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.

THE AGEING OF BRUISES IN LAMBS

A Thesis presented in partial fulfilment
of the requirements for the
Degree of Doctor of Philosophy
at Massey University

Ronald Norman Thornton
1983

ABSTRACT

Bruising in lambs processed for human consumption is a significant economic problem. A reduction in the prevalence of bruises could be achieved relatively efficiently if their important places of occurrence could be identified by ageing these lesions with respect to the known times of occurrence of events of possible aetiological significance. To this end efforts were made to age experimental bruises in lambs by objectively assessing semi-quantitative histopathological data using a mathematical model based on Bayes' theorem of inverse probabilities, by enzyme histochemical and isoelectric focussing studies and by measurement of muscle pH.

The Bayesian method for objective histopathological ageing was developed and tested on data representing 178 bruises. It was successful in identifying bruises as either 1-20 hours or more than 24 hours old. The 'accuracy' with which a bruise of known age could be identified as such depended on the nature and number of tissue samples studied. The degree of 'confidence' with which an individual bruise of unknown age could be aged, however, depended both on the 'accuracy' of the method and on the relative number of bruises estimated to belong to each of the two age categories considered. In general a degree of 'confidence' of 80-90% can be expected in practice, and in this respect the performance of the Bayesian method is superior to that achieved by purely subjective means. A pilot survey involving 107 bruises collected from an export meat works established both the practical value of the objective ageing method and its superiority over alternative epidemiological approaches to the problem of utilising data pertaining to trucking times and holding times in meat works yards. Of the bruises studied, 60% were estimated to have been inflicted within the works, and 40% prior to arrival.

Enzyme studies on bruises aged 4-144 hours old revealed no detectable relationships between observed changes in either histochemical or isoenzyme activities and bruise ages. In light of contradictory published results pertaining to other types of wounds, this lack of success was thought to reflect the relatively mild nature of the tissue reaction in bruises.

Statistically significant relationships could not be demonstrated between absolute or relative muscle pH and the ages of bruises from 4-48 hours old.

A newly recognised condition of 'subcutaneous haemorrhagic speckling' in the carcase adipose tissue of young lambs processed for human consumption was investigated. From histopathological and epidemiological evidence, the primary cause of the lesions was shown to be electrical stunning. However, secondary aetiological factors were proposed as having influenced the prevalence and severity of lesions. Attempts to elucidate the pathogenesis of 'speckling' with the intention of formulating a rational approach to its prevention were unsuccessful.

ACKNOWLEDGEMENTS

This research was conducted using the facilities of the Department of Veterinary Pathology and Public Health at Massey University. I am grateful to Professor B.W. Manktelow for the opportunity of undertaking the study and to others who have offered assistance over the past years. In particular I would like to thank Dr R.D. Jolly and Professor D.K. Blackmore for their guidance and unfailing encouragement throughout.

Invaluable advice on mathematical aspects of the thesis was provided by Professor R.E. Munford of the Department of Physiology and Anatomy. The late Dr R.E. Harris of the Department of Veterinary Clinical Sciences helped plan and execute the epidemiological section of the study on 'subcutaneous haemorrhagic speckling'.

Paraffin sections for light microscopy were prepared by Mrs P.M. Slack and Miss S.L. Malloch, both of the Department of Veterinary Pathology and Public Health. Photographs were processed with the help of Mr T.G. Law. Willing assistance in all practical aspects of animal handling was given by Mr A.T. De Cleene and Mr C.K. Barnett. The thesis was typed by Miss S.J. Shirriffs.

The investigation into 'subcutaneous haemorrhagic speckling' and the pilot bruise survey were conducted at the Hawke's Bay Farmers Meat Co. Ltd and Borthwicks-CWS, Feilding respectively. I am grateful to the employees of both of these companies for their helpfulness in these aspects of the study. Of particular note was the cooperation and enthusiasm of Messrs N. Marsden and P. Dingeman of the former company.

I wish to gratefully acknowledge the funding of this research by the New Zealand Meat Board and the combined Freezing Works Association.

