# Pulmonary Pathology

M. S. Dunnill

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**M. S. Dunnill**
MA MD FRCP FRCPath
Consultant Pathologist
John Radcliffe Hospital
Fellow of Merton College
Oxford

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# Preface

The lungs occupy a unique position in the human body, receiving virtually the entire cardiac output. Of immense structural complexity they are concerned not only with gas transport, mixing and exchange but also with homeostasis; increasingly it is realised that their role as an endocrine organ is important. In writing this text, as well as describing gross and histopathological appearances, I have tried to place considerable emphasis on aetiology, functional changes and clinicopathological correlation. Nowhere has this been more important than in describing the role of tobacco smoking. We may reflect with James I of England, and VI of Scotland, that tobacco smoking is 'A branch of the sin of drunkenness, which is the root of all sins.' One of the most interesting advances in the last ten years has been the elucidation of the role of tobacco smoke in the aetiology of emphysema to add to the already established part that it plays in causation of lung cancer.

My interest in pulmonary pathology stems from a period thirty years ago spent as a locum House Officer to Dr J. E. G. Pearson at Frenchay Hospital, Bristol. Since that time the spectrum of lung disease has undergone a remarkable transformation with virtual elimination of pulmonary tuberculosis and, following the attack on urban air pollution, the decrease in deaths associated with smog. Unfortunately other disorders, notably opportunistic infections in patients on immunosuppressive drugs and the diseases known as interstitial pulmonary fibrosis or fibrosing alveolitis, have taken their place. The lack of knowledge with regard to the aetiology of pulmonary fibrosis provides an interesting contrast with information available on the pathogenesis of glomerular disease. Safe needle biopsy of the kidney together with the application of immunofluorescent and ultrastructural techniques, heralded a considerable advance in understanding glomerulonephritis. Needle or drill biopsy of lung, while often yielding useful information, is hampered by considerable sampling difficulties. It may be that the technique of examining bronchoalveolar lavage fluid, developed following the use of fibreoptic bronchoscopy, and employed to such great effect by Crystal and his colleagues, may herald a similar advance in understanding the pathogenesis of pulmonary fibrosis.

I hope that this book will prove of value not only to pathologists but also to physicians and surgeons with an interest in chest diseases.

I would like to express my thanks to the physicians and surgeons of the Oxford hospitals who have provided me with much of the material on which this book is based. In particular I owe a debt of gratitude to the late Professor P. R. Allison, to Mr A. J. Gunning and to Dr D. J. Lane. Professor Bernard Naylor lent me valuable material for illustrations and, during a period spent in Oxford, gave me valuable advice. Dr Jean Keeling very kindly allowed me access to much paediatric and neonatal pathology which I have used in the section devoted to these subjects. Dr Christopher Wagner and Dr Roger Seal not only supplied me with material from cases of industrial lung disease but also discussed some of the difficult problems associated with its diagnosis. However, the opinions expressed on these matters are my own and any mistakes must be attributed to me. I have enjoyed excellent technical assistance from Mr A. Chaplin, Miss M. Reading, Mrs M. Rychetnikova and Mr Andrew Skinner. Over the years Denis Jerrome has given much assistance with interpretation of electron micrographs. I am particularly

indebted to Mr S. Berrisford and Boehringer Ingelheim Limited for allowing me to reproduce some scanning electron micrographs. Dr R. H. Cowdell, Dr Winifred Gray, Professor D. H. Wright and Dr A. I. Spriggs kindly supplied some material used for photomicrography. Dr Basil Shepstone kindly supplied me with many chest radiographs. Dr B. A. Afzelius, Dr G. M. Green, Dr G. J. Jakab, Dr G. Slavin, Dr Colin Soutar and Dr B. M. Wright have allowed me to reproduce some of their published diagrams. Sylvia Barker kindly supplied some line drawings. Mr Terence Lee and Dr A. H. Tomlinson have graciously given me illustrations of microbiological material. Roy Holton showed considerable patience in taking and printing the photomicrographs. The publishers and in particular Mr Andrew Stevenson and Miss Dinah Bagshaw have offered me every assistance.

I would have been unable to produce this book without the ever present encouragement of my secretary Mrs Rachel Hunt who has typed many manuscript drafts and given constant help. Once again I owe to her an especial debt of gratitude.

Oxford, 1982 M.S.D.

# Contents

# 1

# Pulmonary defence mechanisms

Most agents which are pathogenic to lung are inhaled. It is thus appropriate to examine the type of particle involved and natural defence mechanisms available to deal with those agents that settle at various levels within the air passages and lung parenchyma. Although we are concerned here mainly with infectious agents similar principles apply to particles which result in physical, chemical or immunological damage to lung.

## DISTRIBUTION OF INHALED PARTICLES IN LUNG

The site of deposition of inhaled particles depends on their physical properties as well as on the depth and frequency of respiration. The subject has been extensively reviewed by Hatch (1961) and Green (1973a). Hatch has demonstrated that the depth to which particles penetrate into the respiratory tract and the percentage of particles removed at any particular site vary with the aerodynamic particle size (Fig. 1.1) and with the breathing pattern. The term 'aerodynamic particle size' embraces density and shape as well as physical dimensions. Particles larger than 10 μm diameter are retained in upper air passages. This is mainly the result of turbulence brought about by changes in direction of air flow in the nasal cavity and in the bifurcating bronchial tree. The percentage of particles greater than 5 μm diameter retained in upper airways is very high and then decreases rapidly so that with those between 2 μm and 0.5 μm diameter all but 20–30 per cent reach the alveoli. Curiously enough particles between 0.5 and 0.25 μm tend to be retained in air passages proximal to terminal bronchioles. The highest probability for deposition

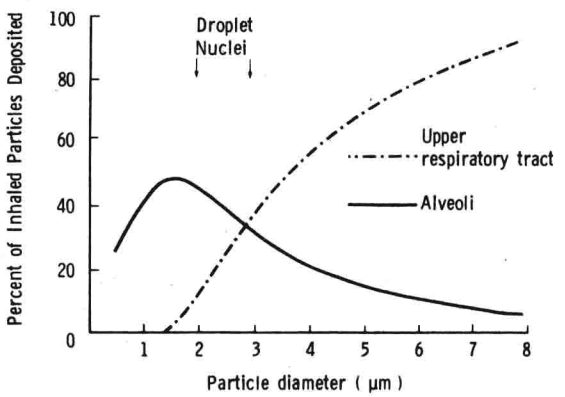



**Fig. 1.1** Graph illustrating relationship between particle size and site of deposition in the lung. Particles with an aerodynamic diameter of 2–3 μm tend to lodge at alveolar level. (Modified from Hatch 1961.)

in alveoli occurs in the size range 1–2 μm where particles are subject to gravity settlement, and below 0.2 μm, where precipitation by diffusion takes place (Hatch, 1961).

