer 1969; Belton et al 1971; Smith et al 1972; Rhodin 1974). These cells occur in groups of two or three, usually in the niches in the alveolar wall. The characteristic feature of these cells is the presence of osmiophilic lamellated bodies also called cytosomes. These cytosomes vary greatly depending not only on methods of preservation but also on the species (Kikkawa and Spitzer 1969; Schock and Pattle 1973). In optimally fixed specimens the cytosomes contain whorls of regularly spaced lamellae (Weibel 1973). Freeze fracture studies and histochemical methods suggest that they contain phospholipi The cytoplasm also contains mitochondria; free ribosomes; a few lysosomes; some granular endoplasmic reticulum; multivesicular bodies and a Golgi complex. These cells are attached to adjacent type I and type II cells by tight junctions and desmosomes.

(c) Type III cells.

The type III cells, also called brush cells, were first

described by Meyrick and Reid (1968), and later by Luciano et al (1969). They are not seen very often in lungs other than of rodents (Weibel 1973). The type III cell presents a small apical surface covered by 0.2 μm micrivilli with blunt ends, containing bundles of fine filaments which extend down into the cytoplasm. The lateral borders of the cells are covered by the type I and II cells. The distinguishing feature of these cells is that they contain no osmiophilic lamellated bodies. The cytoplasm contains numerous glycogen granules and elongated mitochondria. These cells resemble the brush cells seen in the trachea (Rhodin and Dalhamn 1956; Luciano et al 1968).

The epithelium rests on a basement membrane which fuses with that of the capillaries in the thinnest portions of the blood-air barrier.

2-2 G-2 The pulmonary capillary network.

The capillaries form a very dense network in the alveolar septum. They are lined by non-fenestrated endothelium. The organelles are confined to the perinuclear region of the cell and consist of mitochondria; a Golgi complex; and some profiles of endoplasmic reticulum. The attenuated lateral processes of the endothelial cells which contain only pinocytotic vesicles, may be only 20 nm thick. In some areas the basement membranes of the epithelium and endothelium fuse and in these regions the blood-air barrier may be only 0.1 to 0.3 μm thick, consisting only of epithelial and endothelial processes separated by fused

basal laminae. The thinnest areas of the barrier are seen more frequently in the smaller animals (Weibel 1973). True pericytes, as defined by Rhodin (1968) are rarely seen in the lung; however, many thin branching cell processes are seen in the basement membrane. Weibel (1974) has recently discussed this and considers that many of these are in fact processes of pericytes.

2-2 G-3 The interstitial tissues.

The interstitial tissues are in fact the alveolar septum. This connective tissue framework consists of bundles of collagen and elastic fibres running between the capillaries. The bundles are connected to the fibrous framework of the intermediate and conducting zones (Krahl 1964). Sobin and his co-workers have proposed a model for the structure of the septum called the sheet-flow model (Sobin et al 1970, 1972; Rosenquist et al 1973) They consider that the septum is not unlike a "parking garage with floor, ceiling and inter-running support posts". Ryan (1969, 1973) however, suggests that the foetal pattern of two separate networks with support posts between them is more probable. Fibroblasts are infrequently seen in the septum, but many cell processes are seen, many of which probably belong to fibroblasts. Occasionally other wandering cells, macrophages and blood cells, are seen, together with an occasional mast cel: Nerve fibres have also been described in the septum (Meyrick and Reid 1971; Hung et al 1972, 1973 b).

2-2 G-4 Alveolar macrophages.

Macrophages are found not only in the septum, but also on the

surface of the alveoli and bronchioles and higher up the respiratory tract. The origin of these cells has been the source of much debate in recent years (see Bowden 1971 for a review). These cells have the same structure as macrophages elsewhere in the body (Sorokin 1973; Rhodin 1974).

2-2 G-5 Pores of Kohn.

The pores of Kohn are inter-alveolar connections first described by Kohn in 1893 (see Cordingley 1972). There has been much debate as to whether they occur in normal lungs. This problem has now been resolved (Cordingley 1972; Weibel 1973). The best demonstration of the pores however, is seen in the SEM (Kuhn and Finke 1972; Nowell and Tyler 1971; Wang and Thurlbeck 1970)

2-2 H The Pleura.

The pleura is the investing coat of the lung. It consists of two parts, a visceral and a parietal layer. I will consider only the visceral layer.

(a) the visceral pleura.

The visceral pleura consists of mesothelial surface cells covering a connective tissue layer of varying thickness containing blood-vessels and lymphatics. The mesothelial cells with prominent microvilli in the perinuclear region. Most of the organelles are accumulated in this region while the thin lateral processes contain mainly pinocytotic vesicles. The cells are joined by tight junctions. The mesothelium is separated from the connective tissue by a basement membrane.

(b) the connective tissue.

The connective tissue of the pleura contains collagen and elastic fibres, fibroblasts, blood vessels, lymphatics and occasional smooth muscle cells. It is continuous with the framework of the subpleural alveoli. Attempts have been made to relate the structure and thickness of the pleura to the structure of the lung in different animals, but no definite conclusions have been reached (McLaughlin and Tyler 1961 a, b; Tyler et al, 1971).

2-2 I Lymphatics.

Lymphatics in the lung are confined to the peribronchial perivascular and subpleural connective tissues (Krahl 1964; Nagaishi 1971; Sorokin 1973; Rhodin 1974; Murray 1976). No lymphatics have been found in the adult mammalian alveolar septum, but they have been observed in foetal lung (Lauweryns 1971).

2-3 Functional Relationships.

In this section I will discuss some of the functional relationships which have a structural basis and which may be modified in the dolphin lung. The mammalian respiratory system performs two main functions:- (a) respiratory (see Comroe 1974 for a review); and (b) non-respiratory mainly metabolic (see Heineman and Fishman 1969 for a review). The epithelial lining of the respiratory tract is the largest epithelial surface in the body exposed to the environment. There is an elaborate defence mechanism in the respiratcry system to protect the delicate epithelial cells, particularly those in the alveoli, from

noxious agents in the inspired air. This defence mechanism consists of two parts:- (a) air-conditioning or warming and humidifying; (b) filtration and cleansing.

2-3 A Air-conditioning.

The principal function of the upper respiratory tract in all mammals is the air-conditioning of the inspired air so that the air which reaches the alveoli is fully saturated with water vapour and at body temperature (Walker et al 1961; Comroe 1974). The nose, nasopharynx and oropharynx are generally regarded as the sites of the airconditioning. The nasal cavity is the principal site of the conservation of heat and water with its extensive mucosal area over the turbinates. The average adult human, under normal conditions loses 250 ml of water and 350 Kcal of heat per day. This will of course vary depending on the prevailing conditions. In some abnormal situations such as hyperventilation, fever, during anaesthesia and after tracheostomy, there may be excessive heat gained or lost, and the epithelium may become desiccated.

2-3 B Filtration and cleansing.

Filtration is performed by two methods in the nasal cavity, one is mechanical by means of the hairs in the vestibule, the other by precipitation caused by air flowing around the turbinates and causing eddy currents. The particles removed by both methods are trapped then in the mucous covering of the epithelium and transported to the oral cavity by the action of the cilia, or expelled by sneezing.

2-3 B-1 Cleansing of the lower respiratory tract.

Cleansing of the lower respiratory tract is also performed by two methods, one is by the muco-ciliary escalator after deposition of the particles either by gravity or turbulent flow and the other is by phagocytosis of the deposited particules by macrophages in the airways and the alveoli. These mechanisms are a very important part of the defence and have been the subject of much research and excellent reviews are available (Morrow 1973; Green 1973).

An important part of the cleansing mechanism, whether in the nasal cavity, bronchial tree or alveoli is the surface lining layer. This lining layer varies depending upon the region being investigated. In the upper respiratory tract it appears to consist mainly of mucus and to be principally protective, while in the small airways and alveoli it is composed of "surfactants" and appears to be important in maintaining the patency of both airways and alveoli (see Scarpelli 1968; Comroe 1974; Murray 1976 for reviews).

The mucuous lining of the bronchial tree consists of two parts:- a viscous surface layer; and a less viscous deeper layer (Dalhma 1956; Krahl 1964). The cilia beat in the less viscous layer, or hypophase, propelling the more viscous surface layer towards the mouth. Until recently it had been assumed that all of the mucus produced in the bronchial tree and lung was removed either by coughing, sneezing or by swallowing. Recent evidence suggests that not all of the secretions are removed by this method

because the flow rates necessary to achieve this in the trachea would need to be about eight times the rate in the bronchi. The observed rates in the trachea are in fact only twice those in the bronchi and it has been suggested that water is absorbed frc the mucus (Asmundsson and Kilburn 1970; Van As and Webster 1972, 1974; Irvana and Van As 1972). Species differences have also been described in the types of mucus which is secreted by the goblet cells and the glands (Goco et al 1963; Meyrick and Reid 1970; Lamb and Reid 1969, 1970; Spicer et al 1971). The significance of these differences is not yet clearly defined. The surfactant system of the distal lung is an important contributor to the mucuous raft as well as being important in the maintenance of alveolar distension. Von Neergard (1929) was the first to demonstrate that surface forces contributed significantly to the elastic recoil of the lung. The surface lining layer was not demonstrated until Pattle and Macklin, independently, showed its existence in 1955. Since then numerou attempts have been made to visualise electron microscopically the surface lining layer (see Scarpelli 1968 and Weibel 1973 for a review). The most convincing evidence so far has been using freeze-fracture specimens (Untersee et al 1971) and freeze-substitution (Kuhn 1972).

The surface lining layer of the alveoli is composed of a group of phospholipids, of which dipalmitoyl lecithin is a major constituent. These function by lowering the surface tension as the alveoli decrease in size, thus preventing total collapse

of the lung (Scarpelli 1968). The source of the surfactant has been the cause of much debate in the literature some of the confusion being due to the difficulties associated with the preservation of material. Autoradiographic studies have shown that both the type II cells and the Clara cells are involved in the secretion of parts of the surface linings of the alveoli (Buckingham et al 1966; Faulkner 1969; Askin and Kuhn 1971; Etherton et al 1973). It is believed that the surface lining layer of the alveoli is moved towards the respiratory bronchioles by the movements involved in expansion and collapse of the alveoli (see Green 1973). Very little is known about the rates of secretion of surfactant so that it is difficult to estimate how much of the phopholipid found in the tracheo-bronchial secretions arises from the alveoli.

The immunological system is another very important part of the defence mechanisms of the respiratory system. There are lymphatic collections scattered throughout the respiratory tract from the nasal cavity to the bifurcations of the terminal bronchi (Krahl 1964). Immunoglobulins have been recovered from the secretions at all levels of the respiratory system, particularly IgA (Murray 1976). There is still much to be learnt about the function of the immunological system on the lung.

In this section I have attempted to outline the functional aspects of the mammalian lung which have a structural basis. I will return to a more detailed consideration of some of these points when discussing the relevance of my findings in the dolphin lung.

CHAPTER III

THE DOLPHIN RESPIRATORY SYSTEM

THE DOLPHIN RESPIRATORY SYSTEM

3-1 The blowhole and larynx.

The dolphin respiratory tract has been modified from the usual mammalian pattern by the replacement of the nostrils by the blowhole, situated on the dorsal surface of the head, and by locating the larynx in a permanent intra-narial position (Slijper 1962; Green 1972). The blowhole is a relatively simple tube surrounded by a complicated system of accessory air-sacs and associated muscles (Lawrence and Schevill 1956, 1965). No structures similar to the turbinates found in the nasal cavities of other mammals have been reported. The larynx is also a relatively simple tube without vocal cords (Blevins and Parkins 1973). The larynx is held in an intra-narial position by two very powerful muscles attached to the base of the skull, the occipito-thyroid and the palato-pharyngeus. No opposing muscles have been found (Lawrence and Schevill 1965). There is a sphincter muscle, the pterygo-pharyngeus which seals off the nasal cavity and the larynx from the oro-pharynx and the oesophagus. The function of the blowhole, nasal cavities and the larynx is not well understood, but it has been suggested, on the results of cine-radiograms, that this region is the site of sound production in dolphins (Norris et al 1971).

3-2 The trachea and bronchi.

The dolphin trachea is short but wide; tapering somewhat to the points of its bifurcation. A tracheal bronchus arises on the right proximal to the bifurcation (see appendix 1) for more

details). The microscopic structure of the dolphin was first described by Fiebiger (1916) in *Delphinus delphis*. He described: anastomosing rings of hyaline cartilage; internal and external elastic lamellae; a prominent venous plexus in the submucosa; glands; and illustrated but did not describe the epithelium. Wislocki (1929) described the trachea and bronchi of *Tursiops truncatus* as consisting of: anastomosing circular cartilaginous rings without a membranous portion; a high columnar ciliated epithelium with a row of smaller basal cells; a fine elastic lamina forming a network with the basement membrane; a stout elastic lamina situated midway between the epithelium and the cartilages; longitudinally running veins between this internal elastic lamina and the cartilages; hyaline cartilages; a stout elastic tissue membrane enclosing the cartilages, heavier on the outer surface than the inner surface; glands in the tunica propria, which were small and infrequent in the trachea and larger bronchi, but not found in the smaller bronchi; and no muscularis. He did not illustrate his findings in the trachea.

In 1933, LaCoste and Baudrimont described the trachea and lungs of *Phocoena*, which contained: stratified columnar non-ciliated epithelium without goblet cells; a thick elastic lamina dividing the area between the cartilage and the epitheli into two zones; abundant serous glands and lymphatic collections in the inner zone; a plexus of venous sinuses in the outer zone; and hyaline cartilages embedded in a fibro-elastic membra

They commented particularly on the absence of mucous secreting elements. Simple columnar epithelium with areas of pseudostratified epithelium, with numerous tubulo-acinous glands, was described by Bonin and Belanger (1939), but they commented that their material was not well enough preserved to describe the fine detail. Slijper (1962) found that there were no mucous glands or cells in the dolphin trachea or bronchi but said that this may have been due to poor material. In the Beluga, *Delphinapterus leucas*, Kleinenberg et al (1964, translated in 1969) reported plicated stratified epithelium with ducts of glands opening in the base of the plications. They also described glands, a vascular layer, longitudinal elastic lamellae and a muscular layer. Simpson and Gardner (1972) state "ciliated pseudostratified columnar epithelium arises abruptly from the squamous epithelium in the larynx and extends throughout the air-conducting pathways". They also reported that mucous glands were fewer in number than in terrestrial mammals; goblet cells were not often seen; and lymphatic aggregates were sparse. Most authors have described a similar structure for the trachea, extrapulmonary and major intra-pulmonary bronchi.

3-2 The lungs.

The lungs are pyramidal in shape with the bulk lying caudally and dorsally. There is no external lobulation (see appendix 1) The first microscopic description of the dolphin lung was by Königstein (1903) in *Delphinus delphis*. Barbosa (1914)

described the bronchial sphincters in a preliminary report. Fiebiger (1916) described the structure of the bronchial tree and lungs of *Delphinus delphis*. He found plates of cartilage present in the bronchi to their termination. He described a "bronchiolus" which was a bronchus less than 0.5 mm in diameter and contained a series of myo-elastic valves. The valves were composed of rings of smooth muscle fibres situated between the cartilage plates with radial elastic fibres connecting the thin elastic fibres beneath the epithelium to the elastic fibres adjacent to the cartilages. The openings of the alveolar ducts and alveolar sacs off the "bronchiolus" were guarded by a valve. He described a thick alveolar septum with a capillary network on each surface; many elastic fibres around the openings of the alveoli; and elastic fibres in the pleura. Wislocki (1929) reported on the structure of the lung of *Tursiops*. He found a sudden change in the morphology in bronchioles of 0.5 mm in diameter, when myo-elastic valves appeared. Six to eight branches arose from each bronchiole with six to eighteen alveolar sacs arising from each of the branches; each opening was guarded by a valve. Each valve, or sphincter, consisted of: circular smooth muscle fibres, not connected by longitudinal fibres, lying between the elastic fibres of the tunica propria and the longitudinal elastic membrane enclosing the cartilages; and radial elastic fibres connecting the two elastic membranes. At the first valve he noted a change in the epithelium from high ciliated columnar cells to delicate non-ciliated cells resembling endothelium

and the presence of an abundant plexus of capillaries in intimate relationship with this new epithelium. He confirmed Fiebiger's observation (1916) of a thick connective tissue septum in the alveoli, with a separate capillary plexus for each alveolus, and a pleura containing elastic fibres without muscle fibres. Similar findings in other Delphinind lungs have been described by LaCoste and Baudrimont (1926); Neuville (1928) Bonin and Belanger (1939); Belanger (1940); Kleinenberg et al (1964); and Ito et al (1967). Simpson and Gardner (1972) described the alveolar septum of the dolphin lung in the electron microscope. They found: the alveolar septum was 15 to 50 µm thick with capillaries on each surface; type I and type II epithelial cells; a blood-air barrier 300 to 600 nm thick; and intra-alveolar macrophages. They also commented on the relativel poor presentation of their material.

3-4 Functional relationship.

3-4 A Introduction.

Dolphins and whales breathe infrequently, averaging two to four breathes per minute while at the surface. Breathing is of the apneustic type, that is, there is a pause which occurs at the e of inspiration. When a dolphin or whale returns to the surface after a dive, the first breathe or two generally cause a visibl plume of vapour called "the blow" or "the spout". This feature was well known to whalers who used the "blow" to identify the type of whale from a distance (see Slijper 1962; Matthews 1968) The blow occupies only a short period of time, some 0.3 seconds in *Tursiops* (Lawrence and Scheville 1956) to up to one minute in some of the baleen whales. During this

short period one complete cycle of expiration and inspiration occurs. The flow rates necessary to complete the blow in this short period are extremely high. It has been estimated that in the dolphin, *Tursiops truncatus*, a flow of between 30 and 70 litres per second is required, and in the California Gray Whale, *Eschrichtius robustus*, flow rates of 200 litres per second have been recorded (Ridgway 1972; Kooyman et al 1975). It has also been reported that dolphins exchange 80% of their total lung capacity (TLC) with each blow (Irving et al 1941; Ridgway et al 1969). In order to achieve this the flow rate must be maintained even down to 20% TLC. In man and other terrestrial mammals it has been shown that at 20% TLC the flow rate is almost zero (Hyatt et al 1958; Kooyman 1973; Comroe 1974). The limiting factor in terrestrial mammals is the size of the terminal airways at the end of expiration (Hyatt et al 1958; Hughes et al 1970).

Also it has been said, that because dolphins and whales live at sea, they have no need for a filtration and cleansing mechanism, such as is present in terrestrial mammals (Slijper 1962; Cowan 1968). Anybody who has visited the sea-shore, even on a calm day, or ventured to sea, is aware that the atmosphere above the water contains many particles, as well as an aerosol of sea-water. Investigations have shown that even hundreds of miles from land, the following may be present in the atmosphere: salt nuclei; aerosols of sea-water; bacteria; diatoms; pollens; and sand (Zobell and Matthews 1936; Jacobsen 1968; Corn 1968; (First 1973).

Respiratory function in dolphins had been difficult to measure simply because of the technical problems, however, there are now some parameters available. Ridgway et al (1969) have shown in a trained dolphin "Tuffy", that there are at least two patterns of oxygen utilisation; in deep dives to 300 metres which last approximately four minutes, they showed that there was about 80% utilisation of oxygen; during shallow dives to only 20 metres, which lasted longer periods up to seven minutes, there was up to 95% utilisation.

Karandeeva et al (1973) have observed in Black Sea dolphins a similar pattern of utilisation, with the higher utilisation occurring during the longer, but shallower dives. Ridgway et al (1969) have also shown that thoracic compression commences at depths of 20 metres and is almost completed at 100 metres. This was a direct confirmation of the suggestion by Scholander (1940) that marine mammals avoid the bends by forcing the air out of the alveoli during the descent and therefore nitrogen is not absorbed. This explanation, however, is not the complete answer to the question of how dolphins avoid the bends, because Kooyman (1972, 1973) has shown that there is potentially enough nitrogen present in the air in the lungs, if it was absorbed at depths of 20 metres to cause the bends. This would certainly be possible if Ridgway et al (1969) observations are correct.

3-4 B Problems.

These observations pose several interesting problems:-

(a) what is the nature of the visible portion, or plume, of the blow;

(b) how is it possible for the upper airway to perform its

air-conditioning functions;

(c) how is it possible to exchange such a large proportion of the TLC and to maintain such high flow rates;

(d) what mechanisms are there which will allow increased utilisation of oxygen during shallow dives, but which avoid absorption of nitrogen.

I will now consider each of these points in turn in the light of available evidence.

(a) The blow or spout of dolphins and whales.

"But dolphins and whales and all such Cetacea are without gills; and, having a lung are provided with a blowhole. By this they discharge the sea-water which has been taken into the mouth."

Aristotle

The debate concerning the nature of the spout of whales has continued since Aristotle's time until quite recently (see Matthews 1968; Wood 1973). It is now generally agreed that the visible portion of the spout is water vapour which condenses due to the sudden expansion of the expired air. This is said to occur in the tropics as well as in the polar regions (Slijper 1962). Kooyman et al (1975) have suggested that the spout is due to water, but not water vapour. They have said that a small amount of water is left around the blowhole as the animal surfaces, or the blow commences just below the surface, or a small amount of water enters the blowholes as the animal dives. It is

this water which account for the visible plume. Lawrence and Scheville (1956, 1965) have shown that it is not unusual for a small amount of water to enter the blowhole as the animal dives, but that it enters the upper nasal sac and does not enter the larynx. Fraser and Purves (1955) suggested that the blow may be due to mucus which is secreted by the lining of the nasal sacs.

I believe that there is another possibility.

The spout of dolphins is due in part to water trapped as suggested above, in part to the mucus from the nasal sacs, and in part to the respiratory tract mucus being expelled from the lower regions of the respiratory tract. The expiratory effort of the dolphin, although it is due to passive recoil Kooyman (1973) has a force not unlike that found in a cough in terrestrial mammals. I will also show that there is good structural evidence for the production in the dolphin airways of similar amounts of secretion as in the terrestrial mammals.

(b) Functions of the upper airway.

One of the principal functions of the upper airway in terrestrial mammals is the warming and humidification of the inspired air, and the conservation of heat. These functions are also required by the dolphin, particularly in colder climates. It has been shown that although there is a complex series of accessory air sacs related to the nasal cavity, the cavity itself is an uncomplicated tube

without turbinates or similar structures. It would seem unlikely then, that with the high flow rates observed, and the absence of turbinates, that the nasal cavities can function in the air-conditioning. Coulombe et al (1965) have reported that the respiratory water loss for two species of dolphins, *Tursiops truncatus* and *Lagenorhynchus obliquidens*, was only 30 and 70 per cent of the expected loss from a terrestrial mammal of the same body weight. They suggested that the saving occured due to the control of pressure and temperature of the air in the nasal cavities. Krogh (1939) proposed that there was a reduction in the water loss from the respiratory tract in dolphins because of the reduced frequency of breathing. Another function of the upper airway in terrestrial mammals is the filtration of particles from the inspired air. In dolphins this function would still be necessary but cannot be performed, therefore it must be transferred to the lower respiratory tract. It has been shown that these functions can be performed in extreme situations by the trachea in terrestrial mammals (Moritz and Weisiger 1945). I will show that the structure of the dolphin trachea has been modified to perform these functions, and, that the apparent contradictions in the earlier reports of the tracheal epithelium are consistent with these modifications.

(c) Maintenance of high flow rates.

The dolphin respiratory system has cartilage present in the walls of the bronchi to their termination, and a system of

myo-elastic sphincters in the most distal branches. Kooyman (1973) has suggested that the function of the cartilage is to maintain airway patency even at low volumes, and is supported by the evidence of Hyatt et al (1958) that compression of the terminal airways is responsible for the great increase in resistance, and hence decrease in flow rate at low volumes in terrestrial mammalian lungs. Another function for the cartilage is to maintain the patency of the airways as the animals dive so that the air can be forced from the alveoli into the airways. Scholander (1940) was the first to suggest that the air from the alveoli was sequestered away from the absorptive surfaces in the alveoli during diving. This has now been confirmed by direct observation by divers and by television (see Ridgway 1972). The function of the myo-elastic valves has not yet been satisfactorily explained. Slijper (1962) has suggested that they act as a series of taps on a pipe, regulating the return of the air from the bronchial tree during the ascent phase of the dive. Other suggestions that they play a part in sound production have not been substantiated.

I propose that these sphincters close at the end of inspiration trapping the air in the alveoli and allowing relaxation of the external respiratory muscles. If this did not occur the elastic recoil would force the air into the bronchial tree when the external muscle relaxed. During the dive the sphincters act as Slijper has suggested to

control the flow into and out of the alveoli.

(d) Mechanisms to allow additional absorption of oxygen during shallow dives.

This follows directly from the preceding section. Wislocki (1929) showed that at the level of the first myo-elastic valve there was a change in the lining of the bronchial tree from a cuboidal to a squamous type of epithelium, and that in this region capillaries became closely associated with the cells. He suggested that this sphincteric segment therefore had a respiratory function.

I will demonstrate that Wislocki was indeed correct and this segment has structurally all of the requirements for gas exchange to occur. I propose that there is a counter-current blood flow through this segment to enable nitrogen to be returned from the circulation during the ascent phase of the dive.

CHAPTER IV

MATERIALS AND METHODS

MATERIALS AND METHODS.

4-1 Animals.

The bronchial tree and lungs from eight South Australian Bottle-nosed dolphins have been examined. Six of the animals were taken, under license, for research purposes, between August 1971 and May 1972. The remaining two were obtained from Marineland of South Australia; one was found dead in its pool and euthanasia was performed on the other because of serious injury sustained during capture.

All of the animals were caught in nets in Spencer's Gulf, off Port Broughton, and transported to Marineland of South Australia by truck. The six animals caught for research purposes were held in quarantine pools for from one to two days. The other two animals had been in the main pool for two and seven months respectively. The animals were numbered sequentially AD1 to AD8 according to the order in which they were first examined and measured. The details of the general anatomy and measurements of the animals are recorded in appendix 1 and Harrison and Fanning (1974).

4-2 Collection of samples.

The animals were transported from Marineland to the Medical School. Some were given Diazepam 10mgm/100kgm(D) by injection into the dorsal musculature just lateral to the dorsal fin before transportation (Ridgway 1972). Others were given no premedication. General anaesthesia was induced in four animals AD1, 2, 5 and 8 by injection either Phencyclidine 0.2mgm/kgm(PH)

or Pentothal sodium 10mgm/kgm(Pe) intravenously into a tail-fluke vein (Ridgway and McCormick 1971; Ridgway 1972), until the blowhole reflex was abolished. An endotracheal tube was then passed through the mouth and anaesthesia was continued using Halothane, nitrous oxide and oxygen (HGO) with manually assisted respiration. Considerable difficulties were encountered with the techniques . Euthanasia was performed on one animal, AD4, with sodium pentobarb 60mgm/kgm intravenously. One animal, AD7, which did not receive premedication, collapsed and died immediately before anaesthesia was attempted. One animal, AD4, died in its pool and another, AD6, died en-route from Port Broughton in near century heat (T39^{o}C).

Samples obtained and anaesthetics used are summarised in table 1

4-3 Specimen preparation.

Samples were obtained for electron microscopy from six animals, AD1, 2, 3, 5, 7 and 8; and from all except AD6, for light microscopy. The samples for electron microscopy were obtained:- at thoracotomy, under anaesthesia; immediately after death; or following perfusion of the airway or aorta.

4-3 A The following samples were taken:-

The trachea. Complete rings were taken at three levels: just below the larynx; the mid portion; just above the bifurcation. Longitudinal sections were also taken from the ventral, lateral and dorsal aspects at these levels.

Bronchi. Complete rings and longitudinal sections were taken at various levels in the bronchial tree.

TABLE SUMMARY OF ANAESTHETIC AGENTS, SPECIMENS TAKEN & TECHNIQUES USED

SPECIMEN NO.:	AD1	AD2	AD3	AD4	AD5	AD6	AD7	A
Length (cm)	202	201	162	143	201	223	222.5	1
Sex	M	F	M	F	M	F	F	
Anaesthetic agents. see text for abbreviations.	Ph.D. HGO	Pl.D. HGO	P.B.	None	Pl.D. HGO	None	None	P H
Light microscopy	+	+	+	+	+		+	
T.E.M.	+	+	+		+		+	
S.E.M.	+	+			+		+	
Trachea biopsy	+		+				+	
Lung biopsy	+	+	+		+		+	
Airway perfusion of fixative for EM		+			+			
Airway perfusion of fixation for LM	+		+	+			+	
Aortic perfusion		+			+			

Lung. Two types of specimen were obtained from the lung:-

(a) collapsed lung: slabs of lung up to 1 cm thick were cut from the deflated lung and fixed by immersion in fixative.

(b) the lung was perfused with fixative through the trachea or segmental bronchus.

Further processing of the specimens will be considered separately

4-3 B Light microscopy.

Specimens for L.M. were fixed in 10% buffered formalin or in one of the fixatives used for T.E.M.. All samples were dehydrated in alcohol and embedded in pariffin wax. Five micron sections were cut and stained. Special care was required after glutaraldehyde fixation (Rosen et al 1967). The stains used included: haematoxylin and eosin (H&E); Verhoeff and van Geisen (V&VG); Weigert's stain for elastic tissue; Lison's Alcian blue-Chlorantine fast red (Culling 1963); and Alcian blue - periodic acid Schiff (Lamb and Reid 1969).

4-3 C Transmission electron microscopy.

Two techniques were issued to fix the specimens:-

(a) Routine techiques. Specimens were diced into small fragments from 1-2 mm x 6-5 mm for orientation in the trachea, or from 1-3 mm^3 for the lung. These techniques are standard methods of preparation for T.E.M. (Hayat 1970; Glauert 1975). The primary fixatives used were:-

i) 3% or 6% glutaraldehyde in 0.1 M phosphate or 0.1 M cacodylate buffer at pH 7.3 (Sabatini et al 1963);

ii) 3% glutaraldehyde in 45 mM cacodylate buffer at pH 7.3

adjusted to 320 ± 10 m osm with 5% sodium chloride using a Fiske freezing point depression osmometer (Gil and Weibel 1968);

iii) Karnovsky's glutaraldehyde-paraformaldehyde fixative diluted with either 0.1 M phosphate or 0.1 M cacodylate buffer at pH 7.3 to give a final osmolarity of 550 - 650 M osm (Scheenberger-Keeley and Karnovsky 1968).

The time of fixation varied from one to twenty-four hours depending on the specimen.

(b) Perfusion fixation. This technique was used in two of the animals, AD2 and AD5. The fixative used was diluted Karnovsky's in phosphate buffer (iii above). The fixative was perfused through the endotracheal tube after the animal had been given an overdose of sodium pentobarbital. The lung was first allowed to collapse by opening the abdominal cavity and incising the diaphragm. The lung was then reinflated so as to fill the thoracic cavity; this required a pressure of 20 - 30 cm of water. After the lung perfusion was commenced, a T-tube was inserted into the abdominal aorta below the renal arteries and perfusion commenced using the same fixative at a pressure of 120 mm of mercury. Both perfusions were continued overnight. The next day the lungs and heart were removed en-bloc and stored in fixative until the specimens were selected.

(c) Post fixation. The tissues were washed overnight in buffer at pH 7.3 adjusted to 320 M osm. Specimens were post-fixed in approximately 1% osmium tetroxide in phosphate,

cacodylate, or S-collidine buffer, all adjusted to pH7.3 and 320 m osm (Gil and Weibel 1968). Some specimens were block stained with 0.5% uranyl acetate in veronal-acetate buffer (Farquar and Palade 1965) or 1% uranyl acetate in distilled water for two hours.

(d) Embedding. All specimens were dehydrated in alcohol and epoxypropane and embedded in a variety of epoxy resins: Epon 812 (Luft 1961; Weibel, personal communication); Araldite (Luft 1961); Epon-araldite mixture (Ito, personal communication). There was considerable variation in the cutting properties of the blocks obtained. Many modifications to both the formulae and the curing and embedding techniques were tried without achieving a consistent result. Late in 1972 the opportunity arose to try the low viscosity medium described by Spurr (1969). This procedure was further modified in our laboratory (Fanning and Findlay unpublished). The procedure is as follows:-

Tissues which had been stored in a variety of buffers and fixatives at 4^{o}C or at room temperature and freshly fixed tissues from rat and sheep were washed in several changes of fresh 0.1 M cacodylate buffer pH 7.3 and 320 m osm, for two days at 4^{o}C. They were post-fixed in 1% osmium tetroxide in cacodylate buffer pH 7.3 and 320 m osm for two hours, rinsed in water, blocked stained with 0.5% uranyl acetate for two hours. The specimens were rapidly dehydrated in ethanol commencing at 70%, using two changes of five

minutes each of 70%, 80%, 95% and 100% ethanol. During this procedure the specimen vials were placed on an inclined rotator revolving at one revolution per minute. The vials were then placed in a desiccator containing fresh phosphorou pentoxide for one hour. Just enough ethanol to cover the specimens was used. The Spurr medium was purchased from two sources:- Polysciences U.S.A. and Taab laboratories U.K.; each gave identical results. The medium was freshly prepared each time and consisted of:-

ERL 4206	10 g
DER 736	5 g
NSA	26 g
S 1	0.4 g

The constituents were weighed on a balance, mixed by hand and stored in the desiccator. After one hour in the desiccator an equal quantity of the resin mixture was added to the vials, stirred and the vials were then replaced in the desiccator for a further thirty minutes. The vials were then capped with dry caps and replaced on the rotator for four hours, when the resin was replaced completely by fresh mixture, the vials recapped and left on the rotator overnight.

The specimens were then embedded in Beem capsules or flat trays using the same batch of resin that had been stored in the desiccator. It is most important that strict precautions be taken to exclude all traces of water, otherwise

brittle blocks will result. This is seen particularly in the case of gelatine capsules which we were never able to use successfully.

(e) Sectioning. Semithin sections were cut at 0.5 - 1 µm and stained with 1% toluidine blue in 1% borax or Methylene blue-Azure 11 (Richardson et al 1960) for orientation. Silver to gray sections were cut with glass or diamond knives on Cambridge-Huxley or Reichert OmU2 ultra-microtomes collected on coated or naked grids and stained with uranyl acetate and lead citrate.

(f) Microscopy. The sections were examined in a Philips EM 300 electron microscope fitted with one of the following stages:- high resolution; goniometer; or PW 6500 STEM unit; operating at either 60 KV or 80 KV.

4-3 D Scanning electron microscopy.

This part of the study was not commenced until the latter part of 1974. The specimens were selected from those animals where T.E.M. had shown excellent preservation. As all had been stored for some years, additional samples were also processed by the new techniques (see above) to check the results.

The specimens were washed in buffer and dehydrated in graded alcohol and amyl acetate in readiness for critical point drying (Anderson 1951). Because of the large size of some of the specimens (Fig. 51) it was necessary to use a standard rotary shaker operating at slow speed during the dehydration. It was also necessary to prolong the process to two days for these samples.

Critical point drying was performed in a Denton DCP 1 apparatus using liquid CO_2. The technique used was that recommended by Stephens and Evans (1973) of venting the chamber for two minutes in every ten until no trace of amyl acetate was detected. Other samples were allowed to dry in a dust-free atmosphere after the last change of amyl acetate. Other specimens were dehydrated in acetone and then air-dried.

The dried specimens were then trimmed under a dissecting microscope to demonstrate the features required, e.g. brochioles, and to fit the specimen holders. They were then cemented onto the holders using silver conducting paint (Pelco U.S.A). Completed specimens were then coated with carbon and gold-palladium in a Denton DV 502 vacuum evaporator fitted with a tilting omnirotator. A vacuum of 10^{-5} torr was used with 5 second coat of carbon and six inches of 0.008" gold-palladium wire.

The specimens were then examined in the Philips EM 300 with PW6500 STEM or a Siemens Etec Autoscan at Flinders University Medical School, or the Department of Geology, University of Adelaide. All of the pictures used in this work were taken on one of the Etec microscopes due to failure of the detector in the Philips instrument.

4-4 Additional specimens.

A number of other specimens were available for study from a collection obtained during my service in the Anatomy Department at the University of Queensland from 1966 to 1968. These were

collected in conjunction with Professors H.W. Whitting and R.J. Harrison. Most of the material consists of paraffin sections from the trachea and lungs of four Delphinid species:- *Tursops catalania (T.truncatus); Stenalla rosieventris Globicephala scammoni; Peponocephala electra.* Two of the *Tursiops'* lungs were processed for electron microscopy and I have used one of the pictures from this series (Fig. 99). The preservation of the specimens for T.E.M. was not very good.

A corrosion cast of the brochial tree was prepared from the *Peponocephela electra* using the techniques of Tompsett (1956), modified to use Shell Epirez 8859. Details of this specimen are included in appendix 3.

CHAPTER V

RESULTS

RESULTS

5-1 General Anatomy.

Details of the form, colour, measurements and certain of the anatomical features of the eight animals from South Australian waters which formed the basis of this study are included in appendix 1 (Harrison and Fanning 1974). Detailed taxonomic description must await further studies but it has been identified provisionally as *Tursiops truncatus*.

5-2 Gross anatomy of the trachea and lungs.

A general account of the findings in this group of animals is included in appendix 1 (pp 209-210). A brief description of the corrosion cast of the bronchial tree of the Queensland specimen is included as appendix 3. This is a transcript of a demonstration together with a series of photographs of the specimen.

5-3 Techniques.

No account of electron microscopic investigation can be complete without some comments on the techniques used and the reasons for choosing them. In 1966 when I started the study of lung structure the "standard" techniques for TEM were in fact far from standard or even repeatable. Even to attempt to record all of the methods used during the study would fill a volume larger than this without adding to our understanding.

5-3 A Anaesthesia.

The anaesthetic techniques used were those in general used in 1971 (Ridgway 1972); however, we experienced some problems because our animals were smaller and reacted differently to the

agents used. This has caused some concern amongst the veterinary officers charged with the care of the animals in the various marinelands in Australia, and is the subject of a continuing investigation.

5-3 B Fixation.

Considerable variation in the quality of fixation was observed in blocks from the first two animals AD1 and AD2. The best fixation was obtained when one of the diluted Karnovsky's solutions was used. The best technique was airway perfusion. Unfortunately most of the biopsy specimens showed fixation artefacts (Fig. 100). These were the ones chosen for examination of the surface lining layer and the results are not included in the thesis- Dolphins are a protected and endangered species so that I have not felt justified in pursuing this aspect of the study at this time. The specimens which had been stored for several years showed surprisingly little difference from those processed immediately. The main changes observed were some extraction of the phospholipid containing areas, namely membrane and the lamellar bodies of the type II alveolar cells. (Fig. 103, 104). All species fixed in phosphate buffered solutions exhibited electron-dense deposits scattered throughout. If the tissues were adequately rinsed however, these deposits were not seen.

5-4 The trachea.

5-4 A The epithelium.

Three types of epithelium have been found in the dolphin

trachea: proximally there is a transitional type; distally there is respiratory epithelium, but most of the trachea is lined by a stratified columnar to cuboidal type of epithelium. Occasional islands of either respiratory or transitional type are found scattered throughout the trachea (Fig. 1,2,3,and 4). No consistent pattern has been observed apart from the general one already described.

5-4 A-1 Stratified columnar to cuboidal type or microvillous type. Most of the trachea is lined by this type of epithelium, tending to cuboidal cells in the more proximal regions and columnar in the distal regions. No abrupt transitional areas have been seen (Fig. 2 and 3). In younger animals the cells are smaller and the epithelium thinner; about 20 µm. In the older animal it may be up to 60 µm thick.

The surface of this epithelium stains positively with Alcian blue in both the Lison and Alcian-blue/PAS techniques. Occasiona cells in the surface layer also stain blue with these methods, but most of the cells are PAS positive.

In the SEM, this epithelium is seen to consist almost exclusivel of microvillous cells which are 3-8 µm in diameter and irregula ly hexagonal in shape (Fig. 5). The microvilli are heaped up around the margins of the cells to form distinct outlines. Occasionally cells are seen below the level of the surface which are similar in all other respects and probably represent younger cells (Fig. 6). In some areas isolated ciliated cells are found (Fig. 7). In other areas cells with fewer microvilli

protrude above the surface. These cells may well be goblet cells but as yet no attempt has been made to embed these areas for TEM. In the TEM the characteristic features of this epithelium are seen (Fig. 8 and 9).

The basal cells are about 6 μm in diameter, rounded, more electron-dense than the rest of the epithelium, and have a high nucleus-to-cytoplasm ratio. The nucleus is usually densely stained with a much folded outline; however, occasionally nuclei are rounded and pale staining, with a prominent nucleolus. The cytoplasm contains: many free ribosomes; some profiles of endoplasmic reticulum; some round to oval mitochondria up to 0.6 μm in length with prominent transverse cristae. The cells are attached to the prominent basal lamina which is 0.15 μm thick, by half desmosomes and associated tonofilaments. There are some convolutions of the basal plasma membrane but no prominent infoldings. The lateral cell membrane has many long interdigitating processes with contact areas between cells, but no junctional areas. Desmosomes are rarely seen.

The surface cells are from 12 to 20 μm in height (Fig. 8 and 9). The luminal surface of the more proximal cells has short squat microvilli up to 0.2 μm high and 0.15 μm wide (Fig. 11), however, many were 0.1 x 0.1 μm. The more distal cells have longer and thinner microvilli 0.6 - 0.8 x 0.1 - 0.2 μm. The surface cells are divided into four zones: (a) a basal zone containing a large pale nucleus up to 5 μm in diameter and few organelles; (b) a supranuclear zone containing a Golgi complex,

some profiles of smooth and rough endoplasmic reticulum and a few mitochondria; (c) a prominent mitochondrial zone generally packed with oval mitochondria up to 1 μm in length; (d) an apical organelle-free zone containing many cytoplasmic filaments in a layer up to 0.5 μm thick immediately beneath the surface plasma membrane.

In the more distal regions of the trachea the cytoplasm of the surface cells almost reaches the basement membrane, extending long finger-processes between the basal cells (Fig. 9). These cells also have a more prominent supranuclear zone and in some, membrane-bound vesicles up to 0.2 μm in diameter containing pale staining amorphous material or whorls of lamellated material are found (Fig. 11). The cells are attached to the basal cells or basement membrane by desmosomes and tonofilaments. The more proximal epithelium consists of smaller surface cells, differing mainly in the size of the supranuclear zone. The lateral plasma membrane of the surface cells has even more folding and interdigitating processes than the basal cells. Few junctional areas are seen between these processes.

Intermediate cells are present between the basal and the surface cells. They are essentially the same as in other regions of the trachea and will be described shortly.

A feature of the tracheal epithelium which is first seen in the illustrations for this section is the differences which occur in the size of the intercellular spaces. These differences depend on the fixatives used. The intercellular space is

dilated in specimens fixed in isosmolar solutions, for example 3% glutaraldehyde in 45mM cacodylate, but in hyper-osmolar solutions, for example, Karnovsky's, the spaces are almost obliterated.

5-4 A-2 Transitional type.

The epithelium in the most proximal regions of the trachea is statified squamous non-keratinizing to stratified cuboidal in type. It is a transition from the epithelium lining the larynx to that lining most of the trachea. It has several features which make it unique, namely :-

(a) The epithelium varies in thickness from three cells (20 μm) when stretched to five or more cells (50-60 μm) when relaxed (Fig. 12 and 13).

(b) The plasma membrane of the luminal cells has an asymetric trilaminar structure very similar to that found in the urinary bladder.

Thus not only is it transitional in position, but it has some of the features of the transitional epithelium of traditional histology.

The basal cells are similar in size and staining characteristics to those described above. They differ however in having a less folded basal plasma membrane resting on a convoluted basement membrane. There are numerous hemi-desmosomes in contact with the basal lamina, with bundles of tonofilaments extending up to 5 μm towards the luminal surface of the cells (Fig. 16). The lateral processes of these cells contain more tonofilaments

than the basal cells of the microvillous epithelium, but intercellular junctions are not more common.

The intermediate cells range in size from 5 - 6 μm to 8 - 10 μm and in shape from round to ellipsoidal. Their cytoplasm contains round to elongated mitochondria 0.2 to 1.2 μm in size; many free ribosomes; some profiles of smooth and rough endoplasmic reticulum; and membrane-bound droplets up to 1 μm in size containing granular material of varying electron-density, some have an electron-dense core (Fig. 17). These droplets are not seen in every cell.

The surface cells are bulbous, particularly in the contracted state. This is best seen in the L.M. of both paraffin and epoxy sections (Fig. 12 and 13) although it is also seen in T.E.M. (Fig. 14 and 15). These cells are also divided into four zones: (a) a basal or nuclear zone, (b) a supranuclear zone, (c) a mitochondrial zone and (d) an organelle-free zone or terminal web. The principal difference between these cells and the microvillous cells are:-

(a) the shape of the cell, ranging from almost squamous in the stretched state to columnar and bulbous in the contracted state.

(b) in the supranuclear zone there are one or more prominent Golgi complexes containing stacks of flattened cisternae and/or vesicles, some profiles of rough endoplasmic reticulum, and many vesicles ranging in size from 0.1 - 0.8 μm. The smaller vesicles contain electron-dense granular

material, while the larger ones contain dense granular material or whorls of lamellae. These vesicles are found only in the supranuclear zone.

(c) the mitochondria are oval to spherical up to 0.5 μm in diameter with very prominent transverse cristae.

(d) the surface of the contracted epithelium is thrown into a series of irregular folds not unlike the scalloped surface of the urinary bladder. The surface microvilli are short and squat, up to 0.2 x 0.2 μm. On closer examination the plasma membrane of the surface cells is found to consist of an asymmetrical unit membrane. The outer leaflet is more densely staining, 3.4 nm thick with about 8 nm of fuzzy coat on its surface; the inner leaflet is paler staining, 2.3 nm thick; and the central electronlucent lamina is 3.3 nm thick (Fig. 18). This pattern of the unit membrane does not end abruptly but is continued for some distance into the microvillous epithelium of the proximal trachea (Fig. 19). The usual trilaminar membrane about 9.0 nm thick is found in the more distal regions and through out the remainder of the respiratory tract (Fig. 20). These measurements are the average of ten readings. No densitometer measurements have been done, but photographs have been taken in the goniometer of both this membrane and rat urinary bladder, to confirm that this is not an artefact. This will obviously need to be repeated on fresh material. In the L.M. this epithelium has a pale PAS-positive surface

coat with strongly positive granules in the intermediate cells. The S.E.M. shows an irregular surface on which the individual cells are not well defined (Fig. 21 and 22). At higher magnification rows of rugae are seen, rather than the microvilli expected from the TEM.

5-4 A-3 Respiratory epithelium.

Typical respiratory epithelium is found in the most distal part of the trachea and in the major bronchi. In the L.M. the epithelium is pseudostratified ciliated columnar in type with numerous goblet cells (Fig. 4). The goblet cells stain blue with the Lison technique, are PAS positive, and red-blue or blue-red with the Alcian blue-PAS technique.

In TEM only four cell types have been identified: ciliated cells; goblet cells; intermediate cells; and basal cells. Occasional microvillous cells are found, but so far despite an extensive search, no brush cells or dense-core granule cells have been found (Fig. 23).

The basal cells are very similar to those already described in both of the previous types of epithelium. The only difference is that they may be slightly paler staining, they contain fewer tonofilaments and have fewer basal attachments.

The ciliated cells send elongated processes between the basal and intermediate cells to reach the basement membrane. The nucleus is pale-staining, oval, and is found at varying levels within the cell. The cilia are up to 8 μm long with a typical nine-plus-two axial arrangement and basal bodies (Fig. 24).

Between the cilia are microvilli 4 μm long and 0.15 μm wide. In the supranuclear region are found: a Golgi complex; some profiles of endoplasmic reticulum; free ribosomes; and some glycogen granules. In the apical region of the cell are numerous mitochondria 0.2 x 1.5 μm and in some cells densely staining pleomorphic lysosomes. The lateral surface of the cell has a much folded plasma membrane interdigitating with adjacent cells but forming few junctions. Tight junctions are found adjacent to and at the luminal surface.

Goblet cells are seen at all stages of the secretory cycle and are generally more densely staining than the ciliated cells. (Fig. 23 and 25). The apices of some of the cells bulge above the level of the ciliated cells and the surfaces of these cells have microvilli 0.5 x 0.15 μm scattered over them. Most of the cells have a more typical goblet shape with a depressed surface, with microvilli aggregated around the periphery and are packed with membrane-bound mucous droplets up to 1 μm in diameter. These droplets contain granular material of varying electron-density, some having dense cores 0.1 μm in diameter. Some cells are so full of mucous droplets that the membrane cannot be seen and it is difficult to discern any cytoplasmic organelles. Others, usually more darkly staining with protruding apices, contain many free ribosomes, a Golgi complex, round to oval mitochondria with transverse cristae and granules, and smooth and rough endoplasmic reticulum (Fig. 25). These cells also have narrow elongated basal processes which reach the basement

membrane, and many interdigitating lateral processes.

The intermediate cells form a layer between the basal cells and the goblet and ciliated cells. They rarely reach the surface but often show characteristics of either the ciliated or goblet cells. They are generally pale staining rounded cells with a large pale rounded nucleus (Fig. 23).

In the SEM the typical appearance of this epithelium consists of a mat of cilia bent over just above the surface (Fig. 26, 27, 28 and 29). On closer inspection it can be seen that not all of the cilia are pointing in the same direction, there are whole areas or groups of cells with cilia which are bent at various angles to the long axis of the trachea. Between the ciliated cells can be seen either the dome-shaped apices of engorged goblet cells, or the more typical appearance of a central depressed area with surrounding microvilli.

5-4 A-4 Basement membrane.

All three types of epithelium rest on a basement membrane which consists of a denser staining basal lamina 50 nm thick and a layer of collagen fibres, microfibrils and fine elastic fibres (Fig. 10, 16 and 23). The type of epithelium found depends on the area examined, because all three types have been found in the youngest animal in the series (AD8) which was probably less than one year old (see appendix 1).

5-4 B Lamina propria.

The lamina propria consists of connective tissue which in some areas is loosely arranged and in others is more dense. It

consists of collagen and elastic fibres and contains the bodies of the superficial layer of glands. It also contains nerve fibres, plasma cells, and in many areas mast cells.

5-4 C Internal elastic lamina.

This dense elastic lamina marks the boundary of the lamina propria and the submucosa. It ranges in thickness from 100 to 600 µm thick (Fig. 31, 32 and 38) and contains both longitudinal and spiral fibres. The fibres have the usual arrangement of pale staining amorphous fibres surrounded by more densely staining microfibrils. A detailed study of the elastic fibres in dolphins and other mammals is in progress (Fanning and Cleary unpublished data). The internal elastic lamina encloses the bodies of the deeper layer of glands.

5-4 D Submucosa.

This extends from the internal elastic lamina to the cartilage plates. It contains an extensive plexus of veins and arteries and the bodies of the deeper layer of glands. This plexus of vessels consists mainly of spiral and longitudinal thin-walled veins up to 500 µm in diameter (Fig. 31, 32 and 41). These veins contain both elastic fibres and smooth muscle in their walls (Fig. 41). A detailed study of this plexus has not yet been completed. Stout elastic fibres run between the internal elastic lamina and the cartilage and in some areas seems to fuse with the walls of the veins.

5-4 E Glands.

Two layers of glands have been found in the trachea; one lies

between the epithelium and the elastic lamina and the other lies within and extends through the elastic lamina. There does not appear to be any structural difference between the two layers, but they open by separate ducts onto the surface (Fig. 31 and 32). The inner layer (or superficial) is 30 to 40 µm thick and its glands have short ducts lined by simple cuboidal epithelium except near their termination, where it resembles the surface epithelium (Fig. 36). The outermost (or deeper) layer of glands is larger, measuring 50 - 200 µm thick with long coiled ducts lined by stratified cuboidal to columnar epithelium which also assumes the structure of the surface epithelium near its termination.

The glands consist of serous and mucous acini. Few demilunes are found. No quantitative studies have been done but there is an obvious preponderance of serous glands in the more proximal regions and a fairly equal distribution in the distal regions. The serous acini stain positively with PAS and are red/blue with Alcian blue-PAS. The mucous cells stain blue with the Lison technique. Detailed histochemical studies have yet to be done.

The glands have been studied by TEM only.

The serous cells are characterized by densely staining granules up to 2 µm in diameter (Fig. 33). In active cells there are: a prominent Golgi complex; many profiles of dilated rough endoplasmic reticulum; many free ribosomes; and elongated mitochondria 2.5 x 0.2 µm. The apex of the cell contains

secretory granules which vary greatly in size, shape and staining characteristics. A detailed study of this variation was not attempted. The lateral surfaces of the serous cells have many long thin processes which project into the intercellular canaliculi.

The mucous cells contain droplets which vary in size and staining. The smaller droplets, about 0.5 μm, are darker staining, while the larger, up to 2 μm, are lighter. The cells have a prominent Golgi complex consisting of flattened cisternae and/or vesicles up to 50 nm in diameter. The rough endoplasmic reticulum is flattened in the basal region of the cell but dilated with pale-staining granular material in the apical region. There are tubular mitochondria 2.5 x 1 μm with prominent cristae (Fig. 34, 35). Dilated intercellular channels are commonly seen in active glands. In some cells membrane-bound granules 1 to 1.5 μm in diameter containing whorls of densely staining material are found. The nucleus is compressed towards the base of the cell. The mucous and serous cells are joined by tight junctions at their luminal surfaces.

Myo-epithelial cells surround each acinus. They are situated between the secretory cells and the basement membrane, and extend thin processes between the secretory cell (Fig. 36). The myo-epithelial cells have elongated nuclei and are packed with myo-filaments which are parallel to the long axis of the cell. A few spherical mitochondria, free ribosomes and some profiles of endoplasmic reticulum occupy the small area between the

elongated nucleus and the myo-filaments. Desmosomes are occasionally found joining the myo-epithelial and secretory cells. Kultchitzsky cells have not been seen; however, many typical plasma cells are found adjacent to the glands (Fig. 33).

5-4 F Lymphatic collections.

The proximal trachea has longitudal folds along its ventral surface (see appendix 1). In the grooves of this region are found the openings of epithelial-lined crypts (Fig. 30 and 40). These crypts are surrounded by lymphatic tissue collections. The crypts which are lined by pseudostratified ciliated columnar epithelium with goblet cells, extend into and through the internal elastic lamina. Openings into the lumen at the base of the crypts are the ducts of glands which are predominantly serous. Germinal centres are found in many of the larger nodules (Fig. 39, 40). In the younger animals (AD3, 4 and 8), the lymphatic tissue is not as well developed and nodules and germinal centres are scanty; however the crypts are surrounded by lymphocytes. The lymphatic collections are found principally in the junctional region of the larynx and the trachea, but extend distally as far as the second tracheal cartilage.

5-4 G Cartilages.

The cartilages are typical hyaline cartilage embedded in a dense fibro-elastic membrane 100-200 μm thick on the outer surface and 20-50 μm thick on the inner surface. Between the cartilages this membrane is up to 1.5 mm thick (Fig. 38) and consists of longitudinally running elastic and collagen fibres.

5-4 H Nerves.

Intra-epithelial nerve fibres have been found in all three types of epithelium. These fibres are mainly unmyelinated and are found generally in the basal regions between the basal cells. No nerve endings have been identified (Fig. 42, 43 and 44).

5-4 I Adventitia.

The adventitia is continuous with the surrounding connective tissue.

KEY TO LEGENDS AND LABELS TO PLATES.

All micrographs are from lungs fixed by tracheal perfusion unless otherwise stated in the legend. All TEM specimens were block stained with uranyl acetate and sections were stained with uranyl acetate and lead citrate. All SEM photographs are from an ETEC SEM and all specimens were coated with gold-palladium.

COMMON LABELS.

