PRESLAUGHTER AND SLAUGHTER FACTORS AFFECTING MEAT QUALITY IN LAMBS

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ABSTRACT

A plug sampling technique based on a modified muscle biopsy instrument, was developed for the measurement of muscle pH without mutilation of the carcase. It was found possible to routinely obtain muscle tissue samples weighing approximately 2 g and when these were incubated for 24 hours under liquid paraffin at room temperature, followed by homogenisation in a 'Colworth Stomacher', the pH of the solution was found to be an accurate measurement of the ultimate pH of the muscle.

Both the sample method and a direct probe method were found to be well suited for measuring the ultimate pH of muscles. However, the sample method had a higher degree of precision as compared to the probe method when used for measuring pre-rigor pH values.

In a longitudinal survey, in which 1536 lamb carcases were examined, 85.2% of carcases were found to have ultimate pH values below 5.80. Highly significant associations were found between season (summer period) and breed (Perendales) and unsatisfactorily high values of ultimate pH. There was also a highly significant direct correlation between the duration of holding periods of lambs and the ultimate pH of meat, whereas there was a highly significant inverse correlation between wool score and ultimate pH. It was concluded that nutrition plays an important role in the development of high ultimate pH values.

Studies at the meat works indicated that there is a highly significant linear relationship between the number of times lambs are washed prior to slaughter and the ultimate pH of the longissimus muscle. Subsequent resting of animals for varying periods prior to slaughter has no apparent effect on repletion of glycogen stores and may, in some cases, exacerbate

the problem of high pH meat. It was also found that washing of lambs is associated with a highly significant increase in bruising of carcases and that such washing may not necessarily result in a decrease in carcase contamination.

Investigations of the changes of body weights and the weights and nature of ruminoreticular contents during the preslaughter holding period indicated that the ideal time to slaughter lambs, in terms of potential carcase contamination, is 18 to 24 hours after removal from pasture.

It was found that stunning by a 'head-to-leg' electrical method significantly increased the rate of pH decline compared to other methods of slaughter. A further increase in the rate of pH decline was achieved by low voltage stimulation at the time of slaughter. It was concluded that the combined effects of low voltage and high voltage stimulation can cause irreversible contraction and associated toughness in a large proportion of carcases.

Studies of the occurrence of haemorrhages in carcases and organs indicated that these defects are related to the method of stunning. Although blood splash has been reported to be associated with prolonged one stage prothrombin times in lambs, no statistical association was found between this parameter and speckling.

It was found that there is a two to threefold increase in arterial pressure following 'head-only' stunning whereas there was only a moderate increase in venous pressure. On the other hand, stunning by the 'head-to-back' method was followed by a decrease in arterial pressure, but venous pressure increased to levels above 50 mm Hg. Electromyographic studies indicated that there is a significant increase in the intensity of muscular activity following 'head-to-back' stunning as compared to 'head-only' stunning and that there is a correlation between the increased muscular activity and the increase in venous pressure. It is concluded that these events may lead to pressure changes in the microcirculatory bed which are likely to be associated with the occurrence of haemorrhagic defects following stunning by electrical methods.

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CHAPTER ONE

INTRODUCTION AND GENERAL REVIEW OF LITERATURE

THE NEW ZEALAND MEAT INDUSTRY

The growth of the agricultural industry in New Zealand has been reflected by a steady increase in both livestock numbers and the number of cattle and sheep slaughtered for human consumption. During the last few years approximately two million cattle, one million calves, eight million adult sheep and nearly 30 million lambs have been slaughtered annually, whereas the number of pigs slaughtered has remained at a steady level of just under one million per annum (Anon, 1980a). As few pig carcases are exported, the meat export industry in New Zealand is mainly made up of beef and sheep and total export earnings from these two species are now exceeding $2000 million annually or approximately one third of New Zealand's overseas income (Anon, 1982). It would appear that the earnings from beef and sheep are almost equal and in spite of its distance from major world markets, New Zealand has during the last 100 years become the world's largest exporter of sheep meats.

Slaughter of lambs in New Zealand shows a strong seasonal pattern (Figure 1.1). Following lambing in the early spring, the first of the new seasons lambs are delivered to the meat works in late October and early November. These early lambs are usually drafted directly off the ewes and are often referred to as milk lambs. The bulk of lambs is slaughtered from December through to May-June the following year and these animals have usually been weaned for some time and shorn at least once prior to slaughter.

All lambs intended for export together with most of those for the local markets are slaughtered at approximately 50 meat export works scattered throughout the country (Figure 1.2). The lambs are usually transported directly from the farm of origin to the meat works but a small proportion (estimated to be less than ten percent) may pass through saleyards prior to arrival at the works. The majority of lambs are trucked by road and the standard of both vehicles and unloading facilities at the works have been upgraded during the last ten years in order to cope with the high demands

FIGURE 1.1 LAMBS SLAUGHTERED DURING THE 1981/82 SEASON

(Source : New Zealand Freezing Companies Association, Inc.)

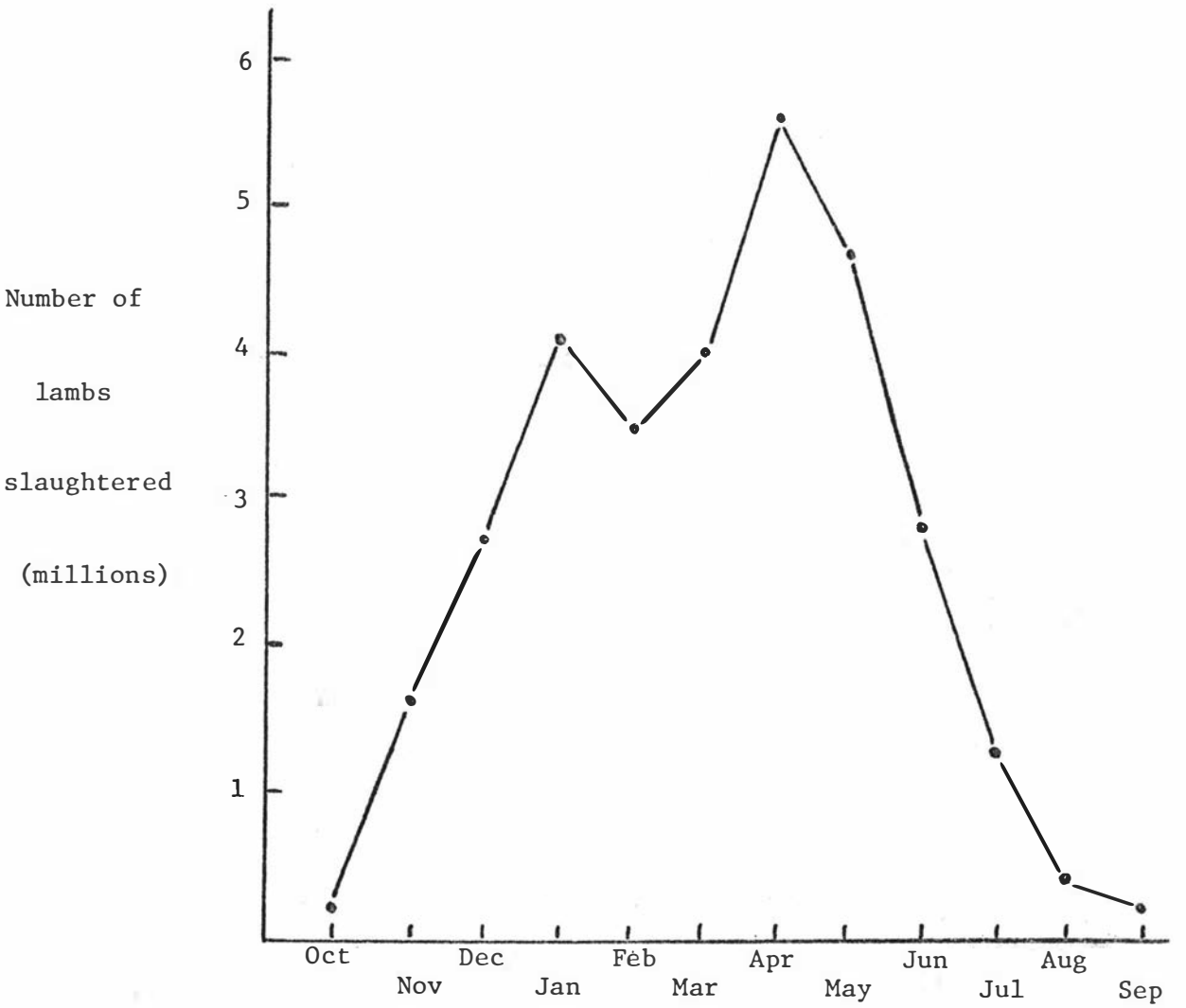
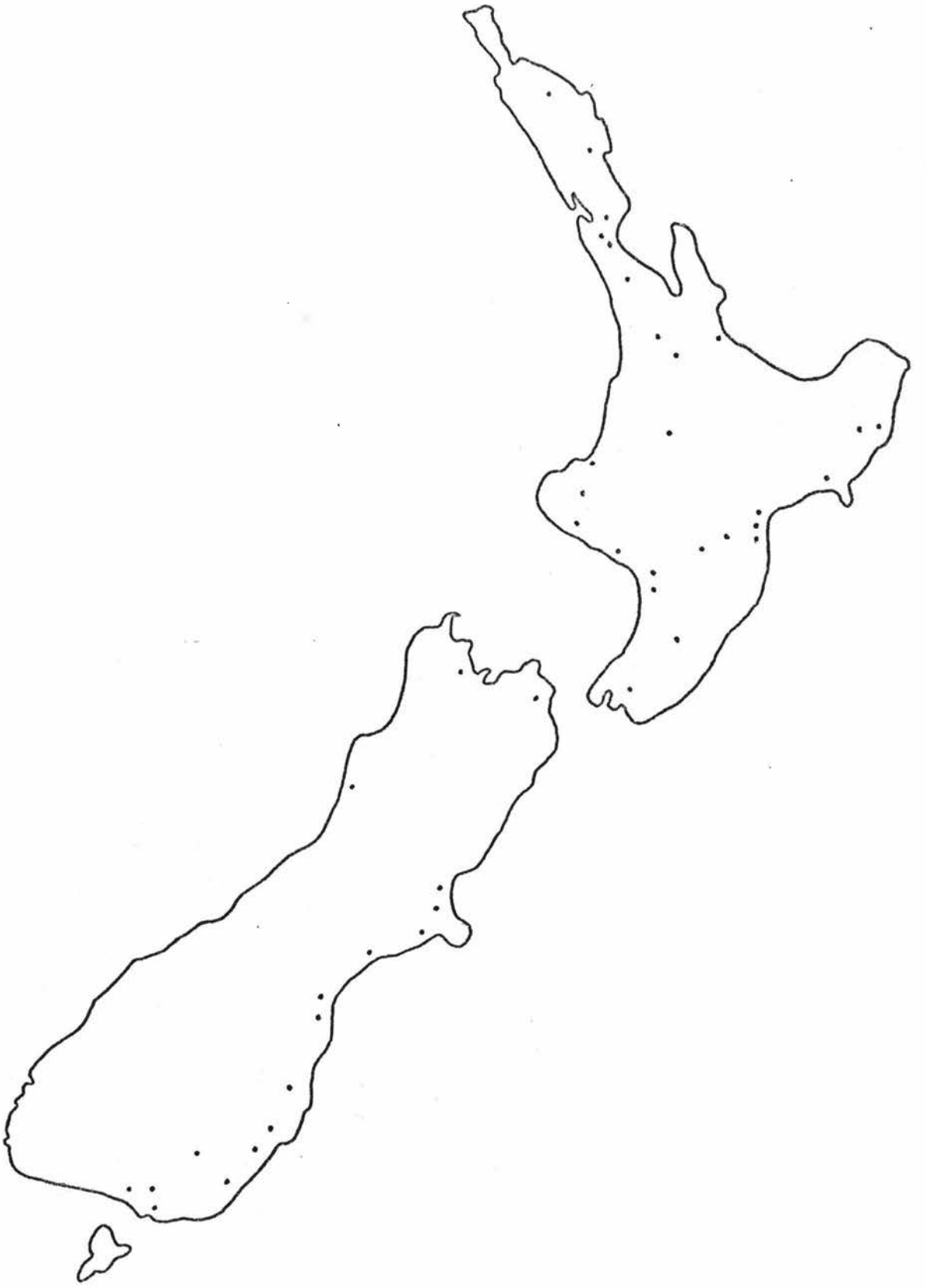


FIGURE 1.2 LOCATION OF MEAT EXPORT WORKS IN NEW ZEALAND

(Source : Meat Division, Ministry of Agriculture and Fisheries)



during the peak of the season when many works receive more than 10,000 lambs per day.

There appears to be no information concerning the average distance travelled by lambs prior to slaughter but in view of the geographical location of meat works within the country (see Figure 1.2) and the increasing transport costs to producers, it would seem unlikely that many lambs travel more than 400 km between farm of origin and the meat works.

After arrival at the meat works both sheep and lambs are subjected to a variety of handling procedures including washing, inspection and sometimes drafting and dagging (clipping of faecally contaminated wool from the hind legs). The animals are then left overnight in covered sheep pens before being slaughtered the following day.

It is now a statutory requirement that stock must be rendered insensible prior to exsanguination (Anon, 1977) and all sheep and lambs are stunned by electrical methods at the export works. However, the method of electrode application and electrical parameters have been changing during the last few years and new methods of electrical stunning are still being developed and introduced in the Industry. The methods of exsanguination have also changed during the last five to ten years and most meat works are now using the thoracic stick described by Blackmore and Newhook (1976).

The dressing and evisceration systems used for sheep and lambs are highly mechanised and new techniques are continuously being introduced. Most of the slaughter chains operate at speeds of approximately eight lambs per minute and the time delay between slaughter of the animal and production of a dressed carcase is usually 20-30 minutes. Many works are operating electrical stimulation systems for accelerated conditioning of carcases so that they are finally frozen within three to four hours of slaughter.

The steady increase in production of lambs for export, has been associated with technological advances in both production and processing. However, it has become evident within the last 20 years, that to compete adequately on world markets, more emphasis must be placed on the meat quality of export lambs.

The quality of meat is determined both by its keeping quality (shelflife) as well as its eating quality (palatability). The shelflife of meat is affected by deteriorative changes taking place in the muscle after slaughter. These include microbial, chemical and physical processes and although there are interrelationships between them, the changes taking place as a result of microbial growth are by far the most important (Ingram and Dainty, 1971; Urbain, 1971). The growth of microorganisms on meat usually results in the development of off-odours or slime and the extent of these potential changes have usually been measured by enumerating the number of organisms on or in the product (Favero *et al.*, 1968; Winter *et al.*, 1971; Nottingham *et al.*, 1975; Yokoya and Zulzke, 1975).

The eating quality of meat is difficult to define as it is related to consumer requirements and therefore varies according to local customs, food preparation and eating habits. The more important factors related to palatability of meat include: aroma, flavour, colour, texture, tenderness and juiciness (Bratzler, 1971) and methods for the measurement of some of these factors such as tenderness and colour have been developed (Macfarlane and Marer, 1966; MacDougall and Rhodes, 1972). However, in spite of these developments it would appear that a trained panel of tasters is still one of the best means for assessment of eating quality of meat. This is because tenderness is multifactorial in nature and the different objective methods usually only measure one characteristic of the meat at a time (Boutou and Harris, 1972).

Research into the quality of meat has traditionally been focused on factors associated with the handling of products after slaughter such as improvements in preservation methods and the effects of different methods of packaging. Recently it has become more apparent that some of the most important factors affecting both eating and keeping quality are related to the handling of animals immediately before and during slaughter. The preslaughter period is defined here as the time between mustering and drafting of animals on the farm until the initiation of slaughter. This latter process usually includes two separate phases, namely stunning which should cause immediate insensibility of the animal, followed by exsanguination.

The work presented in this thesis is related to various aspects of the preslaughter and slaughter period and therefore covers a wide range of topics. The following review of some of the literature in relation to these topics is not intended as a comprehensive analysis of all facets of this subject. It is intended only to give a general overview of some of the important developments in this field which will be expanded and discussed in more detail as specific aspects are dealt with in each chapter.

MICROBIOLOGY OF CHILLED AND FROZEN LAMBS

The remoteness of New Zealand from major meat markets has always been a major factor in the development of the meat industry and it was not before mechanical refrigeration came into use, just a little over a hundred years ago, that lambs from New Zealand could be profitably marketed in the United Kingdom (Critchell and Raymond, 1912). Some of the first shipments of meat were troubled by the appearance of "Dark Spots" which was probably similar to the condition now known as "Black Spot" and reported to be caused by fungal growth (Thornton, 1968). Some of the organisms associated with "Black Spot" have recently been identified (Gill and Lowry, 1981) and factors affecting their growth established (Gill and Lowry, 1982). From these observations it would appear that fungal spoilage of meat only occurs in frozen meat if it is held at temperatures 2-3^{o}C below the freezing point of the product for several months, or if meat with a surface sufficiently dry to inhibit bacterial growth reaches higher temperatures. Such conditions would rarely occur during normal commercial practice as statutory regulations require that all meat must be stored at temperatures below -12^{o}C (Anon, 1969) and prolonged drying of meat surfaces during chilling is usually avoided because it causes loss of weight of carcases. The microflora associated with spoilage of meat is thus usually dominated by bacteria (Scott and Vickery, 1939; Ingram and Dainty, 1971).

The bacterial spoilage of chilled meat has been studied extensively by Australian workers (Empey and Scott, 1939) and they concluded that the "low temperature type" organisms play an important role. They have been termed psychrotrophic organisms by Eddy (1960) who suggested that all organisms capable of growth below 5^{o}C can be included in the group regardless of their optimum temperature for growth. The work by Empey

and Scott (1939) also suggested that the psychrotrophic organisms mainly originate from the skin of animals. It was therefore recommended that animals should be washed prior to slaughter and that care be exercised during the dressing of carcases to avoid contamination of the surface of meat. Similar findings have been reported from the U.S.A. (Ayres, 1955) and the importance of the initial bacterial loads required for development of slime was later stressed by Ayres (1960).

In New Zealand, Nottingham *et al.* (1974) concluded that cleanliness of stock before slaughter is necessary to reduce contamination of beef carcases. In a more recent study, Newton *et al.* (1978) compared microbial loads of the fleece of sheep with that of the surface of carcases throughout the killing season. These workers reported that the total counts on the carcases were an almost constant fraction (0.3%) of those found on the fleeces but there were no significant differences in total counts on any of these surfaces between different seasonal periods. However, some seasonal variation was observed in relation to the data on psychrotrophic counts from fleece surfaces.

