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

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## ABSTRACT

The histology, mitotic index, ultrastructure and Na<sup>+</sup>-K<sup>+</sup>-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<sup>+</sup>-K<sup>+</sup>-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<sup>+</sup>-K<sup>+</sup>-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<sup>++</sup>-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<sup>+</sup>-K<sup>+</sup>-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<sup>+</sup>-K<sup>+</sup>-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.

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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 <sup>+</sup> -K <sup>+</sup> -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 <sup>+</sup> -K <sup>+</sup> -ATPase Cytochemistry	78
3.5.1 Light microscopy	78
3.5.2 Electron microscopy	79
 <u>CHAPTER IV - 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 <sup>+</sup> -K <sup>+</sup> -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 <sup>+</sup> -K <sup>+</sup> -ATPase cytochemistry of the ruminal epithelium. Complete Medium.	78 - 79
3.27	Na <sup>+</sup> -K <sup>+</sup> -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 <sup>+</sup> 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