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CAMPYLOBACTER INFECTION IN INTESTINAL ORGAN CULTURES

A thesis presented in partial fulfilment (50%) of the requirements for the degree of Master of Philosophy in Veterinary Pathology at Massey University Palmerston North New Zealand

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ABSTRACT

Six different media (T199 medium, T199 + 10% foetal calf serum, T8 medium, T8 + 10% foetal calf serum, RPMI medium and RPMI + 10% foetal calf serum) were tested for their ability to maintain foetal lamb intestine in organ culture. T199 medium + 10% foetal calf serum was chosen because it gave more consistent results in maintaining the foetal intestine for a period of six days in culture.

Two groups of foetal lamb intestine were cultured, a control group and a group infected with <u>Campylobacter</u> jejuni. The effects of the microorganisms on the intestinal culture were assessed at 6 hours, 13 hours and 15 hours post-culture.

Light, Transmission and Scanning Electron Microscopy were used to study the pathogenicity of <u>C.jejuni</u> at the cellular level. Light microscopic studies showed that <u>C.jejuni</u> were attached and colonised the tips of the villi and the crypt epithelium of the intestinal cultures at 6 hours, 13 hours and 15 hours. The epithelial cells showed marked necrosis at the tips of the villi. The microorganisms also invaded the cytoplasm of epithelial cells of the villi and the intestinal crypt.

Transmission Electron Microscopy revealed degeneration of the microvilli in the infected cultures. The microorganisms were found attached to the tips of the microvilli of the villous epithelial cells by pilus-like structure. Microorganisms were present within phagolysosomes of macrophages in the lamina propria. Various cytoplasmic changes were observed at 6, 13 and 15 hours post-infection.

Scanning Electron Microscopy confirmed the different changes in the morphology of the infected epithelial cells. The microorganisms were observed adhering to the surface of the epithelial cells at 6 , 13 and 15 hours post-culture.

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TABLE OF CONTENTS

Abstract	ii
Acknowledgements	iii
Table of contents	iv-x
List of tables	xi
List of figures	xii-xxviii
INTRODUCTION	1-3
CHAPTER 1	4-32
LITERATURE REVIEW	
1.1 ORGAN CULTURE	4-12
1.1.1 Types of Organ Culture	5-6
1.1.1.1 culture of mature differentiated organs	5-6
1.1.1.2 <u>culture of embryonic organs</u>	6
1.1.2 Technical Development of Organ Culture	7-9
1.1.2.1 The Hanging drop method	7
1.1.2.2 <u>Maximow's double cover slip method</u>	7-8
1.1.2.3 Roller tube method	8
1.1.2.4 Watch glass method	8-9

1.1.2.5 Trowell's method

1.1.3 Applications of Organ Culture in Virology, Microbiology 10-12 and Toxicology.

1.1.4 Reason for the use of Organ Culture 12 **1.2 INTESTINAL ORGAN CULTURE** 13 - 201.2.1 Research Applications of Intestinal Organ Culture 13 - 1713 - 141.2.1.1 Coeliac disease 1.2.1.2 Ulcerative colitis 14 14 - 151.2.1.3 Cystic fibrosis 1.2.1.4 Studies of carcinogenesis 15 16 - 171.2.1.5 Viruses 17-19 1.2.2 Techniques used for Intestinal Organ Culture 1.2.3 Reason for using Foetal Lamb Intestinal Organ Culture. 20 **1.3 CAMPYLOBACTERIOSIS** 1.3.1 Significance of <u>C.jejuni</u> Infection in Sheep in New Zealand. 22 22-26 1.3.2 Pathogenesis of Campylobacter species. 22 - 241.3.2.1 Sheep 24 1.3.2.2 Calves 25 1.3.2.3 Pigs

