

Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.

EPITHELIAL DEVELOPMENT IN THE FORESTOMACH
OF PASTURE-FED LAMBS (BIRTH TO 8 WEEKS)

A thesis presented in partial
fulfilment of the requirements
for the degree of

Master of Philosophy in Veterinary Science

at

Massey University
New Zealand

KENNETH YERRO ILIO
1983

68550_12

ABSTRACT

The histology, mitotic index, ultrastructure and Na⁺-K⁺-ATPase cytochemistry of lambs reared on pasture were studied during the period of weaning.

Two embalmed mature non-pregnant Romney cross-bred ewes and thirty Romney-cross lambs reared on Massey University sheep farms pastures were used. Five lambs (three in 1981 and two in 1982) were taken from their dams on pasture at each of the following respective ages: within 24 hours of birth, and at 12, 23, 34, 45 and 56 days. Stomach-tissue samples from 1 adult and from the lambs reared during the 1981 lambing season were prepared for histology using Haematoxylin and Eosin, Masson's green trichrome, Periodic-acid-Schiff and Toluidine Blue stains, and for conventional transmission electron microscopy. Tissue samples from the rumens of lambs reared during the 1982 season were used for strontium-capture technique Na⁺-K⁺-ATPase cytochemistry.

Gross dissection of the stomach of one-day-old lambs confirmed that the largest compartment at birth is the abomasum, followed, in decreasing order of size, by the rumen, reticulum and omasum. Progressive development resulted in the forestomach compartments assuming their adult proportions by 56 days of age.

Preliminary histological studies of epithelium taken from the rumen, reticulum, omasum and reticular groove of the adult sheep confirmed it to be a stratified keratinizing epithelium. Five general cell layers were clearly seen: stratum basale, stratum spinosum, stratum granulosum, stratum transitionale and stratum corneum. (In previous studies, the stratum granulosum and the stratum transitionale have been considered as one layer.) Mucopolysaccharides were located in the inter-cellular spaces in the stratum corneum.

Examination of the forestomach mucosa

revealed a number of changes between birth and 56 days of age: (1) an increase in papillary length; (2) starting from 23 days, the development of extensive papillary process-epithelial bulb interactions accompanied by proliferation of blood vessels in the papillary processes; (3) a decrease in epithelial thickness for the first 45 days of age; (4) the appearance of a complete layer of transitional cells by 45 days; (5) the disappearance of glycogen from the epithelium; (6) an increase in the amount of mucopolysaccharide in the intercellular spaces in the stratum corneum; (7) the increase in the number of non-keratinocytes in the basal layer; and (8) the appearance of apoptotic bodies due to single cell death in the basal layer of the epithelium from 45 days of age.

The mitotic index of the epithelium in developing lambs decreased from birth until 23 days of age, had increased at 34 days, but decreased again between 45 and 56 days.

Examination of the ultrastructure of the adult epithelium provided general results consistent with previous studies. However, gap junctions were found in the stratum basale, stratum spinosum and were extensive in the stratum granulosum. Tight junctions (zonulae occludentes) were seen between the cells of the stratum corneum.

Langerhans cells and mast cells/globule leukocytes were classified as non-keratinizing cells. Other non-keratinocytes recognised were the 'indeterminate cells', lymphocyte-like cells and cells similar to Merkel cells.

Completely keratinized cells appeared in the epithelium at about 12 days of age, in association with the increased production of tonofilaments, keratohyalin granules, endoplasmic reticulum protein and membrane-coating granules. Proliferation of mitochondria in the basal layers started at about 12 days of age, and glycogen deposits in the intermediate layers were not found after this age.

Increased folding of the basolateral membrane surfaces of basal cells and progressive thinning of the endothelium of sub-epithelial blood vessels were also observed. Gap junctions in the stratum granulosum became progressively more obvious between birth and 56 days of age. Annular gap junctions were also found.

Na⁺-K⁺-ATPase enzymatic sites were identified from 12 days of age, on the cytoplasmic membranes of lower granular, spinous and basal cells: the reaction products being localised on both the cytoplasmic and inter-cellular-space surfaces of the plasma membranes. Ouabain inhibited the formation of deposits only on the cytoplasmic side. Alkaline phosphatase activity was localised in the stratum corneum. Mg⁺⁺-ATPase was demonstrated in the stratum corneum, the intercellular spaces in the stratum granulosum and stratum transitionale, and in mitochondria.