### Droplet nuclei

When a subject sneezes or coughs vigorously up to a million droplets may be forcibly expelled into the atmosphere. These are approximately 100 μm in diameter but, as evaporation occurs rapidly, residues are left which are known as droplet nuclei; these vary from 0.5–5 μm in diameter and some contain organisms. They remain in the air for several hours before gravitating to the ground. It is these particles which form the main source of pulmonary infection. The majority of particles are between 2 and 3 μm in size. Thus they are ideal for maximum penetration to the respiratory zone of

the lung. Dustborne particles of larger size tend to be retained in the upper respiratory tract.

### Two-stage ventilation and deposition of particles in the lung

While studying the intrapulmonary mixing of gases using aerosols Altshuler et al (1959) formulated a view of lung ventilation as a two-stage operation. They pointed out that with normal breathing at rest only 10 per cent or so of the tidal volume reached the residual volume of the lung and oxygen and carbon dioxide exchange within the acinus occurred by diffusion, as of course did gas transfer between blood and air across the alveolar membrane. The truth of this can easily be checked from figures for tidal air and airway volume. Thus at rest the volume of tidal air is approximately 500–600 ml. The anatomical dead space is between 150 and 200 ml so that only 300–400 ml of inspired gas passes the terminal bronchiole. Yet the total volume of gas in the acini is of the order of 4000 to 5000 ml and so many alveoli are not involved in the 'zone of active replacement' at all.

The system is extremely efficient and results in rapid and effective distribution of gas to the exchange zone in normal subjects. Unfortunately this applies not only to normal air but also to inhaled cytotoxic gases, such as ozone (Coffin et al, 1968) and nitrogen dioxide. In some instances poisonous gases may be partially absorbed in the upper respiratory tract, thus to some extent protecting alveoli; a good example of this is provided by sulphur dioxide where pathogenic effects may be found in larger bronchi which respond by hypersecretion.

## AERODYNAMIC FACTORS IN PARTICLE DEPOSITION

Deposition of inhaled particles may take place by impaction, sedimentation or Brownian movement (Newhouse, Sanchis and Bienenstock, 1976).

(i) *Inertial impaction* occurs primarily in the nares and upper air passages and although it is the principal mechanism for large particle deposition it acts on a wide range of particle sizes. It is the force which tends to cause an air-borne particle to travel along its initial path when the supporting air stream is suddenly deflected, as happens in the nose or at bifurcations of bronchi. The likelihood of particles being deposited in the airways increases with particle size and Stuart (1973) gives a probability of 33 per cent for particles 7 μm diameter, 10 per cent for those of 3 μm but only 1 per cent for those of 1 μm diameter. The probability of inertial impaction, $I$, will vary according to the expression

$$\frac{U_t U \sin \theta}{gR}$$

where $U$ is the velocity of the air stream, $U_t$ the terminal velocity of the entrained particle, $\theta$ the angle of deflection of the air stream, $R$ the radius of the airway and $g$ the gravitational constant.

(ii) *Sedimentation* or gravity settling represents the most important method of deposition of pathogenic particles in alveoli. Due to the two-stage process of ventilation, air flow in the acinus approaches zero and under these conditions particles of 0.5 to 5 μm are deposited as a result of gravitational forces. Any particle allowed to fall in air accelerates to a terminal settling velocity, $U_t$ where gravitational force balances resistance of air.

(iii) *Brownian movement* or particle deposition by diffusion affects very small particles of less than 0.1 μm diameter and is brought about by bombardment of such particles by gas molecules. Muir and Davies (1967) did not consider this to be an effective or important method of deposition as very few such particles appear to reach the alveoli. Stuart draws attention to effectiveness of deposition by diffusion increasing with decreasing particle size but points out that with decreasing size the probability of deposition by sedimentation decreases. This results in a size of minimum deposition which is found for particles of approximately 0.5 μm diameter.

### Physical characteristics of particles

Size, density, shape, solubility, surface structure and surface tension are all of importance in deciding the fate of inhaled particles. The question of aerodynamic size has already been referred to

but it is essential to realise that this is not just a matter of diameter. Shape is important. Thus asbestos fibres which are up to 300 μm in length but of less than 0.1 μm in diameter have been found in alveoli. Beeckmans (1970) and Timbrell (1972) have demonstrated that this is probably due to orientation of their longitudinal axis parallel to the air stream, with the result that their ultimate behaviour may in some ways be likened to spheres of 1 μm diameter. Solubility and surface area are of importance in particles where toxic absorption of pharmacologically active agents takes place. Silica and asbestos may, due to physical and chemical characteristics of the surface of inhaled particles, cause lysosomal damage and cell death (Allison et al, 1967).

## PHYSIOLOGICAL REFLEXES

The cough reflex and bronchoconstriction are two important and direct mechanisms which may immediately affect inhaled substances. Reflex constriction of bronchial smooth muscle occurs via the vagus nerve, is stimulated by direct irritation of upper respiratory passages and is often accompanied by submucosal vasodilatation and oedema. The resultant increase in resistance to gas flow in the airways in itself constitutes a protective mechanism ensuring that it becomes more difficult to inhale irritant substances and that less of these substances reach the respiratory tissue (Nadel, 1963).

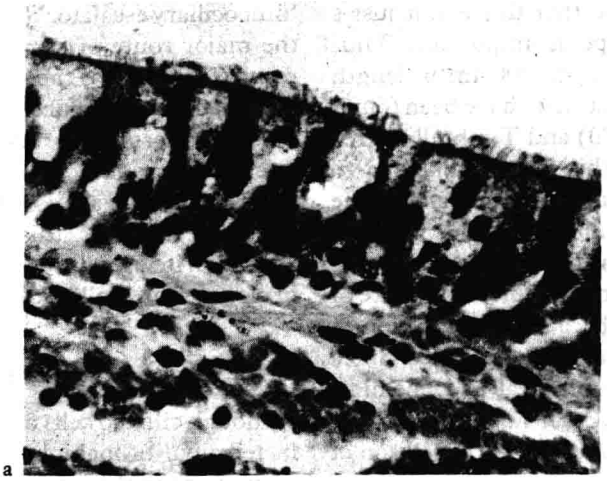
The cough reflex only operates in response to stimuli in larger airways. It is an effective method of clearing excess secretions and foreign bodies from major bronchi in normal lungs. In diseased lungs it may however have the reverse effect (Newhouse et al, 1976). In chronic bronchitis and emphysema for instance coughing may actually force secretions into more distal airways due to the high viscosity of the secretions and local differences in airway resistance.

