CHRONIC BRONCHITIS IN THE DOG

Thesis submitted for the degree of Doctor of Philosophy in the Faculty of Veterinary Medicine, the University of Glasgow.

by

ERIC BRIAN WHEELDON, B.V.M.S., M.R.C.V.S.

Department of Veterinary Pathology

University of Glasgow.

October, 1974.

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

CONTENTS

ACKNOWLEDGMENTS

ACKNOWLEDGMENTS.

I am indebted to Professor W.F.H. Jarrett for his encouragement and advice in my postgraduate studies. I am particularly grateful to him for his support in allowing me full use of the facilities in his department, as a result of which I have been able to study chronic bronchitis in the dog.

Dr. H.M. Pirie has acted as my mentor in this work, giving valuable guidance with regard to techniques and general procedures. In addition, I have to thank him for stimulating my interest in pulmonary function and pathology.

Dr. R.G. Breeze has taken an interest in this work and has made many helpful suggestions and criticisms while the study was in progress.

These three pathologists have all influenced this thesis and I am indebted to them for their encouragement, advice and criticism.

It is not possible to attempt the characterization of a disease without the cooperation of clinical departments, and I was fortunate in having as my colleague Dr. E.W. Fisher, who assisted with clinical aspects of the disease; similarly, Mr. R. Lee contributed the radiological examinations.

James Murphy has been most helpful throughout the study, both in collection of material and in assistance in the post mortem room.

I have been fortunate in having received very able technical assistance throughout this study: Morag Anderson prepared and sectioned material for microscopical examination, including the histochemical manipulations, while Carole McLay prepared material for electron microscopical examination.

Dr. Helen Laird spent a considerable amount of time instructing me in the use of the electron microscope and I would like to thank her for this

training. Similarly, I must thank Dr. M. Grindlay for the bacteriological examinations carried out during the course of this study.

The illustrations and photographs in the thesis were supplied by the members of the photography department: Archie Finnie, Alan May, Colin Wilson and Jim Morrison, to whom I owe a debt of gratitude.

Jan AbuBakar and Teresa Collins typed the manuscript in draft and final form; I am indebted to both of them for their infinite patience and their general help in preparing this thesis.

Finally, I gratefully acknowledge the financial support of the Wellcome Trust; I was in receipt of a Wellcome Research Training Scholarship during the period of this study. In addition, the study was financed in part by a grant from the World Health Organization.

GENERAL INTRODUCTION

GENERAL INTRODUCTION

The main objects of this work were to delineate the features of canine chronic bronchitis and to evaluate its usefulness as a model system for the study of the human disease.

It is not possible to describe chronic bronchitis in the dog without making considerable reference to the disease in man, where it is of great importance. For this reason, the greater part of the first section of this thesis is devoted to a comprehensive review of the literature relating to chronic bronchitis in man. There is a surprisingly large amount of literature on the disease, considering that the aetiology and pathogenesis of the disease are not known; the amount of literature reflects, in large part, the efforts and speculations resulting from the intensive research of the last 25 years. I have restricted the review of the literature in man to emphasising the main theories and factors considered to be important, for subsequent comparison with findings in the dog. The problems of definition are reviewed in detail, since this has been the subject of debate; the disease in the dog has been defined using the criteria applied to man. Chronic bronchitis was known to occur in the dog but there were no detailed, definitive studies in the literature and it is significant that Done (1970) did not include chronic bronchitis in his review of canine pulmonary pathology. References to the disease in the dog merely indicate its existence, so that it is not possible to build up a complete picture. The limited literature relating to the disease in the dog is reviewed. The first section is then completed by a consideration of the desirability of an experimental model system of the disease; the attempts to produce chronic bronchitis are reviewed and the choice of species is discussed in detail.

The second and third sections of the thesis contain a description of chronic bronchitis in the dog in both qualitative and quantitative terms. The second section contains the results of a field survey into chronic bronchitis in dogs in the Glasgow area. These include the epidemiology of

the disease, the clinical picture and a detailed account of the pathological changes in the bronchial tree. This section contains a consideration of the problem of the coughing dog and gives the results of a survey of dogs entering the University of Glasgow Veterinary School (U.G.V.S.); this was an attempt to gain some information on the background number of coughing dogs in the population.

One of the lines of study currently being investigated in human chronic bronchitis is the quantification of the mucus-secreting structures of the bronchial tree in an attempt to determine when the degree of mucous gland hypertrophy becomes significant and clinically detectable. Quantification methods had not been applied to the canine bronchial tree, and the third section describes the application of the Dunnill point-counting method (Dunnill, 1962) to the bronchi of young dogs, adult dogs and dogs with chronic bronchitis. The findings in the three groups are compared and related to those in man.

The hypersecretory state of the respiratory mucins in human chronic bronchitis has been investigated by de Haller and Reid (1965); their histochemical studies on the bronchi of normal subjects and subjects with chronic bronchitis led them to the conclusion that the respiratory mucus in chronic bronchitis is normal in its constituents, but that the proportions of constituents are altered. The fourth section of the thesis contains a histochemical comparison of the bronchi in normal dogs and dogs with chronic bronchitis; the findings are related to those in man.

Ultrastructural studies of the bronchi of all species, including man, are surprisingly few; the ultrastructure of the human bronchus in chronic bronchitis is limited to one, brief account (Watson and Brinkman, 1964). The bronchus of the dog has only been investigated at the epithelial level; there appear to be no ultrastructural descriptions of the lamina propria, including the mucous glands. The fifth section is a fine structural description of the canine bronchus with particular reference to the mucus-secreting structures in chronic bronchitis.

MATERIALS AND METHODS

EXPERIMENTAL ANIMALS

POST MORTEM TECHNIQUES

HISTOLOGICAL AND STAINING METHODS

ELECTRON MICROSCOPE TECHNIQUES

BACTERIOLOGICAL METHODS

RADIOGRAPHIC METHODS

EXPERIMENTAL ANIMALS

Young dogs

Newly-weaned puppies, 6-10 weeks of age and of mixed breeding, were purchased from a commercial source. They were not vaccinated and were kept isolated, being housed indoors at an ambient temperature of 70°C. During their stay, they were fed on commercial dog food (Lassie, Pet Foods Ltd., Melton Mowbray, Leics.) and reconstituted dried milk. These puppies were kept for approximately one month before euthanasia; their lungs were used for studies of normal lung structure in the young dog.

Adult dogs

A group of adult dogs, referred to the University of Glasgow Veterinary School (U.G.V.S.) for euthanasia during the course of the investigation, were used as control animals. These dogs were examined clinically at the time of admission and found to have no clinical signs of respiratory disease. After euthanasia, the lungs were examined immediately during the course of the post mortem examination.

Dogs with chronic bronchitis

A survey of the clinical and pathological files of the U.G.V.S. revealed all cases of canine respiratory disease which had a chronic intractable cough and which had subsequently been diagnosed as having chronic bronchitis. Also, a letter was sent to all practising veterinary surgeons in the Glasgow area in October 1972 (with a reminder in October 1973) requesting notification and referral of all dogs with a cough of two months duration. These cases, together with dogs routinely submitted for other reasons, were brought to the University of Glasgow Veterinary School for a clinical examination by Dr. E.W. Fisher; further radiological

investigations were then carried out by Mr. R. Lee. A proportion of dogs was then sent home (some being subsequently re-admitted) and the remainder either died or were destroyed on the recommendation of the clinician. All dead animals were examined at post mortem.

POST MORTEM TECHNIQUES

Clinical cases were subjected to post mortem examination at various times after first examination at the U.G.V.S. Those dogs which did not die were euthanized by intravenous injection of pentobarbitone sodium (Euthatal, May and Baker, Dagenham, Essex) and immediately exsanguinated by jugular section. In all cases a full post mortem examination was carried out as soon as possible after death; in each instance, a full macroscopic inspection of all organ systems was made and tissues removed for further examination, as necessary. In all cases, the trachea, bronchi, lungs and broncho-mediastinal lymph nodes were examined. After external inspection and palpation, the trachea was opened dorsally along its length and the bronchi of one side of the lung, usually the right, were opened (the left side of the lung was detached at the level of the tracheal bifurcation for fixation *in toto* in formol saline.) Samples for bacteriological examination were obtained either by inserting a swab into a main stem bronchus or by examining the entire intermediate lobe. After being opened and examined, tissue for electron microscopy was obtained from the trachea and bronchi, and then the entire trachea was fixed in formol saline. Subsequently, the fixed lung and trachea were sampled at eleven specified sites (Fig. 1). A complete, transverse section of bronchus from each of these sites was taken, together with a representative portion of trachea and bronchial lymph node making a minimum number of thirteen blocks from each case.

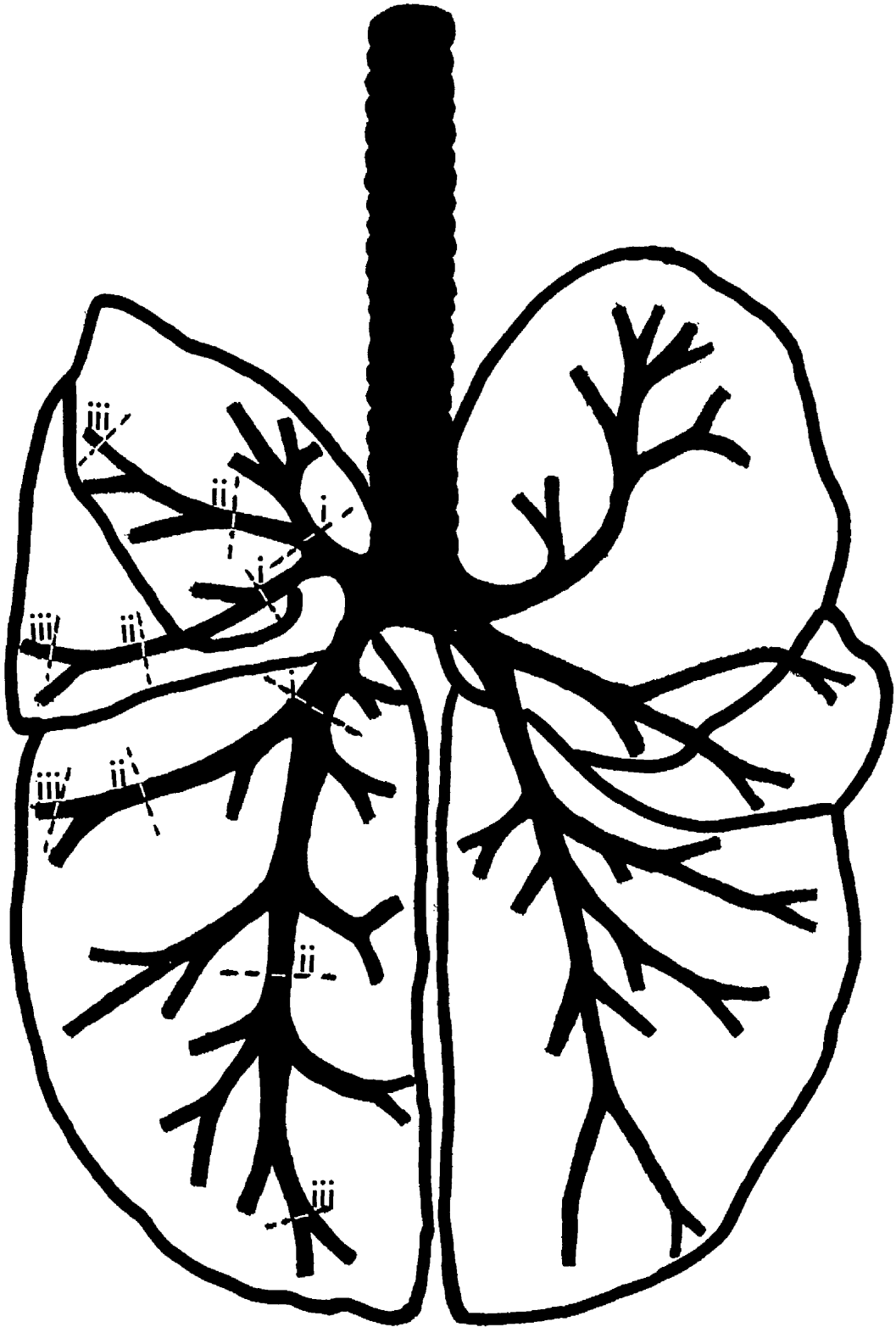


Fig. 1 : Chronic bronchitis: diagram of tracheobronchial tree of the dog indicating the standard sample sites.

HISTOLOGICAL AND STAINING METHODS

Fixation

Tissues for microscopical examination were fixed either by placing samples in fixative, or by perfusing an entire lung with fixative per trachea, or by infusion of fixative into the pulmonary vessels. Most cases were processed either by placing samples in fixative or by infusing fixative into the pulmonary vessels using a syringe. Tissues fixed by perfusing the entire lung were subjected to a constant head of fixative per trachea of 3-5cm for a period of 24-48hr. The fixative used in all cases was formol saline, prepared as follows:-

Formol saline

Formaldehyde (40 per cent)	200ml
Sodium chloride	10g
Sodium sulphate	30g
Distilled water	1800ml

After fixation, tissues were dehydrated and cleared in a double embedding series and finally embedded in paraffin wax under vacuum. Paraffin embedded sections were cut at 6-8 microns on a Cambridge rocker microtome and mounted on glass slides.

Staining

Paraffin sections were routinely stained with haematoxylin and eosin. When particular morphological changes in the bronchi were to be demonstrated more clearly, special stains were employed. These were periodic acid Schiff, alcian blue, the combined alcian blue-periodic acid Schiff technique (Mowry, 1956), Verhoeff-van Gieson, Martius scarlet blue, picro-Mallory and the carbol chromotrope method.

ELECTRON MICROSCOPE TECHNIQUES

Fixation

Small blocks of tissue 1-2mm in size were excised, as soon as possible after euthanasia, from the mucous membrane of the trachea and bronchial tree. The specimens were placed in drops of chilled fixative on blocks of dental wax, chopped into pieces 0·5mm or less in thickness using a grease-free razor blade and then transferred to vials containing chilled fixative at 4°C. The blocks of tissue were left for 1½ hours in 2 per cent or 3 per cent glutaraldehyde at 4°C, rinsed in Sorensen's phosphate buffer and then postfixed for 1 hour in 1 per cent osmium tetroxide. Tissues fixed in paraformaldehyde/glutaraldehyde remained in this fixative for 4-6 hours before being transferred to Michaelis buffer in which they were left overnight. They were then postfixed in 1 per cent osmium tetroxide for 1 hour. Tissues were also fixed in 1 per cent osmium tetroxide in Millonig's phosphate buffer for 1½ hours.

The fixatives were prepared as follows:-

(i) Glutaraldehyde: a stock solution of 25 per cent glutaraldehyde (TAAB Labs.) stabilised at pH 5-6, was used. The fixative was a 2 or 3 per cent solution in 0·067M Sorensen's phosphate buffer, pH 7·2-7·4.

(ii) Osmium tetroxide: 1 per cent osmium tetroxide was made up in Millonig's buffer at pH 7·2-7·4.

(iii) Paraformaldehyde/glutaraldehyde: a mixture of 1·3 per cent paraformaldehyde (BDH, Poole, Dorset) and 1·6 per cent glutaraldehyde was prepared in cacodylate buffer at pH 7·2-7·4. The proportions were:-

Paraformaldehyde	2g
Distilled water	25ml
1N sodium hydroxide	2-3 drops
25 per cent glutaraldehyde	10ml

Cacodylate buffer	115ml
Anhydrous calcium chloride	25mg

The buffers were prepared as follows:-

(i) 0·067M Sorensen's phosphate buffer:

KH_2PO_4 (9·118g/litre)	1 part
Na_2PO_4 (9·512g/litre)	3 parts

pH 7·2-7·4.

(ii) Millonig's phosphate buffer:

$NaH_2PO_4 \cdot 2H_2O$ (2·26 per cent)	83ml
Sodium hydroxide (2·52 per cent)	17ml
Distilled water	10ml
Sucrose	0·54g

Final pH 7·2-7·4.

(iii) Cacodylate buffer: this was prepared from a stock 0·1M solution of sodium cacodylate (21·4g/litre) as a 0·067M solution of 14·331g/litre. A few drops of concentrated hydrochloric acid were used to adjust the pH to 7·2-7·4.

(iv) Michaelis buffer:

Sodium veronal	14·7g
Sodium acetate	9·7g

Embedding

Dehydration was through an ascending series of 70 per cent, 90 per cent and absolute alcohol. The tissue blocks were then rinsed with propylene oxide before being embedded in Araldite, Araldite and Epon, or Epon epoxy-resin preparations in gelatin capsules. Araldite-embedded tissues were left at 57°C for 48 hours and Araldite/Epon embedded tissues at 80°C for 36 hours

to allow the resins to polymerise. Epon-embedded material was kept at 60°C for 24 hours to permit polymerisation.

Three preparations were used:-

(i) Araldite (CIBA-Geigy UK Ltd., Cambridge): equal parts of Araldite resin CY212 and Araldite hardener HY964 were mixed by stirring overnight and stored at 4°C. Before use, 0·6ml of accelerator DY064 (CIBA-Geigy) and 2·4ml of di-n-butyl phthalate (BDH) were added to 57ml of the resin/hardener mixture, and the whole stirred well for 30 minutes. Hardening was at 57°C for 48 hours.

(ii) Araldite/Epon:

Epon 812 (Epicote 812) (Searle Scientific Services, Bucks)	25ml
D.D.S.A.	55ml
Araldite resin CY212	15ml
Di-n-butyl phthalate	4ml

After thorough stirring, the mixture was stored at 4°C. Before use, 1·56 per cent DMP 30 (Searle Scientific Services, Bucks) was added and well mixed. Hardening at 80°C continued for 36 hours.

(iii) Epon:

Epon 812 (Epicote 812)	85·4ml
D.D.S.A.	86·4ml
N.M.A. (T.A.A.G. Labs)	28·2ml

The mixture was thoroughly stirred and kept at room temperature. Before use, 2 per cent DMP 30 was added and well mixed. Hardening was at 60°C for 24 hours.

Staining

Sections 1-1·5 microns in thickness were cut on an L.K.B. Mark III ultratome and mounted on glass slides. They were stained with 1 per cent toluidine blue in 1 per cent borax (Trump, Smuckler and Benditt, 1961). These

sections were then used to locate lesions or orientate specimens for electron microscopical examination.

Ultrathin sections were then cut on the ultratome, mounted on copper mesh grids and double stained with saturated uranyl acetate in methanol (Watson, 1958), then with lead citrate (Reynolds, 1963). Stained sections were examined with an A.E.I. 6B electron microscope.

The stains were prepared as follows:-

(i) Uranyl acetate: this was a 20 per cent solution of uranyl acetate (May and Baker, Dagenham, Essex) in absolute alcohol.

(ii) Lead citrate:

Lead nitrate $Pb(NO_3)_2$	1·33g
Sodium citrate $Na_3(C_6H_5O_7)\cdot 2H_2O$	1·76g
Distilled water	30ml
1N Sodium hydroxide	8ml
pH 12·0-12·1	

BACTERIOLOGICAL METHODS

A sample for bacteriological examination was obtained from each dog either by inserting a swab into a main stem bronchus or by examining the entire intermediate lobe; the sample was submitted to the Bacteriology laboratory for routine examination. Here it was smeared onto horse blood agar and McConky agar; in addition, a direct smear on a glass slide was stained by Gram's method and examined for the presence of bacteria. The agar plates were incubated at 37°C for 18-24 hours and then examined; colonies which had appeared were presumptively identified by films on glass slides and colonial morphology on blood or McConky agar.

In the case of Bordetella bronchiseptica, suspected colonies were inoculated into a short sugar set to confirm their identity. B.bronchiseptica

was considered to be present if there was an alkaline reaction with litmus milk, a positive reaction with stannous citrate, a negative reaction for urea, a negative reaction with dextrose, a negative reaction for lactose and a positive production of catalase.

RADIOGRAPHIC METHODS

All clinical cases of suspected chronic bronchitis were subjected to a radiological examination; a radiograph was taken of the thorax in the lateral position and in the dorso-ventral position.

CHRONIC BRONCHITIS: A REVIEW OF THE LITERATURE

THE DISEASE IN MAN

THE DISEASE IN THE DOG

EXPERIMENTAL MODEL SYSTEMS

THE DISEASE IN MAN

> "Chronic bronchitis refers to the condition of subjects with chronic or recurrent excessive mucous secretion in the bronchial tree. The words 'chronic or recurrent' may be defined as occurring on most days for at least three months in the year during at least two years"
>
> (C.I.B.A. Symposium, 1959).

Chronic bronchitis is primarily a disease of civilised man living in the temperate zones and its importance is now well recognised. Great Britain has the highest mortality rate from the disease of any country in the world (W.H.O., 1971) and within this country the disease is localised by urban and climatic factors. Despite intensive research into the disease in the last 25 years, the mortality rate is still increasing (Crofton, 1970; Registrar General, 1970,1971).

The morbidity of this insidious disease is probably more important than the mortality, when one considers the consequent social problems, National Health Service burden and economic loss to the nation. Bronchitis and emphysema account for more days of certified incapacity for work than any other illness (Health and Personal Social Service Statistics, 1972). This is given some perspective when one compares the sickness benefit figures with the Department of Employment figures for industrial disputes (Fig. 2); thus, in 1971, 13½ million working days were lost through industrial disputes, but 34½ million working days were lost through certified incapacity due to bronchitis and emphysema (Department of Employment Gazette, 1973).

The picture in Scotland reflects the overall situation in Britain; a recent report on chest services in Scotland concedes that "there is no tendency for the rate (of chronic bronchitis) for either sex to fall" (Scottish sub-committee, 1973). Crofton (1970), in a survey of male deaths

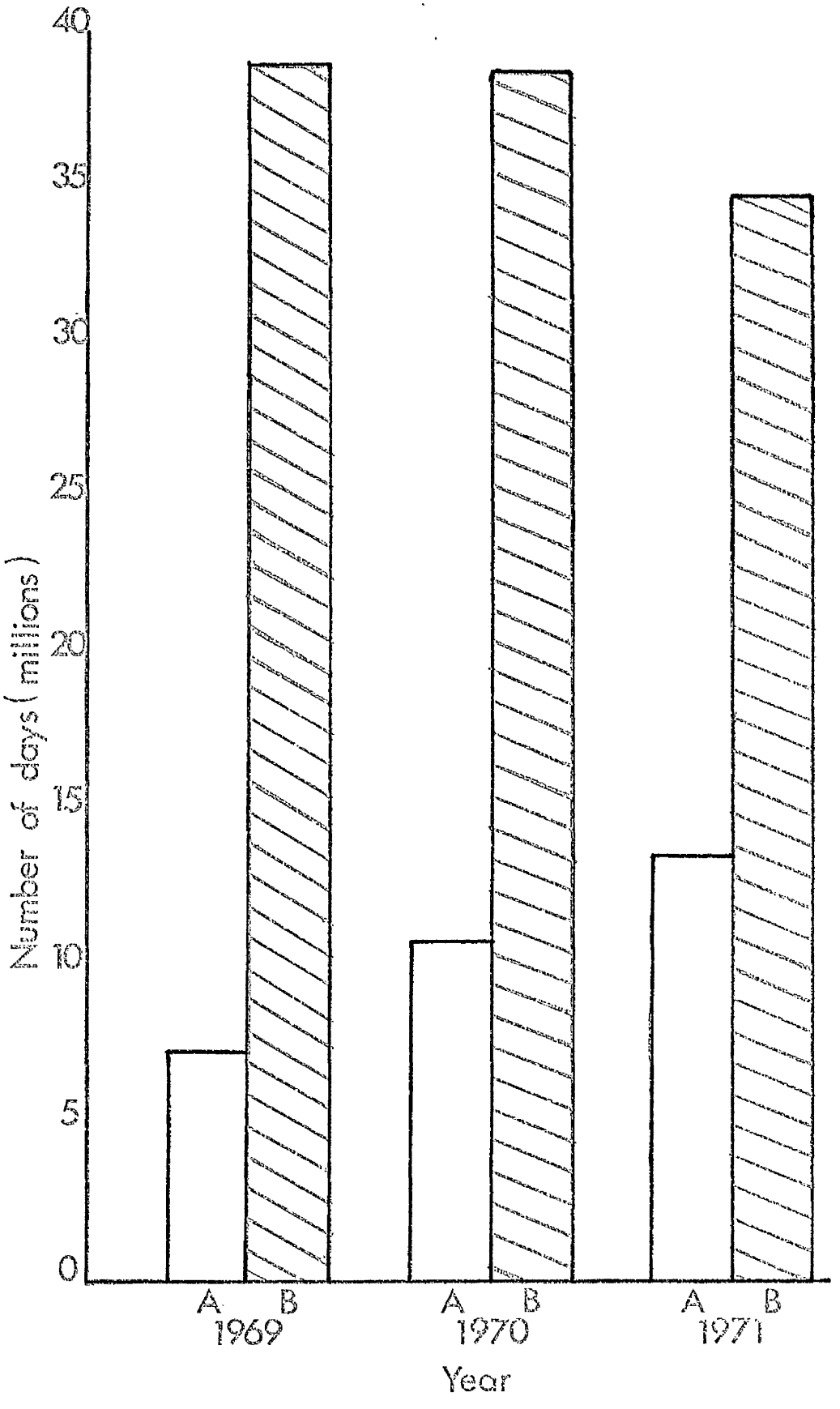


Fig. 2 : Number of working days lost due to:
A Industrial disputes
B Certified incapacity due to bronchitis and emphysema.

in Scotland for the period 1951-1965, found an increasing mortality rate from chronic bronchitis over that time. Glasgow has a high mortality rate from chronic bronchitis; the death rate per million from bronchitis, emphysema and asthma for the years 1968, 1969 and 1970 was 757, 856 and 848 respectively: this mortality level was exceeded only by deaths from each of malignant disease, ischaemic heart disease and cerebro-vascular disease (Report of Medical Officer of Health for Glasgow, 1970).

Classically, chronic bronchitis is a disease affecting middle-aged and elderly males. Deaths from chronic bronchitis occur mainly in patients over the age of 45 years (Scottish sub-committee, 1973) and are commonest after the age of 70 years (Oswald, 1958). The disease is much more frequent in male subjects than female subjects; the overall male to female ratio for deaths from chronic bronchitis in 1970 was just over 3:1 (Registrar General, 1970). This ratio has been increasing since the nineteenth century and the ratio also increases with advancing age (Crofton and Douglas, 1969). The predominance of chronic bronchitis in males is usually ascribed to their more frequent cigarette smoking habits (Crofton and Crofton, 1963). There is also a marked seasonal incidence of the disease; acute exacerbations of bronchitis in subjects with chronic bronchitis occur mainly in the winter (Caird, 1972) and, correspondingly, deaths from chronic bronchitis are more frequent in the winter months. In Scotland, 45 per cent of deaths from bronchitis occur in the first 3 months of the year (Crofton and Douglas,1969).

Although the precise aetiology of chronic bronchitis is not known, there are thought to be 3 main predisposing factors: cigarette smoking, atmospheric pollution and infections of the respiratory tract (Crofton and Douglas, 1969).

An association between cigarette smoking and mortality from chronic bronchitis has been amply demonstrated by a survey of the smoking habits of 40,000 general medical practitioners (Doll and Hill, 1964); this study

revealed a correlation between chronic bronchitis mortality rate and cigarette smoking habit. Similar prospective surveys in the U.S.A. and Canada also confirmed this correlation (United States Public Health Service, 1967). Other studies have investigated the incidence of respiratory signs in cigarette smokers; Higgins (1959) found a clear relationship between cigarette smoking, cough and sputum production. Cigarette smokers were also claimed to have more chest illness than non-smokers (Higgins, 1957). In addition to these clinical effects, there are also physiological disturbances, reflected in impaired pulmonary function tests which indicate a rise in airways resistance, probably at the level of the small airways (Seely, Zuskin and Bouhuys, 1971). Cigarette smoke impairs the pulmonary defence mechanisms, causing ciliostasis (Dalhamn, 1970) and depression of alveolar macrophage activity (Green and Carolin, 1967). Dalhamn (1970) demonstrated the ciliotoxic effects of cigarette smoke, which slowed down mucociliary clearance of inhaled particles. Clearance of foreign material in the airspaces is reduced, because of the depressant effect of cigarette smoke on alveolar macrophage respiration and phagocytic activity (Green and Carolin, 1967). Cigarette smokers have diminished amounts of pulmonary surfactant in bronchial washings (Finley and Ladman, 1972); this may be a consequence of a decreased rate of production due to interference with synthesis (Balint, Bondurant and Kyriakides, 1971) or of an increased rate of removal.

Implication of atmospheric pollution in the pathogenesis of chronic bronchitis was supported by a considerable amount of circumstantial evidence. Consideration of bronchitis mortality in England and Wales led to the realisation that bronchitis death rates increased in proportion with the size of the community (Registrar General, 1956). Daly (1959), in a massive survey of air pollution and mortality in the large towns of England and Wales, found a close association between air pollution and deaths from bronchitis.

Atmospheric pollution is a complex subject. Levels of pollutants in the air vary between countries and within countries, depending on localisation of pollutants and their interaction with particular climates. The air contains many potentially harmful agents, not all of which are man-made. Apart from organic material, such as pollen and fungal spores, the chief pollutants are by-products from industrial processes or energy production. The most important of these are smoke, dust and grit and sulphur dioxide.

Smoke from coal has been recognised as a cause of atmospheric pollution since the seventeenth century (Evelyn, 1661). However, since the "great smog" of 1952 in London, legislative measures (Clean Air Act, 1956) have reduced smoke emission from 2·39 million tonnes in 1952 to 0·77 million tonnes in 1970 (Warren Spring Laboratory, 1972). The levels of grit and dust which are emitted into the atmosphere have been further reduced by the second Clean Air Act (1968) which controls chimney heights and the Clean Air (Emission of Grit and Dust from Furnaces Regulations, 1971). The most important pollutant at present is sulphur dioxide, released from industrial processes and power stations which burn either coal or oil. Although the concentration of sulphur dioxide in urban air has decreased by 29 per cent in the period 1962-72 (Warren Spring Laboratory, 1972), total emission of sulphur dioxide in the United Kingdom has risen from 4·75 million tonnes in 1952 to 5·95 million tonnes in 1970 (Warren Spring Laboratory, 1972). Despite this, the level of sulphur dioxide in the atmosphere of urban areas rarely reaches a level of one part per million. All the experimental investigations into the effects of sulphur dioxide on the respiratory tract have utilized far higher levels than this.

Reid (1963) produced hypersecretion of respiratory mucus in rats by exposing them to sulphur dioxide at levels of 300-400 parts per million for three months; initial exposures to levels of sulphur dioxide of 40 parts per

million produced no significant changes. Sulphur dioxide gas administered to dogs only produced clinical and pathological signs at a level of 500 parts per million (Lulling, et al., 1968; Chakrin and Saunders, 1974). This does not mean that the low levels of sulphur dioxide encountered in urban areas are unimportant, since the effects of such low levels may only become apparent after many years of exposure, or be enhanced by adsorption onto particles (Amdur, 1959).

Dalhamn and Strandberg (1961) exposed rabbits to sulphur dioxide at levels of 100-130 parts per million, and found that 90-95 per cent was absorbed by the nasal mucosa, due to the high solubility of the gas. Inert carbon particles, present in polluted urban air, could act both as a vehicle for sulphur dioxide gas, carrying it past the upper respiratory tract to be deposited in the lungs, and also act as a focus for chemical reactions, such as the conversion of sulphur dioxide to sulphuric acid (Lowther, 1972). A synergistic effect between sulphur dioxide gas and dust particles has also been demonstrated in piglets (Martin and Willoughby, 1971, 1972). Martin and Willoughby (1971) exposed piglets to relatively low levels of sulphur dioxide gas of 33-36 parts per million without effect. Addition of corn dust or corn starch particles to the exposure chamber produced a reduction in the numbers of tracheal goblet cells per unit length of epithelium in piglets after 1 to 6 weeks of continuous exposure (Martin and Willoughby, 1972). It was suggested that the goblet cells had been stimulated to discharge their mucus by the sulphur dioxide gas.

Sulphur dioxide and carbon particles can also affect the immune response (Zarkower, 1972). In mice exposed to concentrations of carbon particles at 2 parts per million, there was a progressive decrease in the overall ability to produce antibody to killed Escherischia coli administered as an aerosol. Mice exposed to the sulphur dioxide and carbon had decreased amounts of antibody in their serum as measured by haemagglutination techniques. There was an initial increase of antibody production in the

spleen and mediastinal lymph nodes as measured by haemolysis-in-gel techniques but this trend was later reversed with marked immunosuppression by 192 days (Zarkower, 1972). The levels of sulphur dioxide used in this experiment can be achieved in urban and industrial environments so that this study may explain the decrease of resistance to pulmonary infections in heavily polluted areas implied by epidemiological studies (Higgins, 1971).

The precise role of infection in the aetiology and pathogenesis of chronic bronchitis is unclear. Early work naturally included bacteriological examination of the sputum in subjects with chronic bronchitis, and some emphasis was placed on the putative role of the isolated organisms, notably pneumococci and Haemophilus influenzae (Stuart-Harris, et al., 1953). Studies of the bacterial flora of the upper and lower respiratory tract in both normal subjects and those with chronic bronchitis led to the realisation that the lower respiratory tract was contaminated by micro-organisms as a result of the chronic bronchitis (Laurenzi, Potter and Kass, 1961). The lower respiratory tract in normal subjects was sterile, despite the presence of potential respiratory pathogens in the upper respiratory tract and saprophytes in the oro-pharynx. In chronic bronchitis subjects, both potentially pathogenic and saprophytic bacteria could be isolated from bronchial mucus, even when the disease was quiescent (Laurenzi, Potter and Kass, 1961). In the event of an acute exacerbation, one of these resident species, principally pneumococci or H. influenzae, predominated and gave rise to an incident of purulent bronchitis or bronchopneumonia. These acute exacerbations were usually presumed to be the result of a viral infection or exposure to an air pollutant (Green, 1970). Thus it would appear that bacteria are an important factor in the pathogenesis of the disease, particularly in exacerbating the effects of other agents. Viral infection of the respiratory tract has been thought to be responsible for acute exacerbations in the established chronic bronchitis subject (Grist, 1967)

and also to predispose to the subsequent development of the disease in subjects repeatedly infected as children (Colley, Douglas and Reid, 1973). Colley, Douglas and Reid (1973) in a prospective cohort study, found that cough and sputum production in young adults were more common in those who had had chest illnesses as infants. Previously, Reid and Fairbairn (1958) had found that men with severe chronic bronchitis in middle age had had more sickness absence due to other respiratory disease in their early career than matched controls.

In addition to these main putative causative agents, several other factors are thought to predispose to chronic bronchitis: these are genetic factors, climatic factors and socio-economic factors.

Although a certain genetic constitution may predispose to the disease (Crofton and Douglas, 1969), the contribution of genetic factors is disputed; for example, the sisters of men with chronic bronchitis tend to have a higher prevalence of bronchitis than the men's wives (Layland, 1964). This may reflect a true genetic disposition, a common cigarette-smoking habit or the shared early environment. Similarly, a survey of the relatives of subjects with chronic bronchitis attending a clinic had revealed that the relatives suffered from bronchitis symptoms three times as often as the relatives of controls (Oswald, Harold and Martin, 1953). Again, the contribution made by common smoking habits was difficult to evaluate.

The ambient climate is thought to be important in the incidence of chronic bronchitis, although there is disagreement as to how climate can best be measured. Stocks (1947) found a positive correlation between high mortality due to chronic bronchitis and low hours of sunshine recorded in 20 towns; this observation, however, made no allowance for smoke in the atmsophere, a particular problem at that time, which could account for increased bronchitis incidence and reduction in the total hours of sunshine. Holland, Spicer and Wilson (1961) examined the admissions to London hospitals

of patients with respiratory disease and found a correlation between increased incidence of respiratory disease and low atmospheric temperature. In support of this claim, the effect of low atmospheric temperature in increasing the incidence of acute respiratory disease was confirmed at R.A.F. recruiting stations (Holland, Spicer and Wilson, 1961). Reid and Fairbairn (1958), in a survey of bronchitic postmen, compared monthly trends in absence from work with levels of temperature, humidity and atmospheric pollution; they found that there was a relationship between increased bronchitis incidence and low temperatures, but this was outweighed by the effects of fog frequency. Gregory (1970), investigating sickness absence at a Sheffield steel-works over a 6 year period, found that temperature appeared to be the dominant factor in monthly sickness rates; he concluded that, whereas smoke pollution appeared to be a precipitating factor in sickness absence, low temperatures were responsible in delaying a return to work. This last observation, a delay in return to work after a bronchitic episode, may reflect another reason for the apparent link between temperature and chronic bronchitis. Holland, Spicer and Wilson (1961) pointed out that people were reluctant to go outdoors in cold weather, particularly if they had respiratory disease and that they not only tended to stay indoors, but also decreased the ventilation and thus enhanced the spread of infection. The effects of low temperature on the mucociliary apparatus have been described by Baetjer (1967), who found that mucociliary clearance varied directly with the ambient temperature. A direct broncho-constricting effect was demonstrated by Wells, Walker and Hickler (1960); they exposed subjects with chronic bronchitis who had a history of exacerbations in cold weather to very cold air and produced an increase in airways resistance, which could be relieved by bronchodilators.

Social factors form the most enigmatic problem in the pathogenesis of chronic bronchitis. Firstly, a clear correlation between the incidence

of bronchitis mortality and descending socioeconomic class, as defined by the Registrar General, was demonstrable in males (Crofton and Douglas, 1969). A similar trend was present in the wives, which tended to confirm the importance of social class (Crofton and Douglas, 1969). However, this factor is inextricably enmeshed with the possibility that occupation is a contributing factor. It is generally considered that bronchitis mortality is higher in people working in dirty and dusty conditions (Stuart-Harris and Hanley, 1957). Lowe (1968), recognising the fact that workers in dirty and dusty conditions also tended to be in the lower socioeconomic classes, tried to take account of this by comparing bronchitis mortality within a single socioeconomic class. He chose social Class IIIe (skilled workers other than mineworkers, transport and clerical workers, and members of the armed forces) and compared the eight industrial occupations with the highest mortality rates for bronchitis with the eight with the lowest; the results indicated that all the occupations with high bronchitis mortality involved exposure to atmospheric pollutants at work (Lowe, 1968). Lowe (1968) concluded that, other factors (particularly cigarette smoking and standard of living) being equal, chronic bronchitis was twice as common in dusty occupations; in addition, cigarette smokers were much more likely to be affected than non-smokers.

Secondly, there is some evidence for an urban factor in the aetiology of chronic bronchitis. Holland and Reid (1965), in a survey of vehicle drivers in London and 3 county towns (Gloucester, Peterborough and Norwich), found that the older men (over 50 years of age) in London had more severe respiratory signs such as chest illness, dyspnoea and sputum production and poorer results for lung function tests than the men in the county towns. Holland and Reid (1965) thought that these differences were at least partly due to different levels of air pollution. It may be that the urban environment has other factors operating to exacerbate the situation,

such as overall population density, housing density and localised overcrowding. All these characteristics of urban life would promote, singly or in combination, the transmission and perpetuation of infections, particularly of the respiratory tract.

From the review of the literature so far, it is easy to build a theoretical, composite picture of the subject with chronic bronchitis. That subject will be male, 40-50 years of age or over and will smoke cigarettes. He will live in a large town and work in a semi-skilled or unskilled capacity at a dirty and dusty occupation. It is likely that several of his male relatives will have clinical signs of bronchitis or at least have a cough and sputum production. He may have had chest illness as an infant and is likely to have a history of episodes of respiratory infection into his early working life. The subject will have had clinical signs of cough and sputum production, particularly in the morning, over a number of years; this will have been regarded as normal and dismissed as a 'smoker's cough'. However, over the years, these signs will have increased in severity and winter chest infections will have become more common and more intractable; the subject with chronic bronchitis will find himself increasingly incapacitated and reliant upon antibiotic therapy. His general medical practioner has been consulted too late in the course of the disease and can only dispense antibiotics to minimise the increasingly frequent acute exacerbations. The eventual outcome may well be pneumonia, respiratory failure, chronic cor pulmonale and congestive heart failure.

However, it is far from easy to systematically dismantle this composite picture and accurately pinpoint the precise contribution made by each of the various factors implicated in the pathogenesis of chronic bronchitis. An appreciation of the complex nature of the disease, its multiple aetiology with attendant predisposing and exacerbating factors, together with its imperceptible onset and its insidious progress through

very unremarkable stages over a period of years, is vital to an understanding of the disease. Understanding is not helped by the lack of standardization of techniques in the epidemiological surveys. Chronic bronchitis incidence has been measured by mortality rates (Registrar General, 1970 and yearly), numbers admitted to hospital (Holland, Spicer and Wilson, 1961), records of sickness absence due to bronchitis (Reid and Fairbairn, 1958; Gregory, 1970), retrospective questionnaire surveys of clinical signs (Higgins, et al., 1956) and pulmonary function tests (Higgins, 1957). The influence of climate has been measured by hours of sunshine (Stocks, 1947) and mean daily temperature (Holland, Spicer and Wilson, 1961). Atmospheric pollution has been measured by 'fog frequency' (Reid and Fairbairn, 1958), local fuel consumption (Daly, 1959), smoke pollution (Gregory, 1970) and sulphur dioxide levels (Gregory, 1970). Ferris (1973) has stressed the need for comparable field studies which can standardize for cigarette smoking, residence and occupation. Standardized questionnaires and diagnostic criteria are essential for the diagnosis of chronic bronchitis (American Thoracic Society, 1962; Medical Research Council, 1965) and the minimization of observer variation (Fairbairn, Wood and Fletcher, 1959).

The aetiological and predisposing factors are also very difficult to separate satisfactorily; the interrelationship between socioeconomic class and occupation has already been discussed. Similarly, the possibility of a genetic disposition to develop chronic bronchitis and an inherent tendency to smoke cigarettes cannot readily be analysed. The urban factors thought to be important may be a simple reflection of atmospheric pollution, or may involve more complex variables such as housing density in low socioeconomic class communities. The predominance of chronic bronchitis in males has been assumed to be due to cigarette smoking habits (Crofton and Crofton, 1963); it may also be partly explained by the greater incidence of lower respiratory tract illness in male children (Glezen and Denny, 1973).

The current definition of chronic bronchitis is a clinical, descriptive one and so the disease is usually only detected in its advanced stages (Macklem, 1972). Little is known about detection of the early stages of the disease. Recent work by Hogg, Macklem and Thurlbeck (1968) indicated that the early lesions of chronic obstructive lung disease were in the small airways.

The diseases which make up the syndrome of "chronic obstructive pulmonary disease" are chronic bronchitis, emphysema, asthma and bronchiectasis; these all have the common patho-physiological abnormality of increase in resistance to bronchial air flow (Thurlbeck, Henderson and Fraser, 1970). Chronic bronchitis and asthma have clinical, descriptive definitions, whereas emphysema and bronchiectasis have morbid-anatomical definitions (C.I.B.A. Symposium, 1959). Thus, it is difficult to categorize subjects with emphysema clinically, and, conversely, to categorize cases of chronic bronchitis at post mortem examination, due to lack of clinical, diagnostic criteria in the former and pathological criteria in the latter (Heitzman, Markarian and Solomon, 1973).

Although the definition of chronic bronchitis is clinical in nature, the term chronic bronchitis is more applicable to a morbid-anatomical description. The name implies a chronic inflammation of the bronchi; this would include hypertrophy of the mucus-secreting structures, a typical inflammatory response of a mucus-secreting surface (Florey, 1970). This last feature is now accepted as the hallmark of chronic bronchitis and is mainly responsible for the clinical features of cough, sputum production, wheeze and dyspnoea (Crofton and Douglas, 1969). Early definitions of chronic bronchitis were based on individual features, such as dyspnoea (Oswald, 1958) and the incidence of infection (Higgins, et al., 1956). Eventually, cough and sputum production were adopted as the definitive signs. Participants at the C.I.B.A. Symposium of 1959 defined chronic

bronchitis in clinical, descriptive terms of cough and expectoration viz. "chronic bronchitis refers to the condition of subjects with chronic or recurrent excessive mucous secretion in the bronchial tree" (C.I.B.A. Symposium, 1959). The condition is manifested by a chronic productive cough and other possible causes of this, such as tuberculosis, pneumonia and bronchiectasis, must be excluded. The phrase "chronic or recurrent" was arbitrarily suggested to mean "occurring on most days for at least three months in the year, during at least two years". A similar definition was adopted shortly thereafter by the World Health Organisation (W.H.O., 1961) and in the United States of America by the American Thoracic Society (American Thoracic Society, 1962). This definition has since been used for survey work (Bates, Woolf and Paul, 1962) and in standard medical textbooks (Crofton and Douglas, 1969).

Several people have advocated the use of a clinical-descriptive term to replace 'chronic bronchitis'. Laennec (1819, 1826) used the term 'pulmonary catarrh' and Scadding (1963) suggested the term 'chronic bronchial catarrh'. More recently, Anderson and Foraker (1971) have criticised the present definition as merely describing a state of bronchorrhoea and have stressed the need to add a morbid-anatomical component to include epithelial metaplasia, leucocytic infiltration, vascular congestion and oedema.

Most of the research on chronic bronchitis has focussed on the hypersecretory component of the inflammatory response, because of the profound functional effects of mucus hypersecretion, especially cough and narrowing airways. There are surprisingly few full accounts of the pathology of chronic bronchitis in the literature; even Robbins (1967) did not include a description in his standard pathology textbook. The chief description is that by Reid (1954), who examined the bronchial tree of 16 subjects known to have chronic bronchitis, together with bronchial biopsies from 6 subjects with chronic bronchitis and pneumonectomy specimens from 8 subjects with

both bronchial carcinoma and chronic bronchitis. Reid (1954) compared this material with tissue from 10 controls and found changes at the bronchial, bronchiolar and alveolar levels. The main pathological features were: excess mucus in the airways due to hypertrophy of mucous glands and increase in the number of goblet cells, extension of goblet cells into the bronchioles, oedema of the bronchial wall with swelling of the basement membrane and an infiltration by "small cells" (Reid, 1954). In more advanced cases, there was extension of lesions into the terminal airways with plugging of bronchioles by mucus, with peribronchial fibrosis and scarring. Bronchi and bronchioles were often slightly dilated with focal narrowing due to fibrosis. The accompanying alveolar changes were focal areas of pneumonia, with organisation of exudate, oedema, emphysema, collapse and collections of mucus in the alveolar spaces (Reid, 1954). Reid (1954) also described the formation, in advanced cases, of purulent bronchiolitis with abscess formation and narrowing and obliteration of the lumen.

A later description by Reid (1958) emphasised the infiltration of the bronchial wall by "chronic inflammatory cells", the localised nature of the repeated acute inflammatory episodes and the dilatation of the capillaries of the bronchial wall. Reid (1958) also described scarring of the bronchial wall with ulceration and the appearance of "stratified epithelium".

Several specialized techniques have been applied to the bronchial wall as an adjunct to the descriptive pathology; one of these is the histochemical examination of the respiratory tract mucins. The hypersecretory state of respiratory mucins in human chronic bronchitis has been investigated by de Haller and Reid (1965); comparison of material from normal bronchi and bronchi from patients with chronic bronchitis led them to the conclusion that the mucus produced in chronic bronchitis was composed of normal constituents, but that the proportion of these constituents was abnormal.

Thus, in chronic bronchitis, there was an increased number of distended mucous cells, a higher proportion of which contained acid mucosubstances, particularly those which are neuraminidase resistant (de Haller and Reid, 1965).

Another technique has been the application of morphometry to the bronchial wall in an attempt to quantitate the bronchial wall components, particularly the hypertrophied mucous glands. Several techniques are available for this purpose and it has been possible to detect a significant increase in the proportion of bronchial wall occupied by mucous glands in subjects with chronic bronchitis (Reid, 1960).

Finally, an ultrastructural comparison of bronchial epithelium from normal subjects and subjects with chronic bronchitis has been made (Watson and Brinkman, 1964); the results indicated a loss of cilia and an increase in the number of mature goblet cells in diseased subjects.

Chronic bronchitis is an important, but poorly understood, disease of man; it is complex in aetiology and difficult to diagnose consistently. In particular, the pathological changes are not well described, though certain techniques, such as morphometry and histochemistry, have been applied with some success.

THE DISEASE IN THE DOG

Although respiratory diseases in the dog are common and have been investigated by many workers, chronic bronchitis has not been defined or described in clinical and pathological terms consistent with those used in man.

Respiratory disease is a major problem in canine medicine (Wright, et al., 1974). Actual figures for the incidence of respiratory disease are difficult to determine; in a recent survey of dogs at the U.G.V.S., nine per cent of all dogs aged four years and over were reported as having a cough at the time of admission. Canine respiratory infections are particularly common in epizootic forms where large numbers of dogs are housed together, such as in kennels, breeding establishments and research establishments (Appel, et al., 1970). Canine respiratory disease can be an important cause of morbidity and mortality in dogs kept for long term investigations (Snow, et al., 1969); Rubin (1967) is quoted by Snow, et al., (1969) as stating that 17 per cent of 3,650 dogs arriving at the Walter Reed Army Institute of Research subsequently died of respiratory disease.

Diseases caused by infectious agents are well recognised and have been extensively investigated in recent years. The roles of canine distemper virus, canine adenovirus, canine herpesvirus and other agents have been described (Wright and Cornwell, 1968; Wright, Thompson and Cornwell, 1971; Wright, 1973). However, information concerning chronic obstructive pulmonary disease in the dog is scanty. Despite the various attempts to use the dog as a model for chronic bronchitis (Lulling, et al., 1968; Chakrin and Saunders, 1974) there was no satisfactory description of naturally-occurring

Done (1970), in the most recent review of canine pulmonary pathology, did not include chronic bronchitis, although he suggested that it might

assume greater importance as a result of more critical study. Most standard veterinary pathology texts fail to mention the disease in the dog at all (Smith and Jones, 1972; Jennings, 1970) or dismiss it briefly e.g. "chronic bronchitis with polypoid growths on the mucous membrane is occasionally seen in dogs" (Nieberle and Cohrs, 1967). Jubb and Kennedy (1970) provide a fuller account of the post mortem lesions and histopathological findings in chronic bronchitis, but do not single out the dog as a particular problem or give details of the animals upon which the observations were based.

Peacock and Archibald (1968) give a concise clinical picture of chronic bronchitis in the dog and suggest that the disease may be a primary disease or develop as a sequel to a systemic infectious disease or pulmonary parasitic infestation.

Although there are several reports of diseases resembling chronic bronchitis in the dog, all lack the clinical and pathological detail that would enable the disease to be characterized according to the criteria adopted by medical workers. The most relevant reports are those of O'Brien and Kelley (1968) and O'Brien (1974); O'Brien and Kelley (1968) described bronchitis in general clinical terms, stressing the infectious nature of acute bronchitis and the persistent nature of chronic bronchitis.

O'Brien (1974) discussed the possible aetiological mechanisms, radiology, clinical signs and treatment of cases of chronic bronchitis in the dog. The differential diagnosis of their condition was not considered, there was no description of the pathology and no actual cases of chronic bronchitis were referred to in the review.

Chronic respiratory disease in the dog has been noted on several occasions. Although many of the cases in these reports have some features consistent with chronic bronchitis, there is a constant lack of the detailed clinical and pathological data that would enable a precise

diagnosis to be made. The syndromes have been discussed under a variety of names including tracheitis and bronchitis (Rohrbach, 1970), mucoid impaction (Castleberry, 1965) and bronchiectasis (Archibald, Clacken and Bishop, 1955).

Chronic obstructive pulmonary disease in man embraces four syndromes; chronic bronchitis, emphysema, asthma and bronchiectasis. There are few reports of the latter three syndromes in the dog.

Reif and O'Brien (1968) outlined the circumstances in which pulmonary emphysema could be found clinically and radiologically in the dog and stated that diffuse emphysema was an extremely rare disease. Schiefer, Hurov and Seer (1974) have recently described pulmonary emphysema in a dog in association with polyarthritis.

It has been claimed that asthma does not occur in dogs (Halliwell, 1974), although an experimentally induced condition has been defined in dogs sensitised with ragweed extract (Arkins and Hogan, 1973).

Bronchiectasis has been diagnosed from time to time in single animals (O'Brien, Reif and Schryver, 1966).

One of the aims of this thesis was to establish the existence of chronic bronchitis in the dog in such a manner that useful comparisons could be made between the canine and human conditions.

EXPERIMENTAL MODEL SYSTEMS

The possibility of an animal model for chronic bronchitis has not been systematically pursued; this is surprising in view of the complex aetiology of the disease in man and the consequent difficulty in excluding other variables in either retrospective surveys or prospective cohort studies.

The dog is well suited for comparative environmental studies, since it shares man's environment very closely, breathes the same air and lives out its natural life span. This view has also been put forward independently by Nielsen (1968). In addition, the dog is known to be susceptible to tumours associated with atmospheric pollution, such as squamous carcinoma of the bronchus and tonsillar carcinoma (Ragland and Gorham, 1967; Nielsen, 1971). In a comparative study of the mucous-secreting structures of the bronchial tree, de Haller (1969) stressed the need for care in the choice of suitable experimental animals and the value of observations made on naturally occurring chronic bronchitis in other species, citing as examples the dog and horse quoted by Jubb and Kennedy (1970).

A wide variety of animals has been exposed to respiratory irritants in experimental situations and a number of parameters have been measured to assess the effect of these irritants. The influence of cigarette smoking on bronchial clearance in the donkey has been described by Albert, et al., (1971), while the influences of cigarette smoking on histological changes has been investigated in the dog by Hammond, et al. (1970). Long term exposure to sulphur dioxide has been studied in the dog by haematology and pulmonary function tests (Lewis, et al., 1973) and in zynomolgus monkeys (Macaca irus) by these methods and by biochemistry and histology (Alarie, et al., 1972). In addition, a wide range of laboratory animals has been used in studies involving exposure of the respiratory tract to atmospheric

pollutants. This includes the rat (Reid, 1963; Parkinson and Stephens, 1973), mouse (Bowden and Adamson, 1971), rabbit (Dalhamn, 1970), guinea pig (Flint, Maxwell and Renzetti, 1971), Syrian hamster (Asmundsson, Kilburn and MacKenzie, 1973) and the chicken (Battista and Kensler, 1970).

It is obviously very important to have an understanding of the responses of the respiratory tract to irritants, whether these are single gases, particles, aerosols or complex mixtures, such as cigarette smoke. Similarly, the measurement of more than one parameter is desirable if one is to build an understanding of the structural and functional changes taking place. However, a better knowledge of these changes and effects would be possible if there were some standardization in the choice of experimental animal. Such standardization would result in an uniformity of dosage rates and experimental techniques and would allow a comparison of the different effects in one or two species. It is not possible to compare the results in all these species directly, due to wide differences in bronchial tree micro-structure.

Many of the structural and functional changes occurring in chronic bronchitis are considered to be primarily reflections of the increased reactivity of the mucous-secreting elements in the tracheobronchial tree. Consequently, most studies in animal models have concentrated on the changes in the mucous-secreting apparatus of the bronchial tree following experimental procedures. The comparative anatomy of the mucous-secreting structures of the respiratory tract in the different species is poorly documented (de Haller, 1969); the available knowledge suggests that the distribution of secreting elements is very variable and does not always resemble that of man, particularly in small mammals (de Haller, 1969). As well as these anatomical variations in distribution, there are histochemical variations in mucus composition in different species. Thus, although the distribution and morphology of goblet cells and mucous glands are similar

in the rat and mouse, there are very noticeable histochemical differences in the respiratory mucus of the two species (McCarthy and Reid, 1964A). In addition, there may be differences in the composition of mucus at different sites in the bronchial tree of a single species. For example, the epithelial mucosubstances in the bronchial tree of the rat vary between the large and small airways (McCarthy and Reid, 1964A). The significance of these histochemical differences, if any, is not known. Similarly, the significance of the variation in distribution of goblet cells and mucous glands in the different species is also unknown; there appears to be no ready explanation, for example, of why man and the pig have numerous bronchial mucous glands, while the dog has relatively few and the rat has none (de Haller, 1969). Also, the fact that there are two components to the mucous-secreting apparatus (in most species) has not been adequately explained (Yeager, 1971); it has not even been possible to separate the two secretions experimentally (Florey, Carleton and Wells, 1932). Knowledge of the contribution to the total amount of respiratory mucus made by each of the two components, goblet cells and mucous glands, is based on an estimate by Reid (1960) that the mucous glands in man have a volume 40 times greater than that of the goblet cells. This has led to the assumption that the volume of the secretion of the goblet cells is relatively unimportant in comparison to the mucous glands. The estimation by Reid (1960) has been quoted widely (Reid, et al., 1962; Thurlbeck and Angus, 1964; Reid, 1968; Yeager, 1971). It may be, of course, that the goblet cells have a greater turnover of mucus.

There have been only a few attempts to produce experimental bronchitis; these include the early experiment of Florey, Carleton and Wells (1932) using cats, the work of Reid (1963) with rats and the work of Mawdesley-Thomas, Healey and Barry (1971) using rats and sheep. Lulling, et al., (1968) and Chakrin and Saunders (1974) have attempted to produce experimental bronchitis in dogs.

Florey, Carleton and Wells (1932), in their paper on mucous secretion in the trachea, reported an increase in the number of tracheal goblet cells after repeated intra-tracheal administration of formalin to cats over a period of weeks.

Reid (1963) exposed groups of rats to sulphur dioxide gas, at levels from 300-400 parts per million, for five hours a day for five days a week for up to six weeks. This produced an increase in the number of goblet cells, particularly in the peripheral bronchioli, excess mucus in the bronchial lumina and colonisation of the airways and airspaces by naso-pharyngeal commensal organisms. The increased number of goblet cells persisted for three months after the exposure (Reid, 1963).

Mawdesley-Thomas, Healey and Barry (1971) exposed four groups, each of eight rats, to levels of sulphur dioxide gas recorded as 50, 100, 200 and 300 parts per million respectively. The gas was administered for ten periods each of six hours; it was claimed that there was an increased number of goblet cells (as measured by the amount of PAS-positive material) in the bronchial tree. Further, this increase in goblet cells was a dose-related response, as measured by an automated image analyser. Additional investigations by these authors into the effects of cigarette smoke on the bronchial tree of the rat did not demonstrate any variation in the amount of mucus as measured by an automated image analyser. This was due to breath-holding by the rats and this could be overcome by intravenous anaesthesia of the animals (Mawdesley-Thomas, Healey and Barry, 1971).

Experimental exposure of lambs to cigarette smoke resulted in a dose-related response in hyperplasia of the mucous glands, while image analysis of goblet cell numbers and sizes was inconclusive (Mawdesley-Thomas, Healey and Barry, 1971; Mawdesley-Thomas and Healey, 1973). Six groups of lambs were used and each animal smoked four or eight cigarettes daily for periods of three to five weeks.

The groups of animals in the studies by Reid (1963), Mawdesley-Thomas, Healey and Barry (1971) and Mawdesley-Thomas and Healey (1973) had intercurrent respiratory infection. Reid (1963) acknowledged that the rats exposed to sulphur dioxide had bronchiectasis and attributed a lack of uniformity in the response to genetic differences between different strains. Mawdesley-Thomas, Healey and Barry (1971) found that respiratory infection of their rats by Mycoplasma pulmonis was a recurring problem; the effect of such an infection was to produce a marked hyperplasia of goblet cells, thus invalidating the interpretation of the quantitation procedures. The significance of M. pulmonis infection in the rat, in relation to experimentation in general and respiratory tract experiments in particular, has been thoroughly reviewed by Lindsey, et al. (1971). These authors claimed that chronic respiratory disease in rats due to M. pulmonis infection had an incidence ranging from 50 to 100 per cent, that mortality remained low and that many of the lesions could only be detected histologically. The lesions included flattening of bronchial epithelium, "squamous changes", increased mucus production and the formation of simple glands lined by goblet cells (Lindsey, et al., 1971). Asmundsson, Kilburn and MacKenzie (1973) were unable to repeat the work of Reid (1963) because of a high mortality rate in the rats they exposed to sulphur dioxide and the high level of spontaneous respiratory disease in the control rats. Ventura and Goucher (1966) also encountered M. pulmonis infections in rats and noted the resulting hypersecretion of bronchial mucus and goblet cell "metaplasia" (hyperplasia).

Mawdesley-Thomas and Healey (1973) stated that they were unable to count the goblet cells in the airways of some of their sheep; they considered this inability to distinguish individual goblet cells could have been due to low-grade infection, which was present in a proportion of their animals. It is not clear whether this infection was also present in the control animals.

Lulling, et al. (1968) exposed dogs to sulphur dioxide at levels of 500 parts per million for one to three times a week and up to four hours at a time, giving a total of 32 exposures over a period of approximately 18 months. The dogs were examined by bronchography, pulmonary function tests, histopathology and bacteriology. In addition, mucus collected from the dogs was examined by electrophoresis, immunoelectrophoresis and chromatography. The authors thought that both chemical irritation and bacterial infection were necessary to initiate the hypersecretion. They also considered that the bronchial secretions in the dogs exposed to sulphur dioxide were significantly different from the bronchial secretions in man; in particular, the canine bronchial secretions contained 'elements' originating in the plasma. Bacteriological examination of the bronchi revealed a variety of Gram-negative organisms and occasionally streptococci and staphylococci, but not B. bronchiseptica. Histological lesions included mild hypertrophy of mucous glands, bronchiectasis, inflammatory polyps and considerable areas of emphysema (Lulling, et al., 1968).

More recently, Chakrin and Saunders (1974) have described a laboratory model of chronic bronchitis using the dog. In this study, fourteen adult beagle dogs were exposed to levels of sulphur dioxide gas of between 500-600 parts per million for two hour periods twice a week for four to five months. This exposure produced an increase in the number of goblet cells in the small bronchi and an extension of goblet cells peripherally into the bronchioles. There was also hyperplasia of the mucous glands and an excess mucopurulent exudate in the bronchial tree. However, it did not appear that emphysema was such a prominent finding as in the dogs of Lulling, et al. (1968). Both the studies of Lulling, et al. (1968) and Chakrin and Saunders (1974) used exposure levels of 500 parts per million, but in the latter study there were more than 600 exposures of two hours duration, compared to the 32 exposures of one to four hours duration in

the experiment of Lulling, et al. (1968); this may explain why the mucous gland hypertrophy was more pronounced in the study of Chakrin and Saunders (1974). Unfortunately, Chakrin and Saunders (1974) did not include any bacteriological examinations of the bronchi of the dogs exposed to sulphur dioxide. Both Lulling, et al. (1968) and Chakrin and Saunders (1974) asserted that naturally occurring chronic bronchitis was rare in the dog.

The importance of experimental animals in comparative studies on atmospheric pollutants is well recognised. Careful choice of suitable experimental animal species is most important (de Haller, 1969). The rat appears to be unsuitable, since it has no bronchial mucous glands (de Haller, 1969) and is especially prone to M. pulmonis infection (Lindsey, et al.,1971), particularly when subjected to such stress as exposure to high levels of sulphur dioxide. Reid (1967A) has perhaps pertinently observed that work on the human lung has been bedevilled for the last 50 years by unjustified extrapolations from animal work to man.

The various studies referred to above have indicated that a suitable model system for chronic bronchitis using small laboratory animals is not feasible. However, since the domestic dog shares man's environment closely and, as will be shown, is subject to naturally-occurring chronic bronchitis, it would appear to be one of the more useful species to study in a model system. The work of Chakrin and Saunders (1974) has indicated that this model is feasible and the quantification of bronchial wall components, pathology and histochemical studies described below add further support to the idea of using the dog as a model for human chronic bronchitis.

A FIELD STUDY OF CHRONIC BRONCHITIS IN THE DOG

INTRODUCTION

The previous section described in some detail the disease of chronic bronchitis in man and then went on to review the limited literature on chronic bronchitis in the dog. Previous attempts to describe chronic bronchitis in the dog have not used the criteria applied to the disease in man. This field study was an attempt to define chronic bronchitis in the dog using the criteria applied to chronic bronchitis in man.

The current definition of chronic bronchitis in man is as follows: "chronic bronchitis refers to the condition of subjects with chronic or recurrent excessive mucous secretion in the bronchial tree, occurring on most days for at least three months of the year during at least two years" (CIBA Symposium, 1959). This definition is obviously a somewhat arbitrary one; the time of three months during each of two years was originally a suggestion put forward by the members of the CIBA Symposium in 1959, but it was widely adopted thereafter (W.H.O., 1961; American Thoracic Society, 1962). At the same time, epidemiological surveys were being conducted using more complex definitions in the form of questionnaires (Higgins, et al., 1956; Higgins, 1957) and subsequently, a revised questionnaire was issued by the Medical Research Council in an attempt to standardise the recording of clinical signs (M.R.C., 1960). The questions posed were an attempt to determine both the severity and duration of clinical signs.

It was this emphasis on sputum production and the duration of cough, both in the CIBA definition and in the questionnaires, that led to the adoption of similar criteria for chronic bronchitis in the dog.
In view of: a) the difficulty of obtaining accurate histories from owners and b) the shorter life span of the dog, a simplified definition of coughing for two consecutive months in the preceding year was adopted. This minimum requirement of two consecutive months in the preceding year was tentatively proposed after a preliminary investigation of chronic respiratory disease in the dog, which involved a retrospective study of the clinical and pathological files of the U.G.V.S. This study revealed

that all cases which originally had been diagnosed as having chronic bronchitis, and which were subsequently confirmed by re-examination of histopathological material, had coughed for a minimum of two months prior to admission to U.G.V.S. In addition, it was felt that a history of coughing for two consecutive months would exclude most acute respiratory infections and their complications. As in man this definition is made only by excluding other bronchopulmonary diseases, such as tuberculosis and bronchial carcinoma, and cardiac disorders as the sole cause for the signs. This section describes the findings in a series of cases which conformed to this definition and in which chronic bronchitis was confirmed at post mortem examination.

The material for this series of cases was obtained from two sources: (a) the clinical and pathological files of the U.G.V.S. and (b) from a survey of dogs referred to U.G.V.S. with a chronic intractable cough during the last three years.

A retrospective survey of the clinical and pathological files at U.G.V.S. revealed all the cases of canine respiratory disease which had a chronic intractable cough and which had subsequently been diagnosed as having chronic respiratory disease. Twenty nine case histories of chronic respiratory disease were available in the files of this department; these cases had been diagnosed as having a range of bronchopulmonary disease including chronic bronchitis, chronic pneumonia, purulent bronchitis, cararrhal bronchitis and bronchiectasis. Examination of the clinical records of these cases was then undertaken, together with a re-examination of the histopathological material. Subsequently, seventeen of the cases were confirmed as having clinical and pathological evidence of chronic bronchitis, as required by the definition adopted for canine chronic bronchitis.

A letter was then sent to all practising veterinary surgeons in the Glasgow area in October, 1972 (with a reminder in October, 1973) requesting notification and referral of all dogs with a cough of two months' duration

duration. These dogs, together with dogs routinely submitted for other reasons, were brought to the U.G.V.S. for clinical examination by Dr. E.W. Fisher; further radiologic investigations were then carried out by Mr. R. Lee. After examination, these referrals were usually sent home (some of these were subsequently re-admitted) and the remainder either died or were destroyed if this was recommended by the clinicians.

It was not always possible to recommend euthanasia where chronic bronchitis had been diagnosed clinically if the dog was considered fit enough to be sent home. In all, 24 dogs which were destroyed or had died during the survey were subjected to a full post mortem examination. Macroscopic and histopathological examination revealed a variety of causes for the presenting respiratory signs, including primary and secondary pulmonary neoplasia, space-occupying neoplasms in the thorax, acute and sub-acute bronchitis, pneumonia, congestive cardiac failure and chronic bronchitis. Nine cases which conformed to the definition of chronic bronchitis were obtained.

The seventeen cases of chronic bronchitis obtained from the files of the U.G.V.S. together with the nine cases from the survey made up the twenty-six cases of chronic bronchitis which are described in the following pages of this thesis.

EPIDEMIOLOGY, HISTORY AND CLINICAL SIGNS

The 26 dogs with chronic bronchitis were all adults with a history of coughing for a minimum period of two consecutive months in the preceding year. The clinical and pathological features of each case are set out in detail in Appendix 1; the main clinical features are summarised in Table 1. The main features of the series as a whole, are considered below.

District

There was no marked tendency for the dogs with chronic bronchitis to have lived in the city (Fig. 3), despite the fact that chronic bronchitis in man is more common in the urban environment (see The Disease in Man) and that the majority of dogs referred to U.G.V.S. are primarily from Glasgow and district. Thirteen of the dogs lived in the city and the other 13 lived in either a rural or suburban locality.

