GLOBULE LEUCOCYTES AND RESPIRATORY DISEASES IN CATTLE

Stephen Owarioro Akpavie, D.V.M.,M.Vet.Sc.,

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

Department of Veterinary Pathology,
University of Glasgow,
June, 1985

TO MY PARENTS,
DIEMITORO AND ROWENA,
FOR THEIR GUIDANCE.

CONTENTS

CONTENTS

	Page
Contents	i
List of Tables	v
List of Figures	ix
Acknowledgements	xvi
Declaration	xix
Summary	xxi
Abbreviations	xxiv
General Introduction	xxvii
CHAPTER ONE - REVIEW OF THE LITERATURE	
Bovine Parasitic Bronchitis	2
Immunity to Helminth Parasites	8
Mast Cells	12
Globule Leucocytes	17
Discussion	31
CHAPTER TWO - THE PREVALENCE AND DISTRIBUTION OF GLOBULE LEUCOCYTES IN THE RESPIRATORY TRACT OF CATTLE	
Introduction	36
Materials and Methods	37
Results	
The Prevalence and Distribution of Globule Leucocytes in the Respiratory Tract of Different Age Groups of Cattle.	40
Globule Leucocyte Population Density in the Respiratory Tract of Different Age Groups of Cattle.	42
Globule Leucocytes per Centimetre in the Bovine Respiratory Tract.	44
Discussion	45
Tables	49
Figures	60

Page

CHAPTER THREE - CHARACTERISATION OF THE GLOBULE LEUCOCYTE OF THE BOVINE RESPIRATORY TRACT

Introduction 65
Materials and Methods 67
Results
Light Microscopical Observations 71
Histochemical Observations 72
Electron Microscopic Findings 75
Discussion 82
Tables 89
Figures 92

CHAPTER FOUR - THE RELATIONSHIP BETWEEN PULMONARY GLOBULE LEUCOCYTES AND
(i) CLINICAL RESPIRATORY DISEASE
(ii) PULMONARY LESIONS
(iii) GLOBULE LEUCOCYTES IN OTHER MUCOUS MEMBRANES

Introduction 113
Materials and Methods 114
Results
Animals with Clinical Respiratory Disease 117
Animals with Gross Pulmonary Lesions 119
Histological Classification of Lung Lesions 123
Animals with Gross and Microscopic Pulmonary Lesions. 129
The Relationship Between Pulmonary Globule Leucocytes and Globule Leucocytes in Other Mucous Membranes. 132
Discussion 135
Tables 138
Figures 147

Page

CHAPTER FIVE - EXPERIMENTAL INDUCTION OF GLOBULE LEUCOCYTES IN THE RESPIRATORY TRACT OF CATTLE USING DICTYOCAULUS VIVIPARUS.

Introduction 155
General Materials and Methods 156
Experiment 1: Single Exposure of Calves To Dictyocaulus viviparus
Experimental Design 160
Results 160
Experiment 2: Oral Vaccination Followed by Oral Challenge with Dictyocaulus viviparus
Experimental Design 162
Results 164
Experiment 3: Oral Vaccination, Continuous Oral Challenge and Trickle Infections with Dictyocaulus viviparus
Experimental Design 169
Results 171
General Discussion of the Results of Experiments 1, 2 and 3. 177
Tables 181
Figures 186

CHAPTER SIX - GENERAL DISCUSSION AND CONCLUSIONS 208

APPENDIX ONE: Histopathological, Histochemical and Electron Microscopy Techniques 212

REFERENCES 226

LIST OF TABLES

LIST OF TABLES

CHAPTER TWO

TABLE 1: The age groups and number of animals examined.

TABLE 2: The prevalence of globule leucocytes in the bovine respiratory tract.

TABLE 3: The distribution of globule leucocytes along the respiratory tracts of foetuses (Group 1).

TABLE 4: The distribution of globule leucocytes along the respiratory tracts of calves between 0-1 month of age (Group 2).

TABLE 5: The distribution of globule leucocytes along the respiratory tracts of calves between 2-4 months of age (Group 3).

TABLE 6: The distribution of globule leucocytes along the respiratory tracts of calves between 5-11 months of age (Group 4).

TABLE 7: The distribution of globule leucocytes along the respiratory tracts of calves between 12-23 months of age (Group 5).

TABLE 8: The distribution of globule leucocytes along the respiratory tracts of cattle above 2 years of age (Group 6).

TABLE 9: Globule leucocyte population density in the respiratory tracts of different age groups of cattle.

TABLE 10: Globule leucocyte per centimeter in twenty adult bovine respiratory tracts.

CHAPTER THREE

TABLE 11: The size of globule leucocytes in ten adult bovine respiratory tracts.

TABLE 12: Size of globules of the adult bovine respiratory tract globule leucocytes.

TABLE 13: The staining characteristics of globule leucocytes, mucosal mast cells and eosinophils in the bovine respiratory tract.

CHAPTER FOUR

TABLE 14: Globule leucocytes and clinical respiratory disease in the cattle studied.

TABLE 15: Globule leucocyte population density and clinical respiratory disease in the cattle studied.

TABLE 16: The gross pulmonary lesions in the cattle studied.

TABLE 17: The histological diagnosis of the pneumonic lesions found in the cattle studied.

TABLE 18: Globule leucocytes and gross pulmonary lesions in the cattle studied.

TABLE 19: Globule leucocyte population density and gross pulmonary lesions in cattle.

TABLE 20: Globule leucocyte and pulmonary lesions (gross and microscopic) in cattle.

TABLE 21: Globule leucocyte population density and pulmonary lesions (gross and microscopic) in cattle.

TABLE 22: Globule leucocytes in the lungs, the abomasums, the bile ducts and the urinary bladders of the cattle studied.

CHAPTER FIVE

TABLE 23: The experimental design and the GL found after a single exposure of calves to Dictyocaulus viviparus infections.

TABLE 24: The design of the experiment for oral vaccination followed by oral challenge with Dictyocaulus viviparus.

TABLE 25: The results of the experiment involving oral vaccination followed by oral challenge with Dictyocaulus viviparus.

TABLE 26: The design of experiment for vaccination followed by repeated and trickle challenge infections with Dictyocaulus viviparus.

TABLE 27: The results of the experiment involving vaccination followed by repeated and trickle challenge infections with Dictyocaulus viviparus.

LIST OF FIGURES

LIST OF FIGURES

CHAPTER TWO

FIG. 1: Standard sampling sites.

FIG. 2: Globule leucocytes between respiratory epithelial cells.

FIG. 3: A globule leucocyte in the bronchial glandular epithelium.

FIG. 4: A globule leucocyte in the bronchial lumen.

FIG. 5: A globule leucocyte in a bronchial lymph node.

FIG. 6: Globule leucocytes in the trachea of a foetus.

CHAPTER THREE

FIG. 7: Globule leucocytes in a bronchial epithelium.

FIG. 8: A globule leucocyte in the connective tissue of a bronchus.

FIG. 9: Globule leucocytes showing the characteristic cartwheel arrangement of nuclear chromatin.

FIG. 10: A globule leucocyte with two nuclei.

FIG. 11: A globule leucocyte undergoing mitosis.

FIG. 12: A large globule in the cytoplasm of a globule leucocyte.

FIG. 13: Globule leucocytes discharging their granules.

FIG. 14: Globule leucocytes with very small globules.

FIG. 15: Globule leucocytes with very small globules.

FIG. 16: A young calf globule leucocytes with toluidine blue stain, granules stain metachromatic.

FIG. 17: Mucosal mast cells and globule leucocytes stained with toluidine blue.

FIG. 18: Mucosal mast cells stained with astra blue/safranin.

FIG. 19: Globule leucocytes stained with astra blue/safranin.

FIG. 20: Globule leucocytes stained with Martius scarlet blue.

FIG. 21: Globule leucocytes stained with phosphotungstic and haematoxylin.

FIG. 22: Eosinophils and globule leucocytes stained with carbol chromatrope.

FIG. 23: Ultrastructural appearance of the bovine respiratory tract globule leucocytes.

FIG. 24: A globule leucocyte with a cytoplasmic prolongation.

FIG. 25: A globule leucocyte with nuclear indentations.

FIG. 26: A globule leucocyte with a centriole.

FIG. 27: A globule leucocyte with other cytoplasmic organelles.

FIG. 28: A globule leucocyte with a Type 1 globule.

FIG. 29: A globule leucocyte with a Type 2 globule.

FIG. 30: A globule leucocyte in the Stage I of the degenerative process.

FIG. 31: A later stage of Stage I.

FIG. 32: A globule leucocyte in the Stage II of the degenerative process.

FIG. 33: A globule leucocyte in the Stage III of the degenerative process.

FIG. 34: Ultrastructural features of a Mast Cell in the lamina propria and of a globule leucocyte in the epithelium.

FIG. 35: A mast cell with granules with dense homogeneous matrix and an outer light rim.

FIG. 36: A mast cell with both fine and coarse reticulated granules.

FIG. 37: A mast cell with granules made up of both reticular and dense homogeneous matrix.

FIG. 38: A mast cell with crystalloid containing granules.

FIG. 39: A mast cell with a honeycomb type of crystalloid.

FIG. 40: Ultrastructural features of a plasma cell.

FIG. 41: Ultrastructural features of an eosinophil.

FIG. 42: Eosinophil with lamellated granules.

FIG. 43: Ultrastructural features of a secretory cell.

FIG. 44: A secretory cell with well developed rough endoplasmic reticulum.

FIG. 45: A secretory cell with junctional complexes with other epithelial cells.

FIG. 46: Ultrastructural features of a goblet cell.

CHAPTER FOUR

FIG. 47: A calf lung with consolidation of cranial lobes.

FIG. 48: Acute exudative pneumonia.

FIG. 49: Acute proliferative pneumonia.

FIG. 50: Chronic suppurative pneumonia.

FIG. 51: Chronic granulomatous pneumonia.

FIG. 52: Chronic cuffing (lymphocytic) pneumonia.

FIG. 53: Chronic interstitial pneumonia (Farmer's Lung).

FIG. 54: Diffuse fibrosing alveolitis.

FIG. 55: Pulmonary congestion and oedema.

FIG. 56: Interstitial emphysema.

FIG. 57: Pulmonary collapse.

FIG. 58: Pulmonary involvement of lymphosarcoma.

FIG. 59: Pulmonary involvement of pancreatic adenocarcinoma.

CHAPTER FIVE

FIG. 60: Widespread consolidation in the lungs of a calf infected with Dictyocaulus viviparus.

FIG. 61: Adult lungworms in a bronchial lumen.

FIG. 62: Lungworm larvae in a bronchiole.

FIG. 63: Bronchitis in Dictyocaulus viviparus infection.

FIG. 64: The mean weekly respiratory rates of Group 2a calves.

FIG. 65: The mean weekly eosinophilic response of Group 2a calves.

FIG. 66: The mean weekly respiratory rates of Group 2b calves.

FIG. 67: The mean weekly eosinophilic response of Group 2b calves.

FIG. 68: The faecal larval counts of Group 2b calves.

FIG. 69: The mean weekly eosinophilic response of Group 2c calves.

FIG. 70: The gross appearance of a subpleural parasitic nodule.

FIG. 71: The histological appearance of a parasitic nodule.

FIG. 72: A secondary lysosome in a parasitic nodule.

FIG. 73: The histological appearance of a lymphoid nodule.

FIG. 74: Ultrastructure of a pulmonary lymphoid nodule.

FIG. 75: A lymphocyte undergoing mitosis; ultrastructure.

FIG. 76: The mean weekly respiratory rates of Group 3a calves.

FIG. 77: The eosinophilic response of Group 3a calves.

FIG. 78: The mean weekly respiratory rates of Group 3b calves.

FIG. 79: The eosinophilic response of Group 3b calves.

FIG. 80: The mean weekly respiratory rates of Group 3c calves.

FIG. 81: The eosinophilic response of Group 3c calves.

FIG. 82: The faecal larval counts of Group 3c calves.

FIG. 83: The mean weekly respiratory rates of Group 3d calves.

FIG. 84: The eosinophilic response of Group 3d calves.

FIG. 85: The faecal larval counts of Group 3d calves.

ACKNOWLEDGEMENTS

ACKNOWLEDGEMENTS

My very sincere thanks go to Professor W.F.H. Jarrett who made facilities in his department available to me during this study. Many thanks to my supervisor Professor H.M. Pirie who conceived the ideas investigated in this thesis. I wish to thank him as well for his advice, patience and the interest he took in this study. My thanks also go to him for the comments and corrections he made on the draft of this thesis and I also wish to thank him for his very excellent human relationship.

All the technical staff of Veterinary Pathology and Photography departments did an excellent job in the preparation of tissue blocks for microscopy and the preparation of photographs presented in this thesis. I wish to thank them all. Messrs R. Irvine and J. Murphy deserve my thanks for the immense help given during the collection of materials for this study.

My very sincere thanks also go to Dr. Helen Laird who introduced me to the use of the electron microscope and to Dr. Irene A.P. McCandlish who was always available to help answer questions on pathology.

I will not forget to thank Professor J. Armour and the technical staff of the department of Veterinary Parasitology who contributed a lot towards the experimental studies carried out during this investigation.

Members of the academic staff of the department of Veterinary Medicine who examined the clinical cases presented in this thesis also deserve my special thanks.

The work presented in this thesis was carried out when the author was on study leave from the University of Ibadan, Nigeria, on a Commonwealth Academic Staff Scholarship from the Association of Commonwealth Universities. He therefore wishes to thank them for the opportunity and financial help.

Finally I wish to express my very sincere gratitude to Professor B.O. Ikede who provided the opportunity and to Mrs. Janet Morrans and Miss Frances Fagg for typing the thesis.

DECLARATION

DECLARATION

The work described in this thesis is original and has not been submitted in any form to any other University. It was carried out by the author in the Department of Veterinary Pathology, Veterinary School, University of Glasgow under the supervision of Professor H.M. Pirie.

SUMMARY

SUMMARY

A review of the literature concerning the immunity to helminth parasites indciated the involvement of the mucosal mast cell (MMC); and the production of another cell, the globule leucocyte (GL). This cell has been found and characterised in various mucous membranes including those of alimentary tract, the bile duct, the urinary system and the respiratory tract of different animal species.

Globule leucocytes have been observed in the bovine respiratory tract in health and in disease. Large numbers have been seen in the respiratory tracts of adult cattle, but no detailed studies have been done to characterise this cell in the bovine respiratory tract. It was also not known if their presence in the bovine respiratory tract was a coincidental finding or if it indicated a pathological state particularly one following exposure to parasites especially the bovine lungworm Dictyocaulus viviparus. The work reported in this thesis was therefore undertaken in order to understand the importance of the GL in bovine respiratory diseases.

The results of a survey carried out among different age groups of cattle established the presence of GL in all age groups of cattle from foetuses to adults although they were not found in every animal examined. The distributions of GL within the bovine respiratory tract varied in the different age groups examined. Globule leucocytes were more concentrated in the trachea in younger animals as compared to adult animals where they were more concentrated in the bronchi and especially the bronchi of the caudal lung lobes.

With the light microscope, the GL of the bovine respiratory tract was similar to those of other mucous membranes described by other workers. The GL of the bovine respiratory tract were observed to contain both sulphated and carboxylated mucin in the young animal as do mast cells, while in the adult, they contained carboxylated and neutral mucin.

Only two types of globules were seen with the electron microscope in the GL of the bovine respiratory tract and rough endoplasmic reticulum or Golgi apparatus were not seen in the cells examined.

There was no distinct relationship found between the bovine respiratory tract GL and clinical respiratory disease, although the GL population density was more likely to be increased in adult animals with clinical respiratory disease. In addition, both calves and adult animals with gross and microscopic lung lesions were more likely to have high densities of GL in their respiratory tracts.

Globule leucocytes were not found in the urinary bladder of any of the cattle examined. The presence of GL in the bovine respiratory tract did not correspond to their presence in either the bile duct or the abomasum where they were also found, but the percentage of animals with GL in their respiratory tracts as well as in their bile ducts and abomasums increased with age.

Globule leucocytes were found in the respiratory tracts of calves exposed to D. viviparus infection, but the population densities of GL seen in the lungs of these calves were not different from what was normally seen in the respiratory tracts of this age group of cattle.

It was concluded that GL were found in the respiratory tracts of calves infected with D.viviparus but no definite correlation could be demonstrated between the bovine respiratory tract GL and lungworm infections. In addition, it was thought that the presence of large numbers of GL in the lungs of adult cattle might be associated with the age of the animal resulting in their exposure on more than one occasion to other unidentified stimuli.

ABBREVIATIONS

ABBREVIATIONS

Unless specified below, the units, symbols and abbreviations used in this thesis are those recommended in "A Guide For Biological and Medical Editors and Authors" published by the Royal Society of Medicine, London.

AB	Alcian blue.
AB/S	Astra blue/safranin.
AO	Acridine orange.
B	Blue.
BB	Blue brown.
CBPP	Contagious bovine pleuropneumonia.
CC	Carbol chromotrope.
C_c	Caudal lung lobe.
C_cB	Caudal lobe bronchus.
Cr	Cranial lung lobe.
CrB	Cranial lobe bronchus.
DFA	Diffuse fibrosing alveolitis.
D.P.X.	Distrene plasticizer xylene.
ECF-A	Eosinophil chemotactic factor of anaphylaxis.
EOS	Eosinophil.
GL	Globule leucocyte.
H&E	Haematoxylin and eosin.
HETE	Lipo-oxygenase product of arachidonic acid.
HHT	Cyclo-oxygenase product of arachidonic acid.
HMN-NCF	High molecular weight neutrophil chemotactic factor.
IBR	Infectious bovine rhinotracheitis.
L_3	Third stage infective larvae.
l.p.g.	Larvae per gram.
MC	Mast cell.
MMC	Mucosal mast cell.
MSB	Martins scarlet blue.
PAF	Platelet activation factor.
Para	Parasitology.
PGE	Prostaglandin E.

Abbreviations contd.

PGF	Prostaglandin F.
PI	Post infection.
PI_3	Para influenza Type 3.
PTAH	Phosphotungstic acid haematoxylin.
R	Red.
RER	Rough endoplasmic reticulum.
RMCPII	Rat mast cell protease II.
RSV	Respiratory syncytial virus.
SRS-A	Slow reacting substance of anaphylaxis.
TB	Toluidine blue.
TC	Transisitional cell.
Tr	Trachea.
V	Violet.
YO	Yellow orange.
5 - HT	5 - Hydroxytryptamine.

GENERAL INTRODUCTION

GENERAL INTRODUCTION

The importance of respiratory diseases of cattle has long been recognised in Britain. Although contageous bovine pleuropneumonia (CBPP) has been eradicated from Britain and tuberculosis has been controlled, many more respiratory syndromes are recognised in adult cattle in Britain. These were reported by Breeze et al., (1975), to be fog fever, extrinsic allergic alveolitis or 'farmer's lung', diffuse fibrosing alveolitis, re-infections with Dictyocaulus viviparus, thrombosis of the posterior vena cava with pulmonary arterial thromboembolism and chronic suppurative pneumonia; others included pulmonary oedema and interstitial emphysema.

Respiratory diseases also occur in young cattle, and are probably among the most important diseases of young calves in the United Kingdom (Thomas, 1979). At the present time, the important respiratory tract infections of calves are considered to be infectious bovine rhinotracheitis (IBR), (Wiseman et al., 1980) respiratory syncytial virus (RSV), (Pirie et al., 1981), parainfluenza Type III (PI3) as well as D. viviparus infection in grazing animals (Bryson, 1980, Knifton, 1983), and pneumonic pasteurellosis (Andrews, 1983).

Hypersensitivity in cattle has recently been reviewed (Black, 1979), therefore it is not intended in this work to review this topic but to highlight the important link associating hypersensitivity with dypsnoea, acute pulmonary oedema and interstitial emphysema. Anaphylaxis or immediate (Type I) hypersensitivity which normally produced this type of respiratory distress could be initiated experimentally by intravenous injections of albumin and has also been recognised in several clinical situations. These included the administrations of medical substances like antibodies or their vehicle (Leeman et al., 1969), biological products eg. horse serum (Fincher, 1960), heat and formalin denatured bovine serum (Capstick et al., 1970), foot

and mouth disease vaccine (Black, 1977) and vaccines against other pathogens eg. rinderpest (Scott, 1963), Brucella abortus vaccine (Reichel, 1939) and CBPP (Turner and Trethewie, 1961), parasites eg. Hypoderma bovis and H. lineatum (Campbell, 1970), re-infections of previously sensitised cattle with lungworms (Michel and Coates, 1958) and Ascaris suum infestation (Greenway and McCraw, 1970). Other agents causing anaphylaxis in cattle included milk casein autoallergy resulting from reabsorbed milk protein (Campbell, 1970), inspired pollens, dust and moulds (Weil and Reddin, 1943, Nilson, 1963, Pasternak and Brysin, 1965, Pemberton et al., 1977). Similar pulmonary reactions which were thought not to be hypersensitivity reactions could be brought about by pneumotoxic agents (Pirie et al., 1971, Hilderman and Taylor, 1974).

Immediate (Type I) hypersensitivity or anaphylaxis was classically initiated by the interreaction between specific antigens and homocytotropic reaginic antibodies attached to mast cell or basophil surfaces. Mast cells and basophils are an important source of biologically active substances which are mediators of anaphylaxis. Their effects are exerted on smooth muscles and vascular endothelium causing their contraction and extravasation of fluid into tissue spaces. Mediators released during hypersensitivity reactions in cattle are histamine, serotonin or 5-hydroxytryptamine (5-HT), dopamine, slow reacting substances of anaphylaxis (SRS-A), prostaglandin (PG) F and E, and bradykinin (Eyre and Burka, 1978, Black and Burka, 1979). Histamine has been shown to be released from bovine leucocytes (Holroyde and Eyre, 1976).

In some helminth parasite infections of the alimentary tract the phenomenon of "self-cure", a loss of the infection followed by "protection", has long been recognised (Stoll, 1928). The expulsion of worms at the time of self cure has been shown to be an immune phenomenon (Mulligan et al., 1965, Whur, 1966). The authors suggested that self cure was related to induced anaphylaxis resulting from antigen-antibody reactions in immune rats. These resulted in increased capillary permeability with associated

antibody release into the gut of Nippostrongylus brasiliensis infected rats in their experimental system. The antibody could then act directly on the worms to bring about their expulsion. Miller, (1971a) and Murray et al., (1971), observed an increase in the numbers of mast cells during the expulsion of N. brasiliensis from the intestine of immune rats. As well as this increase in cell number, cell divisions, degranulations and movement into the epithelium from the lamina propria were observed. Mast cells in the intestinal epithelium had the characteristics of classic globule leucocytes (GL), (Jarrett et al., 1967a, Murray, 1968, Miller, 1971b). This observation was supported by Miller, (1972), who reported loss of biogenic amines from mast cells undergoing degranulation and suggested that these amines facilitated the rapid translocations of anti-worm antibody into the intestinal lumen as a result of mucosal damage. Apart from his observations on the increase in the number of GL and mast cells, Miller, (1972), reported that both cell types had the same histochemical and electron microscopic characteristics. Earlier Hole, (1937), had suggested that there was a relationship between GL and parasitic infections and Taliaferro and Sarles, (1939), had produced GL experimentally in N. brasiliensis infections in rats. Whur, (1966), also reported an increase in the number of GL in rats infected with N. brasiliensis. In (1967a) Jarrett et al., reported a relationship between mucosal mast cells (MMC) and GL in parasitic infections. They reported the presence of a range of cells which histologically, cytochemically and ultrastructurally showed a gradation from the (MMC) to GL. Murray et al., (1968), Murray, (1968), gave more convincing evidence by using experimental parasitic infections in rats, cattle and sheep to produce episodes of transformation of MMC into GL. It was suggested that the change from MMC to GL was caused by alterations in the relationship between the acid mucopolysaccharide and the basic proteins of MMC granules as well as by their release of biogenic amines.

Globule leucocytes were observed in the respiratory tracts of cattle with farmer's lung and fog fever (Breeze et al., 1975), but their significance was not known. In addition, large numbers

of these cells have been found in the lungs of adult cows in other outbreaks of respiratory disease and it was difficult to interpret the findings (Pirie, personal communication). The association of GL with lungworm infections in cattle has not been studied previously although Mahmoud, (1978), found a relationship between Dictyocaulus filaria infections in sheep and the presence of GL in the ovine respiratory tract. So far, there have been no detailed studies done to investigate the morphology of the GL in the respiratory tract of cattle and their relationship to respiratory disease. The morphology of the GL in the bovine abomasum, in ostertagiasis (Jarrett et al., 1967a, Miller et al, 1967, Murray, 1968) and in the bile ducts in liver fluke infections (Rhako, 1970a), has been investigated and reported. Recently, Lawrence, (1977), reported their presence in the urinary tract of cattle in Schistosoma mattheei infections. In order to understand the importance of GL in the bovine respiratory tract, a study was undertaken to:

(1) Establish the prevalence of the GL in the respiratory tract of all age groups of cattle.

(2) Characterise the cell in the bovine respiratory tract.

(3) Investigate the relationship between the GL and respiratory diseases of cattle.

(4) Observe the relationsip between their presence in the bovine respiratory tract and in other mucous membranes in which they have been reported, e.g. abomasum, bile duct and the urinary bladder of cattle.

(5) Investigate the experimental induction of GL in the bovine respiratory tract as well as the pathology of the respiratory system in animals infected with D. viviparus.

CHAPTER ONE

REVIEW OF THE LITERATURE

(i) BOVINE PARASITIC BRONCHITIS

(ii) IMMUNITY TO HELMINTH PARASITES

(iii) MAST CELLS and

(iv) GLOBULE LEUCOCYTES

REVIEW OF THE LITERATURE.

A. BOVINE PARASITIC BRONCHITIS.

In view of the known link between parasitic infection and globule leucocytes (GL), it seemed important to consider the literature relating to the influence that the bovine lungworm D. viviparus might have on the developement of GL in the bronchopulmonary system of cattle.

Bovine parasitic bronchitis, husk or hoose, is a disease associated with infection by the nematode D. viviparus. The disease has long been recognised and documented (Holmes, 1688, cited by Allan and Johnson, 1960). Parasitic bronchitis usually affects calves during the later half of their first grazing season, although adults could be affected as well (Smythe, 1937, Urquhart et al., 1973). In a short history of husk, Allan and Johnson, (1960), traced the origin of the words husk and hoose and tried to explain how they became associated with lungworm infections in cattle. An increase in the incidence of the disease was attributed to changes in farming practice as well as land enclosures. During the fifties, the pathogenesis and immunity of the disease was investigated in detail by Jarrett et al., (1957a, b, and 1959). The disease was common, widespread and of greatest severity in the wetter parts of the countries where it occurs and in wetter periods (Oakley, 1982). Control of the disease was discussed recently by Selman, (1984). Four stages of the development of the disease were recognised (Jarrett et al., 1957a) and this classification of the pathogenesis is still being used (Urquhart et al., 1973, Pirie, 1978). The stages are (i) penetration phase, (ii) prepatent phase, (iii) patent phase and (iv) post patent phase. The penetration phase occurs during the first seven days of the infection. Respiratory signs are not usually observed during this phase hence it is of little clinical significance. The small intestine is penetrated by the infective third stage larvae of

D. viviparus to enter into the mesenteric lymph nodes for onward migrations to the lungs. The prepatent phase starts from about the seventh day to the 25th day of infection. This phase is very important clinically and results from lesions produced by the fourth and fifth stage larvae as they migrate within the lung parenchyma and ascend the bronchial tree. The patent phase lasts from the 25th day to the 55th day of infection. The developing fifth stage larvae have become adult lungworm and are present in the bronchial and tracheal lumena. Eggs are produced during this phase and clinical signs observed are a result of bronchitis and a pulmonary reaction to aspirated first stage larvae and eggs. The post patent phase is from the 55 day until about the 90th day and is characterised by gradual recovery of most animals from the infection. The number of adult worms in the bronchi diminishes as they are expelled. Clinical signs observed are as a result of previous damage done to the lungs by migrating larvae and worms; or from complications such as pulmonary oedema and emphysema and secondary bacterial infections. In approximately 25 per cent of cases, there is an exacerbation of clinical signs due to development of alveolar epithelial hyperplasia.

Detailed descriptions of the pulmonary pathology of bovine parasitic bronchitis have been given (Jarrett et al., 1957a, b, 1960, Michael and MacKenzie, 1965) and discussed by Pirie, (1978). No pulmonary lesions were observed during the penetration phase. In severe cases, the most striking gross lesion of the prepatent phase was severe interstitial emphysema. Microscopically, alveolitis was produced as a reaction to the presence of migrating larvae by infiltrating eosinophils, macrophages and giant cells. There was bronchiolitis and mild bronchitis with the presence of eosinophils in their epithelia and lumena. Airways obstructed by inflammatory cells and parasites could lead to collapse of related lung lobules. The lesions found during the patent phase mainly affected the caudal lung lobes. Grossly, the lungs were non-collapsed and had diffuse areas of reddish-grey consolidation. The bronchial and bronchiolar epithelium was thickened and their lumina contained adult parasites

lying in frothy white exudate. Microscopically the bronchial and bronchiolar lesions included epithelial hyperplasia, with dysplasia in some regions. Both the epithelium and lamina propria were heavily infiltrated by eosinophils and plasma cells as well as some neutrophils and lymphocytes. Mature parasites were present in the lumena and were surrounded by eosinophils, macrophages, plasma cells, neutrophils and mucus. In the bronchioles epithelial hyperplasia was sometimes accompanied by polypoid projections into their lumena; these polyps could persist for a considerable time and were indicative of pulmonary damage by D. viviparus.

The alveolar changes included the presence of aspirated eggs and first stage larvae surrounded by giant cells, macrophages, eosinophils and neutrophils. Where there had been complications from pulmonary oedema and interstitial emphysema, hyaline membranes with focal alveolar epithelial hyperplasia occurred. Animals sometimes died during this phase in heavy infections.

The post patent phase of husk was characterised by either the presence of very few worms or the absence of adult worms from the consolidated lungs. The most important microscopic lesion in some cases was diffuse alveolar epithelial hyperplasia. Healing with fibrosis was present around the bronchioles and bronchi. There was also broncholitis obliterans. Pulmonary oedema and interstitial emphysema were also present in some animals. No GL were described or found in the mucous membrane of the bronchi at any of the four stages of the disease process, unlike many other parasitic processes in other mucous surfaces.

Detailed reports on the clinical signs of calves infected experimentally with the infective stage of D. viviparus larvae have been published (Jarrett et al., 1957a, Urquhart et al., 1973). The penetration phase was of little clinical significance. The prepatent or alveolar-bronchiolar phase was marked by increased respiratory rates with occasional cough. During the later part of this phase, heavily infected calves sometimes died gasping for breath with short shallow respiration. Harsh bronchial respiration

with emphysematous crackles were detectable on auscultation. Laterly, animals became anorexic, lost weight and respiration was followed by an expiratory grunt.

During the patent phase the clinical signs became very severe and reached peaks. Coughing was very frequent, rhonchi and emphysematous crackling could be heard over harsh bronchial breathing. The head and neck of the dyspnoeic calf was held straight out with the tongue protruding each time it tried to cough. A steady improvement of clinical signs began at the later part of this phase.

The post patent phase was associated with recovery and elimination of most of the adult worms. Severely affected animals could develop a sudden severe exacerbation of the clinical disease and show signs of acute respiratory distress. Some of the affected animals died after two to four days. Emphysematous crackles, rhonchi as well as rales were occasionally heard in these animals when they were auscultated. It was emphasized that because of the variations in the degree of infection that occurred in the field situation the wide range of clinical signs described above were seen in different outbreaks.

The re-infection syndrome was characterised by severe acute respiratory distress with persistent coughing seen in adult animals. The pathology as re ported by Jarrett et al., (1960), Jarrett and Sharp, (1963), Michel et al., (1965), Pirie et al., (1971), Breeze et al., (1975), was similar to those described above, but lymphoreticular nodules were present. These nodules 3-4 mm in diameter were within the lung parenchyma or beneath the pleura. They were raised above the lung surface and were greyish-red with a greenish yellow centre. Microscopically the central portion was made up of parasitic debris and eosinophils surrounded by giant cells, macrophages, proliferating lymphoreticular tissue and when related to bronchioles hyperplastic bronchiolar epithelium. The reaction was as a result of previous exposure to the parasite and the development of immunity (Pirie et al., 1971). Again there was no mention of the presence of GL in

the respiratory tract.

The complex nature of the epidemiology of parasitic bronchitis is reflected by the large amount of literature on the subject. For the disease to occur, susceptible animals have to ingest the infective third stage larvae (L_3) of the parasite D. viviparus.

The important features of D. viviparus which influence the number of free living larvae on pasture have been summarised by Oakley, (1982). Among them were the high susceptibility to desiccation, rapid development to the infective stage in summer temperatures, persistent but slower development at winter temperatures, sluggish mobility of infective larvae, their limited migration from faecal pats and their apparent reliance on other factors such as diarrhoea and dissemination by fungi to aid their spread on herbage.

Overwintered larvae as the source of infection for young grazing cattle has been reported (Jarrett et al., 1955, Michel and Parfitt, 1956, Allan and Baxter, 1957, Guptar and Gibbs, 1970, Oakley, 1977, Duncan et al., 1979, Jørgensen, 1980a and b). Other factors reported to be responsible for the propagation of D. viviparus infection include carrier animals (Michael and Shand, 1955, Jarrett et al., 1957b, Cornwell, 1959, Cornwell and Berry, 1960, Gupta and Gibbs, 1970, 1975, Oakley, 1977 and 1979). The carrier animals are thought to have immature worms which resume development when environmental conditions improve. Survival of larvae in soil was reported by Nelson, (1977), Duncan et al., (1979) and Armour et al., (1980). Smythe, (1937) and later Selman, (1984), associated the use of farm manure from infected areas on grass with disease outbreaks on farms that had been hitherto free. Hay and stock purchased from infected farms and moved to farms that were free of infection with D. viviparus have also been associated with the spread of the infections (Smythe, 1937). Recently, Robinson, (1962), showed that D. viviparus larvae may be transmitted by the violent discharge of the sporangia of the

coprophilous fungus Pilobolus spp; experimental evidence to support this observation has also been reported by Jørgensen, (1982). Earlier Morozov, (1958), reported that lungworm larvae were carried into the soil by coprophagous beetles, and that larvae had been found in earthworms (Oakley, 1981). The possibility that Psychoda spp. which may carry larvae of Oesophagostomum and Ostertagia spp. may be involved in the spread of D. viviparus has also been suggested (Jacobs et al., 1968, cited by Oakley, 1982). It was pointed out that immunity to D. viviparus infection in cattle developed rapidly but resistance to infection was dependent on the level of the first challenge dose to which the animal was exposed (Michel and Parfitt, 1956). In addition the persistence of immunity depends on animals being re-exposed to challenge at regular intervals.

The difficulty of preventing cattle from being exposed to the agent of parasitic bronchitis has recently been reviewed (Selman, 1984). Prevention of the disease was therefore not very feasible and control measures were practiced to help reduce losses in any form. Control measures practiced were (i) vaccination, (ii) use of clean pasture to graze animals, (iii) limited exposure to infected pasture, (iv) use of anthelmintics to prevent lung damage and (v) zero grazing to reduce larval challenge.

All the above systems apart from vaccination using the X-irradiated lungworm larvae vaccine were impracticable either because of the high cost or because of the management difficulties involved. Because of the known association between GL and parasitic infection, any study on the link between GL in the bovine respiratory tract and respiratory disease in cattle must take into account the information that has just been reviewed on D. viviparus.

An important aspect of GL activity is considered to be their involvement in immune resistance to helminth parasites. Therefore it is pertinent to look at some of the literature related to immunity to helminth parasites.

B. IMMUNITY TO HELMINTH PARASITES

Most of the reports on the mechanisms of immunity to helminth parasites have been from experimental studies on a few animal parasites. These namely were the intestinal parasites N. brasiliensis of rats, Trichostrongylus columbriformis of ruminants, Trichinella spiralis of swine and the gastric parasite Haemonchus contortus of sheep. Because vaccination with irradiated lungworm larvae against the bovine lungworm was very successful it is perhaps not surprising that the mechanisms of immunity against this parasite has not been studied in detail. According to Soulsby, (1979), the immune response to helminth parasites is comparable to that against any other infective agent although their varied and at times extended developmental cycles as well as the differing locations which they inhabit, both imply that a wide range of responses could be expected. The contribution made by both specific and non-specific effector systems to protective immunity varies considerably for each helminth infection and indeed may vary for different parts of the life cycle of an individual helminth.

The immunity to helminth parasites at mucosal surface is affected by both humoral and cellular responses. The presence of the secretory immunoglobulin A (IgA) at mucosal surfaces is recognised in both man and animals. This immunoglobulin has been demonstrated in bovine lungs (Mach and Pahud, 1971, Morgan et al., 1977). Immunoglobulin A is thought to be produced locally in the plasma cells in the lamina propria at mucosal surfaces and plasma cells containing IgA have been shown in calves both healthy and diseased (Allan et al., 1979) and in sheep (Scicchitano et al., 1984). Immunoglobulin A then, crosses either intracellularly or intercellularly through the epithelium to the mucosal surface. Because IgA is more resistant to digestion it could therefore survive longer and presumably be useful over an extended period of time. It could act by attaching to receptors on the surface of parasites thus preventing them from interacting with mucosal epithelial cells. It could probably take part in the alternative

pathway by activating complement and also take part in antibody dependent cell mediated cytotoxicity as suggested in the review by Bazin, (1977). Immunoglobulin M (IgM) was another immunoglobulin which may be secreted across the epithelium of a mucous membrane (Bazin, 1977, Wakelin, 1984). Jones et al., (1970), observed that IgM had some protective value against N. brasiliensis infection. However Immunoglobulin G (IgG) was the major immunoglobulin found in the sera of animals infected with N. brasiliensis (Jones et al., 1970). Attachment of IgG to the parasite's surface might be followed by release of lysosomal enzymes or other cytotoxic factors from inflammatory cells causing the destruction of the parasite i.e. antibody dependent cell mediated cytotoxicity (Wakelin, 1984). Another important immunoglobulin in the immunity to helminth infection is Immunoglobulin E (IgE) which is produced by plasma cells in lymphoid aggregate associated with mucosal surfaces. Immunoglobulin E is a homocytotropic reaginic immunoglobulin which has been associated with mast cells and basophils in the self-cure phenomenon of helminth infections; this topic was review by Jarrett and Miller, (1982).

The involvement of lymphocytes in the helminth immunity studies referred to above was with regard to their activation, division and production of antibodies. However, it is considered by some workers that there are other cells which are also very important in cellular immunity in particular mast cells, basophils and eosinophils. Eosinophils are thought to be involved in the antibody dependent cell mediated cytotoxicity. In this system, IgG antibodies attach to antigenic determinants on the parasite and eosinophils adhered via surface receptors for IgG. Subsequently, the attached eosinophils spread out along the surface of the parasite, degranulate and damage the integument of the parasite e.g. the schistosomule in Schistosoma mansoni infection (Butterworth et al., 1974, Ogilvie et al., 1979); Askenase, (1980), reviewed this subject.

Mast cells and basophils have several characteristics in common. The two most prominent being (i) cell surface receptors for IgE and (ii) metachromatic staining cytoplasmic granules which

are sites for storage of vasoactive amines. Taliaferro and Sarles, (1939), Jarrett et al., (1967a,b), Miller, (1971b) and Murray et al., (1971) have all reported an increase in the number of MMC during the expulsion of N. brasiliensis in the rat. Also, it has become known that helminth infections are one stimulant which induces a high concentration and a longstanding IgE response (Jarrett and Miller, 1982). Immunoglobulin E has a unique area with a complementary receptor on the plasma membrane of mast cells and basophils. During helminth infections the self-cure phenomenon, which is an allergic reaction, results in the loss of adult worms from mucosal surfaces. The reaction is thought to be brought about by the combination of specific antigen with IgE bound to mast cells. This combination led to the release of active substances, vasoactive amines, from mast cells. These substances such as histamine, prostaglandins and slow reacting substances of anaphylaxis (SRS-A) then acted on the smooth muscles of the mucous membranes to bring about contractions leading to peristalsis and finally loss of the adult parasites (Askenase, 1980). Another hypothesis was that by Murray et al., (1971), in which the released substances caused a change in the mucous membrane with resultant mucosal leakage and transportation of antibodies onto the mucosal surface from the lamina propria.

Worm expulsion in re-infection was much more immediate and explosive in tempo than that in primary expulsion and might be more dependent upon the release of anaphylactic mediators from local MMC (Askenase, 1980).

Although some workers (Jarrett et al., 1967b, Ogilvie and Jones, 1971), do not agree with the leak hypothesis proposed by Murray et al., (1971), there is still general agreement that mast cells are involved during the expulsion stage of helminth infection (Review by Ogilvie and Jones, 1971). Jarrett et al., (1967a), Murray, 1968, Murray et al., (1971), Miller, (1971a and b), have reported the derivation of GL from MMC as a result of their degranulation and movement into the epithelium with a change in their granule content to basic proteins. The end product of the

anaphylactic reaction during worm expulsion was therefore the GL.

Occasionally, large numbers of the GL have been seen in the respiratory tract of cattle in outbreaks of respiratory disease in adult animals (Breeze et al., 1975). The prevalence of parasitic bronchitis in cattle has been pointed out already in this review. Since studies on infection with bovine lungworm and the proliferation of both mast cells and GL have previously not been reported it was not possible to associate the large numbers of GL present in the respiratory tract of adult animals with infection with the bovine lungworm or any particular bovine respiratory tract disease.

The literature concerning mast cells and GL in bovine mucous membranes and particularly the respiratory tract will now be reviewed taking into consideration information from other species.

C. MAST CELLS

The first phase of research on mast cells was started in 1863 by Von Recklinghansen (cited by Archer, 1980), who noticed the granular cell in the mesentery of frogs. The cell was later described by Ehrlich in 1877 as a cell concerned with nutrition and named it as 'Mastzellen'. The mast cell which was identified by its metachromatic granules was found in all vertebrates, although wide variations occurred in distribution, morphology and granule constituents (Enerback, 1966, Miller and Walshaw, 1972). The subject has been recently reviewed by Jarrett and Haig, (1984).

The mast cell because of its occurrence, function, morphology and histochemical properties has been divided into two subpopulations (Enerback, 1966, Jarrett and Haig, 1984), however Befus et al., (1984), do not agree with this division into mucosal and connective tissue mast cells. They argued that not enough studies have been done on the mast cells in different animal species to provide acceptable nomenclature of this kind for mast cell subpopulations. The description that follows of the mast cell covers therefore what had generally been said about both subpopulations.

The review by Benditt, (1958) and Benditt and Lagunoff, (1964), described the light microscopic appearance of the mamalian mast cell as an ovoid or irregularly elongated cell that averaged 12.6 μ in diameter. The nucleus was usually round and resembled that of the plasma cell with the cartwheel appearance of the chromatin pattern; the nucleus was usually obscured by the densely packed cytoplasmic granules. These granules were metachromatic and in the rat were between 0.5 and 2.0μ in diameter.

A large number of reports are available on the fine structure of both the human and the rat mast cell, while only a few reports describe those of other species. The rat and hamster mast cells have the same electronmicroscopic features (Smith and Lewis, 1957). With the electron microscope Smith and Lewis, (1957),

Fernando and Movat, (1963), Miller et al., (1967), Taichman, (1970) and Enerback and Lundin, (1974), were able to identify the mast cell because of the numerous electron dense granules contained in its cytoplasm. The cell was often found to be elongated or spindle shaped and finger like processes of the double cell membrane were a characteristic feature, although these were not observed by Enerback and Lundin, (1974). The nucleus was large, often eccentric and had an irregular outline caused by indentation of its surface by surrounding cytoplasmic granules. The nuclear membrane was double layered and had nuclear pores but nucleoli were rarely found. The cytoplasmic granules were of uniform electron density although an occasional granule was reticulated. They were bounded by a smooth surfaced trilaminar membrane and averaged 0.5 to 1.0μ in diameter. Endoplasmic reticulum and ribosomes were sparse and a Golgi region was seen in some cells. The mitochondria were small and small vacuoles were also found in the cytoplasm. Jarrett et al., (1967a), reported that mitosis was a regular occurrence in the mast cells of rats. The fine structure of the guinea pig and human mast cells were similar to that of the rat but Hibbs et al., (1960), Brinkman, (1968), Dobbins et al., (1969), Orr, (1977) and Caulfield et al., (1980), all observed cystaline structures in the form of scrolls or whorls in human mast cells and Taichman, (1970), in guinea pig mast cells.

The mast cell granule content in various animal species has been studied and reviewed by Benditt,(1958), Benditt and Lagunoff, (1964), Miller, (1980), Enerback, (1981) and Jarrett and Haig, (1984).

The rat mast cell has been most extensively studied. The granules have been shown to contain sulphated glycosaminoglycan (heparin), basic proteins, monoamine, histamine, IgE and a protease. The morphology, occurrence of the cell and the presence or absence of some of these substances had previously led to the sub-division of mast cells into mucosal and connective tissue mast cells.

The origin of the mast cell has been a matter of speculation for a very long time. The association of mast cell origin with T-lymphocytes was made by Burnet, (1965), who noted the accumulation of mast cells in the cortex of the thymus of a small number of NZB mice with autoimmune haemolytic anaemia. He speculated that because the mast cells resembled a clone, that some mast cells might be T-cell dependent. Miller, (1971a), Miller and Walshaw, (1972), showed that during immune response to N. brasiliensis infection in the rat, MMC appeared to be derived from lymphoid like cells which differentiated locally into lymphoblasts and then into MMC. More recently, Ishizaka et al., (1976), showed that mast cells can be derived in vitro from suspensions of thymocytes or lymph node cells. Later work in the seventies by Ruitenberg and Elgersma (1976), Ruitenberg et al., (1979), has shown that the intestinal MMC response to Trichinella spiralis infection in rats was thymus dependent. Befus et al., (1979) and more recently Haig et al.,(1982), have shown that lymphocytes from helminth infected rats released factors that caused pronounced mastocytosis. As a result of their observations Haig et al., (1982), Jarrett and Haig, (1984), proposed the same origin for all mast cell populations and suggested that mast cell precursor cell migrated from the bone marrow and that their differentiation in tissues was a secondary phenomenon.

Wasserman, (1979), enumerated the mast cell dependent mediators of inflammation and their various functions. These included vasoactive, bronchoconstrictor and chemotactic mediators such as histamine, slow reacting substances of anaphylaxis (SRS-A) or leukotrenes, serotonin or 5-hydroxytryptamine (5-HT), platelet activation factor (PAF), prostaglandins, eosinophil chemotactic factor of anaphylaxis (ECF-A), higher molecular weight neutrophil chemotactic factor (HMW-NCF), lipid chemotactic factors (HETE and HHT), heparin, kallikrein of anaphylaxis and proteases.

A biphasic inflammatory response provoked by mast cell mediators according to this review was by generation of spasmodic and vasoactive mediators. These then establish a vasodilatory

(humoral) phase while the release of the chemotactic mediator caused a cellular response. Apart from the lysosomal enzymes released by leucocytes during inflammation, mast cells can also release proteases and lysosomal enzymes that might themselves alter the ground substance. Mast cells are therefore an important component for any inflammatory reaction.

Although the mucosal leak phenomenon associated with mast cell degranulation in the rejection of intestinal nematode infections in laboratory rodents was proposed some time ago and has been accepted by many workers (Mulligan et al., 1965, Murray et al., 1971, Miller, 1971b), other investigators (Keller, 1971, Uber et al., 1980), have refuted the fact that self-cure depended on mast cells. The role of mast cells in the defence against gut parasites has recently been reviewed by Ferguson and Miller, (1979) and Askenase, (1980). Both reviews have indicated that although mast cells may not be as important as was previously thought in the primary rejection of intestinal nematode infection, they are invariably very necessary during re-infections. The fact that MMC could also influence globlet cells that produce and secrete mucus might be of importance, since Nawa and Miller, (1979), Uber et al., (1980), have shown correlation between worm expulsion and mucus secretion.

Woodbury and Neurath, (1978), purified a mast cell protease, rat mast cell protease II (RMCPII) from the gut of rats. Because of the dramatic increase in the IgA levels in alimentary tract mucous which coincided with the increase in both RMCPII in serum and the mast cell contents of the small intestinal mucosa, they supported the opinion that mast cells were involved in the first active immune response at mucosal surface. An increase in mast cell protease has also been shown to be coincident with mast cell proliferation in N. brasiliensis infection in rats (Uber et al., 1980). Woodbury and Miller, (1982), showed convincingly for the first time that mucosal mast cells responded to infection with the nematode N. brasiliensis and suggested that because RMCPII was more effective than chymotrypsin in releasing epithelial cells from

intestinal slices, there was the possibility that this enzyme promoted the separation and shedding of epithelial cells *in vivo*. This report has recently been supported by Woodbury *et al.*, (1984), who demonstrated the fact that the systemic finding of RMCPII in serum coincided with the immune expulsion of *N. brasiliensis* and *T. spiralis* hence mucosal mast cells were functionally active during the immune elimination of primary nematode infection. The involvement of mast cells in intestinal helminth parasite infection has therefore been clearly established.

Taliaferro and Sarles, (1939), Murray *et al.*, (1971), Miller, (1971a and b) and MacDonald *et al.*, (1980), have all established increases in MMC numbers during *N. brasiliensis* infection in the rat.

A relationship between GL and mast cells has also been established (Jarrett *et al.*, 1967a and b, Miller *et al.*, 1967, Miller 1971b and 1972 and Murray *et al.*, 1971b), who demonstrated in the alimentary tract that discharging mucosal mast cells moved into the epithelium to become the cells morphologically recognised as GL. This relationship has recently been confirmed by Huntley *et al.*, (1984), who reported the derivation of the GL from isolated mucosal mast cells in sheep infected with *Ostertagia circumcincta*. They provided ultrastructural morphological and histochemical evidence to demonstrate the sequence of transformation from the mucosal mast cell to the globule leucocyte.

Detailed studies on mast cells and globule leucocytes in the respiratory tract of this species have not been carried out.

D. THE GLOBULE LEUCOCYTE

It is apparent from the above review that not only do MMC proliferate and GL increase in number during parasitic infections but they are actively involved in the immune reaction against the parasite. It is therefore proper to look into the features of the GL as has been reported by earlier investigators.

The GL is a cell which is usually found within the epithelium of the mucous membranes of many vertebrates including man. Weill, (1919), first published the description of this cell in the alimentary tract epithelium of the dog, cat and mouse. Since then other workers have observed and described them in a variety of organs and animal species.

They have been reported in the alimentary tract of cattle (Keasbey, 1923, Duran Jorda, 1945, Jarrett et al., 1967a, Miller et al., 1967, Murray et al., 1968, Rakko, 1970a and b, Blazek, 1971 and Lawrence, 1977). Several workers have also observed them in the alimemntary tract of the sheep (Keasbey, 1923, Duran Jorda, 1945, Kent, 1952, Sommerville, 1956, Dobson, 1966a and b, Zipper, 1966, Miller et al., 1968, Rahko 1969, Knight, 1980 and Gregory and Nolan, 1981).

Other ruminants in which GL have been reported in the alimentary tract were the goat, (Hill, 1951 and Rahko, 1972) and the deer, (Zipper, 1966 and Blazek, 1971). In the small intestine of domestic animals (Lim, 1922, Dawson, 1943, Takeuchi et al., 1969 and Finn and Schwartz, 1972), have observed them in the cat, while Dawson, (1927a,b), observed them in the dog.

Globule leucocytes have been reported in the rat (Taliaferro and Sarles, 1939, Kent, 1966, Whur, 1966, 1967, Whur and Johnston, 1967, Whur and White, 1970, Ruitenberg and Elgersma, 1979, 1980). Their presence in another laboratory rodent the mouse has also been reported (Carr, 1967, Crandall et al., 1974). Toner, (1965), Asdrubali, (1969) and Holman, (1970, 1972), all observed them in the intestine of the fowl.

In addition to the alimentary tract, GL have been described in the respiratory tract of several species. In the respiratory tract of cattle, GL have been observed by Blazek, (1971), Breeze et al., (1975), Pirie et al., (1976), Breeze et al., (1976) and recently Lawrence, (1977) and Allan et al., (1983). In the sheep respiratory tract Kent, (1966), Zipper, (1966), Mahmoud, (1978) and Mahmoud and Pirie, (1982), have observed them. Another ruminant in whose respiratory tract GL have been described is the deer (Zipper, 1966 and Blazek, 1971). The cell had also been observed in the respiratory tract of the rat (Taliaferro and Sarles, 1939, Kent, 1966, and Jeffery and Reid, 1975).

The presence of GL in the urinary and reproductive tracts has also been reported. Lawrence, (1977), observed them in the urinary tract of cattle while Kirkman, (1947, 1950), Cantin and Veilleux, (1972) and Hoyes et al., (1974), described them in the urinary tract of the rat. Their presence in the reproductive tract was observed by Kellas, (1961), who reported their presence in the uteri of cattle, sheep and goats and in wild species such as the Giraffa Camelopardalis (Linneaus), Ourebia Ourebi (Zimmermann), Rhynchotragus Kirkii thomasi (Neumann), Raphicerus Campestris neumanni (Matschie) and Sylvica pragrimmia (Linnaeus). Their presence in the pregnant uteri of goats was again reported by Tokashiki et al in (1981).

The GL of the sheep and rat have received most attention from investigators probably because the laboratory rodent was readily available and both animals were not very expensive to work with in experimental models. Most detailed descriptions of the morphology and histochemistry of the GL available were made on material from the sheep and the rat. Detailed descriptions of the GL of the digestive tract of cattle are scarce. No detailed descriptions of the GL of the bovine respiratory tract is available in the literature as far as the author is aware although that of the sheep has been studied (Mahmoud, 1978). The literature so far does not indicate marked differences between the GL of different animal species. The following description is therefore based on

the information available from the species in which the cell has been observed and described.

Several authors have described in some detail the light microscopic appearance of the GL. The descriptions of the GL in the digestive tract of cattle were made by Jarrett et al., (1967a), Miller et al., (1967), Murray et al., (1968) and of the GL of the digestive tract of the sheep Kent, (1952) and goat Rahko, (1972). Globule leucocytes in the digestive tract of the rat were studied and described in detail by Jarrett et al., (1967a). Takeuchi et al., (1969), gave details of a light microscopic study on GL in the cat's digestive tract. In addition, in cattle, goat and in the sheep Kellas, (1961), described GL in the uteri and Mahmoud, (1978),described them in the respiratory tract of the sheep. A detailed description of GL in the urinary tract of rats was made by Kirkman, (1950).

Globule leucocytes were generally found within the epithelium of mucous membranes in the various systems referred to above between the epithelial cells; their average diameter was about 12.8μ. The nuclei of these cells were usually ovoid or spherical and had distinct nuclear membranes. The cartwheel distribution of the nuclear chromatin characteristic of plasma cell was very common and the nuclei were eccentric. Keasbey, (1923), observed binucleate cells in sheep gastric mucosa. The cytoplasm of the cells was filled with refractile, acidophilic globules (granules) which showed variation in number and size. The granules generally appeared spherical or nearly so, but sometimes however, they seemed to have coalesced. The number of granules in a single cell ranged from about 5-30 (Keasbey, 1923, Kent, 1952, Kellas, 1961). All authors observed some globules of the GL to have a dark staining outer zone while others were found to contain one or more sharply defined vacuoles or a mass of smaller, closely packed vacuoles in the very large globules. Although cup shaped globules were observed by Keasbey, (1923), these have not been reported by others. The cytoplasm was never abundant and was very difficult to examine, although Keasbey, (1923), reported the presence of

pseudopodial prolongations. Kent, (1952), demonstrated both mitochondria and Golgi complex in the cytoplasm of sheep GL. The mitochondria were in the form of granules or short rods and were sometimes found to be applied to the globules or free in the cytoplasm in immature cells. The Golgi material on the other hand occurred in form of strands scattered throughout the cytoplasm. Centrospheres were observed by Kirkman, (1950), in a clear area of the cytoplasm devoid of globules and opposite one pole of the nucleus.

Although mitosis was not reported by most workers, Weill, (1919), observed them in the mouse; Kent, (1966), observed them in the rat, and Jarrett et al., (1967a), also saw them in the rat especially in animals treated with colchicine four hours before death. Keasbey, (1923), attributed her inability to observe mitosis to the fact that the nuclei of most cells were frequently hidden by the globules. Nuclear changes such as pyknosis and karyorrhexis were uncommon occurences although they have been reported (Kent,1952).

Diverse reports on the staining characteristics of the globules of the GL and hence their chemical components are available in the literature. These differences have either been attributed to the fixatives or staining methods that were used (Enerback, 1966, Takeuchi et al., 1969). Keasbey, (1923), observed the globules of the GL of the sheep to have great resemblance to the erythrocyte. On the basis of this she undertook a series of staining reactions that led her to the conclusion that the globules contained iron. In 1950, Kirkman undertook a comprehensive study of the cytochemical properties of the globules of the GL of the urinary tract of the rat. He found some relationship between the granules of the mast cell and the GL; both granules stained metachromatically with thionin, toluidine blue and brilliant cresyl blue although with varying degrees. However he enumerated seven different reasons which led him to conclude that a close genetic relationship between mast cells and GL of the urinary tract of the rat was unlikely. Neither he nor Dawson, (1943), studying GL in

the gall bladder of cats were able to confirm the findings of Keasbey since neither haemoglobin or any iron containing product of haemoglobin was present in the GL they examined.

Although Kent, (1952), reported the presence of the enzyme phosphatase and the amino acid arginine in the globules of the GL of the sheep, staining reactions for them in the rat urinary tract were negative (Kirkman, 1950). Again the xanthoproteic test for tyrosine was negative in the sheep intestinal GL granule while this was positive for those of the urinary tract GL (Kirkman, 1950, Kent, 1952). The metachromatic property of the globules of the GL was confirmed by Kent, (1952), thus establishing the presence of acid mucopolysaccharide in GL.

Later studies of the chemical composition of the GL granules in cattle, sheep and the rat confirmed the presence of acid mucopolysaccharide which was identified to be sulphated because of the positive reaction with astra blue stain (Jarrett et al., 1967a, Miller et al., 1967 and Murray et al., 1968). These investigators as well as Kirkman, (1950), Dobson, (1966a) and Rahko, (1970b), noted that the staining reaction of the acid mucopolysaccharide moiety present in the globules was reduced in the larger globules. It should also be emphasised that although Carnoy's fixative was the best for the demonstration of this component in mast cells (Enerback, 1966), Miller et al., (1967), observed that in the bovine and sheep some GL granules were not adequately fixed by this method and corrosive formol was found to give the most adequate preservations. Good preservation of GL in cattle was also reported by Rahko, (1970b), who used formalin and Bouin's fixative.

The presence of basic proteins but not 5-hydroxytryptamine (5-HT) was also established (Jarret et al., 1967a). The absence of 5-HT from the rat GL granules had earlier been reported by Whur & Gracie in (1967) and was confirmed by Cantin and Veilleux, (1972), in the GL of the urinary tract of the rat. Whur and Gracie, (1967), also observed a negative staining reaction by the globules of the GL with toluidine blue at pH 4.2 and concluded that heparin

was absent from the globules. In the intestine of sheep, Dobson, (1966a), also reported the absence of acid mucopolysaccharide from the globule of the GL. This was because the globules gave a negative reaction to alcian blue stain.

In 1972 Rahko, looking at the components of the GL in dicrocoeliasis and fascioliasis in goats reported that the granules of the GL did not stain with toluidine blue at pH 4.0 and 0.5, alcian blue at pH1.0 and astra blue/safranin, but stained lightly with alcian blue at pH 2.5, astra blue and periodic acid-schiff (PAS) at pH 2.5 even though they were stainable with biebrich scarlet at pH 10.5. He therefore concluded that the mucous substance of the goat's GL consisted of both neutral mucin and carboxy mucin and was different from the sulphamucin of cattle and mice. However he did not rule out the possibility of the presence of sulphate groups in the goat's GL granules. His second conclusion was that the goat's GL globules contained strongly basic proteins.

The histochemical properties of the ovine respiratory tract GL were the same as those of the cattle, sheep and rat intestinal GL (Mahmoud, 1978, Mahmoud and Pirie, 1983).

In the cat, Takeuchi et al., (1969), reported that histochemically, the granules of the GL do not have uniform composition. They reported differences between the GL of the small and large intestine and also between GL in the same location. With alcian blue/PAS staining sequence on paraffin sections, the GL of the small intestine stained very faintly if at all, while in the colon, the granules may show a PAS-positive core surrounded by a capsule stained with alcian blue. This according to Takeuchi et al., (1969), indicated that the core of the globule contained neutral mucosubstance while acidmucosubstance was present in the capsule. The capsular acid mucosubstance was identified as sulphated by the high iron diamine reaction. These workers also suggested the presence of phospholipids because of the affinity of the granules for osmium and the positive reaction with Bakers acid

hematein staining method. The GL did not also stain metachromatically with toluidine blue or Giemsa stains.

The fine structure of GL has been described by various authors. Jarrett et al., (1967a), Miller et al., (1967), Murray et al., (1968), gave a detailed description of the GL in the digestive tract of cattle. The description of the GL in the digestive tract of sheep also was given by Jarrett et al., (1967a), Miller et al., (1967), Murray et al., (1968). In the digestive tract of the rat the fine structure of the GL was described by Kent, (1966), Whur and Johnston, (1967), Jarrett et al., (1967a), Miller et al., (1967), and Murray et al., (1968). In the digestive tract of the mouse Carr, (1967), reported on the fine structure of the GL with particular reference to the globules it contained. Takeuchi et al., (1969), Finn and Schwartz, (1972), described the GL of the cat's alimentary tract while Toner, (1965) and Holman (1972), described them in the alimentary tract of the fowl. The fine structure of the GL has also been described in the respiratory tract of sheep, (Mahmoud, 1978) and in the rat (Kent, 1966). They have also been described in the urinary tract of the rat by Cantin and Veilleux, (1972) and Hoyes et al., (1974).