TABLE OF CONTENTS

ABSTRACT	ii
ACKNOWLEDGEMENTS	iv
TABLE OF CONTENTS	v
LIST OF TABLES	xv
LIST OF FIGURES	xx
INTRODUCTION	1
CHAPTER I: REVIEW - THE AGEING OF WOUNDS	3
I. FACTORS AFFECTING THE TIME-COURSE OF THE TISSUE REACTION TO INJURY	3
1. The effect of type and severity of injury on the time-course of the ensuing tissue reaction	4
2. The effects of species of animal and type of tissue affected on the time-course of the tissue reaction to injury	4
3. The effects of subject age, multiple wounding and observer variation on the rate of the tissue reaction to injury	5
II. THE GROSS APPEARANCE OF BRUISES	6
III. BLOOD DEGRADATION PRODUCTS AND TISSUE CONDUCTIVITY IN BRUISES	7
IV. HISTAMINE AND SEROTONIN LEVELS IN WOUNDS	8
V. THE HISTOPATHOLOGY OF WOUNDS	8
A. The histopathology of wounds involving skeletal muscle	8
1. Degenerative changes in muscle fibres following injury	8
2. The leucocytic reaction following injury to muscle	9
3. Fibroplasia and vascular changes following injury to muscle	10
4. Regenerative changes in muscle fibres following injury	11
B. The histopathology of wounds involving adipose tissue	13
1. Degenerative changes in adipocytes following injury	13
2. The leucocytic reaction following injury to adipose tissue	14
3. Fibroplasia and vascular changes following injury to adipose tissue	15

C. The histopathology of wounds involving skin 16

1. Degenerative changes in skin following injury 16

2. The leucocyte reaction following injury to skin 17

3. The histochemistry of acid glycosaminoglycans in skin wounds 18

4. Fibroplasia and vascular changes following injury to skin 19

VI. ENZYMIC ACTIVITY IN WOUNDS 20

A. Technical considerations in measuring tissue enzyme activities 20

B. The nature of enzyme changes in wounds 21

C. The time course of enzyme changes in wounds 22

D. Observations on specific enzyme activities in wounds 23

1. The glycolytic pathway 24

(a) Aldolase 24

(b) Glycogen phosphorylase 24

(c) Lactate dehydrogenase 25

2. The citric acid cycle 26

(a) Succinate dehydrogenase 26

3. The pentose phosphate pathway 27

(a) Glucose-6-phosphate dehydrogenase 27

4. Hydrolases 28

(a) Acid phosphatase 28

(b) Alkaline phosphatase 29

(c) Non-specific esterase 30

(d) Leucine aminopeptidase 31

5. Miscellaneous enzymes 31

(a) Creatine phosphokinase 32

(b) Myosin adenosine triphosphatase 32

CONCLUSION 33

CHAPTER II: AGEING BRUISES ACCORDING TO THEIR GROSS AND HISTOPATHOLOGICAL CHARACTERISTICS 34

INTRODUCTION 34

MATERIALS AND METHODS 34

I. IMPACT BRUISES 34

1. Experimental animals 34

2. Production of bruises 35

3. Processing of bruised tissues 36

4. Examination and scoring of tissue sections 37

5. Analyses of data and methods used to estimate the ages of bruises 39

(a) The Bayesian probability model 40

(b) Linear regression analysis 42

II. WOOL PULL BRUISES 43

1. Production of bruises 43

2. Analysis of the histopathological data 43

RESULTS 44

I. GROSS AND MICROSCOPIC APPEARANCE OF IMPACT BRUISES 44

1. Gross appearance of bruises 44

2. The histopathology of bruises 44

II. SELECTION OF HISTOPATHOLOGICAL FEATURES SUITABLE FOR AGEING IMPACT BRUISES MATHEMATICALLY 50

1. Initial selection of histopathological features 50

2. Further selection of histopathological features according to results of chi-square analyses on data in contingency tables 51

3. Relationships between bruise ages and scores for histopathological features whilst controlling for the effects of haemorrhage 52

4. Histopathological features eventually chosen for use when estimating the ages of bruises 52

III. DEVELOPMENT OF A MATHEMATICAL MODEL FOR AGEING IMPACT BRUISES OBJECTIVELY 54

A. Developing the Bayesian probability model 54

1. The performance of the Bayesian probability model using data from muscle 58

2. The performance of the Bayesian probability model when used to place bruises into one of only two broad age categories 60

3. The performance of the Bayesian probability model using data from adipose tissue 61

4. The effects of re-scoring of tissue sections on bruise age estimates 61

B. Developing the multiple regression model 63

C. Assessment of the performance of the Bayesian probability model when applied to data not previously used to develop the model 64

1. Investigation into the advantages of considering more than one sample per bruise 67

(a) The effect of duplicate within-bruise sampling on the 'accuracy' of ageing bruises 67

(b) The 'accuracy' of ageing bruises when there was concordance between age estimates from muscle and adipose tissue 68