Age

The age distribution of the dogs in the series is given in Fig. (4); none of the dogs was under three years of age, despite the disproportionately high number of young dogs which enters the U.G.V.S. (vide infra). The histogram indicates a spread throughout the adult age range.

Sex

Despite the preponderance of human male subjects with chronic bronchitis, the 17 males and 9 females in this series represents a male-female ratio of 1.9:1. Although this is a male to female ratio of approximately 2:1, this is virtually the sex ratio of the background population referred to the U.G.V.S. (Lauder, 1974).

Breed or type

The breeds or types of dog represented in this series are set out in Table (2). Although thirteen varieties of dog are represented, these do not form the most common breeds and types referred to the U.G.V.S. (Fig. 5). All the dogs tended to be of the smaller breeds and types (Schneider-Leyer, 1964)

of which the spaniels were the largest (Fig. 6). It will be demonstrated below (see Coughing in Adult Dogs) that other larger breeds of dogs constitute a significant proportion of the referrals to the U.G.V.S.; for example, alsatians and labradors together comprised 20 per cent of all admissions over the 18 month period of the survey described below. The fact that only smaller breeds and types of dogs were affected appears to be of significance.

Condition

Fifteen or the 26 dogs were considered to be obese and 5 were recorded as having a markedly enlarged abdomen.

Season

The seasonal distribution of the cases of chronic bronchitis is given in Fig. (7), together with the seasonal distribution of a human series extracted from the Registrar-General's figures (Registrar-General, 1966). In both instances, mortality is heaviest in the winter months. The human series demonstrates a peaking in January, whereas in this much smaller series of dogs, there were no cases in January, despite a definite winter incidence. This may be due to the small number of dogs or reflect adminstrative factors.

Onset and duration of illness

The clinical picture was dominated by the chronic intractable cough. At the time of presentation, the dogs were said to have been coughing for periods of two months to two years, with the majority in the two to six months range (Fig. 8). The time of onset and rate of progress was very difficult to ascertain from the owner's history - most cases had had a gradual, insidious onset before attracting the owner's attention. In a few cases, there was a definite history, either of the cough originating after the dog had returned from kennels (2 cases) or the dog had been treated for 'pneumonia' (3 cases). In these cases, it was often reported that the cough had never really cleared up.

Clinical signs

The nature of the cough was obviously difficult to describe in a standardised form, but retching with production of sputum occurred in eleven of

the dogs. The chronic cough varied in type and intensity - in some cases there were bouts of coughing with cyanosis and subsequent exhaustion for up to an hour afterwards. It was not usually possible to assess the productivity of the cough since any mucus coughed up was usually swallowed. In exceptional cases, expectoration of mucus occurred, with irregular sputum production.

The temperature was usually normal; only four dogs had a temperature in excess of 102^{0}F at the time of admission. On auscultation, nearly all cases had adventitious sounds of emphysematous crackling and rhonchi were also detected. On initial observation of the majority of these cases, there appeared to be hyperpnoea (increased depth of breathing).

The co-existence of valvular endocardosis and chronic bronchitis may complicate the clinical picture; in such cases the contribution to clinical signs may be difficult to gauge. In this series, seven of the dogs, (6,10, 13,17,19,21,23) were stated to have a systolic murmur. Fourteen of the dogs were subsequently recorded as having valvular endocardosis and in five of the dogs (6,7,8,15,21) the endocardosis was stated to be severe in degree. In general, if the murmur was caused by a degree of incompetence sufficient to produce pulmonary oedema and coughing, then, except terminally, the murmur was gross and was accompanied by tachycardia and a fast, weak pulse. In contra-distinction, except in terminal right heart failure, the chronic bronchitics had a marked sinus arrhythmia. The pulse volume was good but often misleading because of the conformation, as in short-legged, fat dogs (see Breed/Type).

Radiology

Radiographic examination of the thorax was often of limited value in confirming the diagnosis of chronic bronchitis. There was frequently little or no radiological abnormality even though clinical signs were marked. When changes were observed, these took the form of a generalised increase in broncho-vascular markings extending to the periphery of the lung fields with delineation of the bronchial walls resulting in parallel and annular linear opacities (Figs. 9,10). Changes were often masked or difficult to

differentiate from changes resulting from co-existing cardiac disease and the general increase in lung markings observed in ageing dogs. Twelve of the dogs in the series were reported as having evidence of bronchial thickening on routine radiological examination.

Bacteriology

Thirteen of the dogs had samples of lung or bronchial swabs submitted for bacteriological examination; Bordetella bronchiseptica was isolated from seven of these cases. Proteus spp. was isolated from one case and in the other five cases there were no significant findings.

POST MORTEM FINDINGS

The detailed post mortem findings in each case are tabulated in Appendix 1, and the main observations are summarised in Table 3.

Macroscopic findings.

Macroscopic examination of the lower respiratory tract revealed anthracosis in 25 of the cases. This appeared as black speckling distributed diffusely beneath the pleura of all the lung lobes. In addition, there was pronounced black pigmentation of all the lymph nodes, particularly the subcarinal lymph nodes. The degree of anthracosis was severe in 11 of the cases and mild in the other 14 cases.

The lungs were often very pale cream-grey, unless there was a concurrent pneumonia, in which case the lobes were darker than normal, mottled red-blue, heavy and congested.

Emphysema, though frequently encountered, was usually mild, appearing as rims of pale pink distended lung tissue around the edges of all the lung lobes (Fig. 11). These distended areas were very soft in consistency and crackled when palpated: close inspection revealed enlarged air spaces. Eight of the dogs were recorded as having macroscopic evidence of emphysema.

On opening the lower respiratory tract, the tracheobronchial tree was found to contain variable amounts of excess mucus. Pooling of mucus was often very noticeable at the level of the tracheal bifurcation (Fig 12); the mucus was viscous in character, often clinging tenaciously to the underlying mucosa, and was admixed with varying amounts of purulent material. The strands of mucus were very difficult to demonstrate satisfactorily because they tended to be washed off the underlying mucosa and to be contaminated by blood, oedema fluid and water during the examination procedure. For these reasons, photographic demonstration of material fixed in formol saline was preferred to demonstration of fresh material: Figs. 13 and 14 illustrate severe obliteration of the bronchial lumen by thick, rope-like strands of mucus.

The tracheobronchial mucosa had a slightly roughened surface, having lost the smooth, glistening character of the normal lining. In severe cases, the mucosa was thickened and had a velvet-like surface (Fig. 15). Seven of the cases had polypoid proliferations of the bronchial wall which were visible to the naked eye; these appeared as smooth-surfaced, roughly-spherical nodules up to 3 mm. in diameter which projected into the lumen of the lobar or segmental bronchi (Figs. 15,16), especially at bifurcations.

Microscopic findings

The normal bronchus is essentially a thin-walled conducting tube with an oval or circular cross-section. The lamina propria is narrow and mucous glands are sparsely distributed in the bronchial wall (Fig. 17). In the dogs with chronic bronchitis, the bronchial tree had undergone several characteristic changes, all of which could be seen in advanced cases. The bronchial wall was diffusely thickened by varying degrees of cellular infiltration, oedema and fibrosis. The regular outline seen in the cross-section of normal bronchus was replaced by folding of the mucosa to give an irregular outline in cross-section (Fig. 18). The epithelium appeared thickened over this folded mucosa and often had a rather ragged appearance (Fig. 18). The lamina propria was thickened by oedema and cellular infiltration (Figs. 18,20); in addition, the mucous glands were dramatically increased in number and size (Figs. 19,20) to completely surround the bronchial lumen. As a result, the bronchial lumen contained varying amounts of excess mucus (Fig. 20); this accumulation of mucus was mixed with large numbers of neutrophils and macrophages. Consequently, the bronchial lumen was stenosed by the thickening of the bronchial wall, folding of the bronchial mucosa and excess amounts of mucus.

The bronchial epithelium often appeared thickened, with an increased number of layers of cell nuclei. The appearance of normal bronchial epithelium is seen in Fig. 21; the ciliated columnar cells rest on the basement membrane, and interspersed between them at regular intervals can be seen the nuclei of undifferentiated basal cells and goblet cells. The goblet cells are in various stages of synthesis and secretion and some are discharging granular mucosubstances out into the lumen between the cilia. By contrast, in the cases of chronic bronchitis, there was an increase in the number of undifferentiated basal cells and, in some cases, of the more differentiated columnar cells. As a result, the columnar cells were often two or more cells deep and rested on two or three layers of basal cells (Fig. 22).

Basal cell proliferation was the dominant feature at certain sites in the epithelium; the respiratory epithelium of columnar ciliated cells and goblet cells was lost and replaced by squamous-type, dedifferentiated epithelium (Fig. 23). These areas were often quite small (up to 1 mm.) and merged gradually or abruptly into normal ciliated epithelium; they were particularly common over folds of the mucosa and over areas where there was intense cellular infiltration of the lamina propria (Fig. 24). The proliferating basal cells had then either become attenuated and eroded, producing defects in the epithelium (Fig. 25), or irregularly piled up (Fig. 23). The lamina propria was then exposed by these defects in the epithelium (Fig. 25) and neutrophils often appeared to pass through these into the lumen (Fig. 26). Excess mucus also gathered over this non-ciliated epithelium and often appeared to be adherent to areas where the epithelium had been lost (Fig. 27).

The epithelial goblet cells appeared to be much more numerous in chronic bronchitis; however, they were much thinner in outline than those of the normal epithelium. They often appeared as slender bands in sections stained for mucosubstances (Fig. 52). Thus, the regularly-distributed, evenly-staining, plump, flask-shaped goblet cells of the normal epithelium (Fig. 28) were replaced by many more slender, unevenly-granular cells.

In some sites, the goblet cells had been totally exhausted and were seen as apical remnants of mucus between the columnar cells (Fig. 29). In more advanced stages, where there was epithelial hyperplasia and basal cell proliferation, goblet cells diminished over areas of epithelium, particularly over folds, and tended to proliferate in epithelial recesses (Fig. 27). Neutrophils could often be seen between epithelial cells, particularly when there was a superimposed episode of acute inflammation (Fig. 22).

Numerous plasma cells and lymphocytes were found in the lamina propria together with aggregates of macrophages. This cellular infiltrate varied in intensity within the bronchial tree of individual cases and even in individual sections of bronchial wall. Where there was an acute inflammatory reaction, there were increased numbers of neutrophils and pronounced dilatation of capillaries. These engorged capillaries often contained neutrophils and lymphocytes. The lamina propria was swollen by the cellular infiltrate and by generalised oedema (Figs. 18,26); the oedema was often particularly noticeable in the sub-epithelial region (Fig. 30). The thickened lamina propria tended to become very folded, giving a more irregular outline to the cross-section of the bronchus (Fig. 18). This folding often occurred where the cellular infiltrate was most severe and varied in degree from case to case. In mild cases, the lamina propria had only a light inflammatory infiltrate, but in more severe cases there was also pronounced capillary dilatation, marked oedema and a heavier cellular infiltrate. In these more severe cases, the epithelium was more likely to have undergone degenerative change with basal cell proliferation. Sloughing and attenuation of epithelium was found in these more advanced cases with complete loss of epithelium over very severely affected folds. Focal polypoid proliferations were to be found on the severely affected folds and these appeared as nodules of oedematous, loose connective tissue infiltrated by plasma cells, lymphocytes, macrophages, varying numbers of neutrophils and fibroblasts (Fig. 31). There was pronounced sub-epithelial oedema over these proliferations together with multiple foci of epithelial dedifferentiation which were often completely eroded over much of the

polyp surface. In a few cases, the polypoid proliferations were visible to the naked eye (Figs. 15,16); seven of the cases had macroscopic polyps and fourteen of the cases had microscopic polyps (Tables 3a,3b).

Marked hyperplasia of the mucous glands was seen in all the twenty six cases. The characteristic features were an increase in the number and size of mucous glands; the acini proliferated (Fig. 32) and then became enlarged and distended, often with neutrophils and excess secretion visible in their lumen. The result was a greatly increased volume of mucous secreting tissue in the bronchial wall (Fig. 33). The proliferating, branched gland acini were surrounded by varying numbers of plasma cells and lymphocytes (Fig. 34); in most of the cases this peri-acinar infiltrate was very noticeable. The mucous glands extended peripherally along the airways beyond the distal limits of the bronchi and could be seen, often in considerable numbers, in small bronchioles where they would not normally be found (Fig. 35).

The muscularis mucosa of the bronchus was influenced largely by the changes taking place in the other bronchial wall components, most particularly in the lamina propria and the mucous glands. Infiltration, irregular thickening and folding of the lamina propria caused distortion of the smooth muscle layer (Fig. 26). In addition, the enlarged hyperplastic mucous glands could often be seen to be disrupting the muscle bundles. There was no evidence that the amount of bronchial smooth muscle increased in cases of chronic bronchitis (see Quantitation section) although the muscle appeared abundant in sections from some of the cases (see Appendix 1).

The thickening of the bronchial wall seen in Figs. 18,19 and 20 was repeated at the bronchiolar level. The walls of the bronchioles were often markedly thickened by a cellular infiltrate; in addition, the bronchiolar lumen contained mucus, necrotic cell debris and varying numbers of cells, primarily macrophages and neutrophils. Mucous gland acini were visible in the walls of many bronchioles (Fig. 35) and the overall effect was a narrowing of the lumen (Fig. 36).

The appearance of the alveolar airspaces depended on the presence or absence of pneumonia. Nine of the cases had microscopic evidence of a superimposed acute pneumonia or a chronic pneumonia; five of the cases had macroscopic evidence of pneumonia though this was known to be terminal in only two of the cases. The peribronchial alveoli were often affected by the chronic inflammation of the bronchi and were distorted by inflammatory changes and peribronchial fibrosis.

Anthracosis was seen in the lungs of 25 of the dogs in the series. The degree of anthracosis varied markedly in the cases; characteristically, heavy focal deposits were seen in most of the lungs in perivascular and peribronchiolar sites.

Emphysema, though often present, was frequently mild and was seen, histologically, as localised sub-pleural emphysema with destruction of alveolar walls (Fig. 37). Attenuation and rupture of alveolar walls was the prominent feature, with the formation of large air-filled spaces beneath the pleura in more severe cases (Fig. 38). The pleura itself was frequently distorted by this process. Macroscopic evidence of emphysema was recorded in eight of the cases; microscopic evidence of emphysema was seen in a total of 17 of the cases.

Three dogs were recorded as having congestive cardiac failure and two of the dogs had cor pulmonale. In man, cor pulmonale is a recognised late development in cases of chronic bronchitis, emphysema and combined chronic bronchitis-emphysema (White, 1973). Cor pulmonale is defined as "hypertrophy of the right ventricle resulting from diseases affecting the function and/or the structure of the lung, except when these alterations are the result of diseases that primarily affect the left side of the heart or of congenital heart disease" (World Health Organisation, 1961). The right sided heart failure is thought to be partly due to constriction of pulmonary vessels, but other factors may be involved (Crofton and Douglas, 1969). In the case of dog 19, the presence of cor pulmonale was confirmed by weighing the ventricles according to the technique described by the World Health Organisation

(World Health Organisation, 1961). Dog 3 had a grossly enlarged and flabby right ventricle, though there were no endocardial or myocardial lesions present. In addition, there was chronic venous congestion of the liver. Dog 17 had marked ascites, with three litres of fluid recovered from the abdomen at post mortem examination, together with chronic venous congestion of the liver. There was endocardosis of both atrioventricular valves; this endocardosis was severe on the left side but of only moderate degree on the right side. Dog 19 had cor pulmonale, which was confirmed by weighing the ventricles, but did not have either ascites or chronic venous congestion of the liver. Lastly, dog 21 had ascites and hydrothorax (200 ml. of fluid recovered from the body cavities) together with chronic venous congestion of the liver. In this case, there was severe endocardosis of both atrioventricular valves.

COUGHING IN ADULT DOGS

The coughing adult dog is a well-recognised clinical problem; acute attacks of coughing are usually ascribed to the syndrome known as "kennel cough", but chronic coughing, which is a continuing problem, is less well understood (Veterinary Record, 1974). Although persistent coughing is the outstanding clinical feature of chronic bronchitis in the dog, other diseases can give rise to this clinical sign. The other possible causes of chronic coughing to consider are: pulmonary tuberculosis, Filaroides osleri infection, primary and secondary lung tumours, chronic pneumonia and valvular endocardosis (Wheeldon, et al., 1974).

The greatest problem in differential diagnosis is posed by valvular endocardosis. This disease is particularly frequent in older dogs, where it can be a cause of systolic murmur. Persistent coughing in the older dog is often attributed to endocardosis, particularly when a systolic murmur is present. However, since it became apparent from this study that chronic bronchitis and valvular endocardosis could co-exist in the same subject (Table 3), it was important to try to establish the precise contribution made by each of the syndromes to the persistent coughing. For this reason, valvular endocardosis will be considered in some detail. In addition, an attempt has been made to establish the incidence of systolic murmur and cough in a sample of the background population of dogs entering the U.G.V.S., using the Termatrex Data Retrieval System (Jonker Corporation, Gaithersburg, Maryland, U.S.A.) currently in use.

Using the Termatrex Data Retrieval System, it is possible to extract retrospective information on the population of dogs examined. This population of dogs is unlikely to be representative of the background population since, in the United States of America where figures for background population are known from licence registers, Robinson (1968) found that the selected population referred to a Veterinary Hospital had a disproportionate number of young dogs and uncommon breeds compared to the background/...

background population. Although figures for the background population of dogs in the Glasgow area are not available, a breakdown of the selected population referred to the U.G.V.S. can be used to find the ages and breeds of the Hospital population, together with the incidence of certain clinical signs such as cough and systolic murmur within that population. Over a twenty month period from January 1972 to August 1973, 2,707 dogs were admitted to U.G.V.S. for study. Details of the clinical history of this group were then extracted from the Termatrex System. The age distribution of the group, together with the number of dogs coughing in each age group is given in Table 4 ; it can be seen that the percentage of dogs with a cough at the time of admission rises with age.

The number of animals in each of twenty main breeds or types is given in Table 5 . These twenty breeds or types represented 2,460 of the 2,707 dogs which were admitted during the survey period; the remaining 247 dogs represented minority groups and breeds. 180 (7.3 per cent) of the 2,460 dogs in this selected group were recorded as having a cough at the time of admission; the breeds or types which had a frequency of cough in excess of this overall mean of 7.3 per cent included: poodle (11 per cent), fox terrier (11 per cent), other types of terrier (10.3 per cent), boxer (10.3 per cent), spaniel-type (10.1 per cent) and border collie (10 per cent). It has been demonstrated in this field study that poodles and terriers are particularly liable to develop chronic bronchitis. The breeds and types quoted above did not comprise the breeds or types most frequently admitted; these were crossbreds (22.3 per cent), alsatian (10.7 per cent), labrador (10.1 per cent) and poodle (9.3 per cent).

The increase with age in the number of dogs with a cough is accompanied by an increase in the number of dogs with a distinct systolic murmur. Valvular endocardosis is uncommon in dogs under four years of age (Pirie, 1967) so the age groups susceptible to valvular endocardosis were selected from the study group of 2,707 dogs to determine the frequency of cough with concurrent systolic murmur. The groups were: four

to/...

to eight years of age, eight to twelve years of age and twelve years of age and over (Table 6). The number of dogs in each age group is given in Table 6 : dogs aged four years and over comprised 41 per cent of the total survey population. Only a small proportion (4 per cent) of dogs in the four to eight year age group had a distinct systolic murmur, but this figure rose markedly to 19 per cent of dogs aged twelve years and over. It can be seen that, in the dogs aged twelve years and over, 19 per cent had a distinct systolicmurmur and 19 per cent had a cough; however, only 7.4 per cent (5 dogs) had concurrent cough and systolic murmur.

Valvular endocardosis is the commonest heart lesion in the dog (Detweiler, et al., 1962; Pirie, 1967). Incidence figures range from 8.1 per cent (Detweiler and Patterson, 1965) to 42 per cent of all dogs (Das and Tashjian, 1965). These overall percentages are not truly representative, since the lesion has a marked age incidence; it is uncommon in dogs under five years of age, and rapidly becomes more prevalent in older dogs (Detweiler and Patterson, 1965). Valvular endocardosis is the commonest cause of systolic murmur in the dog; Detweiler and Patterson (1965) found a systolic murmur incidence of 8.3 per cent among 4,831 dogs. The prevalence of systolic murmur also increases with age, rising to over 30 per cent in dogs over 13 years of age (Pomerance and Whitney, 1970). Ettinger and Suter (1970) described the progressive nature of the disease from the early, mild, incidental lesion of mitral incompetence, fully compensated for by the heart, through to the stages of cardiac decompensation and congestive heart failure. The description of the cough in these latter stages was detailed, although no data was presented about the number of cases examined or the presence or absence of chronic bronchitis. Ettinger and Suter (1970) described a nocturnal, deeply-resonant cough, often beginning in the early morning hours, progressing to coughing spasms with retching. Dyspnoea and tachypnoea developed and the cough became paroxysmal, with bouts of hollow, rasping coughing precipitated by excitement. The dog became orthopnoeic (unable to breathe while lying down) and this was manifested as nocturnal restlessness. In the terminal stages, the decompensating heart could not prevent the development of gross pulmonary oedema and the eventual signs of right heart failure, including hepatomegaly, ascites and oedema. Ettinger and Suter (1970) claimed that the serious clinical manifestations of valvular endocardosis seemed to be more frequent in toy and medium-sized breeds (up to 40lb. bodyweight).

The relationship between coughing and heart disease in the dog is difficult to evaluate, since there have been few clinical reports of these points/...

points and there is little data available. Despite the great prevalence of valvular endocardosis in older dogs, only a proportion of these develop clinical signs, including cough and systolic murmur. Ettinger and Suter (1970) asserted that the clinical signs described above (phlegm production with progressive, paroxysmal cough) were early signs of left heart failure. However, although coughing in dogs with a systolic murmur is often attributed to valvular endocardosis, there do not appear to be any correlative studies in the literature to determine if this is really the case. Many of the cases of chronic bronchitis in this study were initially considered to have cardiac disease by the referring veterinarian because there was a systolic murmur and cough. In addition, although coughing can be a sign of heart disease, the mechanism by which heart disease stimulates the cough receptors in the wall of the trachea and bronchi is not known, although the presence of pulmonary congestion and oedema could be important. Despite the fact that the mechanism of cough is obscure, paroxysmal nocturnal cough and dyspnoea (so called "cardiac asthma") are well recognised signs of left ventricular failure in man (Houston, Joiner and Trounce, 1968). In fact, Turner (1969) has stated that "pulmonary congestion from cardiac failure may mimic chronic bronchitis in elderly persons".

As regards the other conditions which may produce a chronic cough in the dog, pulmonary tuberculosis is not a common condition in the dog at the present time; in 1972 there was one case out of 598 dogs (0.2 per cent) admitted to the U.G.V.S. Although there is often a chronic intractable cough, the presenting sign may be breathlessness or tachypnoea (Hawthorne, et al., 1957) and there are usually other marked clinical signs, including prolonged pyrexia and wasting; there may also be characteristic features on radiographic examination.

Filaroides osleri infection is seen in young dogs and in such cases the dogs are bright, and there are usually no other clinical signs apart from the continuous cough (Wheeldon, et al., 1974), although Lauder and Lawson (1959) have described a characteristic expiratory wheeze.

Radiographic examination might also reveal typical worm-nodules at the bifurcation of the trachea.

Primary lung tumours are not common in the dog; Nielsen (1965) found an incidence of 0.6 per cent (11 dogs) in 1850 necropsies at the University of Connecticut, and Stünzi (1973) an incidence of 1 per cent (88 dogs) in 8,650 dogs at the Veterinary Pathology Institute at Zurich. Secondary lung tumours are much more common. The main clinical presenting sign in both primary and secondary pulmonary neoplasia is tachypnoea without cough (Wheeldon, et al., 1974). Radiographical examination is of value in confirming the presence of pulmonary neoplasms.

Chronic pneumonia presents difficulties in differential diagnosis, since exudate in the bronchial tree regional to the focus of pneumonia may produce clinical signs similar to chronic bronchitis. For this reason, on clinical grounds, a degree of pneumonia is considered to be present in cases of chronic bronchitis if there is no response to antibiotic therapy (Fisher, 1973).

Although mitral insufficiency due to valvular endocardosis is widespread in dogs, there do not appear to have been any studies to determine the degree of severity of valvular damage which is necessary to produce pulmonary congestion and subsequently a cough. If this information were available, it would enable a more precise assessment of the importance of valvular endocardosis as a cause of coughing in older dogs. For this reason, it was decided to investigate the prevalence of cough and systolic murmur in dogs to determine if there was an association.

Both of the clinical signs of cough and systolic murmur increase in frequency with the age of the population; if the valvular endocardosis producing the systolic murmurwere also responsible for the cough, one would expect a considerable degree of overlap, resulting in a similar figure for the number of dogs with cough and systolic murmur. However, this does not appear to be the case; 9 per cent of the dogs aged four years and over had a cough and 6 per cent had a murmur, but only 1.5 per cent had both cough and murmur co-existing. Thus, only one quarter of the dogs with/...

with a cough had a systolic murmur.

It is obvious that valvular endocardosis was not responsible for most cases of coughing in the adult dogs admitted during this period.

DISCUSSION

It will be recalled, from the first section of this thesis, that chronic bronchitis is an important problem in man and that a poorly documented disease resembling chronic bronchitis had been reported in the dog. When this investigation began, it was not known whether chronic bronchitis in the dog could be described in terms of a definition along the lines of that currently used in man viz. "Chronic bronchitis refers to the condition of subjects with chronic or recurrent excessive mucus secretion in the bronchial tree, occurring on most days for at least three months in the year during at least two years (C.I.B.A. Symposium, 1959). As a result of this study, it has been possible to demonstrate: (i) a disease with the pathological feature of mucus hypersecretion in the bronchial tree in the dog, and (ii) that the dogs in this series belong to a group of animals which can be defined using the duration of cough.

Although respiratory disease is common in the dog, it was felt that a minimum period of two months would eliminate acute and sub-acute respiratory diseases. The remaining chronic respiratory diseases can then be defined; the diseases which present as a chronic intractable cough have been reviewed in the previous sub-section (Coughing in Adult Dogs). Because coughing in adult dogs is frequently attributed to valvular endocardosis, an attempt has been made to assess the prevalence of cough in the population of dogs entering the U.G.V.S. It has been possible to demonstrate that the majority of coughing in a group of adult dogs aged four years and over entering the U.G.V.S. did not occur in those dogs with a distinct systolic murmur, i.e. cough and systolic murmur co-existed in only a small proportion of the dogs in the sample population. In this series, the duration of cough was not known, but it was obvious that coughing in the majority of dogs was not attributable to the effect of valvular endocardosis.

Difficulties in collection of material were encountered in this study; dogs are not usually referred to the U.G.V.S. because they have an intractable

treatment or cure is thought unlikely. More cases were submitted as a result of the letters to general practitioners; however, because the disease is so lengthy in course, in many cases the dogs could only be examined and sent home. A dog with chronic bronchitis may live for years providing the owner is prepared to tolerate the persistent coughing. During this time the dog may succumb to other diseases such as chronic renal disease or neoplasia. In only a few cases was it legitimate to advise euthanasia. This series is composed largely of dogs where euthanasia was the outcome; of 22 dogs in the series, 17 were euthanized and only five actually died. In the prospective survey, many of the dogs in which chronic bronchitis was diagnosed clinically were sent home after treatment and only a fraction of these were returned for necropsy. For these reasons, cases of chronic bronchitis were difficult to obtain for thorough histopathological study. Although future studies could probably be based on clinical diagnosis, it was important initially to compile a series of cases which were confirmed at post mortem examination.

The prevalence of chronic bronchitis in dogs living in rural areas is not known; in this series, 13 of the dogs came from outside the city of Glasgow and many of these were referred to U.G.V.S. from outlying rural areas. It is not possible to state whether or not there is an urban incidence for chronic bronchitis in the dog on the basis of this small series: at this stage, one can only assert that dogs living in rural areas can develop chronic bronchitis. In man, chronic bronchitis is much more prevalent in urban areas (see The Disease in Man); this may be due simply to higher levels of atmospheric pollution or to more complex "urban factors". The recent study by Crofton (1970) of mortality from lung cancer and bronchitis in urban and rural areas in Scotland indicated that the increase in mortality rate was proportionately greater in rural areas over the period 1951-1965. Crofton, however, suggested that rural cigarette smoking trends might be responsible for this disparity.

The spread of chronic bronchitis throughout the adult age range in this series of dogs partly reflects a tendency for the dogs in this series

to be euthanized - the cough failed to respond to repeated attempts at treatment and eventually, usually for a variety of social and domestic reasons, the owners requested euthanasia.

There did not appear to be a sex incidence for chronic bronchitis in the dog; this is in contrast to the situation in man, where there is a definite male to female ratio which increases with age to around six to one at age 55-64 (Crofton and Douglas, 1969). This preponderance of the disease in human male subjects is usually ascribed to the greater frequency of cigarette smoking (Crofton and Crofton, 1963). It would appear from this series of canine chronic bronchitis that both sexes are equally at risk.

The breed and type distribution of the dogs affected by chronic bronchitis was found to be markedly different from the overall breed distribution of the dogs referred to the U.G.V.S. The breed distribution of the bronchitics consisted of terriers and small terrier-type mongrels, spaniels, shetland sheep dogs, poodles and one case each in a corgi, pug and miniature pinscher. This differed from the breed distribution of the overall input into the U.G.V.S. which includes a significant proportion of the larger breeds of dogs, such as alsatians and labradors.

There is no ready explanation for this apparent tendency for chronic bronchitis to occur in small breeds of dogs. The observation that several of the dogs in the series were obese to some degree is also of unknown significance. It may be that both small size and obesity affected airflow in the airways. Ventilation of the lungs may have been impaired either by a variation in the dimensions of lungs in small dogs such as airway diameter compared to distance from the hilus, which is known to affect particle deposition (Reid, 1973) and/or by fat accumulations over the thorax and abdomen and in the abdomen.

There was a general tendency for the chronic bronchitics to come to post mortem examination in the winter months; this was a similar situation to that of man where most deaths occur in the winter months. Because the series was small, the seasonal incidence was not as clear cut in the dogs as in man - several cases occurred in the summer months and there were no

cases recorded in January. This latter feature may have been due to administrative factors, with a possible disruption of referred material at that time of the year. However, in both man and in the dog, chronic bronchitis tends to surface clinically in the winter months.

The clinical signs of chronic bronchitis in the dog, set out in detail in Appendix 1, are consistent with a progressive condition, of insidious onset, withmucous hypersecretion in the tracheobronchial tree. The dog's owner cannot pin-point the onset of the condition accurately and the history usually refers to a minimum duration of noticeable coughing. Expectoration of sputum, a classical feature of the disease in man, does not generally occur as dogs usually swallow expectorated mucus; in exceptional cases gagging with sputum production did occur. Because of insidious onset, the early stages of the disease are ill defined in both man and in the dog. In man, the classical history is of a patient, usually male, who has a slight morning cough (thought by many people to be normal) which he associates with smoking. The cough is ignored but eventually comes to be regarded as a nuisance. The cough progresses over a period of years with steady deterioration; winter colds become more frequent and then do not clear up as before. The result is a patient with a cough for most of the winter with intermittent purulent sputum. Later this cough fails to clear with the onset of warmer weather; the patient has an almost continuous cough producing at times up to two cupfuls of sputum every day. At this stage the patient is a chronic bronchitic having marked exercise intolerance, with breathlessness and a distressing cough. Usually it is only at this late stage that a doctor is consulted, and by this time successful treatment is no longer possible. Further exacerbations may occur, usually in the winter months, including the possibility of pneumonia, cor pulmonale leading to congestive heart failure and respiratory failure. Because euthanasia is a frequent sequel to the disease in the dog, the end-stage complications seen in man are not encountered so frequently.

The contribution to coughing in adult dogs by valvular endocardosis has been considered (see "Coughing in Adult Dogs"); it was shown that in a group of adult dogs the majority of coughing could not be attributed to the presence

of valvular endocardosis. In this series of chronic bronchitics, all the dogs had a cough, half of them had evidence of endocardosis at post mortem and only one third of them had a distinct systolic murmur. So in both series, coughing in most of the dogs could not be attributed to a heart lesion. Because of this frequency of valvular endocardosis in middle-aged and elderly dogs, it is also difficult in many cases to assess the precise contribution to cardiac failure made by chronic bronchitis and valvular endocardosis; congestive cardiac failure is often attributed to valvular endocardosis of the right atrioventricular valves. Of the four cases of congestive cardiac failure, only dog 21 would appear to have had sufficiently severe endocardosis of the right atrioventricular valves to have accounted for the cardiac failure.

The radiological findings in the dogs in this study are in broad agreement with the findings in man. Heitzman, Markarian and Solomon (1973) have pointed out the difficulty of establishing radiographic criteria for the diagnosis of chronic bronchitis in man. They cite Simon (1959) who found that 50 per cent of a series of patients with clinical chronic bronchitis failed to exhibit plain film radiographic changes. In this study in the dog, half the dogs did not have plain film radiographic changes.

There was no clear correlation of symptoms or severity of post mortem lesions with the duration of cough. Thus, if the duration of cough is compared with cough and sputum production, it will be seen from Table 1 that dogs which had been coughing for long periods did not have the most severe coughing. Moreover, sputum production was not limited to dogs with a long history of coughing. Dogs which had been coughing for only two or three months often had a more severe cough than dogs with a history of coughing for up to a year; similarly, sputum production was recorded in dogs which had coughed for the minimum period of two months but was not recorded in many dogs which had coughed for six months or more.

Post mortem examination of the dogs revealed a corresponding lack of correlation of the severity of lesions (Table 3). The characteristic polypoid proliferations seen on the bronchial mucosa were found in dogs with a

history of coughing for a minimum of two months. Conversely, dogs which had been coughing for six months or more did not always have polyps. Thus, it would appear that these polyps are not a common feature of advanced cases of chronic bronchitis.

The amount of mucus present in the airways was not always easy to assess objectively; however, comparison of the amount of mucus with the severity of cough and the duration of cough did not reveal any clear-cut correlation. One would have thought that polyps and large amounts of mucus would have caused severe airway obstruction, giving rise to more severe coughing.

The mucous glands did not increase in amount with increasing duration of cough; dogs which had been coughing for only three months appeared to have more numerous mucous glands than some of the dogs which had coughed for over six months.

The presence of emphysema did not correlate with any of the above factors; it was recorded in dogs which had been coughing for a minimum of only two months. It was not a feature of the lungs of dogs which had had a particularly severe cough; nor was it particularly prevalent in dogs which had bronchial polyps and/or large amounts of mucus in the airways.

This lack of correlation of symptoms and post mortem findings with the duration of cough precludes any possible generalisations as to the possible progressive course of the disease in the dog. It is important to remember that the stated duration of cough is based entirely on the owner's history; because this was in part a retrospective study, the duration of cough for each dog (Table 1) is a conservative figure. Consequently, the progressive nature of the disease may have been masked.

As regards the aetiology of chronic bronchitis in man, three factors are considered to be important; atmospheric pollution, infections of the respiratory tract and cigarette smoking. The possible contribution of atmospheric pollution to chronic obstructive pulmonary disease in the dog is not well described. The possible effect of air pollution on animals was first considered as a result of the unusual smog episode at Donora, Pennsylvania in the U.S.A. in 1948. A retrospective survey of farm animals and household pets was undertaken 2 - 4 months after the smog episode to

investigate the effects, if any, of the smog on exposed animals. This survey was merely a series of histories, obtained from the owners, relating to the episode - no formal clinical or pathological studies were undertaken. On this basis, 15.5% of dogs exposed to the smog episode were affected in some way (U.S. Public Health Service, 1948).

Catcott, McCammon and Kotin (1958) examined the lungs of 51 dogs which had lived in the Los Angeles area for periods of 4 months to 18 years and concluded that "no characteristic or pathognomonic pattern of morphological changes in the tracheobronchial epithelium, subepithelial glands or in pulmonary parenchyma of the dogs under study could be detected."

By contrast, Conway (1971), in a random sample of 45 dogs living in the Dublin area for periods of 6 months to 12 years, described macroscopic changes in 41 (91%) of the dogs; these macroscopic changes were discoloration (affecting particularly the right side) and atelectasis of the right apical lobe. All the dogs affected had emphysema; in addition, bronchial changes were seen in some of the cases - mucous gland hypertrophy and goblet cell hyperplasia. Despite these findings, only one dog had clinical evidence of respiratory abnormality viz occasional coughing. Other findings were anthracosis (in four cases associated with 'lung cancer'), chronic bronchiolitis, mucus accumulation in the alveoli, peribronchial fibrosis, bronchiolar muscle hypertrophy and epithelial changes - metaplasia, hyperplasia and sloughing. These extensive changes are comparable to those produced by Catcott, McCammon and Kotin (1958) in dogs exposed to synthetic smog in an inhalation chamber.

Ohtani (1959) carried out an epidemiological and pathological study of 829 dogs; in his summary, he mentioned the existence of proliferative change of bronchopulmonary epithelium in 14 (1.7%) of the dogs and 7 cases of "metaplastic" proliferation.

Reif and Cohen (1970) made a retrospective radiographic analysis of the chest radiographs in 1,007 dogs at the University of Pennsylvania Veterinary School; they claimed that significant differences existed between the chest

radiographs of middle aged and old dogs from urban and rural areas. These dogs were not examined clinically or at post mortem; nevertheless, the authors speculated that the radiographic changes in the lungs of urban dogs indicated pathological lesions of: "chronic alveolar inflammation", chronic and subacute "pleuritis" and pleural fibrosis, alveolar septal thickening, focal alveolar destruction associated with focal fibrosis, and subacute to chronic bronchitis and bronchiolitis.

The role of infection in the pathogenesis of human chronic bronchitis, particularly in childhood, is now receiving a great deal of attention. Such infections are more common in urban areas (Colley and Reid, 1970) and cough and sputum production is more common in those people who had chest illnesses when they were infants (Colley, Douglas and Reid, 1973). In the dog, respiratory infections are well recognised in the young animal,- distemper and adenovirus infections for example (Wright, 1973), but the possible contributions made by these infections or "kennel cough" in the pathogenesis of canine chronic bronchitis has yet to be determined.

The possible significance of cigarette smoking in canine chronic bronchitis should not be dismissed immediately, as there is evidence that "passive smoking" - that is to say the involuntary inhaling of smoke by a non-smoker when in the presence of a cigarette smoker - can occur in confined spaces. Investigations have shown that noxious agents in tobacco smoke can accumulate in poorly-ventilated confined spaces such as rooms, railway carriages and motor cars, to exceed air quality safety limits; such accumulations have been demonstrated for carbon monoxide (Russell, Cole and Brown, 1973) and nicotine (Horning, et al., 1973). However, the amount absorbed is well below levels which typically prevail in smokers over long periods; for each hour of passive smoking in extreme conditions of smoke accumulation, the amount of carbon monoxide absorbed by non-smokers was equivalent to having actively smoked a single cigarette (Russell, Cole and Brown, 1973).

The role of "passive smoking" in respiratory disease has also been investigated; Norman-Taylor and Dickinson (1972) found higher prevalence rates for respiratory disease among children with parents who smoked. Colley

(1974) found that the prevalence of cough in a group of children was associated with the parents' smoking habits; the highest incidence of respiratory disease was in children both of whose parents smoked, the lowest incidence where neither of the parents smoked, and an intermediate incidence where one of the parents smoked. Colley (1974) was uncertain whether passive smoking, genetic factors or cross infections were important; certainly, a strong association existed between parental phlegm production and chest symptoms in the children. Thus, the possibility that the dog is affected to some degree by cigarette smoking in the home is as yet undetermined; such an associated might partly explain the occurrence of chronic bronchitis in rural dogs in this series.

The significance of emphysema in this series of dogs with chronic bronchitis is difficult to evaluate. The CIBA Symposium (1959) defined emphysema as a condition of the lung characterised by an increase beyond normal in the size of the airspaces, from either dilatation or destruction. This option of either dilatation or destruction has resulted in some workers accepting dilatation as evidence of emphysema (Reid, 1967B), whilst other groups prefer to include destruction in their definition (World Health Organisation 1961; American Thoracic Socy., 1962). Pathological diagnosis of emphysema requires the lung to be examined in a distended state (Silverton, 1963), and Gough and Wentworth (1960) have evolved techniques employing thin slices of lung lobes from lungs fixed under pressure to study the disease. At the beginning of this study, dog lungs were fixed whole using a constant pressure apparatus based on the technique employed by Heard (1960). Heard (1960) described a method of lung fixation using endotracheal fixation with formalin supplied at a constant pressure of 25-30 cm. for 72 hours. Attempts to reproduce this method of fixation in dog lungs were not successful; fixative poured into the trachea at a constant pressure of 25-30 cm. produced severe distortions of both airways and lung parenchyma. In particular, flushing of exudate, flattening of bronchial epithelium and, in severe cases, flattening of bronchial wall occurred in these cases. A series of dog lungs fixed at lower pressures revealed that gross distortion

was only avoided at pressure of around 2-3 cm. Even then, washing of exudate from airways and distortion of epithelium were seen on histological examination of this material. Since the study was primarily one of chronic bronchitis, it was decided to abandon this technique and inject fixative into the vascular bed of the lung. By using this alternative, it was hoped to avoid washing exudate out of the airways and distorting pulmonary structure whilst achieving adequate fixation. Because of this, emphysema, which was present in many of the cases, could not be assessed critically.

Emphysema has not been studied systematically in the dog; references to the disease are brief and there are no descriptions of naturally-occurring emphysema based on pressure fixation. Emphysema and pulmonary fibrosis have been recorded in association with polyarthritis in a beagle (Schiefer, Hurov and Seer, 1974).

The dog has been used as an experimental model for the study of artificially-induced emphysema. Pushpakom, et al. (1970) injected papain, a powerful proteolytic enzyme, into the trachea of eight dogs and produced a disease similar to human panlobular emphysema both morphologically and functionally. Other workers have used papain (Weinbaum, et al., 1972), leucocyte homogenates (Marco, et al., 1971) and cigarette smoke (Hernandez, et al., 1966) to induce experimental emphysema.

The dog's lung is characterised by its lack of supporting connective tissue, having a thin membranous pleura and no secondary lobulation (McLaughlin, Tyler and Canada, 1961). This lack of intrapulmonary connective tissue architecture may explain the gross distortions with constant pressure fixation, since in man the lung has a thick pleura, a degree of secondary lobulation and well-defined interlobular septa (McLaughlin, Tyler and Canada, 1961). Van Allen and Lindskog (1931) showed that collateral respiration, i.e. a drift of air into adjacent lobules across septa , occurs in dog lung at normal physiological pressures. This did not take place in most of the human lungs studied and in the calf and pig, where there is abundant interlobular connective tissue Interpassage of air between lobules was only achieved at pressures much higher than those encountered under normal physio-

logical conditions (Van Allen and Lindskog, 1931). If this compensating action of collateral respiration is important in reducing pressure in certain lung lobes, for example in lobes whose supplying bronchi are blocked by mucus, then this may explain why emphysema was encountered chiefly around the edges of the lung lobes. The pulmonary lobules along the border of a lung lobe have the least chance of collateral respiration since they constitute the narrow edge of the lobe and have little connecting interface with other lobules.

The polypoid proliferations on the bronchial wall encountered in several of the cases (Fig. 15,16)have not been described in human chronic bronchitis. However, Chakrin and Saunders (1974) describe similar formations in the bronchial tree of dogs with experimental chronic bronchitis. These "polyps" are essentially localised proliferations of loose connective tissue with varying numbers of infiltrating cells, and are not in any way neoplastic in nature.

The changes undergone by bronchial epithelium have been described in detail; in man epithelial changes in chronic bronchitis are not well described. Changes in human bronchial epithelium are limited to a consideration of bronchial neoplasia and consequently tend to be limited to the relevant specialist journals (Chang, 1957; Valentine, 1957; Auerbach, et al., 1967). The reduction in numbers of ciliated epithelial cells with subsequent replacement by a less specialised form of epithelium is an important factor in considering the ability of the bronchial epithelium to transport increased amounts of mucus. The mucociliary apparatus is thus both overloaded with increased numbers of goblet cells, and impaired by the failure of the ciliated epithelium. The ciliated cells become reduced in number and are replaced by increased numbers of goblet cells. At this time, the basal layer of undifferentiated cells increases from a single layer to approximately 3-5 cells in thickness. (Fig. 24) Eventually the ciliated cells and goblet cells are lost, leaving this dedifferentiated epithelium; this is referred to as "squamous metaplasia" (Valentine, 1957). Such areas are localised over the bronchial wall and tended, in the dogs with chronic bronchitis, to be common

over mucosal folds, in areas of epithelium overlying foci of intense cellular infiltration, and over the surface of "polyps". In severe cases, this undifferentiated epithelium appeared to become attenuated and actual erosions in bronchial epithelium could be seen.

The proliferation of basal cells seen in certain areas of the epithelium is presumably a response to increased loss of epithelium. Greenberg and Hillms (1962) sutured plastic grafts into the tracheal wall of 14 dogs and found that the surface was colonized initially by a layer of basal ("reserve") cells. Columnar cells appeared after 14 days and ciliated cells and mucus-secreting acini were present after 21 days (Greenberg and Hillms, 1962). The appearance of goblet cells is not mentioned by the authors, but they presumably are derived from the intermediate (differentiating), low columnar cells. Knowledge of the origin, secretory cycle and turnover of goblet cells is scanty and is based almost entirely on studies of intestinal goblet cells. Subbuswamy (1973) has suggested that goblet cells in the gut may develop from Paneth cells or have a common stem cell. It seems likely that, in the bronchi, the goblet cells are derived from the basal cells; Freeman (1962) found that the fine structure of "resting" goblet cells in the gut, i.e. goblet cells after secretion of mucinogen granules, was indistinguishable from the fine structure of the columnar absorbing cells. Lane and Gordon (1974) traumatised the tracheal epithelium of the rat and found that basal cells had proliferated and differentiated to form cells with recognisable secretory granules by 60 hr. post injury. It appears that, in the event of prolonged irritation to the bronchial epithelium, basal cells tend to proliferate and a greater proportion of these then differentiate into goblet cells rather than ciliated cells.

The stimulus by which the mucous glands hypertrophy can either be irritation of surface epithelium which causes glandular secretion, or vagal stimulation (Florey, Carleton and Wells, 1932). The relative contributions of both mechanisms in chronic bronchitis is not known.

The possible role of immune deficiency states in the aetiology of chronic bronchitis has not been widely considered. All the mucosal surfaces of the body, including the respiratory tract, are protected by local antibody - lgA (Tomasi, 1968). The lgA found in secretions differs from serum lgA; secretory lgA exists in a dimeric form, with two molecules of lgA held together by a J (junction) molecule and a "secretory piece" molecule. The lgA is synthesized in plasma cells present in the lamina propria whilst the secretory piece is believed to be synthesized in the epithelial cells (Tomasi, 1968). Porter and Allen (1972), working with the intestinal mucosa of the pig, proposed that secretory piece was produced specifically in the epithelial goblet cells. The secretory piece is not now thought to be important in the actual transport of lgA to the surface as had been supposed; its function is now believed to be to resist digestion of lgA by enzymes (Tomasi, 1967) and possibly also to bind lgA to the mucus spread over the epithelial surface.

Selective lgA deficiency is the commonest immunodeficient state, occurring in one in 500 to 700 randomly selected persons (Johansson, 1968). In a survey of children with recurrent upper respiratory tract infections, Buckley, Dees and O'Fallon (1968) found an incidence of lgA deficiency of one in 200 children.

The possible clinical significance of lgA deficiency has been considered by Ziegler, Penny and Hughes (1973) who investigated a group of eight patients with selective lgA deficiency and found that all had respiratory and gastrointestinal signs. These included bronchitis, asthma , steatorrhea and diarrhoea. The patients still possessed other immunological capacities including allergy and autoimmunity, and had evidence of asthma, hay fever, thyrotoxicosis, gluten-enteropathy and allergic alveolitis.

More recently, Webb and Condemi (1974) described a woman with selective lgA deficiency and chronic obstructive lung disease; a subsequent

investigation of her family revealed an increased incidence of both IgA deficiency and chronic obstructive lung disease. Webb and Condemi (1974) considered that a selective IgA deficiency could lead to increased suppuration in the lungs with increased release of proteolytic enzymes from leucocytes. It had already been shown (Mass et al., 1972) that such enzyme release could produce emphysema.

Studies of immunoglobulin levels in chronic obstructive lung disease are few and the results are contradictory. Biegel and Krumholtz (1968) found elevated IgA levels in the serum of patients in respiratory failure as a result of advanced chronic obstructive lung disease. Falk, Siskind and Smith (1970) measured the serum immunoglobulin levels in 49 patients with chronic bronchitis and emphysema and found normal or raised serum immunoglobulin levels.

Falk, Okinaka and Siskind (1972) studied IgA levels in serum and bronchial washings of six patients with chronic obstructive lung disease. One of the six had complete IgA deficiency, while bronchial washings from the six patients contained only two thirds of the amount of IgA that the controls had.

Thus, present evidence indicates that serum immunoglobulin levels, and in particular serum IgA levels in patients with chronic obstructive lung disease are normal or elevated. However, Falk, Okinaka and Siskind (1972) suggested that these patients might be unable to transport immunoglobulin, particularly IgA, into bronchial washings, although their small sample numbers were not statistically significant.

Medici and Buergi (1971) examined 34 subjects with chronic bronchitis and chronic asthmatic bronchitis and estimated both IgA levels in sputum and the degree of damage to the bronchial epithelium. This epithelial damage was assessed by histochemical and biochemical criteria, i.e. lactic dehydrogenase, fibrinogen and deoxyribonucleic acid fibre content in the sputum. This study revealed that sputum IgA levels were increased in patients with mild to moderate bronchitis of short duration, whilst this response was absent in cases of advanced bronchitis of long duration.

Moreover, immunodiffusion studies suggested that amount of secretory piece were reduced in this latter group of severely affected patients. Medici and Buergi (1971) suggested that this was due to damage to the bronchial epithelium, resulting in impaired transport of lgA into the bronchial washings. The overall result would then be a relative lgA deficiency in the bronchial mucus blanket which would render the patient more susceptible to disease.

Any lgA present in the bronchial washings would probably be in the single monomer form due to lack of available secretory piece; consequently, the lgA which was present would be more rapidly degraded by enzymes in the mucus (Medici and Buergi 1971).

The secretory piece molecule is known to contain sialic acid (Tomasi, 1967) and dogs with chronic bronchitis have increased numbers of goblet cells which contain sialic acid-rich mucosubstances (vide Histochemistry section). The presence of sialic acid-rich mucosubstances has often been interpreted as implying an increased viscosity of the bronchial mucus with resulting impaired ciliary movement and decreased mucous blanket flow (Reid, 1969). It may be, however, that the histochemical techniques are demonstrating a more fundamental change, viz. accumulated secretory piece molecules not incorporated into lgA dimer. This concept has not yet been evaluated in man or the dog for it would be necessary to know not only the levels of lgA in the bronchial mucus, but also the proportion of lgA molecules with secretory piece incorporated.

Crofton and Douglas (1969) have stated that clinically, "the term chronic bronchitis is a convenience rather than a straitjacket" and this raises the possibility that, though chronic bronchitis may be one syndrome, it may be composed of several disorders with common clinical and pathological manifestations. Summarising their views on the aetiology, Crofton and Douglas (1969) comment "it is thought that infection is seldom a mitigating factor but that, once smoking or atmospheric pollution have induced the chronic cough and sputum, the patient's bronchial tree most readily becomes infected,

at first in acute exacerbations and later perhaps chronically. At present it is thought that the initial stimuli to increased mucus production in the respiraoty tract, giving rise to chronic cough and sputum, are most often cigarette smoking and pollution of the atmosphere by smoke."

Recent advances in immunology have not yet been fully applied in chronic bronchitis but the few reports available, mentioned above, raise the possibility of analternative aetiological mechanism.

Conventionally, cigarette smoke and atmospheric pollution have been thought to result in loss of cilia and altered viscosity of mucus due to quantitative constituent change. If the mucus change is interpreted in immunological forms, rather than solely mechanical or solely histochemical, it is possible to hypothesise that cigarette smoking and atmospheric pollution affect the secretion of intact dimer IgA molecules in such a way that the ability of the respiratory tract to mount immunological defence is impaired. This is not to deny the altered viscosity due to sialic acid-rich mucosubstances, but this would be an effect rather than a cause.

Chronic bronchitis in clinical terms could be regarded therefore as a clinical syndrome encompassing: persons with inherited IgA and IgA secretory piece deficiency, smokers with induced IgA secretory piece deficiency and persons exposed to heavy atmospheric pollution (Sulphur dioxide can affect serum immunoglobulins - Zarkower, 1972). The continuation of chronic bronchitis would be the result of continued IgA deficiency, of which repeated infection would be a sign, rather than recurrent infections.

The overall effect of chronic bronchitis is one of narrowing of the airways; the bronchial wall is thickened by cellular infiltrate, oedema and mucous gland hypertrophy, whilst the stenosed lumen tends to have accumulations of mucus. The mucociliary apparatus is severely impaired, not only by the increased amounts of mucus, but also by widespread epithelial changes which reduce the area of ciliated epithelium. The result is a narrowed airway with accumulations of mucus; the airway is thus pre-

disposed to further infection and normal respiration is impaired.

The disease which has been delineated in this field study closely resembles the disease of chronic bronchitis in man both clinically and pathologically. In both species, the disease is characterised by an insidious onset and a progressive course; it presents clinically as a chronic intractable cough and pathologically has a hypersecretion of mucus in the respiratory tract. Much remains to be learned of the epidemiology and pathogenesis of this disease, both in man and in the dog; it seems likely that further comparative and veterinary studies of the disease may help to elucidate these factors.

Case	Age (years)	Sex	Breed or type	Condition	Pyrexia	Cough	Duration of cough (months)	Production of sputum	Systolic murmur	Sinus arrhythmia	Month of death	Radiological Evidence
1	9	F	Manchester Terrier	Dull & very fat Enlarged abdomen	-	++	4	Yes	-	+	February	Yes
2	10	M	Cairn Terrier	Bright; very fat	-	+	9	No	-	+	November	Yes
3	15	F	Terrier type	Dull; fair condition	-	+	3	No	-	-	February	No
4	6	M	W. Highland Terrier	Bright; very fat abdomen	-	+	3	No	-	-	December	No
5	3	M	W. Highland Terrier	Dull; thin	-	++	2	Yes	-	-	November	No
6	10	M	Mongrel	Bright; fair condition	-	++	2	Yes	+	+	November	No
7	9	M	Bull Terrier	No clinical data available							December	Not known
8	12	M	Terrier type	Bright	+	+	2	No	-	-	April	No
9	6	M	Shetland Sheep dog	Bright; fat	+	+	18	No	-	+	July	No
10	7	M	Poodle	Bright; fat	-	++	6	Yes	+	-	July	Yes
11	5	M	Corgi	Bright; good condition	-	++	6	Yes	-	-	December	Yes
12	5	F	Cocker Spaniel	Bright; good condition	+	+	3	No	-	+	December	Yes
13	12	M	Pug	Bright; fat	-	++	6	No	+	+	November	No
14	9	M	Shetland Sheep dog	Extremely fat	-	++	12	Yes	-	+	February	Yes

Continued on next page.

Case	Age (years)	Sex	Breed or type	Condition	Pyrexia	Cough	Duration of cough (months)	Production of sputum	Systolic murmur	Sinus arrhythmia	Month of death	Radiological Evidence
15	12	M	W. Highland	Dull and fat; enlarged abdomen	-	++	6	No	-	+	June	No
16	13	F	Springer Spaniel	Dull and very fat enlarged abdomen	-	++	24	No	-	+	February	Yes
17	14	M	Terrier type	Dull and thin	-	++	3	No	+	+	March	No
18	8	M	Poodle	Fat; enlarged abdomen	-	+	4	No	-	+	March	No
19	9	F	Shetland Sheep dog	Very fat	+	++	3	Yes	+	-	May	Yes
20	4	M	Cocker Spaniel	Dull; fair condition	not known	++	2	Yes	-	-	June	Yes
21	5	M	Poodle	Dull; thin	-	++	3	No	+	-	February	No
22	6	F	Jack Russell Terrier	Fair condition	-	++	3	Yes	-	+	February	No
23	11	M	Mongrel	Very fat	-	++	2	No	+	+	April	Yes
24	6	F	Bull Terrier	Very fat	-	+	8	Yes	-	-	March	Yes
25	14	M	Bull Terrier	Fat	not known	+	3	No	-	-	September	Not known
26	7	F	Miniature Pinscher	Very fat	-	++	3	Yes	-	+	November	Yes

Table 1: Chronic bronchitis: summary of the main clinical findings in the 26 cases of chronic bronchitis.

(Cough graded + to ++)

- Not found

Breed or Type	Number of Animals
West Highland Terrier	3
Shetland Sheepdog	3
Spaniel (Cocker and Springer)	3
Bull Terrier	3
Poodle	3
Terrier type	3
Mongrel	2
Jack Russell Terrier	1
Manchester Terrier	1
Miniature Pinscher	1
Cairn Terrier	1
Corgi	1
Pug	1

Table (2): Chronic bronchitis; the breeds and types of the 26 cases of chronic bronchitis.

Case	Duration of cough (months)	Excess mucus in bronchi	Polyps: Macro	Polyps: Micro	Amount of mucous glands	Anthracosis	Pneumonia	Emphysema	Valvular Endocardosis	Diagnoses at post mortem	Bacteriology
1	4	+	++	++	++	++	-	-	-	Chronic bronchitis	NSF
2	9	+	-	-	++	+	-	-	-	Chronic bronchitis	NSF
3	3	++	-	-	+++	++	+	++	-	Congestive cardiac failure; chronic bronchitis and emphysema; terminal acute pneumonia; cor pulmonale	NSF
4	3	+	-	+	++	+	+	-	+	Chronic bronchitis	NSF
5	2	+	+	++	++	+	-	+	+	Chronic bronchitis and emphysema	NSF
6	2	+	-	-	++	+	-	+	+	Chronic bronchitis; severe valvular endocardosis	NSF
7	-	++	++	++	++	+	-	+	+	Chronic bronchitis; severe valvular endocardosis	NSF
8	2	++	+	++	++	++	-	-	+	Chronic bronchitis; severe valvular endocardosis	NSF
9	18	++	++	++	+++	-	+	-	-	Chronic bronchitis; pneumonia	Proteus
10	6	++	-	-	+++	+	+	-	-	Chronic bronchitis	Negative
11	6	++	+	++	+++	+	-	+	-	Chronic bronchitis	Negative
12	3	+	+	++	+++	+	-	+	-	Chronic bronchitis and emphysema	NSF
13	6	+	-	-	++	++	+	+	+	Chronic bronchitis	NSF
14	12	++	-	++	++	++	-	+	+	Chronic bronchitis and emphysema	Bordetella bronchiseptica

Continued on next page.

Case	Duration of cough (months)	Excess mucus in bronchi	Polyps: Macro	Polyps: Micro	Amount of mucous glands	Anthracosis	Pneumonia	Emphysema	Valvular Endocardosis	Diagnoses at post mortem	Bacteriology
15	6	+	-	++	++	+	-	-	+	Chronic bronchitis; severe valvular endocardosis	NSF
16	24	+	-	-	++	++	-	+	+	Chronic bronchitis; renal amyloidosis; pulmonary embolism	NSF
17	3	+	-	+	++	++	+	+	+	Congestive cardiac failure; chronic bronchitis	NSF
18	4	+	-	+	++	++	-	+	+	Chronic bronchitis; valvular endocardosis	Bordetella bronchiseptica
19	3	+	-	-	+++	++	-	+	+	Cor pulmonale; chronic bronchitis	Bordetella bronchiseptica
20	2	++	-	-	++	+	-	+	-	Chronic bronchitis	NSF
21	3	+	-	+	++	+	+	-	+	Congestive cardiac failure chronic bronchitis severe endocardosis	Bordetella bronchiseptica
22	3	+	-	+	++	+	+	++	-	Chronic bronchitis and emphysema	NSF
23	2	+	-	-	++	++	-	+	-	Chronic bronchitis and emphysema	Bordetella bronchiseptica
24	8	++	-	+	+++	+	-	+	+	Chronic bronchitis and emphysema	NSF
25	3	+	-	-	++	++	-	+	-	Chronic bronchitis	Bordetella bronchiseptica
26	3	++	-	++	+++	+	+	-	-	Chronic bronchitis terminal pneumonia	Bordetella bronchiseptica

Table 3: Chronic bronchitis: Summary of the main postmortem findings in the 26 cases of chronic bronchitis (Bronchial mucus, polyps, anthracosis and emphysema graded + to ++; mucous glands graded + to +++)
NSF no significant findings; - not found.

Age (Years)	Number admitted	% of total	Number with cough	Cough		% of dogs in group with cough
				Occasional	Frequent	
0-1	777	28.7	37	25	12	4.8
1-2	369	13.7	24	13	11	6.5
2-4	461	17.0	25	16	9	5.5
4-8	593	21.9	42	30	12	7.1
8-12	439	16.2	41	30	11	9.5
12+	68	2.5	13	8	5	19.0
Total	2707	100.0	182	122	60	6.7

Table 4 : from Termatrex input for January 1972 - August 1973.

The number of dogs admitted to University of Glasgow Veterinary School (U.G.V.S.) during this period is given for each of the age groups indicated. The number in each age group with a cough at the time of admission is also noted and related to the total number of dogs in each group. The presence of cough at the time of admission does not necessarily imply that this was the reason for referral by the veterinarian.

Breed or type of dog	Number of dogs	% of total number of admissions	Cough		% of group with cough on admission
			Occasional	Frequent	
Poodle	228	8.4	16	9	11
Fox Terrier	64	2.4	7	0	11
Terrier-type	136	5.0	4	10	10.3
Boxer	97	3.6	7	3	10.3
Spaniel	69	2.5	5	2	10.1
Border Collie	10	0.4	1	0	10.0
Cocker Spaniel	62	2.3	3	2	8.1
Crossbreds	548	20.2	24	19	7.9
W. Highland Terr.	134	5.0	8	2	7.5
Toy/Miniature type	94	3.5	5	2	7.5
Shetland Collie	81	3.0	5	1	7.4
Giant breeds	15	0.6	1	0	6.7
Alsatian	263	9.7	8	6	5.3
Collie-type	158	5.8	6	2	5.0
Cairn Terrier	100	3.7	4	1	5.0
Dachshund	39	1.4	1	1	5.0
Labrador	248	9.2	8	4	4.8
Beagle	32	1.2	0	1	3.0
Pekingese	40	1.5	1	0	2.5
Corgi	42	1.6	0	1	2.4
TOTAL	2460	91.0	114	66	7.3

Table 5 : Distribution of twenty selected breeds and types on 2,460 canine admissions to University of Glasgow Veterinary School from January 1972 to August 1973.

(The twenty breeds and types comprised 91 per cent (2,460 dogs) of the study population. The proportion of breed or type as a percentage of the study population is noted, together with figures for coughing within the breed or group.)

Age group (years)	Total number of dogs	Total number of dogs with distinct systolic murmur	Dogs with a cough				Total number of dogs with cough	Total number of dogs with cough and systolic murmur
			Occasional cough	Occasional cough + systolic murmur	Frequent cough	Frequent cough + systolic murmur		
4-8	593	21 (4%)	30	1	12	1	42 (7%)	2 (0.3%)
8-12	436	35 (8%)	30	4	11	5	41 (9%)	9 (2.1%)
12+	68	13 (19%)	8	3	5	2	13 (19%)	5 (7.4%)
TOTAL	1097	69 (6%)	68	8	28	8	96 (9%)	16 (1.5%)

(Figures in parentheses indicate percentage of total number of dogs in the age group).

Table 6: Incidence of cough and systolic murmur in three age groups of the survey population of dogs admitted to University of Glasgow Veterinary School (January 1972 - August 1973).

In each group, the numbers of dogs having a cough, a distinct systolic murmur and cough and distinct systolic murmur are noted.

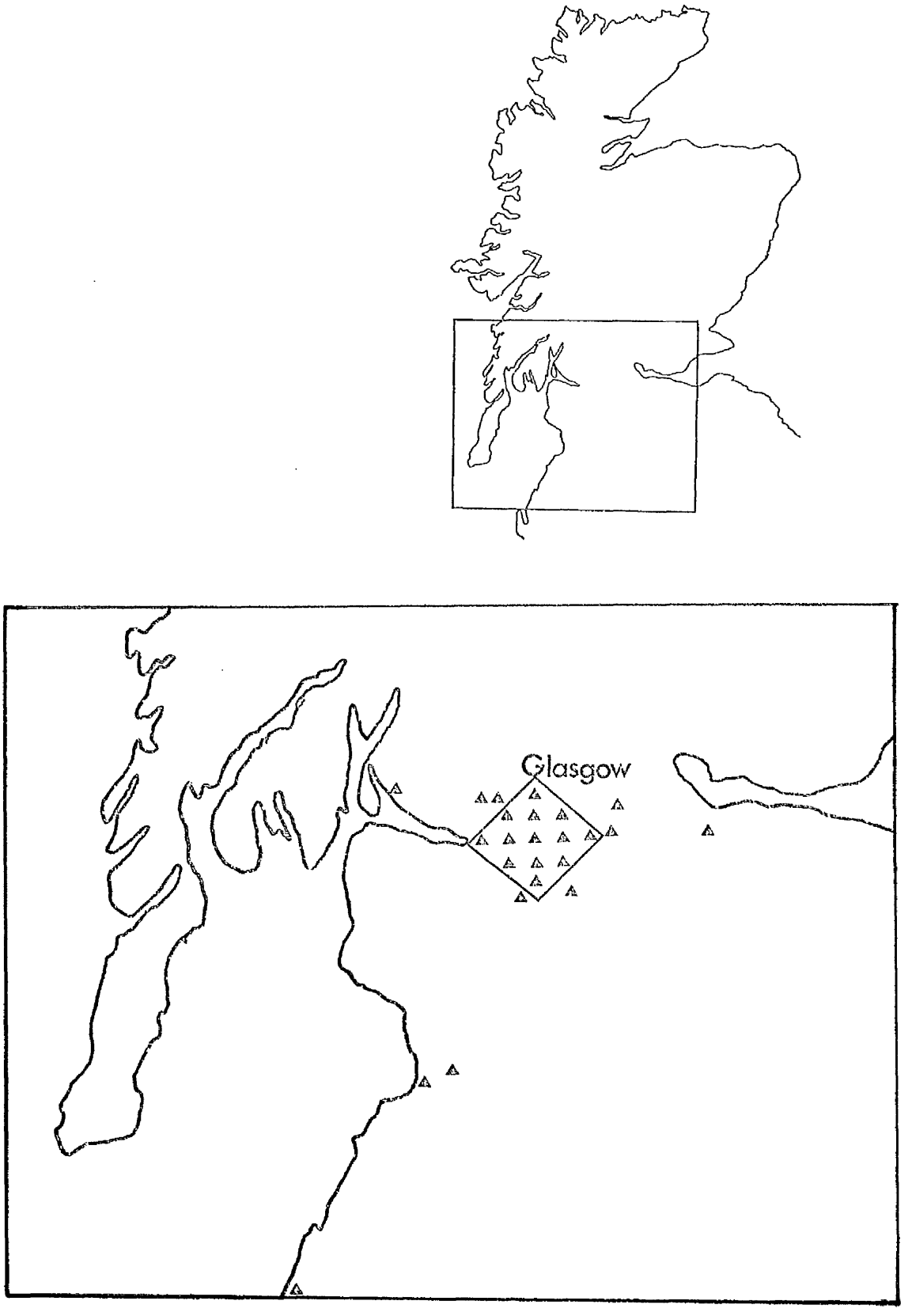


Fig. (3): Chronic bronchitis: District of origin of 26 cases of chronic bronchitis in the dog. Map of Scotland (top right) has inset (square) which is enlarged in lower half of page. The urban environs of Glasgow are represented by the diamond and cases of chronic bronchitis are represented by triangles. Thirteen of the cases had lived within the city of Glasgow, eleven had been referred from Central and Southern Scotland. Two of the cases (not shown) were referred from England (Stourbridge, Worcestershire and Byfleet, Surrey).

Fig. 4 : Chronic bronchitis: Age distribution of 26 cases of chronic bronchitis in the dog.