Globule leucocytes were easily recognised within the epithelium by their characteristic large, round electron dense, cytoplasmic inclusions. They were found sandwiched between either columnar, cuboidal, transitional, goblet or enterochromaffin cells. They were usually separated from neighbouring cells by an intercellular space of variable dimension and small irregular protrusions (pseudopodia) of the cytoplasm were sometimes present in this space. No junctional complexes were observed between GL and epithelial cells. The cell was usually round or oval with a nucleus which was somewhat variable in shape. It could be large and ovoid or idented by the cytoplasmic granules and was usually eccentric. The nuclear chromatin was uniformly distributed throughout the nucleoplasm and a nucleolus was sometimes present. (Takeuchi et al., 1969).

Most workers agreed with the fact that the cytoplasm of the GL was pale and contained sparse granular endoplasic reticulum, although Whur and Johnston, (1967), reported them to be abundant in the GL of rats with few globules and Cantin and Veilleux,(1972), observed some in the GL of the urinary epithelium of normal rats. The presence of numerous free ribosomes, Golgi apparatus, few mitochondria, microtubules and membrane bound vacuoles within the cytoplasm have been reported. The Golgi complex occupied a central position in the cytoplasm near the nucleus and was made up of stacks of smooth surfaced cisternae slightly curved around a single or a pair of centrally located centrioles and did not show any evidence of globule formation. Miller et al., (1967), did not observe Golgi complexes in cattle GL. The mitochondria were thick, short and their cristae were parallel, well defined and with a pale matrix.

The granules contained in the cytoplasm varied in size and structure. Their structure varied from homogenous dense membrane bound bodies to vesicles containing rod-shaped crystalloid bodies (Whur and Johnston, 1967). The globules were round, had close fitting membranes which varied considerably in thickness and had been found connected with the endoplasmic reticulum. Most of the investigators have reported four main types of globules. The most common type of globule was that which was made up of homogenous electron dense material. A second type of globules was that whose dense homogenous matrix was spotted with irregular areas of electron lucidity or opacity (moth eaten). A third type was that with multivesicular bodies. In these, a small part of the globule was filled with vesicles alone, while electron dense material was localised beside the vesicles. The fourth form of globule was that with vacuoles, some of which contained crytaline material.

Other less common forms of globules have been observed. Murray et al., (1968), in the sheep, bovine and rat digestive tract, observed globules in which small areas or rim of less electron density and more granular matrix, separated the dense, homogenous material from the surrounding membrane, and granules in which the perigranular membrane had been lost. Kent, (1966), in

the digestive and respiratory tract of rats and Carr, (1967), in the intestine of the mice, observed empty globules. Carr, (1967), also described different forms of crystalline structures that could be found in the GL of the mouse intestine. These according to her were either rhomboid or rectangular although she remarked that this difference could be the result of different planes of sectioning. The rhomboid crystals had no organized fine structure and at high magnifications were seen to consist of amorphous granular material. The rectangular crystals commonly showed a strong striation which was always parallel to the long axis of the crystal and had a mean periodicity of 45A°. These types of crystalloid structures were also observed by Cantin and Veilleux, (1972), lying free in the cytoplasm of the GL of the rat's urinary tract epithelium.

The origin, fate and function of the GL have also been a matter of great discussion. Weill, (1919), in his article on the leucocyte elements in the alimentary mucosa of mammals described a cell found in the epithelium of the alimentary tract of the dog, cat and mouse which he called 'schollenleukozyten'. He regarded this cell, because of its microscopic appearance, to be of lymphocyte origin and the globules contained to be endogenous secretion granules. Keasbey, (1923), observed these cells in large numbers in the ruminant abomasum. The cell because it had similar morphology and staining characteristics, possessing a high reducing substances and a peroxidase as do the red blood cells, was considered to be one of the erythrocyte series. In 1927 Dawson, examining the lymph nodes from irradiated dogs observed a series of transitional stages from unmodified lymphocytes to normoblast-like cells with a few globules and to the fully differentiated GL. He concluded that these leukocytes occupied an intermediate position between red blood cells and leucocytes. Their origin was therefore again linked to lymphocytes. Kirkman, (1950), performed a series of staining reactions on the GL of the urinary tract of the rat, from which he was able to indicate differences between the GL and the eosinophil, the mast cell, the adrenal gland macrophages, intracellular globules of ceroid, and red blood cells. He proposed

a connective tissue origin for GL and suggested that further investigations should be carried out to elucidate the relationship between GL, plasma cells and Russell body cells. Kent, (1952), using the staining characteristics of the GL, was able to establish differences between GL, eosinophils, Russell body cells, and erythrocytes. He supported the theory of lymphocytic origin because, according to him, in the gastrointestinal tract, GL originated from small lymphocytes. These lymphocytes were usually in the lamina propria, but sometimes migrated into the epithelium in large numbers and eventually passed out into the lumen where presumably they were destroyed. This theory was later supported by Kent et al., (1954), when they used corticotropin and cortisone to reduce the number of lymphocytes and GL in the tracheal mucosa of the rat. Also Kent et al., (1956), showed a reduction in the numbers of GL in the intestine of X-irradiated and hypophysectomised rats. Using the electron microscope Toner, (1965), observed a relationship between the GL and lymphocytes in terms of their diffuse endoplasmic reticulum, mitochondria, Golgi apparatus and vacuole containing bodies. He therefore supported Kent's theory of a lymphocyte origin for the GL.

While studying the immune response to Oesophagostomum columbianum infection in sheep, Dobson, (1966b), using immunofluorescent techniques, demonstrated that the GL and plasma cell contained inclusions which fluoresced the same way and that both cells had pyroninophilic cytoplasms. She also showed that the number of GL increased as that of plasma cells decreased and attributed a change from plasma cell to GL for this relationship. She therefore concluded a lymphocyte origin for the GL. Whur and Johnston, (1967), observed an increase in the number of GL in the external muscular layer and submucosa of the intestine of the rat infected with N. brasiliensis. Because of the distribution of these cells within the mucous membrane, they thought it indicated that the majority of them migrated from the lamina propria into the epithelium. Again because of the reported relationship between GL and lymphocytes, they postulated that GL were modified plasma cells migrating into the site of antigenic stimulus. Still on the origin

of the GL, Takeuchi et al., (1969), suggested that in the cat because there were fine structural as well as staining differences between mast cells and GL, they were probably not related. He also suggested that because comparison between GL and other cells had failed to identify intermediate forms, an unknown pleuripotential precursor cell might transform into a GL. He suggested that a type of this transformation occurred in small lymphocytes after stimulation with phytoagglutinins.

Whur and White, (1970), failed to demonstrate immunoglobulin in GL of the small intestine of rats using fluorescent microscopy and concluded that they were not related to plasma cell or Russell body cells. Holman, (1970), agreed with the observation of Toner (1965). He reported similar fine structure in the GL and the lymphocyte in the small intestine of the chick.

In the late sixties, publications relating the origin of GL to MMC appeared in the literature. Jarrett et al., (1967a), Miller et al., (1967) and Murray et al., (1968), all postulated that the GL was an end stage cell resulting from degranulation process of the MMC. Globule leucocytes and MMC were found in large numbers during parasitic infections especially during self cure. Using the electron microscope and aided by histochemical studies, these authors were able to observe various stages in the formation of GL from the MMC. They argued that the slight differences in staining characteristics observed were as a result of the loss in some of the amine contents of the mucosal mast cell during their degranulation and movement from the lamina propria into the epithelium. Ruitenberg and Elgersma, (1979), did not agree with this hypothesis because according to them, GL were seen to be undergoing mitosis during T. spiralis infection in the rat, and the major increase in the number of GL in animals with an intact thymus was paralleled by the increase in intestinal mast cells; the number of mast cells was always lower than that of GL and that the increase in GL started earlier. On the basis of this, they put up another hypothesis that the GL was an intraepithelial cell independent of the MMC. Earlier on, Finn and Schwartz, (1972), had

suggested the possibility of the GL being a specific cell type since they were able to find a neoplasm of this cell in the cat's intestine. Ruitenberg et al., (1979), supported their observations when they showed that although the MMC response to T. spiralis infection was not thymus dependent, that of GL was. They did not find evidence for the hypothesis that the subepithelial intestinal mast cell was the precursor for the intraepithelially related GL. However, because both the MMC and GL shared various characteristics and taking into account the distribution of GL within the epithelium during T. spiralis infection in rats, Ruitenberg and Elgersma, (1980), did not totally discard the mucosal mast cell origin of the GL.

More recently, Kamiya et al., (1983), published a preliminary observation on the absence of GL in mast cell deficient W/W^v anaemic mice after T. spiralis infections. They did not observe proliferation of GL 5-35 days post infection in W/W^v anaemic mice while there was an increase in the number of GL in control mice which had sufficient numbers of mast cells 10 days post infection with a peak of mast cells at 14 days post infection. Huntley et al., (1984), finally confirmed the link between GL and MMC proposed earlier by Jarrett et al., (1967a), Miller et al., (1967) and Murray et al., (1968). They showed convincingly both with the electron microscope and by histochemical studies that isolated mast cells, transitional cells (TC) and GL from the intestine of sheep infected with O. circumcincta and H. contortus had a link. Apart from the grad ation of granular size from the small granules of the MMC through mixed large and small granules of the TC to the very large granules of the GL, all three cell types contained proteoglycan, serine esterase, dopamine and surface as well as intracellular immunoglobulin. As with the grad ation in granular size, their granular contents decreased as the granules got bigger. The hypothesis of Jarrett et al., (1967a), Miller et al.,(1967) and Murray el al., (1968), of degranulating mucosal mast cells losing their amine content and moving into the epithelium to become GL therefore holds.

With the problem of the origin of the GL concluded at least for now, their fate and function still remained unclear. Various propositions have been made as regards what happens to the GL after formation. Dawson, (1943), observed GL apparently discharging their granules into the lumen of the cat's intestine. Kirkman, (1950), suggested that GL passed into the lumina of the urinary tracts of rats although he did not find them there. This suggestion was later echoed by Kent, (1952), who presumed that the cells were then destroyed in the lumen. Whur, (1967), Whur and Johnston, (1967), observed that the occurrence of GL was related to the onset of immune reactions against worms in the intestine of rats infected with N. brasiliensis and suggested that the cells were concerned with the transportation of antibodies into the intestinal lumen. In the urinary tract of baboons fed magnesium deficient (nephrocalcinogeni c) diet, DuBruyn and Liebenberg, (1974), observed GL undergoing degeneration followed by their development into calcified microliths.

Various functions have been atributed to the GL. They have been assigned the role of assimilation of food substances (Weill, 1919). In 1923 Keasbey, concluded that the globules contained haemoglobin hence the GL had the same function as red blood cells, while Duran Jorda, (1945), thought the globules were erythrocytes being synthesized within the cell in the abomasum of cattle and sheep. The relationship between the GL and parasitic infections was first suggested by Hole, (1937). Later Taliaferro and Sarles, (1939), produced GL experimentally in rats infected with N. brasiliensis. Since then other workers have reported the association between parasites and GL (Kirkman, 1947, Sommerville, 1956, Dobson, 1965, Whur, 1966, Jarrett et al., 1967a and b, Miller et al., 1967, Murray et al., 1968 and Mahmoud, 1978).

These reported associations led to various suggestions as to other functions of the GL. Dobson, (1966b), showed by immunofluorescence that the globules of the GL contained condensation of globulin. This observation was confirmed by Mayrhofer et al., (1976). Whur, (1967), observed the occurrence of

GL to be related to immune reaction to the parasite N. brasiliensis infections in the rat, he proposed the role of antibody production and transportation for the GL. Carr, (1967), also supported this role of antibody production, although the kinetics by which the GL produces or concentrates its globulin and transports it has yet to be clarified. The recent confirmations of the presence of immunoglobulins in GL, their proven relationship to MMC through the TC (Huntley et al., 1984), and also the fact that both cells have been shown to be abundant during parasitic infection (reviewed by Gregory, 1979), lends some support to the hypothesis of Whur, (1967), that the GL play a role in immune reactions to parasites.

E. DISCUSSION.

Anaphylaxis or type 1 hypersensitivity in cattle affecting the respiratory tract produces severe respiratory distress associated with dypsnoea, acute pulmonary oedema and interstitial emphysema. This phenomenom can be initiated by the intravenous injection or inhalation of natural or medicinal products. During hypersensitivity reactions, mast cells and basophils release biologically active substances which exert their effects on smooth muscles and vascular endothelial cells. The resultant effect on these tissues is to cause their contraction and extravasation of fluid into tissue spaces.

In helminth parasitic infections, the phenomenon of 'self cure' (Stoll, 1928), has been linked with induced anaphylaxis (Mulligan et al, 1965, Whur, 1966). Jarrett et al., (1967a and b), Miller, (1971a), Murray et al., (1971), all observed an increase in the numbers of mast cells in the intestine of rats infected with N.brasiliensis during self cure. Apart from detecting an increase in the number of mast cells, Murray et al., (1971), suggested that mast cells moved into the intestinal mucosal epithelium to become GL. This observation strengthened the link between GL and parasitic infections. Earlier on, Hole, (1937), had suggested a relationship between GL and parasitic infections and Taliaferro and Sarles, (1939), had produced GL experimentally in rats infected with N.brasiliensis, while Whur and Johnston, (1967), had observed an increase in the number of GL in the muscular layer and submucosa of the intestine of rats infected with N.brasiliensis.

The GL which is a cell found within the mucous membrane of many vertebrates is characterised by the presence of large numbers of eosinophilic, refractile globules in its cytoplasm.

Jarrett et al., (1967a), Miller et al., (1967) and Murray et al., (1968), all postulated that the GL was an end stage cell resulting from the degranulating process of the MMC which then moved into the epithelium from the lamina propria during self-cure.

Before this time, the lymphocyte origin of the GL was suggested by Weill, (1919), who first described the cell. He also suggested that the globules which the cell contained were endogenous secretory products. Other investigators have studied the morphology, histochemistry and ultrastructural features of this cell and have suggested different origins. Keasbey, (1923), suggested the erythrocyte origin for the GL because according to her, the cell had similar histochemical properties as the red blood cell. Dawson, (1927b), agreed with, the lymphocytic orgin suggested by Weill because he observed transitional stages from lymphoblasts to GL in the lymph nodes of irradiated dogs. Kirkman, (1950), did not agree with the view because the results of the histochemical studies which he performed on the GL of the rat's urinary tract enabled him to differentiate the cell from other cell type to which it had been associated. His results were confirmed by Kent, (1952), who although observed differences between the GL and the eosinophil, the Russel body cell and erythrocytes, supported Weill's theory. This was because the lymphocytes which were usually in the lamina propria, sometimes migrated into the epithelium in large numbers to become GL.

Kent et al, (1954,1956), supported this hypothesis when they used corticotropin and cortisone as well as X-rays and hypophysectomy to reduce the number of GL in the intestine of rats. Lymphocyte origin for the GL was also supported by Toner, (1965), when he showed that the GL and lymphocyte had similar ultrastructural features.

Other investigators, Dobson, (1966) and Whur and Johnson, (1967), working with different parasites suggested the plasma cell origin of GL and so linked GL to lymphocytes although Whur and White, (1969), later refuted this hypothesis. This was because according to them, the GL of the rat's intestine did not contain immunoglobulin. Kirkman, (1950), proposed a connective tissue origin for the GL and this was supported by Finn and Schwart, (1972), who reported a neoplasm of the GL in the cat's intestine.

Takeuchi et al., (1969), did not support the MMC origin proposed earlier by Jarrett et al., (1967a), Miller et al., (1967) and Murray et al., (1968), because they were able to demonstrate fine structural differences between the GL and mast cells in the cat's intestine. They then proposed a pleuripotent precursor cells as the origin of the GL. Ruitenberg and Elgersma, (1979), also disagreed with the mast cell origin of the GL because they observed GL to be undergoing mitosis during T.spiralis infections in the rat. Ruitenberg et al., (1979), showed that although GL response in the intestine of rats infected with T.spiralis was thymus dependent, that of the mast cell was not.

Although various authors have confused the story as to the origin of the GL, more recent studies by Kamiya et al, (1983) and Huntley et al, (1984), still indicated and supported the theory of MMC origin as postulated by Jarrett et al., (1967a), Miller et al., (1967) and Murray et al., (1968).

The recent confirmation of the presence of immunoglobulin in GL (Huntley et al., 1984), the fact that the cell was related to mast cell (Huntley et al., 1984), and abundant during parasitic infections, strengthens the fact that the GL plays a role in parasite immunity as was suggested by Whur and Johnston, (1967), Jarrett et al., (1967a and b).

Large numbers of GL have been observed in the respiratory tract of cattle with various respiratory problems (Breeze et al., 1975), but their significance was not known. Also large numbers of this cell have been found in the lungs of adult cows in other outbreaks of respiratory diseases (Pirie, personal communications) and it was difficult to interpret the findings.

Globule leucocyte proliferation have been associated with parasitic pneumonia in sheep, (Mahmoud, 1978). This type of association has not been reported for lung worm infection in cattle, neither has there been a study carried out to investigate

the relationship between GL and bovine respiratory diseases.

The importance of bovine lungworm infection in countries where it occurs was indicated in the review on bovine parasitic bronchitis. The mechanism of immunity against this parasite has not been studied in detail since vaccination against the bovine lungworm infection has been very successful. Also studies on bovine lungworm infection and mast cell and GL proliferation have not been previously reported and hence the association between the large numbers of GL in the lungs of adult cattle and lungworm infection was not possible.

The GL has been characterised in the bile duct, abomasum and the urinary bladder of cattle where the occurence has been associated with parastic infections (Jarrett et al., 1967a,b, Miller et al., 1967, Murray, et al., 1968, Rahko 1970 and Lawrence, 1977).

Similar detail studies have not been made on pulmonary GL in cattle, therefore the work reported in this thesis was undertaken.

CHAPTER TWO

THE PREVALENCE AND DISTRIBUTION OF GLOBULE LEUCOCYTES IN THE RESPIRATORY TRACT OF CATTLE

THE PREVALENCE AND DISTRIBUTION OF GLOBULE LEUCOCYTES IN THE RESPIRATORY TRACT OF CATTLE

A. INTRODUCTION

Several studies in cattle have described the presence of a cell whose cytoplasm was packed with large, refractile, eosinophilic granules. This cell was found within mucous membranes including the mucous membrane of the respiratory tract; it was ovoid or spherical and had a round nucleus which was usually eccentric.

In the previous chapter it was pointed out that a similar cell called the 'globule leucocyte' (GL) had been described in the epithelia of other animal species (Weill, 1919, Keasbey, 1923, Kent, 1952 and 1966). The occurence of this cell has been associated with parasitic infections (Kirkman, 1950, Taliaferro and Sarles,1939, Whur, 1966, Jarrett et al., 1967a,b, Miller et al., 1967, Murray et al., 1968, Mahmoud, 1978). The presence of this cell in the bovine respiratory tract has also been reported in cattle with farmer's lung, fog fever, diffuse fibrosing alveolitis and atopic rhinitis (Breeze et al., 1975, Pirie et al., 1976, Allan et al.,1984). In addition large numbers of this cell have been found in the lungs of cows in other outbreaks of respiratory disease and it was difficult to interpret the finding. Because the cell was found in large numbers in the respiratory tract of some adult cattle, and its occurence in the respiratory tract of young cattle had not been described, a study of the prevalence of the GL in the bovine respiratory tract of all ages of cattle was carried out. The cell was then characterised by both light and electron microscopy as well as by histochemical studies which will be described later.

A light microscopic study of the tracheobronchial tree of all age groups of cattle from foetuses to adult animals was carried out. As a result of this study the GL was found in the respiratory tract of all age groups of cattle in the mucous membrane from the trachea to the bronchioles. Apart from within the epithelium,

GL were found in the glands, their ducts, the connective tissue and in the tracheal and bronchial associated lymphoid tissues.

These observations on the prevalence and distribution of GL in the bovine respiratory tract are described in this chapter.

B. MATERIALS AND METHODS

1. Animals

In this study, all the animals used were those presented to the University of Glasgow Veterinary School for both clinical as well as post-mortem examinations. The trachea, bronchi, bronchioles and lung parenchyma, bronchial and mediastinal lymph nodes were examined in six age groups of cattle. The age groups and the number of animals examined are shown in Table 1.

Group 1 animals were made up of 19 foetuses between the ages of three and nine months which were obtained from their dams at slaughter. Two sets of twin foetuses were included in this group.

Group 2 animals were 25 calves that were between zero and one month of age, none of which had a clinically diagnosed respiratory problem.

Group 3 animals were 20 calves between two and four months of age, nine of which had been clinically diagnosed to have respiratory problem.

Group 4 was made up of 24 calves between five and eleven months old. Twelve of these animals were also diagnosed as having had clinical respiratory problem.

The animals in Group 5 were made up of 27 calves between the ages of 12 and 23 months. Again nine of these had clinical respiratory problems.

Group 6 animals were all over 24 months old. A total of 34 animals were examined in this group, ten of which also had clinical respiratory problem.

Animals of both sexes were examined in each group. The division of animals into age groups was based on the herd management practices in Scotland. Animals in Groups 1 to 3 were considered to have been either raised indoors and have been reared parasite free or born outside in circumstances unlikely to involve exposure to parasites. Those in Groups 4 to 6 were considered to have been moved outside to graze at some time in their lives and therefore might have been exposed to parasites particularly Dictyocaulus viviparus.

2. Tissues

At necropsy, tissues for light microscopy were obtained from 10 standard sites. Tissues were collected from the trachea and the right lung as shown in Fig. 1. They were obtained not more than three hours after death of animal. Tissue blocks were also obtained from the bronchial and mediastinal lymph nodes.

3. Fixation

The blocks of tissue about 2-3 mm thick were collected in two fixatives. These were: 10% buffered neutral formalin and Carnoy's fixatives. The details of these fixatives are in Appendix 1. Tissues were fixed for at least 24 hours, post fixed in corrosive formol, dehydrated and cleared in a double embedding series. Tissues blocks were finally embedded in paraffin wax and cut at 5-6μ in a rotary microtome.

4. Staining Proceedures

Haematoxylin and eosin (H&E) stain was the routine stain used for the identification of GL. Other staining techniques were carried out to confirm that the cell identified with H & E

was the GL. The details of these techniques will be described in the next chapter.

5. Identification and Assessment of Prevalence of Globule Leucocytes.

Globule leucocytes in the bovine respiratory tract were identified in H & E stained sections. The cell was easily identified by its characteristic intracytoplasmic globules. The bovine respiratory tract GL were usually round or oval and had eccentric nuclei which had a cartwheel chromatin pattern. The cytoplasmic globules were round, refractile and stained eosinophilic with H & E stain.

In each group, an animal was considered positive when a GL was identified in any section of the respiratory tract. The distribution of GL along the respiratory tract of cattle was obtained by examining the respiratory tract of each animal in each age group. The density of GL was assessed as follows: One, two, or three pluses (+) were assigned to each region of the respiratory tract depending on the number of GL present in the whole histological section examined from that position of the respiratory tract. One plus (+) indicated that less than 10 GL were found, two pluses (++) indicated the presence of up to 19 GL while three pluses (+++) indicated that 20 or more GL were found.

The variation in GL density among the groups of cattle examined was investigated as follows: the numbers and percentages of animals in each age group with one, two or three pluses were obtained by examining each portion of the respiratory epithelium collected at necropsy. The highest number of pluses assigned to any portion examined even if they occurred in only one section indicated the group to which the animal was categorised.

6. Quantification of Globule Leucocytes in the Respiratory Tract of Adult Cattle

To quantify the GL in adult animals (Group 6), GL were calculated per unit length of the tracheal and bronchial epithelium in the various sections collected from 20 animals. The standard unit length was taken as one centimetre (cm) and the GL per cm in the respiratory tract of each animal was calculated as follows. All GL in a transverse section from six standard sites of the trachea and bronchi were counted by examinations of the epithelium with the high dry objective (x40). The circumference of the epithelium around the lumen in each section was measured by projecting the slide using a Leitz projector with objective (x2.5) and eye piece (x10) on to a screen. The distance from the projector to the screen was kept constant in each slide examined. The basement membrane around the lumen of the sections was measured on the screen using a map measure. The circumference of the epithelium was converted to cm after calibrating the map measure using a Leitz 2 mm long micrometre slide. Globule leucocytes per cm was then calculated and expressed as GL per cm.

C. RESULTS

1. The Prevalence and Distribution of Globule Leucocytes in the Respiratory Tract of Different Age Groups of Cattle

The GL was usually found within the respiratory tract epithelium between epithelial cells (Fig. 2) they were either in the infranuclear or supranuclear positions when compared with the epithelial cell nucleus. They were also present in the duct and glandular epithelium of trachea and bronchi (Fig. 3). Occasionally GL were found in tracheal and bronchial lumina (Fig. 4) as well as in the connective tissue peribronchial lymphoid aggregates in the lamina propria and in bronchial lymph node Fig. 5.

Differentiation between the GL and eosinophilic leucocytes which were occasionally found in the intraepithelial positions was

usually easy. Apart from the obvious presence of the lobed nucleus of the eosinophil, its cytoplasmic granules were smaller than those of the GL. The eosinophils were also comparatively smaller than the GL.

The cytochemistry of the GL will be discussed in the next chapter but it will suffice to say here that special staining techniques were carried out to confirm that the cell described above was similar to that described by Miller et al., (1967).

Although GL were present in the respiratory tract of all age groups of cattle, they were not found in every animal examined (Table 2). In foetuses (Group 1) 47.36% of 19 foetuses examined had GL in their respiratory tract. The cells were few, contained few globules and were scattered within the tracheal and bronchial epithelium (Fig 6). They were not usually found in bronchioles, neither were they found in the tracheal and bronchial glands, their ducts or in the connective tissue of the lamina propria. Globule leucocytes were concentrated in the tracheal epithelium (Table 3).

The situation in animals under one month old (Group 2) was different from that stated for foetuses.

Of the 25 calves examined, 76.00% of them had GL in their respiratory tract (Table 2). The cell was found within the epithelium of the trachea and bronchi. They were absent from bronchioles, tracheal and bronchial glands, their ducts and the connective tissues of the lamina propria. Although few GL were present in the respiratory tract of this group, they were concentrated in the tracheal epithelium (Table 4).

In animals between two and four months of age (Group 3) 50.00% of the 20 animals examined had GL in their respiratory tract (Table 2). The cells were evenly distributed between the trachea and bronchi (Table 5). Occasionally, a GL was found in the bronchiolar epithelium, but GL were not found in the connective tissue of the lamina propria or in the ducts and epithelium of the

tracheal and bronchial glands.

Of the 24 animals examined in group 4, 58.33% had GL in their respiratory tract (Table 2). In this group, GL seemed to be more concentrated in the bronchi (Table 6). They were usually absent from the tracheal and bronchial glands and their ducts and from the connective tissues of the lamina propria.

The prevalence of GL in the respiratory tract of animals in the remaining two groups of animals examined was similar (Table 2). Of the 27 animals in Group 5, 85.18% had GL in their respiratory tract. The cell was found in the epithelium of the trachea, bronchi and bronchioles. Globule leucocytes in this age group were more concentrated in the bronchi although they seemed to be more numerous in the smaller bronchi of the caudal lung lobes (Table 7). Apart from their presence in the bronchioles, GL were found occasionally in the tracheal and bronchial glandular epithelium their ducts and the connective tissues of the lamina propria.

Although 91.17% of the 34 animals in Group 6 had GL in their respiratory tract (Table 2), they had more GL in their epithelia compared to Group 5 animals (Table 9). Globule leucocytes were also commonly found in their tracheal and bronchial glands, their ducts and connective tissues of the lamina propria. As was stated for Group 5 animals, GL seemed to be concentrated in the bronchi, especially the smaller bronchi of the caudal lung lobes (Table 8).

2. Globule Leucocyte Population Density in the Respiratory Tract of Different Age Groups of Cattle.

Globule leucocytes were present in the respiratory tract of more than 40.00% of animals examined in all age groups of cattle (Table 2). In the foetuses and animals under one month old, GL were found to be present and more concentrated in the tracheal epithelium although a few were found in the bronchi (Tables 3,4). In these age group of cattle, no histological section of the respiratory tract examined had more than 19 GL. Of the nine

foetuses with GL in their respiratory tract, only 33.33% had up to 20 GL in any section of their respiratory tract examined while 36.84% had as many in their respiratory tract in Group 2 animals (Table 9).

In Group 3 animals GL were evenly distributed within the respiratory tract epithelium. The population density of GL within the respiratory tract epithelium was the same in the trachea and bronchi (Table 5). Only 10.00% of animals positive for GL in this group had more than 20 GL in any sections of their respiratory tract, 30.00% of them had up to 19 GL while 60.00% had less than 10 GL in any section of their respiratory tract examined (Table 9). There was no marked difference in GL population density between the cranial and caudal lung lobes from the tissues and the number of animals examined in this age group (Table 5).

The situation regarding GL population density in Group 4 animals was different from those of Groups 1,2 and 3 animals. Globule leucocytes were found to be more concentrated in the bronchi (Table 6) as opposed to the situation in Groups 1 and 2 animals where they were observed to be concentrated in the trachea and in Group 3 animals where they were evenly distributed throughout the respiratory tract epithelium. Of the 14 animals that had GL in their respiratory tract in this group, 7.14%, 42.85% and 50.00% had more than 20, less than 19 and less than 10 GL in any sections of their respiratory tract examined (Table 9). The number of animals with GL in their respiratory tract and the distribution of GL population density was comparable to those of Groups 1 and 3 animals (Table 9).

Although the distribution of GL within the respiratory tract epithelium of Groups 5 and 6 animals were similar to that of Group 4 animals. Many more animals in these groups had more than 20 GL in sections of their respiratory tract examined (Tables 7 and 8). In Group 5 animals, 17.39% of the 23 animals with GL in their respiratory tract had more than 20 GL, 30.43% had less than 19 GL and 52.17% less than 10 GL in any section of their respiratory

tract examined (Table 9). In group 6 animals, 54.83% of the 31 animals positive for GL in their respiratory tract had more than 20 GL, 38.70% less than 19 GL and 6.45% less than 10 GL in any portion of their respiratory tract examined (Table 9).

Group 6 animals therefore not only had a large number of animals with GL (91.17%) but also had more animals (54.83%) with more than 20 GL present in their respiratory tract. Another striking observation was that both Groups 5 and 6 animals seemed to have their GL concentrated in the respiratory epithelium of their caudal lung lobes (Tables 7,8) and in the smaller bronchi.

3. Globule Leucocytes per centimetre in the Bovine Respiratory Tract.

The results obtained for the quantification of GL in the respiratory tracts of 20 adult animals (Group 6) are presented in Table 10. The mean for each animal, the standard error as well as the standard deviations for each animal are also presented in this table. As few as 0.21 GL/cm were counted in the section of one of the standard sites (C_cB_1) examined in animal 89978 while as many as 129.47 GL/cm were counted in the standard site C_cB_2 examined in animal 89724. The results of GL quantification presented in this table also indicated that GL tended to be more concentrated in the smaller bronchi of the caudal lung lobes. This confirms the observation on the distribution of GL along the bovine respiratory tract. For each animal the mean value of GL/cm varied from 3.53 ± 1.24 GL/cm for animal 89978 to 57.12 ± 18.52 GL/cm for animal 89018.

D. DISCUSSION

Some details of the light microscopical appearance of the GL in mucous membranes of the bovine animal have been given by the investigators referred to in the Review of the Literature.

In the digestive tract of cattle, Jarrett et al., (1967a), Miller et al., (1967) and Murray et al., (1968), reported and described the GL. In the bile duct of cattle, Rahko, (1970a,b), described the GL while Kellas, (1961), described them in the bovine uterus and Lawrence, (1977), in the urinary bladder.

The cell was usually found within the epithelia of the organs in which it occurred. It was found between epithelial cells, had an ovoid, eccentric nucleus and was characterised by the presence of large numbers of intracytoplasmic globules. The cytoplasmic globules were refractile and stained eosinophilic with H&E stain.

The globules were observed histochemically to stain metachromatic with toluidine blue as do the granules of the mast cell, red with biebrich scarlet at pH 8.0, 9.0 and 10.0, blue with astra blue/safranin and alcian blue stains.

Various investigators have reported the presence of large numbers of GL in the bovine respiratory tract although the significance of their presence was not known (Blazek, 1971, Breeze et al., 1975, Pirie et al., 1976, Breeze et al., 1976, Lawrence, 1977 and Allan et al., 1983). These reports were based on the light microscopical appearance of the cells in H&E stained sections of the bovine respiratory tract.

This present study on the respiratory tract of cattle in age groups ranging from foetuses to adults revealed the presence of similar cells within the mucous membrane of the respiratory tract of all the age groups of cattle examinied. The cytoplasm of the cell contained large numbers of eosinophilic, refractile globules

in H&E stained sections and the cell had a nucleus which was round and usually eccentric.

The cytoplasmic globules gave variable results when stained with toluidine blue. Some of the small globules stained metachromatic while the large ones did not. Similar observations were reported by Miller et al., (1967). The globules stained bright red with Martius scarlet blue and with biebrich scarlet at pH 8.0, 9.0 and 10.0.

Globule leucocytes were found in the mucous membranes of the respiratory tracts of all age groups of cattle, but they were not found in every animal examined. Fo rty seven per cent of all foetuses examined had GL in the sections of their respiratory tracts examined while between 50.00% and 76.00% of calves between the ages of zero and 11 months had GL in their respiratory tract. The number and percentages of animals with GL in their respiratory tract increased as the calves got older. Eighty five per cent of all the animals above one year old examined had GL in their respiratory tract. The results differ from the findings of Taliaferro and Sarles, (1939), Kirkman,(1950), Whur, (1966), Jarrett et al., (1967a and b), Miller et al., (1967), Murray et al., (1968), Takeuchi et al., (1969) and Mahmoud, (1978), all of whom associated the presence of GL in the mucous membranes of the various organs which they studied to be associated with parasitic infections.

The distribution of GL within the respiratory tract from the trachea to the bronchi was not uniform in the bovine animal. Globule leucocytes were more concentrated in the trachea of the foetus and calves up to the age of one month, while the distribution was even in the respiratory tracts of animals up to four months old. In older animals i.e. in calves more than five months old, GL were observed to be more concentrated in the bronchi and especially in the smaller bronchi of adult cattle. Also in the adult cattle GL tended to be more concentrated in the smaller bronchi of the caudal lobes although large numbers were found in

the bronchi of the cranial lobes. This observation agrees with that of Mahmoud, (1978), who also observed large numbers of GL in the caudal lobes of adult sheep when compared to the cranial lobes.

The population density of GL within the respiratory tracts of the age groups of animals examined also varied. Fewer GL were found in the lungs of calves when compared to the adults. No calf up to one month old was found to have more than 19 GL in the sections of the respiratory tract epithelia examined. Seven calves between the ages of two and 23 months old had more than 20 GL in the sections of the respiratory tract examined while 16 (55.17%) of the adult cattle examined had up to and more than 20 GL in the sections of their respiratory tract examined. As few as 0.21% GL/cm and as many as 129.47% GL/cm were counted in the sections of the respiratory tracts of adult animals with a mean value ranging between 3.53 ± 1.24 GL/cm and 57.12 ± 18.52 GL/cm. These values obtained in this study were comparable to those obtained by Mahmoud, (1978), in the respiratory tract of adult outdoor sheep.

The large number of GL found in the respiratory tract of adult sheep was associated with parasitic pulmonary infections (Mahmoud, 1978).

The results of the present study did not show any obvious relationship between pulmonary parasites and the presence of GL in the bovine respiratory tract as found by Mahmoud, (1978), in the ovine respiratory tract. However he also observed that the presence of parasites or their lesions in the respiratory tract did not always result in the appearance or presence of GL in the ovine respiratory tract. In the animals studied in this investigation GL were found in cattle with no pulmonary parasites.

Although there was no observed association between the appearance or proliferation of GL and pulmonary parasitic infections in the bovine animal, the large numbers of GL found in the respiratory tracts, their concentration in the caudal lung lobes of adult cattle and the previous association with repeated exposure to parasites (Whur and Johnston, 1967), initiated thoughts

about the probable association of adult animals being repeatedly exposed to lung worm infections and the presence of GL in their respiratory tracts.

This hypothesis was investigated in the next part of this study.

TABLE 1: The age groups and number of animals examined.

GROUPS OF CATTLE EXAMINED DURING
GLOBULE LEUCOCYTE STUDY

GROUP	AGE (MONTHS)	TOTAL NO. OF ANIMALS EXAMINED
1	Foetuses	19
2	0 - 1	25
3	2 - 4	20
4	5 - 11	24
5	12 - 23	27
6	> 24	34

TABLE 2: The prevalence of globule leucocytes in the bovine respiratory tract.

PREVALENCE OF GLOBULE LEUCOCYTES IN
BOVINE RESPIRATORY TRACT

GROUP	AGE (MONTHS)	NO. EXAMINED	NO. +ve GL (%)
1	Foetuses	19	9 (47.36)
2	0 - 1	25	19 (76.00)
3	2 - 4	20	10 (50.00)
4	5 - 11	24	14 (58.33)
5	12 - 23	27	23 (85.18)
6	> 24	34	31 (91.17)

GL = Globule leucocyte
% = percentage
+ve = positive

TABLE 3: The distribution of globule leucocytes along the respiratory tracts of foetuses (Group 1).

DISTRIBUTION OF GLOBULE LEUCOCYTES ALONG
THE RESPIRATORY TRACT OF FOETUSES (GROUP 1)

CASE NO.	AGE	TRACHEA		CRANIAL LOBE		CAUDAL LOBE	
		Tr_1*	Tr_2*	CrB_1*	CrB_2*	C_cB_1*	C_cB_2*
F1	Foetus	++	–	+	–	–	–
F2	"	–	–	–	–	–	–
F3	"	–	–	–	–	–	–
F4	"	+	++	–	–	+	–
F5	"	++	++	–	–	–	–
91244F	"	–	–	–	–	–	–
91267F	"	+	–	–	–	–	–
91543F	"	+	+	–	–	–	–
91854F	"	+	–	–	–	–	–
93859F	"	+	–	–	–	–	–
94544F	"	+	+	–	–	–	–
94632F	"	–	–	–	–	–	–
95178F	"	–	–	–	–	–	–
96628Fa	"	–	–	–	–	–	–
96628Fb	"	–	–	–	–	–	–
96909Fa	"	–	–	–	–	–	–
96909Fb	"	+	–	–	–	–	–
97735F	"	–	–	–	–	–	–
97825F	"	–	–	–	–	–	–

*Tr_1, Tr_2, CrB_1, CrB_2, C_cB_1, C_cB_2 - Sample sites see fig. 1.

TABLE 4: The distribution of globule leucocytes along the respiratory tracts of calves between 0-1 month of age (Group 2).

DISTRIBUTION OF GLOBULE LEUCOCYTES ALONG THE RESPIRATORY TRACT OF CALVES BETWEEN 0 - 1 MONTH OF AGE (GROUP 2)

CASE NO.	AGE	TRACHEA		CRANIAL LOBE		CAUDAL LOBE	
	(DAYS)	Tr_1*	Tr_2*	CrB_1*	CrB_2*	C_cB_1*	C_cB_2*
MicroB/FR	30	+	+	-	-	+	-
MicroB/DB	30	+	+	-	-	-	-
MicroB/Ti	30	++	-	+	-	+	-
MicroB/1c	7	++	+	-	-	+	-
MicroB/BL	30	+	-	+	-	-	-
MicroB/2c	7	+	-	-	-	-	-
M1	30	-	-	-	-	-	-
M8	30	-	+	-	-	-	-
M9	30	+	-	-	-	-	-
M107	30	+	-	-	-	-	-
83737B	7	+	-	+	-	-	-
83737/469	2	++	++	+	-	+	-
89822	7	+	-	+	-	+	+
91624A	30	-	-	-	-	-	-
91624B	30	-	-	-	-	-	-
90317	4	++	+	+	-	+	-
91522C	8	+	-	-	-	-	-
92900	14	-	-	-	-	-	-
93406/207	3	-	-	-	-	-	-
94231	6	++	+	-	-	-	-
94232	10	-	-	-	-	-	-
94233	10	+	-	-	-	-	-
94238	30	+	-	-	-	-	-
95036	21	++	-	-	-	-	-
95086	30	++	+	-	-	+	-

*Tr_1, Tr_2, CrB_1, CrB_2, C_cB_1, C_cB_2 - Sample sites see fig. 1.

TABLE 5: The distribution of globule leucocytes along the respiratory tracts of calves between 2-4 months of age (Group 3).

DISTRIBUTION OF GLOBULE LEUCOCYTES ALONG THE RESPIRATORY TRACT OF CALVES BETWEEN 2 - 4 MONTHS OF AGE (GROUP 3)

CASE NO.	Age	TRACHEA		CRANIAL LOBE		CAUDAL LOBE	
	(MONTHS)	Tr_1*	Tr_2*	CrB_1*	CrB_2*	C_cB_1*	C_cB_2*
83737	3	-	-	-	-	-	-
89629	2	-	+	+	-	+	-
89686	4	-	-	-	-	-	+
89767	3	-	-	-	-	-	-
89781	3	-	+	-	+	-	+
89983	3	-	-	-	-	-	-
90328	2	-	-	+	-	+	-
90365	2	-	-	-	-	-	-
90512	2	-	-	-	-	-	++
90728	3	+	-	+	-	-	+
92785	$2\frac{1}{2}$	++	+	-	-	-	-
92891	3	-	-	-	+	-	-
93190	4	-	-	-	-	-	-
94856	3	+	++	-	-	-	-
94966	2	-	-	-	-	-	-
95410	$1\frac{1}{2}$	-	-	-	-	-	-
95494	4	+++	+++	++	+++	++	+
97119	3	-	-	-	-	-	-
96819	3	-	-	-	-	-	-
96988	3	-	-	-	-	-	-

*Tr_1, Tr_2, CrB_1, CrB_2, C_cB_1, C_cB_2 - Sample sites see fig. 1.

TABLE 6: The distribution of globule leucocytes along the respiratory tracts of calves between 5-11 months of age (Group 4).

DISTRIBUTION OF GLOBULE LEUCOCYTES ALONG THE RESPIRATORY TRACT OF CALVES BETWEEN 5 - 11 MONTHS OF AGE (GROUP 4)

CASE NO.	AGE (MONTHS)	TRACHEA		CRANIAL LOBE		CAUDAL LOBE	
		Tr_1*	Tr_2*	CrB_1*	CrB_2*	C_cB_1*	C_cB_2*
89795	10	+	+	++	++	++	++
90275	10	-	-	-	-	-	-
90363	8	-	-	+	++	+	+
91525	8	-	-	-	+	-	++
91611	11	-	-	+	+	+	+
91632	7	-	-	-	-	-	-
92748	10	-	-	-	-	-	-
92791	5	-	-	-	-	-	-
93755	10	+	-	-	+	-	+
94446	5	-	-	-	-	-	-
94447	5	-	-	-	-	-	-
94462	5	+	-	-	-	-	+
95269	11	+	-	-	+	+	+
95274	6	-	-	-	-	-	+
95350	10	-	-	-	+	-	-
95381	6	-	-	-	-	-	-
95439	6	-	-	-	-	-	-
95597	6	-	-	-	-	-	-
95701	6	++	++	+	+	++	+++
95776	9	-	-	-	+	-	+
96187	10	-	-	-	-	-	-
96263	7	+	-	+	++	-	-
96443	11	-	-	-	-	-	++
96498	7	-	+	-	+	-	-

*Tr_1, Tr_2, CrB_1, CrB_2, C_cB_1, C_cB_2 = Sample sites see fig. 1.

TABLE 7: The distribution of globule leucocytes along the respiratory tracts of calves between 12-23 months of age (Group 5).

DISTRIBUTION OF GLOBULE LEUCOCYTES ALONG THE RESPIRATORY TRACT OF CALVES BETWEEN 12-23 MONTHS OF AGE (GROUP 5)

CASE NO.	AGE (MONTHS)	TRACHEA		CRANIAL LOBE		CAUDAL LOBE	
		Tr_1*	Tr_2*	CrB_1*	CrB_2*	C_cB_1*	C_cB_2*
Para 10	20	++	++	++	++	++	++
89030	18	-	-	-	-	-	-
89103	18	+	-	+	-	+	-
89165	18	++	++	+++	++	+++	+++
89450	12	+	+	++	++	++	++
89556	18	-	-	-	+	-	++
89823	12	+	+	+	-	-	+
90677	18	+	-	-	++	+	-
90841	18	-	-	-	+	-	-
90898	18	+	-	-	+	-	+
91205	12	+	-	+	+	-	-
91579	18	-	-	-	-	+	-
91647	12	-	-	-	-	-	-
91753	12	-	-	-	-	-	+
92493	15	-	-	+	++	+	++
92841	18	-	-	+	+	-	++
93872	12	-	-	-	+	-	-
94721	18	+	-	+	+	+	+
94756	12	-	-	-	-	-	-
95144	12	-	-	-	-	-	+++
95306	12	-	+	-	++	-	+++
95401	12	-	-	-	-	-	+++
95431	12	-	-	+	+	+	+
95777	15	-	-	-	+	-	-
96496	12	-	-	-	-	-	-
97406	15	++	-	+	+++	+	+
97943	12	-	-	+	-	-	+

*Tr_1, Tr_2, CrB_1, CrB_2, C_cB_1, C_cB_2 - Sample sites see fig. 1.

TABLE 8: The distribution of globule leucocytes along the respiratory tracts of cattle above 2 years of age (Group 6).

DISTRIBUTION OF GLOBULE LEUCOCYTES ALONG THE RESPIRATORY TRACT OF CATTLE ABOVE 2 YEARS OF AGE (GROUP 6)

CASE NO.	AGE (MONTHS)	TRACHEA		CRANIAL LOBE		CAUDAL LOBE	
		Tr_1*	Tr_2*	CrB_1*	CrB_2*	C_cB_1*	C_cB_2*
MB1	96	++	++	++	+++	++	+
MB2	42	-	-	+	++	-	-
87203	60	-	-	+	+	+	+
88323	24	+	+	+	++	+	+
88529	96	-	+	+	+	+	+
89018	36	+	+	+++	+++	++	++
89139	108	+	-	++	++	++	++
89317	72	++	+	++	++	++	+
89348	Adult	+	+	++	++	++	++
89515	72	+	+	+	++	-	+++
89724	120	+	+	+	++	-	+++
89978	84	-	-	++	+	+	++
90375	28	-	+	++	++	++	+++
90862	72	-	-	++	-	++	+
91267	36	+	-	++	+++	-	+++
91434	72	+	-	+++	++	+++	+++
91439	36	++	+	-	-	-	++
91589	42	++	++	++	++	++	++
91858	24	-	-	-	++	-	+
91896	60	+	++	++	++	++	++
92583	96	++	+	+++	+++	+++	+++
92793	72	-	-	-	-	-	-
92860	24	-	-	-	-	-	-
93119	228	-	+	+	-	-	+++
93859	108	+++	+++	+++	+++	+++	+++
93878	48	+	+	++	++	+++	+++
94406	24	-	-	-	-	-	-
94419	108	+	+	-	+++	++	+++
94632	48	++	++	+++	+++	+	+++
94880	120	+++	++	+++	+++	+++	+++

Continued on next page:

DISTRIBUTION OF GLOBULE LEUCOCYTES ALONG THE
RESPIRATORY TRACT OF CATTLE ABOVE 2 YEARS OF AGE (GROUP 6)

CASE NO.	AGE (MONTHS)	TRACHEA		CRANIAL LOBE		CAUDAL LOBE	
		Tr_1*	Tr_2*	CrB_1*	CrB_2*	C_cB_1*	C_cB_2*
95233	84	-	-	++	++	-	+++
95432	72	++	++	+++	+++	+++	+++
96141	84	-	-	-	-	-	-
96737	36	-	-	+	+++	-	+++

*Tr_1, Tr_2, CrB_1, CrB_2, C_cB_1, C_cB_2 - Sample sites see fig. 1.

TABLE 9: Globule leucocyte population density in the respiratory tracts of different age groups of cattle.

GL POPULATION DENSITY IN THE RESPIRATORY TRACTS
OF DIFFERENT AGE GROUPS OF CATTLE

GROUP	AGE (MONTHS)	NO. EXAMINED	NO. +ve GL	GL POPULATION DENSITY (%) (+)	(++)	(+++)
1	Foetuses	19	9	6 (66.67)	3 (33.33)	0
2	0 - 1	25	19	12 (63.16)	7 (36.84)	0
3	2 - 4	20	10	6 (60.00)	3 (30.00)	1 (10.00)
4	5 - 11	24	14	7 (50.00)	6 (42.85)	1 (7.14)
5	12 - 23	27	23	12 (52.17)	7 (30.43)	4 (17.39)
6	> 24	34	31	2 (6.45)	12 (38.70)	17 (54.83)

GL = Globule leucocyte
% = Percentage
+ve = Positive

TABLE 10: Globule leucocyte per centimeter in twenty adult bovine respiratory tracts.

GLOBULE LEUCOCYTES PER CENTIMETRE (GL/CM) IN TWENTY ADULT BOVINE RESPIRATORY TRACTS.

CASE NO.	TRACHEA		CRANIAL LOBE		CAUDAL LOBE		MEAN ± SE*	S.D. **
	Tr_1	Tr_2	CrB_1	CrB_2	C_cB_1	C_cB_2		
88323	1.72	0.69	1.02	19.62	0.67	10.00	5.62 ± 3.16	7.75
89018	3.85	3.37	74.87	118.93	76.10	65.60	57.12 ± 18.52	45.37
89139	0.98	-	6.27	24.17	8.45	63.00	20.57 ± 11.28	25.57
89317	10.18	3.88	18.13	8.93	11.32	6.67	9.85 ± 1.97	4.84
89348	3.56	1.09	35.24	53.33	6.84	100.00	33.33 ± 15.7	38.65
89515	0.31	-	19.55	65.63	8.10	108.72	40.46 ± 20.48	45.79
89724	-	-	1.77	14.53	-	129.47	48.59 ± 40.60	70.33
89978	-	-	6.28	3.85	0.21	3.77	3.53 ± 1.24	2.49
90375	-	0.58	5.00	28.81	7.01	46.33	17.55 ± 8.69	19.43
90862	-	-	4.59	-	17.76	8.23	10.19 ± 3.92	6.80
91267	0.86	-	3.08	26.81	-	-	10.25 ± 8.30	14.38
91434	0.35	0.41	46.91	16.33	65.60	94.96	37.43 ± 15.68	38.42
91439	29.17	8.33	-	-	-	19.77	19.09 ± 6.02	10.43
91589	2.60	3.14	2.81	40.00	6.25	51.60	17.73 ± 9.01	22.08
91858	-	-	-	12.10	0.39	4.29	5.59 ± 3.44	5.96
91896	2.17	9.82	6.83	24.55	24.61	17.50	14.25 ± 3.84	9.42
92583	0.87	2.62	27.12	88.89	67.86	31.40	36.46 ± 14.44	35.38
93859	31.61	47.45	68.55	89.58	30.86	74.29	57.06 ± 9.85	24.13
93878	1.58	0.68	4.74	41.18	17.44	45.28	18.48 ± 8.21	20.12
95432	4.30	3.04	21.28	111.63	30.65	126.67	49.60 ± 22.48	55.07

*S.E. = Standard error. ** S.D. = Standard deviation.
Tr_1, Tr_2, CrB_1, CrB_2, C_cB_1, C_cB_2 = Standard sites.

FIG. 1: Standard sites of sampling from the respiratory tract and lungs of cattle.

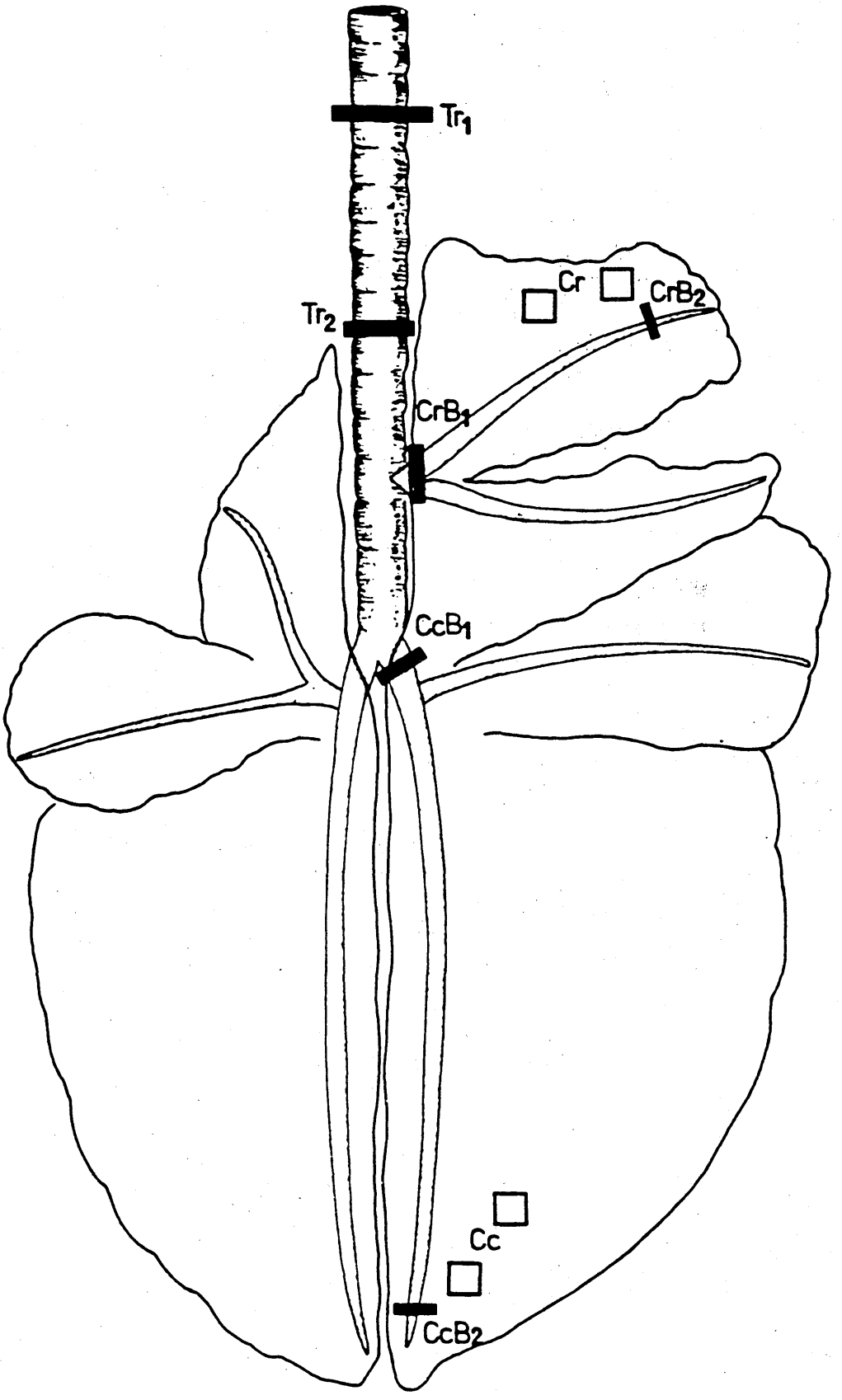

Tr1
Tr2
Cr
CrB2
CrB1
CcB1
Cc
CcB2

FIG. 2: Globule leucocytes within the bovine respiratory tract epithelium between epithelial cells.

H&E x 140

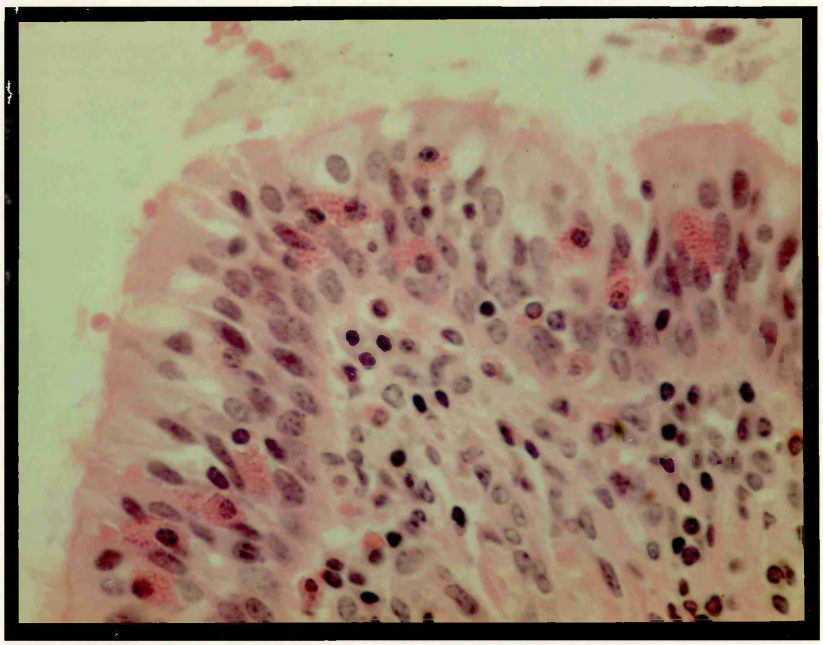
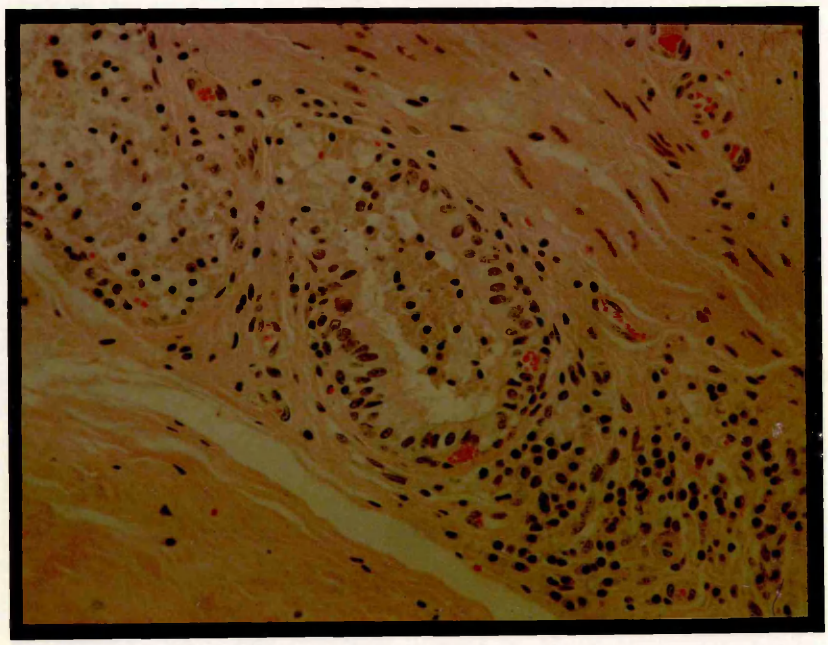


FIG. 3: A globule leucocyte within the glandular epithelium of a bronchus.

H&E x 140

FIG. 4: A globule leucocyte in the bronchial lumen.