It is concluded that the ultrastructural features and Na⁺-K⁺-ATPase cytochemistry of the epithelium at 12 days of age appeared to be similar to those found in older animals. However, structural (and presumably functional) maturity did not appear to be complete until after 45 days of age at which stage the stratum transitionale had become complete and the mitotic index and the thickness of the epithelium had become stable. The increase of non-keratinocytes suggests the increasing immunocompetence of the epithelium.

Tight junctions and extruded contents of membrane-coating granules in the intercellular spaces of the stratum corneum could provide a barrier to the diffusion of solutes across the epithelium. The development of gap junctions and the presence of Na⁺-K⁺-ATPase enzymatic sites in the membranes are consistent with the absorptive and transport functions of the epithelium, particularly the active transepithelial movement of sodium ions. Future studies could well show hormones, hormone-like substances and antibiotics to be important in the development of the forestomach epithelium in ruminants.

ACKNOWLEDGEMENTS

I wish to thank the governments of New Zealand and the Republic of the Philippines for giving me the opportunity to study for this degree.

My sincere gratitude is also extended to Professor R. E. Munford for providing the opportunity and facilities to undertake the study and to my supervisor, Dr. E. J. Kirk for his advice and assistance during this study.

I would also like to thank Mr. P. Whitehead for his willing help with the supply of animals, as well as Mr. Bill Morris, Mr. Doug Stewart and Mr. Matthew Ratumaitavuki for assisting in the collection of the lambs.

Thanks are also extended to Mrs. Pam Slack and Mr. Doug Hopcroft for their assistance and advice in the electron microscope work; to Mr. Mervyn Eirtles, Mrs. Irene Hall and Mr. Roy Sparkman for their help in the preparation of slides for histology; to Mr. Neil Ward for the supply of materials; to Mr. Tom Law for allowing the use of his darkroom and to Mr. Walt Abel for the use of his computer.

Thanks are also given to T. C. Balassu and P. D. Buckley for their support during the preparation of this thesis.

CONTENTS

| | <u>Page No.</u> |
|--|-----------------|
| Abstract | ii |
| Acknowledgements | v |
| List of Contents | vi |
| List of Figures | x |
| List of Tables | xii |
| INTRODUCTION | 1 |
| CHAPTER I - <u>REVIEW OF LITERATURE</u> | 6 |
| 1.1 Early Studies on the Ruminant Stomach | 6 |
| 1.2 Functional Anatomy of the Ruminant Stomach | 8 |
| 1.2.1 Gross anatomy | 10 |
| 1.2.1.1 The rumen and reticulum | 10 |
| 1.2.1.2 The omasum | 12 |
| 1.2.1.3 The abomasum | 14 |
| 1.2.2 Forestomach mucosal form and architecture | 14 |
| 1.2.2.1 Rumen | 15 |
| 1.2.2.2 Reticulum | 17 |
| 1.2.2.3 Omasum | 17 |
| 1.2.3 Blood supply and drainage, lymphatics and innervation | 18 |
| 1.2.3.1 Arterial supply | 18 |
| 1.2.3.2 Venous drainage | 19 |
| 1.2.3.3 Lymphatics | 19 |
| 1.2.3.4 Innervation | 19 |
| 1.3 Histology | 20 |
| 1.3.1 Rumen | 20 |
| 1.3.2 Reticulum, reticular groove and omasum | 22 |
| 1.3.3 Abomasum | 23 |
| 1.4 Embryology | 23 |
| 1.4.1 Organogenesis | 23 |
| 1.4.2 Histogenesis | 25 |