C	cilia or ciliated cells
G	Golgi
G.C.	goblet cell
B.C.	basal cell
N	nucleus
R	rugae
M.V.	microvilli
AD	alveolar duct

alv.	alveolus
b.v.	blood vessel
b.l.	basal lamina
b.b.	basal bodies
cap.	capillary
des.	desmosome
el	elastic fibres
E.el.	external elastic lamina
I.el.	internal elastic lamina
i.c.	intercellular space
g.	granules
m.g.	mucous granules
s.g.	serous granules
lym.	lymphatic
lys.	lysosome
m.	mitochondria
n.v.	nerve
s.m.c.	smooth muscle cell
s.m.b.	smooth muscle bundle
t.f.	tonofilaments
t.w.	terminal web
r.e.r.	rough endoplasmic reticulum
s.e.r.	smooth endoplasmic reticulum

Plate 1

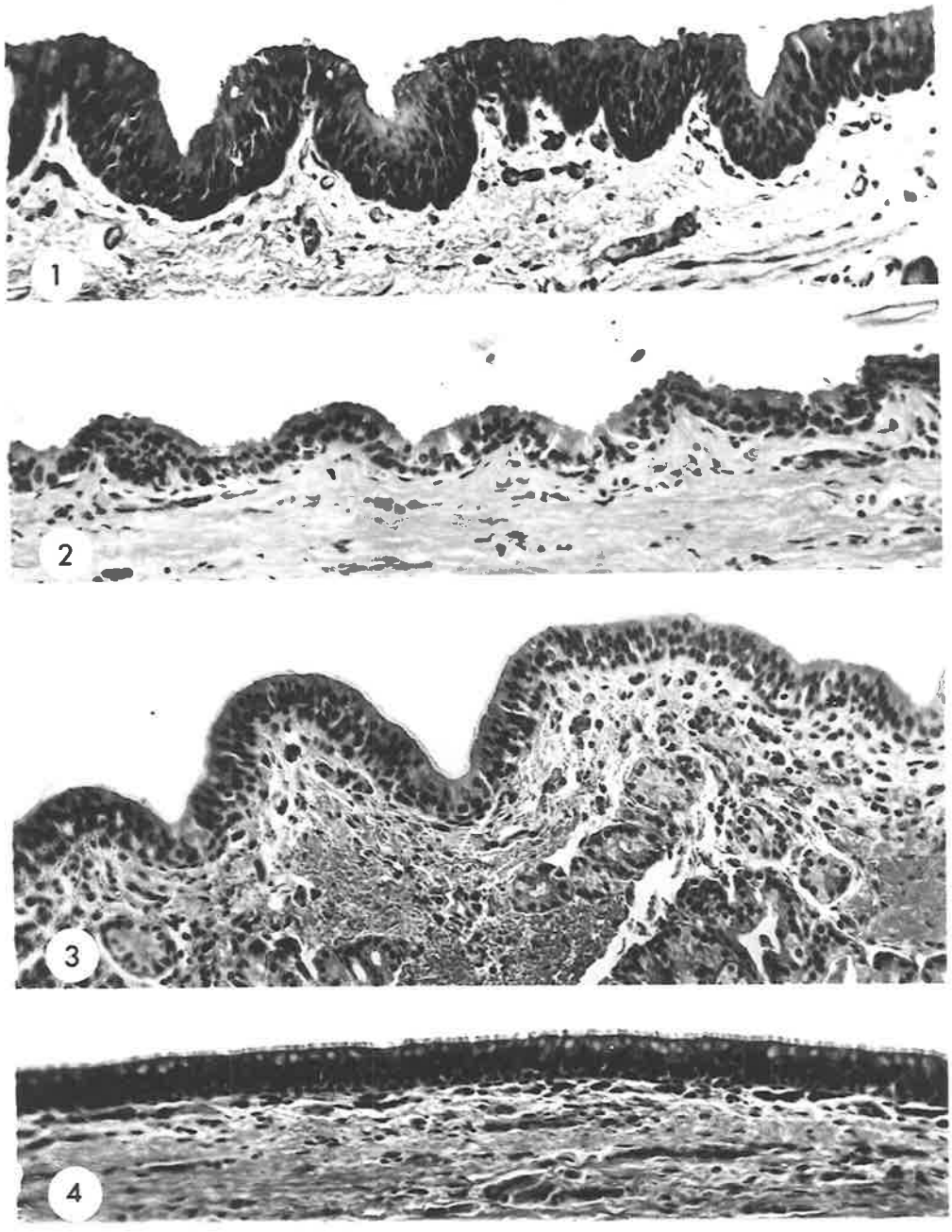


Fig. 1. AD.7, Trachea dorsal surface showing transitional epithelium.
Fig. 2. AD.7, Trachea dorsal surface showing stratified cuboidal epithelium.
Fig. 3. AD.3, Trachea ventral surface showing stratified to pseudo-stratified columnar epithelium with cilia, but no goblet cells.
Fig. 4. AD.7, Trachea dorsal surface distally showing pseudo-stratified ciliated columnar epithelium with goblet cells
All sections H and E 200 ×

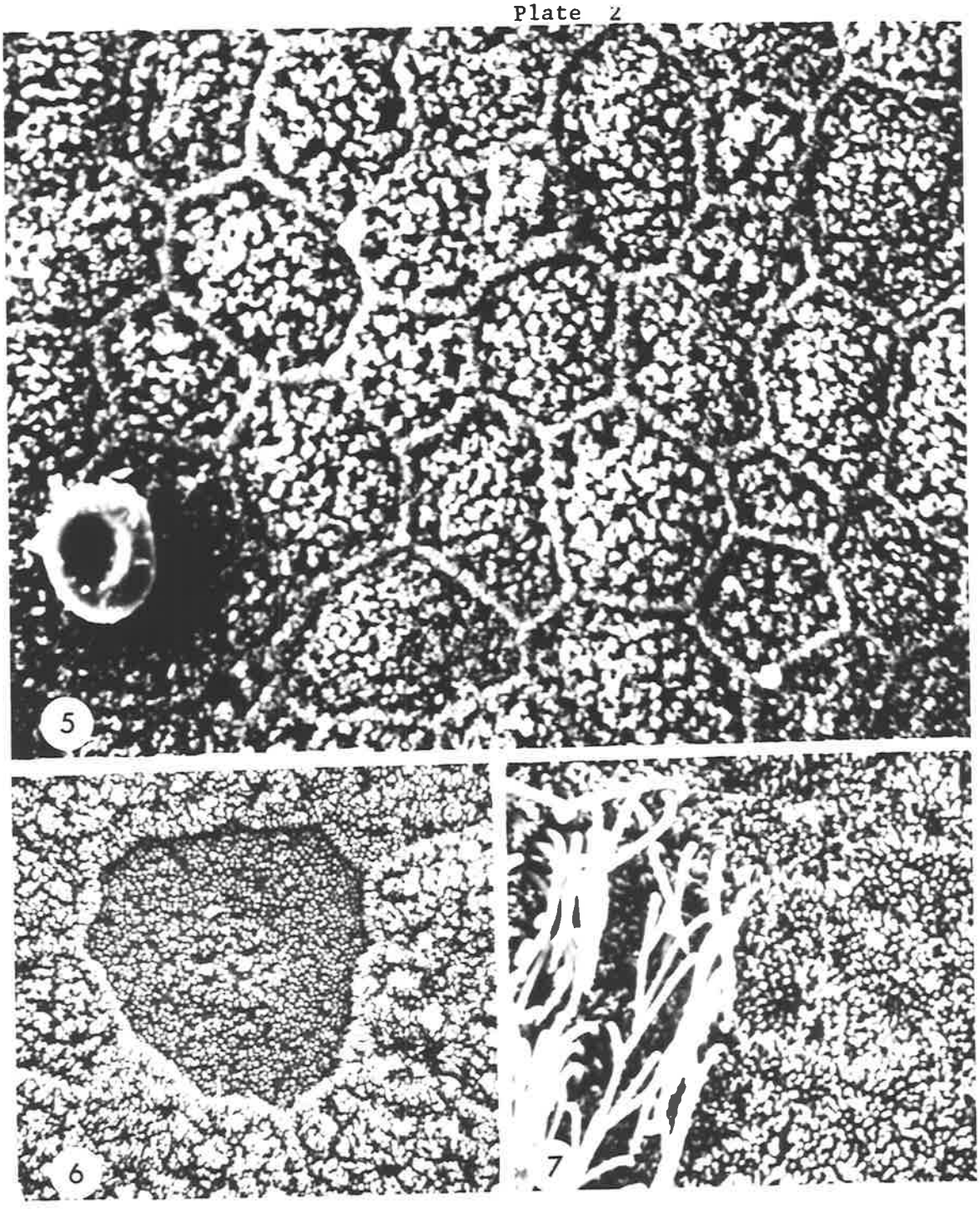
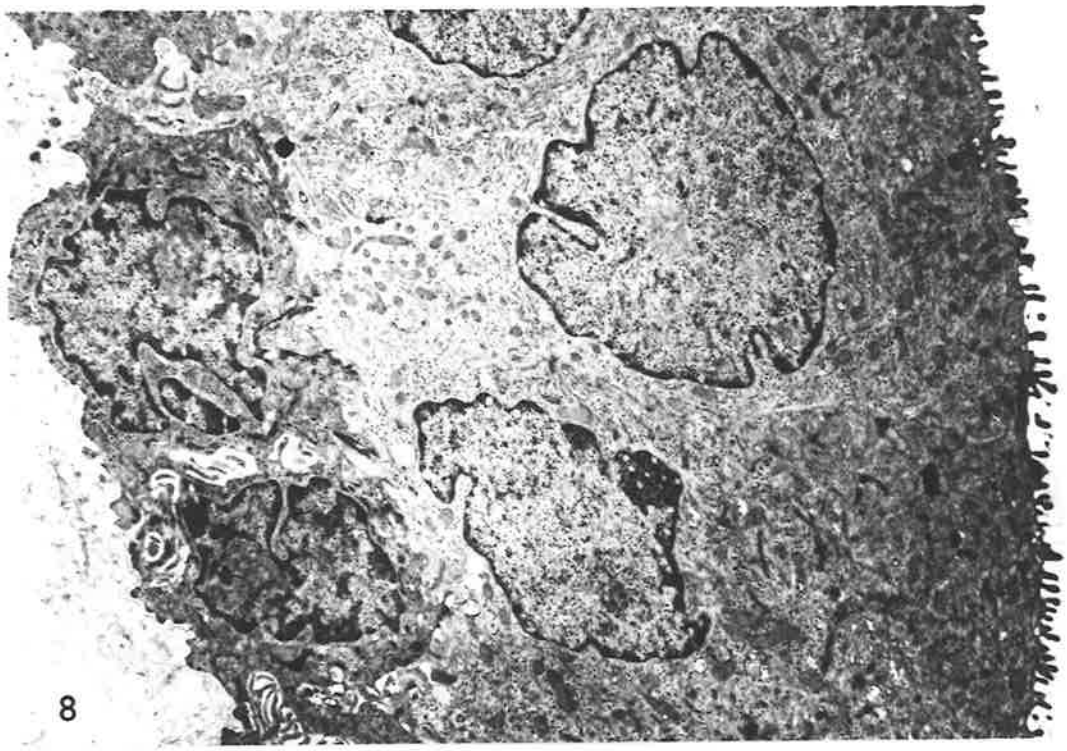


Fig. 5 AD.5, Proximal trachea. Note the microvilli heaped up around the cell boundaries. A red blood cell is seen in one corner. SEM 1700 ×

Fig. 6. AD.5, Area adjacent to Fig.5 showing a cell depressed below the surface with fewer microvilli. SEM 2000 ×

Fig. 7. AD.5, Middle third of the trachea showing an isolated cell with cilia. SEM 2000 ×

Plate 3



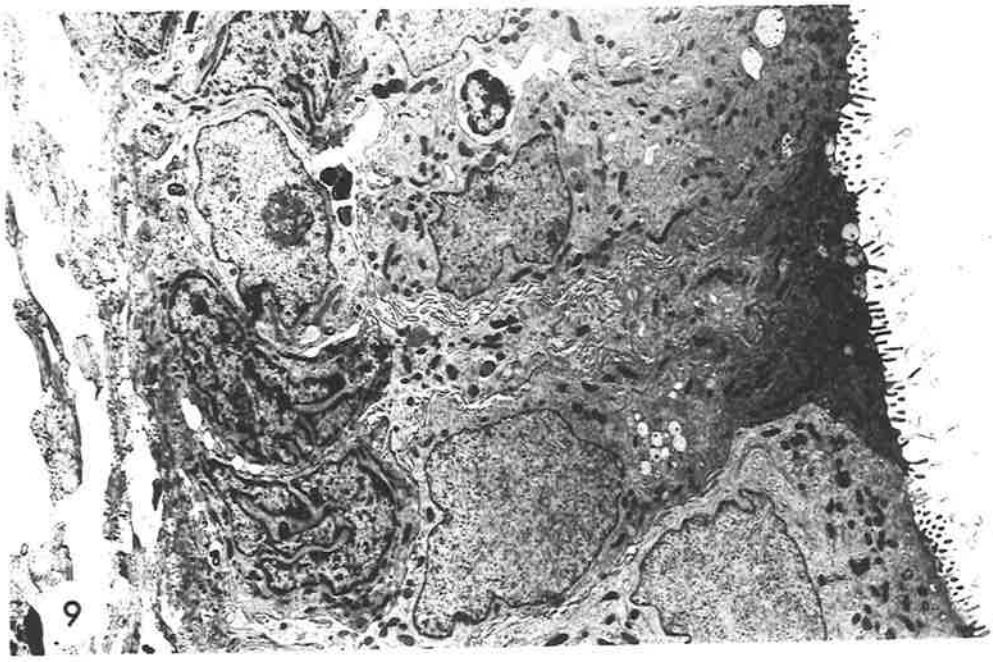


Fig. 8. AD. 2, Proximal trachea showing pseudostratified low cuboidal epithelium with some short squat microvilli, which may be rugae. TEM 6600 ×

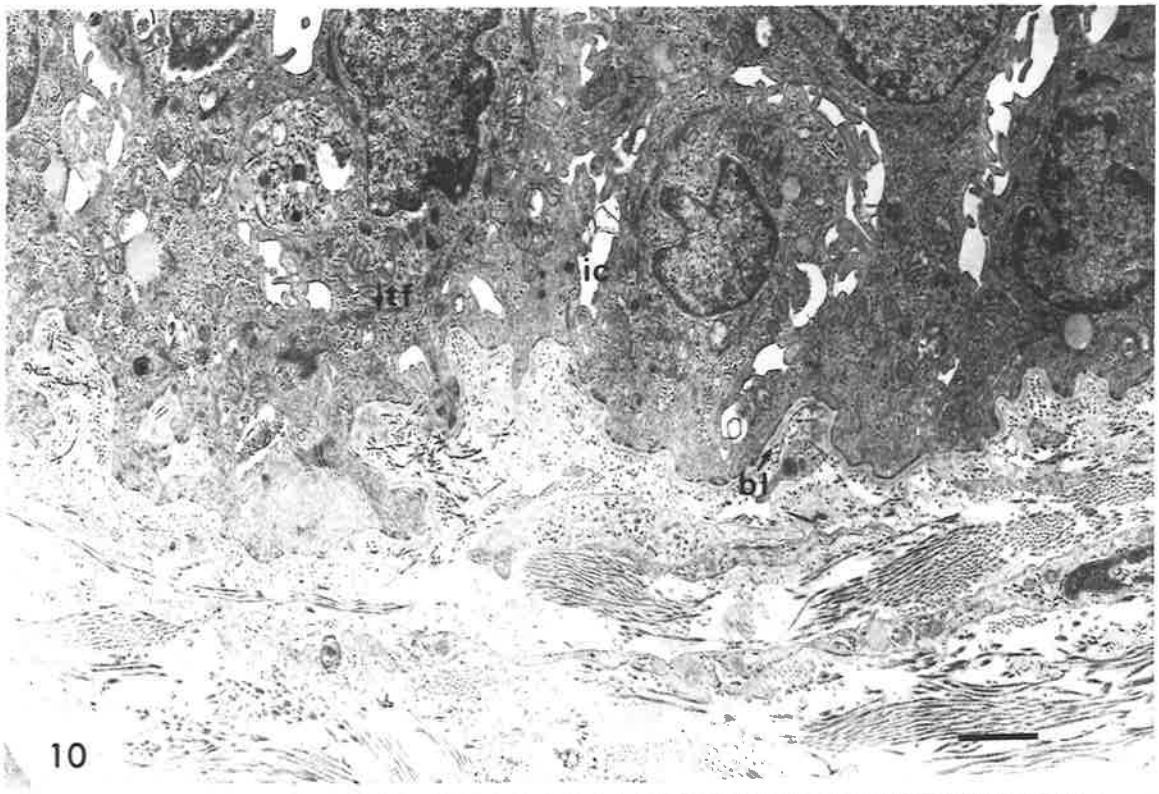
Fig. 9. AD. 5, Distal trachea almost at the junction with the respiratory epithelium. The cells are tall and reach the basement membrane. The microvilli are long and thin. TEM 3500 ×

Plate 4.

Fig. 10. AD. 2, Proximal trachea showing basal region of microvillous epithelium.
Note: basal cell with half desmosomes; tonofilaments (tf); the intercellular space (ic) with interdigitating processes; the prominent basal lamina (bl); and the connective tissue of the lamina propria TEM 10,000 x bar = 1 μm

Fig. 11. AD. 2, Surface cell from same area as Fig. 10.
Note: the squat microvilli on the surface; four zones are clearly seen, the terminal web (w), mitochondrial (m), supranuclear with Golgi (G) and some darker staining granules (g); and the nucleus (N).
TEM 15,400 x bar = 1 μm

Plate 4



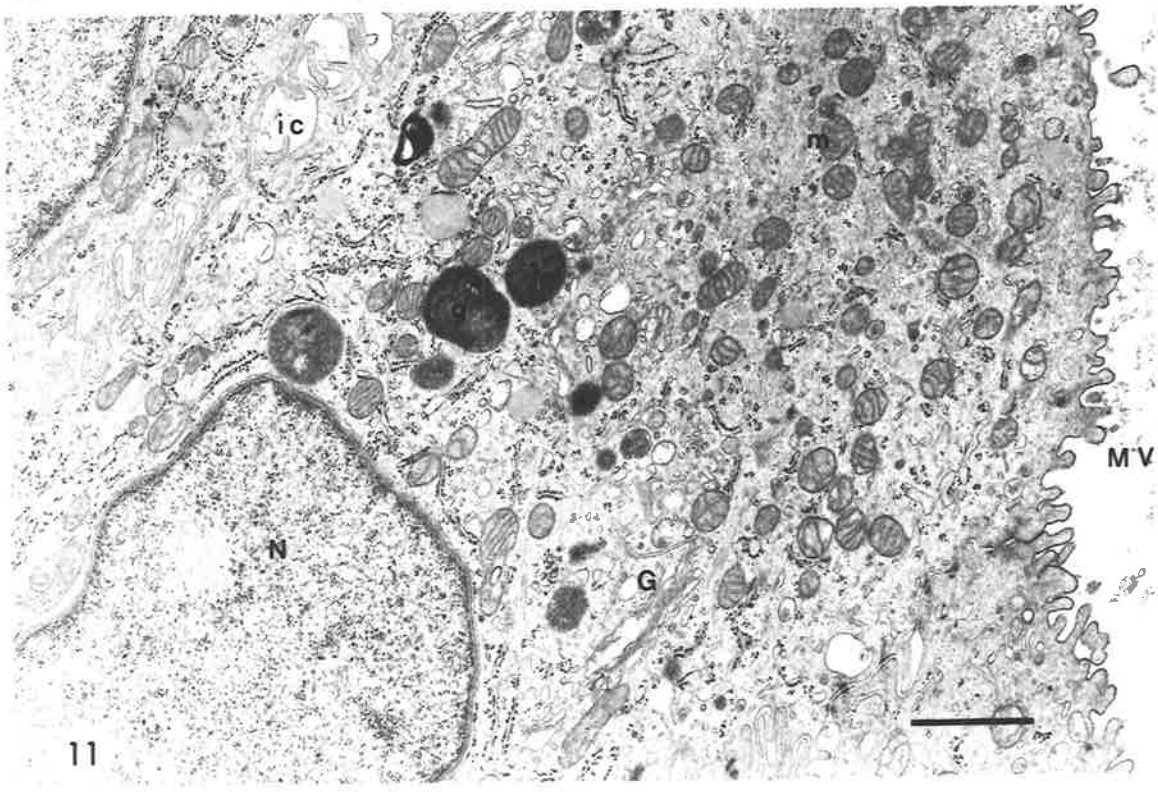


Plate 5.

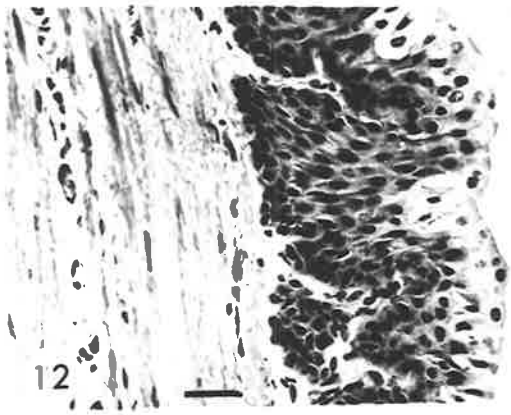
Fig. 12. AD.7, Proximal trachea, ventral aspect showing transitional epithelium. H and E 360 x bar = 20 µm.

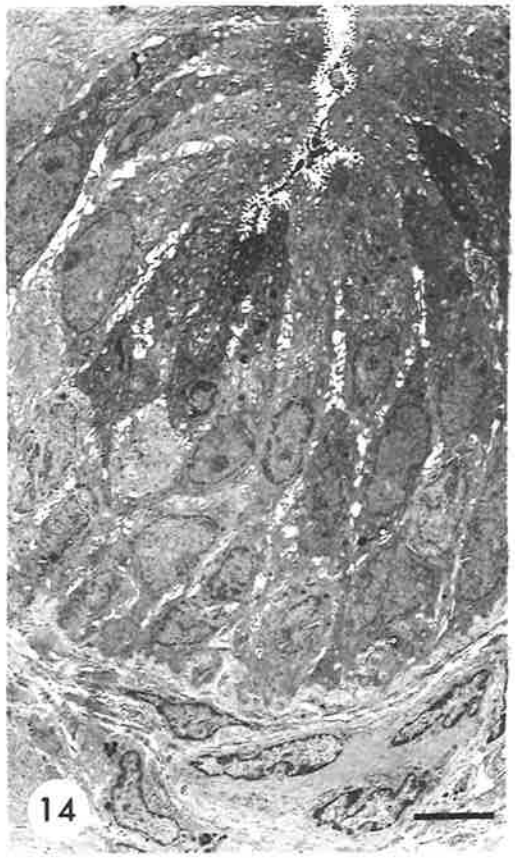
Fig. 13. AD. 2, Proximal trachea, ventral aspect showing transitional epithelium. Epoxy resin 1 µm sections MB/AZ 400 x bar = 20 µm.

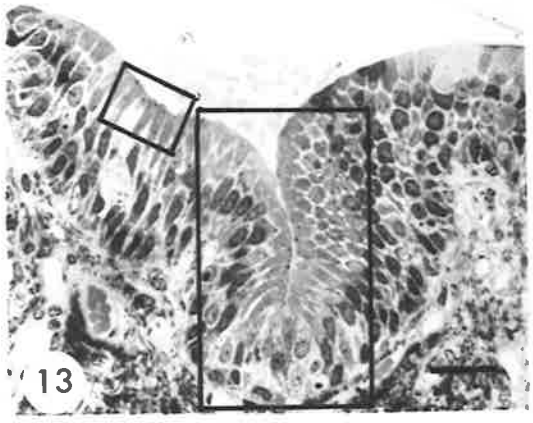
Fig. 14. AD. 2, Thin section of large area outlined in Fig. 13.
Note: compression of surface cells and the differing sizes of the intercellular spaces. 2000 x bar = 5 µm.

Fig. 15. AD. 2, Thin section of smaller outlined area in Fig. 13.
Note: the surface cells with terminal web (tw); microvilli; and the mitochondrial zone. 10,000 x bar = 1 µm.

Plate 5







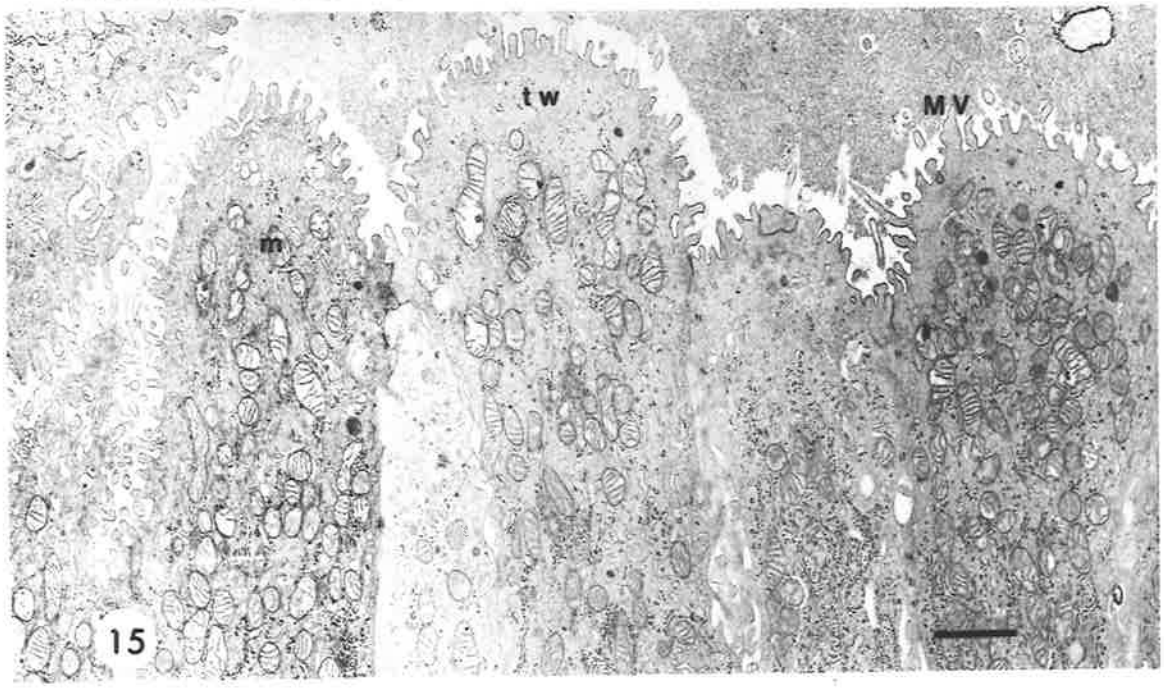
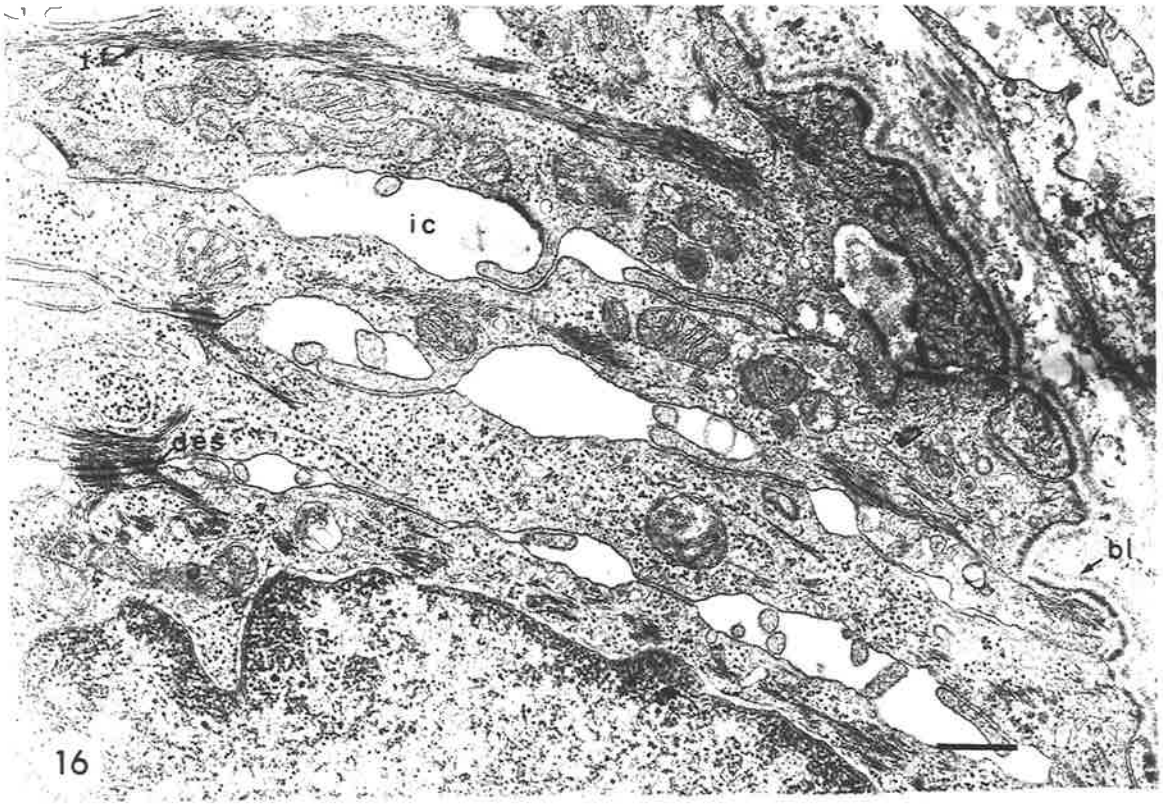


Plate 6.

Fig. 16. AD. 2, Proximal trachea, ventral aspect showing basal cells.
Note: tonofilaments; the intercellular space with many interdigitating processes, but only occasional desmosomes are seen. 20,000 x bar = 0.5 µm.

Fig. 17. AD. 2, Area adjacent Fig. 16 showing intermediate cell containing secretory granules, some with an electron-dense core. Adjoining cells have no droplets. 20,000 x bar = 0.5 µm.

Plate 6



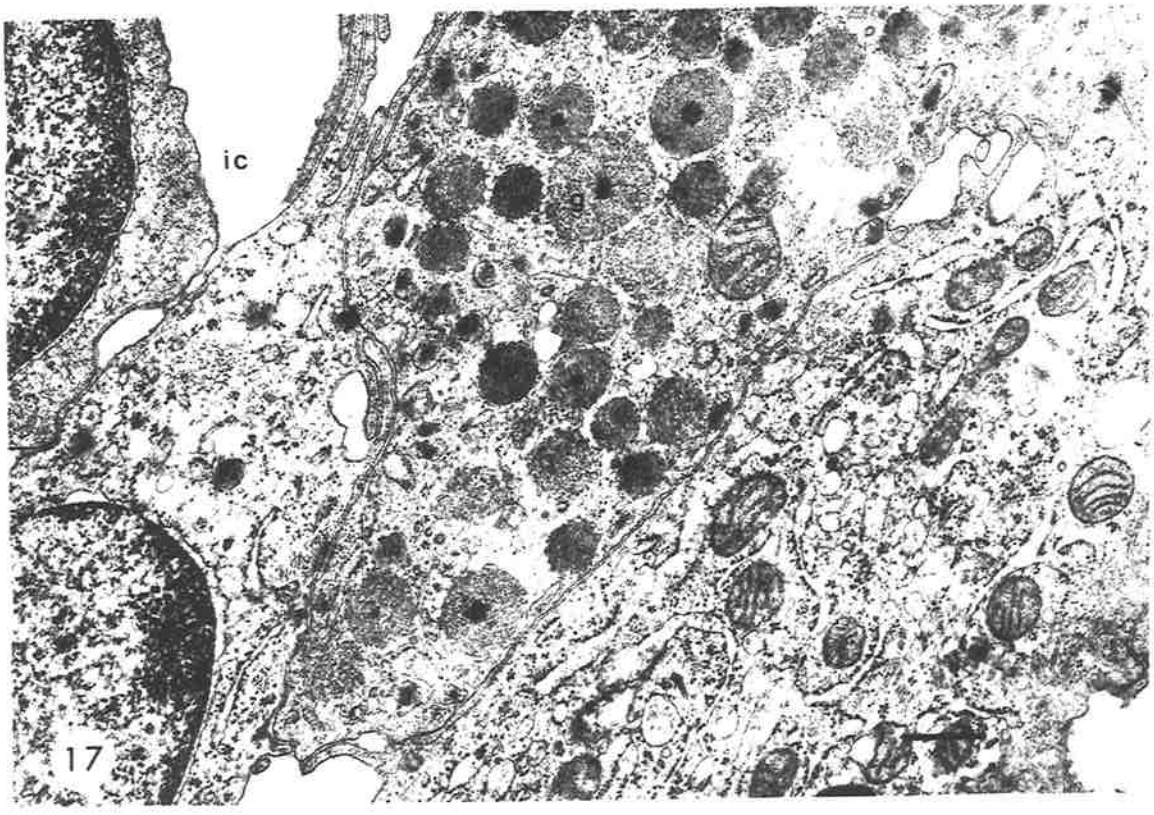


Plate 7.

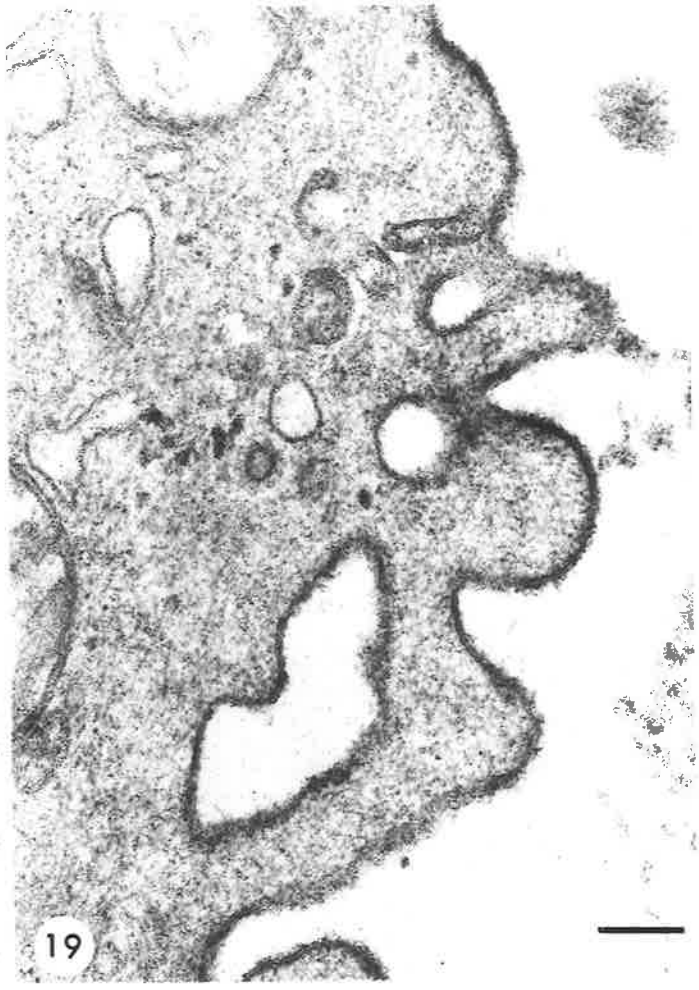
Figs. 18, 19, 20. All from AD. 2, showing different types of unit membrane in different regions.

Fig. 18. Transitional type with an symmetric membrane, outer leaf 3.4 nm with 8 nm fuzzy coat, inner leaflet and core each 2.3 nm. 110,000 x bar = 100 nm.

Fig. 19. Transitional type with the usual symmetric membrane 9.0 nm thick. 110,000 x bar = 100 nm.

Fig. 20. Microvillous type with the usual symmetric membrane (lower magnification) 84,000 x bar = 100 nm.

Plate 7



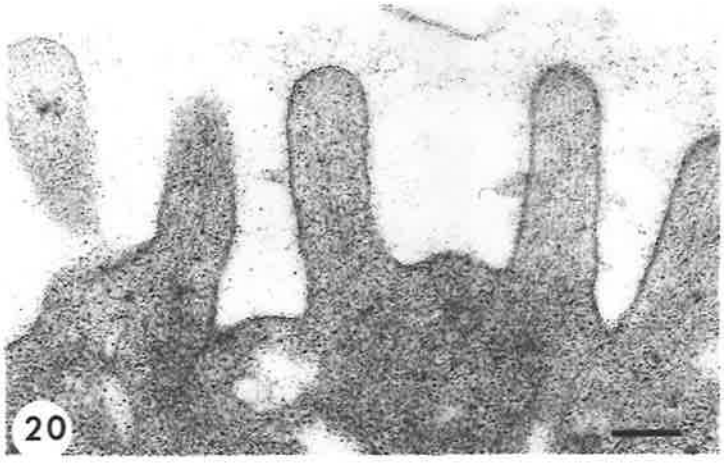
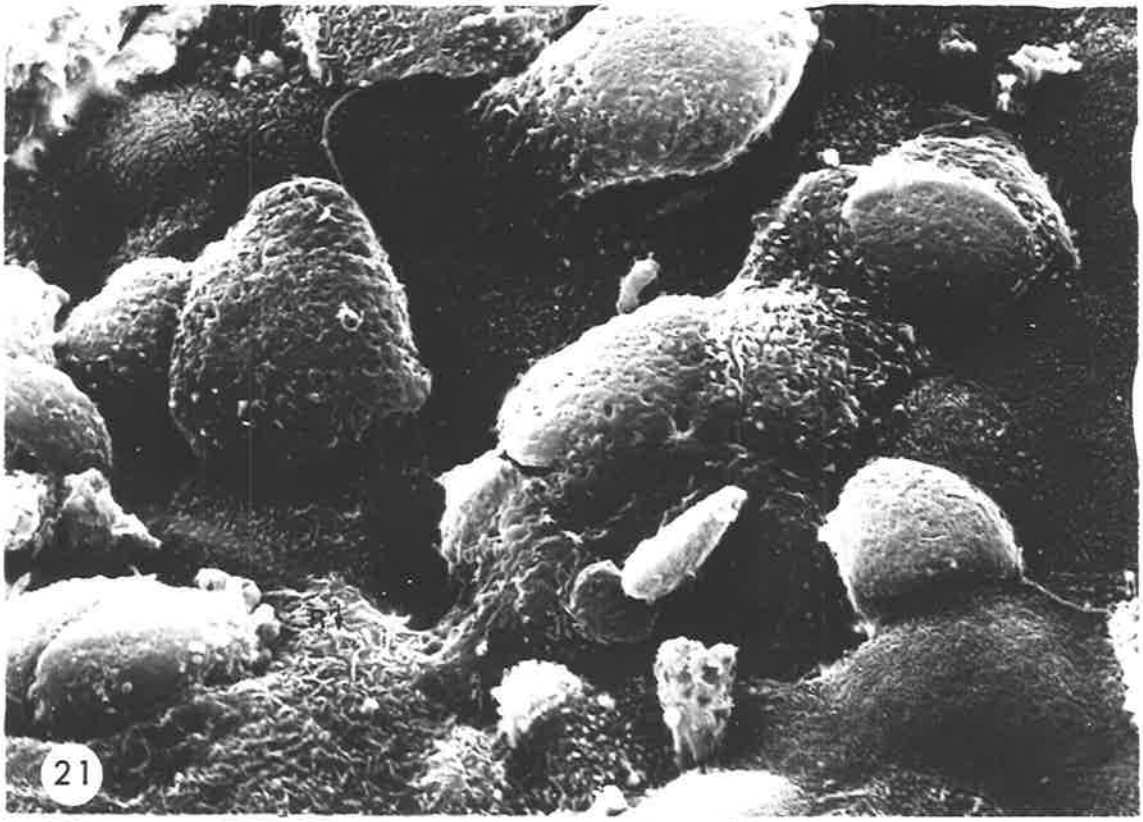


Plate 8.

Fig. 21. AD. 2, Proximal trachea, dorsal aspect showing transitional epithelium. Note the irregular outline of the surface cells and the rugae (R) on their surface. SEM 2,900 x

Fig. 22. AD. 2, An adjacent area from the same specimen as Fig. 21 showing rugae. SEM 8,000 x

Plate 8



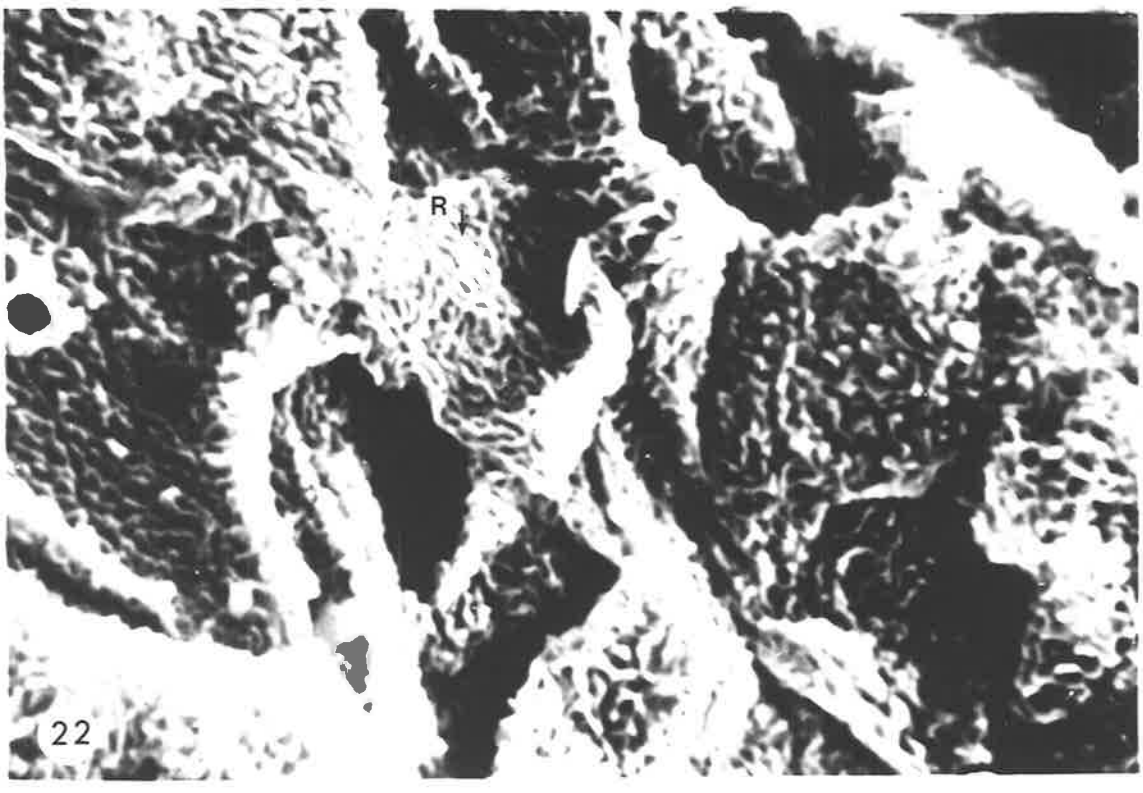


Plate 9.

Fig. 23. AD. 5, Distal trachea. The epithelium is pseudo-stratified ciliated columnar with goblet cells. Note: ciliated cells (C) have occasional lysosomes (lys) and many mitochondria; all the goblet cells (GC) have indented apices and are in the early synthetic stage, with mucous droplets; a nerve is seen well into the epithelium (nv); it is difficult to positively identify intermediate cells; pale staining basal cells (BC); a thin walled blood vessel in the lamina propria. 2000 x bar = 1 μm.

Plate 9

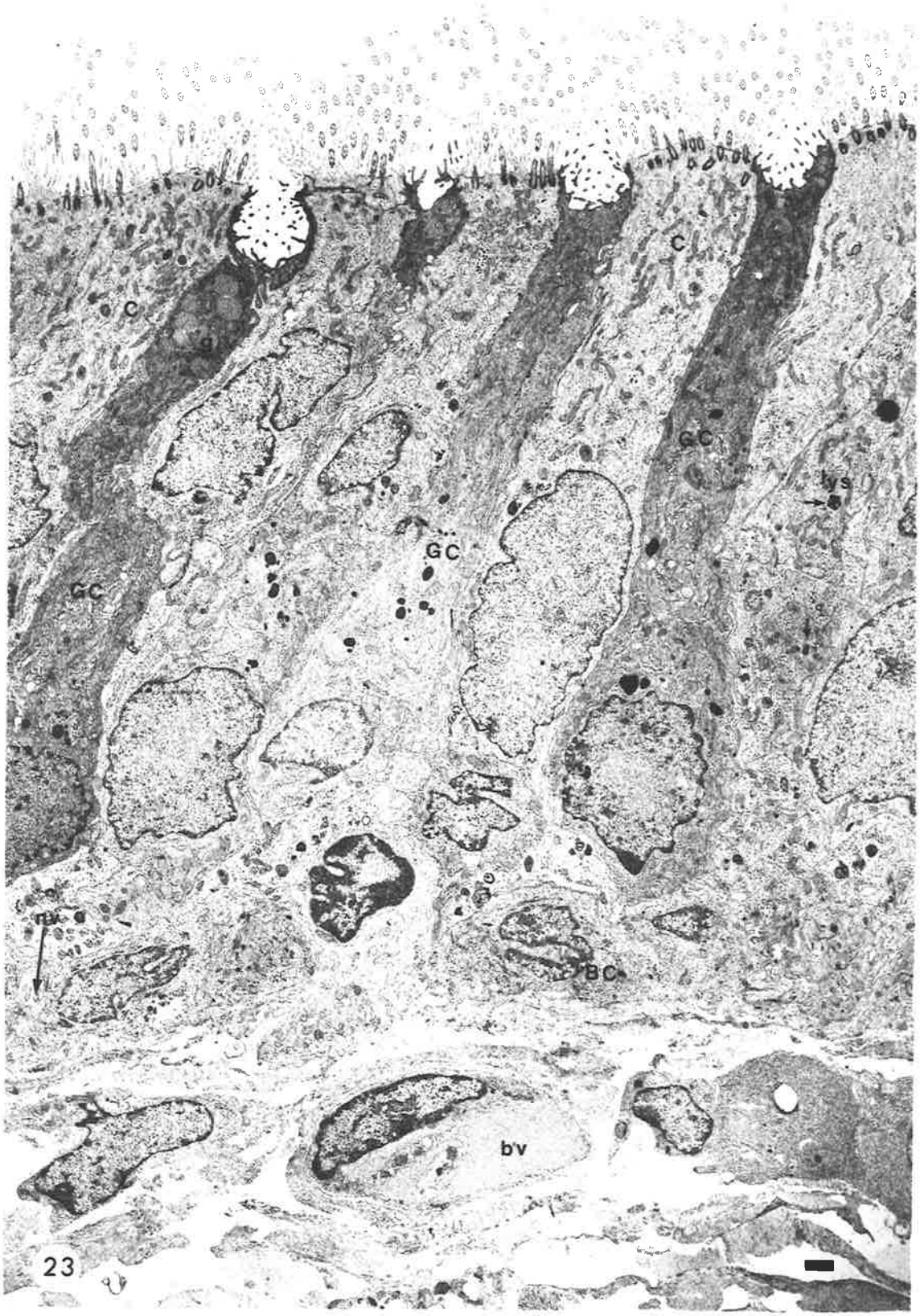
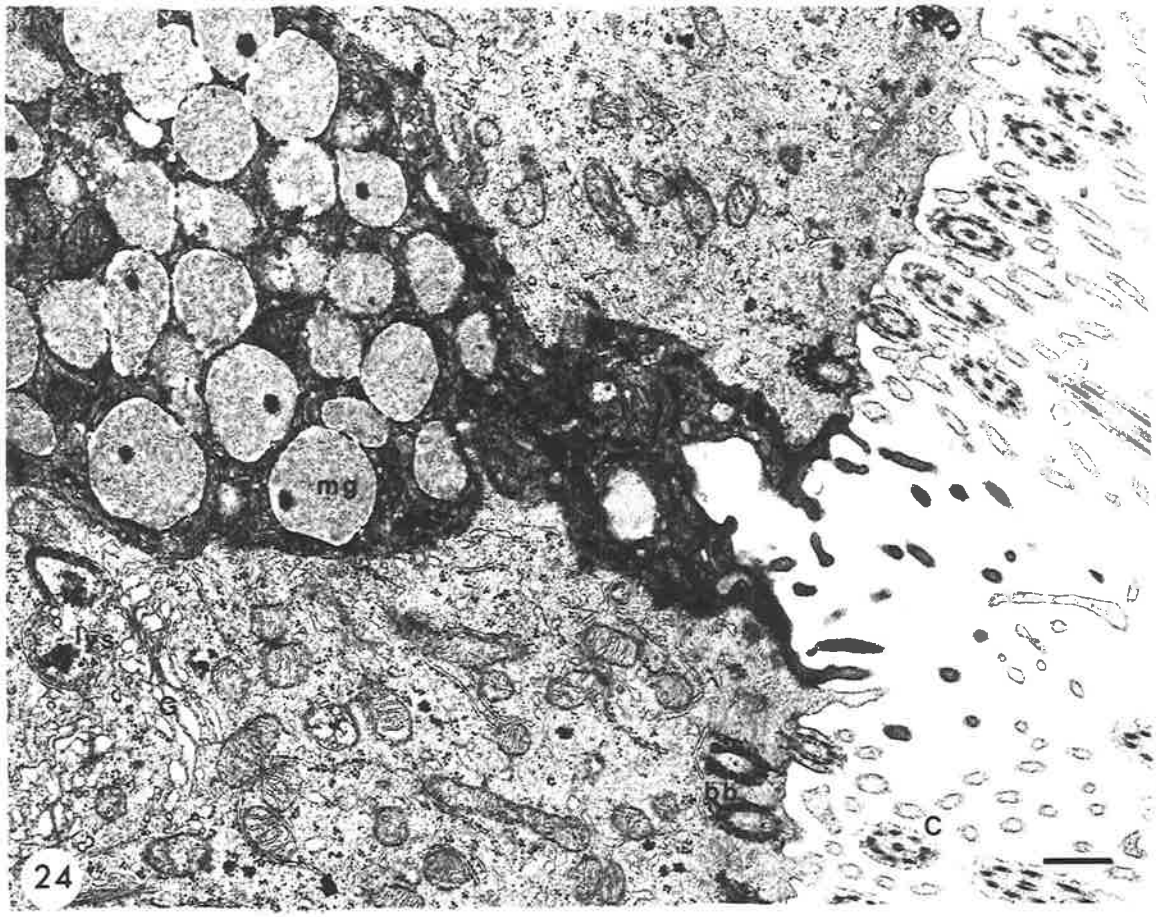


Plate 10.

Fig. 24. AD, 2. Distal trachea showing apex of goblet cell and ciliated cells. The goblet cell is darker staining than the ciliated cells and has an indented apex. Some of the mucous granules have small electron-dense cores. The cilia have the typical 9+2 arrangement of filaments and basal bodies (bb) are present. 19,000 x bar = 0.5 μm.

Fig. 25. AD, 2. Distal trachea showing several goblet cells. Note: the Golgi complex; pale staining mucous droplets; and the plications of the lateral cell membranes (↓) 20,000 x bar = 0.5 μm.

Plate 10



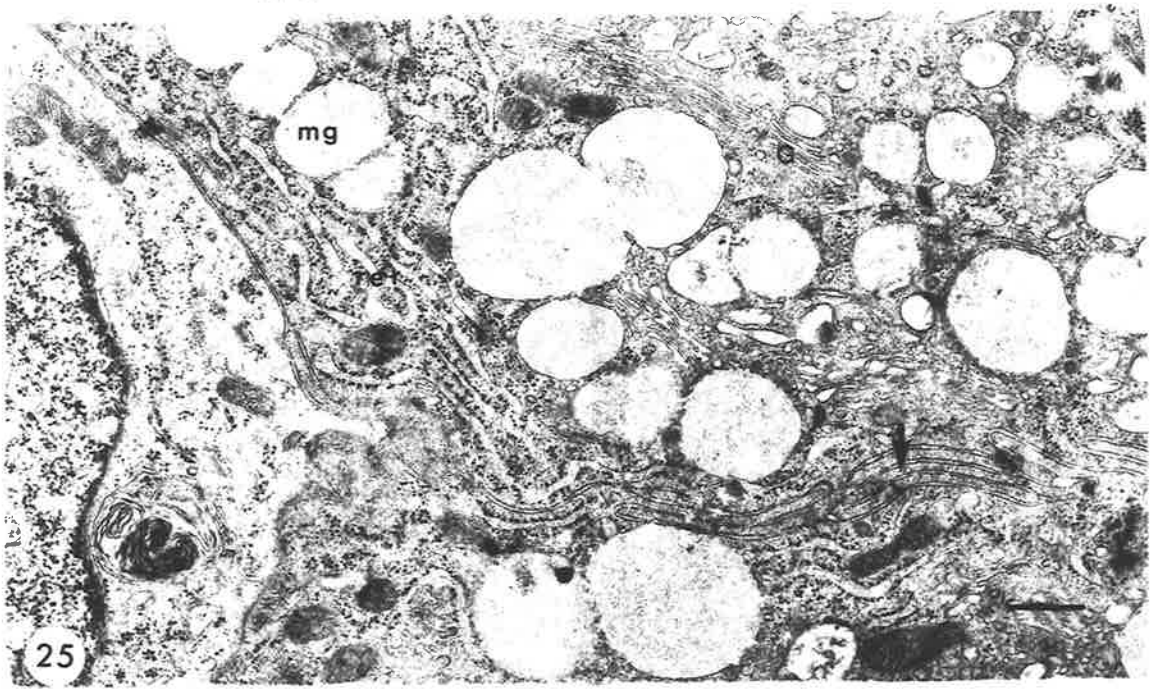


Plate 11.

Fig. 26. AD, 2. Middle third of trachea showing an area of microvillous cells amongst the ciliated ones. The cells with the bulbous apices may be goblet cells. SEM 3,600 x

Fig. 27. AD, 2. Distal trachea showing ciliated epithelium with the orifices of several ducts (↓). Some mucous particles are seen on the surface. SEM 1,200 x

Plate 11

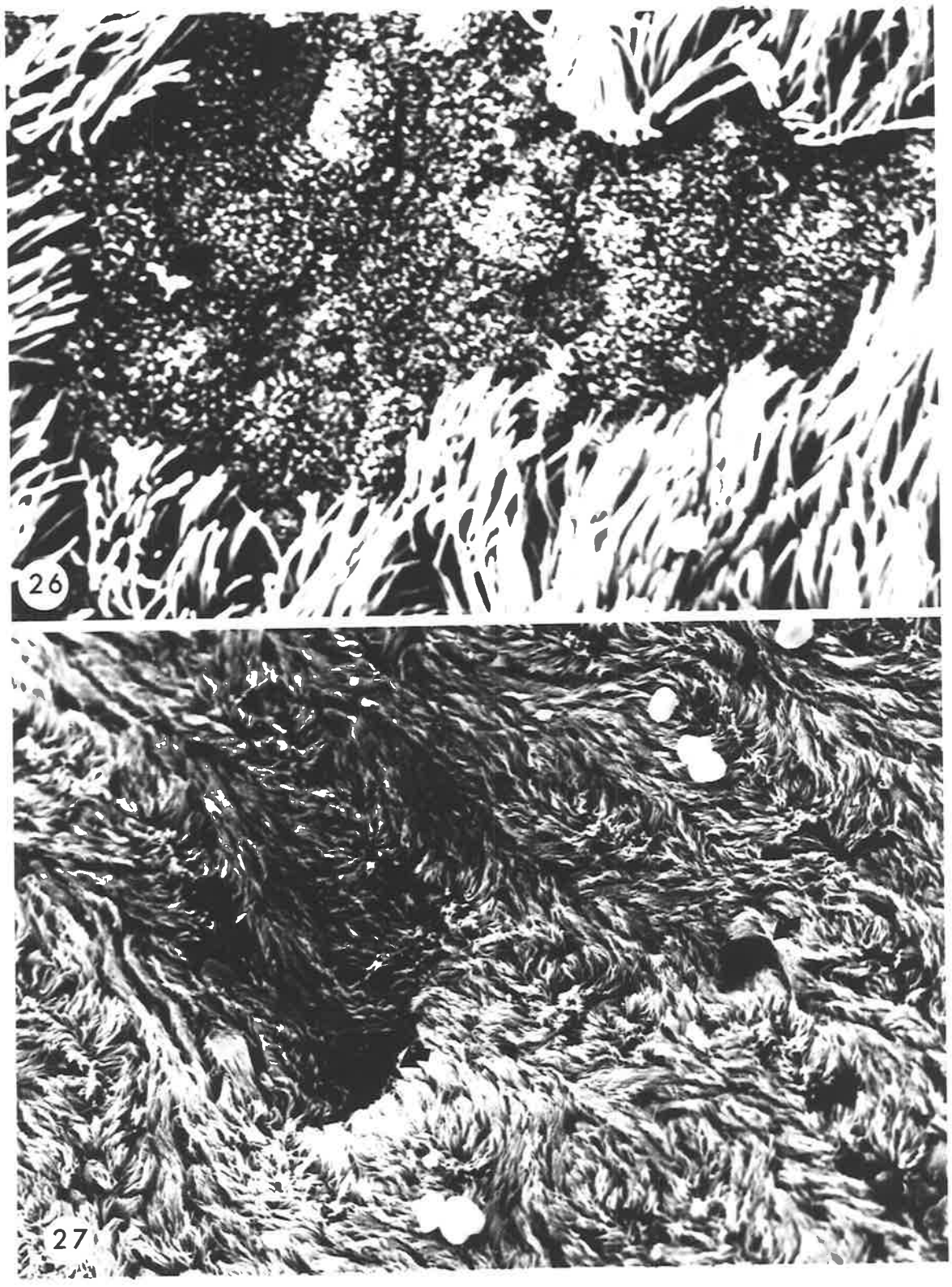


Plate 12

Fig. 28 AD.2 ,Distal trachea at higher magnification showing goblet cells bulging above the surface,some microvillous cells and ciliated cells.
SEM 3,200 ×

Plate 13.