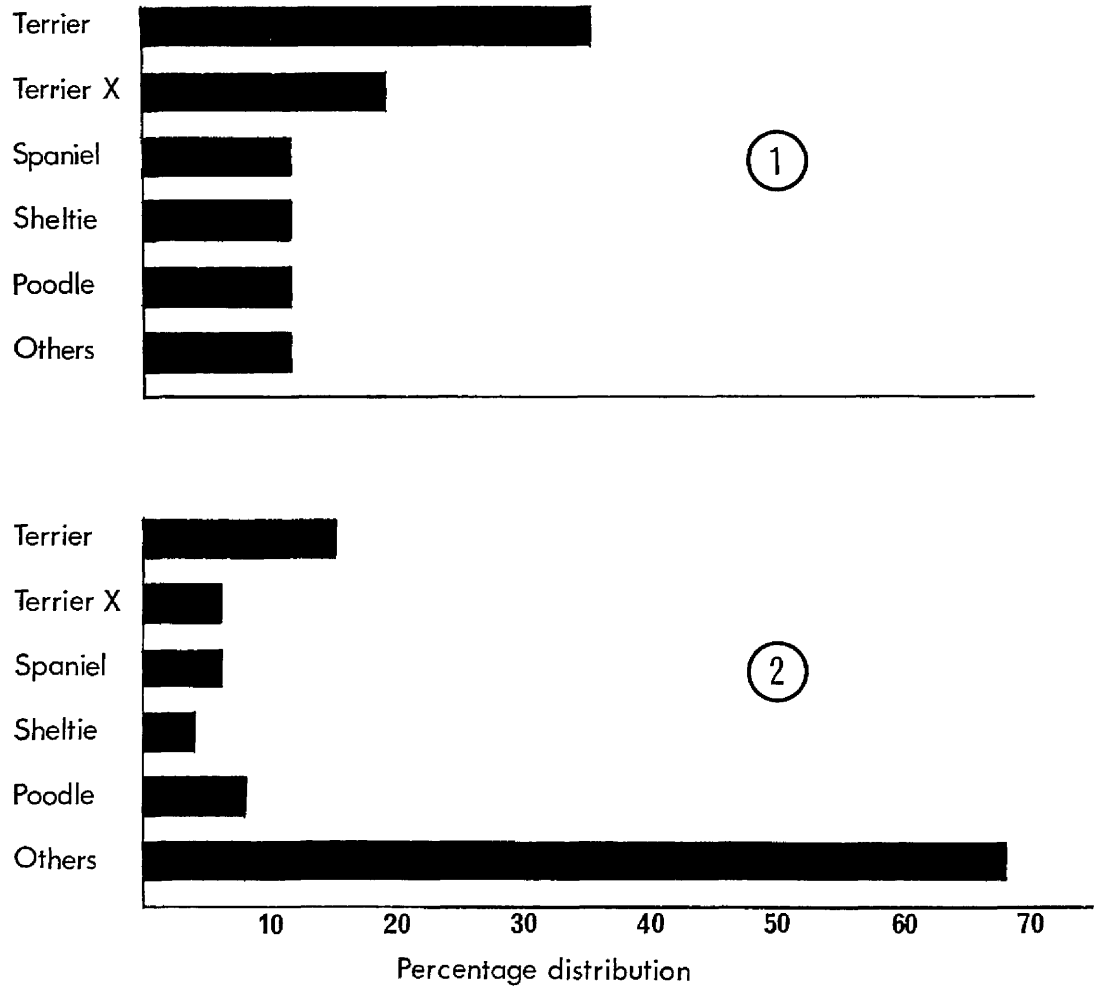


Fig. 5 : Chronic bronchitis: The percentage distribution of breeds and types in :
(1) 26 cases of chronic bronchitis
(2) 1337 cases referred, for any reason, to the University of Glasgow Veterinary School during 1972.

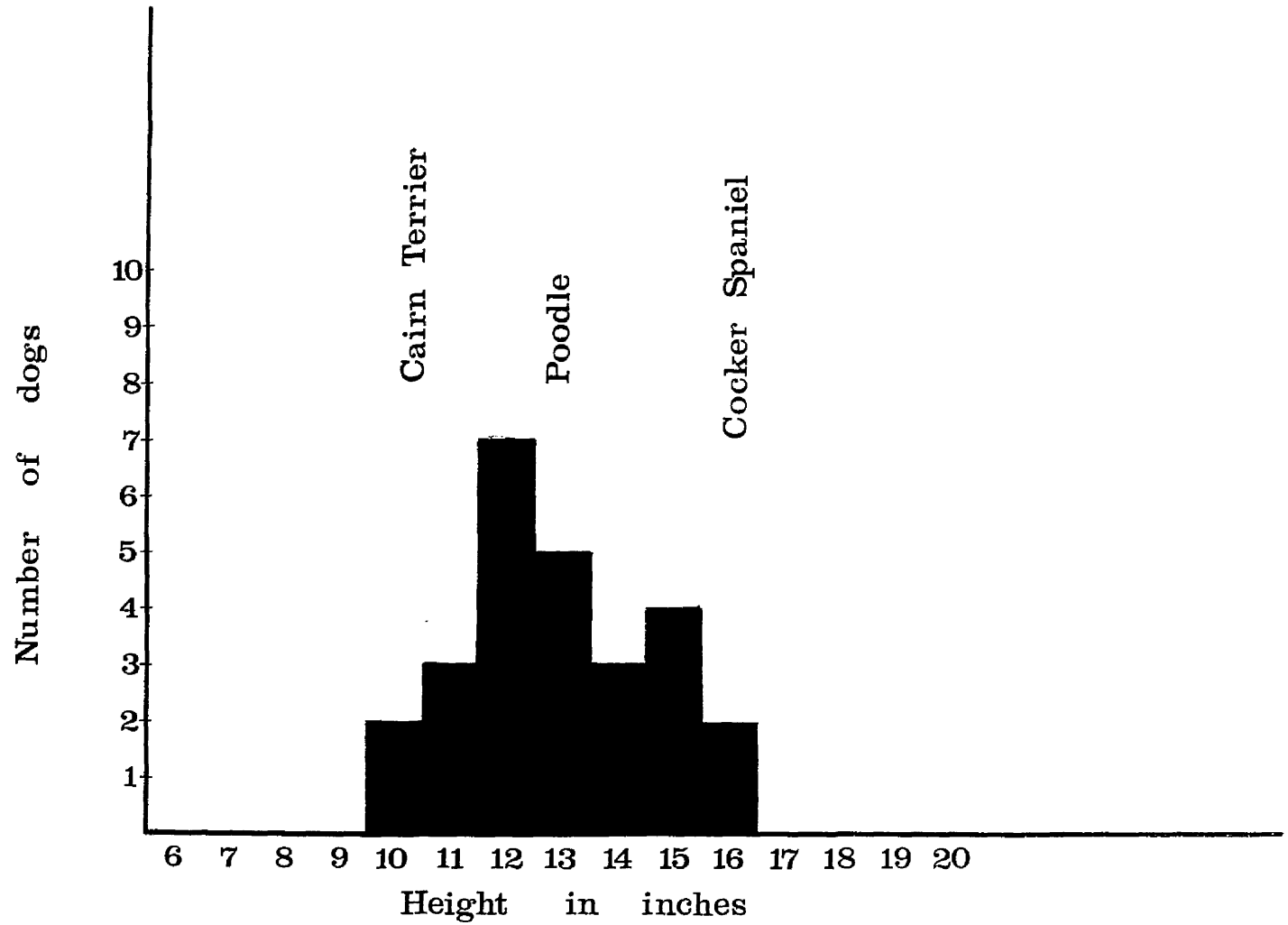


Fig. 6 : Chronic bronchitis: Heights of 26 dogs with chronic bronchitis.

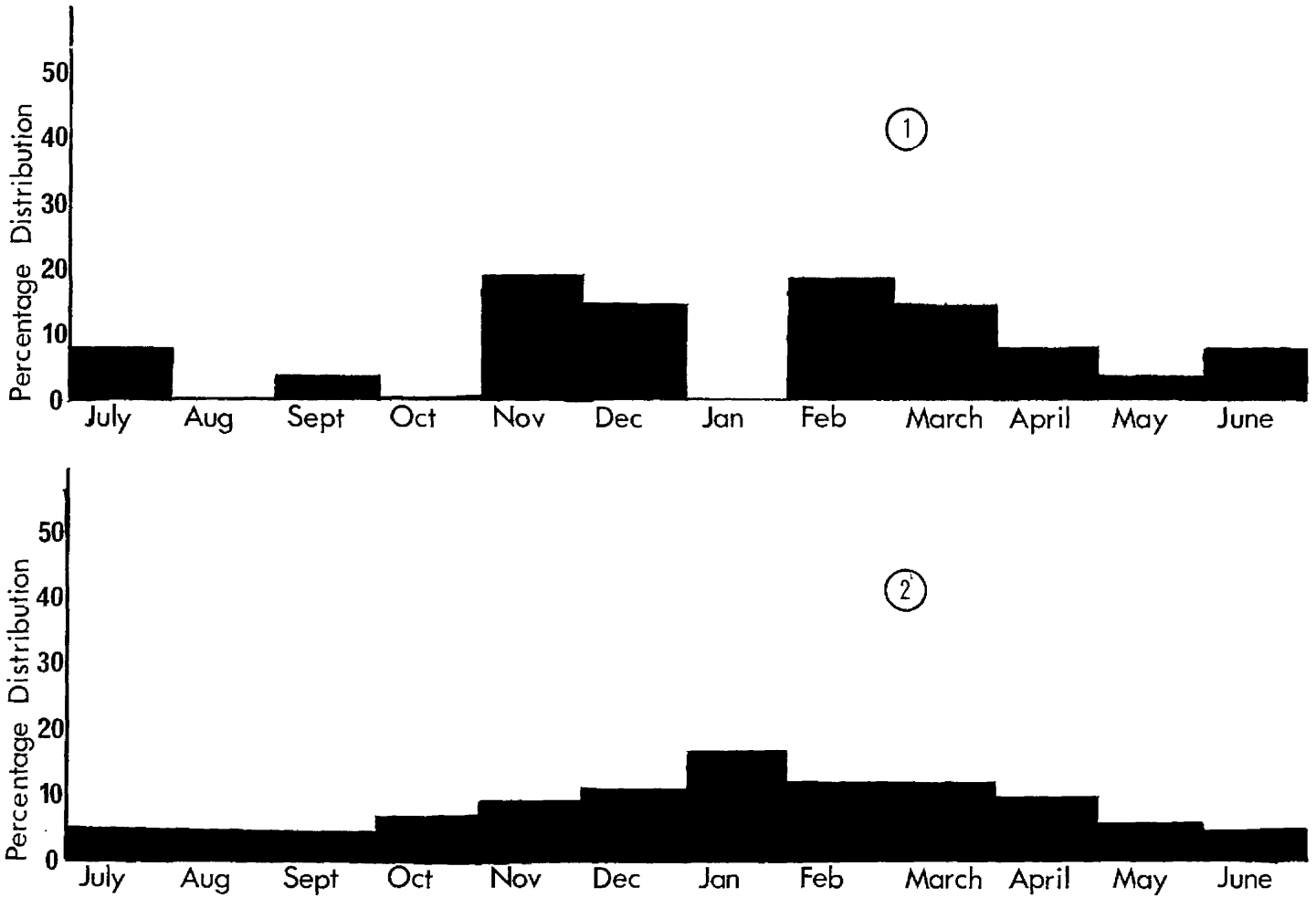


Fig. 7 : Chronic bronchitis: Seasonal distribution of mortality due to chronic bronchitis in :
(1) 26 cases of chronic bronchitis in the dog
(2) Man in England and Wales, 1966 (Registrar General's figures)

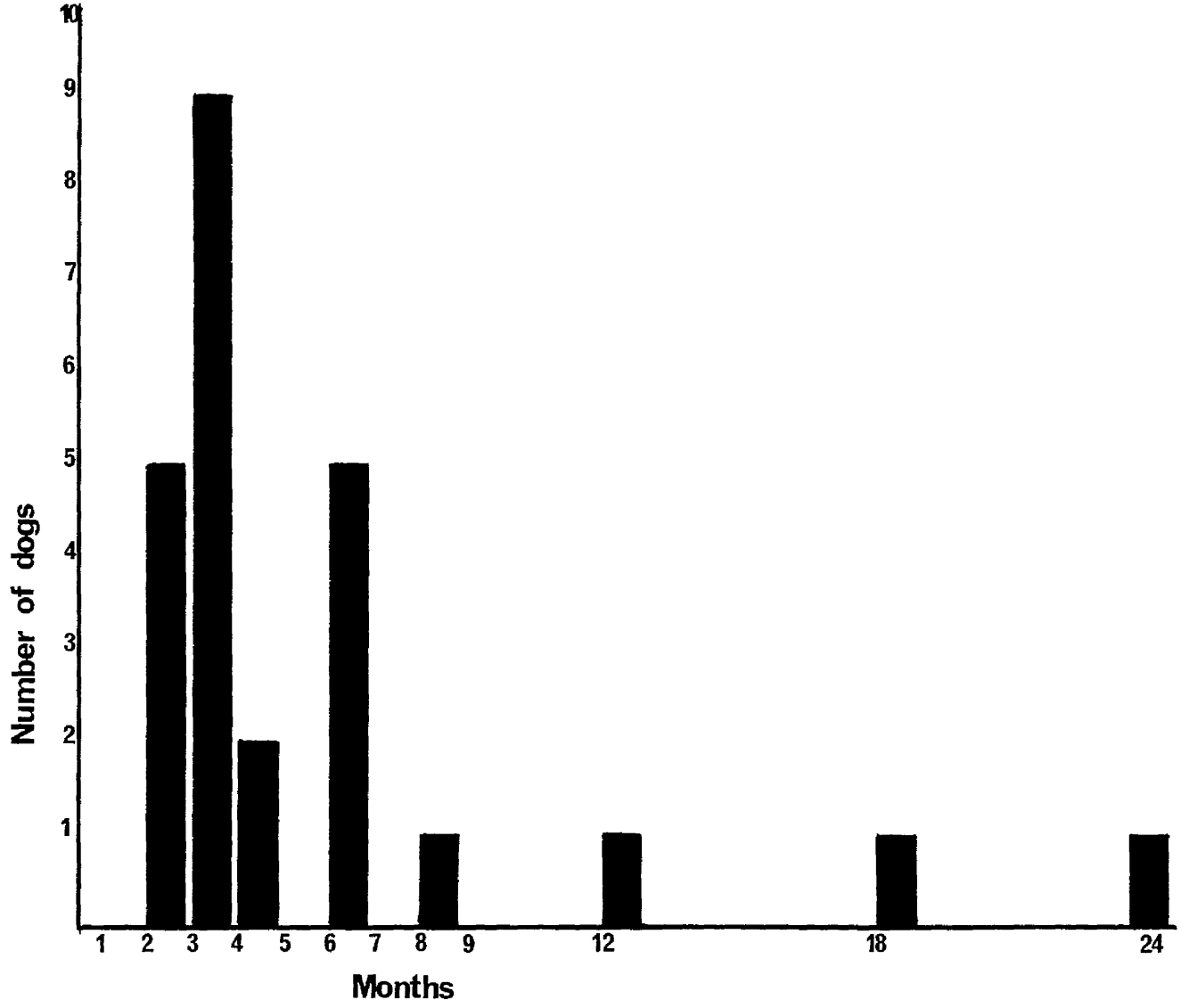
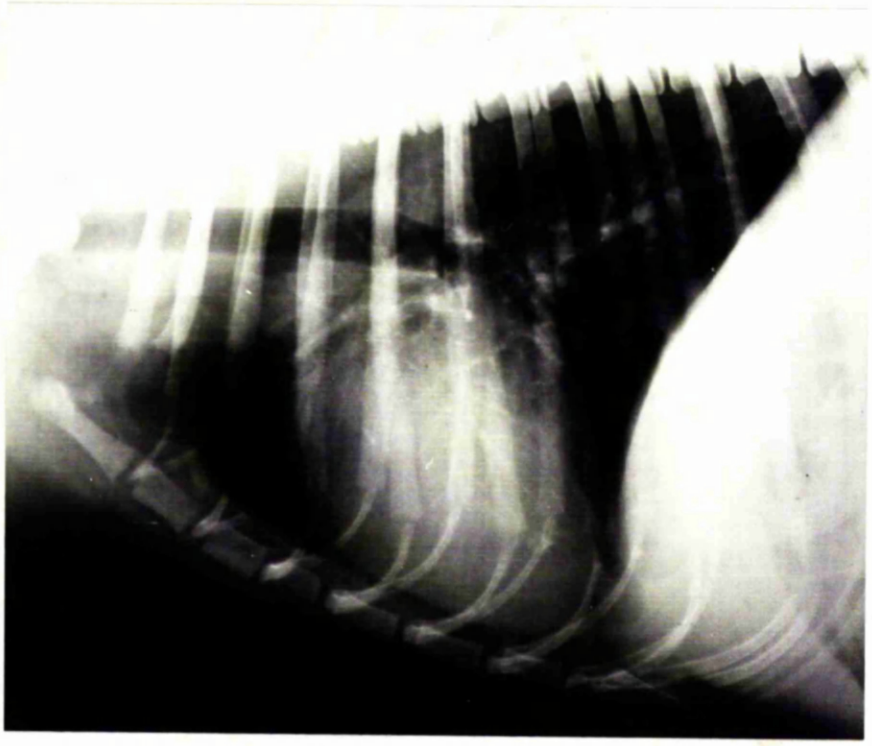


Fig. 8 : Chronic bronchitis: Duration of cough in 25 cases.

Fig. (9): Chronic bronchitis: Lateral radiograph of normal canine thorax.

Fig. (10): Chronic bronchitis: Lateral radiograph of thorax of dog with chronic bronchitis. Prominent bronchial markings appear as circular and linear opacities (arrows) over lung field.



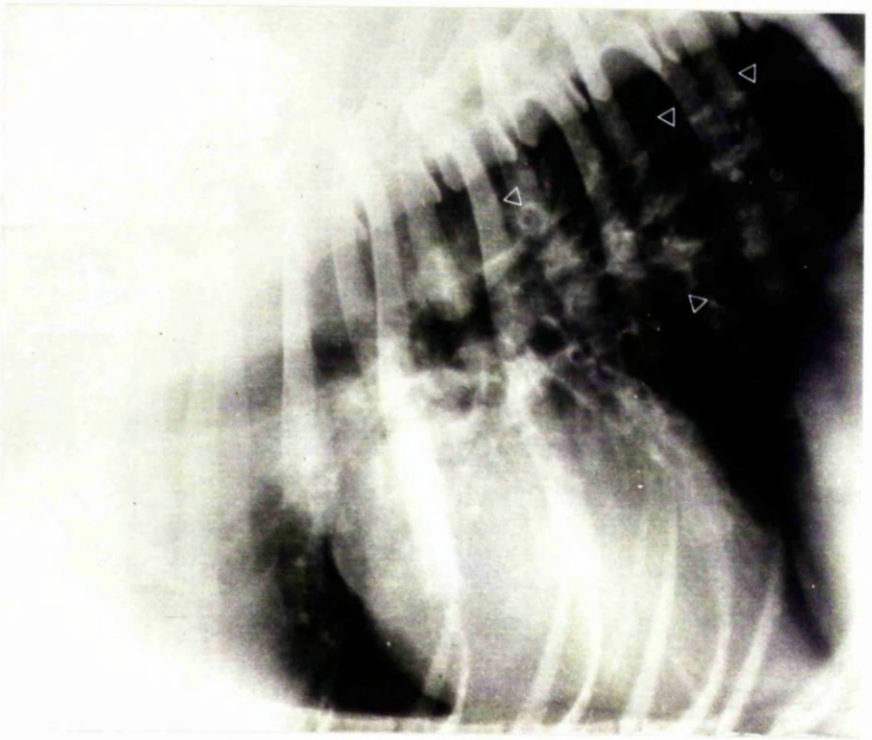


Fig. (11): Emphysema: anterior lobes from the lungs of a dog with chronic bronchitis. Pale distended areas of emphysema can be seen around the edges of all lobes. (Case no.22).

Fig. (12): Chronic bronchitis: Lungs of a dog with chronic bronchitis with a large pool of mucus at the tracheal bifurcation. A strand of mucus (arrow) can be seen lifted by forceps.

(Case no.26).

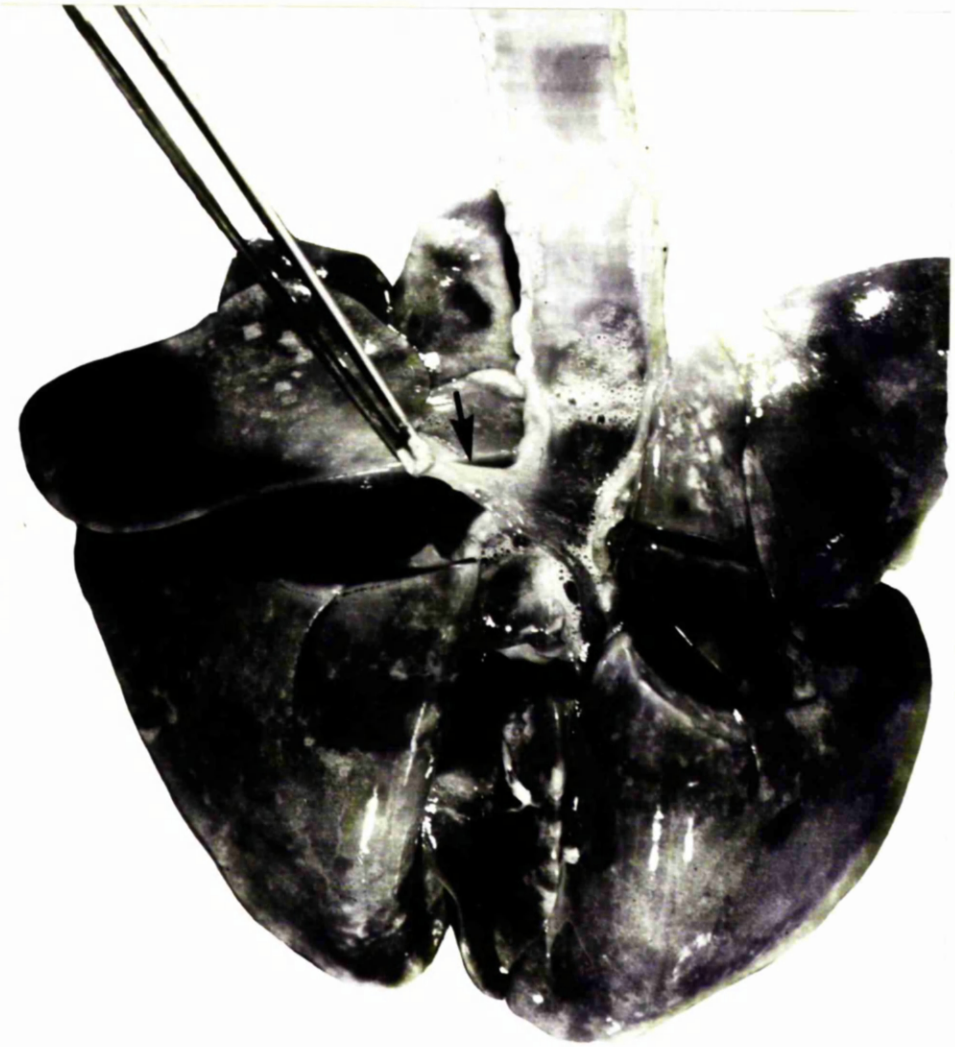
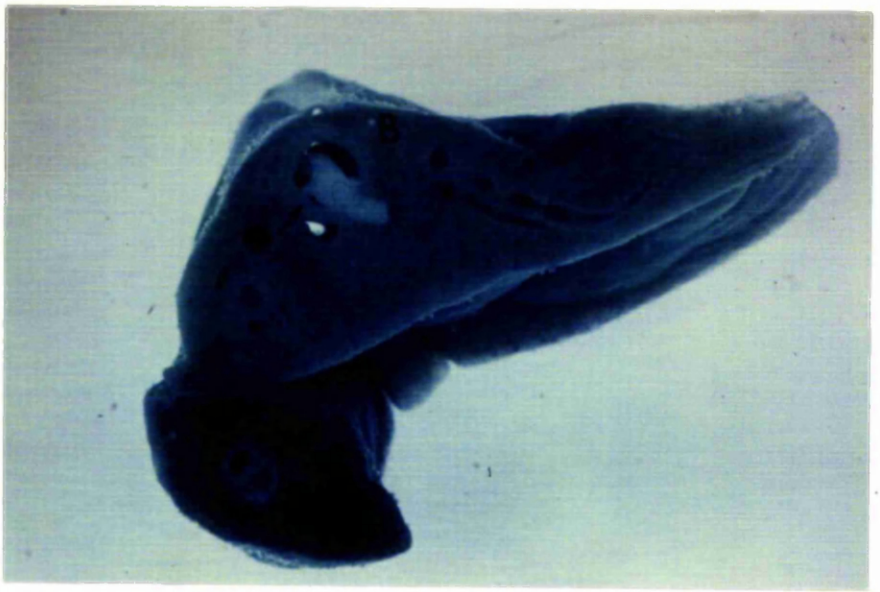


Fig. (13): Chronic bronchitis: Transverse slice of lung lobe fixed whole in formol saline, sectioned and immersed in water. The lobar bronchus (B) contains a thick strand of mucus (arrow).

Fig. (14): Chronic bronchitis: Cardiac lobe of lung fixed whole in formol saline before opening the lobar bronchus. A large plug of mucus (arrow) can be seen lifted out of the lumen.

(Case no.24).



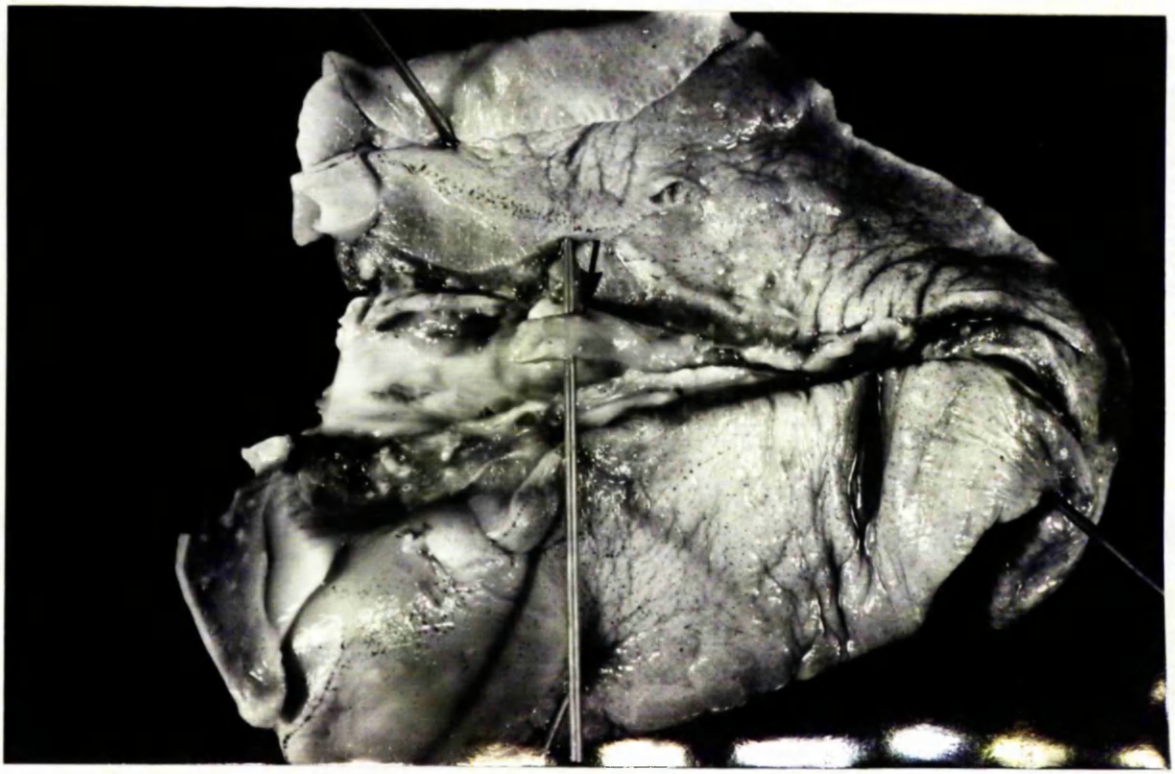


Fig. (15): Chronic bronchitis: Detail of bronchial mucosa with a large polyp (arrow) and many smaller polyps (open arrows).

Fig. (16): Chronic bronchitis: Lungs of a dog with chronic bronchitis with large polypoid proliferations (open arrows) visible in the lobar bronchus of the diaphragmatic lobe.

(Case no. 9).

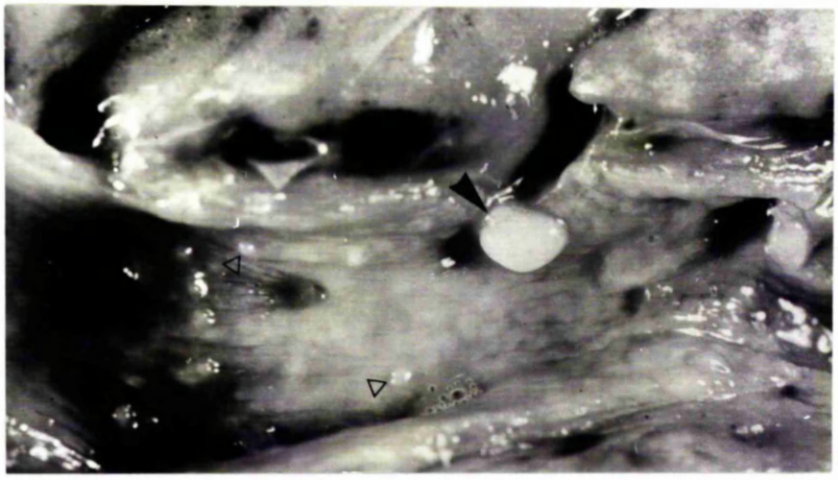
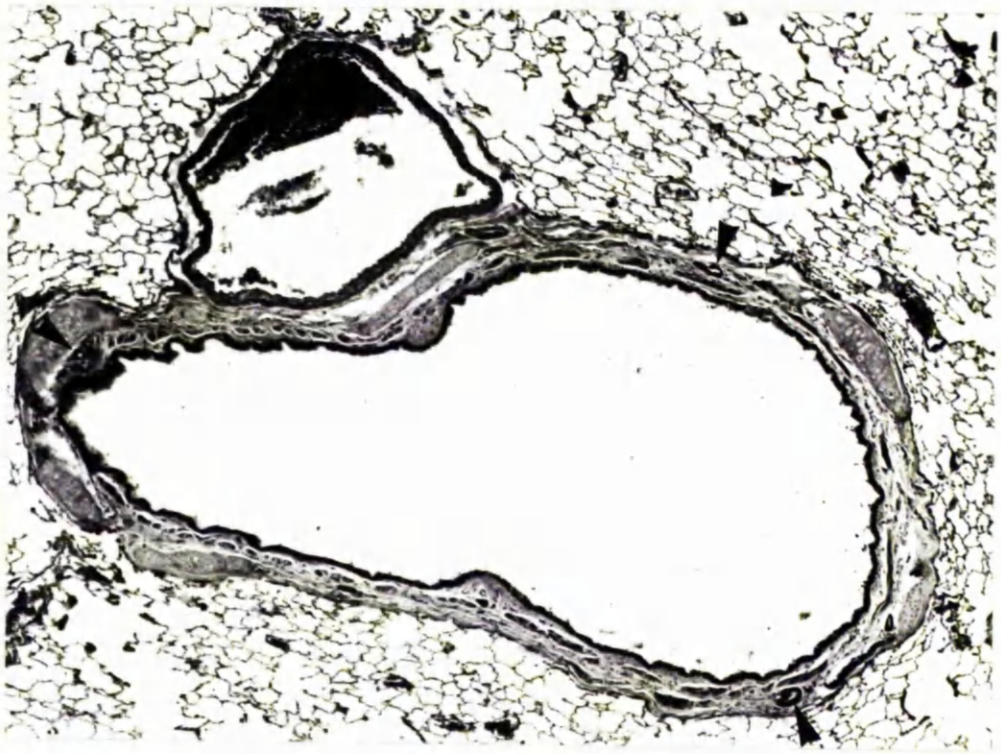


Fig. (17): Canine bronchus: Cross-section of normal bronchus. The normal bronchus is a thin-walled tube with pseudostratified epithelium and a fairly narrow lamina propria. Mucous glands (arrowed) are sparsely distributed.

(HE X 50).

Fig. (18): Chronic bronchitis: Cross-section of bronchus - compare with Fig. (17); the epithelium is hyperplastic and has a ragged appearance with clefts and defects. The mucosa is thrown into folds and there is a heavy cellular infiltrate into both the lamina propria and sub-mucosa. There is an increase in the number and size of mucous glands (arrowed). Mucus and inflammatory cells are present in the lumen.

(HE X 50).



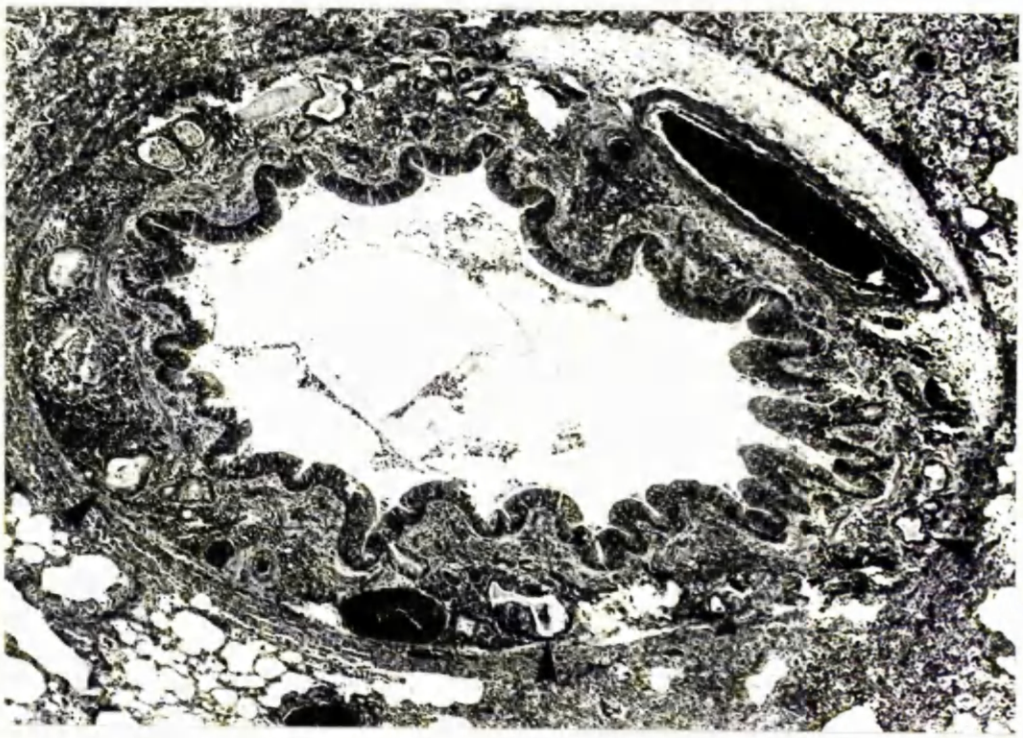
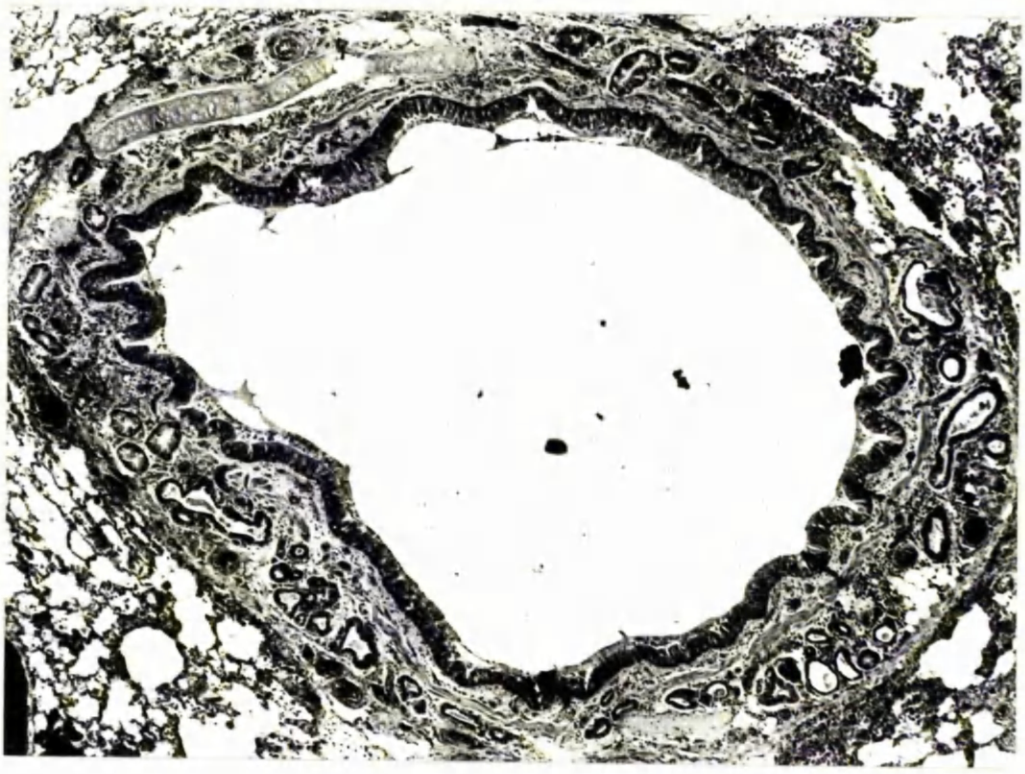


Fig. (19): Chronic bronchitis: cross-section of bronchus- compare with Fig. 17; the mucous glands are particularly numerous, although the cellular infiltrate is not marked.

(HE X 50).

Fig. (20): Chronic bronchitis: cross-section of bronchus - compare with Fig. 17; there is a large plug of mucus in the lumen.

(PAS X 50).



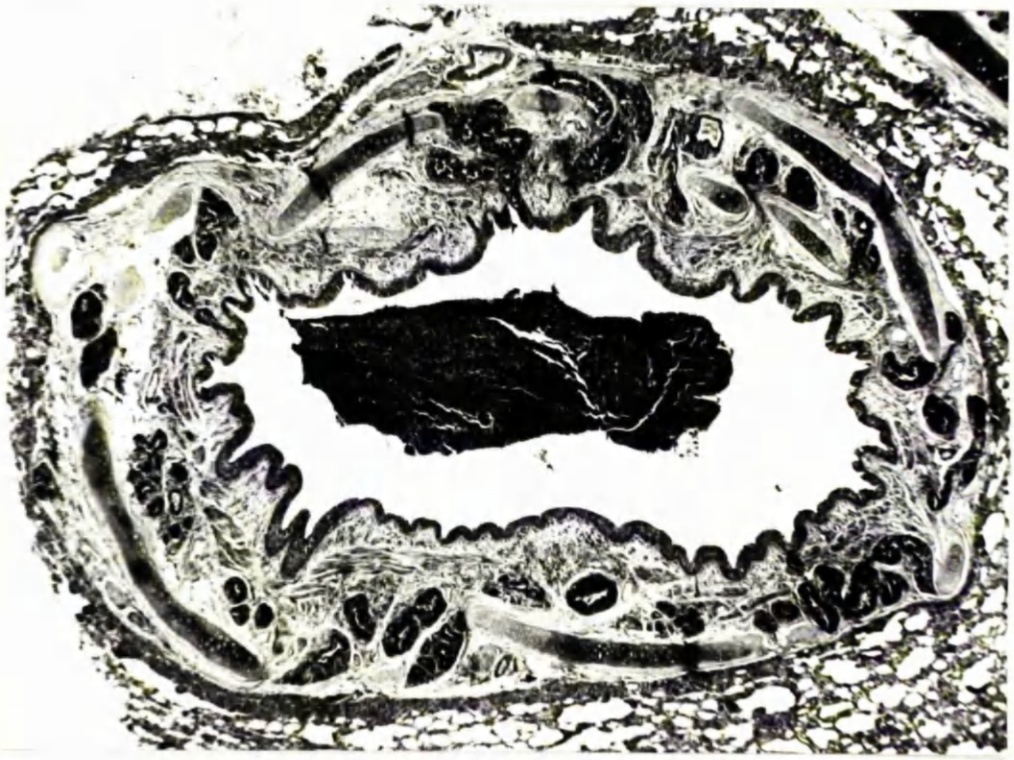


Fig. (21): Bronchial epithelium: normal bronchial epithelium with ciliated columnar cells (*) resting on basement membrane (double arrows). Goblet cells (single arrows) can be seen interspersed between the columnar cells and some are discharging granular mucosubstances (open black arrows) into the bronchial lumen. A single layer of basal cell nuclei can be seen above the basement membrane. (Toluidine blue, 1μ section X 1200).

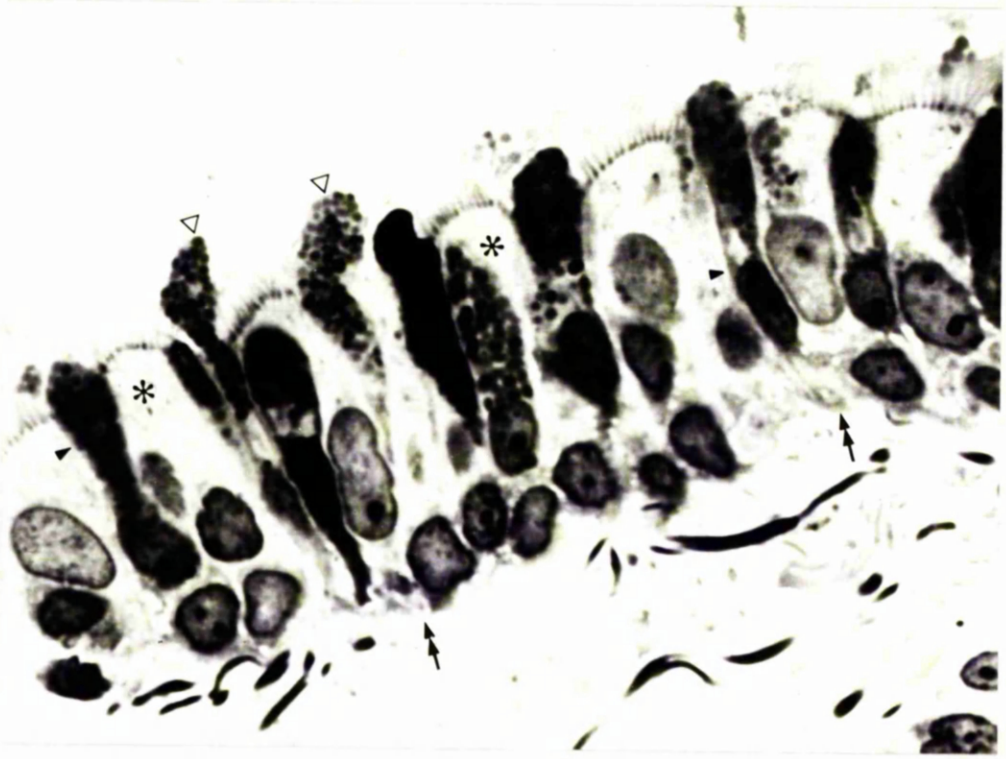
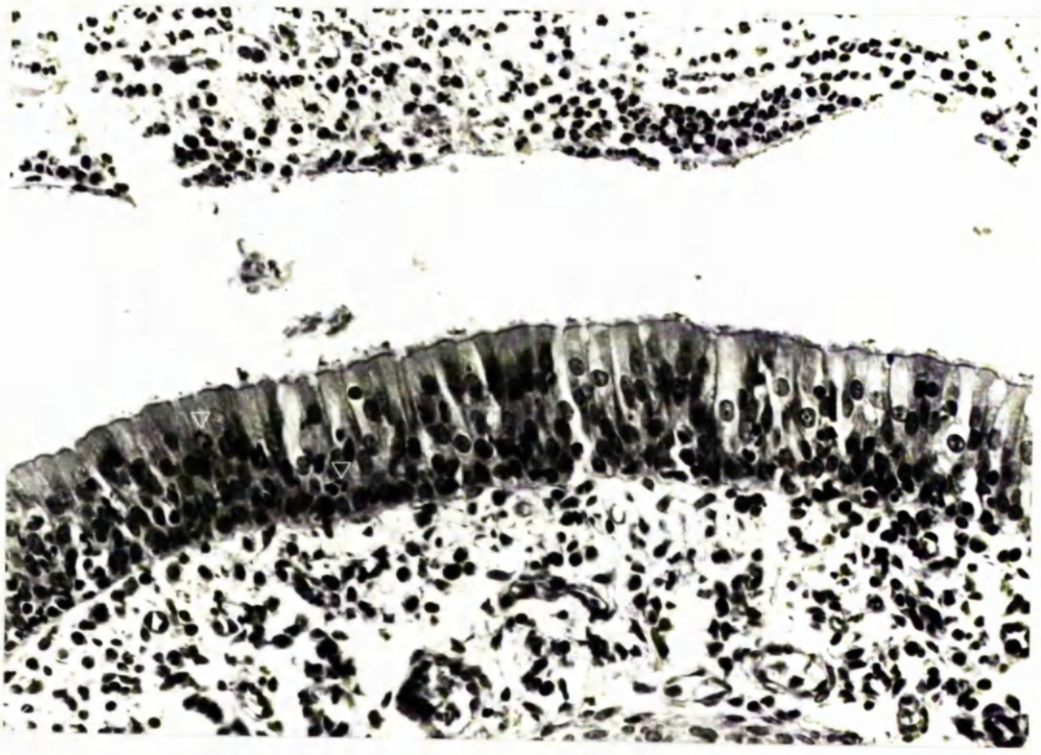


Fig. (22): Chronic bronchitis: intact bronchial epithelium from a dog with chronic bronchitis. Ciliated columnar cells are still present but appear more numerous judging by the number of nuclei present in the middle regions of the epithelium. In addition, the basal cell nuclei are also increased in number. The underlying lamina propria is very oedematous and has been infiltrated by plasma cells and lymphocytes. There are considerable numbers of neutrophils migrating through the epithelium (open arrows) and there is a cellular exudate in the lumen.

(HE X 350).

Fig. (23): Chronic bronchitis: Longitudinal view of small bronchus with bulging of bronchial wall due to inflammatory reaction. The normal bronchial epithelium (arrowed) is replaced by proliferating basal cells which have piled up to form clumps in some areas (double arrows) and have formed thin sheets in other areas (double arrows). The epithelium is broken at two sites (open arrows). The subjacent lamina propria has a cellular infiltrate with pronounced dilatation of capillaries.

(PAS X 110).



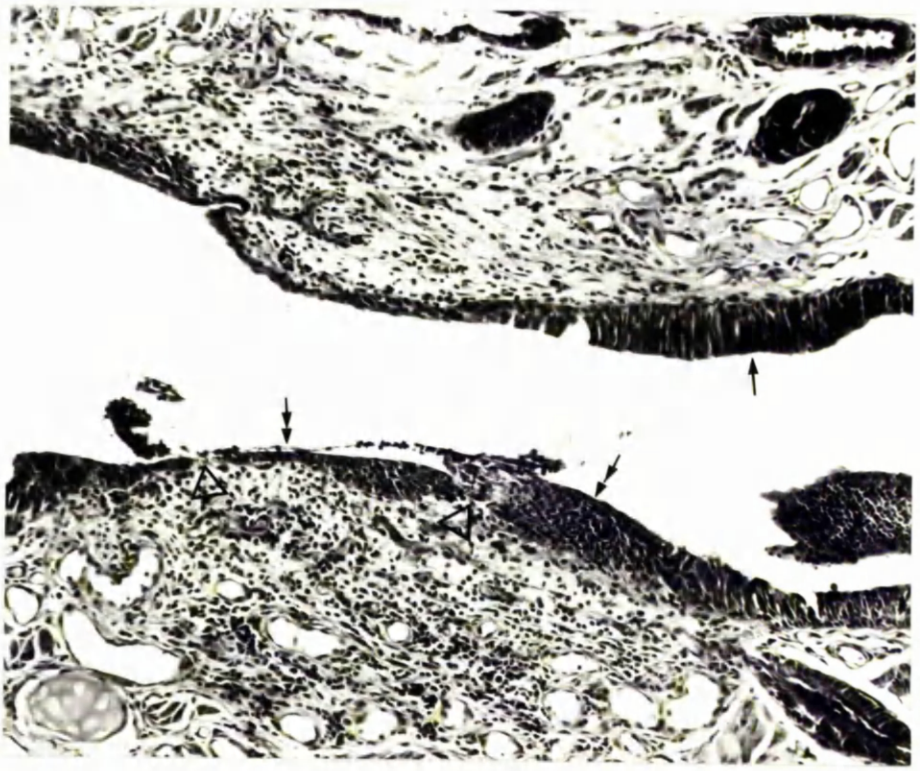
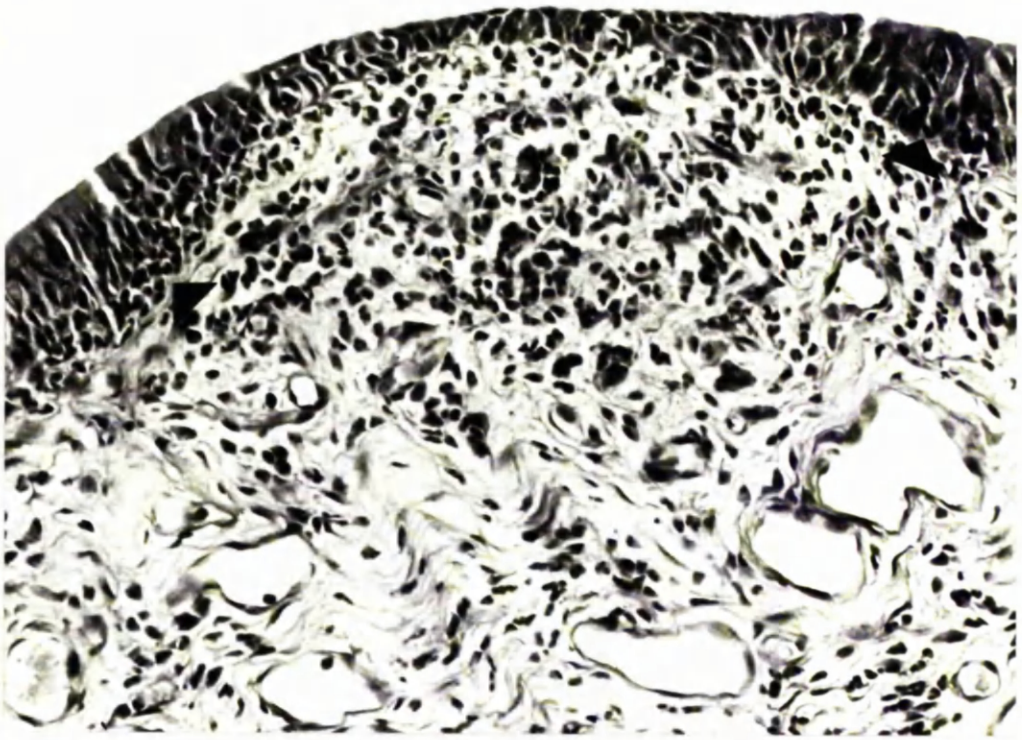


Fig. (24): Chronic bronchitis: Focal infiltration of inflammatory cells into the bronchial lamina propria. At either side, the respiratory epithelium has increased numbers of basal cells (arrows); these undifferentiated cells form the epithelium over the infiltrated area. Below this attenuated epithelium, there is sub-epithelial oedema, and a heavy infiltration of neutrophils, plasma cells and lymphocytes with congestion and dilatation of capillaries.

(HE X 350).

Fig. (25): Chronic bronchitis: Complete loss of epithelium over a section of bronchial wall with bulging of the underlying lamina propria. There is a heavy cellular infiltrate in this area, particularly around the mucous gland acini.

(HE X 110).



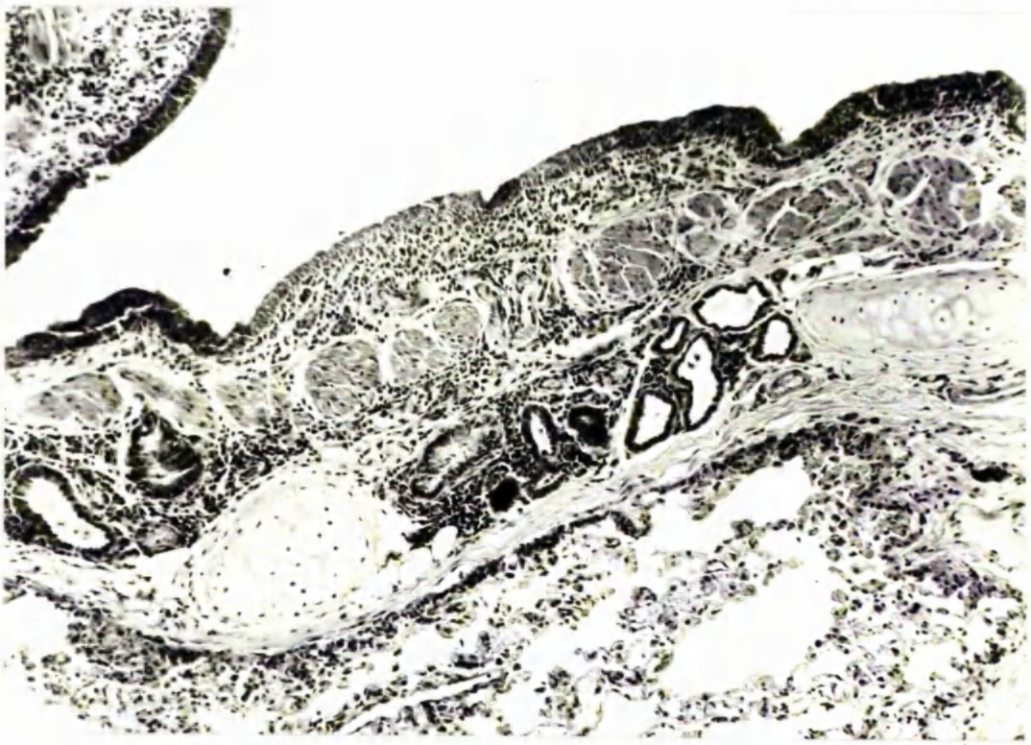
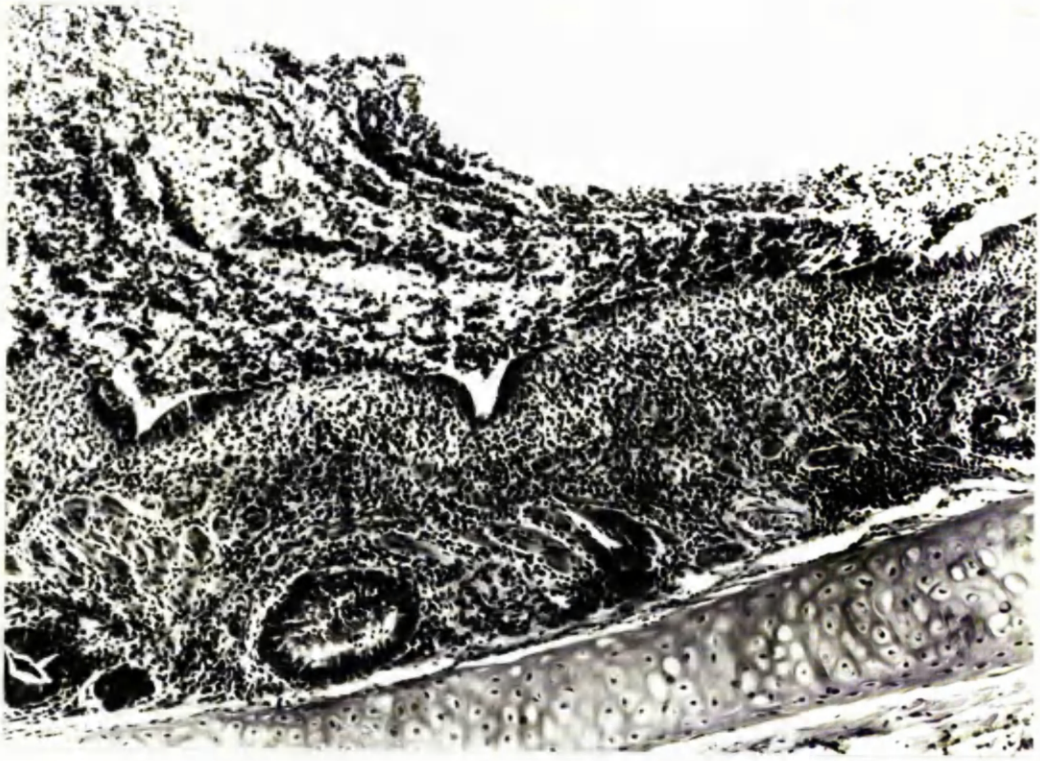


Fig. (26): Chronic bronchitis: Intense cellular infiltration of bronchial wall with oedema of lamina propria and disruption of muscle layer. Widespread erosion of the epithelium has occurred and large numbers of cells can be seen in the bronchial lumen.

(HE X 110).

Fig. (27): Chronic bronchitis: Low power view of segment of bronchial wall with epithelial hyperplasia (arrowed), attenuation of epithelium (open arrow) and complete loss of epithelium (double arrow). A large tenacious plug of mucopus appears to be adhering to sites where the epithelium has been lost. Goblet cells are particularly prominent in epithelial recesses (*).

(PAS X 200).



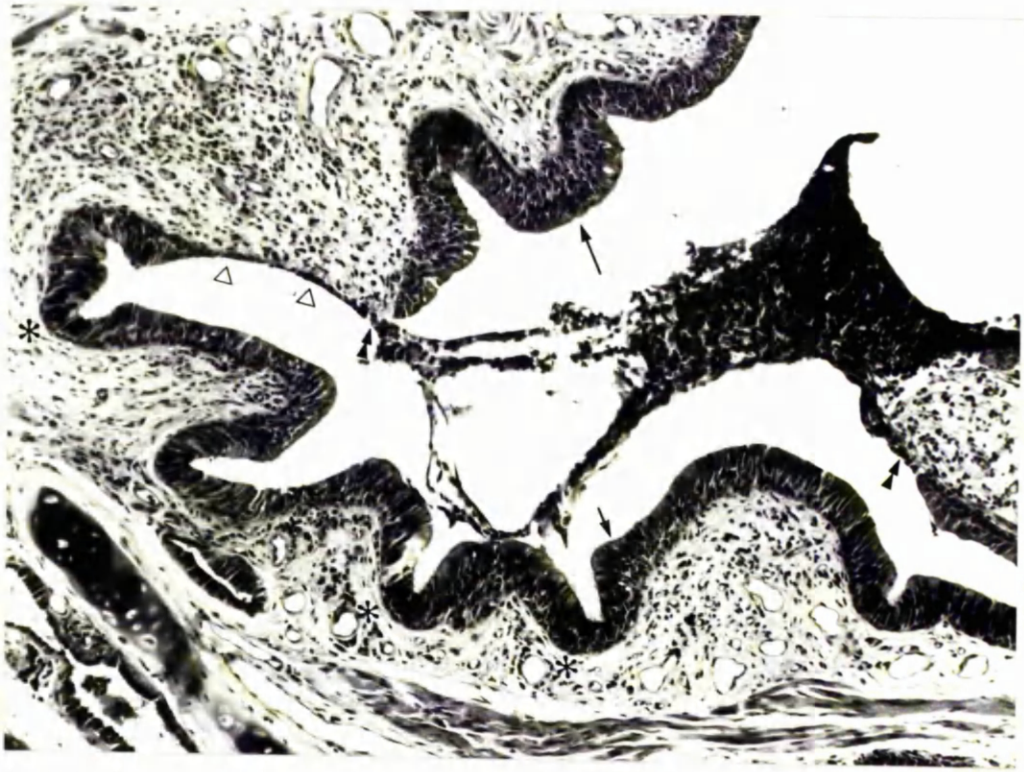
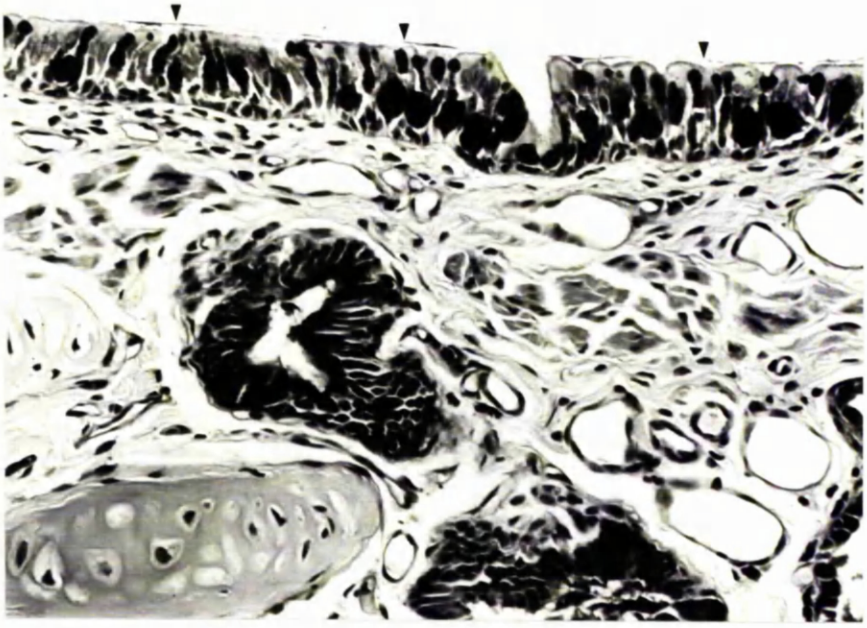


Fig. (28): Normal bronchus: the epithelium has a regular distribution of goblet cells. In this section, the goblet cell secretions are seen as dark staining globules in the epithelium; a range of forms can be seen from plump, flask-shaped cells, set deep in the epithelium to more slender undulating forms. A fine layer of mucus can be seen in the lumen resting on the tips of the cilia (arrows).

(PAS X 350).

Fig. (29): Chronic bronchitis: epithelial goblet cells are poorly visualized by mucosubstance-selective staining. The goblet cells are represented by small apical dark-staining residues of mucosubstance along the luminal border of the epithelium (arrows). Debris can be seen trapped on the surface of the epithelium.

(PAS X 350).



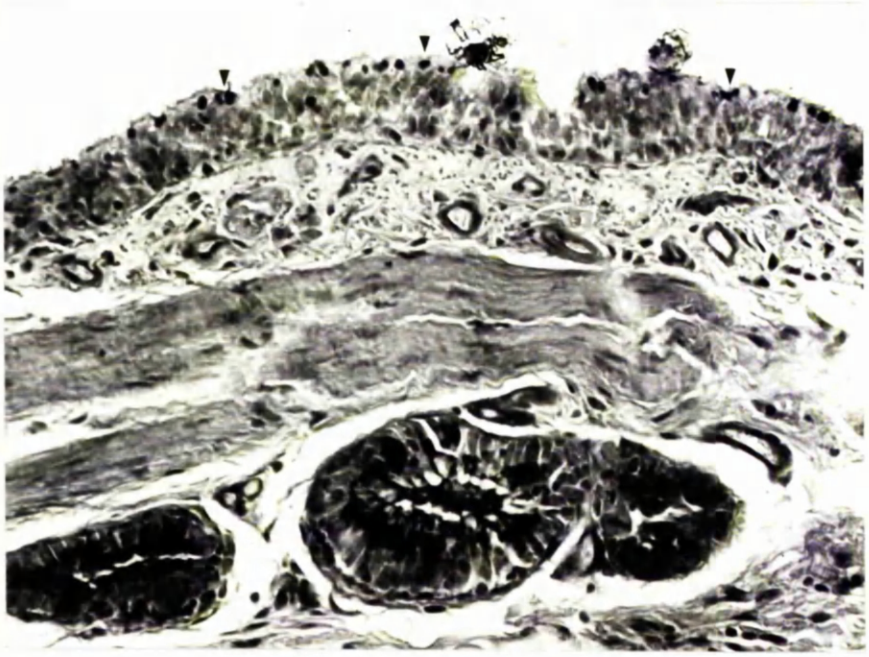
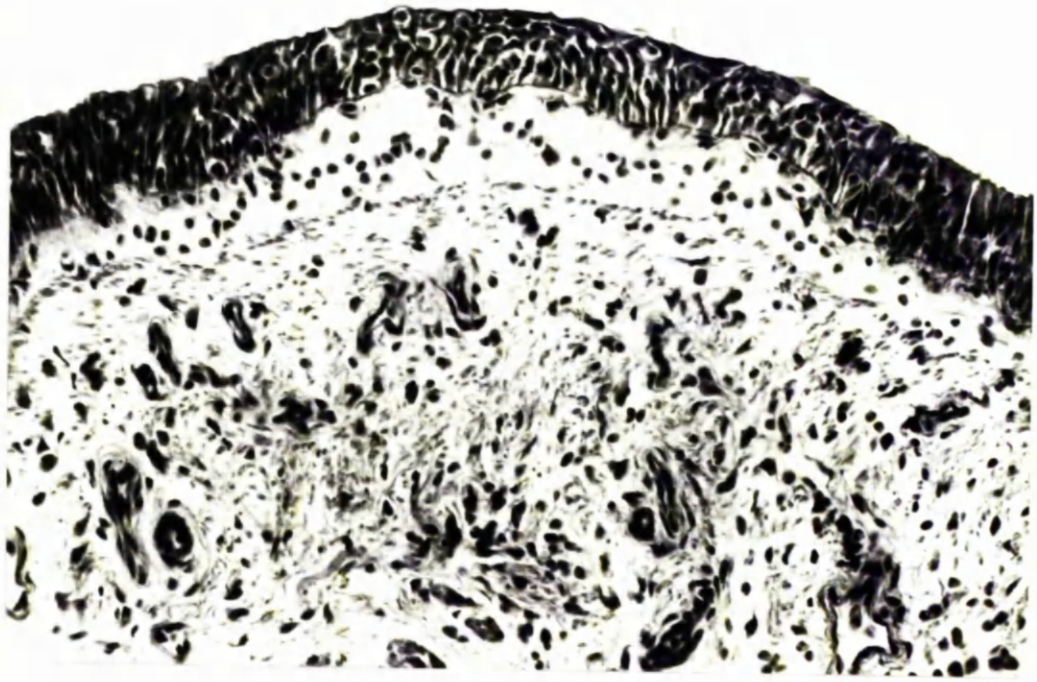


Fig. (30): Chronic bronchitis: severe sub-epithelial oedema beneath an area of epithelial dedifferentation.

(HE X 350).

Fig. (31): Chronic bronchitis: fold of bronchial mucosa with intense oedema and a cellular infiltrate of plasma cells, neutrophils and lymphocytes. The bronchial epithelium is intact and differentiated at each side, but over the fold itself there is marked dedifferentiation with basal cell proliferation, and piling up of basal cells (arrows). The epithelium is attenuated over the top of the fold with complete erosion at the centre (open arrow).

(HE X 300).



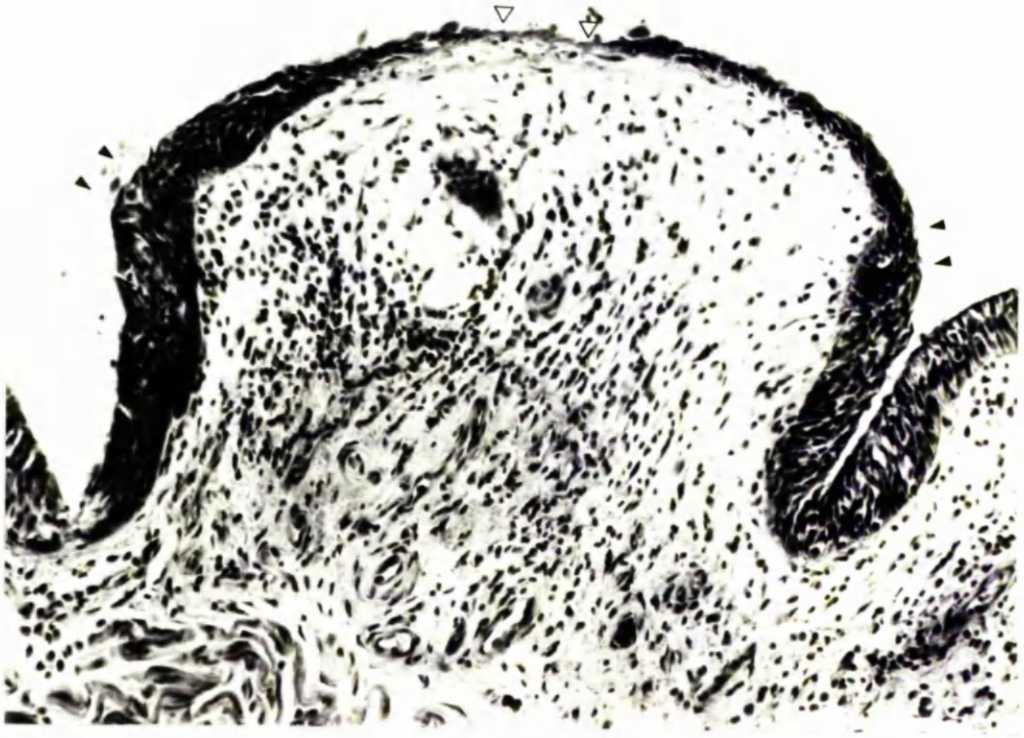


Fig. (32): Chronic bronchitis: proliferation of acini in a bronchial mucous gland. There are a large number of small acini present and these are surrounded by considerable numbers of lymphocytes and plasma cells. The epithelium has very thin goblet cells, and a large number of basal cell nuclei. A thick layer of mucus and cell debris can be seen resting on the epithelium.