2. Comparisons between the 'accuracies' of interpretive and Bayesian methods of ageing bruises 69

IV. APPLICATION OF THE BAYESIAN PROBABILITY MODEL TO WOOL PULL BRUISES 70

1. Gross pathology 70

2. Histology 70

V. THE 'CONFIDENCE' OF AGEING BRUISES OF UNKNOWN AGES BY APPLICATION OF THE BAYESIAN PROBABILITY METHOD 70

(a) 'Accuracy' of the Bayesian probability model 71

(b) The 'confidence' of ageing individual bruises 71

1. Method of calculating the 'confidence' with which individual bruises have been aged 71

2. Method of calculating the 'confidence' with which single bruises have been aged 76

VI. PRACTICAL APPLICATION OF THE BAYESIAN AGEING METHOD: THE AGEING OF BRUISES IN A PILOT STUDY AT A MEAT WORKS 78

INTRODUCTION 78

MATERIALS AND METHODS 78

RESULTS 79

1. Gross characteristics of bruises 79

2. Histopathological characteristics of bruises 79

3. Ageing of bruises using the Bayesian probability method 79

4. Bruise ages considered in relation to holding times in meat works yards 80

5. Epidemiological data 83

(a) Between-farm clustering of bruises 83

(b) Trucking distance 83

(c) Holding times in meat works yards 84

(d) Carcase position of bruises 84

DISCUSSION 87

I. EXPERIMENTAL ANIMALS 87

II. PRODUCTION OF BRUISES 87

III. GROSS APPEARANCE OF BRUISES 88

IV. HISTOPATHOLOGY OF BRUISES 88

1. Haemorrhage and fibrin 89

2. Histopathological changes in muscle fibres 90

3. Fat necrosis 91

4. The cellular exudates 91

5. Tissue repair 92

6. Haemosiderin 93

7. Mast cells and free histamine 93

8. Acid glycosaminoglycans 94

V. MATHEMATICAL METHODS AVAILABLE FOR PROCESSING HISTOPATHOLOGICAL DATA 94

1. Logical models 95

2. Statistical models 96

(a) Bayesian probability model 96

(b) Discriminant function analysis 97

(c) Data matching models 97

(d) Regression analysis 97

VI. ESTIMATING THE AGES OF BRUISES MATHEMATICALLY USING SCORES FROM SEVERAL HISTOPATHOLOGICAL PARAMETERS 99

1. Selection of histopathological features 100

2. Developing the Bayesian probability model 102

(a) Data base 103

(b) Selection of indicants used for age estimation 103

(c) Age classification and the potential performance of the model 104

3. Scoring variation 105

4. Developing the multiple linear regression model 105

5. Assessing the realistic 'accuracy' of the Bayesian probability model 106

6. Validation of the Bayesian probability model 108

7. The 'confidence' of ageing bruises of unknown ages by application of the Bayesian probability model 108

VII. PRACTICAL APPLICATION OF THE BAYESIAN AGEING METHOD TO BRUISES IN A PILOT MEAT WORKS STUDY 110

VIII. WOOL PULL BRUISES 112

CHAPTER III: ENZYMIC ACTIVITY IN BRUISED MUSCLE 114

MATERIALS AND METHODS 114

1. Enzyme histochemistry 114

2. Isoenzyme studies 115

(a) Preparation of tissues 115

(b) Isoelectric focussing 116

RESULTS 117

I. ENZYMES OF THE GLYCOLYTIC PATHWAY 117

1. Aldolase 117

(a) Enzyme histochemistry 117

(b) Isoenzyme studies 118

2. Lactate dehydrogenase 118

(a) Enzyme histochemistry 118

(b) Isoenzyme studies 121

II. ENZYMES OF THE CITRIC ACID CYCLE 122

1. Succinate dehydrogenase 122

(a) Enzyme histochemistry 122

(b) Isoenzyme studies 122

III. ENZYMES OF THE PENTOSE PHOSPHATE PATHWAY 124

1. Glucose-6-phosphate dehydrogenase 124

(a) Enzyme histochemistry 124

(b) Isoenzyme studies 124

IV. HYDROLYTIC ENZYMES 126

1. Acid phosphatase 126

(a) Enzyme histochemistry 126

(b) Isoenzyme studies 128

2. Alkaline phosphatase 128

(a) Enzyme histochemistry 128

(b) Isoenzyme studies 130

3. Leucine aminopeptidase 130

(a) Enzyme histochemistry 130

(b) Isoenzyme studies 132

4. Non-specific esterase 132

(a) Enzyme histochemistry 132

(b) Isoenzyme studies 132

V. MISCELLANEOUS ENZYMES 134

1. Creatine phosphokinase 134

(a) Enzyme histochemistry 134

(b) Isoenzyme studies 134

2. Myosine adenosine triphosphatase 136

(a) Enzyme histochemistry 136

(b) Isoenzyme studies 136

DISCUSSION 137

Enzyme histochemistry 137

Isoenzyme studies 139

CHAPTER IV: pH CHANGES IN BRUISED MUSCLE 142