| | | |
|---|---|----|
| 1.5 | Functional Organisation of the Forestomach Epithelium | 27 |
| 1.5.1 | The ultrastructure of the forestomach epithelium | 28 |
| 1.5.2 | Proposed model for transport pathways in the epithelium | 31 |
| 1.5.3 | Histochemistry | 33 |
| 1.5.4 | Epithelial cell differentiation | 34 |
| | 1.5.4.1 Keratinization | 34 |
| | 1.5.4.2 Mitotic index | 35 |
| 1.6 | Establishment of the Ruminant State | 37 |
| 1.6.1 | Anatomical development | 39 |
| 1.6.2 | Histological development | 40 |
| 1.6.3 | Physiological development | 42 |
| | 1.6.3.1 Absorption and transport of solutes | 43 |
| | 1.6.3.2 Metabolism | 44 |
| CHAPTER II - <u>MATERIALS AND METHODS</u> | | 47 |
| 2.1 | Animals | 47 |
| 2.2 | Gross Dissection | 47 |
| 2.3 | Tissue Sampling | 48 |
| 2.4 | Histological Techniques | 49 |
| 2.5 | Electron Microscopic Techniques | 50 |
| | 2.5.1 Conventional electron microscopy | 50 |
| | 2.5.2 Scanning electron microscopy | 52 |
| 2.6 | Mitotic Index | 53 |
| 2.7 | Na ⁺ -K ⁺ -ATPase Cytochemistry | 53 |
| 2.8 | Illustrations | 55 |
| 2.9 | Statistical Methods | 55 |
| CHAPTER III - <u>RESULTS</u> | | 56 |
| 3.1 | Gross Dissection | 56 |
| 3.2 | Histology | 58 |
| | 3.2.1 General observations | 58 |
| | 3.2.2 Histological appearance at different ages | 61 |
| | 3.2.2.1 Birth to 24 hours | 61 |
| | 3.2.2.2 12 days | 62 |
| | 3.2.2.3 23 days | 63 |

| | <u>Page No.</u> |
|--|-----------------|
| 3.2.2.4 34 days | 63 |
| 3.2.2.5 45 days | 64 |
| 3.2.2.6 56 days | 64 |
| 3.2.3 Changes in the number of layers of the epithelium in different ages | 65 |
| 3.3 Mitotic Index | 65 |
| 3.4 Electron Microscopy | 66 |
| 3.4.1 General observations | 66 |
| 3.4.2 Observations on the cytology of epithelial cells | 67 |
| 3.4.2.1 Cells of the stratum basale | 67 |
| 3.4.2.2 Cells of the stratum spinosum | 70 |
| 3.4.2.3 Cells of the stratum granulosum | 71 |
| 3.4.2.4 Cells of the stratum transitionale | 72 |
| 3.4.2.5 Cells of the stratum corneum | 73 |
| 3.4.3 Observations on the ultrastructure of the epithelium in different ages | 74 |
| 3.4.3.1 Birth to 24 hours | 74 |
| 3.4.3.2 12 days | 74 |
| 3.4.3.3 23 and 34 days | 77 |
| 3.4.3.4 45 and 56 days | 78 |
| 3.5 Na ⁺ -K ⁺ -ATPase Cytochemistry | 78 |
| 3.5.1 Light microscopy | 78 |
| 3.5.2 Electron microscopy | 79 |
| CHAPTER IV - <u>DISCUSSION</u> | 81 |
| 4.1 Classification and Nomenclature | 81 |
| 4.2 Organisation of the Forestomach Mucosa and Epithelium as Related to their Functions | 87 |
| 4.3 Keratinization | 91 |
| 4.4 Epithelial Non-keratinocytes | 94 |
| 4.4.1 Langerhans cells | 94 |
| 4.4.2 Indeterminate cells | 97 |
| 4.4.3 Intraepithelial lymphocytes | 97 |
| 4.4.4 Mast cells and globule leukocytes | 98 |
| 4.4.5 Other non-keratinocytes | 99 |

| | | |
|-------|--|-----|
| 4.5 | Structural Changes from Birth to 56 days of Age | 102 |
| 4.5.1 | Gross anatomical development of the forestomach | 102 |
| 4.5.2 | Gross and histological development of the forestomach mucosa | 102 |
| 4.6 | Mitotic Index | 110 |
| 4.7 | Ultrastructural Features of the Epithelium during Development | 113 |
| 4.8 | Na ⁺ -K ⁺ -ATPase Cytochemistry | 118 |
| | CHAPTER FIVE - <u>CONCLUSIONS</u> | 124 |
| | APPENDIX I | 128 |
| | APPENDIX II | 129 |
| | APPENDIX III | 132 |
| | APPENDIX IV | 135 |
| | APPENDIX V | 137 |
| | REFERENCES | 139 |