Fig. 29. AD, 2. Distal trachea showing a small collection of cells with a microvillous surface between the ciliated cells. These cells may be goblet cells. SEM 3,700 x

Fig. 30. AD, 2. Distal trachea showing goblet cells, one of which (↓) may have just discharged its contents, or be incompletely filled. SEM 8,000 x

Plate 13

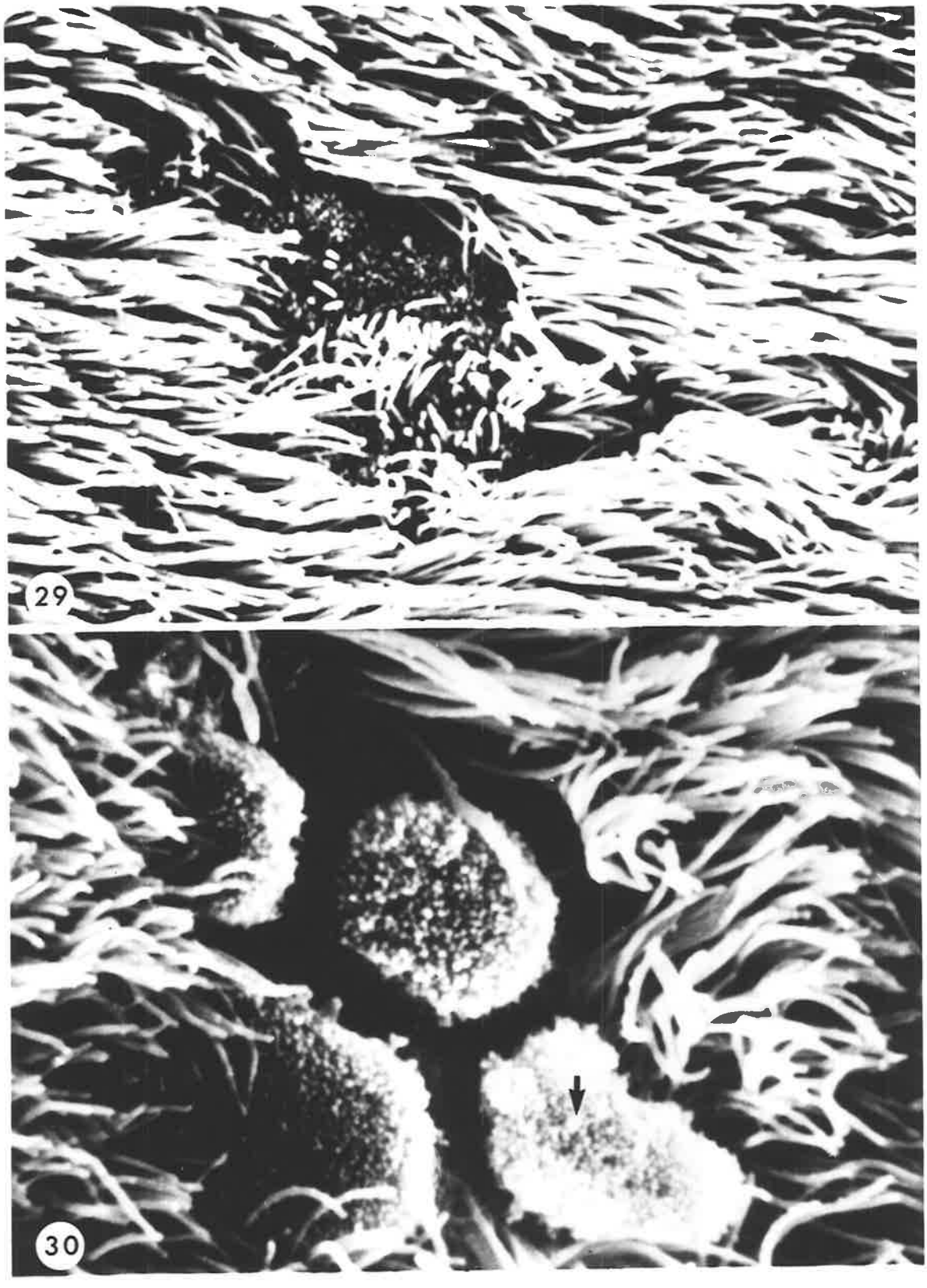
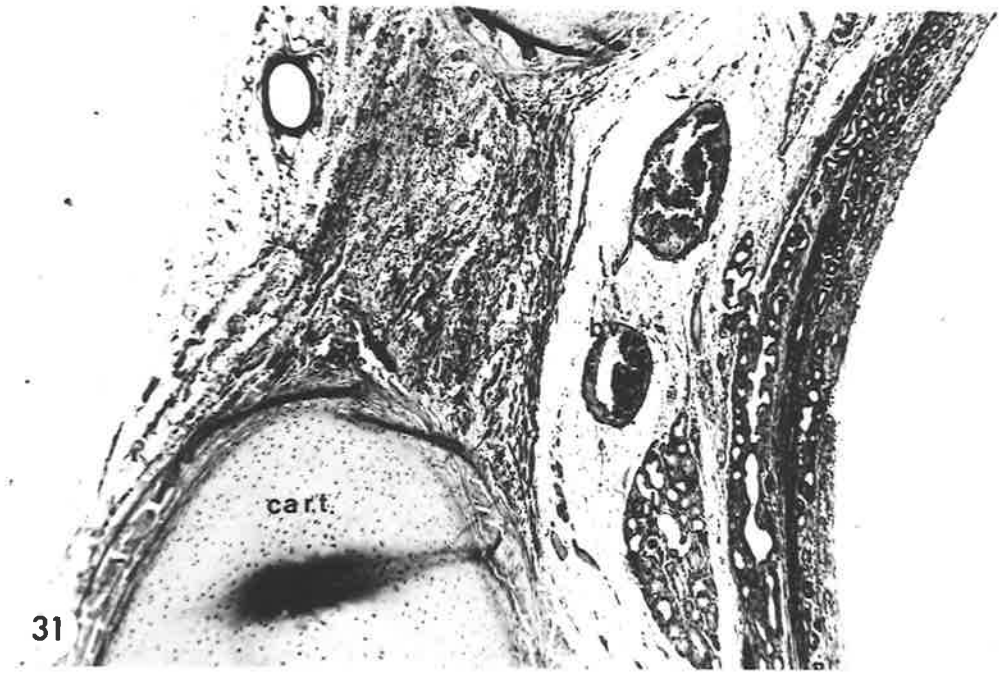


Plate 14

Fig. 31. AD. 2, Middle third of trachea stained for elastic tissue.
Note: the hyaline cartilage contains no elastic fibres (cart); the stout external elastic lamina (E el) fuses with the perichondrium at the edges of the cartilage; the glands (gl) are divided into two layers by the internal elastic (I el) lamina, with further aggregations in the gaps between the cartilage; a few large vessels (bv) are present on the submucosa. V and VG 40 ×

Fig. 32. AD. 3, Middle third of the trachea.
Note: the epithelium is non-ciliated (MV) and has no goblet cells; only the deeper layer of glands is present, and they are mainly serous; thick walled muscular vessels deep to the glands.
H and E 80 ×

Plate 14



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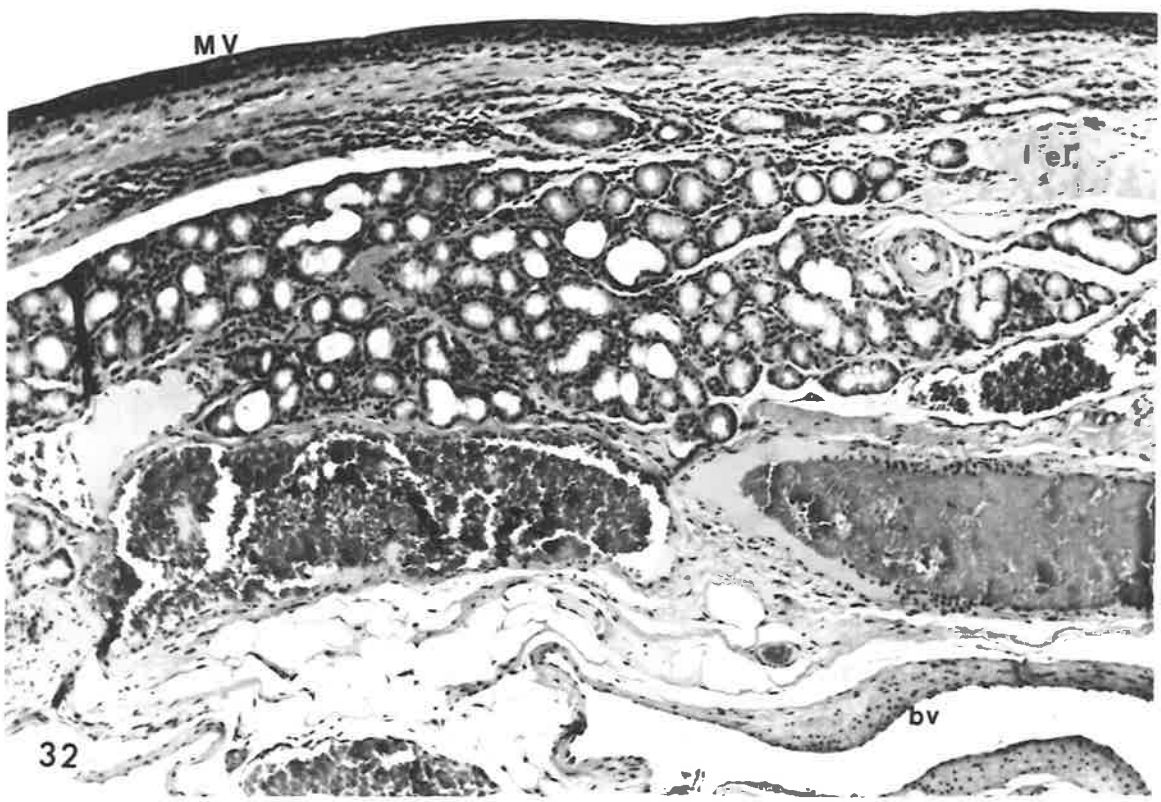

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Plate 15

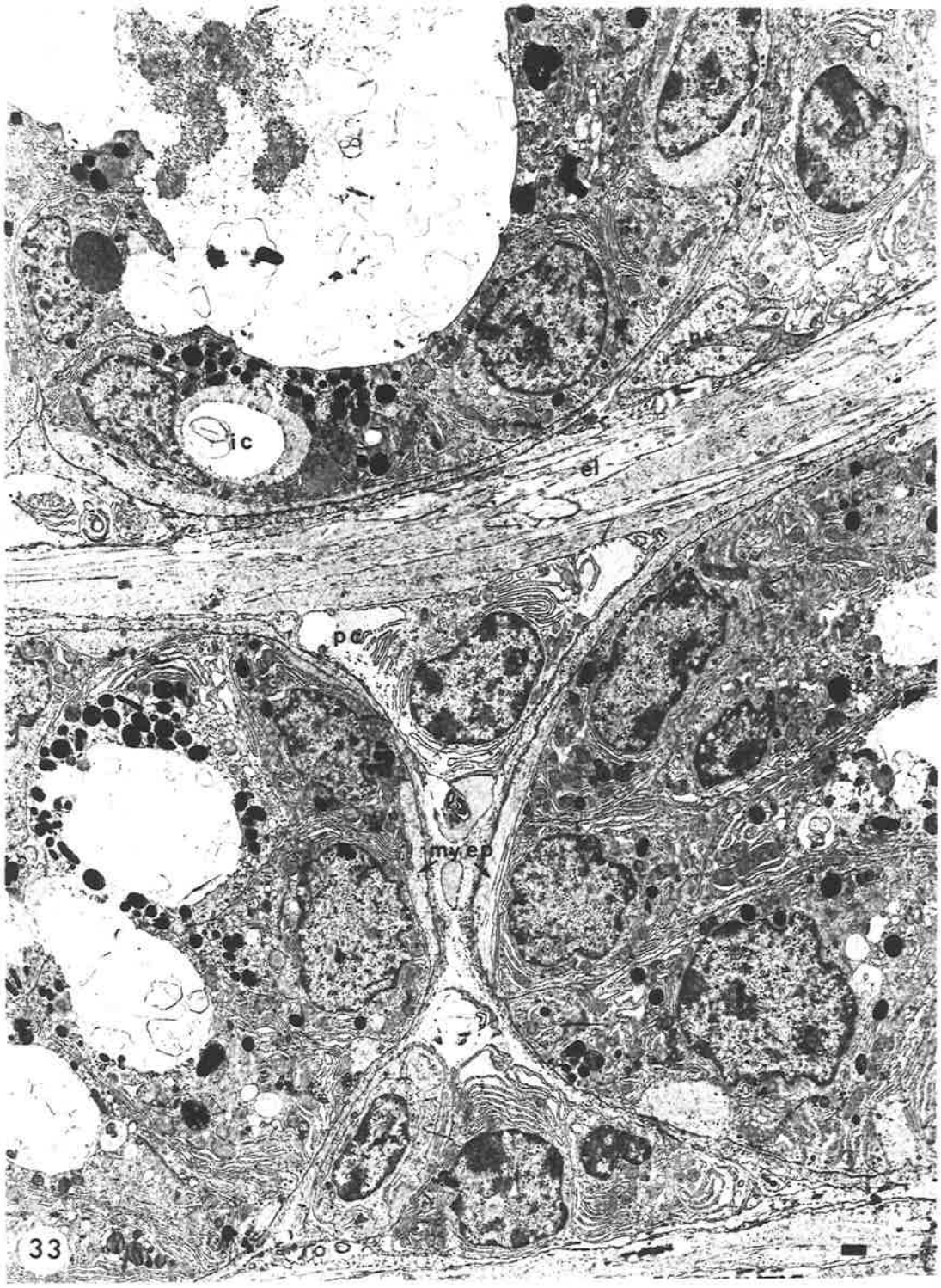


Fig. 33. AD.5,Serous tracheal gland. Note:elastic lamellae surrounding the acini(el);dilated intercellular canaliculi(ic); myo-epithelial cells(my ep);plasma cells(pc);and nerve fibres(nv). 4,000 × bar = 1μm.

Plate 16

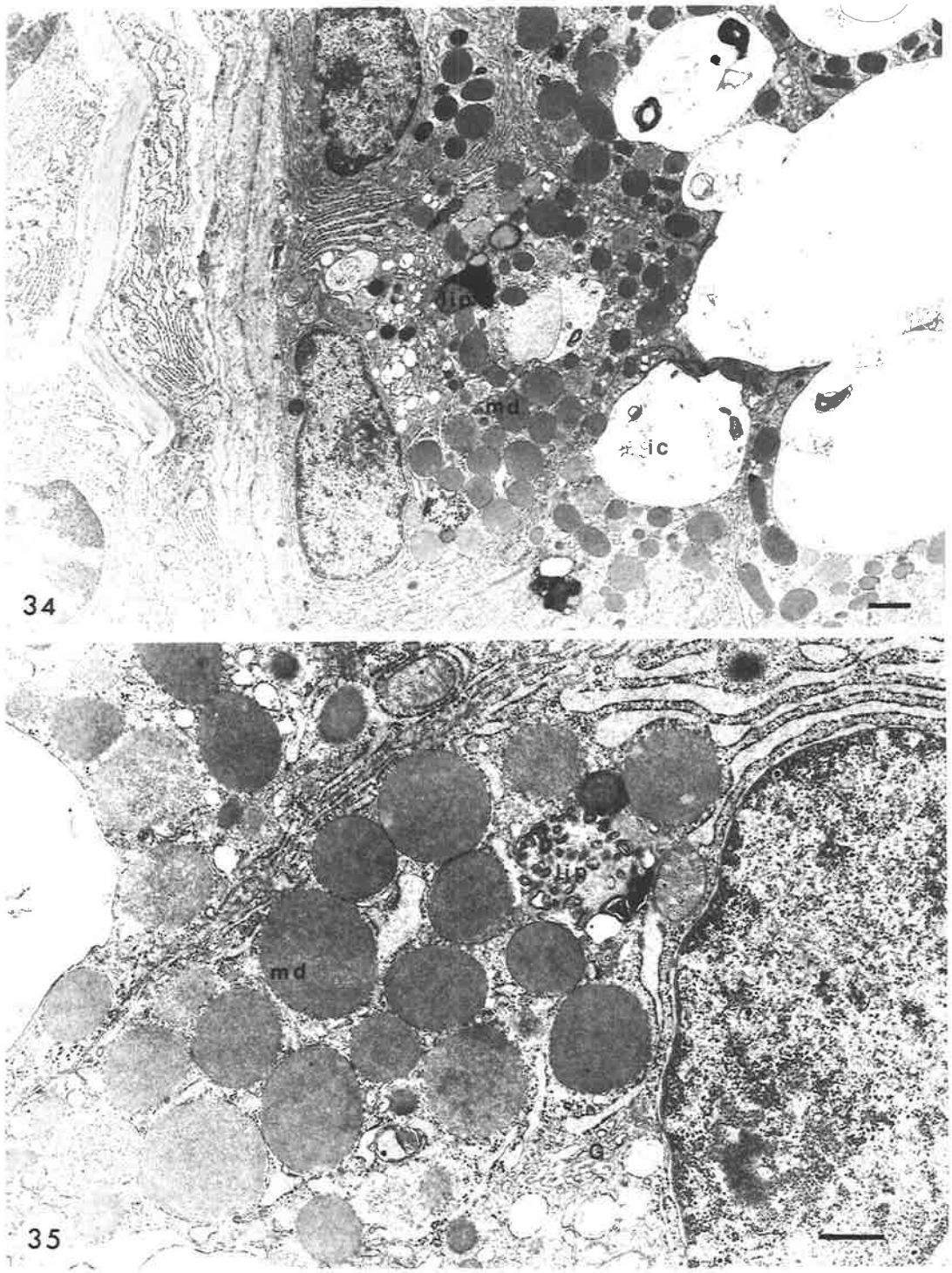


Fig. 34. AD. 5, Trachea, mucous secreting gland. Note: dilated intercellular canaliculi(ic); mucous droplets of varying size and density(md); lipochondria(lip). 6,600 × bar = 2μm

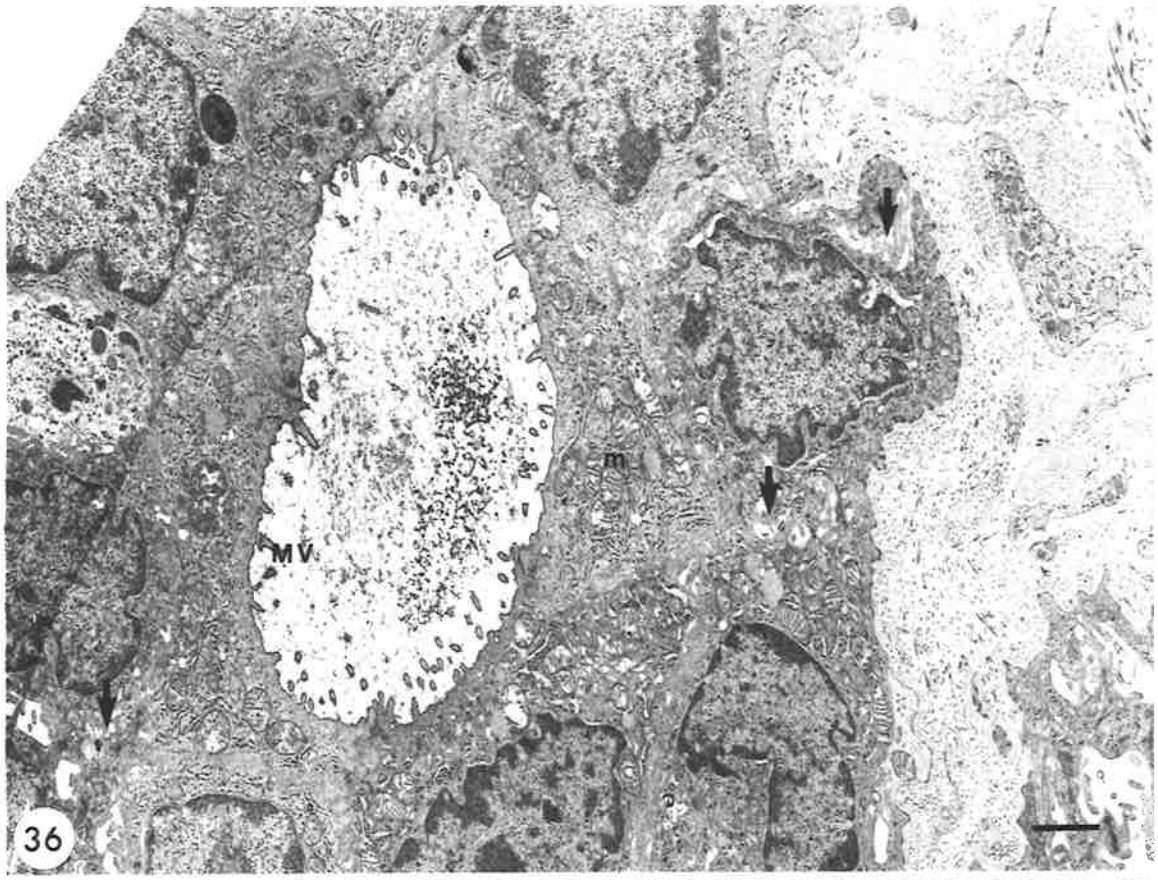
Fig. 35. AD. 5, Higher magnification of Fig. 34. 20,000 × bar = 0.5μm

Plate 17.

Fig. 36. AD, 2. Proximal trachea showing a duct of a gland.
Note: microvilli on the surface; mitochondria; and the extensive plication of the lateral and basal surface membrane (↓). 8,000 x bar = 1 μm.

Fig. 37. AD, 5. Part of a myo-epithelial cell around the periphery of an acinus of a gland.
Note: the myofilaments (mf).
40,000 x bar = 200 nm.

Plate 17



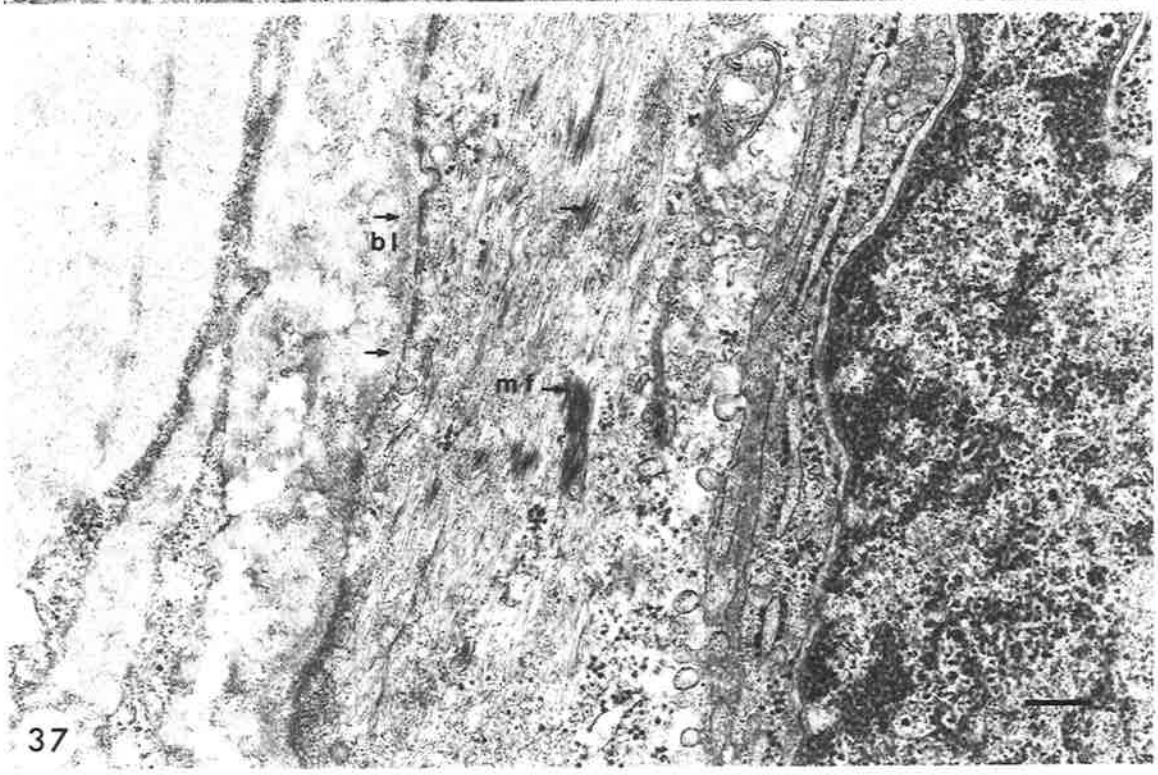


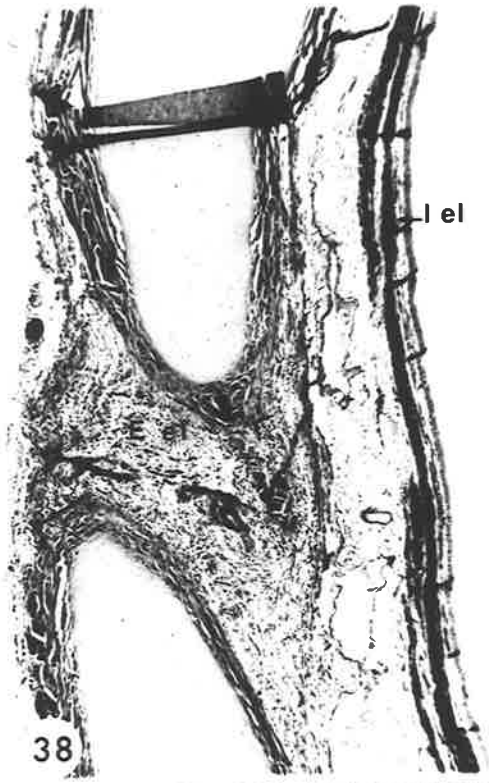
Plate 18.

Fig. 38. AD, 5. Proximal trachea stained for elastic tissue.
Note: the thick internal elastic lamina which appears to be a double lamina in some areas where it encloses the glands; the external elastic lamina; and the vascular plexus. Compare with Fig. 31.
V and VG 100 x

Fig. 39. AD, 7. Ventral aspect of the laryngo-tracheal junction.
Note: crypts surrounded by lymphatic collections with germinal centres. H and E 7 x

Fig. 40. AD, 7. High power of portion of a crypt showing: ciliated epithelium, thinning over the germinal centres to squamous; typical lymphatic collections with germinal centres (gc). H and E 360 x

Plate 18



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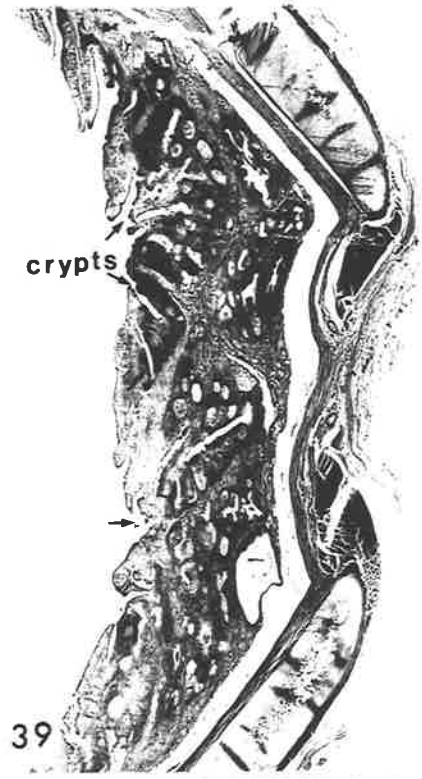

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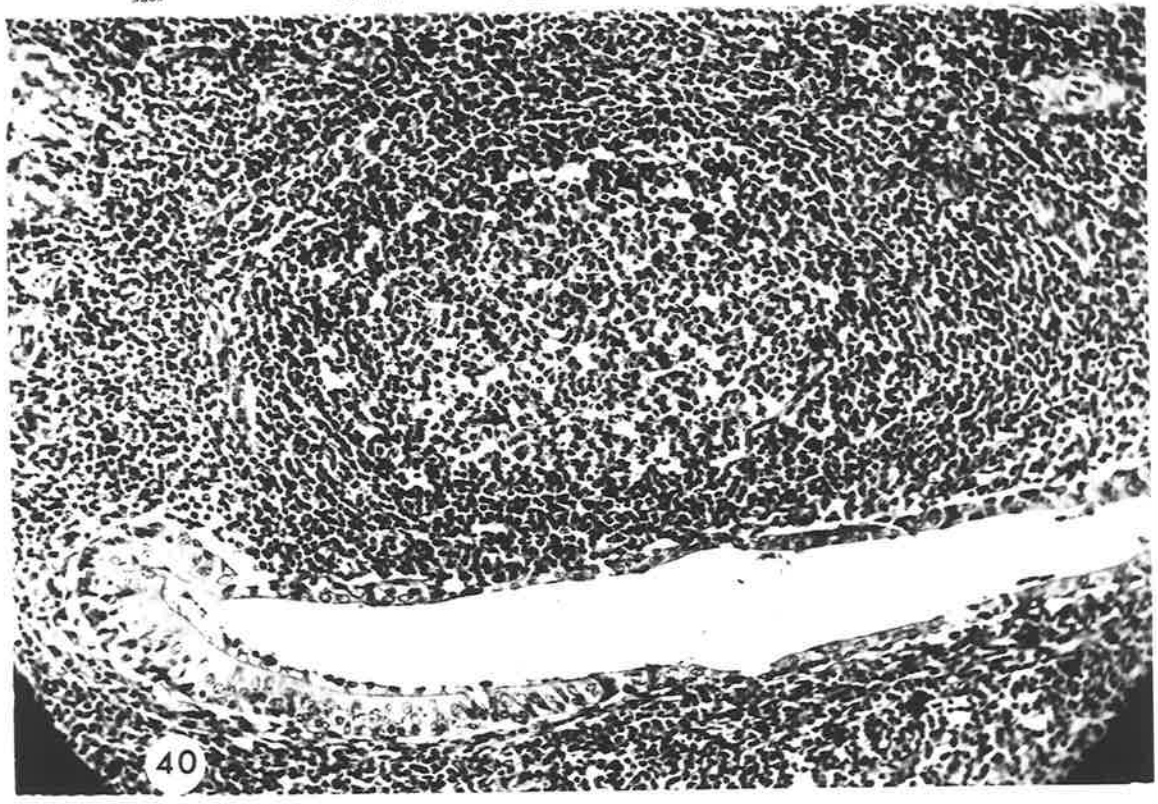

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Plate 19

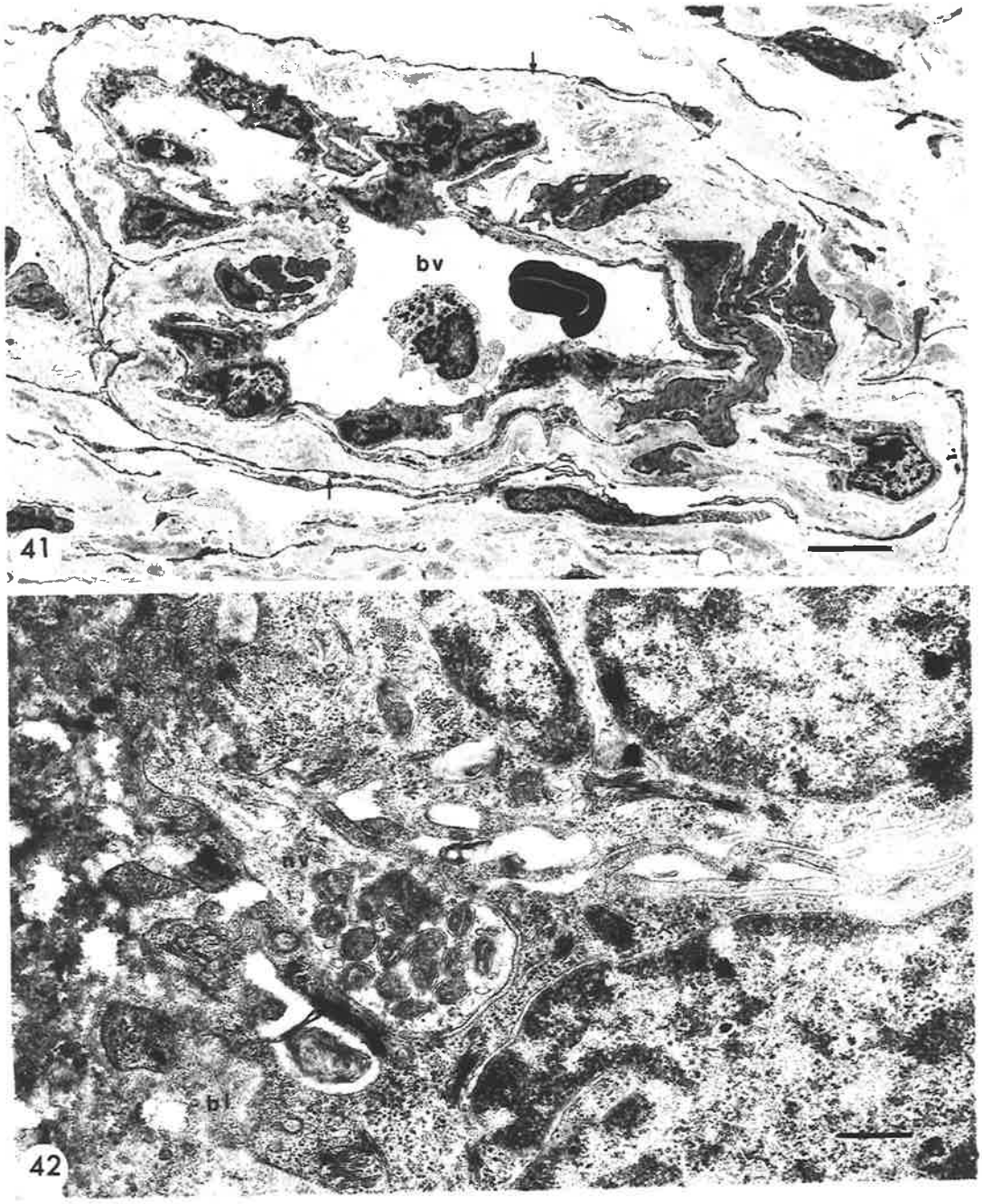


Fig. 41. AD.5, Distal trachea showing a thin walled vein in the submucosa. Note the smooth muscle cells and the thin processes of the pericytes(↓). 2,600 × bar = 5μm

Fig. 42. AD.2, Proximal trachea showing a nerve entering the epithelium 20,000 × bar = 0.5μm

Plate 20

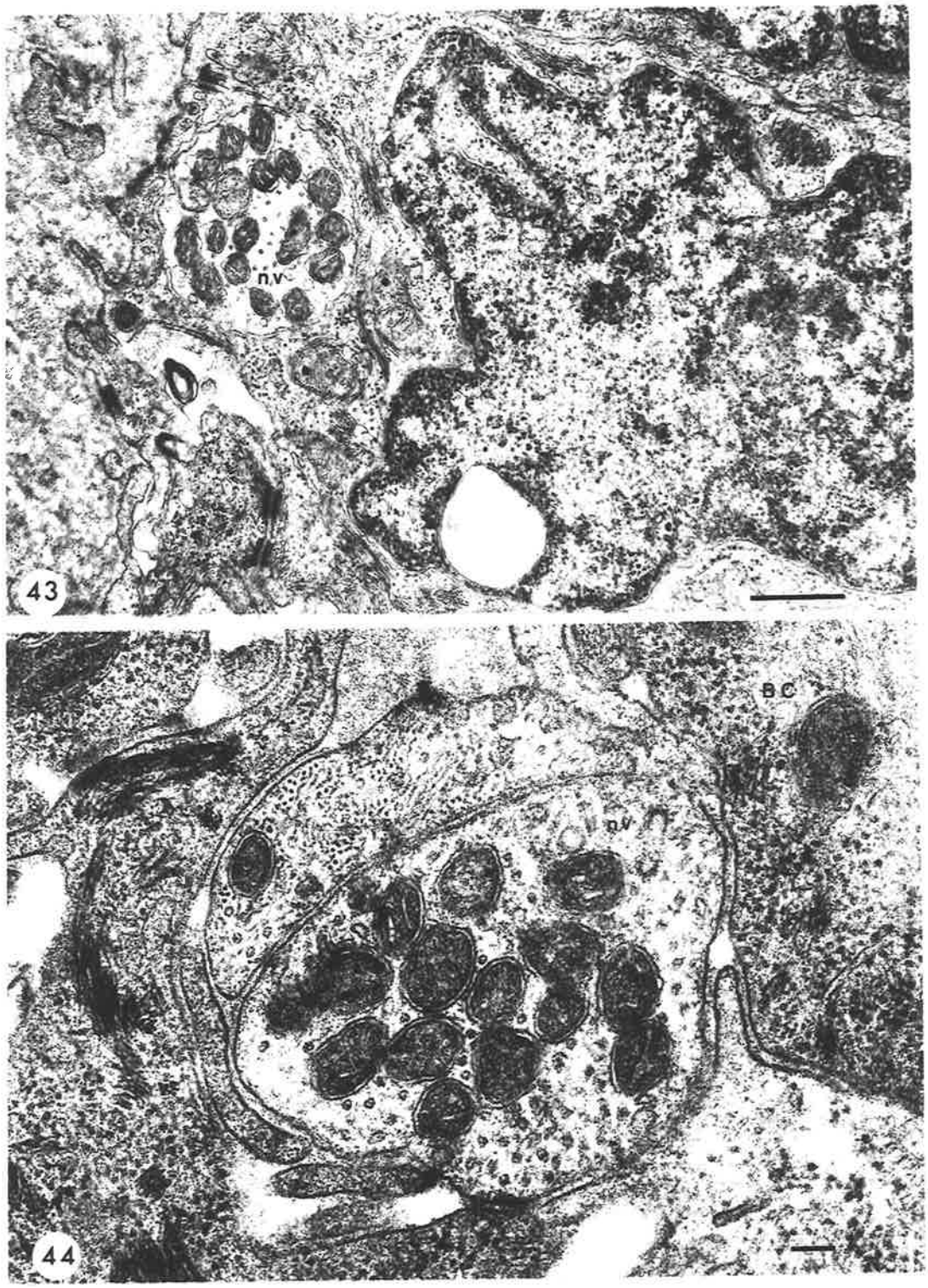


Fig. 43. AD.5,Nerve fibre in the basal layer of the epithelium adjacent to Fig.9. 31,000 × bar = 0.5μm

Fig. 44. AD.5,Detail of a nerve in the epithelium. 70,000 × bar =100nm

5-5 The bronchi.

5-5 A Extra pulmonary bronchi.

The extra pulmonary bronchi have essentially the same structure as the distal portion of the trachea. I have not observed the transitional epithelium beyond the bifurcation of the trachea, but islands of the microvillous epithelium are commonly found. I have divided the intra-pulmonary bronchi into two groups: secondary and tertiary. The secondary bronchi range in size from 1 cm down to several millimetres when the tertiary bronchi commence, however, the change from secondary to tertiary bronchus is marked not by this change in size but by changes in microscopic structure (Fig. 51, plate 23).

5-5 B Secondary bronchi.

The secondary bronchi differs from the extrapulmonary bronchi and the trachea mainly in the difference in the size of the components of the wall, not their arrangement. The epithelium is pseudostratified ciliated columnar with abundant goblet cells and is 40 µm thick proximally decreasing to 20 µm distally (Fig. 45). Glands are less frequent and are generally found between the cartilages and are not found in bronchi less than 0.5 cm in diameter. The lamina propria is 50-100 µm thick proximally with a distinct elastic component. It is difficult to distinguish as a separate layer distally where it has few elastic fibres (Fig. 46 and 47). The internal elastic lamina also decreases from 60 µm to 15 µm and is composed of thin elastic fibres running spirally and longitudinally. The venous plexus is composed of veins about 60 µm in size. Numerous

arterioles up to 60 μm are found between the cartilages.

5-5 C Tertiary bronchi.

The tertiary bronchi differ from the secondary bronchi in the following ways: (1) they are smaller, generally less than 0.4 cm in diameter; (2) there are smooth muscle bundles in the walls; (3) the venous plexus is replaced by a mixed vascular plexus in the submucosa; (4) the fusion of the dense internal elastic lamina and the fine elastic network of the lamina prorpia; and (5) the absence of glands and the replacement of the goblet cells by non-ciliated cells.

I will now consider these differences in more detail.

5-5 C-1 Size.

The size of the bronchi is not a reliable guide to its structure and is recorded only for completemess.

5-5 C-2 Smooth muscle.

Smooth muscle has been conspicuous by its absence so far in the dolphin bronchial tree. Initially only a few cells are found in the submucosa between the cartilages (Fig. 48 and 49). As the tertiary bronchi decrease in size to 100 to 50 μm the smooth muscle cells increase in number and form distinct circular bundles which can be seen in the SEM as ridges (Fig. 52).

5-5 C-3 Vascular plexus.

The change in the venous plexus is one of the most obvious features of the tertiary bronchi and can be seen in unfixed and fixed specimens with the aid of a hand lens. The plexus arises abruptly, suggesting that it has a different blood

supply as well as a different structure. It is composed of arterioles, veins and capillaries, each in about equal proportions, and it extends throughout the submucosa(Fig. 45, 46 and 47). The vessels are less than 25 μm in diameter with the usual mammalian structure.

5-5 C-4 Elastic tissue.

In sections stained for elastic tissue, the most obvious difference is the fusion of the two elastic layers to form one much thinner layer in the lamina propria (Fig. 46 and 47). The elastic fibres are not lost, however, but are dispersed throughout the submucosa, forming a fine network of fibres extending from the new internal elastic lamina, just beneath the basement membrane to the thick external elastic lamina surrounding the cartilages.

5-5 C-5 Epithelium.

The epithelium decreases from 20 μm to less than 10 μm in a few millimetres and changes from pseudostratified ciliated columnar to simple cuboidal with occasional basal cells. No goblet cells are found, their place being taken by non-ciliated cells of at least three types (Fig. 50, 52, 53, 54 and 60).

The ciliated cells are pale-staining cuboidal cells with shorter cilia (about 5 μm long) than the tracheal cells. They have fewer mitochondria and their round, pale-staining nuclei lie in the centres of the cells.

The non-ciliated cells are taller and darker staining than the ciliated cells, with a broad base resting on the basement mem-

brane. The apices of these cells are often dome shaped and often protrude above the surface (Fig. 73). Two different types of cells can be distinguished by the granules which they contain:

(a) Junctional zone cells.

This cell type is found in the junctional region of the secondary and tertiary bronchi. I have only seen an occasional cell distally. Three types of granules are found in the cytoplasm of these cells: (1) many small granules and vesicles 0.1 to 0.15 μm in diameter of moderate electron density, some having very dense cores; (2) tubular granules 0.04 x 0.25 μm of varying density; and (3) some pale staining spherical granules 0.5 μm in diameter (Fig. 53 and 54).

(b) Secretory cells.

These cells are first found in the junctional zone and then distally. They have been divided into two groups according to the type of granules present: (1) Typical Clara-type cells which have many pale-staining membrane-bound droplets 0.5 to 1 μm in diameter; a dome-shaped apex with microvilli 0.1 x 0.5 μm on its surface; abundant profiles of smooth endoplasmic reticulum in the supranuclear cytoplasm; a prominent Golgi complex; and numerous oval mitochondria up to 5 μm in size (Fig. 55). (2) the other cell type is more numerous and contains dark-staining membrane-bound granules 0.1 to 0.2 μm in diameter; equal proportions of rough and

smooth endoplasmic reticulum; many free ribosomes; and many microvilli 0.1 x 0.5 μm on its free surface (Fig. 57 and 58).

The basal cells are similar to those in the more proximal bronchi but contain fewer tonofilaments (Fig. 56). No brush cells have so far been found in the material examined, but mast cells are very frequently found in the submucosa and within the epithelium. Mast cells are seen throughout the dolphin respiratory system but appear to be most numerous in the terminal airways. I will therefore digress and describe their structure before proceeding down the bronchial tree further.

5-5 C-6 Mast cells.

Dolphin mast cells are ovoid cells up to 20 μm in length with many short, blunted processes and several longer thinner processes up to 5 μm in length (Fig. 59 and 60). The nucleus is indented and has very densely staining peripheral chromatin. The cytoplasm is packed with membrane bound vesicles up to 0.8 μm in diameter. These vesicles contain one or more of three types of material: pale finely granular material; dense coarse granules; and whorls of lamellae. Microtubules are also found in some cells (Fig. 60).

5-6 The intermediate zone.

The intermediate zone in the dolphin is composed of a sphincteric segment which gives rise to the alveolar ducts and thence to the alveolar sacs.

5-6 A The sphincteric segment.

The sphincteric segment ranges in length from 1-3 mm with two valves (Fig. 61 and 67) to 10 mm or more with twenty or more valves. Detailed studies of this segment with regard to distribution, number of valves and length have not yet been done. The sphincters or valves which guard each opening from this segment are situated between the cartilage plates.

The sphincteric segment is characterised by the presence of the sphincters and the following: cartilage plates in its wall; the absence of ciliated epithelium; the presence of thin walled blood vessels in its walls; and alveoli arising directly from its wall.

5-6 A-1 Size.

As the tertiary bronchi decrease further in size to about 0.5 to 0.25 mm the muscle bundles become more prominent (Fig. 48, 49, 61 and 67). This can be seen in low power L.M. and in the SEM distinct ridges can be seen (Fig. 52 and 72). These muscular bundles form definite sphincters in bronchi less than 0.25 mm in diameter (Fig. 61, 62, 63, 64, 67, 75 and 76) and are best seen in specimens of collapsed lung (Fig. 70).

5-6 A-2 Sphincters.

The myo-elastic sphincter consists of circularly-running smooth muscle fibres and radially running elastic fibres. (Fig. 61, 62, 63 and 64). The smooth muscle fibres are typical mammalian in structure with many longtitudinally running myo-filaments; scattered fusiform bodies, dense bodies 0.5 - 1 μm; some mitochondria; and many pinocytotic vesicles near the

surface membrane (Fig. 79 and 80). Nexus regions are rarely seen. The elastic fibres are radially arranged attaching to the edges of the cartilages peripherally and merging with the fine elastic fibres of the lamina propria near the basal lamina (Fig. 63, 64, 79 and 80).

5-6 A-3 Epithelium.

The epithelium changes from ciliated to non-ciliated cells as the muscle bundles become more prominent and is then composed of two cell types: (a) cuboidal cells with microvilli, and (b) squamous cells. This change is best seen in the SEM (Fig. 71 and 72). The cuboidal cells are somewhat flattened up to 10 μm in diameter and 6 to 8 μm high. The cells have many microvilli 0.1 x 0.5 μm on their apcial surface (Fig. 74 and 78), a convoluted nucleus, ovoid mitochondria, up to 0.7 μm long, many free ribosomes, and many small vesicles (Fig. 78). The squamous cells are very similar to the type I cells of the alveolus and will be described below. The epithelium occasionally appears to be stratified in this segment. The basal cells appear to be either differentiating microvillous cells or squamous cells. There is a prominent basement membrane beneath the epithelium. Occasional multilamellar bodies, similar to those in the type II alveolar cells, are found in the more distal microvillous cells.

5-6 A-4 Submucosa.

The remainder of the wall of the sphincteric segment between the epithelium and the cartilage consists of a plexus of thin-walled blood vessels (Fig. 81, 83 and 85). This plexus appears

to be separate from the plexus in the wall of the tertiary bronchus, and to be supplied by branches of the pulmonary artery (see later). The plexus consists of thin-walled venules and capillaries which have only endothelial cells and a few pericytes in their walls (Fig. 79 and 85). In some areas the basement membranes of the blood vessels and the epithelium fuse and in these areas the blood-air barrier may be only 200 nm thick.

5-6 A-5 Cartilage.

Cartilage is an important component of the dolphin bronchial tree, extending from the larynx to the openings of the alveolar ducts. I have studied the cartilage in some detail only in this sphincteric segment, but it is not significantly different from that in the remainder. The cartilage is typical mammalian hyaline cartilage (Fig. 85 and 86). The chondrocytes have a centrally placed nucleus with peripherally distributed chromatin, and in some there is a prominent nucleolus The cytoplasm contains: a Golgi complex; some tubular mitochondria 0.7 x 0.15 μm; free ribosomes; and some rough endoplasmic reticulum. The cell surface is scalloped with many fine processes up to 1 μm long extending into the matrix. The matrix contains fibrils ranging in size from 10 to 25 nm without crossbanding; occasional typical collagen fibres; and many granules 10 to 30 nm in diameter, in the amorphous matrix. The perichondrium contains fibroblast-like cells and occasional elastic fibres. No elastic fibres have been found in the cartilage matrix.

5-6 B Alveolar ducts and alveolar sacs.

The sphincteric segment terminates by dividing into alveolar ducts, which in turn give rise to alveolar sacs and alveoli. I have not yet attempted to reconstruct this portion of the lung, but a general impression of the arrangement can be seen in the SEM and low power LM (Fig. 67 and 69) and in the corrosion specimen (see appendix 3).

The walls, or pillars, of the alveolar ducts and sacs consist of a fibro-elastic framework which is continuous with that of the sphincteric segment. The lining epithelium is cuboidal to squamous cells with an occasional cell resembling the type II cells of the alveoli. The openings of the alveolar ducts from the sphincteric segment are always guarded by a myo-elastic valve, but, although smooth muscle fibres are often found in the walls of the ducts, valves have not been observed (Fig. 67, 68, 88 and 89). The walls of the alveolar sacs are generally thinner and seldom contain smooth muscle fibres.

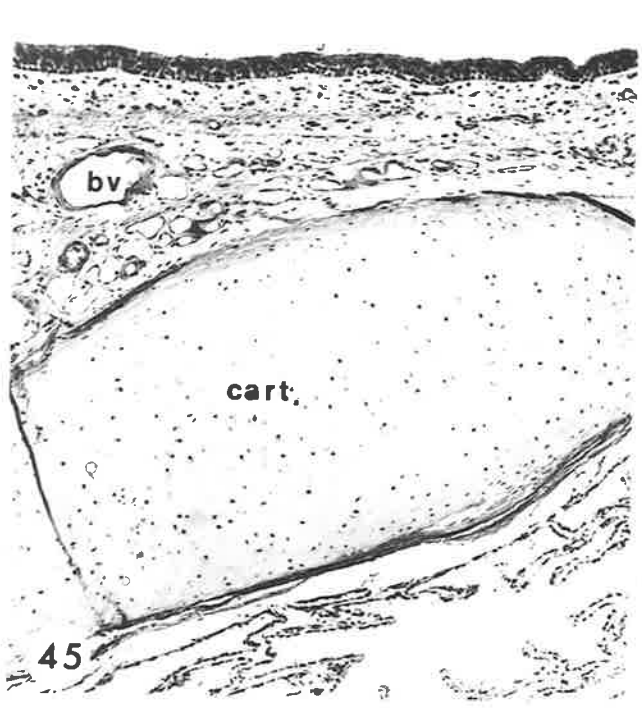
Plate 21.

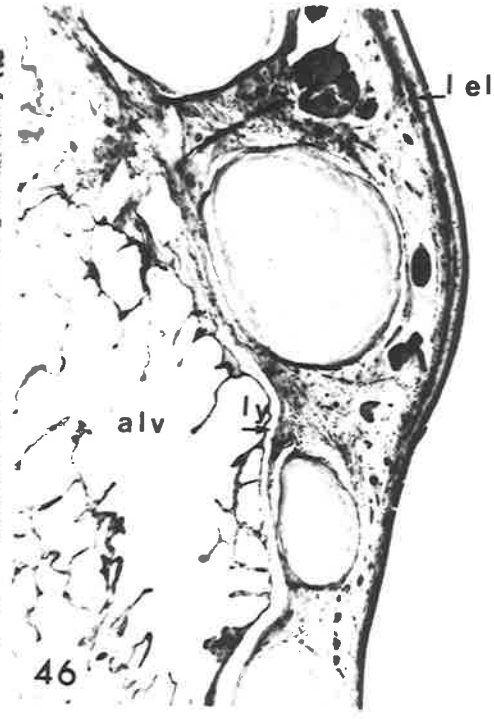
Fig. 45. AD, 1. Distal region of secondary bronchus.
Note: pseudo-stratified, ciliated columnar epithelium with goblet cells; hyaline cartilage; the prominent vascular plexus; the alveoli adjoining the adventitial layer. H and E 150 x

Fig. 46. AD, 5. Transitional zone of secondary to tertiary bronchus.
Note: the smaller cartilage plate in the tertiary bronchus; the sudden change in size of the vessels in the submucosa; the merging of the prominent elastic lamina in the submucosa with the thinner elastic lamina immediately beneath the epithelial layer.
V and VG 40 x

Fig. 47. AD, 5. Higher power of Fig. 46. showing the fusion of the two elastic lamellae.
Note: also the lymphatic vessel in the peribronchial tissue (lv) V and VG 200 x

Plate 21





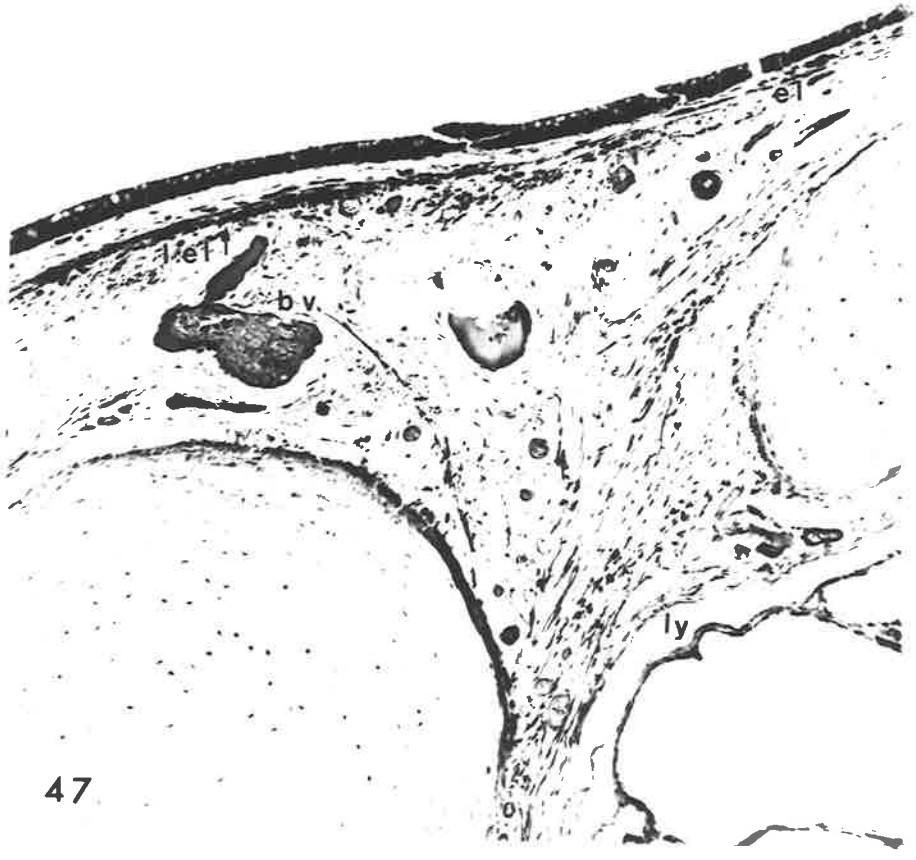


Plate 22

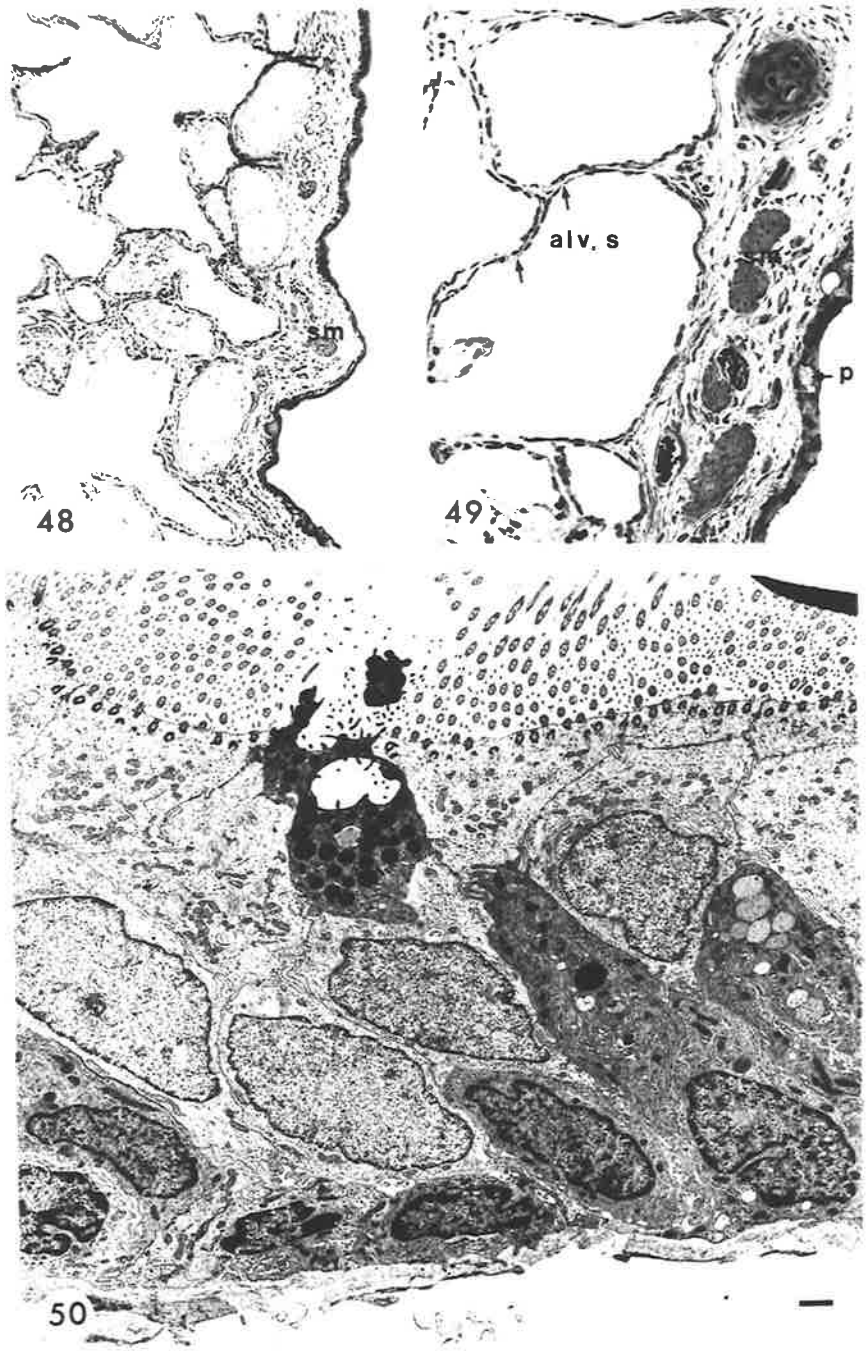


Fig. 48 . AD. 1, Tertiary bronchus. Note: smooth muscle bundles; the decrease in thickness of the epithelium. H. and E. 75 ×

Fig. 49. AD. 5, Tertiary bronchus. Note: smooth muscle bundles; cuboidal epithelium containing intra-epithelial parasites(p); alveolar septa with double capillary networks(alv s). 1μm plastic section MB. and Az. 300 ×

Fig. 50. AD. 5, Distal end of secondary bronchus showing pseudostratified low columnar to cuboidal epithelium with cilia and goblet cells. TEM 4,400 × bar = 1μm.