H&E x 88



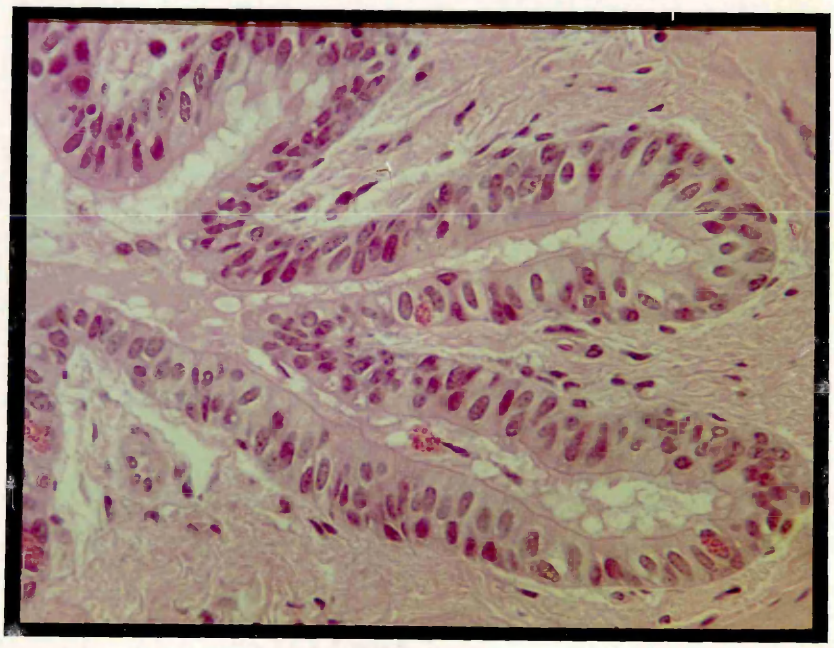
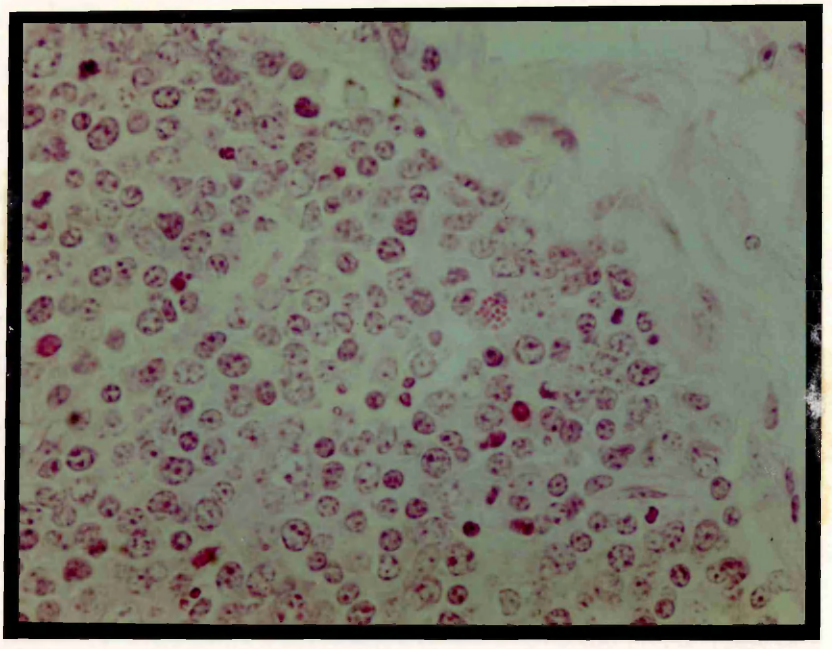


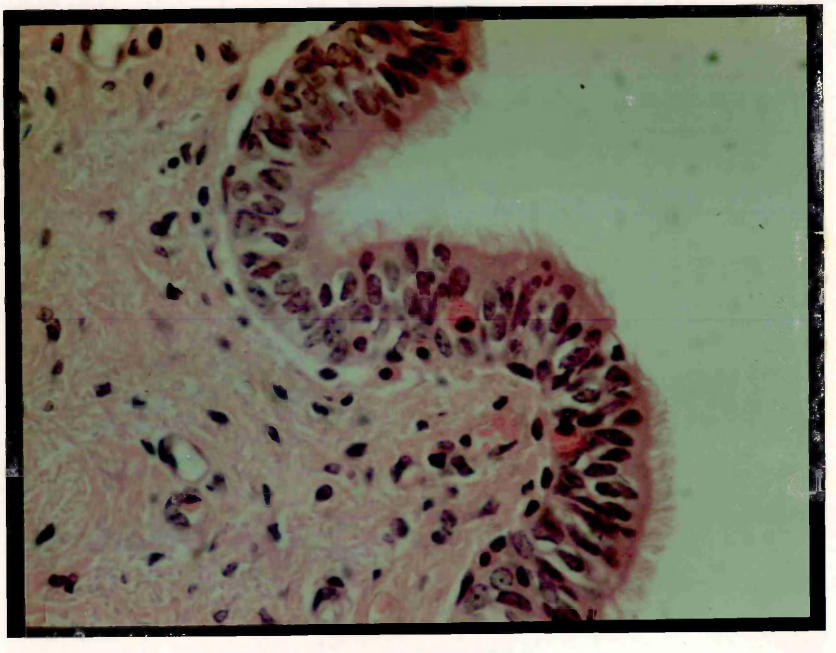
FIG. 5: A globule leucocyte in a bronchial lymph node.

H&E x 140

FIG. 6: Globule leucocytes within the tracheal epithelium of a bovine foetus.

H&E x 140





CHAPTER THREE

CHARACTERISATION OF THE GLOBULE LEUCOCYTE OF THE BOVINE RESPIRATORY TRACT: MORPHOLOGICAL, HISTOCHEMICAL AND ULTRASTRUCTURAL FEATURES

CHARACTERISATION OF THE GLOBULE LEUCOCYTE OF THE BOVINE RESPIRATORY TRACT: MORPHOLOGICAL, HISTOCHEMICAL AND ULTRASTRUCTURAL FEATURES.

A. INTRODUCTION

The occurrence of GL in the respiratory tract of all age groups of cattle was reported in Chapter 2. The cell type was not found in the respiratory tract of every animal examined although large numbers were present in the respiratory tract of most adult animals.

Globule leucocytes have been reported in various mucous membranes and investigators have observed them in the respiratory tract of several animal species. Their presence in the respiratory tract of cattle has been resported by Blazek, (1971), Breeze et al., (1975), Pirie et al., (1976), Breeze et al., (1976), Lawrence, (1976), and recently Allan et al., (1983). In the respiratory tract of sheep, Kent, (1966), Zipper, (1966), Mahmoud, (1978) and Mahmoud and Pirie, (1982), have described them also. Another ruminant in whose respiratory tract they have been observed is the deer, (Zipper, 1966, and Blazek, 1971). One of the few other species in which GL have been described in the respiratory tract was the rat, Taliferro and Sarles, (1939), Kent, (1966) and Jeffery and Reid, (1985), have described them.

Although GL have been observed in the respiratory tract of cattle, no detailed descriptions of the light microscopic observations are available in the literature.

Various authors have undertaken comprehensive histochemical studies of the GL in the epithelia of different species and have reported certain differences between them. The globules of the GL in some species seem to contain an acid mucopolysaccharide moiety (Kirkman, 1950, Jarrett et al., 1967a, Miller et al., 1967, Murray et al., 1968, Takeuchi et al., 1969, Rahko, 1970b and Mahmound and Pirie, 1983). The presence of both neutral and acid

mucopolysaccharide in the globules of the GL was suggested by Takeuchi et al., (1969), for the GL of the cat. The presence of acid mucopolysaccharide in the globules of the GL was usually associated with the metachromatic staining of the globules when stained with toluidine blue at PH 4.0.

Other staining methods that were used were alcian blue at PH 1.0 and 2.5, astra blue/safranin at PH 0.3 and acridine orange. Enerback, (1966), suggested that Carnoy's fluid was the best fixative for the demonstration of the mucosubstances in the granules of the mast cell but Miller et al., (1967), observed that in the bovine and the sheep, GL granules were not adequately fixed with Carnoy's fluid. They found that corrosive formol was most adequate for their granules. Other workers could not demonstrate metachromasia or characteristic staining with alcian blue or astra blue in the granules of the GL of the rat (Whur and Gracia, 1967), sheep (Dobson, 1962), goat (Rahko, 1972) and in the cat (Takeuchi et al., 1969).

Another substance observed to be present in the globules of the GL was basic protein. In the gastrointestinal tract of cattle, sheep and rat, Jarrett et al., (1967a), demonstrated the presence of basic proteins in the granules of the GL. Basic proteins were also found to be present in the sheep respiratory tract GL (Mahmoud,1978, Mahmoud and Pirie, 1983), and in the GL of the bile duct of goats (Rahko, 1972).

Again, although histochemical studies on the granules of the GL have been done in the bovine gastrointestinal tract, such studies have not been reported for the GL of the respiratory tract of cattle. Detailed morphological and histochemical studies were therefore undertaken for the GL of the bovine respiratory tract to observe any similarities or differences that could be present between them and and those in other mucous membranes of cattle. A second objective was to compare them with the other cells to which they had been thought to be related especially the mucosal mast cell (MMC) and the eosinophilic leucocyte.

The fine structure of the GL in various epithelia of several species has been studied. The only reports of the fine structure of the GL in the respiratory tract were those of Mahmoud,(1978), for the sheep respiratory tract and Kent, (1966), for the respiratory tract of the rat. To compare the GL of the bovine respiratory tract with those of the sheep and rat and with the fine structure of the bovine gastrointestinal GL, an electron microscopic study into the fine structure of the GL of the bovine respiratory tract was undertaken.

In this chapter, observations on the morphologic, histochemical and electron microscopic features of the GL of the bovine respiratory tract are reported.

B. MATERIAL AND METHODS

1. Animals.

The animals used in this study belong to the same groups outlined in Table 1, Chapter 2.

2. Histological Methods

(a) Tissue Samples.

Tissue blocks were obtained from the respiratory tract and pulmonary parenchyma of the animals. The number of samples and the sites were described in Section B of Chapter 2. In addition to these, tissue blocks were taken from the bile duct and the abomasum of each animal examined.

(b) Fixation

The fresh tissue block about 2-3 mm in size were collected in two fixatives. These were: 10% buffered neutral formalin and Carnoy's fixatives as in Section B of Chapter 2. The details of the fixatives are in Appendix 1. Tissues were fixed for at least 24 hours, post fixed in corrosive formol, dehydrated and cleared in a double embedding series. Tissue blocks were embedded in paraffin wax and cut at 5-6μ in a rotary microtome.

(c) Staining Procedures

For routine examinations and identification of GL, haematoxylin and eosin (H & E) stain was used. To examine special features of the globules of the GL, special staining techniques were used. The staining techniques employed were Martius scarlet blue (MSB), carbol chromotrope (CC), acridine orange (AO), biebrich scarlet (BS), toluidine blue (TB), alcian blue (AB), astra blue/safranin (AB/S) and phosphotungstic acid haematoxylin (PTAH). The preparation of reagents and staining techniques are shown in Appendix 1.

3. Measurement of Globule Leucocytes and their Globules.

To study the size of GL in the respiratory tract of cattle, samples from the lungs of ten adult cattle were examined. Adult animals were used because they had been shown (Chapter 2) to contain many GL in their respiratory tracts. Tissues obtained from the standard sites (Fig. 1) of these animals were prepared as above and stained with H & E. Measurements were made on 100 cells in the respiratory tract of each animal. The cell size was assessed by two measurements: the maximum diameter of each cell and the dimension taken at right angle to that. In all 1,000 cells were measured.

For the size variation of globules, the diameter of the largest and smallest globules from 25 GL in the small bronchus of both cranial and caudal lung lobes were taken. Again ten adult (Group 6) animals were used and a total of 100 globules were measured from each animal. The 100 globules measured from one animal was made up of 50 largest and 50 smallest globules. A total of 1,000 globules were measured.

All measurements were done using a Leitz micrometer eye piece calibrated on a standard Leitz 2mm slide micrometer using the x 40 objective.

4. Histochemical Techniques

Formalin fixed tissues were used for histochemical staining

methods to characterise the carbohydrate rich compounds of the GL. The possible presence of acid mucosubstances were investigated by acridine orange (AO) using fluorescent staining according to Hicks and Matthaei, (1958). Acid mucosubstances were further investigated by alcian blue staining at pH 1.0 and 2.5 (AB 1.0, 2.5) according to Enererback, (1966). Some sections were stained with astra blue at pH 0.3 and counterstained with safranin according to Bloom and Kelly, (1960).

To investigate the metachromatic nature of the acid mucopolysaccharides, toluidine blue was used in 0.5 and 0.1 per cent aqueous solutions at pH 4.0 and 0.3 respectively (Enerback, 1966). The presence of basic protein in the granules of the GL was investigated by staining with biebrich scarlet at pH 8.0, 9.0, and 10.0 according to Spicer and Lillie, (1961). The presence of protein in the globules was first established by staining with Martius scarlet blue which colours the globules red and phosphotugstic acid haematoxylin which coloured the globules blue brown.

For fluorescent microscopy, sections stained with acridine orange were examined on a Leitz ortholux microscope fitted with a fluorescence incident light illuminator. The light source was a mercury Osram HB0200 watt burner with a BG38 red suppression filter. A BG12 exciter filter was used in conjunction with position three of the filter turret which contained dichromic beam splitting mirrors and built-in suppression filters TK510 and TK515 respectively. Photomicrographs were taken with a Leitz Orthomat camera system with a x 10 eye piece.

5. Electron Microscopic Techniques

(a) Tissues.

The presence of large numbers of GL in the respiratory tract of adult cattle (Group 6) was reported in Chapter 2. Tissues for electron microscopic study were therefore taken from 12 adult animals.

Small blocks of epithelium from the small bronchi of both the cranial and caudal lung lobes (Sites CrB_2 and CcB_2) and of the lung parenchyma of both lobes (Sites Cr and Cc), as well as tissues from the bile duct and the abomasal folds were taken from the same animal. Tissues were obtained within 30 minutes after the death of the animal. The specimens were placed in drops of chilled fixatives on blocks of paraffin wax, chopped into very small pieces of about 1 mm square and were transfered into small vials containing chilled fixatives at 4°c.

(b) Fixation

The tissue blocks were fixed in paraformaldehyde/ glutaraldehyde fixative for four to six hours at 4°C, rinsed in 0.1M cacodylate buffer containing 0.1M sucrose and left in fresh rinse at 4°C overnight. Blocks of tissue were then post fixed in one per cent osmic acid in Millonigs phosphate buffer for one and a half hours at 4°C. The details of the fixatives and rinsing buffers are in Appendix 1.

(c) Embedding

Dehydration in acetone was through increasing concentrations from 30 per cent to 100 per cent followed by infiltration with acetone diluted araldite and then araldite alone for 30 minutes. Tissue blocks were then embedded in beem capsules containing araldite and hardened in the oven (57-60°C) for 48 hours. Details of the embedding reagents are described in Appendix 1.

(d) Sectioning and Staining

Thick sections approximately 1μ in thickness were cut on an L.K.B. Mark III ultratome using glass knives. Sections were mounted on glass slides and stained with borax buffered methylene blue/ Azure II (Trump, Smuckler and Benditt, 1961). The sections were then examined to locate specific areas of bronchial, bile duct or abomasal epithelium from which ultrathin sections could be made.

Ultrathin sections were then cut on the ultramicrotome, mounted on copper mesh grids and double stained with 20 per cent

uranyl acetate in absolute methanol (Watson, 1958), followed by lead citrate (Reynolds, 1963). Sections were rinsed in distilled water, dried and examined with an AEl 6B electronmicroscope. The preparation and description of stains for thick and ultrathin sections are given in Appendix 1.

C. RESULTS

1. Light Microscopical Observations

The light microscopical appearance of the GL of the bovine respiratory tract was similar to to those in the bile duct and abomasum of cattle as described by the authors referred to earlier (Fig. 7).

The GL was usually within the respiratory tract epithelium and between the epithelial cells. They were found either in the infranuclear or supranuclear positions when compared to the epithelial cell nuclei. They were present in the epithelium of the tracheal and bronchial glands and their ducts. Occasionally GL were found in the connective tissues of the lamina propria (Fig. 8) and also in the bronchial lumen.

The bovine respiratory tract GL were round or oval. The cells varied in size and were between 5 and 16μ in diameter. The majority of these cells were of 8 and 14μ in diameter (Table 11).

The nucleus of the GL, bounded by a nuclear membrane, was usually eccentric, round or ovoid and had the characteristic plasma cell pattern of cartwheel arrangement of chromatin (Fig. 9). Most GL in the respiratory tract, bile ducts, and abomasum, had single nuclei but it was not uncommon to observe GL with two nuclei (Fig. 10). A GL undergoing mitosis was observed in the abomasum of one animal examined (Fig. 11). Occasionally the nucleus of the GL was observed to be pyknotic. Karyorrhectic nuclei were not seen.

The cytoplasm of GL contained many globules which were round, homogeneous, refractile and separated from each other. They

stained red with H&E. The cytoplasm between the globules was sparse and was not observed to contain vesicles or vacuoles. The number and size of the globules contained within a cell varied. It was possible to have as few as four globules and as many as 40 globules in a cell. Globules were between 1 and 7μ in diameter for the larger globules and between less than 1 and 4μ in diameter for the smallest globules (Table 12). The majority of the globules were 1μ and 4μ in diameter. An occasional large globule (Fig. 12) was found in cells which had few globules.

The globules of some GL were observed to have lost their eosinophilic colour and were brown in sections stained with H & E. Also GL were often observed to be discharging their globules into a bronchial lumen (Fig. 13). The globules themselves were occasionally found free. They were not observed to possess either vesicles or vacuoles but the centre of the large globules stained very pale with H & E while the rims stained deeply. In some GL present in the lamina propria and close to the basement membrane the globules were very small and numerous (Fig. 14), but were easily observed when stained with Martius scarlet blue stain (Fig. 15).

2. Histochemical Observations

(a) Fixation

Two fixatives were used in the preservation and study of tissues from the respiratory tract of cattle. The GL of the bovine respiratory tract were not well preserved in tissues fixed in Carnoy's fluid and histochemical studies were carried out only on tissues fixed with 10% buffered neutral formalin and post fixed in corrosive formol.

(i) Carnoy's fluid:

When compared to 10% buffered neutral formalin, Carnoy's fluid did not give satisfactory results in the preservation of the granules of the GL. Most granules were ruptured and most cells had very poor cytological details.

(ii) Buffered neutral formalin (ten per cent):

The results of the histochemical studies undertaken for the staining properties of the GL, (MMC) and eosinophilic leucocyte of the bovine respiratory tract are shown in Table 13. These results are considered in the next paragraph.

(b) Histochemistry of the Globule Leucocyte, Mucosal Mast Cell and Eosinophilic Leucocyte.

Histochemical studies were carried out to investigate the cytoplasmic components of the GL of the bovine respiratory tract. Also to observe any similarities or differences between GL, MMC and the eosinophilic leucocyte, cells to which the GL had been thought to be related. The results of these studies are illustrated in Table 13. The results of the staining methods have been classified according to whether a positive reaction occurred (+), was fairly strong (++), very strong (+++) or did not occur (-) in the globules or granules of the cells examined.

Mucosal mast cells were not usually demonstrated in sections stained with H & E, but GL and eosinophilic leucocytes were always deeply stained. The intracytoplasmic granules of the mucosal mast cell were revealed by certain histochemical techniques and they were smaller than those of the GL.

(i) Mucosubstances.

The mucosubstances present in the globules of the GL, MMC and eosinophilic leucocytes was investigated by acridine orange staining technique, toluidine blue at pH 0.3 and 4.0, Alcian blue at pH 1.0 and 2.5 and astra blue/safranin at pH 0.3.

With acridine orange, the yellow orange fluorescence given by GL was weak, compared to those of the globlet cells or the yellow fluorescence given by the MMC.

Mixed results were obtained when the GL of the bovine respiratory tract were stained with toluidine blue at pH 0.3 or

4.0. In the adult cattle, at pH 0.3 and pH 4.0, the larger globules of the GL were not usually stained although the smaller occasionally showed violet metachromasia. In the young animals, most of the globules of the GL were stained as compared to those of the adult at pH 4.0, when stained at pH 0.3, the small globules gave very deep blue violet metachromasia (Fig. 16) while the larger stained light blue if at all. When large numbers of goblet cells were present along with GL, it was difficult to distinguish between the cells with large numbers of small globules and the goblet cells at pH 4.0.

At any pH used, the MMC granules gave violet metachromasia with toluidine blue (Fig. 17). They were distinguishable from the goblet cells and GL because of their position in the lamina propria and the size of their granules. The eosinophilic leucocytes were not observed to stain with toluidine blue at any pH used.

The granules of the MMC stained strongly with alcian blue at pH 1.0 and 2.5 and with astra blue/safranin at pH 0.3 (Fig. 18). The globules of the GL on the other hand did not stain at any pH when stained with alcian blue. A mixed result was obtained with the globules of the GL when the cells were stained with astra blue/ safranin sequence. In some cells, some of the globules stained blue while they did not stain in other cells (Fig. 19). Eosinophilic leucocyte granules were never stained when alcian blue or astra blue safranin staining techniques were used.

The above histochemical studies indicated that the globules of the GL of the bovine respiratory tract contained both sulphated and carboxylated mucins in young animals while the GL of the adult animal probably contained a low amount of carboxylated mucin mixed with neutral mucin. The adult animal may be negative for sulphated mucin in their GL. The MMC granules in the bovine respiratory tract contained both sulphated and carboxylated mucins in both the young and adult animals. Eosinophilic leucocytes were not shown to contain any type of mucosubstance in their granules.

(ii) Basic proteins

The presence of basic proteins in the globules of the GL, MMC and the eosinophilic leucocytes was investigated by the use of the biebrich scarlet staining technique at pH 8.0, 9.0 and 10.0.

In the buffered neutral formalin fixed tissues post fixed in corrosive formol used, both the GL and the MMC granules gave very bright red colouration. This indicated the presence of basic protein moiety at the site of carbohydrate rich compounds. The eosinophilic leucocyte granules were faintly stained with biebrich scarlet.

Globule leucocytes were differentiated from mucosal mast cells by staining with Martius scarlet blue and phosphotungstic acid. With Martius scarlet blue the GL globules stained bright red while those of the mucosal mast cell were not stained (Fig. 20). The globules of the GL were bluish brown when stained with phosphotungstic acid haematoxylin. Those of mucosal mast cells did not pick up the stain (Fig. 21).

Globule leucocytes were differentiated from eosinophilic leucocytes by staining with carbol chromotrope stain. This is a special stain for the granules of the eosinophilic leucocytes. The granules of the eosinophilic leucocyte stained bright red, those of the GL stained faintly red while those of the mucosal mast cell were not stained (Fig. 22).

3. Electron Microscopic Findings

The ultrastructural features of the GL of the bovine respiratory tract were compared and contrasted with those of the abomasum and bile duct as well as with the bronchial, abomasal and bile duct MMC, plasma cell, eosinophilic leucocytes and other granular cells present in these epithelia.

(a) Globule Leucocytes:

The GL of the bovine respiratory tract, the bile duct and the abomasum were observed in this study to have similar structures.

Ultrastructurally, the GL were easily recognised because of their characteristic large electron dense membrane bound intracytoplasmic globules (Fig. 23). Most GL observed were found sandwiched between epithelial cells and were within the epithelium. The cells were oval or circular in shape and were separated from other epithelial cells by variable intercellular spaces in which in some cases were found the cytoplasmic prolongations of the GL (Fig. 24). GL were never observed to be attached to epithelial cells by cell junctions.

The nucleus of the GL was usually circular, spherical or ovoid and was eccentric. The nucleus was usually indented by the globules and was irregular in outline (Fig. 25). Mitosis were not observed.

The cytoplasm of the GL was very sparse and contained few free ribosomes. Occasionally a centriole (Fig. 26) was seen close to the nucleus. Rough endoplasmic reticulum or Golgi apparatus were not seen in any of the GL observed from the respiratory tract, bile duct or abomasum. The cytoplasm contained few membrane bound vacuoles and mitochondria which were short, thick and had well defined cristae. Few microtubules were present in the cytoplasm of the GL. The structures described above were scattered throughout the cytoplasm (Fig. 27).

The main structures present in the cytoplasm were the large electron dense globules which gave the cell its name. They varied in size and morphology but were evenly distributed within the cytoplasm. The typical globule was about four times the size of a mitochondria, was round and surround by a unit membrane. The bounding membrane was not found to be continuous with either

those of the membrane bound vesicles or microtubules.

Two main types of globules were observed in the bovine tissues examined.

(i) Globules with homogeneous electron dense material which was in close apposition to a surrounding unit membrane (Fig. 28), Type I globule.

(ii) Vacuolated globules which contained crystalloid bodies, Type II globules. The crystalloid bodies were either filamentous structures or cylindrical in form (Fig. 29). Both had an internal lamellated structure and could usually be found in the same cell. Most of the cells seen during this study contained Type I globules.

With the light microscope, GL were observed to undergo degenerative changes. This was confirmed at the ultrastructural level. A series of cells at different stages of the degenerative process leading to ultimate necrosis were observed in the respiratory tract of some animals. In Stage I of the degenerative process (Fig. 30) vacuoles were present in a few globules and later on in this stage, the cytoplasmic globules became extensively vacuolated (Fig. 31). During Stage II the vacuolated globules fused together and were less electron dense (Fig. 32). At this stage, the GL was still bounded by its cell membrane. Finally, the cell membrane ruptured (Stage III) with loss of globules and cytoplasmic contents and the cellular outline became very difficult to follow (Fig. 33). The loss of granular material by vacuolation may explain the loss of eosinophilia observed with the light microscope.

(b) Mast Cells

The mast cells were also easily identified at the ultrastuctural level by their characteristic intracytoplasmic granules (Fig. 34). They were found below the basement membrane

in the lamina propria of the bovine respiratory tract, bile duct and the abomasum as well as in the connective tissue below these epithelia.

The cells varied in shape and size. They were either oval, polygonal, spindle shaped or rectangular. Microvilli were usually observed projecting from the cell surface. The mast cell nucleus was usually large and centrally placed. The nuclear shape also varied and oval or spindle forms were commonly seen. The nucleus was indented by the cytoplasmic granules and it usually had an irregular outline. The nuclear chromatin was condensed and uniformly distributed. Mitotic figures were not observed in the mast cell of any of the organs examined.

The cytoplasmic organelles seen included a few mitochondria scattered throughout the cytoplasm. The mitochondria had pale matrix and parallel cristae. The rough endoplasmic reticulum was difficult to observe although prominent Golgi regions were seen. Free ribosomes were common and uniformly distributed. A few membrane bound vacuoles and microfibril s or fil aments were always present in the cytoplasm.

The characteristic feature of the cytoplasm was the large number of electron dense granules which varied in shape and structure. The granules were either oval or round in shape and could be tightly packed or sparsely arranged. Granules usually had an outer limiting unit membrane. Five types of granules with different internal structures were observed in the mast cells of the mucous membranes examined.

(i) Granules with an inner dense homogeneous matrix surrounded by an outer lighter rim (Fig. 35).

(ii) Granules with fine reticulated matrix (Fig. 36).

(iii) Granules with coarse reticulated matrix (Fig. 36).

(iv) Granules whose matrix were made up of both dense homogeneous and reticulated matrix (Fig. 37).

(v) Granules with crystalloid structures. (Figs. 38 and 39).

Two types of crystalloid structures were observed. One which was rectangular in appearance and in which no substructure was distinguishable (Fig. 38). A second type which had a honeycomb pattern consisting of an array of hexagonal subunits with electron lucent cores and electron opaque rims was observed in cross sections of such inclusions (Figs. 38 and 39).

(c) Plasma Cells

The plasma cell (Fig. 40) was studied ultrastructurally to enable differentiation to be made between it and the GL of the bovine respiratory tract, bile duct and the abomasum. The cell was usually found in the lamina propria and was easily differentiated from the GL because of the complex cytoplasmic rough endoplasmic reticulum. The cell itself was large and had a round or spherical nucleus. A large proportion of the nuclear chromatin was made up of heterochromatin.

Most of the cytoplasmic organelles were very well developed compared to those of the GL and mast cell. The rough endoplasmic reticulum was highly developed and was studded with ribosomes. In some cases, the cisternae of this structure was well distended by materials which it secreted. The Golgi apparatus was also well developed and was found close to the nucleus. It was made up of groups of smooth membrane flattened sacs, vesicles or vacuoles. The mitochondria were scattered throughout the cytoplasm between the rough endoplasmic reticulum. They had parallel cristae and light and dark matrix. Free ribosomes were found distributed throughout the cytoplasm.

(d) Eosinophilic Leucocytes

The eosinophilic leucocytes which were frequently seen in the tissues examined were easily differentiated from the GL and the

mast cells by the presence of their lobed nuclei (Fig. 41). The cytoplasm contained fewer granules compared with those of the GL and mast cells. The granules were large, round or oval and electron dense.

Three types of granules were observed in the cytoplasm of the eosinophils.

(i) Granules with a homogeneous matrix bounded tightly by a unit limiting membrane(Fig.41)

(ii) Granule with lamellated internal structure. The lamellar structures being arranged in a circular form (Fig. 42).

(iii) Granules with both types of structures in their matrix (Fig. 42).

Also present in the cytoplasm were free ribosomes, a few membrane bound vacuoles, mitochrondria which were round or elongated and a Golgi apparatus which was close to one of the nuclear lobes. Rough surfaced endoplasmic reticulum was scarcely seen.

(e) Secretory Cells

Secretory cells were also commonly seen in the epithelium examined. (Fig. 43). Because of their large secretory granules it was possible to confuse secretory cells with GL. These cells could also be differentiated from GL. Although their cytoplasmic granules were similar to those of the GL, secretory cells had well developed rough endoplasmic reticulum (Fig. 44) which in most cases surounded the secreted granules. Free ribosomes were found throughout the cytoplasm. A second distinguishing factor was the well developed Golgi region. This was not found in the GL. In the secretory cell, the Golgi complex was made up of flattened smooth membrane bound sacs and numerous vesicles as well as vacuoles (Fig. 44). A third factor that distinguished the

secretory cell from the GL was the fact that the secretory granules were usually found in the region of the cell close to the lumen and to one side of the nucleus. A fourth factor was the fact that secretory cells were epithelial cells associated with microvilli and had junctional complexes (Fig. 45).

(f) Goblet cells

The goblet cells had the same electron microscopic features as the secretory cells. They differed from the other secretory cells because they contained pale droplets in their cytoplasm (Fig. 46). They were therefore easily differentiated from the GL and other secretory cells.

D. DISCUSSION

In Chapter 2 of this thesis, the occurence of GL in the respiratory tract of all age groups of cattle from foetuses to adults was reported.

With the light microscope, GL in the bovine respiratory tract were observed to be similar to those previously described in the digestive tract of cattle (Jarret et al., 1967a, Miller et al., 1967, Murray et al., 1968, Rahko, 1970a, Blazek, 1971, and Lawrence, 1977).

The bovine respiratory tract GL was usually found within the respiratory tract epithelium and between epithelial cell, although they were occasionally found in the connective tissues of the lamina propria as well as in the bronchial lumen. The observation of GL in the bronchial lumen supports the suggestions of Kirkman, (1950) and Kent, (1952), who presumed that the end of the GL cell cycle occured when the cells were destroyed after they had passed from the epithelium into the lumen. Their presence in the lumen might also be related to the transportation of antibodies as was suggested by Whur and Johnston, (1967).

The GL of the bovine respiratory tract were round or oval and varied in size, their sizes ranging from 5 to 16μ. The cell had a nucleus which was eccentrically located, round or ovoid, indented and had a cartwheel arrangement of chromatin.

Occasionally, GL in the bovine respiratory tract were observed to have more than one nucleus. This observation supports the report of Keasbey, (1923), who observed binucleate cells in the sheep gastric mucosa. A GL in one of the sections of the bovine abomasum examined was observed to be undergoing mitosis. This finding also agrees with those of Weill, (1919), Kent, (1966) and Jarrett et al., (1967a), all of whom observed GL mitotic figures in the mucous membranes of the gastro intestinal tracts of the various animals they worked with. The only indication of degenerative or

necrotic change observed in the nucleus of the GL of the bovine respiratory tract was pyknosis.

The cytoplasm of the bovine respiratory tract GL was packed with globules which were round, homogeneous, refractile, separated from each other and stained eosinophilic with H&E stain. Vacuoles or ves icles were not observed in the cytoplasm of the GL of the bovine respiratory tract. The number of globules contained within the cytoplasm varied and as many as 40 globules could be present in one cell. This number also compares with the findings of Keasbey, (1923), Kent, (1952), and Kellas, (1961), who reported between 5 and 30 globules in the cells studied. The globules themselves varied in size. Globules ranging between 1 and 7μ in size were observed; the majority of which were within 1 and 4μ in size. Cup shaped globules reported by Keasbey, (1923), were not observed in this study, but large globules were observed to stain deeply at the rims and pale at the centre; an observation which had been reported earlier (Keasbey, 1923, Kent, 1952, Kellas, 1961).

In some cells, the globules were observed to have lost the usual eosinophilic colour and were brown when stained with H&E. It was suggested earlier in this chapter that the change in colour was associated with a degenerative process which was later confirmed with the electron microscope; and was thought to be linked with the loss of globule content. Globule leucocytes undergoing degenerative processes have been observed (DuBruyn and Leibenberg, 1974) in the urinary tract of baboons fed a nephrocalcinogenic diet.

Globule leucocytes discharging their globules into the bronchial lumen were observed in the bovine respiratory tract. This observation was previously reported (Dawson, 1943). The cause of this phenomenon was not known, although there is the possibility that it could be associated with immunological responses as was suggested by Whur and Johnston, (1967).

The results of the histochemical studies on the globules of

the GL of different species, have indicated certain differences between them. In some species, Kirkman, (1950), Jarrett et al., (1967a), Miller et al., (1967), Murray et al., (1968), Takeuchi et al., (1969), Rahko, (1970b), Mahmoud, (1978), and Mahmoud and Pirie, (1983), all reported the presence of acidmucopolysaccharide moiety in the globules of the GL of the rat, cattle, sheep and cat urinary, gastrointestinal and respiratory tracts. Takeuchi et al., (1969), also suggested the presence of both neutral and acid mucopolysaccharides in the globules of the GL of the cat's intestine.

In this study, formalin fixed tissues, post fixed in corrosive formol were used for the histochemical studies because the globules of the bovine respiratory tract were not properly preserved in Carnoy's fluid. Miller et al., (1967), reported similar observations for the GL of the bovine and ovine gastrointestinal tracts, and Rahko, (1970b), reported good preservation of bovine GL in formalin fixed tissues.

The presence of mucosubstance in the GL of the bovine respiratory tract was revealed by acridine orange staining. Although the yellow or ange fluorescence given by GL was weak when compared to those of mast cells and goblet cells, it indicated the presence of this substance in the globules. With toluidine blue, large globules in adult cattle did not give violet metachromasia at both pH used although the very small globules occasionally showed this characteristic. The situation was different in the calves and foetuses. All globules at pH 4.0 gave violet metachromasia while it was the small globules that stained deeply at pH 0.3, with the larger ones staining light blue. The observation that the large globules of the GL of the respiratory tract of adult cattle did not stain with toluidine blue corresponds with the report of Miller et al., (1967). Rahko, (1970b, 1972), also demonstrated reduced staining of the globules of the GL of the bovine and caprine bile ducts with toluidine blue stain at pH 4.0 and 0.5.

In this study, the granules of mast cells of the bovine respiratory tract gave very deep metachromasia when stained with toluidine blue at both pH 0.3 and 4.0.

With alcian blue, the globules of the GL of the bovine respiratory tract did not stain at the pH used but those of the mast cell stained deeply blue. The result with astra blue/safranin sequences for the GL was different when compared with their staining reaction to alcian blue. Some globules in some cells stained blue with astra blue/safranin sequence while others did not. The above results agree with the report by Miller et al., (1967), for the bovine and ovine gastrointestinal GL and Rahko, (1972), for the GL of the caprine bile duct affected by liver flukes. The results of his histochemical studies led Rahko, (1972), to suggest the possible absence of sulphated acid mucin from the caprine bile duct GL. He concluded that the mucous substance in the caprine bile duct GL was made up of both neutral and carboxylated mucin.

The results of the histochemical studies carried out in this investigation also indicated that the globules of the bovine respiratory tract GL contained both sulphated and carboxylated mucins in young animals while in the adult animal the globules probably contained a low amount of carboxylated mucin mixed with neutral mucin. The adult bovine animal may therefore be negative for sulphated mucin in the globules of the GL of their respiratory tracts. Also that the mast cells of the bovine respiratory tract contained both sulphated and carboxylated mucin in both the young and adult animals.

Basic protein were present in the globules of the GL and granules of the mast cells of the bovine respiratory tract. The difference in the staining characteristics of the GL and mast cell when Martius scarlet blue and phosphotungstic acid haematoxylin were used may indicate the fact that the percentage of protein moiety present in the globules of the GL was more than that present in the granules of mast cells.

Few reports were available in the literature describing the fine structure of the GL in the respiratory tract. Kent, (1966), reported on the ultrastructural features of the GL of the rat's respiratory tract, while Mahmoud, (1978), reported on that of the sheep. Other reports on the ultrastructural features of the GL in other mucous membranes were given by other investigators referred to in Section D of Chapter 1.

In this study, the GL of the bovine respiratory tract, bile duct and abomasum were observed to have similar ultrastructural features. The GL were easily recognised with the electron microscope by their characteristic large electron dense globules. The cells were found within the epithelium and between epithelial cells. The GL was oval or circular, in shape; had a nucleus that was usually circular, spherical or ovoid, eccentric and indented by globules.

Most of the ultrastructural features of the GL reported by other investigators were observed in the GL of the bovine respiratory tract except; (i) Golgi apparatus and (ii) rough endoplasmic reticulum which were not observed in this study. The absence of Golgi apparatus from the GL reported in this study confirms the observations of Miller et al., (1967), who were also unable to demonstrate Golgi apparatus in the GL of the gastro-intestinal tract of cattle, sheep and rat.

The characteristic feature of this cell is the large number of the cytoplasmic globules. Most investigators who have studied the ultrastructural features of the GL in various other species and mucous membranes, have reported the presence of four main type of globules in the GL. In this study, two main types of globules were observed ultrastructurally in the bovine respiratory tract GL. One had a homogeneous electron dense matrix and the other was a vacuolated globule which contained crystalloid bodies. The moth eaten and the multivesicular body types were not observed in this study. The other type with a less electron dense rim separating the

dense homogeneous material from the surrounding membrane observed by Murray et al., (1968) and the empty globules observed by Kent, (1966) and Carr, (1967), were not seen in this study.

A suggestion was made from the H&E staining characteristic of the globules of the GL indicative of the GL undergoing a degenerative process. This observation was confirmed with the electron microscope. A series of GL at different stages of the degenerative process leading to ultimate necrosis were seen. In all, three stages were recognised. The first stage was that of vacuolation of the globules; the second stage, that of the fusion of globules which have undergone extensive vacuolation; and the third stage was that of rupture of the cell membrane with loss of cellular architecture. These various stages have not been previously described.

The mast cell was easily differentiated from the GL at the ultrastructural level because they were found below the basement membrane of the epithelium. The cytoplasmic granules which are also characteristic of this cell were quite different from those of the GL but were similar to those that have been described for the human, rat and guinea pig mast cells (Hibbs et al., 1960, Brinkman, 1968, Dobbin et al., 1969, Orr, 1977, Caulfield, 1980 and Taichman, 1970).

Five types of mast cell granules were observed in this study. These were: (i) granules with an inner dense homogeneous matrix surrounded by an outer lighter rim; (ii) granules with fine reticulated matrix; (iii) granules with coarse reticulated matrix; (iv) granules with both dense homogeneous and reticulated matrix and (v) granules with crystalloid structures.

The GL was also differentiated from other cell types. The plasma cell was differentiated from the GL by the presence of a complex rough endoplasmic reticulum. The eosinophilic leucocytes was also differentiated from the GL by (i) the presence of a lobed nucleus and (ii) its granules. Very few reports describing the

granules of the bovine eosinophils are available. Murray, (1968), observed eosinophils in the bovine abomasum in ostertagiasis. He did not observe central bars, a feature of eosinophils in many animal species (Bessis, 1964, Hudson, 1967), in the bovine eosinophils. The bovine eosinophil has a reniform nucleus and many round and oval electron dense amorphous granules. In this study, three types of granules were observed in the cytoplasm of the eosinophil of the bovine respiratory tract. The first was similar to the one described by Murray, (1968). The granule had a homogeneous matrix and was bounded tightly by a unit limiting membrane. A second granule was observed to have a lamellated internal structure and a third granule had both types of structures.

Secretory cells were easily differentiated from GL. Apart from their well developed Golgi apparatus and rough endoplasmic reticulum, secretory cells also had junctional complexes with other epithelial cells. The goblet cells on the other hand have pale droplets in their cytoplasm compared with the homogeneous electron dense granules of the secretory cell.

Recent studies by Kamiya et al., (1983) and Huntley et al., (1984), strongly indicated a relationship between GL and mucosal mast cells (MMC). The results of the light microscopical, histochemical and electronmicroscopical studies reported in this chapter also indicated differences between these two cells. In the light of this, apart from the present acknowledged hypothesis of MMC origin for the GL, the possibility of the GL being a specific cell type should always be considered; since GL were found in foetuses which have hitherto not been exposed to parasites, and also, end cells have not been known to undergo cell division.

TABLE 11: The size of globule leucocytes in ten adult bovine respiratory tracts.

SIZE OF GLOBULE LEUCOCYTES IN TEN ADULT BOVINE RESPIRATORY TRACTS

Cell Size μ

CASE NO.	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
89018	-	-	1	1	6	16	28	18	17	5	7	-	1	-	-
89515	-	1	1	1	3	12	32	23	14	5	3	3	2	-	-
89724	-	-	1	6	9	16	23	22	9	7	4	1	1	-	-
90267	-	-	-	-	11	7	26	19	11	10	6	4	6	-	-
91267	-	-	-	2	3	5	38	26	17	7	2	-	-	-	-
91434	-	-	-	-	5	7	33	16	19	13	5	-	2	-	-
91589	-	-	-	3	6	13	21	22	19	10	6	-	-	-	-
93119	-	-	-	11	22	29	27	9	1	1	-	-	-	-	-
94632	-	-	-	1	9	29	26	20	9	4	1	-	1	-	-
95432	-	-	1	-	5	23	21	19	20	6	3	1	1	-	-

TABLE 12: Size of globules of the adult bovine respiratory tract globule leucocytes.

SIZE OF GLOBULES OF THE ADULT BOVINE RESPIRATORY TRACT GLOBULE LEUCOCYTES

CASE NO.	Size of Globule μ 1	1	2	3	4	5	6	7	8
88552	1	80	19	–	–	–	–	–	–
89018	–	17	53	27	3	–	–	–	–
89139	1	19	47	21	8	1	2	1	–
89348	–	36	54	9	1	–	–	–	–
89515	–	8	48	33	8	2	–	1	–
90375	–	9	44	30	10	5	1	1	–
91267	–	47	52	1	–	–	–	–	–
91434	–	32	58	9	1	–	–	–	–
91589	–	32	52	14	2	–	–	–	–
93119	2	75	23	–	–	–	–	–	–

TABLE 13: The staining characteristics of globule leucocytes, mucosal mast cells and eosinophils in the bovine respiratory tract.

STAINING CHARACTERISTICS OF GL, MMC and EOSINOPHILS
OF THE BOVINE RESPIRATORY TRACT.

STAINING METHODS	GL	MMC	EOS
Haematoxylin and Eosin	R +++	-	++
Martius Scarlet Blue	R +++	-	+
Carbol Chromotrope	R +	-	+++
Acridin Orange	YO ++	+++	-
Biebrich Scarlet pH 8.0	R +++	+++	+
Toluidine Blue pH 0.3	V ±	++	-
4.0	V ±	+++	-
Alcian Blue pH 1.0	-	B +++	-
2.0	-	B +++	-
Astra Blue/Safranin pH 0.3	B ±	+++	-
Phosphotungstic acid Haematoxylin	BB +++	-	-

R = Red
YO = Yellow Orange
V = Violet
B = Blue
BB = Blue brown
GL = Globule leucocyte
MMC = Mucosal Mast Cell

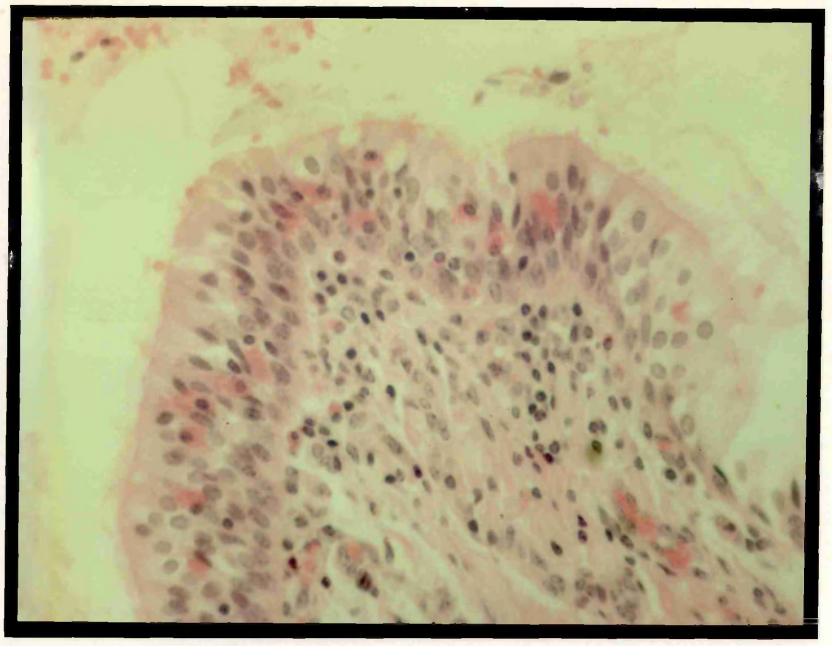
\- = Negative
\+ = Positive reaction
++ = Fairly strong reaction
+++ = Very strong reaction

FIG. 7: Globule leucocytes within the epithelium of a bovine bronchus.

H&E x 88

FIG. 8: A globule leucocyte within the connective tissue in the lamina propria of a bovine bronchus.

H&E x 140



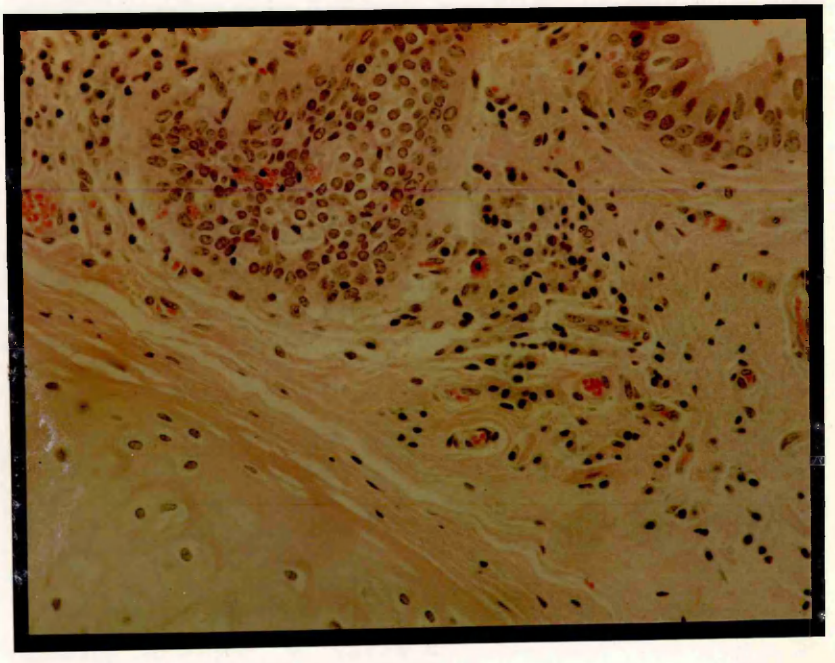
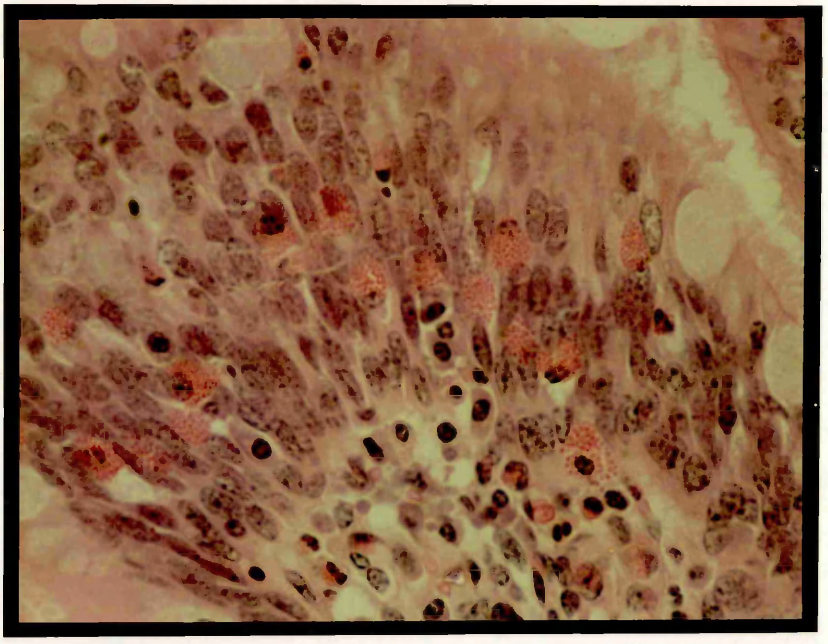


FIG. 9: Globule leucocytes within the bronchial epithelium of a bovine bronchus showing the characteristic cartwheel arrangement of nuclear chromatin.

H&E x 140

FIG. 10: A bovine bronchial globule leucocyte with two nuclei.

H&E x 140



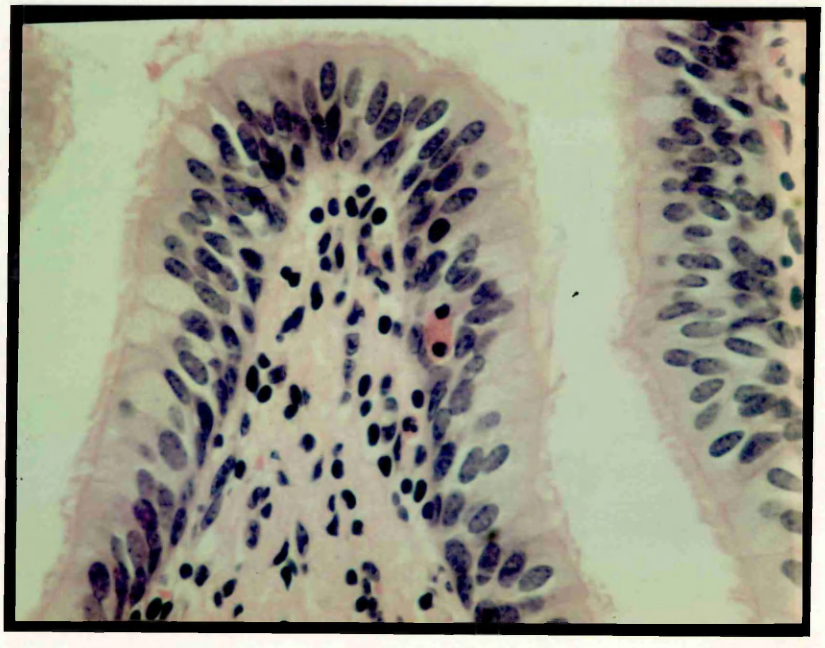
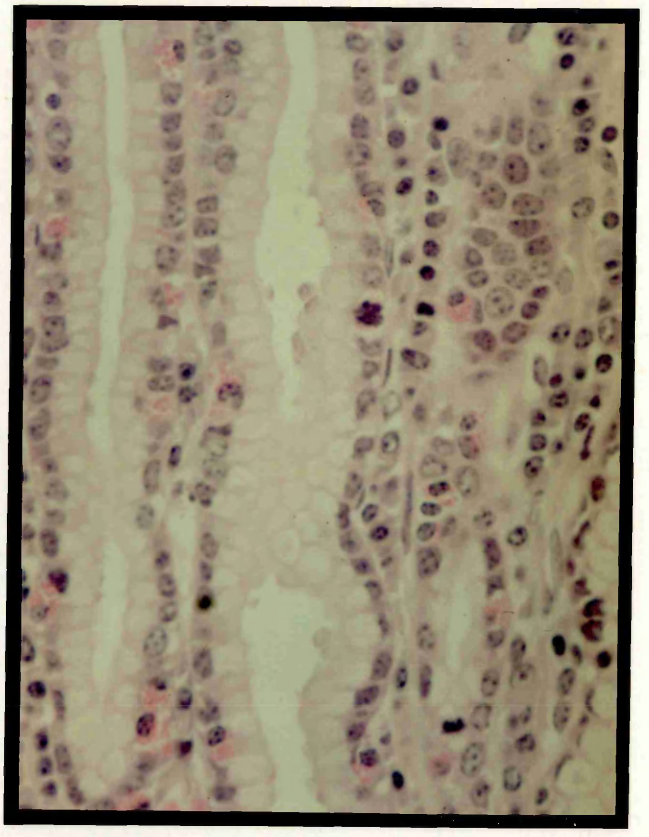


FIG. 11: A globule leucocyte undergoing mitosis in the bovine abomasum.

H&E x 140

FIG. 12: An occasional large globule in the cytoplasm of a bovine bronchial globule leucocyte.

H&E x 140



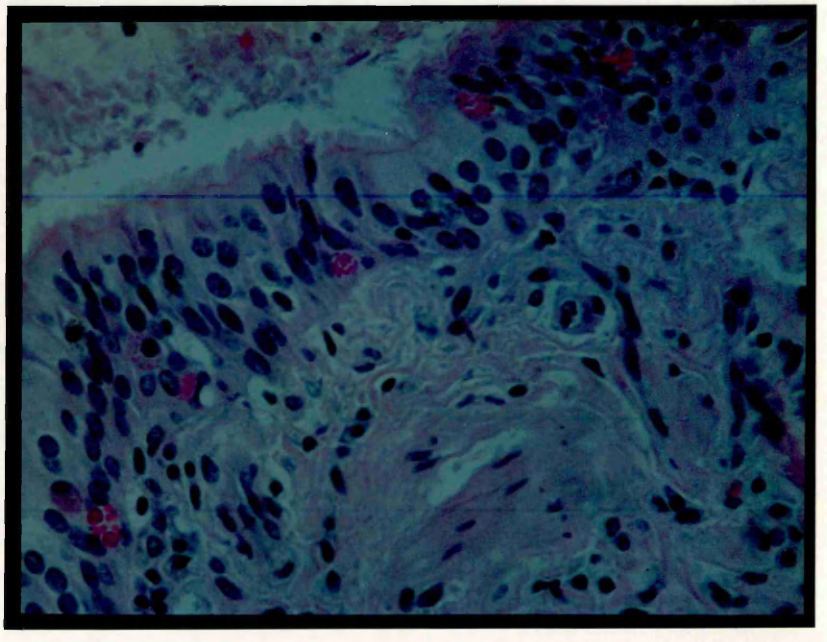
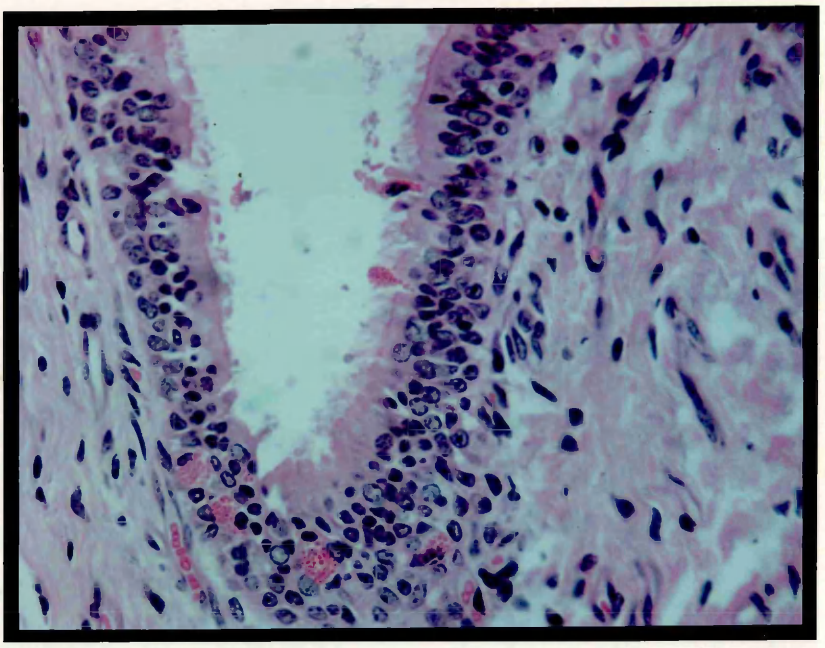


FIG. 13: Globule leucocytes discharging their globules into the bronchial lumen.

H&E x 140

FIG. 14: Globule leucocytes in the lamina propria of the bovine respiratory tract containing numerous very small globules and close to the basement membrane.