(PAS X 250).

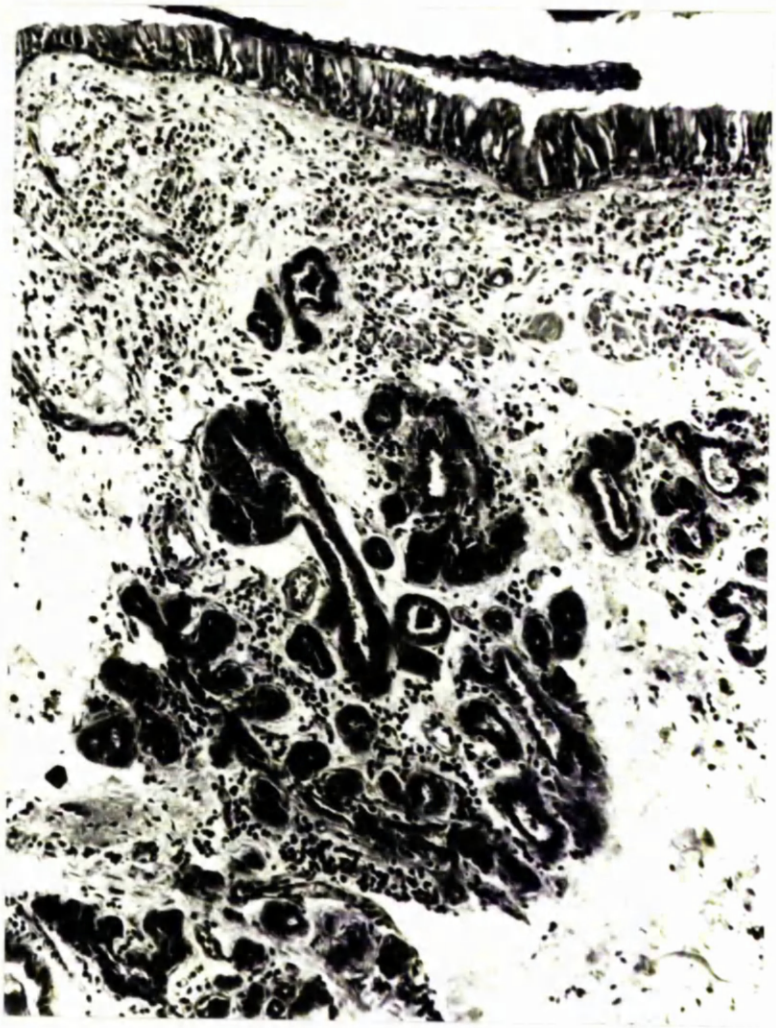
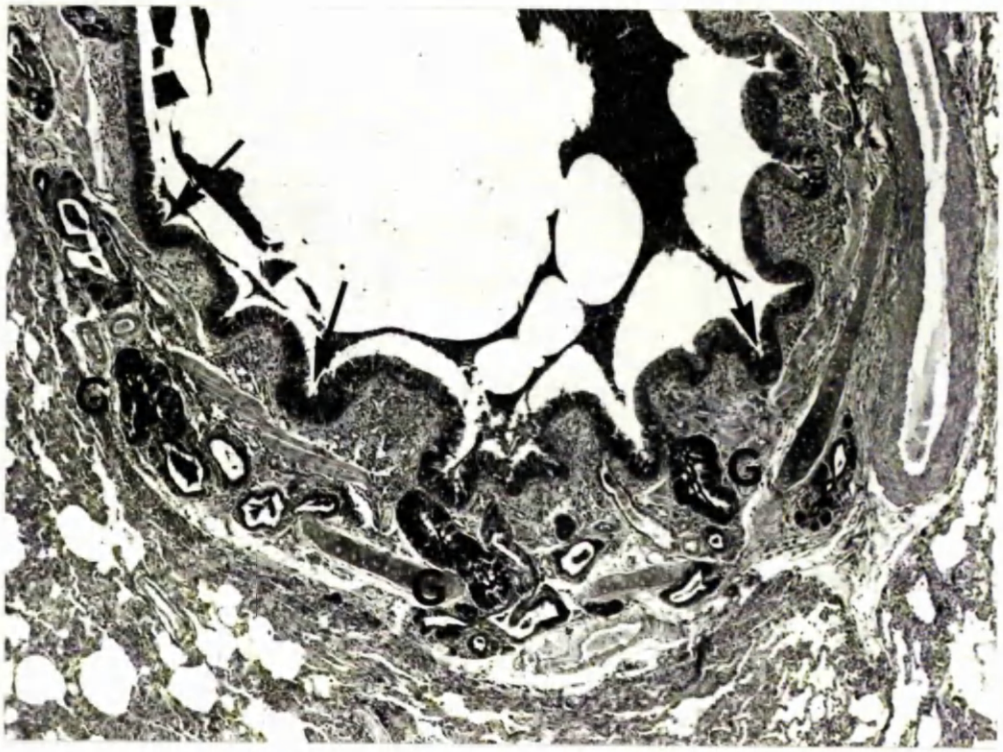


Fig. (33): Chronic bronchitis: Section of bronchial wall illustrating the increase in the number and size of mucous glands (G) and the increase in the number of goblet cells, particularly in the epithelial folds (arrow). There is an inflammatory cellular infiltrate into the bronchial wall giving a folded outline.

(PAS X 110).

Fig. (34): Chronic bronchitis: Mucous gland in chronic bronchitis. The acini are increased in number and size with secretion visible in the larger ones. Large numbers of lymphocytes and plasma cells can be seen around the acini.

(HE X 500).


G
G
G
G

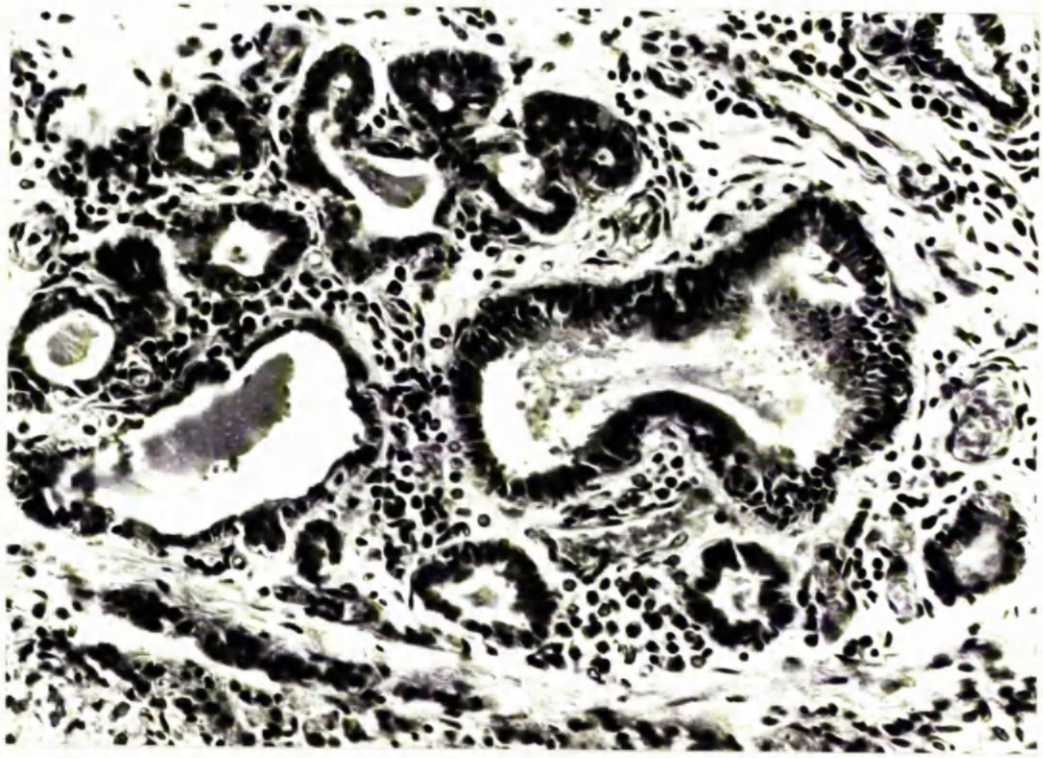
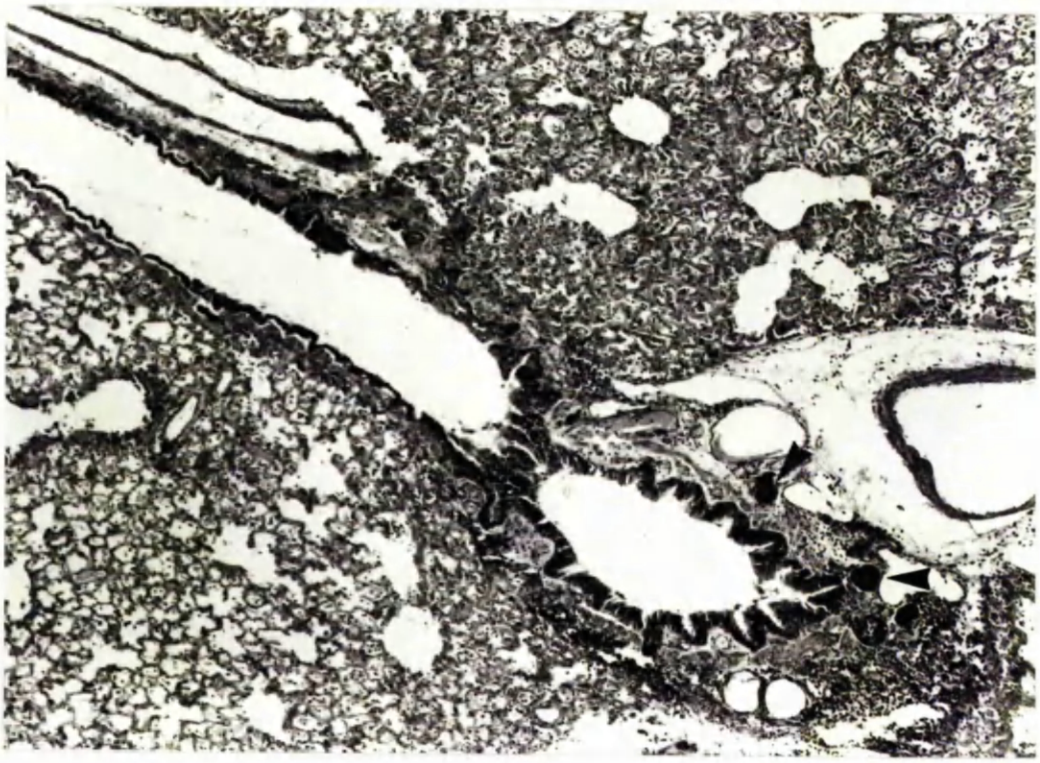


Fig. (35): Chronic bronchitis: Extension of mucous glands into bronchioles. Several small mucous gland acini (arrows) can be seen in the wall of this bronchiole.

(HE X 110).

Fig. (36): Chronic bronchitis: Severe bronchiolitis with intense cellular infiltration of the bronchiolar wall.

(HE X 110).



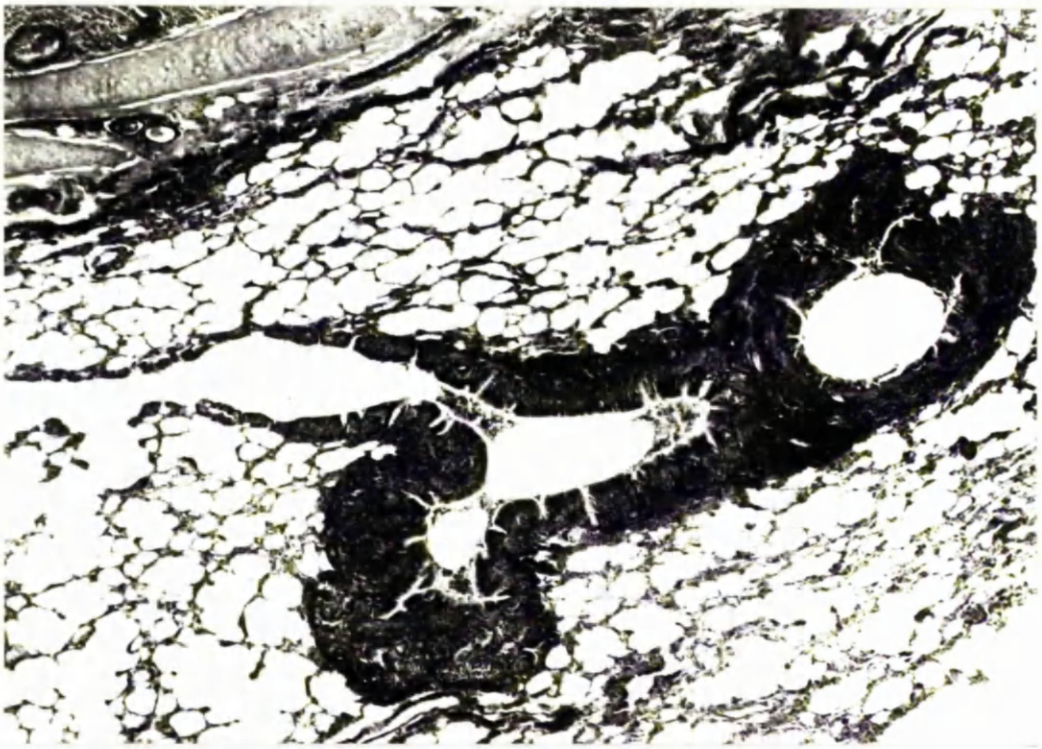
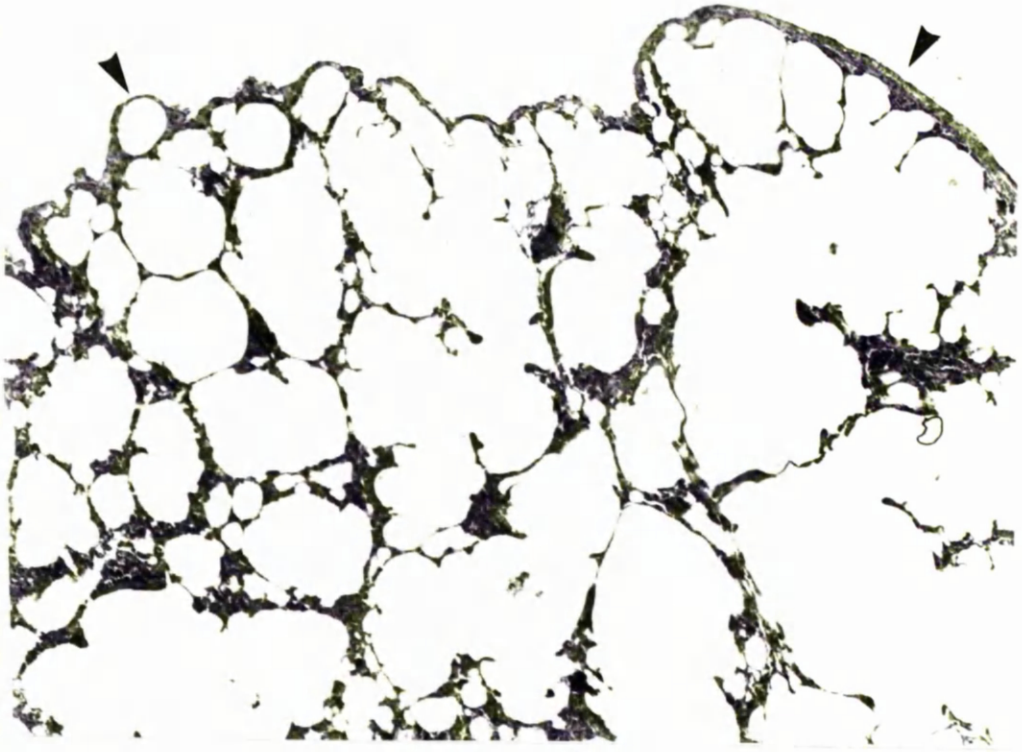


Fig. (37): Emphysema: large coalescing air spaces beneath a bulging pleura (arrows). There is thinning and rupture of alveolar walls with subsequent distortion of architecture and foci of collapse.

(HE X 110).

Fig. (38): Emphysema: severe sub-pleural emphysema. There is almost total destruction of alveolar architecture.

(HE X 110).



MEASUREMENT OF BRONCHIAL WALL COMPONENTS IN YOUNG DOGS,

ADULT DOGS AND DOGS WITH CHRONIC BRONCHITIS

INTRODUCTION AND REVIEW OF THE LITERATURE

MATERIALS AND METHODS

RESULTS

DISCUSSION

INTRODUCTION AND REVIEW OF THE LITERATURE

The bronchial mucous glands in subjects with chronic bronchitis are enlarged: various attempts have been made to apply quantification methods to measure this enlargement, to establish normal limits and to define baselines for the hyperplasia. Three main methods have been used: a one-dimensional method using a ratio of mucous gland thickness to bronchial wall thickness, and two-dimensional methods of the paper cut-out technique and the point-count technique.

Reid (1960) described a technique for the measurement of the bronchial mucous glands in man, expressing the result as a ratio of gland thickness to bronchial wall thickness (Fig. 39). With this ratio, which later came to be known as the Reid index, Reid was able to detect a significant difference in the size of the mucous glands in the bronchi of normal subjects and those in the bronchi of subjects with chronic bronchitis. In normal bronchi, the ratio was 0.26 and in bronchi from subjects with chronic bronchitis the ratio increased to around 0.6; this change was the result of an increase in size and complexity of the mucous glands in the latter group of subjects. Moreover, Reid (1960) was able to correlate sputum production with an increase in the Reid index; thus, the index increased from around 0.48 in patients producing $\frac{1}{2}$-1 fluid ounces of sputum per day, to around 0.65 in patients producing over 2 fluid ounces of sputum per day. However, there was no difference in mucous gland thickness which could be attributed to a past history of cigarette smoking.

Subsequently, other groups of workers (Thurlbeck, Angus and Pare, 1963; Thurlbeck and Angus, 1964; Field, et al., 1966; Field, 1968; Burton and Dixon, 1969) used the Reid index to establish the distribution and reactivity of mucous glands in normal and diseased bronchi. Thurlbeck, Angus and Pare (1963), working in North America, were able to confirm the observations of Reid (1960), notably the increase in the mucous gland to wall ratio in subjects with chronic bronchitis, but, unlike Reid (1960), the

values of the Reid index in the controls and the subjects with chronic bronchitis overlapped, the means for each of these two groups, being 0.37 and 0.5 respectively. In addition, Thurlbeck, Angus and Pare (1963) found that there was no correlation betwen values of the Reid index and sputum production, although they were able to detect a significant increase in values of the Reid index in subjects who had smoked cigarettes. Thurlbeck, Angus and Pare (1963) thought that this difference from the findings in cigarette smokers by Reid (1960) might be attributable to the higher levels of air pollution in the United Kingdom, which might tend to blur the distinction between cigarette smokers and non-smokers.

Field, et al., (1966) found high values for the Reid index in infants and children in a routine survey of 644 consecutive necropsies; 30 per cent of the children under 4 years of age had Reid index values in excess of 0.4, which was considered by Field and her co-workers to be the upper limit of normality in adult subjects. This work was later extended (Field, 1968) to a specific study of the bronchi in children. Eighty six consecutive child necropsies were divided into 4 age groups: infants aged 14 days or less, infants aged 15 days to a year, children 1-4 years and children 5-15 years. From this work, Field (1968) concluded that mucous gland hypertrophy was most common in infants less than 1 year of age; the mean value of the Reid index in this group was 0.36, and for children over 1 year old (i.e. 1-15 years age group) the mean Reid index value fell significantly and was approximately comparable to that in the human foetus. Thurlbeck, Benjamin and Reid (1961) had found that in the foetal trachea the mucous gland to wall ratio reached the adult value (around 0.26) by 25 weeks of gestation, but did not exceed this thereafter.

Thurlbeck and Angus (1964) used the Reid index to assess mucous gland size in a series of 101 random necropsies and described a bell-shaped distribution for Reid index values and mucous gland sizes (Fig. 40). Thurlbeck and Angus (1964) pointed out that, in the original work, Reid (1960) had used for her 'bronchitic' group only those subjects with a history of chronic bronchitis for at least five years together with excessive sputum

production. It was suggested by Thurlbeck and Angus (1964) that the two groups of 'controls' and 'bronchitics' in the material of Reid (1960) represented the two extremes of this bell-shaped distribution; they con - sidered it inevitable that there would be an overlap in the measurements obtained in bronchi from subjects with chronic bronchitis and those without.

A comparison of mucous glands of nasal sinus mucosa and bronchial mucosa in 44 random necropsies by Burton and Dixon (1969) failed to reveal any correlation between the Reid indices for the two sites. Increased values of the Reid index in the bronchi of subjects with chronic bronchitis were not accompanied by an increase in Reid index values for the nasal sinus mucosa. Burton and Dixon (1969) suggested that this discrepancy might be due to the irritant effects of cigarette smoking on the lower respiratory tract, with resultant mucous gland hypertrophy in the bronchi.

The Reid index had proved to be a means of making a rapid assessment of bronchial mucous gland hypertrophy. However, it did not give information on other bronchial wall components, such as smooth muscle, and it was sub- ject to errors in interpretation, in particular in the choice of site for measurement of gland thickness (Bedrossan, Anderson and Foraker, 1971).

The interest generated by Reid (1960) in the quantification of bronchial wall components led to the development of two more major techniques - the paper cut-out method and the point count method. Both represented an advance insofar as they were two-dimensional, as opposed to the one-dimensional approach of the Reid index. These techniques enable an estimation of mucous gland area to be made in sections of bronchus and Dunnill (1962) was able to show that in the complex bronchial wall, the areal proportions of a section are equivalent to the volume proportions (principle of Delesse, 1948).

Restrepo and Heard (1963A) estimated mucous gland areas by the paper cut-out technique. By projecting an entire histological cross-section of bronchus onto a paper card screen, it was possible to outline the various structures, such as mucous glands, on the card with a pen. The outlined images could then be cut out of the card and weighed, to give an estimation

of their absolute area and area relative to the total bronchial wall, providing that the magnification of the projected image was known and the weight of the unit area of card was known. Retrepo and Heard (1963A) confirmed that bronchitics tended to have large mucous glands but, again, there was an overlap between the mucous gland areas of normal subjects and those with chronic bronchitis. Further work with this method (Restrepo and Heard, 1963B) revealed that the mucous gland enlargement extended throughout the bronchial tree and was proportionately greater in the segmental bronchi.

The third method, the point count technique, was outlined by Dunnill (1962) and has been the method of choice in recent papers on quantification (Takizawa and Thurlbeck, 1971A, 1971B; Sobonya and Kleinerman, 1972; Niewoehner, Kleinerman and Knoke, 1972; Matsuba and Thurlbeck, 1972; Hossain, 1973; Heard and Hossain, 1973). This method also involves projection of the image of an entire cross-section of bronchial wall onto a screen; the screen incorporates a grid of regularly-distributed points and so the estimation of relative areas can be made by a comparison of the number of points falling on each area (Fig. 41). The bronchial wall image can either be projected onto a large screen which incorporates a lattice of points or viewed with a microscope fitted with an eyepiece graticule. Dunnill, Massarella and Anderson (1969) used this point count technique to study the bronchi of subjects with status asthmaticus, chronic bronchitis and emphysema. They found an increase in mucous gland volume in the bronchi of patients with status asthmaticus, together with a pronounced increase in the proportion of bronchial wall occupied by smooth muscle (a mean value of 11.9 per cent compared to 4.6 per cent in the bronchi of control subjects). The chronic bronchitic subjects had increased mucous gland volumes but, again, there was an overlap with the mucous gland volumes in the bronchi of normal subjects. The results for mucous gland volume in subjects with emphysema were more variable and there was considerable overlap with normal material. In a survey of 353 random necropsies, Ryder,

Dunnill and Anderson (1971) found a correlation between mucous gland volume, cigarette smoking history and the incidence of emphysema. However, this correlation was not detected by Sobonya and Kleinerman (1972), who examined the bronchi in thirteen male cigarette smokers and eleven male non-smokers using a microscope eyepiece graticule. This latter study utilised selected cases, 18-46 years of age, with no previous history of respiratory disease, whereas Ryder, Dunnill and Anderson (1971) used an unselected series with an age range of 22-95 years.

Similar apparent contradictions with this type of work have appeared with the results for the quantification of smooth muscle. Although Macleod and Heard (1969) could find no difference from normal in the amount of tracheal smooth muscle in subjects with chronic bronchitis, subsequent work by Hossain and Heard (1970), using basal segment and posterior basal segment bronchi (nomenclature based on recommendations of the Thoracic Society, 1950), revealed a significant increase in smooth muscle in the bronchi of patients with chronic bronchitis. This increase in smooth muscle (an approximate doubling) was not confirmed by the work of Dunnill, Massarella and Anderson (1969) or that of Takizawa and Thurlbeck (1971B). However, both these latter two groups of workers did detect an increase in smooth muscle in the bronchi of subjects with "asthma" - patients whose records showed classic spasmodic asthma with a history or a family history of allergy and death in *status asthmaticus*, together with a history of skin sensitivity to a variety of allergens (Takizawa and Thurlbeck, 1971B).

Matsuba and Thurlbeck (1972) were able to use the point count technique to confirm the increased volumes of mucous glands in the bronchi of children first reported by Field, *et al.*, (1966). In addition, they detected an increase in the amount of smooth muscle in the "small airways" of both children and adults; this increase was probably in the bronchioles.

The potential of these techniques for comparative pathology had not been investigated. The considerable body of work outlined in this review has made a significant contribution to our knowledge of the human tracheo-

bronchial tree in health and disease. Perhaps surprisingly, there has been little work on the quantification of bronchial wall components in animals, either with naturally-occurring disease in the domestic animals or with experimental disease in laboratory animals.

Lamb and Reid (1968) measured the dimensions of enlarged tracheal glands in rats which had been exposed to sulphur dioxide gas. More recently, an automated image analyser has been used to measure the areas of goblet cells and mucous glands in sheep exposed to cigarette smoke (Mawdesley-Thomas and Healey, 1973) and the areas of goblet cells in rats exposed to sulphur dioxide gas and cigarette smoke (Mawdesley-Thomas, Healey and Barry, 1971). Work on the dog was limited to one paper dealing with the morphologic features in the lungs of ageing beagle dogs (Robinson and Gillespie, 1973), where the assessments were entirely subjective.

The primary objective of this investigation was to establish normal values for the proportions of bronchial wall components in young and adult dogs and then to determine whether there was a detectable increase in mucous gland volumes in the bronchi of dogs which had been diagnosed as having chronic bronchitis. These findings could then be compared to the findings in human chronic bronchitis for evaluation of the usefulness of the dog in a possible experimental model system. It was decided to include young dogs in the study to see whether the high mucous gland volumes seen in human infants (Field, et al., 1966; Field, 1968; Matsuba and Thurlbeck, 1972) also occurred in the young dog. In addition, by careful selection of sample sites, it would be possible to gain information on the sizes and variations of bronchial wall components at different levels and in different lobes within the bronchial tree in these three representative groups. The findings of this investigation have been described recently in the literature (Wheeldon and Pirie, 1974).

The mucous glands of the dog's bronchus are much less numerous than those of man. Preliminary attempts using the Reid index in the series of dogs were not successful, due to lack of adequate numbers of representative

sample sites. Similarly, the paper cut-out technique has certain disadvantages, most notably a difficulty in assessing bronchial smooth muscle, which consists of thin, interrupted fibres (Hossain and Heard, 1970). Thus, it was finally decided to use the point count method to assess the bronchial wall components in the dog.

MATERIALS AND METHODS

Animals

Fifteen dogs were divided into 3 groups of 5:

Group 1 - five young dogs (1-5), aged 3-5 months, with no previous history of respiratory disease.

Group 2 - five adult dogs (6-10), aged 10-14 years (mean age of 11.8 years) with no clinical signs of respiratory disease.

Group 3 - five adult dogs (11-15), aged 5-11 years (mean age of 7.6 years) which had all been diagnosed clinically as having chronic bronchitis. These dogs had all been coughing for a minimum of two consecutive months in the preceding year. Dogs 12-15 had radiological signs of chronic bronchitis. The pulmonary lesions in these five dogs are described below (vide Pathology- cases 19,21,22,23,26).

Post mortem technique

The dogs were all humanely destroyed by intravenous injection of pentobarbitone sodium (Euthatal, May and Baker, Dagenham, Essex) except for dog 11 which died. The lungs and heart were removed from the thorax immediately after death and the heart dissected away from the lungs. One side of the lungs, usually the right side, was opened for macroscopic inspection of the airways and for the collection of samples for bacteriological and electron microscopical examination. The other, unopened side of the lung was fixed whole in formol saline by infusion of fixative into the pulmonary vessels. After all blood had been flushed from the vessels, the lung was immersed in formol saline for 24-48 hours.

After this period, the fixed lung lobes were sliced to sample the bronchial tree at the sites indicated in Fig. 1. A complete transverse section of bronchus from each of these eleven sites was then trimmed, dehydrated and cleared in a double embedding series and finally embedded

in paraffin wax under vacuum. The paraffin embedded sections were then cut at 6-8 microns: 2 sections were taken from each sample site, one section being stained by haematoxylin and eosin and the other by the combined alcian blue-periodic-acid Schiff technique (Mowry, 1956).

Point counting technique.

Point counting of the bronchial wall at each site was done by examining an entire section of the bronchial wall with a microscope fitted with a graticule (Ernst Leitz, Wetzlar, Germany) in the X10 eyepiece. The graticule was a 1 cm. square, each side of which was divided into 20, giving 21 points along each edge and a grand total of 441 intersections. These intersections were then regarded as the regular lattice of points which could be superimposed on the bronchial wall (Fig 42). In order to get a reasonable number of points falling on all the structures, a X10 objective was used. The points falling on mucous gland, muscle, cartilage and total wall were counted for a field, the grid realigned within the field and the count repeated to give a mean figure. The adjacent field was then brought under the grid and the procedure was repeated until the entire bronchial wall had been counted systematically. In each cross section of bronchial wall examined, the number of points falling on any given component could be expressed as a percentage of the total number of points falling on the entire wall. Figures for each site counted were then referred to as the mucous gland per cent (MGP), the smooth muscle per cent (SMP) and the cartilage per cent (CP). Statistical differences between groups of dogs, lung lobes and sample sites were assessed by the analysis of variance (Goldstein, 1964).

RESULTS

The results for the percentage of bronchial wall occupied by mucous gland, smooth muscle and cartilage at the 11 sample sites in each of the fifteen dogs, together with a mean and standard error value for the eleven sites in each dog are set out in Tables 7, 11 and 15 respectively. In the remaining tables, these results for the three components of the bronchial wall at the eleven sites in each dog are regrouped to give results for individual lobes and also for sampling levels.

Thus, Tables 8, 12 and 16 set out the results for MGP, SMP and CP in individual lobes in each of the dogs. In each case a mean value together with the standard error for each lobe is given. Similarly, Tables 9, 13 and 17 set out the corresponding results for MGP, SMP and CP at the sampling levels (i), (ii) and (iii) in each dog. Lastly, the results of the analysis of variance on the comparisons of MGP, SMP and CP values for lobes and sites within and between groups are set out in Tables, 17, 14 and 18 respectively.

Mucous glands.

The percentage of bronchial wall occupied by mucous glands at each of the eleven sites in the fifteen dogs, together with an overall mean and standard error value in each dog, are set out in Table 7 . The individual mean values for MGP in the young dogs of Group 1 range from 0.8 to 1.9 with an overall mean of 1.1. The individual means for the adult dogs of Group 2 range from 0.8 to 1.7 with a mean of 1.5. The dogs with chronic bronchitis in Group 3 had MGP values which were not only considerably increased compared to the MGP values in Groups 1 and 2, but also had a wider range - from 3.8 to 9.4 with a mean of 6. Analysis of variance revealed no significant difference between the mean MGP values of young and adult dogs ($P>0.05$) but the mean MGP values of the dogs with chronic bronchitis were significantly increased (Fig. 43) compared to both these groups ($P<0.01$). This confirmed the observation that the mucous glands were increased in

size and volume in cases of chronic bronchitis and that there was no difference between adult and young dogs.

The MGP values for lung lobes, together with mean and standard error values for each lobe are set out in Table 8. Analysis of variance failed to reveal any significant difference between the lung lobes within each group, i.e. comparisons of apical, cardiac and diaphragmatic lobes within each group did not reveal any significant differences betwen lobes.

The MGP values are arranged by site in Table 9 together with a mean and standard error value for sites (i), sites (ii) and sites (iii). In all the dogs of each group, the MGP appeared to increase from site (i) to site (iii), i.e. an increase peripherally in the amount of bronchial wall occupied by mucous gland. However, analysis of variance revealed that only in Group 2 was there a significant increase from site (i) to site (iii) Fig. (44).

A comparison of corresponding lung lobes between each of the three groups revealed that for apical, cardiac and diaphragmatic lobes, the mean lobe MGP values in Group 3 were all significantly increased over the other two groups ($P<0.01$ for all 3 lobes). Similarly, a comparison of the three corresponding site means in the three groups revealed that all three site mean MGP values in Group3 were increased significantly over the other two groups. The results for mucous glands in the three groups are summarised in Table 10.

Smooth Muscle.

The proportion of bronchial wall occupied by smooth muscle (SMP values) at each of the eleven sites in the fifteen dogs together with an overall mean and standard error value for each dog is set out in Table 11. The SMP values in the three groups were not significantly different when overall means, lobe means and site means were compared; an apparent increase in SMP values from site (i) to site (iii) in Groups 1 and 2 was significant in Group 2.

The mean SMP values for each dog were much higher than the mean MGP

values, being of the order of 7.0. There was no difference between the mean SMP values in the three groups ($P>0.05$). Similarly, a comparison of mean SMP values for lobes within each of the groups revealed no significant differences ($P>0.05$). A comparison of mean SMP site values within each group revealed an apparent increase in SMP values from site (i) to site (iii) in Groups 1 and 2. In Group 2 there was a significant increase in mean SMP values from site (i) to site (ii) ($P<0.05$) and from site (ii) to site (iii) ($P<0.05$) (Fig. 45). This peripheral increase in the amount of smooth muscle in the bronchial tree was not detected in the dogs of Group 3. A comparison of corresponding lobes and sites between the three groups did not reveal any significant differences. The results for smooth muscle in the three groups are summarised in Table 14.

Cartilage.

The CP value at each of the eleven sites in each dog together with an overall mean and standard error value for each dog is given in Table 15. It will be seen that there is a wide range in values within groups and within individual dogs, resulting in high standard errors for the mean values for each dog. The bronchi of the Group 3 dogs appeared to have less cartilage than the other two groups, while the expected reduction in the amount of cartilage from site (i) to site (iii) was not found in all three groups.

A comparison of mean CP values in the three groups, revealed a significant difference (relative reduction) in the mean CP values in Group 3 compared to Group 1 ($P<0.01$) and Group 2 ($P<0.05$). A comparison of mean CP values for sites in each group revealed a significant decrease in CP values from site (i) to site (iii) ($P<0.05$) in Groups 1 and 3. This reduction in the amount of cartilage in the bronchial wall in the peripheral bronchial tree was not detected in Group 2. A comparison of corresponding lobe CP values in the three groups (Table 16) revealed that the dogs in Group 1 had significantly higher CP values for apical and cardiac lobes compared to the dogs in Group 3 ($P<0.05$). Similarly, a comparison of

corresponding mean site CP values (Table 17) revealed significantly higher CP values in site (i) and site (iii) in the dogs of Group 1 compared to the corresponding sites in the dogs of Group 3 ($P<0.05$). The results for cartilage in the three groups are summarised in Table 18.

DISCUSSION

The clinical hallmark of chronic bronchitis is a chronic cough with production of sputum due to enlargement of the mucous-secreting apparatus of the tracheobronchial tree. The disease picture is thus dependant on the extent and degree of this hypertrophy. Reid (1960) pointed out that diagnosis of chronic bronchitis would be greatly facilitated if this hypertrophy could be measured and used as a diagnostic marker. This would depend on the hypertrophy being a constant feature of the disease and preferably increasing with the severity of the disease. Using the mucous glands, Reid (1960) was able to show that both these factors were operating in her group of patients with chronic bronchitis. However, since that time, no workers have been able to correlate the severity of symptoms with increasing hypertrophy of the mucous glands. Moreover, most subsequent work has demonstrated an overlap in mucous gland values in the bronchi of normal and bronchitis subjects. This is almost certainly due to the bell-shaped distribution for Reid index values and mucous gland thickness in the background population described by Thurlbeck and Angus (1964). The lack of overlap in mean gland values in this experiment could indicate that the samples are from either end of this bell-shaped distribution as was the case with the material of Reid (1960). However, the experiment does demonstrate a detectable increase in mucous gland volume in the bronchi of dogs with chronic bronchitis.

In contrast, the high proportion of mucous glands in infants, first described by Field, et al., (1966) and later by Field (1968) and Matsuba and Thurlbeck (1972), was not found in the young dogs, in which mean MGP values corresponded very closely to those of the adult dogs. The significance, if any, of this difference from the findings in man is not known. It may be that there is an age variation in the other component of the respiratory mucus-secreting apparatus, the epithelial goblet-cells, which were not investigated in this experiment, or in the postnatal growth

of the bronchial tree.

The increase in the proportion of mucous glands in the peripheral bronchi of adult dogs is difficult to compare with the situation in man. The only comprehensive study of mucous gland volumes at differing levels in the bronchial tree of man was undertaken by Restrepo and Heard (1963B), who expressed their findings as absolute areas of mucous glands rather than a relative percentage of total bronchial wall. Takizawa and Thurlbeck (1971A), in a comparison of quantitative methods, compared their findings for mucous gland volumes in major airways with those of Dunnill, Massarella and Anderson (1969), who used segmental bronchi; this comparison revealed a greater proportion of mucous glands in the segmental bronchi.

An increase in the proportion of bronchial smooth muscle in peripheral bronchi was found by Takizawa and Thurlbeck (1971A), when they compared their findings with those of Dunnill, Massarella and Anderson (1969). Matsuba and Thurlbeck (1972) described a greater proportion of muscle in small airways compared to large airways, but this finding may not be comparable, as the 'small airways' were probably bronchioles rather than small bronchi. A similar variation in the distribution of smooth muscle was found in the adult dogs of Group 2 but not in the young dogs, and, again, this could be explained by postnatal growth of the bronchial tree. No increase in smooth muscle was seen in the group of dogs with chronic bronchitis and this is in agreement with the majority of work on this aspect of bronchial quantification in man.

The apparent reduction in cartilage in the bronchi of dogs with chronic bronchitis is almost certainly due to a relative increase in the amount of other connective tissues in the bronchial wall. In man, there is considerable disagreement as to whether there is an absolute reduction in cartilage in the bronchi of subjects with chronic bronchitis. Restrepo and Heard (1964), using the paper cut-out method, compared absolute areas of cartilage from corresponding sites in bronchi from normal subjects and subjects with chronic bronchitis: they failed to detect a significant

difference in the amounts of cartilage in the 2 groups. Recently, Thurlbeck, et al., (1974), using a point count method on macroscopic specimens of dissected bronchi from patients with chronic bronchitis and emphysema, found a diminution of cartilage only in the cases of emphysema. However, Tandon and Campbell (1969), using a similar technique but employing a subjective visual assessment, claimed that atrophy of bronchial cartilage had occurred in a proportion of their cases of chronic bronchitis.

The present position with regard to bronchial cartilage is, at best, undecided; further work with absolute measurements such as planimetry should serve to clarify the situation. The wide variation in CP values in this study is a reflection of the distribution of cartilage in the normal bronchial wall; the irregular cartilage plates are set randomly in the bronchial wall (Miller, 1947), so that there is a wide variation in CP values over a short distance. This would explain why there appears to be no significant decrease in cartilage in peripheral bronchi in the sites for the adult dogs.

Despite widespread use of the point counting technique by many workers, no one has mentioned the possibility of errors due to irregular thickening of the bronchial wall. Thurlbeck and Angus (1964), in their Reid index measurements, considered the lamina propria, smooth muscle and sub-mucosa to be (W-G, Fig. 39) 0.3mm. in all their groups. Because the bronchial wall components are related to the total wall in the point counting method, then any possible differences in the size of the wall will affect the percentages of the components accordingly. Thus, even if there appears to be no increase in the amount of smooth muscle when expressed as a percentage of the bronchial wall, nevertheless there will have been an absolute increase if thickening of the bronchial wall has taken place.

Methods available for quantification of bronchial wall components have been reviewed by Bedrossan, Anderson and Foraker (1971) and Takizawa and Thurlbeck (1971A). The Reid index correlated poorly with planimetry

(Bedrossan, Anderson and Foraker, 1971), but it was found to distinguish between subjects with chronic bronchitis and those without chronic bronchitis (Takizawa and Thurlbeck, 1971A). The great advantage of the Reid index is the speed with which large numbers of cases can be screened in an epidemiological survey. An example of this was the survey of mucous gland hypertrophy in Glasgow reported recently by Scott (1973). Scott (1973) used the Reid index to examine the right main bronchus from 359 consecutive adult necropsies and found a significant relationship between chronic productive cough in life and the size of the mucous glands at necropsy. One hundred and nineteen of the cases were also examined by point counting and Scott (1973) found a close correlation between the Reid index and the point counting method.

The two comparative studies (Bedrossan, Anderson and Foraker, 1971 and Takizawa and Thurlbeck, 1971A) found that the point count technique was an effective method for diagnosing mucous gland hypertrophy. Bedrossan, Anderson and Foraker (1971) considered that the point count and paper cut out methods approached the 'true' values obtained by planimetry. Although planimetry is applicable to assessment of bronchial morphology (Anderson and Foraker, 1962) and is nearly ideal as regards precision (Keuffel and Esser Company, 1963), it has the disadvantages of initial cost outlay and of being tedious and time consuming in operation (Bedrossan, Anderson and Foraker, 1971). The great advantage of the point count method is that it can provide information on other bronchial wall components, such as smooth muscle; the disadvantage is that it too is time consuming. Despite this, the point count method was eventually adopted as the method of choice for this study in the dog. The Reid index is not a suitable technique for the study of dogs' bronchi, because the glands are much less numerous than those of man. A pilot study using the Reid index was abandoned due to lack of sufficient numbers of representative sites. The point count method was eventually modified into a multiple point count method, as first described by Hale, Olsen and Mickey (1968) and the bronchial wall counted piecemeal.

The conversion to a microscope with an eyepiece graticule was preferred to a projection system, which required a screen and black-out facilities.

This study has shown that it is possible to quantify the bronchial mucous glands in dogs diagnosed as having chronic bronchitis and to detect a significant increase in gland volume compared to the glands of control dogs. The large numbers of sites were sampled in an attempt to detect both the degree and extent of diffuse change within the bronchial tree. Much of the work in man suffers from a lack of standardisation, both in techniques and in sample sites. Using the sample sites in this study, it has been possible to map the structures of normal and diseased bronchi in the dog.

Dog	Age	Sample Sites										Mean ± SE
		Apical			Cardiac			Diaphragmatic				
		(i)	(ii)	(iii)	(i)	(ii)	(iii)	(i)	(ii)	(ii)	(iii)	(iii)
1. Young dog	14 wks	0.9	0.9	1.4	1.8	0.7	1.7	0.5	0.7	1.3	1.2	1.7	1.2 ± 0.14
2. Young dog	14 wks	0.7	0.9	0.7	1.6	2.5	0.3	0.3	0.8	1.7	1.2	1.2	1.1 ± 0.20
3. Young dog	14 wks	1.7	0.5	1.6	0.2	0.4	1.1	0.2	0.6	0.8	0.2	1.0	0.8 ± 0.16
4. Young dog	20 wks	0.4	1.1	2.2	0.6	2.6	3.1	0.5	2.5	2.5	1.5	4.1	1.9 ± 0.36
5. Young dog	20 wks	0.6	1.6	0.9	0.3	0.2	1.1	0.3	0.5	1.2	1.3	0.7	0.8 ± 0.14
6. Adult dog	10 yrs	0.4	0.6	2.4	0.5	0.5	0.3	0.2	0.6	1.0	0.9	1.2	0.8 ± 0.19
7. Adult dog	10 yrs	0.4	0.3	1.7	1.2	1.5	2.5	0.3	1.2	1.2	2.0	3.7	1.5 ± 0.30
8. Adult dog	12 yrs	0.7	1.4	1.4	2.5	0.8	3.3	0.2	1.0	2.2	1.9	2.7	1.7 ± 0.29
9. Adult dog	13 yrs	0.4	2.0	3.2	1.4	1.0	2.1	0.5	2.4	2.6	0.9	1.5	1.6 ± 0.27
10. Adult dog	14 yrs	0.2	2.9	1.7	2.0	1.2	3.3	0.5	0.9	1.6	2.1	2.7	1.7 ± 0.60
11. Chronic bronchitic	7 yrs	5.1	8.9	2.5	8.4	4.4	4.7	2.5	5.6	7.4	6.6	7.7	5.8 ± 0.67
12. Chronic bronchitic	11 yrs	7.3	4.6	13.8	0.9	4.2	5.3	4.3	3.6	3.4	2.1	2.6	4.7 ± 1.04
13. Chronic bronchitic	9 yrs	7.8	20.4	6.3	13.1	11.4	10.3	1.6	7.9	15.2	2.0	7.6	9.4 ± 1.67
14. Chronic bronchitic	6 yrs	5.0	6.3	16.2	3.1	7.9	8.2	3.3	3.0	3.6	5.8	6.3	6.3 ± 1.14
15. Chronic bronchitic	5 yrs	3.0	5.0	3.4	3.1	2.3	8.0	4.5	1.9	2.1	2.8	5.8	3.8 ± 0.56

Table (7): percentage of bronchial wall occupied by mucous gland (mucous gland per cent (MGP) values) at three sites in each of three lung lobes in three groups of dogs. An overall mean MGP and standard error value are included for each dog.

Dog	Age	Sample Sites																	
		Apical					Cardiac					Diaphragmatic							
		(i)	(ii)	(iii)	Mean ±	SE	(i)	(ii)	(iii)	Mean ±	SE	(i)	(ii)	(ii)	(iii)	(iii)	Mean ±	SE	
1. Young dog	14 wks	0.9	0.9	1.4	1.1 ±	0.17	1.8	0.7	1.7	1.4 ±	0.35	0.5	0.7	1.3	1.2	1.7	1.1 ±	0.22	
2. Young dog	14 wks	0.7	0.9	0.7	0.8 ±	0.07	1.6	2.5	0.3	1.5 ±	0.64	0.3	0.8	1.7	1.2	1.2	1.0 ±	0.23	
3. Young dog	14 wks	1.7	0.5	1.6	1.3 ±	0.38	0.2	0.4	1.1	0.6 ±	0.27	0.2	0.6	0.8	0.2	1.0	0.6 ±	0.16	
4. Young dog	20 wks	0.4	1.1	2.2	1.2 ±	0.52	0.6	2.6	3.1	2.1 ±	0.76	0.5	2.5	2.5	1.5	4.1	2.2 ±	0.60	
5. Young dog	20 wks	0.6	1.6	0.9	1.0 ±	0.30	0.3	0.2	1.1	0.5 ±	0.29	0.3	0.5	1.2	1.3	0.7	0.8 ±	0.19	
6. Adult dog	10 yrs	0.4	0.6	2.4	1.1 ±	0.64	0.5	0.5	0.3	0.4 ±	0.07	0.2	0.6	1.0	0.9	1.2	0.8 ±	0.17	
7. Adult dog	10 yrs	0.4	0.3	1.7	0.8 ±	0.45	1.2	1.5	2.5	1.7 ±	0.38	0.3	1.2	1.2	2.0	3.7	1.7 ±	0.56	
8. Adult dog	12 yrs	0.7	1.4	1.4	1.2 ±	0.23	2.5	0.8	3.3	2.2 ±	0.74	0.2	1.0	2.2	1.9	2.7	1.6 ±	0.45	
9. Adult dog	13 yrs	0.4	2.0	3.2	1.9 ±	0.81	1.4	1.0	2.1	1.5 ±	0.32	0.5	2.4	2.6	0.9	1.5	1.6 ±	0.41	
10. Adult dog	14 yrs	0.2	2.9	1.7	1.6 ±	0.78	2.0	1.2	3.3	2.2 ±	0.61	0.5	0.9	1.6	2.1	2.7	1.6 ±	0.40	
11. Chronic bronchitic	7 yrs	5.1	8.9	2.5	5.5 ±	1.86	8.4	4.4	4.7	5.8 ±	1.29	2.5	5.6	7.4	6.6	7.7	6.0 ±	0.94	
12. Chronic bronchitic	11 yrs	7.3	4.6	13.8	8.6 ±	2.73	0.9	4.2	5.3	3.5 ±	1.32	4.3	3.6	3.4	2.1	2.6	3.2 ±	0.39	
13. Chronic bronchitic	9 yrs	7.8	20.4	6.3	11.5 ±	8.94	13.1	11.4	10.3	11.6 ±	0.81	1.6	7.9	15.2	2.0	7.6	6.9 ±	2.47	
14. Chronic bronchitic	6 yrs	5.0	6.3	16.2	9.2 ±	3.54	3.1	7.9	8.2	6.4 ±	1.65	3.3	3.0	3.6	5.8	6.3	4.4 ±	0.68	
15. Chronic bronchitic	5 yrs	3.0	5.0	3.4	3.8 ±	1.22	3.1	2.3	8.0	4.5 ±	1.78	4.5	1.9	2.1	2.8	5.8	3.4 ±	0.75	

Table (8): percentage of bronchial wall occupied by mucous gland (mucous gland per cent (MGP) value) at three sites in each of three lung lobes in three groups of dogs. An overall mean MGP and standard error value for each lobe in the three groups of dogs are included.

Dog	Age	Sample sites (i)	(i)	(i)	Mean ± SE	Sample sites (ii)	(ii)	(ii)	(ii)	Mean ± SE	Sample sites (iii)	(iii)	(iii)	(iii)	Mean ± SE
1. Young dog	14 wks	0.9	1.8	0.5	1.1 ± 0.38	0.9	0.7	0.7	1.3	0.9 ± 0.14	1.4	1.7	1.2	1.7	1.5 ± 0.12
2. Young dog	14 wks	0.7	1.6	0.3	0.9 ± 0.38	0.9	2.5	0.8	1.7	1.5 ± 0.40	0.7	0.3	1.2	1.2	0.9 ± 0.22
3. Young dog	14 wks	1.7	0.2	0.2	0.7 ± 0.50	0.5	0.4	0.6	0.8	0.6 ± 0.08	1.6	1.1	0.2	1.0	1.0 ± 0.29
4. Young dog	20 wks	0.4	0.6	0.5	0.5 ± 0.06	1.1	2.6	2.5	2.5	2.2 ± 0.36	2.2	3.1	1.5	4.1	2.7 ± 0.56
5. Young dog	20 wks	0.6	0.3	0.3	0.4 ± 0.10	1.6	0.2	0.5	1.2	0.9 ± 0.32	0.9	1.1	1.3	0.7	1.0 ± 0.13
6. Adult dog	10 yrs	0.4	0.5	0.2	0.4 ± 0.09	0.6	0.5	0.6	1.0	0.7 ± 0.11	2.4	0.3	0.9	1.2	1.2 ± 0.44
7. Adult dog	10 yrs	0.4	1.2	0.3	0.6 ± 0.28	0.3	1.5	1.2	1.2	1.1 ± 0.24	1.7	2.5	2.0	3.7	2.5 ± 0.44
8. Adult dog	12 yrs	0.7	2.5	0.2	1.1 ± 0.70	1.4	0.8	1.0	2.2	1.4 ± 0.31	1.4	3.3	1.9	2.7	2.3 ± 0.42
9. Adult dog	13 yrs	0.4	1.4	0.5	0.8 ± 0.32	2.0	1.0	2.4	2.6	2.0 ± 0.36	3.2	2.1	0.9	1.5	1.9 ± 0.49
10. Adult dog	14 yrs	0.2	2.0	0.5	0.9 ± 0.56	2.9	1.2	0.9	1.6	1.7 ± 0.44	1.7	3.3	2.1	2.7	2.5 ± 0.35
11. Chronic bronchitic	7 yrs	5.1	8.4	2.5	5.3 ± 1.71	8.9	4.4	5.6	7.4	6.6 ± 0.99	2.5	4.7	6.6	7.7	5.4 ± 1.14
12. Chronic bronchitic	11 yrs	7.3	0.9	4.3	4.2 ± 1.85	4.6	4.2	3.6	3.4	4.0 ± 0.28	13.8	5.3	2.1	2.6	6.0 ± 2.71
13. Chronic bronchitic	9 yrs	7.8	13.1	1.6	7.5 ± 3.32	20.4	11.4	7.9	15.2	13.7 ± 2.68	6.3	10.3	2.0	7.6	6.6 ± 1.73
14. Chronic bronchitic	6 yrs	5.0	3.1	3.3	3.8 ± 0.60	6.3	7.9	3.0	3.6	5.2 ± 1.15	16.2	8.2	5.8	6.3	9.1 ± 2.41
15. Chronic bronchitic	5 yrs	3.0	3.1	4.5	3.5 ± 0.48	5.0	2.3	1.9	2.1	2.8 ± 0.73	3.4	8.0	2.8	5.8	5.0 ± 1.19

Table (9): percentage of bronchial wall occupied by mucous glands (mucous gland per cent (MGP) value) at three levels of the bronchial tree in three groups of dogs. Site (i) represents the proximal part of the bronchial tree, site (iii) represents the peripheral bronchi and site (ii) is intermediate. The mean MGP and standard error value for each level are included for each dog.

Group	Comparison between the three groups of the following means:							Comparison within each group of:	
	Mean of eleven counts in each dog	Mean of counts in apical lobes	Mean of counts in cardiac lobe	Mean of counts in diaphragmatic lobe	Mean of counts at site (i)	Mean of counts at site (ii)	Mean of counts at site (iii)	Apical (A) Cardiac (C) Diaphragmatic (D) lobes	Sample sites (i), (ii), (iii)
Young Dogs								A-C N.S. C-D N.S. A-D N.S.	(i)-(ii) N.S. (ii)-(iii) N.S. (i)-(iii) N.S.
Adult Dogs								A-C N.S. C-D N.S. A-D N.S.	(i)-(ii) N.S. (ii)-(iii) N.S. (i)-(iii) N.S.
Chronic Bronchitic Dogs	* *	* *	* *	* *	* *	*	* *	A-C N.S. C-D N.S. A-D N.S.	(i)-(ii) N.S. (ii)-(iii) N.S. (i)-(iii) N.S.

Table (10): results of analysis of variance on the percentage of bronchial wall occupied by mucous glands (MGP values) in three groups of dogs.

* $P<0.05$

** $P<0.01$

N.S. Not significant

Dog	Age	Sample Sites										Mean ± SE
		Apical			Cardiac			Diaphragmatic				
		(i)	(ii)	(iii)	(i)	(ii)	(iii)	(i)	(ii)	(ii)	(iii)	(iii)
1. Young dog	14 wks	4.2	6.9	5.6	6.2	6.6	3.5	5.2	5.5	5.4	4.7	4.5	5.5 ± 0.44
2. Young dog	14 wks	7.3	10.2	12.7	6.9	10.7	8.0	5.5	8.7	5.4	6.8	11.3	8.5 ± 0.73
3. Young dog	14 wks	5.4	9.1	9.7	5.0	9.0	13.9	4.5	10.2	5.3	11.8	8.1	8.4 ± 0.92
4. Young dog	20 wks	3.4	5.0	9.9	3.8	5.1	8.1	5.1	6.1	7.5	7.3	6.4	6.2 ± 0.59
5. Young dog	20 wks	5.4	8.2	10.5	5.2	6.9	6.4	5.2	10.0	10.4	7.6	8.7	7.7 ± 0.62
6. Adult dog	10 yrs	1.8	5.6	6.4	1.9	3.6	4.0	3.5	5.9	8.7	5.6	11.6	5.3 ± 0.88
7. Adult dog	10 yrs	3.6	6.3	5.7	4.1	6.3	5.6	4.5	5.8	4.3	5.1	8.0	5.4 ± 0.37
8. Adult dog	12 yrs	3.4	5.7	9.0	6.9	5.5	6.0	4.5	7.3	6.7	8.4	10.8	6.8 ± 0.63
9. Adult dog	13 yrs	5.0	7.7	14.0	2.7	5.2	6.8	4.2	8.4	6.2	9.1	10.6	7.3 ± 0.96
10. Adult dog	14 yrs	2.3	8.2	12.0	9.8	4.7	8.6	3.5	7.9	8.5	12.1	8.1	7.8 ± 0.95
11. Chronic bronchitic	7 yrs	3.5	6.0	7.4	2.2	5.7	5.2	9.1	3.3	4.3	8.4	5.2	5.5 ± 0.65
12. Chronic bronchitic	11 yrs	9.4	5.1	10.2	4.2	12.1	10.3	8.0	10.5	5.1	14.5	9.4	9.0 ± 0.95
13. Chronic bronchitic	9 yrs	4.2	4.5	4.9	5.5	3.8	6.5	5.9	6.5	6.5	7.5	5.5	5.6 ± 0.34
14. Chronic bronchitic	6 yrs	6.2	4.6	5.3	8.2	5.3	9.0	3.6	3.7	3.2	3.8	7.5	5.5 ± 0.60
15. Chronic bronchitic	5 yrs	4.4	4.7	6.6	6.5	6.0	8.1	4.4	4.6	6.2	7.2	7.1	6.0 ± 0.39

Table (11): percentage of bronchial wall occupied by smooth muscle (smooth muscle per cent (SMP) values) at three sites in each of three lung lobes in three groups of dogs. An overall mean SMP and standard error value are included for each dog.

Dog	Age	Sample Sites														
		Apical				Cardiac				Diaphragmatic						
		(i)	(ii)	(iii)	Mean ± SE	(i)	(ii)	(iii)	Mean ± SE	(i)	(ii)	(ii)	(iii)	(iii)	Mean ± SE	
1. Young dog	14 wks	4.2	6.9	5.6	5.6 ± 0.78	6.2	6.6	3.5	5.4 ± 0.97	5.2	5.5	5.4	4.7	4.5	5.1 ± 0.2	
2. Young dog	14 wks	7.3	10.2	12.7	10.1 ± 1.56	6.9	10.7	8.0	8.5 ± 1.13	5.5	8.7	5.4	6.8	11.3	7.5 ± 1.1	
3. Young dog	14 wks	5.4	9.1	9.7	8.1 ± 1.34	5.0	9.0	13.9	9.3 ± 2.57	4.5	10.2	5.3	11.8	8.1	8.0 ± 1.3	
4. Young dog	20 wks	3.4	5.0	9.9	6.1 ± 1.96	3.8	5.1	8.1	5.7 ± 1.27	5.1	6.1	7.5	7.3	6.4	6.5 ± 0.4	
5. Young dog	20 wks	5.4	8.2	10.5	8.0 ± 1.47	5.2	6.9	6.4	6.2 ± 0.50	5.2	10.0	10.4	7.6	8.7	8.4 ± 0.9	
6. Adult dog	10 yrs	1.8	5.6	6.4	4.6 ± 1.42	1.9	3.6	4.0	3.2 ± 0.64	3.5	5.9	8.7	5.6	11.6	7.1 ± 1.4	
7. Adult dog	10 yrs	3.6	6.3	5.7	5.2 ± 0.82	4.1	6.3	5.6	5.3 ± 0.64	4.5	5.8	4.3	5.1	8.0	5.5 ± 0.6	
8. Adult dog	12 yrs	3.4	5.7	9.0	6.0 ± 1.63	6.9	5.5	6.0	6.1 ± 0.41	4.5	7.3	6.7	8.4	10.8	7.5 ± 1.0	
9. Adult dog	13 yrs	5.0	7.7	14.0	8.9 ± 2.67	2.7	5.2	6.8	4.9 ± 1.19	4.2	8.4	6.2	9.1	10.6	7.7 ± 1.1	
10. Adult dog	14 yrs	2.3	8.2	12.0	7.5 ± 2.82	9.8	4.7	8.6	7.7 ± 1.54	3.5	7.9	8.5	12.1	8.1	8.0 ± 1.3	
11. Chronic bronchitic	7 yrs	3.5	6.0	7.4	5.6 ± 1.14	2.2	5.7	5.2	4.4 ± 1.09	9.1	3.3	4.3	8.4	5.2	6.1 ± 1.1	
12. Chronic bronchitic	11 yrs	9.4	5.1	10.2	8.2 ± 1.58	4.2	12.1	10.3	8.9 ± 2.39	8.0	10.5	5.1	14.5	9.4	9.5 ± 1.5	
13. Chronic bronchitic	9 yrs	4.2	4.5	4.9	4.5 ± 0.20	5.5	3.8	6.5	5.3 ± 0.79	5.9	6.5	6.5	7.5	5.5	6.4 ± 0.3	
14. Chronic bronchitic	6 yrs	6.2	4.6	5.3	5.4 ± 0.46	8.2	5.3	9.0	7.5 ± 1.12	3.6	3.7	3.2	3.8	7.5	4.4 ± 0.7	
15. Chronic bronchitic	5 yrs	4.4	4.7	6.6	5.2 ± 0.69	6.5	6.0	8.1	6.9 ± 0.63	4.4	4.6	6.2	7.2	7.1	5.9 ± 0.6	

Table (12): percentage of bronchial wall occupied by smooth muscle (smooth muscle per cent (SMP) value) at three sites in each of three lung lobes in three groups of dogs. An overall SMP and standard error value for each lobe in the three groups of dogs are included.

Dog	Age	Sample sites (i)	(i)	(i)	Mean	±	SE	Sample sites (ii)	(ii)	(ii)	(ii)	Mean	±	SE	Sample sites (iii)	(iii)	(iii)	(iii)	Mean	±	SE
1. Young dog	14 wks	4.2	6.2	5.2	5.2	±	0.58	6.9	6.6	5.5	5.4	6.1	±	0.38	5.6	3.5	4.7	4.5	4.6	±	0.43
2. Young dog	14 wks	7.3	6.9	5.5	6.6	±	0.55	10.2	10.7	8.7	5.4	8.8	±	1.19	12.7	8.0	6.8	11.3	9.7	±	1.38
3. Young dog	14 wks	5.4	5.0	4.5	5.0	±	0.24	9.1	9.0	10.2	5.3	8.4	±	1.07	9.7	13.9	11.8	8.1	10.9	±	1.26
4. Young dog	20 wks	3.4	3.8	5.1	4.1	±	0.51	5.0	5.1	6.1	7.5	5.9	±	0.58	9.9	8.1	7.3	6.4	7.9	±	0.74
5. Young dog	20 wks	5.4	5.2	5.2	5.3	±	0.07	8.2	6.9	10.0	10.4	8.9	±	0.81	10.5	6.4	7.6	8.7	8.3	±	0.87
6. Adult dog	10 yrs	1.8	1.9	3.5	2.4	±	0.55	5.6	3.6	5.9	8.7	6.0	±	1.05	6.4	4.0	5.6	11.6	6.9	±	1.64
7. Adult dog	10 yrs	3.6	4.1	4.5	4.1	±	0.24	6.3	6.3	5.8	4.3	5.7	±	0.46	5.7	5.6	5.1	8.0	6.1	±	0.64
8. Adult dog	12 yrs	3.4	6.9	4.5	4.9	±	1.03	5.7	5.5	7.3	6.7	6.3	±	0.42	9.0	6.0	8.4	10.8	8.6	±	0.99
9. Adult dog	13 yrs	5.0	2.7	4.2	4.0	±	0.67	7.7	5.2	8.4	6.2	6.9	±	0.72	14.0	6.8	9.1	10.6	10.1	±	1.51
10. Adult dog	14 yrs	2.3	9.8	3.5	5.2	±	2.33	8.2	4.7	7.9	8.5	7.3	±	0.88	12.0	8.6	12.1	8.1	10.2	±	1.07
11. Chronic bronchitic	7 yrs	3.5	2.2	9.1	4.9	±	2.12	6.0	5.7	3.3	4.3	4.8	±	0.63	7.4	5.2	8.4	5.2	6.6	±	0.81
12. Chronic bronchitic	11 yrs	9.4	4.2	8.0	7.2	±	1.55	5.1	12.1	10.5	5.1	8.2	±	1.82	10.2	10.3	14.5	9.4	11.1	±	1.15
13. Chronic bronchitic	9 yrs	4.2	5.5	5.9	5.2	±	0.51	4.5	3.8	6.5	6.5	5.3	±	0.69	4.9	6.5	7.5	5.5	6.1	±	0.57
14. Chronic bronchitic	6 yrs	6.2	8.2	3.6	6.0	±	1.33	4.6	5.3	3.7	3.2	4.2	±	0.47	5.3	9.0	3.8	7.5	6.4	±	1.15
15. Chronic bronchitic	5 yrs	4.4	6.5	4.4	5.1	±	0.70	4.7	6.0	4.6	6.2	5.4	±	0.42	6.6	8.1	7.2	7.1	7.3	±	0.31

Table (13): percentage of bronchial wall occupied by smooth muscle (smooth muscle per cent (SMP) value) at three levels of the bronchial tree in three groups of dogs. Site (i) represents the proximal part of the bronchial tree, site (iii) represents the peripheral bronchi and site (ii) is intermediate. The mean SMP and standard error value for each level are included for each dog.

Group	Comparison between the three groups of the following means:							Comparison within each group of:	
	Mean of eleven counts in each dog	Mean of counts in apical lobes	Mean of counts in cardiac lobe	Mean of counts in diaphragmatic lobe	Mean of counts at site (i)	Mean of counts at site (ii)	Mean of counts at site (iii)	Apical (A) Cardiac (C) Diaphragmatic (D) lobes	Sample sites (i), (ii), (iii)
Young Dogs								A-C N.S. C-D N.S. A-D N.S.	(i)-(ii) N.S. (ii)-(iii) N.S. (i)-(iii) N.S.
Adult Dogs	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	A-C N.S. C-D N.S. A-D N.S.	(i)-(ii) * (ii)-(iii) * (i)-(iii) **
Chronic Bronchitic Dogs								A-C N.S. C-D N.S. A-D N.S.	(i)-(ii) N.S. (ii)-(iii) N.S. (i)-(iii) N.S.

Table (14): results of analysis of variance on the percentages of bronchial wall occupied by smooth muscle (SMP values) in three groups of dogs.