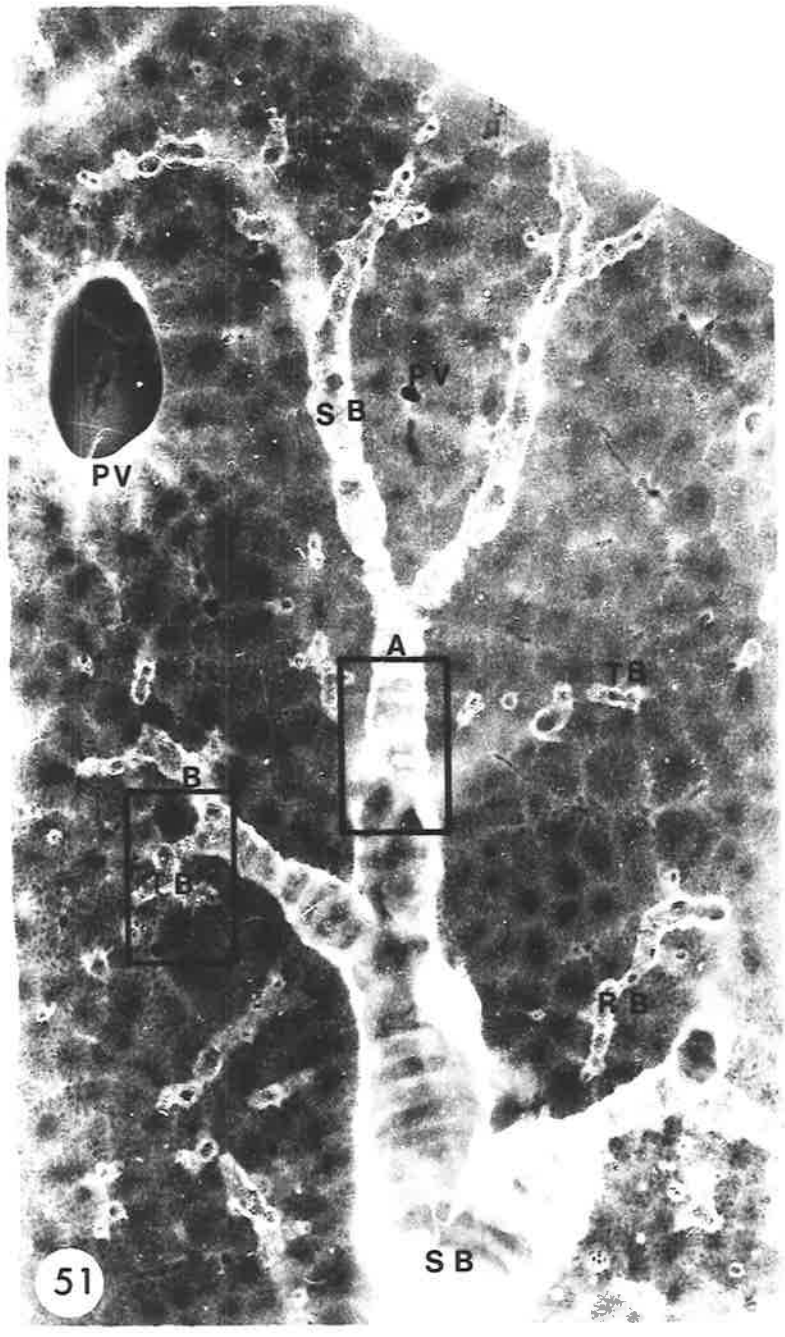


Fig. 51. AD 2 ,Slice of lung in buffer before critical-point drying. After drying the whole slice it was divided into suitably sized sections,mounted on stubs and coated. This was done to avoid loss of orientation. Area A was used for Fig.52.Area B for Figs.69,71,84. Note:the bifurcation of a secondary bronchus(SB); tertiary bronchi(TB);respiratory bronchi(RB)or sphincteric segments;and pulmonary vessels(PV).

Fig. 52. AD. 2, Terminal portion of the secondary bronchus showing ciliated epithelium raised into prominent circular folds. SEM 400 ×

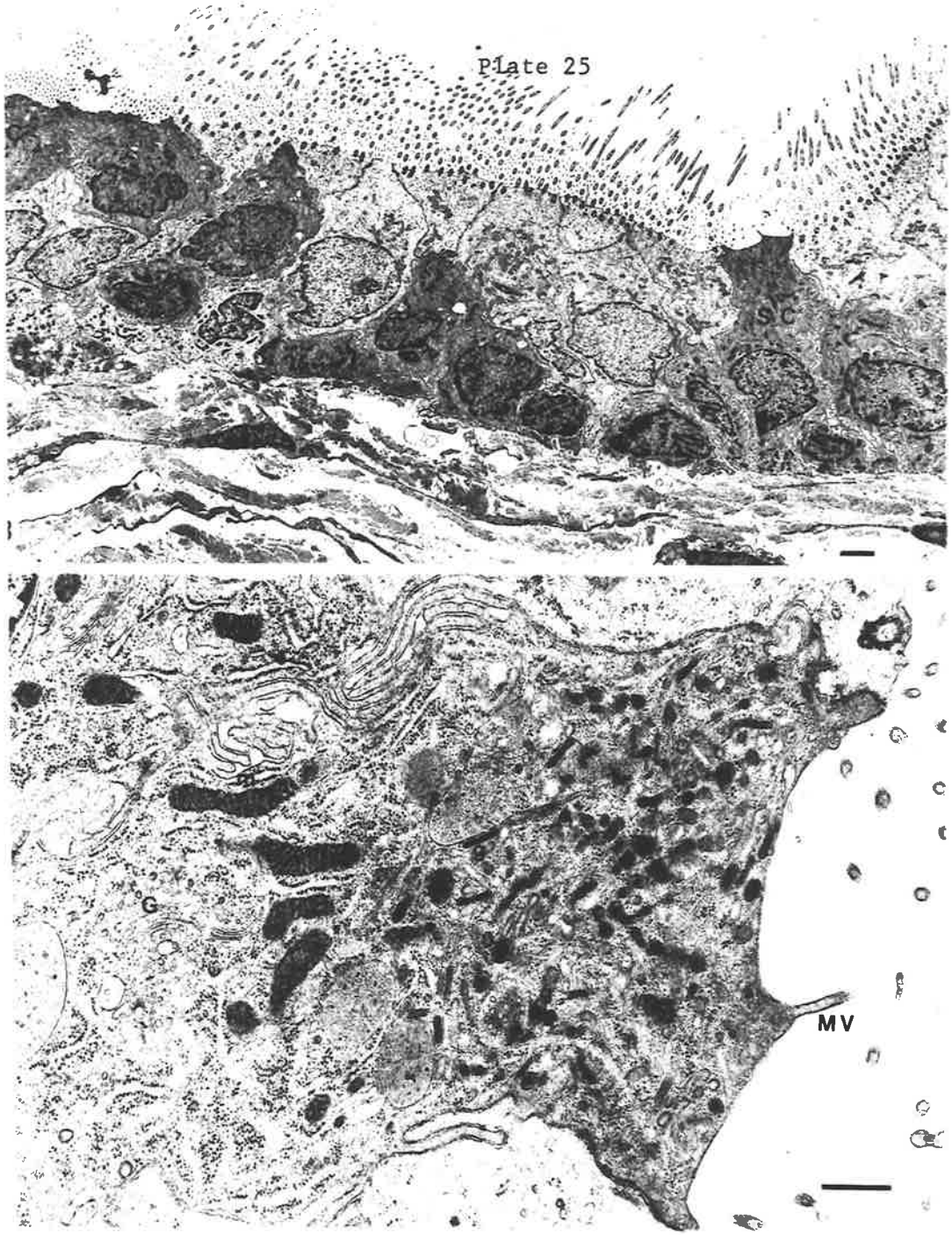


Fig. 53. AD.5, Tertiary bronchus showing low columnar to cuboidal pseudostratified, ciliated epithelium. Some secretory cells (SC) are seen but no goblet cells. 2,600 × bar=2μm.

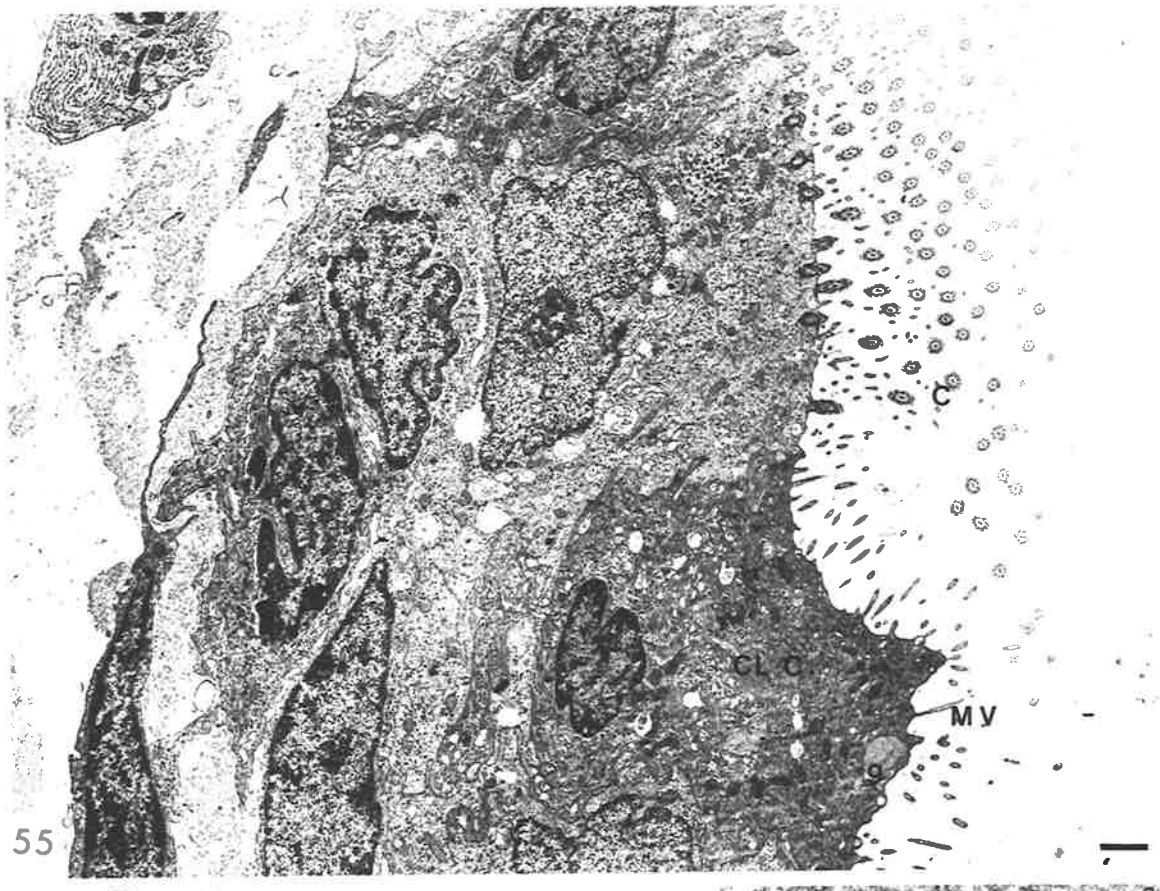
Fig. 54. AD.5, Higher power of a secretory cell from Fig.53. Note: the variation in size and staining of the granules in the apical region of the cell. 20,800 × bar = 0.5μm.

Plate 26.

Fig. 55. AD, 5. Tertiary bronchus showing a Clara cell (CLC).
Note: the stratified epithelium in this section; the bulbous apex of the Clara cell containing only a few secretory granules; and the relatively short cilia.
6,600 x bar = 1 μm.

Fig. 56. AD, 5. Higher magnification of the basal cells.
Note: the plication of the lateral and basal surfaces (↓); the basal attachments with relatively few desmosomes or tonofilaments.
20,000 x bar = 0.5 μm.

Plate 26



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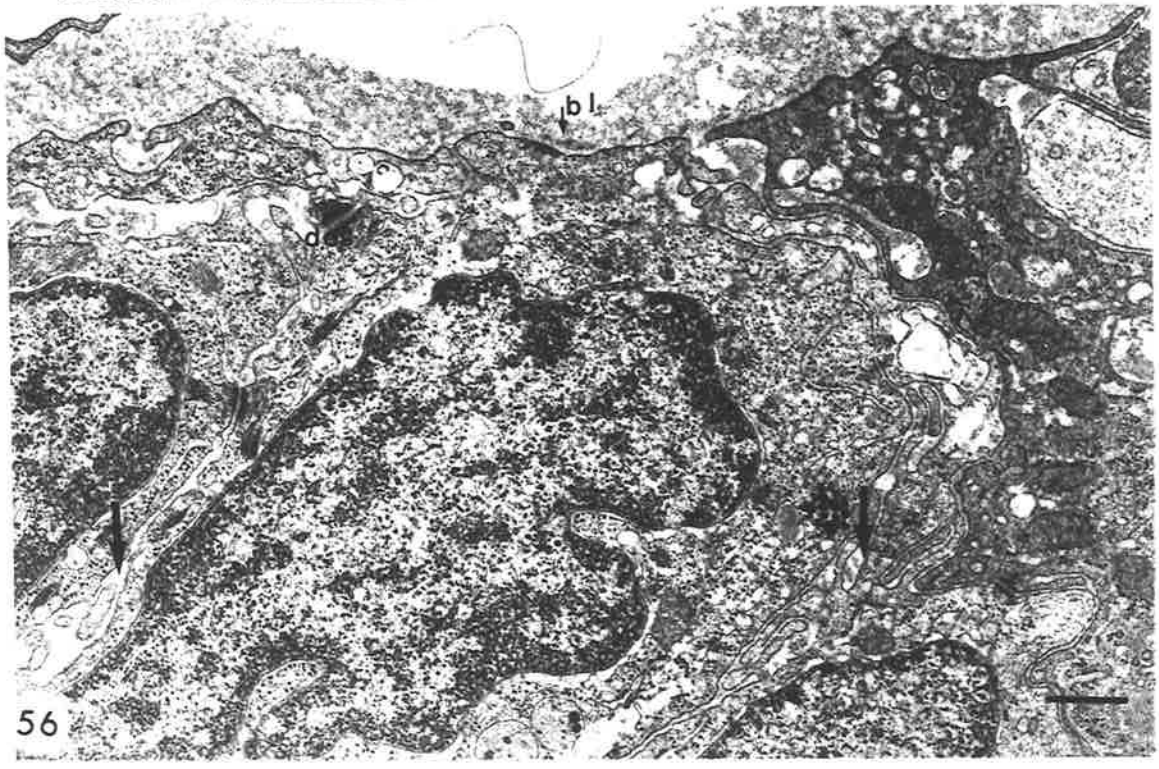

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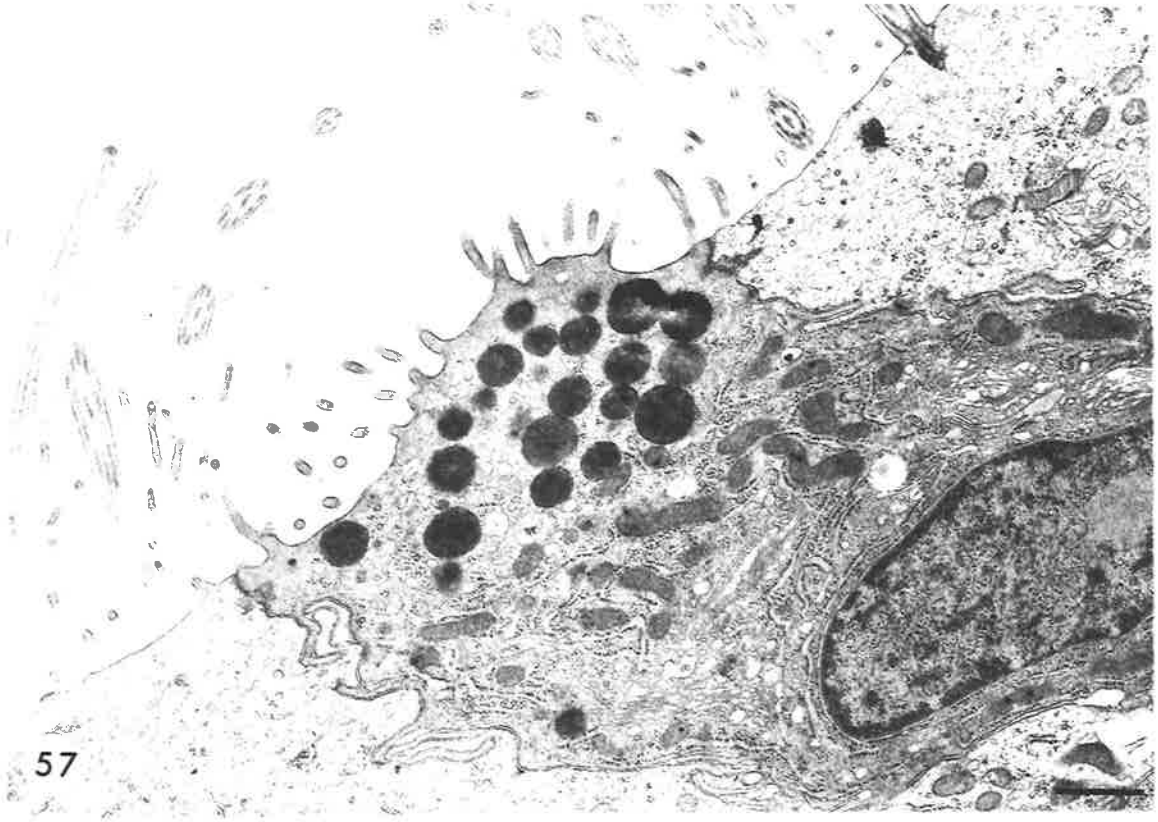
Plate 27.

AD, 5. Tertiary bronchus.
These two figures illustrate two additional types of secretory granules found in the Clara cells.

Fig. 57. This Clara cell contains dense granules with little protrusion of the apical region.

Fig. 58. This Clara cell has a protruding apex which appears to have just discharged its contents. Some pale staining granules with electron-dense cores are still present. Both figures 40,000 x bar = 0.25 μm.

Plate 27



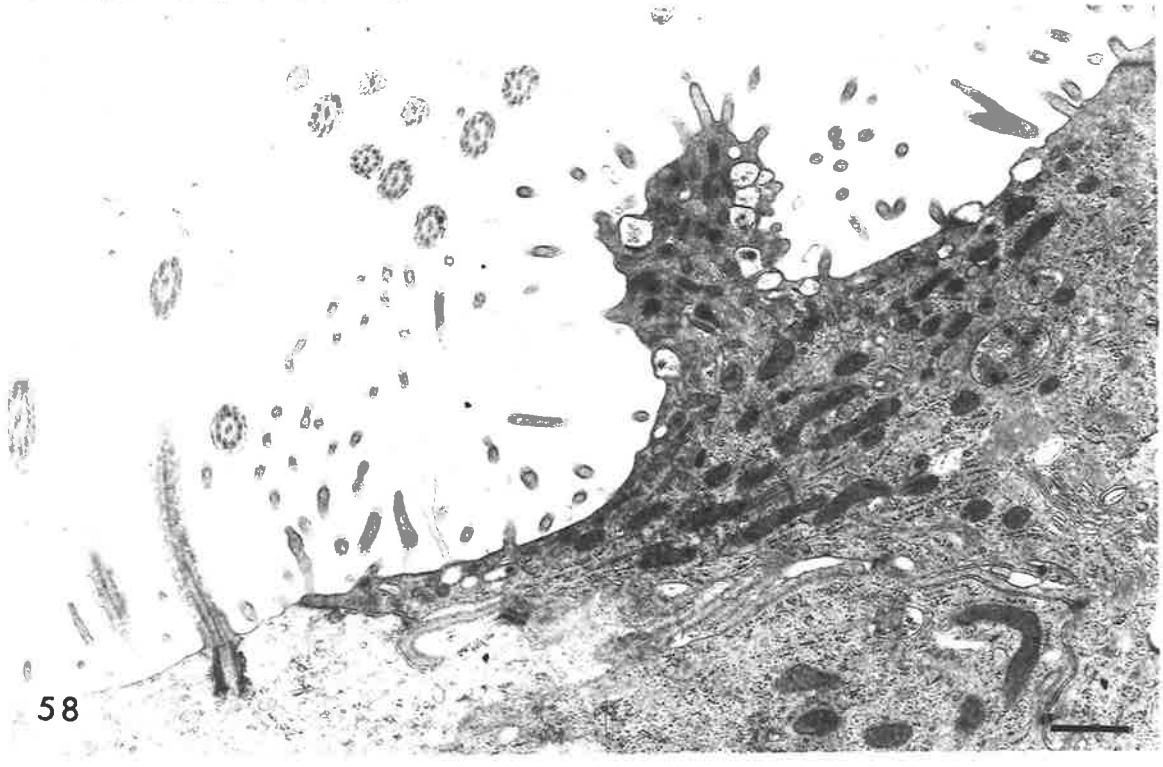
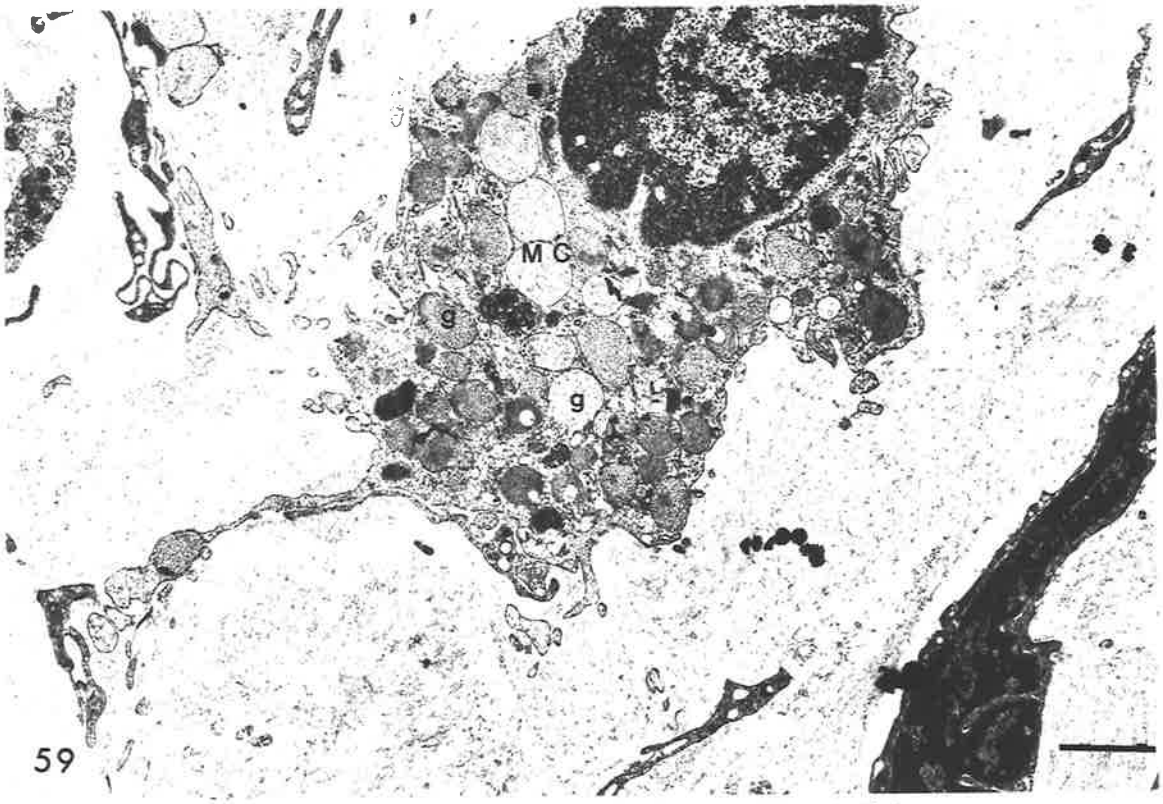


Plate 28.

Fig. 59. AD, 2. Proximal trachea showing a mast cell (MC) in the lamina propria.
Note: the heterogeneity of the granules.
12,600 x bar = 1 μm.

Fig. 60. AD, 5. Tertiary bronchus. A mast cell is almost entirely within the epithelium. This cell contains a mixture of granules including an array of tubules (T) 12,600 x bar = 1 μm.

Plate 28



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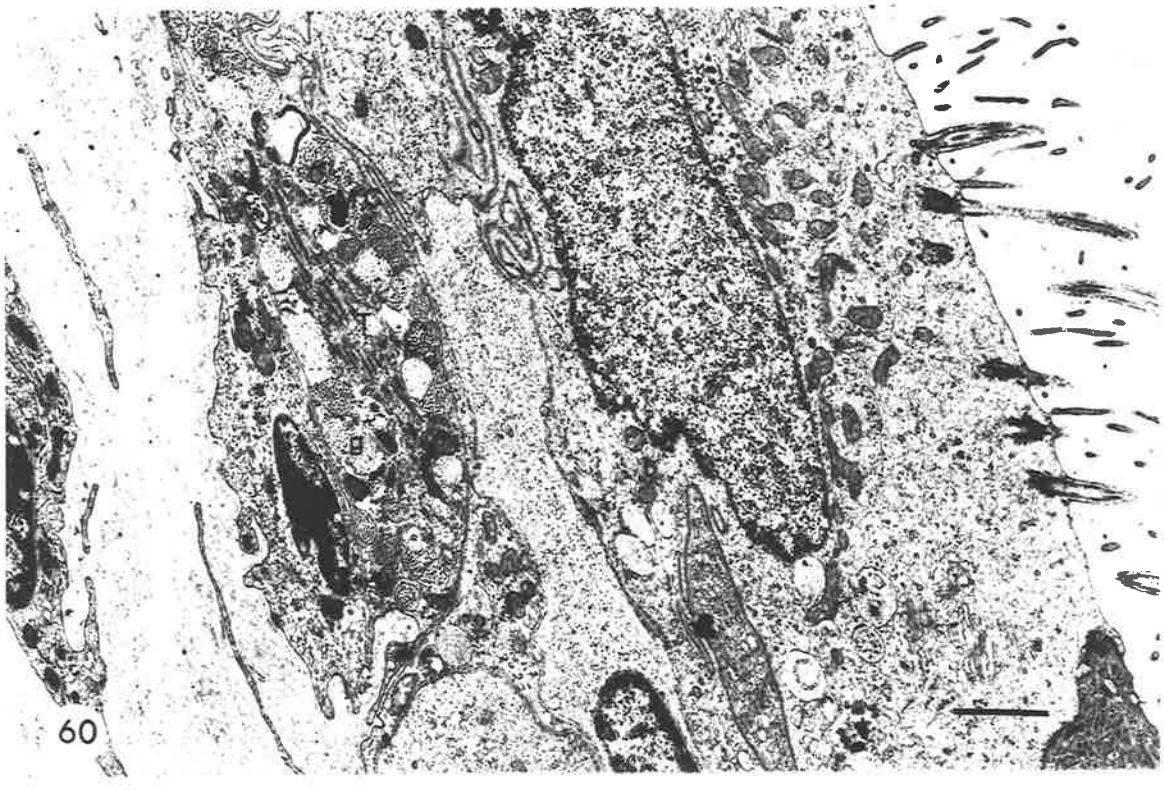


60

Plate 29.

Fig. 61. AD, 1. Right lung.
Note: the myo-elastic valves (my.v.) in the gaps between the cartilages; the thin epithelium; the vascular plexus (↓); and the surrounding alveoli. H. and E. 150 x

Fig. 62. AD, 7. Right lung showing a valve in cross-section. H. and E. 150 x

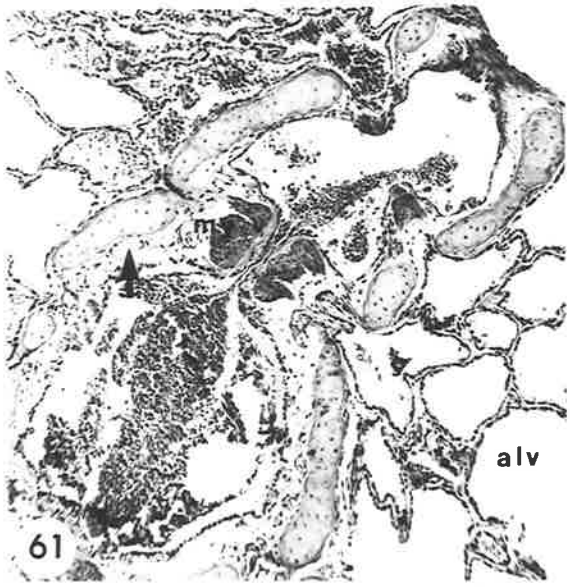
Fig. 63. AD, 3. Left lung stained for elastic tissue.
Note: the fine elastic fibres in the wall away from the valves; the denser elastic fibres around the valve which seem to come from the external elastic lamina and the peri-bronchial tissue; the collection of elastic fibres at the opening of the alveolar duct (↓). Weigert's elastic stain. 150 x

Fig. 64. AD, 3. Left lung stained for elastic tissue showing a cross-section of a valve.
Note: the condensation of the elastic fibres of the lamina propria between the smooth muscle and the cartilages; and the external elastic lamina which is connected by radial fibres is drawn into the lumen of the bronchus. Weigert's elastic stain. 150 x

Fig. 65. AD, 3. Right lung showing large densely stained bodies which appear to be both intra- and sub-epithelial (↓). H. and E. 120 x

Fig. 66. AD, 1. Alveolar septum showing the capillary network on each surface. H. and E. 600 x

Plate 29



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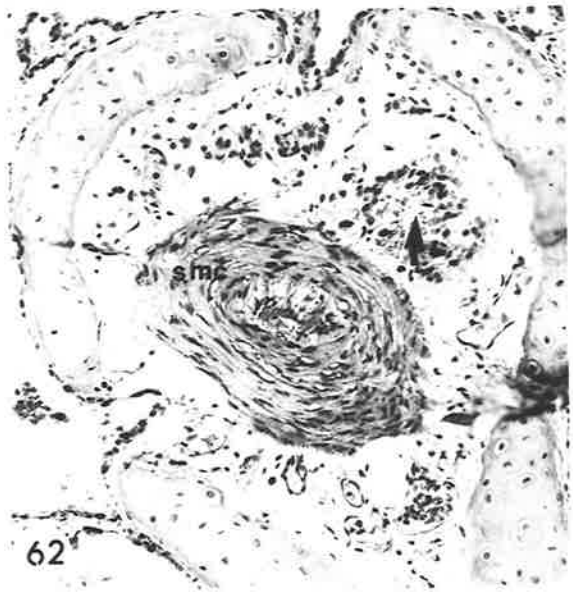

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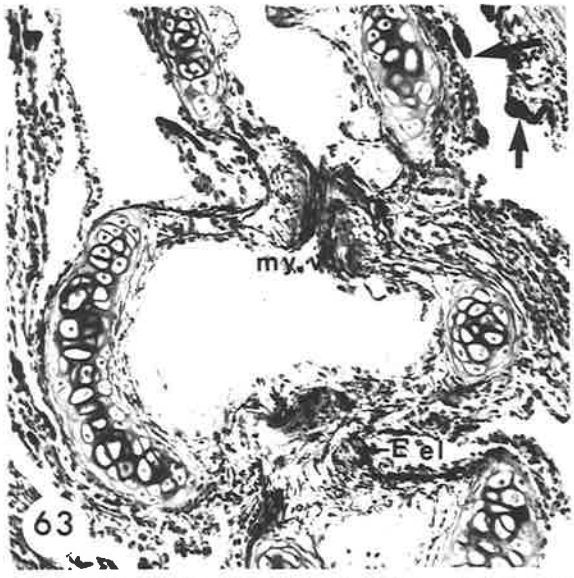

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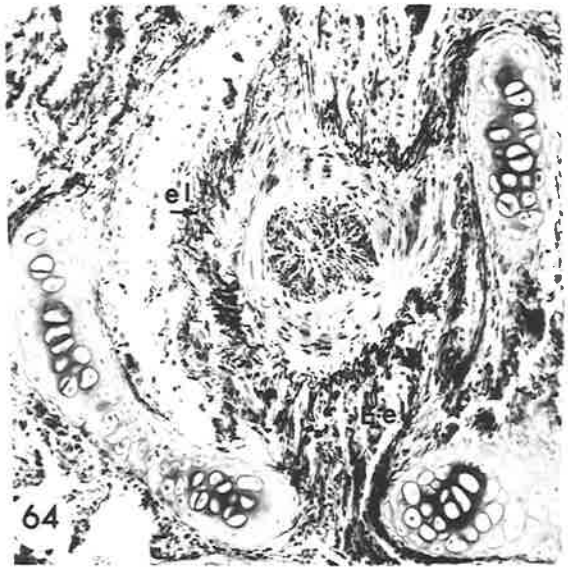

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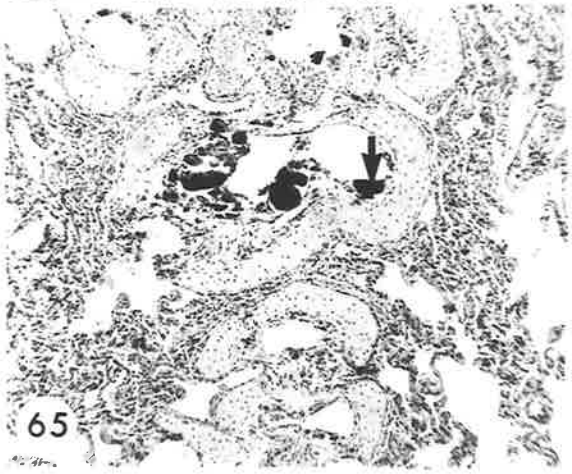
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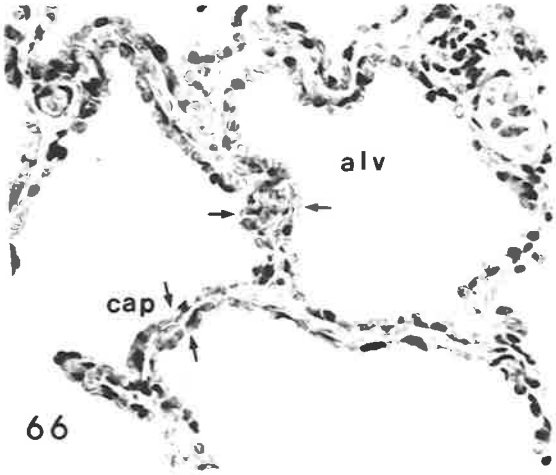


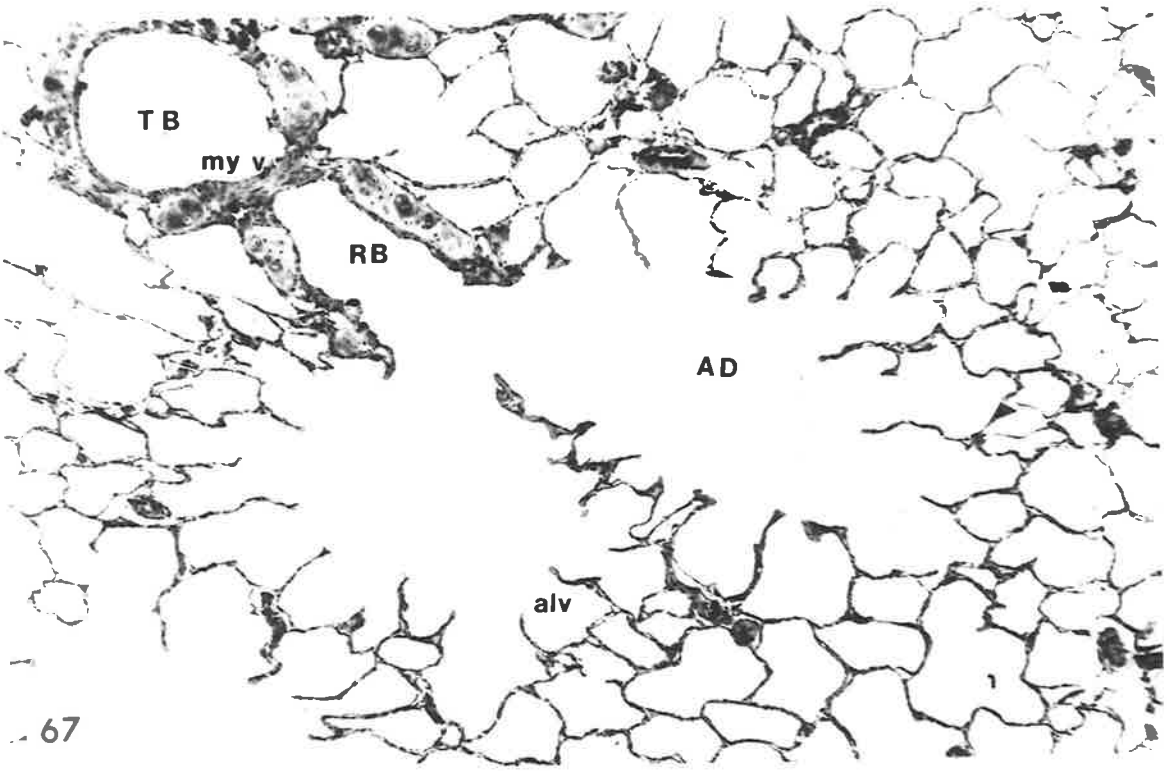
66

Plate 30.

Fig. 67. AD, 8. Section of lung showing; a tertiary bronchus (TB); a respiratory bronchus (RB); alveolar ducts (AD); and alveoli (alv). H. and E. 120 x

Fig. 68. AD, 8. Higher magnification of a similar area showing: cartilage plates around the opening of the alveolar duct; myo-elastic valves (my v); and the alveolar septum. H. and E. 200 x

Plate 30



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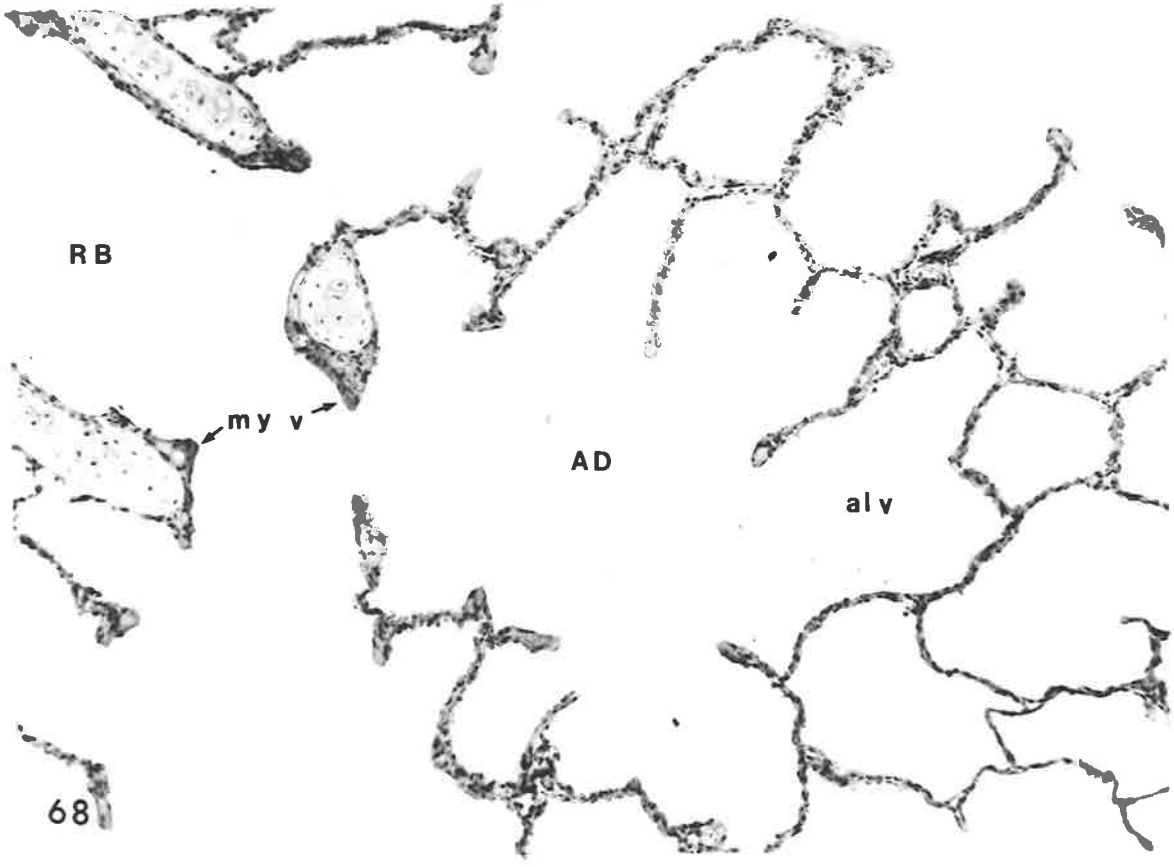

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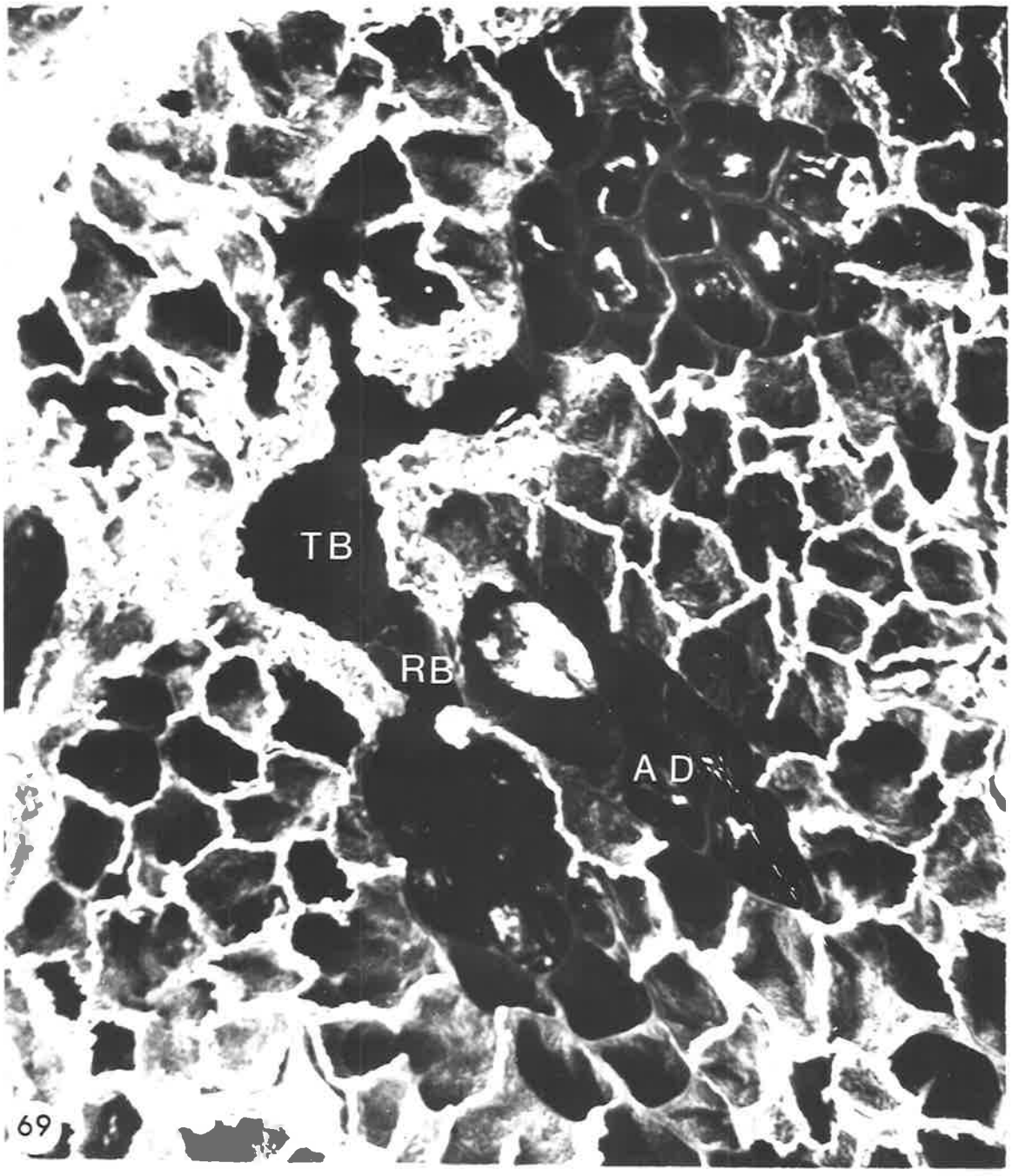


Fig. 69. AD.2, Low power SEM showing tertiary bronchus dividing into two respiratory bronchi. Note: the tubular appearance of the alveolar ducts; the absence of pores of Kohn; and the polyhedral alveoli. 500 ×

Plate 32.

Fig.70. AD, 7. Section of lung fixed in the collapsed state.
Note: the cartilage plates (cp) and the myo-elastic valve pulling the peribronchial tissue into the lumen 100 x

Fig. 71. AD, 2. Junctional region of the tertiary and respiratory bronchi.
Note: the ridges (↓) which may be caused by the vascular plexus; the scattered ciliated cells; the microvillous surface of most of the bronchus. 2,400 x

Plate 32

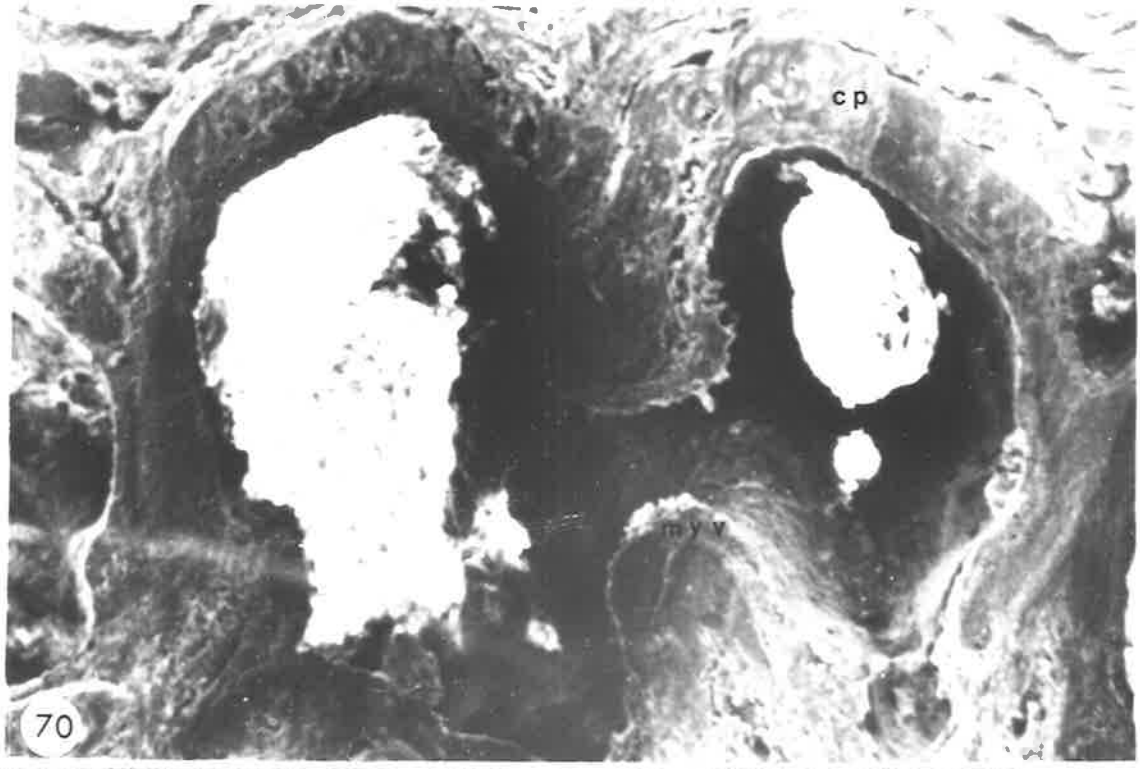


Fig. 72. AD.2, Higher magnification of a similar area to Fig.71 showing ridges raised by the underlying muscle bundles and microvillous cells between the ciliated cells. 4000 ×

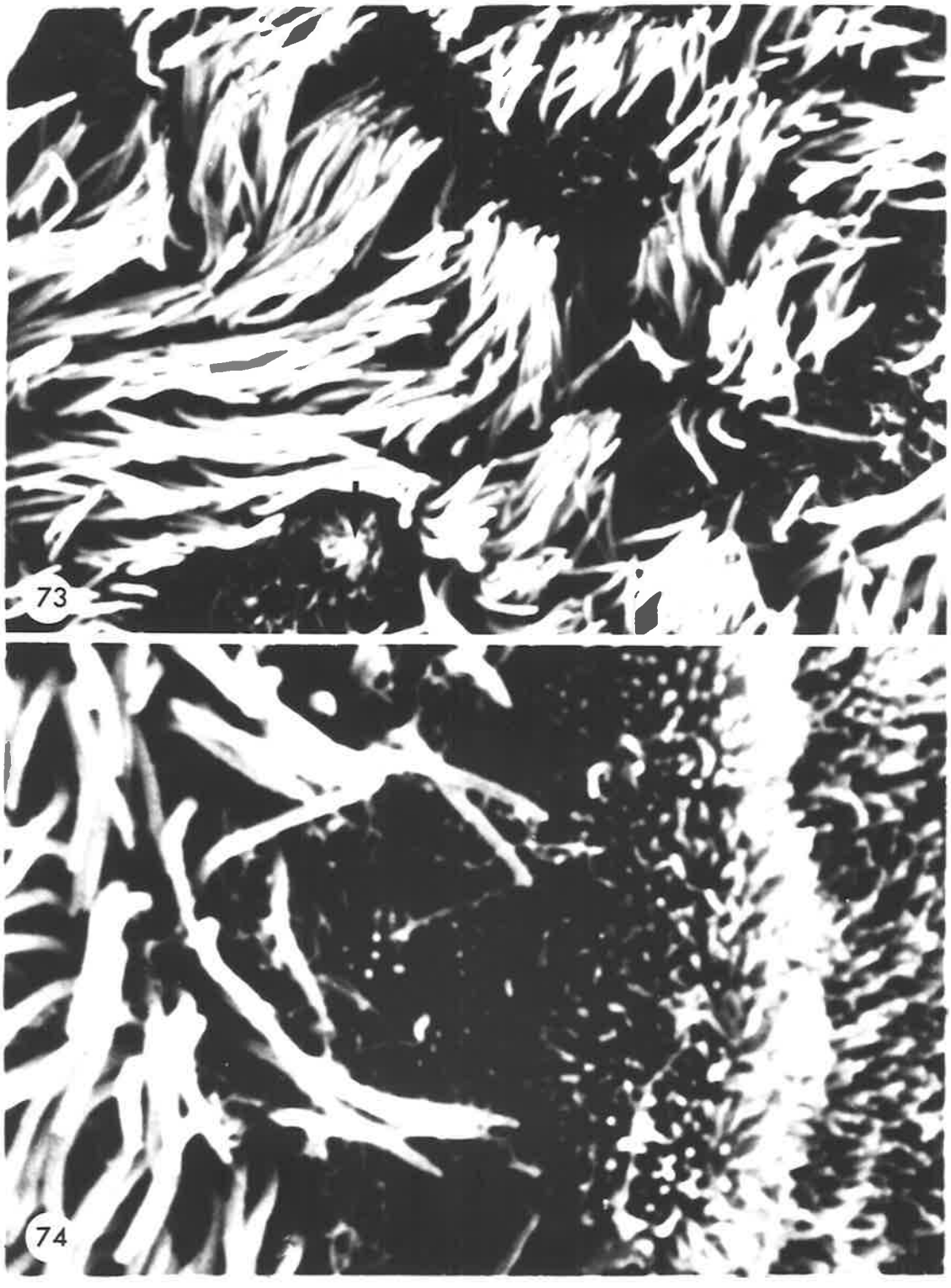


Fig. 73. AD.2, Tertiary bronchus showing ciliated cells and microvillous cells. A Clara cell is present(↓). 8,000 ×

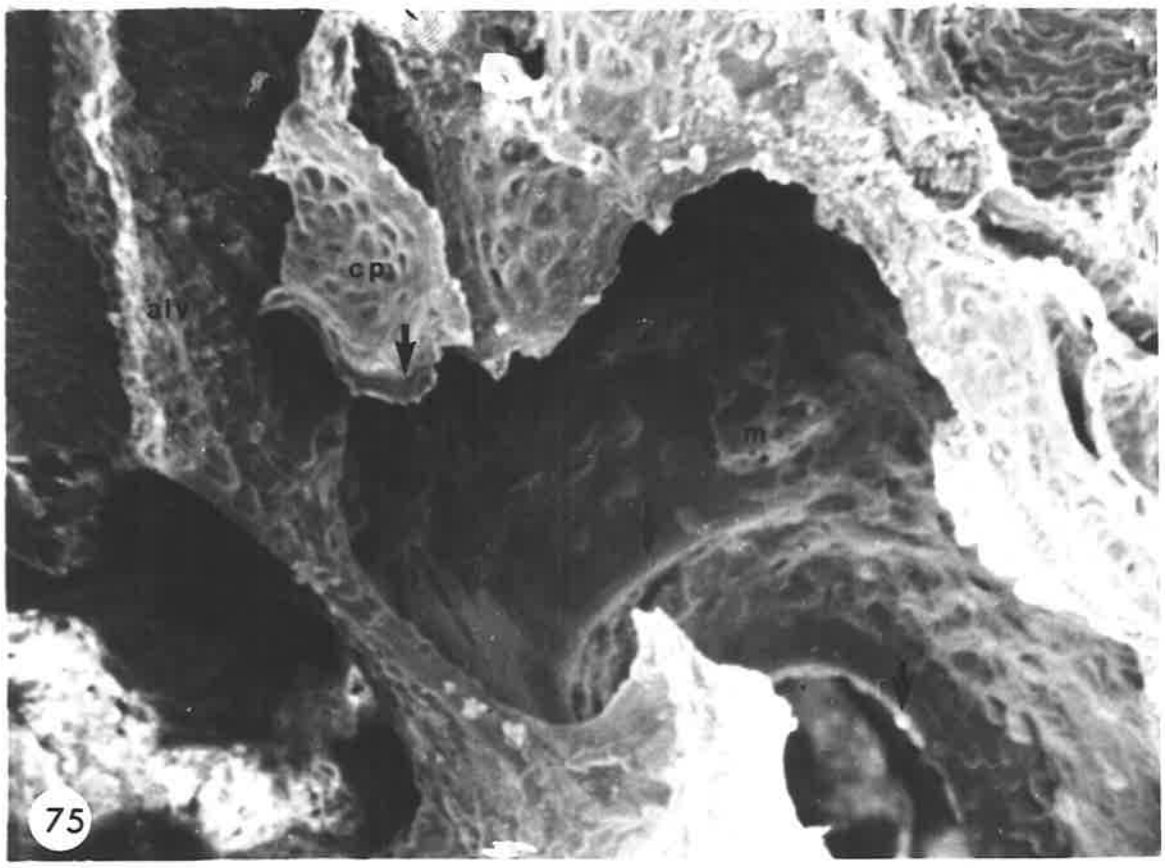
Fig. 74. AD.2, Junction of tertiary and respiratory bronchus showing the change in epithelium. 10,300 ×

Plate 35.