H&E x 140



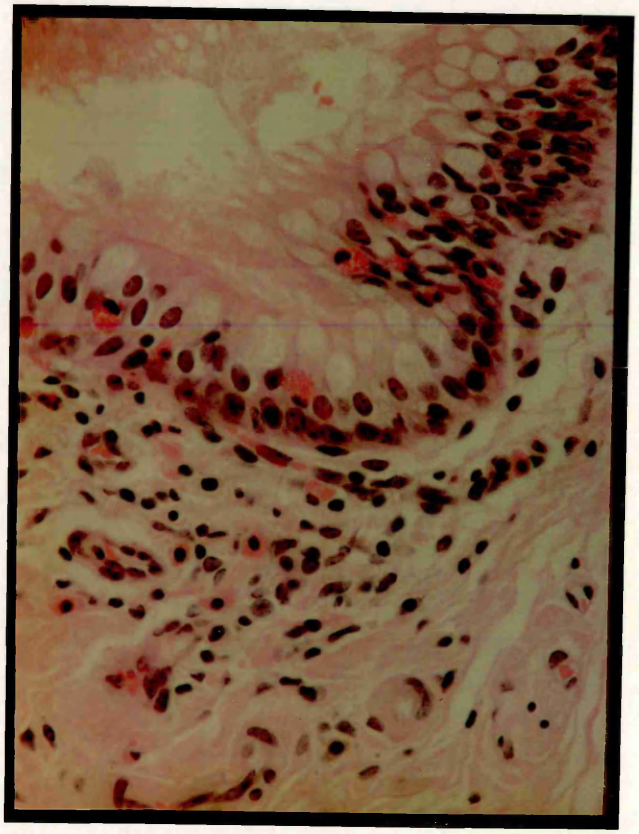
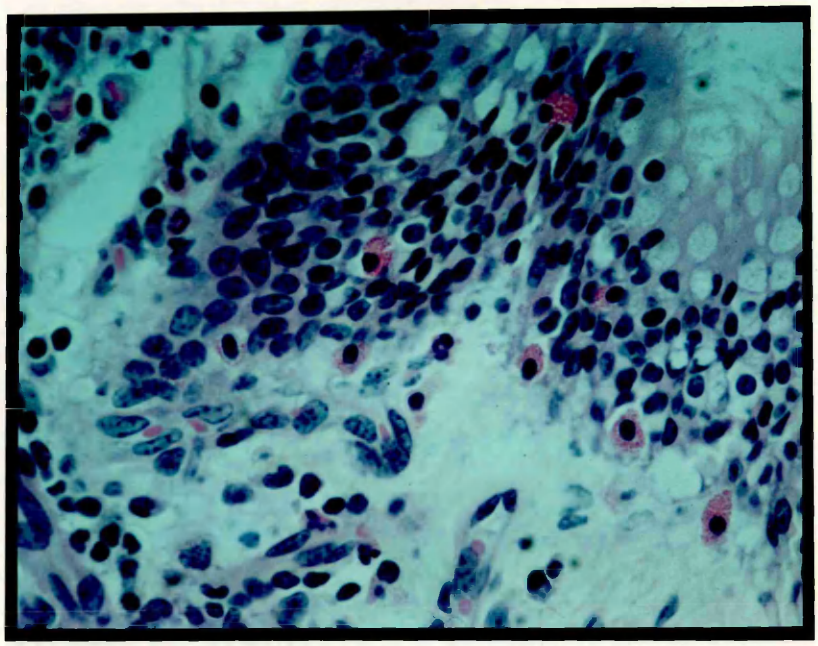


FIG. 15: Globule leucocytes, in the lamina propria of the bovine respiratory tract, containing numerous very small globules which are now easily observed when stained with MSB

MSB x 140

FIG. 16: Globule leucocytes in a four days old calf with violet metachromasia after toludine blue staining at pH 0.3.

x 140



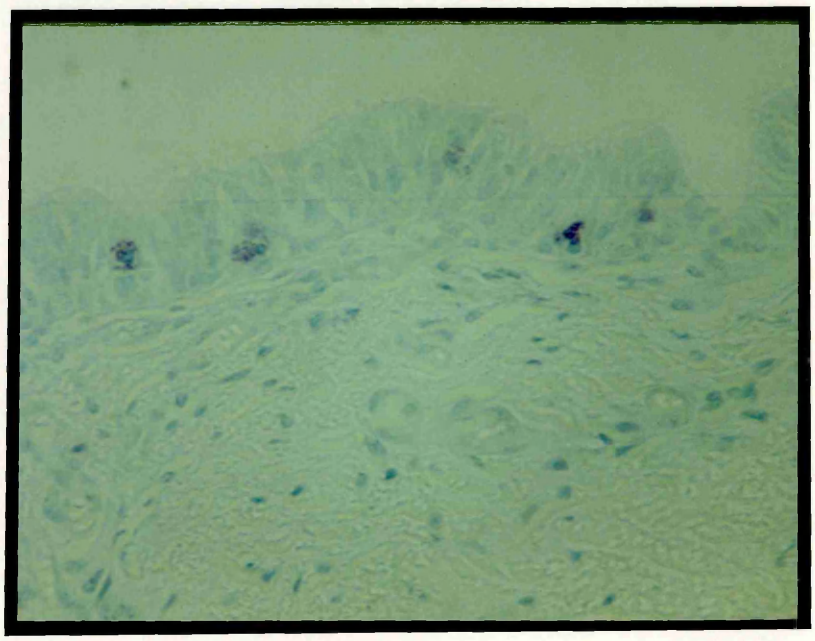
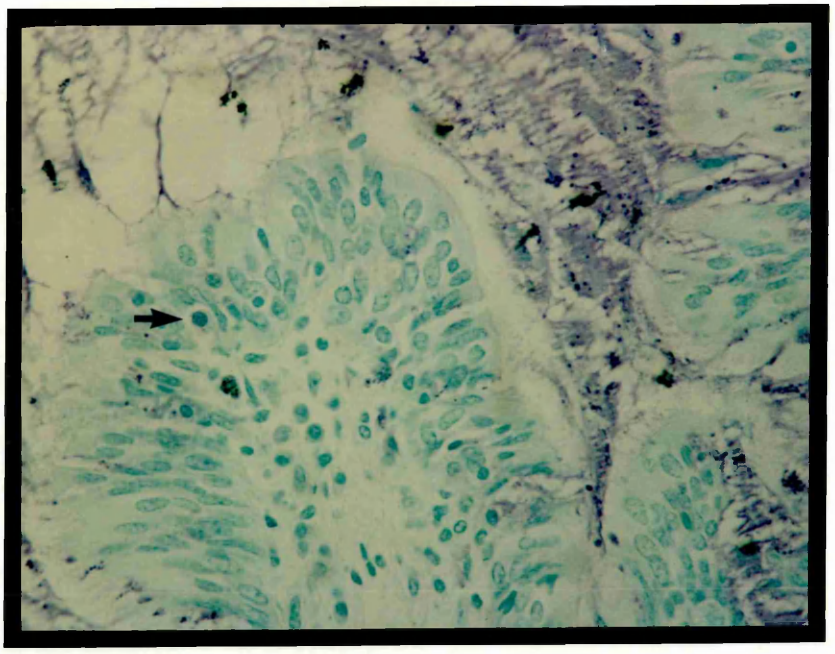


FIG. 17: Mucosal Mast Cells with violet metachromasia after toludine blue staining at pH 0.3. GL not staining (arrow)

x 140

FIG. 18: Mucosal mast cells, with blue staining after astra blue/safranin staining pH 0.3

x 140



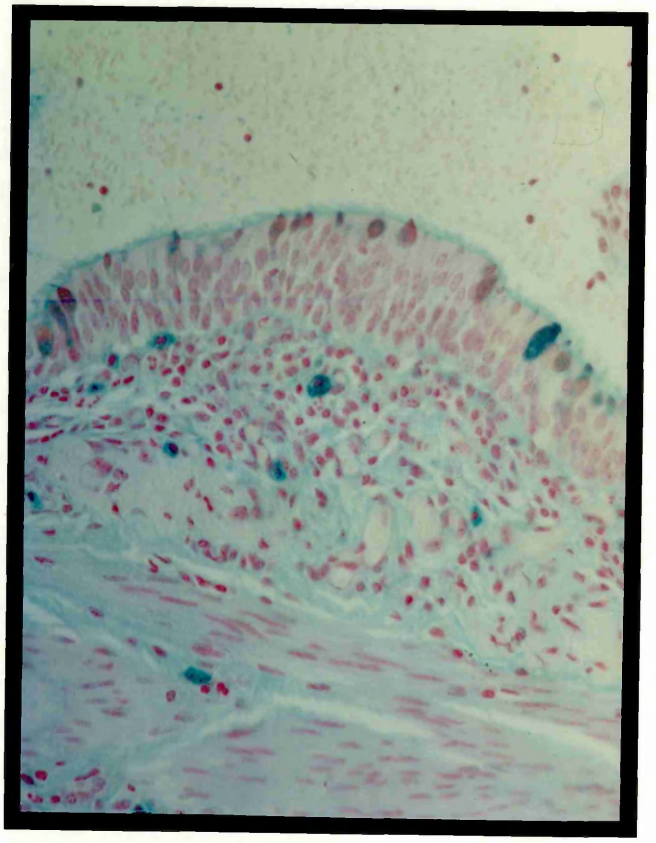
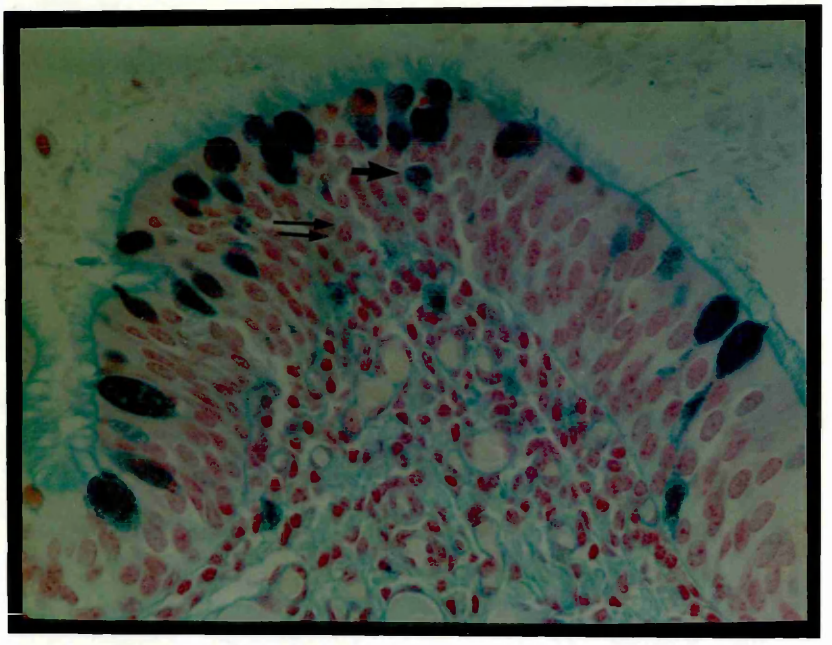


FIG. 19: Globule leucocytes in the bovine bronchial epithelium with globules giving a blue staining reaction with astra blue/safranin (single arrow) and globules not staining (double arrows).

pH 0.3 x 140

FIG. 20: Globule leucocytes in the bovine bronchial epithelium stained red with Martius scarlet blue stain.

x 140



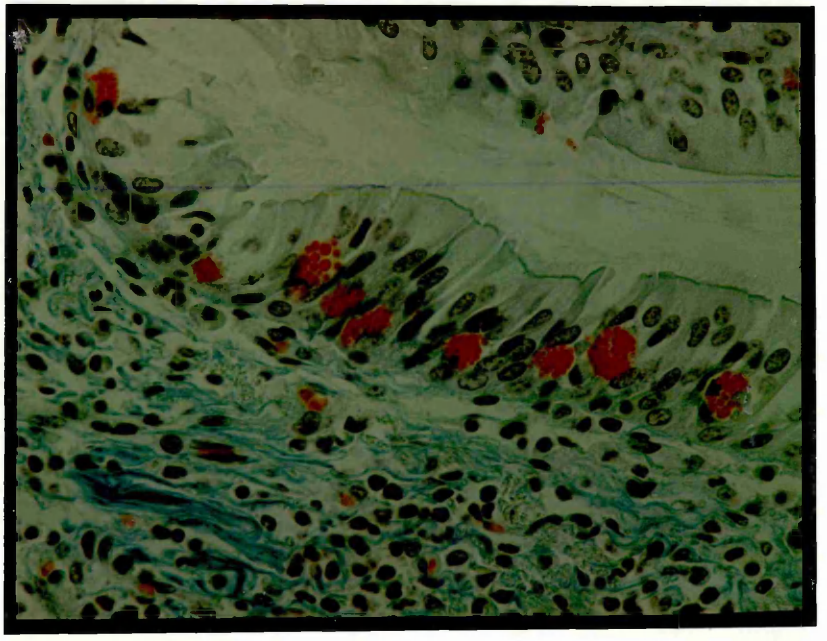
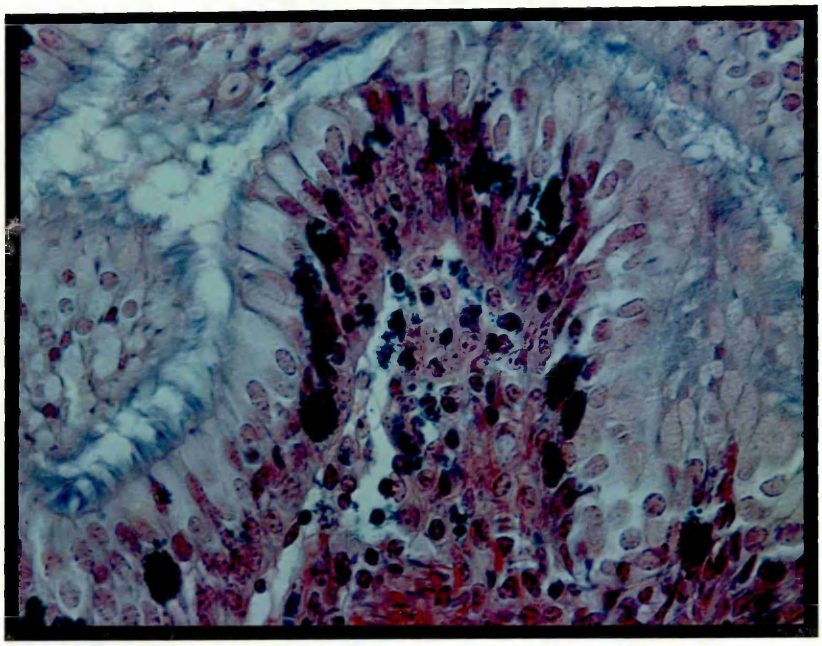


FIG.21: Globule leucocytes in the bovine bronchial epithelium with bluish-brown staining reaction after phospho-tungstic acid haematoxylin stain.

x 140

FIG. 22: Eosinophilic leucocytes in the bovine respiratory tract stained bright red with carbol chromotrope while the globule leucocytes were faintly stained.

x 140



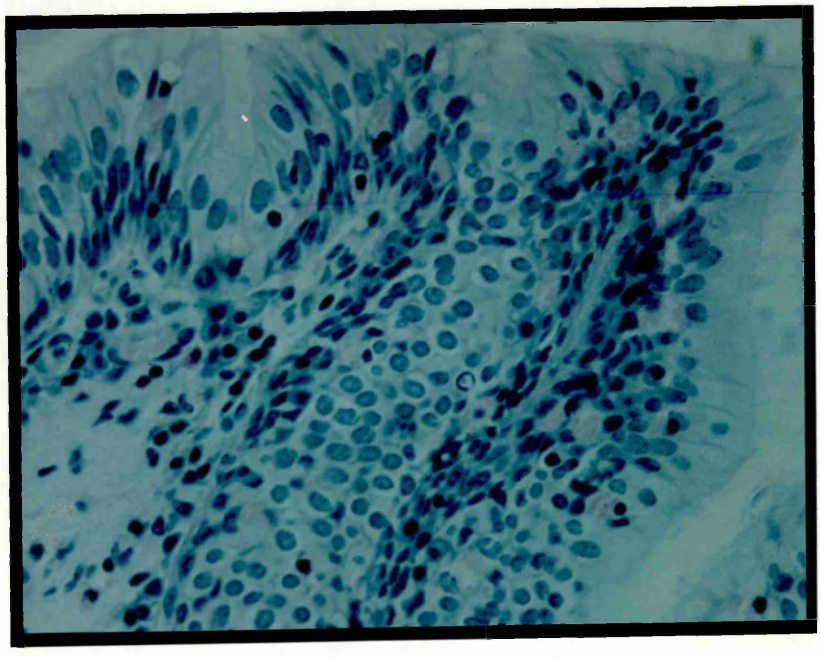
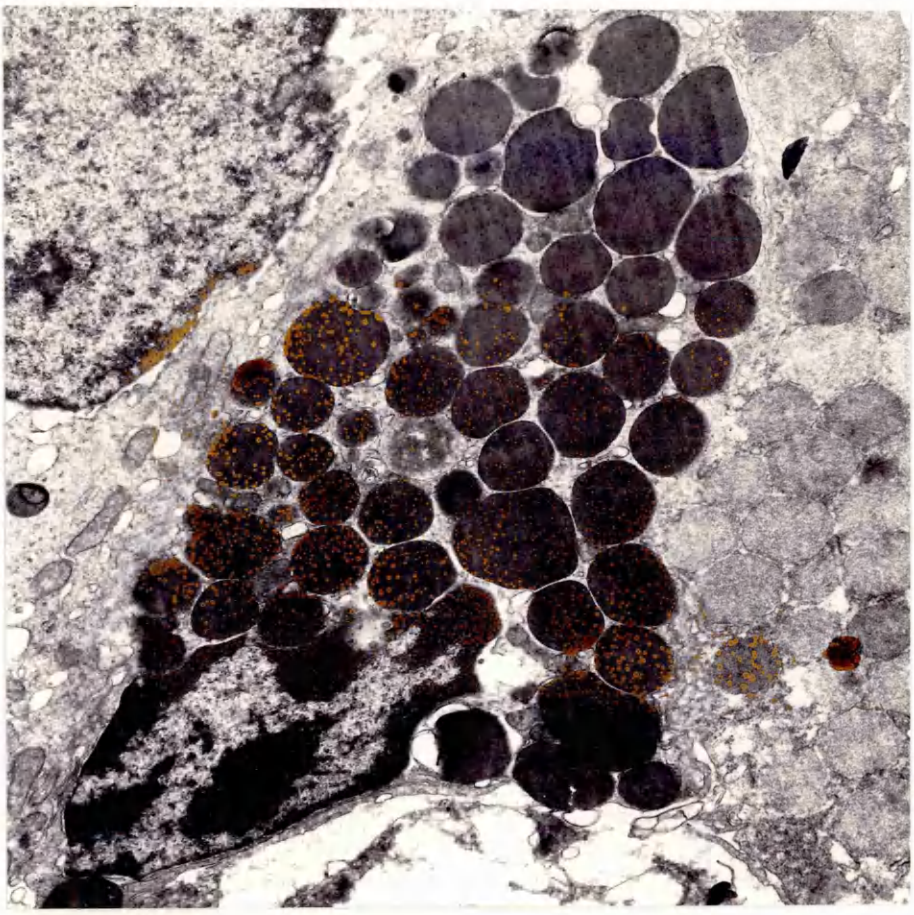


FIG. 23: Electron micrograph of the bovine globule leucocyte between epithelial cells in a bovine bronchus.

x 15,000

FIG. 24: A globule leucocyte in the bovine bronchial epithelium showing a cytoplasmic prolongation.

x 30,000



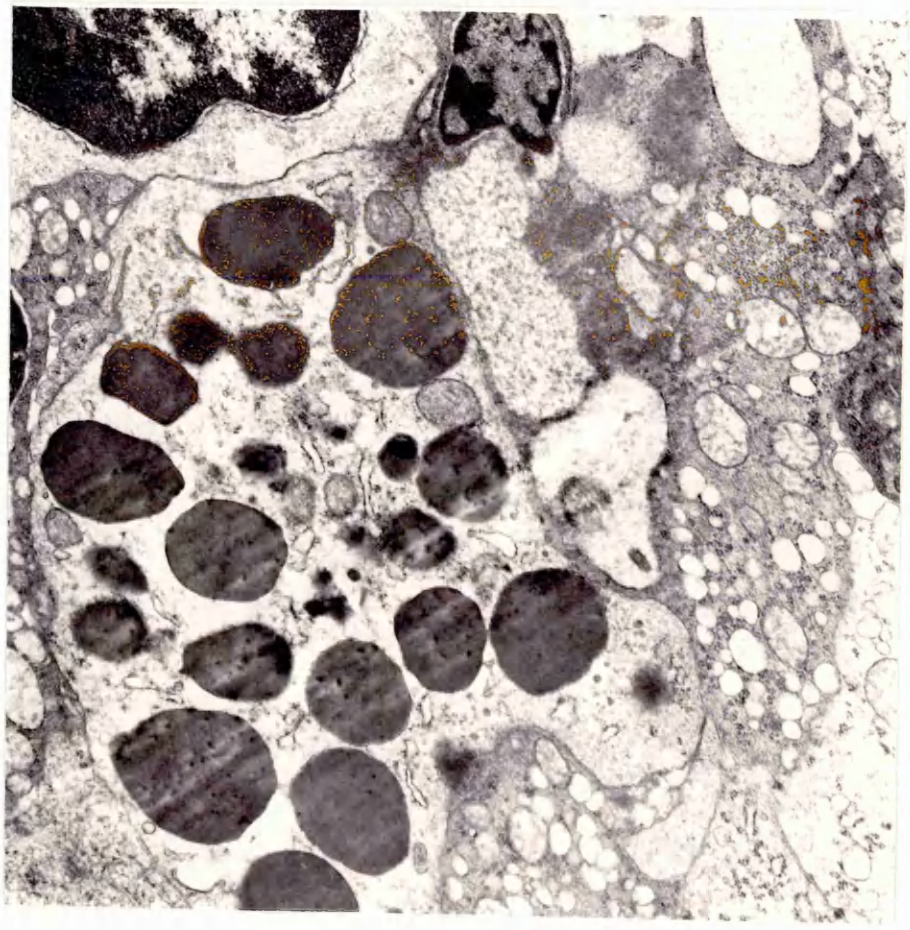
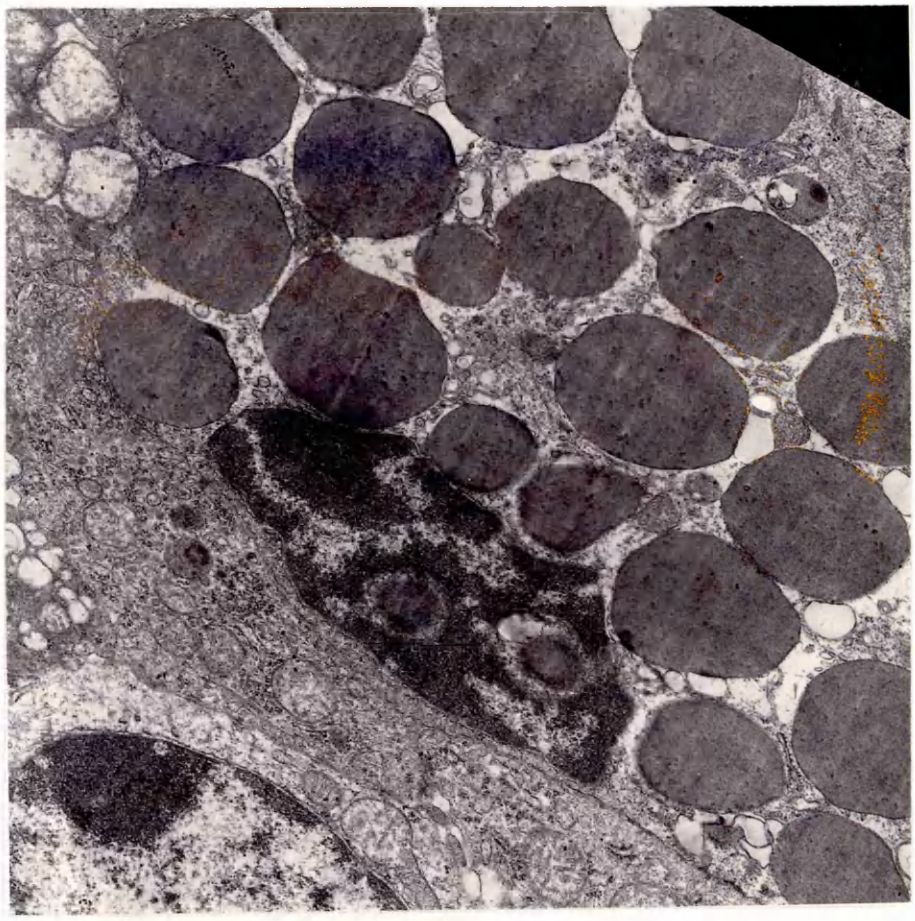


FIG. 25: A globule leucocyte in the bovine bronchial epithelium with irregular nuclear outline resulting from indentations caused by the cytoplasmic globules.

x 15,000

FIG. 26: A globule leucocyte in the bovine bronchial epithelium with a centriole in its cytoplasm.

x 30,000



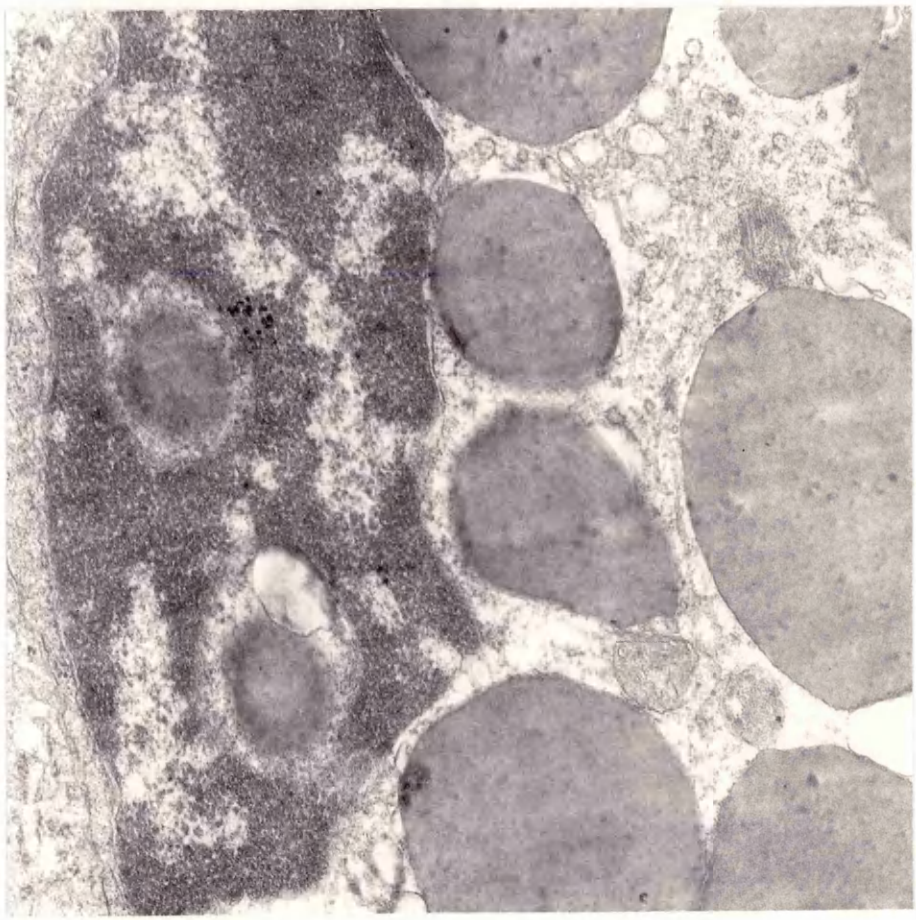
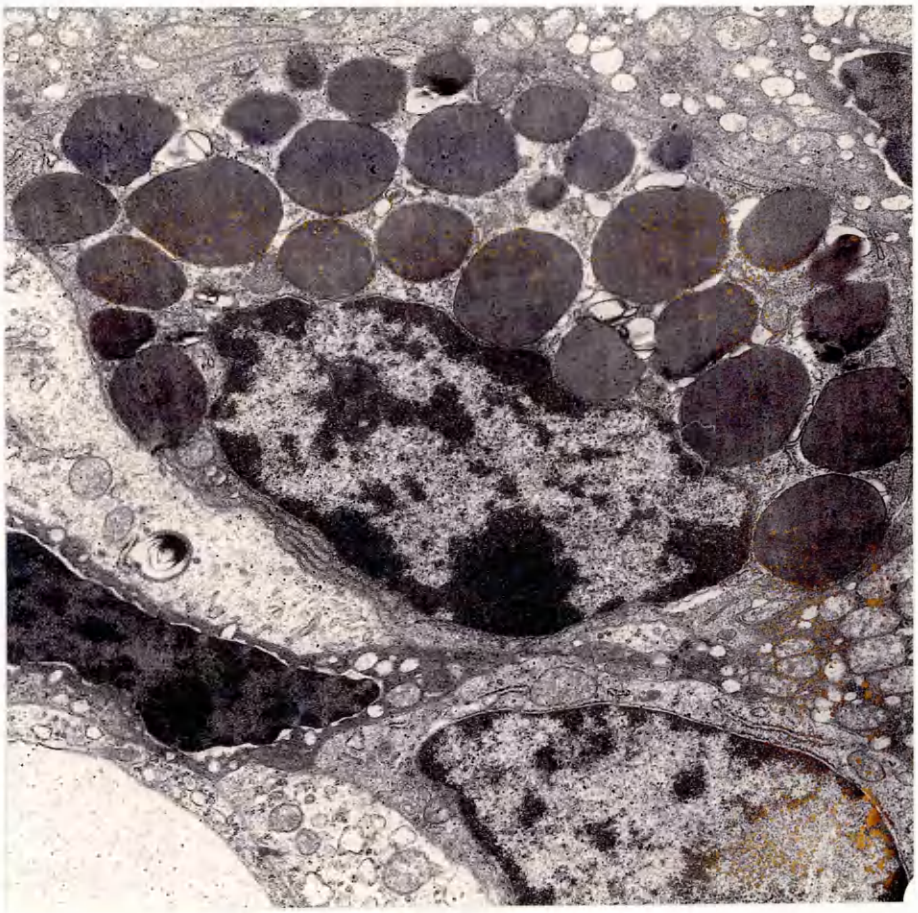


FIG. 27: A globule leucocyte in the bovine bronchial epithelium with various cytoplasmic organelles. (Globules, mitrochodria, a few free ribosomes and membrane bound vacuoles can be seen).

x 11,250

FIG. 28: A globule leucocyte in the bovine bronchial epithelium with Type I globules composed of homogeneous, electron dense membrane bound material.

x 11,250



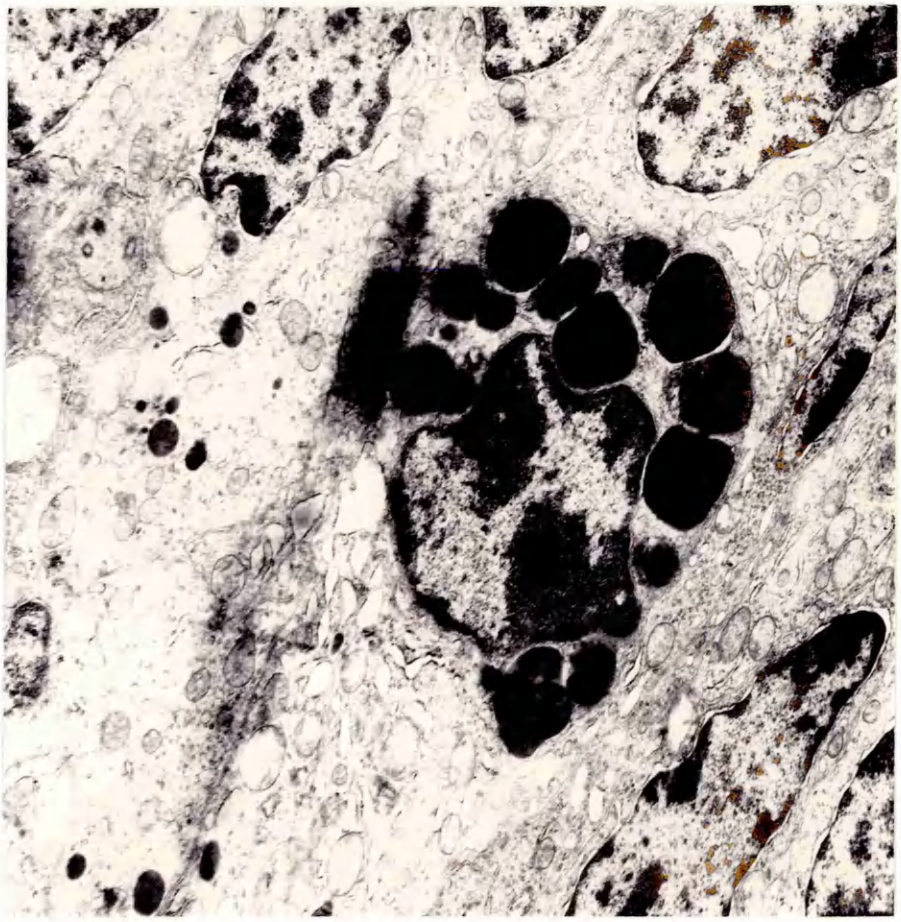
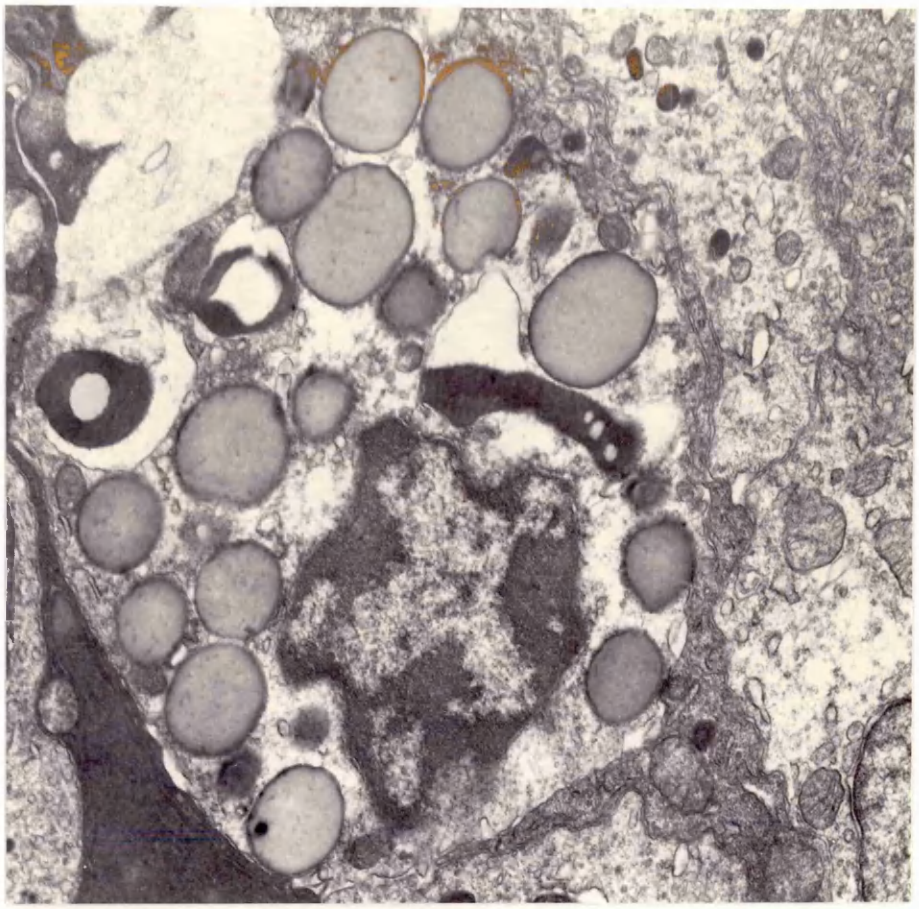


FIG. 29: A globule leucocyte in the bovine bronchial epithelium with Type II globule which is vacuolated and contains a crystalloid body.

x 15,000

FIG. 30: A globule leucocyte in the bovine bronchial epithelium. The Stage I features of the degenerative process; globules have become vacuolated.

x 11,250



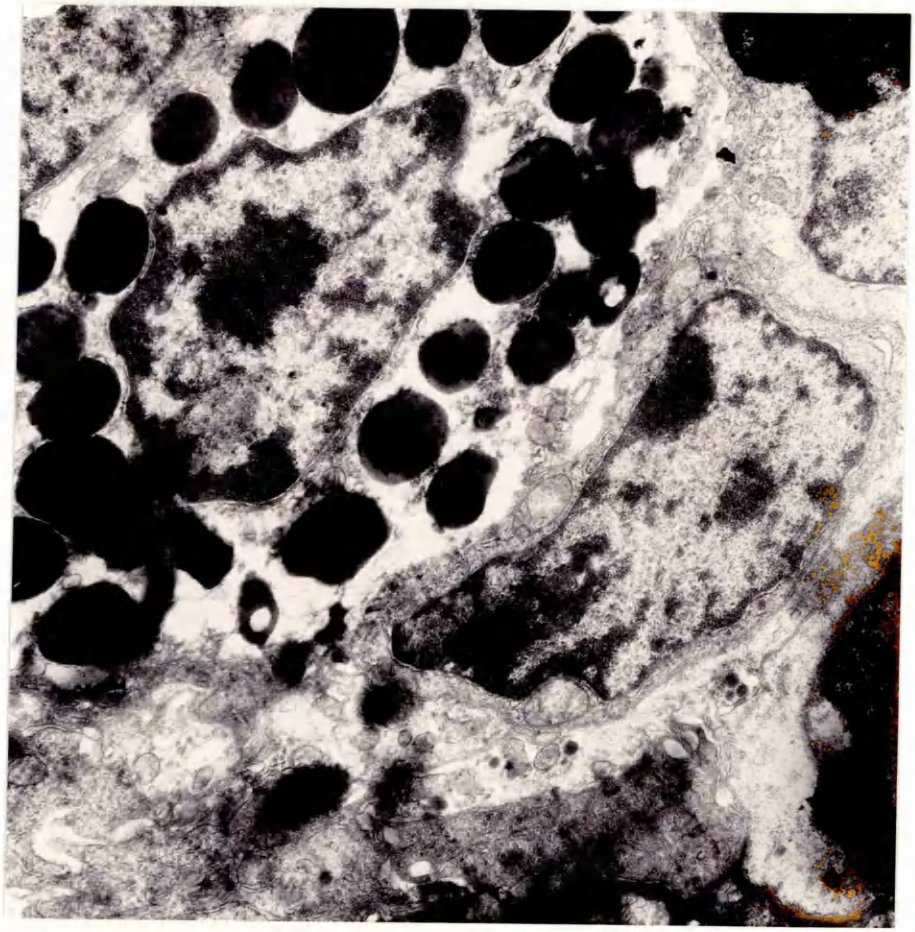
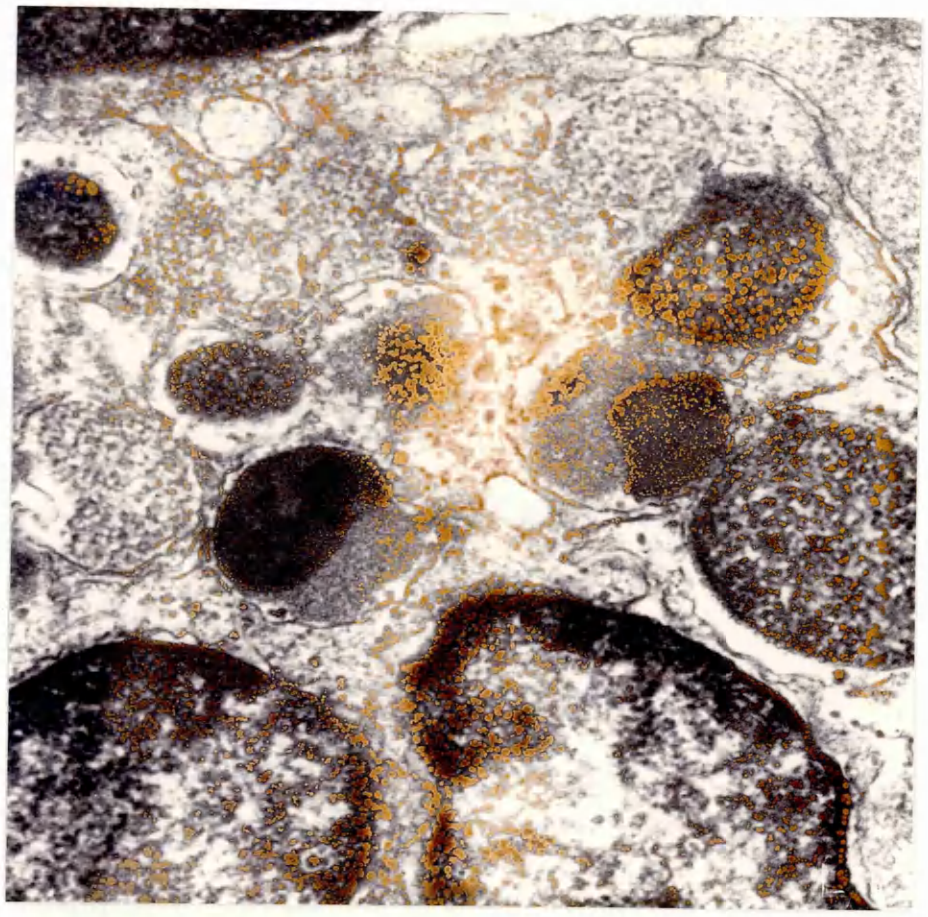


FIG. 37: A mast cell in the lamina propria of a bovine bronchus with cytoplasmic granules made up of both dense homogeneous and reticulated matrix.

x 45,000

FIG. 38: A mast cell in the lamina propria of a bovine bronchus containing a globule with a rectangular substructureless crystalloid and another with a honeycomb crystalloid.

x 15,000



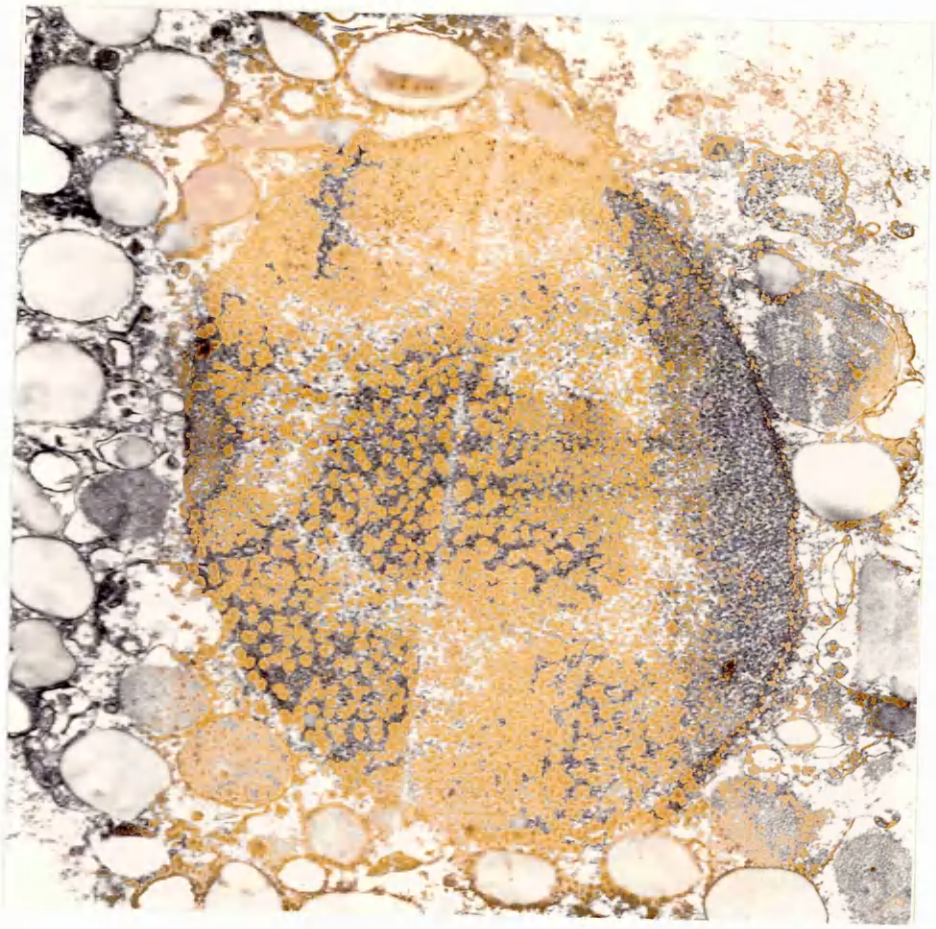
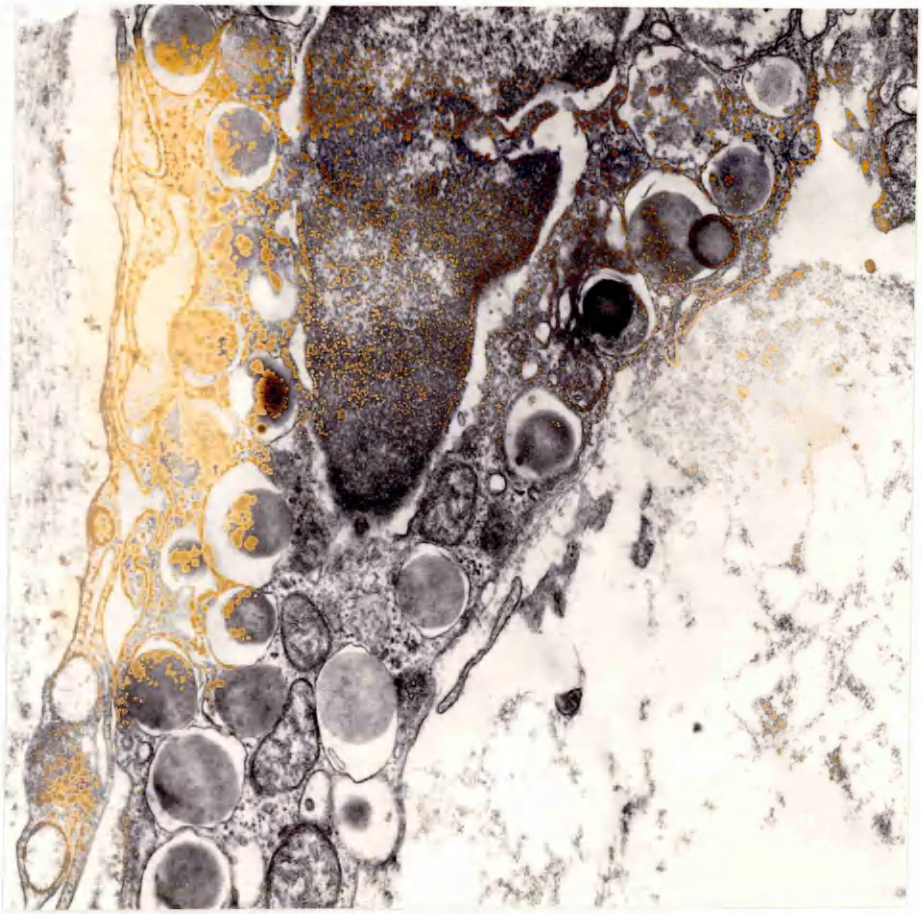


FIG. 39: A mast cell in the lamina propria of a bovine bronchus containing a globule with a honeycomb pattern type of crystalloid.

x 22,500

FIG. 40: A plasma cell in the lamina propria of a bovine bronchus showing an extensive rough endoplasmic reticulum.

x 22,500



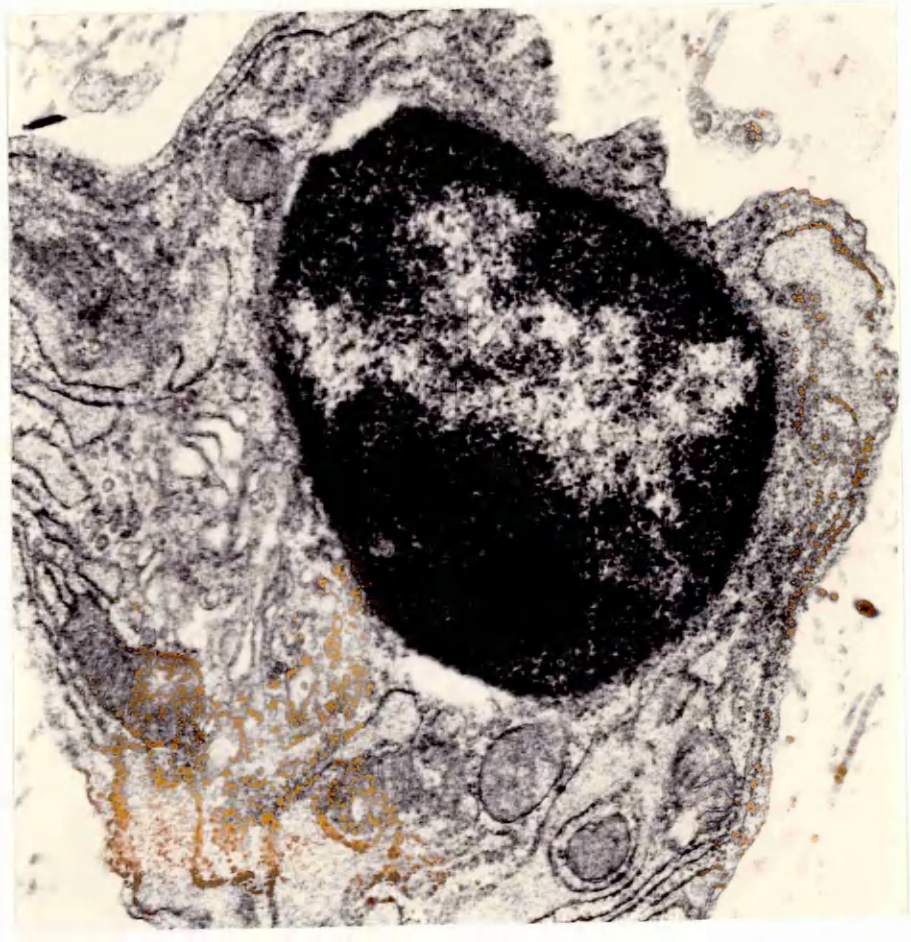
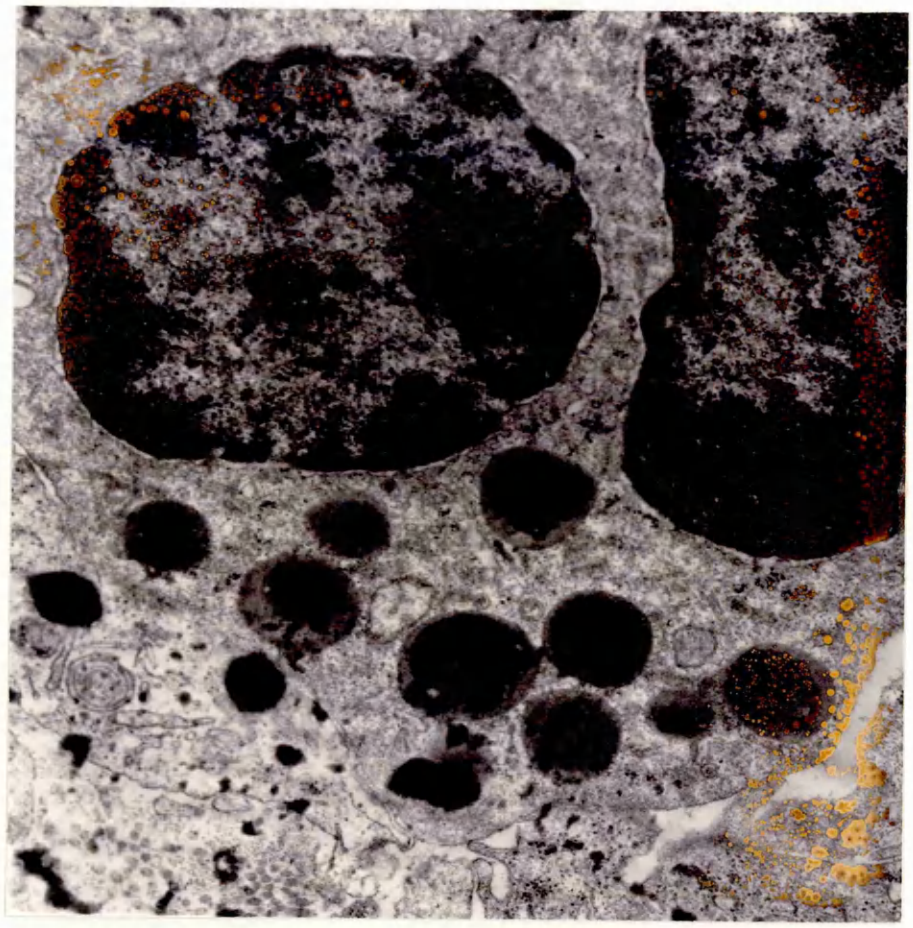


FIG. 41: An eosinophilic leucocyte with a lobed nucleus in the lamina propria of bovine bronchus.

22,500

FIG. 42: An eosinophilic leucocyte in a bovine bronchus with granules containing both dense homogeneous matrix and lamellated internal structure.

x 45,000



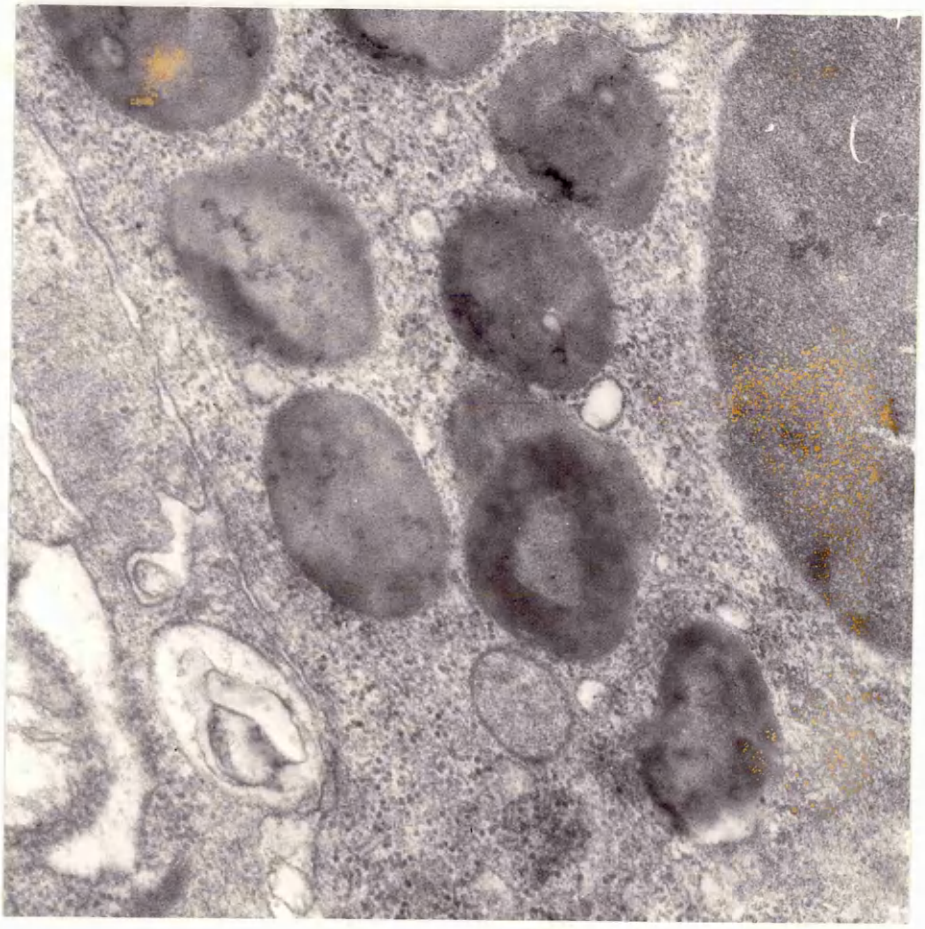
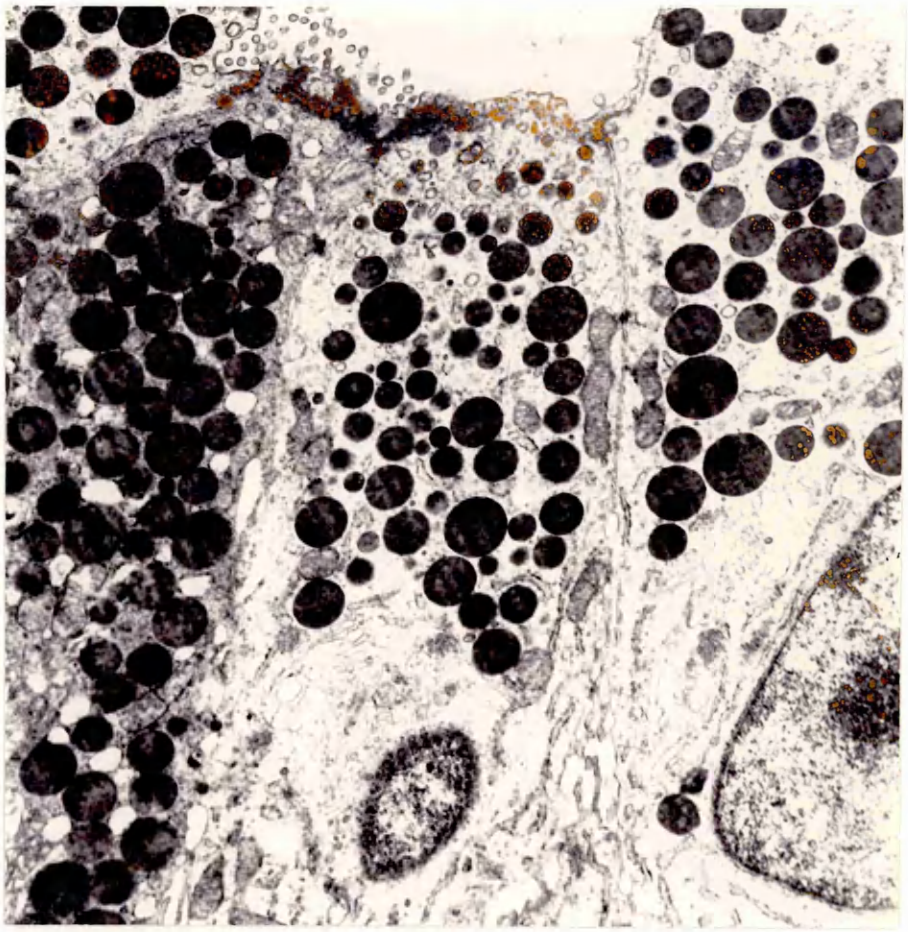


FIG. 43: An electron micrograph of secretory cells in bovine bronchial epithelium. Secretory granules close to the region of the lumen and microvilli could be seen.

22,500

FIG. 44: A secretory cell in a bovine bronchial epithelium. The well developed rough endoplasmic reticulum, a Golgi complex and secretory granules could be seen.

x 22,500



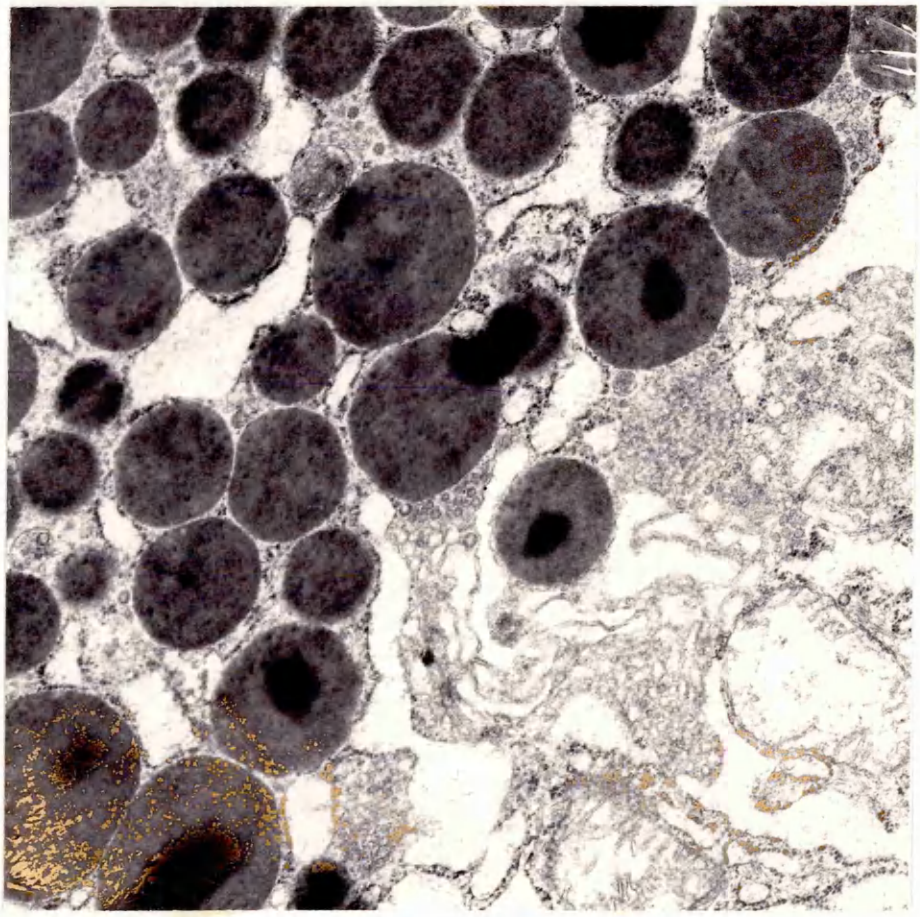
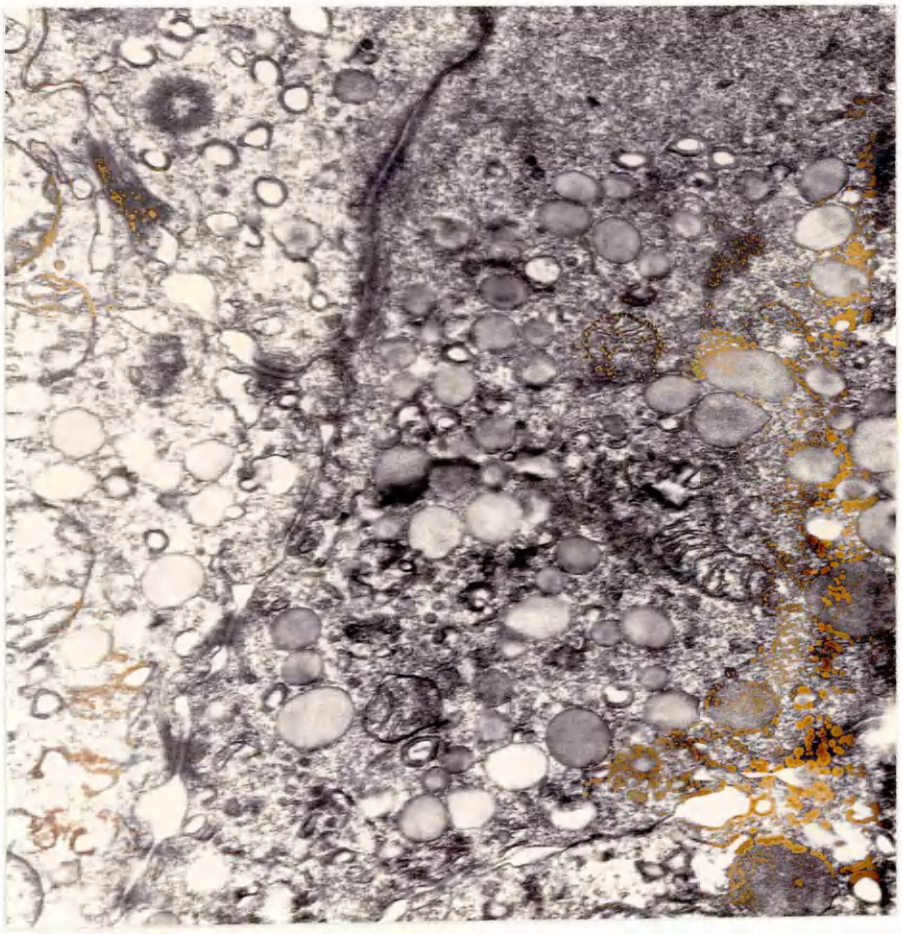


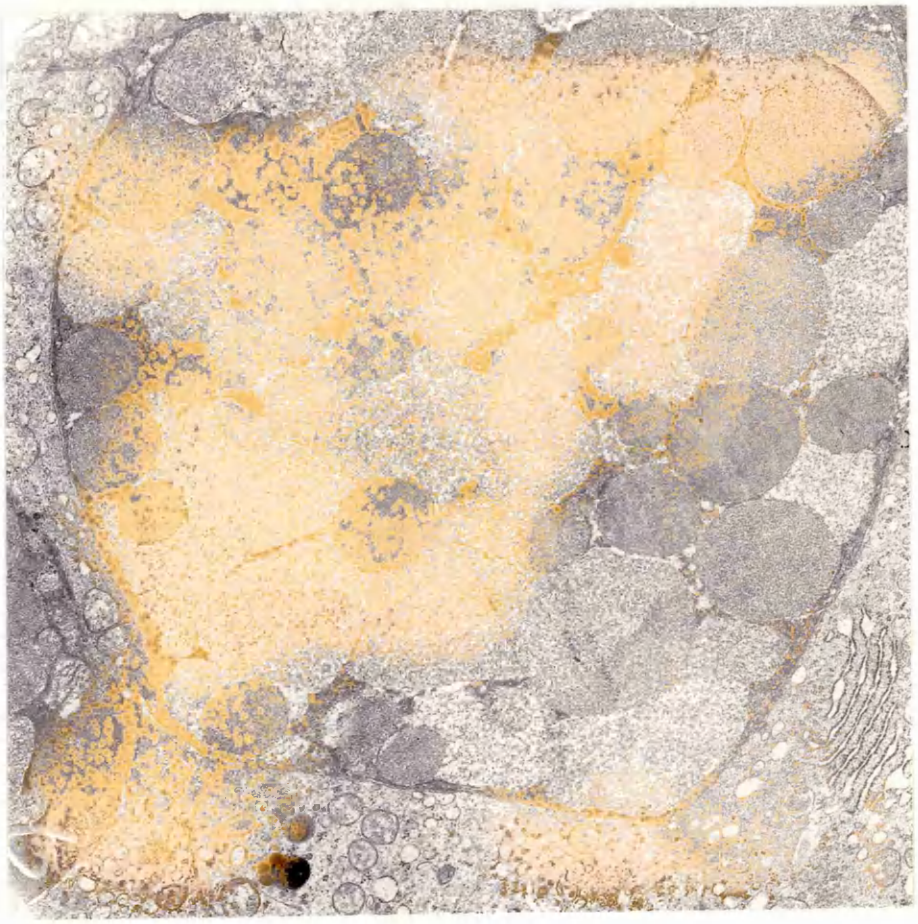
FIG. 45: A secretory cell in a bovine bronchial epithelium showing junctional complexes with other epithelial cells.

x 22,500

FIG. 46: A goblet cell in the bronchial epithelium containing pale cytoplasmic droplets.

x 11,250





CHAPTER FOUR

THE RELATIONSHIP BETWEEN PULMONARY GLOBULE LEUCOCYTES AND

(i) CLINICAL RESPIRATORY DISEASE,

(ii) PULMONARY LESIONS AND

(iii) GLOBULE LEUCOCYTES IN OTHER MUCOUS MEMBRANES

THE RELATIONSHIP BETWEEN PULMONARY GLOBULE LEUCOCYTES AND (i) CLINICAL RESPIRATORY DISEASE, (ii) PULMONARY LESIONS AND (iii) GLOBULE LEUCOCYTES IN OTHER MUCOUS MEMBRANES.