* $P<0.05$

** $P<0.01$

N.S. Not significant

Dog	Age	Sample Sites										Mean ± SE
		Apical			Cardiac			Diaphragmatic				
		(i)	(ii)	(iii)	(i)	(ii)	(iii)	(i)	(ii)	(ii)	(iii)	(iii)
1. Young dog	14 wks	30.8	23.0	17.8	13.6	35.2	11.9	29.1	17.2	19.8	19.5	10.1	20.7 ± 2.4
2. Young dog	14 wks	16.9	25.1	21.0	25.6	16.7	2.1	17.6	14.6	9.3	18.9	12.8	16.4 ± 2.0
3. Young dog	14 wks	27.6	22.6	13.3	18.3	10.6	6.4	15.4	9.5	14.8	10.5	13.3	14.8 ± 1.8
4. Young dog	20 wks	22.2	17.0	0.7	19.4	9.42	2.3	15.8	15.7	13.4	15.7	5.5	12.5 ± 2.1
5. Young dog	20 wks	17.3	14.2	3.8	14.4	10.8	11.0	9.8	9.7	9.3	2.9	1.2	9.5 ± 1.5
6. Adult dog	10 yrs	21.3	10.4	3.8	8.1	2.1	1.6	10.4	4.2	4.4	3.9	0.5	6.4 ± 1.8
7. Adult dog	10 yrs	21.5	20.7	18.1	18.3	4.9	4.4	16.4	17.8	18.1	20.6	6.6	15.2 ± 2.0
8. Adult dog	12 yrs	21.3	10.75	1.6	9.2	15.6	7.9	14.7	10.9	10.6	19.3	12.7	12.2 ± 1.6
9. Adult dog	13 yrs	26.1	17.2	0.4	20.1	28.8	14.8	27.0	27.9	16.3	6.8	7.1	17.5 ± 2.9
10. Adult dog	14 yrs	8.1	4.6	6.1	6.6	10.2	14.4	9.4	8.1	9.5	5.3	9.1	8.3 ± 0.8
11. Chronic bronchitic	7 yrs	11.1	3.1	11.1	2.8	2.6	0.3	9.7	6.1	5.6	1.1	0.6	4.9 ± 1.2
12. Chronic bronchitic	11 yrs	1.6	9.1	0.6	13.9	6.8	2.8	5.5	5.5	12.0	4.5	1.0	5.8 ± 1.3
13. Chronic bronchitic	9 yrs	7.5	1.1	1.1	2.4	1.6	1.3	12.9	4.2	4.2	3.1	0.5	3.6 ± 1.1
14. Chronic bronchitic	6 yrs	20.3	15.1	5.6	15.2	7.8	4.5	14.5	11.2	10.8	8.3	10.1	11.2 ± 1.4
15. Chronic bronchitic	5 yrs	18.0	13.2	3.1	7.3	10.1	1.1	2.8	10.7	6.8	1.2	4.0	7.1 ± 1.6

Table (15): percentage of bronchial wall occupied by cartilage (cartilage per cent (CP) values) at three sites in each of three lung lobes in three groups of dogs. An overall mean CP and standard error value are included for each dog.

Dog	Age	Sample Sites													
		Apical				Cardiac				Diaphragmatic					
		(i)	(ii)	(iii)	Mean ± SE	(i)	(ii)	(iii)	Mean ± SE	(i)	(ii)	(ii)	(iii)	(iii)	Mean ± SE
1. Young dog	14 wks	30.8	23.0	17.8	23.9 ± 3.8	13.6	35.2	11.9	20.2 ± 7.5	29.1	17.2	19.8	19.5	10.1	19.1 ± 3.0
2. Young dog	14 wks	16.9	25.1	21.0	21.0 ± 2.4	25.6	16.7	2.1	14.8 ± 6.9	17.6	14.6	9.3	18.9	12.8	14.6 ± 1.7
3. Young dog	14 wks	27.6	22.6	13.3	21.2 ± 4.2	18.3	19.6	6.4	11.8 ± 3.5	15.4	9.5	14.8	10.5	13.3	12.7 ± 1.2
4. Young dog	20 wks	22.2	17.0	0.7	13.3 ± 6.5	19.3	9.4	2.3	10.4 ± 5.0	15.8	15.7	13.4	15.7	5.5	13.2 ± 2.0
5. Young dog	20 wks	17.3	14.2	3.8	11.8 ± 4.1	14.4	10.8	11.0	12.1 ± 1.2	9.8	9.7	9.3	2.9	1.2	6.6 ± 1.9
6. Adult dog	10 yrs	21.3	10.4	3.8	11.8 ± 5.1	8.1	2.1	1.6	3.9 ± 2.1	10.4	4.2	4.4	3.9	0.5	4.7 ± 1.6
7. Adult dog	10 yrs	21.5	20.7	18.1	20.1 ± 1.0	18.3	4.9	4.4	9.2 ± 4.6	16.3	17.8	18.1	20.6	6.6	15.9 ± 2.4
8. Adult dog	12 yrs	21.3	10.8	1.6	11.2 ± 5.7	9.2	15.6	7.9	10.9 ± 2.4	14.7	10.9	10.6	19.3	12.7	13.6 ± 1.6
9. Adult dog	13 yrs	26.1	17.2	0.4	14.6 ± 7.5	20.1	28.8	14.8	21.2 ± 4.1	27.0	27.9	16.3	6.8	7.1	17.0 ± 4.6
10. Adult dog	14 yrs	8.1	4.6	6.1	6.3 ± 1.0	6.6	10.2	14.4	10.4 ± 2.3	9.4	8.1	9.5	5.3	9.1	8.3 ± 0.8
11. Chronic bronchitic	7 yrs	11.1	3.1	11.1	8.4 ± 2.7	2.8	2.6	0.3	1.9 ± 0.8	9.7	6.1	5.6	1.1	0.6	4.6 ± 1.7
12. Chronic bronchitic	11 yrs	1.6	9.1	0.6	3.8 ± 2.7	13.9	6.8	2.8	7.8 ± 3.2	5.5	5.5	12.0	4.5	1.0	5.7 ± 1.8
13. Chronic bronchitic	9 yrs	7.5	1.1	1.1	3.2 ± 2.1	2.4	1.6	1.3	1.8 ± 0.3	12.9	4.2	4.2	3.1	0.5	5.0 ± 2.1
14. Chronic bronchitic	6 yrs	20.3	15.1	5.6	13.7 ± 4.3	15.2	7.8	4.5	9.2 ± 3.2	14.5	11.2	10.8	8.3	10.1	11.0 ± 1.0
15. Chronic bronchitic	5 yrs	18.0	13.2	3.1	11.4 ± 4.4	7.3	10.1	1.1	6.2 ± 2.7	2.8	10.7	6.8	1.2	4.0	5.1 ± 1.7

Table (16): percentage of bronchial wall occupied by cartilage (cartilage per cent (CP) value) at three sites in each of three lung lobes in three groups of dogs. An overall SMP and standard error value for each lobe in the three groups of dogs are included.

Dog	Age	Sample sites (i)	(i)	(i)	Mean ± SE	Sample sites (ii)	(ii)	(ii)	(ii)	Mean ± SE	Sample sites (iii)	(iii)	(iii)	(iii)	Mean ± SE
1. Young dog	14 wks	30.8	13.6	29.1	24.5 ± 5.5	23.0	35.2	17.2	19.8	23.8 ± 4.0	17.8	11.9	19.5	10.1	14.8 ± 2.3
2. Young dog	14 wks	16.9	25.6	17.6	20.0 ± 2.8	25.1	16.7	14.6	9.3	16.4 ± 3.3	21.0	2.1	18.9	12.8	13.7 ± 4.2
3. Young dog	14 wks	27.6	18.3	15.4	20.4 ± 3.7	22.6	10.6	9.5	14.8	14.4 ± 3.0	13.3	6.4	10.5	13.3	10.9 ± 1.6
4. Young dog	20 wks	22.2	19.4	15.8	19.1 ± 1.9	17.0	9.4	15.7	13.4	13.9 ± 1.7	0.7	2.3	15.7	5.5	6.1 ± 3.4
5. Young dog	20 wks	17.3	14.4	9.8	13.8 ± 2.2	14.2	10.8	9.7	9.3	11.0 ± 1.1	3.8	11.0	2.9	1.2	4.7 ± 2.2
6. Adult dog	10 yrs	21.3	8.1	10.4	13.3 ± 4.1	10.4	2.1	4.2	4.4	5.3 ± 1.8	3.8	1.6	3.9	0.5	2.5 ± 0.8
7. Adult dog	10 yrs	21.5	18.3	16.4	18.7 ± 1.5	20.4	4.9	17.8	18.1	15.4 ± 3.6	18.1	4.4	20.6	6.6	8.6 ± 3.5
8. Adult dog	12 yrs	21.3	9.2	14.7	15.1 ± 3.5	10.8	15.6	10.9	10.6	12.0 ± 1.2	1.6	7.9	19.3	12.7	10.4 ± 3.7
9. Adult dog	13 yrs	26.1	20.1	27.0	24.4 ± 2.2	17.2	28.8	27.9	16.3	22.6 ± 3.4	0.4	14.8	6.8	7.1	7.3 ± 2.9
10. Adult dog	14 yrs	8.1	6.6	9.4	8.0 ± 0.8	4.6	10.2	8.1	9.5	8.1 ± 1.2	6.1	14.4	5.3	9.1	8.7 ± 2.1
11. Chronic bronchitic	7 yrs	11.1	2.8	9.7	7.9 ± 2.6	3.1	2.6	6.1	5.6	4.4 ± 0.9	11.1	0.3	1.1	0.6	3.3 ± 2.6
12. Chronic bronchitic	11 yrs	9.4	4.2	8.0	7.2 ± 1.55	5.1	12.1	10.5	5.1	8.2 ± 1.82	10.2	10.3	14.5	9.4	11.1 ± 1.15
13. Chronic bronchitic	9 yrs	4.2	5.5	5.9	5.2 ± 0.51	4.5	3.8	6.5	6.5	5.3 ± 0.69	4.9	6.5	7.5	5.5	6.1 ± 0.57
14. Chronic bronchitic	6 yrs	6.2	8.2	3.6	6.0 ± 1.33	4.6	5.3	3.7	3.2	4.2 ± 0.47	5.3	9.0	3.8	7.5	6.4 ± 1.15
15. Chronic bronchitic	5 yrs	4.4	6.5	4.4	5.1 ± 0.70	4.7	6.0	4.6	6.2	5.4 ± 0.42	6.6	8.1	7.2	7.1	7.3 ± 0.31

Table (17): percentage of bronchial wall occupied by cartilage (cartilage per cent (CP) value) at three levels of the bronchial tree in three groups of dogs. Site (i) represents the proximal part of the bronchial tree, site (iii) represents the peripheral bronchi and site (ii) is intermediate. The mean CP and standard error value for each level are included for each dog.

Group	Comparison between the three groups of the following means:							Comparison within each group of:	
	Mean of eleven counts in each dog	Mean of counts in apical lobes	Mean of counts in cardiac lobe	Mean of counts in diaphragmatic lobe	Mean of counts at site (i)	Mean of counts at site (ii)	Mean of counts at site (iii)	Apical (A) Cardiac (C) Diaphragmatic (D) lobes	Sample sites (i), (ii), (iii)
Young Dogs		* *	*		*		* *	A-C N.S. C-D N.S. A-D N.S.	(i)-(ii) N.S. (ii)-(iii) N.S. (i)-(iii) *
Adult Dogs				N.S.		N.S.		A-C N.S. C-D N.S. A-D N.S.	(i)-(ii) N.S. (ii)-(iii) N.S. (i)-(iii) N.S.
Chronic Bronchitic Dogs	* **							A-C N.S. C-D N.S. A-D N.S.	(i)-(ii) N.S. (ii)-(iii) N.S. (i)-(iii) *

Table (18): results of analysis of variance on the percentages of bronchial wall occupied by cartilage (CP values) in three groups of dogs.

* - $P<0.05$

** - $P<0.01$

N.S.- Not significant

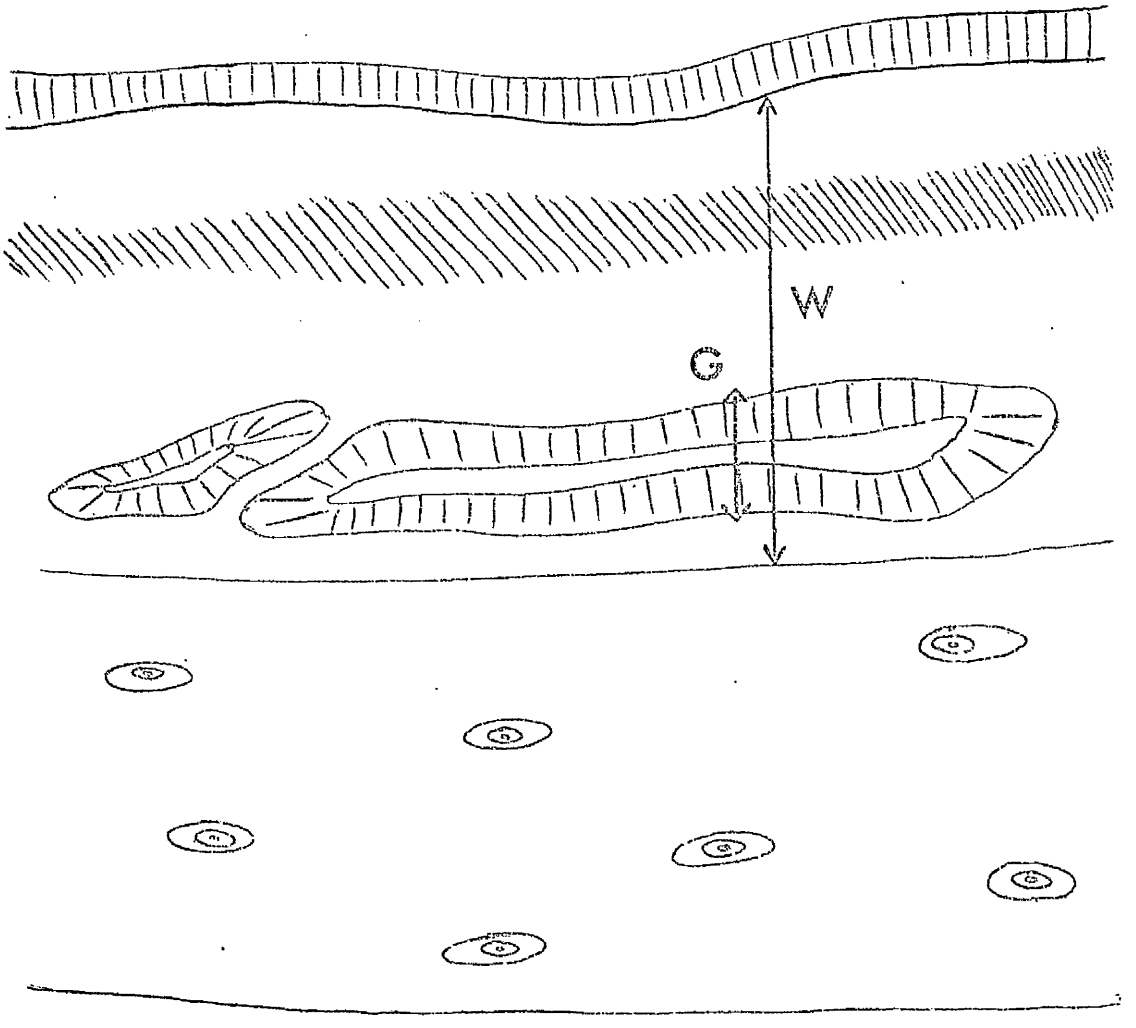
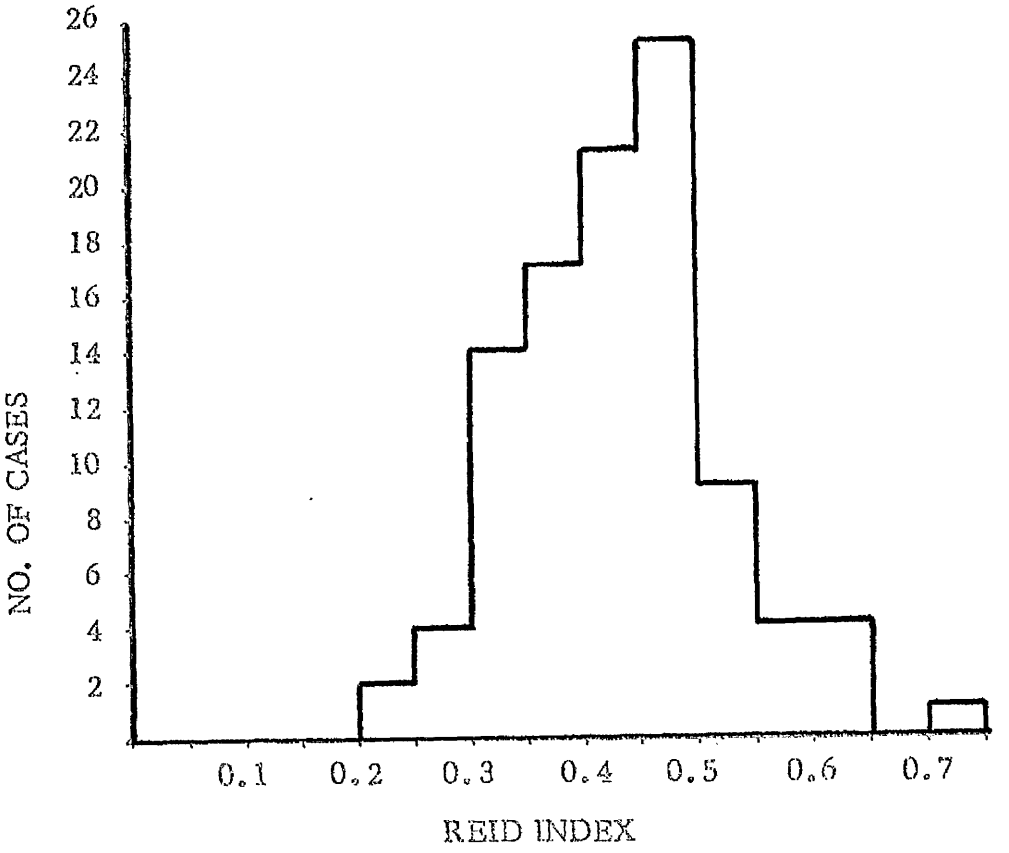
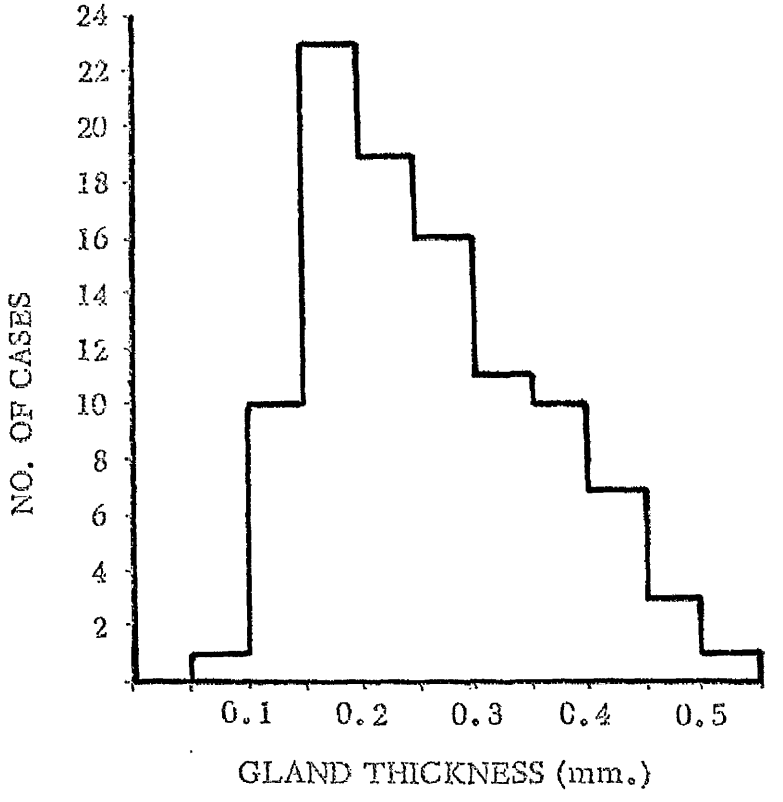


Fig. 39: Chronic bronchitis: Reid index method. Ratio of mucous gland thickness (G) to bronchial wall thickness (measured from basement membrane to inner aspect of the perichondrium) -(W).



A Reid index values in 101 random necropsies.



B Bronchial mucous gland size in 101 random necropsies.

Fig. 40 : A Values of the Reid Index (bronchial mucous gland thickness/ bronchial wall thickness) in 101 random necropsies.
B Thickness of mucous gland size in bronchi of 101 random necropsies.
(From Thurlbeck and Angus, 1964).

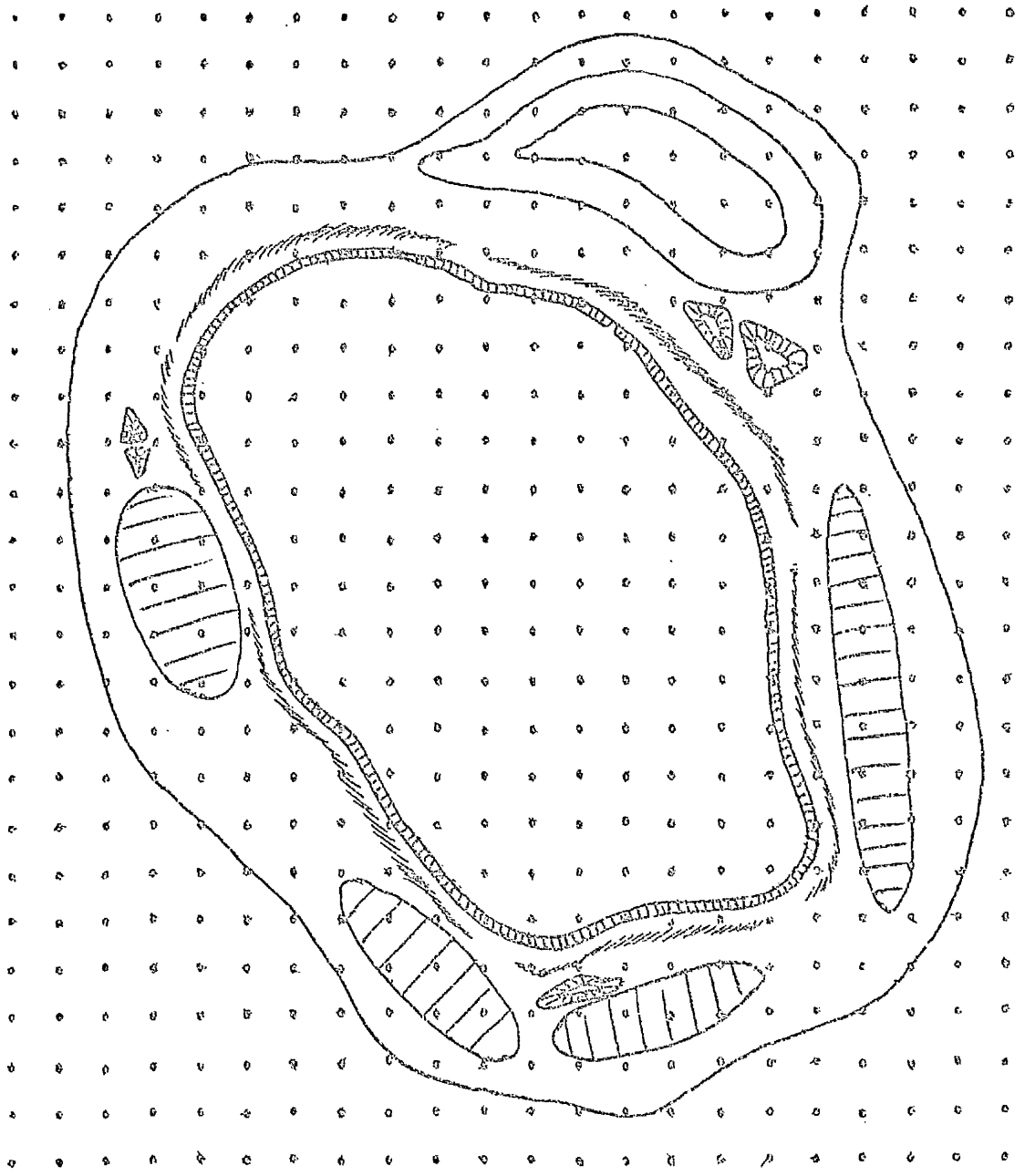
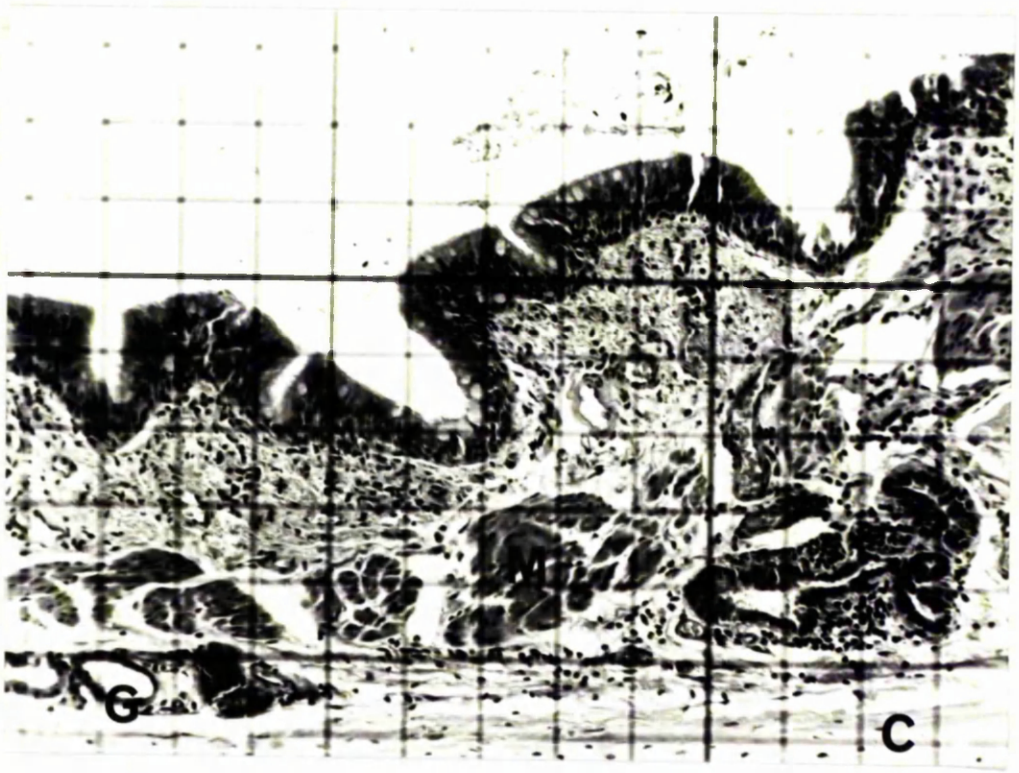


Fig. 41: Chronic bronchitis: Point-count method. A grid of regularly-distributed points superimposed on an entire cross-section of bronchus.

Fig. (42): Chronic bronchitis: view of 441-point graticule superimposed on segment of cross-section of bronchus. Intersections (points) can be seen on mucous gland (G), smooth muscle (M) and cartilage (C).


G
C

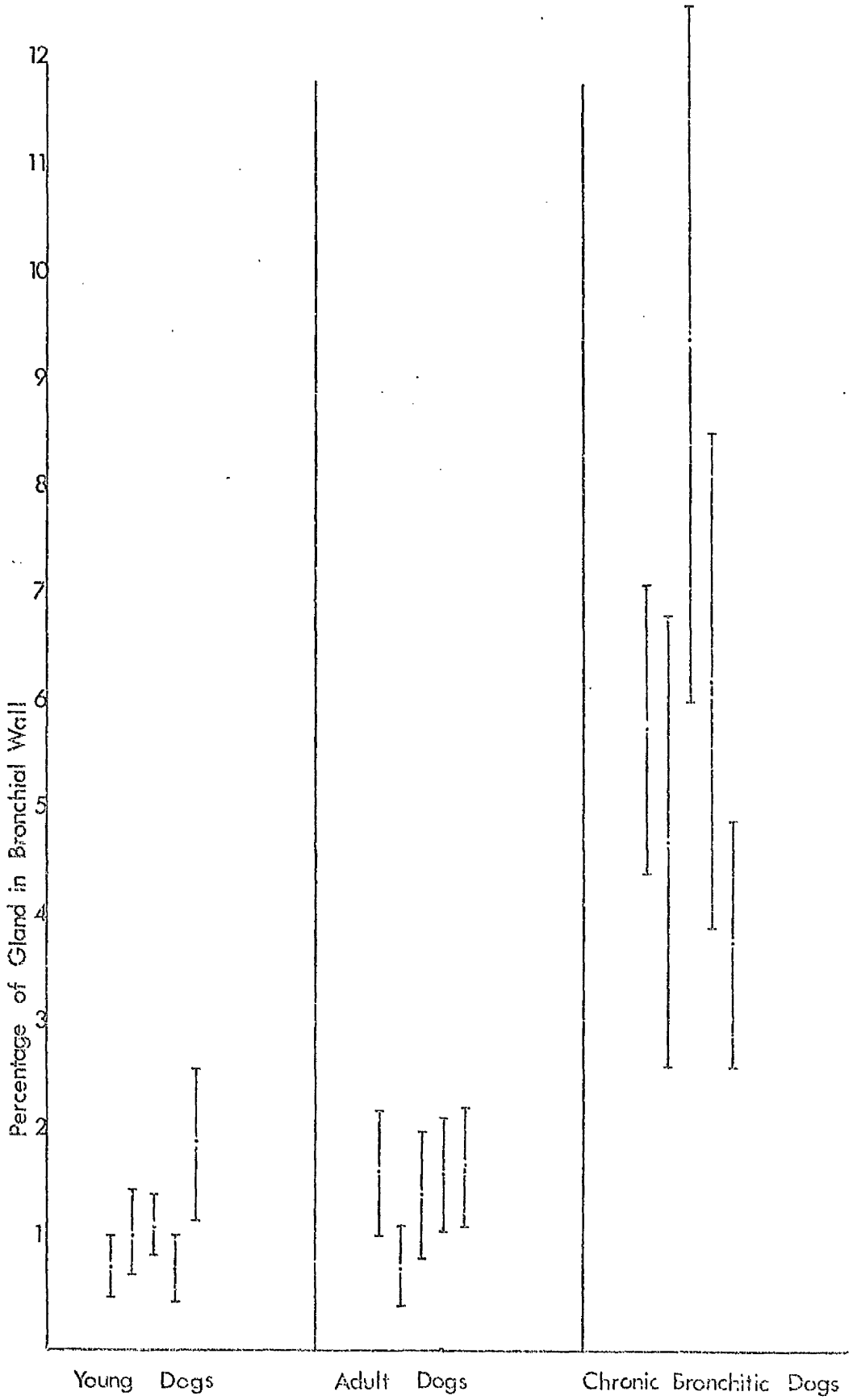


Fig. 43: Chronic bronchitis: A comparison of the mean percentage of mucous glands at 11 sites in the bronchi of 3 groups of dogs. The groups represent young dogs, normal adult dogs and dogs with chronic bronchitis. The mean gland percentage for each dog is illustrated ±2 standard errors.

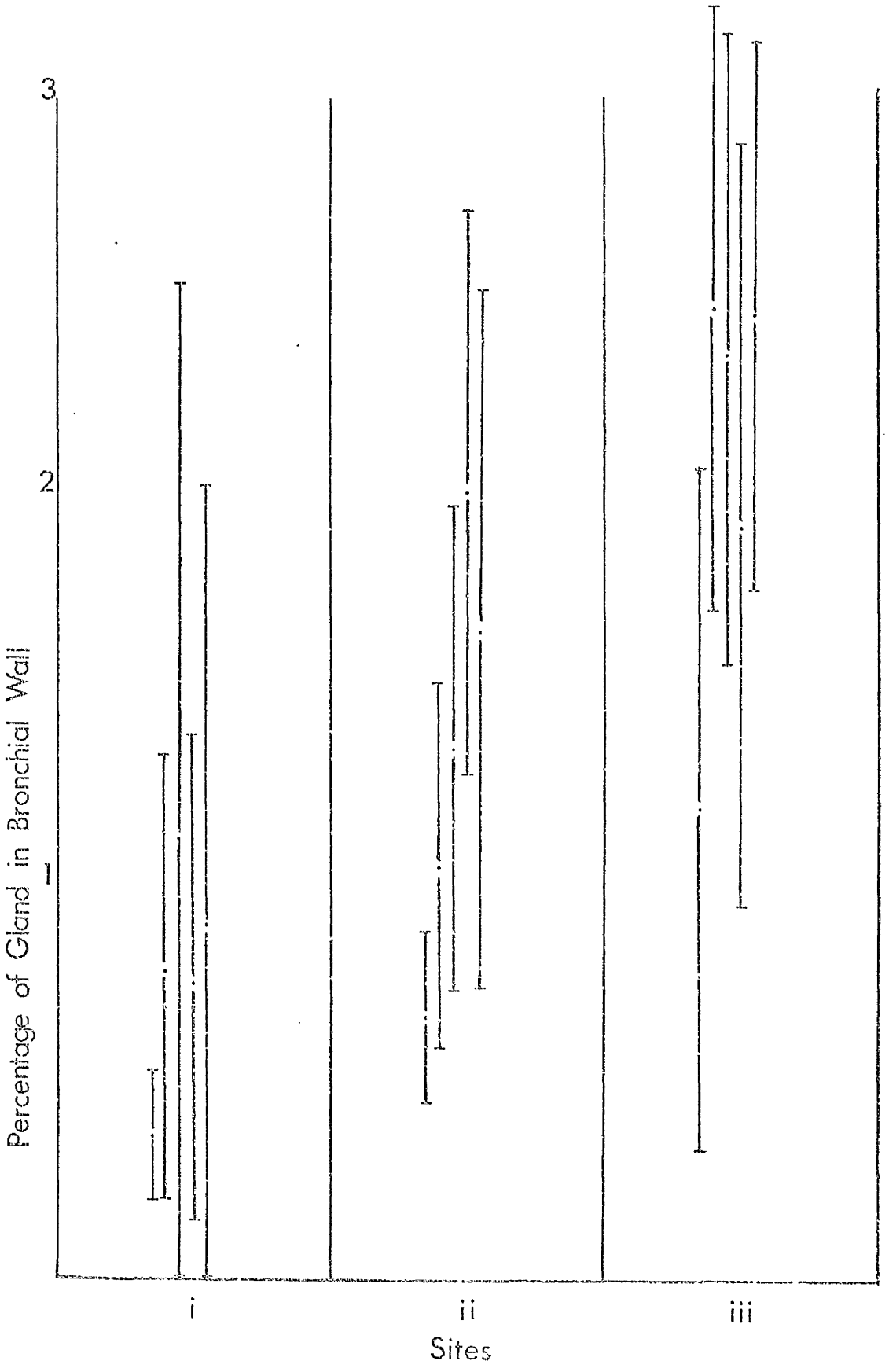


Fig.44 : A comparison of the percentage of mucous glands in the bronchial wall of five normal adult dogs at different levels in the bronchial tree. Site (i) represents the proximal part of the bronchial tree, site (iii) represents the peripheral bronchi and site (ii) is intermediate. The mean gland per cent for each site in each of the dogs is illustrated ± 2 standard errors.

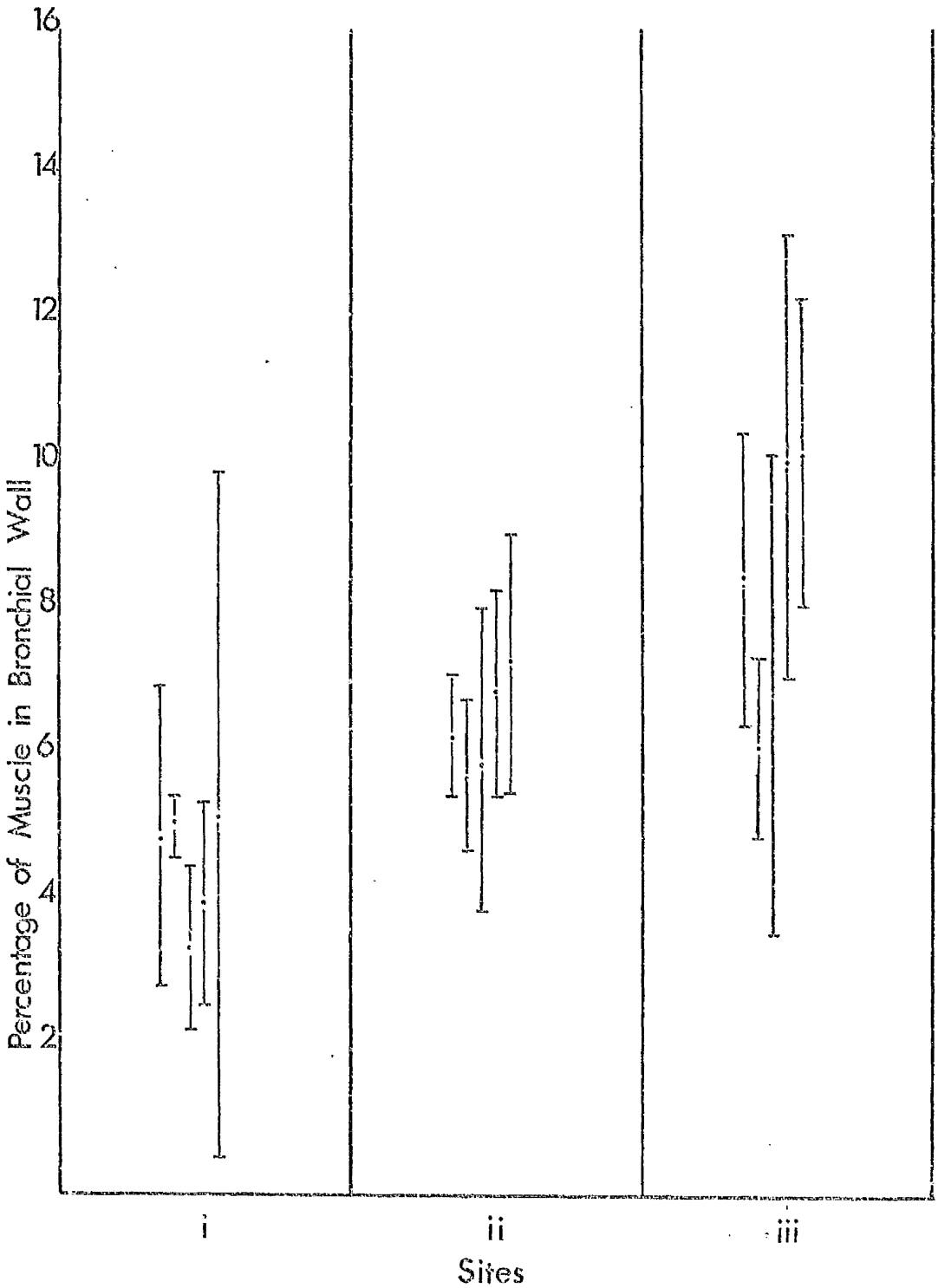


Fig. 45: A comparison of the percentage of smooth muscle in the bronchial wall of five normal adult dogs at different levels of the bronchial tree. Site (i) represents the proximal part of the bronchial tree, site (iii) represents the peripheral bronchi and site (ii) is intermediate. The mean gland percent for each site in each of the dogs is illustrated ±2 standard errors.

A HISTOCHEMICAL STUDY OF MUCOSUBSTANCES IN THE CANINE RESPIRATORY TRACT WITH SPECIAL REFERENCE TO CHRONIC BRONCHITIS

INTRODUCTION

The previous section was an attempt to quantify the increased activity of the mucus-secreting apparatus in chronic bronchitis. In addition to this quantitative change, the possibility exists of an accompanying qualitative change in the respiratory tract mucosubstances.

Because the secretions of the respiratory tract are difficult to collect, it was decided to attempt a histochemical characterisation of the mucosubstances *in situ*. Respiratory tract secretions are readily contaminated by sputum, blood and transudate and cannot be collected in amounts adequate for biochemical anaylysis. Moreover, mucusubstances are very complex and cannot be analysed readily. However, it is possible to partially characterise these mucosubstances by histochemical techniques.

Before beginning this characterisation, it was necessary to review the techniques available for such a study and to analyse the reports of investigations in man and animals in both health and disease. This section deals with the problems involved in characterisation of mucosubstances, including their classification, reviews the literature concerning respiratory tract mucosubstances in normal and diseased states in man and animals, and then goes on to present the findings in a comparison of normal dogs and dogs with chronic bronchitis.

HISTOCHEMISTRY AND CLASSIFICATION OF MUCOSUBSTANCES:

A REVIEW OF THE LITERATURE.

The characterisation of mucosubstances in the tracheobronchial tree is complicated by the inaccessibility of the respiratory mucosa and the small amounts of secretion normally produced. Collection of adequate volumes of respiratory tract mucus is thus particularly difficult, though this has been circumvented to some extent by the development of the canine tracheal pouch (Wardell, Chakrin and Payne, 1970). The pouch consists of a 5-6 cm. isolated segment of functionally normal trachea; the remaining trachea is joined by an anastomosis, while the pouch is flattened and sutured at its caudal end. The cranial end is then brought to the surface to form a stoma into the lumen of the pouch. This allows the repeated collection of millilitre quantities of uncontaminated respiratory tract mucus over periods of several months enabling definite studies on the physical properties and chemical composition of the mucus which is considered to represent normal secretion.

The application of histochemical techniques has allowed the investigation of bronchial mucosubstances *in situ*, within the mucus-secreting cells of the tracheobronchial tree. Histochemical identification avoids contamination by transudation from subepithelial capillaries, which frustrated early attempts to isolate and distinguish respiratory secretions (Florey, Carleton and Wells, 1932) and also contamination by saliva, as in the examination of sputum samples. By the use of a wide range of histochemical techniques, the intracellular mucins can be compared, classified and partially identified (McCarthy and Reid, 1964B).

However, these results can only give a limited characterization; there is little detailed chemical information about the structure of the substances and analytical data, such as is derived by biochemical techniques, is not usually gained by histochemistry. This has led

to discrepancies between histochemical and biochemical terminology. For this reason, Spicer, Leppi and Stoward (1965) have suggested that the term "mucopolysaccharide" should only be used when referring to acidic carbohydrate in connective tissue which has been identified by biochemical techniques. These workers suggested that the term "mucosubstance" be used to designate carbohydrate components of an unknown nature. The term "glycoprotein" was reserved for those substances which have been analysed biochemically.

Spicer and Henson (1967) have described the techniques available for the classification of mucosubstances; these include basic dye stains used singly or in sequence, enzymatic digestion, autoradiography, oxidation procedures, fluorescent antibody methods and chemical modification of tissue. These techniques were first used to identify and localise mucosubstances at various sites in the body, such as the vagina and salivary glands of rodents (Warren and Spicer, 1961), and were later applied to the tracheobronchial tree of man (McCarthy and Reid, 1964B; Lamb and Reid, 1969, 1970), the mouse and the rat (McCarthy and Reid, 1964A). More recently, the methods have been applied to the canine tracheobronchial tree with particular reference to the tracheal pouch (Chakrin, et al. 1970; Spicer, et al. 1971).

The mucosubstances of the respiratory tract are either acid or neutral in nature (Table 19). The neutral mucosubstances comprise Group I in the proposed classification of Spicer, Leppi and Stoward (1965); all are periodate-reactive and can be visualised by the periodic acid Schiff stain. All mucosubstances in Group I lack demonstrable acid groups.

The acid mucosubstances comprise Group II in the classification; they may be divided into sulphated and non-sulphated forms. Sulphated mucosubstances, such as chondroitin sulphate of cartilate, all contain sulphate esters and can be visualised by appropriate histochemical stains or by autoradiography (vide infra). The non-sulphated mucosubstances may

be further classified into hexuronic acid-rich mucopolysaccharides, such as hyaluronic acid, and the sialic acid-rich mucosubstances. There is no evidence that hexuronic acid-rich mucosubstances are present in epithelia; Spicer, et al. (1971) found no evidence of such groups after treating sections of canine bronchus with testicular hyaluronidase in an attempt to visualise hyaluronic acid groups. Thus, acid epithelial mucosubstances are characterised by either sulphate esters or sialic acid groups. Because mucosubstances in connective tissue are customarily called mucopolysaccharides, while those in epithelial sites are called mucins (Spicer, Leppi and Stoward, 1965), the acid epithelial mucosubstances containing sulphate esters and sialic acid residues are referred to as sulphomucins and sialomucins respectively.

Sialic acid-rich mucosubstances can then be further classified according to their reaction with the specific enzyme neuraminidase, which is derived from Vibrio cholerae. It can be seen from Table 19 that sialomucins are either highly susceptible, resistant or poorly susceptible to the enzyme. No biochemical or structural reason for this difference is known (Lamb and Reid, 1969), although Lev and Spicer (1965) suggested that resistance to neuraminidase could be due to: sialic acid occupying a non-terminal position in the molecule; the presence of substituents; ketosidic linkages which are resistant to neuraminidase; the present of additional bonds between sialic acid and the adjacent residue. However, all sialic acid groups are released by acid hydrolysis (Lamb and Reid, 1969), a technique originally used to remove sialic acid from whole sputum (Gibbons, 1963).

MUCOSUBSTANCES IN NORMAL AND DISEASED STATES: A REVIEW

Apart from the work of Spicer, et al. (1971) on man and the dog, there is little published information on the comparative morphology and histochemistry of the mucus-secreting structures in the tracheobronchial tree of man and animals.

Goco, Kress and Brantigan (1963) examined the goblet cells and mucous glands in man, rat, guinea pig, monkey, dog, sheep, pig and horse: they also compared the number, size and distribution of the glands. Since the periodic acid-Schiff (PAS) reaction was the only histochemical stain used, they were unable to compare or classify the mucosubstances present in the different species. Of the animals studied, they considered the pig to have mucous glands most closely resembling those of man. Surprisingly, Goco and his co-workers (1963) claimed that the horse did not possess mucous glands, whereas glands are readily found in trachea and bronchi (personal observations).

Korhonen, Holopainen and Paavolainen (1969) also concluded that of the mouse, rat, guinea pig, rabbit and pig, the mucous glands of the pig most closely resembled those of man. Furthermore, by using a more comprehensive range of histochemical stains (periodic acid Schiff, toluidine blue, azure A, astra blue, methylation, alcian blue with critical electrolyte concentrations, and digestion with diastase and hyaluronidase), they were able to demonstrate that the pig's mucous glands resembled those of man histochemically as well as morphologically.

Spicer, et al. (1971) used a comprehensive range of techniques in a comparison of the histochemistry of mucosubstances in the canine and human respiratory tracts and were able to detect differences in the type of secretion in the two species. The most noticeable was a predominance of sulphomucins in the goblet cells of the canine tracheobronchial tree.

The possible biochemical and histochemical alterations in the nature

of respiratory mucus in diseased states is not well documented. Korhonen, Holopainen and Paavolainen (1969) failed to detect any evidence of qualitative change in respiratory mucus in cases of bronchiectasis; however, they did report the presence of glycogen in epidermoid carcinoma cells, a mucosubstance in bronchoalveolar carcinoma, and carbohydrate-rich compounds in both the stroma of the neoplasms and the inflammatory infiltrates in the cases of bronchiectasis.

Lev and Spicer (1965) compared epithelial mucins in children with hypersecretory diseases such as colitis and cystic fibrosis, and also bronchiectasis and biliary cirrhosis with control subjects; they claimed that subjects with cystic fibrosis had an increase in epithelial sulphomucins.

The composition of bronchial mucus has been more extensively investigated in chronic bronchitis (Reid, 1965; de Haller and Reid, 1965; Lamb and Reid, 1969) where histochemical and enzyme studies have centred around the use of neuraminidase to locate sialic acid residues (de Haller and Reid, 1965). Using neuraminidase digestion followed by the combined alcian blue-periodic acid Schiff technique, Reid (1965) found that subjects with chronic bronchitis had a larger proportion of mucous cells which were completely or partially resistant to neuraminidase. Reid (1965) ascribed this to an increase in neuraminidase-resistant sialomucins; but the possibility also exists that this increase was due to an increase (relative or absolute) in sulphomucins.

Localisation of sulphomucins by autoradiography appears tohave been performed only on normal bronchus; the original report (Reid, et al., 1962) stated that autoradiography was conducted on rat, mouse and human bronchial biopsy material. Rats and mice were injected intraperitoneally with sulphur - 35 as sulphate and killed after two hours. Tissue cultures of human bronchial biopsy material, which cannot be assumed to be from normal subjects since the reason for excision was not stated, were incubated with labelled sulphate for one hour. Both sets of material, the rat and mouse lungs, and the human biopsy cultures were then processed and incubated with photographic films to give autoradiographic localisation of sulphate in the bronchial tree.

Sulphate could then be seen to be present in mucous gland acini, but only in a few of the goblet cells (Reid, et al., 1962).

Spicer, Chakrin and Wardell (1972) have reported briefly on the effect of sulphur dioxide inhalation on respiratory mucins in the dog. In the normal dog, the epithelial goblet cells secrete sulphomucins (Spicer, et al., 1971); after exposure to sulphur dioxide, there was apparently less sulphomucin present in the epithelial goblet cells, despite hyperplasia and hypersecretion of goblet cells. The mucous glands were not as affected by exposure to sulphur dioxide but they were hypertrophied (Spicer, Chakrin and Wardell, 1972).

The present study was intended to investigate the possible alterations in respiratory mucins in dogs with naturally-occurring chronic bronchitis and to confirm the observations of Spicer, et al. (1971) in the normal dog.

MATERIALS AND METHODS

Portions of bronchial wall were taken from six adult dogs and six dogs which had been diagnosed as having chronic bronchitis. All material was taken immediately after post mortem examination, fixed in formol saline solution and processed by the means described in the general Materials and Methods section.

In addition to the standard techniques of haematoxylin and eosin and the combined alcian blue-periodic acid Schiff techniques (Mowry, 1956), sections from selected blocks of tissue were stained by various additional methods. All sections from dogs with chronic bronchitis were compared to equivalent sections from normal adult dogs. Controls for such techniques as neuraminidase digestion were always serial sections to the test section, so that the mucosubstances in the test and control sections were as nearly identical as possible. Similarly, comparison of histochemical techniques for detection and elimination of mucosubstances was performed on serial sections.

The histochemical stains can be divided into those staining neutral or acid mucosubstances, or particularly types of mucosubstances selectively. The details of the staining techniques are given at the end of this section.

I. STAINS FOR NEUTRAL MUCOSUBSTANCES.

1. Periodic acid Schiff (PAS) Technique

Histochemical Result.

PAS positive mucosubstance stained a deep red (magenta)

2. Diastase Digestion

Histochemical Result

Diastase digestion selectively eliminates PAS staining attributable to glycogen.

II. STAINS FOR ACID AND NEUTRAL MUCOSUBSTANCES

1. Alcian blue-periodic acid Schiff Technique (AB-PAS)

Histochemical Result

Acid mucosubstance - blue

Neutral mucosubstance - red

III STAINS FOR SIALOMUCINS

1. Acid Hydrolysis Technique (Lamb and Reid, 1969)

Histochemical Result

Comparison of serial sections reveals loss of staining of all sialic acid groups with resultant loss of alcian blue alcianophilia. The increase in PAS - staining material in the acid hydrolysis section thus represents sites of sialomucin. The remaining alcian blue basophilia is due to other acid epithelial mucosubstances, i.e. sulphomucins.

2. Neuraminidase Digestion (McCarthy and Reid, 1964A)

Histochemical Result

Neuraminidase reacts with neuraminidase-sensitive sialomucins to eliminate metachromasia and alcian blue affinity. Comparison of control and test sections reveals the removal of sensitive sialomucins where there is a colour change from blue to red with AB-PAS stain or from purple to brown with high iron diamine-alcian blue (HID-AB).

IV. STAINS FOR SULPHOMUCINS

1. Alcian blue (pH 2.5) Technique (Spicer, Horn and Leppi, 1967)

Histochemical Result

Sialomucins, hyaluronic acid and weakly acid sulphated mucosubstances stain dark blue.

2. Alcian blue (pH 1.0) Technique (Spicer, Horn and Leppi, 1967)

Histochemical Result

Sulphated mucosubstances are selectively stained.

3. Alcian blue with graded increases in Magnesium Chloride

Histochemical Result

0.1M $MgCl_2$ eliminates staining of hayluronic acid, sialomucins and some weakly acidic sulphomucins.

0.2M $MgCl_2$ gives strong and selective staining of most sulphated mucosubstances.

Various sulphated mucosubstances lose alcianophilia at different levels with increasing $MgCl_2$ concentration, some (including some epithelia) persist to 1.0M $MgCl_2$.

4. High-Iron Diamine Technique (Spicer, Horn and Leppi, 1967)

Histochemical Result

Sulphated mucosubstances are coloured brown-black.

5. High-Iron Diamine Alcian blue (Spicer, 1965)

Histochemical Result

Most sulphated mucosubstances are coloured purple-black; acid mucopolysaccharides lacking sulphate esters (i.e. hyaluronic acid and sialomucins) are unstained by the diamine. The post-staining for 30 minutes in 1 per cent alcian blue in 3 per cent acetic acid colours sialomucins and hyaluronic acid blue.

6. Low Iron Diamine-Alcian blue (Spicer, 1965)

Histochemical Result

Low-iron diamine negative, non-sulphated acid mucosubstances are blue; low iron diamine reactive, non-sulphated and sulphated acid mucosubstances are black.

V. STAINS FOR HYALURONIC ACID

1. Hyaluronidase Digestion (Spicer, Leppi and Stoward, 1965)

Histochemical Result

Basophilia is eliminated by testicular hyaluronidase indicating the presence of hyaluronic acid.

RESULTS.

Using the battery of histochemical techniques outlined above, it was possible to identify the various mucosubstances in the respiratory tract of normal dogs and also to detect fundamental changes in the composition of those mucosubstances in dogs with chronic bronchitis.

Control dogs - Neutral mucosubstances.

The location of neutral mucosubstances was identified by the use of the combined alcian blue-periodic acid Schiff (AB-PAS) technique. The sites of neutral mucosubstances could then be seen in slides used as controls for neuraminidase digestion and acid hydrolysis. Neutral mucosubstances did not comprise a large proportion of mucosubstances staining in the goblet cells and mucous glands in the respiratory tract of normal dogs (Fig.46) The majority of goblet cells and mucous gland cells were strongly alcianophilic.In sections stained by PAS, the majority of goblet cells and mucous cells stained magenta; this appearance could represent the presence of periodate reactive neutral mucosubstances and also periodate reactive acid mucosubstances.

Treatment of serial sections of normal bronchus with diastase digestion was undertaken to determine if any of the magenta staining of the PAS stain was caused by glycogen in the mucous cells. However, there was no detectable diminution in PAS staining which was attributable to loss of glycogen.

Bronchitic dogs - Neutral mucosubstances.

The dogs with chronic bronchitis had very few magenta staining goblet cells or mucous glands with the combined AB-PAS stain; the proportion of alcianophilic staining cells increased overall.

Similarly, treatment of serial sections of bronchitic bronchus with diastase failed to reveal any detectable diminution of PAS staining which could have been attributed to loss of glycogen.

Control dogs - Sialomucins

The presence of sialomucins could be pinpointed by the loss of alcianophilia after treatment with neuraminidase and after hydrolysis. Figs.46 and 47 were from serial sections of bronchus from a normal dog. The test section incubated with neuraminidase had a slight overall loss of alcianophilia and, in addition, the remaining alcianophilia was not as deep blue in colour. This loss in alcianophila represented a loss at those sites of neuraminidase-sensitive sialomucins.

The extent of neuraminidase-resistant sialomucins in normal bronchus could be determined by comparing the loss of alcianophilia in a pair of serial sections, one treated by acid hydrolysis and the other treated by neuraminidase digestion. Since the former method (acid hydrolysis) eliminates all basophilia due to sialomucins, and the latter method of neuraminidase digestion eliminates all basophilia due to neuraminidase-susceptible sialomucins, then a comparison of two serial sections treated by acid hydrolysis and neuraminidase digestion would reveal the loss of alcianophilia due to neuraminidase-resistant sialomucins (Figs. 48,49) This loss of alcianophilia can be seen in Figs 48 and 49 where the loss of staining is the result of the loss of neuraminidase-resistant sialomucins. Thus, it would appear that the normal canine bronchus has both neuraminidase-susceptible and neuraminidase-resistant sialomucins; both forms are present in roughly equal amounts histochemically, but the total amount of sialomucin is small compared to the remaining alcianophilic mucosubstances present after acid hydrolysis (Fig.48).

Bronchitic dogs - Sialomucins.

The results in the dogs with chronic bronchitis were appreciably different from the findings in the control dogs. The results of neuraminidase - digestion on an enlarged mucous gland are illustrated in Figs. 50 and 51. It can be seen that neuraminidase-sensitive sialomucins appeared to comprise most of the mucosubstance within the gland for, after neuraminidase digestion, there was almost total loss of alcianophilia (Fig. 50).

Similarly, a comparison of Figs. 52 and 53 revealed a significant loss of alcianophilia after sections had been subjected to acid hydrolysis and neuraminidase digestion, indicating the presence of an appreciable amount of neuraminidase-resistant sialomucins.

These results with neuraminidase digestion and acid hydrolysis techniques indicated that there were increased amounts of sialomucins, both neuraminidase-susceptible and neuraminidase-resistant, in the respiratory tract of dogs with chronic bronchitis.

Normal dogs - Magnesium chloride extinction series.

The results of the magnesium chloride extinction series on normal bronchial epithelium are seen in Figs. 54 - 59. There was little difference in alcianophilia of goblet cells between 0.1 M (Fig. 54) and 0.2M (Fig. 55) magnesium chloride. Even at 0.5 M magnesium chloride (Fig. 56) the alcianophilia had only begun to fade, indicating abundance and persistent of sulphomucins up to 0.5 M magnesium chloride concentrations. Thereafter, alcianophilia diminished rapidly with increasing concentrations of magnesium chloride; thus alcianophilia markedly diminished by 0.6 M (Fig. 57) concentrations of magnesium chloride and was virtually absent by 0.8 M (Fig. 58) and 1.0 M (Fig. 59) concentrations of magnesium chloride.

The effect of increasing concentrations of magnesium chloride on mucous glands from normal bronchus is seen in Figs. 60 - 63. There was a slight loss of alcianophilia from 0.1 M (Fig. 60) to 0.5 M (Fig. 61); this loss of alcianophilia was marked by 0.6 M (Fig. 62) and was complete by 0.8 M (Fig. 63).

Bronchitic dogs - Magnesium chloride extinction series.

In the case of chronic bronchitics, the magnesium chloride extinction profile appeared to be generally similar (Fig. 64 - 67). The epithelial goblet cells were very attenuated in this field, as can be seen in Fig. 64 at 0.1 M magnesium chloride concentration; nevertheless, there was total loss of alcianophilia in these goblet cell remants at 0.5 M magnesium chloride solution (Fig. 65). There

was even a marked loss of alcianophilia at 0.2 M magnesium chloride, which indicated a lack of epithelial sulphomucins.

Despite this pronounced lack of epithelial alcianophilia, the mucous glands had appreciable alcianophilia up to 0.6 M (Fig. 66) and there were even remnants at 0.8 M magnesium chloride (Fig. 67). This indicated the presence of appreciable amounts of sulphomucins in these glands.

Normal dogs - Sulphomucins

This apparent decrease in epithelial sulphomucins was then examined further by specific sulphomucin stains. The results of the high iron diamine stain on normal bronchus can be seen in Figs.. 68 and 69. There were frequent, regularly-distributed, dark staining, sulphomucin-containing goblet cells in the normal epithelium (Fig. 68 with a high-power detail photomucrograph - Fig 69). A serial section counterstained with alcian blue revealed very few alcianophilic, sialomucin-containing goblet cells (Fig. 70 with a corresponding detail photomucrograph - Fig. 71).

Bronchitic dogs - Sulphomucins

By contrast, in material from dogs with chronic bronchitis, there was an almost complete lack of sulphomucin-containing goblet cells in bronchial epithelium.

Figs. 72 and 73 are sections stained by the low iron diamine (LID) and high iron diamine (HID) techniques respectively. In Fig. 72, a bronchus stained by the LID technique, the larger bronchus has focal areas of dark staining goblet cells in the epithelium. Sulphomucins and many sialomucins are stained black by the LID technique. The high iron diamine (HID) technique stains sulphomucins selectively while leaving nonsulphated acid mucosubstances unstained. Using the HID technique, the same bronchus had virtually no dark-staining, sulphomucin-containing goblet cells, although there is some focal staining of goblet cells in the smaller bronchus, and in the mucous glands. (Fig. 73) Further investigations, using the HID technique to selectively visualize sulphomucins, revealed a substantial reduction in the numbers of sulphomucin-containing goblet cells in the

epithelium (Figs. 74 and 75). However, counterstaining of HID sections with alcian blue revealed a large increase in the numbers of alcianophilic (sialomucin-containing) goblet cells in the bronchitis epithelium; these appeared as numerous, attenuated alcianophilic cells scattered in the epithelium (Fig. 76). Thus, the stains for sulphomucin indicated a marked reduction in epithelial sulphomucins while the stains for sialomucins revealed a marked increase in epithelial sialomucins.

Serial sections from the bronchus of normal dogs and dogs with chronic bronchitis were subjected to digestion with testicular hyaluronidase. In both normal and bronchitic material there was no evidence of the presence of hyaluronic acid groups in the mucosubstances.

DISCUSSION

Our knowledge of the carbohydrate-rich components synthesised in animal tissues comes from both biochemical and histochemical investigations. Discrepancies in nomenclature between the two approaches led to a proposal by Spicer, Leppi and Stoward (1965) of a comprehensive classification of these carbohydrate-rich tissue components using a standardised terminology. Their suggested classification was subsequently adopted by Pearse (1968) and that terminology has been followed in this thesis. The term 'mucopolysaccharide' has not been used, since Spicer, Leppi and Stoward (1965) suggested that it should be reserved for acidic carbohydrate visualised in connective tissue which is known to contain mucopolysaccharide by biochemical methods. Instead, the term 'mucosubstance' has been used to denote a carbohydrate of unkown nature.

The respiratory epithelial mucosubstances are either acid or neutral in nature. The neutral mucosubstances comprise Group 1 in the proposed classification of Spicer, Leppi and Stoward (1965); they are all periodate reactive and can be visualised by the periodic acid Schiff stain.
In human adults up to 14 per cent of mucous cells in the glands have been found to contain neutral mucosubstance (de Haller & Reid, 1965), while neutral mucosubstance is present in only a few of the goblet cells (McCarthy and Reid, 1964B). Neutral-staining goblet cells are rarely found in hypersecretory states such as infected cystic fibrosis and adult chronic bronchitis (McCarthy & Reid, 1964B). It has been suggested that the neutral mucosubstance represents a precursor stage in a cycle of secretion of mucosubstances (de Haller, 1969). Some support for this view has been provided by Spicer, *et al.*, (1971), who described a red staining in the Golgi zone of goblet cells in sections of canine trachea stained by the AB-PAS method; these workers interpreted this as indicating that the mucosubstances in the flattened vesicles of the Golgi zone had not reached a state of full sulphation.

The reactivity to AB-PAS stain in normal dog bronchus indicated the presence of neutral mucosubstances; no appreciable staining due to the presence of glycogen was detected.

The acid mucosubstances comprise Group II of the proposed classification of Spicer, Leppi and Stoward (1965). The acid mucosubstances are divided into sulphated and non-sulphated esters; Spicer, Leppi and Stoward (1965) suggested the terms "sulphomucin" and "sialomucin" for acid epithelial mucosubstances containing sulphate esters and sialic acid respectively.

In man, nearly all goblet cells contain some acid mucosubstances (McCarthy and Reid, 1964); this consists of periodate reactive sulpho- and sialo-mucins (Spicer, Chakrin and Wardell, 1972). The mucous glands contain mostly acid mucosubstances and the majority of this is composed of sialomucins (de Haller and Reid, 1965). In the dog, it would appear from this study that the goblet cells contain largely sulphomucin; this agrees with the work of Spicer, Chakrin and Wardell (1972). The mucous glands in the dog produce sulphomucin and sialomucin as well as neutral mucin.

The sialomucins present in human respiratory mucus are of two types; those which are susceptible to neuraminidase and those which are resistant to it (Spicer and Warren, 1960; Warren and Spicer, 1961). Digestion with sialidase releases sialic acid from some sialomucins to eliminate metachromasia and alcian blue affinity in these mucosubstances. In human subjects with chronic bronchitis, de Haller and Reid (1965) found that there was an increased number of mucous cells, a higher proportion of which contained acid mucosubstance. Of these cells containing acid mucosubstance, a greater proportion were neuraminidase-resistant than in bronchi from normal subjects. Reid (1965) ascribed this increase in neuraminidase-resistant mucosubstance to an increase in neuraminidase-resistant sialomucins, though it is possible that there may have been a relative or absolute increase in sulphomucins.

The canine bronchus appears to have less epithelial sialomucin than

is the case in man. Treatment of serial sections with either neuraminidase or acid hydrolysis revealed only a small decrease in alcianophilia with either method. This indicates that only a small proportion of the epithelial mucins are sialomucins (indicated by acid hydrolysis) and that only a proportion are neuraminidase-sensitive (as indicated by neuraminidase digestion). In the dogs with chronic bronchitis, treatment of sections with neuraminidase digestion and acid hydrolysis revealed a more marked loss of alcianophilia with both techniques. Thus, dogs with chronic bronchitis have greater amounts of sialomucins, both susceptible and resistant to neuraminidase.

The specificity of histochemical stains for sulphate groups has been the subject of some debate. Several histochemical techniques have been used to selectively stain sulphate groups, notably aldehyde-fuchsin-alcian blue (Spicer and Meyer, 1960), alcian blue in aluminium sulphate (Heath, 1961) and the high iron diamine technique (Spicer and Duvenci, 1964). Lamb and Reid (1969) stated that none of these were known to be completely specific; they found that these stains accounted for fewer mucous cells than by staining with alcian blue after acid hydrolysis (which would stain all acid mucosubstances except sialomucins). However, Reid (1968) had previously stated that comparison between autoradiographs of tissue culture fragments after sulphate uptake and a range of stains claiming to identify sulphate revealed that staining identified all of the sulphated cells present in the autoradiographs. In both reports (Reid, 1968; Lamb and Reid, 1969) the authors compared histochemical stains for sulphate with an autoradiograph method using uptake and incorporation of sulphur-35. This paradox is as yet unresolved; since autoradiography was not undertaken in this study, it has not been possible to confirm or deny this theory in the case of the dog.

The high iron diamine (HID) technique did demonstrate the presence of sulphomucins in the goblet cells of the bronchial epithelium in normal dogs. Staining of epithelium by the combined high-iron diamine-alcian blue (HID-AB) technique revealed a predominance of sulphomucin (brown-black

staining) over the sialomucin (blue-green staining). By contrast, in the dogs with chronic bronchitis, staining by the HID technique revealed a reduction in goblet cells staining for sulphomucin, with patchy focal areas of brown-black goblet cells; staining with the HID-AB technique revealed an increase in epithelial sialomucins.

Gibbons (1959), using bovine cervical mucus collected at different stages in the oestrous cycle, has demonstrated that variations in the amount of sialic acid can be related to changes in the internal viscosity of the mucus. Thus an increase in the amount of sialic acid residues in the respiratory mucus in chronic bronchitis could explain the increased viscosity of the mucus (Reid, 1969).

It is possible that this apparent increase in sialomucins could be the result of an increase in mucosubstances containing uronic acid. Spicer, et al., (1971) regarded all mucosubstances not apparently containing sulphate esters as carboxymucins, i.e. they contain sialic acid or uronic acid as these are the only acidic groups that could account for the basophilia. However, Spicer, et al., (1971) subjected sections of canine bronchus to hyaluronidase digestion (which destroys uronic acid groups with subsequent loss of alcianophilia), and found no change in the brown-black staining of the HID technique when compared with control sections. This failure to detect carboxyl groups containing uronic acid in the respiratory mucins was confirmed in this study.

Although, as Spicer, Leppi and Stoward (1965) have pointed out, "histochemical methods (in their present stage of development) provide relatively little detailed chemical information about the structure of carbohydrate-rich substances", nevertheless, the same histochemical methods provide "a clear picture of the structure of the carbohydrates which are responsible for the specific histochemical reactions of the various mucins" (Pearse, 1968). Using a variety of histochemical stains, it has been possible to compare and characterise the mucosubstances of the canine respiratory tract in health and disease. In chronic bronchitis, there appears to be a reduc-

tion in epithelial sulphomucins, together with an increase in sialomucins. This may explain the increased viscosity of respiratory mucus in chronic bronchitis in the dog, since it has been shown by Gibbons (1959) that increased amounts of sialic acid residues in mucus can increase mucus viscosity.

1. NEUTRAL MUCOSUBSTANCES

These are neutral glycoproteins, fucomucins, mannose-rich mucosubstances and immunologically reactive glycoproteins. All react with the PAS stain.

II. ACID MUCOSUBSTANCES

A. Sulphated

1. Connective tissue mucopolysaccharides (periodate unreactive)
    a) Resistant to hyaluronidase
    b) Susceptible to hyaluronidase
2. Epithelial sulphomucins (hyaluronidase resistant)
    a) Periodate unreactive
    b) Periodate reactive

B. Nonsulphated

1. Hexuronic acid-rich mucopolysaccharides (hyaluronic acid, chondroitin)
2. Sialic acid-rich mucosubstances
    a) Connective tissue mucopolysaccharides containing sialic acid.
    b) Epithelial sialomucins

(i) Highly susceptible to Vibrio cholerae sialidase, periodate reactive and metachromatic.