Fig. 75. AD, 2. Respiratory bronchus.
Note: several valves (ǂ); a macrophage (m); the capillary plexuses in the wall of the bronchus and the alveoli. 260 x

Fig. 76. AD, 5. Respiratory bronchus showing portion of a valve.
Note: the squamous epithelium on the surface, the cartilage (cp) and the vessel containing red blood cells (ǂ). 1,600 x

Plate 35



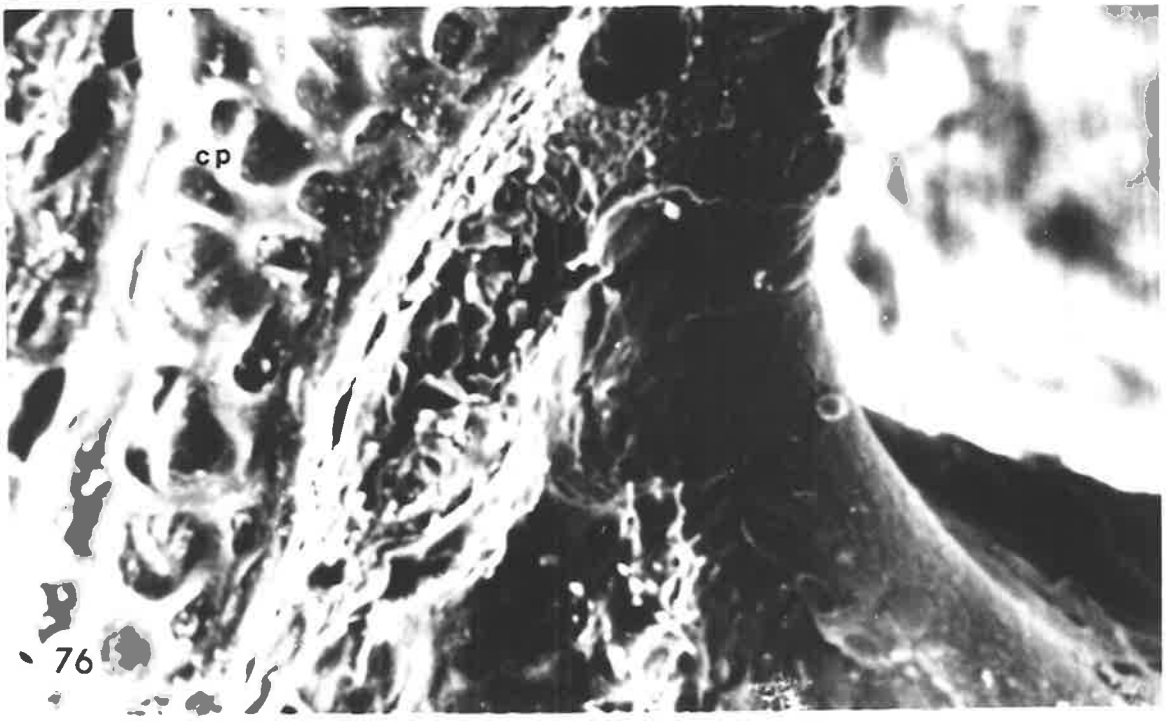
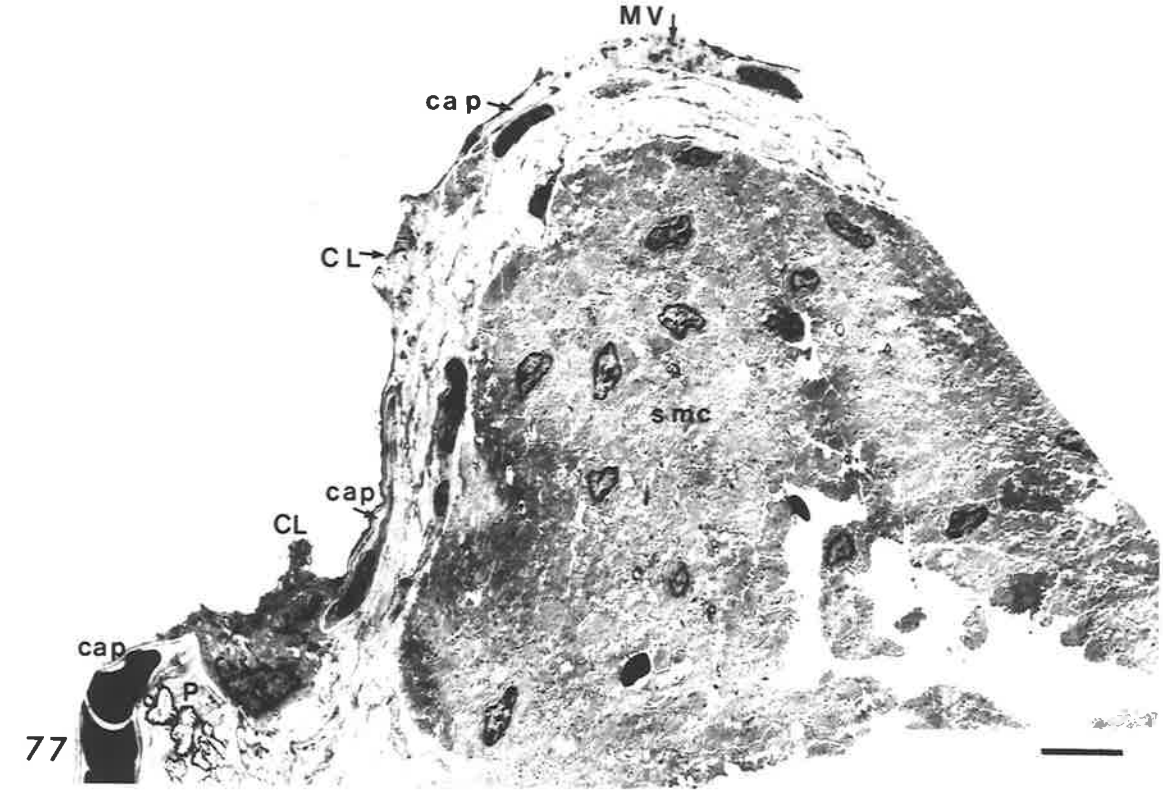


Plate 36.

Fig. 77. AD, 5. Junction of tertiary and respiratory bronchus.
Note: the thick bundle of smooth muscle cut tangentially; the capillaries beneath the squamous epithelium; Clara cells (CL); microvillous cells (MV); and a parasite (P).
2000 x bar = 5 μm.

Fig. 78. AD, 5. Respiratory bronchus.
Note: the capillary covered by squamous epithelium (sq ep); the microvillous cells; and the apparent stratification of the epithelium.
13,500 x bar = 1 μm.

Plate 36



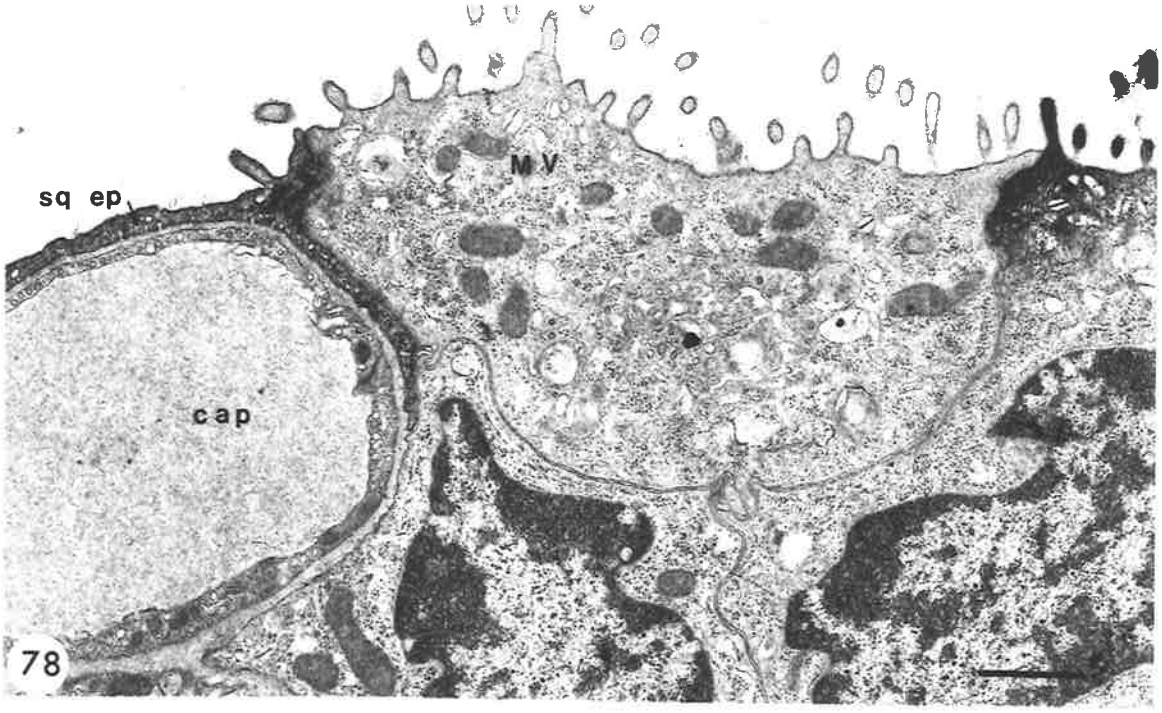
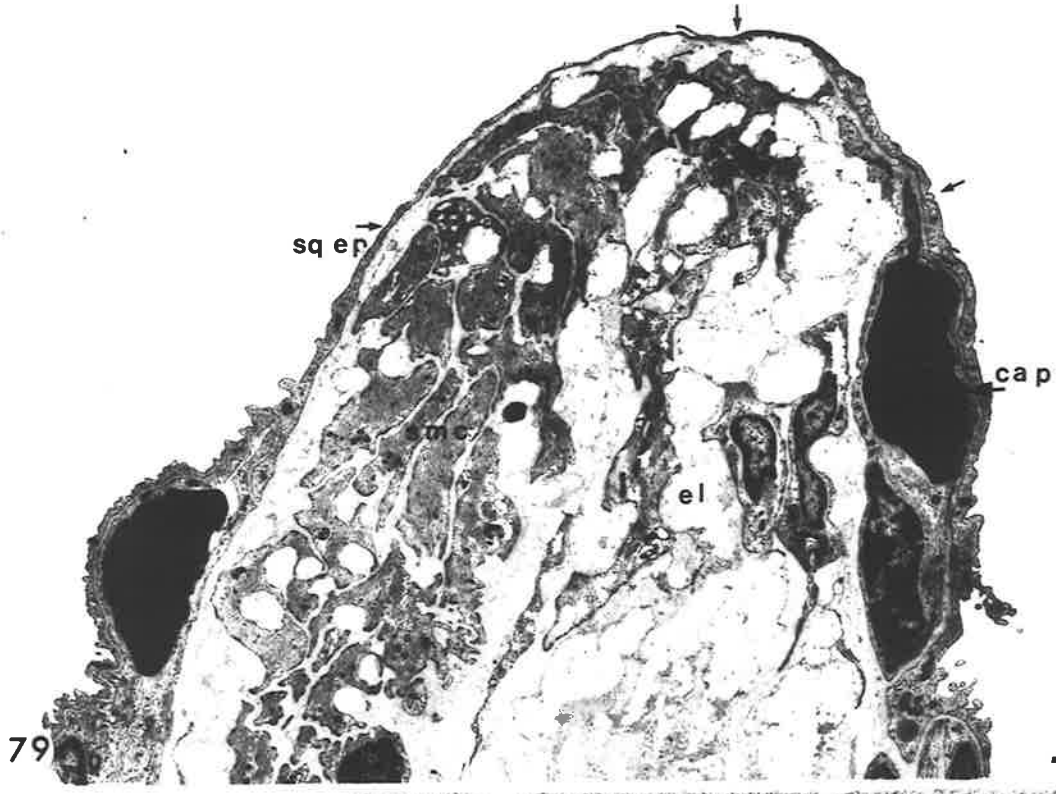


Plate 37.

Fig. 79. AD, 5. Respiratory bronchus showing one of the distal myo-elastic sphincters.
Note: the thin squamous epithelium (sq ep) covering the sphincter and capillaries; the smooth muscle cells; elastic fibres. 6,600 x bar = 1 μm.

Fig. 80. AD, 5. Higher magnification of portion of Fig. 79.
Note: the squamous epithelium has few organelles in its cytoplasm; the smooth muscle cells with (mf) myofilaments and peripheral pinocytotic vesicles (v); and the elastic fibres with central amorphous core and peripheral microfilaments (ǂ). 20,800 x bar = 0.5 μm.

Plate 37



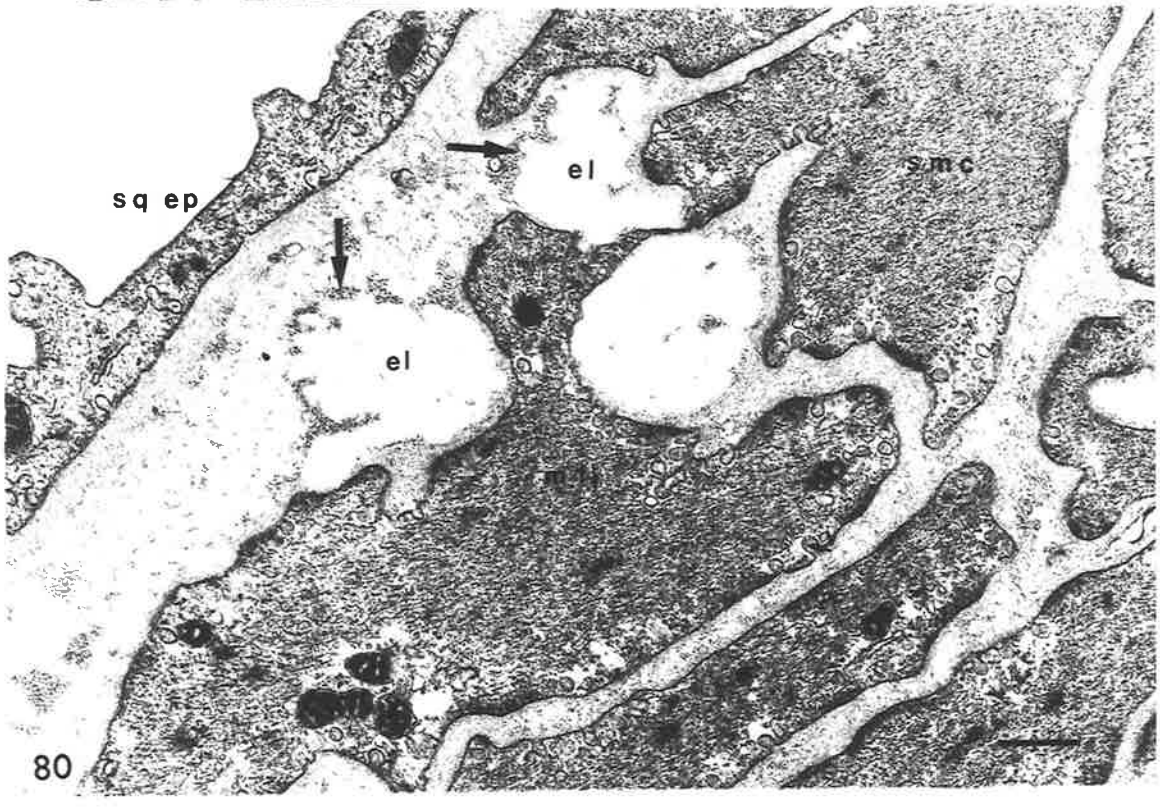


Plate 38.

Fig. 81. AD, 2. Respiratory bronchus, from an air-dried specimen.
Note: the engorged capillary plexus beneath the epithelium; macraphages (M); and some mucous particles (P). 1,400 x

Fig. 82. AD, 2. Higher magnification of portion of Fig. 81.
Note: the red blood cells are more prominent due to shrinkage from the air-drying; the outlines of the squamous cells (↓); and the microvillous cells. 2,200 x

Plate 38

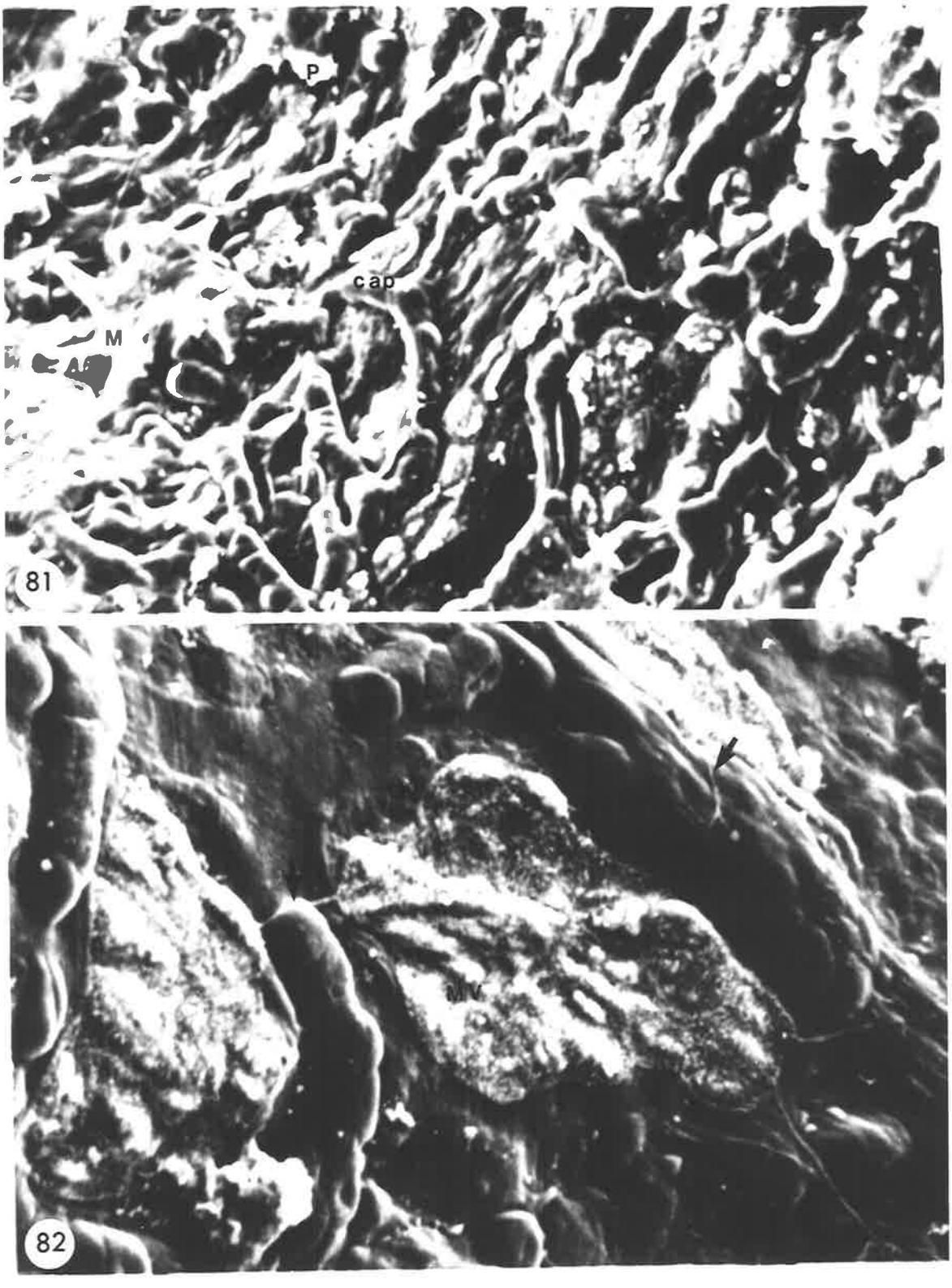
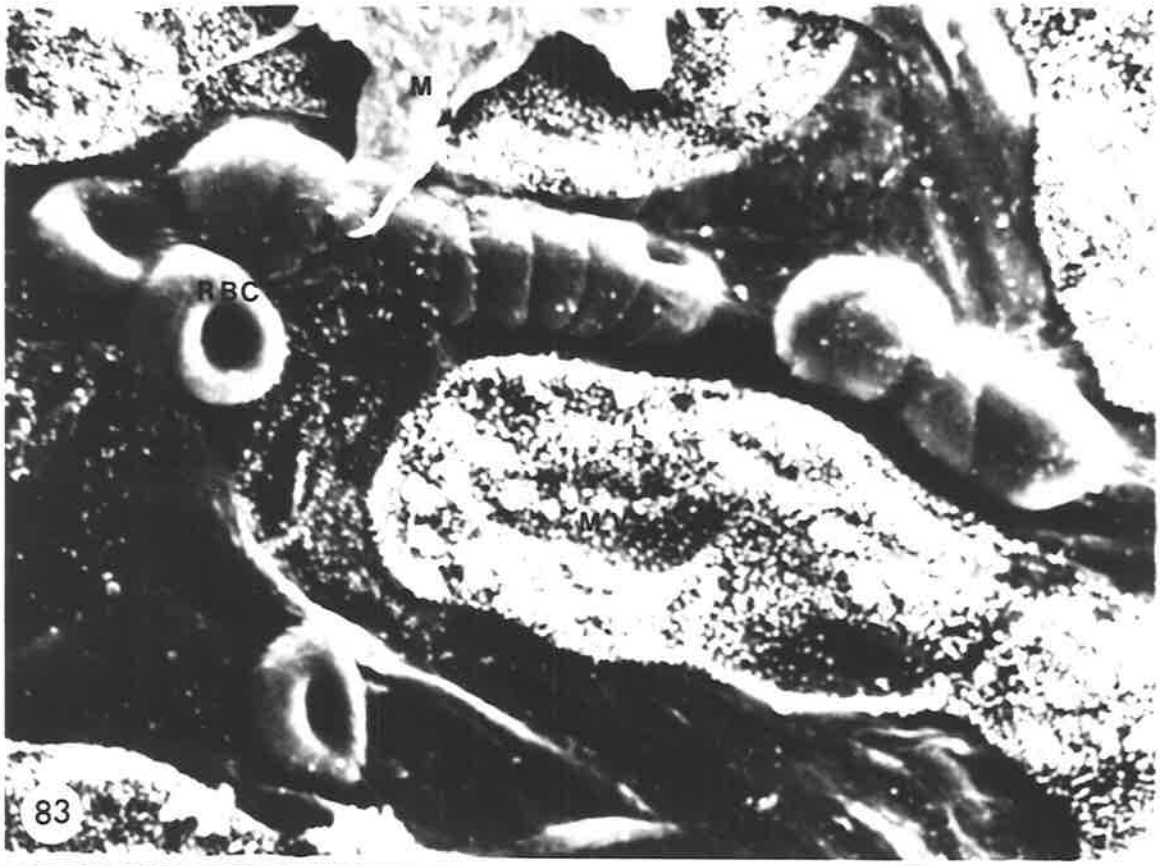


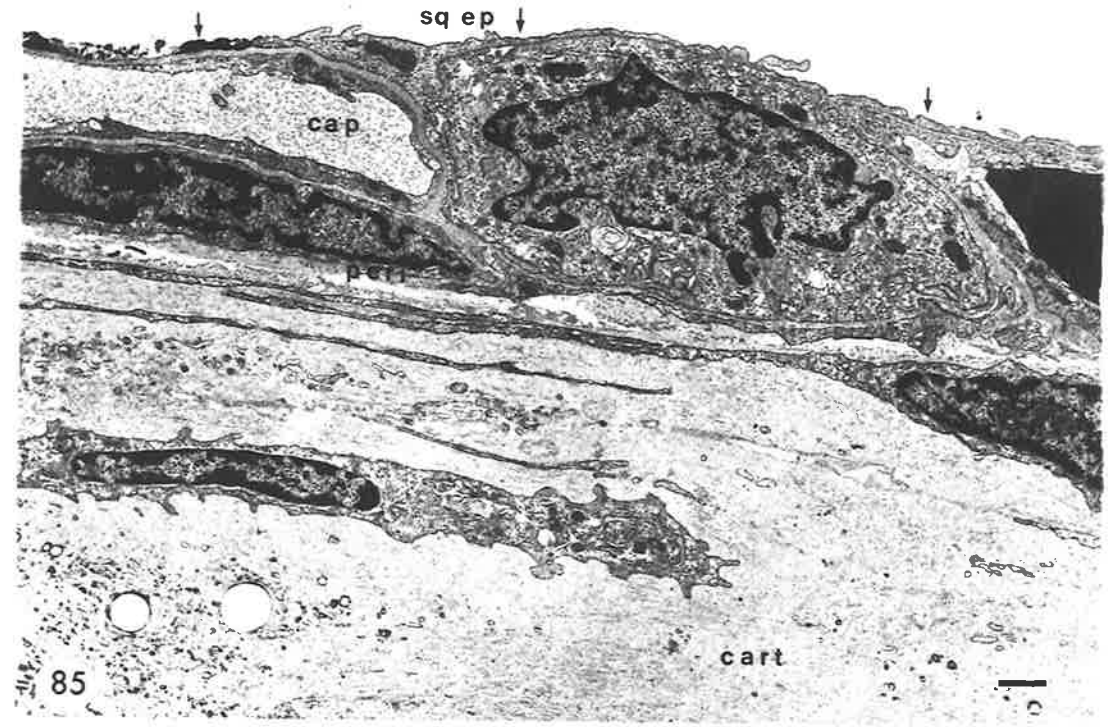
Plate 39.

Fig. 83. AD, 2. Respiratory bronchus (air-dried).
Note: portion of a macrophage (M); microvillous cells; and the biconcave red blood cells clearly outlined by the contraction of the squamous epithelium and the endothelium.
3,200 x

Fig. 84. AD, 2. Respiratory bronchus (critically-point dried).
Note: the better definition of the microvilli on the surface; the mucous particles (P); and the microplicae on the surface of the squamous epithelial cells (mp)
2,400 x

Plate 39





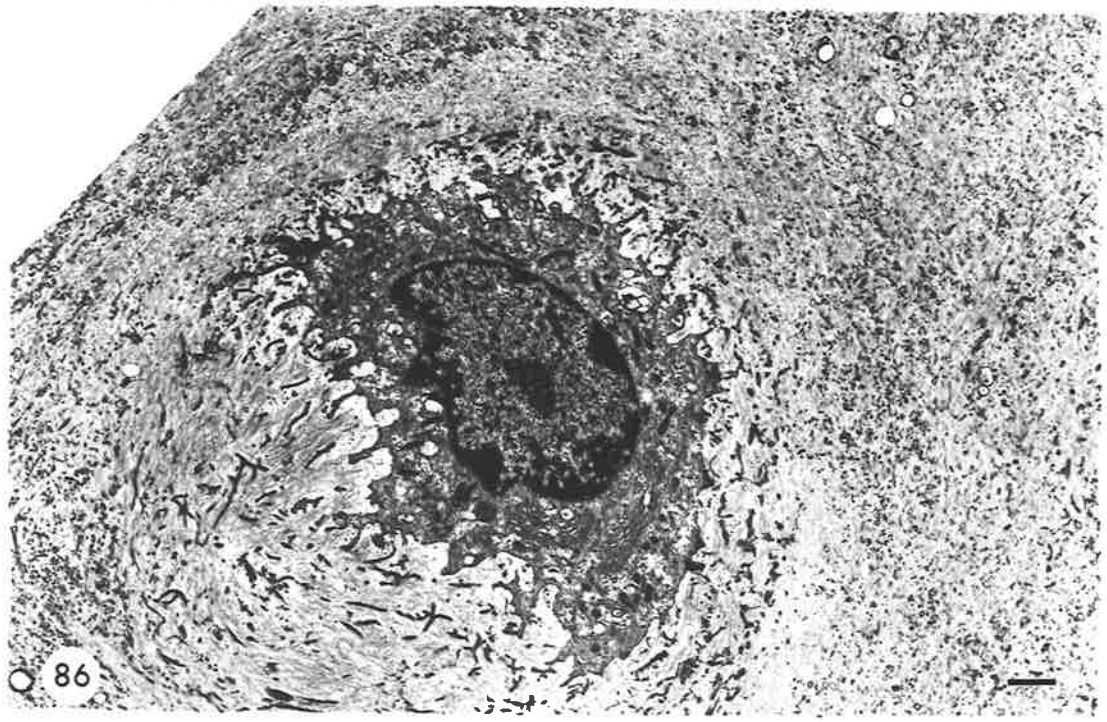


Fig. 85. AD.5,Respiratory bronchus between the valves.Note:the thin lamina propria over the cartilage;the cuboidal cell(C) covered by squamous epithelium;and the capillaries and pericytes(peri). 6,600 × bar = 1μm.

Fig. 86. AD.2,Chondrocyte from the wall of a secondary bronchus. 6,600 × bar = 1μm.

5-7 The respiratory zone.

5-7 A Alveoli.

The alveoli arise from the alveolar ducts, sacs and directly from the sphincteric segment. When alveoli arise from the sphincteric segment, their openings are guarded by myo-elastic valves (Fig. 67, 68 and 69). The size and shape of the alveoli depend on the method of fixation and the area sampled. In the inflated lung specimens the most common shape seen is the brandy balloon in SEM which appear polygonal in LM and TEM. The alveoli in these specimens range in size from 0.1 to 0.25 mm (Fig. 66, 67, 68 and 87). The alveolar septum consists of a connective tissue core 3 to 10 µm thick with a network of capillaries on each surface, covered by an epithelium composed of two types of cells, type I and type II (Fig. 90 and 91).

5-7 A-1 Epithelium.

(a) The type I cells, or squamous cells have their nuclei and most of the cytosplasmic organelles situated in the gaps in the capillary network and thin lateral cytoplasmic processes stretched out over the surface of the capillaries (Fig. 90, 91, 92 and **93**). The organelles consist of: a few mitochondria up to 1 µm in diameter; some profiles of rough and smooth endoplasmic reticulum; free ribosomes; and a Golgi complex. In most areas the epithelial processes are 50 to 100 nm thick and contain many pinocytotic vesicles 5 to 25 nm in diameter, but in some areas the processes are only 30 to 40 nm thick and have only an occasional pinocytot vesicle between the cell membranes (Fig. 93). The type I

cells do not cross the connective tissue core and only extend into adjacent alveoli around the ends of the septum.

(b) Type II cells.

Type II cells are cuboidal cells 10 to 20 μm in size which are found in groups of two or three in the inter-capillary niches, or the junctional regions of the septum (Fig. 87, 91, 94, 95 and 96). The cells contain: characteristic membrane-bound osmiophilic multilamellar bodies, or cytosomes up to 2 μm in diameter; multivesicular bodies 20 to 40 nm in size; many small vesicles 20 to 40 nm in size; profiles of rough and smooth endoplasmic reticulum; free ribosomes; and a prominent Golgi complex (Fig. 97, 98 and 99). The free surface has microvilli 0.1 x 0.4 μm, while the lateral surfaces have prominent infoldings and interdigitating processes. Occasional unmyelinated nerve fibres are found in relation to the basal surfaces of the cells, but no nerve endings have been found.

The type I and type II cells are joined by tight junctions. They rest on a basement membrane 10 to 20 nm thick, which fuses with the capillary basement membrane in the thinnest regions of the blood-air barrier. Type III cells have not ye been found.

5-7 A-2 Capillaries.

The capillary networks on the surface of the alveolar septum appear to be two separate plexuses arranged in many areas so that the gaps on one surface correspond to vessels on the

complimentary surface (Fig. 90 and 91). In the SEM quite large areas of the septum can be seen to be devoid of capillaries (Fig. 87) and in the gaps between the vessels type II cells and the occasional elevation caused by Type I cell nuclei can be found. In TEM these areas of the septum are covered by the lateral processes of the type I cells (Fig. 91). The capillary networks on the alveolar septum are supplied from branches of the pulmonary arteries and drain to tributaries of the pulmonary vein which run in the connective tissue framework of lung. The two networks are usually separate, but occasional trans-septal anastomotic channels are found (Fig. 101). The capillaries are usual non-fenestrated mammalian vessels with few cytoplasmic organelles, mitochondria, Golgi complex and a few profiles of endoplasmic reticulum confined to the perinuclea region. The thin lateral processes generally contain pinocytotic vesicles 20 to 40 nm in diameter, except in the thinnest portion of the blood-air barrier, when along with the type I cells they consist only of the cell membrane and a thin layer of cytoplasm (Fig. 93). The capillaries are surrounded by a basement membrane which sometimes contains a pericyte.

5-7 A-3 Blood-air barrier.

The blood-air barrier is 150 to 250 nm thick in most areas, decreasing to only 120 nm in the thinnest regions. It has not yet been possible to quantitate the diffusion capacity of the dolphin lung.

5-7 A-4 Interstitial tissue.

The interstitial tissue of the septum consists of collagen and

elastic fibres which do not seem to have any preferred orientation. Thin cytoplasmic processes are frequently seen in the interstitial tissue. It is not always possible to identify the cell of origin of these processes. Occasionally the cell body of a fibrobalst is seen and many of these processes almost certainly belong to fibroblasts; however, some have basement membrane surrounding them and are probably nerve fibres. Others may be processes from lymphatic vessels which are found in the junctional region of the septum (Fig. 105).

5-7 B Lymphatics.

The lymphatics drain from the septum to the junctional regions of the septa and thence to the peribronchial vessels. It has been easier to trace these vessels in reverse order, commencing with the peri-bronchial vessels and progressing peripherally. Some of the subpleural alveoli have lymphatics which drain towards the pleural lymph vessels. The lymphatics have a typical mammalian structure. The thin endothelium has many intraluminal processes and infoldings on its inner surface (Fig. 103 and 104). There is no basement membrane surrounding the vessels, which makes them rather difficult to trace for any distance in the septum. In the junctional regions of the septum mast cells, plasma cells, blood vessels, and unmyelinated nerves are found. No nerve endings have been found, but a detailed examination has not yet been undertaken.

5-7 C Macrophages.

Macrophages are occasionally seen on the surface of the alveolar septum, and in the bronchial tree, and also in the interstitial

tissues (Fig. 81, 87 and 111). There are no unusual features in these cells.

5-7 D Lining layer.

The surface lining layer has not been well preserved by the techniques used for most of this study. In some of the sub-pleural alveoli there are collections of flocculent and lamellated osmiophilic material which resembles surfactant (Fig. 96). The biopsy specimens which were collected and fixed using a variety of techniques, including the tricomplex salt method (Dermer 1969) unfortunately show many fixation artefacts and have not therefore been included at this time. They do however confirm the presence of the usual mammalian type of surface lining layer.

5-7 E Blood supply.

A detailed analysis of the blood supply has not been performed as yet, but there are some features which have been observed which are worthy of note at this time. The pulmonary arteries and veins run together from the hilum of the lung, but they do not run with the bronchi. These vessels are enclosed in a separate connective tissue sheath away from the bronchi. At the junction of the secondary and tertiary bronchi the pulmonary artery gives off branches which then run in the peribronchial sheath. Tributaries of the pulmonary vein take a similar course. The vessels supplying the alveolar septum appear to arise from the peribronchial vessels. Branches of the pulmonary artery and vein are seen in the junctional regions of the alveolar septum (Fig. 106). The vessels so far examined have a typical

mammalian pattern.

5-8 The pleura.

The visceral pleura is 100 to 200 μm thick (Fig 107). It consists of fibro-elastic tissue containing blood vessels, lymphatics and nerves (Fig. 107 and 108). The mesothelium is composed of typical squamous cells with thin cytoplasmic processes containing few organelles. In the region of the nucleus some short microvilli 0.1 x 0.1 μm are often found embedded in a granular or amorphous surface coat (Fig. 109). The cells are joined by tight junctions (Fig. 110). Occasional cell containing myo-filaments are also seen beneath the mesothelial cells (Fig. 109 and 110).

5-9 Additional Findings.

This section contains three groups of observations of appearances which may be pathological in origin but which require further investigation. They are included in the main body of the thesis rather than in a separate appendix because some of them may be normal structures.

(a) In all of the animals so far collected, both of the Adelaide and Brisbane series, spherical or ovoid structures up to 25 μm have been found in bronchi less than 1 mm in diameter (Fig. 65, 111, 112 and 113). They are PAS positive and take up Alcian blue with the Lison's stain. They are found beneath the basement membrane and are often surrounded by mast cells. In the TEM they vary greatly both in staining and in structure. In some sections definite substructure is present while in others they appear as masses of calcified debris.

TEM confirms that these bodies are in the lamina propria, with the epithelium often stretched over them. In some areas macrophages, fibroblasts, mast cells and occasionally an increase in connective tissue are found. Mostly however there is little reaction to their presence. In the SEM they are clearly seen as elevations beneath the epithelium (Fig. 111).

(b) the second group are found within macrophages either within the blood vessels or on the surface of the bronchi or alveoli. These bodies appear to be the remnants of phagocytosed cells or parasites (Fig. 114).

(c) the third group is a collection of smaller bodies most prominent in the tracheal epithelium. They are found principally in the inter-cellular space and consist of osmiophilic material arranged in flattened concentric whorls (Fig. 9).

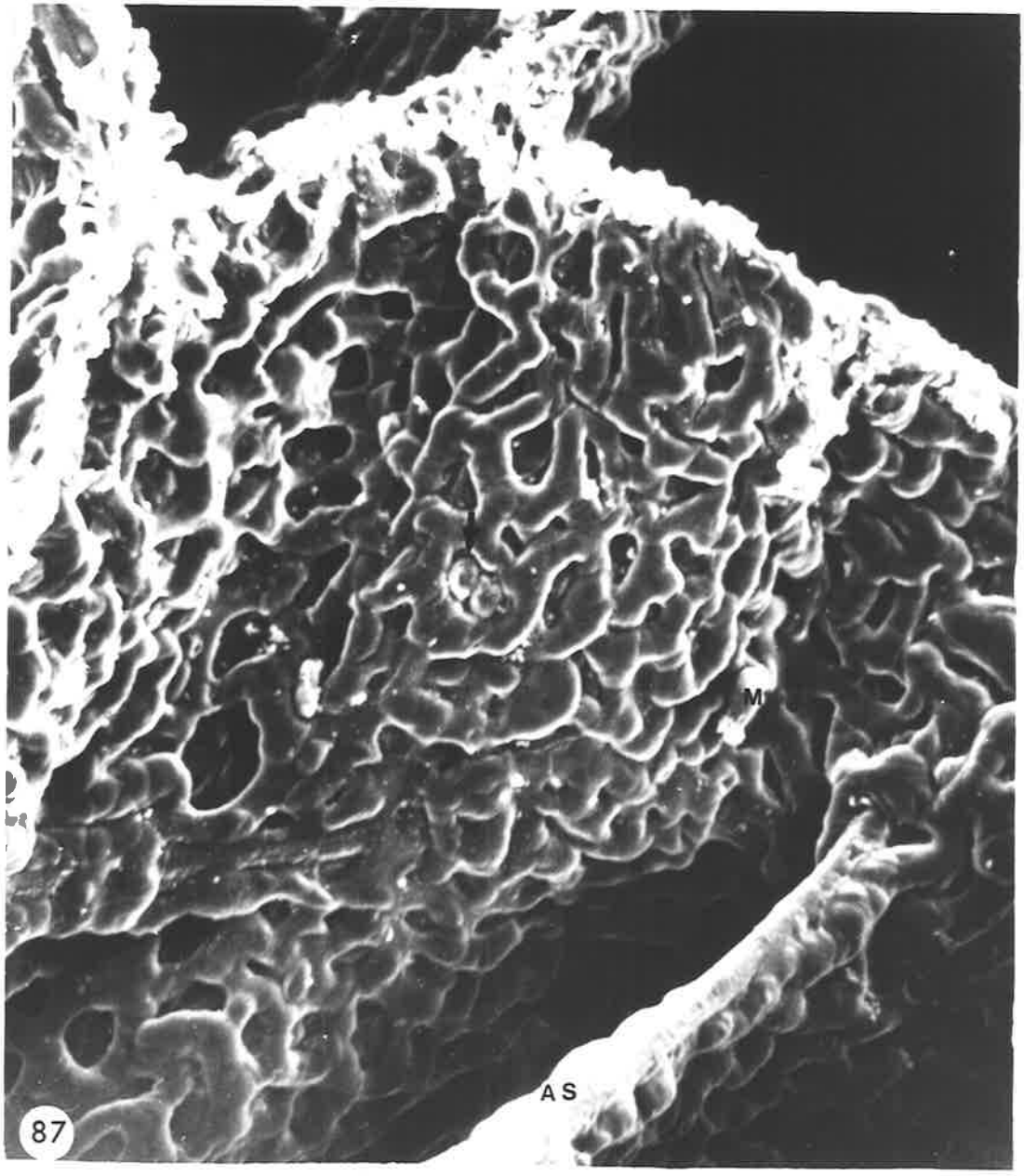


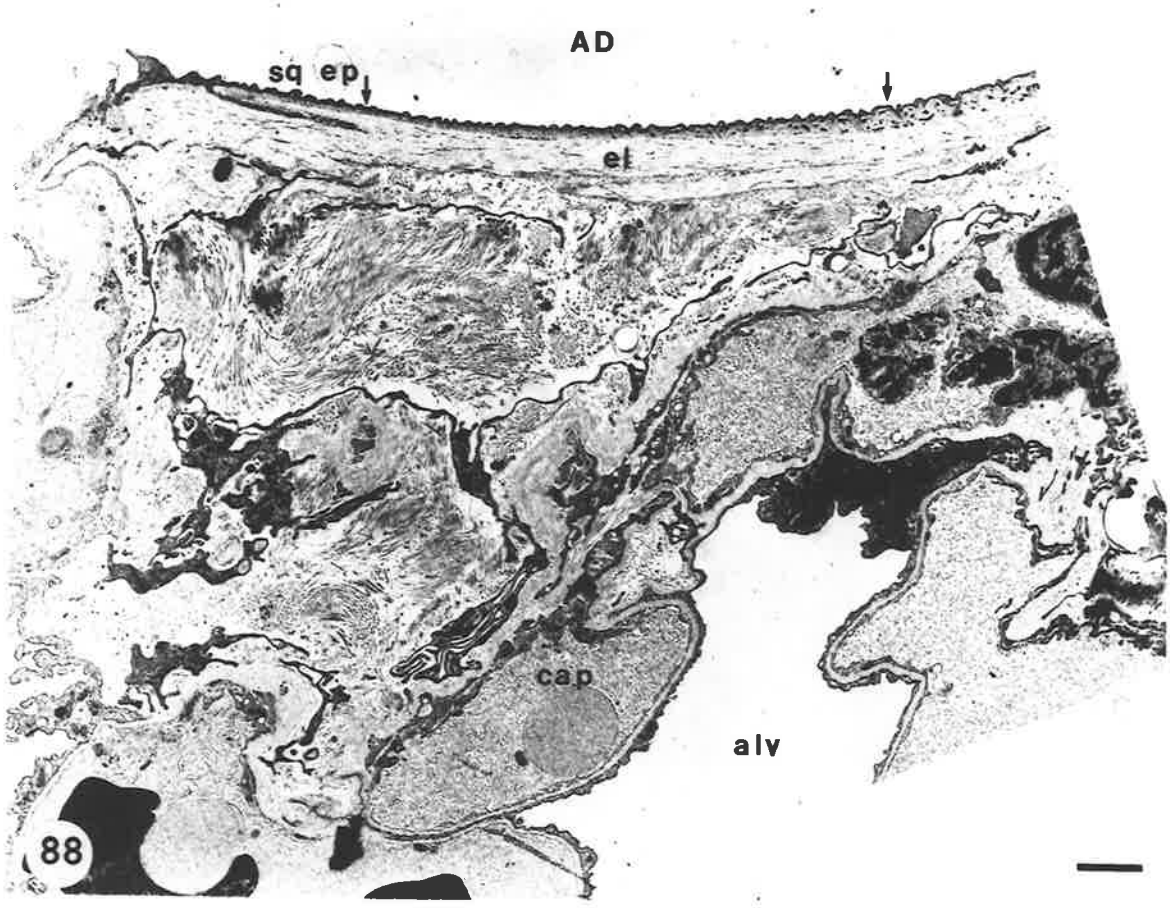
Fig. 87. AD.2, Alveoli. Note:the end of an alveolar septum at its entrance into an alveolar duct,devoid of capillaries(AS); the arrangement of the capillaries;type 11 cells in groups(↑) in the gaps in the capillary plexus;and the alveolar macrophage(M). 1,000 ×

Plate 42.

Fig. 88. AD, 5. Lung (immersion fixed) showing the wall of an alveolar duct in longitudinal section.
Note: the folding of the capillaries (see also Fig. 100); the squamous epithelium on the surface; the elastic fibres; and the bundles of collagen which do not appear to be arranged in any special direction. Compare with Figs. 87 and 89.
3,600 x bar = 2 μm.

Fig. 89. AD, 5. Section of alveolar septum from a perfused lung.
Note: the elastic fibres and possibly one smooth muscle cell in the terminal portion at the entrance into the alveolar sac; the type II cell; and the capillaries. 3,000 x bar = 2 μm.

Plate 42



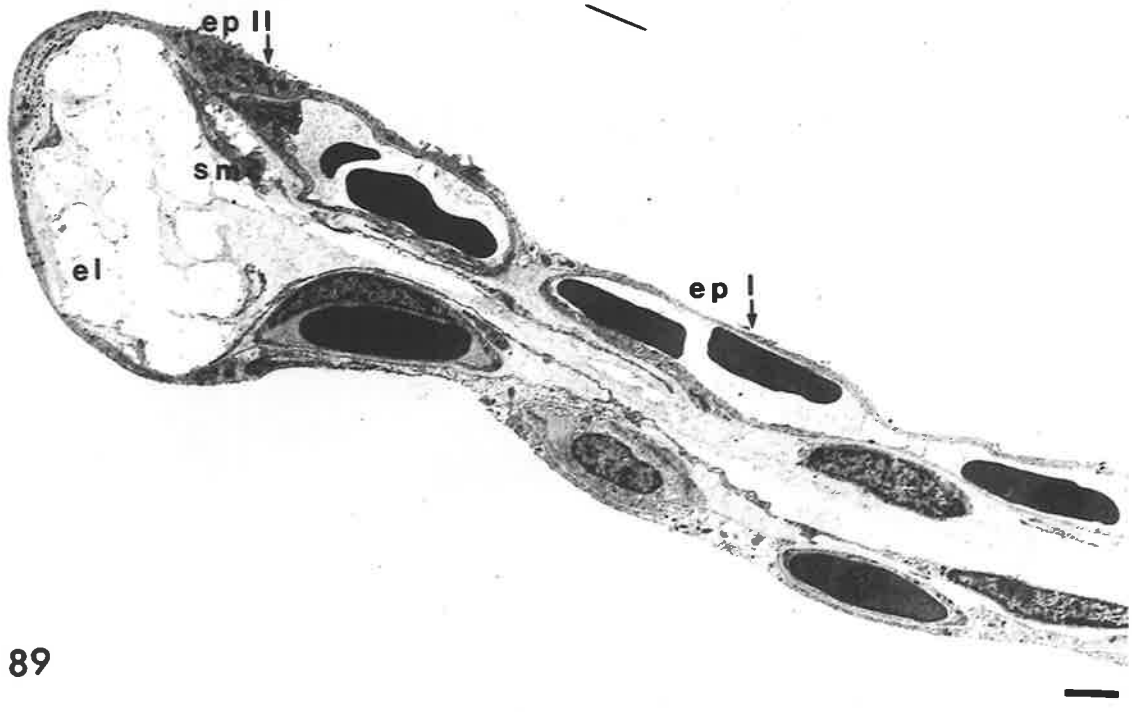


Plate 43

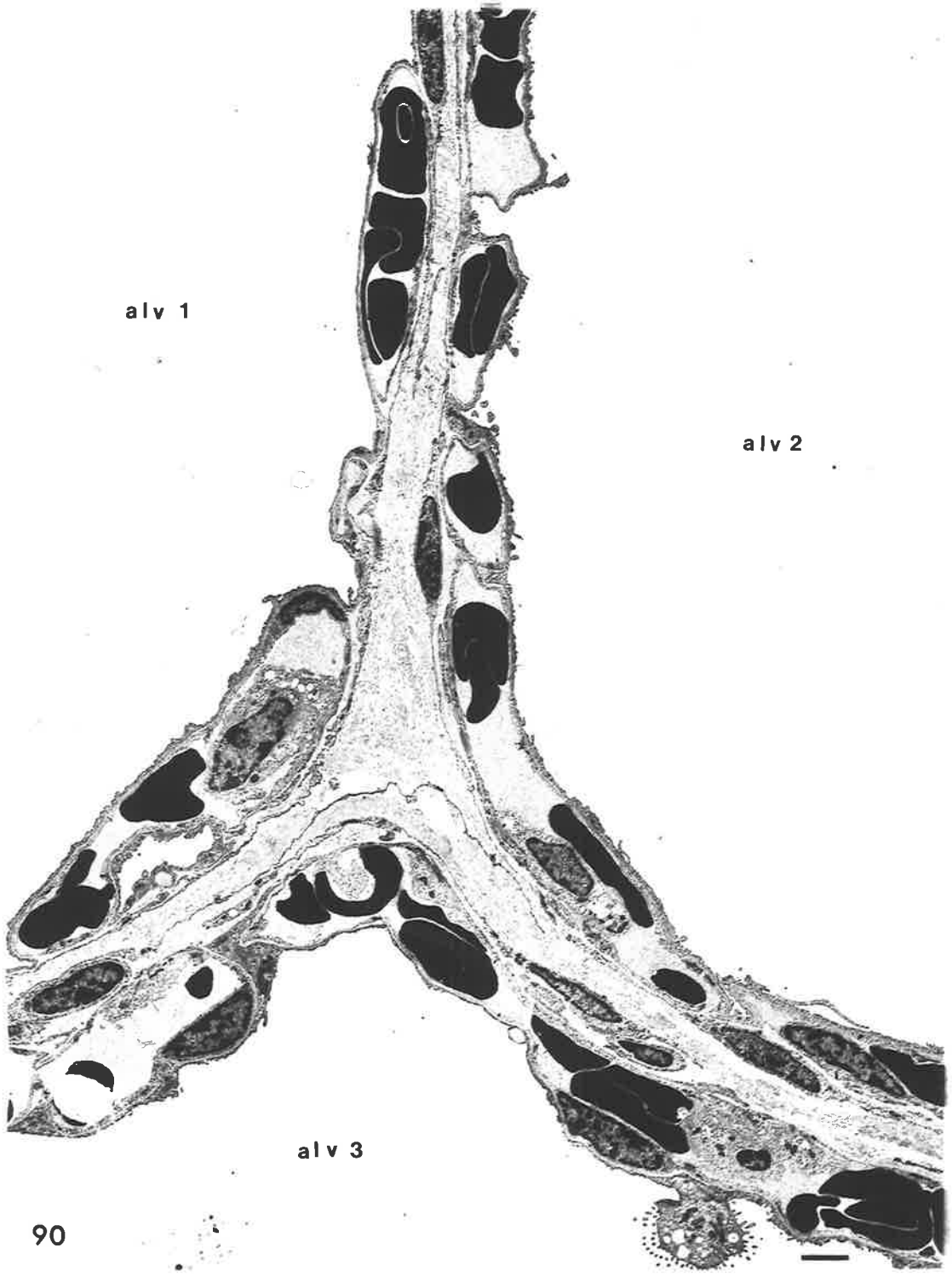


Fig. 90. AD.5,Junctional region of the alveolar septa from three alveoli.Note:there are no lymphatics or blood vessels in this junction;one type 11 cell is present,but no type 1 nuclei can be seen;the arrangement of the capillary networks. 3,800 × bar = 2μm

Plate 44

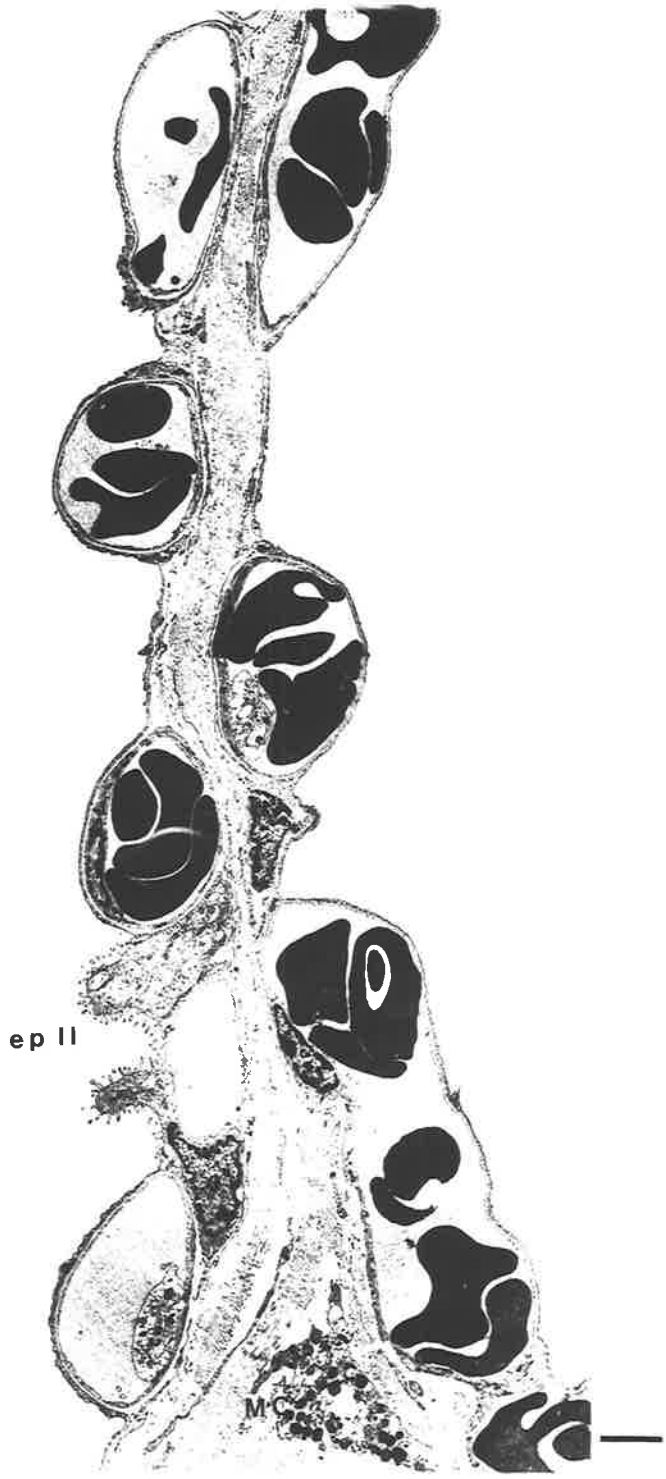


Fig. 91. AD.5,Alveolar septum. Note:the gaps in the capillary network on one surface correspond to capillaries on the other;two type 11 cells;no type 1 cell nuclei;a mast cell(MC);and the absence of elastic fibres from the connective tissue core. 4,000 × bar = 2μm

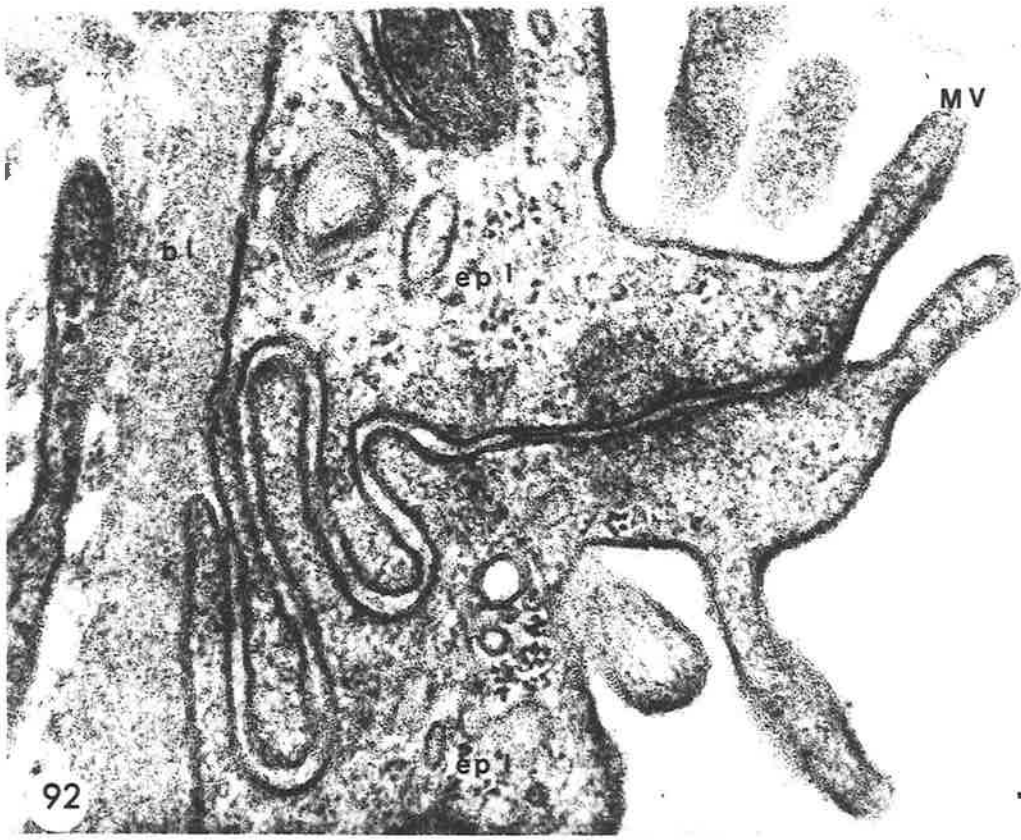
Plate 45.

Fig. 92. AD, 5. Junction of two type I epithelial cells on the surface of the alveolar septum. This is a typical tight junction. A few finger like projections are seen around the junction. 84,000 x bar = 100 nm.

Fig. 93. AD, 5. High magnification of the thinnest part of the blood-air barrier.
Note: the type I epithelial cell consists of a thin layer of cytoplasm and the outer and inner unit membranes; a pinocytotic vesicle (v) is present; the endothelial cell is even thinner; the two basal laminae have fused. 210,000 x bar = 50 nm.

See Fig. 94, 95, 96, 100, 101 and 102 for more details of type I epithelial cells.