1.3.2.4 <u>Dogs</u>	25-26
1.3.2.5 <u>Humans</u>	26
1.3.3. Pathogenicity of <u>Campylobacter</u> species.	26-32
1.3.3.1 Attachment and colonization	27
1.3.3.2 <u>Toxins</u>	27-28
1.3.3.2.1 Endotoxins	28-29
1.3.3.2.2 Enterotoxins	30
1.3.3.2.3 Cytotoxin	30
1.3.3.3 Invasion	30-32
CHAPTER II	33-39
MATERIALS AND METHODS	
2.1 COLLECTION OF FOETUSES	33
2.2 RECOVERY OF FOETAL LAMB INTESTINE FOR ORGAN CULTURE	33
2.3 CULTURE MEDIA	33-34
2.4 SELECTION OF CULTURE CONDITIONS AND MEDIA.	34
2.5 PREPARATION OF THE TEST ORGANISM <u>CAMPYLOBACTER</u> <u>JEJUNI</u> .	34-35
2.6 INTERACTION OF <u>C.JEJUNI</u> WITH FOETAL LAMB INTESTINAL	35-38

2.6.1 Light Microscopy	36
2.6.2 Scanning Electron Microscopy	37
2.6.3 Transmission Electron Microscopy	37-38
2.7 STATISTICAL METHODS	38-39
CHAPTER III	40-55
RESULTS	
3.1 SELECTION OF A SUITABLE MEDIUM FOR OVINE INTEST ORGAN CULTURE.	INAL 40-42
3.1.1 The effect of different Media on the Morphology	of 40-42
primary Intestinal Organ Culture.	
3.1.1.1 T199.	40
3.1.1.2 T199 with 10% foetal calf serum.	41
3.1.1.3 Trowell's (T8)	41
3.1.1.4 Trowell's medium with 10% foetal calf serum.	41
3.1.1.5 RPMI	41-42
3.1.1.6 RPMI with 10% foetal calf serum.	42
3.2 STUDIES OF SOME ASPECTS OF PATHOGENESIS AND PATHOG	ENICITY OF 42

<u>CAMPYLOBACTER</u> JEJUNI USING OVINE FOETAL INTESTINAL ORGAN

CULTURE AS A MODEL.

3.2.1 Light Microscopy	42-46
3.2.1.1 Control	43-44
3.2.1.1.1 Zero time	43
3.2.1.1.2 <u>Six hours</u>	43-44
3.2.1.1.3 Thirteen hours	44
3.2.1.1.4 <u>Fifteen hours</u>	44
3.2.1.2 Infected.	44-46
3.2.1.2.1 <u>Six hours</u>	44-45
3.2.1.2.2 Thirteen hour	45
3.2.1.2.3 Fifteen hours	45-46
3.2.2 Transmission Electron Microscopy.	46-49
3.2.2.1 Control	46-47
3.2.2.1.1 Six hours	46
3.2.2.1.2 Thirteen hours	46
3.2.2.1.3 Fifteen hours	47
3.2.2.2 Infected	47-49
3.2.2.1 Six hours	47_48

3.2.2.2 Thirteen hours	48
3.2.2.3 Fifteen hours	48-49
3.2.3 Scanning Electron Microscopy	49-52
3.2.3.1 Control	49-50
3.2.3.1.1 <u>Six hours</u>	49-50
3.2.3.1.2 Thirteen hours	50
3.2.3.1.3 Fifteen hours	50
3.2.3.2 Infected	50-52
3.2.3.2.1 <u>Six hours</u>	50-51
3.2.3.2.2 Thirteen hours	51
3.2.3.2.3 Fifteen hours	51-52
3.3 STATISTICAL RESULTS.	52-55
CHAPTER IV	56-67
DISCUSSION AND CONCLUSION	
4.1 SELECTION OF MEDIUM	57-59
4.2 PATHOGENICITY OF <u>C.JEJUNI</u> IN SHEEP	59-67
4.2.1 Morphological Changes	60-64

4.2.2 Attachment and Penetration of $\underline{C.jejuni}$ into the epithelial

cells of foetal lamb intestine.	64-67
BIBLIOGRAPHY	68-91
APPENDICES	92-94

Table

- 1.1. Techniques used by other workers for intestinal 19 organ culture, the types of medium used, the period of survival, and the types of species investigated.
- 1.2. Association of various <u>Campylobacter</u> species with 23 different animal species, the clinical symptoms and site of infection.
- 1.3. Limulus gelatin assay and endotoxin concentration 29 in suspensions of <u>Campylobacter</u> species and E.coli.
- 3.1. The effects of <u>Campylobacter</u> jejuni on the height 53 of villi at 6, 13 and 15 hours post-infection.
- 3.2. The effects of <u>Campylobacter</u> jejuni on the width 54 and height of epithelial cells of the villi of control and infected tissues at Zero hours, 6 hours, 13 hours and 15 hours post-infection.