On the basis of these studies and subject to storage conditions, it would appear that spoilage of meat is dependant on initial bacterial load which in turn may be related to the degree of contamination of the skin of animals prior to slaughter. It would also seem likely that when animals are excessively dirty, contamination of meat can be reduced by washing the animals prior to slaughter. In fact, the New Zealand meat industry has during the last ten years introduced a vigorous policy of washing stock prior to slaughter. During the same period, dressing techniques have also improved and this has undoubtedly also played an important part in reducing the risk of transferring dirt from skin to carcases. The effect of either one or both of these factors may have been the reason why no direct correlation could be found between bacterial counts of the fleeces of sheep and counts on meat surfaces in the more recent studies in New Zealand (Newton *et al.*, 1978).

Little attention has been paid to the possible side effects of washing of stock, although it has been shown that washing of lambs greatly increases the risk of bruising of the animals (Petersen, 1978). These studies also indicated that in many cases, washing of lambs has no significant effect on the amount of visible contamination which can be detected on the carcases by regulatory inspection procedures.

The additional handling and time associated with washing and drying of lambs prior to slaughter may also have some other effects on meat quality. Some early studies in pigs indicated that the prevalence of salmonellae in the intestinal tract of these animals at slaughter is greater than it is when they are on the farm (Galton *et al.*, 1954). It has also been shown by Grau and Smith (1974) that the percentage of sheep infected with salmonellae and the number of salmonellae/g of faeces increases with increased holding period in the yards. Although similar studies have not been carried out in New Zealand, it has been shown that the prevalence rate of enteric salmonellae of sheep slaughtered at two meat works was 4.7% (Kane, 1979). The risk of spread of salmonellosis within the crowded environment of the stockyards at the meat works would thus seem substantial.

Recent work in Australia indicates that the important sources of salmonellae contaminating carcases meat includes the contents of the gastrointestinal tract, the mesenteric lymph nodes and the skin of the animals (Peel and Simmon, 1978; Smeltzer *et al.*, 1980; Samuel *et al.*, 1980a; 1980b). The likelihood of contamination from the first source is probably related to the amount and nature of gastrointestinal contents at the time of slaughter but this problem has attracted limited attention in the past.

TENDERNESS OF EXPORT LAMBS

It has been suggested by Bratzler (1971) that tenderness of meat is the most important palatability characteristic and that it is influenced by both ante mortem and post mortem factors. Most of the earlier work on tenderness was carried out in beef and Lawrie (1974) suggested that species, breed and heritability factors may all be important. More recent work in New Zealand has failed to establish significant differences between breeds of cattle with respect to tenderness (Purchas and Barton, 1976) and there appears to be no information available concerning breed differences in sheep. In general, increasing age is associated with decreasing tenderness but is also associated with a decrease in collagen. This apparent contradiction may be explanined by the fact that the collagen in young animals has a higher content of reticulum, and less cross-binding, than collagen in older animals (Hiner and Hankins, 1950; Goll *et al.*, 1963).

These intrinsic factors associated with tenderness are probably of far less importance in lambs as compared to factors related to handling of the carcases during and immediately after slaughter. Investigations in this field were initiated some 20 years ago after complaints were received from some markets about the toughness of New Zealand lambs and this led to a number of exciting discoveries concerning the post mortem changes in muscles. These studies have been reviewed by Locker *et al.* (1975) so only the main features will be discussed in this review.

It was suggested by Locker (1960) that contraction of muscles induces toughness in the meat and this was later confirmed by Marsh and Leet (1966). The relationship between pre rigor shortening of muscles and tenderness (measured in 'Shear Force' values) has been depicted in Figure 1.3. It can be seen that there is a steady decrease in tenderness as muscle shortening increases up to 40%. When muscle shortens beyond 40%, there is an increase in tenderness which has been ascribed to gross stretching and tearing of fibres resulting in progressing weakening of the tissue. This clearly indicates that even small variations in pre rigor shortening of muscles can have a marked effect on tenderness, e.g. an increase from 20% to 30% shortening would result in 'Shear Force' values increasing from approximately 40 (equal to acceptable meat quality) to nearly 80 (very tough meat).

Changes in the degree of shortening of muscles pre rigor are temperature related and the association between these two factors was first reported in beef by Locker and Hagyard (1963). It can be seen in Figure 1.4 that minimum shortening occurs at 15-20^{o}C. There is an increase in shortening when temperatures are higher but this has little practical significance because ambient temperatures at meat works would rarely exceed 25^{o}C. However, muscle shortening also increases at lower temperatures particularly below 10^{o}C and this finding is of considerable importance as meat is usually chilled quickly after slaughter in order to control the growth of microbes. This cold shortening also occurs in sheep meat and the ability of muscles to shorten at low temperatures persists until the muscles go into rigor and there is no longer any adenosine triphosphate (ATP) available for contraction. At this stage in the rigor process no further anaerobic glycolysis occurs. Thus there is no further production of lactic acid and the pH of meat is at its lowest or ultimate value.

FIGURE 1.3 TENDERNESS IN RELATION TO SHORTENING INDUCED BY EXPOSING MEAT SAMPLES TO CHILLING (2°C) DURING ONSET OF RIGOR MORTIS

(Source : Marsh and Leet, 1966)

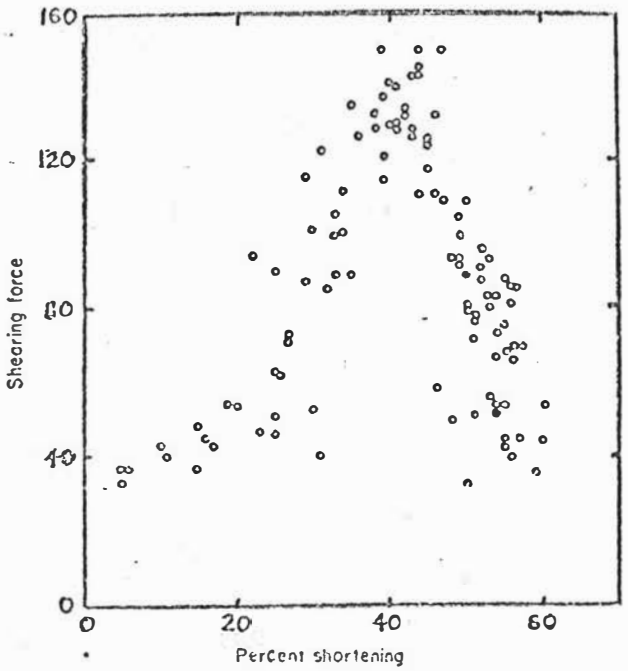
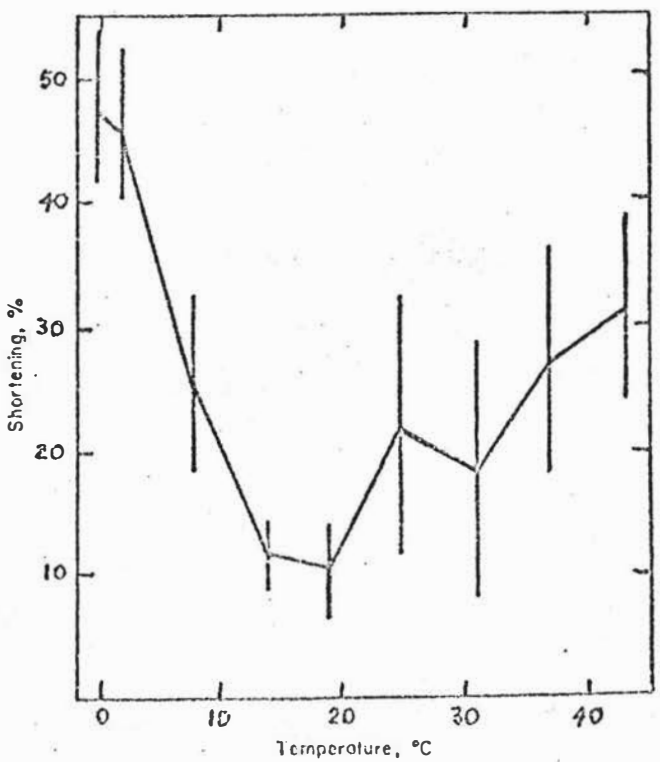


FIGURE 1.4 MEAN ULTIMATE SHORTENINGS OF MUSCLES AT VARIOUS STORAGE TEMPERATURES

(Source : Locker and Hagyard, 1963)



Compared with physiological contraction, cold shortening is extremely slow and develops very little force. It has also been shown by Marsh and Thompson (1958) that if muscles are frozen pre rigor and rapidly thawed, there is drastic shortening accompanied by massive exudation. This so called thaw shortening was shown to be more forceful than cold shortening.

These studies indicate that if shortening of muscles and the accompanying toughness is to be avoided, carcases should be allowed to enter rigor mortis at optimal temperatures (15-20^{o}C) or muscles should be restrained in order to prevent them from contracting. This latter concept of 'posture hanging' was suggested as a practical solution to overcome the problems of toughness and at the same time allow early freezing of carcases (Davey and Gilbert, 1974) but the system has had only limited use in the meat industry. More recently, it was suggested by Davey and Garnett (1980) that cold shortening can be prevented by freezing lamb carcases very rapidly within a few hours of slaughter. If such carcases are stored at -12^{o}C for approximately one month, post mortem glycolysis will continue at a slow rate and the muscles will eventually enter rigor while still in the frozen state. Thus by strict control of both freezing rates and frozen storage times and temperatures, the toughness associated with both cold and thaw shortening can be avoided.

As a result of the earlier studies of tenderness of meat, many meat works introduced new processing methods for lambs which allowed carcases to be held for one to two days at temperatures above 10^{o}C. The first part of this holding period is usually referred to as the conditioning period and the latter part as the aging period. Such processing methods allow the muscles to go into rigor without any appreciable shortening during the conditioning period and structural changes associated with weakening of Z lines within the myofibril occurring during the aging period will further increase tenderness (Davey and Gilbert, 1967).

The additional space and extra labour required for the conditioning and aging process and increased attention to environmental conditions to avoid microbial spoilage were costly to the meat industry. Consequently, when later research indicated that required holding times prior to freezing could be reduced by accelerating the post mortem glycolysis in muscles, by electrical stimulation of carcases, this new process was widely accepted. It was shown in pigs that electrical stimulation can double

the rate of decline of pH post mortem (Hallund and Bendall, 1965) and later work in lambs gave similar results (Carse, 1973). Further studies indicated that 90 seconds electrical stimulation within 30 minutes of slaughter of lambs resulted in mean pH of muscles declining to below 6.0 within one to two hours. Such methods could therefore greatly reduce the required holding period prior to freezing without the associated risks of cold shortening (Chrystall and Hagyard, 1976; Hagyard *et al.*, 1980; Davey and Chrystall, 1980). These methods have been adopted by the meat industry and it has been estimated that 90 per cent of all export lambs are now being subjected to electrical stimulation (Chrystall, 1980).

Since the introduction of electrical stimulation, studies have been carried out overseas to evaluate the tenderness of New Zealand lambs at the point of usage (Hagyard, 1980). These investigations indicated that electrical stimulation (or accelerated conditioning as it is often called) had vastly improved tenderness. However, in some of the samples, a small number of carcases (sometimes up to 20%) were still unacceptably tough. Although this discrepancy between the apparently excellent results obtained in laboratory studies and those obtained from commercially stimulated carcases might be explained by occasional failure of equipment within the works, other factors could also have had an effect. The time between death of an animal, the development of rigor mortis and ultimate pH is dependent on the amount of glycogen in the muscle at the time of death (Bate-Smith and Bendall, 1949). Thus, muscles developing a low ultimate pH would require longer periods prior to freezing or more efficient stimulation to avoid cold shortening as compared to muscles developing a higher ultimate pH. These differences in times between slaughter and the onset of rigor mortis do not appear to have been taken into account in previous studies of electrical stimulation. Such studies have generally been carried out with a small number of animals developing a narrow range of ultimate pH values and it is therefore difficult to assess whether the results are applicable to the total population of lambs slaughtered at the meat works which may have different muscle glycogen reserves.

ULTIMATE pH AND MEAT QUALITY

The ultimate pH of meat is probably the most important objective quality characteristic of the product and meat with a high ultimate pH is usually referred to in the literature as "Dark, Firm and Dry" (DFD) meat or dark cutting meat. Both of these terms refer to the appearance of such meat which is described as being of a darker than usual colour and having a firm and dry texture. The condition has probably been recognised since 1774 but it is only more recently that it has been realised that it is associated with depletion of muscle glycogen prior to slaughter, resulting in the degree of post mortem glycolysis being insufficient to produce enough lactic acid to lower the ultimate pH to the usual level of 5.4 to 5.6 found in meat from normal animals (Lawrie, 1974).

The appearance of DFD meat is generally considered undesirable by consumers and a number of studies have also been carried out to evaluate the eating quality of such meat. Recently Dransfield (1981) reported that laboratory tests indicated that DFD beef is more tender than beef of normal pH but the relation between tenderness and pH depends upon muscle, cooking conditions and method of assessment. It was also reported that DFD had less "beef flavour" and consequently was less preferred and less acceptable than meat of normal pH. Similar findings in sheep meat were reported in a review by Ford and Park (1981) who also reported an increase in "non-meat aroma" which was concurrent with higher ultimate pH values.

DFD meat has poor keeping qualities. It was reported by Nicol *et al.* (1970) that meat stored under low oxygen tension (packed in gas impermeable films) became more rapidly spoilt when the pH was above 6.0 because of the growth of a hydrogen sulphide producing organism and consequent formation of the green pigment sulphmyoglobin. More recently these problems have been extensively investigated in New Zealand (Newton and Gill, 1978; 1980; 1981). Glucose is preferentially utilised by meat spoilage organisms and aerobic spoilage is delayed until the glucose is exhausted and amino acids are used as an alternative substrate. DFD meat contains little or no glucose and amino acids are therefore utilised without delay with the result that spoilage odours can be detected when bacterial densities are still quite low. On the other hand the early spoilage of DFD meat packed in gas impermeable film was found to be

caused by growths of an organism (*Alteromonas putrefaciens*) which can be inhibited by lowering the pH of the meat. Thus spoilage of DFD meat under aerobic and anaerobic conditions would appear to be caused by two different mechanisms.

Economically, the spoilage of DFD meat under anaerobic conditions is probably the most important factor because the export of chilled beef meat packed in gas impermeable films is becoming an increasingly important method of marketing. The desirable shelflife of up to three months for such products can only be achieved under optimal conditions and the ultimate pH of the meat has been the limiting factor. Chilled lamb is also exported from New Zealand but so far the amount of meat has not been substantial and it is not known whether problems associated with DFD meat may be of importance with this type of product.

In view of the importance of the ultimate pH in relation to meat quality, it is not surprising that many studies have been carried out to investigate the effects of preslaughter handling on muscle glycogen reserves because these reserves are directly related to ultimate pH values (Lawrie, 1974). Initial work on the influence of fasting on the depletion of muscle glycogen reserves was carried out in 1877 (Lawrie, 1958) and later studies in beef indicate that limited feeding produces high ultimate pH of meat only when animals are subjected to concurrent stress (Lewis *et al.*, 1962; Carr *et al.*, 1973; Holmes *et al.*, 1973). Paradoxically, Shorthose (1978) reported that the carcases of sheep on higher levels of nutrition have higher ultimate pH values as compared to animals on lower levels of feed intake but the work on which this finding was based involved a very small number of sheep (four animals per group). This rather surprising result may well have been due to the effects of unidentified variables.

The effect of exercise on muscle glycogen reserves have been investigated in man (Rosell and Saltin, 1973), rats (Terjung *et al.*, 1973), pigs (Briskey *et al.*, 1959), cattle (Mitchell and Hamilton, 1933) and sheep (Forrest *et al.*, 1964; Chrystall *et al.*, 1981a). Although these studies indicate that muscle glycogen can be depleted by exercise in mammals, the exercise has to be of a very severe nature before there is an appreciable reduction in muscle glycogen. It would thus, appear, as has previously been pointed out by Lawrie (1958), that neither fasting nor enforced

exercise can result in a marked increase in the ultimate pH of meat under natural circumstances.

The effect of adrenaline injections prior to slaughter on depletion of muscle glycogen in lambs was studied by Hedrick *et al.* (1961). They reported that muscles from adrenalin treated animals were significantly darker in colour and had higher ultimate pH values as compared with muscles of untreated animals. Similar findings have also been reported in beef (Hedrick, 1965) and it was later reported that the effects of adrenalin administration could be abolished by simultaneously injecting the animals with a beta-blocking agent (Ashmore *et al.*, 1973). These findings indicated that glycogen depletion may be induced by adrenalin acting on glycogen phosphorylase and thereby accelerating glycogen metabolism in the muscle. However it has later been found that beta-blocking agents only have a small protective effect when bulls are subjected to stress by mixing them with bulls from other sources (McVeigh and Tarrant, 1981a). This suggests that under such conditions muscle glycogen depletion in cattle is not predominantly mediated by catecholamines.

The majority of studies of the aetiology of DFD meat have been carried out as small laboratory investigations and it has therefore been difficult to use the results of such work to formulate guildelines for handling of stock prior to slaughter. However, it has been suggested that extended holding periods in the stock yards and mixing of different groups of animals can cause high ultimate pH in both beef and pigs (Nielsen, 1979; Augustini, 1981). The lack of more extensive investigations of the causative factors associated with DFD meat may be related to difficulties in objectively identifying the condition during commercial operation at the meat works. In earlier investigations, the colour of the cut muscle surface was used as an indicator of DFD meat. However, the ultimate pH of the muscle is now commonly measured and it has been shown that there is a good correlation between these two paraemeters (Munns and Burrell, 1965; MacDougall and Rhodes, 1972). Such measurements of ultimate pH are often only obtained from one of the larger muscles of the carcase and it would appear that most workers have been using the thoracic and lumbar parts of the longissimus muscle (Anon, 1973). This muscle has previously been referred to as the longissimus dorsi muscle (LD).

The time required for a muscle to reach the ultimate pH is temperature dependent (Bate-Smith and Bendall, 1949) and Marsh (1954) suggested that, with efficient cooling systems, a period of 26 to 36 hours may be required for the completion of all post mortem glycolytic changes in the LD of beef animals. In spite of these earlier observations, which have not been disputed, it would appear that in some later studies of factors related to ultimate pH, measurements obtained only approximately 24 hours after slaughter have been used (Munns and Burrell, 1966; Duchesne, 1978; Puolanne and Aalto, 1980; 1981). It is suggested that the assumption made by these workers that such pH values are identical to the ultimate pH is erroneous and can lead to false conclusions. This belief tends to be confirmed by more recent studies which showed that there are significant differences in chilled beef between mean pH values obtained 24 hours after slaughter and those obtained a further 24 hours later (Petersen, 1982).