INTRODUCTION 142

MATERIALS AND METHODS 142

I. TECHNIQUE FOR MEASUREMENT OF MUSCLE pH 142

II. MEASUREMENT OF BRUISE AND CONTROL MUSCLE pH VALUES 143

1. Experimental animals 143

2. Bruising of animals 143

3. Slaughter of animals and processing of muscle samples 143

RESULTS 144

I. TECHNIQUE FOR MEASUREMENT OF MUSCLE pH 144

II. MEASUREMENT OF BRUISE AND CONTROL MUSCLE pH VALUES 144

1. Bruise pH values 144

2. Bruise pH values relative to those of appropriate controls 147

(a) Selection of appropriate control muscle samples 147

(b) Bruise pH values relative to those of within-muscle controls 148

DISCUSSION 150

CHAPTER V: PETECHIAL HAEMORRHAGE IN THE CARCASE FAT OF SLAUGHTERED LAMBS 153

INTRODUCTION 153

MATERIALS AND METHODS 154

A. Histology 154

B. Immunohistochemistry 154

C. Epidemiological studies 155

1. Statistical analysis of population factors associated with petechiation in carcase fat 155

2. Split-line trial to determine whether lesions occurred at or prior to slaughter 156

3. Factors associated with the process of slaughter 156

D. Pathogenesis of petechiation in adipose tissue 157

1. Measurement of post-stunning systemic blood pressure 157

2. Attempts to produce petechial haemorrhages in inguinal fat 158

RESULTS 159

A. Gross pathology 159

B. Histopathology 159

C. Immunohistochemistry 160

D. Epidemiological studies 160

1. Statistical analysis of population factors associated with petechial haemorrhage 160

2. Split-line trial to determine whether lesions occurred at or prior to slaughter 162

3. Experiment to isolate factors associated with the process of slaughter 162

E. The pathogenesis of petechiation in adipose tissue 164

1. Measurement of post-stunning systemic blood pressure 164

2. Attempts to produce petechial haemorrhages in inguinal fat 165

DISCUSSION 165

1. The primary aetiology of 'speckling' 167

(a) Method of exsanguination 167

(b) The restraining conveyor 168

(c) Electrical stunning 168

2. Secondary factors in the aetiology of 'speckling' 169

3. The pathogenesis of 'speckling' 170

SUMMARY AND CONCLUSIONS 174

I. THE AGEING OF BRUISES 174

1. Histopathology 174

2. Enzyme studies 176

3. pH of bruised muscle 177

II. SUBCUTANEOUS HAEMORRHAGIC SPECKLING 178

APPENDIX: MATERIALS AND METHODS USED IN THE ENZYME STUDIES ON BRUISED MUSCLE 180

BIBLIOGRAPHY 201

LIST OF TABLES

TABLE		
2.1	The ages, numbers and positions of impact bruises inflicted on experimental lambs.	36
2.2	Example of a contingency table used for chi-square analysis. In this case the relationship between bruise ages and scores for macrophage exudation is investigated.	40
2.3	A list of the special stains used on tissue sections from impact bruises, showing the numbers of bruises of each age stained.	45
2.4	Statistical significance of associations between scores from various histopathological features and bruise ages. The results are from chi-square analyses of the respective contingency tables.	51
2.5	Statistical significance of associations between scores for various histopathological features in muscle and bruise ages, whilst controlling for the effects of haemorrhage.	53
2.6	Statistical significance of associations between scores for various histopathological features in adipose tissue and bruise ages, whilst controlling for the effects of haemorrhage.	53
2.7	Probabilities of observing individual scores for selected histopathological features of bruised muscle, calculated for each of six age categories of bruises.	55
2.8	Probabilities of observing individual scores for selected histopathological features of bruised adipose tissue, calculated for each of six age categories of bruises.	56
2.9	The calculations required when using histopathological data to age bruises are shown for one bruise in muscle.	57
2.10	The estimated ages of experimental bruises, as determined by applying the Bayesian probability model to histopathological data for muscle, are compared with their known ages. Macrophages and neutrophils were scored separately rather than as ratios.	59