A. INTRODUCTION

The results of the studies reported in Chapters 2 and 3 established the fact that GL were present in the respiratory tract of all age groups of cattle. These studies also indicated that the GL of the bovine respiratory tract were similar to those described by other investigators in other bovine mucous membranes.

The occurence of the GL in the bovine respiratory tract has been reported in cattle affected by different respiratory diseases. Breeze et al., (1975), reported the presence of GL in the respiratory tracts of cattle with farmer's lung, fog fever and diffuse fibrosing alveolitis. Pirie et al., (1976), again reported their presence in the respiratory tract of cattle given the amino acid L-tryptophan while Allan et al., (1983), reported their presence in the upper respiratory tract of cattle with atopic rhinitis. Large numbers of GL have been observed in the lungs of cows in other outbreaks of respiratory disease and it has been very difficult to interpret the findings (Pirie, personal communication) because it was not known whether the GL were a coincidental finding or indicated exposure to parasites particularly lungworms.

The presence of GL in other mucous membranes such as the alimentary tract and the urinary tract of cattle and rats has been associated with parasitic infection (Kirkman, 1950, Taliaferro and Sarles, 1967, Whur, 1966, Jarrett et al., 1967a & b, Miller et al., 1967 and Murray et al., 1968). The association of GL with parasitic infection in the ovine respiratory tract was studied by Mahmoud, (1978).

So far, there have been no detailed studies done to investigate the relationship between the GL of the bovine respiratory tract and respiratory diseases in cattle although their

presence have been reported in the respiratory tract of cattle suffering from different respiratory diseases.

Because of the known relationship between the GL and parasitic infection, the reported presence of GL in the respiratory tract of cattle and the large numbers found in the lungs of adult cattle, an investigation was undertaken in order to understand the importance of GL in the bovine respiratory tract in relationship to respiratory disease.

An attempt was made to assess their relationship with (i) clinical respiratory disease, (ii) pulmonary lesions, both macroscopic and microscopic and (iii) the study was extended to investigate the possibility of a relationship between pulmonary GL and the presence of GL in other mucous membranes such as the bile duct, abomasum and urinary bladder.

B. MATERIALS AND METHODS

1. Animals

The same age groups of cattle as those in Chapter 2 (Table 1) were used. To investigate the relationship between GL and respiratory disease in cattle the animals of Groups 3,4, 5 and 6 were used since about half the animals in each group had been diagnosed as having had clinical respiratory problems. None of the animals in Group 2 had been diagnosed as having had clinical respiratory problem. Group 1 animals were foetuses obtained from their dam at death and Group 2 animals were less than one month old. To investigate the relationship between the presence of GL in the respiratory tract and their presence in other mucous membranes, the mucous membranes of the bile duct, abomasum and urinary bladder were examined in 92 animals that were found to have GL in their respiratory tract and whose abomasum, bile duct and urinary bladders were collected post-mortem.

2. Post-mortem Examination.

Post mortem, the whole lungs including the trachea and larynx, the liver and urinary bladder were removed from the carcase. These organs were then carefully examined for gross lesions.

3. Histological Methods

(a) Tissue Samples

Tissue blocks were obtained from the respiratory tract and pulmonary parenchyma as described in Section B of Chapter 2, and two sections each were obtained from the bile duct, abomasal folds and the urinary bladder of each animal examined in this study.

(b) Fixation

Fresh tissue blocks about 2-3mm in size were collected in two fixatives: these were 10% buffered neutral formalin and Carnoy's fluid as described in section B of Chapter 2. The details of the fixatives are in Appendix 1.

Tissue blocks were fixed for at least 24 hours, post fixed in corrosive formol, dehydrated and cleared in double embedding series. Tissue blocks were finally embedded in paraffin wax and cut at 5-6μ in a rotary microtome.

(c) Staining Procedures

For routine histopathological examintion of tissues, sections and identification of GL, haematoxylin and eosin (H&E) stain was used. Some special staining techniques were carried out to confirm that the cell identified as GL with H&E was the GL and for the histological classification of lung lesions. The Martius scarlet blue (MSB) and toluidine blue (TB) special staining techniques were frequently used. The preparation of reagents and staining techniques are in Appendix 1.

4. Identification and Quantification of Globule leucocytes

Globule leucocytes in the epithelia of the bovine respiratory tract, bile duct, abomasum and the urinary bladder when present, were identified in H & E stained sections by their characteristic eosinophilic intracytoplasmic inclusions

For each animal, the presence of a GL in any section of the respiratory tract examined, indicated the presence of GL in the respiratory tract of that animal.

The GL population densities in the respiratory tracts of animals examined were obtained by assigning one, two or three pluses (+) to the population of GL present in the section examined. One plus (+) indicated that less than 10 GL were found, two pluses (++) indicated that 10-19 GL were found and three pluses (+++) indicated that 20 or more GL were found. The highest number of pluses assigned to any portion of the sections examined indicated the group to which that particular animal was classified.

To identify the presence of GL in the mucous membranes of the bile duct, abomasum and urinary bladder, two sections were examined from each organ and the presence of GL in any sections indicated their presence in that organ.

5. Clinical Disease

The presence of clinical respiratory disease was assessed by clinicians in the department of Veterinary Medicine at the University of Glasgow and their clinical diagnosis was used to group the animals.

6. Identification of Pulmonary Lesions

Pulmonary lesions were classified as (i) gross or (ii) microscopic. The gross lesions were examined histologically and classified as outlined in the results. The lungs of all animals

without gross lesions were examined for microscopic changes and classified accordingly.

C. RESULTS

1. Animals with Clinical Respiratory Disease

The total number of animals and the proportion of them with clinical respiratory problems are shown in Table 14. The number and percentages with GL in the respiratory tract within each of these is also indicated in Table 14.

In Group 1 and 2, ie foetuses and calves up to one month old, clinical respiratory disease was never diagnosed. Of the 19 foetuses examined, nine (47.37%) had GL in their respiratory tracts while 19 (76.00%) of the 25 calves examined in Group 2 had GL in their respiratory tracts.

In Group 3, nine of the 20 calves examined were diagnosed to have had clinical respiratory problems. Three of these animals (33.33%) had GL in their respiratory tracts. Of the 11 calves which were not diagnosed as having had clinical respiratory problems, seven (63.64%) had GL in their respiratory tracts.

Twentyfour animals were examined in Group 4. Twelve of these were diagnosed to have had clinical respiratory problems. Seven (58.33%) of these had GL in their respiratory tracts while seven (58.33%) of the 12 animals without clinical respiratory problems in this group also had GL in their respiratory tracts.

In Group 5, 27 animals were examined, nine of these had clinical respiratory problems out of which six (66.67%) had GL in their respiratory tracts, eighteen of the animals in this group had no clinical respiratory problems but 17 (94.44%) of these had GL in their respiratory tract.

The respiratory tracts of 34 animals were examined in Group 6, ten of these were diagnosed as having had clinical respiratory problems. Eight animals (80.00%) with clinical respiratory problems had GL in their respiratory tracts. Also in this age group, 23 (95.83%) of the 24 animals that were not diagnosed as having had clinical respiratory problems had GL in their respiratory tracts.

The population density of GL in the animals with and without clinical respiratory disease are given in Table 15. Animals in Groups 1 and 2 were not diagnosed as having had clinical respiratory problems. Six (66.67%) of the nine foetuses without respiratory problems were positive for GL in their respiratory tract and had less than 10 GL in any section of the respiratory tracts examined while the remaining three (33.33%) had more than 10 GL in the sections of the respiratory tract examined. Nineteen animals without clinical respiratory problemns had GL in their respiratory tracts in Group 2. Twelve (63.16%) had less than 10GL while seven (36.84%) had more than 10GL in the sections of the respiratory tracts examined. No animal in these two age groups was found to have more than 20 GL in the sections of the respiratory tracts examined.

Calves with or without clinical respiratory problems were examined in Groups 3,4,5 and 6. In Group 3, nine animals had clinical respiratory problems, three (33.33%) had GL in the sections of their respiratory tracts examined. Of these 2 (66.67%) had less than 10 GL and none had more than 20GL in the sections of their respiratory tract examined. Eleven calves in this group had no clinical respiratory problem. Seven (63.64%) of these had GL in their respiratory tracts. Five (71.43%) had less than 10GL and one (14.29%) had more than 20GL in the sections of their respiratory tracts examined.

Seven (58.33%) of the 12 animals with clinical respiratory problem examined in Group 4 had GL in the section of their

respiratory tract examined. Three (42.86%) had less than 10 GL, in the sections of their respiratory tracts examined and none had more than 20 GL in the sections of the respiratory tracts examined. Of the 12 animals in this group without clinical respiratory problems, seven (58.33%) had GL in their respiratory tracts, five (71.43%) had less than 10 GL, and one (14.29) had more than 20GL in the sections of their respiratory tracts examined.

Nine animals had clinical respiratory problems in Group 5, of these 6 (66.67%) had GL in their respiratory tracts. Three (50%) had less than 10GL and two (33.33%) more than 20 GL in the sections of the respiratory tracts examined. Seventeen (94.44%) of the 18 animals without clinical respiratory problems had GL in their respiratory tracts. Nine (52.94%) of them had less than 10 GL, and 2 (11.76%) more than 20 GL in the sections of the respiratory tracts examined.

In the adult animals (Group 6) eight (80%) of the 10 animals with clinical respiratory problems had GL in their respiratory tracts. None had less than 10 GL but 6 (75%) had more than 20 GL in the sections of their respiratory tracts examined. Twenty four of the animals examined in this group had no clinical respiratory problems and 23 (95.83%) of them had GL in their respiratory tracts. One (4.35%) had less than 10 GL and 12 (52.17%) had more than 20 GL in the sections of their respiratory tracts examined.

2. Amimals with Gross Pulmonary Lesions

In the series of animals examined there were cases with subclinical gross pulmonary lesions and animals with only microscopic pulmonary lesions which had not produced clinical signs. It was decided therefore to look at the occurence of GL in relation to the presence of (i) gross pulmonary lesions whether or not these produced clinical signs and (ii) the presence of pulmonary lesions of any type including microscopic lesions which had not produced clinically significant lung disease.

The number of animals with gross lung lesions and the type of lesions found are given in Table 16. The essential features of the lung lesions and the criteria used for the classification of pneumonias are given in Table 17. The main features of these are as follows.

In Group 1, none of the foetuses examined had gross pneumonic lesions in their lungs although three animals with pulmonary congestion and oedema and fluid in their respiratory tracts were seen.

In Group 2 no animals were diagnosed as having had clinical respiratory problems, but gross lung lesions were observed at post mortem examination. Pulmonary congestion and oedema, which was the most frequently observed lesion was seen in 12 animals. The lungs of two other animals in this group, were found to contain pneumonic lesions. In these animals the cranial, middle, intermediate and the cranial portions of the caudal lobes were usually reddish grey and consolidated (Fig. 47). The lungs of one animal had portions of pulmonary collapse.

Sixteen of the animals examined in Group 3 had pulmonary lesions in their lungs. Fourteen of the lungs had either red or grey consolidation while the others had collapsed and emphysematous lung lobules. Some animals whose lungs were consolidated usually had some degree of interstitial emphysema as well. The bronchial tree contained variable amounts of mucopus and the bronchial and mediastinal lymph nodes were enlarged. The anteroventral portions of the lungs were more affected. In one lung, there were focal areas of grey consolidation in the caudal lobes and adult lungworms were found in the bronchi. Few cases with areas of focal abscessation were observed along with pulmonary consolidation. The consolidated areas of the lungs were firm to touch and granular on the cut surface. The alveolar and interlobular septae were thickened.

In Group 4, 15 of the animals examined had gross pulmonary

lesions in their lung parenchyma. Two had collapsed lungs and one had grey nodules from infiltrating tumour. Of the 12 with pneumonic lesions, four had adult lungworms in their bronchi. The gross lung lesions were similar to those described for Group 3 animals but in one case, there were marked fibrin deposits on the pleura with subsequent adhesions to the thoracic cage.

Group 5 had 20 animals with gross pulmonary lesions. Five of these had adult lungworms in their bronchi. The lungs of two other animals had diffuse yellowish pale foci in the cranial lung lobes. The bronchial walls, the alveolar and interlobular septae of one calf with thymic lymphosarcoma were severely thickened by infiltrating tumour cells. The gross pneumonic lesions observed in 16 animals were also similar to those previously described. One animal had diffuse interstitial emphysema.

In the adult animals, Group 6, 24 animals had gross pulmonary lesions. Three had interstitial emphysema while the lungs of two were collapsed. The observed pneumonic lesions in 12 animals in this group were also similar to those previously described. Another lesion observed in the pneumonic group was the presence of diffuse grey pinpoint foci in their lung lobules. Two animals had pulmonary abscesses and one had an over-inflated lung. One animal with pancreatic adenocarcinoma had secondary pulmonary infiltrates. Two of the animals with pneumonic lesions in their lungs had adult lungworms in their bronchi. The lungs of one animal with very few adult lungworms in its bronchi had no gross pneumonic lesions.

The relationship between GL and gross lung lesions is shown in Table 18. In Group 1 two (66.67%) of the three foetuses with gross pulmonary lesions had GL in their respiratory tracts, while 43.75% or seven of the 16 foetuses without gross pulmonary lesions had GL in the sections of their respiratory tracts examined.

In Group 2, 80% of calves with gross pulmonary lesions and 70% of calves without gross pulmonary lesions had GL in the

sections of their respiratory tracts examined.

Sixteen of the 20 animals examined in Group 3 had gross pulmonary lesions and seven (43.75%) of these had GL in their respiratory tracts. Four animals in this group had no gross pulmonary lesion and three (75%) of them had GL in their respiratory tracts.

In Group 4, 15 of the 24 calves examined had gross pulmonary lesions and of these eight (53.33%) had GL in their respiratory tracts. Of the nine animals in this group without gross pulmonary lesions six (66.67%), had GL in their respiratory tract.

In Groups 5 and 6, 20 out of 27 and 24 out of 34 animals examined had gross pulmonary lesions. Of these 16 (80%) in Group 5 and 21 (87.50%) in Group 6 had GL in their respiratory tracts. All the animals in these groups without gross pulmonary lesions had GL in the sections of their respiratory tracts examined.

The relationship between GL population density and lung lesion is shown in Table 19. One of the two foetuses (50%) with gross pulmonary lesions had less than 10 GL and none more than 20GL in the section of the respiratory tract examined. Seventy one per cent i.e. five of the seven foetuses without gross pulmonary lesions had less than 10 GL while the remaining two (28.57%) had less than 20 GL in the sections of their respiratory tract examined.

Nine (75%) of the calves in Group 2 with gross pulmonary lesions had less than 10 GL in their respiratory tracts and none had more than 20 GL. Three (42.86%) of calves, in this group, without gross pulmonary lesions had less than 10 GL in their respiratory tracts and no animal had more than 20 GL in the sections of the respiratory tract examined.

The situation in Group 3 was similar to that of Group 2 in the lungs of animals with gross pulmonary lesions, but differed in the lungs of animals without gross pulmonary lesions. Five

(71.43%) of the animals with gross pulmonary lesions had less than 10 GL in their respiratory tracts while one (33.33%) without gross pulmonary lesions had more than 20 GL in its respiratory tract.

In Group 4, four (50.00%) of animals with gross pulmonary lesions had less than 10 GL in their respiratory tracts and none had more than 20 GL. Three (50%) of the animals without gross pulmonary lesions in this group had less than 10 GL in their respiratory tracts while one calf representing (16.67%) of those without gross pulmonary lesions in this group had more than 20 GL in its respiratory tract.

Nine (56.25%) of the animals with gross pulmonary lesions in Group 5 had less than 10 GL in their respiratory tracts, and 3 (18.75%) more than 20 GL in their respiratory tracts. Of those without gross pulmonary lesions, two (28.57%) had less than 10 GL and two (28.57%) more than 20 GL in the sections of their respiratory tracts examined.

Two (9.52%) of the adult animals (Group 6) with gross pulmonary lesions had less than 10GL in their respiratory tracts but 11 (52.38%) had more than 20 GL in their respiratory tracts. Of the animals without gross pulmonary lesions in this group, none had less than 10 GL in their respiratory tracts but 4 (40%) had more than 20 GL in their respiratory tracts.

3. Histological Classification of Lung Lesions

Some animals without gross lung lesions had microscopic lesions conforming to the general group listed in Table 16. It was then decided to look at the relationship between GL and all pulmonary lesions whether gross or microscopic. The results of these are shown in Table 20.

Two major classes of pneumonias, acute and chronic were diagnosed histologically.

(a) Acute Pneumonias

In this group of pneumonias, two basic forms exudative and proliferative pneumonia were recognised.

(i) Acute exudative pneumonia.

The histological appearance of acute exudative pneumonia (Fig 48) was characterised by both marked vascular and cellular responses. The pulmonary capillaries were engorged and both the alveolar septae, the alveolar airspace and the bronchiolar lumina were infiltrated by neutrophils and latterly macrophages. The vascular reaction was in a few cases accompanied by the presence of fibrin mixed with proteinaceous fluid in the alveolar spaces.

(ii) Acute proliferative pneumonia

The lesion in this type of pneumonia usually involved the bronchioles and the alveoli. The vascular and cellular responses were similar to those described above. In addition there was hyperplasia of both bronchiolar and alveolar lining cells (Fig. 49). Various forms of syncytia of these cells were seen in some cases. Focal areas of alveolar and bronchiolar epithelial necrosis were also present.

(b) Chronic Pneumonias

Three forms of chronic pulmonary disease were recognised; two forms of chronic pneumonia and diffuse fibrosing alveolitis (DFA). The two basic forms of chronic pneumonias were chronic suppurative and chronic non-suppurative pneumonias. Chronic non-suppurative pneumonia was further classified into four types. These were granulomatous pneumonia (such as Husk), cuffing or chronic lymphocytic pneumonia, chronic interstitial pneumonia such as Farmer's Lung (extrinsic allergic alveolitis) and chronic non-suppurative pneumonias that did not fit into any of these classifications.

(i) Chronic suppurative pneumonia

Histologically, this lesion (Fig. 50) was characterised by marked lobular suppurative necrosis. The bronchial, bronchiolar and alveolar lining epithelium were necrotic and their lumina contained large numbers of neutrophils. The necrotic lobule usually had a centre made up of necrotic debris mixed with inflammatory cells and bacterial colonies. The necrotic centre was surrounded by proliferating fibroblasts, infiltrating plasma cells, macrophages and lymphocytes. The bronchial and bronchiolar walls were markedly infiltrated by plasma cells and macrophages. The alveolar lumena contained numerous macrophages, neutrophils, a few giant cells and their septae were thickened by fibrosis. In some cases there was proliferation of the bronchiolar epithelium with subsequent bronchiolitis obliterans.

(ii) Chronic non-suppurative pneumonias

Most cases of chronic non-suppurative pneumonias progressed from persistent acute exudative pneumonia in which suppuration and damage to lung tissue had not occurred. In such cases, the cellular exudate was replaced by fibrous connective tissues and cells of an immune response.

Chronic granulomatous pneumonia

This lesion was seen in cases of patent husk (bovine parasitic bronchitis). The granulomatous reaction was due to the presence of aspirated eggs and first stage larvae in the alveoli. The lesion was characterised by a dense infiltrate of neutrophils, macrophages and large numbers of multinucleated giant cells. Some foci consisted of dense masses of eosinophils with central areas of necrosis. Apart from the granulomatous reaction around parasites or their eggs, other lesions present were the thickening and hyperplasia of bronchial and bronchiolar epithelia which were also heavily infiltrated by large numbers of eosinophils, plasma cells and some neutrophils (Fig. 51). Bronchiolitis obliterans as well

as proliferation of alveolar epithelium were usually present. The lesion was usually associated with marked interstitial emphysema in very heavy infections.

Cuffing pneumonia

The histological features in the early stages of this condition were the presence, in the alveoli, of many macrophages and some polymorphonuclear cells in oedema fluid. Little fibrin was present. There were diffuse accumulation of lymphocytes and plasma cells in considerable numbers in the peribronchiolar and perivascular connective tissue. In the late stages, the perivascular and peribronchiolar cellular infiltrates were replaced by lymphoid follicles (Fig. 52) which often formed cuffs around the bronchioles compressing their walls and causing stenosis of the lumen.

Farmer's lung

This entity was characterised histologically by interstitial infiltrates of lymphocytes, plasma cells and interstitial cells and intraseptal aggregates of lymphocytes without the formation of germinal centres (Fig. 53). The alveoli contained oedema fluid, neutrophils and macrophages. Neutrophils, lymphocytes and plasma cells were frequently found in the alveolar septa. In chronic cases there was focal alveolar fibrosis with alveolar epithelial hyperplasia and the alveolar epithelium could even be replaced by tall columnar ciliated or mucous secreting cells. Bronchiolitis obliterans was a very common feature.

Chronic pneumonia unclassified:

This group was made up of chronic lung lesions which had no features to enable them to be classified into any of the identified entities described earlier.

Histologically, the lesions were characterised by peribronchial, peribronchiolar and alveolar septal fibrosis with marked macrophage, plasma cell and lymphocytic infiltrates. The bronchial and bronchiolar lumena contained purulent exudate while the alveolar spaces macrophages, neutrophils and oedema fluid.

(iii) Diffuse fibrosing alveolitis (DFA):

The most striking lesions in this condition were present in the respiratory acini. The alveolar septae were severely thickened and distorted by fibrosis and cellular infiltration. The cellular infiltrate usually was made up of mature plasma cells, lymphocytes fibroblasts and interstitial cells. The fibrosing lesion was made up of large amounts of collagen and reticulin in the alveolar walls (Fig. 54). Large numbers of mast cells were also present in the alveolar septal connective tissue in sections stained with toluidine blue.

Most alveoli contained accumulations of large mononuclear cells which were either macrophages or probably desquamated alveolar type 2, pneumocytes. There was diffuse alveolar epithelial hyperplasia and the alveoli were lined by cuboidal cells. Tall columnar cells were seen in some foci and others had mucous secreting cells.

(c) Other Lung Lesions

Five other lung lesions were observed in the lungs of animals examined. These included pulmonary congestion and oedema, pulmonary interstitial emphysema, pulmonary abscess, pulmonary collapse and infiltrating secondary pulmonary tumours.

(i) Pulmonary congestion and oedema:

Histologically the alveolar capillaries were engorged and the alveolar spaces contained granular pink staining precipitate which was sometimes vacuolated in places. A few alveolar macrophages and haemosiderin laden macrophages were usually

present. The alveolar septae were widened both by dilation of alveolar capillaries and by oedema fluid present within the interstitium of the alveolar septae (Fig. 55).

(ii) Pulmonary interstitial emphysema:

The histological appearance of this condition was characterised by the distension of the pulmonary interstitium by the accumulation of air (Fig. 56). The lesion was usually present between the interlobular and alveolar septae and around blood vessels. The alveoli in the affected lobes were also markedly distended and in a few cases ruptured septae were seen.

(iii) Pulmonary abscess:

These were usually single large abscesses involving the caudal lobes of the lungs.

Histologically, there was marked central liquifactive necrosis of lung parenchyma surrounded by a layer of large numbers of macrophages, plasma cells and neutrophils. This layer of inflammatory cells was usually followed by a surrounding thick layer of proliferating fibrous connective tissues. The adjacent lung lesion was compressed and so were the blood capillaries in the vicinity.

(iv) Pulmonary collapse:

The main histological features of the lungs of animals with marked pulmonary collapse was that of chronic bronchial and bronchiolar inflammation with massive destruction of the epithelial lining and accumulation of purulent exudate in their lumena. The walls of the respiratory airways were thickened by proliferating fibrous connective tissue as well as plasma cells and macrophage infiltrates. The adjacent alveoli were collapsed and in close apposition and in some cases separated by small slit-like

spaces (Fig. 57). The alveolar spaces in other lobules were usually distended by the accumulation of air.

(v) Secondary pulmonary neoplasia:

Three types of secondary pulmonary neoplasia were diagnosed histologically. Two of these were lymphosarcomas of different types and the third a pancreatic adenocarcinoma. One of the lymphosarcomas was of thymic origin while the other was multicentric.

Histologically, the lymphosarcomas infiltrated the lungs via the bronchial and septal routes. There was severe massive lymphoblastic infiltration of the bronchial, bronchiolar and alveolar walls as well as the interlobular septae (Fig. 58). Purulent exudate was present in the bronchial and bronchiolar lumena and focal areas of necrosis were present in the lung parenchyma.

In the case of the pancreatic tumour, well differentiated tumour cells in tubular arrangements were found within the lung parenchyma (Fig. 59).

4. Animals with Pulmonary Lesions (Gross and Microscopic)

The relationship between GL in the bovine respiratory tract and pulmonary gross and histological lesions is shown in Table 20. Five of the 19 foetuses examined in Group 1 had pulmonary lesions and two of these (40%) had GL in their respiratory tracts. Sixteen foetuses in this group had no pulmonary lesions but six (37.50%) of these had GL in their respiratory tracts.

In Group 2, 25 calves were examined and 19 of these had pulmonary lesions out of which 13 (68.42%) had GL in their respiratory tracts. All six (100%) calves without pulmonary lesion had GL in their respiratory tracts.

Twenty animals were examined in Group 3, 18 of which had pulmonary lesions and ten (55.55%) of these had GL in their respiratory tract. Two (100%) of the two animals in this group without pulmonary lesions had GL in their respiratory tracts.

Nineteen of the 24 animals examined in Group 4 had pulmonary lesions and 12 (63.15%) of these had GL in their respiratory tract. Two (40.00%) of the five animals that had no pulmonary lesions had GL in their respiratory tract.

In Group 5, 27 animals were examined for GL in their respiratory tracts. Of these, 23 had pulmonary lesions and 19 (82.61%) had GL. All the four remaining animals (100%) in this group without pulmonary lesions had GL in their respiratory tract.

In animals above two years of age ie Group 6, 25 of the 34 animals examined had lung lesions and 22 (88%) had GL in their respiratory tracts. Again all the nine (100%) animals in this group without lung lesions had GL in their respiratory tracts.

The relationship between GL population density in the respiratory tract and lung lesions in shown in Table 21.

In Group 1, one of the two foetuses (50%) that had pulmonary lesions had less than 10 GL and none had more than 20 GL in their respiratory tracts. Six foetuses that had no pulmonary lesions had GL in their respiratory tracts. Four of these (66.67%) had less than 10 GL in the sections of the respiratory tracts examined, while none had more than 20 GL in the sections of their respiratory tracts examined.

Group 2 animals had 13 animals positive for GL in their respiratory tracts out of the 19 animals that had pulmonary lesions. Eight (61.53%) of these had less than 10 GL in the sections of the respiratory tract examined, while none had more than 20 GL in the sections of the respiratory tracts examined. All of the five animals without pulmonary lesions had GL in their respiratory tracts, of these four (66.66%) had less than 10 GL and

none had more than 20 GL in the sections of the respiratory tracts examined.

Eight of the 18 animals with pulmonary lesions in Group 3 had GL in their respiratory tracts. Five of these (62.50%) had less than 10 GL, and only one (12.50%) had more than 20 GL in the sections of their respiratory tracts examined. Both the animals without pulmonary lesion had GL in their respiratory tracts, neither had more than 20 GL and one had less than 10 GL in the sections of their respiratory tracts examined.

In Group 4, of the 19 animals with pulmonary lesions, 12 had GL in their respiratory tracta; six (50.00%) of these had less than 10 GL and one (8.33%) had more than 20 GL in the sections of the respiratory tracts examined. Again two of the five animals in this group without pulmonary lesions had GL in their respiratory tracts none had more than 20 GL. One of these had less than 10 GL .

Nineteen of the 23 animals with pulmonary lesions in Group 5 had GL in their respiratory tracts; 11 of these (57.89%), had less than 10 GL, and four (21.05%), had more than 20 GL in the sections of their respiratory tracts examined. All of the four animals without pulmonary lesions in this group had GL in their respiratory tracts. One (25%), had less than 10 GL and none more than 20 GL in the sections of their respiratory tracts examined.

In the adult animals (Group 6), 22 of the 25 animals with pulmonary lesions had GL in their respiratory tracts. Two of these (9.09%) had less than 10 GL, and 13 (59.09%) had more than 20 GL in the sections of their respiratory tracts examined. All of the nine animals without pulmonary lesions in this group had GL in their respiratory tracts. None of these had less than 10GL in the sections of the respiratory tracts examined but four (44.44%) had more than 20 GL in the sections of their respiratory tracts examined.

5. The Relationship between Pulmonary Globule Leucocytes and Globule Leucocytes in Other Mucous Membranes.

Gross lesions were not observed in the liver, the abomasum and the urinary bladder of Groups 1 and 2. One calf in Group 3 had urolithiasis and the mucous membrane of the urinary bladder was congested and had focal areas of haemorrhage.

In Group 4, three calves had mucosal disease and their abomasal mucosal surfaces were hyperaemic and oedematous. A fourth calf had a ruptured urinary bladder. The serosal surface of the urinary bladder was adhered to the pelvic peritoneum and the mucosal surface adjacent to the tear was hyperaemic.

Eight animals in Group 5 had mucosal disease and similar lesions as described for Group 4 animals were observed in their abomasal mucous membranes. Two animals in this group also had Ostertagia ostertagi infection and apart from the massive oedema present in their abomasum, whitish nodules about 1mm in diameter were found scattered throughout the abomasal mucous membrane. Another animal with ragwort poisoning was observed to have had its liver moderately fibrosed. The bile duct of this liver was not found to be grossly affected.

In the adult animals, Group 6, one case of urinary bladder tumour and another of enzootic haematuria were examined. The urinary bladder tumour was that of a cavernous haemangioma which was grossly haemorrhagic and had focal raised red areas. No gross lesions were observed in the case of enzootic haematuria. Another case of suspected bracken fern poisoning examined did not have any gross lesion in the urinary bladder. In a few cases, the bile ducts of the livers of adult animals were found to be very much thickened and fibrosed. In some cases, they had become calcified and in others, few adult liver flukes (Fasciola hepatica) were observed. Three cases of mucosal disease with similar abomasal lesions to those described previously were seen. A further, three animals with Johne's disease were examined and the basic gross lesion found in their abomasum was that of massive oedema.

The relationship between the presence of GL in the bovine respiratory tract and the presence of GL in the mucous membranes of the abomasum, the bile duct and the urinary bladder, is shown in Table 22.

Two (22.22%) of the nine foetuses with GL in their respiratory tracts had GL in their bile ducts. Globule leucocytes were not found in the abomasums or urinary bladders of any animal in this group.

The abomasums, the bile ducts and the urinary bladders of 14 animals in Group 2 with GL in their respiratory tracts were examined. Two (14.29%) of these had GL in their abomasums and three (21.43%) had GL in their bile ducts. No GL were observed in the sections of the urinary bladders of the animals examined in this age group.

Nine animals in Group 3 with GL in their respiratory tract were examined for GL in their abomasums, bile ducts and urinary bladders. Four (44.44%) of these had GL in their abomasums, while only two (22.22%) had them in their bile ducts. The urinary bladders of these animals were not observed to contain GL.

The abomasums, bile ducts and urinary bladders of 15 animals in Group 4 which had GL in their respiratory tracts were examined for the presence of GL. Eleven of these (73.33%) had GL in their abomasum while seven (46.67%) had GL in their bile ducts. None of the animals in this group examined was found to have GL in its urinary bladder.

In Group 5, of the 20 animals which had GL in their respiratory tracts, 17 (85.%) and 14 (70%) had GL in their abomasums and bile ducts respectively. Again none of the animals examined in this group was found to have GL in its urinary bladder.

Twenty five of the adult animals (Group 6) which had GL in their respiratory tracts were examined for the presence of GL in their abomasums, bile ducts and urinary bladders. Twenty four (96%) of these had GL in their abomasum, while 19 (76%) had GL in their bile ducts. None of the urinary bladders of the animals examined in this group were found to contain GL.

D. Discussion

Globule leucocytes were found in the respiratory tracts of all age groups of cattle, but not in every animal examined. The presence of GL in other mucous membranes in the bovine animal have previously been associated with parasitic infections (Jarrett et al., 1967a, Miller et al., 1967, Murray et al., 1968, Rahko 1970a), in ostertagiasis and fascioliasis.

Large numbers of GL were present in the respiratory tract of adult cattle (Chapter 2), and Breeze et al., (1975), Allan et al., (1983), have reported the presence of large numbers of GL in the respiratory tract of adult cattle with various respiratory diseases. Large numbers of GL have also been found in the respiratory tract of cattle in other outbre aks of respiratory disease of unknown aetiology (Pirie, personal communication).

In 1978, Mahmoud investigating GL in the respiratory tract of sheep, associated the presence of GL with parasitic infections and was able to induce proliferations of GL in the respiratory tracts of animals infected with Ascaris suum. Although the inductions of GL with A. suum was successful, he was however unable to induce GL proliferation in the respiratory tracts of sheep exposed to Aspergillus fumigatus and mouldy hay.

The association between the bovine respiratory tract GL and bovine parasitic pulmonary infections or respiratory diseases have not been studied previously.

In the results presented in Section C of this Chapter, the relationship between GL and respiratory disease and respiratory tract GL and GL in other mucous membranes in the bovine animal were reported. No distinct correlation was found between clinical respiratory disease and the presence of GL in the bovine respiratory tract. Globule leucocytes were present in animals with or without clinically diagnosed respiratory disease.

Infact a higher percentage of animals without clinical respiratory disease were positive for GL in their respiratory tract, although a higher percentage of adult animals that had clinically diagnosed respiratory disease had over 20 GL per section in their respiratory tracts than the clinically normal group.

A total of eight categories of gross lung lesions were seen during this study and eight histopathological diagnoses were made of lung lesions. Again, no correlation was seen between GL and the type of gross pulmonary lesions or between GL population density and the gross lung lesions. Although there was also no correlation between GL and pulmonary lesions (gross and microscopic), there was an observed relationship between GL population density and pulmonary lesions (gross and microscopic). A higher percentage of animals with both gross and microscopic lung lesions were observed to have more GL in their respiratory tracts. Animals without gross and microscopic pulmonary lesions in the younger age groups (Groups 1 to 5) did not have 20 or more GL per section in their respiratory tracts. It could therefore be inferred that pulmonary lesions led to an increase in the number of GL in the respiratory tract of cattle. This increase in the GL population could therefore have been brought about by any agent that was present and caused damage to the bovine lungs. Mahmoud, (1978), observed 14 of the 61 outdoor sheep he examined with parasites in their respiratory tracts to be negative for GL, but the bronchi and bronchioles which had no parasites but had microscopic lesions which according to him were parasitic lesions had many GL in their epithelium. It is possible that the tissue in which GL were not found were examined at the time GL may have left the respiratory tract mucous membrane as suggested by Mahmoud (1978). On the other hand it is possible that the agent that produced the lung lesion around the bronchi which contained many GL was responsible for their presence in that area. The absence of GL from the site of parasite infestation was reported by Sommerville, (1956), who did not find any correlation between the region at which GL were commonly observed and the site of nematode infestation.

Mahmoud, (1978), suggested the possibility of systemic proliferation of GL, since he was able to induce GL in the respiratory tract of sheep infected with Haemonchus contortus. This hypothesis led to the thoughts about a possible relationship between the respiratory tract GL and GL in other mucous membranes.

The presence of GL in the bovine respiratory tract did not usually correspond with their presence in other mucous membranes examined, the abomasum, the bile duct or the urinary bladder. Globule leucocytes were never found in the urinary bladder of animals examined even when present in their respiratory tract or any of the other mucous membranes examined. Animals in Groups 2 and 3 were not thought to have been outside grazing and should be parasite free but GL were found in both the abomasums and bile ducts of these animals.

In Group 1, two foetuses were found with GL in their bile ducts. Unless the systemic response which was suggested by Mahmoud, (1978), could occur via the placenta, GL should not be present in foetuses either in the lungs or any other organ. On the other hand, the occurence of GL in mucous membrane of foetuses could be possible only if the cell itself was of mesenchymal origin. This observation could therefore support the hypothesis of the independency of the GL on the MMC proposed by Finn and Schwartz, (1972) and Riutenberg and Elgersma, (1979).

In Group 4, 5 and 6 the number of animals in whose lungs, abomasums and bile ducts GL were found increased with age. The possible explanation for this could be that as the animals now had access to outside grazing, they became exposed to parasites and GL appeared in these organs or that the increase or proliferation of GL in all mucous membranes was also age dependent.

TABLE 14: Globule leucocytes and clinical respiratory disease in the cattle studied.

GLOBULE LEUCOCYTES AND CLINICAL RESPIRATORY DISEASE IN THE CATTLE STUDIED

GROUP AGE (MONTHS)	TOTAL NUMBER OF ANIMALS EXAMINED	ANIMALS WITH CLINICAL RESPIRATORY DISEASE		ANIMALS WITHOUT CLINICAL RESPIRATORY DISEASE	
		NUMBER EXAMINED	NUMBER +ve GL (%)	NUMBER EXAMINED	NUMBER +ve GL (%)
1 (Foetuses)	19	0	0	19	9 (47.37)
2 (0 - 1)	25	0	0	25	19 (76.00)
3 (2 - 4)	20	9	3 (33.33)	11	7 (63.64)
4 (5 - 11)	24	12	7 (58.33)	12	7 (58.33)
5 (12 - 23)	27	9	6 (66.67)	18	17 (94.44)
6 (> 24)	34	10	8 (80.00)	24	23 (95.83)

GL = Globule leucocyte. +ve = Positive. (%) = Percentage

TABLE 15: Globule leucocyte population density and clinical respiratory disease in the cattle studied.

GLOBULE LEUCOCYTE POPULATION DENSITY AND CLINICAL RESPIRATORY DISEASE IN THE CATTLE STUDIED

GROUP AGE (MONTHS)	ANIMALS WITH CLINICAL RESPIRATORY DISEASE					ANIMALS WITHOUT CLINICAL RESPIRATORY DISEASE				
	NUMBER EXAMINED	NUMBER +ve GL%	GLOBULE LEUCOCYTE POPULATION DENSITY			NUMBER EXAMINED	NUMBER +ve GL%	GLOBULE LEUCOCYTE POPULATION DENSITY		
			+(%)	++ (%)	+++(%)			+(%)	++(%)	+++(%)
1 (Foetuses)	-	0	0	0		19	9(47.37)	6(66.67)	3(33.33)	0
2 (0 - 1)	-	0	0	0		25	19(76.00)	12(63.16)	7(36.84)	0
3 (2 - 4)	9	3(33.33)	2(66.67)	1(33.33)	0	11	7(63.64)	5(71.43)	1(14.29)	1(14.29)
4 (5 - 11)	12	7(58.33)	3(42.86)	4(57.14)	0	12	7(58.33)	5(71.43)	1(14.29)	1(14.29)
5 (12 - 23)	9	6(66.67)	3(50.00)	1(16.67)	2(33.33)	18	17(94.44)	9(52.94)	5(29.41)	2(11.76)
6 (>24)	10	8(80.00)	0	2(25.00)	6(75.00)	24	23(95.83)	1(4.35)	10(43.48)	12(52.17)

GL = Globule leucocyte. +ve = Positive (%) = Percentage.

TABLE 16: The gross pulmonary lesions in the cattle studied.

THE GROSS PULMONARY LESIONS IN THE CATTLE STUDIES

GROUP AGE (MONTHS)	TOTAL NO. OF ANIMALS STUDIED	TOTAL NO. OF ANIMALS WITH GROSS PULMONARY LESIONS	NO.WITH PULMONARY CONGESTION AND OEDEMA	NO.WITH PNEU-MONIAS	NO.WITH PULMONARY COLLAPSE	NO.WITH PULMONARY EMPHYSEMA	NO.WITH OVER-INFLATED LUNGS	NO.WITH PULMONARY ABSCESS	NO.WITH TUMOURS	NO.WITH OTHER LUNG LESIONS
1 (Foetuses)	19	3	3	-	-	-	-	-	-	-
2 (0 - 1)	25	15	12	2	1	-	-	-	-	-
3 (2 - 4)	20	16	-	14	1	1	-	-	-	-
4 (5 - 11)	24	15	-	12	2	-	-	-	1	-
5 (12 - 23)	27	20	-	16	-	1	-	-	1	2
6 (>24)	34	24	1	12	2	3	1	2	1	2

* See Table 17 for a classification of the pneumonia cases.

TABLE 17: The histological diagnosis of the pneumonic lesions found in the cattle studied.

HISTOLOGICAL DIAGNOSIS OF THE PNEUMONIC LESIONS FOUND IN THE CATTLE STUDIED

GROUP AGE (MONTHS)	NUMBER OF ANIMALS WITH ACUTE PNEUMONIAS		NUMBER OF ANIMALS WITH CHRONIC PNEUMONIAS					
				CHRONIC NON-SUPPURATING PNEUMONIAS				
	EXUDATIVE	PROLIFERATIVE	CHRONIC SUPPURATIVE PNEUMONIA	GRANULOMATOUS (HUSK)	CUFFING	FARMER'S LUNG	UN CLASS-IFIED	DIFFUSE FIBROSING ALVEOLITIS
1 (Foetuses)	-	-	-	-	-	-	-	-
2 (0 - 1)	2	-	-	-	-	-	-	-
3 (2 - 4)	5	1	2	1	1	-	-	4
4 (5 - 11)	2	1	3	4	-	-	-	2
5 (12 - 23)	4	-	1	5	2	1	-	3
6 (>24)	2	-	2	2	-	3	2	1

TABLE 18: Globule leucocytes and gross pulmonary lesions in the cattle studied.

GLOBULE LEUCOCYTES AND GROSS PULMONARY LESIONS IN THE CATTLE STUDIED

GROUP AGE (MONTHS)	TOTAL NUMBER OF ANIMALS EXAMINED	ANIMALS WITH GROSS PULMONARY LESIONS		ANIMALS WITHOUT GROSS PULMONARY LESIONS	
		NUMBER EXAMINED	NUMBER +ve GL (%)	NUMBER EXAMINED	NUMBER +ve GL (%)
1 (Foetuses)	19	3	2 (66.67)	16	7 (43.75)
2 (0 - 1)	25	15	12 (80.00)	10	7 (70.00)
3 (2 - 4)	20	16	7 (43.75)	4	3 (75.00)
4 (5 - 11)	24	15	8 (53.33)	9	6 (66.67)
5 (12 - 23)	27	20	16 (80.00)	7	7(100.00)
6 (>24)	34	24	21 (87.50)	10	10(100.00)

GL = Globule leucocyte. +ve = Positive. (%) = Percentage

TABLE 19: Globule leucocyte population density and gross pulmonary lesions in cattle.

GLOBULE LEUCOCYTE POPULATION DENSITY AND GROSS PULMONARY LESIONS IN CATTLE

GROUP AGE (MONTHS)	ANIMALS WITH GROSS PULOMONARY LESIONS					ANIMALS WITHOUT GROSS PULMONARY LESIONS				
	NUMBER EXAMINED	NUMBER +ve GL%	GLOBULE LEUCOCYTE POPULATION DENSITY			NUMBER EXAMINED	NUMBER +ve GL%	GLOBULE LEUCOCYTE POPULATION DENSITY		
			+(%)	++ (%)	+++(%)			+(%)	++(%)	+++(%)
1 (Foetuses)	3	2(66.67)	1(50.00)	1(50.00)	0	16	7(43.75)	5(71.43)	2(28.57)	0
2 (0 - 1)	15	12(80.00)	9(75.00)	3(25.00)	0	10	7(70.00)	3(42.86)	4(57.14)	0
3 (2 - 4)	16	7(43.75)	5(71.43)	2(28.57)	0	4	3(75.00)	1(33.33)	1(33.33)	1(33.33)
4 (5 - 11)	15	8(53.33)	4(50.00)	4(50.00)	0	9	6(66.67)	3(50.00)	2(33.33)	1(16.67)
5 (12 - 23)	20	16(80.00)	9(56.25)	4(25.00)	3(18.75)	7	7(100.00)	2(28.57)	3(42.86)	2(28.57)
6 (> 24)	24	21(87.50)	2(9.52)	8(38.10)	11(52.38)	10	10(100.00)	0	6(60.00)	4(40.00)

GL = Globule leucocyte. +ve = Positive (%) = Percentage.

TABLE 20: Globule leucocyte and pulmonary lesions (gross and microscopic) in cattle.

GLOBULE LEUCOCYTES AND PULMONARY LESIONS
(GROSS AND MICROSCOPIC) IN THE CATTLE STUDIED

GROUP AGE (MONTHS)	TOTAL NUMBER OF ANIMALS EXAMINED	ANIMALS WITH PULMONARY LESIONS		ANIMALS WITHOUT PULMONARY LESIONS	
		NUMBER EXAMINED	NUMBER +ve GL (%)	NUMBER EXAMINED	NUMBER +ve GL (%)
1 (Foetuses)	19	5	2 (40.00)	16	6 (37.50)
2 (0 - 1)	25	19	13 (68.42)	6	6 (100.00)
3 (2 - 4)	20	18	10 (55.55)	2	2 (100.00)
4 (5 - 11)	24	19	12 (63.15)	5	2 (40.00)
5 (12 - 23)	27	23	19 (82.61)	4	4 (100.00)
6 (> 24)	34	25	22 (88.00)	9	9 (100.00)

GL = Globule leucocyte. +ve = Positive. (%) = Percentage

TABLE 21: Globule leucocyte population density and pulmonary lesions (gross and microscopic) in cattle.

GLOBULE LEUCOCYTE POPULATION DENSITY AND PULMONARY LESIONS (GROSS AND MICROSCOPIC) IN CATTLE

GROUP AGE (MONTHS)	ANIMALS WITH PULMONARY LESIONS					ANIMALS WITHOUT PULMONARY LESIONS				
	NUMBER EXAMINED	NUMBER +ve GL%	GLOBULE LEUCOCYTE POPULATION DENSITY			NUMBER EXAMINED	NUMBER +ve GL%	GLOBULE LEUCOCYTE POPULATION DENSITY		
			+(%)	++ (%)	+++(%)			+(%)	++(%)	+++(%)
1 (Foetuses)	5	2(40.00)	1(50.00)	1(50.00)	0	14	6(42.86)	4(66.67)	2(33.33)	0
2 (0 - 1)	19	13(68.42)	8(61.53)	5(38.46)	0	6	6(100.00)	6(66.66)	2(33.33)	0
3 (2 - 4)	18	8(44.44)	5(62.50)	2(25.00)	1(12.50)	2	2(100.99)	1(50.00)	1(50.00))	0
4 (5 - 11)	19	12(63.15)	6(50.00)	5(41.66)	1(8.33)	5	2(40.00)	1(50.00)	1(50.00)	0
5 (12 - 23)	23	19(82.61)	11(57.89)	4(21.05)	4(21.05)	4	4(100.00)	1(25.00)	3(75.00)	0
6 (>24)	25	22(88.00)	2(9.09)	7(31.82)	13(59.09)	9	9(100.00)	0	5(55.56)	4(44.44)

GL = Globule leucocyte. +ve = Positive (%) = Percentage.

TABLE 22: Globule leucocytes in the lungs, the abomasums, the bile ducts and the urinary bladders of the cattle studied.

GLOBULE LEUCOCYTES IN THE LUNGS, ABOMASUMS, BILE DUCTS AND URINARY BLADDERS OF THE CATTLE STUDIED

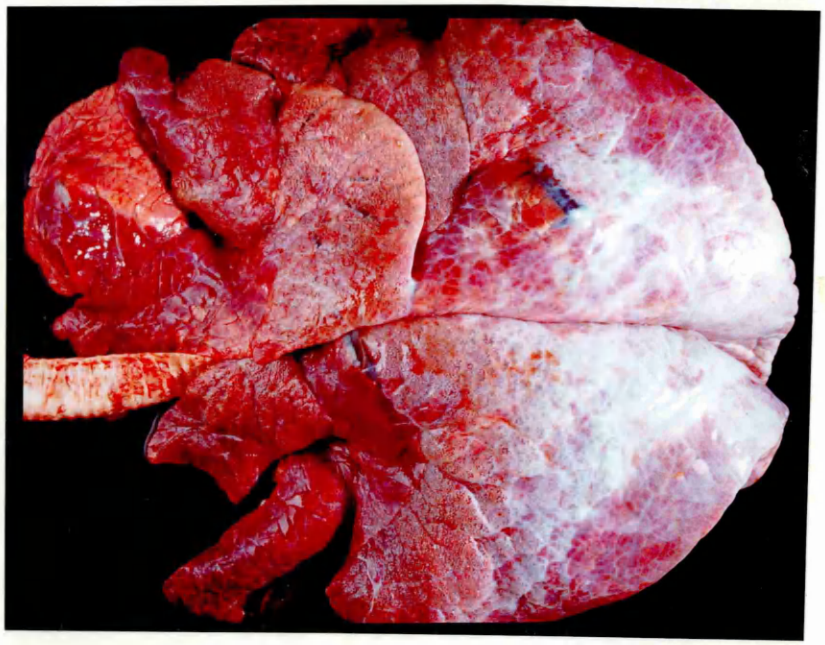
GROUP AGE (MONTHS)	TOTAL NUMBER +ve GL IN THE LUNGS WITH OTHER MUCOUS MEMBRANES EXAMINED	NUMBER +ve GL IN ABOMASUM (%)	NUMBER +ve GL IN BILE DUCT (%)	NUMBER +ve GL IN URINARY BLADDER (%)
1 (Foetuses)	9	0	2 (22.22)	0
2 (0 - 1)	14	2 (14.29)	3 (21.43)	0
3 (2 - 4)	9	4 (44.44)	2 (22.22)	0
4 (5 - 11)	15	11 (73.33)	7 (46.67)	0
5 (12 - 23)	20	17 (85.00)	14 (70.00)	0
6 (> 24)	25	24 (96.00)	19 (76.00)	0

GL = Globule leucocyte. +ve = Positive. (%) = Percentage.

FIG. 47: The lungs of a calf with gross consolidation in the cranial portions and mild emphysema.

FIG. 48: The histological appearance of acute exudative pneumonia showing vascular and cellular responses.

H&E x 35



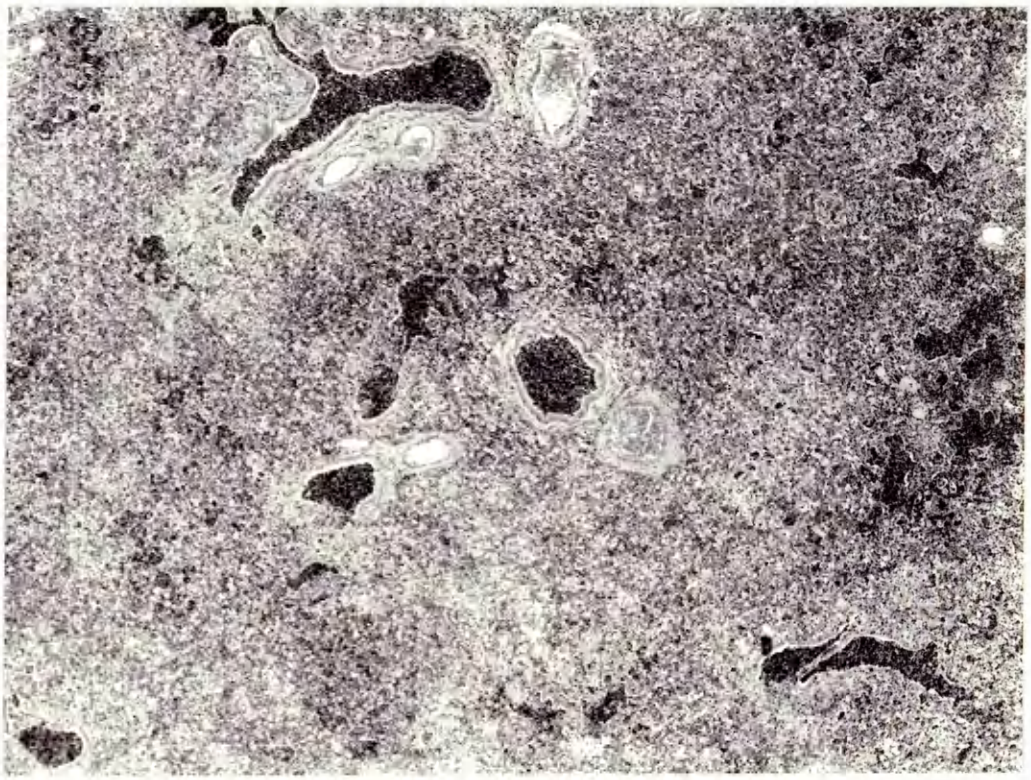
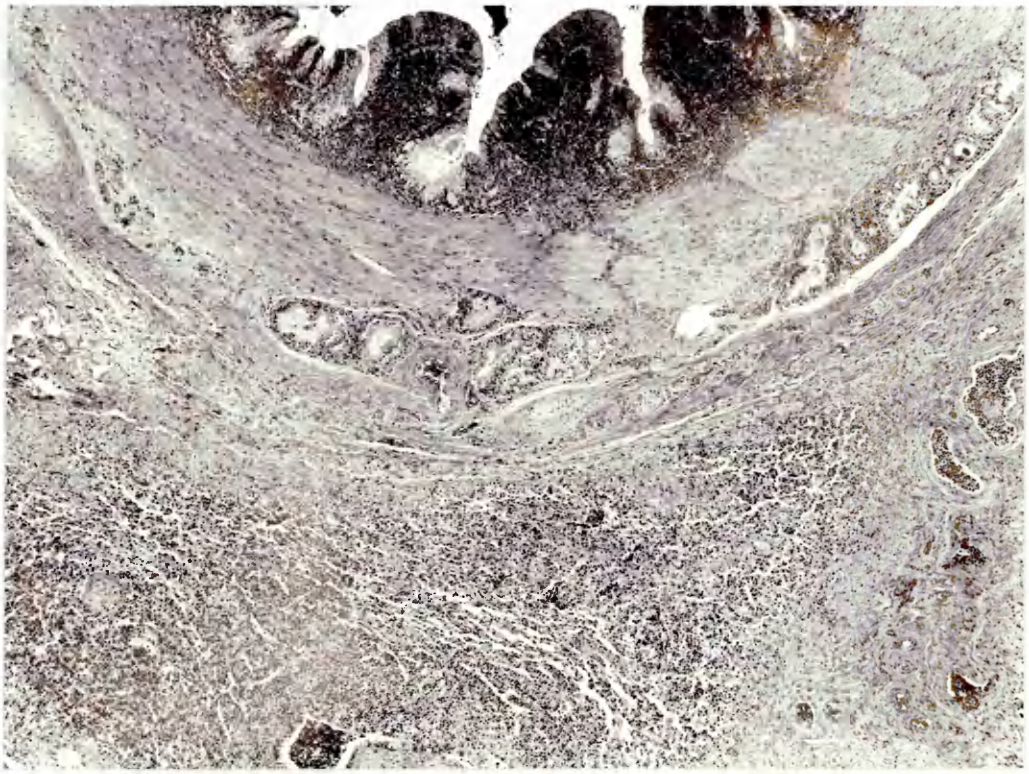


FIG. 51: The histological appearance of chronic granulomatous pneumonia with thickening, hyperplasia and cellular infiltration of bronchial epithelium.

H&E x 35

FIG. 52: The histological appearance of cuffing pneumonia with lymphoid follicles around a bronchiole.

H&E x 35



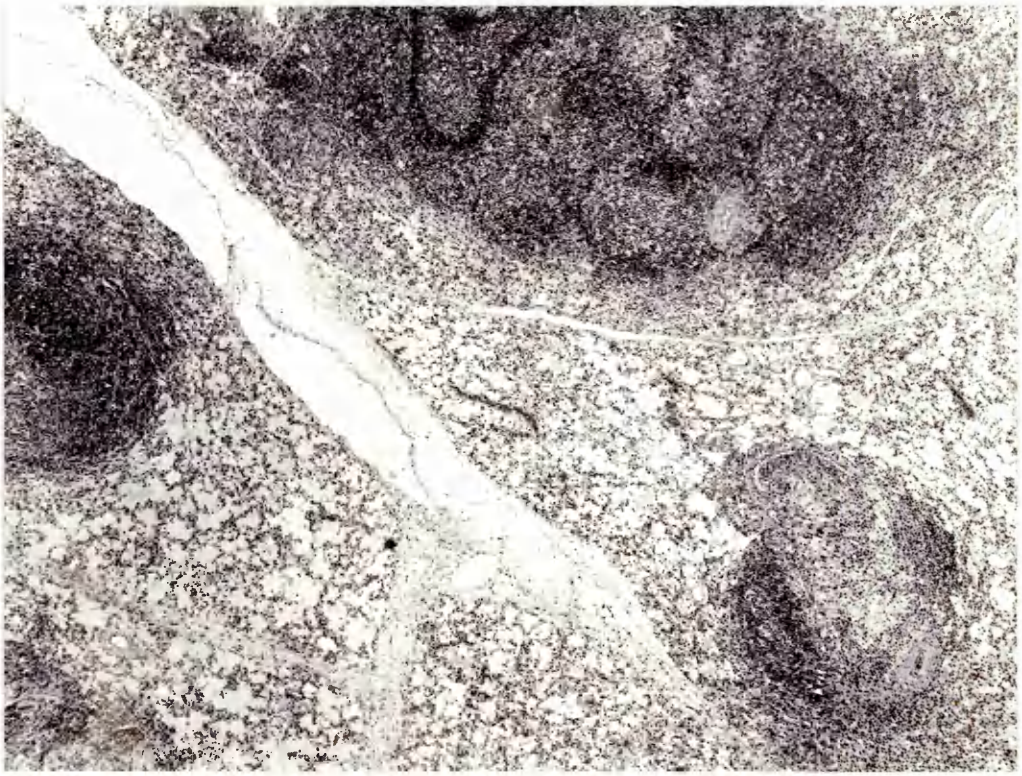
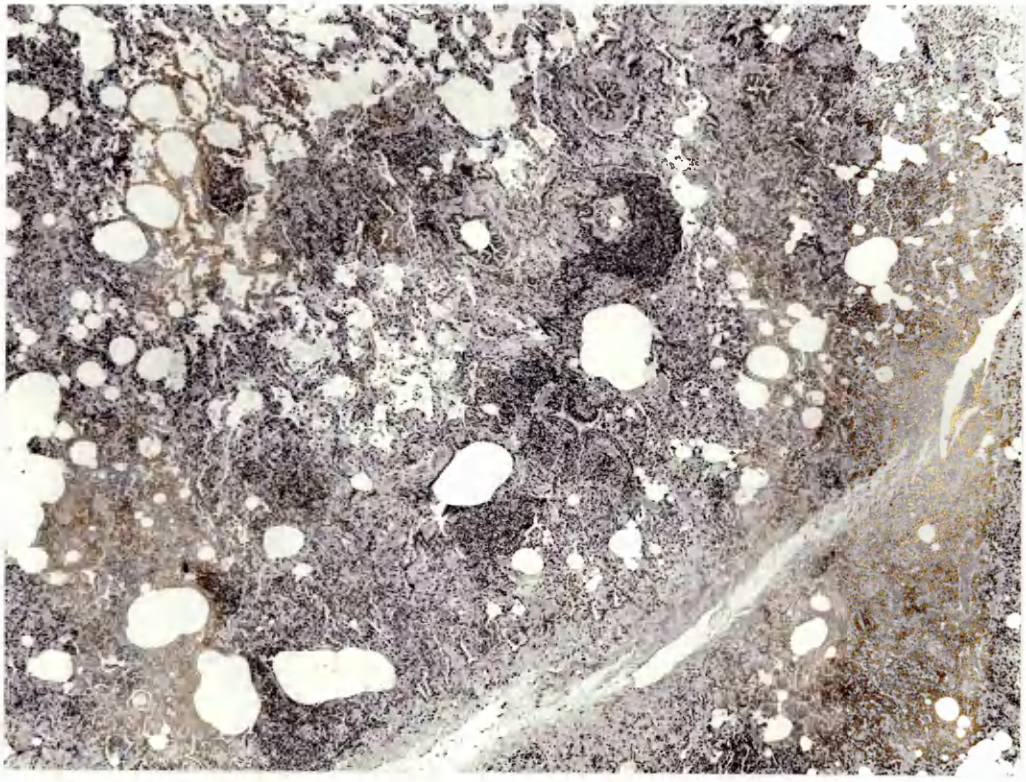


FIG.53: The histological appearance of farmer's lung with intra septal aggregates of lymphocytes without the formation of germinal centres and focal alveolar fibrosis.

H&E x 35

FIG. 54: The histological appearance of diffuse fibrosing alveolitis (DFA) with marked alveolar septal fibrosis.

H&E x 110



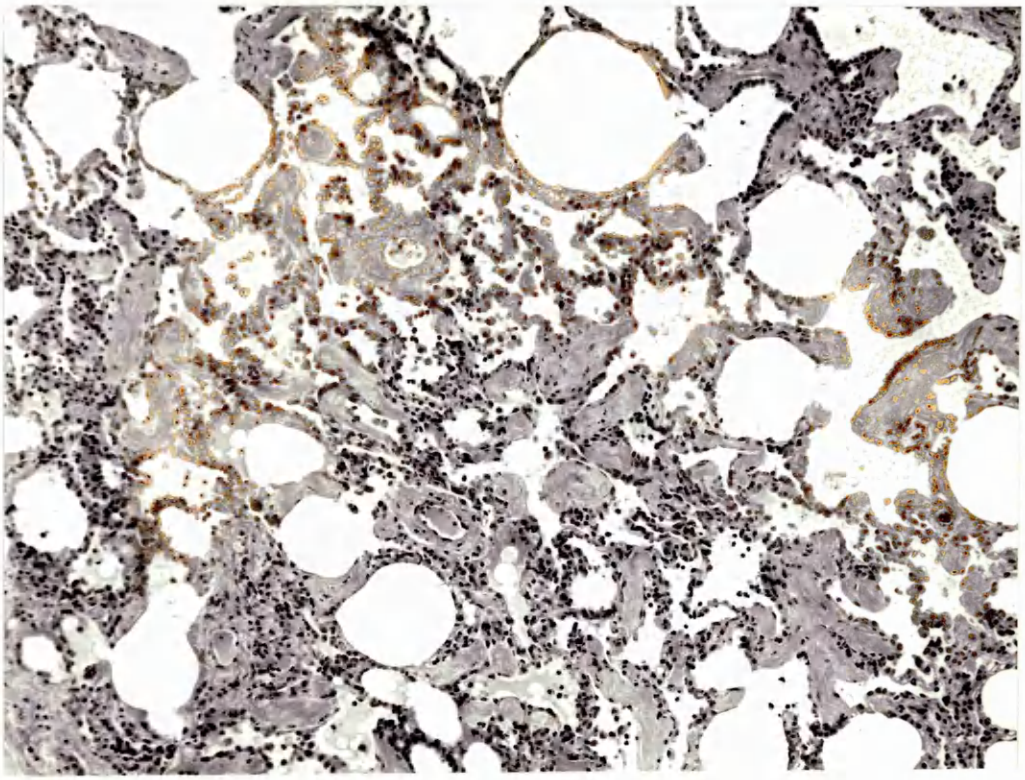


FIG. 55: The histological appearance of pulmonary congestion and oedema with dilated alveolar capillaries and oedema fluid in alveoli.