There are clear advantages if further data on the causes of high ultimate pH of meat are obtained by large scale investigations at the meat works. Not only does this give a better statistical basis on which to analyse data but it also ensures that observations relate to practical conditions in the meat industry. However, before such studies are undertaken, there is clearly a need to develop accurate methods for measuring the ultimate pH of meat under commercial operations at a meat works.

NEW SLAUGHTER METHODS FOR SHEEP AND LAMBS

The methods of stunning and slaughter of sheep and lambs have undergone more changes during the last few years than any other part of the process at the meat works. Until 1977, sheep and lambs were slaughtered by a transverse incision of the extended neck which almost simultaneously severed the trachea, oesophagus, common carotid arteries and jugular veins, and the spinal cord at the occipitoatlantal junction (Blackmore and Newhook, 1976). Although this method was considered both humane and efficient by the meat industry, some overseas observers were critical of the humane aspects of the slaughter method. It was also considered a disadvantage that contamination of head and neck meat from reflux of ruminal contents was difficult to prevent and the extensive incision of skin and tissues of the neck made head skinning a difficult

operation (Blackmore and Newhook, 1976). As a result of these objections, the statutory regulations covering slaughter of stock were changed and now require that all animals must be rendered insensible prior to exsanguination (Anon, 1977).

Since 1977 all sheep and lambs slaughtered in meat works in this country have been stunned by electrical methods. Most meat works initially used methods whereby the electrodes were applied to the head only. Such methods were apparently introduced in the United Kingdom from Germany in the 1930's (Ducksbury and Anthony, 1929; Clark and Tweed, 1932; Warrington, 1974) and were assumed to have induced an effective stun if the animals showed the signs of an electroplectic fit as indicated by a short tonic phase followed by a somewhat longer clonic phase (Croft and Hume, 1956). More recent work employing electroencephalographic techniques in sheep have shown this method of stunning produce reversible insensibility for 18 - 42 seconds and can be considered humane if the animals are slaughtered within 20 - 30 seconds of stunning. Permanent insensibility due to exsanguination occurs within seven seconds (Blackmore and Newhook, 1982; Newhook and Blackmore, 1982).

There is considerable movement associated with the clonic phase following electrical stunning with electrodes applied to the head only. This method was therefore modified to include a third electrode which is either applied to the back of the animal ('head-to-back' method) or to the legs of the animal ('head-to-leg' method). These 'head-to-body' methods appear to produce better immobility of the animals as compared to the traditional 'head-only' method and they also produce permanent cardiac dysfunction (Blackmore and Newhook, 1982). However, this latter effect has been unacceptable to some of the important export markets and most meat works have therefore recently reintroduced electrical stunning by the 'head-only' method. This is frequently followed by a period of electrical stimulation in order to achieve immobilisation during the exsanguination of the animals. This type of electrical stimulation is referred to as low voltage stimulation because peak voltage is usually only 30 - 50 volts as compared to the so-called high voltage stimulation, described earlier, which usually operates at a much higher voltage. A similar distinction between the two types of stimulation has previously been used in beef where methods applying less than 120 volts are referred to as low voltage stimulation and other methods are termed high voltage stimulation (Shaw and Walker, 1977; Bouton *et al.*, 1978).

The different methods of stunning and slaughter can have various effects on meat quality. As mentioned previously, exsanguination resulting in simultaneous severance of the oesophagus commonly causes contamination of the neck and head meat. Thus methods of stunning producing a sufficient length of time of insensibility and stillness to ligate the oesophagus prior to slaughter are therefore preferable. Both rate and extent of bleeding can be affected by methods of slaughter (Blackmore and Newhook, 1976; Kirton *et al.*, 1981b) and it has been suggested that badly bled carcases undergo rapid decomposition (Thornton, 1968). However, recent studies indicate that residual blood content of muscles is not affected by slaughter methods (Warriss, 1977; Warriss and Leach, 1978; Warriss, 1978). Traumatic injuries associated with restraint and handling of the animals during slaughter have also been reported. These include bone fractures in pigs stunned by electrical methods (van der Val, 1976) and bruises in beef and lambs associated with handling during the slaughtering of these animals (Meischke, 1975; Petersen, 1978),

One of the most important defects associated with slaughter of stock is the so called "blood splash" which is described as ecchymoses occurring in muscles and some organs. This defect was studied in sheep by Tweed *et al.* (1931) and they showed that stunning by captive bolt is associated with an increase in arterial pressure which may exacerbate the extent of "blood splash". However, these workers concluded that the increased arterial pressure is not the sole cause of the defect and that other factors such as muscular contractions associated with stunning may play an important role. Similar observations were made in pigs stunned by electrical methods (Mandrup, 1964) and it was suggested that ecchymoses in muscles were not caused by rupture of capillaries but were a result of diapedesis which may be exacerbated by increased blood pressure. More recent work in lambs in New Zealand (Kirton *et al.*, 1978) also failed to confirm that increased arterial pressure was the primary cause of ecchymoses and it has been suggested by Leet *et al.* (1977) that "blood splash" is caused by 'supercontracture' of some muscle fibres, causing severe strain on adjacent blood vessels and consequent rupture of capillaries.

It would appear that "blood splash" in lambs has been of greater importance in New Zealand since the introduction of electrical methods of stunning and it has been shown that both captive bolt and percussion stunning of lambs causes less "blood splash" than electrical stunning

(Blackmore, 1979; Kirton *et al.*, 1981a). It has also been shown that electrical stunning by "head-only" methods increases the rate of "blood splash" as compared to "head-to-back" methods (Kirton *et al.*, 1981b). However, it has been reported that although these latter methods cause few or no ecchymoses in muscles and organs, they are likely to produce petechial haemorrhages in the subcutaneous fat (Petersen and Wright, 1979; Thornton *et al.*, 1979).

Both ecchymotic haemorrhages in muscles and petechial haemorrhages in subcutaneous fat are of considerable economic importance to the meat industry and further studies of the possible causes of these defects are therefore warranted.

RESEARCH NEEDS IN THE MEAT INDUSTRY AND AIMS OF THE PRESENT STUDIES

The introduction of more advanced refrigeration technology in the early nineteen sixties, greatly improved the keeping quality of meat and meat products but its deleterious effect on eating quality of meat was not immediately appreciated. This could have had a disastrous effect on our most lucrative markets had it not been for the successful investigation of this aspect of meat quality (Locker *et al.*, 1975). This incident and others like it occurring within the meat industry during the last two or three decades highlight the problems facing those who are directing and carrying out research related to the quality of meat and meat products.

There is little doubt that research in meat science has become divided into specialised fields as a result of the broad spectrum of basic sciences this subject encompasses (e.g. microbiology, physiology, biochemistry etc.) and the depth of knowledge required of research workers in these specialised fields. Although basic research in narrow fields, such as that related to the biochemical changes involved in muscle contraction, is often required to solve specific problems there is also a need for research on a more broad basis. Such broadly based research cannot rely solely on investigating one particular aspect of the problem but should as far as possible study all effects on meat quality in the context of an industrial setting. For example, in studies of the effects of washing of animals prior to slaughter as a means of controlling contamination of carcases, other possible effects of washing on meat quality also need to be investigated.

With this philosophy in mind the research to be reported upon in this thesis was, wherever possible, based on initial development work in the laboratory which was followed by subsequent application in an industrial setting which was as close to normality as experimental limitations would allow.

Studies were undertaken to investigate the effects on meat quality in lambs of the various current methods which apply from the time the animals leave the farm until slaughter is completed. One of the important quality characteristics measured in these studies was the pH of meat and some of the initial investigations were therefore aimed at developing accurate methods for measuring the pH of muscles. A number of observational studies were designed to identify factors affecting meat quality and further experimental studies of some of these factors were carried out either at the meat works or in the laboratory. It was hoped that by keeping the conditions as close as possible to those prevailing within the meat industry, interpretation of results would be less artificial and the information from such studies would be directly applicable to the meat industry.

CHAPTER TWO

DETERMINATION OF AN APPROPRIATE METHOD FOR MEASURING THE pH OF MEAT

INTRODUCTION

The glycolysis occurring in muscles of an animal after death is associated with a gradual reduction of glycogen, creatine phosphate and later adenosine triphosphate. These chemical changes are accompanied by the formation of lactic acid and a resultant fall of pH in the muscle (Bendall, 1973). The rate and extent of post mortem glycolysis can be assessed by measuring changes in the amounts of these chemical components. However, in field studies involving large numbers of animals, estimation of the various glycolytic intermediates becomes impractical and most workers therefore rely on information on the pH alone when estimating the rate and extent of the post mortem glycolysis. The close relationship between certain aspects of meat quality and the pH of meat (as discussed in the first chapter) also justifies such an approach.

The work and results presented in this chapter pertain to the development and comparison of techniques most suitable for measuring pH of carcase muscles during commercial operations. Conventionally this chapter could be considered a description of the general materials and methods for measuring the pH of muscle. As development of the final method selected depended upon a series of sequential investigations, it was difficult to construct the chapter in the conventional style of introduction, materials and methods, results and discussion. It was therefore felt to be more logical and appropriate to describe in turn each sequential investigation and the results obtained. It is hoped that this less conventional form of presentation provides a clearer appreciation of the development and adoption of the technique used in the further studies which form the major part of this thesis.

The pH is defined as the negative logarithm of the hydrogen ion concentration and it has been suggested (Barth, 1975) that when mean values are calculated, the arithmetic mean of the hydrogen ion concentration should be used rather than using the arithmetic mean

of the pH values. However, Middleton and Rovers (1976) showed that the arithmetic mean of a set of pH data is equal to the negative logarithm of the geometric mean of the hydrogen ion concentrations. The geometric mean puts less emphasis on extreme values, which are likely to occur when measuring the hydrogen ion concentration and it is therefore theoretically a better measure of central tendency in such cases. The use of pH data for calculation of arithmetic mean values and other statistics without transformation to hydrogen ion concentrations would thus appear to be the most valid method of dealing with such data and this procedure has been followed in the present work unless otherwise stated.

As the data on pH from field studies are used to measure the effects of one or several factors, the accuracy of pH measurements becomes an important issue. The accuracy of any test is dependant on the degree of bias of the test as well as on the repeatability of the test. The former can only be evaluated by either measuring standard media of known values or by comparisons with a standard (benchmark) method. On the other hand, the repeatability or precision of different techniques can be directly compared by repeated measurements of test media of unknown values.

The muscles for these studies were obtained from carcases condemned during regulatory meat inspection and a wide range of pH values could therefore be expected. In order to compare the precision of different methods, multiple samples were taken from the muscles. The percentage of the variance of a single observation due to within muscle variation was calculated from a single classification analysis of variance table for each muscle and each method (Sokal and Rohlf, 1969). The within muscle variation reported in this work is thus a direct measure of the precision of the measuring technique used and allows for comparisons between different techniques provided they have been used on the same muscles.

MEASURING THE pH OF SAMPLES

It has been customary to measure pH of muscles at about 20°C in a solution of 5mM sodium iodoacetate at a dilution of one part homogenised muscle to ten of the solution (Bate-Smith and Bendall, 1947 ; Lawrie, 1953; Marsh, 1954; Bendall, 1973). The iodacetate is added to the solution in

order to inhibit the enzyme glyceraldehyde-3-phosphate dehydrogenase and thus to arrest glycolysis (Jeacocke, 1977) so that pH can be measured accurately at any time during the pre rigor period.

Homogenisation of muscle samples in the solution is usually achieved by using a homogeniser similar to that described by Marsh and Snow (1950). Although this is an efficient technique for disintegrating muscle tissue, it is also very time consuming as both the homogeniser and beakers must be cleaned thoroughly between samples to avoid cross contamination. In these studies, a Colworth Stomacher 400* was used to homogenise muscle tissue. Each sample (usually weighing approximately 2g) was placed in a plastic bag and the required amount of solution added by a dispenser. Six to eight bags at a time were placed in the Stomacher for two minutes and the pH measured by inserting a combination glass electrode in the bag and recording values with a Triac pH meter**. It was thus possible to evaluate batches of samples without having to clean any equipment between samples. However, it was thought that the pH of solutions might not remain stable over varying times after homogenisation and it was decided to investigate this problem in a small experiment.

The major part of the LD was excised from one side of 24 lambs and left at room temperature for 24 hours. One sample (approximately 2g) from each muscle was homogenised in 20 ml of a 5mM iodacetate solution. The pH of the solution was measured within five minutes of homogenisation and a number of subsequent readings were obtained during the following eight hour period.

On the basis of the values obtained at the first reading, the animals were divided into two groups of 12 animals in each group:

	Mean	Range
"Low" group	5.72	5.61 - 5.79
"High" group	6.07	5.86 - 6.30

The mean pH for both of these groups as well as for the total group was calculated for each recording time and these data are presented in the diagram in Figure 2.1.

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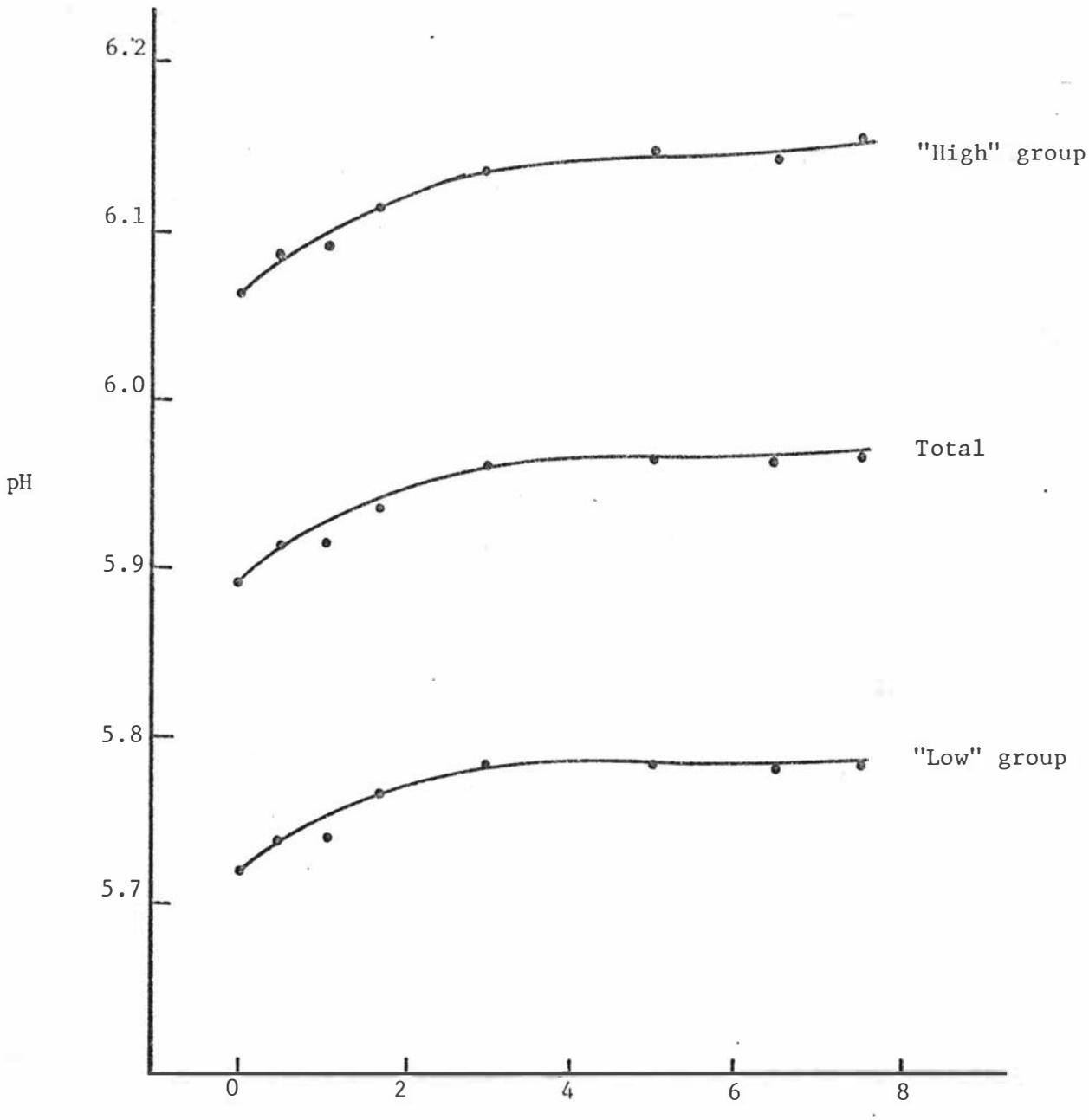
** Triac Controls Ltd., P.O.Box 45-159, Auckland, 8, New Zealand

FIGURE 2.1 THE EFFECT OF TIME ON pH VALUES AFTER IODOACETATE HOMOGENISATION



It can be seen that there is an increase in pH values during the first three hours after homogenisation and that this increase is of the same magnitude in all groups. However, the rate of increase during this period was estimated to be only 0.02 pH units per hour and as samples are always read within half an hour of homogenisation, the change in pH should have little effect on the accuracy of the test.

It has been suggested by Bendall (1973) that iodoacetate inhibition causes some alkalinisation of the muscle homogenate solution but this effect was reported to fall to zero as the pH declined towards the ultimate value. The cause of the increase in pH values in the present experiment does not appear to be associated with differences in actual pH values of the samples as both the "low" and "high" group showed the same increase. It was also suggested by Bendall (1973) that there is a diffusion of carbon dioxide associated with homogenisation in iodoacetate and if such a loss is gradual it may not reach its full effect (estimated to be approximately 0.05 pH units) for several hours after homogenisation and this could explain the present results.

A solution of iodoacetate/water is hypotonic in relation to muscle tissue, thus lowering the ionic strength of muscle suspension in such a solution. This can be avoided by making up the iodoacetate in a 150 mM potassium chloride solution which has the same ionic strength as mammalian muscle. It was suggested by Bendall (1973) that when this latter solution is used different dilutions of muscle suspension may be used without affecting the accuracy of pH measurements. This concept appeared to be of considerable practical importance and it was therefore decided to carry out some comparative pH evaluations of muscle suspension in iodoacetate solution in water and in potassium chloride.