TABLE

2.11	The estimated ages of experimental bruises, as determined by applying the Bayesian probability model to histopathological data for muscle, are compared with their known ages. This time macrophages and neutrophils are scored as ratios.	59
2.12	The percentage 'accuracies' of the Bayesian probability model in ageing bruises using histopathological data from muscle or adipose tissue are shown for the 120 bruises used to develop the model.	60
2.13	The percentage 'accuracies' of the Bayesian probability model in ageing bruises indirectly or directly into two broad age categories are calculated using histopathological data from the 120 bruises used to develop the model.	61
2.14	The estimated ages of experimental bruises, as determined by applying the Bayesian probability model to histopathological data for adipose tissue, are compared with their known ages.	62
2.15	The percentages of scores duplicated after re-scoring the 120 bruises used when developing the Bayesian probability model.	62
2.16	Comparisons between the 'accuracies' of the Bayesian probability model in ageing bruises using the original and new scores for the 120 muscle sections used to develop the model.	63
2.17	The estimated ages of experimental bruises, as determined by applying the multiple regression model to histopathological data for muscle, are compared with their known ages.	65
2.18	The percentage 'accuracy' of the multiple linear regression model is compared with that of the Bayesian probability model, using the same data for muscle.	65
2.19	Comparisons between estimated and actual ages of experimental bruises. Age estimates were made by applying the Bayesian probability model to histopathological data from muscle sections not previously used for developing the model.	66
2.20	Comparisons between estimated and actual ages of experimental bruises. Age estimates were made by applying the Bayesian probability model to histopathological data from adipose tissue sections not previously used for developing the model.	66

TABLE

2.21	The 'accuracy' of the Bayesian probability model when applied to data previously used for its development is compared with that achieved with new data.	67
2.22	The 'accuracy' of the Bayesian probability model in ageing bruises when there was concordance between paired within-bruise samples.	68
2.23	The 'accuracy' of the Bayesian probability model in ageing bruises with concordance between estimates made from muscle and adipose tissue.	69
2.24	The 'accuracy' of the Bayesian probability model in ageing bruises is compared to that of interpretive ageing.	69
2.25	The effect variation in the relative numbers of bruises actually aged 1-20 hours or 24-72 hours old has on the 'confidence' of ageing individual bruises. The 'accuracy' of the model is assumed constant at 0.85 and 0.81 for 1-20 hour and 24-72 hour old bruises respectively.	75
2.26	The 'confidence' with which single or small numbers of bruises can be aged by the Bayesian probability model, using 'accuracy' figures from Tables 2.21, 2.22 and 2.23.	76
2.27	'Confidence' levels of ageing individual bruises calculated after applying the Bayesian probability method to data from muscle, adipose tissue or both.	80
2.28	Bruises aged as 1-20 hours or 24+ hours old are classified according to the known holding times of lambs in meat works yards prior to slaughter.	81
2.29	Results of Student's paired 't' test assessing the significance of differences between mob-specific and appropriate daily average bruising prevalences.	83
2.30	The carcase positions of bruises are compared with their estimated places of occurrence, either before or after arrival at the meat works.	84

TABLE

3.1	Summary of the visually assessed degrees of activity of histochemically demonstrated aldolase in various tissue components of bruised muscle.	119
3.2	Summary of the visually assessed degrees of activity of histochemically demonstrated lactate dehydrogenase in various tissue components of bruised muscle.	120
3.3	Summary of the visually assessed degrees of activity of histochemically demonstrated succinate dehydrogenase in various tissue components of bruised muscle.	123
3.4	Summary of the visually assessed degrees of activity of histochemically demonstrated glucose-6-phosphate dehydrogenase in various tissue components of bruised muscle.	125
3.5	Summary of the visually assessed degrees of activity of histochemically demonstrated acid phosphatase in various tissue components of bruised muscle.	127
3.6	Summary of the visually assessed degrees of activity of histochemically demonstrated alkaline phosphatase in various tissue components of bruised muscle.	129
3.7	Summary of the visually assessed degrees of activity of histochemically demonstrated leucine aminopeptidase in various tissue components of bruised muscle.	131
3.8	Summary of the visually assessed degrees of activity of histochemically demonstrated non-specific esterase in various tissue components of bruised muscle.	133
3.9	Summary of the visually assessed degrees of activity of histochemically demonstrated creatine phosphokinase in various tissue components of bruised muscle.	135
4.1	Results of Student's paired t tests examining levels of significance of pH differences between contra-lateral (C1-C2) and also between within-muscle (C2-C3) controls.	148
4.2	Average muscle pH of bruises and controls, along with results of Student's t tests on pH differences between bruise/control sample pairs.	150