(ii) Slowly digestible with V. cholerae sialidase

(iii) Resistant to V. cholerae sialidase
- sialidase susceptible after saponification
- sialidase resistant after saponification.

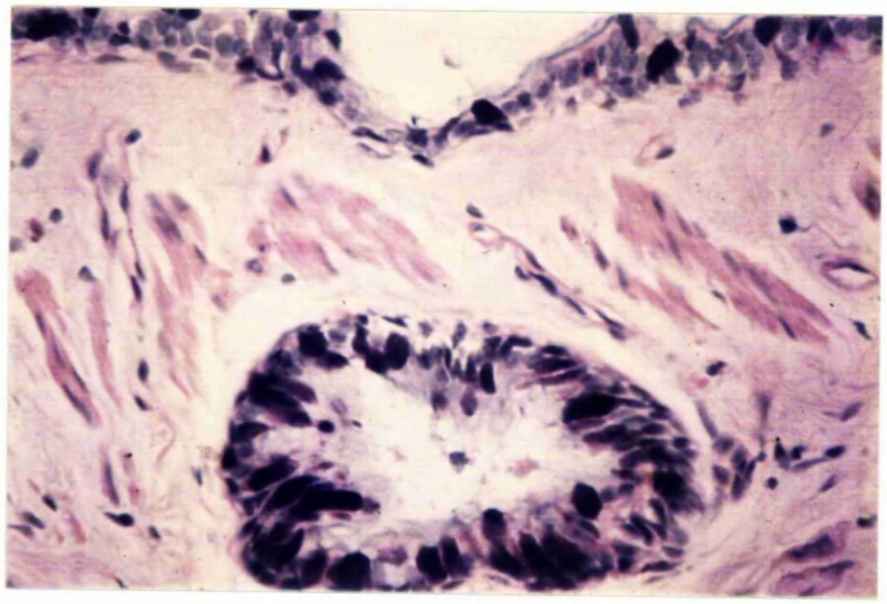
Table 19: Histochemical Classification of Mucosubstances (simplified after Spicer, Leppi and Stoward, 1965).

Fig. 46: Normal bronchus: Neuraminidase digestion followed by staining with alcian blue-periodic acid Schiff technique. There is a slight overall loss in the intensity of alcianophilia compared to Fig. (47); this loss indicates sites of neuraminidase-sensitive sialomucin.

(Neuraminidase, AB-PAS X 500)

Fig. 47: Normal bronchus: Control (Serial) section for Fig. (46) stained by the alcian blue-periodic acid Schiff technique.

(AB-PAS X 500)



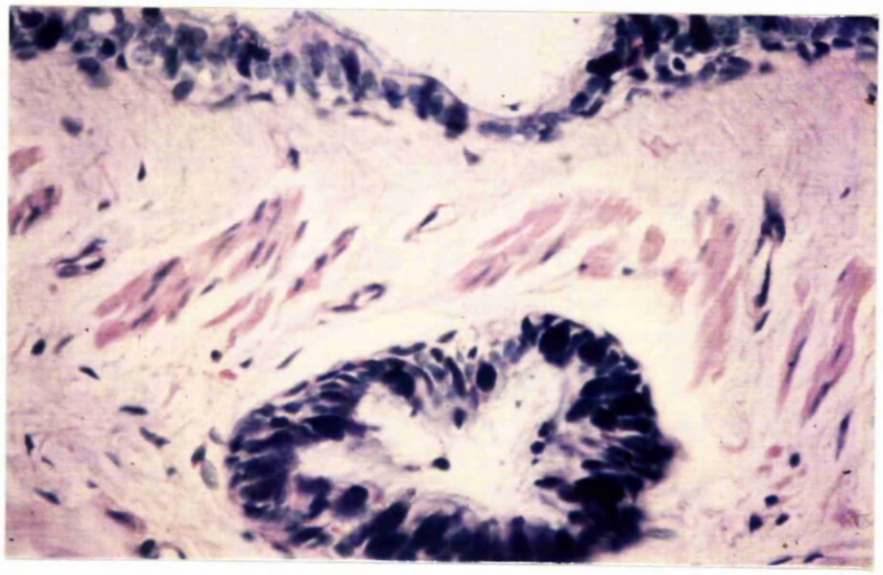
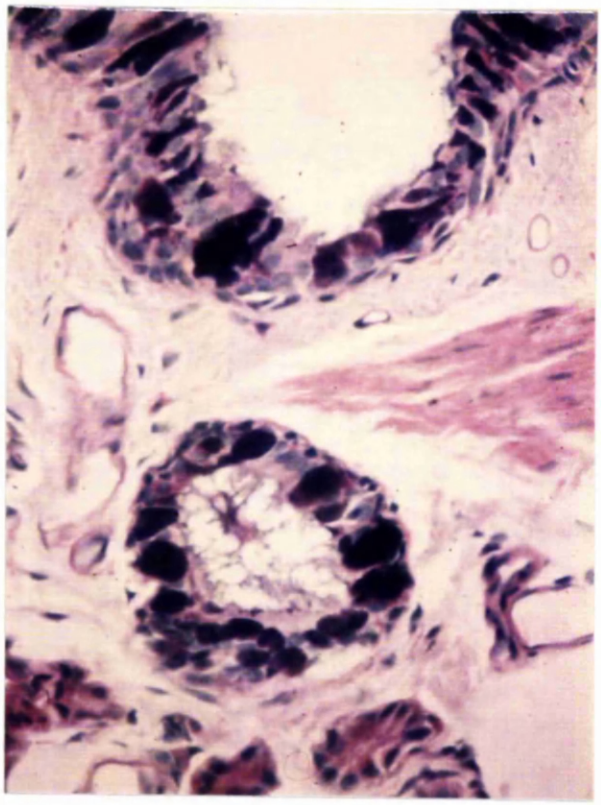


Fig. 48: Normal bronchus: Acid hydrolysis followed by staining with alcian blue-periodic acid Schiff technique. There is a slight overall loss in alcianophilia compared to Fig. 49; this loss indicates the sites of neuraminidase-resistant sialomucin.

(Acid hydrolysis, AB-PAS X 500)

Fig. 49: Normal bronchus: Neuraminidase digestion followed by staining with alcian blue-periodic acid Schiff technique.

(Neuraminidase, AB-PAS X 500)



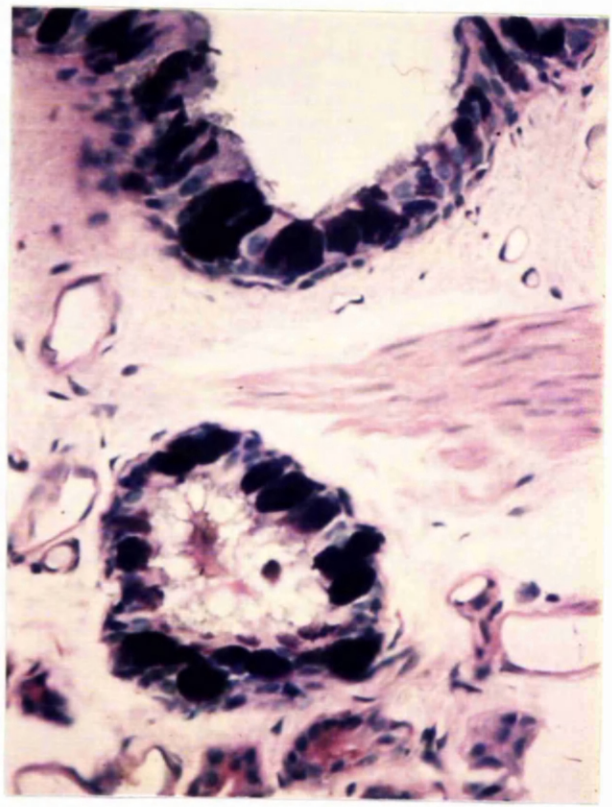
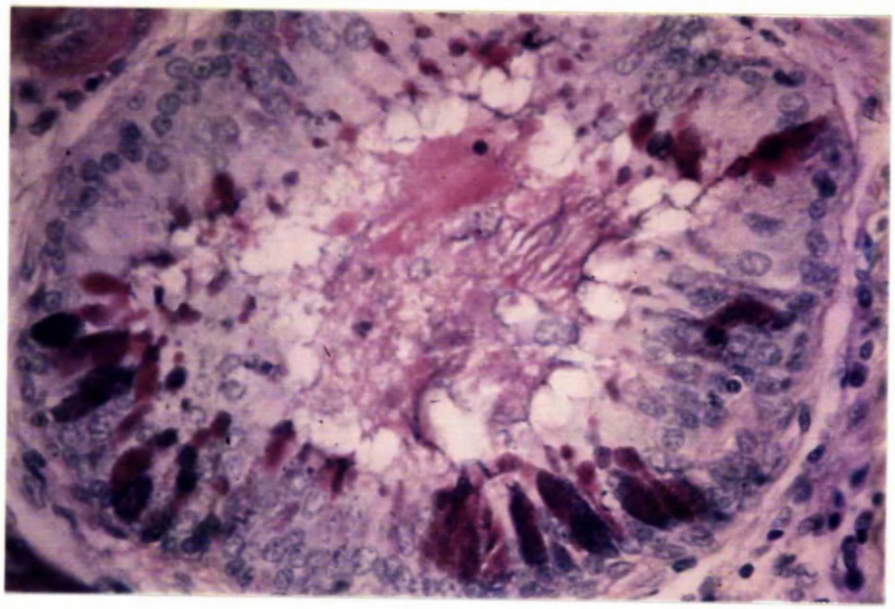


Fig. 50: Chronic bronchitis: Neuraminidase digestion followed by staining with alcian blue-periodic acid Schiff technique. There is a considerable loss of alcianophilia in this mucous gland acinus and very few acinar cells are staining with alcian compared to Fig. 51; this indicates a large proportion of neuraminidase -sensitive sialomucins.

(Neuraminidase, AB-PAS X 700)

Fig. 51: Chronic bronchitis: Control section stained by the alcian blue-periodic acid Schiff technique.

(AB-PAS X 700)



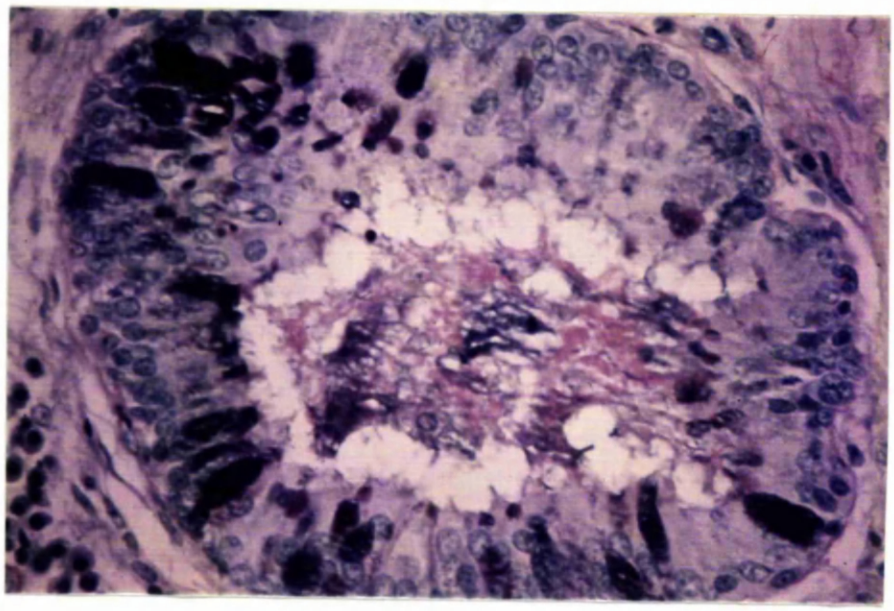
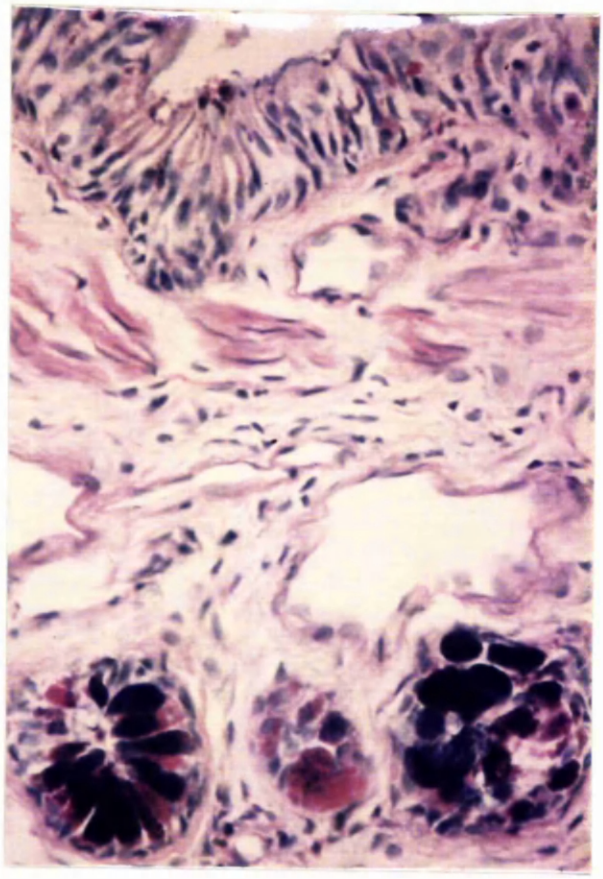


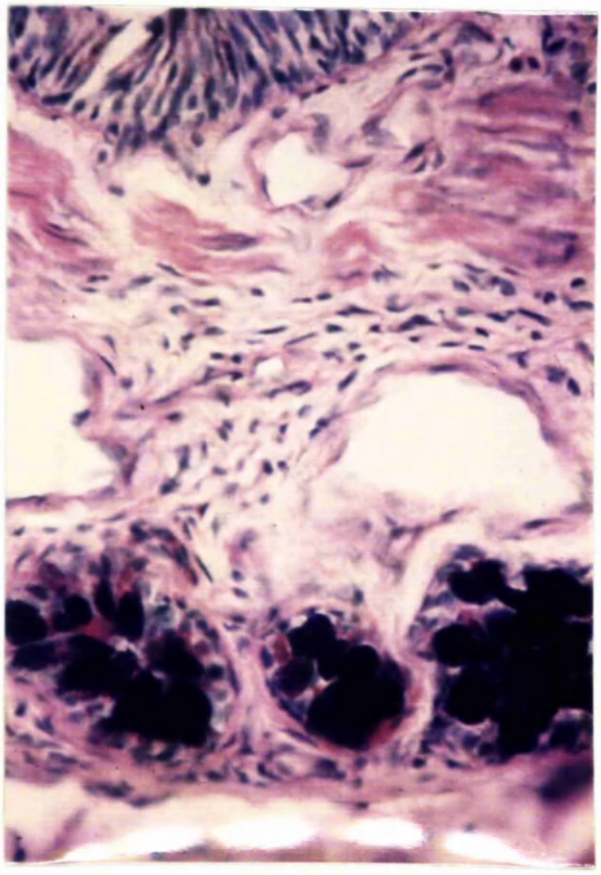
Fig. 52: Chronic bronchitis: Acid hydrolysis followed by staining with alcian blue-periodic acid Schiff technique. The three mucous gland acini have significantly fewer alcianophilic cells, and the attenuated epithelial goblet cells are considerably paler than in Fig. 53. This indicates a large proportion of neuraminidase-resistant sialomucins.

(Acid hydrolysis, AB-PAS X 500)

Fig. 53: Chronic bronchitis: Neuraminidase digestion followed by staining with alcian blue-periodic acid Schiff technique.

(Neuraminidase, AB-PAS X 500)





Figs. 54-59: Normal bronchus: the effect of increasing concentrations of magnesium chloride on epithelium from normal bronchus. Sialomucins are not stained at or above 0·1M $MgCl_2$. Most sulphated muco-substances stain strongly at 0·2M $MgCl_2$. The various sulphomucins lose alcianophilia at different levels with increasing $MgCl_2$ concentrations, some persisting selectively at 1·0M $MgCl_2$.

(Alcian blue-$MgCl_2$ X 350)

Fig.54-0.1M

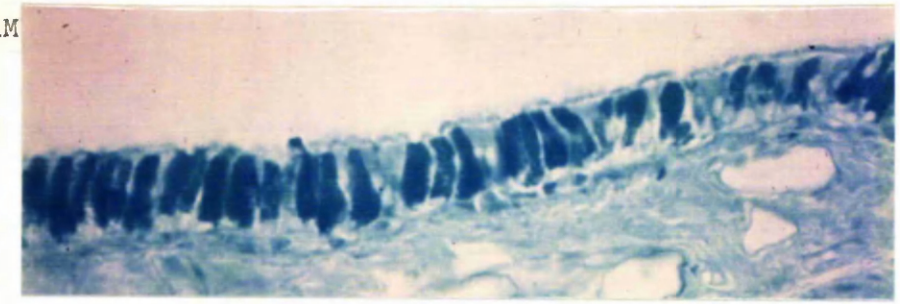


Fig.55-0.2M

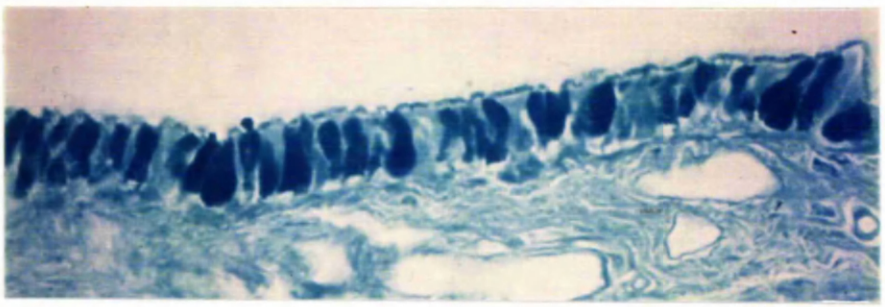


Fig.56-0.5M

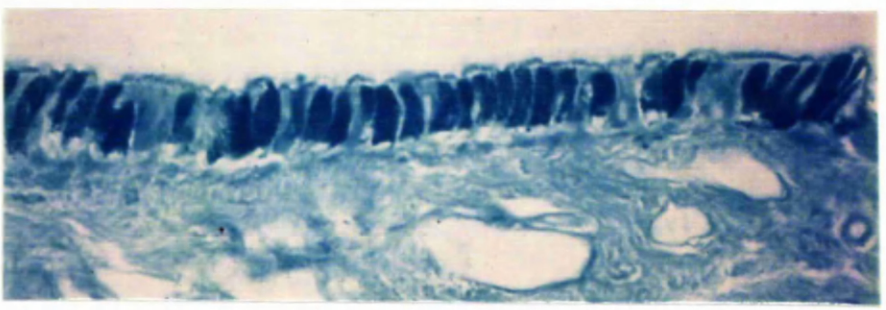


Fig.57-0.6M

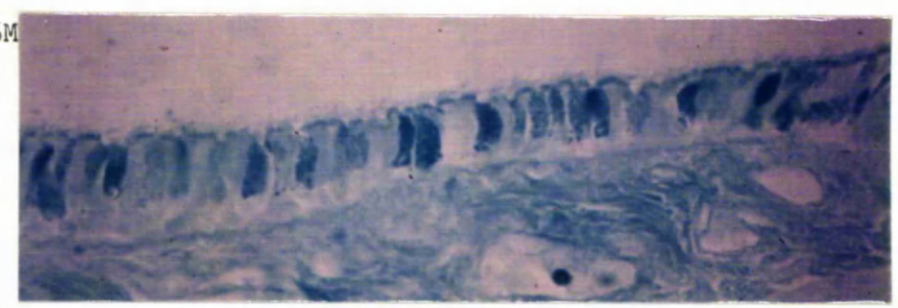


Fig.58-0.8M

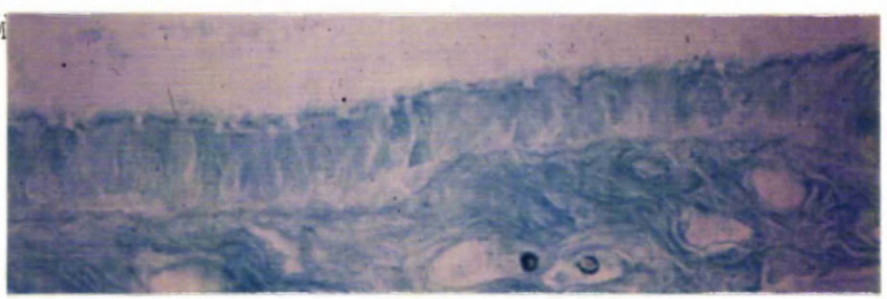
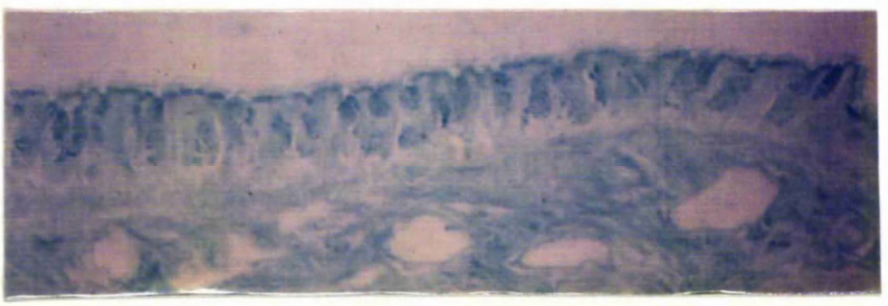


Fig.59-1.0M



Figs. 60-63: Normal bronchus: the effects of increasing concentrations of magnesium chloride on mucous gland from normal bronchus. Sialomucins are not stained at or above 0·1M $MgCl_2$. Most sulphated mucosubstances stain strongly at 0·2M $MgCl_2$. The various sialomucins lose alcianophilia at different levels with increasing $MgCl_2$ concentration, some persisting selectively at 1·0M $MgCl_2$.

(Alcian blue- $MgCl_2$ X 300)

Fig.60-0.1M

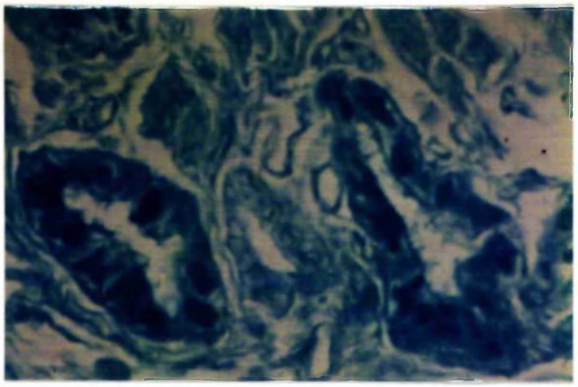


Fig.61-0.5M

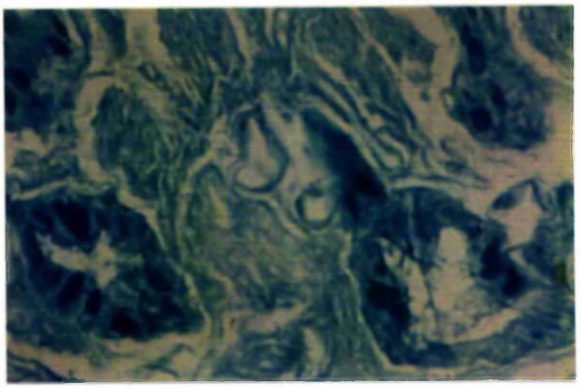


Fig.62-0.6M

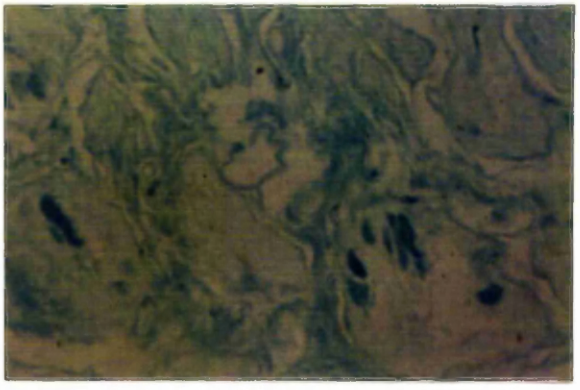


Fig.63-0.8M

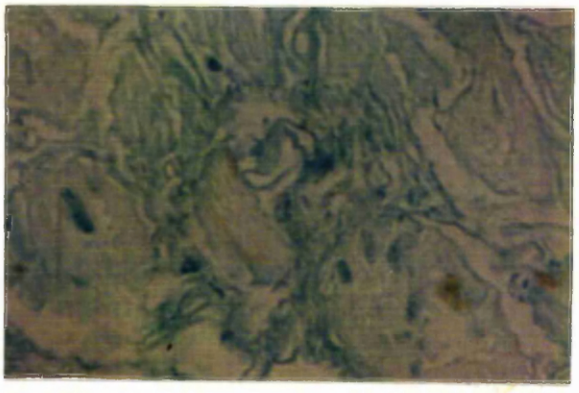


Fig. 64-65: Chronic bronchitis: the effect of increasing concentrations of magnesium chloride on bronchial epithelium and mucous glands in chronic bronchitis. The epithelial goblet cells have faint alcianophilia at 0·1M magnesium chloride concentrations, but this is completely absent at 0·5M magnesium chloride concentrations. The mucous glands, by contrast, have appreciable alcianophilia persisting to 0·5M magnesium chloride concentrations, indicating persistence of sulphomucins. Compare with Figs. 66 and 67.

(Alcian blue-$MgCl_2$ X 250)

Fig.64-0.1M

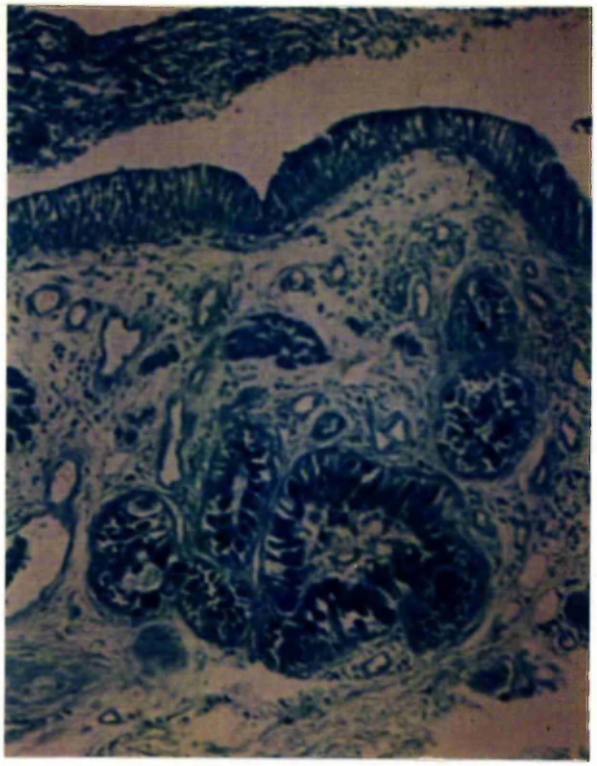
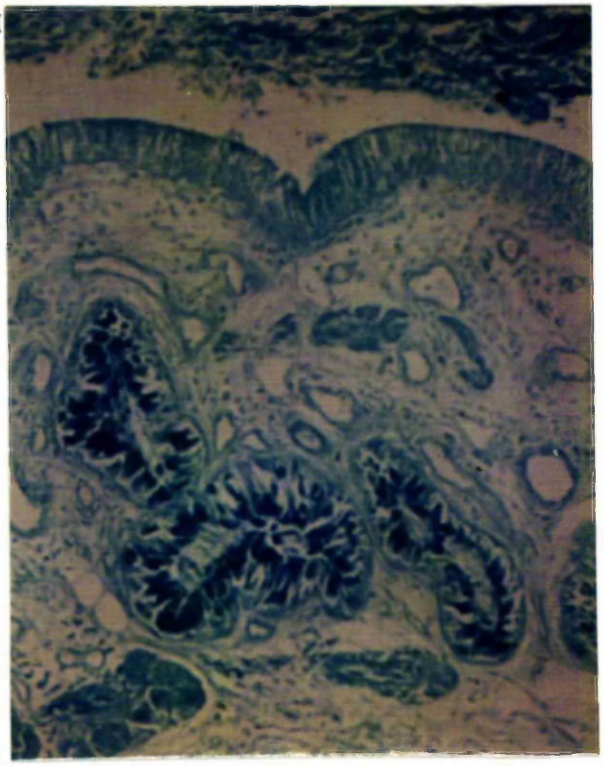


Fig.65-0.5M



Figs. 66-67: Chronic bronchitis: the effect of increasing concentrations of magnesium chloride on bronchial epithelium and mucous glands in chronic bronchitis. Alcianophilia persists in the mucous glands at 0·6M magnesium chloride concentrations but is virtually absent by 0·8M concentrations. Compare with Figs. 64 and 65.

(Alcian blue-$MgCl_2$ X 250)

Fig.66-0.6M

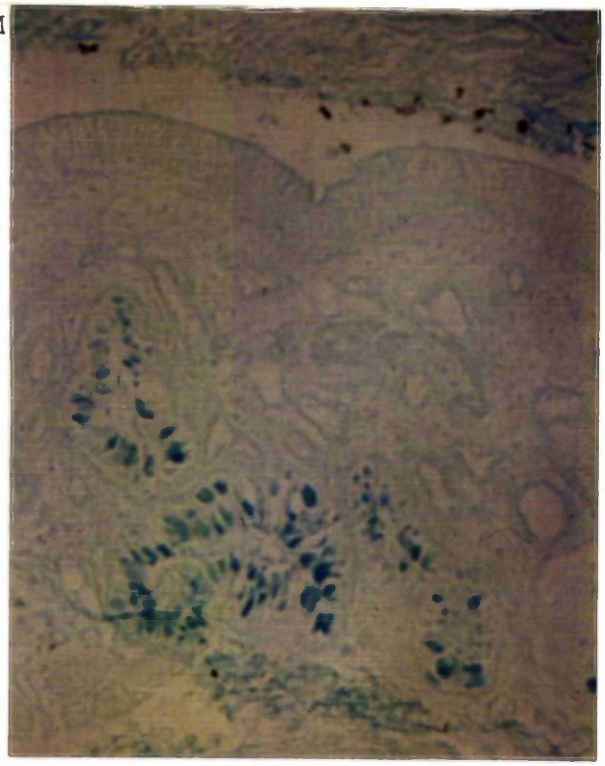


Fig.67-0.8M

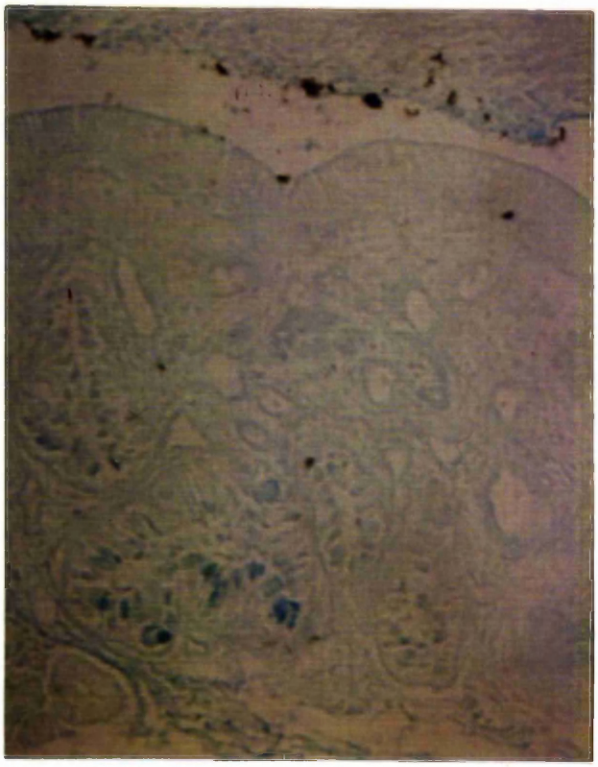
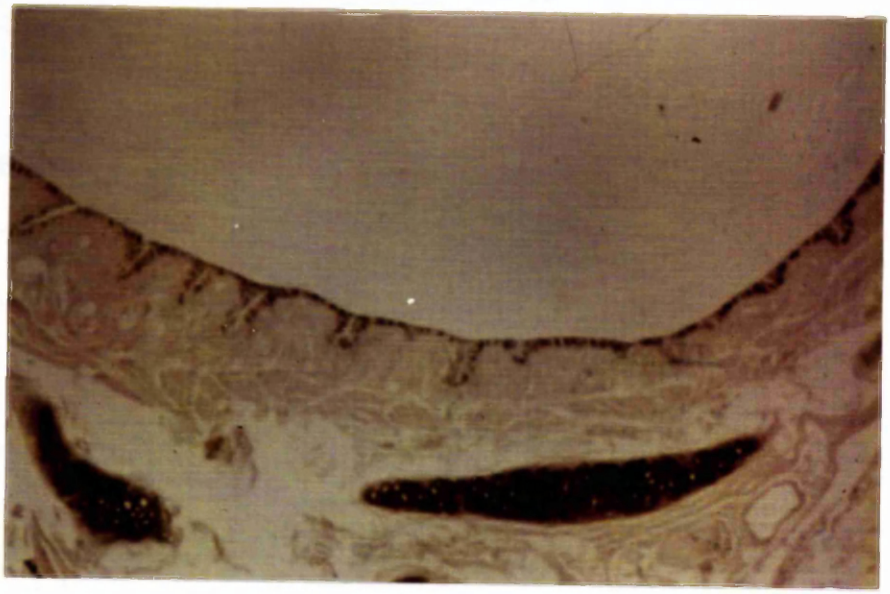


Fig. 68: Normal bronchus: Section of normal bronchus stained by the high iron diamine (HID) technique. The epithelium has many dark staining goblet cells indicating the presence of sulphomucin.

(HID X 110)

Fig. 69: Normal bronchus: Detail of Fig. 68. Numerous dark staining goblet cells are visible in the epithelium.

(HID X 250)



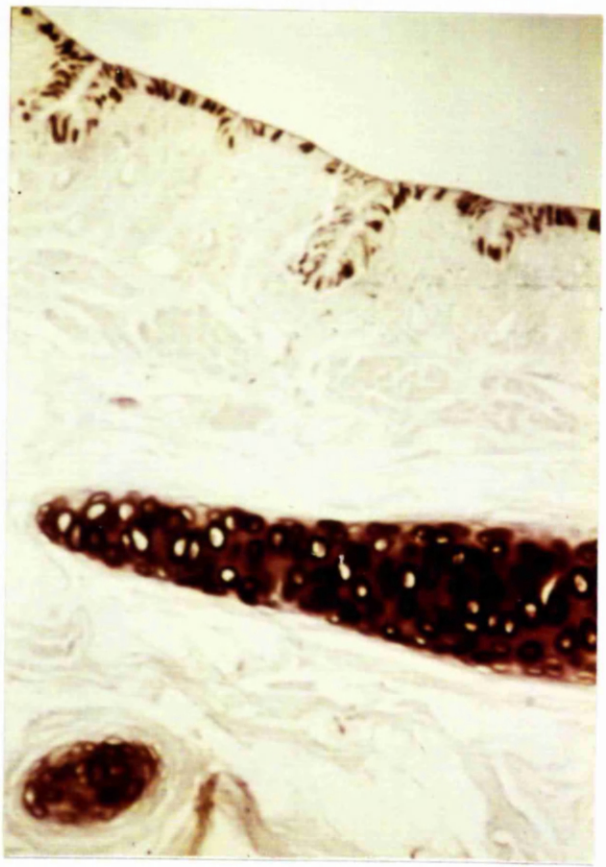
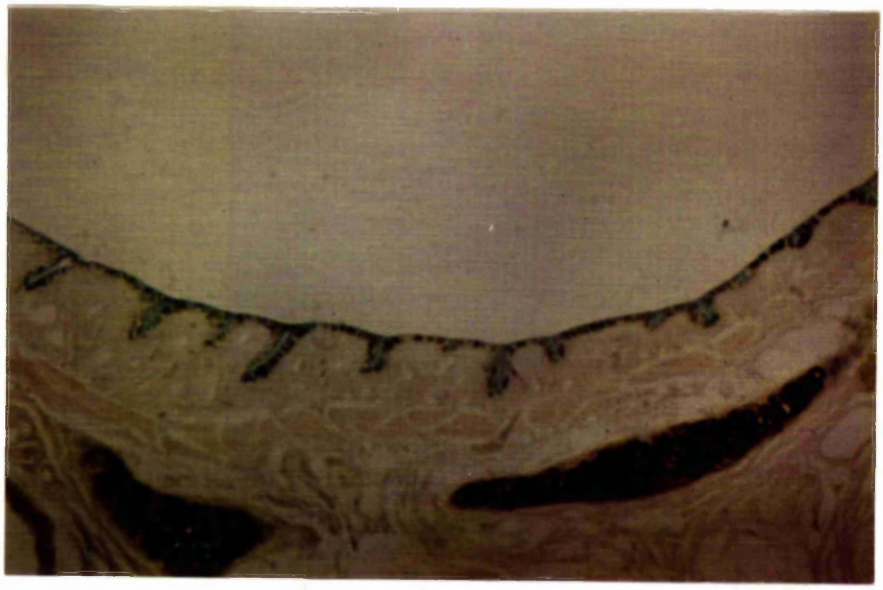


Fig. 70: Normal bronchus: Section of normal bronchus stained by the high iron diamine (HID) technique and counter-stained with alcian blue (AB). This is a serial section to Fig. 68. Alcianophilic goblet cells are rare, indicating little sialomucin in the normal epithelium.

(HID -AB X 110)

Fig. 71: Normal bronchus: Detail of Fig. 70. There is faint alcianophilic staining of cilia and a few of the goblet cells.

(HID -AB X 250)



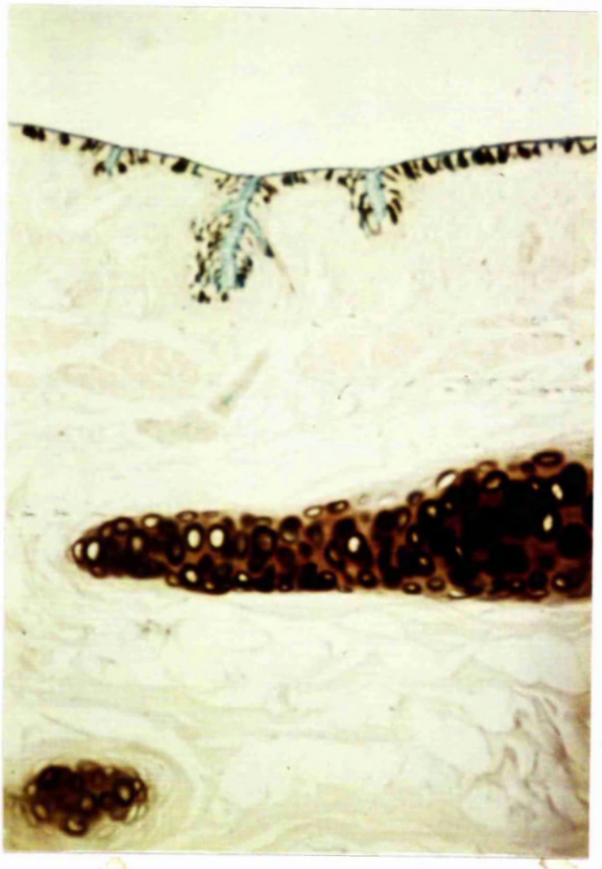
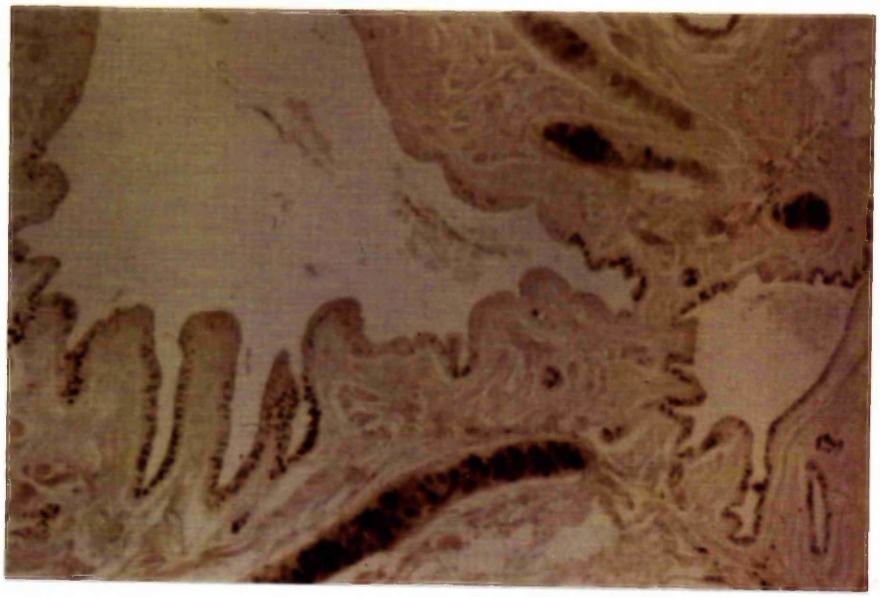


Fig. 72: Chronic bronchitis: Section of bronchus stained by the low iron diamine (LID) technique. Sulphomucins and many sialomucins are stained black. The epithelium has a patchy irregular staining of goblet cells (compare with Fig. 73).

(LID X 110)

Fig. 73: Chronic bronchitis: Section of bronchus stained by the high iron diamine (HID) technique. Sulphomucins are stained black while leaving nonsulphated acid muco-substances unstained. The epithelium in the larger bronchus is unstained (compare with Fig. 72). There is some focal staining of goblet cells in the smaller bronchus, and the mucous glands retain much of their black coloration.

(HID X 110)



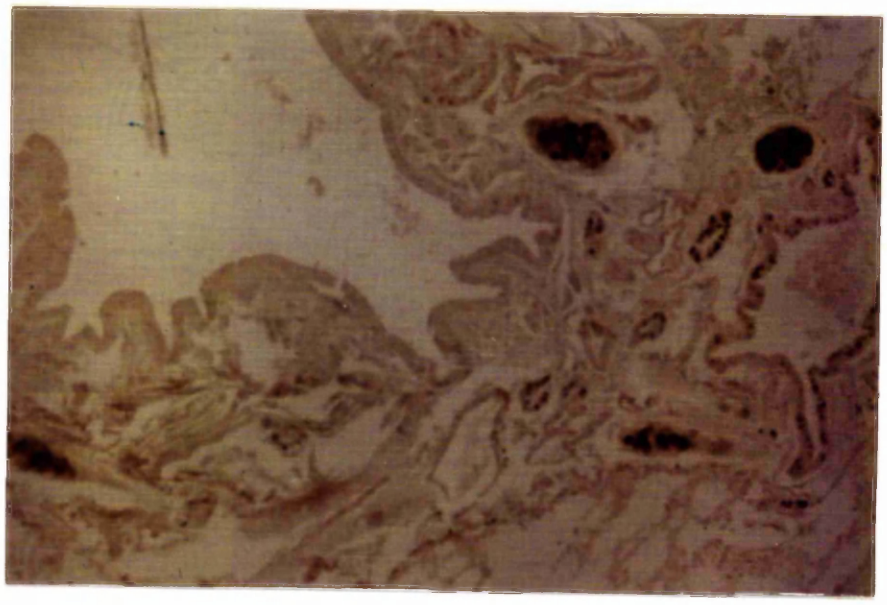
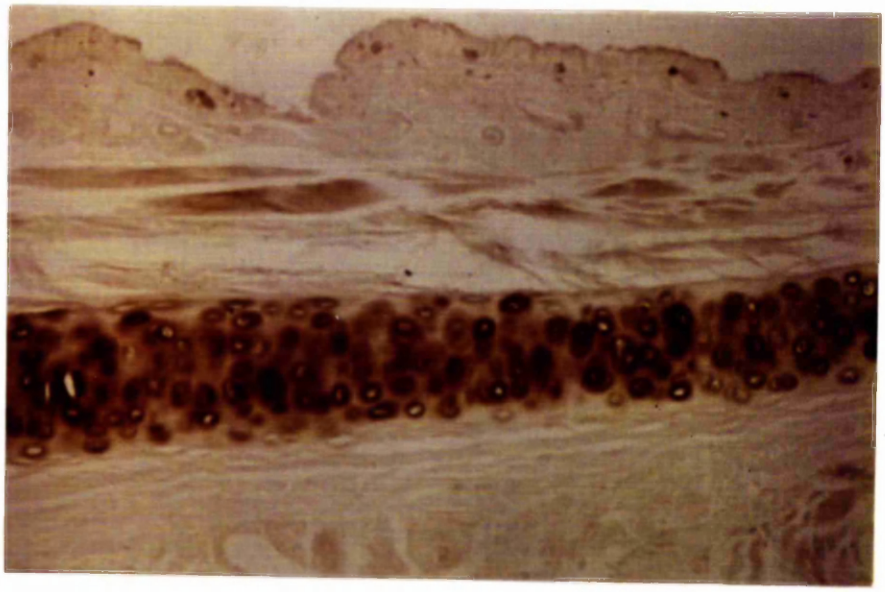


Fig. 74: Chronic bronchitis: detail of bronchial epithelium stained by the high iron diamine (HID) technique, illustrating the marked reduction in dark-staining sulphomucin in the goblet cells. Compare with Fig. 69.

(HID X 250)

Fig. 75: Chronic bronchitis: detail of bronchial epithelium stained by the high iron diamine (HID) technique illustrating the marked reduction in dark staining sulphomucin in the goblet cells. In this field there are a few small foci of goblet cells containing dark-staining sulphomucin (open arrows). Compare with Fig. 69.

(HID X 250)



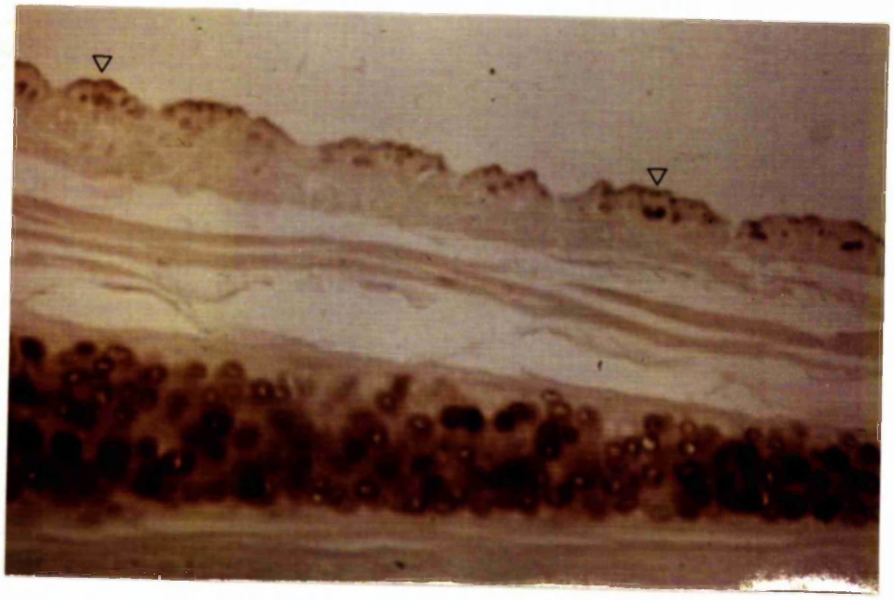


Fig. 76: Chronic bronchitis: detail of bronchial epithelium stained by the high iron diamine (HID) technique and counterstained with alcian blue (AB). There is an absence of dark staining, sulphomucin-containing goblet cells and large numbers of attenuated alcianophilic sialomucin - containing goblet cells. Compare with Fig. 71.

(HID-AB X 250)

S T A I N I N G T E C H N I Q U E S

I. STAINS FOR NEUTRAL MUCOSUBSTANCES

1. Periodic acid Schiff (PAS) Technique

1. Bring section to water
2. Rinse in 70 per cent alcohol - 2 minutes
3. Place in solution A - 15 minutes
4. Rinse in 70 per cent alcohol - 2 minutes
5. Place in solution B - 10 minutes
6. Rinse in 70 per cent alcohol - 2 minutes
7. Wash in water until free of alcohol.
8. Place in solution C - 30 minutes
9. Wash in water to intensify - 5 minutes
10. Stair in haematoxylin and blue - $1\frac{1}{2}$ minutes
11. Wash and blue in Scotts Tap Water Substitute
12. Dehydrate, clean and mount.

Histochemical Result

P.A.S. positive mucosubstance stained a deep red (magenta)

Solution A	Alcohol Periodic acid	
	Periodic acid	0.4 g (50% W/V; 1cc contains 0.5g)
	Distilled water	10 ml
	M/5 Sodium acetate buffer	5 ml
	Absolute ethyl alcohol	35 ml
Solution B	Acid Reducing Rinse	1 g
	Potassium iodide	1 g
	Sodium thiosulphate	1 g
	Distilled water	20 ml
	Absolute ethyl alcohol	30 ml
	2N HCl	0.5 ml
Solution C	Fuchsin sulphite (Schiffs Reagent).	
	Dissolve 2 g of basic fuchsin in 400 ml boiling water.	

Cool to 50°C and filter. Add to the filtrate 10 ml. of 2N HCl and 4 g potassium metabisulphite. Stopper and leave in a cool place overnight. Add 1 g of decolorising charcoal and filter promptly. Add up to 10 ml or more of 2N HCl in small amounts until the mixture, when allowed to dry in a thin film on a slide, does not become pink. This solution should be kept in a dark well-stoppered bottle in a dark cupboard. It will keep for two months.

All three solutions are kept in a refrigerator in dark bottles.

2. Diastase Digestion

1. Bring two serial sections to water
2. Rinse in distilled water
3. Digest one slide on preheated diastase solution at 37°C for one hour.
4. Wash in water for 5 minutes.
5. Stain both sections with periodic acid Schiff.

Histochemical Result

Diastase digestion selectively eliminates PAS staining attributable to glycogen.

Enzyme Solution

Dissolve 100 mg of diastase (Sigma Chemicals, London) in 100 ml of pH 6.0 buffer and use immediately.

The buffer is made up as follows:

Na Cl	8 g
$Na_2 HPO_4$	282 mg
$HaH_2 PO_4$	1.97 g
Double Distilled water	1000 cc

II. STAINS FOR ACID AND NEUTRAL MUCOSUBSTANCES

1. Alcian blue-periodic acid Schiff (Mowry, 1956)

1. Take section to water
2. Stain in filtered alcian blue(1 per cent alcian blue in 3 per cent

acetic acid) for 30 minutes.

3. Wash in tap water for 2 minutes
4. Rinse in distilled water
5. Stain in 0.5 per cent periodic acid for 10 minutes
6. Wash in tap water for 5 minutes
7. Rinse in 70 per cent alcohol
8. Stain in solution A for 10-15 minutes
9. Rinse in 70 per cent alcohol
10. Stain in solution B for 5-10 minutes
11. Rinse in 70 per cent alcohol
12. Wash in water until clear of alcohol
13. Stain in solution C for 30 minutes
14. Wash in water to intensify for 5 minutes
15. Stain in haematoxylin for 1½ minutes
16. Wash in Scott's tap water substitute for 1-3 minutes
17. Wash in water
18. Dehydrate, clear through graded alcohols, and mount

Histochemical Result

Acid mucosubstance - blue

Neutral mucosubstance - red

III. STAINS FOR SIALOMUCINS

1. Acid Hydrolysis Technique (Lamb and Reid, 1969)

1. Bring two serial sections to water
2. Place one section in Coplin jar containing 0.1N sulphuric acid at 80°C for one hour.
3. Rinse both sections and stain with combined AB-PAS method

Histochemical Result

Comparison of serial sections reveals loss of staining of all sialic acid groups with resultant loss of alcian blue alcianophilia. The increase in PAS-staining material in the acid hydrolysis section thus represents sites of sialomucin. The remaining alcian blue basophilia

is due to other acid epithelial mucosubstances i.e. sulphomucins.

2. Neuraminidase Digestion (McCarthy and Reid, 1964)

1. Bring two serial sections to water and dry.
2. Flood one section with neuraminidase enzyme solution, cover and incubate at 37°C overnight. Flood control section with 4 per cent calcium chloride, cover and incubate overnight.
3. Wash carefully in distilled water
4. Stain both sections by the combined AB-PAS method.

Histochemical Result

Neuraminidase reacts with neuraminidase-sensitive sialomucins to eliminate metachromasia and alcian blue affinity. Comparison of control and test sections reveals the removal of sensitive sialomucins where there is a colour change from blue to red with AB-PAS stain or from purple to brown with High iron diamine-alcian blue (H.I.D.-A.B.)

Enzyme solution

Neuraminidase (Wellcome Research Laboratories, Beckenham, Kent) (sialidase, receptor-destroying enzyme, R.D.E.) is a filtrate from Vibrio cholerae; stored in 25 ml bottle at 4°C until use. The working solution is composed of eight parts of enzyme preparation to one part of 4 per cent calcium chloride solution.

IV. STAINS FOR SULPHOMUCINS

1. Alcian blue (pH 2.5) Technique (Spicer, Horn and Leppi, 1967)

1. Bring sections to water
2. Stain in alcian blue (1 per cent alcian blue in 3 per cent acetic acid) for 30 minutes
3. Wash in running water for 5 minutes
4. Dehydrate in alcohol, clear in xylene and mount.

Histochemical Result

Sialomucins, hyaluronic acid and weakly acid sulphated mucosubstances stain dark blue.

2. Alcian blue (pH 1.0) Technique (Spicer, Horn and Leppi, 1967)

1. Bring section to water
2. Stain in alcian blue (1 per cent alcian blue in 0.1N hydrochloric acid - pH 1.0) for 3 minutes.
3. Blot dry with filter paper without rinsing.
4. Dehydrate in two changes of absolute alcohol and one of equal parts absolute alchohol and xylene, clear in xylene.

Histochemical Result

Sulphated mucosubstances are selectively stained.

3. Alcian blue with graded increases in Magnesium Chloride

1. Bring sections to water
2. Stain in 0.1 per cent alcian blue in 0.05M sodium acetate buffer at pH 5.7 with $MgCl_2$ added to a level of 0.1M, 0.2M, 0.5M, 0.6M, 0.8M, 0.9M and 1.0M for 30 minutes.
3. Wash in water for 5 minutes.
4. Quickly dehydrate up the graded alcohols to xylol and mount

Histochemical Result

0.1M $MgCl_2$ eliminates staining of hyaluronic acid, sialomucins and some weakly-acidic sulphomucins

0.2M $MgCl_2$ gives strong and selective staining of most sulphated mucosubstances, including those metachromatic with azure A at pH 0.5

Various sulphated mucosubstances lose alcianophilia at different levels with increasing $MgCl_2$ concentration, some (including some epithelia) persist to 1.0M $MgCl_2$

4. High-Iron Diamine Technique (Spicer, Horn and Leppi, 1967)

1. Bring section to water
2. Stain in high-iron diamine stock solution at room temperature for 24 hours
3. Rinse quickly in water
4. Dehydrate, clear and mount.

Histochemical Result

Sulphated mucosubstances are coloured brown-black

Diamine solution

Dissolve 120 mg of N,N-dimethyl-M-phenylene-diamine-$(HCl)_2$ (Eastman Kodak Co., Liverpool) and 20 mg of N,N-dimethyl-p-phenylenediamine-HCl (Sigma Chemicals, London) simultaneously in 50 ml of distilled water. When the reagents are dissolved, pour this solution immediately into a Coplin jar containing 0.9 ml of standard ferric chloride solution (60 per cent W/V) and 2-3 ml HCl. Thus 50 ml of staining solution contains 180mg of Fe^{+++} The pH of the high iron diamine solution is approximately 1.7

5. High Iron Diamine-Alcian blue (Spicer, 1965)

    1. Bring solution to water
    2. Stain in fresh diamine solution at room temperature for 24 hours
    3. Rinse quickly in water
    4. Stain in 1 per cent alcian blue in 3 per cent acetic acid (pH 2.5) for 30 minutes
    5. Dehydrate through 95 per cent and absolute alcohol, clear and mount

Histochemical Result

Most sulphated mucosubstances are coloured purple-black; acid mucosaccharides lacking sulphate esters (i.e. hyaluronic acid and sialomucins) are unstained by the diamine. The post-staining for 30 minutes in 1 per cent alcian blue in 3 per cent acetic acid colours sialomucins and hyaluronic acid blue.

6. Low-Iron Diamine-Alcian blue (Spicer, 1965)

    1. Bring section to water
    2. Stain in fresh diamine solution at room temperature for 24 hours
    3. Rinse quickly in water
    4. Stain in 1 per cent alcian blue in 3 per cent acetic acid (pH 2.5) for 30 minutes
    5. Dehydrate, through 95 per cent and absolute alcohol, clear and mount.

Histochemical Result

Low-iron diamine negative, non sulphated acid mucosubstances are blue; low iron diamine reactive, non-sulphated and sulphated acid mucosubstances are black.

Diamine solution

Dissolve 30 mg of M-diamine (Eastman Kodak Co., Liverpool) and 5 mg of p-diamine (Sigma Chemicals, London) simultaneously in distilled water and pour immediately into a Coplin jar containing 0.33 ml of ferric chloride solution (60 per cent W/V) and 2-3 ml of HCl.

V. STAINS FOR HYALURONIC ACID

1. Hyaluronidase Digestion (Spicer, Leppi and Steward, 1965)

1. Bring two serial sections to water
2. Rinse in distilled water
3. Incubate one section with hyaluronidase at 37°C for 2-6 hours. Incubate the other section in buffer solution at 37°C for 2-6 hours.
4. Wash in running water for 5 minutes
5. Stain with alcian blue pH 1.0 or 2.5
6. Dehydrate, clear and mount.

Histochemical Result

Basophilia is eliminated by testicular hyaluronidase indicating the presence of hyaluronic acid.

Enzyme solution

Prepare a 0.05 per cent of testicular hyaluronidase (Sigma Chemicals, London) in a pH 5.5 buffer (94 ml of 0.1M KH_2PO_4 and 6 ml of 0.1M Na_2HPO_4).

AN ELECTRON MICROSCOPICAL STUDY OF THE CANINE BRONCHUS

WITH SPECIAL REFERENCE TO CHRONIC BRONCHITIS

INTRODUCTION AND REVIEW OF THE LITERATURE

Early descriptions of the ultrastructure of the tracheobronchial tree were based on studies of small laboratory animals. The first exhaustive description of the tracheobronchial epithelium was by Rhodin and Dalhamn (1956) who described the tracheal epithelium of the rat. Previous studies had examined the respiratory epithelium of the mouse (Karrer 1954; Harford, Hamlin and Parker, 1955) and rat (Policard, Collet and Giltaire-Ralyte, 1955). However, these previous studies had been concerned primarily with the effects of viral infections on airway epithelium (Karrer, 1954; Harford, Hamlin and Parker, 1955) or were limited to a consideration of the bronchiolar epithelium (Karrer 1954; Policard, Collet and Giltair-Ralyte, 1955)

Rhodin and Dalhamn (1956) examined the normal tracheal epithelium of rats as part of an experiment involving exposure to sulphur dioxide. Four kinds of cell were identified in the tracheal epithelium: ciliated cells, goblet cells, "brush" cells and basal cells. Both ciliated cells and goblet cells had "filiform" projections on their surfaces; these differed from the "brush" on the surface of the "brush" cells, which was considered to resemble in dimensions and ultrastructure the brush border of the intestinal epithelium and thus function as an absorptive structure. (Rhodin and Dalhamn, 1956). These workers considered that basal cells represented lymphocytes or white blood cells; further, they could find no evidence of differentiation of any of the four epithelial cell types into other forms.

Later, after a comparison of the tracheal epithelium in man and the rat, Rhodin (1959) thought that high power examination of the "filiform" projections was puzzling and suggested that they might represent differentiating ciliated cells. Sections of the human trachea contained "brush cells", all of which contained structures resembling basal corpuscles and single root fibres, suggesting that cilia were developing (Rhodin, 1959).

Rhodin (1959) now modified his view and considered that the basal cells might be the precursors of the other epithelial cells.

Studies of the normal bronchopulmonary tissue of the pig by Baskerville (1970A, 1970B) also revealed four types of cells in the bronchial epithelium: ciliated cells which had cilia and microvilli along their apical borders, goblet cells which also possessed apical microvilli, basal cells and intermediate cells. Baskerville (1970A) described brush cells in the bronchioles of the pig, similar to the brush cells described earlier by Watson and Brinkman (1964); however these were not observed in the bronchi.

Watson and Brinkman (1964) have examined the bronchial epithelium from normal subjects and subjects with chronic bronchitis. They described four types of epithelial cells in the human bronchus: ciliated cells, brush cells goblet cells and basal cells. Watson and Brinkman (1964) described the presence of "pellicular structures" (microvilli) interspersed between the cilia along the apical border. The epithelial cells in the bronchi of subjects with chronic bronchitis had no absolute differences from those of normal subjects; rather, the differences were a matter of degree. Watson and Brinkman (1964) listed five features found in the epithelium of subjects with chronic bronchitis: a marked reduction in the number of "pellicular structures" (microvilli), altered mitochondria with a reduction in cristae, numerous secretion droplets in the apical cytoplasm of ciliated cells, an increase in the number and size of intercellular spaces particularly in the region of the basal cells, and lastly, an increase in the osmiophilic ("myelinic") granules of the ciliated cells. Watson and Brinkman (1964) could find no evidence of a decrease in the number of cilia in the ciliated cells.

Knowledge of the ultrastructure of the canine bronchus is based on the work by Frasca, et al. (1968A, 1968B) who described the bronchial epithelium of normal dogs and dogs that had been exposed to cigarette smoke. Control dogs had a pseudostratified bronchial epithelium composed of five main cell types: ciliated cells, goblet cells, basal cells, migratory cells and "special type" cells (Frasca et al., 1968A). The ciliated cells possessed both cilia and long slender cytoplasmic processes (microvilli) along their apical borders.

The appearance of goblet cells was varied and ranged from the typical flask-shaped cell containing mucous droplets to the immature forms with cytoplasmic processes along the apical border and granules in the apical cytoplasm which resembled the larger mucous granules of the mature goblet cell. The "special type cells" contained characteristic curved rod inclusions, and the "migratory" cells occurred just above or below the basement membrane, and were characterised by numerous large cytoplasmic vacuoles. A single row of ovoid basal cells was seen; mitotic figures in these basal cells were rare.

In dogs which had been subjected to experimental cigarette-smoking for 44 days, there was an apparent increase in the number of goblet cells, a decrease in the number of cilia and a marked increase in the number of cytoplasmic processes on the apical border of the ciliated cells, together with protrusions of apical cytoplasm into the bronchial lumen (Frasca, et al. 1968B). In dogs that had been smoking cigarettes for 420 days there were more striking changes, the most obvious being an increase in the number of epithelial cell layers to produce an epithelium three to six cells deep. Goblet cells and ciliated cells were completely absent, being replaced by poorly differentiated cuboidal and low columnar cells. The apical cytoplasm of these cells was thrown into short stubby projections. Between these cells and the basement membrane were two to five layers of cells; these were obvious basal cells when next to the basement

membrane but were of intermediate form in the higher layers. Cytoplasmic extrusions of the basal cells frequently extended through the basement membrane into the underlying connective tissue.

The lamina propria of the human bronchus was later studied by Brinkman, Brooks and Bryant (1969); their description included a brief examination of the mucous glands. They described an elaborate endoplasmic reticulum and large number of ribosomes in the mucous cells; as the gland developed, the mucous granules moved centrally toward the gland duct whilst the nucleus was pushed against the cell base and eventually became flattened by the accumulating secretions (Brinkman, Brooks and Bryant, 1969)

The findings of these investigations are summarised in Table 20. Because of the variation in nomenclature and species, and the limited number of studies in the dog, it was decided to examine the bronchus from both normal dogs and dogs with chronic bronchitis in an attempt to determine changes occurring at the ultrastructural level due to naturally-occurring chronic bronchitis.

MATERIALS AND METHODS

Portions of bronchial tree were taken from four control dogs and from two dogs which were diagnosed as having chronic bronchitis. The histopathological and electron microscopical techniques have been detailed in the general Materials and Methods section above.

RESULTS

Normal bronchial epithelium

The normal canine bronchial epithelium was pseudo-stratified and composed of at least five cell types. Three of the cell types present in the canine bronchial epithelium can be seen in Fig. (77); these are the ciliated columnar cells with cilia and microvilli along their apical border, goblet stages in varying stages of synthesis and secretion, and basal cells lying immediately above the basement membrane. A fourth type of cell was seen in some of the sections (Fig. 78); this was characterized by the absence of cilia along its apical border and the presence of numerous well-developed cytoplasmic processes. Lastly, intermediate-type cells could be seen in various stages of differentiation deep in the epithelial layer.

The ciliated cells, goblet cells and non-ciliated cells were all joined to adjacent cells by tight junctions at their luminal surface. In addition, the lateral aspects of these cells had slender cell processes which interdigitated with each other. The intercellular spaces were very narrow and infrequent near the lumen but became much wider around the basal cells (Fig. 77).

The ciliated cells were columnar in outline and extended from the basement membrane to the luminal surface. The basal part of the cell was narrowed and rested on the basement membrane between the basal cells. The supranuclear region in the apical portion of the cell contained numerous mitochondria and, in some cells, very prominent vacuoles (Fig. 77). The cilia were arranged regularly along the apical border and between the cilia were numerous slender cytoplasmic processes (microvilli). In some cases, these microvilli were seen to branch (Fig. 78).

Atypical cilia were a relatively common finding in the control material; they were present in all the dogs examined, including the young dogs. In such cases, in addition to the row of normal cilia, there were cilia which appeared to have multiple sets of axial filaments together with

extra filaments. In these compound cilia, the sets of axial filaments could be seen to be running in different planes. These compound cilia had varying amounts of excess cytoplasm giving a swollen, irregular outline. Sometimes, a large bulging cilium could be seen to be formed from several basal bodies (Fig. 79); in these cases the large mass of cytoplasm projecting into the lumen contained a variety of bizarre arrangements of axial filaments cut transversely and obliquely. A more frequent finding was the presence of multiple sets of filaments lying in the lumen among the normal cilia (Figs. 78, 79). When sectioned transversely, these appeared as multiple complete or incomplete sets of axial filaments surrounded by cytoplasm giving a multiple rosette appearance (Fig. 80).

Goblet cells in the young and adult dogs were observed in various stages of development and discharge. Prior to and during discharge, goblet cells were present in the upper part of the epithelium and were arranged along the luminal surface. The goblet cells were flask-shaped and packed tightly with mucinogen granules; cytoplasm and cytoplasmic organelles such as mitochondria were pushed to the cell periphery (Fig. 81). When the cell had begun to discharge, a thin apical cap of cytoplasm, covered by microvilli, was breached, and the mucinogen granules were discharged singly or in clumps (Fig. 82).

Bronchitic epithelium

In chronic bronchitis, the epithelium had a more ragged apical border; the cilia were reduced in number, and cytoplasmic protrusions were a common feature of many cells (Fig. 83). In some sections, the number of cilia along the apical border often appeared to be reduced from ten to twelve per cell in control epithelium to as few as one or two in epithelium (Fig. 83). Despite the cytoplasmic protrusions, the cell junctions appeared to be intact (Figs. 83,84,85). In addition, the microvilli often appeared to be reduced in number. Atypical cilia were seen in the bronchitic material but these did not appear to be any more common than in the controls.

In chronic bronchitis, the goblet cells appeared to be more numerous;

it was frequently possible to see two goblet cells side by side which was an uncommon feature in control epithelium. In these cases, the goblet cells contained increased numbers of mitochrondria (Figs. 83,84,85); these were often interspersed between the mucinogen granules (Fig. 84) or formed a peripheral layer around the cell borders (Fig. 85). The goblet cells were not tightly packed with granules before discharge; very often it appeared that small numbers of loosely packed granules were discharged along with cytoplasm and organelles, (Fig. 85). Mitochondria could be seen extending up to the luminal surface in considerable numbers (Fig. 85).

No qualitative changes were seen in the other epithelial cells. The non-ciliated cells were not seen more frequently in the bronchitic epithelium. Neither did basal cells appear to be any more numberous. Neutrophils could be seen migrating between the epithelial cells, although cell junctions were seen regularly in the bronchitic epithelium, and appeared to be intact.

Normal mucous glands

The mucous glands in the lamina propria were composed of groups of secretory cells surrounded by a basement membrane. The cells themselves possessed numerous finger-like projections around their edges which formed complex interdigitations with adjacent cells (Figs. 86,87). These secretory cells were surrounded by cytoplasmic processes resting on the basement membrane (Fig. 88); these were processes of myoepithelial cells.