H&E x 110

FIG. 56: The histological appearance of interstitial emphysema with distension of interlobular and alveolar septae.

H&E x 35

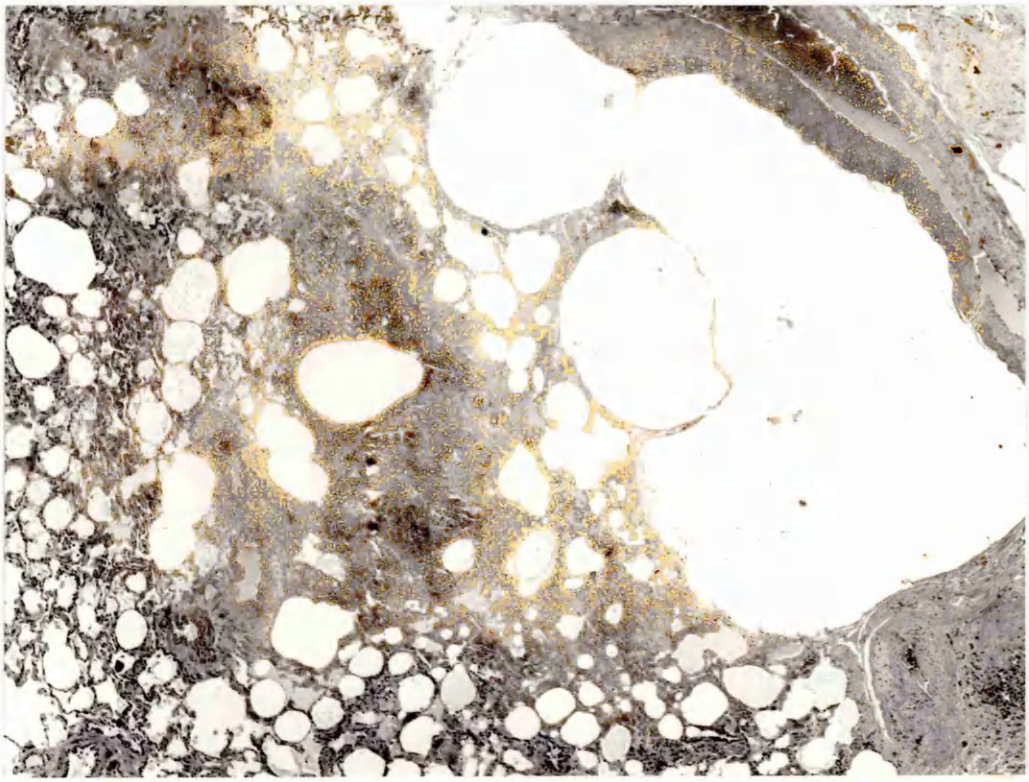
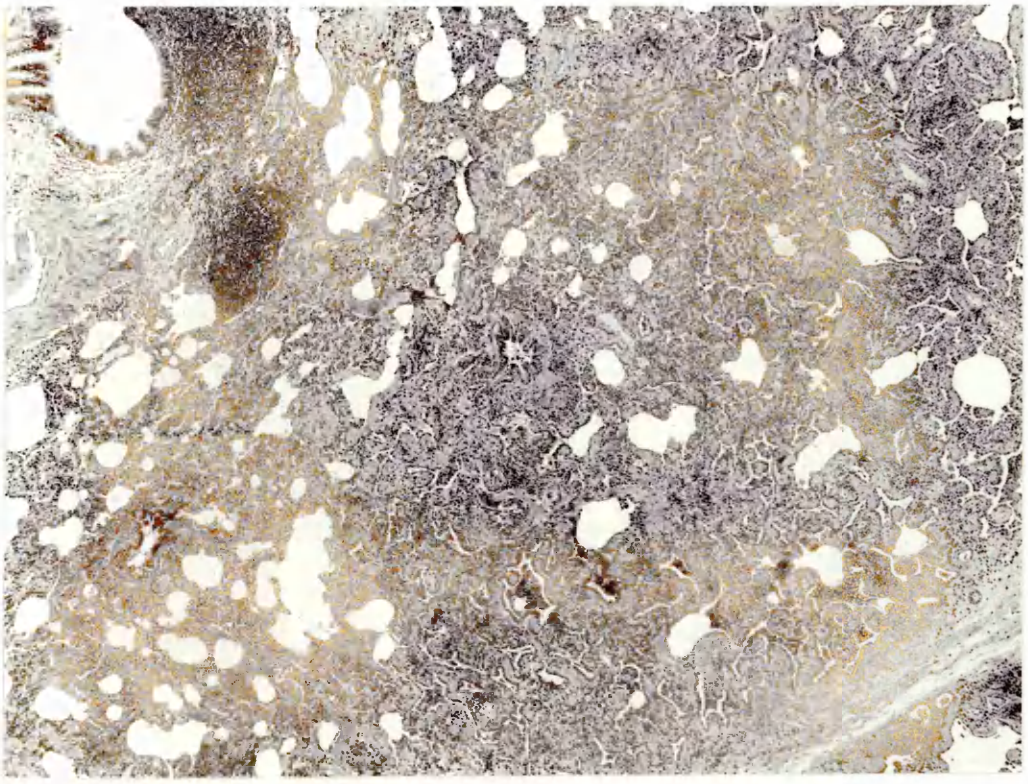


FIG. 57: The histological appearance of pulmonary collapse, showing collapsed alveoli in close apposition and some separated by small slit-like spaces.

H&E x 35

FIG. 58: Histological appearance of pulmonary involvement in lymphosarcoma with massive lymphoblastic and lymphocytic inf iltration of the bronchial wall, alveolar and interlobular septae.

H&E x 35



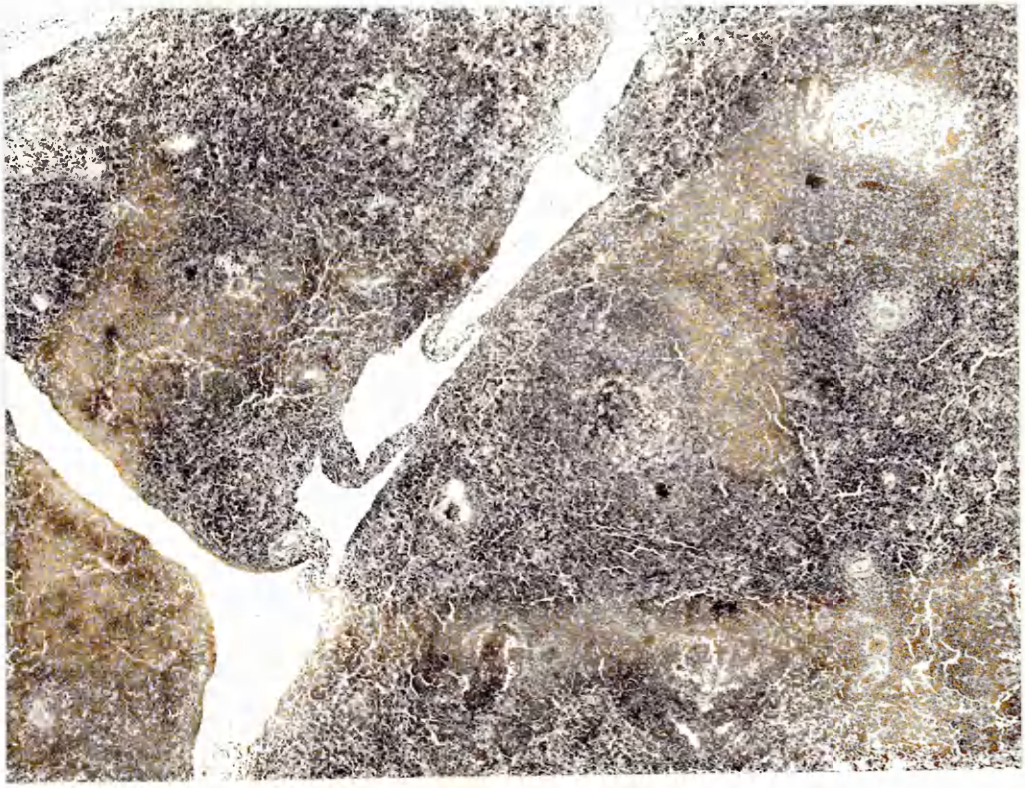
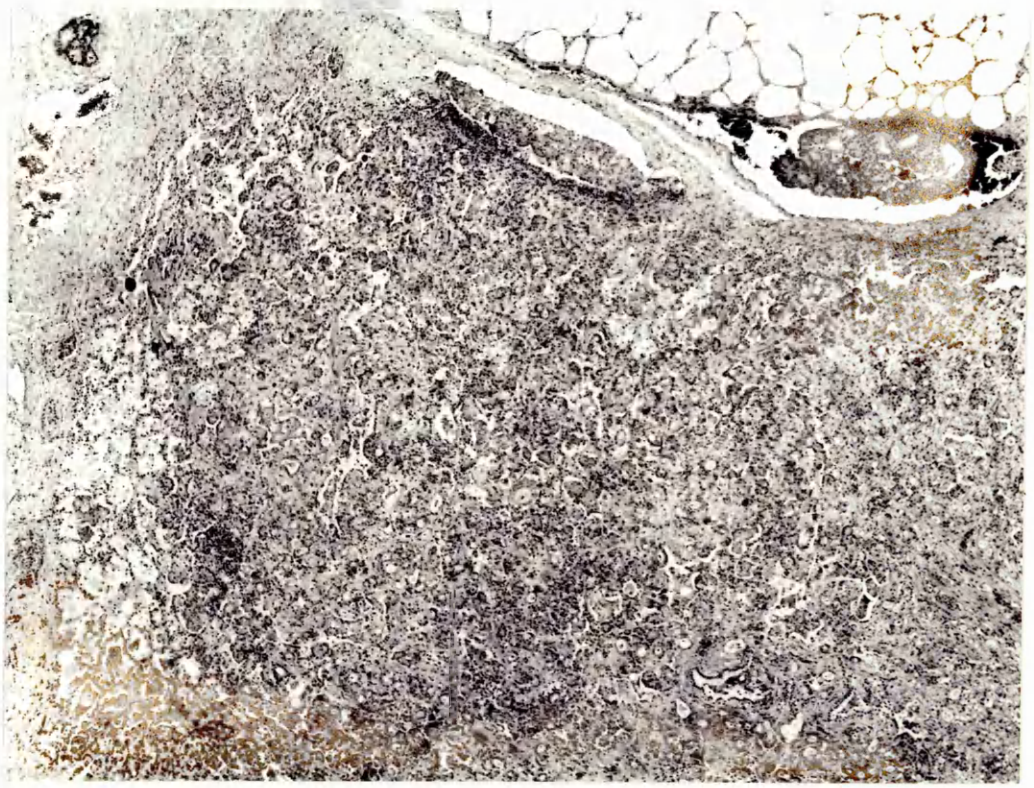


FIG. 59: The histological appearance of pulmonary involvement in pancreatic adenocarcinoma showing tumour cells in lung parenchyma and lymphatics.

H&E x 35



CHAPTER FIVE

EXPERIMENTAL INDUCTION OF GLOBULE LEUCOCYTES IN THE RESPIRATORY TRACT OF CATTLE USING DICTYOCAULUS VIVIPARUS

EXPERIMENTAL INDUCTION OF GLOBULE LEUCOCYTES IN THE RESPIRATORY TRACT OF CATTLE USING DICTYOCAULUS VIVIPARUS

A. INTRODUCTION

In Chapter 2 of this thesis, the occurrence, prevalence and distribution of GL in the bovine respiratory tract was described. In that same chapter, the results (Section C) indicated that although GL were present in the respiratory tracts of all the age groups of cattle examined, they were not however present in the lungs of every animal. Globule leucocytes were found to be more concentrated in the respiratory tracts of adult animals (Group 6) but the significance of their presence was not known.

In chapter 4 of this thesis, the relationship between GL and clinical respiratory disease and the presence of pulmonary lesions was reported. There was no relationship found between the presence of GL in the bovine respiratory tract and clinical respiratory disease in young animals, but their numbers were increased in calves with pulmonary lesions. Adult animals with clinical respiratory disease were more likely to have large numbers of GL in their respiratory tracts than those without although the difference was not clear cut.

The presence of GL in the gastrointestinal and urinary tracts of rats and cattle has been associated with parasitic infection (Taliaferro and Sarles, 1939, Kirkman, 1950, Whur, 1966, Jarrett et al., 1967a & b, Murray, 1968), and similarly in the gastrointestinal tract of sheep (Sommerville, 1956, Dobson, 1966). In the bile ducts of cattle (Rahko, 1970a, 1971), of goats (Rahko, 1972) and of sheep (Rahko, 1970b), GL were associated with liver fluke infestation. This association with parasitism was also studied in the respiratory tract of sheep (Mahmoud, 1978).

The presence of large numbers of GL in the respiratory tract of the adult cattle examined during the survey, the previous association of GL with parasitic infections particularly with

repeated exposure to parasites, led to thoughts about the possibility of an association between repeated exposure of adult cattle to D. viviparus infection and the common finding of GL in the lungs of adult cattle. Current views of the epidemiology, pathogenesis and importance of bovine parasitic bronchitis were briefly reviewed in Chapter 1.

In N. brasiliensis infection in the rat, GL have been reported to increase during self cure (Whur, 1966). In order to understand the relationship between respiratory tract GL and lungworm infections in cattle, three experiments were performed to investigate the following objectives.

1. Was there a GL response in the respiratory tract during lungworm infection in cattle?

2. Was the response related to immunity?

3. Was the response related to self cure as had been shown with other parasites?

4. Was the response influenced by repeated exposure?

5. Was there any relationship between the type of lung lesion and the presence of GL?

B. GENERAL MATERIALS AND METHODS

To investigate the above objectives, three experiments were performed. All the animals used in the experiments had been raised parasite free and were housed throughout the duration of the experiments. The infective third stage larvae (L_3) of D. viviparus and the vaccine Dictol used were obtained from Glaxovet, Harefield, England. The pens in which the animals were kept were thoroughly cleaned every other day.

1. Animals

Two to three month old calves were used in these experiments. The animals were purchased at two or three months of age from a farm known to be free of lungworm and had been raised parasite free.

2. Parasitological Methods

(a) Faecal Examination.

Faecal samples were collected from calves and examined for the larvae of D. viviparus using two techniques. A modified McMaster flotation technique (Gordon and Whitlock, 1939) and a modified Baermann method (Henriksen, 1965).

(ii) Modified McMaster Method:

From each sample of faeces 3 g was weighed then homogenised with 42 ml of water and the resultant suspension passed through a coarse mesh seive of 250 µm aperture size. The filtrate was then mixed thoroughly and 15 ml of this withdrawn into each of two flat bottomed centrifuge tubes. These were then centrifuged at 2,000 revolutions per minute (rev/min) for two minutes. The supernatants from each tube were discarded and the faecal deposit at the bottom of the tubes broken up by rotary ag itation. One of the tubes was then filled to its former level with saturated Nacl solution and inverted several times to ensure proper mixing of the salt solution and the faeces to make a suspension. Both chambers of a McMaster slide were quickly filled from this suspension using a pipette. The number of larvae under the etched areas of the slide were then counted after a short period had been allowed for larvae to float to the surface of the suspension. The results of the count was multipled by 50 to give the number of larvae per gramme of faeces according to the following calculation.

3g of faeces suspended in 42 ml of water was equivalent to 1g of faeces in 15 ml of the suspension.

The total volume under one chamber of the McMaster slide is 0.15 ml. Therefore the number of larvae in one chamber multiplied by 100 gives the number of larvae per gram (l.p.g.) of faeces.

The number of larvae in two chambers multiplied by 50 gives the number of l.p.g. of faeces.

(ii) Modified Baermann:

With the Baermann technique, 10 g of faeces were placed in gauze and suspended in warm water overnight. The gauze and faeces were then discarded after this period. Part of the supernatant of the suspension in the "conical flask" was then syphoned off to concentrate it. The remainder was then further concentrated by centrifuging at 2,000 rev/min. for ten minutes in a bench centrifuge. The supernatant was again discarded and the sediments properly broken up and water added to make up to 10 ml. The larvae present in 1 ml of this final 10 ml volume were counted as above and expressed as l.p.g. of faeces.

(b) Preparation of Larvae Inocula

The stock solution of infective third stage larvae (L_3) of D. viviparus from Glaxovet was made up to 50 ml and mixed properly. The total number of larvae present in 40 x 0.025 ml aliquots of this solution was then counted to give the number of larvae in 1 ml of this solution.

The grand total present in 50 ml was then obtained by multiplying this total by 50. Once the number of larvae in 1 ml was known, the volume necessary to provide the required inoculum was pipetted out using a digital pipette and was made up to about 10 ml for appropriate dosing of calves.

(c) Recovery of Lungworms from Lungs for Worm Count.

Two techniques were used for the recovery of worms from the lungs post-mortem.

In the first technique the air passages i.e. trachea, bronchi were opened up completely and visible worms removed and placed in a petri dish containing warm water. The lungs were then transferred to buckets containing about 5 l of warm water and left there for about three hours. The lungs were again checked for the presence of worms in the respiratory tract before being discarded. The buckets were left to stand for one hour, decanted and the sediment transferred to glass cylinders for final concentration. Worms were then left at 4.0°C to relax and become disentangled to facilitate counting.

In the second technique i.e. the modified perfusion technique (Inderbitzen, 1976), the pericardial sac was incised and reflected to expose the pulmonary artery. A two centimetre incision was then made in the pulmonary artery and a rubber tube introduced. The tubing was fixed in situ by double ligatures. The remaining great vessels were tied off and water from the main water supply allowed to enter the lungs via the pulmonary artery. The lung washings were collected via the trachea into buckets and concentrated by passing through a 37µm aperture sieve. Parasites obtained in this way were again allowed to relax overnight at 4°C before counting.

3. Post-mortem Examinations.

Post-mortem, the lungs including the trachea and larynx were removed from the carcass and carefully examined for gross lesions. Fresh tissue blocks were obtained from the respiratory tract and pulmonary parenchyma as described in Section B of Chapter 2. Tissues were fixed and processed as outlined in Section B of Chapter 2.

Haematoxylin and eosin (H&E) was the routine stain used for the identifaction of GL. Other staining techniques were carried out to confirm that the cell identified with H&E was the GL as in Chapter 3.

4. Globule Leucocyte Population Density

The GL population density in the respiratory tracts of these experimental animals was investigated as described in Section B of Chapter 2.

C. EXPERIMENT 1: SINGLE EXPOSURE OF CALVES TO DICTYOCAULUS VIVPARUS

This preliminary experiment was performed to see if a single exposure of calves to D. viviparus would stimulate proliferation of large numbers of GL in their respiratory tracts.

1. Experimental Design

A total of seven, two to three month old calves were used in this experiment. The calves were dosed orally with doses ranging from 3,000 to 10,000 infective larvae of D. viviparus and the infections allowed to persist from 17 to 75 days as shown in Table 23. The respiratory rates and rectal temperatures of the calves were checked before infection and every other day for the remaining period of the experiment.

2. Results

(a) Clinical Observations

The single challenge infections in calves were successful and animals developed clinical signs of respiratory damage. The frequency of coughing increased from the 11th day post infection (PI). The cough was dry and explosive. The respiratory rate increased from about 25 to 76 per minute by 20 days PI. The coughing and hyperpnoea continued until animals were killed. The

rectal temperature remained within normal range throughout the duration of the experiment. Animals were reluctant to move during the later part of the infection.

(b) Pathology.

At post-mortem examination the animals were observed to have developed typical lesions of lungworm infection. The lesions observed were similar to those previously described by Jarrett et al., (1957). Both gross and microscopic lesions observed were similar in all experimental animals.

(i) Gross Pathology:

There was a red or grey consolidation of the cranial lung lobes which were in most cases collapsed. Consolidation in the caudal lobes was focal but widespread (Fig. 60) and the cut surfaces of the lung lobes were granular in appearance. Occasionally, interstitial emphysema was present in the caudal lobes. The pleura was usually thickened, rough and sometimes adherent to the thoracic cage. The respiratory tract i.e. trachea, bronchi and bronchioles contained large amounts of mucopus mixed with froth and large numbers of adult D. viviparus (Fig. 61). The bronchial and bronchiolar epithelia were thickened in chronic cases and the mediastinal as well as bronchial lymph nodes were oedematous and enlarged.

(ii) Histopathology:

The histological classification of lung lesions was that of either acute exudative or chronic non-suppurative pneumonia. The acute lesions showed marked congestion with neutrophilic infiltrations of alveolar and bronchiolar lumina and into the epithelium which was both dysplastic and hyperplastic. Sections of larvae were found in the alveolar and bronchiolar lumina (Fig. 62).

The chronic lesion was that of massive plasma cell and eosinophilic infiltrates into the lung parenchyma. Peribronchial, subpleural and parenchymal lymphoid aggregates as well as epithelial hyperplasia were present. The bronchial and bronchiolar

lamina propria contained large numbers of both plasma cells and eosinophils (Fig. 63).

(c) Globule Leucocyte Populations in the Respiratory Tract.

The GL response during lungworm infection in calves with a single exposure to D. viviparus was as shown in Table 23.

Only one of the calves infected with D. viviparus for 28 days had a few GL, less than 10, in the respiratory epithelium examined, while all the other calves which were infected for either 17, 27 or 28 days had no GL in their respiratory tracts. All calves infected for between 37 and 75 days had GL in their respiratory tract. The two calves infected for 37 days had between 10-19 GL per section in their respiratory tract while the one infected for 75 days had less than 10 GL in the epithelium of the sections of its respiratory tract examined. There did not seem to be any relationship between the infective dose and GL proliferations (Table 23).

D. EXPERIMENT 2: ORAL VACCINATION FOLLOWED BY ORAL CHALLENGE WITH DICTYOCAULUS VIVIPARUS

In the preceding experiment the effects of a single exposure of calves to D. viviparus on GL response in the bovine respiratory tract was investigated. The results did not indicate clearly that a single exposure of calves to D. viviparus resulted in the proliferation of large numbers of GL. In this second experiment, the GL response when immune animals were challenged was investigated.

1. Experimental Design

The details and design of experiment are shown on Table 24.

Ten three months old calves were used. These animals had been reared and housed as described in section B of this chapter.

The animals were divided into three Groups 2a, 2b and 2c according to their body weights.

Group 2a - This group was made up of four calves (Para 35, 39, 50 and 57) which were vaccinated with Dictol and then challenged. Each animal received two doses of Dictol orally with an interval of 28 days between them and they were then challenged orally with 30 infective third stage larvae of D. viviparus per kg body weight. Thereafter, one calf was killed at two weeks and then one every other 10 days.

Group 2b - This group was also made up of four animals (Para 21, 38, 46 and 53) which were not vaccinated but which were infected at the same time when Group 2a animals were challenged. Each animal received 30 infective third stage larvae of D. viviparus per kg body weight as Group 2a. Thereafter one calf was killed at two weeks and then one every other 10 days as in Group 2a.

Group 2c - This group was made up of two calves (Para 56 and 59) which were used as vaccinated immune controls. The calves were vaccinated as in Group 2a and one calf was killed four days after the last dose of vaccine. The remaining calf in this group was killed when Group 2a and 2b calves were challenged i.e. 28 days after the last dose of vaccine.

(a) Clinical Observations.

Animals were examined clinically from challenge for their respiratory rates, coughing index and rectal temperatures. Haematological examinations were also carried out. Animals were bled prior to first vaccination and then twice a week.

Faecal samples were examined twice before the beginning of the experiment and daily from day 15 post challenge.

At post-mortem examination the total number of worms from the left lung was counted.

(b) Electron Microscopic Observations

Lymphoid and parasitic nodules were processed as described in Section B, Chapter 3 for electron microscopic studies.

2. Results

(a) Clinical Observations

Vaccination was observed to have protected animals in Group 2a from the severe respiratory disease usually produced by D. viviparus infections in calves that have not been previously immunized.

Although the frequency of coughing increased as was reported for animals in Experiment 1, there was a reduction in its severity. The cough was also dry and explosive. The respiratory rate of the calf in Group 2a, Para 35, killed 15 days post challenge was not affected as it remained within 36 respirations per minute. The respiratory rates of the other animals in this group were found to increase four days post challenge and the respiratory rate of Calf Para 50 remained elevated until it was killed (Fig. 64).

The haematological results demonstrated an increase in the number of eosinophils one week after the first dose of vaccine in some calves. In calf Para 57 the number peaked two weeks after the first dose of vaccine and one week after the second dose of vaccine. The number of eosinophils within the circulation then dropped and started to rise again and was highest between one and three weeks post challenge when Para 57 had 2.71 x 10^9/l and Para 50, 3.05 x 10^9/l (Fig. 65).

The haematological results did not indicate changes in both the total white cell counts (WBC) and packed cell volumes (PCV). These kept within normal ranges. The WBC values were between 8.00x10^9/l and 11.00x10^9/l while the PCVs were between 22% and 33%.

The daily faecal examinations from day 15 post infections were negative for this group of animals although a few stunted adult worms were found in their lungs post mortem.

Calf Para 35 killed 15 days PI had 4 worms in the left lung, Calf Para 50 killed 25 days PI had 191 worms while Para 57 and Para 39 killed at 35 and 45 days PI had 9 and 2 worms present in their left lungs respectively.

All the parameters examined clinically, were similarly affected in Group 2b animals. The coughing was very explosive, dry and very frequent. The respiratory rates of all animals were markedly increased and one animal Para 46 had a respiratory rate of 73 per minute (Fig. 66). The respiratory rates of animals in this group started to increase about the second week after infection and remained high until the animals were killed.

The haematological results of the animals in this group also showed a marked eosinophilic response as shown in Fig. 67. Few eosinophils ($0.83 \times 10^9/1$) were observed in the blood of one of the animals Para 38 prior to infection. After infection a marked eosinophilic response was observed for Para 38 ($1.74 \times 10^9/1$) and Para 46 ($1.47 \times 10^9/1$) although this was not as marked as that observed for the immune animals in Group 2a.

The PCV and total WBC counts remained within normal limits throughout the duration of the experiment. The PCV ranged between 23% and 37% while the total WBC counts were between $5.00 \times 10^9/1$ and $13.00 \times 10^9/1$.

Larvae were found in the faecal samples collected from the animals in this group. Larvae were found from the 17th day PI until the calves were killed (Fig. 68). The faeces of one animal Para 53 which was killed two weeks PI was negative for larvae. The number of larvae found in the faeces increased from about the 20th day PI and peaked at about the 28th day for Para 46 then started to fluctuate and dropped to low levels at about the 35th day PI for Para 38 (Fig. 68).

The respiratory rate of one animal Para 59 in Group 2c, the vaccinated controls, remained within normal range; that of the second animal Para 56 became elevated and it was hyperpnoeic during the vaccination period. The calf coughed but not frequently.

The haematological results indicated an eosinophilic response to vaccination which dropped to normal levels before the animals were killed (Fig. 69). The highest eosinophil response was observed between one and two weeks after the first dose of vaccine.

The PCV and WBC's of both animals also remained within the normal range. The PCV for animal Para 59 were between 23 and 31% and the WBC were between $7.00 \times 10^9/l$ and $8.00 \times 10^9/l$. The PCV for animal Para 56 were between 32 and 34% and the WBC between $7.00 \times 10^9/l$ and $19.00 \times 10^9/l$.

(b) Pathology

Both the gross and microscopic lesions observed in the lungs of these groups of calves were similar to those described for the calves in Experiment 1. Lesions were also similar in all groups of calves. In addition parasitic and lymphoid nodules similar to those described by Jarrett and Sharp, (1963), Pirie et al.,(1971), were present in the lungs of the vaccinated and challenged animals. Nodules were not usually found in the lungs of animals that received straight infection (Table 23). Dictyocaulus viviparus were found in the respiratory tract and lung parenchyma of the animals examined; the number varied with the type of treatment received (Table 25). Few worms were recovered from the lungs of Group 2a animals that were vaccinated before challenge when compared to the number of larvae used in the challenge dose. Large numbers of worms were found in the respiratory tract of calves that were not vaccinated Group 2b. A very few stunted worms were found in the calves that were only vaccinated Group 2c (Table 25). Apart from the variation in the numbers of worms found in the respiratory tract of calves of different treatment groups, the worms found in the vaccinated group were usually very small, stunted and infertile.

Parasitic nodules

Grossly the parasitic nodules were raised above the lung surface, about 2mm or more in diameter and greyish in colour (Fig. 70). On pressure, the nodules yielded a small greenish plug.

Microscopically the centre of the nodule was necrotic and contained a degenerating parasite. The necrotic centre was usually surrounded by proliferating fibrous connective tissue, lymphocytes, eosinophils, macrophages, plasma cells and a few giant cells (Fig. 71). Ultrastructurally the parasitic nodules were made up of dying cells, lymphocytes, macrophages and eosinophils. Secondary lysosomes which were either of parasitic origin or cellular debris were observed in macrophages (Fig. 72).

Lymphoid nodules

The number of lymphoid nodules varied between the lungs and the lung lobes. The majority were between 2.00 and 4.00mm in diameter and grey or pinkish in colour. These either bulged slightly or were easily seen through the pleura in which case they bulged from the surface of the lung when the parenchyma was incised.

With the light microscope, the nodules were made up of actively proliferating lymphoreticular tissue sometimes arranged in a distinct follicular pattern (Fig. 73).

Ultrastructurally the lymphoid nodule was seen to be made up solely of proliferating lymphoid cells which were arranged close together (Fig. 74). In a few cases, lymphocytes undergoing mitosis were observed (Fig. 75).

c. Globule Leucocyte Population Density in the Respiratory Tract

The GL response in the respiratory tract of the immune and non-immune calves exposed to D. viviparus infection was as shown in Table 25.

All the calves in Group 2a except one, Para 39 had less than 10 GL in the sections of their respiratory tracts examined. One calf Para 39 in this group whose challenge infection lasted for 45 days had more than 10 GL in the section of the respiratory tract examined.

Group 2b animals which were not vaccinated but infected with D. viviparus had less than 10 GL in the sections of their respiratory tracts examined. The length of time for which the infections lasted did not seem to affect the number of GL present in the respiratory tract.

In Group 2c, of the two animals which received only vaccine, one animal Para 59 which was killed 28 days post vaccination had less than 10 GL in the sections of the respiratory tract examined. The other animal Para 56 killed four days after the last dose of vaccine had no GL in the sections of the respiratory tract examined (Table 25).

E. EXPERIMENT 3: ORAL VACCINATION, CONTINUOUS ORAL CHALLENGE AND TRICKLE INFECTIONS WITH DICTYOCAULUS VIVIPARUS.

The preceeding experiments were performed in order to investigate the GL response in the respiratory tract of non-immune calves receiving a single exposure to D.viviparus infection and in immune calves which had been re-exposed once to D.viviparus infection. The results of both experiments did not clearly indicate a GL response in the non-immune and immune calves. A third experiment was then performed to investigate whether or not there was a GL response in the respiratory tract of immune calves repeatedly exposed to D.viviparus infection and after self-cure.

1. Experimental Design

The details and design of the experiment are shown in Table 26.

Eight, three months old calves were used in this experiment. The animals had been reared and housed as described in Section B of this Chapter. These animals were divided into four groups 3a, 3b, 3c and 3d each consisting of two calves, according to their body weights.

Group 3a

This group was made up of two calves Para S36 and S38 which were used as environmental controls. Each of these animals was housed together with one animal from each of the remaining three groups. The animals were not vaccinated or challenged but were treated every fortnight with Panacur at 7.5mg per kg body weight when D.viviparus larvae were found in the faeces of one animal Para S36 during the period when other animals were being vaccinated or trickle challenged. This was to prevent the establishment of infection in the lungs of these calves. The animals were killed at the end of the experiment.

Group 3b

This group was also made up of two calves, Para S34 and S35 which were each housed together with one animal from each of the other groups. These calves were vaccinated with two doses of Dictol with 28 days interval between them and challenged three times using the oral route at monthly intervals. The challenge dose was 2,000 third stage infective larvae (L3) of D.viviparus. Animals were then left for six weeks from the time of the last challenge to recover from the infections before they were killed.

Group 3c

The group was made up of two calves, Para S33 and S71, each of which was housed with one calf from each of the other groups. These calves received 500 L3 of D.viviparus a week for two weeks. They were rested for another two weeks and dosed again with another 500 L3 of D.viviparus a week for another two weeks. This procedure was intended to mimic vaccination with Dictol using live virulent larvae. Calves were then rested for a week and treated with Panacur at 7.5mg per kg body weight to remove adult worms from their lungs. Calf Para S71 was challenged the following week with 2,000 L3 of D.viviparus. Calf Para S33 died one day after treatment and so received no challenge. The challenge using the same dose of L3 of D.viviparus was repeated on two more occasions with a one month interval to the remaining calf. This calf was again left for six weeks from the time of last challenge to recover from infection before being killed.

Group 3d

This group was made up of two calves, Para S23 and S77, each of which was housed with one calf from each of the other groups. The calves in this group were given a trickle challenge infection of 130 L3 of D.viviparus per day three times a week with an interval of at least a day. This dose was given for five weeks. Calves were rested for two weeks and challenged with

1,000 L3 of D.viviparus. One calf was killed a week later because it suffered from severe respiratory distress. The remaining calf was rested for another five weeks and challenged twice with 600 L3 of D.viviparus for two weeks consecutively. This calf was killed the day following the last 600 third stage infective larvae challenge dose because it also was suffering from very severe respiratory distress.

(a) Clinical Observations

Animals were examined clinically prior to infection and throughout the duration of the experiment. Respiratory rates were taken every other day together with the coughing index and rectal temperatures. Haematological examination was done by bleeding the animals once a week. Faecal samples were collected from animals weekly before infection and throughout the duration of the experiment and examined for first stage larvae of D.viviparus as described in section B of this Chapter.

(b) Pathology

Lungs and tissue blocks from the lungs were examined as described in section B of Chapter 2. Parasitic and lymphoid nodules were examined with the electron microscope as detailed in section B of Chapter 3 and section B of this Chapter.

2. RESULTS

(a) Clinical Observations

The rectal temperatures and respiratory rates (Fig.76) of Group 3a calves remained within normal limits although they were noticed to cough occasionally.

The haematological results indicated a rise in the number of eosinophils between the 12th and 14th week during which time the eosinophil count was $0.76 \times 10^9/l$ for Para S36 and $2.68 \times 10^9 l$ for

Para S38 (Fig. 77). A second peak was observed for Para S38 four weeks later when it rose to $1.39 \times 10^9/l$ after which a gradual drop to $0.20 \times 10^9/l$ was observed for both animals at the time they were killed (Fig. 77). There was no appreciable effects on the PCV and total WBC counts throughout the duration of the experiment.

Faecal examination during the period of vaccination and trickle challenge of other groups revealed the presence of about one larvae of D.viviparus per gram of faeces for calf Para S36 five weeks from the start of infection and vaccination. Larvae were never observed again in this animal after this date throughout the duration of this experiment. No larvae were seen in the faeces of calf Para S38 throughout the duration of the experiment.

The results of clinical observations for Group 3b calves indicated some differences from those of Group 3a. The respiratory rates were moderately elevated, 53 per minute for Para S34 four weeks after the first dose of vaccine and 52 per minute six weeks after the first dose of vaccine for Para S35 (Fig. 78). Respirations continued to fluctuate within the raised limits until the animals were challenged 8 and 12 weeks from the first dose of vaccine. The respiratory rates then rose to 56 and 65 respirations per minute for Para S35 and ParaS 34, 13 and 16 weeks from the first dose of vaccine. The respiratory rates in both animals fell to about 44 respirations per minute at the time they were killed (Fig. 78).

The coughing index was observed to have increased for both animals after vaccination and after challenge for Para S34 but not Para S35. There was no appreciable effect on the rectal temperatures of these animals as it remained between 37°C and 40°C.

The haematological results of this group also indicated a marked rise in the numbers of circulating eosinophils (Fig. 79). The first appreciable rise of $1.14 \times 10^9/l$ and $1.18 \times 10^9/l$ for

Para S34 and Para S35 were observed eight weeks from the time they received the first dose of vaccine. A second higher rise of $3.86x10^9/l$ and $1.9x10^9/l$ for animals Para S35 and Para S34 were observed 14 and 15 weeks after the first dose of vaccine ie. five and six weeks after the first challenge dose and two and three weeks after the second challenge dose. A much higher rise $4.23x10^9/l$ was observed for animal Para S35 four weeks from the third challenge dose. The eosinophil response for animal Para S34 $0.99x10^9/l$ for the third challenge dose occurred much later than that of Para S35 and was lower than the first rise observed after the first dose of vaccine. The eosinophil count for both animals then fell gradually until they were killed (Fig. 79). Again there was no appreciable effect on the PCV or total WBC counts of these calves throughout the duration of the experiments.

Larval examination of the faecal samples obtained from these animals did not reveal the presence of larvae until the 13th week from the start of vaccination and four weeks from the start of challenge when a few larvae, 6 l.p.g. were found in the faeces of animal Para S34. The number of larvae in the faeces of this animal increased to 102 l.p.g. of faeces two weeks later and fell gradually until it became negative 11 week from the time of first challenge. No larvae were found in the faeces of animal Para S35.

The results of the clinical observation for Group 3c animals were similar to those of Group 3b animals. Although the rectal temperatures were not affected there was marked elevation of the respiratory rates. The respiratory rates were 62 and 41 respirations per minute for animals Para S71 and Para S33 two weeks from the start of exposure to D.viviparus L3 (Fig. 80). This increase continued until it was 94 and 76 respirations per minute for animals Para S71 and Paras S33 seven and eight weeks from the start of infection. The respiratory rate of animal Para S71 which was killed at the end of the experiment remained high and fluctuated during this period (Fig. 80). The coughing indices of both animals were observed to have increased 14 days from the start of exposure to D.viviparus and they continued to fluctuate

throughout the duration of the experiment although animal Para S71 coughed frequently during this period.

The haematological results of this group did not indicate appreciable changes in the total WBC and PCV but showed an increase in the number of circulating eosinophils for animal Para S71 (Fig. 81). Animal Para S33 did not have an eosinophilic response and died eight weeks from the start of exposure to D.viviparus L3. The eosinophilic response of animal Para S71 started seven weeks post exposure to D.viviparus infection when it rose to $1.55 \times 10^9/1$. It then dropped gradually to very low levels and rose again to $0.91 \times 10^9/1$ four weeks later. Two other eosinophil peaks were observed 14 and 19 weeks from the begining of exposure to D.viviparus and fell gradually until the calf was killed (Fig. 81).

Faecal examinations for larvae in this group of animals gave positive results three weeks post exposure to D.viviparus when the faeces of animal Para S71 contained 2 l.p.g. and four weeks for animal Para S33 when 10 l.p.g. of faeces were seen (Fig. 82). The faeces of animal Para S33 continued to be positive for larvae until the animal died while those of Para S71 became negative nine weeks post exposure to D.viviparus infection. The faeces of this animal became positive 14 weeks post exposure to D.viviparus although very few larvae were found for another six weeks. Faeces became negative again for another two weeks before becoming positive and fluctuated between weeks before the animal was killed. As many as 821 l.p.g. of faeces were obtained from animal Para S71 five weeks post exposure (Fig. 82).

The respiratory rates of Group 3d calves were comparable to those of Group 3c. The animals were quite hyperpnoeic and as many as 81 and 82 respirations per minute were observed for animals Para S23 and Para S77, 12 and 5 weeks post exposure to D.viviparus L3 (Fig. 83). The coughing indices of both animals were increased. The coughing index increased six days post exposure for animal Para S23 and 10 days for animal Para S77. The rate of coughing of both animals continued to fluctuate until each was

killed. Again, there were no obvious effects on the rectal temperatures of the animals taken during the duration of this experiment.

As in the other groups the effect of the trickle infections with D.viviparus L3 on the haematological findings in these animals was related to an increase in the number of circulating eosinophils. The total WBC and PCV of both animals were unaffected by the trickle infection. Both animals responded in terms of eosinophils to the infection but a higher response was observed in animal Para S23 which was infected for a longer period (Fig. 84). Both animals responded at eight and nine weeks following exposure to D.viviparus L3. Animal Para S77 was killed shortly after this response but a second response ($2.76 \times 10^9/l$) was also observed in animal Para S23, 14 weeks post exposure and shortly before it was killed.

The faeces of both animals were positive for D.viviparus larvae four weeks post exposure (Fig. 85). Larvae were first observed in the faeces of animal Para S23 three weeks post exposure to D.viviparus infection. Both animals passed D.viviparus larvae in their faeces until when they were killed.

(b) Pathology

The gross, microscopic and the ultrastructural pathological changes of these animals were similar to those of Experiments 1 and 2 described in Sections C and D of this Chapter. Parasitic and lymphoid nodules were seen in the lungs of these animals (Table 27).

(c) Globule Leucocyte Population Density in the Respiratory Tract

The GL responses in the respiratory tract of the calves vaccinated and repeatedly challenged or trickle challenged are shown in Table 27.

One calf Para S38 in Group 3a which was used as environmental control had more than 20 GL in the sections of the respiratory tract examined while the other calf Para S36 had less than 10 GL in the sections of the respiratory tract examined.

In Group 3b, one of the animals Para S34 which was vaccinated and repeatedly challenged had 20 or more GL in the sections of the respiratory tract examined, but the remaining calf Para S35, like the other calf in the control group had less than 10GL in the sections of its respiratory tract examined.

Only one calf Para S71 in Groups 3c and 3d which were exposed to L3 of D.viviparus had GL and these were less than 10 GL in the section of the respiratory tract examined. There were no GL observed in the sections of the respiratory tract of the other animals in Group 3c or the two calves in Group 3d.

Repeated exposure of calves to D.viviparus infection did not therefore lead to an increase in the numbers of GL in their respiratory tracts, and the duration of infection did not have any effect on GL response.

D. DISCUSSION

An association, between GL in mucous membranes and parasitic infections in animals has been reported by many of the workers enumerated in the review of the literature. Globule leucocytes have been seen in the respiratory tracts of cattle and large numbers were usually present in the respiratory tracts of adult animals. In the respiratory tract of sheep, Mahmoud, (1978), associated the occurence of GL with parasitic infections. The presence of large numbers of GL in the respiratory tract of adult cattle during some outbreaks of respiratory diseases has been difficult to explain; hence experiments were carried out to investigate the relationship between the bovine respiratory tract GL and lungworm infection in cattle.

The globule leucocyte response in calves exposed once to D.viviparus infection was investigated in the first experiment. The single infections in calves were successful and typical clinical signs and lung pathology similar to those described previously (Jarrett et al., 1957, Michel et al., 1965), were observed.

Small numbers of GL were seen in the respiratory tracts of animals infected for 28 or more days, although animals infected for 37 days had many more GL in their respiratory tracts than the animals infected for 75 days. Mahmoud, (1978), observed GL in the respiratory tracts of sheep exposed to D.filaria for as long as 10 weeks. He did not however observe GL in the lungs of animals killed four weeks after challenge infection and which had been exposed for a total of eight weeks. He attributed the presence of GL in the respiratory tract of sheep to the adult worms or their larvae found in the bronchial lumen.

The results reported in Chapter 2 of this thesis indicated that GL were present in the age group of cattle that had been kept indoors and hence should be free of parasites. The GL population density observed in the respiratory tract of calves exposed once to

D.viviparus and had adult worms in their bronchial lumen in this study, did not differ from what would normally be expected for this age group of cattle as reported earlier. The results of this experiment did not indicate any relationship between the infective dose and GL response.

In the second experiment the GL response when immune animals were challenged was investigated. The clinical disease was similar to that seen in Experiment 1, although there was a reduction in the severity of the clincal signs in vaccinated and challenged animals. The haematological results were also similar to those reported by Michel et al, (1965). The total WBC counts and PCV of animals were not affected, but there was an eosinophilic response to both vaccination and infection. However, the response to vaccination was quite small when compared to challenge or straight infection.

The pulmonary pathology observed in the lungs of animals in this experiment was similar to that in Experiment 1. In addition lymphoid and parasitic nodules similar to those described by Jarrett and Sharp, (1963), Michel et al., (1965), Pirie et al., (1971), were present in the lung parenchyma of vaccinated and challenged animals. Ultrastructurally the lymphoid nodules showed proliferating lymphoid cells and mitotic figures, and the parasitic nodules, dying cells and secondary lysosomes in macrophages.

The GL response in these immune animals re-exposed to D.viviparus challenge was not different from that of animals that have not been immune or those of animals that were vaccinated alone. Small numbers of GL were found in the respiratory tracts of both vaccinated and unvaccinated calves. Although one vaccinated and challenged calf killed six weeks after challenge had a higher GL population density when compared to the unvaccinated calf; the GL population density seen in the immune animals was not more than would be expected for this age group of cattle (Section C, Chapter 2).

Again there was no difference in terms of GL response between vaccinated and unvaccinated animals. Mahmoud, (1978),

observed GL in the respiratory tracts of sheep six weeks after a second exposure to D.filaria infection. The results of this present experiment indicated that GL were present in the respiratory tract of calves 15 days PI and 15 days post challenge in the vaccinated animal in calves exposed to D.viviparus infection.

In the third experiment, the relationship between GL response in the respiratory tract of immune animals and repeated exposure as well as during "self-cure" in D.viviparus infection in cattle was investigated.

The results of the clinical examination of the animals in this experiment were similar to those reported by Michel et al., (1965). An eosinophilic response was observed for both control animals. The explanations for this could be the fact that, one animal from each experimental group were being kept together and the control animals may have picked up larvae from the faeces of the calves that were being given normal larvae or trickle infected with normal larvae even though the pens in which they were kept were thoroughly cleaned every other day. It is also possible that the control calves picked up larvae from the hay on which they were being fed. In the vaccinated animals, the eosinophilic response continued to increase with each challenge but the response fluctuated in the calves that were given normal larvae to mimic vaccination. In the trickle infected group, the eosinophilic response was first seen, one or two weeks post challenge and the response to the second challenge was higher than the first. The eosinophilic response was an anamnestic response as suggested by Mackenzie and Michel, (1964), Michel et al., (1965). Calves that had a good eosinophilic response in the groups that received normal larvae or trickle infection survived longer and produced fewer larvae in their faeces (Figs. 82 and 85).

Although animals were repeatedly exposed to D.viviparus infection and killed at the time of "self-cure", only a few GL were

present in the respiratory tracts of calves which were given normal larvae and trickle vaccinations before challenge. Three animals in these groups had no GL in their respiratory tracts.

Large numbers of GL were found in the respiratory tracts of one control and one vaccinated and challenged animal. The vaccinated calf with large numbers of GL in its respiratory tract had poor eosinophilic response while the control calf which might have picked up larvae from the faeces of other animals or the hay on which it was being fed had a good eosinophilic response. One animal in the groups of calves that received normal larvae to mimic vaccination, had large numbers of lymphoid nodules in its lung parenchyma but few GL in its respiratory tract while the control calf with a moderate number of nodules in its respiratory tract had large numbers of GL in its respiratory tract. The nodules in the lungs of controls calves have resulted from reaction to killed larvae as these animals were treated with panacur.

These results did not therefore indicate any relationship between repeated exposure of calves to D.viviparus and the development of GL in the respiratory tract, and also the duration of exposure and "self-cure" in D.viviparus infection did not have any effect on the GL response.

In conclusion, although GL were found in the respiratory tracts of calves infected with D.viviparus, it was not possible to demonstrate a clear cut correlation between their presence or population density and the method or duration of exposure to D.viviparus. The reasons for this could be as follows: (i) that the inflammatory reaction during lungworm infections resulted in marked epithelial alterations producing an epithelial environment unsuitable for GL; (ii) that the immune reaction of the respiratory tract in calves might be different from those of other mucous membranes; (iii) that the immune response to D.viviparus may be different from that of other parasites; (iv) that the large numbers of GL in adult cattle seen during the survey may be age dependent and (v) that GL development in the bovine respiratory tract may result from non-parasitic antigenic stimuli.

TABLE 23: The experimental design and the GL found after a single exposure of calves to Dictyocaulus viviparus infections.

EXPERIMENT 1: SINGLE EXPOSURE OF CALVES TO
DICTYOCAULUS VIVIPARUS INFECTION

ANIMAL NUMBER	CHALLENGE DOSE	DURATION OF INFECTION (Days)	GL POPULATION DENSITY	PULMONARY NODULES
Para 24	10,000	17	-	-
Para 71	4,000	27	-	-
Para 72	5,000	28	+	-
Para 73	5,000	28	-	-
Para 81	5,000	37	++	-
Para 82	6,000	37	++	-
Para 410	3,000	75	+	-

GL = Globule leucocyte
+ = Positive
- = Negative

TABLE 24: The design of the experiment for oral vaccination followed by oral challenge with Dictyocaulus viviparus.

EXPERIMEMT 2: ORAL VACCINATION FOLLOWED BY ORAL CHALLENGE WITH DICTYOCAULUS VIVIPARUS

Day	0	28	32	56	71	81	91	101
Group 2a	V*	V*	-	C	K	K	K	K
Group 2b	-	-	-	C	K	K	K	K
Group 2c	V*	V*	K	K	-	-	-	-

V* = Vaccination with Dictol
C = Challenge
K = One animal killed

TABLE 25: The results of the experiment involving oral vaccination followed by oral challenge with *Dictyocaulus viviparus*.

EXPERIMENT 2: ORAL VACCINATION FOLLOWED BY ORAL CHALLENGE WITH DICTYOCAULUS VIVIPARUS

GROUP	ANIMAL NUMBER	VACCINATION	CHALLENGE DOSE	DURATION OF INFECTION (DAYS)	WORM COUNT (LUNGS)	GL POPULATION DENSITY	NODULES
2a	Para 35	+	3,150	15	4	+	++
2b	Para 53	-	2,310	15	322	+	+
2a	Para 50	+	3,450	25	191	+	++
2b	Para 21	-	3,000	25	214	+	-
2a	Para 57	+	3,720	35	9	+	++
2b	Para 46	-	4,800	35	1095	+	-
2a	Para 39	+	5,100	45	2	++	+
2b	Para 38	-	4,200	45	9	+	-
2c	Para 56	+	-	-	ND	-	-
2c	Para 59	+	-	-	5	+	-

GL = Globule leucocyte

TABLE 26: The design of experiment for vaccination followed by repeated and trickle challenge infections with Dictyocaulus viviparus.

EXPERIMENT 3: VACCINATION FOLLOWED BY REPEATED AND TRICKLE CHALLENGE INFECTIONS WITH DICTYOCAULUS VIVIPARUS

GROUP	ANIMAL NUMBER	TYPE OF VACCINATION	NUMBER OF CHALLENGES	CHALLENGE DOSE	DURATION OF INFECTION (MONTHS)
3a	Para S36	-	-	-	-
3a	Para S38	-	-	-	-
3b	Para S34	Vaccine*	3	2,000 L_3	4
3b	Para S35	Vaccine*	3	2,000 L_3	4
3c	Para S33	Normal Larvae (500 L_3 x 4)	-	-	2
3c	Para S71	Normal Larvae (500 L_3 x 4)	3	2,000 L_3	6
3d	Para S23	Normal Larvae (130 L_3 x 15)	3	a) 1,000 L_3 b) 600 L_3	4
3d	Para S77	Normal Larvae (130 L_3 x 15)	1	1,000 L_3	3

* Dictol

TABLE 27: The results of the experiment involving vaccination followed by repeated and trickle challenge infections with Dictyocaulus viviparus.

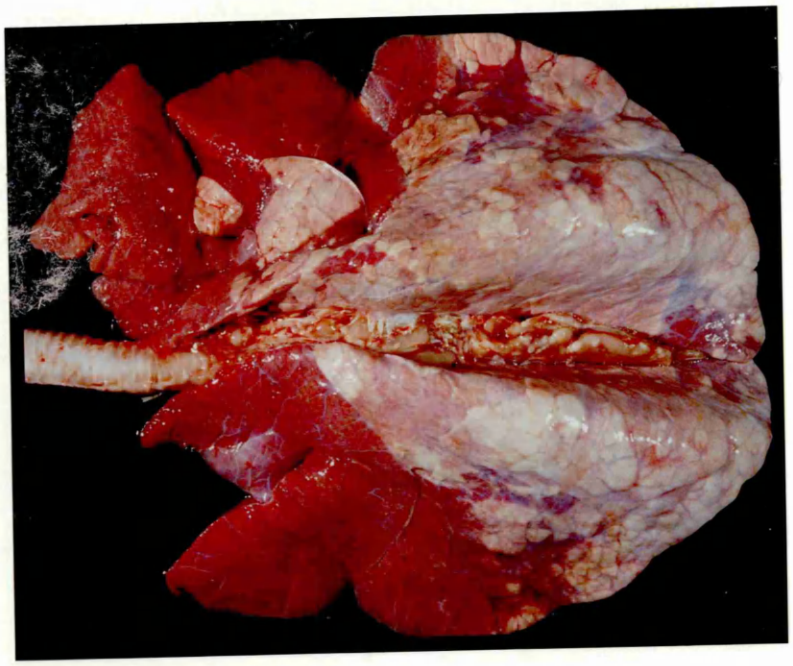
EXPERIMENT 3: VACCINATION FOLLOWED BY REPEATED AND TRICKLE CHALLENGE INFECTIONS WITH DICTYOCAULUS VIVIPARUS

GROUP	ANIMAL NUMBER	TYPE OF VACCINATION	NUMBER OF CHALLENGES	CHALLENGE DOSE	DURATION OF INFECTION (MONTHS)	GL POPULATION DENSITY	NODULES
3a	Para S36	-	-	-	-	+	+
3a	Para S38	-	-	-	-	+++	++
3b	Para S34	Vaccine*	3	2,000 L_3	4	+++	+
3b	Para S35	Vaccine*	3	2,000 L_3	4	+	+
3c	Para S33	Normal Larvae (500 L_3 x 4)	-	-	2	-	-
3c	Para S71	Normal Larvae (500 L_3 x 4)	3	2,000 L_3	6	+	+++
3d	Para S23	Normal Larvae (130 L_3 x 15)	3	a) 1,000 L_3 b) 600 L_3 x 2	4	-	+
3d	Para S77	Normal Larvae (130 L_3 x 15)	1	1,000 L_3	3	-	-

* Dictol

FIG. 60: Focal but widespread consolidation in the caudal lobes of the lungs of a calf infected with Dictyocaulus viviparus.

FIG. 61: Adult lungworms mixed with mucopus and froth in the right bronchus of a calf.



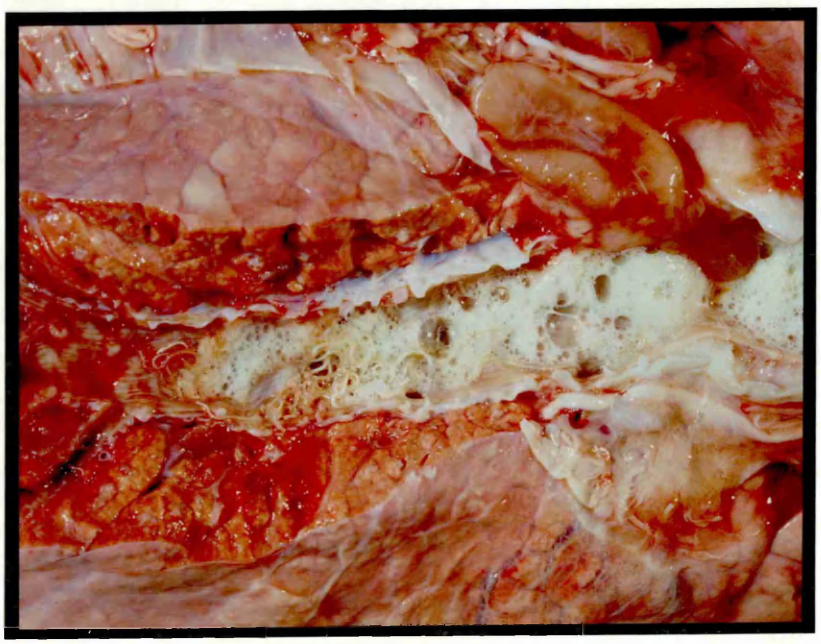
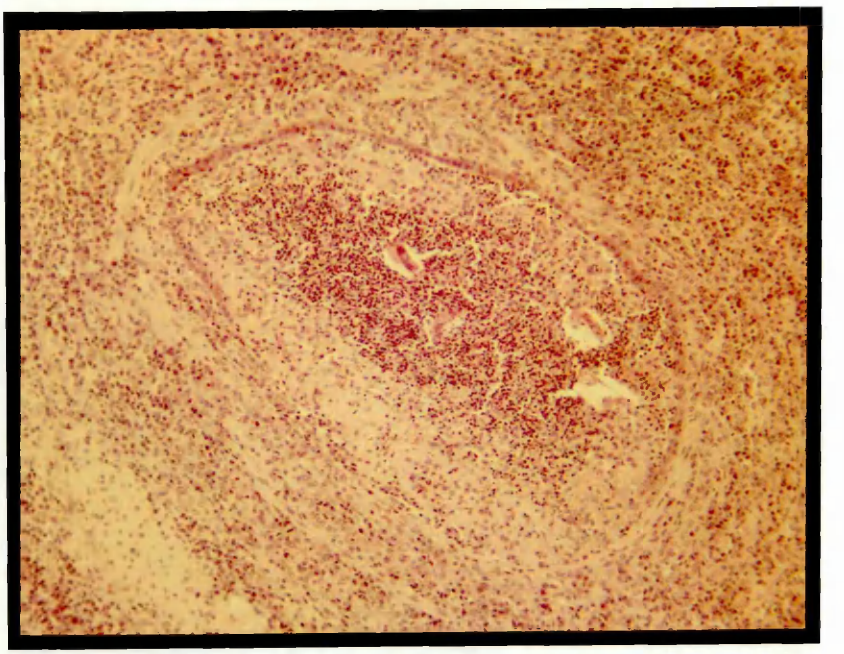


FIG. 62: Sections of lungworm larvae mixed with cellular exudate in bronchiole of a calf.

H&E x 88

FIG. 63: The histological appearance of bronchitis showing the presence of large numbers of plasma cells and eosinophils in the bronchial epithelium.

H&E x 88



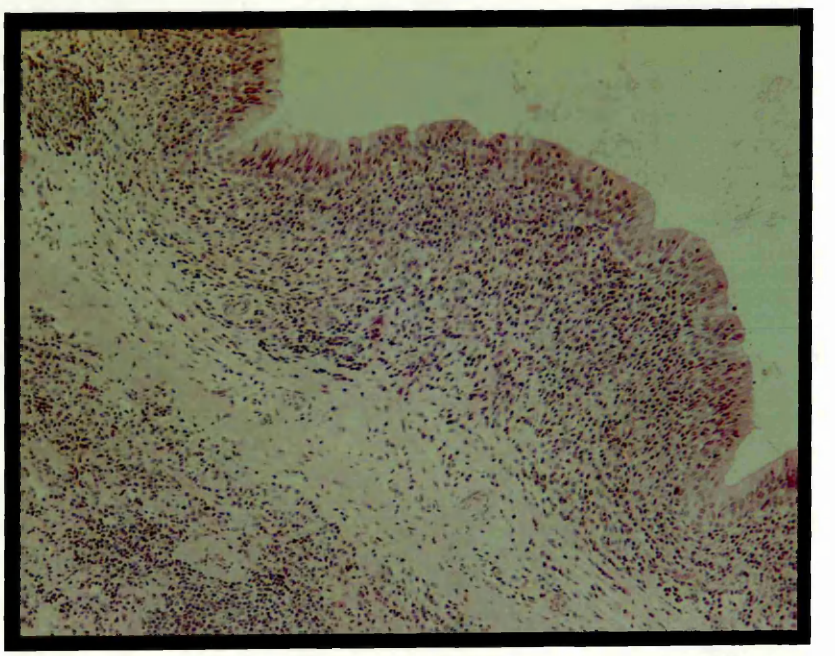


FIG. 64: The mean weekly respiratory rates of Group 2a calves that were vaccinated with Dictol and challenged with Dictyocaulus viviparus.