(xii)

LIST OF FIGURES

Figure

Following Page

- 2.1. Sterilised plastic petridish (90 X 15 mm) with 33 phosphate buffered saline (PBS) (pH 7.4) at room temperature, containing pieces of foetal lamb intestine prepared for organ culture.
- 2.2. Organ culture dish (60 X 15 mm) with a piece of 33 foetal lamb intestine.
- 2.3. Modular incubator chamber showing organ culture 34 dish containing pieces of foetal lamb intestine in T199 medium + 10% foetal calf serum.
- 3.1. Foetal lamb intestine after 3 days culture in T199 40 medium. The villi (v) are dome-shaped and are lined by cuboidal epithelial cells (C). Their nuclei (N) are oval to round and occupy the base of the cells. (H&E X200).
- 3.2. Foetal lamb intestine after 3 days culture in T199 40 medium .The epithelial lining cells are squamous in appearance (C). Their nuclei are elongated to ovoid in shape and occupy most of the cytoplasm. (H & E X200).

- 3.3. Foetal lamb intestine after 6 days culture in T199 41 medium + 10% foetal calf serum. The epithelial lining cells (C) are well preserved. The absorptive cells (C) are columnar with oval-shaped nuclei occupying the base. The intestinal crypts (Cr) seen here are well developed. (H & E X200).
- 3.4. Foetal lamb intestine after 3 days culture in 41 Trowell's medium (T8). The villi (V) are shorter and wider than normal and have a dome-shaped appearance. The enterocytes (C) are more cuboidal towards the apical part of the villi. The intestinal crypts (Cr) are smaller than in uncultured intestinal tissue. (H & E X200).
- 3.5. Foetal lamb intestine after 6 days culture in 41 Trowell's medium (T8) + 10% foetal calf serum. The villi (V) have an uneven surface and are reduced in height. The enterocytes (C) are cuboidal to round in shape and show a number of vacuoles (small arrow) in the apical part of the cytoplasm. (H & E X200).
- 3.6. Foetal lamb intestine after 3 days culture in 42 RPM1. The villi (V) are tall and and thin with an irregular surface (arrow). The epithelial cells (C) are squamous with small round nuclei occupying most of the cytoplasm. The intestinal crypts (Cr) are shallower and narrower than normal. (H & E X200).

- 3.7. Foetal lamb intestine after 6 days culture in RPM1 + 10% foetal calf serum. The villi (V) are severely stunted and have a dome-shaped appearance. The absorptive cells (C) are round with uneven surfaces. Their nuclei (N) are round and occupy the entire cytoplasm. (H & E X200).
- 3.8. Uncultured foetal lamb intestine from a 98 day old 43 foetus. The villi (V) are tall and pointed with prominent microvilli (MV). The epithelial lining cells (C) are columnar with an even striated border, and oval elongated nuclei (N) occupying their bases. (H & E X400).
- 3.9. Morphology of an intestinal gland from uncultured 43 foetal lamb tissue. The intestinal glands (Gl) are healthy and lined by simple columnar cells (C). (H & E X400).
- 3.10. Control foetal lamb intestine after 6 hours 43 culture in T199 medium + 10% foetal calf serum. The morphology of the intestinal villi (V) is well preserved. The epithelial lining cells (C) are columnar with round to elongate nuclei (N). The intestinal crypts (Cr) are well maintained and lined with simple columnar cells. (H & E X200).
- 3.11. Cross section of control foetal lamb intestine 43 after 6 hours culture in T199 medium + 10% foetal calf serum. The villous epithelium (V) is well preserved. The absorptive lining cells (C) are simple columnar in shape, with round to elongate nuclei (N). (H & E X400).