## MUCOCILIARY TRANSPORT SYSTEM

Ciliated cells (Fig. 1.2) are present from the region of the terminal bronchiole to the larynx. The 'mucociliary escalator' (Kilburn, 1967) constitutes the major route of removal of particles from the respiratory zone of the lung via the bronchial tree. Mucus-secreting goblet cells are not normally present in terminal bronchioles but may be found in chronic bronchitis. Normally the epithelium of these small airways is lined by a surfactant-like substance and, if this is replaced by mucus, alteration in viscosity and surface tension can result in closure with collapse of distal lung parenchyma.

### Ciliated cell structure

Although ciliated cells may be relatively infrequent in terminal bronchioles they represent the commonest cell in the trachea and main bronchi where they predominate over goblet cells in the ratio of at least 5:1. Mitochondria are abundant near the apex of these cells and the nucleus occupies a basal position. Lysosomes are prominent. The most striking feature however is provided by the luminal aspect of the cell surface where there are both cilia and microvilli. Approximately 200 cilia each measuring 6 μm in length and 0.3 μm in diameter are found per cell (Rhodin, 1966). Cross section of a cilium (Fig. 1.3) reveals that the central core or axoneme is composed of a constant arrangement of two longitudinal microtubules enclosed in a sheath which is itself surrounded by a matrix containing nine double microtubules. This is the so called nine plus two structure. The outer double units are made up of subunit A which is slightly larger than the other, B, subunit. The A subunits are notable because they possess short diverging projections (Afzelius, 1969), made up of the protein dynein and thus known as dynein side arms, which are involved in ciliary motility. It is thought that bending of cilia is related to sliding of microtubules with respect to one another and that this process is facilitated by the dynein side arms (Summers and Gibbons, 1973). The outer membrane of the cilium is continuous with the plasma membrane of the cell. The fibres however continue into the outer portion of the cell cytoplasm, become triplet in nature and are associated with a densely staining structure known as the basal body from which rootlets pass further into the cytoplasm (Fig. 1.4). The precise functional significance of this complex structure is ill-understood but Sleigh (1969) has



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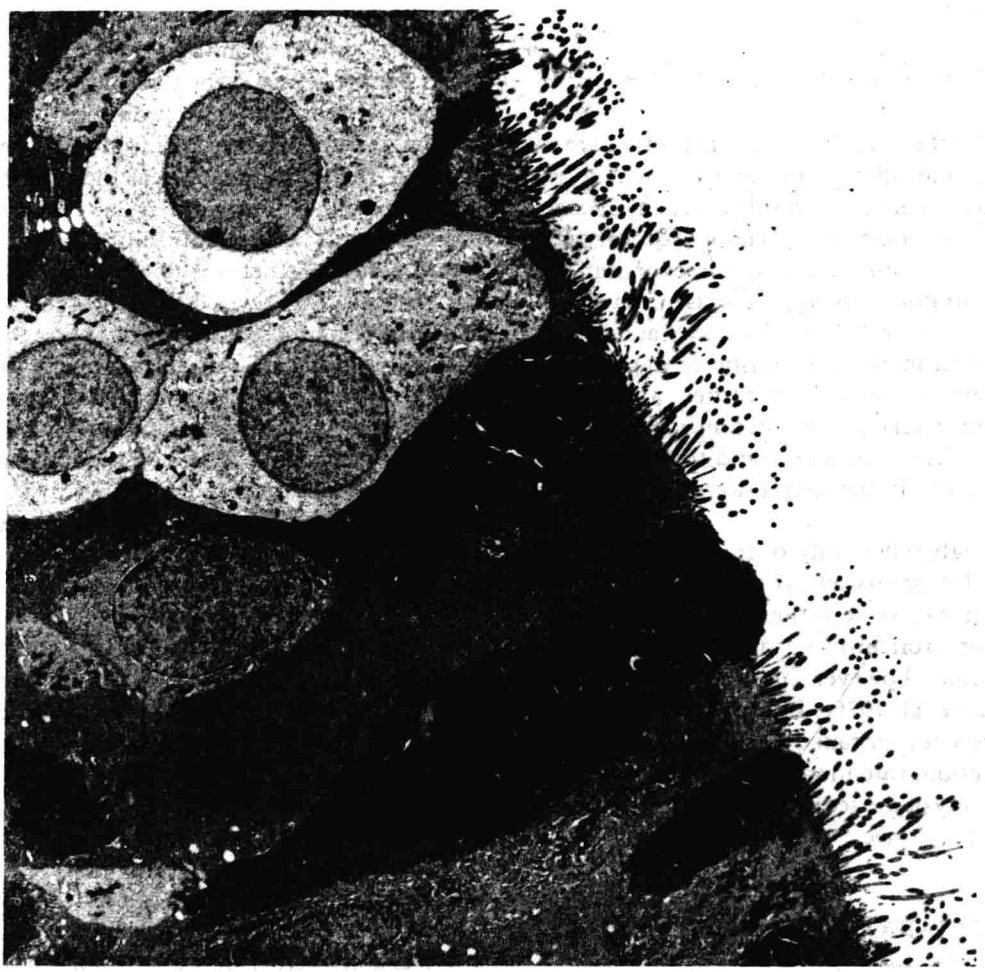

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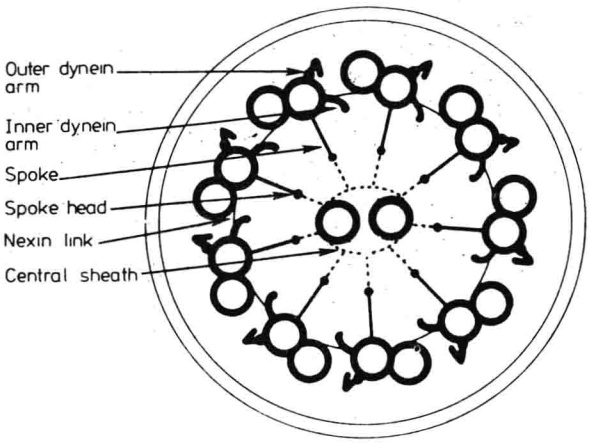
**Fig. 1.2** (a) Normal bronchial mucosa composed of columnar ciliated cells, goblet cells and basal cells. Haematoxylin and eosin × 350. (b) Electron micrograph of bronchial mucosa showing ciliated cells interspersed with goblet cells. × 2750.