TABLE

5.1	Outline of procedures applied during attempts to produce petechial haemorrhages by the direct electrical stimulation of nerves innervating inguinal fat.	158
5.2	The prevalences of petechiation in the carcase fat of two groups of lambs formed from a single mob. The lambs in Group B were killed 20 hours after those in Group A.	162
5.3	Experiment to isolate factors associated with the process of slaughter.	163

LIST OF FIGURES

FIGURE		Following page
2.1	The weight, and plastic tube, along with the hand vice used to inflict impact and wool pull bruises respectively. The wooden ruler is 40cm long.	35
2.2	An example of the score sheets used when studying histopathological changes in bruises.	Page 38
2.3	The number of neutrophils in the infiltrate exceeds that of macrophages, as was generally the case in bruises up to 20 hours old. This bruise was 16 hours old. (Paraffin section, H+E x 260)	48
2.4	The number of macrophages in the infiltrate exceeds that of neutrophils, as was generally the case in bruises 24 hours and older. This bruise was 60 hours old. (Paraffin section, H+E x 260)	48
2.5	Myoblast-like cells indicative of early regenerative change. This bruise was 36 hours old. (Paraffin section, H+E x 500)	48
2.6	Endothelial hypertrophy in a 60 hour old bruise. (Paraffin section, H+E x 260)	48
2.7	Hypertrophy of fibroblasts in a 60 hour old bruise. (Paraffin section, H+E x 500)	49
2.8	Haemosiderin positive macrophages in a 72 hour old bruise. (Paraffin section, Perl's stain, x 160)	49
2.9	A diagrammatic and figurative representation of the performance of the Bayesian probability model in ageing a population of bruises of ages n_x actually 1-20 hours old and n_y actually 24-72 hours old.	Page 72
2.10	Percentages of the total number of bruised mobs plotted against differences between mob-specific and appropriate daily average bruising prevalences. This shows the tendency for bruises to cluster within certain mobs of lambs rather than to occur with even distribution in all mobs slaughtered.	Page 85

FIGURE		Following page
2.11	Percentage bruising in lambs surveyed plotted against trucking distance to the meat works.	Page 85
2.12	Percentage bruising in lambs surveyed plotted against holding time in meat works yards.	Page 86
2.13	Areas into which carcases were divided when classifying bruises according to carcase position.	Page 86
3.1	Bruise 4 hours old. Variation in aldolase staining according to fibre type in apparently normal muscle. x 320	118
3.2	Bruise 48 hours old. Decreased and increased aldolase staining in necrotic muscle fibres. x 320	118
3.3	Bruise 144 hours old. Moderately positive aldolase staining of fibroblasts in connective tissue septum. x 320	119
3.4	Bruise 144 hours old. Enhanced aldolase staining of fibroblasts in connective tissue septum. x 320	119
3.5	Aldolase zymogram of bruised muscle, leucocyctes, plasma and erythrocytes after isoelectric focussing at 25 watt constant power for 1.5 hours in PAG with ampholine buffer range pH 3.5-9.5. The gel was incubated in aqueous substrate medium for 30 minutes at 37^{0}C.	119
3.6	Bruise 4 hours old. Variation in lactate dehydrogenase staining according to fibre type in apparently normal muscle. x 320	119
3.7	Bruise 24 hours old. Decreased lactate dehydrogenase staining in necrotic muscle fibres. x 320	120
3.8	Bruise 24 hours old. Variably positive lactate dehydrogenase staining in the leucocyte exudate. x 320	120
3.9	Bruise 24 hours old. Increased lactate dehydrogenase staining of intramuscular leucocytes. x 320	121

FIGURE		Following page
3.10	Bruise 24 hours old. Moderately positive lactate dehydrogenase staining of fibroblasts in connective tissue septum. x 500	121
3.11	Bruise 48 hours old. Strongly positive lactate dehydrogenase staining of fibroblasts in connective tissue septum. x 320	121
3.12	Lactate dehydrogenase zymogram of bruised muscle, leucocytes, plasma and erythrocytes after isoelectric focussing at 25 watt constant power for 1.5 hours in PAG with ampholine buffer range pH 3.5-9.5. The gel was incubated in aqueous substrate medium for 30 minutes at 37°C.	121
3.13	A representative densitometer scan of electro-focussed lactate dehydrogenase isoenzymes obtained from bruised muscle, showing the 25 bands common to each sample of bruised muscle. This particular bruise was 4 hours old. The gel had ampholine buffer range pH 3.5-9.5.	121
3.14	Bruise 4 hours old. Variation in succinate dehydrogenase staining according to fibre type in apparently normal muscle. x 320	121
3.15	Bruise 48 hours old. Decreased succinate dehydrogenase activity in necrotic muscle fibres. x 320	123
3.16	Bruise 144 hours old. Enhanced succinate dehydrogenase staining in degenerative muscle fibre. x 320	123
3.17	Succinate dehydrogenase zymogram of bruised muscle, leucocytes, plasma and erythrocytes after isoelectric focussing at 25 watt constant power for 1.5 hours in PAG with ampholine buffer range pH 3.5-9.5. The gel was incubated in aqueous substrate medium for 30 minutes at 37°C.	123
3.18	Bruise 4 hours old. Absence of glucose-6-phosphate dehydrogenase activity in apparently normal muscle fibres. x 320	123