A 10-15 cm section of the LD was removed from one side of four sheep carcases within one hour of slaughter. These sections were held for 24 hours at room temperature after which period, surface tissue was removed from the muscles (2-3mm) and the remainder of each muscle was ground in a household mincer and then mixed thoroughly in a polythene bag. From each homogenised muscle section, ten samples of between 1-2.5g were taken. These ten samples were divided into two groups of five ensuring that the distribution of sample weights was similar in both groups. All the samples from one sample group were homogenised in 20 ml 5mM

iodoacetate/water solution whereas the samples from the other group were homogenised in 20 ml 5mM iodoacetate/150 mM potassium chloride solution. The pH values of all solutions were obtained within 30 minutes of homogenisation.

Table 2.1 gives the results of the pH values obtained from using either an iodoacetate/water mixture or an iodoacetate/potassium chloride mixture as solutions in which the muscle was homogenised. It can be seen that the within muscle variation is slightly higher when using the latter solution. Thus it would appear that the addition of potassium chloride to the iodoacetate solution does not increase the precision of pH measurements when different suspensions of muscle ranging from 1:8 to 1:20 are used. However, it will also be noted that the pH values obtained after homogenisation in the iodoacetate/potassium chloride solution were generally lower than those obtained after homogenisation in the iodoacetate/water solution. This problem was further investigated by comparing ultimate pH values obtained by the two different methods from a further 14 muscles from sheep and the relationship between the two sets of values is shown in Figure 2.2.

The data conform to the following relationship:

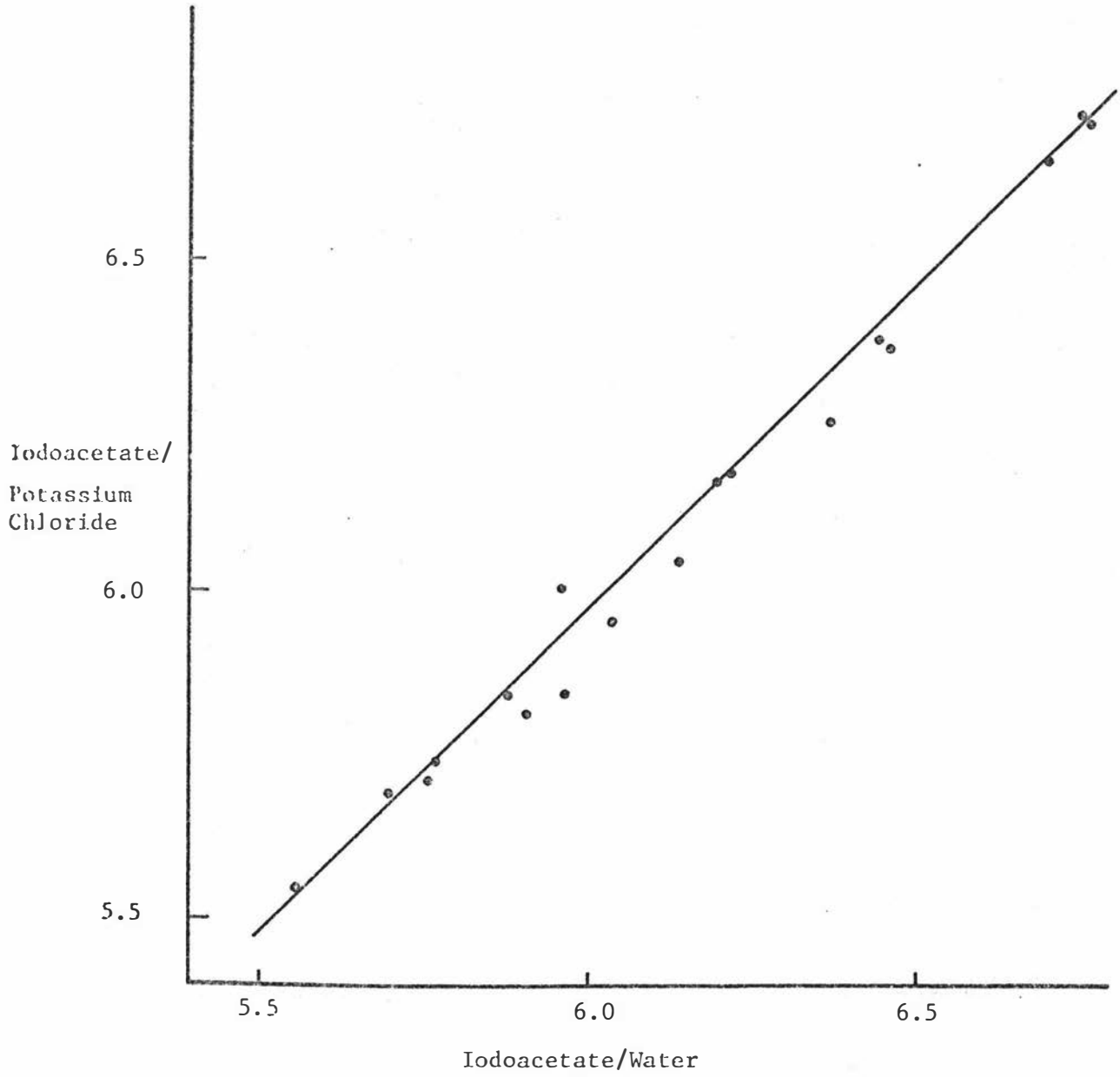
$$\text{pH (iodoacetate/KCl)} = 0.97\ (\text{iodoacetate/}H_2O) + 0.16$$
$$r = 0.993$$

In a previous survey Bendall (1973) reported on 14 pairs of values from *M.sternomandibularis* from beef in which the correlation coefficient (r) was 0.978. It would thus appear that it is possible in both sheep and beef to accurately adjust for the slightly lower iodoacetate/potassium chloride values compared with the iodoacetate/water values but as there appears to be no difference in the precision of the two methods it seems preferable to use the iodoacetate/water technique as it is the most commonly accepted method.

TABLE 2.1 MEAN ULTIMATE pH VALUES OF FIVE SAMPLES WEIGHING FROM 1.0g TO 2.5g FROM FOUR SHEEP

Animal	Iodoacetate/H_2O		Iodoacetate/KCL	
	Mean	± S.E.	Mean	± S.E.
1	5.76	0.008	5.70	0.009
2	5.88	0.011	5.84	0.016
3	6.04	0.015	5.95	0.027
4	6.37	0.007	6.25	0.014
Components of variance %				
Among muscles	98.6		96.47	
Within muscles	1.04		3.53	

FIGURE 2.2 RELATIONSHIP BETWEEN MUSCLE pH MEASURED AFTER HOMOGENISATION IN IODOACETATE/WATER OR IODOACETATE/POTASSIUM CHLORIDE



Although the addition of iodoacetate to samples is only required when measuring pH during the pre rigor period, it is also customary to use it when measuring ultimate pH. Many of the studies presented in this thesis only required assessment of ultimate pH but it was decided to homogenise such samples in iodoacetate as this allowed direct comparisons to be made with pre rigor pH values as well as with ultimate pH values reported by other workers.

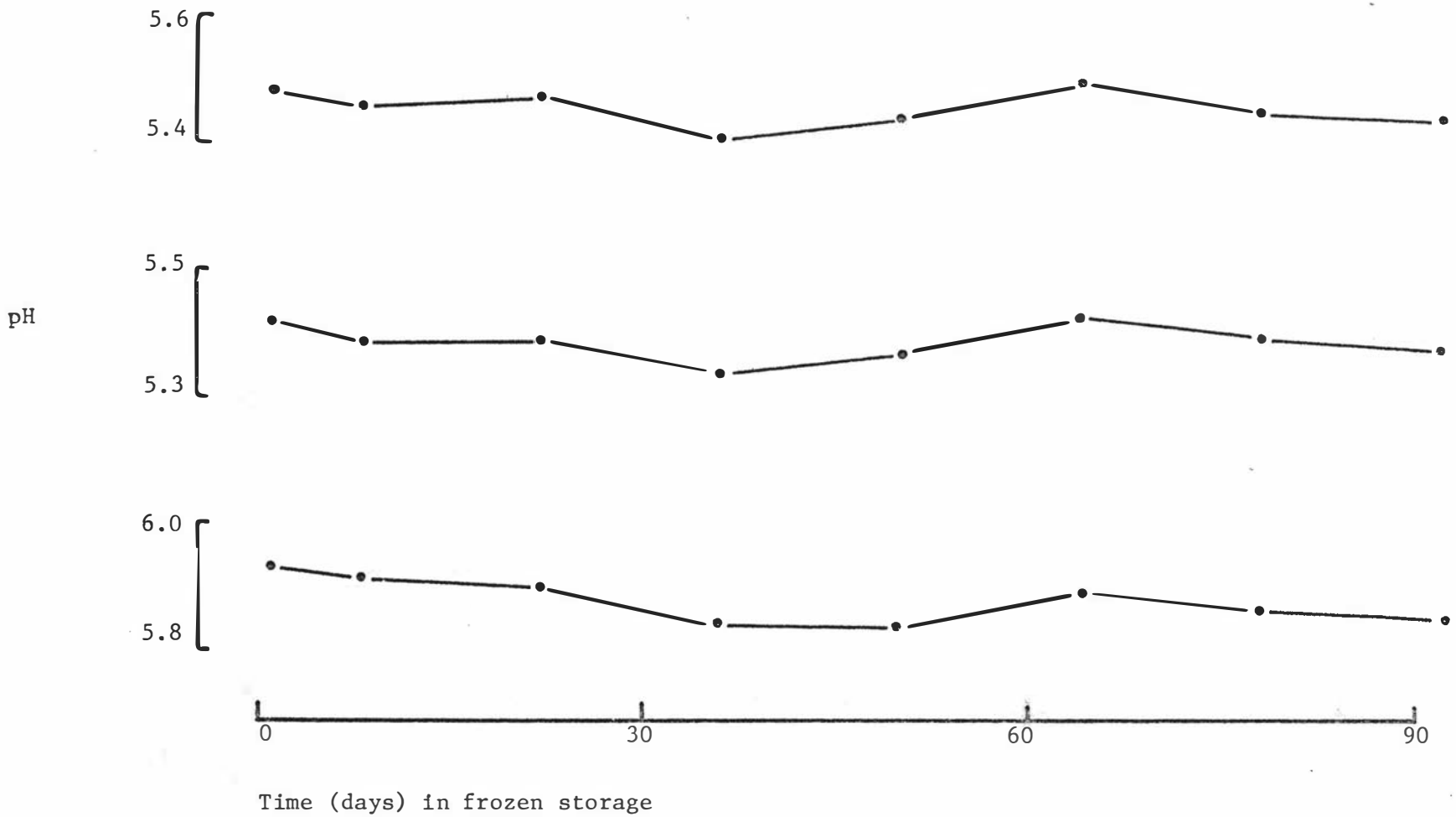
The homogenisation of muscle samples and subsequent measurements of the pH includes a number of procedural steps each of which can result in erroneous data. Although the likelihood of some errors can be reduced by strictly following a set of rules for the technique such as those outlined in Appendix 1, it is desirable to be able to check periodically the accuracy of the technique. It was therefore decided to attempt to design a "quality control"system.

The entire LD from both sides of three sheep were removed shortly after slaughter and then left at room temperature overnight. The following day superficial connective tissue was trimmed from the muscles which were then minced and the tissue from the three animals was mixed separately in three polythene bags. The mince was separated into portions each weighing approximately 2g. The pH of two portions from each animal was measured and the remainder portions were frozen on trays at -18°C. After completion of freezing, 20 portions from each animal were kept in frozen storage at - 18°C whereas the remainder (80-100 portions per animal) were kept in frozen storage at -35°C.

During the following three months, two samples from each animal were removed from frozen storage (-18°C) every two weeks and the pH of these samples measured. Two different pH meters were used and two persons were involved in this work but samples examined on the same day were all done by the same person using the same equipment

The results of these pH measurements have been recorded in Figure 2.3 and it can be seen that the variations with time follows similar patterns in samples from the three animals. These fluctuations did not appear to be related to the operator. This is probably an indication that the true pH of the meat samples did not change during the period of frozen storage and the small deviations occurred as a result of some unavoidable bias associated with the pH measurements.

FIGURE 2.3 MEAN pH OF CONTROL SAMPLES DURING A THREE MONTHS FROZEN STORAGE PERIOD



It is therefore possible to use the remainder of the frozen samples to assess the extent of bias whenever a new batch of samples of unknown value is measured.

The degree of bias which can be tolerated in a series of pH measurements is a somewhat arbitrary decision. However, it is suggested that for every new batch of samples being evaluated, replicate samples from two batches of frozen samples of known values should also be evaluated. The value of any of these samples should be within the range of +/- 0.06 pH unit of the mean of values previously recorded when evaluating this particular control sample.

This "quality control" system was not developed before some difficulties had been experienced with contaminated water during a study presented elsewhere in this thesis. It is believed that the use of such a system is imperative to ensure that the pH measurements are accurate.

INDICATOR MUSCLES FOR ULTIMATE pH

Both the rate and the extent of pH decline is subject to considerable variation between muscles of meat animals (Lawrie, 1962; Martin and Fredeen, 1974; Linke *et al.*, 1976) and it has been stated by Davey and Graafhuis (1981) that the pH value of a single muscle does not represent the general condition of the carcase. On the other hand, it is obviously not possible to measure the pH of all carcase muscles and it has therefore become customary to use as general indicators only, some of the large muscles. The LD is the most commonly used muscle for this purpose and this choice has a number of advantages. In cattle this muscle is more frequently affected by the DFD condition (Tarrant, 1981) it represents the largest single muscle mass of the animal and it is also easy to identify.

Although larger carcase muscles such as the LD are well suited for experimental work, their use may be limited because samples usually cannot be removed without mutilating the carcase. However, there are some smaller muscles which can be removed from a carcase without significantly affecting its commercial value and it was therefore decided to compare the pH of these with that of the LD.

The following muscles (or part of these) from condemned sheep carcases were used for this study:

(a) A 3-5cm length of the *M. longissimus atlantis* excised from the insertion of the muscle on the caudal end of the wing of the atlas to the origin of part of the muscle from the articular processes of the second to third or fourth cervical vertebrae. The cranial part of this muscle is usually visible on fully dressed sheep where the major vessels and the trachea have been removed with some of the muscles of the ventral part of the neck. The caudal part of the muscle is more difficult to distinguish from the larger *M. longissimus capitis* as they both originate from the articular processes of the cervical vertebrae.

(b) The thoracic part of the *M. longissimus colli* excised from the bodies of the first five thoracic vertebrae.

(c) The *M. Masseter* which was freed from its insertion on the mandibule and a cut was made through the muscle at a point just below its origin from the zygomatic arch.

(d) The costal part of the diaphragm excised from the ribs.

(e) The lumbar part of the diaphragm removed from the lumbar vertebrae.

(f) A 10-15cm section of the LD excised from the eighth thoracic vertebrae and caudally to a point approximately lateral to the second or third lumbar vertebrae.

The muscles were removed from both sides of the animals within one hour of slaughter and stored in polythene bags at room temperature. The following day, a sample of approximately 2g was obtained from each muscle (i.e. two samples per animal) and the pH of these samples was measured.

TABLE 2.2 COMPARISON OF ULTIMATE pH BETWEEN SOME SMALLER CARCASE MUSCLES AND THE LD OF SHEEP

		LD		Comparison muscle		
	No. of Comparisons	Within Muscle Variation %	Range of Means	Within Muscle Variation %	Range of Means	Correlation Coefficient
(a) *M.longissimus atlantis*	10	0.57	1.75	0.81	1.44	0.96
(b) *M.longissimus colli*	22	1.72	1.32	8.32	0.86	0.79
(c) *M.masseter*	32	2.61	1.75	18.85	0.86	0.77
(d) Diaphragm costal part	19	1.53	1.77	1.56	1.05	0.84
(e) Diaphragm lumbar part	53	3.63	1.44	2.67	1.37	0.66

A good indicator muscle shows a small degree of within muscle variability and in the present study this was measured by taking two samples from each muscle (one from each side of the animal) and then calculating the within muscle variation, Indicator muscles should also exhibit a wide range of ultimate pH values when they are obtained from animals subjected to different types of preslaughter treatment. Furthermore, as pH values obtained from the LD are most frequently quoted in the literature, there should preferably be a high degree of correlation between the pH of the smaller muscles and that of the LD. In Table 2.2 a comparison is made between the smaller muscles and the LD from the same animals according to these three criteria.

It can be seen in Table 2.2 that the *M. longissimus atlantis* has a high degree of correlation with the LD and also a small within muscle variation as well as a resonable wide wide range of pH values. Unfortunately, the *M. longissimus atlantis* is very difficult to identify and it was found that the excision of this muscle was so time consuming that it would not be possible to use it during commercial operations at a meat works.

Both the *M. longissimus colli* and the *M. masseter* are easy to identify and remove from the carcase, particularly the latter. However, both of these showed a poor correlation with the LD, a narrow range of pH values and a relative high within muscle variation. They were therefore considered unacceptable as indicator muscles.

In regard to ultimate pH values the diaphragm is very uniform, the within muscle variation of this muscle being of the same magnitude as that of the LD from the same animals. The lumbar part of the diaphragm exhibited a wide range of pH values but these did not appear to correlate very well with those from the LD. This is perhaps not surprising because the two muscles have quite different functions.

The results of this study indicate that it may be possible to make some prediction about the ultimate pH of the LD by measuring the ultimate pH of some of the smaller and less expensive muscles. However, it is believed that such predictions would be too inaccurate for use in studies of the effects of preslaughter handling on ultimate pH and it was therefore decided to confine all further examination to the LD.

EFFECT OF INCUBATION METHOD AND SAMPLE SIZE ON ULTIMATE pH

Since removal of samples from the LD may be difficult without mutilating the carcase, the minimum amount of muscle required for accurate pH measurements is of some interest. Furthermore, when the ultimate pH is measured such samples must be held for sufficient time for the end point of post mortem glycolysis to be reached. It seems likely that conditions during this incubation period may affect the ultimate pH because Leet and Locker (1973) have shown that superficial layers of muscle exposed to air maintain high pH values for long periods.

An experiment was therefore designed to investigate the effect of incubation method and sample size on ultimate pH.

The major part of the LD from both sides of 16 sheep was excised and transferred to the laboratory within two hours of slaughter. The ultimate pH of each muscle was measured using the following techniques.

(a) A 5-10cm section of the muscle was left at room temperature for 24 hours after which period two samples were obtained from the centre part of the muscle section. One of the samples weighed approximately 2g(large sample) and the other weighed 0.2 - 0.5g (small sample). Both samples were homogenised in 5mM iodoacetate solution and the pH measured within 30 minutes of homogenisation.