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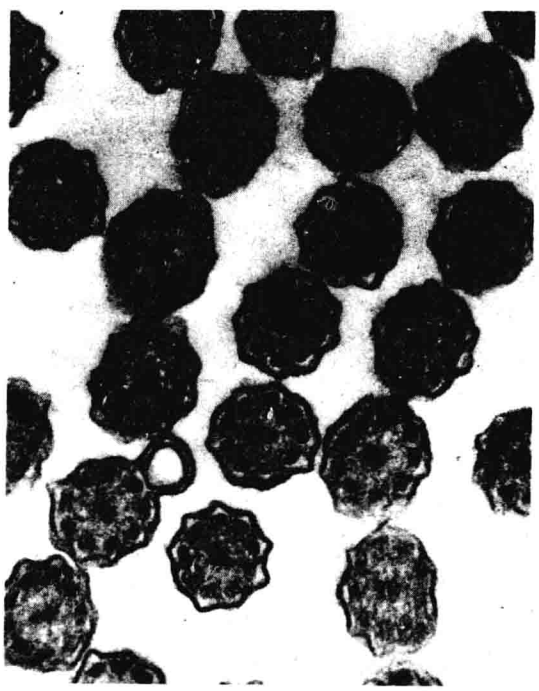

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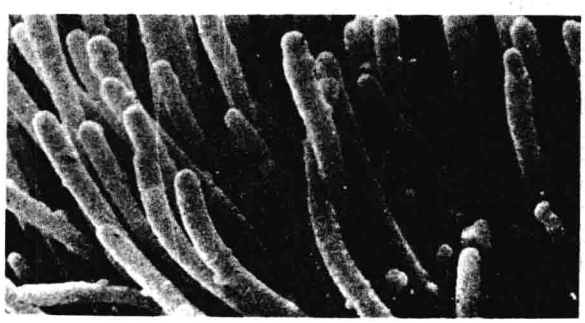

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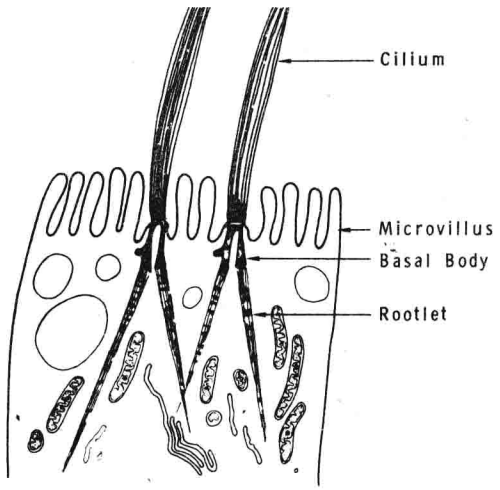
suggested that the peripheral fibres are concerned with contraction deriving their energy from adenosine triphosphate and the central fibres have a co-ordinating function.

## Ciliary function

Cilia do not all beat at the same time, that is their action is not synchronous. Instead they beat one after another, a process referred to as metachronism. (The Shorter Oxford Dictionary defines metachronism as 'an error in chronology consisting in placing an event later than its real date'). To be effective cilia have to beat in a fluid medium with the correct milieu as regards pH, salt concentration and temperature. They beat at the incredible rate of up to 25 beats per second which results in carbon particles placed on the trachea being moved up to 3.5 cm in a minute (Hilding, 1957; Kilburn, 1967). In human bronchial explants obtained by bronchoscopic biopsy Dulfano et al (1981) found an average cilia beat frequency of 12.8 per second which was unaffected by age or smoking history. This represents an 'intrinsic' frequency and clearly may be modified in situ. Each beat has two components, an effective forward stroke and a slower recovery stroke which takes approximately twice the time of the forward stroke. Asmundsson and Kilburn (1970) have demonstrated that there is a pronounced velocity gradient from small to large bronchi. Thus in the dog they found that mucociliary transport rates averaged 1.6 mm per min in distal bronchi, 4.0 mm per min in segmental bronchi and 8.3 mm per min in lobar bronchi.

Functional and histological studies have shown that the fluid medium in which cilia of the respiratory tract act is composed of sol and gel fractions (Lucas and Douglas, 1934; Bang and Bang, 1961; Kilburn, 1967). The sol component is 5 μm thick and adjacent to the cell surface, in contact with the base of the cilia and with the

**Fig. 1.3** (a) Diagram of cross section of a cilium. (Reproduced by permission of Dr B. A. Afzelius and the Editor of *Thorax*.) (b) Electron micrograph showing cross section of cilia. × 65 000. (c) Scanning electron micrograph of cilia. Photo Lennart Nilsson, copyright Boehringer Ingelheim. Reproduced by permission of Boehringer Ingelheim Ltd.

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microvilli (Fig. 1.5). Kilburn (1968) has suggested that the sol layer is secreted by Clara cells and Type II pneumocytes. He has postulated that absorption of this layer occurs via microvilli on the cell surface. This would appear to be necessary because, as the layer is of constant thickness, if the sol was not absorbed there would be a great convergence of fluid in the larger bronchi and trachea. It is also possible that the sol layer undergoes little movement and is actually secreted by microvilli of cells on which it lies.