FIGURE		Following page
3.19	Bruise 24 hours old. Punctate glucose-6-phosphate dehydrogenase activity in degenerative muscle fibre. Intramuscular leucocytes show a moderately positive reaction. x 320	125
3.20	Bruise 48 hours old. Faintly positive glucose-6-phosphate dehydrogenase staining of fibroblasts in connective tissue septum. x 500	125
3.21	Glucose-6-phosphate dehydrogenase zymogram of bruised muscle, leucocytes, plasma and erythrocytes after isoelectric focussing at 25 watt constant power for 1.5 hours in PAG with ampholine buffer range pH 3.5-9.5. The gel was incubated in aqueous substrate medium for 30 minutes at 37^{o}C.	125
3.22	Bruise 4 hours old. Absence of acid phosphatase activity in apparently normal and degenerative muscle fibres. x 125	125
3.23	Bruise 48 hours old. Absence of acid phosphatase activity in necrotic muscle fibres. Intramuscular macrophages show a positive reaction. x 320	127
3.24	Bruise 24 hours old. Apparently positive acid phosphatase staining of a necrotic muscle fibre, actually due to infiltrating macrophages. x 320	127
3.25	Bruise 144 hours old. Absence of acid phosphatase staining in interstitial polymorphs, and a variably positive reaction in macrophages. x 320	127
3.26	Bruise 48 hours old. Acid phosphatase staining of fibroblasts in connective tissue septum. x 320	127
3.27	Acid phosphatase zymogram of bruised muscle, liver and erythrocytes after isoelectric focussing at 25 watt constant power for 1.5 hours in PAG with ampholine buffer range pH 3.5-9.5. The gel was incubated in aqueous substrate medium for 30 minutes at 37^{o}C.	128

FIGURE		Following page
3.28	A representative densitometer scan of electro-focussed acid phosphatase isoenzymes obtained from homogenates of bruised muscle, showing the four isoenzyme bands represented in all the muscle samples. This particular bruise was 144 hours old. The unmarked peaks represent haemoglobin or unidentified protein.	128
3.29	Bruise 24 hours old. Apparently positive alkaline phosphatase staining of a necrotic muscle fibre, actually due to infiltrating macrophages. x 500	129
3.30	Bruise 24 hours old. Strongly positive alkaline phosphatase staining of capillary endothelium. Neutrophils are also strongly positive. x 125	129
3.31	Bruise 24 hours old. Variable alkaline phosphatase staining of infiltrating macrophages. x 500	129
3.32	Alkaline phosphatase zymogram of bruised muscle, small intestine and liver after isoelectric focussing at 25 watt constant power for 1.5 hours in PAG with ampholine buffer range pH 3.5-9.5. The gel was incubated in aqueous substrate medium for 30 minutes at $37^{o}C$.	129
3.33	Bruise 144 hours old. Apparently positive leucine aminopeptidase staining of a necrotic muscle fibre, actually due to infiltrating macrophages. x 320	131
3.34	Bruise 4 hours old. Positive leucine aminopeptidase staining in the absence of infiltrating macrophages. x 320	131
3.35	Bruise 144 hours old. Moderate leucine aminopeptidase staining of fibroblasts in connective tissue septum. x 500	131
3.36	Leucine aminopeptidase zymogram of bruised muscle homogenates after isoelectric focussing at 25 watt constant power for 1.5 hours in PAG with ampholine buffer range pH 3.5-9.5. The gel was incubated in aqueous substrate medium for 30 minutes at $37^{o}C$.	131