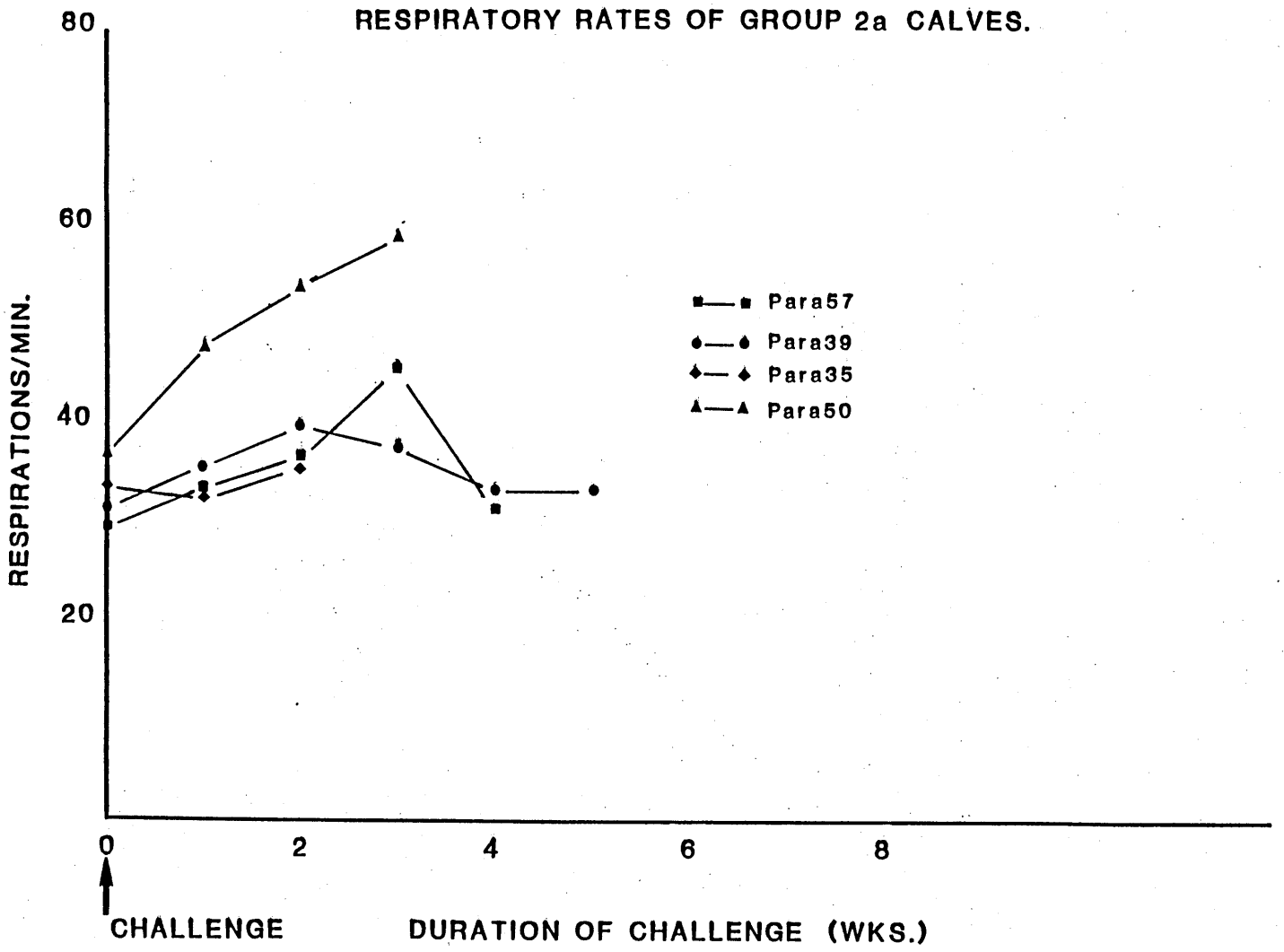

RESPIRATORY RATES OF GROUP 2a CALVES.
80
60
40
20
RESPIRATIONS/MIN.
Para57
Para39
Para35
Para50
0
2
4
6
8
CHALLENGE
DURATION OF CHALLENGE (WKS.)

FIG. 65: The mean weekly eosinophilic response of Group 2a calves that were vaccinated with Dictol and challenged with Dictyocaulus viviparus.

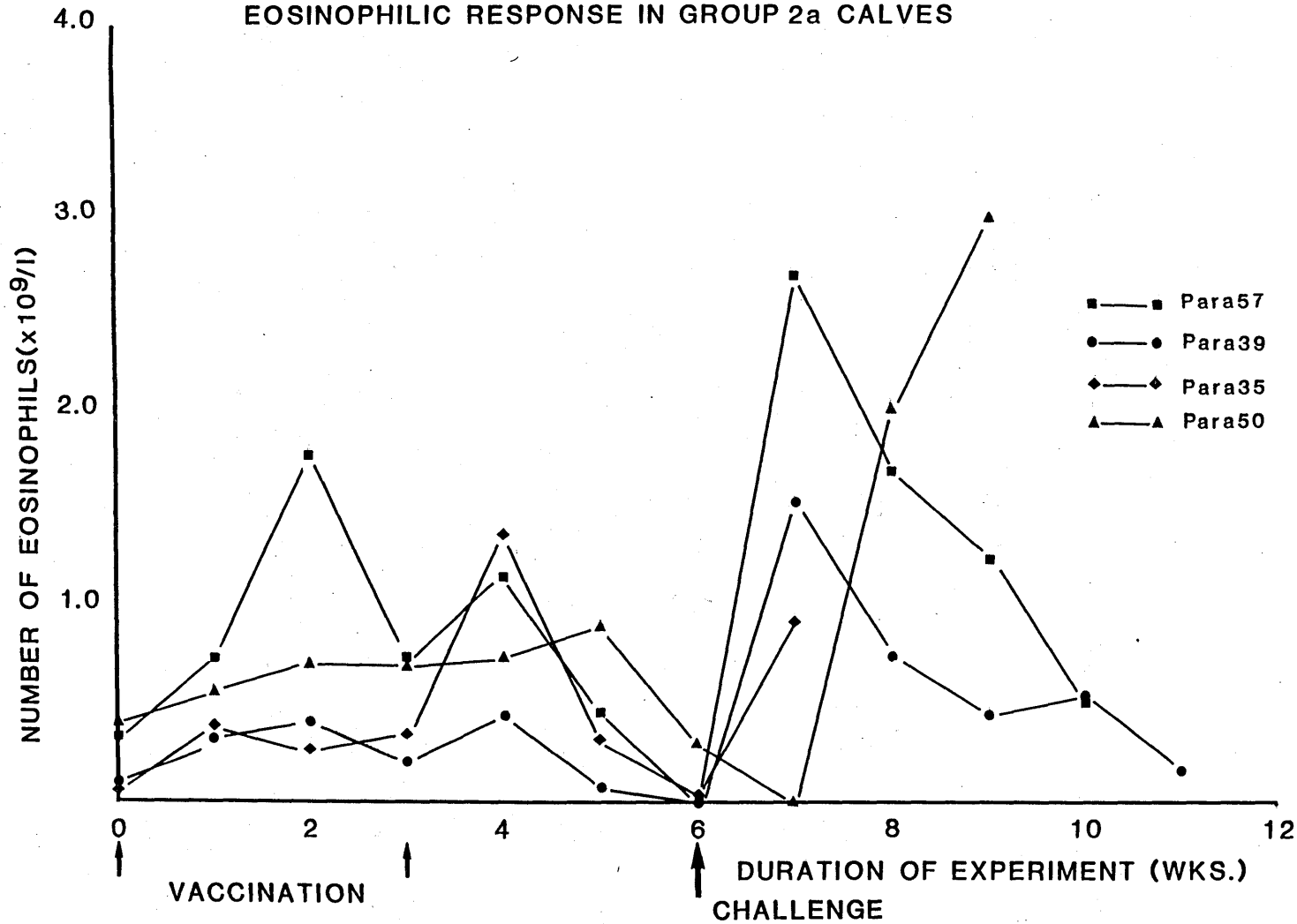

EOSINOPHILIC RESPONSE IN GROUP 2a CALVES
NUMBER OF EOSINOPHILS(x10⁹/l)
4.0
3.0
2.0
1.0
0
2
4
6
8
10
12
VACCINATION
CHALLENGE
DURATION OF EXPERIMENT (WKS.)
Para57
Para39
Para35
Para50

FIG. 66: The mean weekly respiratory rates of Group 2b calves that were not vaccinated but were infected with Dictyocaulus vivparus.

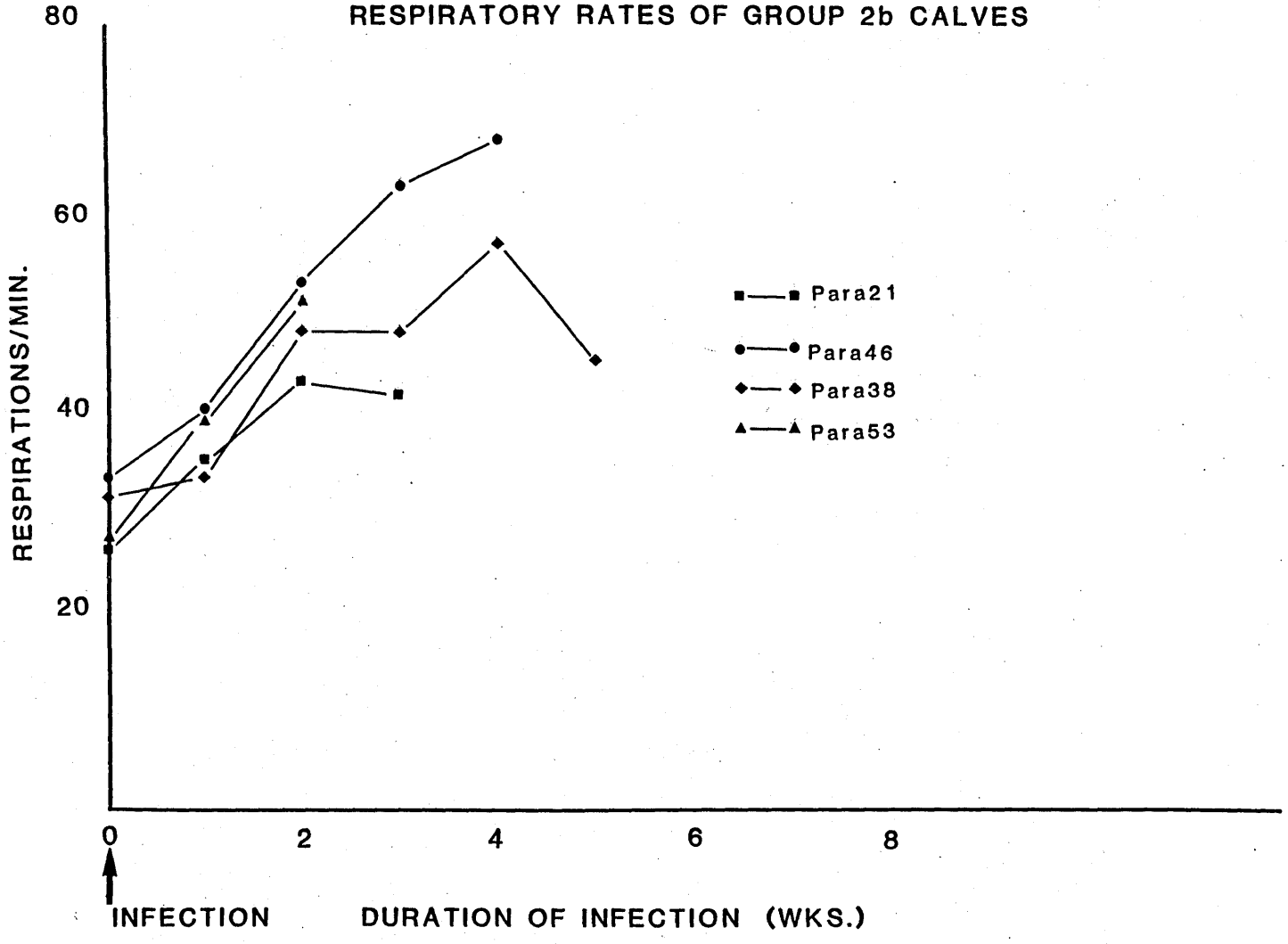

RESPIRATORY RATES OF GROUP 2b CALVES
80
60
40
20
RESPIRATIONS/MIN.
Para21
Para46
Para38
Para53
0
2
4
6
8
INFECTION
DURATION OF INFECTION (WKS.)

FIG. 67: The mean weekly eosinophilic response of Group 2b calves that were not vaccinated but were infected with Dictyocaulus viviparus.

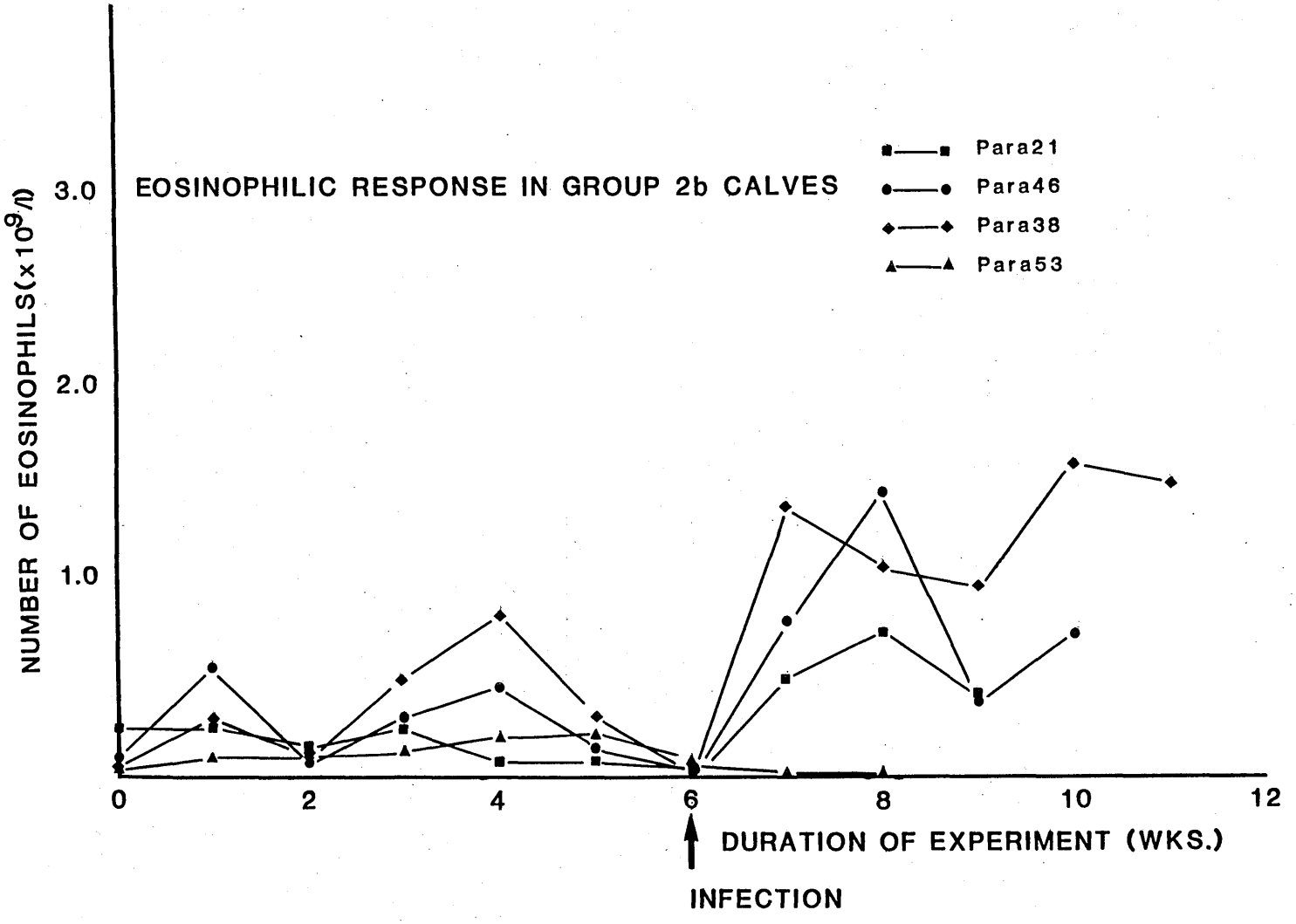

EOSINOPHILIC RESPONSE IN GROUP 2b CALVES
Para21
Para46
Para38
Para53
NUMBER OF EOSINOPHILS(x10⁹/l)
3.0
2.0
1.0
0
2
4
6
8
10
12
DURATION OF EXPERIMENT (WKS.)
INFECTION

FIG. 68: The faecal larval counts of Group 2b calves that were not vaccinated but were infected with Dictyocaulus viviparus.

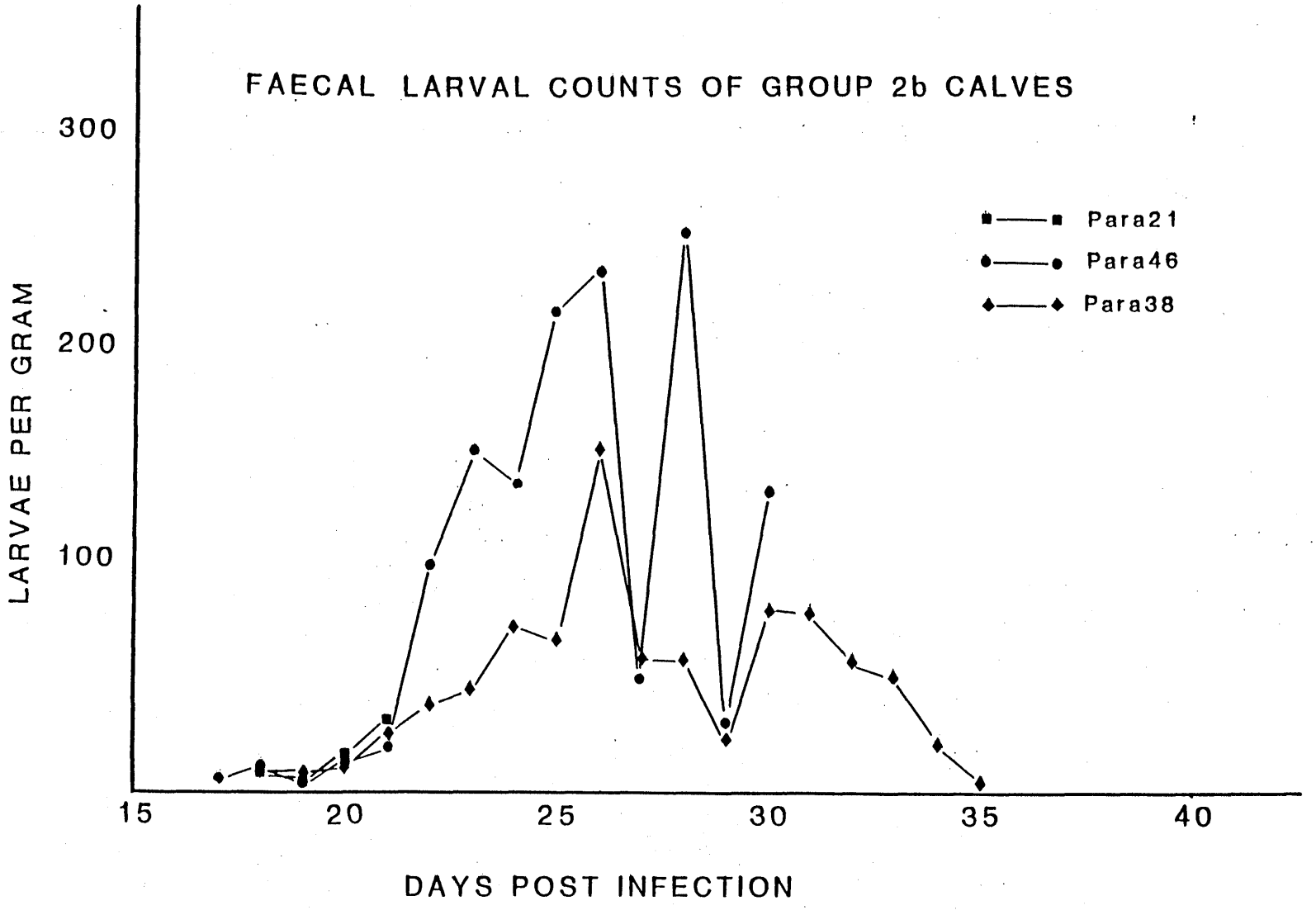

FAECAL LARVAL COUNTS OF GROUP 2b CALVES
LARVAE PER GRAM
300
200
100
15
20
25
30
35
40
DAYS POST INFECTION
Para21
Para46
Para38

FIG. 69: The mean weekly eosinophilic response of Group 2c calves that were only vaccinated with Dictol.

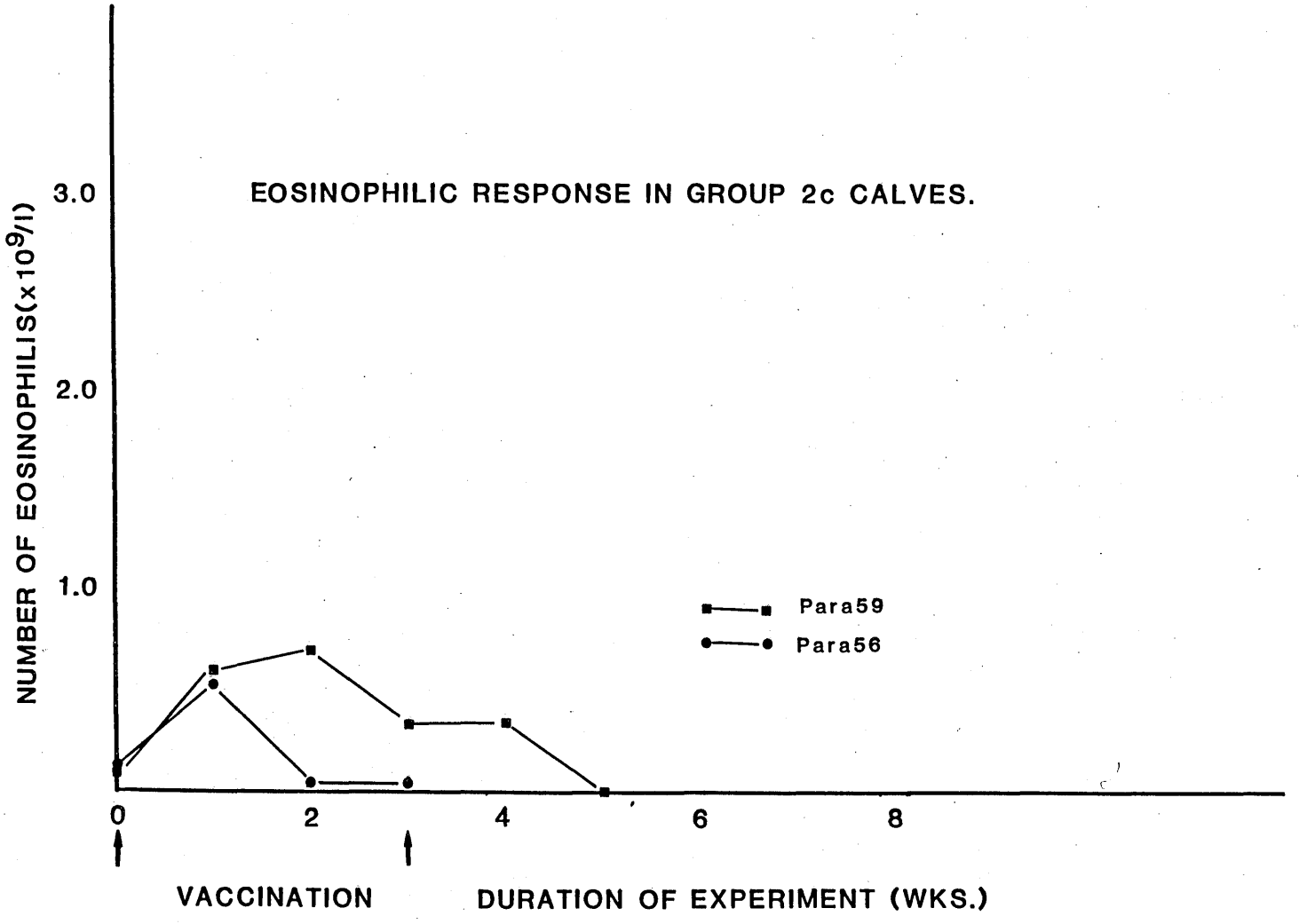
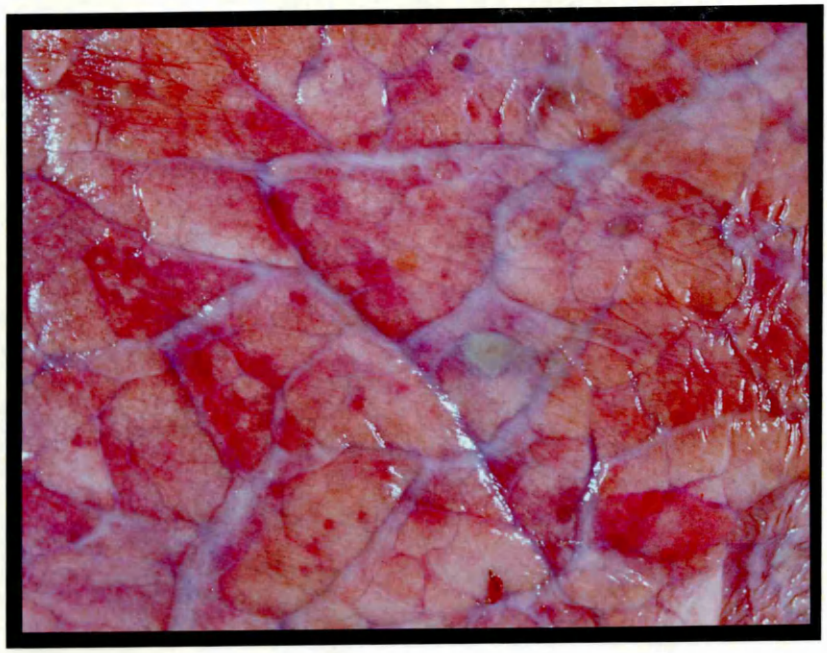

EOSINOPHILIC RESPONSE IN GROUP 2c CALVES.
NUMBER OF EOSINOPHILIS(x10⁹/l)
3.0
2.0
1.0
Para59
Para56
0
2
4
6
8
VACCINATION
DURATION OF EXPERIMENT (WKS.)

FIG. 70: The gross appearance of a subpleural parasitic nodule in the lungs of a calf vaccinated with Dictol and challenged with Dictyocaulus viviparus.

FIG. 71: The histological appearance of a pulmonary parasitic nodule illustrating a degenerating parasite in the necrotic centre.

H&E x 88



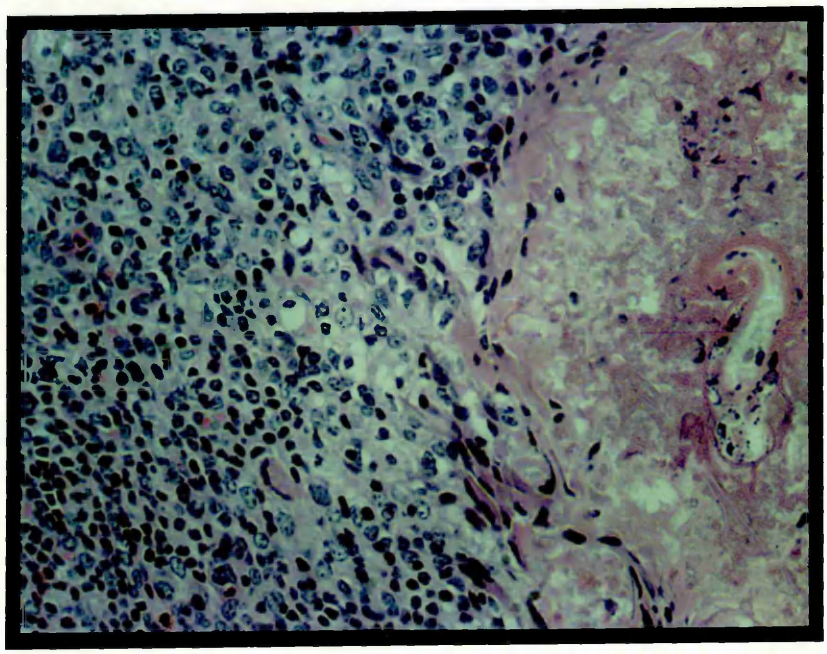
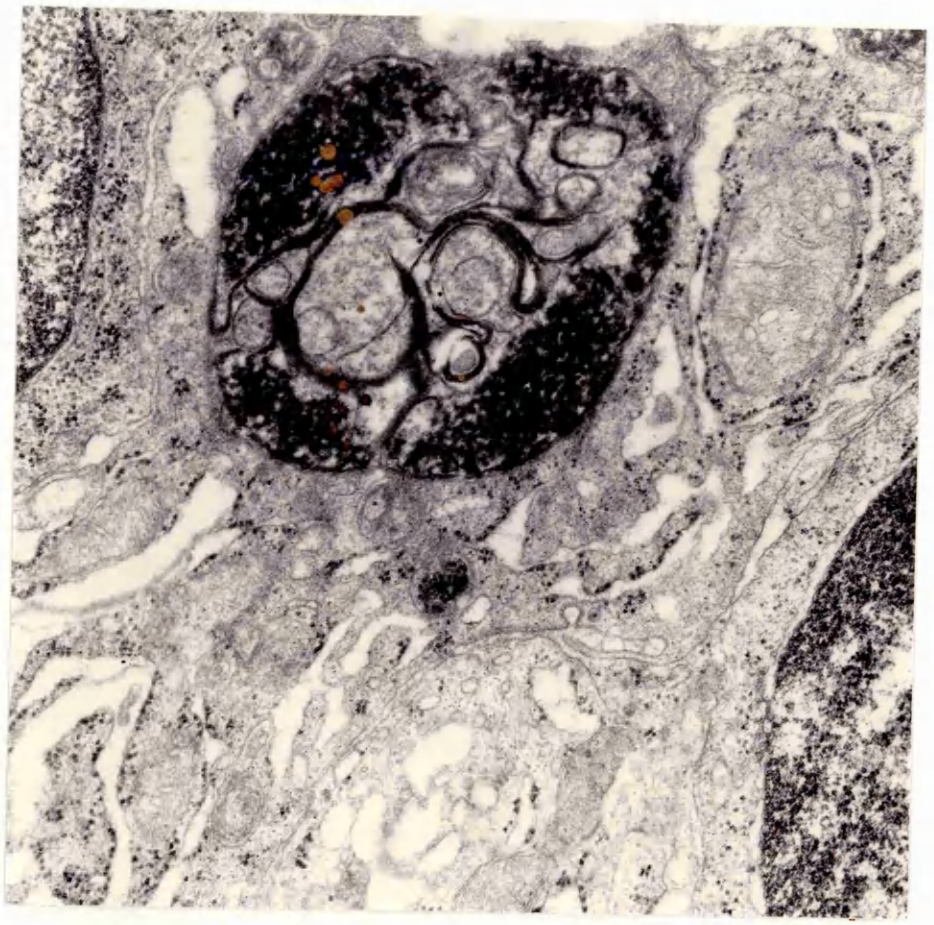


FIG. 72: A pulmonary parasitic nodule showing a secondary lysosome.

x 22,500

FIG. 73: The histological appearance of a pulmonary lymphoid nodule showing proliferating lymphoreticular tissue arranged in a distinct follicular pattern.

H&E x 120



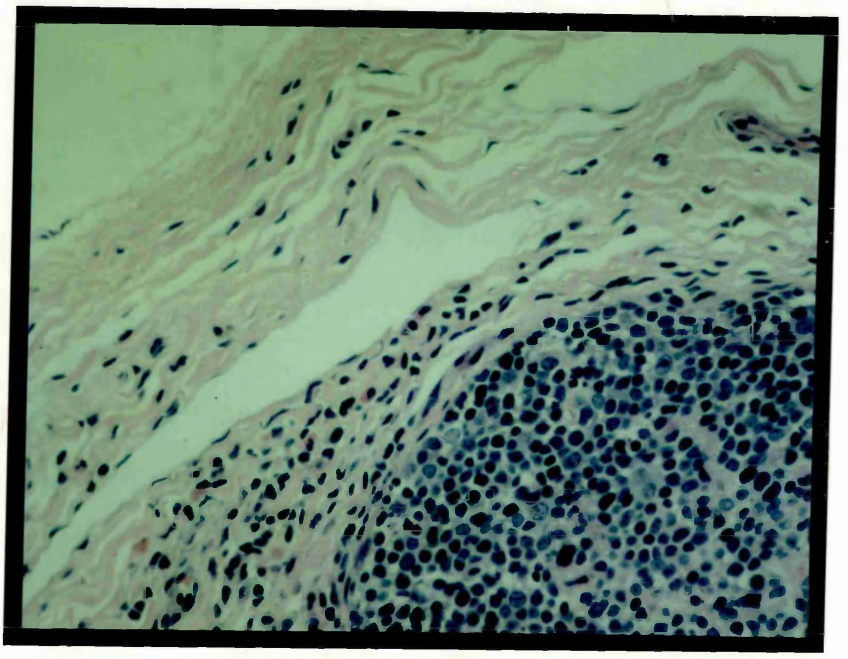
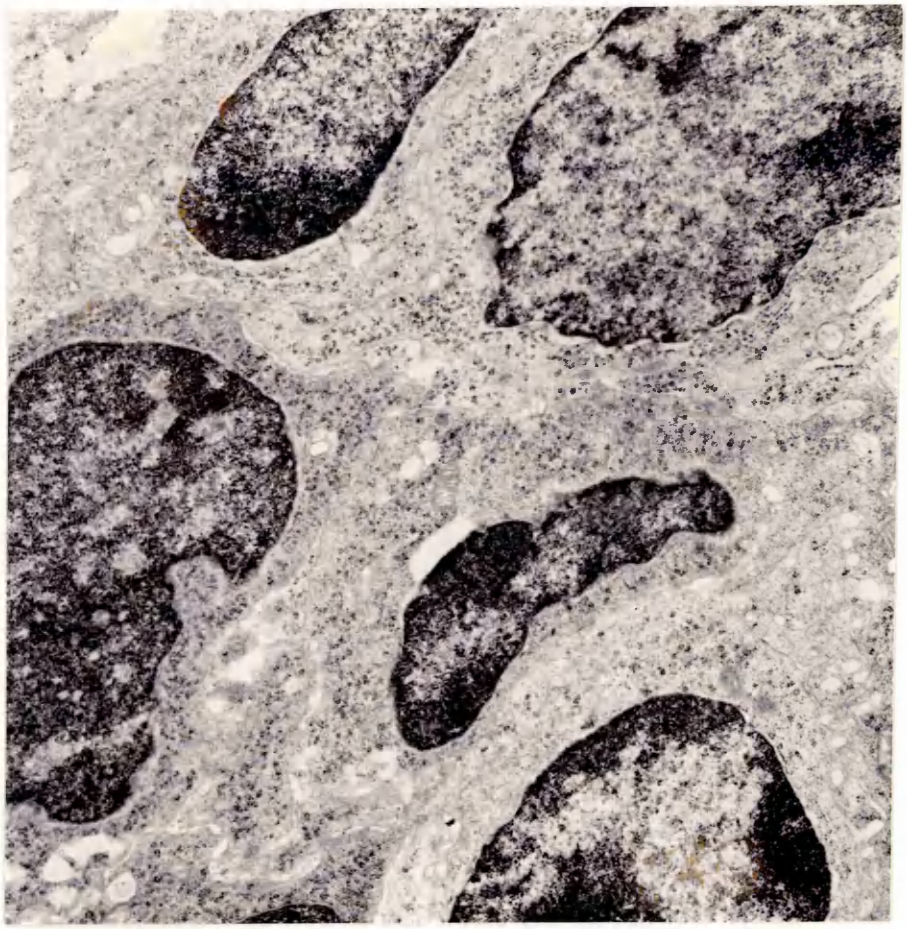


FIG. 74: A pulmonary lymphoid nodule showing the close arrangement of lymphocytes.

x 11,250

FIG. 75: A lymphocyte in a lymphoid nodule undergoing mitosis.

x 11,250



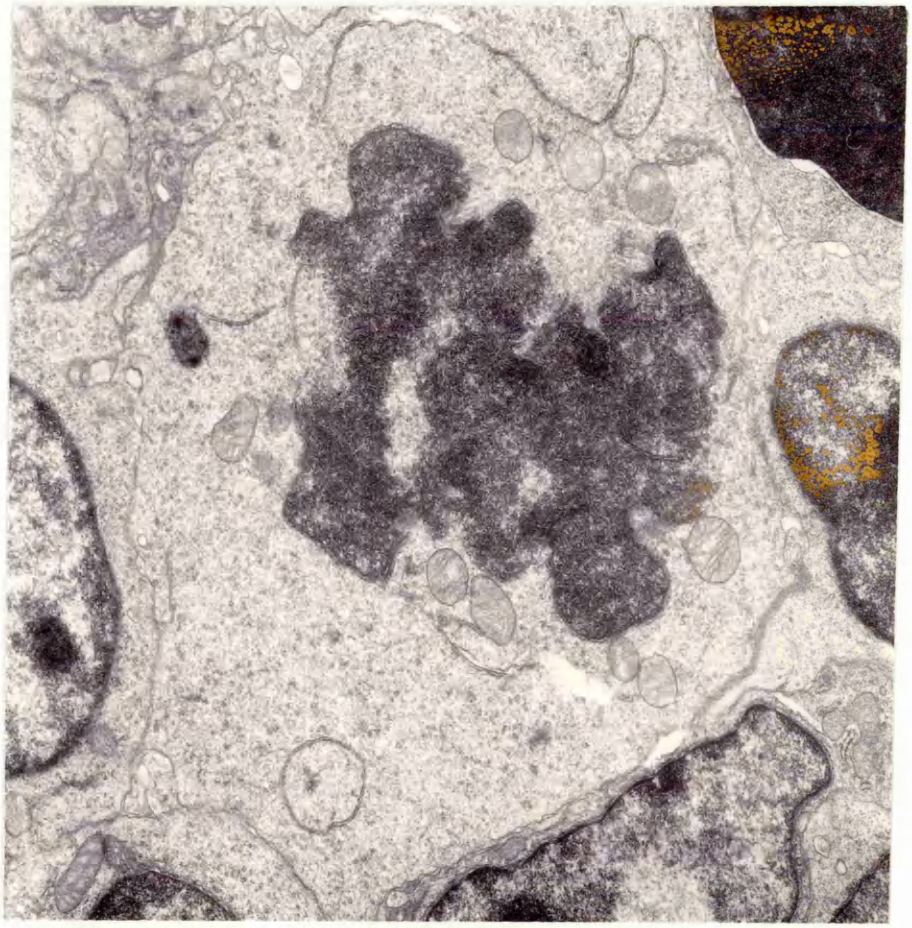


FIG. 76: The mean weekly respiratory rates of Group 3a calves that were used as environmental controls.

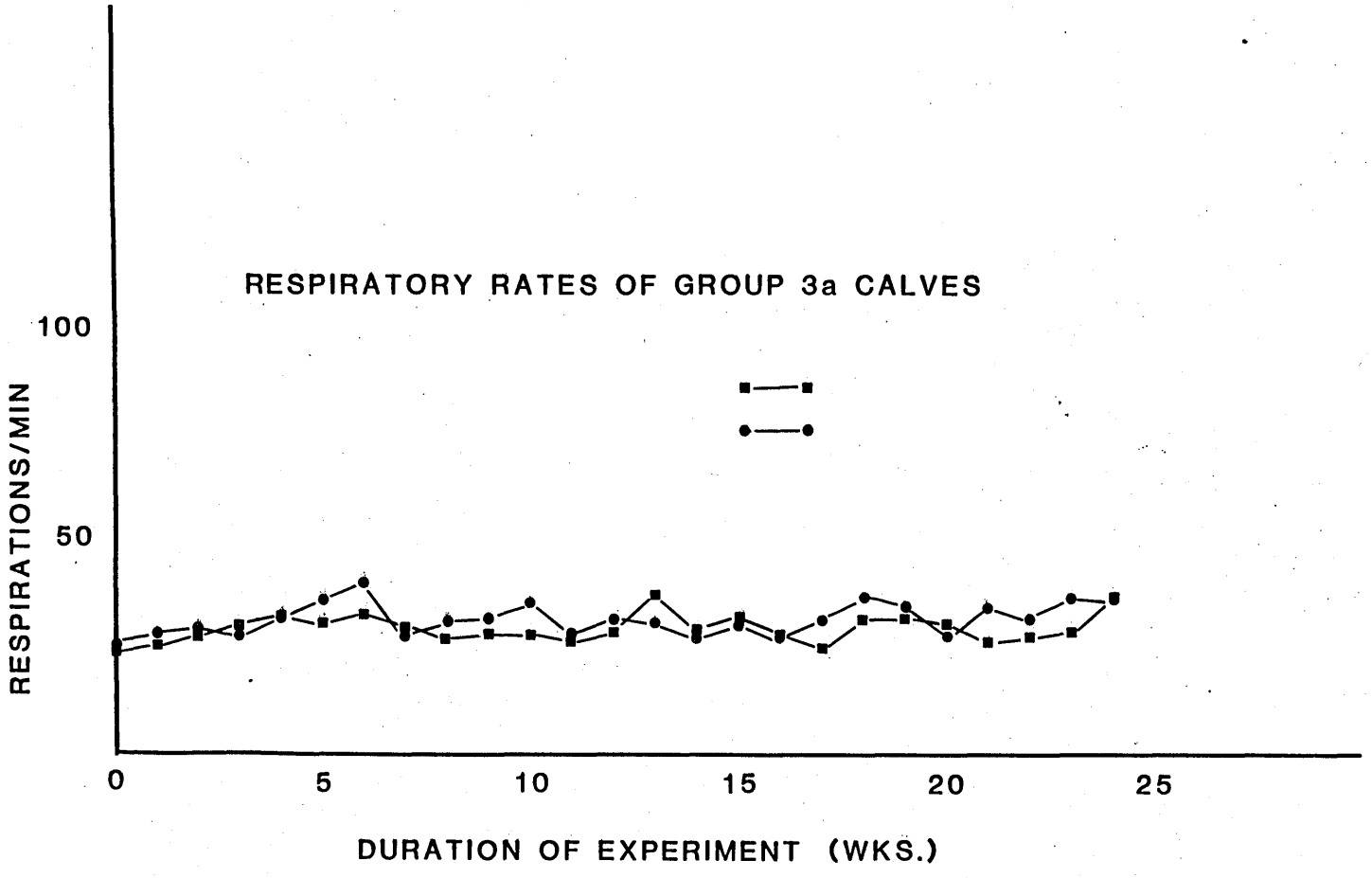

RESPIRATORY RATES OF GROUP 3a CALVES
RESPIRATIONS/MIN
100
50
0
5
10
15
20
25
DURATION OF EXPERIMENT (WKS.)

FIG. 77: The eosinophilic response of Group 3a calves that were used as environmental controls.

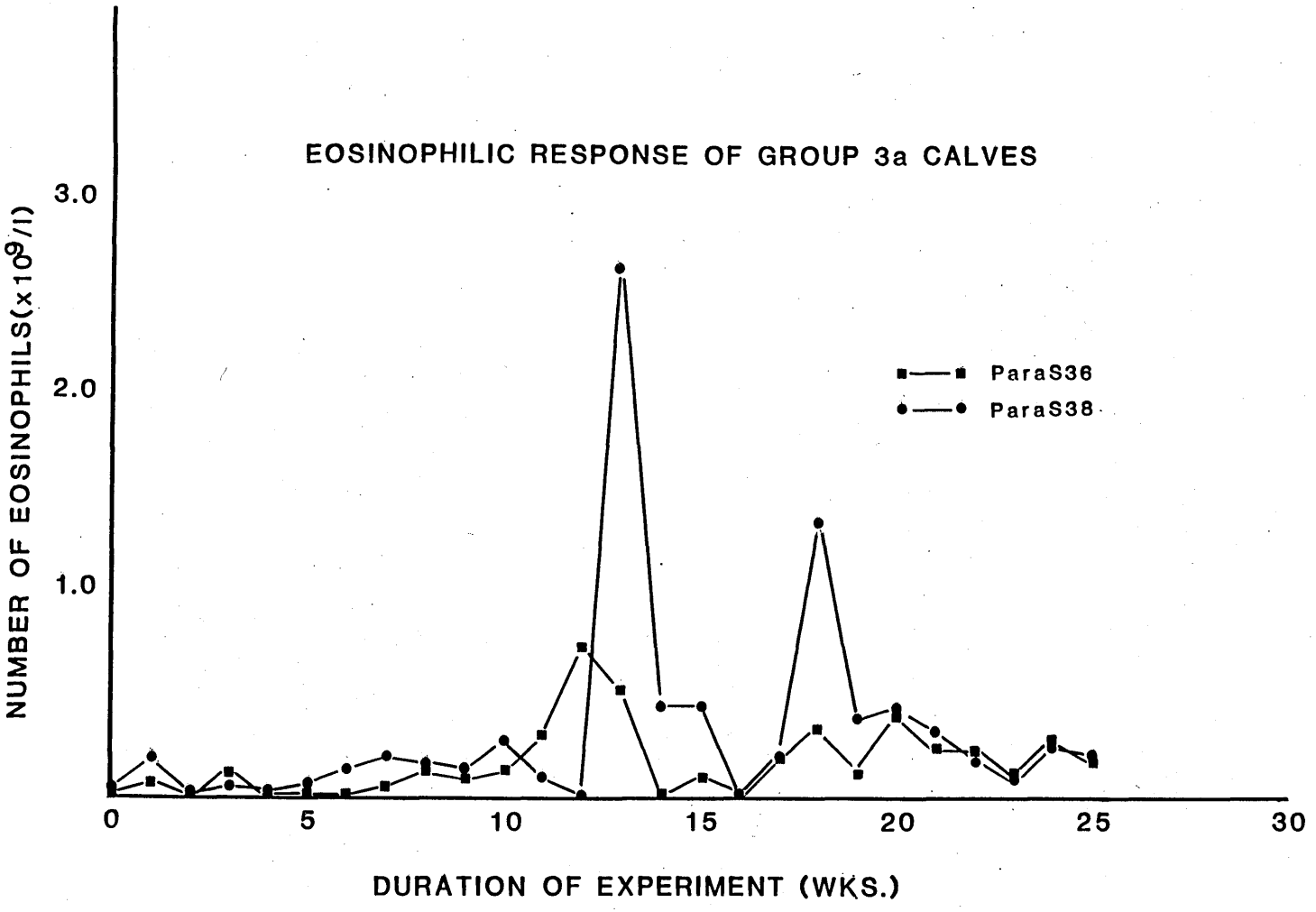

EOSINOPHILIC RESPONSE OF GROUP 3a CALVES
NUMBER OF EOSINOPHILS(x10⁹/l)
3.0
2.0
1.0
0
5
10
15
20
25
30
DURATION OF EXPERIMENT (WKS.)
ParaS36
ParaS38

FIG. 78: The mean weekly respiratory rates of Group 3b calves that were vaccinated with Dictol and repeatedly challenged with Dictyocaulus viviparus.

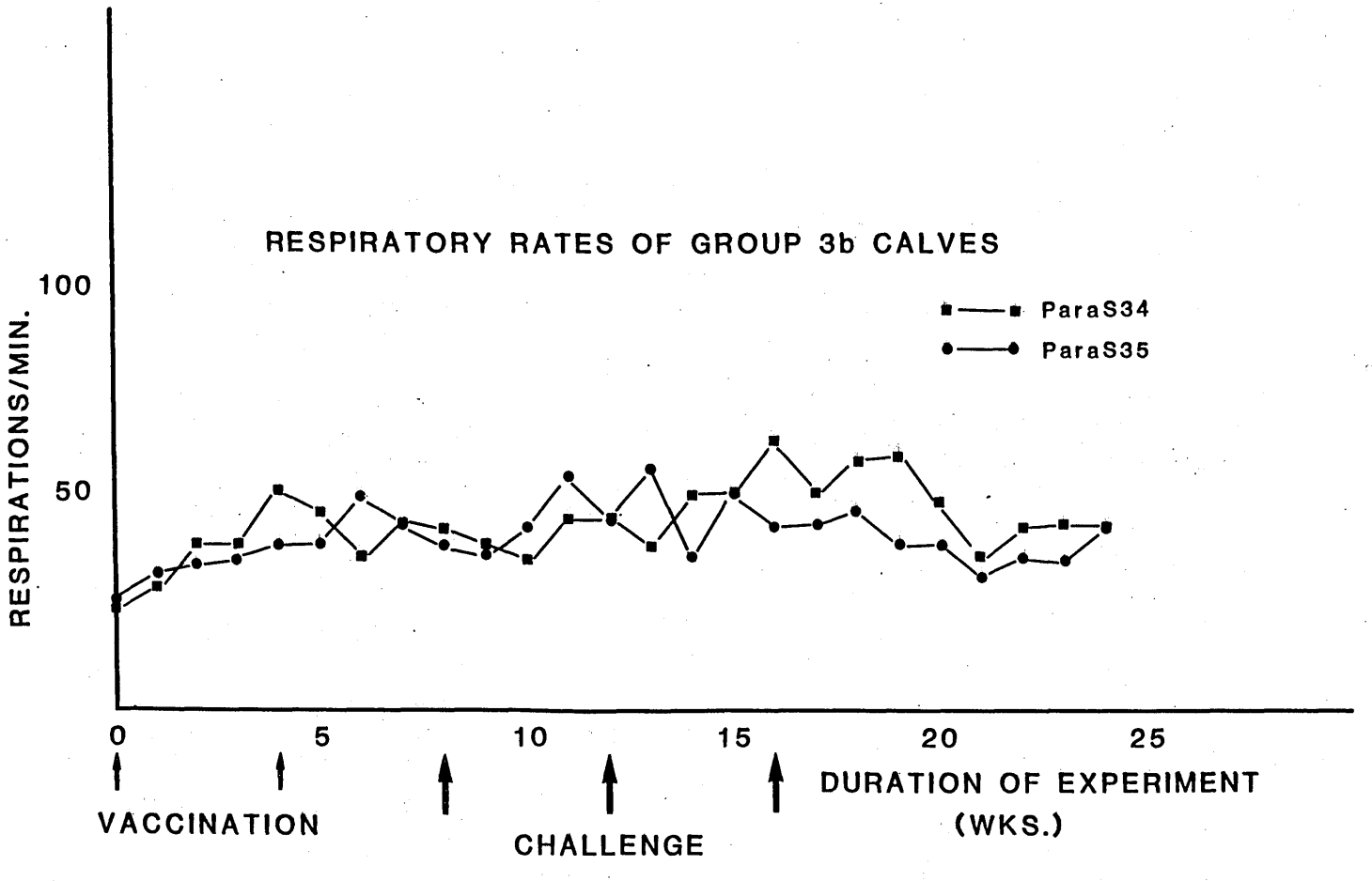

RESPIRATORY RATES OF GROUP 3b CALVES
RESPIRATIONS/MIN.
100
50
ParaS34
ParaS35
0
5
10
15
20
25
VACCINATION
CHALLENGE
DURATION OF EXPERIMENT
(WKS.)

FIG. 79: The eosinophilic response of Group 3b calves that were vaccinated with Dictol and repeatedly challenged with Dictyocaulus viviparus.

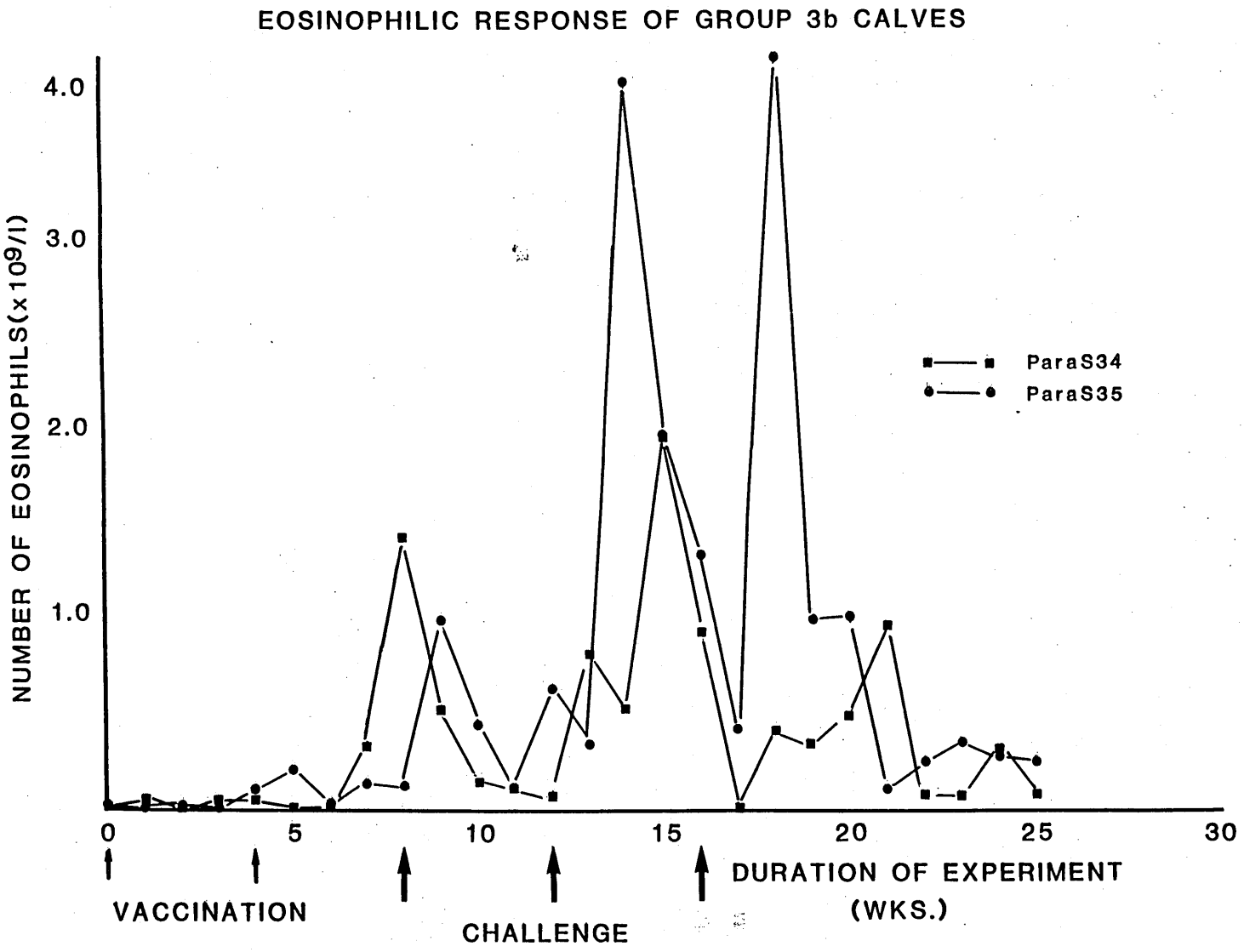

EOSINOPHILIC RESPONSE OF GROUP 3b CALVES
NUMBER OF EOSINOPHILS(x10⁹/l)
4.0
3.0
2.0
1.0
0
5
10
15
20
25
30
ParaS34
ParaS35
VACCINATION
CHALLENGE
DURATION OF EXPERIMENT
(WKS.)

FIG. 80: The mean weekly respiratory rates of Group 3c calves that were given 500 infective third Stage larvae of Dictyocaulus viviparus to simulate vaccination with Dictol and then repeatedly challenged with Dictyocaulus viviparus.

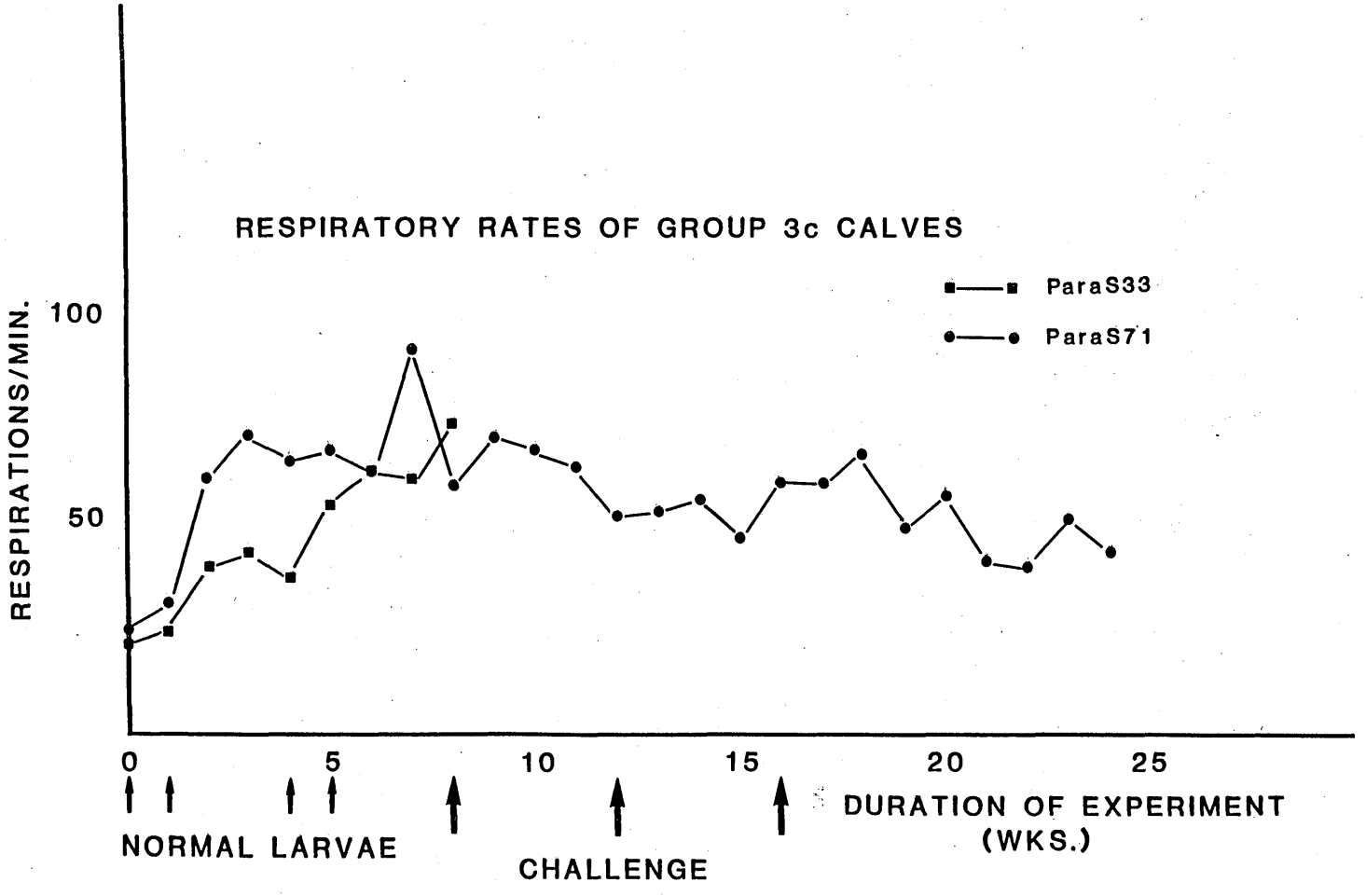

RESPIRATORY RATES OF GROUP 3c CALVES
ParaS33
ParaS71
RESPIRATIONS/MIN.
100
50
0
5
10
15
20
25
NORMAL LARVAE
CHALLENGE
DURATION OF EXPERIMENT
(WKS.)

FIG. 81: The eosinophilic response of Group 3c calves that were given 500 infective third stage larvae of Dictyocaulus viviparus to simulate vaccination with Dictol and repeatedly challenged with Dictyocaulus viviparus.

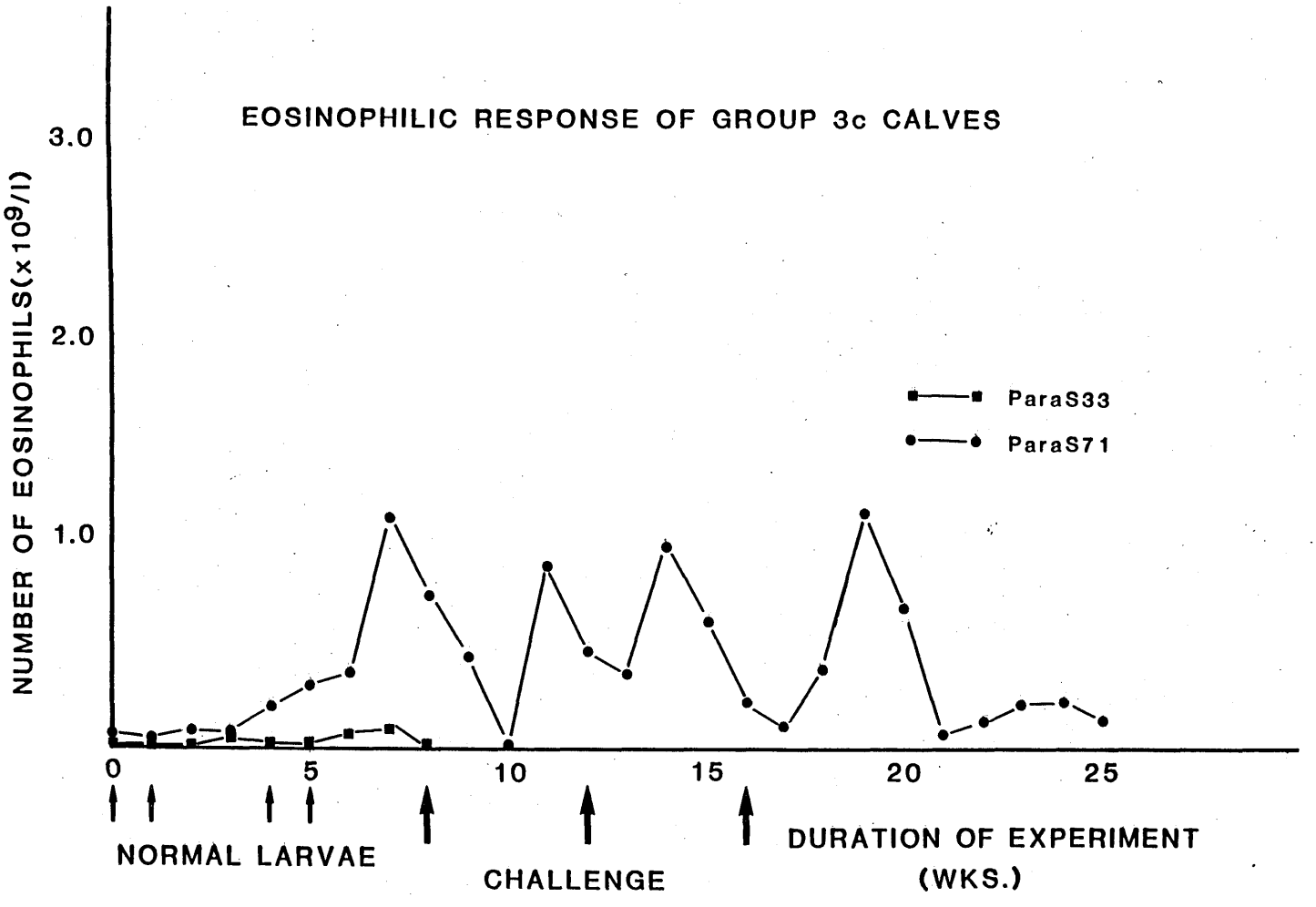

EOSINOPHILIC RESPONSE OF GROUP 3c CALVES
NUMBER OF EOSINOPHILS(x10⁹/l)
3.0
2.0
1.0
ParaS33
ParaS71
0
5
10
15
20
25
NORMAL LARVAE
CHALLENGE
DURATION OF EXPERIMENT
(WKS.)