The gel layer is formed by secretions from goblet cells and mucous glands. It measures only 2 μm in

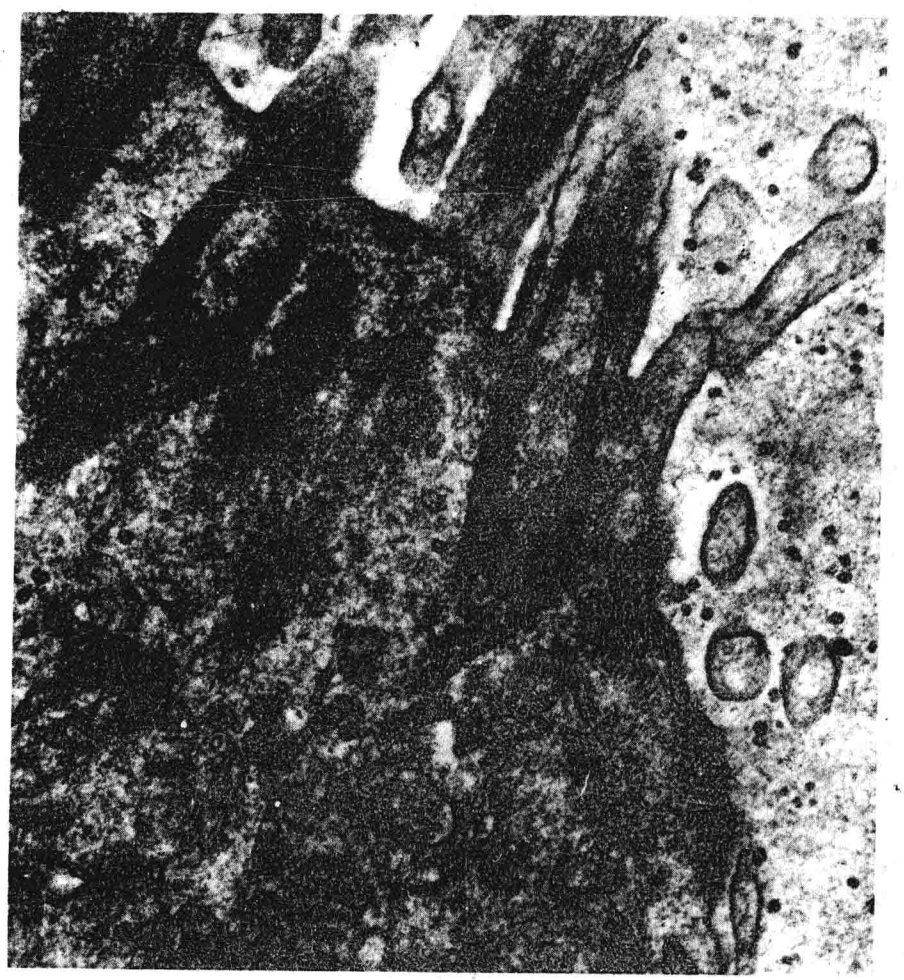

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**Fig. 1.4** (a) Diagram of luminal surface of ciliated cell. (b) Electron micrograph of luminal aspect of ciliated cell. × 80 000.

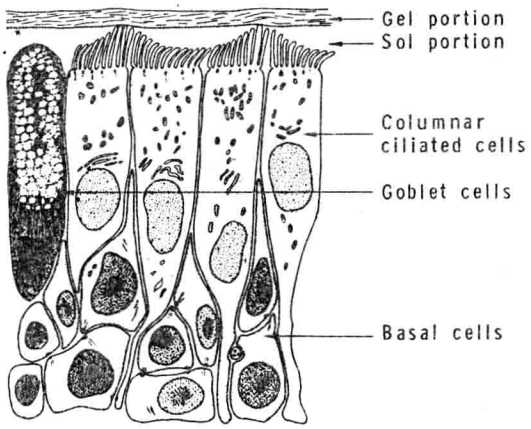


**Fig. 1.5** Diagrammatic representation of bronchial mucosa showing cilia lying in sol with their tips touching the gel portion of the fluid covering the respiratory tract.

thickness and has much greater viscoelasticity than the sol layer. The cilia are very effective in moving this gel which they only touch at the peak of their forward stroke. The gel appears to be impermeable to water and thus prevents dehydration of underlying cells in normal subjects. It also provides some protection against the action of noxious gases.

## Congenital ciliary immotility: Kartagener's syndrome

It has recently become apparent that a syndrome characterised by ciliary immotility may be related to disorders of the microtubular system of ciliated cells. In these conditions there is often both infertility due to sperm immobility and bronchial infection consequent upon ineffective mucociliary clearance (Eliason et al, 1977; Waite et al, 1978). Afzelius (1976) showed that these patients lack dynein side arms in their cilia. He also drew attention to defective or absent dynein side arms in patients with Kartagener's syndrome — situs inversus, sinusitis and bronchiectasis (Kartagener, 1933). He postulated that the situs inversus could well be due to failure of control embryonic epithelial cilia normally exert over positioning of organs. If true, the sidedness of organs in these subjects would be random and situs inversus would be expected in half the patients, which is in fact the observed frequency (Lancet, 1978).

A word of caution needs to be inserted here. Random examination of cilia from 'normal' subjects often reveals cilia which do not conform to the classical nine plus two structure. Sometimes this is due to sections being taken at the tip or base of the cilium, or due to the presence of compound cilia which are found in regenerating mucosa. Not infrequently, however, occasional cilia sectioned in the mid-shaft region may show displaced or absent peripheral microtubules and dynein arms. In order to be certain of a definite and consistent abnormality samples taken at several sites are needed.

## Interference with ciliary action

Cilia are essential for efficient clearance of mucoid exudates in the bronchial tree, particularly in those bronchi distal to regions where the cough reflex operates. If cilia are not present due to regenerative or metaplastic changes in the lining epithelium there is a likelihood of small airway plugging by mucus. The effectiveness of ciliary action is impaired if the exudate is purulent (Dulfano and Adler, 1975) and thus more viscous and less elastic with resultant slowing of transport towards proximal larger airways.