(xiv) Page

- 3.12. Control foetal lamb intestine after 6 hours 4 culture in T199 medium + 10% foetal calf serum. The intestinal glands (Gl) are well maintained and lined by simple columnar epithelium (C). (H & E X400).
- 3.13. Control foetal lamb intestine after 13 hours 44 culture in T199 medium + 10% foetal calf serum. The villous epithelial cells (C) are intact and well preserved. The epithelial lining cells (C) are columnar to cuboidal in shape with round to elongate nuclei (N). Some of the absorptive cells (C) have cytoplasmic vacuoles (arrow). (H & E X400).
- 3.14. Control foetal lamb intestine after 15 hours 44 culture in T199 medium + 10% foetal calf serum. The mucosal epithelium is well preserved. (H & E X200).
- 3.15. Foetal lamb intestine cultured in T199 medium + 44 10% foetal calf serum, infected with approximately 10⁶ CFU/ml of <u>Campylobacter jejuni</u>, showing changes that have occurred 6 hours postinfection . Mild necrosis (arrow) can be seen at the tips of the villi, with a slight shedding of epithelial cells. The microorganisms (MO) are attached to the surface of the villous and crypt epithelia (long arrow). (Warthin Starry Stain X200).
- 3.16. Foetal lamb intestine cultured in T199 medium + 44 10% foetal calf serum, infected with approximately 10⁶ CFU/ml of <u>C.jejuni</u>, showing changes that have

occurred 6 hours postinfection .The epithelial cells at the tips of the villi are disorganised (arrow) and show exfoliation. Some also show cytoplasmic vacuolation (small arrow). (H & E + Warthin Starry Stain X200).

- 3.17. Foetal lamb intestine cultured in T199 medium + 10% foetal calf serum, infected with approximately 10⁶ CFU/ml of <u>C.jejuni</u>, showing changes that have occurred 6 hours postinfection. The epithelial cells (C) at the tips of the villi are slightly damaged. A few of the epithelial cells (C) show cytoplasmic vacuolation (arrow), nuclear swelling (NS), and chromatin margination (CM). (H & E + Warthin Starry Stain X200).
- 3.18. Foetal lamb intestine cultured in T199 medium + 10% foetal calf serum, infected with approximately 10⁶ CFU/ml of <u>C.jejuni</u>, showing changes that have occurred 13 hours postinfection. The cells at the tips of the villi show a moderate necrosis. Spiral to rod-shaped organisms (MO) can be seen attached to the villous epithelial surface. A few organisms can also be seen within the epithelial cells (thin arrow). (Warthin Starry Stain X200).
- 3.19. Foetal lamb intestine cultured in T199 medium + 10% foetal calf serum, infected with approximately 10⁶ CFU/ml of <u>C.jejuni</u>, showing changes that have occurred 13 hours postinfection. The necrotic epithelial cells (C) are being shed from the tips of the villi. The infected cells are round with swollen nuclei (N). The uninfected epithelial lining cells (small arrow) appear unchanged. (H &

(xvi) Page

44

45

E + Warthin Starry Stain X200)

- 3.20. Foetal lamb intestine cultured in T199 medium + 10% foetal calf serum, infected with approximately 10⁶ CFU/ml of <u>C.jejuni</u>, showing changes that have occurred 13 hours postinfection. The infected epithelial cells show moderate vacuolation (arrow) and nuclear swelling (NS). The microorganisms (MO) are attached to the external surface of the villi and the crypt epithelium . (Warthin Starry Stain X200).
- 3.21. Higher magnification of Figure 3.20, showing the morphology of the epithelial cells 13 hours postinfection. The absorptive cells (C) show cytoplasmic vacuolation (V) and necrosis at the tips of the villi. Microorganisms (MO) can be observed attached to the epithelial cells. (Warthin Starry Stain X400).
- 3.22. Foetal lamb intestine cultured in T199 medium + 10% foetal calf serum, infected with approximately 10⁶ CFU/ml of <u>C.jejuni</u>, showing changes that have occurred 15 hours postinfection. Severe necrosis and exfoliation of the epithelial cells (C) can be seen at the tips of the villi. Spiral microorganisms (MO) appear to be colonising the external surface of the cells. (Warthin Starry Stain X200).
- 3.23. Foetal lamb intestine cultured in T199 medium + 10% foetal calf serum, infected with approximately 10⁶ CFU/ml of C.jejuni, showing changes that have