Plate 45



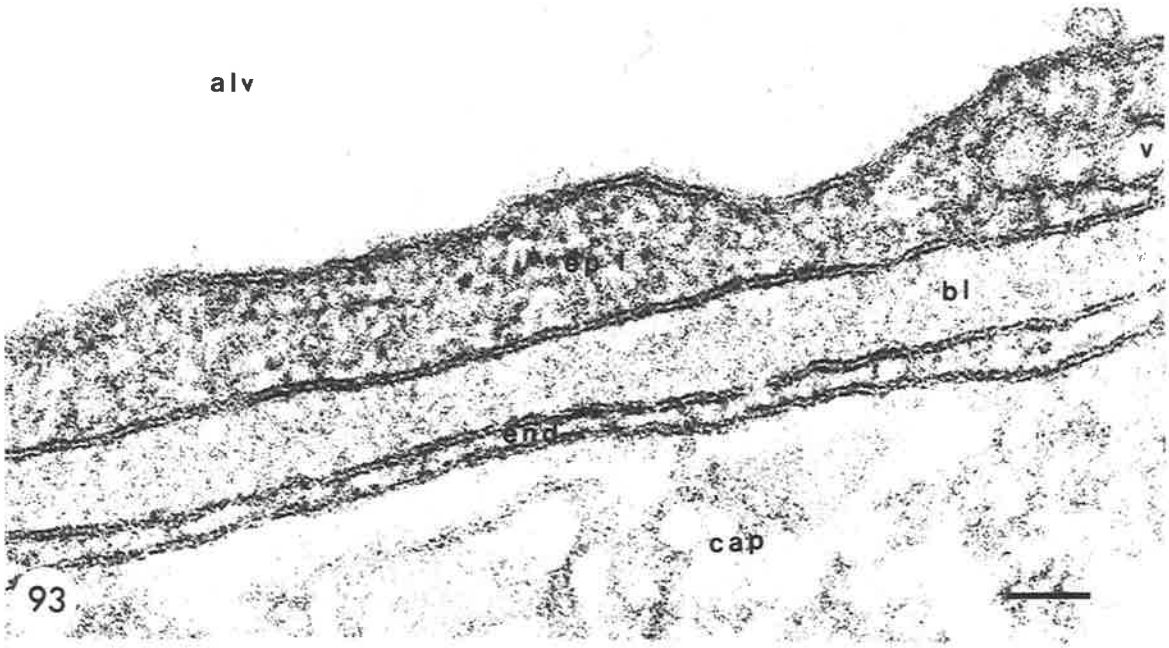
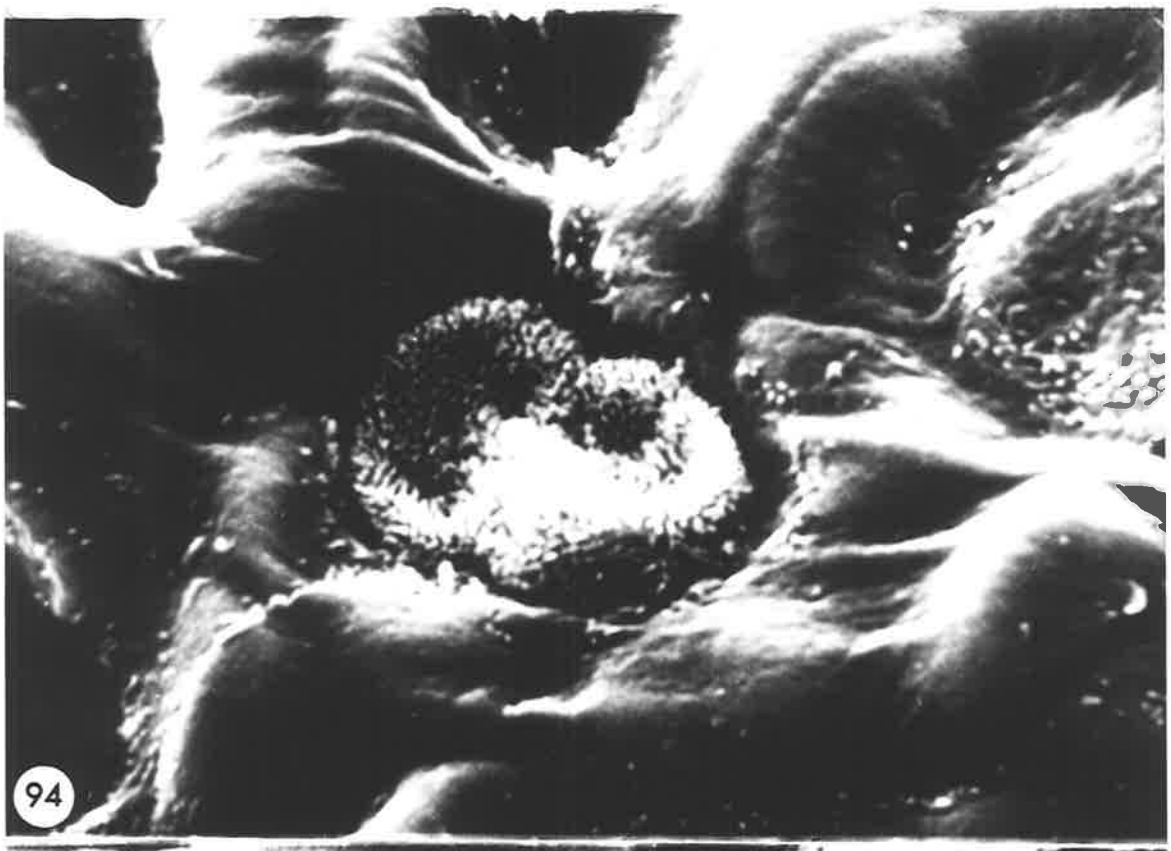


Plate 46.

Fig. 94. AD, 2. Higher magnification of the two type II cells in the centre of Fig. 87. 4,000 x

Fig. 95. AD, 2. Type II epithelial cells.
Note: these two cells are each separate and in niches between the capillaries; the microvilli on their surface; a junctional region between type I cells (↓); and the surface of the type I cells. 10,400 x

Plate 46



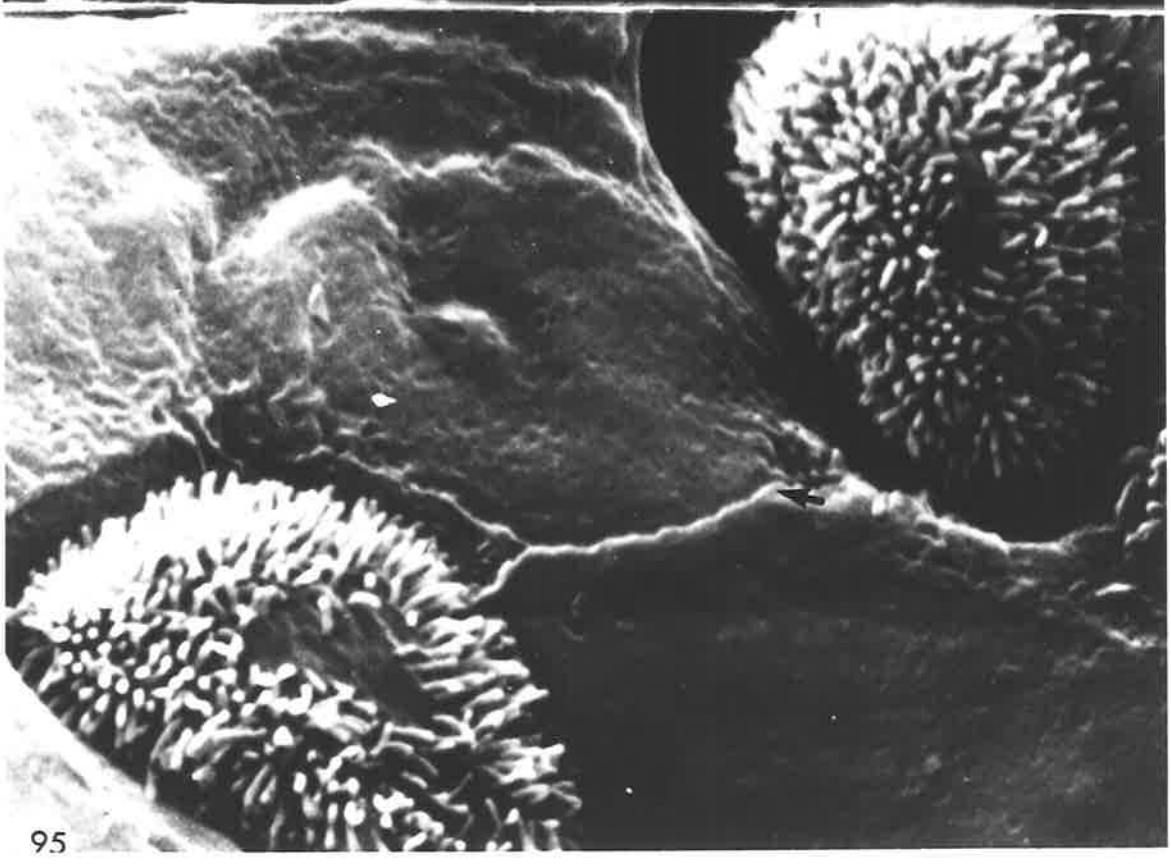
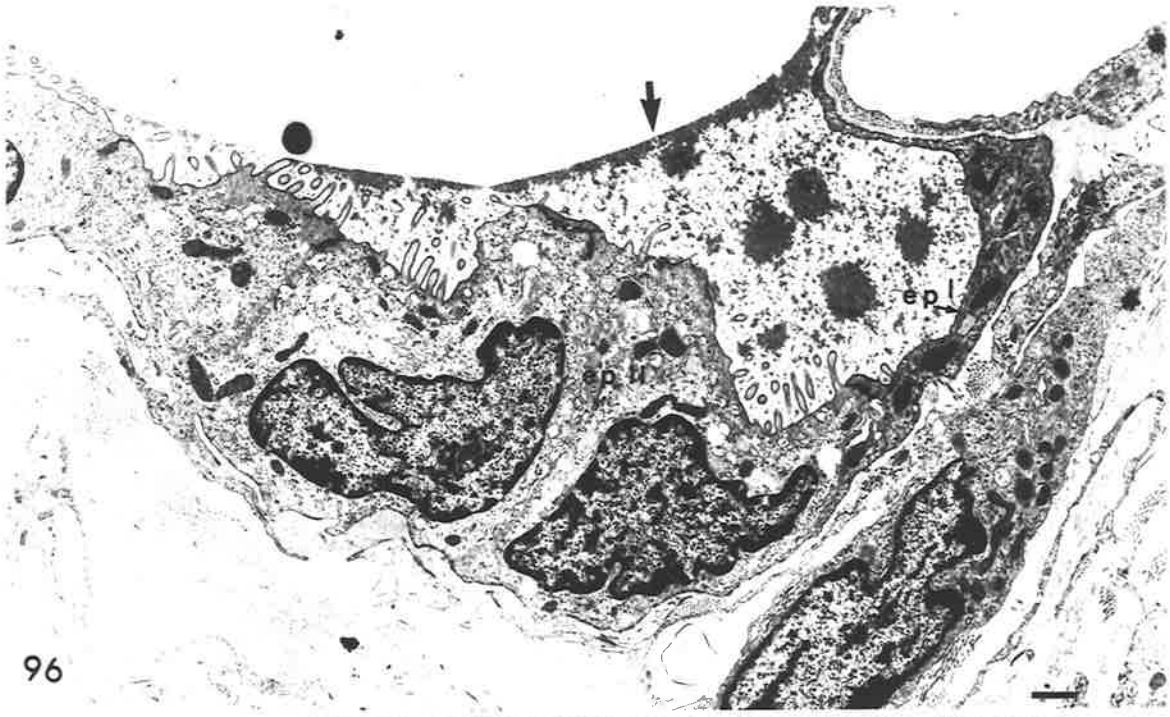


Plate 47.

Fig. 96. AD, 5. Subpleural region of the lung showing an alveolus adjacent to a respiratory bronchus.
Note: flocculent material which may be surfactant, has been retained and fills the niche (↓); the type I cell cytoplasm contains some mitochondria and profiles of endoplasmic reticulum; several type II cells are present. 5,400 x bar = 1 μm.

Fig. 97. AD, 2. A type II epithelial cell.
Note: the cytosomes (cyt); the microvilli; mitochondria; and the vesicles and free ribosomes. 20,800 x bar= 0.5 μm.

Plate 47



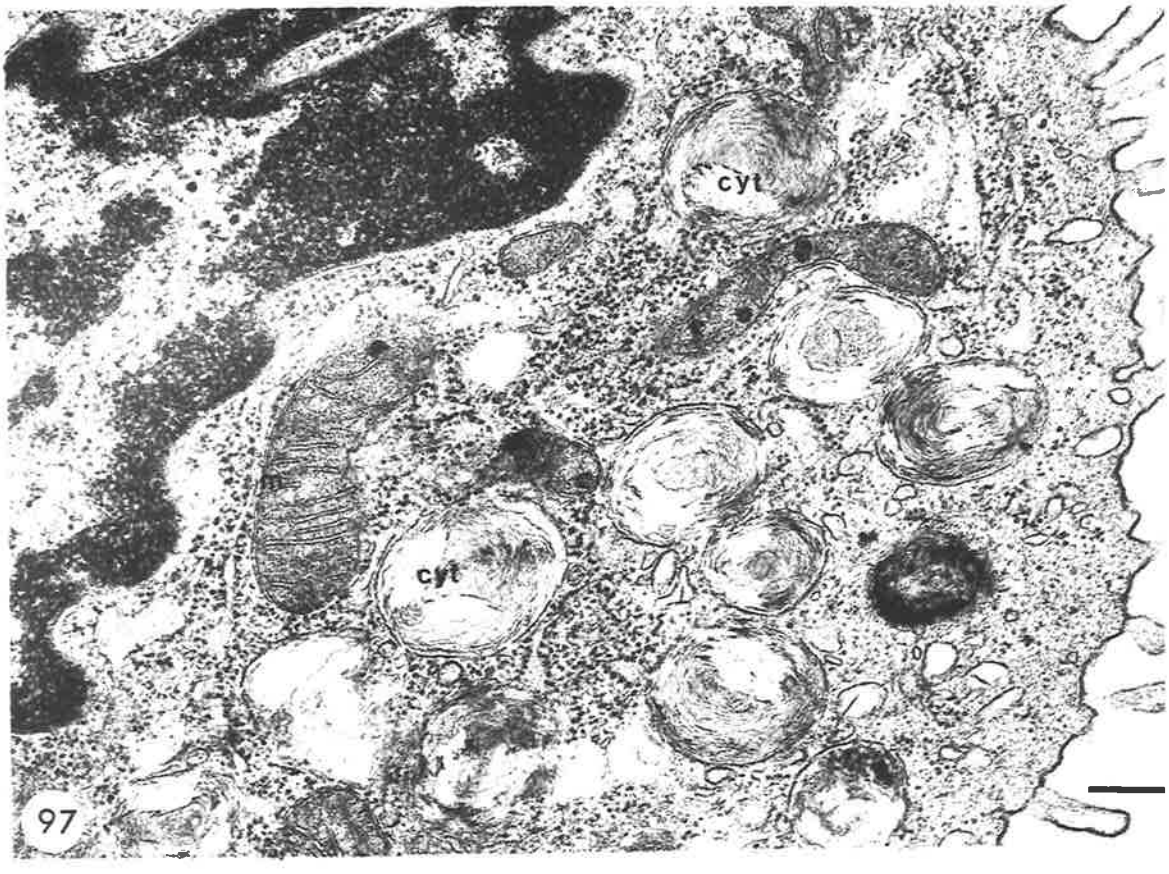
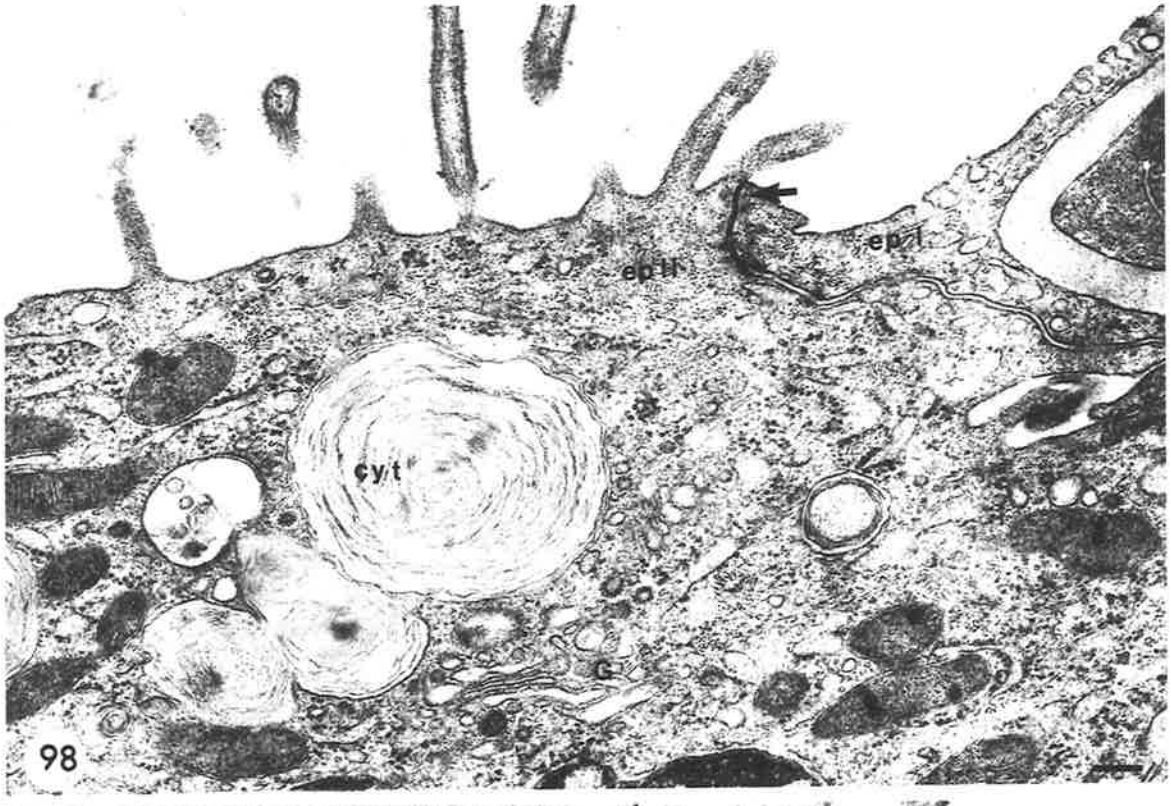


Plate 48.

Fig. 98. AD, 5. Type II epithelial cell.
Note: the tight junction (↓) formed with the adjoining type I cell; the Golgi; the cytosomes which appear to fusing to form one large one; and the microvilli.
30,000 x bar = 0.2 µm.

Fig. 99. D, 12. Brisbane series. Type II epithelial cell with a cytosome discharging its contents onto the surface. 32,000 x bar = 0.2 µm.

Plate 48



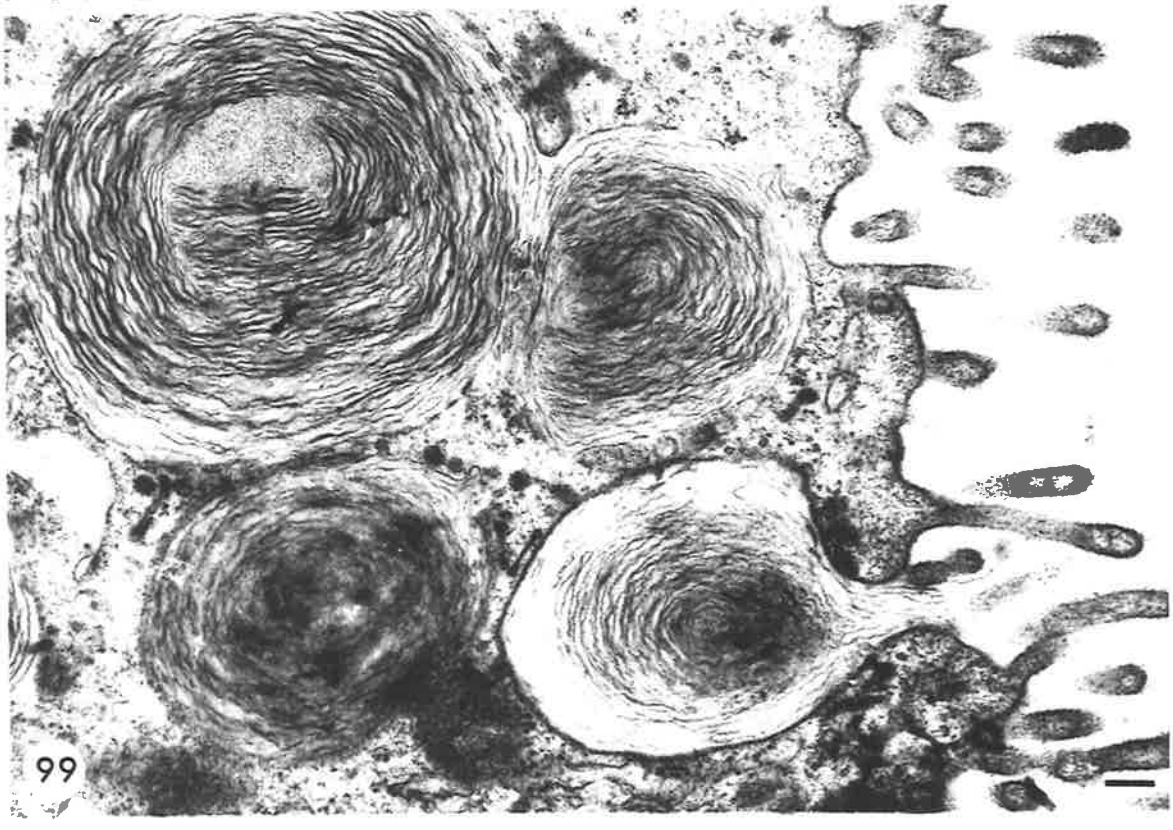
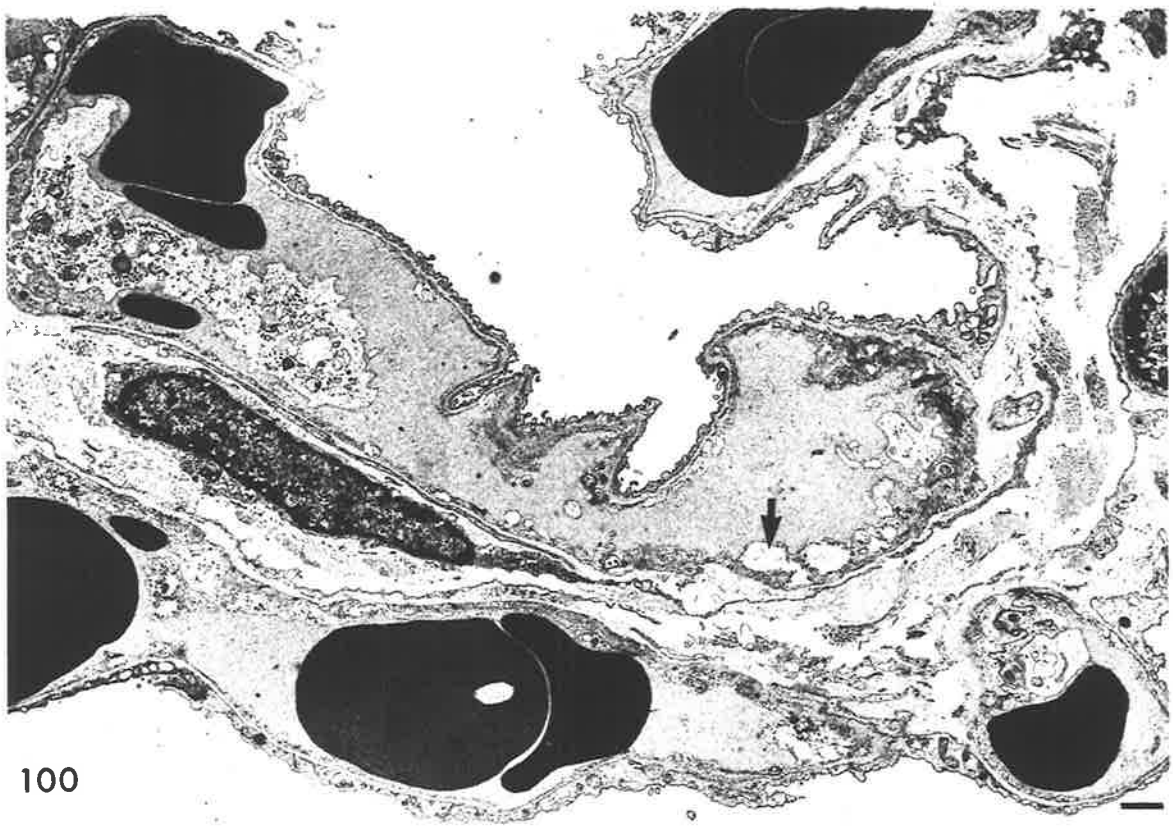


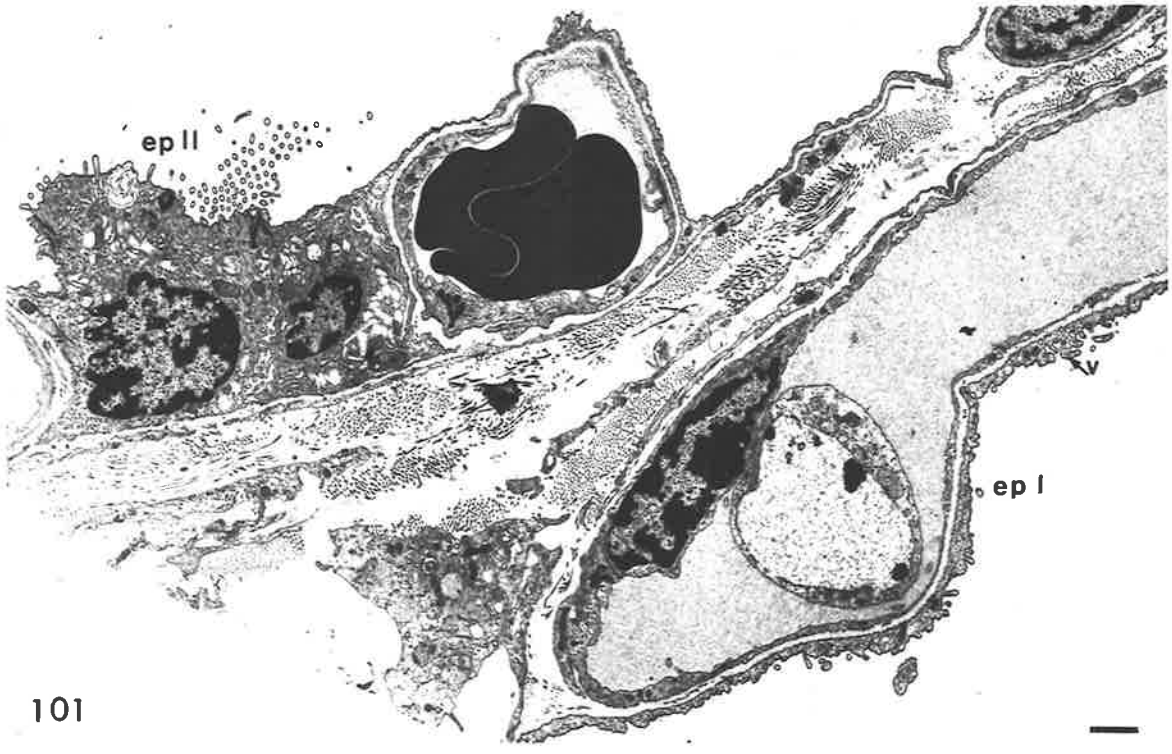
Plate 49.

Fig. 100. AD, 2. Lung (immersion fixed).
Note: there is some vacuolation (↓) of the endothelial cells, particularly on the septal surface indicating poor fixation by this method; although there is folding of the septum and capillaries, the blood-air barrier is not greatly thickened. 52,000 x bar = 1 μm.

Fig. 101. AD, 2. Lung (perfusion fixed).
Note: the two type II cells with extensive plication of their lateral and basal membranes; many cell processes are seen in the septum; and many pinocytotic vesicles are seen in one of the type I cells. 6,500 x bar = 1 μm.



100



101

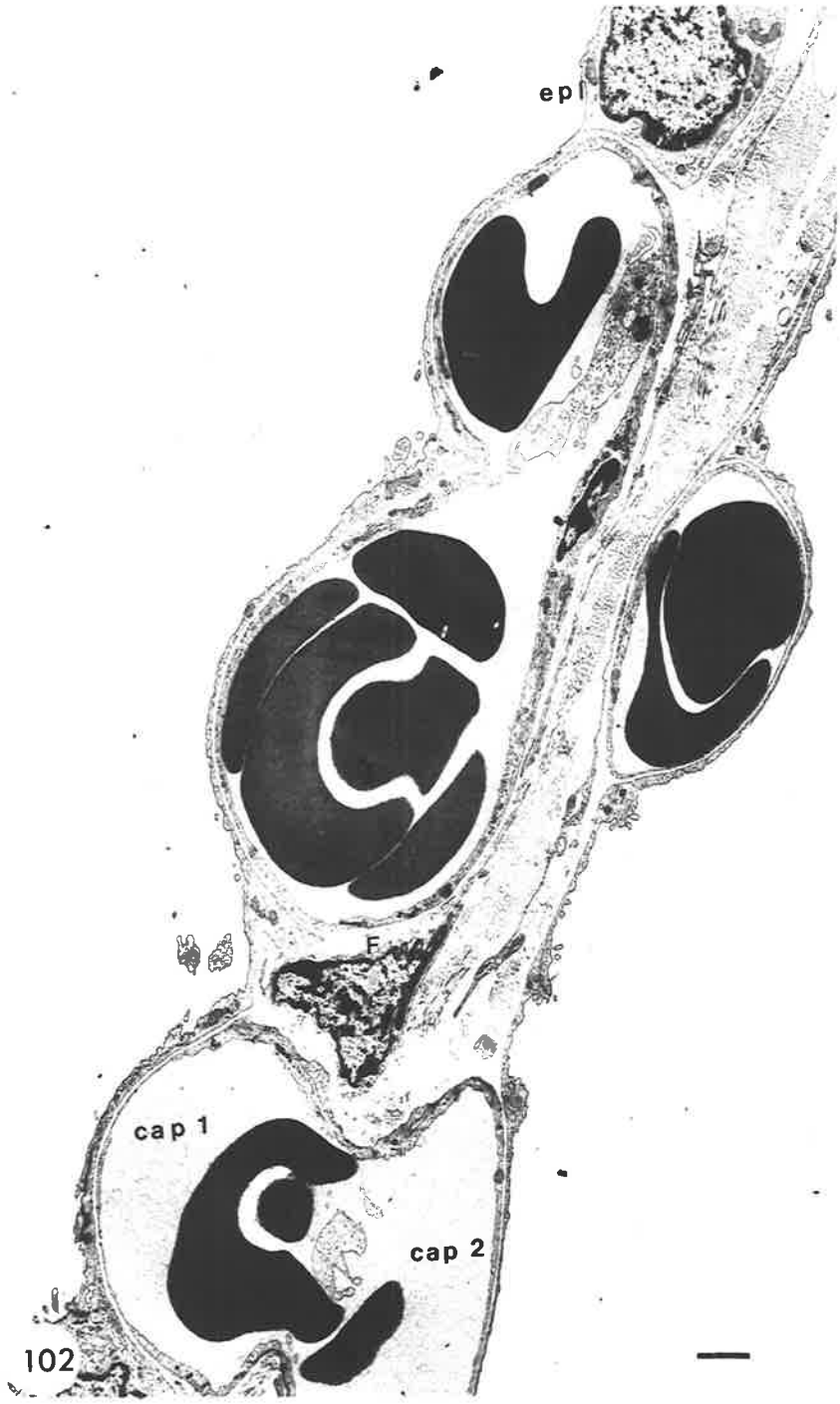


Fig. 102. AD.2,Alveolar septum showing a trans-septal anastomosis between the two capillary networks.A nucleus of a type 1 cell is present at the top of the picture and a fibroblast(F) towards the bottom. 6,800 × bar = 1μm.

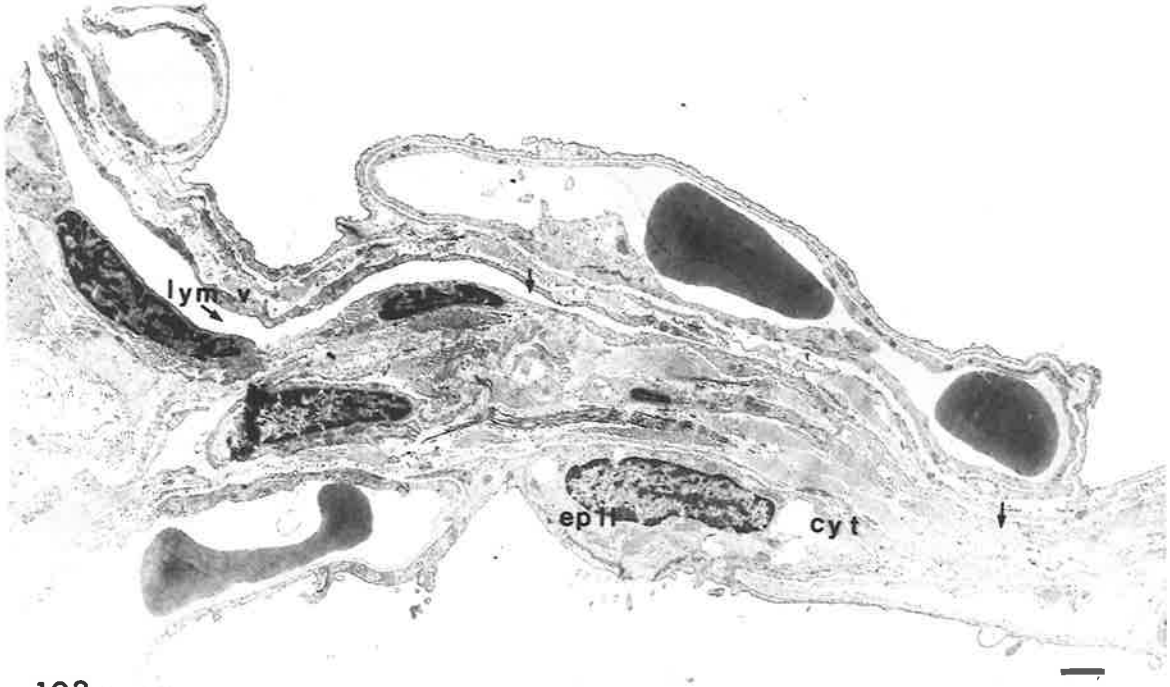
Plate 51.

The specimens from which Figs. 103 and 104 were obtained were taken from material being prepared for SEM. They were embedded in Spurr's resin after an overnight wash in cacodylate buffer (see chapter 4).

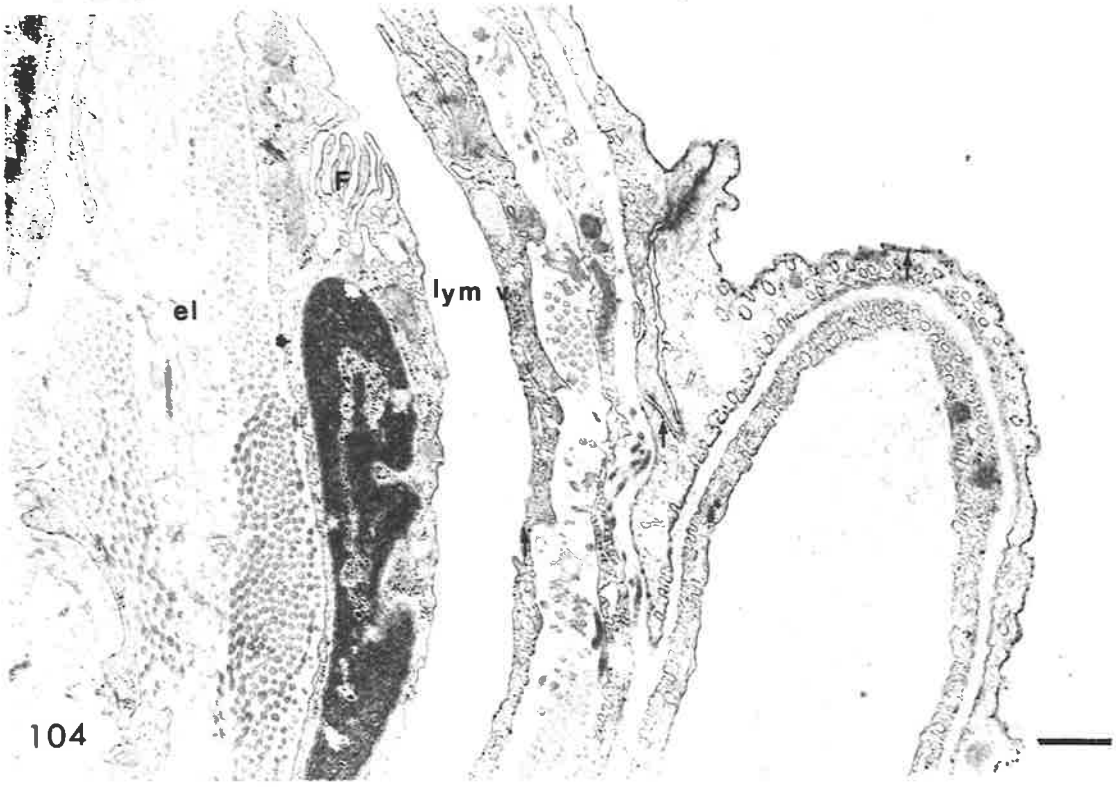
Fig. 103. AD, 5. Alveolar septum showing a lymphatic vessel (lym v) running from the peribronchial tissue on the left along the septum to the right. The only evidence of the prolonged period of storage is the extraction of the contents of the cytosomes (cyt) in the type II epithelial cell. 5,3000 x bar = 1 μm.

Fig. 104. AD, 5. Higher magnification of portion of the lymphatic in Fig. 103.
Note: the fine electron-dense granules (↓) in the type I cell and the endothelial cell; the endothelium of the lymphatic has many folds and finger-like projections (F); some fine elastic fibres are present in the peribronchial tissue.
20,800 x bar = 0.5 μm.

Plate 51



103



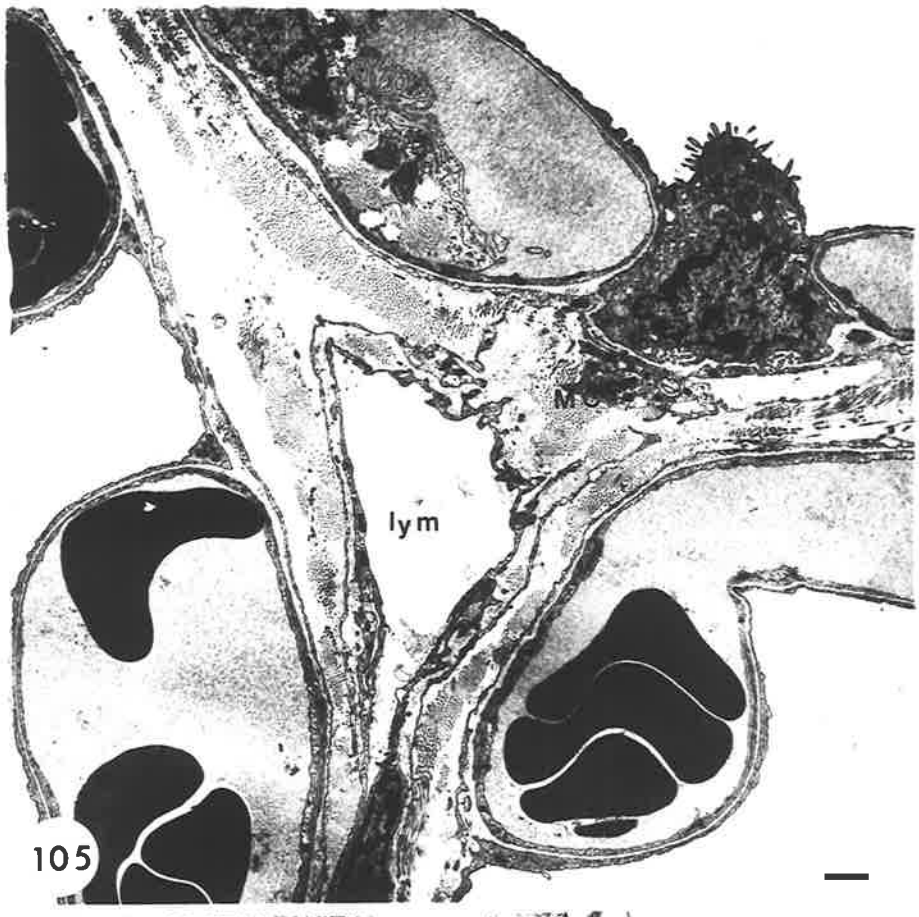
104

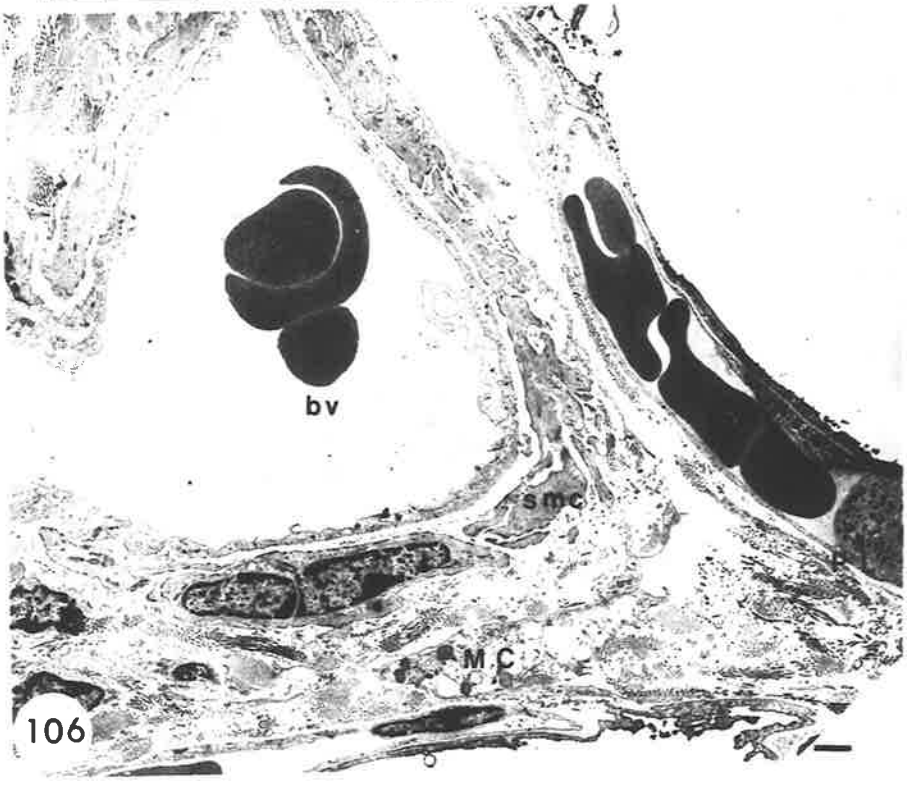
Plate 52.

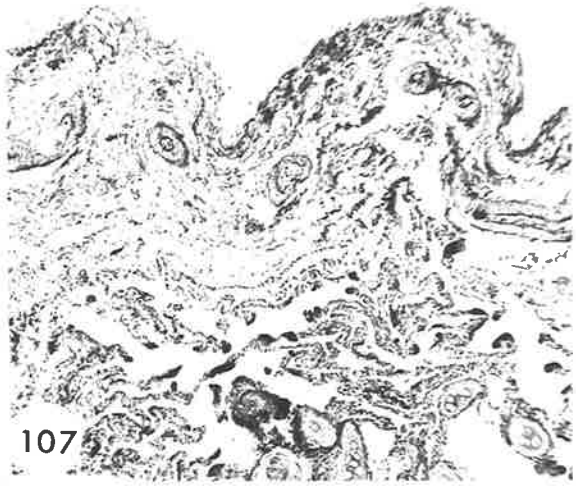
Fig. 105. AD, 2. Junctional region of alveolar septum.
Note: the lymphatic in the centre extending along the septum towards the bottom of the picture; portion of a mast cell (MC); a type II cell; and the capillaries. 5,300 x bar = 1 μm.

Fig. 106. AD, 5. Junctional region from a specimen prepared as for Figs. 103 and 104.
Note: the large muscular vessel; portion of a mast cell, with some loss of granular content; and a polymorphonuclear leucocyte (PL) in a capillary. 4,400 x bar = 1 μm.

Plate 52







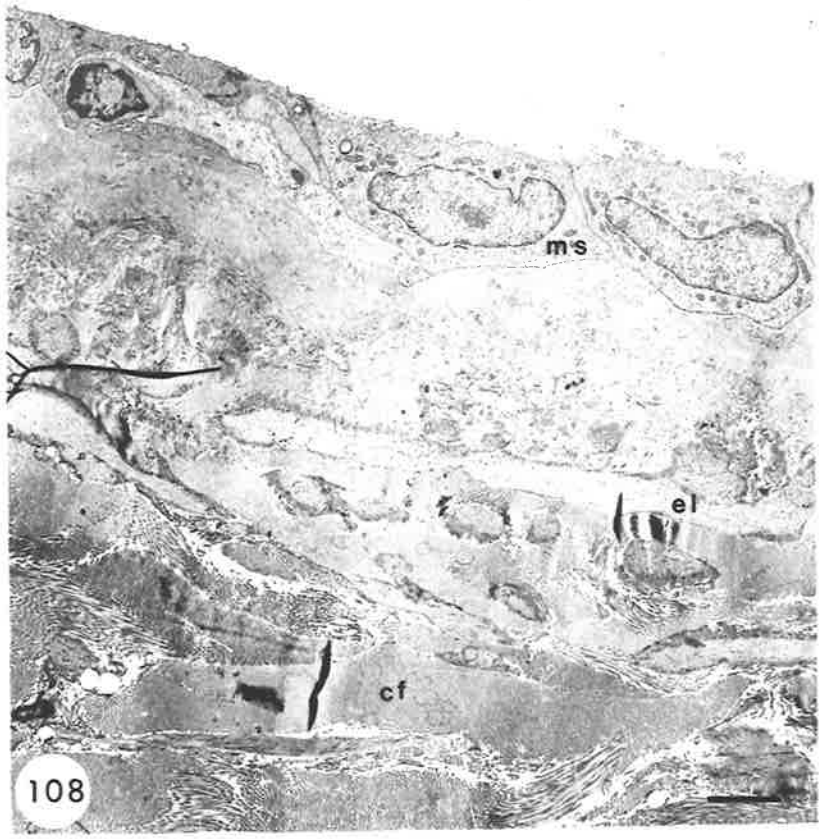


Fig.107. AD.1,Section of collapsed lung showing pleural surface. Note:the thick connective tissue layer with collagen and elastic fibres;the thick walled arteries;and the collapsed alveoli. V.and VG. 240 ×

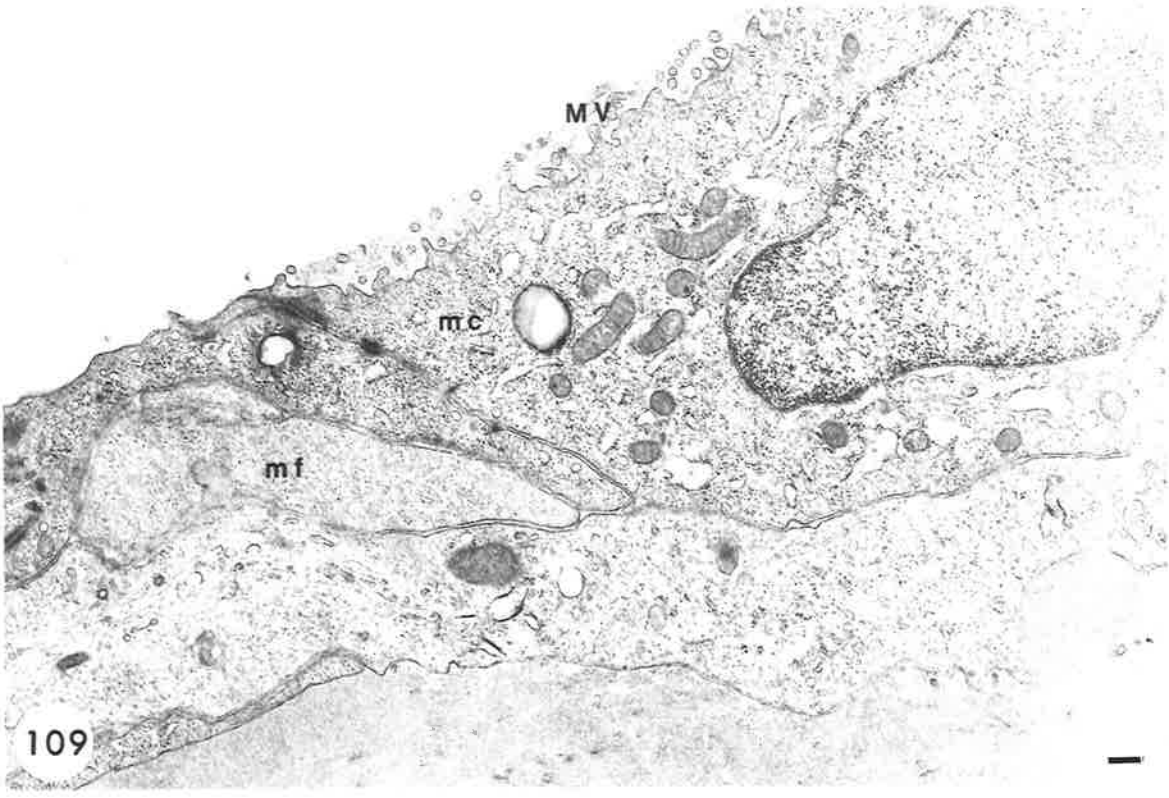
Fig.108. AD.5,Pleural surface of the lung.Note:the mesothelial cells (mc);the elastic fibres;and the thick bundles of collagen fibres(cf). 9,000 × bar = 1μm.

Plate 54.

Fig. 109. AD, 5. Pleura.
Note: there are two types of cell in some regions, the typical mesothelial cell (mc), and a cell containing many myofilaments (mf); the microvilli on the surface are embedded in a layer of granular material. 40,000 x bar = 100 nm.

Fig. 110. AD, 5. Pleura.
Note: the junctions (J) between the mesothelial cells; the absence of microvilli; and the cell containing the myofilaments. 84,000 x bar = 100 nm.

Plate 54



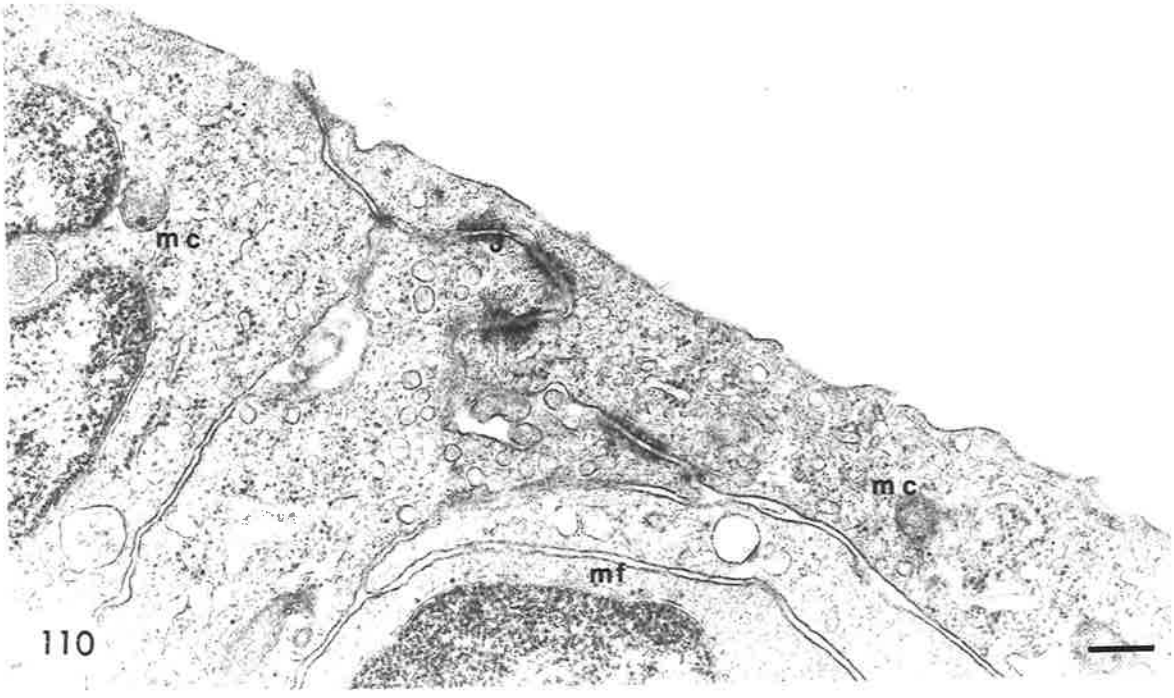


Plate 55.

Fig. 111. AD, 2. Junction of tertiary and respiratory bronchus.
Note: the large elevation caused by a parasite; the microvillous epithelium is intact despite several irregular projections; scattered ciliated cells; and some red cells. SEM 3,000 x

Fig. 112. AD, 5. TEM of a similar parasite nodule to that in Fig. 110.
Note: the calcified areas (Ca); the area which lookslike cuticle (c); the macrophage (M) on the surface of the airway; and the increase in connective tissue around the nodule. 4,400 x bar = 1 μm.

Plate 55

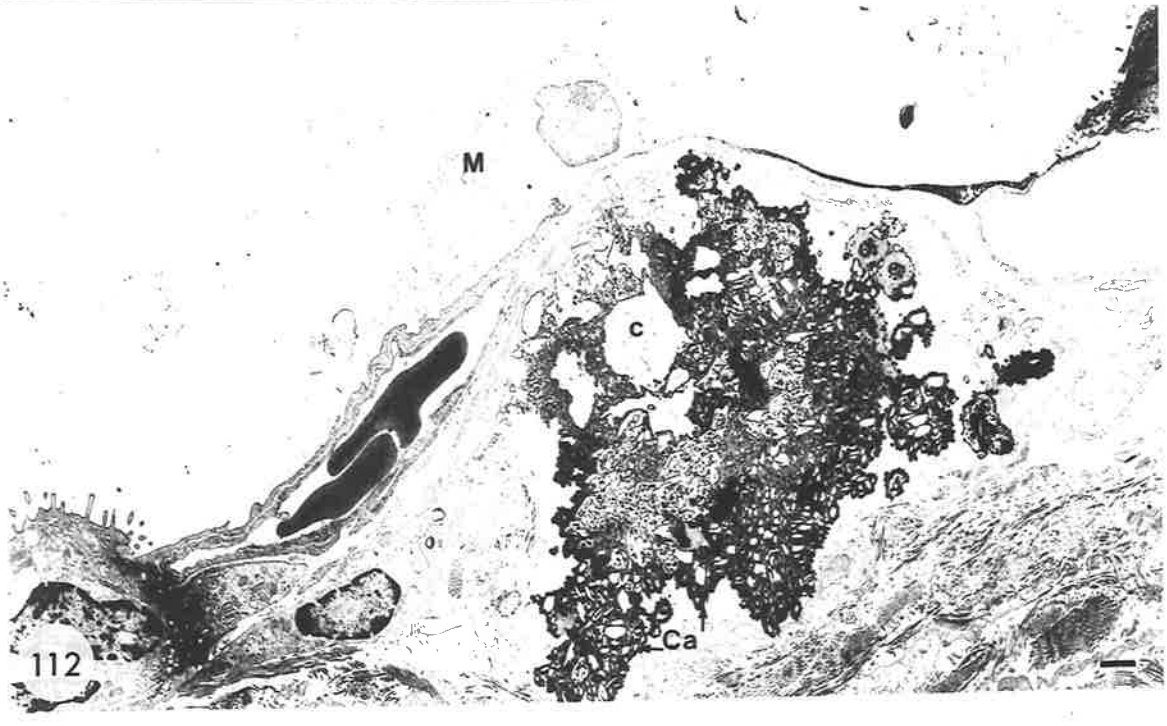
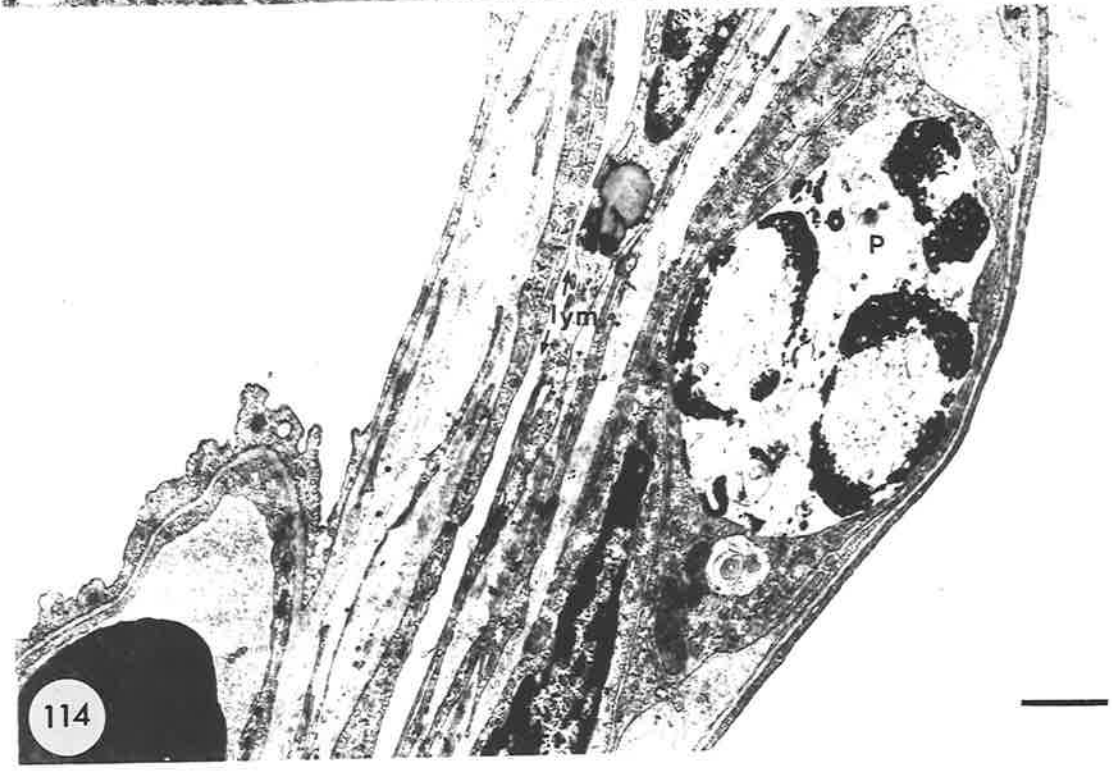


Plate 56.