Factors influencing control of ciliary action are not well understood but oxygen is essential as under anoxic conditions cilia do not function at all. However the level of oxygen in inspired air is critical as inhalation of 100 per cent oxygen is itself ciliotoxic in man (Sackner et al, 1975) as is 7.5 per cent carbon dioxide (Marin and Morrow, 1969). Cigarette smoke is known to interfere with ciliary co-ordination and severely to retard the mucociliary clearance mechanism in vitro (Dalhamn, 1959), in experimental animals (Wanner et al, 1975) and probably in vivo in man. Cilia from smokers may sometimes have clubbed and deformed ends (Fig. 1.6). In fact all air pollutants probably have these effects though most experimental work has been performed using sulphur dioxide which markedly impairs ciliary transport of mucus in the trachea (Andersen et al, 1974; Hirsch et al, 1975) in both animals and man. Anaesthetics are important agents in the causation of ciliary dysfunction. These need not necessarily be inhaled as intravenous barbiturates have been

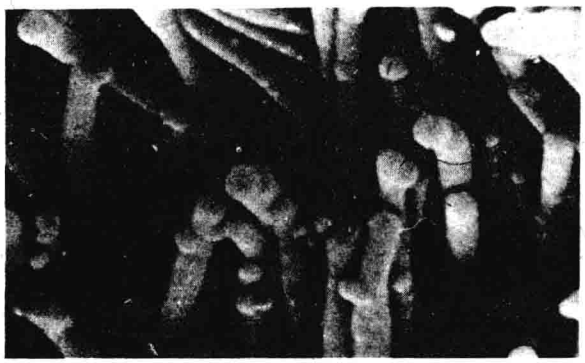

**Fig. 1.6** Scanning electron micrograph of cilia with clubbed and deformed ends. Photo by Lennart Nilsson, copyright Boehringer Ingelheim. Reproduced by permission of Boehringer Ingelheim Ltd.

shown to possess a ciliotoxic action (Landa, Hirsch and Lebeaux, 1975). It should perhaps be noted here that in a scanning electron microscopic study of bronchiolar epithelium Ebert and Terracio (1975) did not find any ciliary abnormality in smokers but did find increased goblet cell numbers and a deficit in the number of Clara cells.

## ALVEOLAR CLEARANCE MECHANISMS

Particles deposited in alveoli must be conveyed to the level of terminal bronchioles before they can contact cilia, enter lymphatics or perivascular interstitial tissues. Alveoli are lined by a thin fluid lipoprotein film of surfactant. It is probable that this fluid film moves along alveolar walls in a proximal direction towards terminal bronchioles (Green, 1973a) though the mechanism whereby this takes place is not understood. It has been suggested (Green, 1973b) that the alveolar fluid may be pulled proximally by traction from the mucociliary escalator with which it is in continuity.

### Alveolar macrophages (Fig. 1.7)

Most important in processing particles inhaled into alveoli is the action of alveolar macrophages. These are derived from bone marrow. Brunstetter et al (1971) demonstrated this in an elegant series of marrow transplant experiments in genetically-related but not identical mice. Irradiated RF/Al(−) mice were given marrow transplants from RF/Al(+) mice which carried a specific esterase marker. Eight weeks after transplantation alveolar macrophages from RF/Al(−) mice showed this esterase marker indicating their origin from the transplanted marrow cells. This was confirmed by Godleski and Brain (1972) also using mouse irradiation chimeras and employing an antigenic marker to identify donor haemopoietic cells. In man the origin of alveolar macrophages has been demonstrated in patients receiving marrow transplants from histocompatible donors of the opposite sex. Thomas et al (1976) were able to show in such cases, where the patient's own marrow had been totally destroyed by irradiation before transplantation, that some lung macrophages were of the same sex as the donor. Yet some were of the same sex as the recipient. It seems that alveolar macrophages are originally derived from marrow but that once in the lung local proliferation can occur (Golde, Byers and Finley, 1974). Certainly in experimental animals these cells have been shown to divide in pulmonary interstitial tissue (Bowden and Adamson, 1972).

Alveolar macrophages possess specific cell membrane surface receptors for IgG and the $C_3b$ fragments of the third component of complement (Reynolds et al, 1975). In infections alveolar macrophages are activated (Mackaness, 1970; North, 1970; Mackaness, 1971) probably by interaction with T lymphocytes so that they become more capable of killing and digesting phagocytosed inhaled pathogens. This property is a general phenomenon and to some extent not specific for individual organisms though in most instances the macrophage is likely to be more effective against the organism which has been used to induce activation. Thus Stubbs et al (1973) found that macrophages which were activated by infection with *Listeria monocytogenes* dealt with Listeria organisms much more efficiently than macrophages from mice infected with BCG. Powell and Muse (1977) demonstrated the importance of antibody-mediated opsonisation. In a beautifully illustrated scanning electron microscope study, they showed that addition of specific anti-*Mycoplasma pneumonia* serum to an in-vitro system containing *M. pneumoniae* and macrophages was needed to mediate phagocytosis of the organisms which did not occur in its absence.

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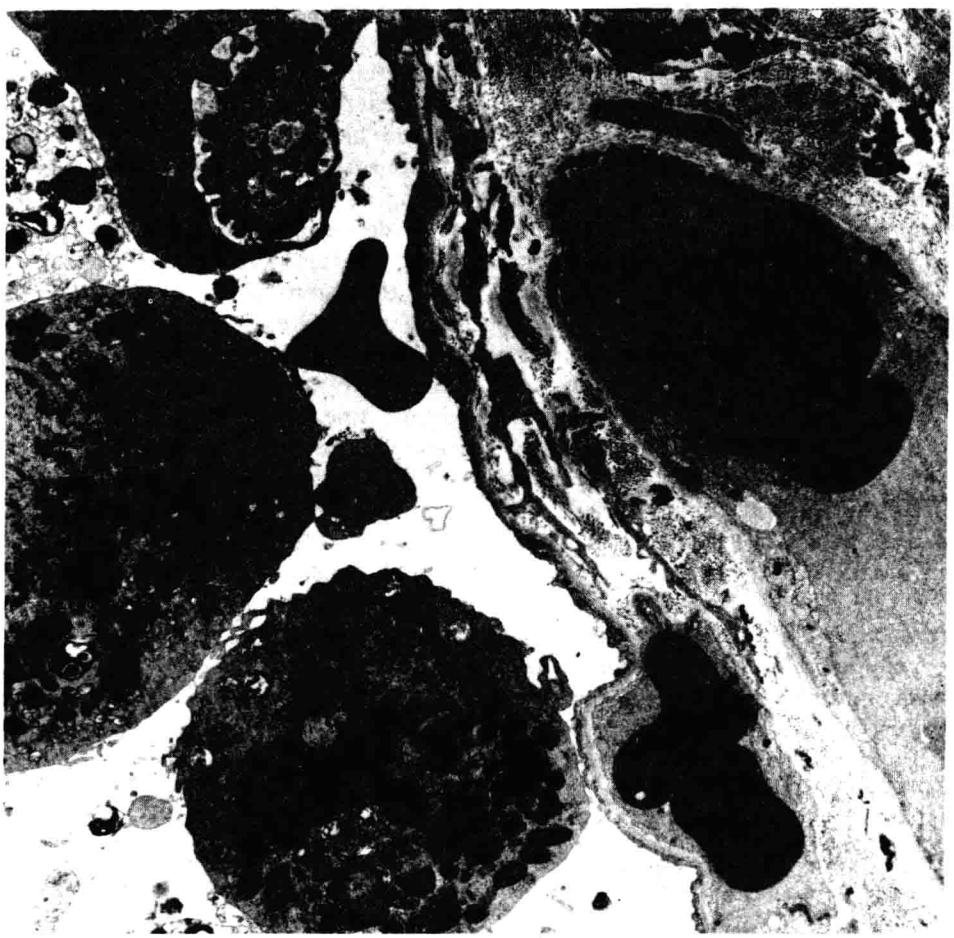