45

45

46

occurred 15 hours postinfection . The infected cells (C) have marked cytoplasmic vacuolation (arrow). Severe exfoliation of the necrotic cells (NC) can be seen. (Warthin Starry Stain X200).

- 3.24. Control foetal lamb intestine after 6 hours 46 culture in T199 medium + 10% foetal calf serum, showing the brush border of the intestinal epithelial cells. The normal structure of the microvilli (MV) can be observed and the fuzzy coat of the glycocalyx is clear (arrow). (TEM X48,600).
- 3.25. Control foetal lamb intestine after 6 hours 46 culture in T199 medium + 10% foetal calf serum, showing well preserved epithelial cells (C), nuclei (N), microvilli (MV), terminal web (TW), junctional complex (JC), dense supranuclear mitochondria (M), rough endoplasmic reticulum (ER) and occasional golgi apparatus (G). (TEM X11,200).
- 3.26. Ultrastructure micrograph of control foetal lamb 46 intestine cultured for 6 hours in T199 medium + 10% foetal calf serum showing an occasional microvesicle (MV) under the terminal web and large digestive vacuoles (DV). A few dense lysosomes (L) are present. (TEM X11,200).
- 3.27. Control foetal lamb intestine after 13 hours 46 culture in T199 medium + 10% foetal calf serum. The epithelial cells show a well preserved brush border (arrow), prominent lysosomal organelles (L) in the apical cytoplasm and multifocal aggregations of glycogen granules (Gl). (TEM X13,500).

Figure

46

47

- 3.28. Ultrastructure micrograph of control foetal lamb intestine after 13 hours culture in T199 medium + 10% foetal calf serum. The microvilli (MV) are uniform in length and well preserved. The terminal webs (TW) and mitochondria (M) can be observed. (TEM X31,800).
- 3.29. Control foetal lamb intestine after 15 hours 47 culture in T199 medium + 10% foetal calf serum. The microvilli (MV) are more numerous and closely packed than after 6 hours and 13 hours culture. Occasional lysosomal-like structures (L) are present in the apical and supranuclear cytoplasm. Moderate numbers of microvesicles (Mv) are present. Many glycogen granule aggregations (Gl) can be observed. (TEM X21,200).
- 3.30. Higher magnification of Figure 3.29 showing the microvillous coat (glycocalyx) (Gx). Free ribosomes (R) are present in the apical portion of the cytoplasm. The mitochondria (M) are large, well preserved and located in the supranuclear region. (TEM X72,100).
- 3.31. Organ culture of foetal lamb intestine in T199 medium + 10% foetal calf serum, infected with approximately 10⁶ CFU/ml of <u>C.jejuni</u>, 6 hours postinfection, showing one microorganism (MO) in close proximity to the brush border. The brush border is intact and irregular in shape. Free ribosomes (R) are present in the apical cytoplasm of the absorptive cells. (TEM X31,800).

- 3.32. Higher magnification of Figure 3.31 showing the attachment of the microorganism (MO) to the tip of the microvillus (MV) by a blurred pilus-like structure (arrow). The microvillus is directed towards the microorganism. The filamentous core (F) and the fuzzy coat are obscured. (TEM X72,100).
- Ultrastructure micrograph of foetal 3.33. lamb 48 intestine cultured in T199 medium + 10% foetal calf serum, infected with approximately 10⁶ CFU/ml of C.jejuni, 6 hours postinfection, showing a macrophage in the lamina propria. The macrophage has two phagolysosomal vacuoles containing degenerated oval and spiral-shaped microorganisms (MO). (TEM X21,300).
- 3.34. Organ culture of foetal lamb intestine in T199 medium + 10% foetal calf serum, infected with approximately 10⁶ CFU/ml of <u>C.jejuni</u>, showing the changes that have occurred 13 hours postinfection. The microvilli (MV) are irregularly distributed and are both shortened and elongated. Numerous vacuoles (arrow) are present in the apical cytoplasm. (TEM X15,300).
- 3.35. Higher magnification of Figure 3.34 showing an elongation of the microvilli (MV). An occasional dilation of the endoplasmic reticulum (ER) can be observed. Free ribosomes (R) are distributed in the apical cytoplasm. (TEM X31,800).