(b) A large and a small sample was obtained shortly after transfer of the muscle to the laboratory. Both samples were left exposed to air for 24 hours before measuring the pH in the same manner as before.

(c) Samples were obtained as under (b) but were then incubated under anaerobic conditions for 24 hours using the BBL Gas Pak System*.

(d) Samples were treated similarly to those under (c) but anaerobic conditions were achieved by overlaying the samples with liquid paraffin**. This treatment was used only on the 12 muscles from the last six sheep in the experiment.

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* Becton, Dickinson and Co., Cockeysville, MD21030, U.S.A.

** 29436 Paraffin Liquid, BDH Chemicals New Zealand Ltd., P O Box 1246 Palmerston North, New Zealand.

TABLE 2.3 EFFECT OF INCUBATION METHOD AND SAMPLE SIZE ON ULTIMATE pH

Incubation Method		Sample size	ULTIMATE pH For n = 20 Mean	Range	For n = 12 Mean	Range
(a)	Muscle Section	large	5.81	5.43 - 6.42	5.88	5.51 - 6.45
		small	6.06**	5.43 - 6.56	6.18**	5.71 - 6.66
(b)	Aerobic Incubation	large	5.88**	5.41 - 6.54	5.97**	5.56 - 6.59
		small	6.29**	5.50 - 6.86	6.45**	6.11 - 6.84
(c)	Anaerobic Jar	large	5.83	5.47 - 6.44	5.92	5.48 - 6.50
		small	6.13**	5.48 - 6.64	6.30**	5.96 - 6.71
(d)	Liquid Paraffin	large			5.92	5.46 - 6.53
		small			6.27**	5.94 - 6.60

** These means are highly significantly different ($P < 0.01$) from the mean of large samples from muscle sections.

In this study, the pH measured on large samples from muscle sections was considered to be the closest value to the true ultimate pH of the muscle and was therefore used as a benchmark. The mean value of this group was compared to mean values of other groups where the pH was measured after different treatment (Table 2.3) and the significance of differences between mean values was evaluated by the t-test. In these analyses, the data from the first 20 muscles, where only one type of anaerobic incubation was used, were treated separately from the data from the last 12 muscles which includes samples that were also incubated under liquid paraffin.

It can be seen in Table 2.3 that when large samples are incubated under anaerobic conditions, the mean values of these are not significantly different to the mean values obtained from large samples from muscle sections. However, it would appear that both sample size and incubation method have a highly significant effect on pH values.

The results of this study indicate the necessity for avoidance of oxygen during incubation of samples and for defining the dilution of the homogenate in which the pH is measured. The former can apparently be achieved by overlaying of samples with liquid paraffin. This technique has also been successfully used in assay of other biochemical parameters of meat where anaerobic conditions are required (Bendall,1975).

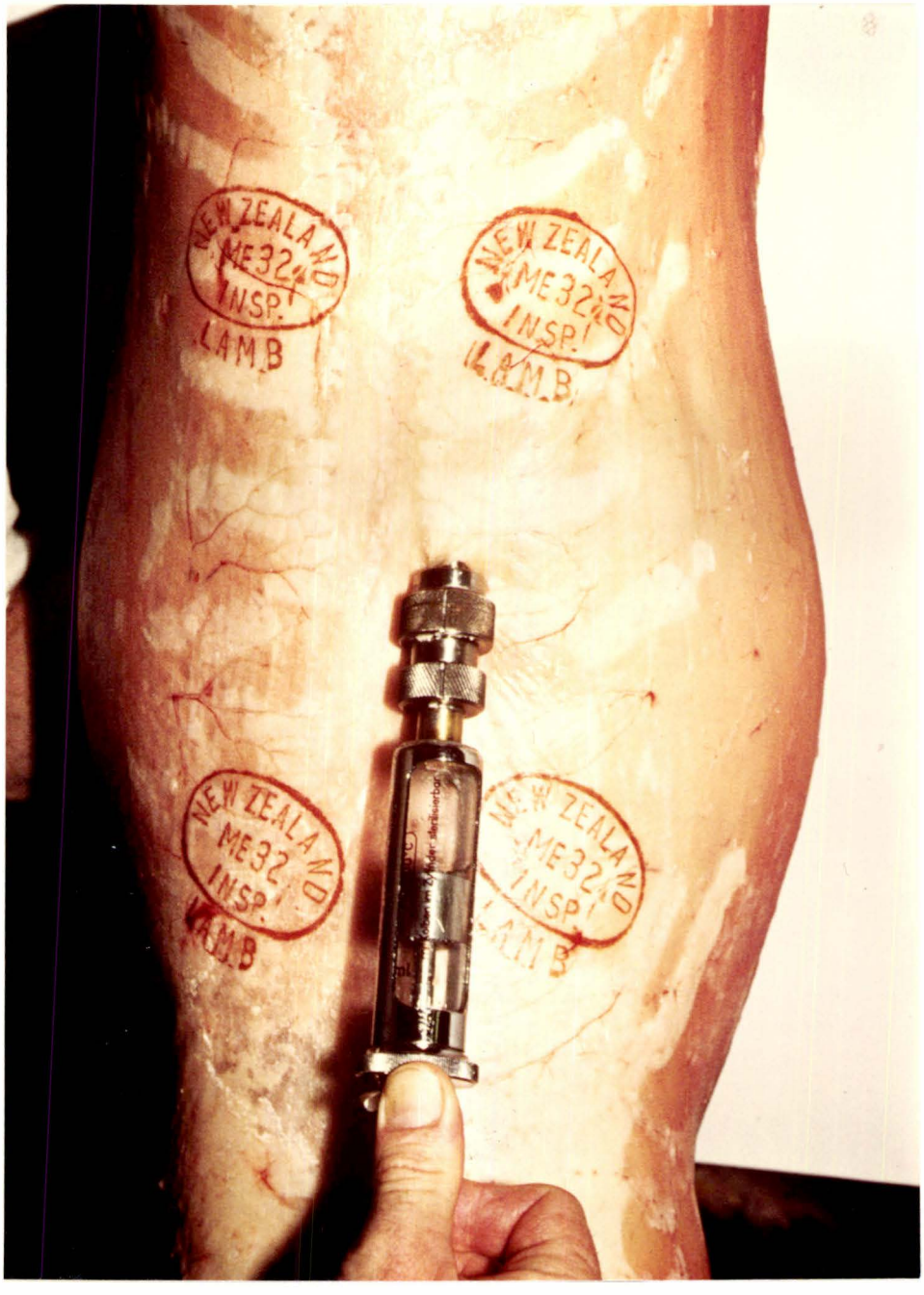
THE PLUG SAMPLING TECHNIQUE

Although it is possible to measure the pH of small samples using special techniques (Heffron and Hegarty, 1971), such methods are much more time consuming compared to the Stomacher method described earlier in this chapter. However, the use of the latter method requires samples weighing 1-2g in order to achieve enough homogenate in the polythene bags for reliable measurements. It is also important to be able to obtain such samples without mutilating the carcases when this technique is used in field studies.

Muscle biopsy techniques have previously been developed for sampling live animals (Bergstrom, 1962; Fisler and Drenick, 1972; Schmidt *et al.*, 1972) but such techniques are only capable of obtaining 200-300mg of muscle tissue. A modified surgical biopsy instrument was therefore developed for obtaining plug samples of the LD from sheep and lamb carcases.

PLATE 2.1 BIOPSY INSTRUMENT CONSISTING OF A MODIFIED CANNULA AND TROCAR ATTACHED TO A 20 ml SYRINGE

PLATE 2.2 PLUG SAMPLING OF A LAMB CARCASE



The instrument consists of a modified trocar and cannula. The inner hollow trocar has a cut-out section (50 x 8mm) with sharpened edges, a solid pointed tip and is attached to a 20ml syringe. This trocar fits into a cannula with an identical cut-out section (Plate 2.1).

The plug samples were obtained while the carcases were in a hanging position suspended by the hindlegs. The biopsy instrument was inserted in the LD at right angles to the muscle surface in the region of the 12th rib, four to five cm lateral to the midline (Plate 2.2). When the tip of the trocar had reached the centre of the muscle, the instrument was pushed caudally, parallel to the vertebral column until the cut-out sections of the tubes were well inside the muscle. The plunger of the syringe was withdrawn, producing a vacuum inside the inner tube which was then rotated 180° inside the outer tube cutting off the portion of muscle sucked into the tubes.

In order to evaluate the accuracy of the plug sampling technique, two experiments were carried out. In the first of these, plug samples were obtained from both sides of 22 sheep carcases, and incubated overnight under aerobic conditions. After removal of the plug sample, a 10-15cm long section of the entire LD at the site where the plug sample was taken was also removed. The pH of the plug samples was measured after 24 hours incubation and these values were compared to pH values measured in 2g samples from the muscle sections.

In a second experiment, plug samples and muscle sections were obtained from 12 sheep in a similar manner except that care was taken to ensure that a plug sample of approximately 2g was obtained. The plug samples were placed in 5ml plastic beakers and overlaid with liquid paraffin immediately after removal from the carcases and incubated under these conditions for 24 hours at room temperature.

The results of the two experiments are recorded in Table 2.4 and it can be seen that there is a highly significant correlation between results obtained by plug sampling and those obtained from samples from muscle sections. However, the table shows that in the first experiment involving aerobic incubation there is a highly significant difference between the means of the two groups and that the values obtained by the plug sampling technique in this experiment are generally higher than those

TABLE 2.4 COMPARISON OF ULTIMATE pH BETWEEN PLUG SAMPLES AND MUSCLE SECTION SAMPLES

	Aerobic incubation		Anaerobic incubation (liquid paraffin)	
	Muscle section	Plug sample	Muscle section	Plug sample
Sample weights (g)				
Mean		1.09		1.87
Range		0.56-1.79		0.83-2.76
pH				
Mean	6.11	6.18	5.93	5.90
Range	5.53-6.87	5.61-7.06	5.60-7.04	5.59-6.93
Variance components (%)				
Among animals	95.3	97.3	96.0	97.1
Within animals	4.7	2.7	4.0	2.9
t	2.994 ($P < 0.01$)		1.546 ($P > 0.05$)	
r	0.97 ($P < 0.01$)		0.99 ($P < 0.01$)	

obtained from samples from muscle sections. It will also be noted that the weights of the plug samples are generally smaller (mean = 1.09g) than the samples used from muscle sections.

The results of the second experiment in which incubation was anaerobic indicate that there is no significant difference between means of pH values obtained when plug samples weighing approximately 2g and overlaid with liquid paraffin were compared to similarly sized samples from muscle sections. It can also be seen that the variation within animals of pH values is of the same magnitude for the two different sampling methods.

The results of this study indicate that it is possible to obtain an accurate measurement of the ultimate pH of the LD of sheep, by the use of a plug sampling technique. The accuracy of such methods is apparently related to the ability to define the dilution of the homogenate in which the pH is measured and to the avoidance of oxygen during incubation. The overlaying of samples with liquid paraffin offers a practical solution to this latter problem and with some experience samples weighing approximately 2g can be routinely obtained.

COMPARISON OF SAMPLE METHOD AND DIRECT PROBE METHOD FOR MEASURING pH OF DIFFERENT MUSCLES

Because of the development of lightweight portable pH meters, it has become common to measure pH by inserting probe electrodes into the muscles and take direct measurements. However, there is apparently little published information on the precision of such methods in comparison to the more traditional sample method. The present study was designed to investigate this problem and at the same time compare the pH of the LD with that of two other carcase muscles.

All pH measurements were carried out at room temperature and the following two methods were used:

(a) A sample method whereby approximately 2g of muscle tissue was homogenised in 20ml of 5mM neutral iodoacetate solution. The pH of this solution was measured using a combination glass electrode attached to a Triac pH meter.

(b) A direct probe method using an Ingold, Lot 406-M4 electrode* and the same pH meter as before.

In the first part of this study, the major part of the LD and two other muscles (*M.biceps femoris* (BF) and *M.supraspinatus* (SS)) was removed from one side of five sheep. After incubation for 24 hours at room temperature, the pH was measured in six similar predetermined locations in all muscles by the two methods.

TABLE 2.5 ULTIMATE pH MEASURED IN THREE MUSCLES FROM FIVE SHEEP
(six measurements per muscle)

		LD	BF	SS
Sample Method	Mean	6.02	6.09	6.12
	Range	1.37	1.35	0.93
	Within muscle variation (%)	1.30	1.08	6.39
Direct Probe Method	Mean	6.06	6.13	6.10
	Range	1.37	1.33	0.86
	Within muscle variation (%)	1.02	2.55	9.21

BF = *M.biceps femoris*
SS = *M.suprasinatus*

The results of these measurements are recorded in Table 2.5 and it can be seen that the means are very similar for the three muscles and the two methods. The ranges are also similar for the LDs and the BFs regardless of method used whereas the SS exhibited a much smaller range of mean values.

The within muscle variation for each method and each muscle was also calculated and it can be seen in Table 2.5 that they are of the same magnitude in the LD and the BF regardless of method used. It will also be noted that there is considerably more within muscle variation in the SS and this may, at least in part, be explained by the smaller variation in

* Dr W. Ingold AG, CH-8902 Urdorf, Zurich, Switzerland.

pH of this muscle between animals. Consequently the within muscle variation contributes a larger percentage to the total variation.

The carcases in this study were selected to give a wide range of ultimate pH values of muscles. It is suggested that a good indicator muscle should depict such a range and that a reliable method should have the least within muscle variation. It would thus appear that both methods are equally suitable for measuring ultimate pH and that the LD and the BF are superior to the SS as indicator muscles. It is interesting that the ultimate pH of the BF in different locations was found to be so similar, expecially in view of the fact that the caudal thigh muscles of sheep have been associated with variations in the proportions of different fibre types at different depths of the muscles (Sivachelan and Davies, 1981). However, care was taken to measure the pH at the same depth in all locations which may account for the present results.

In the second experiment, the LD and the BF from one side of two condemned sheep were obtained at the meat works. The pH decline of these muscles was followed by obtaining replicate measurements with both methods at 2,4,6,9,12 and 24 hours after slaughter. These results were divided on an arbitrary basis into two groups depending on whether the muscle was thought to be still in the pre rigor state or whether the ultimate pH had been reached. Each group was further subdivided into two subgroups depending on whether results had been obtained by the sample method or by the direct probe method. The percentage within group variation, for all groups, was calculated as previously described. In this study, the within group variation represents the variation between replicate measurements obtained by any of the two methods.

TABLE 2.6 PERCENTAGE WITHIN MUSCLE VARIATION OF REPLICATE POST MORTEM pH MEASUREMENTS OF FOUR MUSCLES

	Pre rigor measurements	Post rigor measurements
Sample method	1.75	1.39
Direct Probe Method	9.78	2.15

Table 2.6 indicates that when using the sample method, the within group variation is of the same magnitude, both during decline of the pH and when it has reached its ultimate level. The within group variation of the probe method is also small when measuring ultimate pH but is much greater during the pre rigor state. Thus the probe method is considerably less precise compared to the sample method when used during the pre rigor period when the pH is still declining. Although the reasons for this are not clear, one contributing factor may be variations in the rate of glycolysis in different but adjacent parts of the muscle. The direct probe, measuring the pH of a small part of the tissue only, might be able to detect this variation, whereas the sample method which measures the pH of a larger amount of homogenised muscle would not.

The direct probe provides a rapid and convenient method of measuring pH and it would also appear that this method has a high degree of precision when measuring ultimate pH. The time required for a muscle to reach the ultimate pH is temperature dependent (Bate-Smith and Bendall, 1949) and it has been suggested by Marsh (1954) that with efficient carcase cooling systems, a period of 26 to 36 hours may be required for the completion of all post mortem glycolytic changes in the LD of beef animals. More recently, Tarrant and Mothersill (1977) reported that the time required the pH to fall to 6.0 in some major muscles of beef chilled at 3°C ranged from 2.2 to 13.6 hours, varying with the muscle examined and its depth in the carcase. It would thus appear that with the use of modern refrigeration techniques, a period of at least 24 hours is required before the ultimate pH of the LD can be measured by direct probe methods. In most cases, further processing of carcases will proceed within this period and it is therefore often necessary to use other techniques such as the plug sampling method when studies of ultimate pH of meat are carried out at meat works.

Although the direct probe method is not as precise as the sample method for pre rigor measurements of pH it could still be the method of choice when simultaneously monitoring the post mortem pH decline of carcases at the meat works. In such cases the time required for obtaining plug samples and measuring the pH of these samples will be a limiting factor on the sample size which can be obtained. Furthermore, repeated plug sampling of the same carcase can be difficult and may eventually mutilate the carcase.

THE EFFECT OF SAMPLE LOCATION ON ULTIMATE pH

In the past, the majority of workers have used the LD for studies of ultimate pH in meat and it has been suggested that because of variations within this muscle, more than one sample is required for reliable assessment of pH. This particular problem was investigated in both sheep and beef cattle carcases.

In the first part of this investigation, the major part of the LD was removed from both sides of 13 sheep shortly after slaughter. After incubation for 24 hours, the muscles were divided into five approximately equal sized sections (Plate 2.3) and the pH of a 2g sample from each section was determined.

TABLE 2.7 MEAN pH VALUES FROM THE LD OF 13 SHEEP

Location*	Left Side	Right Side
a	5.83	5.81
b	5.82	5.80
c	5.83	5.81
d	5.78	5.79
e	5.81	5.78
Mean	5.81	5.80

*Locations a - e correspond to those in Plate 2.3

The mean ultimate pH from individual animals varied from 5.43 to 6.43 and values within some animals varied as much as 0.2 - 0.3 pH units between the ten different sites, although in the majority of cases such variations were only in order of 0.1 pH unit. However, when the mean values for each location from the 13 sheep were compared (Table 2.7), the differences between these were small and non significant

In another study, samples were obtained from one LD of 24 beef carcases approximately 24 hours after slaughter. A 2g sample was removed from a medial, central and lateral location in the muscle adjacent to the 12th rib. The pH values of these samples were determined after 24 hours incubation under liquid paraffin.

PLATE 2.3 THE LD FROM BOTH SIDES OF SHEEP

(Sample locations are indicated by horizontal lines. Section 'a' is at the level of the 8th thoracic vertebra. Section 'e' is at the level of the 6th lumbar vertebra.)