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**Fig. 1.7** (a) Scanning electron micrograph of macrophages in an alveolus. Photo by Lennart Nilsson, copyright Boehringer Ingelheim. Reproduced by permission of Boehringer Ingelheim Ltd. (b) Electron micrograph of macrophages in alveolar space lying adjacent to alveolar wall. × 5000.

The energy required for phagocytosis may well be related to ATPase activity which is located predominantly in the plasma membrane (Cross et al, 1971) and is dependent on the presence of sodium, potassium and magnesium ions.

The mechanism whereby organisms are destroyed once phagocytosis has occurred depends upon lysosomal enzymes. Peroxidase metabolism is stimulated by phagocytosis. Gee et al (1971) have demonstrated two hydrogen peroxide utilising pathways within macrophages in vitro and have discovered an enzyme, d-aminoacid oxidase, which is needed for generating hydrogen peroxide.

## Factors influencing macrophage effectiveness

As well as such seemingly obvious causes of impairment of macrophage function as irradiation, immunosuppressive and cytotoxic drugs certain more specific agents have been incriminated. Thus metabolic acidosis has been shown in the experimental situation significantly to depress the ability of the lung to kill bacteria (Goldstein, Green and Seamans, 1970). This may well be a factor in the frequent occurrence of pulmonary infections in conditions such as diabetic ketosis and renal failure.

In a series of classical experiments Green and Kass (1964a) showed that bacterial clearance from lung was dependent on alveolar macrophages and was influenced by various diverse factors. The term bacterial clearance appears to have been coined by Laurenzi et al (1964) who quantified the rate at which inhaled staphylococci disappeared from the lung. Green and Kass (1964a and b) showed that, using nuclide labelled organisms, although the viability of inhaled organisms declined, bacterial matter was still present in the lung. Further, by means of immunofluorescent studies, the organisms could be demonstrated within alveolar macrophages. Certain factors were potent in delaying bacterial clearance (Green and Kass, 1964a). Thus intoxication with ethanol, unrelated to respiratory depression, delayed the rate of removal of inhaled staphylococci. This phenomenon was dose-related. Similar effects were obtained with hypoxia and starvation and to a much lesser degree with corticosteroid therapy. Cold also markedly delayed bacterial clearance when the organisms employed were Staphylococcus albus or Proteus mirabilis (Green and Kass, 1965). The experiments on ethanol intoxication bear out early clinical observations on lowering of resistance to pulmonary infection that occurs in alcoholics (Parkinson, 1909; Pickerell, 1938).

Human alveolar macrophages require both oxidative and glycolytic energy sources for maximal particle ingestion. The extreme and unique sensitivity of human alveolar macrophages to low partial pressures of oxygen has been elegantly demonstrated in vitro by Cohen and Cline (1971). They found that phagocytosis of heat-killed Candida albicans was inhibited by iodoacetate, sodium fluoride, potassium cyanide and oxygen tensions of less than 25 mmHg. They considered that increased susceptibility to pneumonia found in some patients with chronic bronchitis or atelectasis might well be related to sub-optimal phagocytosis, or more likely lack of intracellular killing, by macrophages in areas of lung with depressed oxygen tension.

The depressant effect of cigarette smoke on macrophage activity is of great importance. Using an in vitro system Green and Carolin (1967) found that a water-filterable factor in cigarette smoke quantitatively inhibited inactivation of *Staphylococcus albus* by alveolar macrophages. They showed that nicotine, acetaldehyde, formaldehyde and cyanide in doses comparable to those found in smoke had little effect on macrophage activity. Alterations have been demonstrated in alveolar macrophages removed from animals exposed to cigarette smoke. Rats exposed for 60 days to concentrations comparable to those in human smokers (Davies, Sornberger and Huber, 1977) were found to have macrophages which were twice the normal volume, had markedly reduced surface to volume ratios and exhibited an increase in volume density of cytoplasmic lipid inclusions accompanied by a decrease in lysosomal density. In contrast Matulionis and Traurig (1977) using mice, emphasised the increase in number, size and density of lysosomal bodies and found increased activity of β-glucuronidase and glucosaminidase. They suggested that increased activity of macrophage hydrolases might reflect initial changes leading to permanent lung damage.

The precise mechanism whereby cigarette smoke

interferes with macrophage action is not understood but experiments by Mostafa, Cross and Tyler (1971) have proved that cadmium, which is present to some degree in cigarettes, adversely affects respiration of macrophages by inhibiting mitochondrial oxygen uptake, uncoupling oxidative phosphorylation and inhibiting ATPase activity. Clinical studies on students and nurses have supported these in vitro experiments. Haynes, Krstulovic and Loomis (1966) folowed 191 male students, 48 per cent of whom were smokers and 52 per cent non-smokers. The incidence and severity of both mild and severe respiratory tract infections were significantly greater in smokers than non-smokers. Indeed the incidence of severe lower respiratory infection in smokers was up to nine times that in non-smokers. Similar findings were reported by Parnell, Anderson and Kinnis (1966) in nurses where it was found that the number of days off work due to respiratory illness per nurse per year was much greater in smokers than non-smokers.