FIG. 82: The faecal larval counts of Group 3c calves that were given 500 third stage infective larvae of Dictyocaulus viviparus to simulate vaccination with Dictol and then repeatedly challenged with Dictyocaulus viviparus.

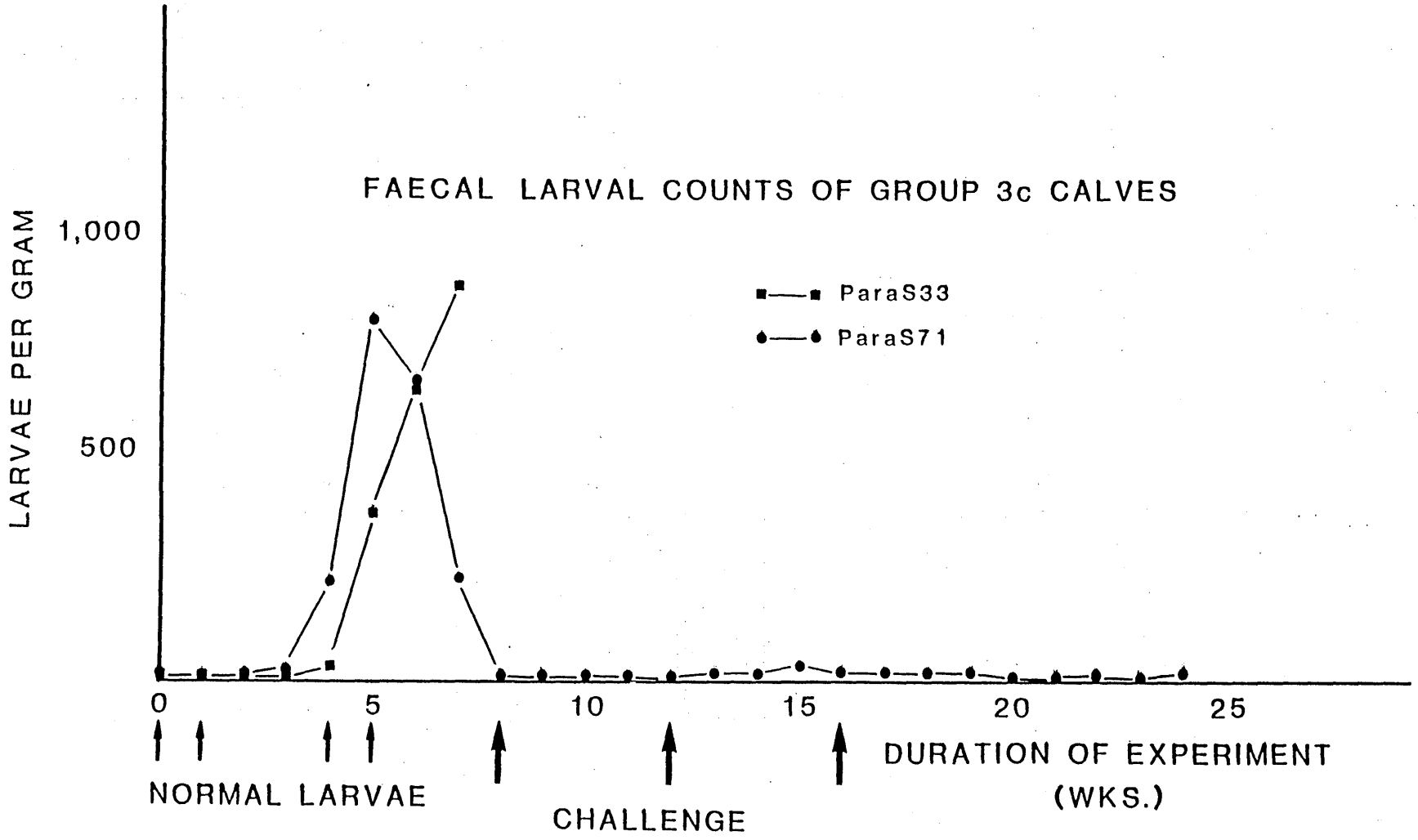

FAECAL LARVAL COUNTS OF GROUP 3c CALVES
LARVAE PER GRAM
1,000
500
ParaS33
ParaS71
0
5
10
15
20
25
NORMAL LARVAE
CHALLENGE
DURATION OF EXPERIMENT
(WKS.)

FIG. 83: The mean weekly respiratory rates of Group 3d calves that were trickle infected with Dictyocaulus viviparus to simulate vaccination with Dictol and then repeatedly challenged with Dictyocaulus viviparus.

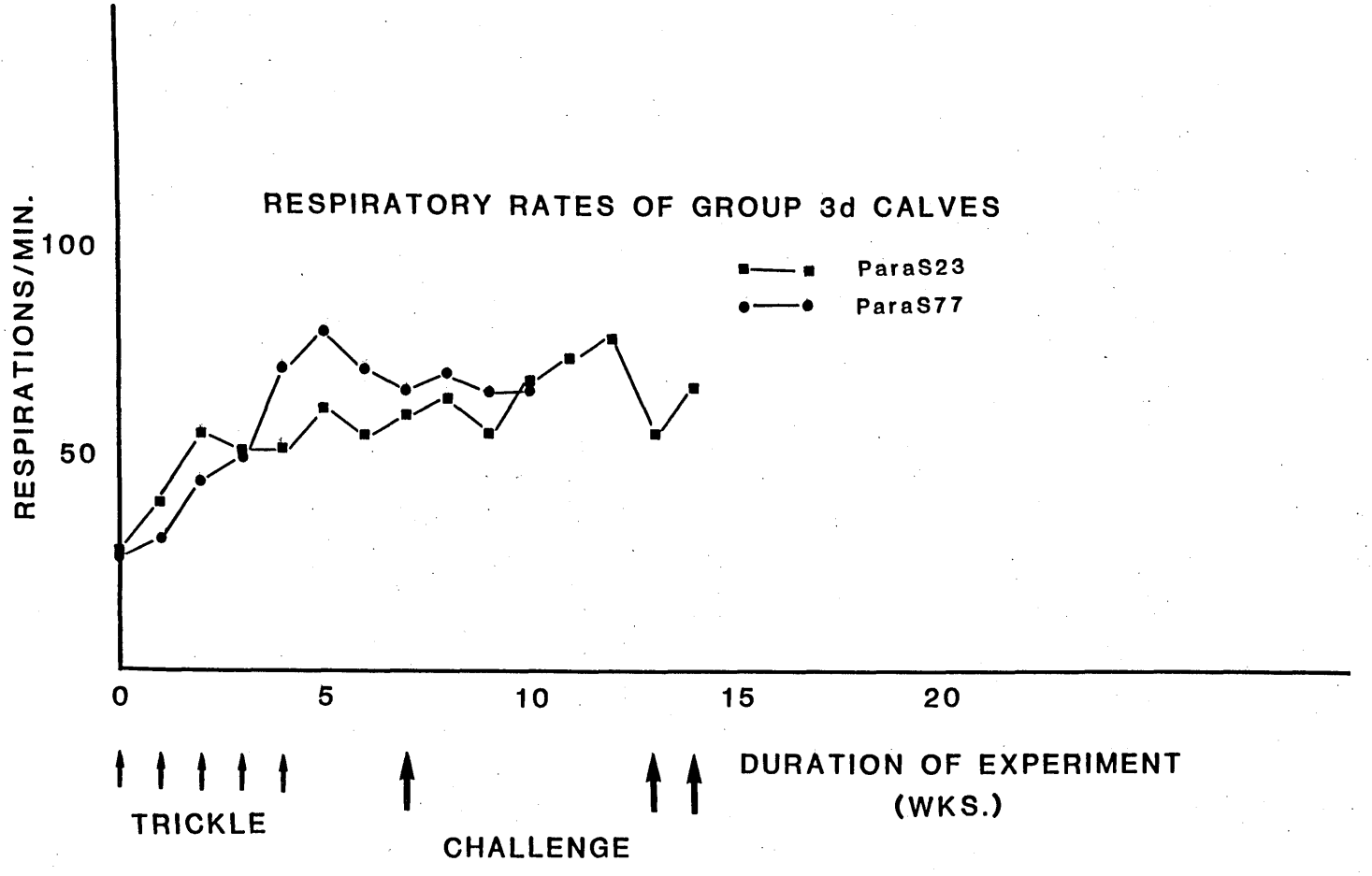

RESPIRATORY RATES OF GROUP 3d CALVES
RESPIRATIONS/MIN.
100
50
ParaS23
ParaS77
0
5
10
15
20
DURATION OF EXPERIMENT
(WKS.)
TRICKLE
CHALLENGE

FIG. 84: The eosinophilic response of Group 3d calves that were trickle infected with Dictyocaulus viviparus to simulate vaccination with Dictol and then repeatedly challenged with Dictyocaulus viviparus.

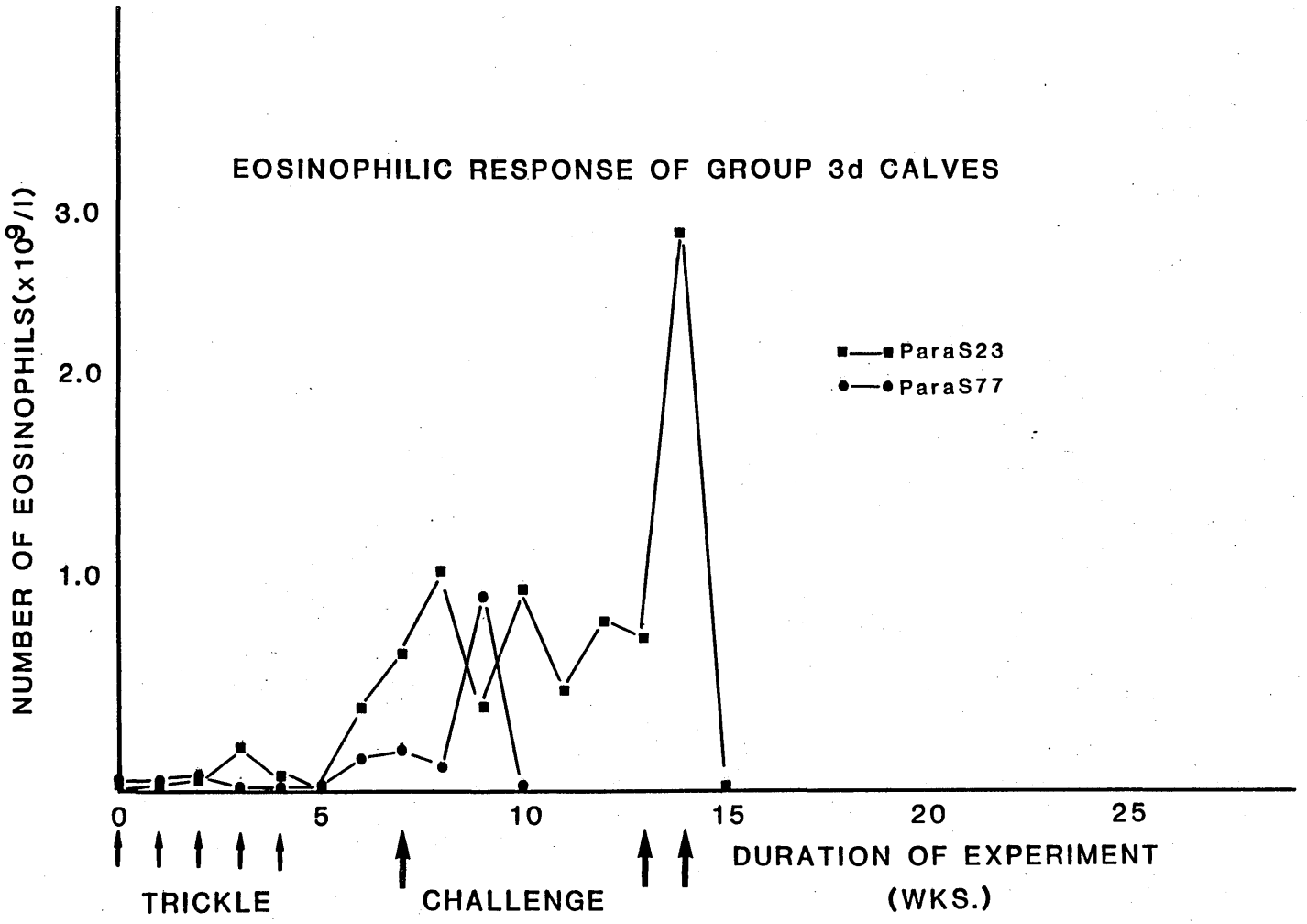
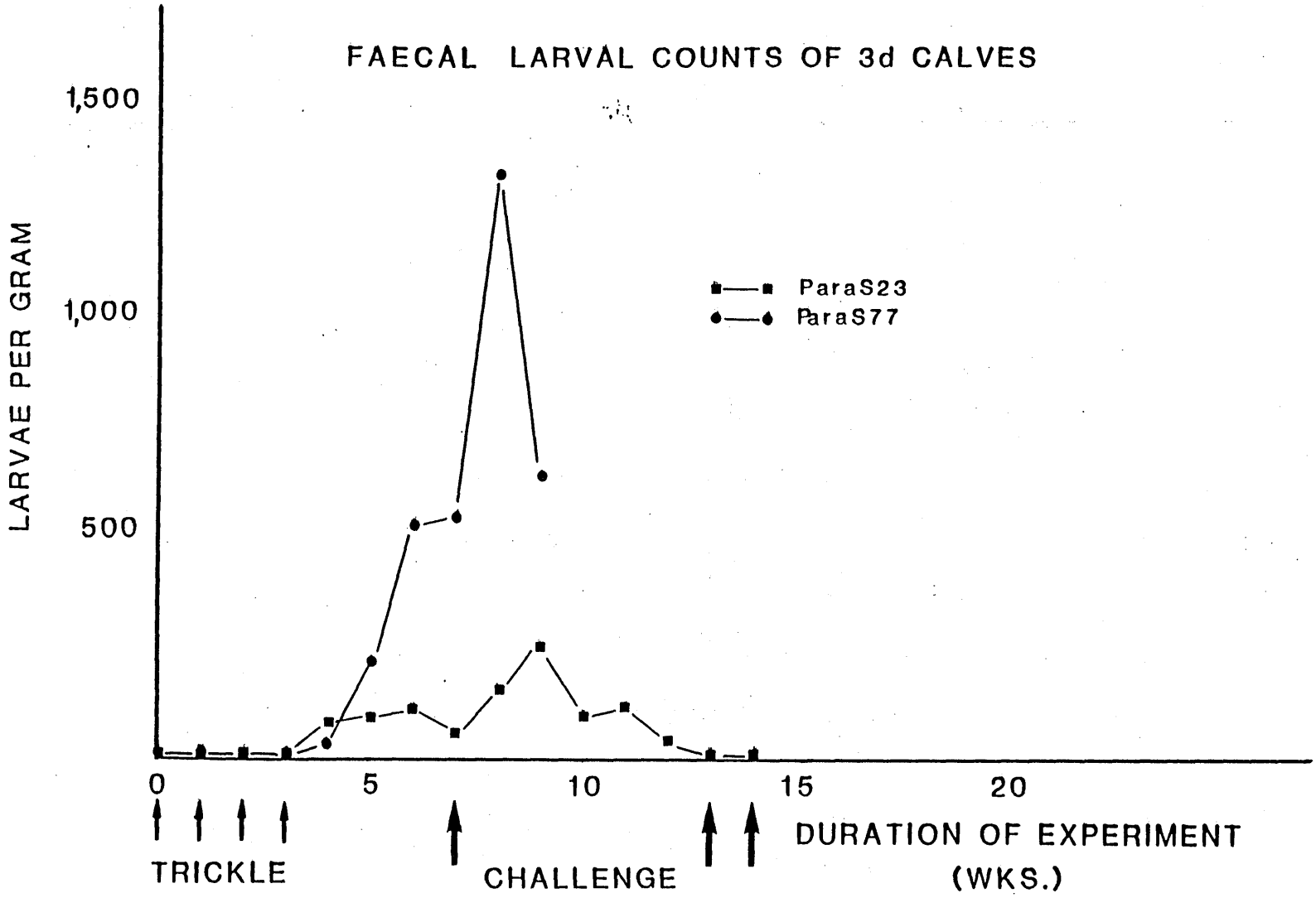

EOSINOPHILIC RESPONSE OF GROUP 3d CALVES
NUMBER OF EOSINOPHILS(x10⁹/l)
3.0
2.0
1.0
ParaS23
ParaS77
0
5
10
15
20
25
DURATION OF EXPERIMENT
(WKS.)
TRICKLE
CHALLENGE

FIG. 85: The faecal larval counts of Group 3d calves that were trickle infected with Dictyocaulus viviparus to simulate vaccination with Dictol and then repeatedly challenged with Dictyocaulus viviparus.


FAECAL LARVAL COUNTS OF 3d CALVES
LARVAE PER GRAM
1,500
1,000
500
ParaS23
ParaS77
0
5
10
15
20
TRICKLE
CHALLENGE
DURATION OF EXPERIMENT
(WKS.)

CHAPTER SIX.

GENERAL DISCUSSION AND CONCLUSIONS.

GENERAL DISCUSSION AND CONCLUSIONS

The literature concerning the globule leucocyte (GL), mast cells (MC) and helminth parasitic infections was reviewed in Chapter 1.

The GL is a cell found within the epithelium of mucous membranes; and is characterised by the presence of numerous cytoplasmic globules. The globules are homogeneous, refractile and stain eosinophilic with haematoxylin and eosin (H&E) stain.

Various origins have been suggested for the GL. Wiell, (1919), Kent, (1952), Toner, (1965), suggested the lymphocyte origin for the GL while Kirkman, (1950) and Finn and Schwartz, (1972), supported the connective tissue origin of GL. Other workers (Jarrett et al., 1967a, Miller et al., 1967, Murray, et al., 1968, Murray et al., 1968) hypothesised that the GL was an end stage cell that resulted from the mucosal mast cell (MMC) degranulation process during "self cure" in helminth parasitic infections. This hypothesis was recently supported by Kamiya et al., (1983) and Huntley et al., (1984).

The occurrence of GL in the mucous membranes of the alimentary tract, including the bile ducts, urinary tract and the respiratory tract, has been associated with various factors such as parasitic infections (Hole, 1937, Kirkman, 1950, Whur, 1966, Jarrett et al., 1967a and b, Murray, 1968, Murray et al., 1968, Lawrence, 1977, Mahmoud, 1978), and reviewed recently by Gregory, (1979). The occurrence of GL has also been associated with pregnancy (Weill, 1920, Kent, 1952, Kellas, 1961), and nutrition (Cantin, 1972, Cantin and Veilleux, 1972, DuBruyn and Liebenberg, 1974), irradiation (Dawson, 1972b) and neoplasia (Finn and Schwartz, 1972).

The presence of GL in the bovine respiratory tract has been reported by various workers (Blazek, 1971, Breeze et al., 1975, Pirie et al., 1976, Lawrence, 1977, and Allan et al., 1983). Large numbers of this cell have been found in the respiratory tracts of

cows in outbreaks of respiratory disease and it was difficult to interpret the findings (Pirie, personal communication). Because the cell was found in large numbers in the respiratory tract of some adult cattle and its occurrence in the respiratory tract of young cattle had not been previously described, a study was carried out to investigate the prevalence of the GL in the bovine respiratory tract.

Six age groups of cattle ranging from foetuses to adults were examined for the presence of GL in their respiratory tracts. Globule leucocytes were present in the respiratory tract of all age groups of cattle but not in every animal examined. Fewer GL were found in the respiratory tracts of foetuses and calves where they were more concentrated in the trachea. The respiratory tract of adult animals contained many more GL and the cells were more concentrated in the bronchi.

With the light microscope, the GL observed in the bovine respiratory tract was similar to those which have been described in other mucous membranes by other workers. The staining reactions of the GL of the bovine respiratory tract observed in this study indicated that the globules of the GL in the young animal contained both sulphated and carboxylated mucin as do the MMC, while in the adult they contained carboxylated and neutral mucin. Rahko, (1972), reported the same for the GL of the bile ducts of adult goats and Takeuchi et al., (1969), for the GL of the cat's intestine. The presence of basic proteins in GL and MMC of the bovine respiratory tract was confirmed in this study.

Although many of the ultrastructural features of the GL described in various organs by investigators were seen in the GL of the bovine respiratory tract; no Golgi apparatus and rough endoplasmic reticulum (RER) were seen in the GL studied in this investigation. Also only two types of globules were seen with the electron microscope. The absence of Golgi apparatus and RER from the bovine respiratory tract GL, agrees with the observations of Miller et al, (1967).

A GL undergoing mitosis and GL undergoing degenerative changes were observed with the light microscope and the degenerative process was confirmed with the electron microscope.

Mast cells were easily differentiated from GL because of their positions in the lamina propria and the ultrastructural feature of their granules. Eosinophils, plasma cells and secretory cells were also easily differentiated from the GL because of the ultrastructural features of their granules and their cytoplasmic organelles.

Because GL were found in large numbers in the lungs of adult cows in outbreaks of respiratory disease, an attempt was made to assess the relationship between GL in the respiratory tract and respiratory diseases as well as pulmonary pathology.

Also because it has been suggested that a mast cell and GL response in one organ (e.g. abomasum) might have a systemic effect leading to an increase in mast cells and GL in another organ (Mahmoud, 1978), the presence of GL in the mucous membranes of the abomasum, bile duct and urinary bladder in relation to their presence in the respiratory tract of cattle was investigated.

There was no relationship found between the respiratory tract GL and clinical respiratory disease but the GL population density increased in calves with pulmonary lesions but not in calves without pulmonary lesions. Globule leucocytes were found in the bile ducts of foetuses and the number of animals with GL in their respiratory tract as well as their bile ducts and abomasums increased with age. The presence of GL in the bile ducts of foetuses may be because GL belonged to a different cell line, of mesenchymal origin and independent of MC; and that their increase in number in the abomasum and bile duct of adult animals may be associated with the repeated exposure of these animals to parasites or other antigens. Globule leucocytes were not found in the urinary bladder of any of the animals examined although GL may develop in the urinary bladder of cattle in schistosoma infections (Lawrence, 1977).

A globule leucocyte response has been induced in mucous membranes by a variety of parasitic infections (Whur, 1966, Jarrett et al., 1976a, Murray, 1968, Murray et al., 1968 and Mahmoud, 1978). The large numbers of GL in the respiratory tract of adult cattle led to the concept of a probable association with repeated exposure of adult animals to lung worm infections. A study was therefore carried out to investigate GL reponse in bovine lungworm infection in calves.

Globule leucocytes were found in the respiratory tracts of calves which were either (i) exposed once to D. viviarus infection, (ii) immune to D. viviparus and challenged once with D. viviparus, (iii) immune to D. viviparus and challenged repeatedly with D. viviparus or (iv) trickled and challenged repeatedly with D. viviparus. The population densities of GL seen in the lungs of these calves were not different from what was normally seen in the respiratory tracts of this age group of cattle. The results of this investigation do not therefore agree with those of Mahmoud, (1978), who associated the presence of GL in the lungs of sheep with parasitic infections.

It was concluded that although GL were found in the respiratory tracts of calves infected with D. viviparus, no correlation could be demonstrated between the bovine respiratory tract GL and lungworm infections. In this connection, it is relevant to note that Jarrett et al., (1957a and b), Pirie et al., (1971), did not associate GL with either experimental or natural infections of cattle with D. viviparus, and Mahmoud, (1978), did not observe GL in the lungs of some populations of outdoor sheep with parasitic pulmonary lesions. It is not therefore correct to assume that the large numbers of GL found in the lungs of adult cattle are due to lungworm infections. However, the presence of large numbers of GL may be associated with the age of the animal resulting from their exposure on more than one occasion to other unidentified stimuli.

APPENDIX I

HISTOPATHOLOGICAL, HISTOCHEMICAL, AND ELECTRON MICROSCOPY TECHNIQUES

HISTOLOGICAL TECHNIQUES

A. FIXATIVE

1. Buffered Neutral Formalin - ten per cent solution. (Luna, 1968).

Formaldehyde (40 per cent)	100 ml.
Distilled water	900 ml.
Sodium phosphate monobasic	4 g.
Sodium phosphate dibasic anhydrous	6.5 g.

2. Carnoy's Fluid (Culling, 1963).

Absolute alcohol	60 ml.
Chloroform	30 ml.
Glacial acetic acid	10 ml.

3. Corrosive Formol, Sublimate-Formol or Mercuric Chloride-Formalin

Saturated aqueous solutions of Mercuric Chloride	900 ml.
Formaldehyde (40 per cent)	100 ml.

B. STAINING TECHNIQUES

Fluorescent staining of acid mucins

(i) Acridine orange method (Hicks and Matthaei, 1958).

Preparation of Stain:

1. Acridine orange 0.1 g.
2. Distilled water 100 ml.

Method:

1. Dewax sections and bring to water.
2. Treat with 4 per cent aqueous iron alum for 15 minutes.
3. Wash well in water.
4. Acridine orange solution 1½ minutes.
5. Wash in water and blot dry.
6. Mount direct in a D.P.X. type mountant.

Results

Using a B G 12 (5mm) exciter filter and TK510 and TK515 suppression filters

1. Acid mucins - Orange red
2. Background - dull green.

Stain for acid mucins

(i) Alcian blue pH 2.5 or pH 1.0 (Steedman, 1950, Lison, 1954).

Preparation of stain

1. Alcian blue 1 gm in either
2. 3 per cent acetic acid 100 ml. yielding a pH of 2.5 or
3. 0.1N hydrochloric acid 100 ml. yielding a pH of 1.0.

Method

1. Dewax sections and bring to water. (If the pH of stain employed is critical, rinse in the appropriate pH (e.g. the solvent for the dye).
2. Alcian blue for 5 minutes.
3. Wash in water or if the pH of staining is critical, omit and blot dry instead.
4. Counterstain with 0.5 per cent aqueous neutral red or 0.1% safranin for 2-3 minutes.
5. Wash in water.
6. Rinse in absolute alcohol.
7. Clear in xylene and mount as desired.

Results

1. Acid mucins - blue.
2. Nuclei - red.

(ii) Modified Astra blue/Safranin method pH 0.3 (Bloom and Kelly, 1960).

Preparation of Stain

1.	Astra blue	1 g.
2.	0.7N Hydrochloric acid	100 ml.
3.	Safranin	1 g.
4.	Distilled water	100 ml.

Method

1. Dewax sections and bring to water.
2. Stain with astra blue solution (1% in 0.7N Hydrochloric acid) for 30 minutes.
3. Rinse in 0.7N hydrochloric acid for 30 minutes.
4. Wash in water.
5. Counter stain with 1 per cent safranin diluted 1:10 just before use) for 30 seconds.
6. Rinse in water.
7. Dehydrate through alcohols to xylene.
8. Mount in D.P.X.

Results

1. Nuclei - red.
2. Acid mucins - blue.
3. Background tissue - pink.

Stain for Basic Protein.

(i) Biebrich scarlet (BS) (Lillie, 1954).

Preparation of Stain

1. Biebrich scarlet 1 g.
2. Distilled water 100 ml.
3. Laskey glycine-sodium hydroxide buffer (pH 9.9), 49 ml; made from 9 ml $N(Na)_2$ OH + 20 ml N glycine + 11ml Distilled water.

Method

1. Dewax sections and bring to water.
2. Stain in a solution of 1% biebrich scarlet in glycine buffer at appropriate pH for 20 minutes.
3. Wash in water or blot and dehydrate in absolute alcohol.
4. Clear in xylene and mount.

Results

1. Basic protein - red.
2. Background stain - pale orange.

Stain for Eosinophils

(i) Carbol chromotrope

Preparation of Stain

1. Phenol 1 g.
2. Chromotrope 2 R 1 g.
3. Methylated spirit 5 ml.
4. Distilled water 50 ml.

Method

1. Dewax section and bring to water.
2. Stain in haematoxylin for 5 minutes.
3. Rinse in water.
4. Blue in Scotts tap water substitute.
5. Wash in water.
6. Stain in carbol chromotrope for 30 minutes.
7. Rinse in water.
8. Dehydrate through alcohol to xylene and mount.

Results

1. Eosinophil granules - red.
2. Red blood cells - orange.

Routine Stain

Haematoxylin (Ehrlich, 1886).

Preparation of Solution

1.	Haematoxylin	2 g.
2.	Absolute alcohol	100 ml.
3.	Glycerin	100 ml.
4.	Distilled water	100 ml.
5.	Glacial acetic acid	10 ml.
6.	Potassium alum	15 g approximately.

Eosin

Preparation of Solution

1.	Eosin Y.	1 g.
2.	Distilled water	100 ml.
3.	A crystal of thymol.	
4.	Acetic acid	0.5 ml.

Haematoxylin and Eosin Staining Method for Paraffin Sections

1. Dewax in xylol, hydrate through graded alcohols to water.
2. Remove fixation pigments if necessary by treatment with Lugol's iodine and 5% sodium thiosulphate.
3. Stain in Ehrlich haematoxylin for 30 minutes.
4. Wash well in running tap water until sections blue (5 minutes).
5. Differentiate in 1 per cent acid alcohol (1% HCL in 70% alcohol) 5 - 10 seconds.
6. Wash in tap water until sections are again blue (5 mins).
7. Stain in 1% eosins Y for 10 minutes.
8. Wash in running tap water for 1 - 5 minutes.
9. Dehydrate through alcohols, clear in xylol. Mount in D.P.X.

Results

1. Nuclei - blue-black.
2. Cytoplasm - varying shades of pink.
3. Muscle fibres - deep pinky red.
4. Collagen - pale pinky red.
5. Red blood cells - orange red.
6. Fibrin - deep pink.

Stain for Proteins (Fibrin)

(i) Martius scarlet blue (MSB), (Lendrum et al., 1962).

Preparation of solutions

(a)

1.	Martius yellow	0.5 g.
2.	Phosphotungstic acid	2.0 g.
3.	95 per cent alcohol	100 ml.

(b)
1. Brilliant crystal scarlet (Acid red 44) 1.0g.
2. Glacial acetic acid 2.0 g.
3. Distilled water 100 ml.

(c)
1. Phosphotungstic acid 1.0 g.
2. Distilled water 100 ml.

(d)
1. Methyl blue (Acid blue 93) 0.5 g.
2. Glacial acetic acid 1.0 ml.
3. Distilled water 100 ml.

(e)
1. Glacial acetic acid 1.0 ml.
2. Distilled water 100 ml.

Method

1. Dewax sections and bring to water.
2. Remove mercury pigment with iodine, thiosulphate treatment.
3. Stain nuclei by the celestin blue-haematoxylin sequence.
4. Differentiate in 1 per cent acid alcohol.
5. Wash well in tap water.
6. Rinse in 95 per cent alcohol.
7. Stain in Martius yellow solution (2 minutes).
8. Rinse in distilled water.
9. Stain in brilliant crystal scarlet solution (10 minutes).
10. Rinse in distilled water.
11. Treat with phosphotungstic acid solution until no red remains in the collagen.
12. Stain in distilled water.
13. Stain in methyl blue solution until collagen is sufficiently coloured.
14. Rinse in 1 per cent acetic acid.
15. Dehydrate through alcohols.
16. Clear in xylene and mount in D.P.X.

Results

1. Nuclei - blue.
2. Erythrocytes - yellow.
3. Muscle - red.
4. Collagen - blue.
5. Fibrin - red (early fibrin may colour yellow and very old fibrin blue).

(ii) Phosphotungstic acid haematoxylin (PTAH). (Mallory, 1879, 1900).

1. Haematoxylin	1 g.
2. Phosphotungstic acid	10 g.
3. Distilled water	1000 ml.

Method

1. Dewax sections and bring to water.
2. Mordant in 4 per cent iron alum for 20 - 60 minutes.
3. Wash well in distilled water.
4. Stain in PTAH for 1 - 16 hours.
5. Rinse briefly in 95 per cent alcohol.
6. Dehydrate in absolute alcohol, clear in xylene and mount in synthetic resin medium.

Results

1. Nuclei, fibrin (if freshly formed) striations of muscle, myofibrils, astrocytes and their processes and fibroglia - blue.
2. Collagen, matrix of bone and cartilage - orange-red or brownish-red to deep brick-red.

Toluidine Blue Stain for Acid Mucin. (Enerback, 1966).

(i) Toluidine blue pH 4.0.

Preparation of Stain

Toluidine blue	0.5 g.
McIvaines buffer (pH 4.0)	100 ml.
made from 33.3 ml 0.1M citric acid	
+ 66.7 ml 0.2M disodium phosphate.	

Method.

1. Dewax sections and bring to water.
2. Stain in 0.5 per cent toluidine blue in McIvaine's buffer for 45 seconds.
3. Rinse in water.
4. Blot dry.
5. Dehydrate through alcohols to xylene and mount.

Results

1. Mast cell granules and mucin - purple red.
2. Background - blue.

(ii) Toluidine blue at pH 0.3 (Enerback, 1966).

Preparation of Stain

1. Toluidine blue	0.1 g.
2. Hydrochloric acid (0.7N)	100 ml.

Method

1. Dewax sections and bring to water.
2. Stain in 0.1 per cent toluidine blue in 0.7N acid for 10 minutes.
3. Rinse in 0.7N hydrochloric acid for 10 minutes.
4. Rinse in distilled water.
5. Dehydrate rapidly to xylene and mount.

Results

1. Mast cell granules and mucins - purple.

ELECTRON MICROSCOPY TECHNIQUES

A. FIXATIVES

(i) Paraformaldehyde/glutaraldehyde fixation (Karnovsky, 1965).

Preparation of Fixative

2 g of paraformaldehyde is dissolved in 25 ml. of distilled water. The solution is heated to 60°-70°C shaking continuously. Add 1-3 drops of 1N NaOH still shaking until the solution is clear or slightly turbid. Allow the solution to cool then add 5 ml. of 50% glutaraldehyde solution (or 10 ml. of 25% solution) and make up to 50 ml with 0.1M cacodylate buffer - pH 7.4 - 7.6. Final pH should be 7.2. 25 mg. of anlydrous calcium chloride are added. Dilute this solution with another 100 ml. of buffer.

The above solution is a mixture of 1.3% paraformaldehyde and 1.6% glutaraldehyde.

Method of Fixation.

1. Fix for $4\frac{1}{2}$ - 6 hours at 4°C.
2. Rinse for 2 minutes in 0.1M cacodylate rinsing solution and then leave overnight in fresh rinse at 4°C.

(ii) Osmic acid (In Millonig's phosphate buffer).

Preparation of Solution

1. Stock acid solution - monosodium phosphate 2.26%.
2. Stock alkali solution - Sodium hydroxide 2.52%.
3. Buffer:-

NaH_2 PO4	83 ml.
NaOH	17 ml.
Distilled water	10 ml.
Sucrose	0.54 g.
pH 7.2 - 7.4.	

1 g. of Osmic acid is added to this buffer.

Method of Fixation

1. As a primary fixative fix for 1½ hours at 4°C.

   NOTE: Very small pieces of material only, should be fixed in this way.

2. As a post fixative, fix for 1 hour at 4°C.

B. BUFFERS

1. Cacodylate Buffer.

   0.1M solution of sodium cacodylate (21.4 g/l) adjusted to pH 7.7 - 7.6 by addition of a few drops of conc. HCl.

2. Cacodylate Rinsing Solution

   Add 0.1M sucrose (32.2 g/l) and pH to 7.2 - 7.4.

3. Millonig's Phosphate Buffer

$NaH_2 PO_4$	83 ml.
NaOH	17 ml.
Distilled water	10 ml.
Sucrose	0.54 g.

   pH 7.2 - 7.4

C. EMBEDDING RESIN

1. Araldite

   Stock Mixture:- Equal parts of araldite resin (CY212) and araldite hardener (H7964).

   Heat both to 55°C and mix by hand or leave at room temperature and mix overnight on the mixer. This mixture can be left indefinitely.

Before use:-	Mix stock mixture	58 ml.
	Accelerator (HY960)	0.6 ml.
	Dl-n-Butyl phthalate	2.0 ml.

This mixture should be mixed well for at least half an hour before use.

Curing 48 hours at 57°C.

D. THICK SECTION STAINING

Methylene Blue/Azure II (Trump, Smuckler and Benditt, 1961).

1. Pre-treat with 1% periodic acid for 5 minutes (not always necessary).
2. Rinse briefly in water.
3. Stain for 5 - 15 minutes (or as necessary) in a freshly prepared solution of equal parts of:-
   1% Azure II
   1% Methylene blue
   1% borax
   Heat but do not allow to dry.
4. Rinse in water.
5. Dry and mount.

E. THIN SECTION STAINING

1. Uranyl Acetate (Watson, 1958).
   20% solution in absolute methanol.

2. Lead Citrate (Reynolds, 1963).

Lead nitrate ($Pb(No_3)_2$)	1.33 g.
Sodium citrate ($Na_3(C_6H_5O_7).2H_2O$)	1.76 g.
Distilled water	30 ml.

Each salt is dissolved in 15 ml of distilled water and when disolved completely mixed together in a 50 ml volumetric flask. The resultant precipitate is shaken for about 1 minute and then left to stand for 30 minutes with intermitent shakings to ensure complete conversion of lead nitrate to lead citrate.

0.8 ml of 1N sodium hydroxide is added and the suspension is diluted to 50 ml with distilled water and mixed by inversion. The lead citrate dissolves and the staining solution is ready for use.

pH 12 ± 0.1

Staining with Uranyl acetate and lead citrate

1. Spin the uranyl acetate for 5 minutes.
2. Float grids on blobs of the stain for 1 minute.
3. Wash grid (i) conc. methanol.
   (ii) 50% methanol - twice.
   (iii) Distilled water - twice.
4. Blot dry.
5. Float the grids on blobs of lead citrate for 1 minute.
6. Wash the grid in (i) 0.02N sodium hydroxide.
   (ii) Distilled water - twice.
7. Blot dry.

REFERENCES

REFERENCES

Allan, D. & Baxter, J.T. (1957)
Veterinary Record 69, 717-718

Allan, E.M., Gibbs, H.A., Wiseman, A. (1983)
Veterinary Record 112, 222-223

Allan, D. & Johnson, A.W. (1960)
Veterinary Record 72, 3, 42-45

Allan, E.M., Pirie, H.M. & Wheeldon, E.B. (1977)
Folia Veterinaria Latina VII, 133-144

Andrews, A.H. (1983)
The Veterinary Annual 23rd Issue, Edited by Gunsell, C.S.G. & Hill, F.W.G., pp. 48-55, Scientechnica, Bristol

Archer, R.K. (1980)
Journal of Royal Society of Medicine 73, 318-319

Armour, J., Al Saqur, I.M., Bairden, K., Duncan, J.L. & Urquhart, G.M. (1980)
Veterinary Record 106, 184-185

Asdrubali, G. & Mughetti, L. (1969)
Atti della Societa Italiana delle Scienze Veterinarie 22, 601-606

Askenase, P.W. (1980)
Springer Seminar, Immunopathology 2, 417-442

Bazin, H. (1977)
In Immunity in Parasitic Diseases, Inserm 72, 185-200

Befus, A.D., Denburg, J. & Bienenstock, J. (1979)
In The Mast Cell, Its Role in Health and Disease. Edited by Pepys, J. and Edwards, A.M., Proceedings of an International Symposium, Davos, Switzerland, pp. 115-122, Pitmans (Publishing) Ltd., London

Befus, D., Denburg, J. & Bienenstock, J. (1984)
Immunology Today 5, 8, 218-219

Benditt, E.P., (1958)
Annals New York Academy of Science 73, 204-211

Benditt, E.P. & Lagunoff, D. (1964)
Progress in Allergy 8, 195-223

Bessis, M. (1964)
'Electron Microscopic Anatomy' Edited by Kurtz, S.M., Academic Press, New York and London

Black, L. (1977)
Veterinary Record 100, 195-198

Black, L. (1979)
Veterinary Bulletin 49 1, 1-9

Black, L. & Burka, J.F. (1979)
Veterinary Bulletin 49, 5, 303-308

Blazek, K. (1971)
Zentralblatt für Veterinarmedizin 18B, Heft 2, 103-112

Bloom, G. & Kelly, J.W. (1960)
Histochemie 2, 48-57

Breeze, R.G., Pirie, H.M., Dawson, C.O., Selman, I.E. & Wiseman, A. (1975)
Folia Veterinaria Latina V, 1, 95-128

Breeze, R.G., Wheeldon, E.B. & Pirie, H.M. (1976)
Veterinary Bulletin 46, 5, 319-337

Brinkman, G.L. (1968)
Journal of Ultrastructure Research 23, 115-123

Bryson, D.G. (1980)
Thesis, The Queen's University of Belfast

Burnet, F.M. (1965)
Journal of Pathology 89, 271

Butterworth, A.E., David, J.R., Frank, D., Mahmoud, A.A.F., David, P.Pt., Sturrock, R.F. & Houba, V. (1977)
Journal of Experimental Medicine 145, 136-150

Campbell, S.G. (1970)
The Cornell Veterinarian LX 1, 240-253

Cantin, M. & Veilleux, R. (1972)
Laboratory Investigation 27, 495-507

Capstick, P.B., Pay, T.W.F., Beadle, G.G., Bandau, R. & Boge, A. (1970)
European Commission for the control of foot and mouth disease standing technical committee, Brescia, Italy, pp. 213-218, FAO, Rome

Carr, K.E. (1967)
Journal of Anatomy 101 4, 793-803

Caulfield, J.P., Lewis, R.A., Hein, A. and Austen, K.F. (1980)
Journal of Cell Biology 85, 299-312

Cornwell, R.L. (1959)
Veterinary Record 71, 562

Cornwell, R.L. & Berry, J. (1960)
Veterinary Record 72, 30, 595-598

Crandall, R.B., Crandall, C.A. & Franco, J.A. (1974)
Experimental Parasitology 35, 275-287

Culling, G.F.A. (1963)
Handbook of Histopathological Techniques, Second Edition,
Butterworths, London

Dawson, A.B. (1927a)
Anatomical Record 35, 99-107

Dawson, A.B. (1927b)
Anatomical Record 36, 1-29

Dawson, H.L. (1943)
Anatomical Record 85, 135-155

Dobbins, W.O. III., Tomasini, J.T. & Rollins, E.L. (1969)
Gastroenterology 56, 2, 268-279

Dobson, C. (1965)
Australian Journal of Science 28, 11, 434

Dobson, C. (1966a)
Australian Journal of Agricultural Research 17, 955-966

Dobson, C. (1966b)
Nature, London 211, 875

DuBruyn, D.B. & Liebenberg, N.V.D.W. (1974)
South African Journal of Science 70 249-251

Duncan, J.L., Armour, J., Bairden, K., Urquhart, G.M. & Jorgensen,
R.J. (1979)
Veterinary Record 104, 274-278

Duran-Jurda, F. (1945)
Veterinary Journal 101, 191-194 + 5 plates

Ehrlich, P. (1877)
Archiv fur Mikroskopische Anatomie Und Entwicklungsmechanik 13,

Ehrlich, P. (1886)
Zeitschrift für Mikroskopische Mikroskopie und für Mikroskopsche Technik 3, 150

Ellis, G. (1972)
In A Guide for Biological and Medical Authors, pp. 1-36 Royal Society of Medicine, London

Enerback, L. (1966)
Acta Pathologica et Microbiologica Scandiavica 66, 303-312

Enerback, L. (1981)
Monographs in Allergy 17, 222-232

Enerback, L. and Lundin, P.M. (1974)
Cell Tissue Research 150, 95-105

Ferguson, A. & Miller, H.R.P. (1979)
In the Mast Cell; Its Role in Health and Disease, Edited by Pepys, J. & Edwards, A.M., Proceedings of an International Symposium, Davos, Switzerland, pp. 159-165., Pitmans (Publishing) Ltd., London

Fernando, N.V.P. & Movat, H.Z. (1963)
Experimental and Molecular Pathology 2, 450-463

Fincher, M.G. (1960)
The Allied Veterinarian XXXI 5, 135-140

Finn, J.P. & Schwartz, L.W. (1972)
Journal of Comparative Pathology 82, 323-326

Gordon, H. McL & Whitlock, H.V. (1939)
Journal of the Council for Scientific and Industrial Research, Australia 12-50

Greenway, J.A. & McCraw, B.M. (1970)
Canadian Journal of Comparative Medicine 34, 227-255

Gregory, M.W. (1979)
Veterinary Bulletin, 49 11, 821-827

Gregory, M.W. & Nolan, A. (1981)
Research in Veterinary Science 30, 385-387

Grupta, R.P. & Gibbs, H.C. (1970)
The Canadian Veterinary Journal 11, 8, 149-156

Haig, D.M., McKee, T.A., Jarrett, E.E.E., Woodbury, R. & Miller, H.R.P. (1982)
Nature, London 300, 5888, 188-190

Henriksen, S.A. (1965)
Nordisk Veterinaer Medicin 17, 446-454

Hibbs, R.G., Burch, G.E. & Phillips, J.H. (1960)
American Heart Journal 60, 1, 121-127

Hicks, J.D. & Matthaei, E. (1958)
Journal of Pathology & Bacteriology 75, 473-476

Hilderman, E. & Taylor, P.A. (1974)
Canadian Veterinary Journal 15, 6, 173-175

Hill, K.J. (1951)
Journal of Anatomy 85, 215-220

Hole, N.H. (1937)
Journal of Comparative Pathology 50, 299-302

Holeman, J. (1970)

Acta Veterinaria Brno 39, 385-390

Holman, J. (1972)
Acta Veterinaria Brno 41, 235-239

Holroyde, M.C. & Eyre, P. (1976)
Immunology 31, 167-170

Hoyes, A.D., Bourne, R. & Martin, B.G.H. (1974)
Journal of Anatomy 117, 214

Hudson, G. (1967)
Experimental Cell Research 46, 121-128

Huntley, J.F., Wallace, G.R. & Miller, H.R.P. (1982)
Research in Veterinary Science 33, 58-63

Inderbitzen, F. (1976)
Thesis Institut für Parasitologie der Universität Zürich,
(Stitzerland)

Ishazaka, T., Okudira, H., Mauser, L.E. & Ishizaka, K. (1976)
Journal of Immunology 116, 747

Jarrett, E.E.E. & Haig, D.M. (1984)
Immunology Today 5, 4, 115-119

Jarrett, W.F.H., Jarrett, E.E.E., Miller, H.R.P. & Urquhart, G.M.
(1967b)
In Reaction of Host to Parasitism, Edited by Soulsby, E.J.L. pp.
191-197, Academic Press, New York

Jarrett, W.F.H., Jennings, F.W., McIntyre, W.I.M., Mulligan, W.,
Sharp, N.C.C. & Urquhart, G.M. (1959)
American Journal of Veterinary Research 522-526

Jarrett, W.F.H., Jennings, F.W., McIntyre, W.I.M., Mulligan, W.,
Sharp, N.C.C. & Urquhart, G.M. (1960)
British Veterinary Association Annual Congress, 1-4

Jarrett, W.F.H., Jennings, F.W., McIntyre, W.I.M., Mulligan, W. & Urquhart, G.M. (1957a)
British Veterinary Association, Annual Congrerss, Cambridge 1-40

Jarrett, W.F.H., McIntyre, W.I.M. & Urquhart, G.M. (1957b)
Journal of Pathology & Bacteriology LXXIII, 183-193

Jarrett, W.F.H., McIntyre, W.I.M., Urquhart, G.M. & Eleanor, J.B. (1955)
Veterinary Record 67, 44, 820-824

Jarrett, E.E.E. & Miller, H.R.P. (1982)
Progress in Allergy 31, 178-233

Jarrett, W.F.H., Miller, H.R.P. & Murray, M. (1967a)
Veterinary Record 80, 16, 505-506

Jarrett, W.F.H. & Sharp, N.C.C. (1963)
Journal of Parasitology 49, 2, 177-189

Jeffery, P.K. & Reid, L. (1975)
Journal of Anatomy 120, 295-320

Jones, V.E., Edwards, A.J. & Ogilvie, B.M. (1970)
Immunology 18, 621

Jørgensen, R.J. (1980a)
Veterinary Parasitology 7, 153-167

Jørgensen, R.J. (1980b)
Acta Veterinaria Scandanavia 21, 658-676

Jørgensen, R.J. (1982)
Veterinary Parasitology 10, 331-339

Kamiya, M., Oku, Y., Fukumoto, S. & Ooi, H. (1983)
Japanese Journal of Veterinary Research 31, 133-140

Karnovsky, M.J. (1965)
Journal of Cell Biology 27, 137a

Keasbey, E.L. (1923)
Folia Heamatologica 29, 155-171

Kellas, L.M. (1961)
Acta Anatomica 44, 109-130

Keller, R. (1971)
Parasitology 63, 473-481

Kent, J.F. (1952)
Anatomical Record 112, 91-115

Kent, J.F., (1966)
Anatomical Record 156, 439-454

Kent, J.F., baker, B.L., Ingle, D.J. & Li, C.H. (1954)
Proceeding Society of Experimental Biology and Medicine 86, 635

Kent, J.F., Baker, B.L., Pliske, E.C. & Van Dyke, J.G. (1956)
Proceeding Society of Experimental Biology and Medicine 19, 154-155

Kirkman, H. (1947)
Anatomical Record 3, 349

Kirkman, H. (1950)
American Journal of Anatomy 86, 91-127

Knifton, A. (1983)
The Veterinary Annual, 23rd Issue, Edited by Gunsell, C.S.G. &
Hill, F.W.G., pp. 56-60 Scientechnica, Bristol

Knight, R.A. (1980)
Journal of Parasitology 66, 5, 844-845

Lawrence, J.A. (1977)
Research in Veterinary Science 23, 239-240

Leeman, W., De Weck, A.L. & Schneider, C.H. (1969)
Nature, London 223, 621-623

Lendrum, A.C., Fraser, D.S., Slidders, W. & Henderson, R. (1962)
Journal of Clinical Pathology 15, 401

Lim, R.K.S. (1922)
Quarterly Journal of Microbiological Science 66, 187-212

Lillie, R.D. (1954)
Histopathologic Technic & Practical Histochemistry, The Blakiston company, Inc. New York

Lison, L. (1954)
Stain Technology 29, 131

MacDonald, T.T., Murray, M. & Ferguson, A. (1980)
Experimental Parasitology 49, 9-14

Mach, J.P. & Pahud, J.J. (1971)
Journal of Immunology 106, 552

Mahmoud, G.S. (1978)
PhD Thesis, University of Glasgow

Mahmoud, G.S., Pirie, H.M. (1982)
Zentralblatt fuer Veterinaermedizin Reiche C Anatomica Histologia embryologia 11, 205-212

Mallory, F.B. (1897)
Journal of Experimental Medicine 2, 529

Mallory, F.B. (1900)
Journal of Experimental Medicine 5, 15

Mayrhofer, G., Bazin, H. & Gowans, J.L. (1976)
European Journal of Immunology 6, 537-545

Michel, J.F., Mackenzie, A., Bracewell, C.D., Cornwell, R.L., Elliot, J., Herbert, C.N., Holman, H.H. & Sinclair, I.J.B. (1965)
Research in Veterinary Science 6, 344-395

Michel, J.F. & Parfitt, J.W. (1956)
Veterinary Record 68, 706-710

Michel, J.F. & Shand, A. (1955)
Veterinary Record 67, 14, 249-266

Miller, H.R.P. (1971a)
Laboratory Investigation 24, 5, 339-347

Miller, H.R.P. (1971b)
Laboratory Investigation 24, 5, 348-354

Miller, H.R.P. (1980)
Biology Cellulaire 39, 229-232

Miller, H.R.P., Murray, M. & Jarrett, W.F.H. (1967)
In Reaction of Host to Parasitism Edited by Soulsby, E.L., pp. 198-210, Academic Press, New York

Miller, H.R.P. & Walshaw, R. (1972)
American Journal of Pathology 69, 1, 195-206

Morgan, K.L., Bradley, P. & Bourne, F.J. (1977)
In Respiratory Disease in cattle, Edited by Martin, W.B. pp. 440-451, Martinus Nijhoff - The Hague/London

Morozov, Y.F. (1958)
Helminth Abstracts 30, 1588

Mulligan, W., Urquhart, G.M., Jennings, F.W. & Neilson, J.T.M. (1965)
Experimental Parasitology 16, 341-347

Murray, M. (1968)
Thesis, University of Glasgow

Murray, M. (1972)
In Immunity to Animal Parasites. Edited by Soulsby, E.L., pp. 155-190, Academic Press, New York

Murray, M., Jarrett, W.F.H. & Jennings, F.W. (1971)
Immunology 21, 17-31

Murray, M., Jennings, F.W. & Armour, J. (1970)
Research in Veterinary Science 11, 417-427

Murray, M., Miller, H.R.P. & Jarrett, W.F.H. (1968)
Laboratory Investigation 2, 222-234

Nawa, Y. & Miller, H.R.P. (1979)
Cell Immunology 42, 225

Nelson, A.M.R. (1977)
Veterinary Record 10, 24

Nilsson, S.A. (1963)
Acta Veterinaria Scandinavia 4, Supplement 1, pp. 1-304

Oakley, G.A. (1977)
Veterinary Record 101, 187-188

Oakley, G.A. (1979)
Veterinary Record 104, 460

Oakley, G.A. (1981)
Research in Veterinary Science 30, 255-256

Oakley, G.A. (1982)
British Cattle Association Proceedings for 1982-83, pp. 97-115, Beecham Animal Health

Ogilvie, B.M. & Jones, V.E. (1971)
Experimental Parasitology 29, 138-177

Ogilvie, B.M., MacKenzie, C.D. & Love, R.J. (1979)
American Journal of Tropical Medicine and Hygiene 26, 61

Orr, T.S.C. (1977)
Scandinavian Journal of Respiratory Diseases. Supplement 98

Pasternak, N.I. & Brysin, V.G. (1965)
Veterinariya Moscow 7, 68-69

Pemberton, D.H., White, W.E. & Hore, D.E. (1977)
Australian Veterinary Journal 53, 201-207

Pirie, H.M. (1978)
In Respiratory Diseases in Cattle, Edited by Martins, W.B., pp. 102-116, Martinus Nijhoff, The Hague/Boston

Pirie, H.M. Breeze, R.G., Selman, I.E. & Wiseman, A. (1976)
Veterinary Record 98, 259-260

Pirie, H.M., Doyle, J., McIntyre, W.I.M. & Armour, J. (1971)
In the Pathology of Parasitic Diseases, pp. 91-104, Purdue University Press, Indiana

Pirie, H.M., Petrie, L., Pringle, C.R., Allan, E.M. & Kennedy, G.J. (1981)
Veterinary Record 108, 411-416

Rahko, T. (1969)
Pathologic Veterinaria 6, 244-256

Rahko, T. (1970a)
Acta Veterinaria Scandinavia 11, 219

Rahko, T. (1970b)
Nytt, Mag. Zool. 18, 111-112

Rahko, T. (1972)
Acta Veterinaria Scandinavia 13, 575-584

Reichel, G.Pa. (1939)
Journal of American Veterinary Medical Association 94, 418-420

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

Robinson, J. (1962)
Nature, London 193, 353-354

Ruitenberg, E.J. & Elgersma, A. (1976)
Nature, London 264, 258-260

Ruitenberg, E.J. & Elgersma, A. (1979)
British Journal of Experimental Pathology 60, 246

Ruitenberg, E.J. & Elgersma, A. (1980)
British Journal of Experimental Pathology 61, 285-290

Ruiterberg, E.J., Elgersma, A. & Kruizinga, W. (1979)
International Archives of Allergy and Applied Immunology 60,
302-309

Ruitenberg, E.J., Elgersma, A., Kruizinga, W. & Leenstra, F. (1977)
Immunology 33, 581-587

Scicchitano, R., Husban, A.J. & Cripps, A.W. (1984)
Immunology 52, 529-537

Scott, G.R. (1963)
Journal of Hygiene, Cambridge 61, 193-203

Selman, I.E. (1984)
The Veterinary Annual 24th Issue, Edited by Grunsell, C.S.G. &
Hill, F.W.G., pp. 80-34, Scientechnica, Bristol

Smith, D.E. & Lewis, Y.S. (1957)
Journal of Biophysical and Biochemical cytology 3, 1, 9-14

Smythe, R.H. (1937)
Veterinary Record 39, 49 1221-1232

Sommerville, R.I. (1956)
Australian Veterinary Journal 32, 237-240

Soulsby, E.J.L. (1979)
In Advances in Veterinary Science and Comparative Medicine, Edited
by Brandly, C.A., & Cornelus, C.E. 23, 71-102

Steedman, H.F. (1950)
Quarterly Journal of Microscopical Science 91, 477

Spicer, S.S. & Lillie, R.D. (1961)
Stain Technology 36, 365

Stoll, N.R. (1982)
Journal of Parasitology 15, 147-148

Taichman, N.S. (1970)
Journal of Ultrastructural Research 32, 284-292

Takeuchi, A., Jervis, H.R. & Sprinz, H. (1969)
Anatomical Record 164, 79-100

Taliaferro, W.H. & Sarles, M.P. (1939)
Journal of Infectious Diseases 64, 157-188

Thomas, L.H. (1979)
Booklet 2181, Maff Publications, Middlesex

Toner, P.G. (1965)
Acta Anatomica 61, 321-330

Tokashiki, S., Kawashima, Y., Kudo, N., Hashimoto, Y. & Sugimura, M. (1981)
Japanese Journal of Veterinary Science 43, 725-732

Trump, B.J., Smuckler, E.A. & Benditt, E.P. (1961)
Journal of Ultrastructural Research 5, 343

Turner, A.W. & Trethewie, E.R. (1961)
Australian Veterinary Journal 37, 1-8

Uber, C.L., Roth, R.L. & Levy, D.A. (1980)
Nature, London 287, 226-228

Urquhart, G.M., Jarrett, W.F.H., McIntyre, W.I.M., Poynter, D. & Peacock, R. (1973)
In Helminth Diseases of Cattle, Sheep and Horses in Europe, Edited by Urquhart, G.M. & Armour, J. Part 1, pp. 23-31

Wakelin, D. (1984)
In Immunity to Partasites, pp. 1-27 Edward Arnold (Publishers) Ltd., London

Wasserman, S.I. (1979)
In The Mast Cell, Its Role in Health and Disease, Edited by Pepys, J. and Edwards, A.M. Proceedings of an International Symposium, Davos, Switzerland, pp. 9-20, Pitmans (Publishing) Ltd., London

Watson, M.L. (1958)
Journal of Biophysical and Biochemical Cytology 4, 475

Weill, P. (1919)
Archives of the Mikroskopic Anatomy 93, 1-81 + 2 plates

Weill, P. (1920)
Archives of the Micrscopic Morphology 17, 77-82

Weil, A.J. & Reddin, L. Jr. (1943)
Journal of Immunology 47, 345-352

Whur, P. (1966)
Journal of Comparative Pathology 76, 57-65

Whur, P. (1967)
Journal of Comparative Pathology 77, 271-277

Whur, P. & Gracie, M. (1967)
Expenrienta 23, 655-657

Whur, P. & Johnston, H.S. (1967)
Journal of Pathology & Bacteriology 93, 81-85

Whur, P. & White, R.G. (1970)
International Archive of Allergy 38, 185-195

Wiseman, A., Msolla, P.M., Selman, I.E., Allan, E.M. & Pirie, H.M. (1980)
Veterinary Record 107, 436-441

Woodbury, R.G. & Miller, H.R.P. (1982)
Immunology 46, 487

Woodbury, R.G., Miller, H.R.P., Huntley, J.F., Newlands, G.F.J., Palliser, A.C. & Wakelin, D. (1984)
Nature 312, 450-452

Woodbury, R.G. & Neurath, H. (1978)
Biochemistry 17, 20, 4298-4303

Zipper, J. (1966)
Zentralblatt Für Veterinar Medizin Reihe 13A, 329-336