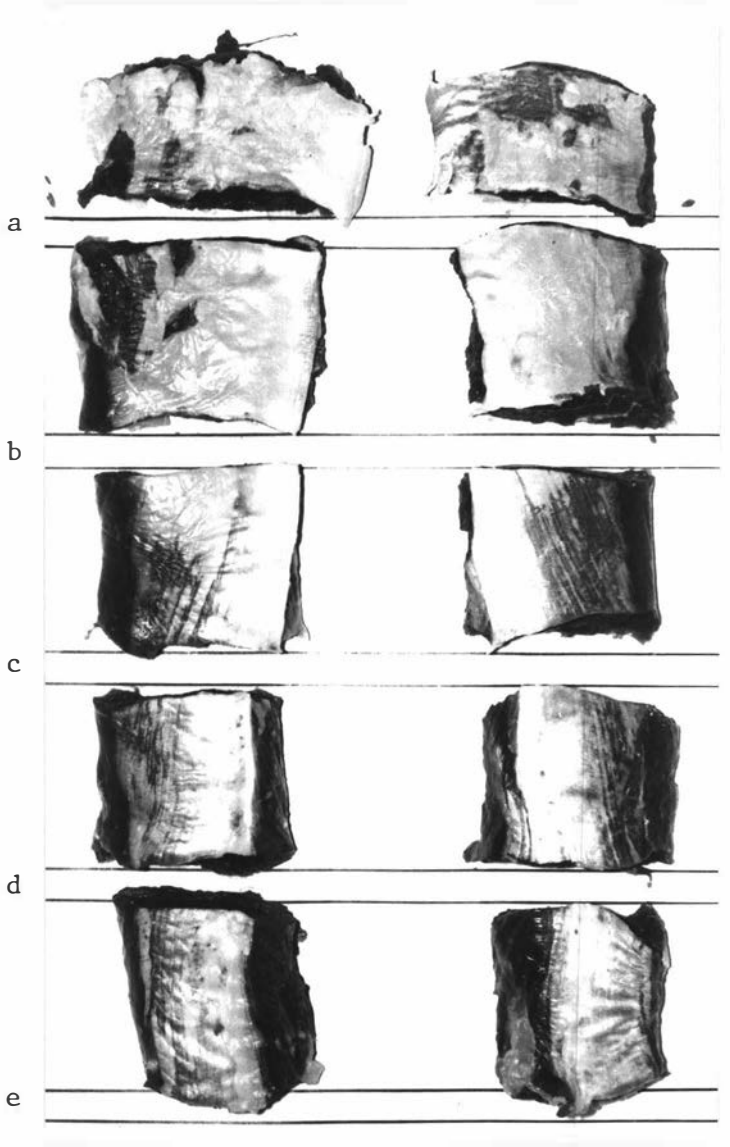


TABLE 2.8 MEAN pH VALUES FROM DIFFERENT LOCATIONS OF THE LD IN 24 CATTLE

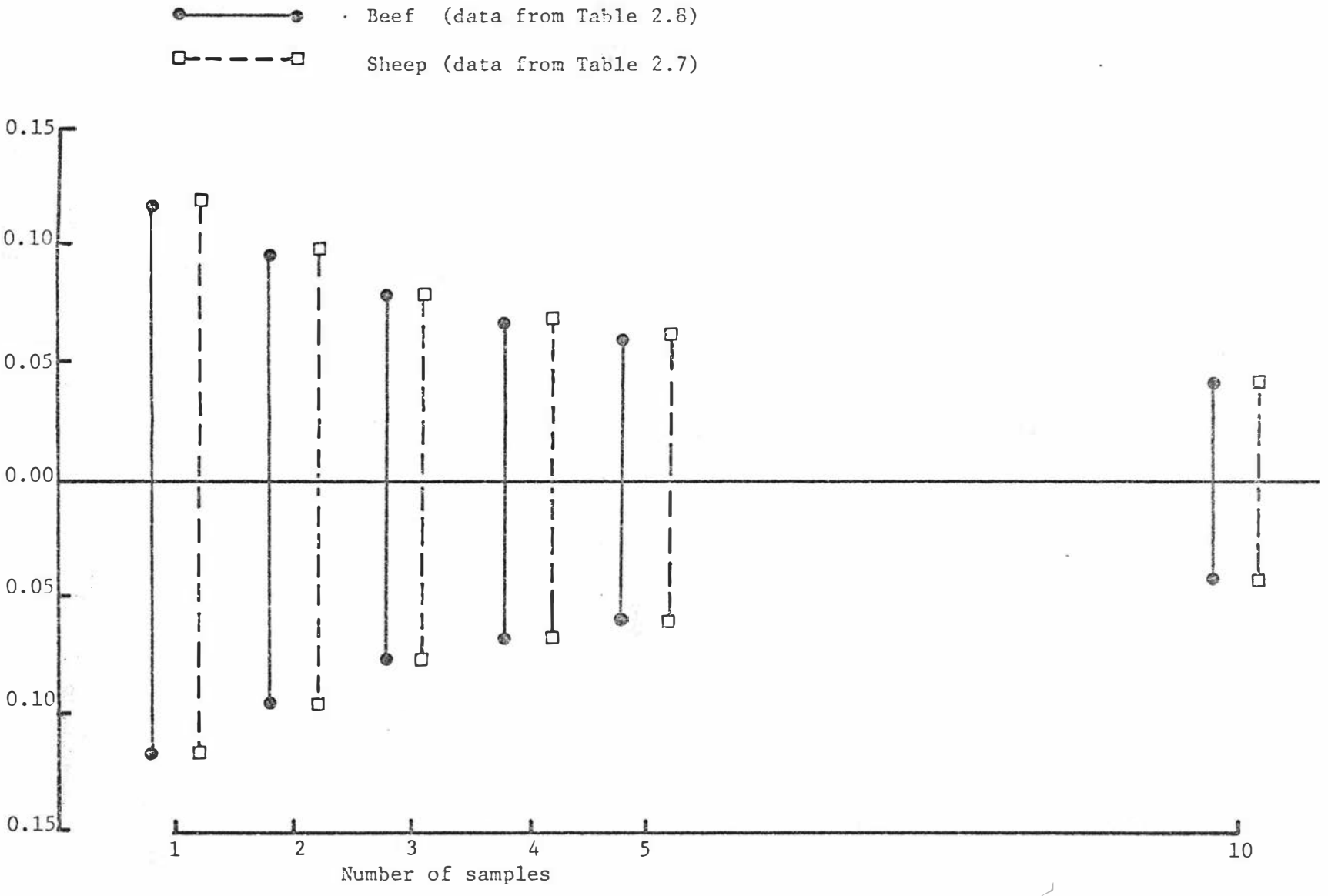
Medial	5.80 +/- 0.051
Central	5.79 +/- 0.053
Lateral	5.78 +/- 0.052

The within muscle variation appeared to be of the same magnitude as in the previous study and it can be seen in Table 2.8 that the differences between mean values of the three sample locations were small and non-significant.

The observed differences between different sites within the LD of both sheep and cattle would thus appear to occur at random and are probably caused by both real randomly distributed differences between actual muscle tissue as well as a randomly distributed error associated with sampling, homogenisation and measurements of pH values. The latter factor is the only one under control of the investigator.

Although these two studies indicate that there is no advantage in sampling the LD over a wider area when measuring the ultimate pH of this muscle, it is possible to reduce the error associated with such measurements by increasing the number of samples. In Figure 2.4 the confidence limits of the means of the pH values obtained in the two studies are indicated for different numbers of samples (n). The standard error of the mean for different values of n was estimated from a standard deviation based on the within variance of all groups in each study and confidence limits could then be estimated in the usual manner (Sokal and Rohlf,1969). It will be noted that the decrease in error associated with measuring more samples is relatively small and that nearly ten samples would be required to bring the confidence limits within +/- 0.05 pH units. It is therefore believed that in studies on factors affecting the ultimate pH of carcase, it is more important to increase the number of animals per group than it is to increase the number of samples per animal.

FIGURE 2.4 CONFIDENCE LIMITS (95%) OF MEANS OF SAMPLES FROM THE LD



GROUP SIZE REQUIRED FOR STUDIES OF ULTIMATE pH

One of the advantages of carrying out studies on pH at the meat works is that large groups of animals can usually be obtained for comparative studies without any additional cost to the investigator. However, the time required for obtaining samples and measuring their pH must also be considered in the planning of group sizes for such studies.

In most of the studies at the meat works, comparisons are made between two or more groups of animals with respect to one or several factors. It is therefore important to know how big a group must be obtained in order to be able to show that a true difference is significant ($P<0.05$) with a defined probablility that the significance will be found if it exists. In order to be able to answer this question it is necessary to have some information about the variability of the data and how far apart one might expect group means to be.

During the evaluation of the plug sampling technique, data were obtained from several lines of lambs and these are presented in Table 2.9. It will be noted that these lines can be divided into four distinct groups both with respect to the mean values as well as the number of animals with values above 6.0. From each of these groups, one line of animals was selected for statistical analysis (Line Nos.2,4,6 and 8). The distributions of pH values in these four lines are shown in Figure 2.5 indicating the apparent normal distribution of values within lines, whereas the distribution of the combined values from the four lines appears to be skewed to the right. It is therefore possible to use simple analysis of variance techniques for comparisons of differences between lines.

Samples of 6, 12 and 24 values were drawn at random from these four lines. The significance of differences between mean values of neighbouring groups (i.e. 2-4, 4-6 and 6-8) and between mean values two groups apart (2-6 and 4-8) were tested using the F-test and these have been summarised in Table 2.10.

TABLE 2.9 COMPARISONS BETWEEN ULTIMATE pH OF DIFFERENT LINES OF SHEEP

(Ranked according to mean ultimate pH)

Line No.	No. Sampled	Mean	Standard Deviation	Range	% ≧ 6.00
1	48	5.53	0.116	5.29 - 5.89	0
2	48	5.58	0.132	5.32 - 5.94	0
3	45	5.61	0.110	5.44 - 5.87	0
4	46	5.70	0.220	5.34 - 6.38	9
5	45	5.73	0.254	5.32 - 6.43	11
6	48	5.87	0.180	5.52 - 6.42	19
7	24	5.90	0.117	5.70 - 6.12	21
8	48	6.05	0.255	5.50 - 6.63	52

FIGURE 2.5 DISTRIBUTION OF ULTIMATE pH VALUES OF SHEEP LD

(Values from Table 2.9)

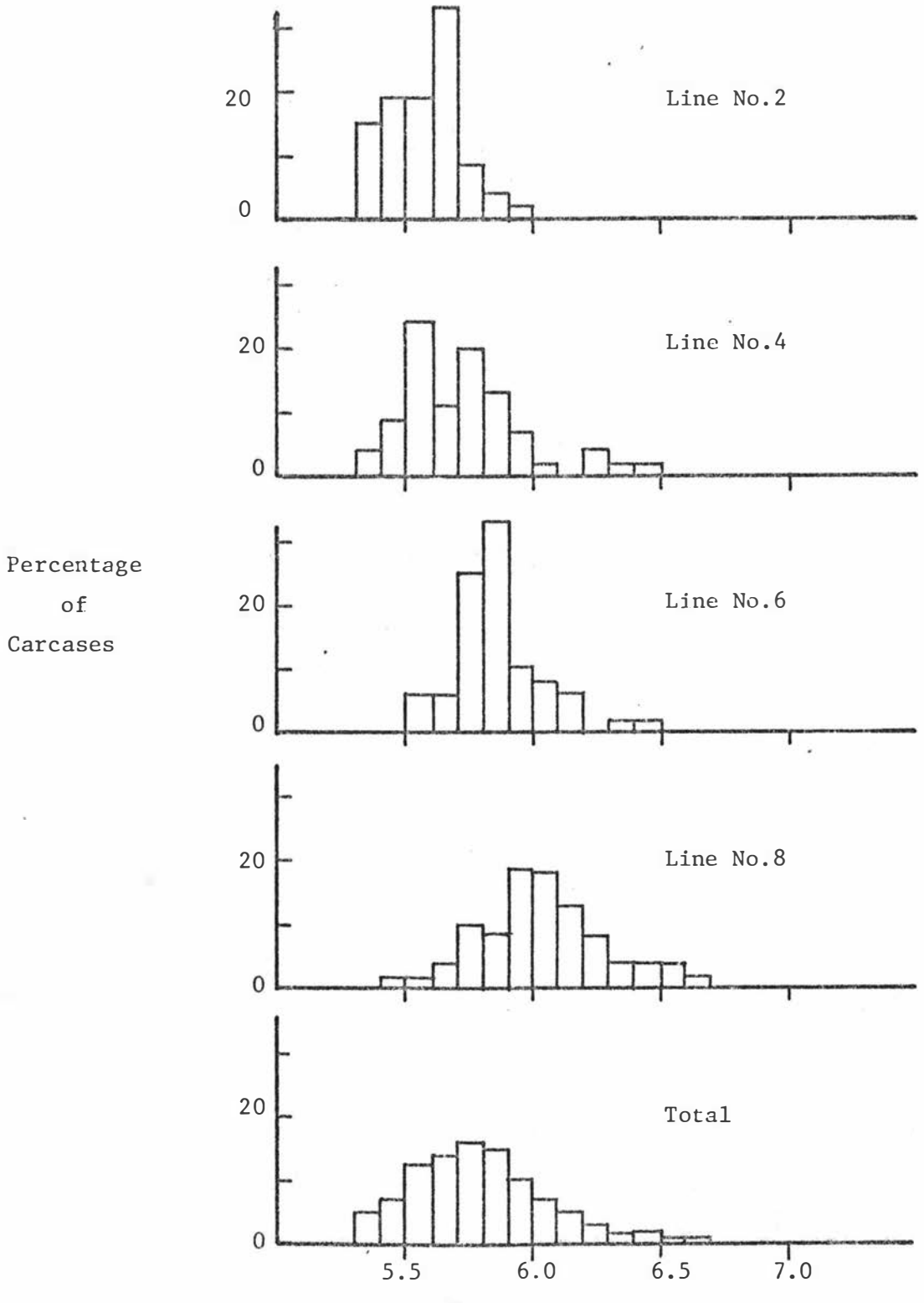


TABLE 2.10 PROPORTION OF COMPARISONS BETWEEN LINES 2, 4, 6 AND 8 (TABLE 2.9) SHOWING SIGNIFICANT DIFFERENCES AT THE 0.05 PROBABILITY LEVEL

Group Size	Neighbouring Groups	Two Groups Apart
n = 24	3/3	3/3
n = 12	3/9	6/6
n = 6	1/9	5/6

It can be seen that with a sample size of 24, there was always a significant difference between the means. When sample size was decreased to 12, the differences between neighbouring samples were only significant in three out of nine comparisons but all the comparisons between samples being two groups apart were significant. With samples of six values, one out of nine comparisons between neighbouring groups was significant and five out of the six comparisons between samples being two groups apart were significant.

Another approach to the problem is to use the iterative solution method suggested by Sokal and Rohlf (1969). The appropriate formula is:

$$n \geq 2 \left(\frac{s}{d}\right)^2 \times \left(t_{a(v)} + t_{2(1-P)(v)}\right)^2$$

where n = number of animals per sample

s = standard deviation of the population

d = smallest true difference that is desired to detect

v = degrees of freedom of the sample standard deviation with a groups and n animals per group

a = significance level

P = desired probability that a difference will be found to be significant

$t_{a(v)}$ and $t_{2(1-P)(v)}$ = values from a two tailed t-table with v degrees of freedom and corresponding to probabilities of a and 2 (1-P), respectively.

Table 2.9 indicates that the standard deviations of the populations (lines) from which animals were sampled varied from approximately 0.12 to 0.25 and the above calculations were therefore performed using three different standard deviations (0.12, 0.18 and 0.24). It will also be

noted that the difference between the arbitrary groups in this table is approximately 0.2 pH units and this value was therefore used for d. As it was decided arbitrarily that it would be desirable to have four out of five studies showing significant differences if means differed by at least 0.2 units, the value of P was fixed at 0.80 and a significance level of 5% was used ($a = 0.05$).

For the first step of the calculations for each of the three standard deviations chosen, the number of groups was fixed at four ($a = 4$) and the number of animals per group was 12. Where the calculated number deviated considerably from the arbitrarily chosen number, a second calculation was performed trying the calculated number for sample size but leaving the number of groups unchanged.

Table 2.11 indicates the sample sizes required from lines of lambs to be 80% certain of detecting a 0.2 unit difference between two of the four means at the 5% level of significance. It can be seen that if the standard deviation is 0.12, a sample of six animals is required, whereas with standard deviations of 0.18 and 0.24, samples of 13 and 23 respectively are required. These results are compatible with the results in Table 2.10.

During these preliminary studies, it was found convenient to collect samples in multiples of six, using numbered trays of a similar type to those used for tissue cultures. The results of the present calculations indicate that when six samples only are collected from each line, significant differences between lines are not likely to be detected unless the means of the two lines are far apart (0.3 - 0.4 pH units) or the standard deviations of the data are very small (0.12 or less). It would thus appear that 12 to 24 animals per group is required for most comparisons in order to assure that significant differences between group means can be detected.

TABLE 2.11 CALCULATION OF SAMPLE SIZE

(Iterative Solution)

Standard Deviation(s)	Iterative Step	Total No. of Animals	No. of Groups(a)	Animals per Group (n)	v = a(n-1)	Calculated n
0.12	1	48	4	12	44	5.9
0.12	2	24	4	6	20	6.2
0.18	1	48	4	12	44	13.3
0.24	1	48	4	12	44	23.6
0.24	2	96	4	24	92	23.1

DISCUSSION

The use of a single muscle for determination of the pH status of a carcase involves considerable extrapolation and the choice of such a muscle is therefore very important. Because of its size, high commercial value and uniformity, the LD has been the muscle of choice in the past. The present results also indicate that the LD usually exhibits a wider range of pH values and less within muscle variability as compared to other muscles. It may thus be concluded that in field studies where usually only one muscle can be evaluated, the LD is the best indicator of the pH status of the carcase.

In spite of the potential wealth of information about post mortem pH changes in muscles which could be obtained at meat works very few studies have set out to measure such changes. This is probably because of the difficulties involved in measuring pH of carcases during commercial slaughter and dressing of stock. Most of the work on post mortem pH changes has therefore been carried out in laboratories using small number of animals and under conditions which are often far removed from those prevailing at a meat works.

The development of direct probe methods for measuring pH of muscles has made it possible to obtain values at a high rate during commercial operations but this method would appear to be less precise during the pre rigor period and is often not suitable for obtaining ultimate pH values. In New Zealand, sheep and lambs are usually frozen within a few hours of slaughter and the ultimate pH would not have been reached by that time. Although beef meat is routinely chilled for 24 hours prior to freezing, it is doubtful whether post mortem glycolytic changes will have ceased during this period (Petersen, 1982). It is interesting to note that several investiagations of high pH meat in beef (Munns and Burrell, 1966; Poulanne and Aalto, 1980) have suggested that the condition is more prevalent in the leaner grades. The LD of animals in these grades have faster cooling rates because of the reduced fat cover. If the pH of the LD in such animals is measured by direct probe methods within 24 to 36 hours, it is likely that glycolytic changes will not be complete and pH values will be falsely high because they have not reached their ultimate.