FIGURE		Following page
3.37	Bruise 4 hours old. Negative or slightly positive non-specific esterase staining of apparently normal muscle fibres. x 320	133
3.38	Bruise 24 hours old. Unchanged and increased non-specific esterase staining in necrotic muscle fibres. x 320	133
3.39	Bruise 48 hours old. Strongly positive non-specific esterase staining in interstitial fibroblasts. x 320	133
3.40	Esterase zymogram of bruised muscle after isoelectric focussing at 25 watts constant power for 1.5 hours in PAG with ampholine buffer range pH 3.5-9.5. The gel was incubated in aqueous substrate medium for 15 minutes at 25^{o}C.	133
3.41	Esterase zymogram of bruised muscle after isoelectric focussing at 25 watts constant power for 2.5 hours in PAG with ampholine buffer range pH 4.5-6.5. The gel was incubated in aqueous substrate medium for 15 minutes at 25^{o}C.	134
3.42	A representative densitometer scan of electro-focussed non-specific esterase isoenzymes obtained from bruised muscle, showing the 17 peaks represented in all the muscle samples. This particular bruise was 24 hours old. The gel had an ampholine buffer range of pH 4.5-6.5	134
3.43	Bruise 144 hours old. Moderate diffuse and strong punctate creatine phosphokinase staining in apparently normal muscle fibres. x 500	135
3.44	Bruise 24 hours old. Unchanged creatine phosphokinase staining in severely necrotic muscle fibres. x 320	135
3.45	Bruise 48 hours old. Decreased creatine phosphokinase staining in necrotic muscle fibres. x 320	135
3.46	Bruise 144 hours old. Strongly positive creatine phosphokinase activity in the leucocytic exudate. The necrotic fibres show decreased activity. x 320	135

FIGURE		Following page
3.47	Bruise 48 hours old. Strongly positive creatine phosphokinase staining of fibroblasts in connective tissue septum. x 320	135
3.48	Creatine phosphokinase zymogram of bruised muscle, leucocytes, plasma and erythrocytes after isoelectric focussing at 25 watt constant power for 1.5 hours in PAG with ampholine buffer range pH 3.5-9.5. The substrate was incorporated in a 2% agarose gel which was applied to the electro-focussed gel for 30 minutes at 37°C.	135
3.49	Creatine phosphokinase zymogram of bruised muscle, leucocytes and erythrocytes after electrophoresis at 250 volts for 90 minutes in cellulose acetate plates. The substrate was applied to a second cellulose acetate plate as a 0.5% noble agar gel. This plate was firmly applied to the first and the pair incubated for 20 minutes at 37°C.	136
3.50	Bruise 48 hours old. Variation in adenosine triphosphatase staining in normal muscle fibres according to fibre type in apparently normal muscle. x 50	136
3.51	Bruise 24 hours old. Variation of adenosine triphosphatase staining according to fibre type is maintained in areas of muscle necrosis. x 125	136
3.52	Bruise 24 hours old. Very mild and therefore equivocal adenosine triphosphatase staining of fibroblasts in connective tissue septum. x 500	136
4.1	Sequential pH measurements of normal muscle sampled 1 hour *post mortem* and homogenised in 5mM sodium iodoacetate.	Page 145
4.2	Sequential pH measurements of normal muscle sampled 24 hours *post mortem* and homogenised in 5mM sodium iodoacetate.	Page 145
4.3	Ranges of fore and hind limb pH values in 3 hour *post mortem* samples of bruises of different ages. Hind limb values are offset to the right for clarity.	Page 146

FIGURE		Following page
4.4	Ranges of fore and hind limb pH values in 24 hour *post mortem* samples of bruises of different ages. Hind limb values are offset to the right for clarity.	Page 146
4.5	Ranges of bruise/control pH differences for 3 hour *post mortem* fore and hind limb muscle samples of bruises of different ages. Hind limb values are offset to the right for clarity.	Page 149
4.6	Ranges of bruise/control pH differences for 24 hour *post mortem* fore and hind limb muscle samples of bruises of different ages. Hind limb values are offset to the right for clarity.	Page 146
5.1	Petechial haemorrhages occurring in adipose tissue but not in muscle.	159
5.2	Discrete haemorrhage in adipose tissue and its associated fascia. (Paraffin section, H+E x 65)	
5.3	A capillary thrombus with closely associated leucocytes. (Paraffin section, picro-Mallory x 1250)	159
5.4	The weekly incidence of petechiation in carcase adipose tissue in comparison with that of conventional bruising.	Page 161
5.5	Changes in arterial pressure following electrical stunning using a 'Thornton Mk II' head-to-back current applicator.	164
5.6	Changes in arterial pressure following electrical stunning using a 'Paralec' head-only current applicator.	164