(xx) Page

47

48

- 3.36. Foetal lamb intestine cultured in T199 medium + 10% foetal calf serum, infected with approximately 10⁶ CFU/ml of <u>C.jejuni</u>, showing the changes that have occurred 13 hours postinfection. The apices of the absorptive cells can be observed with vesiculation (V) of the microvilli. Some of the microvilli have degenerated and sloughed (arrow). (TEM X15,300).
- 3.37. Organ culture of foetal lamb intestine in T199 medium + 10% foetal calf serum, infected with approximately 10⁶ CFU/ml of <u>C.jejuni</u>, showing the changes that have occurred 13 hours postinfection. Curved microorganisms (MO) can be seen in close association with the microvilli. The nuclei (N) of the epithelial cells show margination of the nuclear chromatin (arrow). (TEM X21,200)
- 3.38. Foetal lamb intestine cultured in T199 medium + 10% foetal calf serum, infected with approximately 10⁶ of <u>C.jejuni</u>, showing changes that have occurred 13 hours postinfection. Swollen mitochondria (M) are seen with a loss of cristae in the mid portion of a villous epithelial cell. Free ribosomal granules (R) and medium sized phagocytic vacuoles (V) can be observed. (TEM X15,300).
- 3.39. Apical portion of villous epithelial cell 48 cytoplasm of foetal lamb intestine cultured in T199 medium + 10% foetal calf serum, infected with approximately 10⁶ CFU/ml of <u>C.jejuni</u>, 13 hours postinfection, showing free ribosomes (R) with a

(xxi) Page

48

48

moderate number of endoplasmic reticuli (ER) and an occasional golgi apparatus (G). Variable-sized cytoplasmic phagocytic vacuoles (V) are present. (TEM X7,800).

- 3.40. Organ culture of foetal lamb intestine in T199 medium + 10% foetal calf serum, infected with approximately 10⁶ CFU/ml of <u>C.jejuni</u>, showing changes that have occurred 13 hours postinfection. The villous epithelial cells (C) are disorganised. The microvilli (MV) are degenerated and an occasional epithelial cell shows an extrusion of cytoplasm, (arrow). Margination of the nuclear chromatin (long arrow) and a variation in nuclear shape (N) can be observed. (TEM X7,800).
- 3.41. Organ culture of foetal lamb intestine in T199 medium + 10% foetal calf serum, infected with approximately 10⁶ CFU/ml of <u>C.jejuni</u>, showing changes that have occurred 13 hours postinfection. The absorptive cells (C) show cytoplasmic budding (arrow) and degeneration of the microvilli (MV). A large intracytoplasmic autophageal vacuole (V) can be seen, containing disrupted organelles and undifferentiated debris. Most of the mitochondria (M) have accumulated near the apex. (TEM X11,200).
- 3.42. Organ culture of foetal lamb intestine in T199 medium + 10% foetal calf serum, infected with approximately 10⁶ CFU/ml of <u>C.jejuni</u>, showing changes that have occurred 15 hours postinfection. Numerous microorganisms varying in shape, (curved (c), spiral (s), and round (r)) are present on the luminal surface. Some of the microorganisms are in

48

48

49

close contact with the microvilli (arrow). The microvilli are shorter than those in the control cultures. (TEM X21,200)