The plug sampling method seems well suited for estimation of ultimate pH and the use of the Colworth Stomacher for the processing of samples is rapid, effective and simple when compared with other methods of homogenisation. Although the collection of plug samples may be slightly more cumbersome than direct probe methods, it can also be performed with minimal damage to the carcase and incubation temperatures of the samples can be completely controlled. It is therefore possible to sample carcases and obtain ultimate pH levels while they are being processed and this technique should be a valuable tool in investigating causes of high pH meat.

The present studies indicate that there is little benefit in taking more than one sample per animal when investigating ultimate pH in sheep and beef. It would also appear that in order to demonstrate statistically significant differences between groups of animals (when there is a true difference) such groups should usually comprise at least 12 animals and if differences are small or variability expected to be high, 24 animals may be required per group.

CONCLUSIONS

1. The pH measurements of muscle samples homogenised in an iodoacetate/water solution are slightly higher (approximately 0.05 pH units) than the pH of similar samples homogenised in an iodoacetate/potassium chloride solution but there is no difference between the precision of the two methods.

2. As there is a high degree of correlation between the two methods using different solutions and the iodoacetate/water technique is most commonly used by other workers, it was adopted for the present studies.

3. It is necessary to use samples weighing not less than approximately two grams and incubated under anaerobic conditions (paraffin overlay) in order to obtain an accurate measurement of the ultimate pH of a muscle.

4. These findings led to the design of a plug sampling technique which allowed accurate measurement of muscle pH without carcase mutilation.

5. Both the sample method and the direct probe method are well suited for measuring ultimate pH of muscles. The precision of the latter method is considerably less when used for measuring the pre rigor pH value.

6. The ultimate pH values of the LD varied considerably among animals but the within muscle variation is small. This muscle is thus well suited as an indicator muscle of the ultimate pH of carcases.

7. The site of sampling within the LD was found to be of little importance in relation to measurements of the ultimate pH. Thus multiple sampling from the same muscle has only limited advantages.

8. Sample sizes of 12 to 24 are required to assure a high probability that significant differences between group means can be detected.

CHAPTER THREE

CROSS-SECTIONAL STUDIES OF ULTIMATE pH IN LAMBS

INTRODUCTION

The occurrence of DFD beef has been investigated in many different countries. Munns and Burrell (1966) in a four year study found that eight percent of steers slaughtered in a Canadian meat packing plant exhibited characteristics of DFD and an investigation in Finland (Poulanne and Aalto, 1980) indicated that the occurrence of the defect could be over 20 percent. In a more recent survey covering 19 countries, Tarrant (1981) reported that the occurrence of DFD meat was estimated at one to five percent in the carcasses of steers and heifers, six to ten percent in cows and 11 to 15 percent in young bulls.

These and other similar surveys of beef have also provided the basis for suggestions regarding the causes and prevention of DFD meat (Augustini *et al.*, 1979; Hedrick, 1981). Recommendations include measures to reduce the excitement of cattle during loading, transporting and holding prior to slaughter by the provision of adequate loading facilities and holding pens and the avoidance of mixing together of groups of cattle of different origin prior to slaughter.

It appears that similar studies on the DFD syndrome have not been carried out in sheep and lambs and there is little available information about the occurrence and possible causes of meat of high ultimate pH in this species in New Zealand or other countries. The reasons for paying less attention to this possible problem in sheep and lambs as compared to beef is probably related to differences in the usual methods of processing and handling of the two types of meat. In many countries it is customary to store beef at chilling temperatures, just above freezing, for long periods. Under such conditions microbial spoilage of the products is particularly likely to occur and would be exacerbated by a high pH. On the other hand, meat from sheep and lambs is usually frozen shortly after slaughter and the ultimate pH of these products is therefore of less importance in relation to possible spoilage. However, the New Zealand meat industry is currently showing considerable interest in

exporting chilled lamb. Under such conditions, the ultimate pH of the meat and its effect upon shelflife will become a much more important issue. Furthermore, recent studies have indicated that there is a relationship between high ultimate pH of meat and undesirable flavours (Dransfield, 1981; Ford and Park, 1981).

For these reasons it was decided to investigate the prevalence and potential causes of high ultimate pH in lamb carcases at a meat export works. The findings from these studies are described and discussed in this chapter.

MATERIALS AND METHODS

Part 1 : Seasonal investigation in lambs

These studies were carried out during the 1981/82 season at a meat export works in the southern part of the North Island. A total of 1536 lambs from 64 farms were included in the investigation which was divided into four sampling periods :

Early summer :	28 November - 22 December
Summer :	24 January - 3 February
Autumn :	18 March - 25 March
Winter :	2 June - 9 June

During each of these sampling periods, 16 lines of lambs were selected using the following criteria :

1. All animals in the line were from the same farm and handled in a similar manner during the last few days prior to slaughter.
2. Each line contained at least 100 lambs.
3. All lambs in each line were of a similar age and breed, or cross, and with the same wool cover.

Furthermore, an attempt was made to select the lines in such a manner that within each of the four sampling periods, lambs from a number of different breeds and from different locations within the works catchment area were included.

For each line, information was obtained on the distance between the farm of origin and the meat works (travel distance), breed of animals, the

time spent in the stockyards prior to slaughter (holding period), weight of fleece (wool score) and whether or not the animals were washed prior to slaughter.

After slaughter, dressing and grading, 24 carcases were selected from each line for sampling. The first and the last five lambs of a line were always excluded from the sample and only carcases graded in one of the four major grading classes described in Table 3.1 were selected.

TABLE 3.1 CLASSIFICATION OF EXPORT LAMBS

(Using criteria from the New Zealand Meat Producers Board)

Grade symbol	Weight Range (kg)	Fat Cover	*Distribution of Carcases (%)
PM	13.0 - 16.0	medium	38
PL	8.0 - 12.5	medium	10
YM	13.0 - 16.0	light	17
YL	8.0 - 12.5	light	26
OTHERS	Includes carcases outside the above weight and fat cover ranges.		9

* Based on data from the New Zealand Meat Producers Board covering all carcases graded for export during the 1979/80 killing season.

From lines where the major proportion of the carcases were in the same grade, only carcases of that grade were included in the sample. Whereas samples from lines of mixed grades consisted of carcases in two different classes.

Within one hour of slaughter, approximately two g of muscle tissue was obtained from the LD of each animal using the plug sampling technique described in Chapter Two. Samples were overlaid with liquid paraffin, incubated at room temperature and the ultimate pH was measured by the method described in Appendix I.

For each day samples were taken, meteorological recordings were obtained from the Grasslands Division of the Department of Scientific and Industrial Research (D.S.I.R.), Palmerston North, which is located approximately 25 km

from the meat works where these studies were carried out. These recordings covered the 24 hour period prior to slaughter of the animals and included information on air temperatures, relative humidity, rainfall and air speed.

Statistical analysis of data

During a preliminary analysis of data from this study, it was found that there was a lack of normality of data on ultimate pH from some lines of lambs. There were also apparent differences between lines with respect to variability of the data (heterogeneity of variances). Both of these factors are important in relation to statistical analysis of data, and in particular, the homogeneity (equality) of variances is an important pre-requisite for analysis of variance (Sokal and Rohlf, 1969). Although the consequences may not be too serious when the data base is of considerable size as in this study, it was considered advantageous to perform a logarithmic transformation of the ultimate pH values before carrying out further statistical analysis. Such a procedure has been shown to alleviate the problems associated with both non-normality of data and heterogeneity of variances (Sokal and Rohlf, 1969).

The experimental design used in these studies did not make it possible to obtain equal numbers of observations for all factors under investigation e.g. breed and grade. Such study designs are termed unbalanced or non-orthogonal and special methods for analysis of variance have been designed for these problems (Hull and Nie, 1981). These and many other statistical methods have been incorporated in statistical package programmes specially designed for computers (Nie *et al.*, 1975; Hull and Nie, 1981). For the present problem, a regression model was chosen which allowed for adjustments of the sum of squares of all effects of the three factors in the study (season, breed and grade). Thus, for example, the main effect of season was being adjusted for effects of both breed and grade as well as for effects of any of the interactions between these factors. This model also allowed for inclusion of covariates in the analysis and initially all three independent, numerical variables were included (travel distance, holding period and wool score). However, as a preliminary analysis of variance indicated that there was no significant effect of travel distance on ultimate pH and since this variable was found not to differ significantly during the survey, it was decided to exclude travel distance from the final statistical analysis.

Part 2 : Ultimate pH of condemned sheep

It was decided not to include carcases of all grades in the seasonal investigation of ultimate pH in lambs because this could have resulted in scattering of data into too many different groups making valid statistical evaluation of this factor difficult. However, it was later found desirable to be able to compare ultimate pH values from the seasonal study with values from carcases having been graded outside the four major grading classes described in Table 3.1. The objective of the second part of these studies was to compare the ultimate pH values of two groups of carcases known to differ considerably with respect to the amount of subcutaneous fat (fat cover).

Some of the data obtained in the investigations described in Chapter Two were also used in this study. The majority of meat samples used for the comparative studies of techniques for measuring ultimate pH of the LD were obtained from sheep carcases condemned during regulatory meat inspection and records were kept of the reasons for condemnation of individual carcases. The two most common reasons for condemnation were neoplasia and emaciation and mean ultimate pH values of these two groups were calculated using the mean values of individual carcases obtained in the previously described studies in Chapter Two.

Part 3 : Ultimate pH of heavy lambs

The objective of the last part of these studies was to obtain ultimate pH values of lamb carcases classed by the meat graders as being heavier and having larger amounts of subcutaneous fat as compared to the carcases used in the first part of this study.

The lambs for this study were derived from a feeding trial carried out by the Department of Animal Science, Massey University. During a two months period, the animals were randomly allocated to four different types of pasture feeding and at the end of this period, all lambs were slaughtered at a local meat export works. The ultimate pH of the LD was measured for each carcase as previously described (Appendix I) and data on grade and weights of individual carcases were also recorded.

Data on carcase weights and ultimate pH from the four groups were treated by analysis of variance and the significance of differences between means evaluated by the 'Least Significant Range Test' as described by Sokal and Rohlf (1969).

RESULTS

Distribution of ultimate pH values

The distribution of ultimate pH values within individual lines of lambs varied considerably (Figure 3.1) but in most cases the distribution of values appeared to be normal or at least approaching normality. It can also be seen in Figure 3.1 that there were apparent differences between lines with respect to the variability of values within the lines. In general, the variability of ultimate pH values increased with increases in mean ultimate pH and there was a highly significant direct correlation between these two statistics ($P < 0.01$ for $n = 64$).

The frequency distributions of ultimate pH of the LD in lambs during the four different sampling periods are shown in Figure 3.2 and it can be seen that these distributions are all skewed to the right.

The normality of a distribution can be examined by graphic methods (Sokal and Rohlf,1969) and the normal probability graph of the 1536 pH values in the study is shown in Figure 3.3A. If a variable is normally distributed, this graph will tend to form a straight line. The shape of the probability graph for the 1536 lambs indicates that this distribution is either skewed or a mixture of two distributions. On the other hand, the probability graph for the 1310 values below 5.80 indicates that the distribution of this group is only slightly skewed to the left, whereas the distribution of values, equal to, or above 5.80 is strongly skewed to the right (Figure 3.3 B and C).

Season

The means and distributions of the ultimate pH values during the four different sampling periods are shown in Table 3.2. The lowest mean (5.55) was recorded during the first sampling period (early summer) and the highest mean (5.69) was recorded during the second period (summer), whereas the overall mean was 5.60. These differences between seasonal sampling periods were also reflected in the distribution of the proportion of carcases having high ultimate pH values and it will be noted that 7.2 percent of all carcases had values equal to, or above 6.00.