The secretory cells contained large pools of pale, granular material lying free in the cytoplasm (Figs. 86,87). This material was probably glycogen lying in pools in the cytoplasm; the cytoplasm contained abundant rough endoplasmic reticulum arranged in narrow parallel arrays in the cytoplasm (Fig. 87). In addition, these cells had a well-developed Golgi apparatus (Fig. 87); secretory vesicles could usually be seen budding off the apparatus.

Bronchitic mucous glands

The mucous glands in subjects with chronic bronchitis were noticeably enlarged; the secretory cells were packed tightly together so that the cytoplasmic interdigitations were not so prominent (Fig. 89). The cells appeared swollen by large accumulations of material in the cytoplasm between the arrays of endoplasmic reticulum (Fig. 90). The large accumulations of pale staining, granular staining material, encountered in normal mucous glands, and thought to be glycogen, was rarely encountered in the bronchitic mucous glands.

The tight cell junctions around the apices of the acinar cells appeared to be intact in the bronchitic mucous glands (Fig. 89,90) and the apical cytoplasm contained accumulations of secretory granules (Fig. 89). The gland lumen became much larger in the duct system of the gland (Fig. 91); in this region of the gland, the gland consisted of a single layer of columnar cells resting on the basement membrane (Fig. 91). The secretory granules appeared to be less frequent in this region, although the Golgi. apparatus was just as prominent (Fig. 91).

DISCUSSION

The ultrastructural changes occurring in human chronic bronchitis have been described by Watson and Brinkman (1964); theirs was a brief report and limited to the bronchial epithelium. Ultrastructural changes in the bronchial tree of the dog after exposure to cigarette smoke have been described by Frasca et al. (1968B). These workers described a reduction in the number of cilia in the bronchial epithelium of dogs exposed to cigarette smoke, whereas Watson and Brinkman (1964) could find no such reduction in human bronchitic epithelium. In this study, there appeared to be a definite reduction in the number of cilia in a proportion of the cells seen in the bronchitic epithelium. It may be that this reduction is only of a focal nature; the extent of this reduction, together with the possible functional significance, requires further studies, including quantification. Watson and Brinkman (1964) also described a reduction in the number of microvilli, whereas Frasca, et al. (1968B) observed a "marked increase" in the number of microvilli. The material in this study was not quantified, but, overall, there appeared to be some reduction in the number of microvilli. Greenwood and Holland (1972) in a scanning electron microscope study of the respiratory tract, described the distribution of ciliated cells into groups which were interspersed with non-ciliated cells; both types of cells were covered by microvilli on their apical surface.

Recently, Ailsby and Ghadially (1972) noted the presence of atypical or compound cilia in the bronchial epithelium of a man with a bronchial carcinoma. They considered such atypical cilia to be a feature of bronchial epithelium with impaired clearance ability and they speculated that the compound cilia might have been associated with the smoking history of the patient. Compound cilia were not seen commonly in longitudinal sections in the material from young dogs, but they were a not uncommon finding in transverse sections in the material. The significance of these compound cilia is not known; because of their occurrence in young dogs, they may

represent imperfect formation in the routine renewal of ciliated epithelium, and thus be a regular feature of bronchial epithelium. Friedmann and Bird (1971) described compound cilia as a variation of the normal structure and considered that they were due to the precocious regeneration of the cilia. Nevertheless, Friedmann and Bird (1971) considered that compound cilia were more frequent in diseased or inflamed respiratory epithelium. The epithelial goblet cells in the bronchitic epithelium were characterised by their small amounts of secretory granules and their tendency to form cytoplasmic protrusions into the bronchial lumen. Secretory granules were not discharged in a group but appeared to be lost singly. Instead of the cytoplasmic organelles being pushed to the cell periphery as in normal goblet cells, organelles and granules were mixed throughout the cell; in particular large numbers of mitochondria were present in all parts of the goblet cells. The protoplasmic protrusions seen in the apical regions of the goblet cells could indicate poor fixation, although the mitochondria and cell junctions appeared to retain their normal structure. It seems likely that the goblet cells were attempting to increase their rate of discharge into the lumen, and that because of this, the cycle of secretion build-up with subsequent discharge into the lumen was lost.

Most of the ultrastructural studies of the airways have been on small laboratory animals and in nearly all the species studied the emphasis has been on the airway epithelium (Rhodin and Dalhamn, 1956; Watson and Brinkman, 1964; Baskerville, 1970B). Consequently, the mucous glands have not been widely studied; Brinkman, Brooks and Bryant (1969) referred briefly to the mucous glands in their paper, while Meyrick and Reid (1970) have described the structure of these glands in detail. However, the material used in this latter study was all derived from people who had smoked cigarettes; in sixteen of these cases the diagnosis was (bronchial) carcinoma and in the other two it was chronic bronchitis. Consequently, although the paper by Meyrick and Reid (1970) is the most extensive study of the mucous glands, it is impossible to determine which of the features are present in normal

mucous gland and which are the result of cigarette-smoking and chronic bronchitis.

The findings of this study confirm several features described by other workers in epithelium under similar circumstances; the epithelial mucociliary apparatus is deranged by the possible reduction in the number of cilia and the abnormal mode of secretion from the numerous goblet cells. The mucous glands appeared to have an increased rate of metabolism with reduced pools of granular material (which is probably glycogen) and increased amounts of material in the endoplasmic reticulum. These pools of granular material, very common in the control tissue, were stated to be pools of glycogen by Frasca et al. (1968A), although the justification for this is not clear. The overall picture in the bronchial mucous glands in chronic bronchitis was an increase in the synthesis of mucosubstances.

Reference	Species	Site	Cell types observed	Comments
Rhodin and Dalhamn (1956)	Rat	Trachea	Ciliated cell Goblet cell Brush cell Basal cell	Filiform (microvilli) on apical surfaces of goblet cells and ciliated cells.
Rhodin (1959)	Rat	Trachea		Basal cells may be precursors of other cell types
	Man	Trachea	Ciliated cell Goblet (mucous) Serous cell Brush cell	
Watson and Brinkman (1964)	Man	Bronchus	Ciliated cell Brush cell Goblet cell Basal cell	Pellicular structure (microvilli) between cilia
Frasca (1968A)	Dog	Bronchus	Ciliated cell Goblet cell Basal cell Migratory cell Special type cell	Cytoplasmic processes - (microvilli) between cilia
Baskerville (1970AB)	Pig	Bronchus	Ciliated cells Goblet cells Basal cells Intermediate cells	Microvilli among cilia

Table (20): summary of the ultrastructural studies of cell types of the epithelium of the normal tracheobronchial tree in man, rat, dog and the pig.

Fig. 77: Normal bronchus: cross-section of normal bronchial epithelium with the basement membrane (arrowed) and the bronchial lumen (BL) indicated. Three cell types are represented: the ciliated cell (CC), goblet cell (GC) and the basal cell (BC).

(OsO_4 X 6,000)

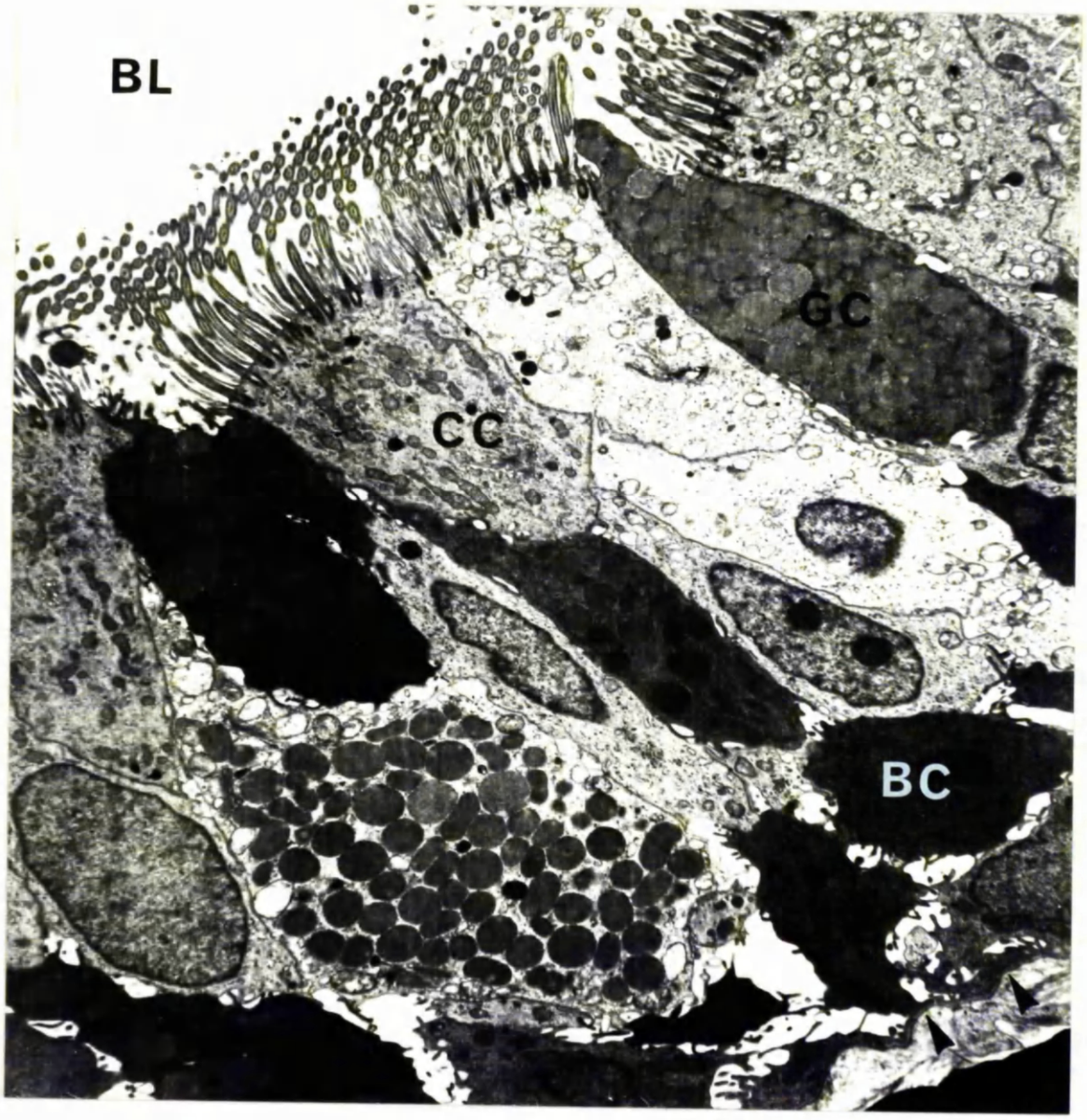

BL
GC
CC
BC

Fig 78: Normal bronchus: normal epithelium illustrating the apical border of three cells. Two of the cells have cilia (C) along their apical border. The middle cell has no cilia along its apical border but has well-developed microvilli (MV). This cell has groups of filaments (arrows) in its cytoplasm which resemble the cilia present in the bronchial lumen.

(OsO_4 X 12,000)

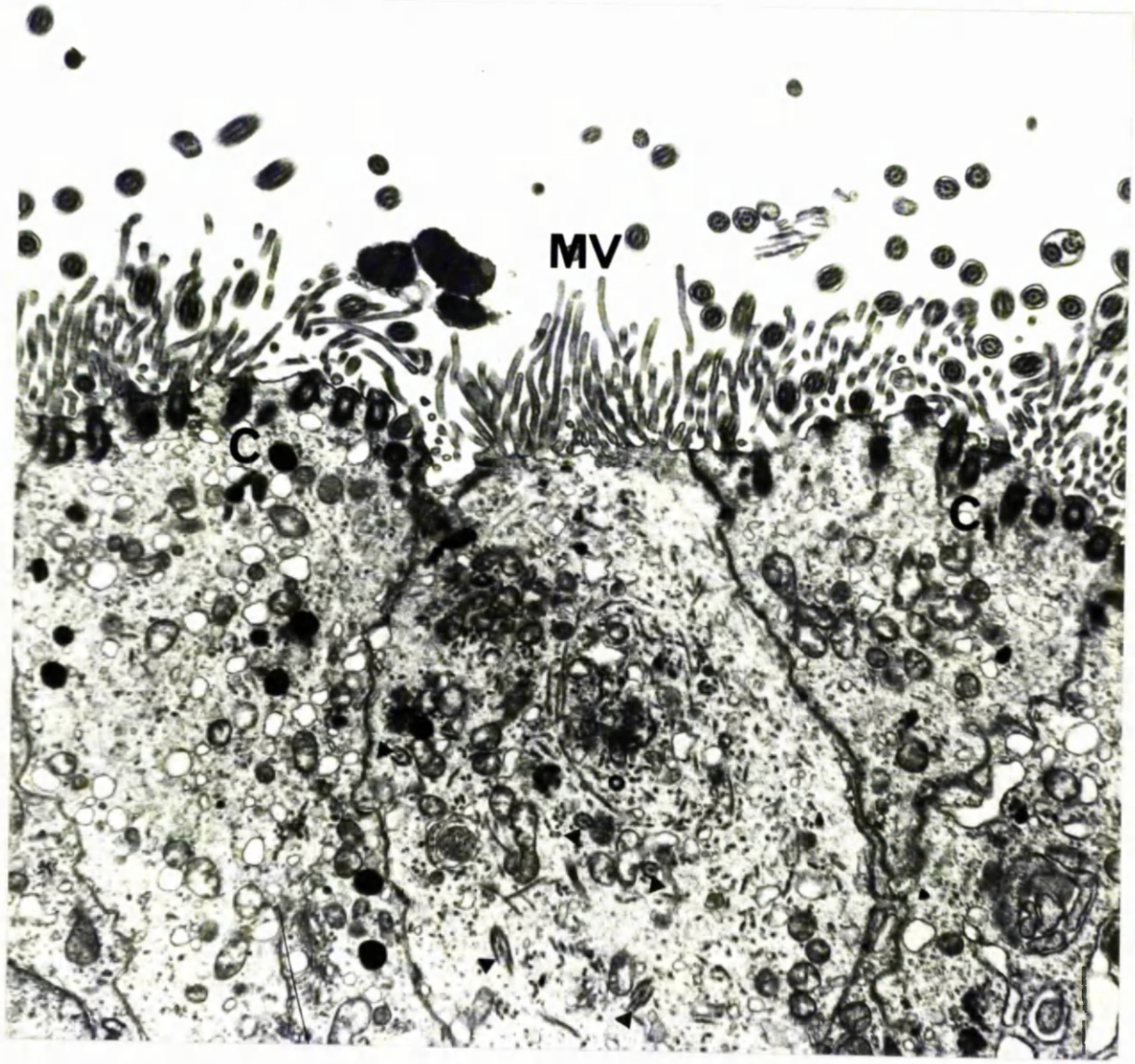
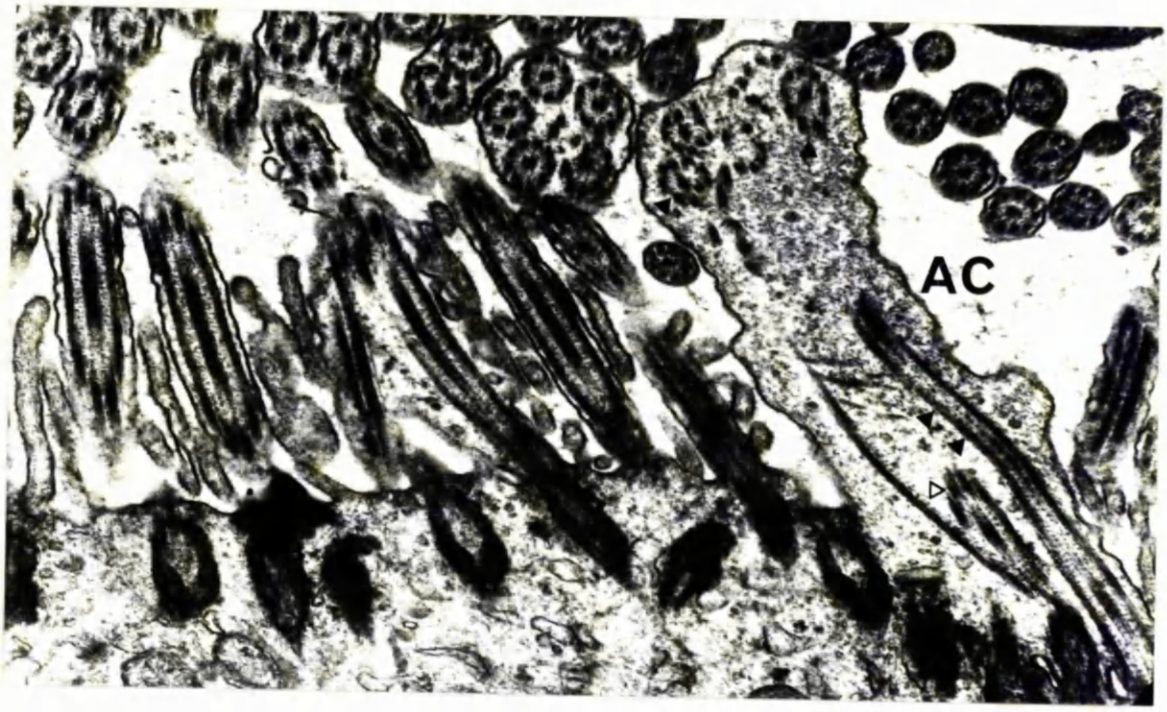

MV
C
C

Fig. 79: Normal bronchus: apical border of a ciliated cell illustrating cilia projecting into the bronchial lumen. There is a large atypical cilia (AC) originating from several basal bodies; it contains multiple sets of filaments cut transversely (single arrow) longitudinally (double arrow) and obliquely (open arrow). The bronchial lumen contains numerous cilia cut transversely; several of these contain multiple sets of filaments.

(OsO_4 X 30,000)

Fig. 80: Normal bronchus: transverse section of an atypical cilia; there are multiple sets of axial filaments and many of the sets are incomplete, lacking the normal "9+2" configuration (arrowed).

(OsO_4 X 60,000)


AC

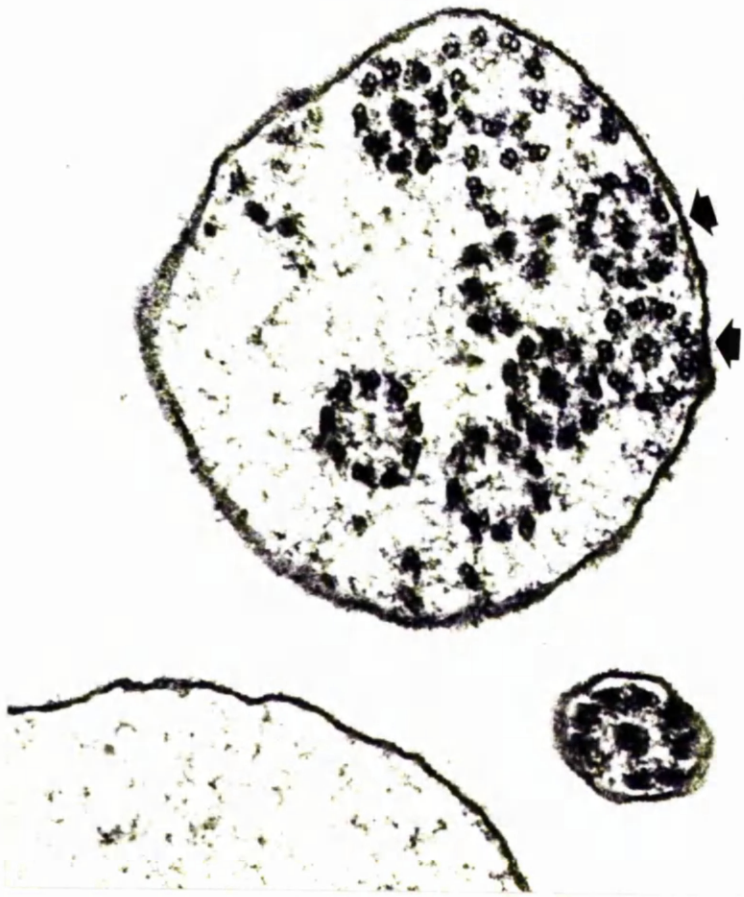
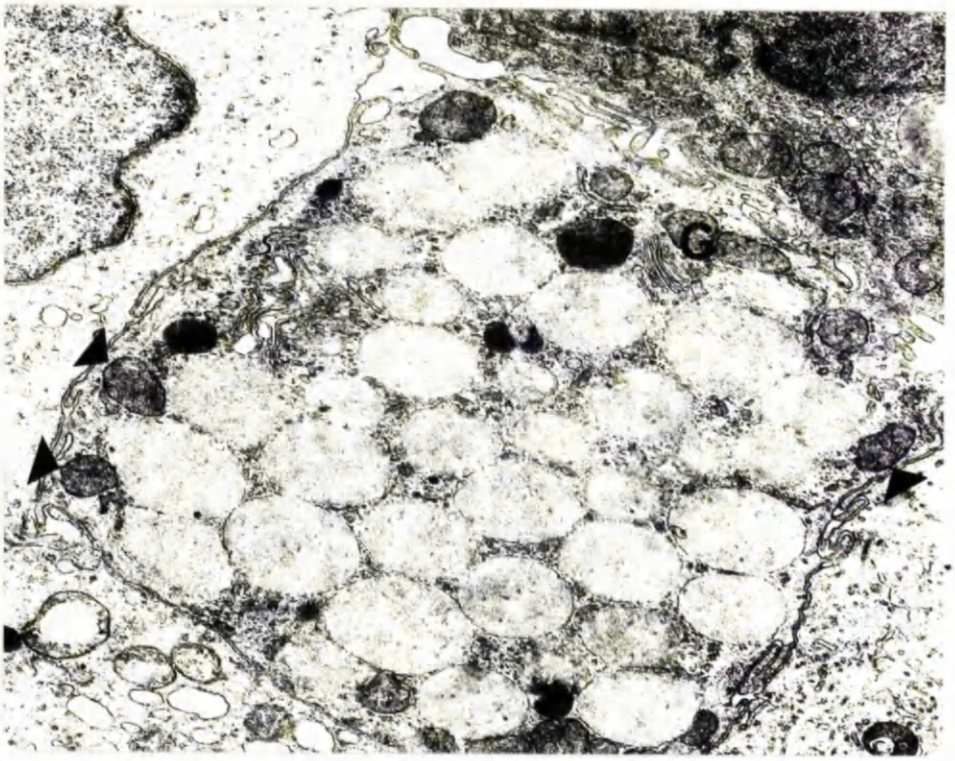


Fig. 81: Normal bronchus: transverse section of goblet cell. The cytoplasm contains numerous large mucinogen granules, while the organelles - mitochondria (arrowed) and Golgi complex (G) - are pushed to the cell periphery.

(OsO_4 X 15,000)

Fig. 82: Normal bronchus: detail of discharging goblet cell (GC) between two ciliated cells (CC). The goblet cell has discharged a large globule composed of mucinogen granules The goblet cell has only a thin rim of cytoplasm around tl neck region and this contains few organelles.

(OsO_4 X 20,000)


G

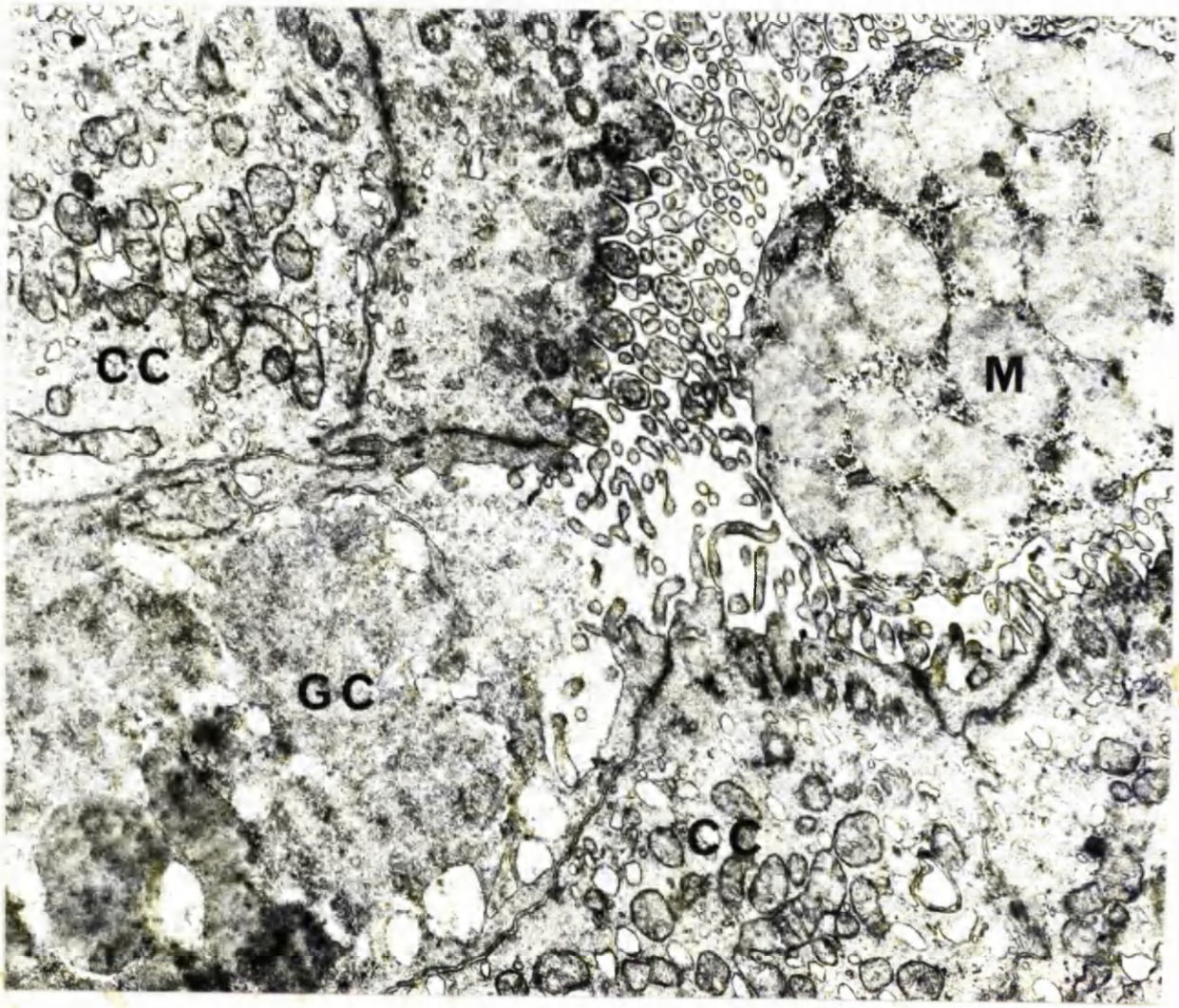

CC
M
GC
CC

Fig. 83: Bronchitic epithelium: section of epithelium containing three goblet cells (G). All three goblet cells contain large numbers of mitochondria and relatively few mucinoger granules (compare with Figs. 77,81). The apical border has numerous cytoplasmic protrusions. The cilia appear to be reduced in number in this section (compare with Fig. 77).

(OsO_4 X 6,000)

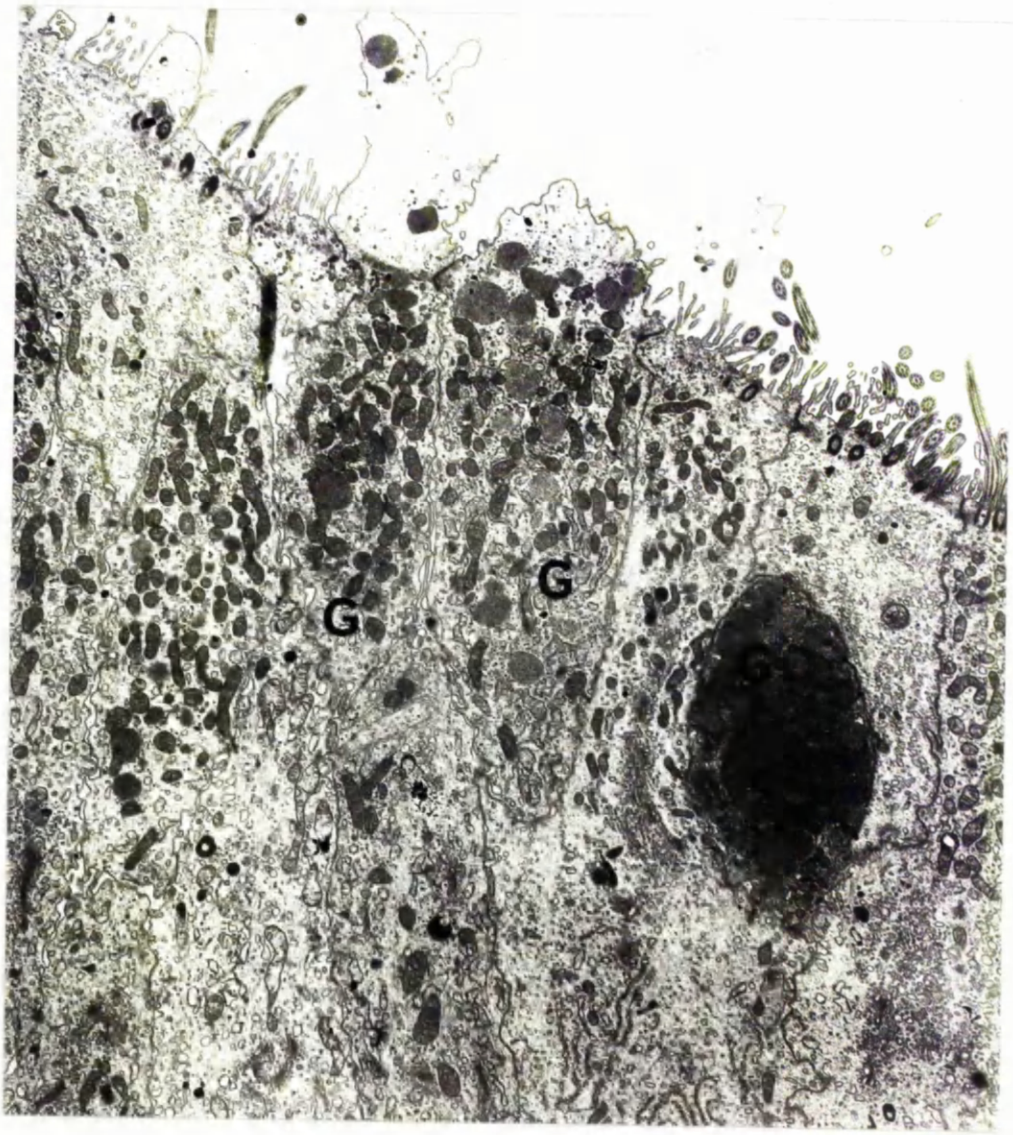

G
G
G

Fig. 84: Bronchitic epithelium: detail of goblet cell. Although the goblet cell has reached the apical surface and is secreting, the mucinogen granules (*) are very sparse and loosely packed (compare with Figs. 77,81).

(OsO_4 X 12,000)

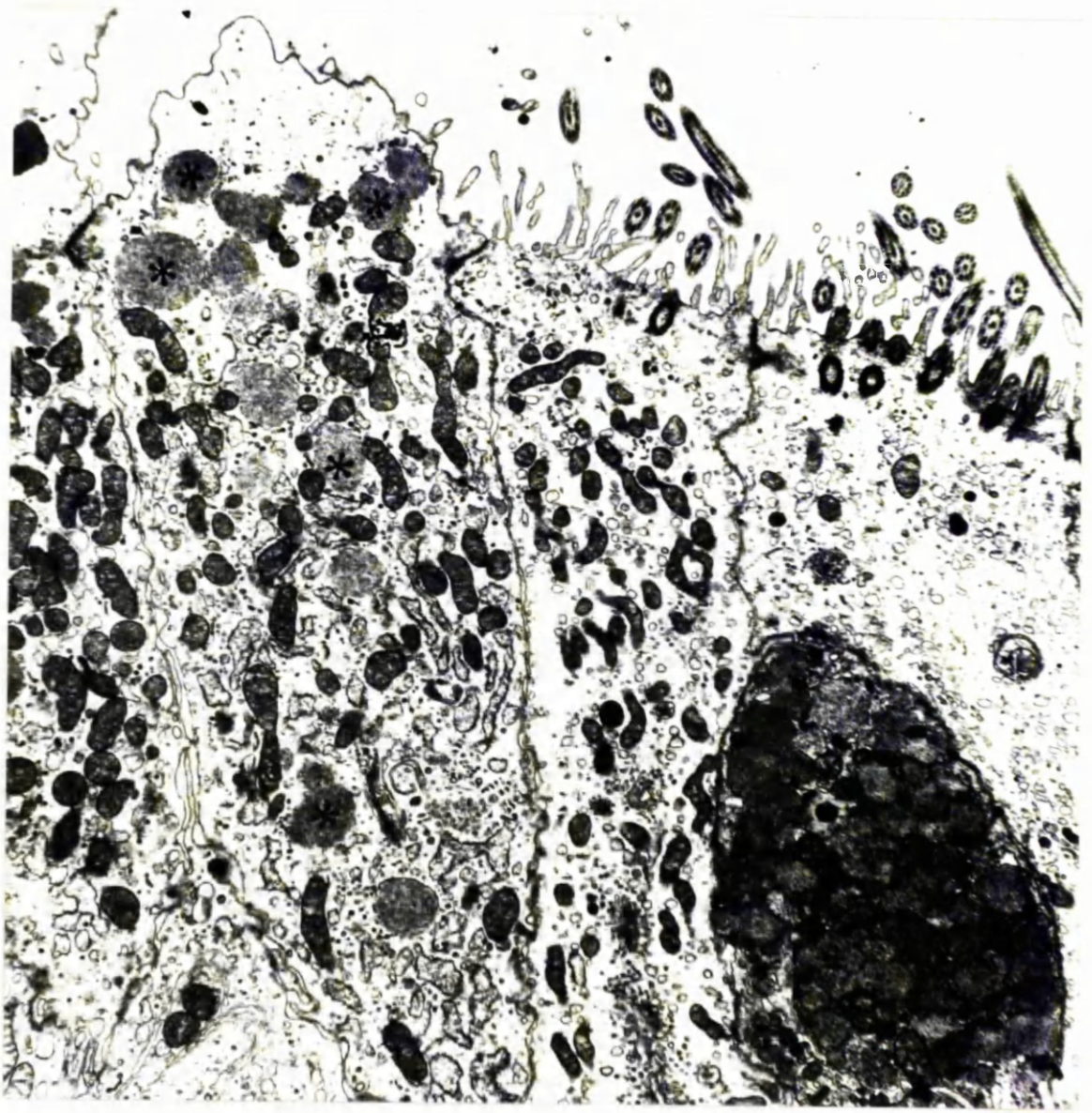


Fig. 85: Bronchitic epithelium: detail of bronchitic epithelium. Mucinogen granules (*) are sparse and are being secreted singly. The goblet cells contain large numbers of mitochondria (large arrows). Despite the cytoplasmic protrusions of the goblet cell, the intercellular junctions (small arrows) appear to be intact.

(OsO_4 X 16,000)

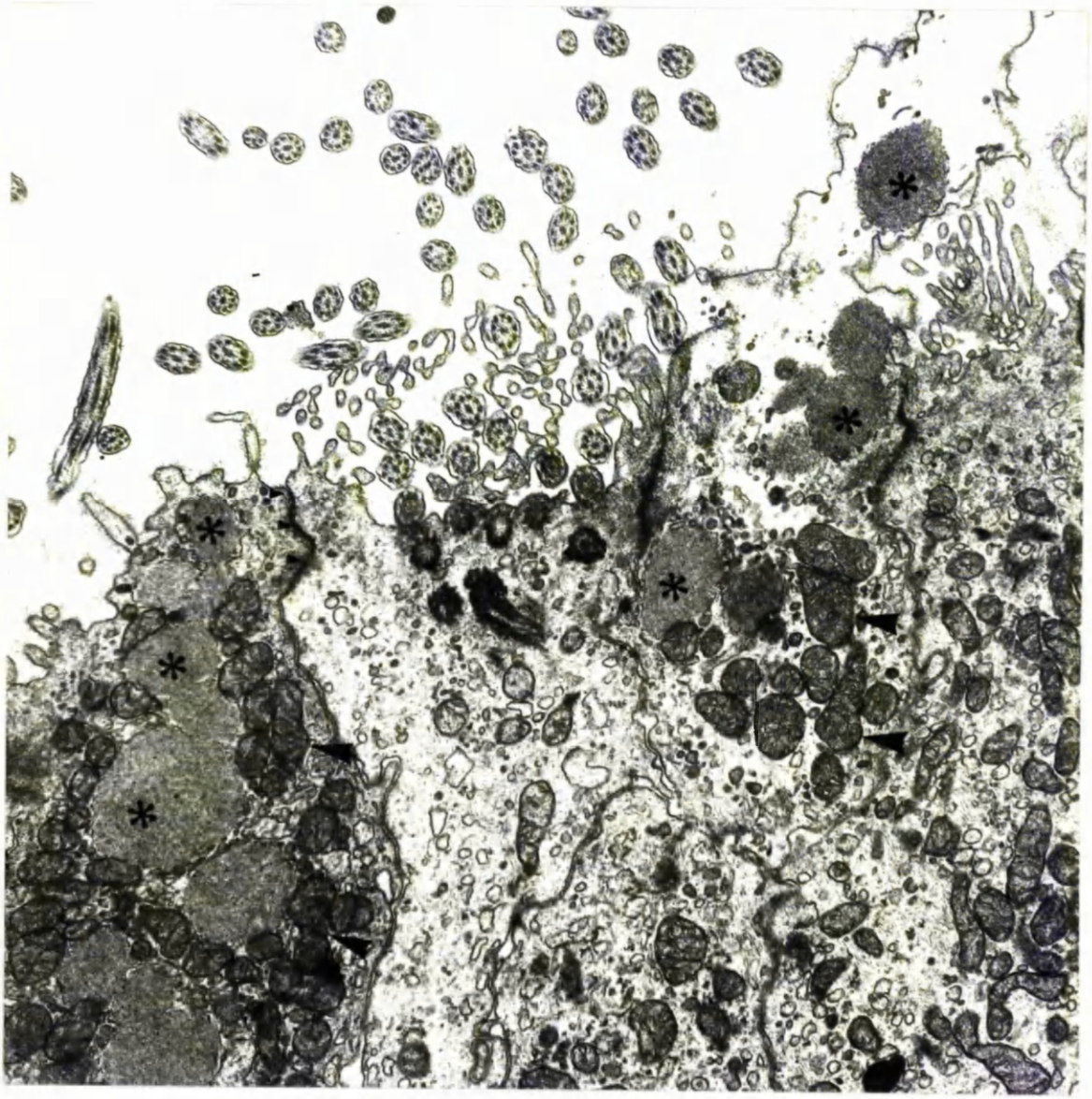


Fig. 86: Normal mucous gland: detail of mucous gland illustrating the basement membrane (arrowed) and the pronounced intercellular interdigitations. The large pools of pale granular material (*) are probably glycogen.

(OsO_4 X 12,000)

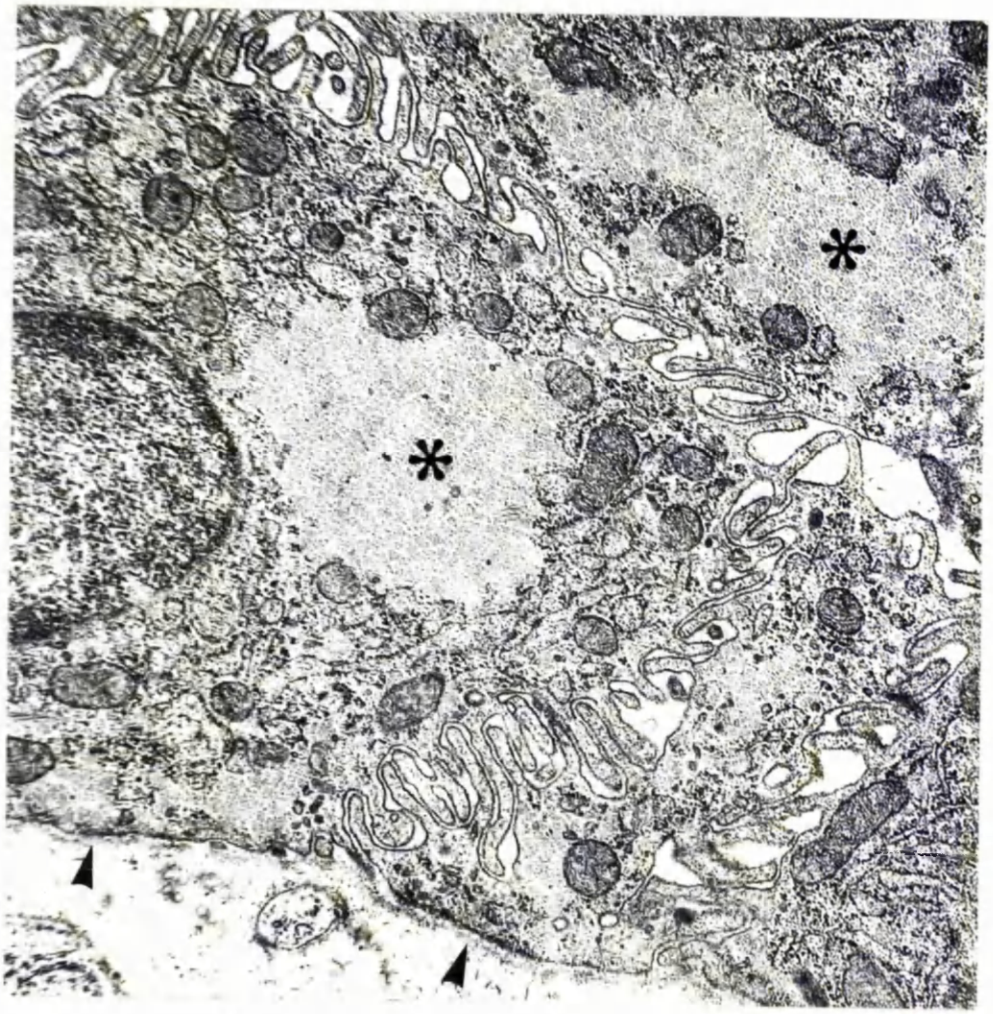


Fig. 87: Normal mucous gland: detail of mucous gland illustrating arrays of rough endoplasmic reticulum (arrowed). The Golgi apparatus (G) is visible in the adjacent cell, together with several membrane-bound vesicles (*) of synthesized material

(OsO_4 X 30,000)

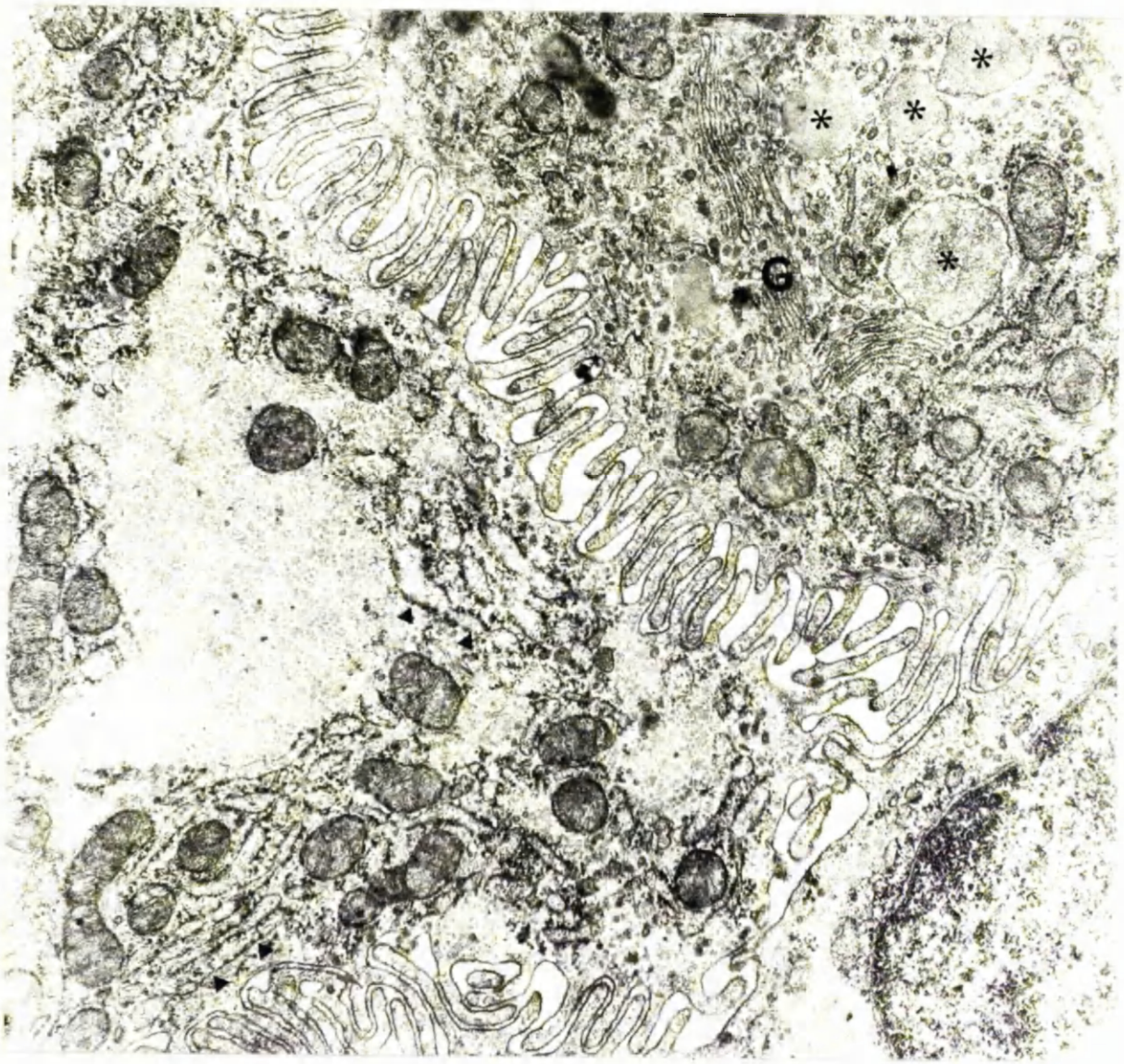

G

Fig. 88: Normal mucous gland: view of mucous gland surrounded by basement membrane (arrowed). Several myoepithelial processes (M) can be seen interspersed between the secretory cells, resting on the basement membrane.

(OsO_4 X 10,000)

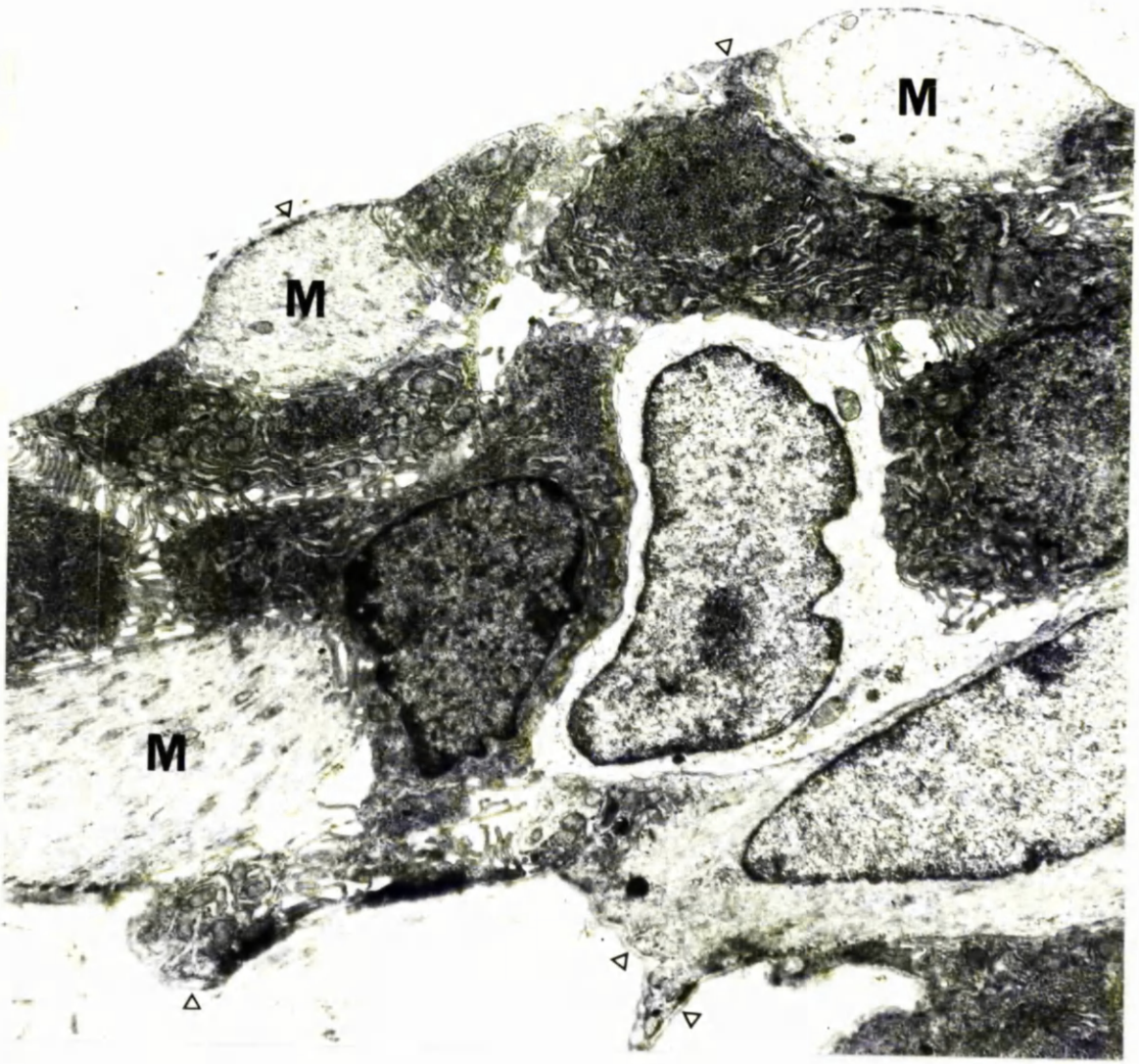

M
M
M

Fig. 89: Bronchitic mucous gland: secretory acinus with the gland lumen delineated by arrows. The secretory cells have tight junctions around their apices (open arrows), while the apical cytoplasm contains considerable numbers of secretory granules (*). The arrays of endoplasmic reticulum are grossly distorted; the normal parallel arrays are distended by excess secretion.

(OsO_4 X 10,000)

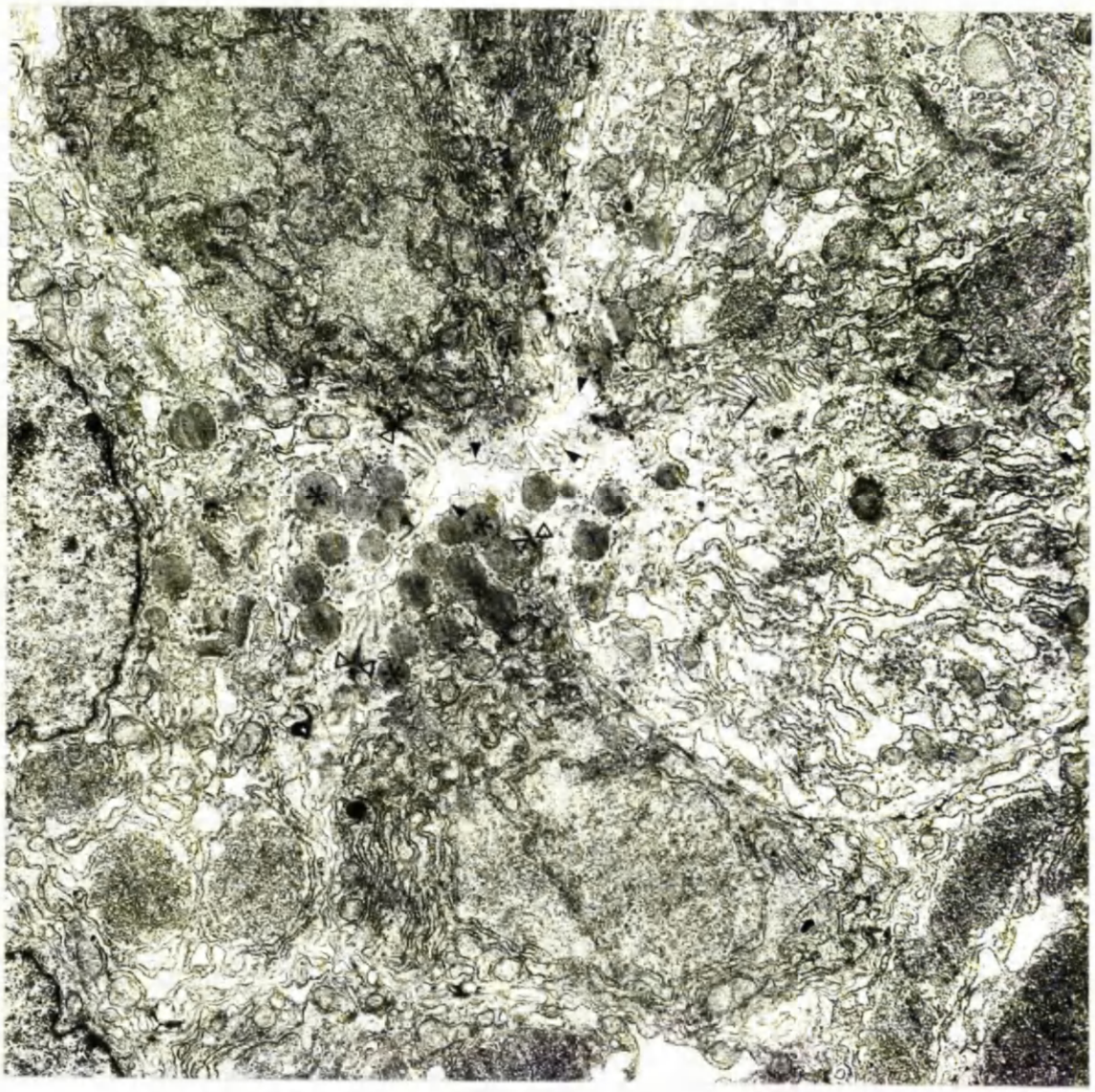


Fig. 90: Bronchitic mucous gland: detail of secretory cell, illustrating the gross distension between the arrays of endoplasmic reticulum. Compare with the narrow parallel arrays indicated by the arrow.

(OsO_4 X 20,000)

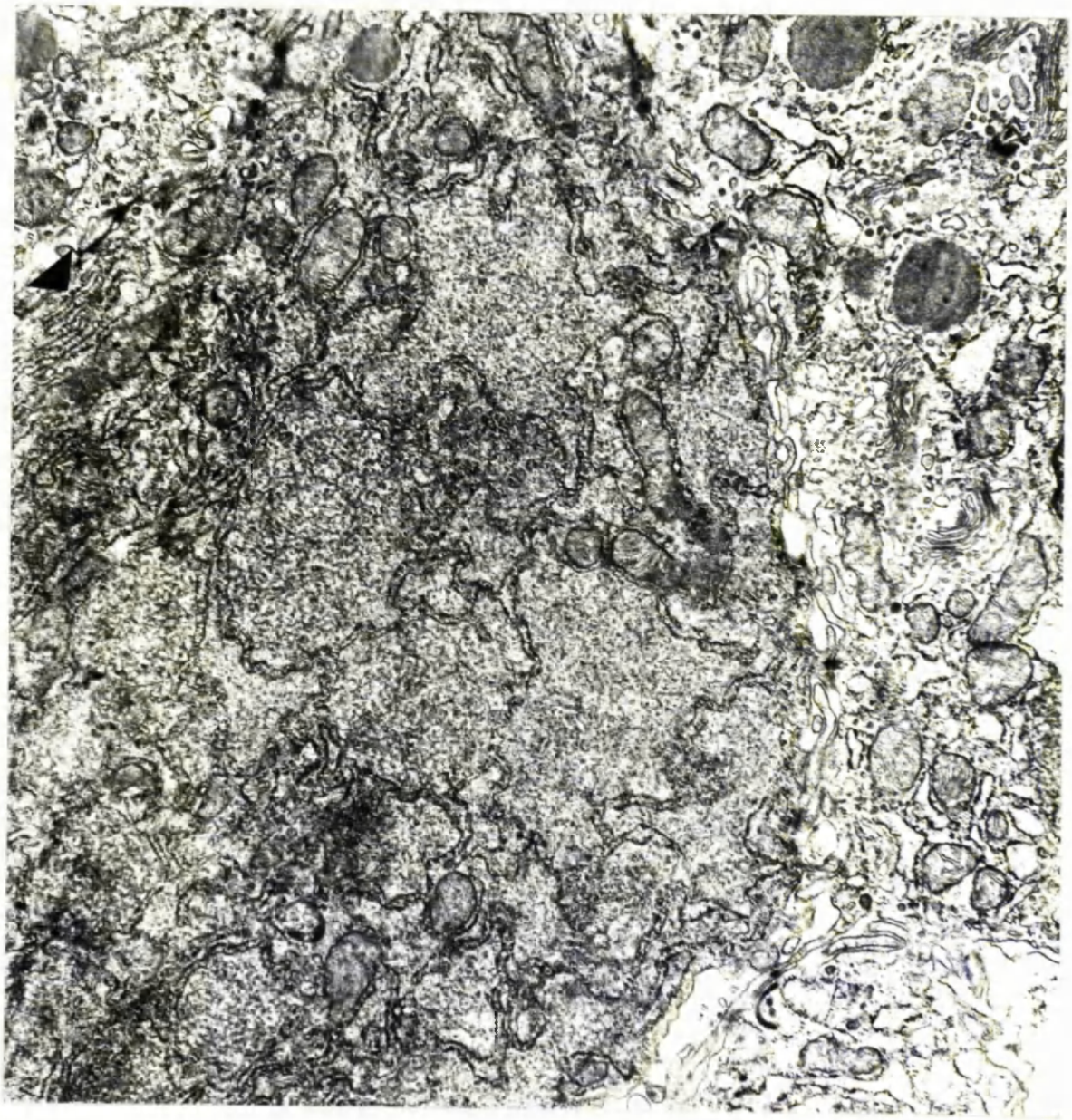


Fig. 91: Bronchitic mucous gland: detail of secretory acinus; the gland lumen (L) is indicated. The cytoplasmic interdigitations appear very tight and compacted, while tight junctions between the secretory cells are clearly visible (arrows). The Golgi apparatus (G) is very prominent in two of the cells.

(OsO_4 X 20,000)

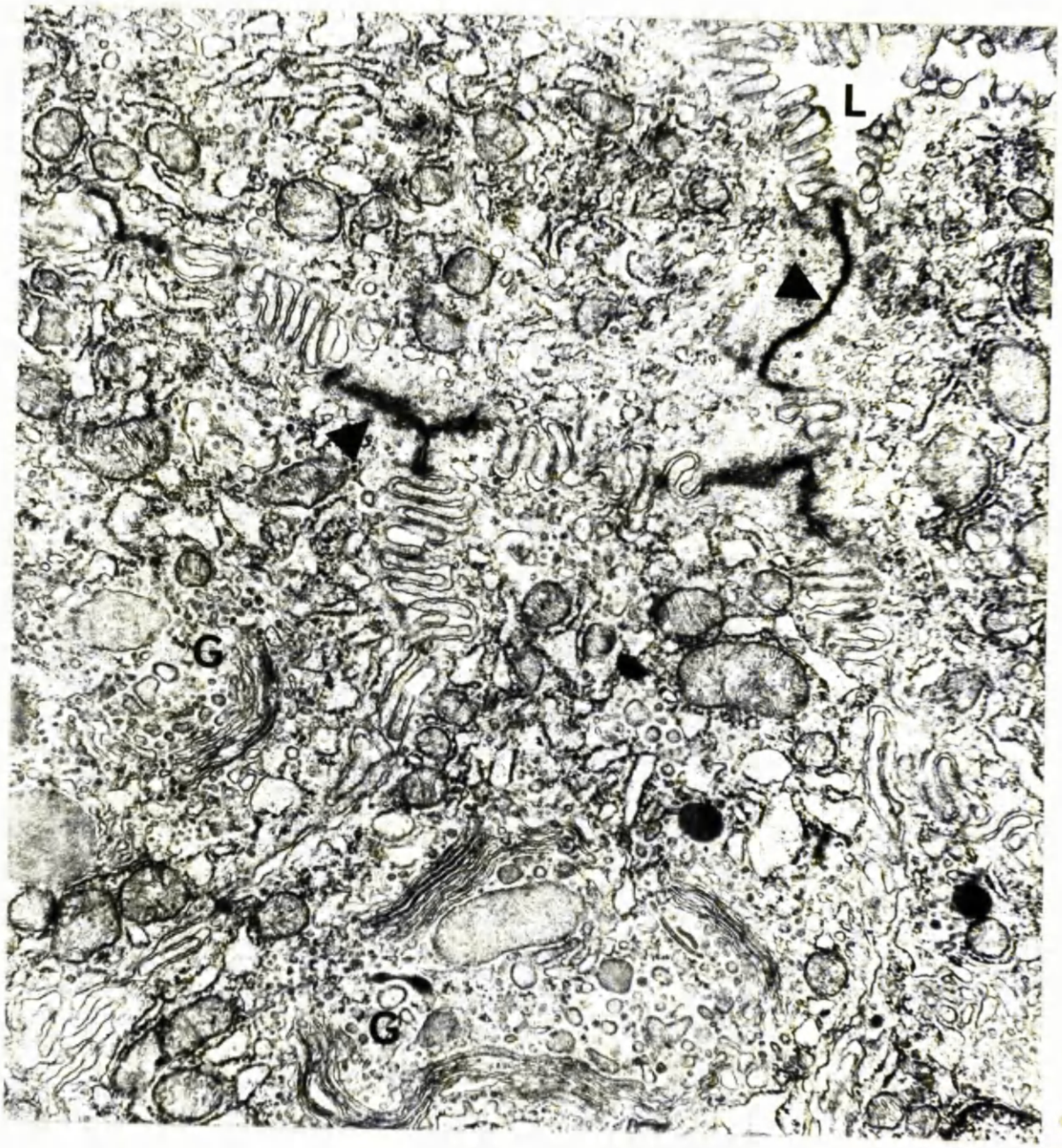
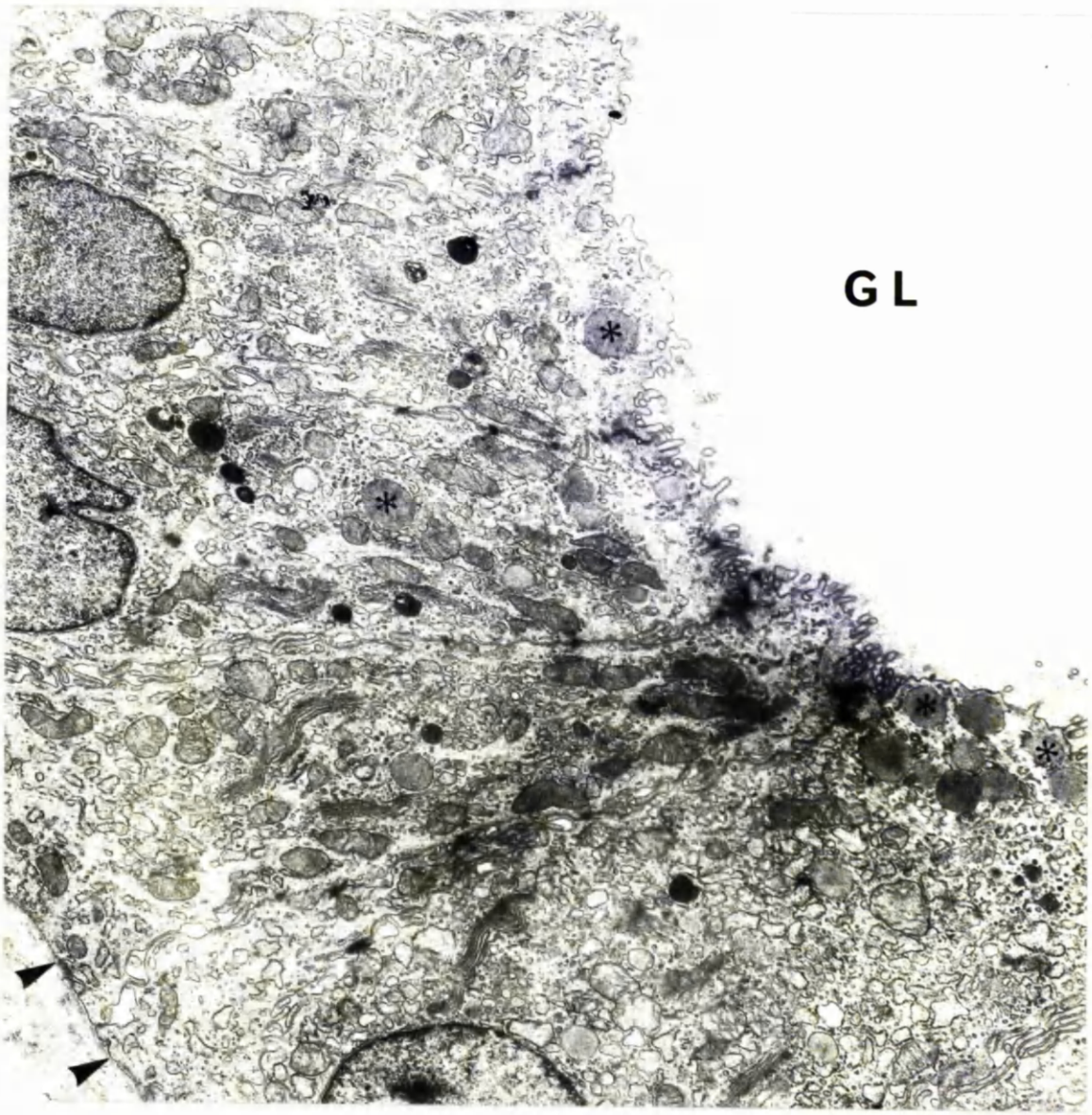

L
G
G

Fig. 92: Bronchitic mucous gland: section through mucous gland illustrating basement membrane (arrows) and gland lumen (GL). There is only a small number of secretory granules (*) at this level in the gland.

(OsO_4 X 10,000)


GL

CONCLUSIONS

CONCLUSIONS

At the beginning of this thesis, considerable reference was made to chronic bronchitis in man, where the disease is of great importance. Because the disease in man has been studied so intensively, the literature review was extended to include an outline of the human disease, so as to provide a reference for the findings in the dog.

The results of the epidemiological, clinical and pathological investigations indicate that there exists in the dog a disease entity closely comparable to chronic bronchitis in man. This disease in the dog has been defined using the clinical and pathological criteria applied to man and includes a requirement of coughing for a minimum of two consecutive months in the preceding year. Chronic bronchitis in the dog is a disease characterised clinically by a chronic intractable cough and pathologically by evidence at post mortem of mucus hypersecretion in the tracheobronchial tree.

In addition, changes occur in the airways of the dog which have their counterpart in man. It has been possible to apply the technique of quantification used in man to demonstrate a significant increase in the volume of mucous glands in the bronchi of dogs with chronic bronchitis. There are also characteristic changes at the histochemical and ultrastructural level.

Because the dog shares man's environment very closely, it has potential as an experimental model system. Using the dog it should be possible to assess the precise contributions made to the aetiology and pathogenesis of chronic bronchitis by the many factors considered to be important.

Our knowledge of the diseases which constitute chronic obstructive lung disease in the dog have been poorly understood up till now, based as they are on largely anecdotal material. It has been demonstrated that heart disease is not responsible for the majority of coughing in adult dogs, as had been widely supposed. Consequently, it should now be possible to separate, define and characterise these diseases of the chronic obstructive lung disease complex using the criteria applied to man.

REFERENCES

REFERENCES

Ailsby, R.L. and Ghadially, F.N. (1972) Journal of Pathology, 109, 75.

Alarie, Y., Ulrich, C.E., Busey, W.M., Krumm, A.A. and MacFarland, H.N. (1972) Archives of Environmental Health, 24, 115.

Albert, R.E., Alessandro, D., Lippman, M. and Berger, J. (1971) Archives of Environmental Health, 22, 12.

Amdur, M.O. (1959) International Journal of Air Pollution, 1, 170.