- 3.43. Organ culture of foetal lamb intestine in T199 medium + 10% foetal calf serum, infected with approximately 10⁶ CFU/ml of <u>C.jejuni</u>, showing changes that have occurred 15 hours postinfection. A marked shortening of the microvilli (MV) can be seen.Occasional lysosome-like structures (L) are present in the mid portion of the epithelial cytoplasm. (TEM X15,300).
- 3.44. Higher magnification of a portion of Figure 3.42 49 showing attachment of the microorganisms (MO) to the tip of a microvillus by a plaque-like structure (arrow). The microvilli are directed towards the microorganism. The filamentous core (F) and terminal web (TW) are obscured. (TEM X48,600).
- 3.45. Organ culture of foetal lamb intestine in T199 medium + 10% foetal calf serum, infected with approximately 10⁶ CFU/ml of C.jejuni, showing changes that have occurred 15 hours postinfection. The exfoliation of a villous epithelial cell (C) can be observed, with disruption and loss of the surface coat. The cell at the lower left margin (arrowed a) is in the process of being extruded. It has lost microvilli (MV) and contains multiple aggregations of glycogen particles (Gl). The epithelial cell in the upper part of the micrograph (arrowed b) appears to be completely detached from The cytoplasm contains the epithelium. dark amorphous material (arrow) and a limited number of

cytoplasmic vacuoles (V). (TEM X7,800).

- 3.46. Organ culture of foetal lamb intestine in T199 49 medium + 10% foetal calf serum, infected with approximately 10⁶ CFU/ml of <u>C.jejuni</u>, showing changes that have occurred 15 hours postinfection. An oval-shaped microorganism (MO) can be seen lying free in the interstitium of the submucosal layer. (TEM X21,200).
- 3.47. Scanning electron micrograph (SEM) of the mucosal surface of control foetal lamb intestine after 6 hours culture in T199 medium + 10% foetal calf serum. The epithelial cells (C) are polygonal in shape and are regularly arranged. The outline of each cell is defined by either a shallow furrow (x) or a deep depression (d). (SEM X4620).
- 3.48. Higher magnification of the epithelial cells of Figure 3.47. Heavily packed, rod-shaped microvilli (MV) can be seen covering the polygonal-shaped epithelial cells. The average distance between each microvillus is 0.1-0.2 um. Mucus blankets are not apparent. (SEM X14000).
- 3.49. SEM of the tips of the villi of control foetal lamb intestine after 13 hours culture in T199 medium + 10% foetal calf serum. The surface is divided into polygonal units (epithelial cells) and is well defined by furrows. A goblet cell pit (G) can be observed as an oval hole, surrounded by absorptive cells. The apices of the epithelial cells are covered by small, densely packed nodules

representing the tips of the microvilli (MV). (SEM X3080).

- 3.50. SEM showing the surface of the intestinal villi 50 of control foetal lamb intestine after 15 hours culture in T199 medium + 10% foetal calf serum. The surface is divided into polygonal units, separated by furrows (f). Nodular-shaped microvilli (MV) cover the tips of the villi. A goblet cell (G) can be seen surrounded by absorptive cells. (SEM X3300).
- 3.51. Higher magnification of an absorptive cell of 50 control foetal lamb intestine after 15 hours culture in T199 medium + 10% foetal calf serum. The absorptive cell (C) is covered with densely packed, rod shaped microvilli (MV). (SEM X16,500).
- 3.52. SEM of the epithelial cells of an organ culture of foetal lamb intestine in T199 medium + 10% foetal calf serum, infected with approximately 10⁶ CFU/ml of <u>C.jejuni</u>, showing changes that have occurred 6 hours postinfection. The epithelial cell surfaces (C), have a coarse appearance and appear disorganised when compared with the six hour control (Figure 3.38). Some of the absorptive cells show either a moderate loss of microvilli (small arrow) or a severe denudation (large arrow) and some of them are exfoliated (Ex). (SEM X3080).
- 3.53. Higher magnification of the epithelial cells of 51 Figure 3.52. The epithelial cells (C) are covered unevenly by microvilli (MV). Some of the

epithelial cells show a moderate loss of microvilli (arrow). (SEM X4840).