LIST OF FIGURES

| <u>Figure No.</u> | <u>TITLE</u> | <u>Between Pages</u> |
|-------------------|--|----------------------|
| 1.1 | Diagrammatic representation of the stomach of the sheep | 10 - 11 |
| 2.1 | Tissue sampling sites | 49 - 50 |
| 3.1 | Diagrammatic representation of the stomach of the adult sheep; right side | 56 - 57 |
| 3.2 | Reticulo-rumen of sheep to show position of internal structures | 57 - 58 |
| 3.3 | Diagrammatic representation of the stomach of a one-day-old lamb (actual size). | 58 - 59 |
| 3.4 | Scanning electron micrograph of the abnormal structure found in the reticulum of a one-day-old lamb killed in 1982 | 58 - 59 |
| 3.5 | Light micrographs of papillae taken from different locations in the forestomach of the adult | 59 - 60 |
| 3.6 | Light micrographs of epithelia taken from the forestomach of the adult | 60 - 61 |
| 3.7 | Light micrographs of the forestomach mucosa taken from one-day-old lambs | 61 - 62 |
| 3.8 | Light micrographs of the ruminal mucosa taken from 12-day-old lambs | 62 - 63 |
| 3.9 | Light micrographs of the ruminal mucosa taken from 23-day-old lambs | 63 - 64 |
| 3.10 | Light micrographs of the ruminal mucosa taken from 34-day-old lambs | 63 - 64 |
| 3.11 | Light micrographs of the ruminal mucosa taken from 45-day-old lambs | 64 - 65 |
| 3.12 | Light micrographs of the ruminal mucosa taken from 56-day-old lambs | 64 - 65 |
| 3.13 | Mean mitotic indices in the ruminal epithelium in different ages | 65 - 67 |
| 3.14 | Semi-diagrammatic representation of the ruminant forestomach epithelium | 66 - 67 |
| 3.15 | Electron micrograph of the epithelium taken from the forestomach of the adult sheep, stratum basale. | 67 - 68 |
| 3.16 | Electron micrographs of the forestomach epithelium taken from the adult. Stratum Basale | 68 - 69 |

| | | |
|------|---|-----------|
| 3.17 | Electron micrographs of the epithelium taken from the forestomach of the adult sheep. Stratum Basale. | 69 - 70 |
| 3.18 | Electron micrographs of the epithelium taken from the forestomach of the adult sheep. Stratum Spinosum. | 70 - 71 |
| 3.19 | Electron micrographs of the epithelium taken from the forestomach of the adult sheep. Stratum Granulosum. | 71 - 72 |
| 3.20 | Electron micrographs of the epithelium taken from the forestomach of the adult sheep. Stratum Transitionale. | 72 - 73 |
| 3.21 | Electron micrographs of the epithelium taken from the forestomach of the adult sheep. Stratum Corneum. | 73 - 74 |
| 3.22 | Electron micrographs of the epithelium taken from one-day-old lambs | 74 - 75 |
| 3.23 | Electron micrographs of the epithelium taken from the forestomachs of 12-day-old lambs | 76 - 77 |
| 3.24 | Electron micrographs of the epithelium taken from the forestomachs of 23- and 34-day-old lambs. | 77 - 78 |
| 3.25 | Electron micrographs of the epithelium taken from the forestomachs of 45- and 56-day-old lambs. | 78 - 79 |
| 3.26 | Na ⁺ -K ⁺ -ATPase cytochemistry of the ruminal epithelium. Complete Medium. | 78 - 79 |
| 3.27 | Na ⁺ -K ⁺ -ATPase cytochemistry of the ruminal epithelium. Controls. | 78 - 79 |
| 4.1 | Schematic representation of three models for transepithelial volume flow. | 87 - 88 |
| 4.2 | Schematic representation of the transport model for Na ⁺ based on the results of the present study and in accord with the model proposed by Mills, Ernst and DiBona (1977) | 122 - 123 |

LIST OF TABLES

| <u>Table No.</u> | TITLE | <u>Between Pages</u> |
|------------------|---|----------------------|
| I | Normal values of mitotic index of the ovine ruminal epithelium | 36 - 37 |
| II. | Proportions of the compartments of the stomach of grazing lambs as percentages of weight of the whole stomach | 40 - 41 |
| III | Number of light-staining cells in the forestomach epithelium in selected ages expressed as a percentage against the number of basal cells | 65 - 66 |
| IV | Number of individual cell layers in the ruminal epithelium in different ages | 65 - 66 |
| V | Mitotic Indices (%) of the ruminal epithelium taken from pairs of lambs at different ages | 65 - 66 |