Fig. 113. AD, 5. Parasite.
Note: the parasite appears to be surrounded by epithelial cells ( ); much of the area is occupied by cuticle-like material (c); and a mast cell (MC). 6,600 x bar = 1 μm.

Fig. 114. AD, 5. Section of alveolar septum.
Note: the cell in the capillary containing a large inclusion body, this may well be a parasite within a circulating macrophage; and lymphatic present in the centre of the septum.
11,200 x bar = 1 μm.

Plate 56



CHAPTER VI

DISCUSSION

DISCUSSION.

6-1 Techniques.

I will not discuss the techniques used in this investigation at great length. When I commenced in 1967, there was some debate about the suitability of some of the fixatives and their buffers, however most of these methods are now to be found in any of the many texts available (see Hayat 1970 and Glauert 1974 for example). One interesting feature of the techniques arose in the later phase of the work when experiments were being done with the old material and the new embedding technique. It was not possible, due to the cost, to avoid the use of phosphate buffers for the perfusions of the dolphins. I found that overnight washing of the samples in cacodylate buffer avoided the electron-dense granular deposits which had been reported by Gil and Weibel (1968), provided there was no phosphate in the osmium tetroxide solution. When samples were processed in the later phases, as controls for the SEM specimens, electron-dense deposits were again found, despite overnight washing in buffer. If, however, the washing was continued for 48 hours with several changes, the deposits were not found. This is interesting in the light of a recent report by O'Hara and Braunschweig (1975) who found electron-dense deposits after any primary fixation which contained phosphate buffer. Further experiments are obviously required to clarify this problem.

The modification of the Spurr embedding technique proposed in this investigation has proved particularly successful in our

laboratory and has been used to embed a wide range of tissues, including brain slices, aorta, nuchal ligament, cell and tissue pellets, kidney and liver (Fanning and Findlay, unpublished results).

The SEM results are not as good as I would like, but the difficulties in obtaining fresh material have so far prevented any further attempts. I hope that in the near future I will be able to obtain some more animals and repeat both the SEM and the histochemical studies of the surface lining layer of the lung.

6-2 Trachea.

6-2 A Functions of the upper airway performed in the trachea.

Several of the functions of the upper airway of terrestrial mammals must be performed in the trachea of dolphins because of the different arrangement of the blowhole, pharynx and larynx. These functions are: the air-conditioning of the inspired air, both the warming and humidification, and the filtration and cleansing; and the conservation of heat and water.

The most striking differences between the usual mammalian pattern of the respiratory tract and the dolphin are found in the region proximal to the bifurcation of the trachea. I have discussed the arrangement of the blowhole, nasal cavities and larynx in Chapter 3-1 and 3-4. The trachea and larynx join almost at right angles and in this junctional region Fanning and Harrison (1972 and appendix 2) have described a structure

very similar to the pharyngeal tonsil of terrestrial mammals. The trachea does not lie in the horizontal plane but follows the curve of the vertebral column. When the dolphin is at rest, in a situation of neutral buoyancy with the lungs full and the blowhole at, or just below, the surface of the water, the trachea is orientated so that the distal end is higher than the lower end (see the illustrations in Green 1972). This is in fact the usual situation at the surface during the blow. This orientation of the trachea means that secretions tend to flow towards the junctional region with the larynx by gravity. This is important because there are no cilia present in the epithelium lining of the trachea.

The other important gross anatomical observation is that the narrowest parts of the upper respiratory tract of the dolphin are the orifices of the blowhole and the larynx, whilst the widest part of the airway is the proximal trachea (Slijper 1962; Kooyman 1973; and appendix 2). This means that on inspiration turbulent flow with eddy currents occurs in the proximal trachea extending at least some of the distance down the trachea. This has two effects: (a) it brings the inspired air into contact with the warm moist mucosa, facilitating warming and humidification; and (b) it results in the deposition of a very large proportion of the particle load by gravity and contact with the lining layer (Kotin 1968; Stuart 1973). I will discuss the warming and humidification in the section on the venous plexus. There are several points concerning the deposition of the inspired particles which I will

discuss now before passing onto a detailed discussion of the epithelium.

A large proportion of the particles in the atmosphere above the sea in the 0.5 to 20 μm range is salt nuclei and aerosols of sea-water (First 1973). Deposition of these particles in the proximal trachea will occur mainly due to gravity and turbulent flow, resulting in local salt (sodium chloride) concentrations greater than 4%. It has been shown in terrestrial mammals that salt concentrations above 4% result in paralysis of cilia and then death of cilia in the trachea (Scudi et al 1951; Proctor 1964). It has also been shown that deposition of noxious substances, including high levels of salt, result in an increase in secretion by the tracheal mucosa and glands (Dalhmann 1956; Kotin 1968).

6-2 B Epithelium.

I have described three types of epithelium lining the dolphin trachea. The larynx is lined by typical stratified squamous non-keratinised epithelium which changes to a transitional type at the junction of the larynx and trachea. Most of the trachea is lined by an epithelium which has a microvillous surface and only occasionally are ciliated cells seen. Distally, in the crypts of the laryngo-tracheal junction, and in the ducts of the glands, typical respiratory epithelium is found. I have not been able to establish the pattern of distribution with any more certainty than this because of lack of additional material containing the whole tracheal epithelium intact.

The significance of possible differences in distribution did not become apparent until quite late in the investigation, by which time much of the material had been used for other purposes and had lost its orientation. Further investigation is required.

6-2 B-1 Transitional type.

This epithelium is found lining the most proximal part of the trachea. This epithelium is distinguished by: (a) basal cells with numerous tono-filaments and basal attachments; (b) several layers of intermediate cells, some containing secretory granules (c) surface cells, which may be squamous or cuboidal depending on the state of contraction of the epithelium, and which have an asymmetric unit membrane on their surface which is arranged as rugae rather than as microvilli. This type of epithelium is found in the young animals as well as the older ones, and also occasional islands are found in the distal trachea. The exact distribution has not yet been established.

The asymmetric unit membrane consists of a thicker, more densely staining outer leaflet, which is easily demonstrated in thin sections and can be followed using the goniometer stage. The asymmetric membrane is also found in the surface cells of the proximal regions of the microvillous epithelium. It is therefore a regional rather than a special feature of the transitiona epithelium. I have not yet been able to establish the extent of this type of membrane.

A similar type of asymmetric unit membrane has been described in the mammalian urinary bladder and ureter (Hicks 1965; Koss

1968; Firth and Hicks 1973; Rhodin 1974; Noack et al 1975). The membrane is thought to present a barrier to the urine, which is hypertonic (Hicks 1966 a). It is not unreasonable to suggest that the dolphin trachea has developed a similar membrane in response to the increased salt concentrations encountered in its proximal regions. The intermediate cells of the dolphin epithelium are secreting a product, which may very well be the same as the intermediate cells of the bladder epithelium, namely membrane precursors (Hicks 1966 b). The basal cells, with tonofilaments, basal attachments and plicated lateral surfaces are well adapted to withstand the stretching and contractions that occur during the blow and diving.

6-2 B-2 Microvillous epithelium.

Most of the trachea is lined by an epithelium which is distinguished in the SEM by its microvillous surface and the absence of, or the presence of only occasional, cilia. In the TEM this epithelium presents a range of appearances from a stratified cuboidal epithelium proximally to a pseudostratified epithelium distally. Most of the epithelium is characterised by surface cells which have: an organelle free terminal web, or zone; a perinuclear zone packed with endoplasmic reticulum and Golgi; between these two zones is a mitochondrial zone; and prominent folding and reduplication of the lateral plasma membrane. These are the characteristics of a transporting epithelium such as is found in mammalian gallbladder (Berridge

and Oschman 1972; Rhodin 1974).

Some recent evidence has suggested that there is re-absorption of water from the mucous raft in terrestrial mammalian trachea (Asmundsson and Kilburn 1970; Van As and Webster 1973; Irvana and Van As 1972). There is also evidence that there is active transport of sodium and chloride ions across the tracheal epithelium of dogs *in vitro* (Marin et al 1974; Olver et al 1975). In the dolphin there is evidence that the loss of respiratory water is not as great as would be expected from a terrestrial mammal of the same body weight (Coulombe et al 1965). Krogh (1939) suggested that the reduction in respiratory water loss in dolphins was due to their reduced respiratory frequency, but Coulombe et al (1965) have proposed that pressure changes in the blowhole account for the reduction. I propose that there is active re-absorption of water by the tracheal epithelium from the tracheal secretions. This re-absorption becomes more important in relationship to the clearance mechanism I propose for the trachea, namely that the secretions from the trachea gravitate to the larynx-tracheal junction and are then expelled in the "blow". This means that unless there is some mechanism for re-absorption of water all of the water in the tracheal secretions is lost during the "blow". This does not happen. I propose therefore that the microvillous epithelium has developed in response to two factors: (a) the need for an epithelium to withstand the high salt concentrations present in the trachea; and (b) to remove water from the tracheal secretions and so reduce the respiratory loss. This is very

important in an animal living in the marine environment (Krogh 1939).

6-2 B-3 Respiratory epithelium.

Respiratory epithelium is found in the distal trachea and the bronchi. It is typical pseudostratified ciliated columnar epithelium with numerous goblet cells. I have found only four main types of cell: ciliated cells; goblet cells; basal cells; and intermediate cells. I have not found brush cells or small granule cells in the specimens so far examined. These are very similar findings to those described for other large animals, where only the four major cell types have been found (Frasca et al 1968 a; Greenwood and Holland 1972; Rhodin 1974). Differences in the types of secretion found in the goblet cells in different species and regions on the trachea-bronchial tree have been described (Lamb and Reid 1969; Spicer et al 1971). I have not yet been able to determine the types of secretion in dolphin goblet cells. I would be surprised if there were no regional differences or species differences in the dolphins.

6-2 B-4 Earlier findings.

Earlier descriptions of the epithelium lining the trachea and bronchi of dolphins were hampered by poor preservation and by being limited to small samples, often of indeterminant site. I have been able to re-examine some of the specimens used in an earlier investigation and found that we had similar problems (Fanning and Whitting 1969). Other authors have found:

stratified columnar non-ciliated epithelium without goblet cells (LaCoste and Baudrimont 1926, 1933); simple columnar epithelium with pseudo-stratified in some areas (Bonin and Belanger 1939); stratified ciliated columnar epithelium (Wislocki 1929); pseudo-stratified ciliated columnar epithelium with few goblet cells (Simpson and Gardner 1972); epithelium without cilia or goblet cell (Slijper 1962); and typical respiratory epithelium (Fanning and Whitting, 1969).

I believe that the differences between the present description and the earlier ones can be explained by the regional variations I have described, or by poor fixation. The other possible explanation for the findings is that some disease process has caused pathological changes to be induced. Most of the material that has been examined both in my investigations, and others, has been obtained from animals caught in the wild, although some specimens have been obtained from marinelands or similar sources. I will now review some of the possible pathological changes that can occur and describe how the dolphin findings differ.

6-2 B-5 Possible pathological changes.

Numerous authors have described examples of metaplasia of the epithelium in the respiratory tract in normal animals and in response to a variety of environmental insults. Sorokin(1973) has said the following of squamous metaplasia: "This change is arguably within the epithelium's normal expressive range; since islands of stratified squamous cells normally occur in exposed parts of the pseudostratified lining of the upper airway and

remain stable for a lifetime." Metaplasia is defined as the reversible replacement of one differentiated epithelium by another differentiated epithelium, which is usually less specialised. Metaplasia is commonly found in the ducts of the pancreas, salivary glands and the bile ducts, usually in response to chronic irritation (Robbins 1974).

Squamous metaplasia has been found in the human lung at the bifurcation of the bronchi even in children (Sanderud 1958; Auerbach et al 1960). It is most commonly found, however at bronchial bifurcations of the lungs of cigarette smokers, of bronchitics and in bronchiectasis (Auerbach et al 1957, 1961, 1962; Sanderud 1958; Knudtson 1960; Kierszbaum 1965; Spencer 1968). Squamous metaplasia has also been found around the openings of tracheo-bronchial glands in normal swine, dogs and rabbits (Wang et al 1972). Numerous experimental studies have tried to establish the relationship between squamous metaplasia and carcinoma of the lung. Some of these studies and the agents used include: vitamin A deficiency (Wong and Buck 1971; Harris et al 1972); cigarette smoke (Auerbach et al 1967; Frasca et al 1968a, 1968b); sulphur dioxide (Asmundsson et al 1973); aldehyde vapours (Dahlgren et al 1972); and various known carcinogens (Gould et al 1971; Harris et al 1971, 1972; Port et al 1973). Squamous metaplasia has also been reported following prolonged endotracheal intubation and in permanent tracheostomy after laryngectomy (Griffith and Friedberg 1964; Friedberg et al 1965; Sara 1967). After injury

squamous metaplasia may accompany healing (Wilhelm 1953, 1954; Hilding 1965; Hilding and Hilding 1966). The epithelium in all of these experimental studies shows a range of changes from slightly abnormal to frank neoplasia. In vitamin A deficiency the TEM showed keratinisation of the surface cells, an increase in the number of desmosomes and an increase in tonofilaments (Harris et al 1971, 1972; Wong and Buck 1971; Port et al 1973). In SEM, vitamin A deficiency of the epithelium showed changes ranging from loss of cilia through a microvillous surface to rugae before the development of typical squamous cells (Port et al 1973). Microvillous cells were found after cigarette smoke exposure in dogs (Frasca et al 1968 a, 1968 b).

The dolphin epithelium looks completely normal. There is no increase in desmosomes, nor any evidence of keratinisation of the surface. Except in a few areas to be discussed under additional findings, there is not even an inflammatory infiltrate. I have found no evidence that the animals were vitamin A deficient but no measurements of nutritional state were made. The animals had not been exposed to any of the known carcinogens used in the above studies. All of these points lead me to believe that I am dealing with an epithelium which has adapted to the environment within the expressive range demonstrated for its type.

6-2 C Glands.

I have described mixed mucous and serous glands in the submucosa and lamina propria of the dolphin trachea. I have not yet

attempted to define the different types of secretion, or the distribution of the serous and mucous glands. This has been attempted in several terrestrial mammals (Meyrich and Reid 1970 a; Lamb and Reid 1970; Spicer et al 1971). The type of secretion may be very different in the proximal regions from that in the distal regions, particularly in view of the salt concentrations I have discussed. The earlier reports have varied in the types and number of glands found (Fiebiger 1916; Wislocki 1929; LaCoste and Baudrimont 1933; Fanning and Whitting 1969; Simpson and Gardner 1972). I believe that the explanation lies in the regional variations which I have described and in the relatively poor fixation of the material. There may also be some species difference which have not yet been considered due to the present difficulty in obtaining specimens.

The glands have a dual function in the dolphin trachea. They provide copious secretions to wash the deposited material, including salt,towards the larynx simply by gravity and flow of fluid. They are also the source of the moisture which is required for the humidification of the inspired air. I suspect that the secretion will be found to consist of low viscosity glycoproteins with a high water content, but this will have to await further study for confirmation.

6-2 D Venous plexus.

I have described a rich plexus of large veins with musculo-elastic walls in the submucosa of the trachea, extending down into the major bronchi. I have not established the blood supply

to this plexus and to my knowledge nothing is known of its source of supply. The plexus has two main functions; (a) it supplies the heat to warm the inspired air, and may function in the conservation of heat; (b) it acts as a space-occupying region during the dive to prevent total collapse of the trachea.

(a) the warming of the inspired air is due to the pool of blood at body temperature contained by the plexus. It is not yet clear how the conservation of heat occurs, but the mechanism suggested by Coulombe et al (1965) of increase in pressure could apply equally to the trachea and the nasal cavities. It may be that there is a counter-current flow of blood in the plexus, and this could account for the removal of heat without the need for pressure increases to occur.

(b) during diving the volume of air in the lungs is compressed due to the increase hydrostatic pressure so that at depths of greater than 100 metres the volume is less than that of the trachea. I have demonstrated that the trachea is compressible and does not collapse, but it is also possible that engorgement of the venous plexus provides the fine control necessary to prevent total collapse, or damage to the mucosal surface by sudden changes in the shape of the trachea. As the heart rate returns to normal during the blow the blood will be quickly removed returning the trachea to its maximum size necessary for rapid exchange of air (see appendix 2, Fanning and Harrison 1972).

6-2 E The internal elastic lamina and the fibrocartilaginous layer.

The two elastic laminae provide the additional support which is required to withstand the explosive forces developed during the blow and during diving. I have demonstrated that the excised trachea does collapse slightly, but it can be deformed by pressure and springs back into shape. This may be due to the elastic laminae or to the cartilage.

6-2 F Lymphatic collections.

Lymphatic collections are found only in the laryngo-tracheal junction and in the subpleural regions (Fanning and Harrison 1972, appendix 2). In the laryngo-tracheal junctional region there are epithelial lined crypts with the ducts of mixed mucous and serous glands opening at their bases, surrounded by large aggregates of lymphatic tissue containing germinal centres. This arrangement is typical of the mammalian tonsil (Rhodin 1974). In many areas the epithelium over the germinal centres is attentuated and infiltrated with lymphocytes suggesting an active immunological function. All of the animals in this series were obtained from the sea, not from aquaria, suggesting that this is a normal occurrence. Also, the younger animals showed a lesser development of these tonsillar areas.

Lymphatic collections are usually found in the terrestrial mammalian respiratory system in the laryngeal sinuses and throughout the bronchial tree at bifurcations (Macklin 1955; Krahl 1964; Beinstock et al 1973). In dolphins lymphatic collections have only been described scattered throughout the inner zone of the trachea (LaCoste and Baudrimont 1933) and

in the subpleural collections (Neuville 1928; Wislocki 1929). Although the nasal cavities and larynx have been extensively investigated, there are no reports of lymphatic collections in these regions (Lawrence and Scheville 1956, 1965; Kleinenberg et al 1964; Green 1972; Blevins and Parkins 1973). I have examined a large number of additional specimens of the laryngo-tracheal junction, kindly sent to me by many workers, and have found lymphatic collections in all of them.

The junctional region is the most dependent part of the upper respiratory tract where secretions may drain, not only from the trachea, but also from the larynx. The trachea performs many of the functions of the nasal cavities, including the handling of inhaled material. I have therefore proposed that these lymphatic collections have a similar function to the tonsils in terrestrial mammals in the protection of the upper airway (Fanning and Harrison 1972, appendix 2).

6-2 G Nerves.

Intra-epithelial nerve fibres are very commonly found at all levels of the dolphin conducting system. I have been unable to find any nerve endings, but that does not mean that they are absent. It is only recently that the original observations of Rhodin and Dalhmann (1956) has been re-investigated by Hung et al (1973 a, 1973 b) and others. The function of the nerve fibres found in the epithelium and submucosa is not yet clear, but some are thought to come from chemoreceptors and others from stretch and pressure receptors (Fillenz and Wood 1972). In the

dolphin it is possible that there may be more pressure receptors but only further investigation will clarify this.

6-2 H Summary.

The dolphin trachea has adopted to the functions required of it in the marine environment in the following ways:-

6-2 H-1 Protection of the airway.

Protection of the airway is afforded by: the tonsil-like lymphatic collections on the ventral aspect of the laryngo-tracheal junction; a specialisation of the surface plasma membrane of the surface cells in the proximal epithelia; and abundant mucous and serous glands in the submucosa.

6-2 H-2 Air-conditioning of the inspired air.

The filtration and cleansing functions and the warming and humidification are aided by the turbulent flow which is produced by air passing from the narrow larynx into the wide trachea at high rates.

6-2 H-3 Tracheal clearance and conservation of water.

The trachea is cleared in the absence of cilia by gravity and the flow of secretions towards the laryngo-tracheal junction. The secretions are then expelled by the explosive forces of the "blow". Water is re-absorbed from the tracheal secretions by the microvillous epithelium lining most of the trachea.

6-3 The secondary bronchi.

The secondary, or intra-pulmonary bronchi have very similar structures and functions to the trachea and main bronchi. They are completely encircled by hyaline cartilage plates; have a

thick internal elastic lamina, a rich vascular plexus consisting mainly of similar vessels to those in the trachea; are lined by respiratory epithelium; have numerous mixed mucous and serous glands proximally, decreasing in number as the bronchi decrease in size. I have been unable to find any smooth muscle cells in the secondary bronchial wall, except in blood vessels. Except for the differences in the epithelium, the structure I have described agrees with the earlier accounts of this segment of the bronchial tree (Wislocki 1929; LaCoste and Baudrimont 1933; Simpson and Gardner 1972).

I think that the more proximal, and larger, branches function in the air-conditioning of the inspired air in the same manner as the trachea and its branches. In the smaller branches the functions are similar to those in terrestrial mammals, namely conduction of the air. I have no explanation for the absence of smooth muscle from this segment.

Summary.

The secondary bronchi have a very similar structure to their terrestrial counter-parts except that they have no muscle.

6-4 The tertiary bronchi.

6-4 A Terminology.

Most of the earlier accounts of the bronchial tree and lung of dolphins have been confused by attempts to find homologues of the terrestrial mammalian structures and to transpose the terminology exactly. I have not done this. The tertiary bronchus is the name I have given to a small segment of the airway, between the secondary bronchi and the sphincteric

segment, which has a distinctive structure.

6-4 B Structure.

The tertiary bronchi range in length from several centimetres to only 1 to 2 mm with most being between 1 and 4 mm long. They are distinguished by: the presence of smooth muscle bundles; the absence of goblet cells and glands; and a re-arrangement of the vascular and connective tissue networks between the epithelium and the fibro-cartilaginous layer.

6-4 B-1 Connective tissue and muscle.

The change which occurs in the connective tissue compartments of the tertiary bronchus in dolphins is abrupt. I have found no smooth muscle by LM or TEM in the walls of the secondary bronchi. There is only one report in the literature of smooth muscle in the dolphin bronchial tree, except in the sphincteric segment, and that is by Kleinenberg et al (1964) in the trachea of the Beluga, *Delphinapterus leucas*. They only used LM and have no illustrations. The absence of smooth muscle is striking because in terrestrial mammals there is a distinct geodesic network of smooth muscle, commencing at the trachea and extending throughout the bronchial tree (Macklin 1929). In the dolphin the first appearance of smooth muscle is as discrete bundles situated in the gaps between the cartilage plates. These bundles are circularly arranged and are not connected by spiral or longtitudinal fibres. The change in the elastic laminae is not quite so abrupt, as it occurs over the length of the tertiary bronchus. The internal elastic lamina does decrease in size as the bronchi decrease in size but it still

forms a distinct lamina until the commencement of the tertiary bronchus. The lamina then rapidly decreases in size, becomes more diffuse and then fuses with the thin lamina immediately beneath the epithelium. This new elastic tissue layer is spread throughout the area between the epithelium and the cartilages as a network of elastic fibres (see Figs. 45, 46 and 47). The network does seem to form two layers around the smooth muscle bundles, and more distally forms part of the myo-elastic sphincters in the sphincteric segment.

6-4 B-2 Vascular plexus.

The change in the vascular plexus is also abrupt. The large vessels which were present in the trachea become smaller as the bronchi decrease in size but still retain their myo-elastic walls. At the level of the tertiary bronchus this plexus disappears completely and is replaced by a plexus of typical mammalian arteries, arterioles, veins, venules and capillaries. It is significant that the pulmonary artery and vein branches which have been situated in a separate sheath some distance away from the bronchi, appear to join the connective tissue sheath of the tertiary bronchi as this change in vascular plexus occurs. This suggests to me that the new plexus is supplied by the pulmonary vessels and the larger plexus by branches from the bronchial arteries, however, perfusion of the vasculature will be required to resolve this.

6-4 B-3 Epithelium.

The changes in the epithelium and glands are not so abrupt but are just as important. The ciliated cells are not as tall

as those in the trachea but are similar in all other respects. I have described two types of non-ciliated cell in the tertiary bronchus (a) Clara type cells and (b) junctional cell

(a) Clara type cells.

There are two types of cells in this group, one is the typical Clara type cell with bulbous apical cytoplasm and pale staining granules, the other which is more numerous, contains dark staining granules. Clara cells were first described by Clara (1937) in the rat, although Kolliker (1881) had described the non-ciliated cells. It is now generally agreed that there is a group of secretory cells present in the terminal airways of terrestrial mammalian lungs. The classical Clara cell is one member of this group, now called bronchial secretory cells (see section 2-2 C-1 for a review).

(b) The junctional cell.

This cell has some of the features of the small granule cell described in other species (Sorokin 1973), but it also has many of the features of the secretory cell, and it reaches the surface. I suspect that this cell is also a member of the secretory cell group, but some neuro-endocrinological function cannot be ruled out at this time. The SEM appearance of the tertiary bronchus are very similar to those described for the terminal bronchiole of large terrestrial mammals (Greenwood and Holland 1972; Castleman et al 1975; Ebert and Terracia 1975; Mariassy et al 1975). Despite the similarities further investigation

will be required to determine whether or not the tertiary bronchus is homologous with the terminal bronchiole.

6-5 The sphincteric segment.

This segment is only found in dolphin lungs. It is difficult to define where the tertiary bronchus ends and the sphincteric segment begins, but the first sphincter usually occurs in bronchi between 1 and 1.5 mm in diameter. The segment varies in length from several millimetres with one or two sphincters to up to twenty sphincters. Even though this segment derives its name from the sphincters, they are not the most important feature present. Wislocki (1929) was the first to recognise the respiratory function of this segment, but it was not until it was examined electron microscopically that this was confirmed (Fanning and Harrison 1972, and appendix 2). In this investigation we called the sphincteric segment the Respiratory Bronchus. Ito et al (1967) also referred to the respiratory character of the sphincteric segment, but they were somewhat confused by the absence of epithelium in their material.

The respiratory nature of this segment is seen in the SEM where the whole of the surface of the bronchus is covered by a capillary plexus which can be clearly seen beneath the epitheliu

The epithelium consists of squamous cells covering the capillaries and cuboidal microvillous cells in the gaps between. The squamous cells are very similar to type I alveolar cells, with thin lateral processes which in some area are only 100 nm thick. In the proximal parts of the segment they do not contain secretory products but in the distal regions they occasionally

contain inclusions similar to those in type II alveolar cells. The significance of these cells is not clear, but they are similar to cells described by Castleman et al (1975) in primate lung. The myo-elastic sphincters consistof bundles of circular smooth muscle fibres situated in the gaps between the cartilage plates. The elastic fibres in the submucosa form two laminae on either side of the bundles with radially running fibres connecting them and attaching to the perichondrium. This means that when the sphincters contract it reduces the size of the lumen and draws the cartilages together, thus decreasing the length of the sphincteric segment. The smooth muscle cells and the elastic fibres have no unusual features.

The nerve supply to the sphincteric segment has not been determined as yet, but nerve fibres are frequently seen in close relationship to both the epithelium and the smooth muscle fibres. I will defer further discussion until after the section on the alveoli (See section 6-6 B).

6-6 The respiratory zone.

6-6 A Alveolar septum.

The most obvious difference between the dolphin lung and the usual mammaliam pattern is found in the structure of the alveolar septum. In the usual mammalian alveolar septum there is a single capillary network with a connective tissue support. The capillaries are exposed to the air on either side of the septum which means that gas exchange can occur from either surface (Krahl 1964; Sorokin 1973; Weibel 1973; Rhodin 1974). In the dolphin however there is thick connective tissue with a

separate capillary plexus on each surface. Thus gas exchange is only possible on only one surface of the capillary.

6-6 A-1 Marine mammals.

This arrangement of the alveolar septum with two capillary networks is only found in Cetaceans and Sirenians. The Sirenian lung was first reported by Pick (1907) and later by Wislocki (1935). The Cetacean lung has been the subject of numerous reports not only in the Dolphins but also in the great whales (Feibiger 1916; LaCoste and Baudrimont 1926, 1933; Neuville 1928; Wislocki 1929, 1942; Haynes and Laurie 1937; Bonin and Belanger 1939; Wislocki and Belanger 1940; Belanger 1940; Murata 1951; Goudappel and Slijper 1958; Baudrimont 1959; Engel 1966; Ito et al 1967; Fanning and Whitting 1969). Simpson and Gardner (1972) reported on the TEM of the dolphin lung but had some problems with fixation. The structure of the alveolar septum of other aquatic mammals has been extensively reviewed by Kooyman (1973) and by Dennison and Kooyman (1973), and has a typical mammalian pattern. Simpson and Gardner (1972) however found a double capillary network in the lung of the Steller sea lion, *Eumetopiuas jubata*, and I have observed a similar structure in the South Australian sea lion, *Neophoca cinerea* (Fanning unpublished observations). It seems therefore that the double capillary network is confined to the totally aquatic groups of aquatic mammals, the Sirenia and the Cetacea.

6-6 A-2 Terrestrial mammals.

The mammalian alveolar septum is covered by two layers of

capillaries during foetal life, but after birth, when the alveoli develop, the capillaries become stretched out to form a single network (Krahl 1964). There has been some debate recently about the disposition of the capillaries (Ryan et al 1969; Ryan 1973; Weibel 1973). Certainly, there is good evidence to support the theory that functionally the capillary network is single (Sobin et al 1970; Weibel 1973; Sobin personal communication).

6-6 A-3 Capillaries.

In the dolphin lung the foetal pattern has persisted in the adult alveolar septum. I believe that the occasional trans-septal anastomoses only tend to confirm this interpretation. The capillaries have a typical mammalian pattern and the cell processes observed in the connective tissue may be processes of pericytes (Weibel 1974). There are no unusual features in the capillary network.

6-6 A-4 Epithelium.

The surface of the alveoli is lined by type I and type II cells No type III cells have been found.

(a) Type I cells.

The type I cells are thin attenuated cells covering the surface of only one alveolus, except at the openings into the alveolar ducts or sacs. These cells may be so attenuated in some areas that the cell process consists only of two plasma membranes with the thinnest layer of cytoplasm separating them. Weibel has described a similar thinning of the type I epithelium in the Etrucsan

shrew (Weibel 1971, 1973; Weibel et al 1971). He has suggested that this is an adaptation allowing the maximum possible gas exchange per unit area of membrane.

(b) Type II cells.

The type II cells contain the characteristic osmiophilic lamellar bodies which has been reported to be surfactant (Sorokin 1967, 1973; Kikkawa and Spitzer 1969; Belton et al 1971; Smith et al 1972; Weibel 1973). I have not counted the number of type II cells, but they do not appear to be less numerous in the dolphin lung than in any other mammal I have examined, including rats, marsupials, sheep and humans It has been stated that the lungs of marine mammals are deficient in surfactant (Greenfield quoted by Boyd 1976 as a personal communication). This report referred to the Weddell seal, *Leptonychotes weddelli*, and I have been unable to find any reports of surfactant measurements in dolphin lungs. I have however shown, in some of the subpleural alveoli, flocculent material and myelin figures, which are reported to be preumptive evidence for surfactant (Weibel 1973). I have performed some of the histochemical reactions which have been reported as demonstrating surfactant, but the fixation of the specimens leaves much to be desired, as does the specificity of the tests. I have therefore decided not to include the results in the main body of this work, eventhough they do support my other evidence. I would be surprised if lung washings and more specific histochemical reactions do not show a usual

mammalian surfactant system in the dolphin lung.

6-6 A-5 Connective tissue.

The connective tissue core of the alveolar septum contains both collagen and elastic fibres, but I have been unable to find any smooth muscle fibres except around the openings of the alveolar ducts and the alveolar sacs. There does not appear to be any preferred orientation of the connective tissue fibres. There is still considerable debate concerning the usual arrangement of the connective tissue framework of the terrestrial mammalian lung and its relationship to the capillary network (Ryan et al 1969; Ryan 1973; Sobin et al 1970, 1972, 1974; Rosenquist et al 1973; Meyrick and Reid 1970 b; Weibel 1973). Recent investigations also have shown that some of the interstitial cells in the alveolar septum may be contractile (Kapanci et al 1974). The arrangement of the connective tissue fibres and the demonstration of contractile cells is important in the terrestrial mammalian lung because elastic recoil of the lung, or compliance, is an important factor in emptying the lung. It has been shown that the increase in expiratory flow in marine mammal lungs is due almost entirely to increase in the elastic recoil (Dennison et al 1971; Leith et al 1972; Kooyman 1973; Kerem et al 1975). It will therefore be important to establish whether this increase is due solely to the increase in connective tissue, or whether the large number of interstitial cell processes present in dolphin alveola septum play any part.

6-6 A-6 Lymphatics.

The presence of lymphatic vessels in the alveolar septum,

away from the junctional regions and peribronchial spaces is an important but not unexpected finding. The structure of the vessels is similar to that described by Leak and co-workers (see Leak 1976 for a review). It has been shown that lymphatics are rarely, if ever, encountered away from the peribronchial, subpleural or paraseptal connective tissues in adult mammalian lungs (Lauweryns 1971). Lymphatics are present in the foetal and newborn lung (Lauweryns 1971) and in the dolphin where this foetal pattern continues into adult life I expect to find lymphatics.

6-6 A-7 Inter-alveolar communications.

I have found no evidence of inter-alveolar communication, such as pores of Kohn. Recent investigations have confirmed that such communications do occur in normal mammalian lungs (Cordingley 1972; Castleman et al 1975). The pores are believed to be responsible in part for collateral ventilation, but what effect, if any, their absence will have on the function of the dolphin lung must await further investigation.

6-6 B Function of the terminal airway and the respiratory zone.

6-6 B-1 General.

The dolphin lung is the most highly modified of all mammalian lungs. Dolphins are not particularly good divers, the deepest dive recorded was by a Pilot whale which reached 2000 feet and remained submerged for 13 minutes (Wood 1973). The deepest and apparently the most successful divers are the true seals, followed by the baleen whales, then the sea lions and dolphins (Kooyman and Andersen 1969; Kooyman 1973). A feature of all

marine mammal lungs is the increase in the amount of cartilage found in the terminal airways. The group with the most extensive cartilaginous reinforcement are the dolphins, where the cartilage extends to the openings of the alveolar ducts, but a similar distribution is found in some seals and sea lions (Kooyman 1973). The dolphins are the only group with well developed sphincters in their terminal airways. Other groups have sphincter mechanisms usually situated at the level of the alveolar ducts (Kooyman 1973).

6-6 B-2 Dolphins.

Dolphins exhibit three main types of activity, each of which I consider to be associated with a different attitude, or configuration of the respiratory tract. These types of activity are (a) surface activity consisting of respiration at the surface either after diving or at rest, (b) the normal, or usual activity which consists of swimming at or near to the surface, diving to depths of 20 to 30 metres and various play activities during feeding and so on, (c) deep diving.

I will discuss these different attitudes adopted by the respiratory tract and then return to a discussion of the problem of "the bends".

(a) Surface activity.

During surface activity the lungs are held in the inflated position, because dolphin respiration is apneustic, with the breathe holding occurring on inspiration. The pause varies from about 20 seconds to up to 3 to 4 minutes during which

At this depth the pressure in the lungs is already at three times atmospheric pressure (Ridgway et al 1969). I consider that the sphincters contract at the end of inspiration trapping the air in the alveoli, but as the pressure begins to rise during the dive air is forced through the sphincters into the bronchial tree and trachea. Alveolar collapse in terrestrial mammalian lungs results in folding of the alveolar septum and a considerable reduction in diffusing capacity (Gil and Weibel 1972; Weibel 1973; Weibel et al 1973). Most of the reduction in diffusing capacity is due to folding of the capillary walls with consequent thickening of the blood-air barrier. In the dolphin lung much of the folding can occur in the gaps between the capillaries. Most of the blood-air barrier is only 200 nm thick, which is considerably thinner than that for terrestrial mammals of similar size and is approaching that of the smallest mammal, the Etruscan shrew (Weibel 1973). This means that in the inflated condition the size of the blood-air barrier could compensate for the increased thickness of the alveolar septum. In the partially collapsed lung the blood-air barrier could increase by a factor of two or even three and still retain a good diffusion capacity. Further investigation of the diffusing capacity of the dolphin lung is required to clarify this point.

The respiratory surface in the sphincteric segment, which has been called the respiratory bronchus (Fanning and Harrison 1972, appendix 2) would aid in this function of

retaining diffusing capacity of the collapsing lung. I consider that the blood flow in the respiratory zone is from the pulmonary artery through the alveolar capillary plexus and then to the capillary plexus in the respiratory bronchus. This may aid in the absorption of oxygen, but I consider that the most important function of this counter-current is in the removal of nitrogen during the ascent of both shallow and deep dives.

(c) Deep diving.

It has now been demonstrated that the alveoli have collapsed completely by a depth of 100 metres (Ridgway et al 1969). It was Scholander (1940) who first suggested that there was collapse of the alveoli during a deep dive. The increase oxygen utilisation of 80% even during deep diving can be explained by an increase in the diffusing capacity due to the thin blood-air barrier and the double capillary network. This will obviously need to be investigated using the stereological techniques proposed by Weibel (1973).

6-6 B-3 Dolphins and the "bends".

Dolphins like most marine mammals do not appear to suffer from the bends. The bends, or Caisson disease, is caused by nitrogen dissolved in the blood at high partial pressure coming out of solution when the partial pressure is reduced. The condition occurs in two situations. Firstly, when nitrogen is absorbed under conditions of increased pressure, for example a deep sea diver returning too rapidly to the surface. Secondly, nitrogen absorbed at normal atmospheric pressure at ground level comes

out of solution during high altitude flying. The usual explanation given for marine mammals' resistance to this condition is that proposed by Scholander (1940; 1964): the animals dive with only the nitrogen contained within the lung, and as they dive, the alveoli collapse thus the nitrogen cannot be absorbed. Recent information suggest that the reason is not as simple as first proposed. It has been calculated in seals, that if all of the nitrogen contained in the lung were absorbed, the nitrogen level in the tissues would reach potentially dangerous levels (Kooyman 1972). It is also well known that seals dive after expiring part of the air in the lungs, and that they do not utilise as much of the oxygen as do dolphins (Kooyman 1973). Dolphins dive with the lung full of air and they utilise 80 to 90% of the oxygen. This means that the potential for the development of problems due to dissolved nitrogen is greater in dolphins than in seals. Kooyman (1973) has discussed this problem and proposed five possible mechanisms for keeping nitrogen levels low in the tissues. These mechanisms are: (1) greater tolerance, such as greater solubility of nitrogen in tissues and blood of marine mammals than in other mammals; (2) widespread distribution of the nitrogen in the tissues; (3) restriction of the length and depth of dives; (4) the presence of special nitrogen absorbing tissue; and (5) prevention of the absorption on nitrogen. To these five I propose to add a sixth: (6) a mechanism for removal of the nitrogen from the blood.

Most of the data which is available on diving in marine mammals has been obtained from seals, particularly the Weddell seal

(Kooyman 1973). The seal has the least modified lung of all marine mammals, but has the best developed diving reflexes. Seals dive after exhalation of about half the total lung capacity whereas dolphins dive with full lungs. In seals during deep dives the alveoli collapse excluding the air from the pulmonary circulation this reducing the absorption of nitrogen. Kooyman (1973) has shown that during deeper dives there is more widespread distribution of blood, that there is less peripheral vasoconstriction than during shallow dives. He suggests that this would account for the absence of the "bends" in seals.

Dolphins have the most highly modified respiratory system, but they have diving reflexes which seem to be not as well developed. Ridgway (1972) has shown that the heart rate in the bottlenosed dolphin, *Tursiops truncatus*, at the end of inspiration is 80 to 90 beats per minute, but slows to 30 to 40 beats per minute after a few seconds and remains at that rate until the next "blow" whether it be in 20 seconds or four minutes. Similar changes in the heart rate have been observed in the Killer whale, *Orcinus orca* (Spencer et al 1967). Ridgway (1972) has reported that the other parts of the diving reflexes, namely peripheral vasoconstriction and redistribution of blood flow to the brain and heart are intact. This means that it is possible for the dolphin to absorb sufficient nitrogen for it to develop the bends unless there is a mechanism for its removal.

I have proposed that the blood flow to the capillary plexus in the respiratory bronchi is from the alveoli to the respiratory bronchi, that is a counter-current flow. This means that there is the opportunity during the dive, and the surfacing to remove nitrogen in the respiratory bronchus. This mechanism would be most effective during surfacing when the air returning into the respiratory bronchus would contain nitrogen at a lower partial pressure than that in the circulation.

This mechanism is not a new development in the dolphin because a similar mechanism is found in birds to maximise the absorption of oxygen (Schmidt-Neilsen 1971). This may also be true in dolphins, for not only is nitrogen being removed, but there is also maximum utilisation of the inspired oxygen (Ridgway et al 1969).

The better development of a basic mechanism such as this is not confined to the respiratory system. Andersen (1966) has shown that the diving reflex is present in all vertebrates, but is most highly developed in the diving vertebrates.

6-6 C Nerve supply.

I have shown many unmyelinated nerve fibres in the epithelium of the trachea and bronchi, but there do not appear to be as many in the distal bronchi. These fibres may be pressure receptors (Hirsch and Kaiser 1969). Similar fibres have been found in the terminal airways of terrestrial mammals (Hung et al 1972, 1973 a, 1973 b; Jeffrey and Reid 1973). I have not yet made a detailed study of the distal airways for nerves, but many fibres have been found in relationship to the myo-elastic sphincters. This is one area which requires further investigatio

6-6 D Surfactant.

It has been shown in terrestrial mammals that there is a certain critical opening pressure below which the alveoli collapse. This pressure depends on many factors, one of which is surfactant (Comroe 1974). Little is known of the dynamics of the lung of dolphins at the alveolar level, but it has been shown that they exchange 80% of the total lung capacity during the blow, and that they collapse during diving. Scholander (1964) has pointed out that there are no problems overcoming the critical opening pressure during the ascent phase of the dive due to the increased pressure from the increased hydrostatic pressure. At the surface, during the blow, the question of the presence of the surfactant and the reduction of the critical opening pressure is important, because for 80% of the total lung capacit to be exchanged there must be almost total collapse of the alveoli. I have presented strong circumstantial evidence for the presence of surfactant, but further investigation is necessa

6-6 E Summary.

The terminal airways and alveoli have adapted to the marine environment in the following ways:

6-6 E-1 Gas exchange.

The available area for gas exchange has been increased by the development of an additional respiratory area in the terminal bronchus and by reducing the thickness of the blood-air barrier in the alveoli. This allows increased utilisation of the inspired oxygen over that observed in the terrestrial mammals, and affords a mechanism for the removal of excess nitrogen.

6-6 E-2 Blood supply.

The blood supply to the respiratory bronchus is derived from the pulmonary circulation after it has passed through the alveoli, thus forming a counter-current circulation of the blood and the air.

6-6 E-3 Sphincters.

A system of sphincters is present in the respiratory bronchus. They have two functions. Firstly, they close to retain the air in the alveoli at the end of inspiration; and secondly, they control the collapse and re-inflation of the alveoli during and after a dive.

6-6 E-4 Connective tissue.

The development of cartilage reinforcement of the terminal airways and thick connective tissue core to the alveolar septum. The cartilages prevent collapse of the airway and thus aid emptying of the lungs. The alveolar septum provides additional support to resist the pressure associated with diving and

provides the increased elastic recoil needed to empty the lungs.

6-7 Additional findings.

A number of unusual bodies are found in the epithelium and submucosa of all the animals used in this investigation. Some are calcified and all are surrounded by an infiltrate of cells, particularly mast cells. Changes associated with acute inflammation are absent. This type of reaction has been reported in parasite infestations in the gastro-intestinal tract (Murray at al 1968). The mast cells are often called globule leucocytes in such infestations (Kent 1966; Murray et al 1968).

Parasite infestation of the lung of Pinnipeds and less frequently of Cetaceans by lung worms has been reported (Simpson and Gardner 1972; Dailey and Brownell 1972). Dailey has suggested that these bodies may represent a stage in the life cycle of a pulmonary parasite, as yet unidentified (Dailey 1976 personal communication). He has suggested that fresh material be examined in an effort to identify the parasite.

Overview.

I embarked on this investigation with the aim of providing a detailed account of the submicroscopic structure of the entire bronchial tree and lung of the dolphin. That this task is beyond me at present is obvious from this thesis. I have chosen therefore to concentrate on three areas in particular, namely: the trachea; the terminal airways; and the alveoli. Even those are incomplete in some aspects, as mentioned in the text. Further research on dolphins will be handicapped by the logistical and technical problems I have experienced and the added difficulties introduced by the Marine Mammal Protection Acts passed in many countries during the last few years. Associated with the legal problems are many emotive problems related to the supposed intelligence of these creatures.

Many of the questions which I have raised could be answered by using material from mass strandings, which are commonplace in some parts of the world. Some of these questions include:

1) the exact distribution of the three types of epithelium I have described in the trachea.
2) structural and biochemical studies of the lining layer of the bronchial tree and lung, and the site of production of the secretions.
3) estimation of the diffusing capacity of the lung.
4) the blood supply to the trachea and the respiratory bronchus and alveoli.
5) Physiological studies should be possible using the trained animals available in the many marine parks around the world.

I will be able to investigate some of these problems on the material I have available but most will require fresh specimens.

Appendix 1.

Appendix 1 consists of a paper by Harrison and Fanning (1974) entitled "Anatomical observations on the South Australian Bottle-nosed Dolphin *Tursiops truncatus*" and an additional plate illustrating the parasites found in the stomach of AD. 3. Unfortunately reprints of this paper are not available and I have had the paper copied for inclusion.

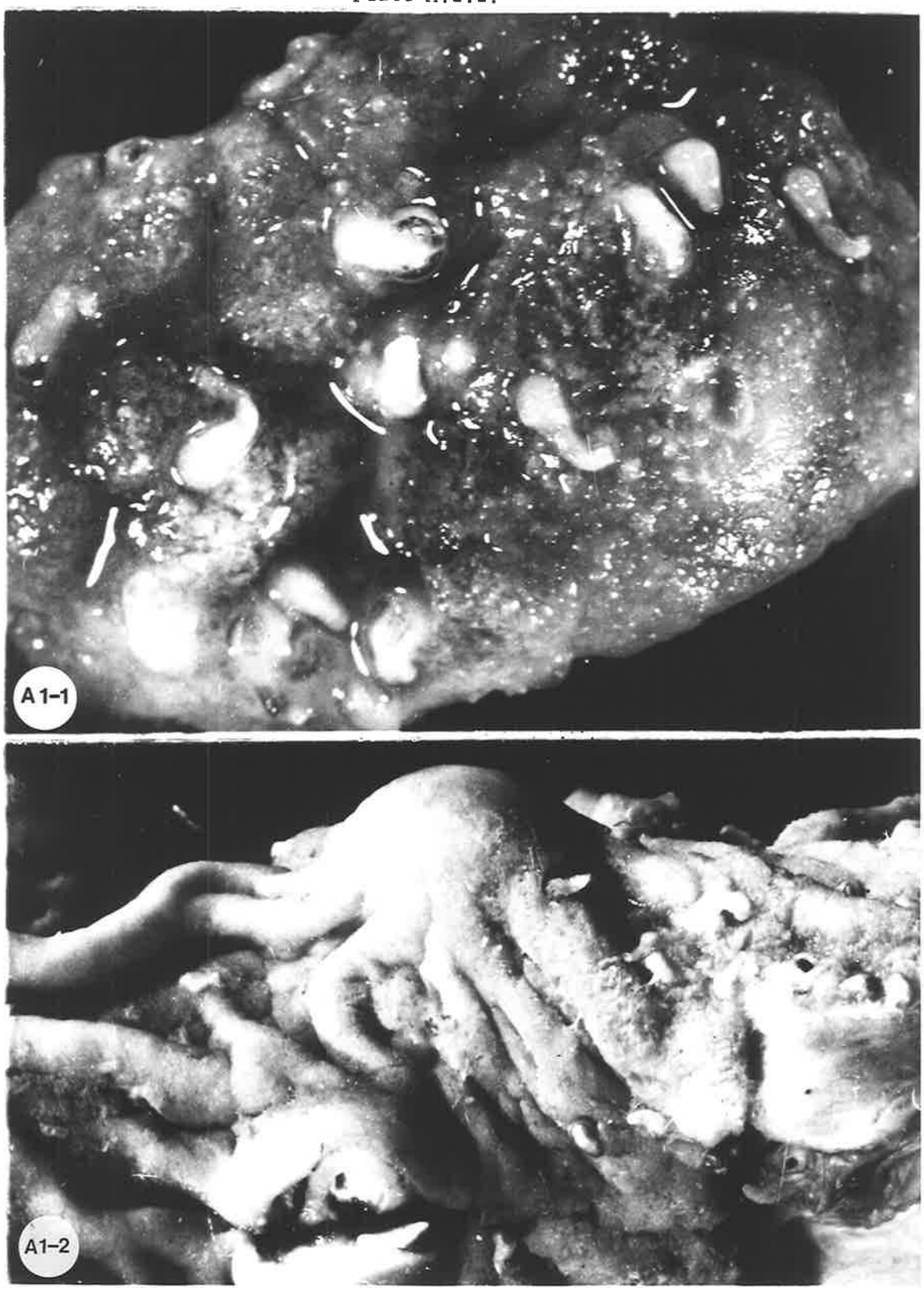
Harrison, R. J. & Fanning, J. C. (1974-1975). Anatomical observations on the South Australian bottlenosed dolphin (Tursiops truncatus). *Investigations on Cetacea, 5,* 203-217.

Plate A.1

Fig. A.1. AD, 3. Small piece of the pyloric stomach before fixation showing the parasites, *Corynosoma cetaceum*, on the the surface.

Fig. A.2. AD, 3. Fixed specimen of the pyloric stomach showing the typical raised fibrotic nodules due to invasion of the mucosal surface by the parasite.

Plate A.1.1.



Appendix 2.

Appendix 2 is a copy of a paper published by Fanning and Harrison (1972) entitled "The structure of the trachea and lungs of the South Australian Bottlenosed Dolphin".

The Structure of the Trachea and Lungs of the South Australian Bottle-nosed Dolphin

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I. Introduction

There is little detailed knowledge of the fine structure of the trachea and lungs of Cetacea. The gross and microscopic anatomy of the trachea was first described by Weber (1886). Narath (1901) has also described

the gross anatomy of the bronchial tree of *Delphinus*. Fiebiger (1916) has illustrated the epithelium and glands of the common dolphin *Delphinus delphis*. Ciliated columnar epithelium without goblet cells has been described by Wislocki (1929) in *Tursiops truncatus*, and Lacoste and Baudrimont (1933) have described stratified columnar non-ciliated epithelium, without goblet cells, glands and lymphatic collections in *Phocoena*. Simple columnar epithelium with areas of pseudo-stratified epithelium, and numerous tubulo-acinar glands has been described by Bonin and Bélanger (1939). Slijper (1962) and Simpson and Gardner (1972) have maintained that all the tracheal epithelial cells in Cetacea were ciliated and that glands were not present.

The microscopic structure of the lung was first described by Fiebiger (1916). He noted that cartilage surrounded the bronchi to their termination, that there were a series of myoclastic sphincters in the terminal bronchi, and that the alveolar septum consisted of a thick connective tissue core with capillaries on each surface. This was subsequently confirmed by Lacoste and Baudrimont (1926), Neuville (1928), and Wislocki (1929). Simpson and Gardner (1972) have recently described the ultrastructure of the alveolar septum in the pilot whale and Pacific white-striped dolphin. This present paper reports a gross, light microscopic and electron microscopic study of the trachea and lungs of the South Australian *Tursiops*, the appearances and general anatomy of which have been described by Harrison and Fanning (1974).

II. Materials and Methods

The lungs and trachea from eight animals have been examined. All were caught under licence in nets in the Spencer Gulf, South Australia, between August 1971 and May 1972 and were transported alive to Adelaide. They were anaesthetized by intravenous injection into the tail fluke vein. The general anaesthetic agents and techniques which have been used are summarized in Table I but are essentially those described by Ridgway and McCormick (1971) and Ridgway (personal communication). Specimens for electron microscopy were obtained from six animals (Nos. AD 1, 2, 3, 5, 7 and 8) and for light microscopy from all except AD 6 which died during transportation. Specimens of lung for electron microscopy from all animals were obtained at thoracotomy, diced into small fragments and immersed in one of the following fixatives: 3% or 6% glutaraldehyde in 0·1 M phosphate buffer (G.P.) or in 45 mM or 0·1 m cacodylate buffer (G.C.), at pH 7·3 (Sabatini *et al.*, 1963), glutaraldehyde–paraformaldehyde diluted

TABLE I

Spec. No.	Length (cm)	Sex	Anaesthetic agents (see footnote)	Trachea Length (cm)	Trachea Width (cm) Cranial end	Trachea Width (cm) Caudal end	Distance of origin(s) of tracheal bronchi from upper end of trachea (cm) Left	Distance of origin(s) of tracheal bronchi from upper end of trachea (cm) Right
AD 1	202	M	Ph D H.G.O.	12·5	4·5×3·7		8·0	6·5
AD 2	201	F	Ph D H.G.O.	13	4·5×3	4×2	—	6·5
AD 3	162	M	P.B.	8	4×3	3·5×2	—	4
AD 4	143	F	None Necropsy	6·5	2·2×1·5	2·9×1·4		
AD 5	201	M	Ph D H.G.O.	12	4·5×3	4×2	—	6
AD 6	223	F	None Necropsy	12·0	4·3×3·4	4·2×2·6		6
AD 7	222·5	F	Necropsy Died just before anaesthetic	12	4·3×2·1	4×2·0		
AD 8	160	F	Pe H.G.O.	6·5	3·5×1·5	3·6×1·6		

PH: Phencyclidine (intravenous injection) Ridgway, personal communication). H.G.O Halothane, Nitrous Oxide 80% Oxygen 20% (Ridgway and McCormick, 1971); P.B. Pentabarbital sodium (intravenous); Pe Pentothal Sodium (intravenous) (Ridgway and McCormick, 1971); D Diazepam (intramuscular) 10 mgm./100 Kgm. (Ridgway, personal communication).

either with 0·1 M phosphate (K.P.) or with 0·1 M cacodylate buffer (K.C.) (Schneeberger-Keeley and Karnovsky, 1968).

The trachea and lungs of AD 2 and AD 5 were fixed *in situ* by the instillation of 1% glutaraldehyde–1% paraformaldehyde in 0·1 M phosphate buffer (K.P.) at pH 7·3 under 20 cm of water pressure. These animals were also perfused through the aorta at 120 cm of water pressure with the same fixative. Specimens of trachea and lung from AD 2 and AD 5 were removed after overnight fixation *in situ*. Specimens of trachea from AD 1, 3 and 7 were fixed in 3% or 6% G.P. and G.C. Specimens were washed overnight in buffer, at pH 7·3 and 320 m osm.

Some specimens were further block-stained with 0·5% uranyl acetate veronal acetate buffer (Farquhar and Palade, 1965).

All were dehydrated in graded ethanol and epoxypropane and e bedded in one of the following epoxy resins: Epon 812 (Weib personal communication); araldite (Luft, 1961) or Epon-arald mixture (Ito, personal communication).

Semi-thin sections were cut at 0·5–1 μ and stained with 1% Toluidi blue in 1% borax or Methylene blue–azure II (Richardson, *et a* 1960) for orientation. Silver to grey sections were cut with glass diamond knives on Cambridge-Huxley or Reichert Om U2 ultr microtomes, collected on naked or coated grids, stained with urar acetate and lead citrate and examined in a Philips EM 300 electr microscope at 60 KV.

For light microscopy, specimens were fixed in 10% phospha buffer formaldehyde, except for AD 2 and 5 (see above), and we embedded in paraffin and sections cut at 5–7 μ. Material fixed glutaraldehyde-containing fixative required special attention duri the sectioning and subsequent staining (Rosen *et al.*, 1967). The b preservation of tissue was obtained using 1% glutaraldehyde–1 paraformaldehyde in phosphate buffer. All electronmicrographs a their description apply to this material.