- 3.54. Organ culture of foetal lamb intestine in T199 51 medium + 10% foetal calf serum, infected with approximately 10⁶ CFU/ml of <u>C.jejuni</u>, showing changes that have occurred 6 hours post infection. A filament-shaped microorganism can be observed attached to the tips of the epithelial cells. The infected epithelial cells (C) show a severe loss of microvilli. (SEM X8,250).
- 3.55. Organ culture of foetal lamb intestine in T199 medium + 10% foetal calf serum, infected with approximately 10⁶ CFU/ml of C.jejuni, showing changes that have occurred 13 hours postinfection. The infected epithelial cells (C) are disorganised and have lost their normal polygonal pattern and their microvilli when compared with the 13 hour culture (Figure 3.49). control 0ne normal epithelial cell (arrow) remains covered with densely packed rod-shaped microvilli (MV). (SEM X7,700).
- 3.56. Organ culture of foetal lamb intestine in T199 medium + 10% foetal calf serum, infected with approximately 10⁶ CFU/ml of <u>C.jejuni</u>, showing changes that have occurred 13 hours postinfection. The infected epithelial cells (C) are swollen and protrude towards the luminal surface. A layer of thick white mucus (mu) can be observed covering the surface of the infected cells. Pits and holes are present (arrow) and represent goblet cells (G). Occasional spiral-shaped (s) to rod-shaped (r)

51

52

microorganisms can be seen within the mucus. (SEM X12,100).

- 3.57. Organ culture of foetal lamb intestine in T199 medium + 10% foetal calf serum, infected with approximately 10⁶ CFU/ml of C.jejuni, showing changes that have occurred 13 hours postinfection. The surface of the villous epithelium is roughened and irregular and covered with thick mucus (arrow). epithelial cells (C) show a sever The loss of microvilli. Spiral (s), filamentous (f) and ring-shaped (r) microorganisms can be observed attached to the surface of the infected epithelial cells. (SEM X6600)
- 3.58. Higher magnification of Figure 3.57. The 51 infected epithelial cells (C) are swollen and protrude into the luminal surface. They show a severe loss of microvilli and are covered with thick, white strands of mucus. (SEM X12,100).
- 3.59. Organ culture of foetal lamb intestine in T199 medium + 10% foetal calf serum, infected with approximately 10⁶ CFU/ml of C.jejuni, showing changes that have occurred 15 hours postinfection. The epithelial cells (C) are severely disorganised, round to elongate in shape (arrow) and some show a loss of microvilli (MV). severe A few are exfoliated (Ex). The external surface of the epithelial cells is roughened and covered with thick strands and plugs of white mucus (M). Occasional spiral-shaped microorganisms (MO) are attached to the surface of the epithelial cells. (SEM X2640).

Figure

- 3.60. Higher magnification of Figure 3.59 showing the irregular, ridged surface of the absorptive epithelial cells (arrow). A severe loss of microvilli can also be observed. (SEM X9,900).
- 3.61. Organ culture of foetal lamb intestine in T199 medium + 10% foetal calf serum, infected with approximately 10⁶ CFU/ml of C.jejuni, showing changes that have occurred 15 hours postinfection. Necrosis and exfoliation of some of the epithelial cells can be observed at the tips of the villi (arrow). The lamina propria (L) is exposed and protrudes above the remaining epithelial cells (C). of the intact epithelial cells show Some а disorganised pattern (long arrow) when compared to the 15 hour control culture (Figure 3.50). Occasional shreds and plugs of mucus (MU) cover the desquamated cells (small arrow). (SEM X6050).
- 3.62. Histogram comparing the means of epithelial 52 villus heights in foetal lamb intestinal organ cultures infected with <u>C.jejuni</u>, and controls, at 6,13 and 15 hours post-infection.
- 3.63. Histogram comparing the means of epithelial cell 53 widths in foetal lamb intestinal organ cultures infected with <u>C.jejuni</u>, and controls, at 6,13 and 15 hours post-infection.
- 3.64. Histogram comparing the means of epithelial cell 53 heights in foetal lamb intestinal organ cultures infected with <u>C.jejuni</u>, and controls, at 6,13 and 15 hours post-infection.

52