### Effect of virus infections on macrophage function

It is a well known clinical fact that viral infections of the lung, and in particular influenza, are frequently complicated by secondary bacterial infection. It appears that acute viral infection alters host resistance to bacteria. This hypothesis is borne out by experimental studies. Mice infected with influenza virus A show an impaired capacity to destroy or remove inhaled staphylococci (Sellers et al, 1961). This is a local phenomenon, as it does not affect the fate of staphylocci in other organs when infection occurs by the intravenous route, and it only lasts seven to ten days. Jakab and Green (1976) found that this inhibition of local defence mechanisms was due to a defect in the alveolar macrophage system. They infected mice with a sublethal dose of aerosolized Sendai virus and seven days later challenged both normal and virus-infected mice with an aerosol of Staphylococcus aureus. In normal non-virus-infected lungs decreasing numbers of staphylococci were found in phagocytic cells at 6, 12 and 24 hours after challenge. In virus-infected cells on the other hand increasing numbers of macrophages showed intracellular clumps of staphylococci (Fig. 1.8). They considered that the defect in defence present in virus-infected cells was primarily related to intracellular processing of ingested organisms rather than to a defect in phagocytosis.

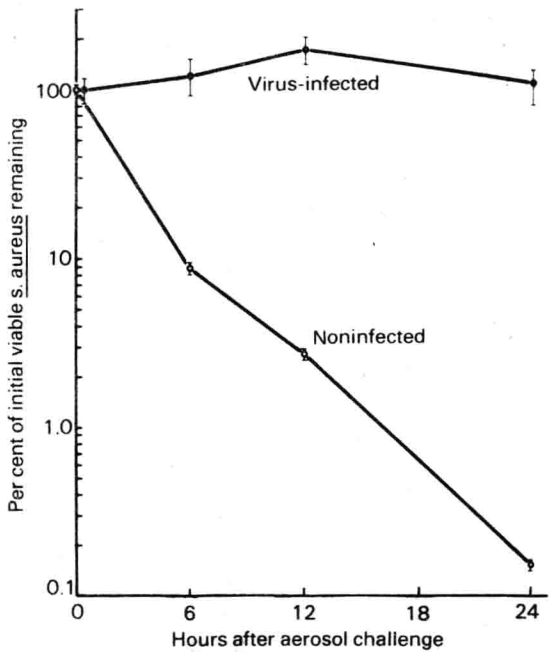


**Fig. 1.8** Intrapulmonary killing of *Staph. aureus* in Sendai virus infected and uninfected mice. The killing is seriously impaired in virus infected animals. Reproduced by permission of Dr G. J. Jakab, Dr G. M. Green and the Editor of the *Journal of Clinical Investigation.*

## LYMPHATICS IN ALVEOLAR CLEARANCE

Removal of particulate matter from alveoli does not occur entirely via macrophages migrating to the region of the terminal bronchiole and joining the mucociliary escalator. There is some evidence that at least a minor route for alveolar clearance is via lymphatic capillaries and this presupposes transport of particles through alveolar epithelium and across pulmonary interstitial tissue (Lauweryns and Baert, 1977). This is of course the principal route for removing fluid from alveoli at the time of the first breath (Aherne and Dawkins, 1964) and in adult life. The method whereby

particulate matter arrives in the interalveolar tissue is disputed but there is some evidence for transcellular vesicular transport across alveolar cells rather than carriage by macrophages through cell junctions. Corrin (1970) noted that finely divided thorium and carbon administered endotracheally to rats was found in small quantities in both Type I and Type II pneumocytes. Some was also found in interstitial macrophages. Lauweryns and Baert suggest that some particulate matter arrives in the pulmonary interstitial tissue via the transcellular route when it is phagocytosed by macrophages which then migrate into local lymphatics. This must be a relatively unimportant method as lymphatics are very scanty in the acinus except at the level of the terminal bronchiole.

## IMMUNE DEFENCES

Immune defence in the respiratory tract is largely dependent upon locally secreted antibody of the IgA class. In lung, lymphoid collections have been described in bronchi which are analogous to Peyer's patches in the small intestine. This bronchial-associated lymphoid tissue, or BALT, has been investigated in detail by Bienenstock and his colleagues (Bienenstock, Johnston and Perey, 1973a and b; Bienenstock, Clancy and Perey, 1976). They have shown that T lymphocytes form approximately 27 per cent of the cells in this tissue, some replicating locally and others being part of a circulating pool. There are no plasma cells or germinal centres but 50 per cent of the lymphocytes have surface markers for immunoglobulin. By means of a radio-labelling technique they have demonstrated that many of the lymphocytes reach the bronchial lumen while others become IgA-producing cells in the lamina propria of the bronchial mucosa. This work was conducted in mice but the distribution of bronchial lymphoid tissue and immunoglobulin-producing cells in man has been extensively studied by Soutar (1976; 1977a and b) using cell-counting techniques on immunofluorescent preparations. Immunoglobulin-containing cells were mostly present in bronchial seromucinous glands but were also found in the lamina propria of the trachea and large bronchi, with IgA-containing cells predominating, but were scanty in small airways and apparently absent in alveoli. Studies on cases of fatal chronic bronchitis revealed a deficiency of IgA-containing cells whereas in those with 'incidental' chronic bronchitis there was no such deficiency. Soutar suggested that those who die from chronic bronchitis are deficient in plasma cells containing IgA but those with 'incidental' bronchitis are normal in this respect. Examination of sputum in patients with chronic bronchitis during acute infections adds some support to this. He found that in approximately 30 per cent of such subjects there was a very low IgA content, thus upholding the hypothesis that IgA secretion is impaired in a proportion of these patients.

The effect of smoking on local production of immunoglobulin is complex but there appears to be little doubt that it results in fairly profound alterations. Soutar (1976) found that smokers who did not suffer from clinical bronchitis had significantly more IgA-containing cells in their lobar bronchi than non-smokers. He also examined the tracheobronchial lymph nodes and found that active germinal centres containing IgM-producing cells were increased in numbers in patients who had been smokers but who had not suffered from clinical bronchitis. Roszman and Rogers (1973) have demonstrated that nicotine and the water-soluble fraction from whole cigarette smoke both have a very considerable suppressive effect on immunoglobulin production by lymphoid cells in culture. The cell and immunoglobulin content of bronchial washings from smokers and non-smokers has been carefully examined by Reynolds and Newball (1976) who have shown that in smokers there is an increase in IgG content relative to IgA and also that the lavage fluid contains increased numbers of cells but that the proportion of lymphocytes is fewer than in normal subjects. There is as yet no unifying hypothesis that reconciles these diverse but apparently well established findings.

There is evidence that concentration of IgA in nasal secretions or washings correlates well with resistance to infection, and in particular viral infection, of the respiratory tract whereas concentrations of various classes of antibody in serum bear little relation to such resistance (Perkins et al, 1969a and b). The structure and function of IgA