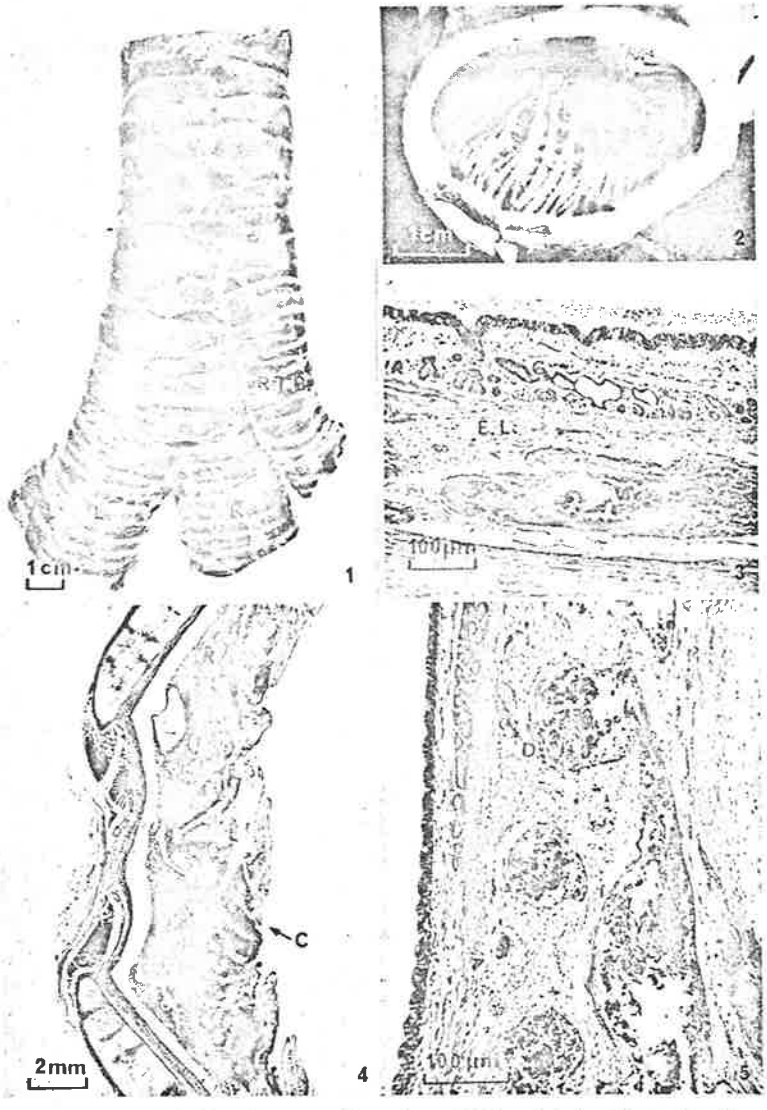
III. The Trachea

A. Gross structure

The trachea is relatively short but wide (see Table I). It contai 14–16 interlocking hyaline cartilaginous rings, a few of which a complete circles, particularly at the cranial end. Most rings are i complete, but all have branches and anastomoses (Fig. 1). In the fre state the trachea collapses dorso-ventrally. The bronchus to the ap of the right lung arises cranial to the bifurcation. In AD 1 the bronch to the left apex also arose from the trachea. There are longitudir folds of mucosa on the ventral aspect extending from the larynx in the cranial 3–4 cm of the trachea (Fig. 2). In the intervening clefts a the elongated to circular openings of epithelial-lined crypts. The ope ings vary from 0·1 mm in diameter to 1 mm in their longest dimensio

B. Microscopic structure

The outer layer of the trachea contains typical hyaline cartilag embedded in a dense fibro-elastic membrane 100–200 μm thick on t outer surface, 20–50 μm thick on the inner surface and up to 1·5 m



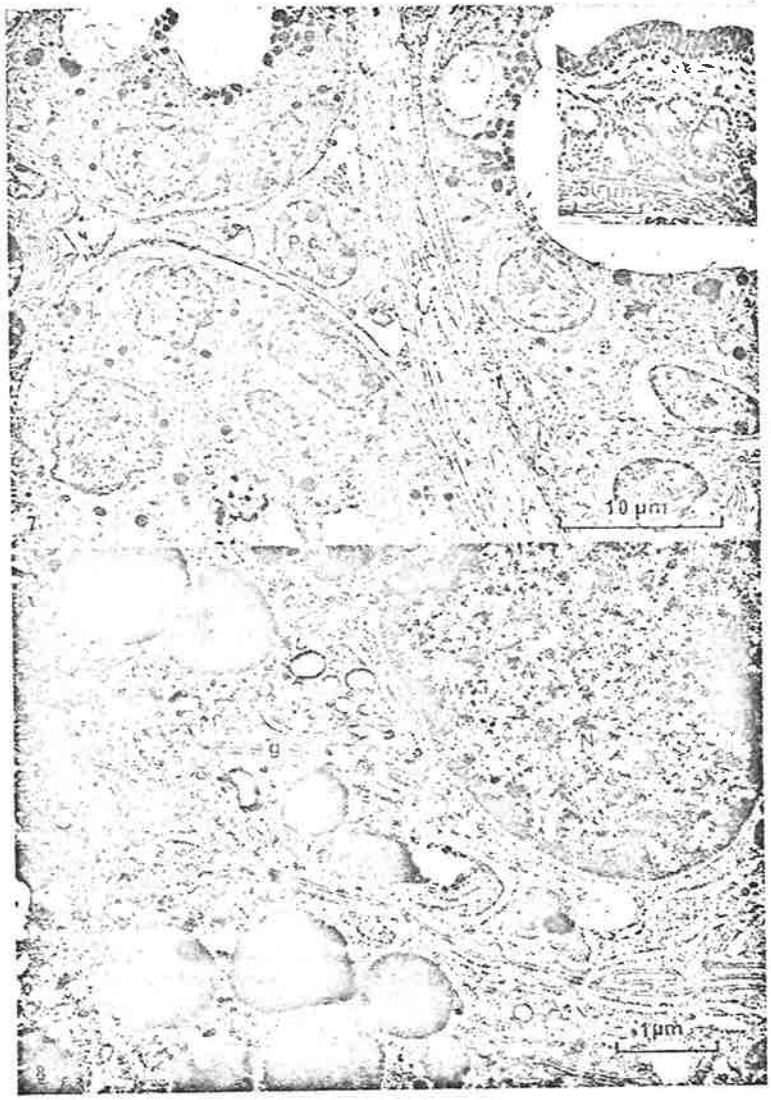
FIGS 1–5: *Fig. 1.* AD 6 Dorsal aspect of trachea. Origin of right (R) tracheal bronchus (R.T.B.); L=left. *Fig. 2.* AD 2 Internal view of larynx and upper trachea from caudal aspect showing longitudinal folds and openings of crypts (C). *Fig. 3.* AD 7 Ventral trachea. Vein (V). Elastic lamina (EL) Glands (G). H & E. *Fig. 4.* AD 7 Ventral aspect of junction of larynx and trachea. Note lymphatic collections (L) surrounding crypts (C). H & E. *Fig. 5.* AD 8 Lateral trachea. Note large thin-walled veins (V), duct (D) of deep layer of glands extending into elastic lamina. H & E.

thick between the cartilages. This membrane is continuous with t perichondrium (Figs 3, 4, 5).

The mucosa contains a dense fibro-elastic layer 100–600 μm thi with predominantly longitudinal and spiral fibres. Embedded in t layer are the bodies of the compound tracheal glands and an extensi plexus of veins, arteries, arterioles and lymphatics. This plexus consi mainly of spiral to longitudinally oriented large thin-walled elas veins up to 500 μm in diameter (Figs 3 and 5). The crypts on the ventı aspect of the cranial end of the trachea are lined by pseudostratifi ciliated columnar epithelium containing goblet cells. The cryp extend into the fibro-elastic layer and are surrounded by dense lympha tissue containing lymphatic nodules 1–2 mm in diameter (Fig. 4). T lymphatic tissue is found chiefly in the gap between the cricoid cartila and the first tracheal cartilage, but it extends cranially into the lary and caudally into the upper trachea. The epithelium lining the cryp adjacent to the nodules is thin and infiltrated with lymphocytes. Coı pound glands, which are mainly serous-secreting, open into the cryp In the younger animals the lymphatic tissue is not well-developed aı lymphatic nodules are scanty. Two layers of active, compound, mix mucous and serous glands are present throughout the trachea. O layer is 30–40 μm thick and has short ducts lined by simple cuboid epithelium. The other layer is thicker, measuring 50–200 μm. It exten into the fibro-elastic layer, and has ducts lined by stratified cuboid to columnar epithelium which is sometimes ciliated and contai occasional goblet cells (Figs 3, 4, 5, 6).

Electron microscopy of these glands reveals mucus-secreting ce with many membrane-bound mucin droplets up to 2 μm in diamet The droplets vary in their staining reaction and smaller ones tend to more densely stained than larger ones. The cytoplasm contains fr ribosomes, smooth endoplasmic reticulum, granular endoplasm reticulum and many mitochondria. The mitochondria range fro 1–2 μm in diameter to $1 \times 2{\cdot}5$ μm in size with prominent cristae aı occasional granules about 35 nm in diameter. The rounded nucleus located towards the base of the cell with a Golgi apparatus adjacent it (Fig. 8).

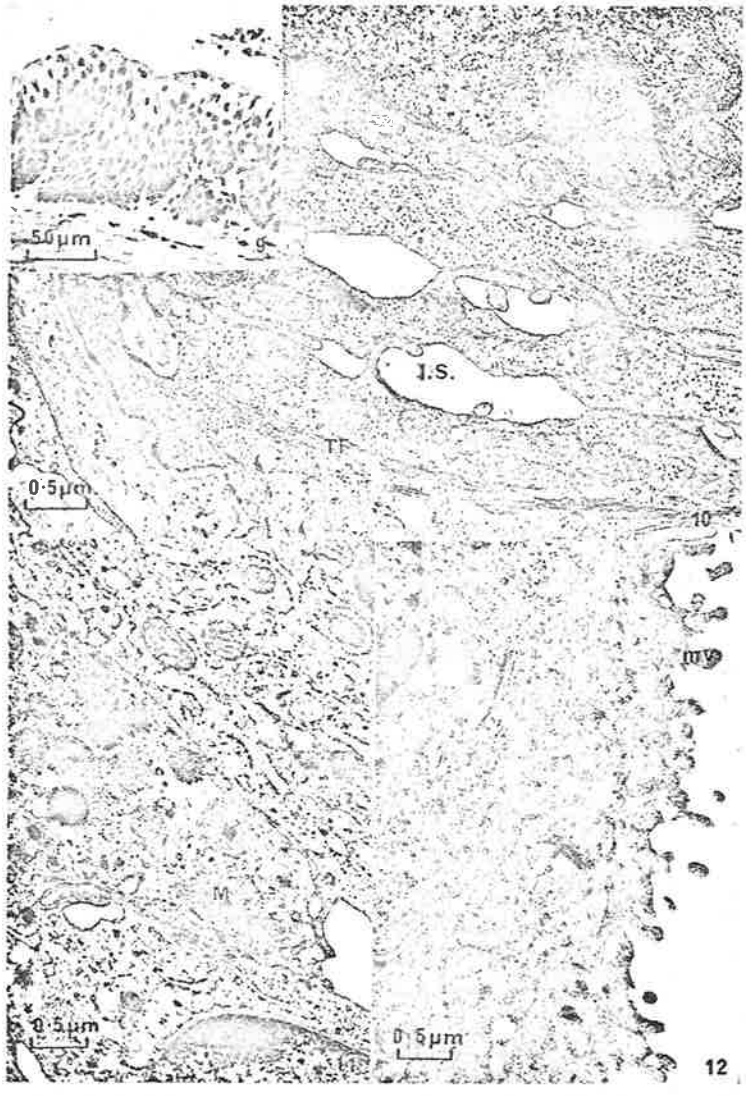
The apex of some serous cells contains large electron-dense membran bound spherical granules up to 2 μm in diameter (Fig. 7). Granul endoplasmic reticulum, a few free ribosomes and elongated mitochondı up to $0{\cdot}2 \times 2{\cdot}5$ μm are present. The nucleus is densely stained. A Gol apparatus is present. Myoepithelial cells surround each acinus, lyi between the secretory cells and the basement membrane (Fig. 7 These cells have an elongated nucleus, and have cytoplasmic process extending between the acinar cells. The cytoplasm is packed wi



FIGS. 6–8: *Fig. 6.* AD 3 Proximal trachea. Superficial layer of glands in the mucosa. H & E. *Fig. 7.* AD 5 Serous gland. Note plasma cell (P.C.). *Fig. 8.* AD 5 Mucous gland. Golgi apparatus (g) nucleus (N) mucus droplets (M) of varying electron density.

myofilaments which run in the long axis of the cell and its processes. A few spherical mitochondria, free ribosomes and endoplasmic reticulum occupy the spaces between myofilaments and nucleus.

Desmosomes are occasionally seen between the myoepithelial cells and the acinar cells. Plasma cells are seen in the connective tissue and



FIGS. 9–12: *Fig. 9.* AD 7 Proximal trachea ventral aspect. Transitional type of epitheliu
H & E. *Fig. 10.* AD 2 Proximal trachea ventral aspect. Basal cells. Note tonofilaments (T I
intercellular space (IS). *Fig. 11.* AD 2 As for Fig. 10. Intermediate cells. Note mucin dropl
(M) some with electron-dense core. *Fig. 12.* AD 2 as for Fig. 10. Apical cell with short sq
microvilli (mv) and many interdigitating processes but few desmosomes.

symphatics surrounding the glands (Fig. 7). The mucosa consists of loose connective tissue containing fibroblasts, lymphocytes, macrophages and plasma cells (Figs 3, 4, 5 and 6). There is a longitudinal elastic lamina (10–50 μm) enclosing a plexus of thin-walled veins up to 20 μm in diameter immediately beneath the basal lamina of the epithelium. There are three main types of non-keratinized epithelium. Towards the laryngeal end, the epithelium is stratified cuboidal (Fig. 9). This epithelium varies in thickness from three cells (20 μm), when stretched, to four cells (50 μm) when relaxed. The basal cells are polygonal and more densely stained, with numerous hemidesmosomes in contact with the basal lamina and with many tonofilaments (Fig. 10). There are intercellular spaces up to $0{\cdot}5 \times 1{\cdot}5$ μm in all layers of the relaxed epithelium. The intercellular spaces contain many elongated interdigitating microvilli up to $0{\cdot}1 \times 1{\cdot}0$ μm. There are also intercellular bridges with few desmosomes (Figs 10, 11, 12).

The intermediate cells show round or elongated mitochondria 0·2–1·2 μm in size, free ribosomes, smooth and granular endoplasmic reticulum and lipid droplets of varying electron density up to 1 μm in size. The surface cells in the relaxed state are bulbous, with short squat microvilli $0{\cdot}15 \times 0{\cdot}25$ μm and with tight junctions between adjacent cells (Fig. 12).

Much of the trachea is lined by stratified cuboidal epithelium tending to a columnar type in the more distal regions. This epithelium is characterized by surface microvilli ranging from $0{\cdot}15 \times 0{\cdot}25$ μm to $0{\cdot}1 \times 1{\cdot}5$ μm, and by terminal bars. Some cells show membrane-bound mucus droplets and a Golgi apparatus similar to the goblet cells seen in other regions. The basal cells are similar to those described above with less prominent membrane attachments and tonofilaments.

Some areas of the trachea, particularly in the more distal regions, are lined by a pseudostratified ciliated columnar epithelium with goblet cells up to 50 μm thick. In this epithelium four cell types are seen: basal cells, intermediate cells, goblet cells and ciliated cells. The basal cells are small, more densely stained, polygonal cells, about 5 μm in size, applied to the basal lamina. The nucleus is rounded, the cytoplasm is scanty with few organelles and some tonofilaments. The intermediate cells show features of both the basal cells and either ciliated or goblet cells. The apices of the goblet cells have microvilli $0{\cdot}05 \times 0{\cdot}15$ μm with tight junctions and desmosomes between adjacent cells. The cells contain varying numbers of membrane-bound mucus droplets up to 0·6 μm in diameter some of which have electron-dense cores up to 0·1 μm in diameter. There are many free ribosomes, a Golgi apparatus, round to oval mitochondria up to 0·5 μm in diameter and smooth and granular

endoplasmic reticulum. The ciliated cells have cilia up to 8 μm lor with a characteristic nine-plus-two axial filament complex. In the apic region there are numerous mitochondria up to $0{\cdot}2 \times 1{\cdot}5$ μm, fr ribosomes, smooth endoplasmic reticulum and densely staining ple morphic lysosomes up to 1 μm in diameter. Numerous nonmyelinat free nerve fibres are seen in each type of epithelium. No nerve endin have been identified.

IV. Bronchi

A. GROSS STRUCTURE

The bronchi give off branches at acute angles; they do not divi dichotomously. The anastomosing rings and plates of cartilage in t trachea continue down to the smallest branches of the bronchial tr The distribution of the bronchi has been reported for *Delphinus* Narath (1901).

B. MICROSCOPIC STRUCTURE

The extra-pulmonary bronchi have essentially the same structu as the trachea. The intra-pulmonary bronchi can be divided into t groups characterized by size and distinctive histological features. T major intra-pulmonary bronchus is between 0·5–1 cm in diamet It is lined by pseudostratified ciliated columnar epithelium up 40 μm thick containing goblet cells. Electron microscopy reveals structure similar to the epithelium in the trachea. Glands are seen or in the larger bronchi and are confined to the regions between cartilag The elastic lamina in the mucosa is thinner, up to 40 μm, but s consists of predominantly spiral and longitudinal fibres. The outerm layer of the adventitia of the bronchus is continuous with the fib elastic framework of the alveoli. The most striking differences betwe the extra-pulmonary bronchi and intra-pulmonary bronchi are t absence of glands in the latter and the decrease in size of the veno plexus between the cartilage and the internal elastic lamina. The plex consists of many thin-walled spirally-running veins up to 40 μm diameter. Numerous arterioles of a similar diameter are also seen. T small intrapulmonary bronchus is between 1–2 mm in diameter. characteristic features are a sudden change in epithelium and t presence of smooth muscle in its wall.

The epithelium lining the bronchial tree gradually becomes thinn as the bronchi decrease in size. However it is still pseudostratifi ciliated low columnar to cuboidal in type, with goblet cells, until t bronchi are 1–2 mm in diameter when it becomes stratified cuboic

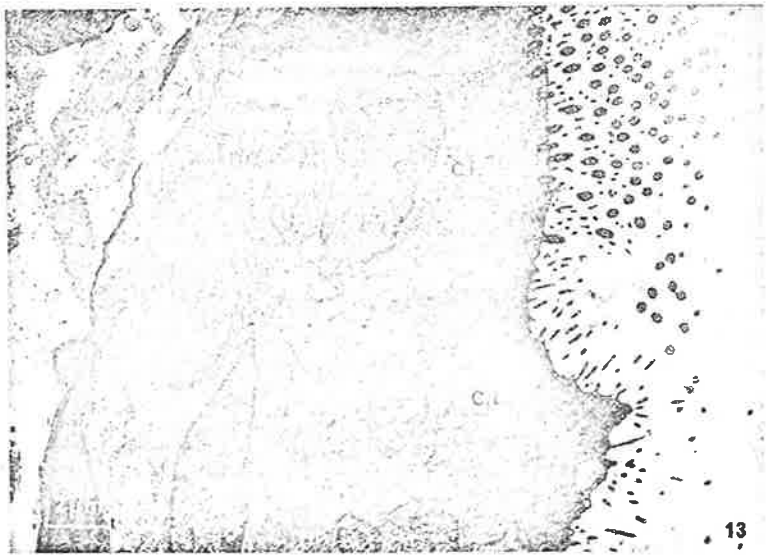


FIG. 13. AD 5 Small intrapulmonary bronchus. Stratified cuboidal epithelium with basal cells (BC) and surface cells either ciliated (Ci) or Clara type (CL).

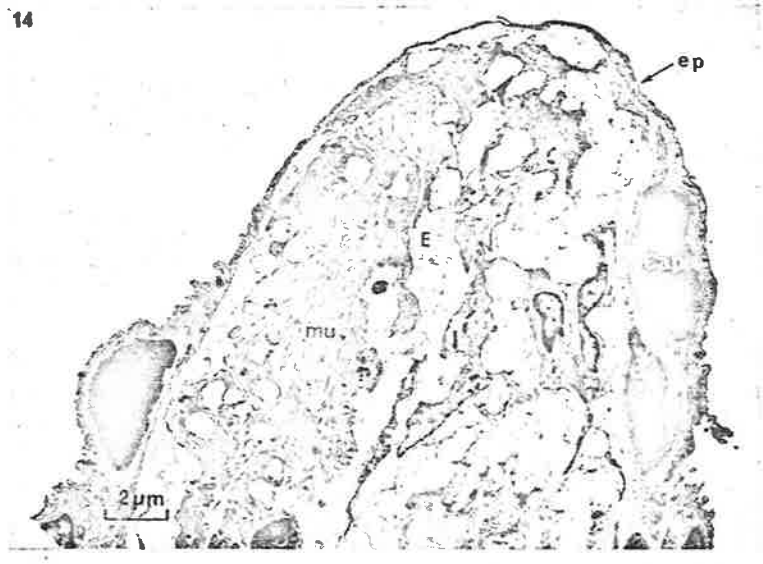


FIG. 14. AD 5 Myoelastic sphincter. Smooth muscle cells (mu), elastic fibres (E), capillary (Cap.), squamous epithelium (ep). The sphincter is covered only by thin squamous epithelium. Capillaries lie immediately beneath the epithelium.

(Fig. 13). This epithelium is 10–15 μm thick, composed of flattene basal cells, ciliated cells and Clara-type cells. Electron microscop reveals that the basal cells are flattened, more densely stained cell applied to the basal lamina where numerous hemi-desmosomes an tonofilaments are seen, and they contain few organelles. The ciliate cells are more numerous proximally. They have many cilia, mitc chondria 0·1 × 1·0 μm, free ribosomes, endoplasmic reticulum and Golgi apparatus (Fig. 13). The Clara-type cells bulge above the surfac have microvilli 0·1 × 0·5 μm, and many membrane-bound lipid drop lets 0·5–1 μm at their apical surfaces. They contain smooth endoplasmi reticulum, a perinuclear Golgi apparatus and numerous round t oval mitochondria about 0·5 μm in diameter. All cells in this epitheliu show interdigitating processes between adjacent cells. Smooth muscle first seen in bronchi about 2 mm in diameter. The bundles are discret circularly orientated, located in the gap between cartilages and l between the elastic laminae in the mucosa. The internal elastic lamin is only 20 μm thick but the external elastic lamina is thicker, up to 1(μm containing fibres up to 3·5 μm in diameter, and is attached to th perichondrium at the edges of the cartilages. Many fine elastic fibr run between the muscle fibres interconnecting the two elastic lamina

V. The Lungs

A. Gross structure

The lungs show no external evidence of lobulation and are pyramid in shape with the bulk lying dorsally and caudally (Harrison an Fanning, 1974).

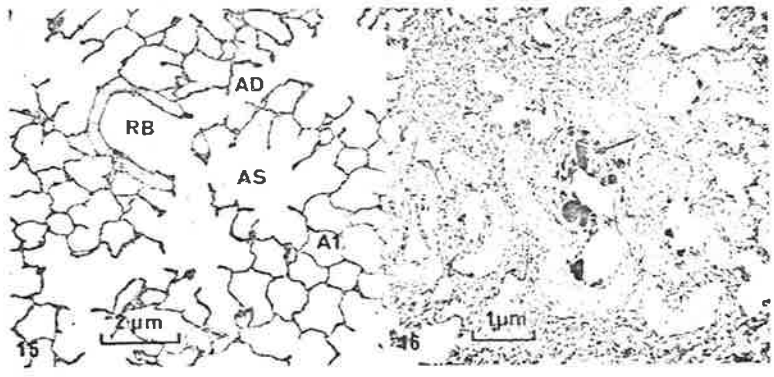
B. Microscopic structure

The respiratory division of the lung is made up of four parts: termin bronchi, alveolar ducts, alveolar sacs and alveoli (Fig. 15).

The terminal bronchus is less than 1 mm in diameter; it gives o numerous alveolar ducts before terminating by dividing into from tw to four alveolar ducts. The alveolar ducts are about 0·7 × 0·2 mm in si and appear to give rise either directly to alveoli or to alveolar sa which then give off alveoli.

The terminal bronchus is surrounded by anastomosing and inte locking cartilaginous plates arranged as rings and connected by th fibro-elastic membrane which extends throughout the bronchial tre The elastic lamina immediately beneath the epithelium consists fine fibres. It is connected to the deeper lamina by radially and spiral running fibres. Between the laminae there is a plexus of thin-walle

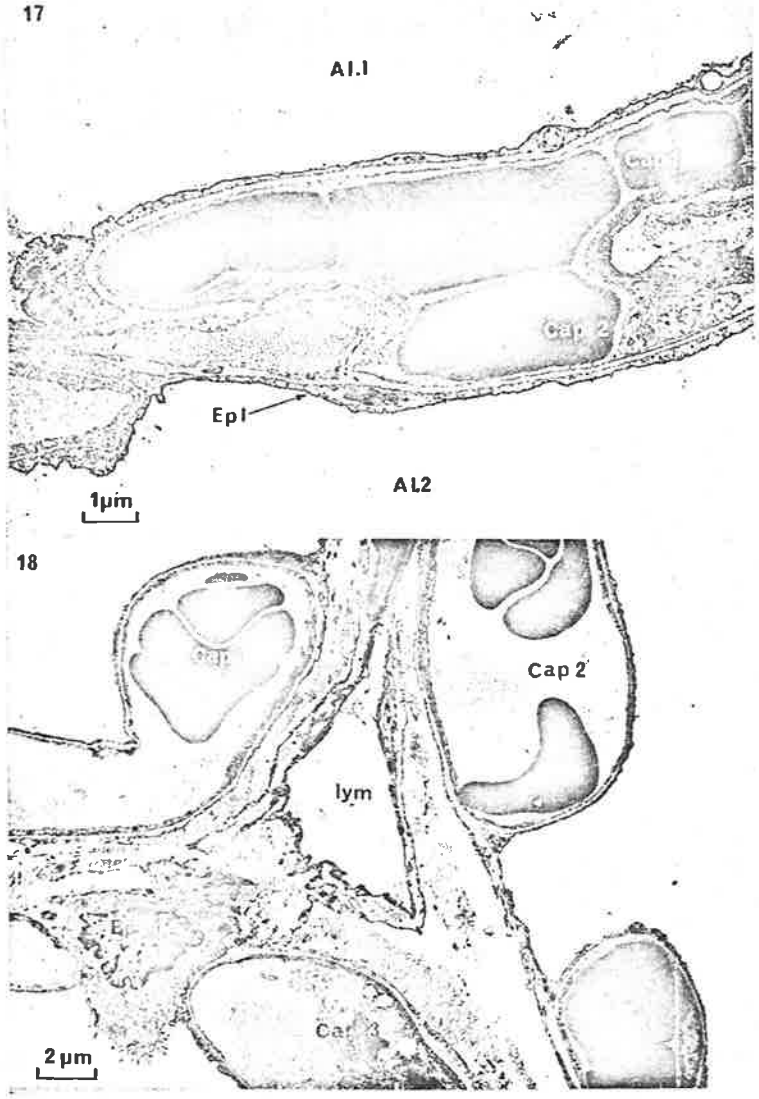
veins up to 20 μm in diameter. The muscle bundles are prominent and form definite sphincters in bronchi less than 1 mm in diameter. The sphincters are found between the cartilage rings, and at the opening of each alveolar duct, with the most distal sphincter at the termination of the bronchus. The sphincters are composed of smooth muscle fibres arranged circularly, between the two elastic laminae. The elastic fibres connecting the two laminae are more frequent in the region of the sphincter and appear to form part of it (Fig. 14). Numerous nerve



FIGS. 15–16: *Fig. 15.* AD 2 Left lung respiratory bronchus (RB) giving rise to alveolar duct (AD) alveolar sac (AS) and alveoli (Al). H & E. *Fig. 16.* AD 3 Right lung. Large densely staining subepithelial spherical bodies (arrow). H & E.

fibres are found in relation to the sphincters. Electron microscopy of the sphincter reveals typical elastic fibres from 0·2–3·5 μm in diameter between and around the smooth muscle fibres. The smooth muscle fibres are up to 5 μm in diameter, the cytoplasm is packed with myofilaments, with some mitochondria, ribosomes and pinocytotic vesicles.

Several changes in structure occur in the terminal bronchus beyond the first sphincter; the epithelium changes from low cuboidal to squamous; the tissue over the cartilages is thin, consisting of only capillaries, a few fibroblasts, collagen and elastic fibres; the capillaries in this region come very close to the squamous epithelium (Fig. 14). These changes occur gradually as the bronchus extends to its termination. The squamous epithelium over the sphincters approaches 0·3 μm in its thinnest regions. In the areas where capillaries are seen the basal laminae fuse and the blood–air barrier may reach 200 nm in thickness, consisting only of epithelium, fused basal laminae and capillary endothelium (Fig. 14). Arising from this segment of the terminal bronchus are alveolar ducts, alveolar sacs and in some areas alveoli. Each of the openings is guarded by a sphincter.



FIGS 17–18: *Fig. 17.* AD 5 Tran-septal anastomoses of capillaries – alveoli (A1 & 2 Capillaries (Cap 1 & Cap 2). Type I epithelium (Ep 1). *Fig. 18.* AD 2 Lymphatic (Lym) septal junction. Type II cell (Ep 11) seen between two capillaries.

The walls of the alveolar ducts consist of a fibro-elastic framework, continuous with the walls of the alveoli and the bronchi, and lined by an attenuated squamous epithelium. Electron microscopy reveals only collagen fibres, elastic fibres up to 0·2 μm in diameter, squamous epithelium 100 nm thick, and an occasional smooth muscle fibre.

The alveoli are polygonal in shape, 0·1–0·2 mm in diameter. They may arise directly from a terminal bronchus, from an alveolar duct or an alveolar sac. The alveolar septum consists of a connective tissue core 3–10 μm thick with blood vessels, lymphatics, fibroblasts and plasma cells. On each surface of the septum there is a network of capillaries 3–5 μm in diameter covered by the alveolar epithelium (Figs 17, 18). The two capillary networks appear to be separate, being supplied from branches of pulmonary arteries, and draining into small tributaries of pulmonary veins at the junction of alveoli. However occasional connections through the septum are seen away from these junctions (Fig. 17). The capillaries have the typical structure of non-fenestrated capillaries with tight junctions. Occasional pericytes are seen (Fig. 18). The alveolar epithelium consists of two cell types resting on a basal lamina; type I (squamous or membranous pneumonocyte) and type II (cuboidal or great alveolar cell). No type III cells (brush cells) have been identified.

The type I cells are thin and confined to one alveolus except at the free border where they are continued into the adjacent alveolus or alveolar duct. The nuclei are found mainly in the gaps between capillaries. In the perinuclear region are seen a few mitochondria up to 1 μm in diameter, granular and agranular endoplasmic reticulum, some free ribosomes and a Golgi apparatus. Away from the nucleus the cytoplasm becomes abruptly attenuated to between 30–40 nm in its thinnest areas. The cytoplasm of the type I cell in this region contains many pinocytotic vesicles 5–25 nm in diameter and few organelles.

The type II cells are relatively infrequent in the inflated lung specimens. They tend to occur in groups of two or three in niches at the junction of adjacent septa (Fig. 18), and singly in the gaps between capillaries. These cells are characterized by the presence within the cytoplasm of membrane-bound multilamellar osmiophilic bodies or cytosomes up to 2 μm in diameter. They also have numerous mitochondria, granular and agranular endoplasmic reticulum, free ribosomes, multivesicular bodies 20–60 nm in diameter, small vesicles 20–40 nm, and a Golgi apparatus. There are extensive foldings of the basal parts of the cell membrane occasionally extending into the gap between type I and type II cells. The type II cells form tight junctions with adjacent type I cells and with each other. The apical surface has microvilli 0·1 $\times$ 0·4 μm. Occasionally the cytosomes are fused with the

surface membrane. The type I and type II cells rest on a basal lamir 10–20 nm thick, which fuses with that of the capillaries in many area

The blood–air barrier consists of the thin type I cell, the fused bas laminae and the scanty cytoplasm of the endothelial cell. The thinne regions are only 120 nm across but most of the barrier is 150–250 n thick. Some areas of the septum are covered only by squamous ep thelium and in these areas the septum is only about 3 μm thick.

Mast cells have been identified in the alveolar septum. They a scattered throughout the bronchial tree but not as abundantly as other mammals. They are ovoid, 20 μm in length, with many sma processes on their surface and several larger processes up to 5 μm length. The cytoplasm is packed with membrane-bounded vesicles u to 0·7 μ in diameter which may contain fine pale granules, dense coar granules or lamellar whorls. Free macrophages are found in the lumir of the alveoli and applied to the surface cells. They contain cell debr and foreign particles. Lymphatics are found in the junction of the wal of the alveoli and occasionally in the septum (Fig. 18).

The pleura is 100–200 μm thick. It consists of fibroelastic tissue co taining blood vessels and lymphatics and has no unusual features.

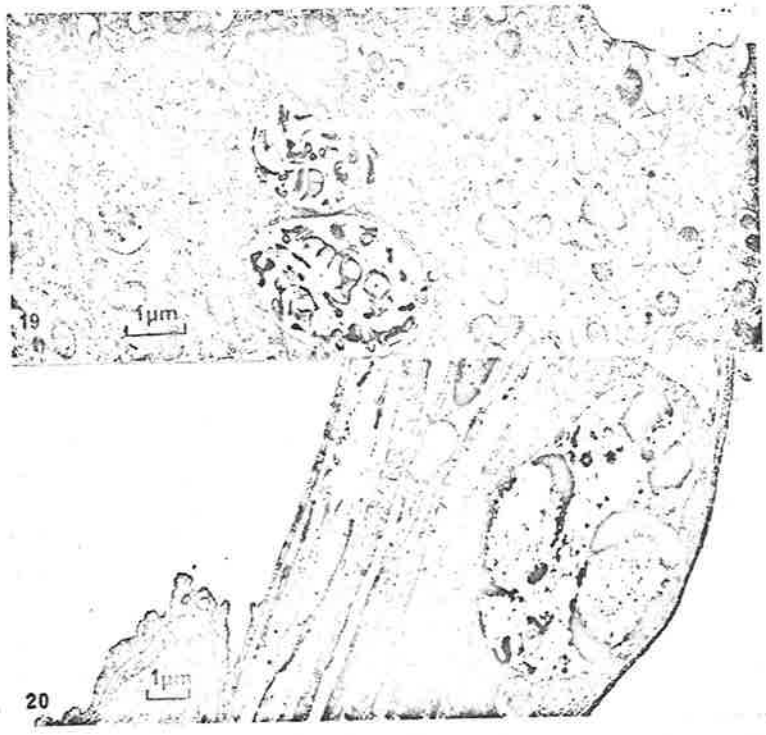
VI. Additional Findings

Also present throughout the bronchial tree and lung are numero ovoid or spherical bodies up to 25 μm in diameter (Figs 16, 19, 2C There appear to be at least three types of these bodies. The larg group, from 5–25 μm in diameter, are found mainly in the small bronchi. They are PAS positive and by electron microscopy appear have various elements arranged in a substructure. The second group a smaller. They are most prominent in the tracheal epithelium where th frequently lie close to the surface of the most superficial cells but a also present in intermediate and basal epithelial cells. They conta osmiophilic elements which may be arranged in more or less flatten concentric whorls (Fig. 19). The third group are found within macr phages either free in the alveoli or within the capillaries (Fig. 2C Identification of these structures has not been possible and although t larger objects found mainly in the smaller bronchi may be pathologic or possibly surfactant, appearances in some of the epithelial ce resemble those shown in "dust" cells (Rhodin, 1963, p. 92).

VII. Discussion

The existence of a left apical bronchus arising from the trachea has n previously been described in any cetacean. Lymphatic tissue a

lymphatic nodules have been described by Lacoste and Baudrimont (1933) in the lamina propria of *Phocoena*. Simpson and Gardner (1972) state that lymphoid aggregates are sparse and without nodule formation. In our specimens, which have been obtained directly from the sea, and not from aquaria, lymphatic tissue in the trachea is prominent. It is situated on the ventral surface between the cricoid cartilage and



FIGS 19–20: *Fig. 19.* AD 5 Trachea. Intracellular inclusions in transitional epithelium *Fig. 20.* AD 5 Lung. Macrophage containing inclusions at capillary lumen.

the first tracheal ring. This is the most dependent part of the respiratory conducting system, and the area in which secretions and foreign material would collect. We believe that these lymphatic collections have a similar function to the pharyngeal tonsil of other mammals.

Wislocki (1929) has described the trachea of the Atlantic *Tursiops truncatus* and has reviewed the available literature. He considered that the tracheal glands were sparse and serous secreting, and denied the presence of mucus-secreting glands. Lacoste and Baudrimont (1933) have described mixed serous and mucus-secreting cells in the trachea of *Phocoena*. Simpson and Gardner (1972) state that mucous glands are

fewer than in terrestrial mammals although one animal (a *Tursiop* in their series did show plentiful mucous glands. In an earlier stud Fanning and Whitting (1969) have described mucous glands in fo delphinid genera including *Tursiops*. The present study shows th there are both mucous and serous glands in the trachea of the Sou Australian *Tursiops*. Wislocki (1929) and Simpson and Gardn (1972) state that there is ciliated columnar epithelium without gobl cells lining the trachea. Both Lacoste and Baudrimont (1933) ar Bonin and Bélanger (1939) have described stratified, non-ciliate columnar epithelium.

Fanning and Whitting (1969) have described pseudo-stratifie tracheal ciliated columnar epithelium with goblet cells in four delphini genera, including *Tursiops*. In the present study three main types epithelium have been described lining the trachea: a stratified cuboid type which resembles transitional epithelium, with bulbous surfa cells in the relaxed state; a stratified cuboidal to columnar epithelium and pseudostratified ciliated columnar epithelium with goblet cell

The exact distribution of the three types of epithelium should b examined in detail. Transitional epithelium is clearly present at th proximal end, occurring in islands over the gaps between the cartilag though occasional islands are found over the cartilages and as fa distally as the carina.

Ridgway *et al.* (1969) have shown that in a deep dive to 300 metr the alveoli of the dolphin lung collapse and the inspired air, approx mately seven litres, is compressed to a volume of 200–260 ml, which much less than the volume of the trachea, bronchi and air sinuses. Th extensive venous plexuses in the submucosa of the trachea togethe with the thoracic and vertebral retia, could well become engorged o deep dives and could help prevent complete collapse of the trache Venous plexuses in the lining of the middle ear and auditory tube seals have had a similar function ascribed to them by Ramprasha *et al.* (1972).

Because the dolphin trachea collapses dorso-ventrally when excise does so on diving, and shortens and elongates during expiration an inspiration, it is tempting to postulate that the transitional type ep thelium is present at sites of maximal movement. It seems equall likely that a mobile transitional epithelium could be related to area where engorgement of the subjacent venous plexus would cause d formation on stretching.

The large intra-pulmonary bronchi are also lined by pseud stratified columnar epithelium with both ciliated cells and gobl cell sand surrounded by irregular rings and plates of cartilage. A muscl layer is absent.

In the usual mammalian pattern, muscle bundles are seen connecting the dorsal ends of the cartilage plates in the trachea and in the intrapulmonary bronchus. The muscle becomes a definite spirally running layer between the cartilage and epithelium in the smaller intrapulmonary bronchi. When the cartilage plates are lost in bronchi of less than 1 mm in diameter spiral muscle forms the outer layer of the bronchiole (Krahl, 1964).

In our specimens smooth muscle first appears in bronchi 1–2 mm in diameter as circularly arranged bundles alternating with the cartilage plates. At first these muscle bundles do not form complete sphincters; however in bronchi less than 1 mm in diameter sphincters are seen. Myoelastic sphincters or valves in the dolphin lung were first described by Barbosa (1914) and later by Feibiger (1916), Lacoste and Baudrimont (1926) and Wislocki (1929). They have subsequently been described by numerous authors. There has been much discussion about the lining of this portion of the bronchial tree. Electron microscopy confirms the statement by Wislocki (1929) that there is a change from the cuboidal epithelium lining the bronchus at the level of the first sphincter to a squamous type of epithelium with closely related capillaries. The terminology for the distal part of the conducting system and the divisions of the respiratory portion of the lung has been confused by attempting to relate it to the usual mammalian pattern. Krahl (1964) says that the usual pattern is terminal bronchus, bronchiole, terminal bronchiole, respiratory bronchiole, alveolar duct, alveolar sac and alveoli. A bronchus becomes a bronchiole when it loses its cartilage and is generally less than 1 mm in diameter. A respiratory bronchiole gives rise directly to alveoli.

On the basis of our findings in the most distal branches of the bronchial tree (namely cartilage still present in a bronchus less than 1 mm; muscle sphincters; low cuboidal epithelium changing to squamous in some areas; alveoli arising directly from the bronchus) it would be not unreasonable to describe the segment as a respiratory bronchus. The function of the sphincters has been the subject of much debate. Lacoste and Baudrimont (1926) and Baudrimont (1959) thought that the valves closed as the dolphin dived, trapping air in the alveoli and thus preventing their collapse. Also, as the pressure increases with the depth of the dive more gas would dissolve in the blood.

Ridgway (1972) has stated that alveolar collapse occurs at about 100 metres. It would seem more likely that the sphincters act by controlling the flow of air into and out of the alveoli. The latter function was first suggested by Goudappel and Slijper (1958). The sphincters could also prevent a sucking in of alveolar tissue as the dive becomes deeper.

The structure of the interalveolar septum of alveolar lungs, an of the epithelial lining has recently been reviewed by Weibel (197 and 1973). The usual mammalian pattern for the alveolar septum i a capillary network exposed to the air on each side of the septur supported by a fibrous tissue core which varies in thickness, bein thinnest in the smallest mammal (the etruscan shrew) and thickes in the monkey and dog (Weibel, 1973). The structure of the cetacea lung with its double capillary network was first described by Feibige (1916) and subsequently by Lacoste and Baudrimont (1926), Neuvill (1928) and Wislocki (1929), Lacoste and Baudrimont (1933), Wislock (1935), Wislocki and Bélanger (1940), Bélanger (1940), Wislock (1942), Murata (1957), Baudrimont (1959) and Ito *et al.* (1967) Simpson and Gardner (1972) published the first electron micrc graphs of cetacean lung. They have described the alveolar septum a being 15–50 μm thick; typical type I and type II cells; a blood–ai barrier 300–600 nm thick: and intra-alveolar macrophages. The state, however, that some of their material shows evidence of fixatio artefacts.

In the present study we endeavoured to achieve optimal fixation c the lung and trachea by using intratracheal instillation. We hav demonstrated that in the inflated lung the alveolar septum is 3–10 μn thick with a plexus of capillaries on each surface. The two capillar networks are however not completely independent because we hav been able to demonstrate trans-septal anastomoses away from the septa junctions. The significance and extent of these anastomoses will have t await further study by injection techniques and serial sectioning. Linin the alveoli are typical type I and type II cells. The blood–air barrier i the inflated lung averages 150–250 nm with areas as thin as 120 nm This data agrees with that published by Weibel (1973) for other mammal of this size.

Lymphatic vessels have not previously been described in mammalia alveolar septum. In the dolphin lung with a thick connective tissue cor to the alveolar septum it would not be unreasonable to expect to fin lymphatic vessels. Slijper (1962) has stated that macrophages ar scarce in the dolphin lung, while Simpson and Gardner (1972) hav described numerous macrophages in their material. In this presen study using animals obtained directly from their natural environmen we have found many free macrophages.

Although there is a relative increase in connective tissue, both carti lage and collagen and elastin, throughout the dolphin trachea and lung the general pattern agrees with the characteristic mammalian pattern There are, however, some developed tracheal features which even if no allowable as adaptations particular to diving could be advantageous t

an animal under pressure. These are: a collapsible trachea; areas of transitional epithelium which could allow for distortion of shape; large internal sub-cartilaginous venous sinuses which might engorge to occupy partially the space filled by air previous to a dive. On reduction of pressure when surfacing, return of blood to a heart freed from a diving bradycardia could reduce rapidly the volume of the tracheal venous sinuses. Together with a return of normal tracheal diameter, an intra-tracheal venous collapse would be related to expansion of contained gases and could also prepare for a sudden, maximal flow of tidal air.

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Appendix 3.

Appendix 3 consists of a partial transcript of a demonstration presented at the Fifth Annual Meeting of the Anatomical Society of Australia and New Zealand held in Adelaide in May 1967 by J.C. Fanning and H.W. Whitting entitled:"The Bronchial Tree of the Hawaiin Porpoise or, Many Toothed Blackfish". I have included three plates illustrating the specimen.

Appendix 3.

The Bronchial Tree of the Hawaiin Porpoise or Many Toothed Black-fish *(Peponocephela electra)*.

Presented as a demonstration at the Fifth Annual Meeting of the Anatomica Society of Australia and New Zealand in May 1967 by J.C. Fanning and H.W. Whitting, School of Anatomy, University of Queensland.

Specimen

The animal was stranded on the northern coast of New South Wales in January 1967. It survived for three weeks at the Tweed Heads Sea-aquarium. Post mortem examination revealed gross parasite infestation with secondary intraperitoneal infection. The animal first identified as a false killer whale *(Pseudorca crassidens)* was later found to belong to the genus Peponocephela - a Delphinid Odontocete Cetacean. It was a male, four years old, weighing about 200 pounds and was 83 inches long.

The Finished Cast.

The bronchial tree is asymmetrical. On the left a large bronchus arises from the lateral aspect of the left main bronchus 2.5 cm. from the tracheal bifurcation. This appears to correspond to the left upper lobe bronchus of man. The next branch is dorsal and corresponds to the apical (superior) branch of the lower lobe. The remaining branches arise as dorsal, lateral, medial and terminal divisions. On the right side there is a large eparterial bronchus which corresponds to that of man. The Bronchus to the lobule of Wristberg

arises from the apical branch of the eparterial bronchus. The next branch is a large dorsal bronchus. The main bronchus then gives off ventral, dorsal, lateral, medial, and terminal divisions.

Plate A. 3,1,

Fig. A. 3, 1, Cast of bronchial tree and lung before trimming. The plastic has penetrated to the alveolar ducts. Although no external evidence of lobulation was visible, there is a suggestion of an upper and lower lobe septum on each side (↓).

Plate A.3.1.

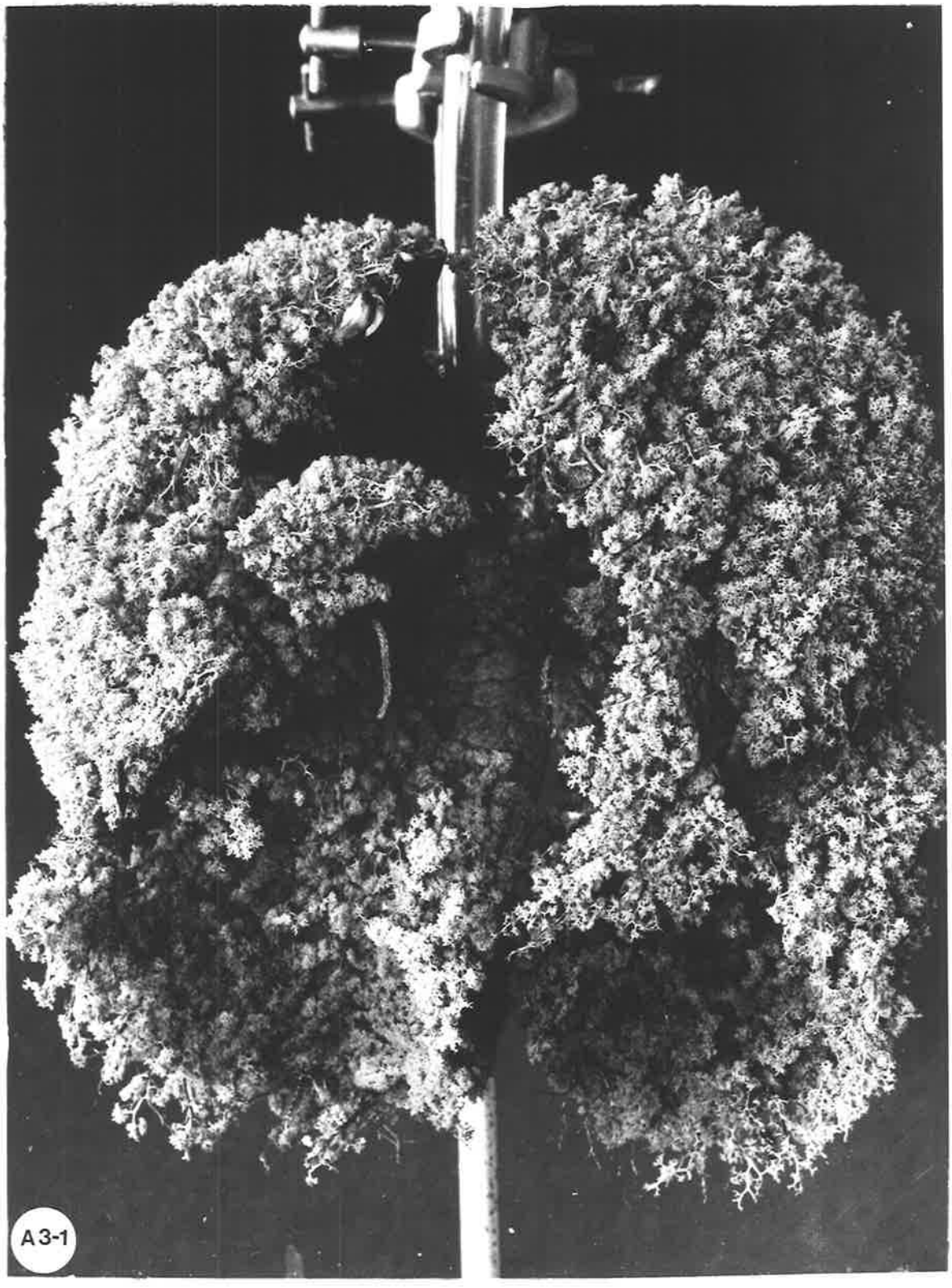


Plate A. 3, 2,

Fig. A. 3, 2, Untrimmed cast showing the respiratory bronchi (RB) and the alveolar ducts (AD).

Fig. A. 3, 3, Cast after trimming the alveolar ducts to reveal the branching of the larger bronchi (SB).

Plate A.3.2.

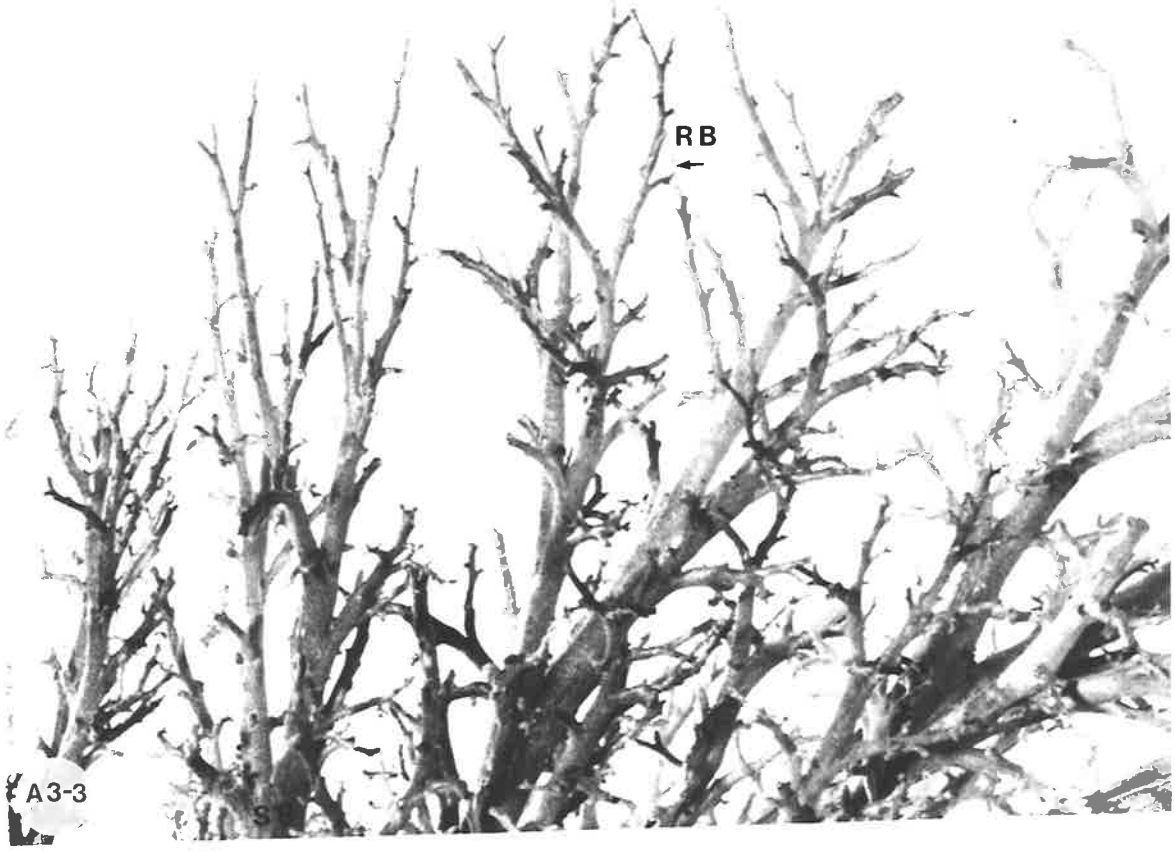
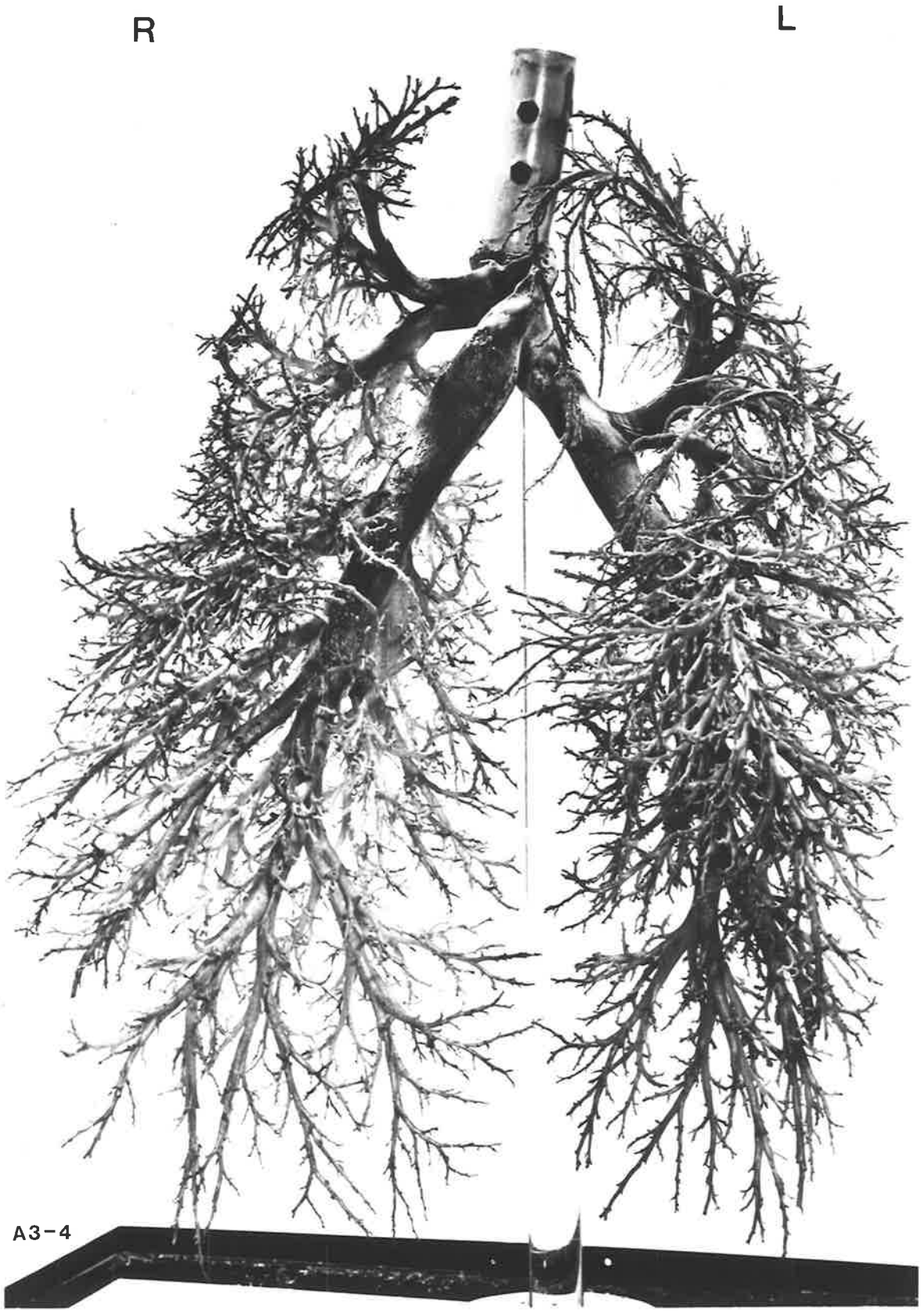


Plate A. 3,3,

Fig. A. 3,4, Trimmed cast of the trachea and bronchial tree. The specimen moved slightly during the curing of the plastic which accounts for the twisting of the branches to the right lung.

Plate A.3.3



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