American Thoracic Society (1962) American Review of Respiratory Disease, 85, 762.

Anderson, A.E. and Foraker, A.G. (1962) American Journal of Medicine, 32, 218.

Anderson, A.E. and Foraker, A.G. (1971) Annals of Internal Medicine, 75, 789.

Appel, M., Pickerill, P.H., Menegus, M., Percy, D.H., Parsonson, I.M. and Sheffy, B.E. (1970) Gaines Veterinary Symposium "The Newer Knowledge about Dogs", New York: Gaines Dog Research Centre.

Archibald, J., Clacken, T. and Bishop, E.J. (1955) North American Veterinarian, 36, 565.

Arkins, J.A. and Hogan, M.R. (1973) Journal of Allergy and Clinical Immunology, 52, 278.

Asmundsson, T., Kilburn, K.H. and McKenzie, W.N. (1973) Laboratory Investigation, 29, 41.

Auerbach, O., Hammond, E.C., Kirman, D., Garfinkel, L. and Stout, A.P. (1967) Cancer, 20, 2055.

Baetjer, A.M. (1967) Journal of Applied Physiology, 23, 498.

Balint, J.A., Bondurant, S. and Kyriakides, E.C. (1971) Archives of Internal Medicine, 127, 740.

Baskerville, A. (1970A) Research in Veterinary Science, 11, 150.

Baskerville, A. (1970B) Zentralblattfur Veterinarmedizin, 17, 796.

Bates, D.V., Woolf, C.R. and Paul, G.I. (1962) Canadian Medical Services Journal, 18, 211.

Battista, S.P. and Kensler, C.J. (1970) Archives of Environmental Health, 20, 326.

Bedrossan, C.W.M., Anderson, A.E. Jr. and Foraker, A.G. (1971) Thorax, 26, 406.

Biegel, A.A and Krumholtz, R.A. (1968) American Review of Respiratory Disease, 97, 217.

Bowden, D.H. and Adamson, I.Y.R. (1971) Archives of Pathology, 92, 279.

Brinkman, G.L., Brooks, N. and Bryant, V. (1969) American Review of Respiratory Disease, 99, 219.

Buckley, R.H., Dees, S.C. and O'Fallon, W.M. (1968) Paediatrics, 42, 50.

Burton, P.A. and Dixon, M.F. (1969) Thorax, 24, 180.

Caird, F.I. (1972) Practitioner, 209, 767.

Castleberry, M.W., Ferrell, J.F., Jones, L.D. and Garvin, C.H. (1965) Journal of the American Veterinary Medical Association, 146, 607.

Catcott, E.J., McCammon, C.J., Kotin, P. (1958) Journal of the American Veterinary Medical Association, 133, 331.

Chakrin, L.W., Baker, A., DeSanctis, N., Wardell, JR., J.R. and Spicer, S.S. (1970) Pharmacologist, 12, 264.

Chakrin, L.W. and Saunders, L.Z. (1974) Laboratory Investigation, 30, 145.

Chang, S.C. (1957) Cancer, 10, 1246.

C.I.B.A. Symposium, (1959) Thorax, 14, 286.

Clean Air Act, (1956) H.M.S.O., London.

Clean Air Act, (1968) H.M.S.O., London.

Clean Air (Emission of Grit and Dust from Furnaces) Regulations (1971) H.M.S.O., London.

Colley, J.R.T. and Reid, D.D. (1970) British Medical Journal, 2, 213.

Colley, J.R.T., Douglas, J.W.B. and Reid, D.D. (1973) British Medical Journal, 3, 195.

Colley, J. (1974) British Medical Journal, 2, 201.

Conway, D.A. (1971) Irish Veterinary Journal, 25, 142.

Crofton, E. and Crofton, J. (1963) British Medical Journal, 2, 1161.

Crofton, J.C. and Douglas, A.D. (1969) Respiratory Diseases, Blackwell Scientific Publications, Oxford and Edinburgh.

Crofton, E.C. (1970) British Journal of Preventive and Social Medicine, 24, 110.

Dalhamn, T. and Strandberg, L. (1961) International Journal of Air and Water Pollution, 4, 154.

Dalhamn, T. (1970) Archives of Environmental Health, 21, 633.

Daly, C. (1959) British Journal of Preventive and Social Medicine, 13, 14.

Das, K.M. and Tashjian. R.J. (1965) Veterinary Medicine, 60, 1209.

Delesse, A. (1848) Annales des Mines, 13, 378.

Department of Health and Social Security (1972) Health and Personal Social Service Statistics, H.M.S.O., London.

Department of Employment (1973) Department of Employment Gazette, H.M.S.O., London.

Detweiler, D.K. (1962) Small Animal Clinician, 2, 79.

Detweiler, D.K. and Patterson, D.F. (1965) Annals of the New York Academy of Science, 127, 322.

Doll, R. and Hill, A.B. (1964) British Medical Journal, 1, 1399.

Done, S.H. (1970) Journal of small Animal Practice, 11, 655.

Dunnill, M.S. (1962) Thorax, 17, 320.

Dunnill, M.S., Massarella, G.R. and Anderson, J.A. (1969) Thorax, 24, 176.

Ettinger, S.J. and Suter, P.F. (1970) Canine Cardiology, London : Saunders.

Evelyn, J. (1661) Fumifugium; Or The Smoke of London Dissipated. National Smoke Abatement Society, 1933 edit.

Fairbairn, A.S., Wood, C.A. and Fletcher, C.M. (1959) British Journal of Preventive and Social Medicine, 13, 175.

Falk, G.A., Siskind, G.W. and Smith, J.P. (1970) Journal of Immunology, 105, 1559.

Falk, G.A., Okinaka, A.J. and Siskind, G.W. (1972) American Review of Respiratory Disease, 105, 14.

Ferris, B.G. (1973) Bulletin de Physio-Pathologie Respiratoire, 9, 1121.

Field, W.E.H., Davey, E.N., Reid, L. and Roe, F.J.C. (1966) British Journal of Diseases of the Chest, 60, 66.

Field, W.E.H., (1968) British Journal of Diseases of the Chest, 62, 11.

Finley, T.N. and Ladman, A.J. (1972) New England Journal of Medicine, 286, 223.

Fisher, E.W. (1973) Personal communication.

Flint, G.L., Maxwell, K.W. and Renzetti, A.D. (1971) Archives of Environmental Health, 22, 366.

Florey, H., Carleton, H.M. and Wells, A.Q. (1932) British Journal of Experimental Pathology, 13, 269.

Florey, H. (1970) General Pathology, London : Lloyd-Luke.

Frasca, J.M., Auerbach, O., Parks, V.R. and Jamieson, J.D. (1968A) Experimental and Molecular Pathology, 9, 363.

Frasca, J.M., Auerbach, O., Parks, V.R. and Jamieson, J.D. (1968B) Experimental and Molecular Pathology, 9, 363.

Freeman, J.A. (1962) Anatomical Record, 144, 341.

Friedman, I. and Bird, E.S. (1971) The Laryngoscope, 81, 1852.

Gibbons, R.A. (1959) Biochemistry Journal, 73, 209.

Gibbons, R.A. (1963) ibid 89, 380.

Glezen, W.P. and Denny, F.W. (1973) New England Journal of Medicine, 288, 498.

Goco, R.V., Kress, M.B. and Brantigan, O.C. (1963) Annals of the New York Academy of Sciences, 106, 555.

Goldstein, A. (1964)Biostatistics: An Introductory Text. London:Collier-MacMillan

Gough, J. and Wentworth, J.E. (1960) Recent Advances in Pathology ed. Harrison. C.V. 7th Edition. London:Chruchill.

Green, G. and Carolin, D. (1967) New England Journal of Medicine, 276, 421.

Green, G. (1970) Archives of Internal Medicine, 126, 500.

Greenberg, S.D. and Willms, R.K. (1962) Archives of Pathology, 73, 53.

Greenwood, M.F. and Holland, P. (1972) Laboratory Investigation, 27, 296.

Gregory, J. (1970) Atmospheric Environment, 4, 453.

Grist, N.R. (1967) Scottish Medical Journal, 12, 408.

Hale, F.C., Olsen, C.R. and Mickey, M.R. Jr. (1968) American Review of Respiratory Disease, 98, 978.

Haller, R. de and Reid, L. (1965) Medicina thoracalis, 22, 549.

Haller, R. de (1969) Development of the Mucous-Secreting Elements. In The Anatomy of the Developing Lung ed. Emery. Ch.6. London:Heinemann.

Halliwell, R.E.W. (1973) Immune mechanisms in dermatological hypersensitivities. In Veterinary Annual ed. Grunsell,C.S.G. and Hill,F.W.G. Bristol:Wright.

Hammond, E.C., Auerbach, O., Kirman, D. and Garfinkel, L. (1970) Archives of Environmental Health, 21, 740.

Harford, C.G., Hamlin, A. and Parker, E. (1955) The Journal of Experimental Medicine, 101, 577.

Hawthorne, V.M., Jarrett, W.F.H., Lauder, I., Martin, W.B. and Roberts, G.B.S. (1957) British Medical Journal, 1, 675.

Heard, B.E. (1960) American Review of Respiratory Diseases, 82, 792.

Heard, B.E. and Hossain, S. (1973) Journal of Pathology, 110, 319.

Heath, I.D. (1961) Nature, 191, 1370.

Heitzman, E.R., Markarian, B. and Solomon, J. (1973) Radiologic Clinics of North America, 11, 49.

Hernadez, J.A., Anderson, Jr.,A.E., Holmes, W.L. and Foraker, A.G. (1966) American Review of Respiratory Disease, 93, 78.

Higgins, I.T.T., Oldham, P.D., Cochrane, A.L. and Gilson, J.C. (1956) British Medical Journal, 2, 904.

Higgins, I.T.T. (1957) ibid, 2, 1198.

Higgins, I.T.T. (1959) ibid, 1, 325.

Higgins, I.T.T. (1971) Archives of Environmental Health, 22, 584.

Hogg, J.C., Macklem, P.T. and Thurlbeck, W.M. (1968) New England Journal of Medicine, 278, 1355.

Holland, W.W., Spicer, C.C. and Wilson, J.M.G. (1961) Lancet, 2, 338.

Holland, W.W. and Reid, D.D. (1965) Lancet, 1, 444.

Horning, E.C., Horning, M.G., Carroll, D.I., Stillwell, R.N. and Dzidic, I. (1973) Life Science, 13, 1331.

Hossain, S. and Heard, B.E. (1970) Journal of Pathology, 101, 171.

Hossain, S. (1973) American Review of Respiratory Disease, 107, 99.

Houston, J.C., Joiner, C.L. and Trounce, J.R. (1968) A Short Textbook of Medicine 3rd. edn. English Universities Press : London.

Jennings, A. (1970) Animal Pathology. London:Bailliere, Tindall and Cassell.

Johansson, S.G., Hogman, C.F. and Killander, J. (1968) Acta pathologica et microbiologica Scandinavia, 74, 519.

Jubb, K.V.F. and Kennedy, P.C. (1970) Pathology of Domestic Animals. London: Academic Press.

Karrer, H. (1954) Journal of Applied Physiology, 25, 1461.

Keuffel and Esser Company (1963) Instruction Manual, Compensating Polar Planimeters.

Korhonen, L.K., Holopainen, E. and Paavolainen, M. (1969) Acta histochemica, 32, 57.

Laennec, R.T.H. (1819, 1826) De l'Auscultation Mediate (2 vols.), Brosson and Chaude, Paris.

Lamb, D. and Reid, L. (1968) Journal of Pathology and Bacteriology, 96, 97.

Lamb, D. and Reid, L. (1969) Journal of Pathology, 98, 213.

Lamb, D. and Reid, L. (1970) ibid, 100, 127.

Lane, B.P. and Gordon, R. (1974) Proceedings of the Society for Experimental Biology and Medicine, 145, 1139.

Lauder, I. and Lawson, D.D. (1959) Veterinary Record, 71, 1096.

Lauder, I. (1974) Personal communication.

Laurenzi, G.A., Potter, R.T. and Kass, E.H. (1961) New England Journal of Medicine, 265, 1273.

Lawther, P.J. (1972) Air Pollution and Tobacco Smoke. In Clinical Aspects of Inhaled Particles, ed. Muir,D.C.F. Ch.2, p.p.21-39. London:Heineman.

Layland, W.R. (1964) M.D. Thesis, University of Sheffield, quoted by Stuart-Harris, C.H. (1965) Scottish Medical Journal, 10, 93.

Lev, R. and Spicer, S.S. (1965) American Journal of Pathology, 46, 23.

Lewis, T.R., Moorman, W.J., Ludmann, W.F. and Campbell, K.I. (1973) Archives of Environmental Health, 26, 16.

Lindsey, J.R., Baker, H.J., Overcash, R.G., Cassell, G.H. and Hunt, C.E. (1971) American Journal of Pathology, 64, 675.

Lowe, C.R. (1968) Proceedings of the Royal Society of Medicine, 61, 98.

Lulling, J., Prignot, J., Lievens, P., Meersseman, F. and Masson, P.L. (1968) Naunyn-Schmiedeberg's Archiv fur Pharmakologie und experimentelle Pathologie, 261, 1.

Macklem, P.T. (1972) American Journal of Medicine, 52, 721.

Marco, V., Mass, B., Meranze, D.R., Weinbaum, G. and Kimbel, P. (1971) American Review of Respiratory Disease, 104, 595.

Martin, S.W. and Willoughby, R.A. (1971) Journal of the American Veterinary Medical Association, 159, 1518.

Martin, S.W. and Willoughby, R.A. (1972) Archives of Environmental Health, 25, 158.

Mass, B., Ikeda, T., Meranze, D.R., Weinbaum, G. and Kimbel, P. (1972) American Review of Respiratory Disease, 106, 381.

Matsuba, K. and Thurlbeck, W.M. (1972) American Review of Respiratory Disease, 105, 908.

Mawdesley-Thomas, L.E., Healey, P. and Barry, D.H. (1971) In Inhaled Particles III (ed. W.H. Walton) Unwin Bros. Ltd., Surrey, England.

Mawdesley-Thomas, L.E. and Healey, P. (1973) Archives of Environmental Health, 27, 248.

Medical Research Council (1960) British Medical Journal, (2), 1165.

Medical Research Council Committee (1965) Lancet, 1, 775.

Medici, T.C. and Buergi, H. (1971) American Review of Respiratory Disease, 103, 784.

Meyrick, B. and Reid, L. (1970) Journal of Anatomy, 107, 281.

Miller, W.S. (1947) The Lung, 2nd edn., Charles C. Thomas, Springfield, Illinois.

Mowry, R.W. (1956) Journal of Histochemistry and Cytochemistry, 4, 407.

McCarthy, C. and Reid, L. (1964A) Quarterly Journal of Experimental Physiology, 49, 81.

McCarthy, C. and Reid, L. (1964B) Quarterly Journal of Experimental Physiology, 49, 85.

McLaughlin, R.F., Tyler, W.S. and Canada, R.O. (1961) American Journal of Anatomy, 108, 149.

MacLeod, L.J. and Heard, B.E. (1969) Journal of Pathology, 97, 157.

Nieberle, K. and Cohrs, P. (1967) (4th edn.) Textbook of the Special Pathological Anatomy of Domestic Animals. Stuttgart:Fisher.

Nielsen, S.W. (1965) Paper presented at International Conference on Lung Tumours in Animals, University of Perugia, Italy, June 1965. Cited by Nielsen, S.W. (1968) Comparative Pathology of Pulmonary Diseases In the Lung ed. Liebow, A.A. and Smith, D.E. Ch. 15. Baltimore : Williams and Wilkins.

Nielsen, S.W. (1971) Journal of the American Veterinary Medical Association, 159, 1103

Niewoehner, D.E., Kleinerman, J. and Knoke, J.D. (1972) American Review of Respiratory Disease, 105, 586.

Norman-Taylor, W. and Dickinson, V.A. (1972) Community Medicine, 128, 32.

O'Brien, J. (1973) Chronic Bronchitis in the Dog. In Veterinary Annual ed. Grunsell, C.S.G. and Hill, F.W.G. Bristol : Wright.

O'Brien, J., Reif, J. and Schryver, H. (1966) Journal of the American Veterinary Medical Association, 149, 1317.

O'Brien, J. and Skelley, J.F. (1968) Bronchiectasis. In Current Veterinary Therapy III. Philadelphia : Saunders.

Ohtani, H. (1969) Bulletin of the Azabu Veterinary College, 19, 1.

Oswald, N.C., Harold, J.T. and Martin, W.J. (1953) Lancet, 2, 639.

Oswald, N.C. (1958) Recent Trends in Chronic Bronchitis. Lloyd Luke, London.

Parkinson, D.R. and Stephens, R.J. (1973) Environmental Research, 6, 37.

Pearse, A.G. Everson (1968) Histochemistry: Theoretical and Applied, Vol.1. London : Churchill.

Pennock, P.W. and Archibald, J. (1968) Diseases of the Respiratory System. In Canine Medicine. ed. Catcott, E.J. Wheaton, Illinois : American Veterinary Publications, Inc.

Pirie, H.M. (1967) Journal of small Animal Practice, 8, 175.

Pittman, J.G. and Cohen, P. (1964) New England Journal of Medicine, 271, 403.

Policard, A., Collet, A. and Giltaire-Ralyte, L. (1955) Bronches, 5, 187.

Pomerance, A. and Whitney, J.C. (1970) Cardiovascular Research, 4, 61.

Porter, P. and Allen, W.D. (1972) Journal of the American Veterinary Medical Association, 160, 512.

Pushpakom, R., Hogg, J.C., Woolcock, A.J., Angus, A.E., Makclem, P.T. and Thurlbeck, W.M. (1970) American Review of Respiratory Disease,102,778.

Ragland, W.L. and Gorham, J.R. (1967) Nature, 214, 925.

Registrar-General (1956) Statistical Review for the Year 1953, London, H.M.S.O.

Registrar-General (1966) Statistical Review of England and Wales for 1964, H.M.S.O., London.

Registrar-General (1970) Annual Report for Scotland No. 116 Part 1 - Mortality Statistics, H.M.S.O., Edinburgh.

Registrar-General (1971) Statistical Review of England and Wales Part 1 - Medical Tables, H.M.S.O., London.

Reid, L. (1954), Lancet, 1, 275.

Reid, L. (1958) Chronic Bronchitis and Hypersecretion of Mucus. In Lectures on the Scientific Basis of Medicine Vol. 8. Ch.14. London : Athlone Press.

Reid, L. (1960) Thorax, 15, 132.

Reid, L. (1963) British Journal of Experimental Pathology 44, 437.

Reid, L. (1965) Medicina thoracalis, 22, 61.

Reid, L. (1967A) The Embryology of the Lung. In Development of the Lung (C.I.B.A. Foundation Symposium) ed. de Reuck A.V.S. and Porter, R. London : Chruchill.

Reid, L. (1967B) The Pathology of Emphysema. London : Lloyd-Luke.

Reid, L. (1968) Bronchial Mucus Production in Health and Disease. In The Lung ed. Liebow, A.A. and Smith, D.E. Ch.8. Baltimore : Williams & Wilkins.

Reid, L. (1973) Development and Anatomy of the Lung In Respiratory Diseases I, Medicine, 13.

Reid, D.D. and Fairbairn, A.S. (1958) Lancet, 1, 1147.

Reid, L., McCarthy, C., Duvenci, J. and Gibbons, R.A. (1962) Nature, 195, 715.

Reif, J.S. and Cohen, D. (1970) Archives of Environmental Health, 20, 684.

Reif, J.S. and O'Brien, J.A. (1968) Pulmonary Emphysema. In Current Veterinary Therapy III. Philadelphia : Saunders.

Report of the Medical Officer of Health for Glasgow (1970).

Restrepo, G. and Heard, B.E. (1963A) Journal of Pathology and Bacteriology, 85, 305.

Restrepo, G. and Heard, B.E. (1963B) Thorax, 18, 334.

Restrepo, G. and Heard, B.E. (1964) American Review of Respiratory Disease, 90, 395

Reynolds, E.S. (1963) Journal of Cell Biology, 17, 208.

Rhodin, J. (1959) Annals of Otology, Rhinology and Laryngology, 68, 964.

Rhodin, J. and Dalhamn, T. (1956) Zeitschrift fur Zellforschung, 44, 345.

Robbins, S.L. (1967) Pathology. 3rd edn., W.B. Saunders Co., Philadelphia.

Robinson, G.W. (1968) Journal of the American Veterinary Medical Association, 152, 1383.

Robinson, N.E. and Gillespie, J.R. (1973) American Review of Respiratory Disease, 108, 1192.

Rohrbach, J.A. (1970) Journal of small Animal Practice, 11, 679.

Rubin, H. (1967) cited by Snow, H.D., Donovan, M.L., Washington, J.O. and Funkalsrud, E.W. (1969) Archives of Surgery, 99, 126.

Russell, M.A.H., Cole, P.V. and Brown, E. (1973) Lancet, 1, 576.

Ryder, R.C., Dunnill, M.S. and Anderson, J.A. (1971) Journal of Pathology, 104, 59.

Scadding, J.G. (1963) British Medical Journal, 2, 1425.

Schiefer, B., Hurov, L. and Seer, G. (1974) Journal of the American Veterinary Medical Association, 164, 408.

Schneider-Leyer, E. (1964) Dogs of the World, London : Popular Dogs.

Scott, K.W.M. (1973) American Review of Respiratory Disease, 107, 239.

Scottish sub-committee (1973) The Future of the Chest Services in Scotland. Report of Scottish Standing Medical Advisory Committee, Scottish Home and Health Department, Edinburgh : H.M.S.O.

Seely, J.E., Zuskin, E. and Bouhuys, A. (1971) Science, 172, 741.

Silverton, R.E. (1964) The Journal of Medical Laboratory Technology, 21, 187.

Simon, G. (1959) British Journal of Radiology, 32, 292.

Smith, H.A. and Jones, T.C. (1972) Veterinary Pathology. Philadelphia: Lea and Febiger.

Snow, H.D., Donovan, M.L., Washington, J.O. and Funkalsrud, E.W. (1969) Archives of Surgery, 99, 126.

Sobonya, R.E. and Kleinerman, J. (1972) American Review of Respiratory Disease, 105, 768.

Spicer, S.S. (1965) Journal of Histochemistry and Cytochemistry, 13, 211.

Spicer, S.S., Chakrin, L.W. and Wardell, J.R. (1972) Federal Proceedings, 31, (2), 663.

Spicer, S.S. and Duvenci, J. (1964) Anatomical Record, 149, 333.

Spicer, S.S. and Henson, J.G. (1967) Methods for Localizing Mucosubstances in Epithelial and Connective Tissue. In Methods and Achievements in Experimental Pathology, Vol.2 - Investigative Techniques. Ed. Bayusz,E. and Jasmin, G. New York : Karger.

Spicer, S.S., Horn, R.G. and Leppi, T.J. (1967) Histochemistry of connective tissue mucopolysaccharides. In The Connective Tissue, International Academy of Pathology Monograph No.7. p.p.251-303. Baltimore : Williams and Wilkins Co.

Spicer, S.S., Leppi, T.J. and Stoward, P.J. (1965) Journal of Histochemistry and Cytochemistry, 13, 599.

Spicer, S.S. and Meyer, D.B. (1960) American Journal of Clinical Pathology, 33, 453.

Spicer, S.S. and Warren, L. (1960) Journal of Histochemistry and Cytochemistry, 8, 135.

Spicer, S.S., Chakrin, L.W., Wardell, J.R. and Kendrick, W. (1971) Laboratory Investigation, 25, 483.

Stocks, P. (1947) General Register Office Studies in Medical and Population Subjects No. 1. H.M.S.O. : London.

Stuart-Harris, C.H., Pownall, M., Scothorne, C.M. and Franks, Z. (1953) Quarterly Journal of Medicine, 22, 121.

Stuart-Harris, C.H. and Hanley, T. (1957) Chronic Bronchitis, Emphysema and Cor Pulmonale, Wright:Bristol.

Stunzi, H. (1962) Schweizer Archiv fur Tierheilkunde, 104, 135.

Stunzi, H. (1973) Pathologia et microbiologia, 39, 358.

Subbuswamy, S.G. (1973) Journal of Pathology, 111, 181.

Takizawa, T. and Thurlbeck, W.M. (1971A) American Review of Respiratory Disease, 103, 774.

Takizawa, T. and Thurlbeck, W.M. (1971B) ibid, 104, 531.

Tandon, M.K. and Campbell, A.H. (1969) Thorax, 24, 607.

Thoracic Society (1950) Thorax, 5, 222.

Thurlbeck, W.M. and Angus, G.E. (1964) Thorax, 19, 436.

Thurlbeck, W.M., Angus, G.E. and Pare, J.A.P. (1963) British Journal of Diseases of the Chest, 57, 73.

Thurlbeck, W.M., Benjamin, B. and Reid, L. (1961) British Journal of Diseases of the Chest, 55, 54.

Thurlbeck, W.M., Henderson, J.A. and Fraser, R.G. (1970) Medicine, 49, 82.

Thurlbeck, W.M., Pun, R., Toth, J. and Frazer, R.G. (1974) American Review of Respiratory Disease, 109, 73.

Tomasi, T.B. (1968) New England Journal of Medicine, 279, 1327.

Trump, B.J., Smuckler, E.A. and Benditt, E.P. (1961) Journal of Ultrastructure Research, 5, 343.

Turner, R.W.D. (1969) Diseases of the Cardiovascular System. In The Principles and Practice of Medicine ed. Davidson, Sir S. p.p. 123-298, Edinburgh and London : Livingstone.

United States Public Health Service (1948) Air Pollution in Donora, Pa. Epidemiology of the Unusual Smog Episode of October, 1948. Public Health Bulletin No. 306. Washington: U.S. Public Health Service.

United States Public Health Service (1967) The Health Consequences of Smoking. A Public Health Service Review. Washington : U.S. Department of Health, Education and Welfare.

Valentine, E.H. (1957) Cancer, 10, 272.

van Allen, L.M. and Lindskog, G.E. (1931) Surgery, Gynecology and Obstetrics, 53, 16.

Ventura, J. and Goucher, S. (1966) Archives of Environmental Health, 13, 593.

Veterinary Record, (1974) Editorial comment, 94, 454.

Wardell, J.R., Chakrin, L.W. and Payne, B.J. (1970) American Review of Respiratory Diseases, 101, 741.

Warren, L. and Spicer, S.S. (1961) Journal of Histochemistry and Cytochemistry, 9, 400.

Warren Spring Laboratory (1972) National Survey of Air Pollution, 1961 - 1971 Vol. 1. H.M.S.O., London.

Watson, J.H.L. and Brinkman, G.L. (1964) American Review of Respiratory Diseases, 90, 851.

Watson, M.L. (1958) Journal of Biophysical and Biochemical Cytology, 4, 475.

Webb, D.R. and Condemi, J.J. (1974) Annals of Internal Medicine, 80, 618.

Weinbaum, G., Marco, V., Ikeda, T., Mass, B., Meranze, D.R. and Kimbel, P. (1974) American Review of Respiratory Disease, 109, 351.

Wells, R.E., Walker, J.E. and Hickler, R.B. (1960) New England Journal of Medicine, 263, 268.

Wheeldon, E.B. and Pirie, H.M. (1974) American Review of Respiratory Disease, (in press).

Wheeldon, E.B., Pirie, H.M., Fisher, E.W. and Lee, R. (1974) Veterinary Record, 94, 466.

White, R. (1973) Medicine - Cardiovascular Diseases, 4, 18, 1128. London : Medical Education (International) Ltd.

W.H.O. (1961) Chronic Cor Pulmonale, Technical Report Series No.213, Geneva : World Health Organisation

W.H.O. (1971) Geneva. Annual Report.

Wright, N.G. (1973) Journal of small Animal Practice, 14, 241.

Wright, N.G. and Cornwell, H.J.C. (1968) Research in Veterinary Science, 9, 295.

Wright, N.G., Thompson, H. and Cornwell, H.J.C. (1971) Research in Veterinary Science, 12, 162.

Wright, N.G., Thompson, H., Cornwell, H.J.C. and Taylor, D. (1974) Journal of small Animal Practice, 15, 27.

Yeager, H. (1971) American Journal of Medicine, 50, 493.

Zarkower, A. (1972) Archives of Environmental Health, 25, 45.

Ziegler, J., Penny, R. and Hughes, D.G. (1973) Australian and New Zealand Journal of Medicine, 3, 565.

APPENDIX I

CLINICAL AND POST MORTEM FINDINGS IN INDIVIDUAL CASES OF CHRONIC BRONCHITIS.

CASE - 1: SUBJECT - Manchester Terrier: AGE - 9 years: SEX - F

History: This dog had been coughing for 3 - 4 months. It became very breathless after exertion.

Clinical examination: On admission it was dull and very obese with a markedly enlarged abdomen. A constant cough with expiratory snore which became worse after exercise, with retching and production of phlegm. The respiratory rate was 30/minute with widespread emphysematous crackling.

The pulse rate was 130/minute and there was sinus arrythmia.

The temperature was normal.

Radiology: The lung field showed a loss of translucency associated with excessive pulmonary markings. The heart shadow was normal.

Post mortem examination: (i) Tracheobronchial tree:

There were numerous polyps in the lower trachea and extending down into the lobar bronchi. These projected into the lumen and caused stenosis. Scanty mucopus was present in the major airways.

There was marked autolysis of the epithelium with sloughing.

In the lamina propria large polyps were present, some of which were pedunculated, with dense fibrous connective tissue cores; many had intense fibroblast activity with oedema and prominent capillaries. Large accumulations of plasma cells, neutrophils and macrophages were present.

The mucous glands were numerous but not distended. Many acini were surrounded by small foci of lymphocytes and plasma cells; a few acini contained plasma cells.

The muscle layer appeared disrupted by polyp formation.

(ii) Other significant findings:

There was severe autolysis and sloughing of bronchiolar epithelium. A few gland acini were present in the bronchioles.

There was patchy alveolar oedema. A moderate degree of anthracosis was seen.

CASE - 2: SUBJECT - Cairn Terrier: AGE - 10 years: SEX - M

History: A history of coughing for between 6 and 9 months.

Clinical examination: This dog was easily exhausted but appeared bright. It was very fat. There was a marked cough on expiration together with prolonged bouts of coughing. A serous nasal discharge was also present.

The respiratory rate was 70/minute with forced expirations and a noticeable abdominal effort. The breathing was harsh and bronchovesicular with sibilant rhonchi.

Pulse rate 130/minute with a poor volume. There was sinus arrythmia and muffling of the heart sounds.

Temperature normal.

Radiology: A slight overall loss of translucency was present, suggestive of bronchitis. Also an increase in peribronchial markings.

Post mortem examination: (i) Tracheobronchial tree:

Pinkish viscid mucopus was present in all the bronchi. There was a slight dilatation of bronchial lumena with flattening of the bronchial epithelium. There was autolysis with sloughing of the epithelium in places.

No polyp formation was seen in the lamina propria. There was congestion with capillary engorgement and oedema but cell infiltration was minimal with a light patchy infiltrate of plasma cells, lymphocytes and macrophages.

All mucous glands were slightly distended with abundant secretions. A few small groups of plasma cells were clustered around the gland acini.

Many of the plates of cartilage had foci of calcification.

The muscle layer appeared normal.

(ii) Other significant findings:

A few gland acini were present in the bronchioles.

There was a patchy alveolar oedema. There was a foreign body reaction with many neutrophils and macrophages around globules of radiographic contrast material. There was mild anthracosis.

The kidneys had a mild focal glomerulosclerosis.

CASE - 3: SUBJECT - Terrier cross: AGE - 15 years: SEX - F

History: This dog had been coughing for approximately 3 months after a gradual onset.

Clinical examination: On admission the dog was dull and in fair condition - it had had abdominal distension for one week prior to admission. Marked dyspnoea present with a pronounced expiratory effort and abdominal breathing.

The respiratory rate was 50/minute; breathing was bronchial in character with rales and sibilant rhonchi. There was a purulent nasal discharge.

A pulse rate of 160/minute with very poor volume. A distinct fluid thrill could be elicited on ballottement of the abdomen.

The temperature was normal but the dog was anorexic and had polydipsia.

Radiology: There was a patchy loss of translucency over the lung fields which could have been due to venous congestion.

Post mortem examination: (i) Tracheobronchial tree:

All the major bronchi were dilated and filled with abundant mucopus.

Marked subepithelial oedema was seen at many sites; this had led to sloughing of epithelium in some areas. Neutrophils could be seen migrating through the epithelium.

The lamina propria had a heavy infiltration of plasma cells and lymphocytes with oedema of the superficial lamina propria.

The mucous glands were hypertrophied with numerous acini many of which contained secretion and neutrophils.

The smooth muscle appeared prominent in all sections.

Many of the cartilage plates had foci of calcification.

(ii) Other significant findings:

All bronchioles were plugged with neutrophils.

There was an acute purulent pneumonia with macrophages and many neutrophils in the alveoli. A moderate degree of anthracosis was seen and there was widespread emphysema.

Congestive cardiac failure was present with an enlarged right heart suggesting early cor pulmonale. There were no obvious valvular or myocardial lesions present. The liver had chronic venous congestion.

CASE - 4: SUBJECT - West Highland Terrier: AGE - 6 years: SEX - M

History: This dog had previously been admitted with a history of eczema, thirst and polyuria. It was treated at that time with cortisone and discharged. It was readmitted 7 months later with a history of cough and panting for several months. Breathing had been laboured for 3 months.

Clinical examination: On admission, the dog was bright and in good condition with a very fat abdomen. Emphysematous crackling heard over both lung fields together with oedematous rales.

Respiratory rate 40/minute.

Pulse rate of 120/minute with a poor volume. The heart sounds were muffled by respiratory sounds but there was no obvious murmur.

Temperature normal.

Radiology: The heart shadow was enlarged and the complete lung field had a loss of translucency with thickening of the pleural lines.

Post mortem examination: (i) Tracheobronchial tree:

A moderate amount of greyish viscid mucopus was present in the smaller bronchi. Many of the smaller bronchi appeared to be plugged by mucus and neutrophils.

The epithelium had foci of goblet cell proliferation; in other areas of epithelium, migrating neutrophils were seen. The epithelium overlying polyps was frequently dedifferentiated.

There was early polyp formation in the lamina propria, with oedema and flattening of the epithelium, and a cellular infiltration of neutrophils, macrophages, lymphocytes and small numbers of plasma cells.

Mucous glands were numerous with moderate distension and occasional neutrophils present in acini.

The smooth muscle and cartilage both appeared normal.

(ii) Other significant findings:

Mucous gland acini were present in many bronchioles. Many bronchioles were plugged by mucus and neutrophils. There was a chronic pneumonia present with macrophages in the alveoli, alveolar epithelial hyperplasia

and an influx of plasma cells and neutrophils. Anthracosis was mild.

There was mild endocardosis of both atrioventricular valves. The kidneys had a mild chronic interstitial nephritis and a proliferative glomerulonephritis.

CASE - 5: SUBJECT - West Highland Terrier: AGE - 3 years: SEX - F

History: A history of continual cough and wheeze since the dog had been acquired 2 months previously.

Clinical examination: On admission the dog was dull and rather thin. Continual coughing with a noticeable abdominal expiratory effort; on occasion, there was retching with production of phlegm.

A respiratory rate of 30/minute with the respirations very laboured after exercise. There was emphysematous crackling heard over both lung fields.

Pulse rate 100 - 110/minute.

Temperature was normal.

Radiology: There was a patchy loss of translucency involving the complete lung field.

Post mortem findings: (i) Tracheobronchial tree:

All bronchi contained mucopus and had pronounced folding of their mucosa, giving a roughened surface.

Some sloughing of epithelium was noted, especially over polyps; in addition, ulceration and dedifferentiation could be seen in other areas. Goblet cells appeared particularly numerous in some bronchi.

The lamina propria had many small polyps - these were areas of oedematous connective tissue with congested capillaries, fibroblasts, neutrophils, plasma cells and macrophages.

The mucous glands were markedly hypertrophied and had excess mucus in their acini. Small numbers of plasma cells surrounded many of the acini.

The smooth muscle appeared thickened.

(ii) Other significant findings:

Many bronchioles were filled with mucus. All the lung lobes had a moderate degree of emphysema. There was very little anthracosis.

The kidneys had a mild focal suppurative nephritis.

CASE - 6: SUBJECT - Terrier cross: AGE - 10 years: SEX - M

History: There had been a fairly sudden onset of retching and choking 2 months previously.

Clinical examination: At the time of admission, this dog was bright and in fair condition. The dog had a paroxysmal choking cough which often lasted for up to 5 minutes with production of phlegm; a nasal discharge was also present.

Respiratory rate was 40/minute; emphysematous crackling and oedematous rales over the lung fields. The pharynx was inflamed and there was a catarrhal exudate.

Pulse rate was 120/minute with a good volume but marked sinus arrhythmia. A systolic murmur was present on the right side. The dog was cyanotic.

Temperature was normal.

Radiology: The thorax had an overall patchy loss of translucency which was suggestive of pneumonia. There appeared to be an overall thickening of the lung field.

Post mortem findings: (i) Tracheobronchial tree:

The bronchi contained mucus with macrophages, neutrophils and lymphocytes.

The epithelium was sloughed in some areas; migrating neutrophils were seen in other areas.

In the lamina propria there was a light infiltration of plasma cells and lymphocytes, with marked congestion of blood vessels and patchy oedema. Numerous hyperplastic mucous glands present with moderate distension of acini with excess mucus. Large numbers of plasma cells and lymphocytes around the acini.

The smooth muscle and cartilage appeared normal.

(ii) Other significant findings:

All bronchioles were plugged with mucus and neutrophils. There was also severe peribronchiolar cuffing by plasma cells and lymphocytes.

Anthracosis was only light and patchy in distribution.

There was severe endocardosis of both atrioventricular valves.

CASE - 7: SUBJECT - Bull Terrier: AGE - 9 years: SEX - M

History: The duration of cough was not known in this dog but it exceeded two months.

Clinical examination: No data available.

Radiology: No data available.

Post mortem examination: (i) Tracheobronchial tree:

A large amount of thick mucopus was present in all the bronchi. In addition the bronchial mucosa was thickened with many large polyps (up to 5mm in diameter) protruding into the lumen. The lobar bronchus of the right diaphragmatic lobe was particularly severely affected.

In the epithelium were many goblet cells and migrating neutrophils were seen frequently. Epithelial hyperplasia was noted in some areas with ulceration and dedifferentiation over the polyps.

Many large polyps were present, especially in the larger bronchi; heavy infiltrates of lymphocytes and plasma cells with oedematous fibrous tissue and prominent capillaries were found in these polyps. There was widespread subepithelial oedema with foci of lymphocytes and plasma cells.

The mucous glands were hyperplastic with moderate distension with mucus secretion. Heavy accumulations of plasma cells and lymphocytes were present around the acini.

Muscle and cartilage both appeared normal.

(ii) Other significant findings:

Mucous glands were noted extending into the bronchioles.

There was mild focal emphysema and moderate anthracosis. Anthracosis was particularly heavy around the bronchioles.

There was severe endocardosis of both atrioventricular valves.

CASE - 8: SUBJECT - Terrier cross: AGE - 12 years: SEX - M

History: This dog had a history of coughing for 2 months. There was an ulcerated swelling on the angle of the left jaw.

Clinical examination: An intermittent cough was present which was particularly severe when the dog was excited.

Respiratory rate 48/minute.

Pulse rate 120/minute.

Temperature was 104°F on admission.

The left tonsil was enlarged.

Radiology: The heart appeared huge on radiographic examination.

Post mortem examination: (i) Tracheobronchial tree:

A considerable amount of mucopus and oedema fluid were present in the bronchi. Several small red polyps were seen on the bronchial mucosa, projecting into the lumen.

Large numbers of goblet cells and migrating neutrophils could be seen in the epithelium. There was ulceration and dedifferentiation of epithelium over the polyps, with flattening and attenuation of epithelial cells.

The lamina propria contained many polyps - in these there was congestion of capillaries and heavy infiltration of plasma cells. The bronchial wall had a moderately heavy infiltration of neutrophils with clumps of lymphocytes.

The mucous glands were hyperplastic, distended with mucus and surrounded by numerous clumps of plasma cells.

Smooth muscle and cartilage both appeared normal.

(ii) Other significant findings:

Many of the bronchioles contained mucus. The lung parenchyma were diffusely oedematous. There were moderately heavy anthracotic deposits, particularly around the bronchioles.

There was severe valvular endocardosis of both atrioventricular valves. There was a squamous carcinoma of the tonsil and the swelling on the angle of the left jaw was a cutaneous mastocytoma.

CASE - 9: SUBJECT - Shetland Sheep Dog: AGE - 6 years: SEX - M

History: This dog had been coughing and wheezing for 18 months since an episode of "pneumonia" which had never really cleared up. On admission this dog was bright and rather fat.

Clinical examination: There was a moist wheezy cough and occasionally sustained bouts of coughing; these lasted for up to an hour and exhausted the dog. The dog also had a purulent nasal discharge.

Respiratory rate 30-40/minute with inspiratory snoring and a forced expiration with abdominal effort. Sonorous rhonchi were heard on inspiration and sibilant rhonchi on expiration.

Pulse rate 105/minute with marked sinus arrhythmia.

The temperature fluctuated between 100 - 104°F with intermittent pyrexia while under observation.

Radiology: Radiologic examination revealed a patchy loss of translucency especially around the hilus which was suggestive of pneumonia.

Post mortem examination: (i) Tracheobronchial tree:

Thick mucopus was present in the nostrils, nasal cavity, naso-pharynx and tracheobronchial tree. Polyps were present on the mucosa of the pharynx and bronchi, particularly at the bronchial bifurcations; these polyps were up to 3mm in size. In addition the bronchial lumina appeared stenosed.

Moderate numbers of neutrophils were seen migrating through the epithelium.

There were many polyps with prominent capillaries and abundant fibrous tissue. The lamina propria appeared thickened and fibrosed with aggregates of lymphocytes.

The mucous glands were hyperplastic with marked distension; many acini contained mucus, neutrophils and macrophages.

The muscle layer was distorted and interrupted by fibrosis and acinar distension.

(ii) Other significant findings:

Neutrophils and macrophages were present in many of the bronchioles. All the lung lobes were mottled, grey-red and very oedematous.

This dog had distemper, probably contracted after admission to U.G.V.S. There were numerous eosinophilic inclusion bodies in the respiratory epithelium together with a generalised proliferative pneumonia. A bacterial pneumonia had superimposed on this; neutrophils were numerous in the airspaces and bacteria were visible on the ciliated epithelium.

CASE - 10: SUBJECT - Poodle: AGE - 7 years: SEX - M

History: This dog had had an intermittent cough for six months.

Clinical examination: On admission the dog was bright and fat. Coughing was spontaneous and frequent. At times white phlegm was produced.

Respiratory rate 48/minute with pronounced rhonchi and rales.

Pulse rate 156/minute with a poor volume. A mild systolic murmur was also heard. This dog became cyanotic very easily.

Temperature was normal.

The dog was also polydipsic.

Radiology: Examination of lung fields revealed a loss of translucency especially around the major bronchi indicating some degree of peribronchial cuffing.

Post mortem examination: (i) Tracheobronchial tree:

A copious mucopurulent exudate present in the bronchi, with plugging of some bronchi. The mucosa was congested and slightly roughened.

The epithelium contained a few migrating neutrophils.

In the lamina propria there was marked congestion of capillaries and a heavy infiltrate of plasma cells, together with some neutrophils and lymphocytes.

Mucous glands were hyperplastic and distended by mucus; large numbers of plasma cells around gland acini.

The smooth muscle layer appeared thickened.

(ii) Other significant findings:

Mucous glands and plasma cells were present in the bronchiolar walls. There were focal areas of chronic pneumonia with macrophages and plasma cells. Pulmonary vessels were congested and there was a patchy pulmonary oedema. Anthracotic deposits were present around blood vessels.

CASE - 11: SUBJECT - Corgi: AGE - 5 years: SEX - M

History: This dog had a history of coughing six months previously; this had been treated and had temporarily cleared. The coughing subsequently recurred three months later.

Clinical examination: On admission the dog was bright and in good condition. There was very severe spontaneous coughing, with occasional retching and production of clear fluid.

Respiratory rate was 40/minute; emphysematous crackling and intermittent rhonchi were heard over both lung fields.

Pulse rate was 130/minute.

Temperature was normal.

Radiology: There was evidence of chronic bronchitis with thickening of bronchi and possible bronchiectasis. In addition, the heart shadow was considerable enlarged, particularly the right ventricular region.

Post mortem examination: (i) Tracheobronchial tree:

All bronchi were markedly dilated and the trachea and bronchi contained mucoid, catarrhal exudate. The bronchial mucosa was roughened, granular and congested. The bronchial mucus was mixed with neutrophils and macrophages. The epithelium had sloughed off in some areas; other areas had numerous goblet cells and migrating neutrophils, with epithelial dedifferentiation and erosion over polyps.

There were numerous large polyps filled with neutrophils and macrophages; in the lamina propria there was a heavy cellular infiltrate of plasma cells, lymphocytes and neutrophils, together with congestion of blood vessels and numerous fibroblasts.

Numerous mucous glands with some distension and large numbers of adjacent plasma cells.

The smooth muscle layer was severely disrupted by the cellular infiltrate.

(ii) Other significant findings:

The bronchioles were severely distorted by a heavy cellular

infiltrate of plasma cells and lymphocytes with narrowing of the lumen.

There were large pale raised areas of emphysema, particularly in the diaphragmatic lobes. Emphysema was largely subpleural in distribution and had caused collapse of neighbouring alveoli.

There was a mild degree of pulmonary oedema and a little anthracosis.

CASE - 12: SUBJECT - Cocker Spaniel: AGE - 5 years: SEX - F

History: This dog had had pneumonia when it was a puppy. It had been coughing for several months at the time of admission.

Clinical examination: Coughing easily induced.

It was bright and in good condition.

Respiratory rate was 18/minute with inspiratory rhonchi and excessive abdominal effort.

Pulse rate was 114/minute with sinus arrhythmia.

Temperature was 103°F.

Radiology: Examination revealed evidence of bronchial thickening together with lymph node enlargement at the tracheal bifurcation.

Post mortem examination: (i) Tracheobronchial tree:

All the bronchi filled with mucus, neutrophils and sloughed epithelial cells. The mucous membrane at the tracheal bifurcation was thickened and roughened with several polyps up to 3mm in diameter. The tracheal and bronchial mucosa was very congested. The smaller bronchi had many small nodules, up to 1mm, on the mucosa, resulting in partial obstruction.

There was sub-epithelial oedema and thickening of the lamina propria by an infiltrate of plasma cells and lymphocytes. Many polyps were seen in which there were numerous engorged capillaries, oedematous fibrous tissue and infiltrating plasma cells and lymphocytes.

Mucous glands were very hyperplastic with distension of acini by mucus.

Many of the cartilage plates had foci of calcification.

(ii) Other significant findings:

In the bronchioles there was thickening and folding of the mucosa, an intense cellular infiltrate and a thickened layer of smooth muscle. There was intense oedema and congestion of the lung parenchyma and many haemosiderin-laden macrophages were observed in the alveoli.

There was a mild degree of anthracosis.

CASE - 13: SUBJECT - Pug: AGE - 12 years: SEX - M

History: This dog had been treated for a year for "heart cough". In addition, the dog had had a gagging "throat" cough in the six months prior to admission. The dog was bright and very fat at the time of admission.

Clinical examination: Persistent coughing was induced on the slightest movement. This cough was often paroxysmal and led to exhaustion.

Respiratory rate was 50/minute with sonorous rhonchi over both chest fields. The dog rapidly became cyanotic with exercise. There was also halitosis and a bilateral serous nasal discharge.

Pulse rate was 170/minute with sinus arrhythmia. A systolic murmur was detected on the left side.

Temperature was normal.

Radiology: Examination revealed increased density around the tracheal bifurcation together with evidence of pulmonary oedema. The heart was enlarged and rounded with elevation of the trachea.

Post mortem examination: (i) Tracheobronchial tree:

The major airways all contained scanty, thick, yellow pus and mucus. There was sub-epithelial oedema and migrating neutrophils.

In the lamina propria there was a minimal infiltration of neutrophils, plasma cells and macrophages, particularly in the tips of the folds. There was a heavier infiltrate in the trachea with neutrophils and epithelial dedifferentiation, ulceration and erosion.

The mucous glands were hyperplastic, with distension of some acini and peri-acinar plasma cells and lymphocytes.

The smooth muscle layer appeared thickened in some sections. Foci of calcification were noted in many cartilage plates.

(ii) Other significant findings:

There were numerous gland acini in the bronchiolar walls. In the dorsal region of the diaphragmatic lobes there were superficial areas of

pneumonia - this was a patchy proliferative pneumonia with fibrosis of alveolar walls and collapse of airways. In addition there was mild subpleural emphysema. There was a moderate degree of anthracosis, especially in the subpleural regions.

There was a mild degree of endocardosis.

CASE - 14: SUBJECT - Shetland Sheep Dog: AGE - 9 years: SEX - M

History: This dog had been coughing for one year after a gradual onset. This cough worsened in the three weeks prior to admission.

Clinical examination: On admission, the dog was very fat.

Coughing with production of mucus. The dog tended to become breathless very easily.

Respiratory rate was 30/minute with emphysematous crackling heard over both lung fields. In addition there was a bilateral serous nasal discharge.

Pulse rate was 90/minute with a poor volume and marked sinus arrhythmia.

Temperature was normal.

Radiology: Examination revealed a diffuse mottling with an increase in density over all the lung field, suggestive of bronchitic or pneumonic change. There was also evidence of emphysematous bullae especially in the diaphragmatic lobes. The heart shadow appeared slightly enlarged.

Post mortem examination: (i) Tracheobronchial tree:

All the major bronchi contained viscid mucopurulent exudate. Many bronchi appeared moderately dilated and some smaller bronchi had roughened mucosae.

The epithelium was sloughed in some areas, with flattening and dedifferentiation over the polyps; many migrating neutrophils were present in other areas. Epithelial goblet cells were very numerous, and were found in clumps in the mucosal folds.

In the lamina propria there were many large oedematous folds filled with macrophages and lymphocytes. The tips of these folds were filled with neutrophils.

The mucous glands were hyperplastic with a moderate amount of acinar distension: clumps of lymphocytes and plasma cells were found around the acini.

(ii) Other significant findings:

The bronchioles were distorted and narrowed by a heavy cellular

infiltrate of plasma cells and lymphocytes. Many mucous glands were present in the walls.

There was moderately severe emphysema and fairly large deposits of anthracotic material.

There was mild endocardosis of left atrioventricular valve.

CASE - 15: SUBJECT - West Highland Terrier: AGE - 12 years: SEX - M

History: A history of pneumonia two years prior to admission. This was followed by two "asthma-like" attacks and bronchitis was diagnosed six months prior to admission, with the dog coughing up mouthfuls of yellow fluid. In the week prior to admission, breathing became more laboured and coughing increased.

Clinical examination: The dog was fat and rather dull with an enlarged abdomen. Coughing spontaneously especially when disturbed.

Respiratory rate was 35-50/minute which readily rose to approximately 80/minute when the dog was disturbed. There was a respiratory wheeze with marked inspiratory and expiratory emphysematous crackling over both lung fields. Faint rhonchi were also detected.

Pulse rate was 110/minute with marked sinus arrhythmia.

Temperature was normal.

Radiology: The lungs had a radiological appearance consistent with pneumonia. The heart appeared to be slightly enlarged.

Post mortem examination: (i) Tracheobronchial tree:

All bronchial walls appeared to be thickened and a small amount of greenish pus was present in the segmental bronchi. The lobar bronchi contained a mixture of mucus, neutrophils and cell debris.

The epithelium was sloughed in some areas; erosion and ulceration of epithelium was noted particularly over polyps.

Polyps were widespread in all bronchi and were characterised by fibroblast proliferation and oedema. In the lamina propria there was a cellular infiltrate of plasma cells and lymphocytes; large numbers of neutrophils were seen near the polyps.

The mucous glands were hyperplastic and several acini were markedly distended with retained secretion.

The layer of smooth muscle was severely interrupted by a cellular infiltrate. Foci of calcification were seen in the plates of cartilage.

(ii) Other significant findings:

Many gland acini were present in the bronchiolar walls. There was also widespread alveolar oedema and perivascular anthracosis.

CASE - 16: SUBJECT - Spaniel: AGE - 13 years: SEX - F

History: There was a history of intermittent coughing for two years.

Clinical examination: The dog was dull on admission, but very fat, with an enlarged abdomen. Frequent coughing with paroxysms of harsh coughing particularly at night. (Coughing was not associated with exercise).

Respiratory rate was 45/minute with harsh respiratory sounds - emphysematous crackling and rhonchi on auscultation. There was also hyperpnoea and the dog had halitosis.

Pulse rate was 100/minute with sinus arrhythmia.

Temperature was normal.

Radiology: There was radiological evidence of peribronchial thickening.

Post mortem examination: (i) Tracheobronchial tree:

All the segmental bronchi contained mucopus, particularly the bronchi of the diaphragmatic lobes.

There was severe autolysis of the epithelium.

There was a very mild cellular infiltrate of plasma cells and lymphocytes in the lamina propria; these cells were grouped mainly around the mucous gland acini which were hyperplastic but were not very distended. All the blood vessels were engorged and prominent.

The smooth muscle layer appeared to be thickened at some sites.

(ii) Other significant findings:

The bronchioles were filled with mucus. The bronchiolar epithelium was sloughed in a few areas, and there were many goblet cells present. These goblet cells were forming large clumps at some sites. The lamina propria was infiltrated by small aggregates of plasma cells and lymphocytes. Mucous gland acini were present in all the bronchioles examined.

All lung lobes were very dark in colour with oedema and congestion and there were pulmonary emboli in the large vessels. In addition, there was marked anthracosis. Sections revealed alveolar oedema, congestion of vessels and many thrombi present. There was a moderate degree of endocardosis of the left atrioventricular valve.

The kidneys were severely affected by renal amyloidosis.

CASE - 17: SUBJECT - Terrier cross: AGE - 14 years: SEX - M

History: Gradual onset of coughing over several months.

Clinical examination: The dog had lost weight and was thin and rather dull on admission. Frequent coughing leading to exhaustion. The dog also had a serous nasal discharge.

Respiratory rate was 80/minute with hyperpnoea and harsh respiratory sounds on auscultation.

Pulse rate was 120/minute with sinus arrhythmia and poor volume. There was a distinct systolic murmur.

Temperature was normal.

Radiology: Not available.

Post mortem examination: (i) Tracheobronchial tree:

The bronchi contained excess, yellow, viscid mucus which extended down to the segmental bronchi. This mucus contained neutrophils and haemosiderin-laden macrophages.

The epithelium contained migrating neutrophils and many goblet cells, particularly in the epithelial folds.

In the lamina propria there was a relatively light, patchy infiltrate of neutrophils and lymphocytes mainly confined to the sub-epithelial regions. There were many small polyps in some areas, and also heavy cellular infiltrate with congestion of capillaries, epithelial dedifferentiation and erosion.

The mucous glands were hyperplastic and distended with mucus. Many of the acini were surrounded by plasma cells and lymphocytes.

The smooth muscle appeared thickened in some areas.

(ii) Other significant findings:

The bronchioles contained mucus and there were also mucus gland acini in their walls. In all the lung lobes there was oedema and congestion with focal areas of acute pneumonia. There was congestion of alveolar capillaries and many neutrophils in the alveoli. There were large focal anthracotic deposits present in the lung parenchyma.

There was severe endocardosis of left atrioventricular valve of heart - the right side was affected but to a lesser degree. There was ascites and chronic venous congestion of the liver.

CASE - 18: SUBJECT - Poodle: AGE - 8 years: SEX - M

History: A history of coughing with breathlessness for approximately four months.

Clinical examination: At the time of admission the dog was fat and had an enlarged abdomen. Intermittent coughing on examination.

Respiratory rate was 30/minute.

Pulse rate was 130/minute with sinus arrhythmia.

Temperature was normal.

Radiology: The right side of the heart appeared to be enlarged on radiographic examination.

Post mortem findings: (i) Tracheobronchial tree:

Moderate amounts of mucus were present in the bronchi together with large numbers of neutrophils.

The epithelium contained many goblet cells, particularly in the epithelial folds. There were also areas of epithelial hyperplasia and dedifferentiation; in other areas large numbers of neutrophils were observed migrating through the epithelium.

There was a very heavy infiltrate of neutrophils in the lamina propria with areas of sub-epithelial oedema. A few small polyps were noted; these contained mostly neutrophils and macrophages.

Mucous glands were moderately hyperplastic and some acini were distended with neutrophils. There were a few lymphocytes and plasma cells around the acini, and large numbers of macrophages around some glands.

The muscle layer appeared to be thickened.

(ii) Other significant findings:

Gland acini could be seen extending down into the bronchioles but there was little inflammation of the bronchioles. All the lung lobes were heavy and congested. There was intense congestion of all the blood vessels, together with marked anthracosis.

There was a moderate degree of endocardosis of both atrioventricular valves.

CASE - 19: SUBJECT - Shetland Sheep Dog: AGE - 9 years: SEX - F

History: This dog had been coughing for several months after a gradual onset.

Clinical examination: It was very fat at the time of admission.

A spontaneous persistent cough with occasional retching and production of white and yellow phlegm. The dog was coughing all day and all night.

Respiratory rate was 35/minute with noticeable abdominal effort. There was also hyperpnoea and emphysematous crackling was heard on auscultation.

Pulse rate was 100/minute with a distinct systolic murmur.

Temperature was 103°F.

Radiology: Dense lung markings and bronchial thickening were visible on the radiographs; these were highly suggestive of chronic bronchitis.

Post mortem examination: (i) Tracheobronchial tree:

The bronchial tree contained mucus mixed with neutrophils and macrophages.

The epithelium was flattened and there were areas with migrating neutrophils. Large numbers of goblet cells were present but these were often empty. The epithelium was totally eroded at a few sites. Numerous neutrophils appeared to be entering the lumen at sites where the epithelium was totally eroded.

The mucous glands were very hyperplastic and many acini were very distended and contained neutrophils. Large numbers of plasma cells were seen around the acini.

(ii) Other significant findings:

There were numerous gland acini and marked infiltrations of plasma cells in the bronchiolar walls. All the lung lobes were pale and cream-grey in colour with marked anthracosis. There was mild emphysema around the edges of the anterior lobes.

There was right ventricular hypertrophy; this was confirmed by weighing the ventricles. There was mild atrioventricular endocardosis.

CASE - 20: SUBJECT - Cocker Spaniel: AGE - 4 years: SEX - M

History: A history of coughing for eight to ten weeks with dyspnoea in the ten days prior to admission.

Clinical examination: This dog was in reasonable condition but very dull at the time of admission. There was frequent coughing with occasional retching and production of sputum. A copious purulent nasal discharge was also present.

Respiratory rate was 48/minute with marked dyspnoea and rhonchi were heard on auscultation.

Pulse rate was 144/minute and of poor volume.

Temperature not recorded.

Radiology: The lung fields had a loss of translucency, especially over the diaphragmatic lobes; also a marked increase in broncho-vascular markings indicating peribronchial inflammation. The heart was bilaterally enlarged.

Post mortem findings: (i) Tracheobronchial tree:

All the major airways were filled with an abundant foam, mucus and pus mixture. This mixture also contained neutrophils and macrophages.

There was widespread sloughing of epithelium. Dedifferentiation, ulceration and neutrophil infiltration were observed in other areas.

In the lamina propria there was a moderate infiltrate, mostly macrophages, with some lymphocytes and neutrophils. The capillaries were congested, particularly in the sub-epithelial region and many contained neutrophils and macrophages.

The mucous glands were hyperplastic and distended; many acini contained mucus and were surrounded by plasma cells and large numbers of lymphocytes.

The muscle layer was markedly thickened.

(ii) Other significant findings:

There were plasma cells and macrophages in the bronchiolar walls. Anthracosis was only light and primarily peribronchiolar in distribution. There was severe oedema of all lung lobes.

CASE - 21: SUBJECT - Poodle: AGE - 5 years: SEX - M

History: This dog had been coughing for several months.

Clinical examination: Frequent coughing. It was thin and dull at the time of admission.

Respiratory rate of 60/minute with hyperpnoea and increased respiratory sounds.

Pulse rate of 160/minute with a poor volume. A distinct systolic murmur present on auscultation. The mucosae were pale and cyanotic and there was preputial oedema.

Temperature was normal.

Radiology: The lung field appeared abnormally opaque. The trachea appeared raised but the heart was obscured by dense pulmonary tissue and possibly fluid.

Post mortem examination: (i) Tracheobronchial tree:

Variable amounts of mucus were present in the bronchi.

Sloughing of epithelium was observed at some sites; in other areas there were increased numbers of goblet cells and migrating neutrophils. Foci of sub-epithelial oedema also present.

In the lamina propria, there was a mild cellular infiltration of lymphocytes and plasma cells, with focal accumulations particularly in the tips of the mucosal folds. A few small polyps could be seen.

Mucous glands were hyperplastic and distended in certain areas; many acini were surrounded by plasma cells.

Muscle and cartilage both appeared normal.

(ii) Other significant findings:

There were a few gland acini in the bronchioles. The lung lobes were pale and mottled with moderate focal anthracosis. There was marked oedema and pneumonia with neutrophils and macrophages in alveoli.

In addition, there was congestive cardiac failure with severe bilateral endocardosis, a dilated globular heart, fluid in the thorax and abdomen, chronic venous congestion of the liver and preputial oedema.

Mild chronic interstitial nephritis was also noted.

CASE - 22: SUBJECT - Jack Russell Terrier: AGE - 6 years: SEX - F

History: This dog had been coughing and wheezing for several months. In addition, there had been a "gagging" noise in the throat for ten days prior to admission.

Clinical examination: Coughing frequently with occasional production of sputum.

Respiratory rate 30/minute with hyperpnoea and pronounced abdominal effort. Rhonchi and emphysematous crackling heard over the lung fields.

Pulse rate was 120/minute with sinus arrhythmia.

Temperature was normal.

Radiology: No abnormalities were detected on examination of the lung field. The right side of the heart appeared slightly enlarged.

Post mortem examination: (i) Tracheobronchial tree:

Considerable amounts of mucus were present in the airways, particularly in the segmental bronchi. The lobar bronchi had polypoid proliferations on their walls.

Many of the epithelial goblet cells were exhausted and numerous neutrophils were migrating into the lumen. There were areas of sub-epithelial oedema and accumulations of leucocytes in the lobar bronchi. The segmental bronchi were much more severely affected with oedema, neutrophils and plasma cells. Polyp formation was seen with dedifferentiation and attenuation of epithelium and severe oedema of the lamina propria.

Mucous glands were hyperplastic and distended with retained mucus. The glands in the smaller bronchi were surrounded by plasma cells.

(ii) Other significant findings:

Many bronchioles were plugged by neutrophils, macrophages and lymphocytes. A few gland acini were present in the bronchioles. The lungs were deep red and there was marked anthracosis. In the anterior lobes there was emphysema, particularly peripherally. There were foci of chronic pneumonia adjacent to the segmental bronchi with congestion of vessels and cuffing of airways by clumps of plasma cells and macrophages.

CASE - 23: SUBJECT - Mongrel: AGE - 11 years: SEX - M

History: A history of persistent coughing for several weeks prior to admission.

Clinical examination: On admission the dog was found to be very obese. The dog was coughing frequently; the cough was harsh, dry and non-productive. There was a serous nasal discharge.

Respiratory rate was 30/minute.

Pulse rate was 120/minute with a poor volume. There was a sinus arrhythmia and a distinct systolic murmur.

Temperature was normal.

Radiology: Over the lung field there was a marked increase in linear markings indicative of chronic bronchitis. Pulmonary oedema was present and there was also possibly some fluid in the thoracic cavity.

Post mortem examination: (i) Tracheobronchial tree:

Thick tenacious mucopus was present in all the lobar bronchi.

The epithelium contained large numbers of goblet cells. Migrating neutrophils were frequently seen.

In the lamina propria there was a moderate cellular infiltrate composed mainly of plasma cells and lymphocytes. Many neutrophils could be seen in sub-epithelial capillaries.

Mucous glands were numerous, particularly in the smaller bronchi. Some acini were distended and some contained neutrophils, while many were surrounded by plasma cells and lymphocytes.

(ii) Other significant findings:

All lung lobes were very pale and there was severe anthracosis; emphysema was moderate but widespread. The bronchioles were moderately affected and a few gland acini were visible.

Mild chronic pyelonephritis was also noted.

CASE - 24: SUBJECT - Staffordshire Bull Terrier: AGE - 6 years: SEX - F

History: This dog had been coughing for 8 months after a gradual onset.

Clinical examination: It was very fat at the time of admission. Coughing frequently. This dog tended to retch and produce white phlegm.

Respiratory rate 36/minute.

Pulse rate was 120/minute.

Temperature was normal.

Radiology: There was an increase in linear markings throughout the lung field, and this was indicative of bronchitis.

Post mortem findings: (i) Tracheobronchial tree:

All the lobar bronchi were plugged by large amounts of tenacious clear mucus mixed with neutrophils.

Some areas of the epithelium contained clumps of migrating neutrophils; other areas had epithelial dedifferentiation and erosion with pronounced sub-epithelial oedema. There were large numbers of exhausted goblet cells present.

The lamina propria was very oedematous and had a heavy cellular infiltrate, mostly neutrophils, extending deep into the submucosa. Oedematous folds of the submucosa were forming small polyps in some areas.

The mucous glands were numerous with some distension, and there were neutrophils in some acini. The glands were surrounded by plasma cells.

(ii) Other significant findings:

There was a very severe bronchiolitis and peribronchiolitis. A few foci of mild emphysema were observed in the apical and cardiac lobes. Anthracosis of lungs and bronchial lymph nodes was minimal.

CASE - 25: SUBJECT - Bull Terrier: .AGE - 14 years: SEX - M

History: A history of sporadic coughing over several months. The dog had been discharged from kennels with a dry hacking cough 7 days prior to euthanasia.

Clinical examination: This dog was submitted for examination immediately after euthanasia. It was markedly obese. No clinical examination was carried out at U.G.V.S.

Radiology: Not available.

Post mortem examination: (i) Tracheobronchial tree:

The lobar bronchi contained relatively small amounts of tenacious mucopus, together with many neutrophils.

There were areas of epithelium with many migrating neutrophils, particularly in the tips of the folds. Numerous goblet cells were seen in the folds of the mucosa, many of them exhausted.

In the lamina propria, there was a mild infiltrate of plasma cells and neutrophils in the tips of the folds. The capillaries were dilated and many contained neutrophils.

Glands were slightly increased in number with distension of acini with mucus and neutrophils. Many acini were surrounded by plasma cells and groups of neutrophils.

(ii) Other significant findings:

The bronchioles appeared to have focal areas of epithelial hyperplasia. The lung lobes were pale in colour, but were markedly anthracotic; there were foci of chronic pneumonia with clumps of plasma cells in the alveoli and fibrosis of alveolar walls. In all the lung lobes there was mild to moderate subpleural emphysema. In all bronchioles there were mucous gland acini and these were often surrounded by plasma cells.

CASE - 26: SUBJECT - Miniature Pinscher: AGE 7 years: SEX - F

History: This dog began coughing in kennels 3 months prior to admission; the coughing had then got steadily worse.

Clinical examination: The dog was very fat at the time of admission. A frequent cough especially at night, with retching at times.

Respiratory rate of 30/minute with hyperpnoea and pronounced emphysematous crackling and rhonchi.

Pulse rate of 120/minute with sinus arrhythmia.

Temperature was normal.

Radiology: Examination revealed a loss of translucency in the lung fields probably due to bronchial thickening and parenchymal change.

Post mortem examination: (i) Tracheobronchial tree:

The bronchi contained strands and plugs of mucus.

The epithelium was hyperplastic in places and contained many migrating neutrophils. Goblet cells were numerous but were thin and attenuated and appeared exhausted. Subepithelial oedema was seen, together with epithelial dedifferentiation and flattening.

There was a heavy infiltration of lymphocytes and plasma cells into the lamina propria. Blood vessels were congested and there was oedema and swelling of mucosal folds forming small polyps.

The mucous glands were very hyperplastic with marked proliferation at some sites; other areas had distended acini with accumulations of mucus. Many plasma cells and lymphocytes were clustered around blood vessels and glands.

(ii) Other significant findings:

There were masses of mucus and neutrophils in the bronchiolar lumina, with mucous glands and plasma cells in the bronchiolar walls. There was a terminal acute pneumonia with all lung lobes mottled grey-red. The lungs were very congested and the alveoli contained numerous macrophages and neutrophils. Many of the macrophages were distended by phagocytosed micropinocytotic inclusions of mucus.