FIGURE 3.1 FREQUENCY DISTRIBUTION OF ULTIMATE pH OF THE LD IN LAMBS FROM TWO DIFFERENT LINES

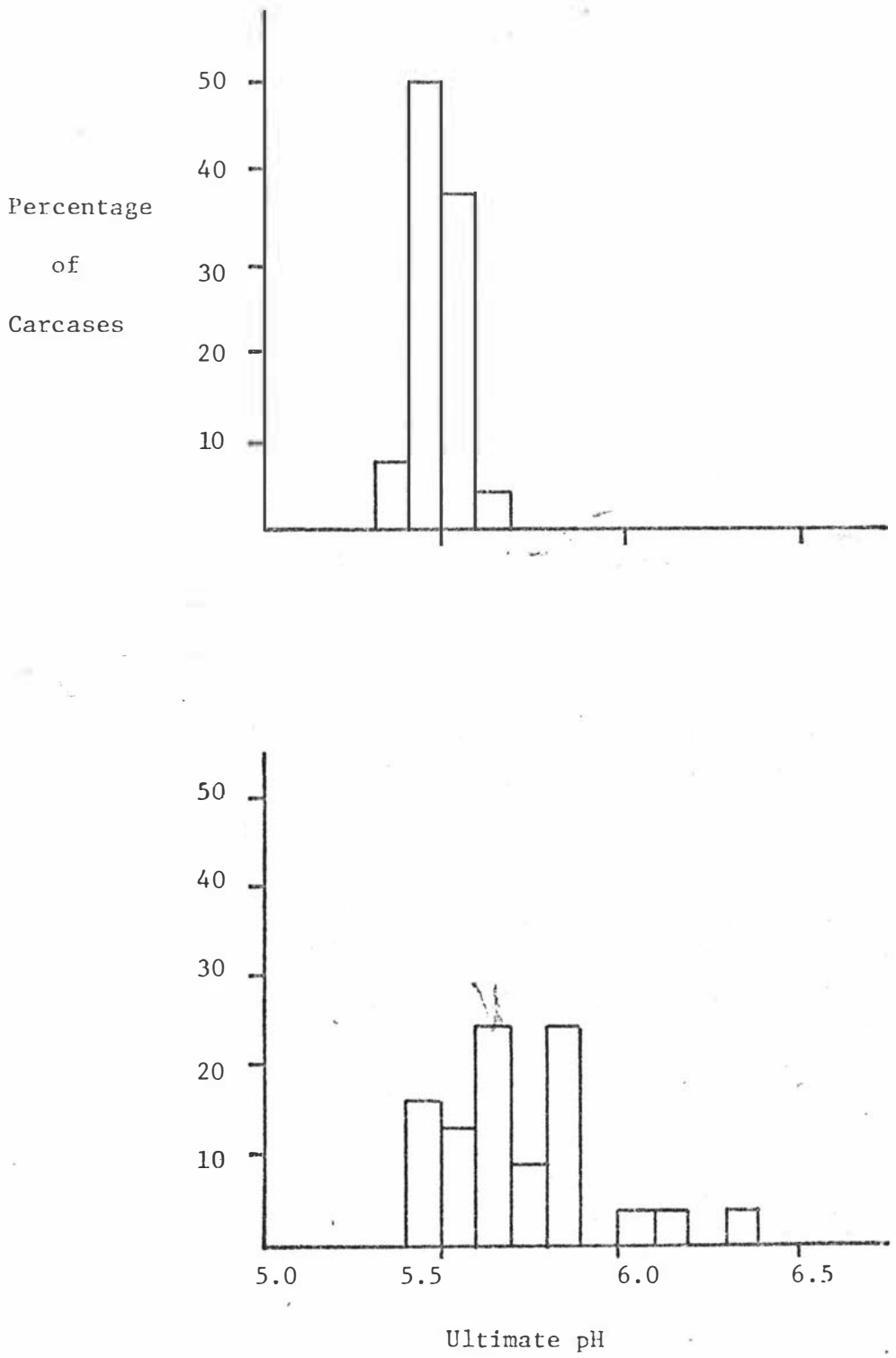


FIGURE 3.2 FREQUENCY DISTRIBUTION OF ULTIMATE pH IN THE LD OF 1536 LAMBS

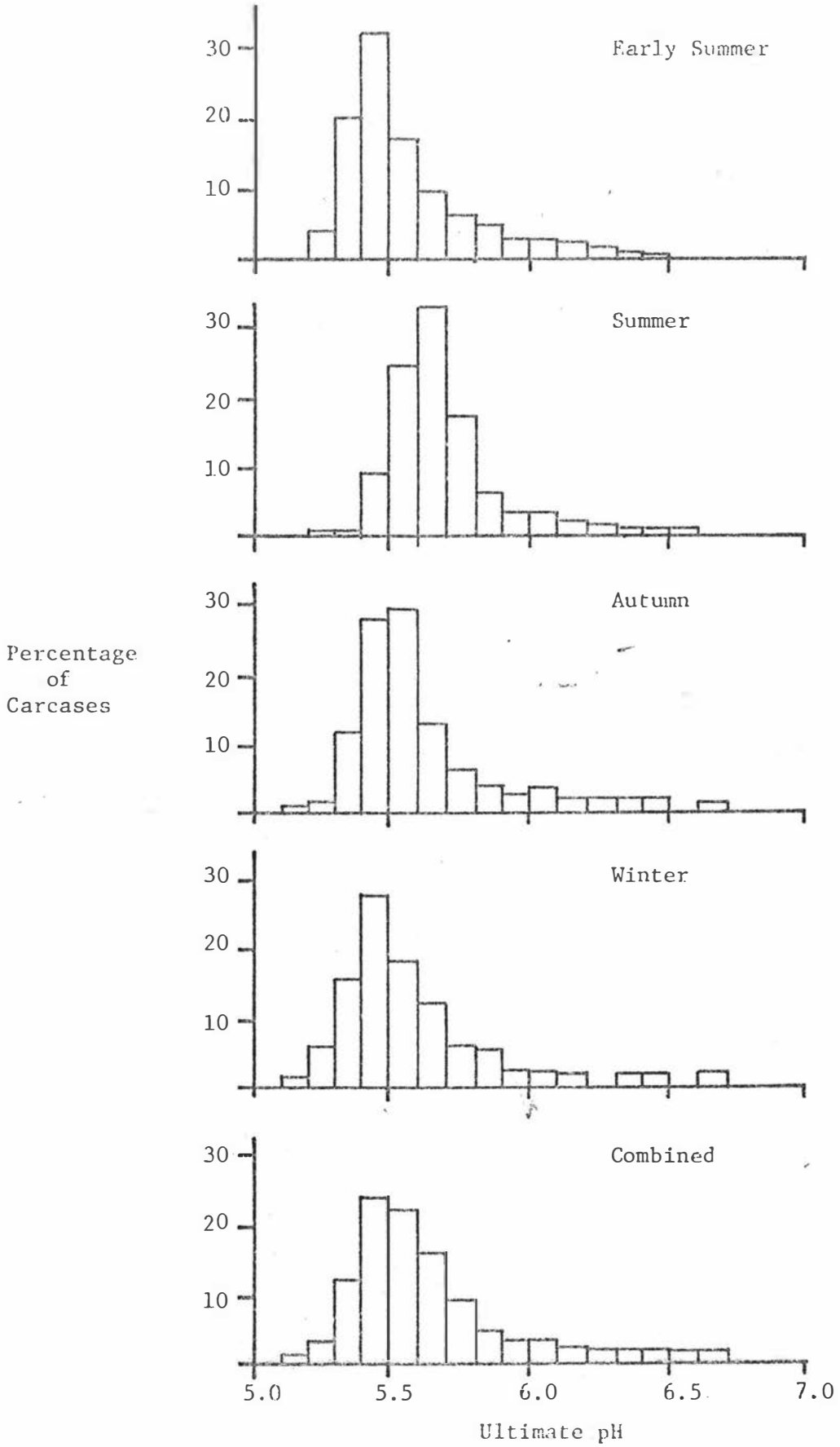


FIGURE 3.3 NORMAL PROBABILITY GRAPHS OF THE ULTIMATE pH DISTRIBUTION

A : values from 1536 lambs
B : 1310 values below 5.80
C : 226 values $\geq$ 5.80

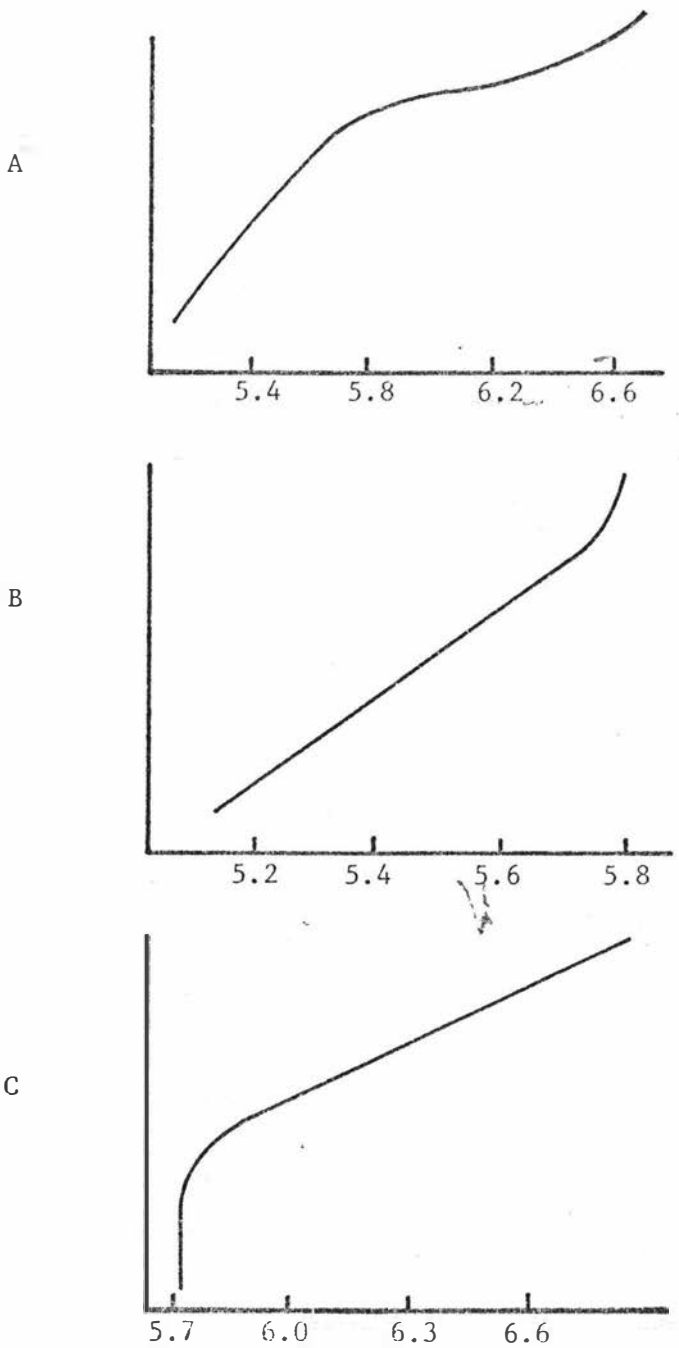


TABLE 3.2 THE MEANS AND DISTRIBUTIONS OF ULTIMATE pH VALUES DURING THE FOUR DIFFERENT SAMPLING PERIODS

		Early Summer	Summer	Autumn	Winter	Total
No. samples		384	384	384	384	1536
Mean		5.55	5.69	5.69	5.57	5.60
± S.E.		0.111	0.010	0.012	0.013	0.006
Percentage Distribution of Values	<5.80	87.5	81.5	87.0	85.2	85.3
	5.80 - 5.99	6.8	10.4	5.2	7.6	7.5
	≥6.00	5.7	8.1	7.8	7.3	7.2

TABLE 3.3 LINES WITH MEAN ULTIMATE pH ABOVE 5.9

Line No.	12	22	28	43	54
Sampling Period	Early Summer	Summer	Summer	Autumn	Winter
No. of Carcases ≧ 6.00	15	8	10	9	13
Mean Ultimate pH	6.01	5.93	5.99	5.92	6.07
Breed	Perendale	Romney X	Perendale	Romney X	Perendale X
Travel Distance (km)	20	30	20	10	50
Holding Period (hrs.)	20	22.5	24	25	23.5
Wool Score (kg)	1.00	0.20	0.30	0.80	1.15
Grades	PL/YL	PM/YL	PM/PL	PL/YL	PL/YL

It is interesting to note that approximately half of the carcases with ultimate pH values equal to, or above, 6.00 originated from five of the 64 lines in the survey. It can be seen in Table 3.3 that the lambs in three of these lines were Perendales or Perendale crosses whereas the lambs in the other two lines were Romney crosses. It will also be noted that lines with high mean ultimate pH values were found during all four sampling periods and at no time during this study did there appear to be a cluster of lines with either low or high mean ultimate pH values. Thus some of the factors affecting ultimate pH in lambs are likely to be related to breed associated factors or to treatment of specific groups of animals rather than general differences in the handling of animals during different periods.

Breed

Although several different breeds were recorded during the study, it was found possible to divide these into four different groups in the following manner.

1. Romney: All animals in a line were considered to be of the Romney breed.
2. Romney Crosses : These were lines consisting of lambs considered to be Romney crosses excluding any crosses with the Perendale breed.
3. Perendale : This group included both lambs considered to be of the Perendale breed as well as any crosses with that breed.
4. Mixed : This group comprised all animals which could not be classed in any of the other three groups.

The distribution of carcases within these four groups of breeds is shown in Table 3.4 and it can be seen that nearly half of the carcases were classed as Romney Crosses and the remainder were almost equally divided between the three other groups. The lowest mean ultimate pH (5.55) was found in the mixed group and the highest in the Perendale group (5.76).

Grade

Table 3.5 indicates that approximately half of the carcases in this study were in the PM grade and the rest of the carcases were unequally distributed between the three other grading classes. The lowest mean ultimate pH was observed in the PM lambs (5.57) whereas the three other

TABLE 3.4 DISTRIBUTION OF BREEDS AND THEIR MEAN ULTIMATE pH VALUES

Breed	No. of Carcases	Distribution of Carcases (%)	Mean Ultimate pH ± S.E.
Romney	264	17	5.57 ± 0.012
Romney Crosses	744	48	5.58 ± 0.008
Perendale	240	16	5.76 ± 0.019
Mixed	288	19	5.55 ± 0.009
TOTAL	1536	100	5.60 ± 0.006

TABLE 3.5 DISTRIBUTION OF GRADES AND THEIR MEAN ULTIMATE pH VALUES

Grade	No. of Carcases	Distribution of Carcases (%)	Mean Ultimate pH ± S.E
PM	786	51	5.57 ± 0.007
PL	294	19	5.63 ± 0.014
YM	51	4	5.63 ± 0.037
YL	405	26	5.63 ± 0.012
TOTAL	1536	100	5.60 ± 0.006

groups all had mean values of 5.63 (the small differences between mean values of these three groups are not noticeable in Table 3.5 as mean values have been recorded only to the second decimal point). Because of the small differences in mean ultimate pH between the latter three groups, it was decided to pool the data from these groups for further statistical analysis and the following recoding of data was introduced :

Grade Symbol	Description	Percentage of Carcases
"Heavy"	All PM lambs	51
"Light"	PL,YM and YL lambs	49

A comparison with the data previously presented in Table 3.1 indicates that the distribution of carcases between these two groups in this study is very similar to the distribution of these groups in the total number of lambs slaughtered for export in New Zealand during the 1979/80 killing season.

Washing

Information about washing of the lambs prior to slaughter indicated that lambs in four of the lines were washed twice, in 48 once, and in two not washed. No information was available about the remainder ten lines. Although all four lines having been washed twice had a higher mean ultimate pH compared to other lines, it was considered that some of the groups were too small to be included in further statistical analysis of data. However, because these results indicated that washing lambs prior to slaughter may affect ultimate pH, this question was explored in separate studies reported in Chapter Four.

Travel distance, holding period and wool score

Some of the statistics associated with the three independent numerical variables in this study have been recorded in Table 3.6. The travel distance varied for individual lines from two to 350 km with a mean for all lines of 73 km. There was considerable variation between lines within all four sampling periods as indicated by the high standard errors of the means but there were no significant differences between sampling periods associated with travel distance.

The holding periods at the meat works, prior to slaughter, varied from 16 to 47 hours with a mean of 24.7 hours. The shortest holding periods were recorded during the early summer period and the longest periods were

TABLE 3.6 MEANS AND THEIR STANDARD ERRORS OF TRAVEL DISTANCE, HOLDING PERIOD AND WOOL SCORE DURING THE FOUR SAMPLING PERIODS

Sampling Period	Travel Distance (km)	Holding Period (hours)	Wool Score (kg)
Early Summer	45 ± 13.3	21.2 ± 0.44	0.91 ± 0.047
Summer	75 ± 20.0	24.1 ± 0.83	0.59 ± 0.071
Autumn	87 ± 26.0	23.2 ± 0.46	0.81 ± 0.051
Winter	86 ± 19.2	30.2 ± 2.28	0.99 ± 0.015
TOTAL	73 ± 2.1	24.7 ± 0.15	0.83 ± 0.009
F Values	0.88	8.99**	4.81**

** Indicates highly significant differences between means in the same column ($P < 0.01$).

reported during the winter period. These differences in holding periods between the four sampling groups were highly significant ($P< 0.01$).

There were also highly significant differences between the four sampling groups with respect to the fleece weight of the lambs (wool score). This statistic varied from 0.20 to 1.70 kg for individual lines and the highest mean wool score was recorded during winter whereas the lowest was recorded during the summer period.

Analysis of variance for three factors and two covariates

The main results of the analysis of variance for the three factors and the two covariates have been recorded in Table 3.7. It can be seen that both breed and season had a highly significant effect on ultimate pH and there were highly significant effects of the interactions of season by breed, season by grade and season by breed by grade. There was also a highly significant direct correlation between holding periods and ultimate pH whereas there was an inverse correlation between wool score and ultimate pH. This latter regression was also highly significant.

The adjusted means of ultimate pH for season and breed and for season and grade have been recorded in Tables 3.8 and 3.9 respectively. Some of these values differ slightly from those presented in Tables 3.2, 3.4 and 3.5 for two reasons. Firstly, the mean values in Tables 3.8 and 3.9 are calculated on the basis of the logarithmic transformation of the raw data which would tend to reduce slightly some of the higher mean values. Secondly, these mean values have been adjusted for the effects of both covariates (holding period and wool score). It was therefore considered that these values represented the best estimate of the combined effects of season, breed and grade.

It can be seen in Table 3.8 that the effect of season on ultimate pH is mainly related to the high values recorded during the summer period whereas the mean values for the other three sampling periods were very similar. The effect of breed would also appear to be related to only one of the four groups of breeds as it will be noted that the Perendale group had considerably higher mean ultimate pH than any of the other three groups. The interaction between season and breed would appear to be related to the higher means recorded in Perendales during three of the four sampling periods. Thus the effect of season is generally exaggerated by the effect of breed. Although the 'heavy' group of lambs had a lower mean

TABLE 3.7 ANALYSIS OF VARIANCE FOR THREE FACTORS AND TWO COVARIATES

Source of Variation	Degrees of Freedom	Mean Square	F Value	Significance of F
Within Cells	1505	0.00023		
Regression	2	0.00185	7.9500	<0.001
Season	3	0.00580	24.9211	<0.001
Breed	3	0.00457	19.6259	<0.001
Grade	1	0.00031	1.3486	0.246
Season by Breed	8	0.00191	8.2253	<0.001
Season by Grade	3	0.00089	3.8327	0.009
Breed by Grade	3	0.00053	2.2930	0.076
Season by Breed by Grade	7	0.00063	2.6922	0.009

Regression of the two covariates:

Covariate	Regression Coefficient	T Value	Significance of T
Holding period	0.00028	2.6481	0.008
Wool Score	-0.00519	-3.4661	0.001

TABLE 3.8 COMBINED ADJUSTED MEANS OF ULTIMATE pH FOR SEASON AND BREED

	Early Summer	Summer	Autumn	Winter	All Season
Romney	-	5.64	5.49	5.53	5.57
Romney Crosses	5.50	5.69	5.64	5.52	5.58
Perendale	5.75	5.83	5.61	5.91	5.76
Mixed	5.55	5.62	5.57	5.44	5.56
All Breeds	5.57	5.68	5.59	5.56	5.60

TABLE 3.9 COMBINED ADJUSTED MEANS OF ULTIMATE pH FOR SEASON AND GRADE

	Early Summer	Summer	Autumn	Winter	All Season
'Heavy'	5.54	5.67	5.55	5.53	5.57
'Light'	5.60	5.69	5.64	5.59	5.62

ultimate pH than the 'light' group of lambs, there was no statistically significant effect of grade. However, there was a highly significant interaction between season and grade and this is probably explained by the fact that differences between the two grading groups were smaller during the summer period as compared to the three other sampling periods.

Climatic conditions

The climatic conditions during the 24 hour periods preceeding slaughter and sampling of lambs in the first part of these studies have been summarised in Table 3.10. It can be seen that the variations in air temperatures were very small during the first three sampling periods, whereas there was a considerable decrease in both maximum and minimum temperatures during the last sampling period (winter). It will also be noted that the mean rainfall during the summer period was much lower as compared to the three other sampling periods.

Ultimate pH of condemned sheep

It can be seen in Table 3.11 that the mean ultimate pH of 20 carcases condemned for neoplasia was 5.72 with only three carcases having values above 6.00 whereas the mean ultimate pH of those carcases condemned for emaciation was 6.35 and 16 of the 20 carcases in this group had values above 6.00. This difference between the mean ultimate pH values was highly significant ($P<0.01$).

Ultimate pH of heavy lambs

The lambs used in this part of the investigation were generally heavier and had a larger amount of subcutaneous fat as compared to the lambs used in the seasonal study of ultimate pH in lambs. There were no apparent differences in the distribution of grades between animals grazed on the four different types of pasture. However, there were significant differences between the means of carcase weights of these four groups and it can be seen in Table 3.12 that the mean weights varied from 16.25 kg to 19.97 kg. The ultimate pH values of these lambs were generally much lower as compared to those recorded previously in the seasonal study and there were significant differences between the mean ultimate pH values of the four groups. It will also be noted in Table 3.12 that the group with the lowest mean carcase weight had the highest mean ultimate pH value.

TABLE 3.10 MEAN CLIMATIC CONDITIONS DURING THE 24 HOUR PERIODS PRIOR TO SLAUGHTER AND SAMPLING

Sampling Period	Air Temperature °C Max.	Air Temperature °C Min.	Relative Humidity %	Rainfall mm	Air Speed km/hr
Early Summer	21.0	14.1	82	4.9	14.4
Summer	22.9	13.1	73	0.7	15.7
Autumn	19.7	11.3	73	4.5	10.9
Winter	14.5	4.3	94	1.8	7.1

TABLE 3.11 COMPARISON OF ULTIMATE pH IN SHEEP CONDEMNED AT A MEAT WORKS FOR NEOPLASMS OR EMACIATION

		Neoplasms	Emaciation
Number of Carcases		20	20
Mean Ultimate pH		5.72	6.35
± S.E.		0.047	0.080
Minimum		5.43	5.66
Maximum		6.24	7.14
Number of Carcases	>6.0	3	16

F_s (1.38) 43.13

TABLE 3.12 THE EFFECT OF LAMBS FEEDING DIFFERENT TYPES OF PASTURE ON CARCASE WEIGHTS AND ULTIMATE pH OF THE LD

	PASTURE TYPES			
	A	B	C	D
Carcase Weights (kg) Mean ± S.E.	19.97^{a} ±0.621	18.81^{ab} ±0.566	17.66^{bc} ±0.463	16.25^{c} ±0.466
Ultimate pH Mean ± S.E.	5.32^{a} ±0.028	5.27^{a} ±0.014	5.27^{a} ±0.014	5.41^{b} ±0.046

Mean values with the same superscript in the same row are not significantly different ($P > 0.05$).

DISCUSSION

Definition of DFD meat

The distribution of ultimate pH values of lamb carcases is of considerable interest because it can provide some information about the proportion of carcases which can be expected to yield DFD meat. Like many other similar conditions, DFD meat was initially not well defined. With the discovery of the relationship between colour and ultimate pH (Munns and Burrell, 1965; MacDougall and Rhodes, 1972) more attention was paid to the latter factor as it is well defined and can be measured by relatively simple techniques.

There appears to be no information on the correlation between colour and ultimate pH in muscles from sheep and lambs. However, during the investigations reported in Chapter Two, it was observed that muscles with high ultimate pH values generally were darker in colour. It may therefore be presumed that there is a relationship between colour and ultimate pH in muscles from sheep and lambs similar to that reported in beef.

It has been suggested by Tarrant (1981) that beef quality deteriorates when the ultimate pH is equal to, or above, 5.8. However, it would appear that in relation to shelflife of meat products, most workers have used 6.0 as the critical value (Nicol *et al.*, 1970; Gill and Newton 1978). It can be seen in Table 3.2 that 7.2 percent of the carcases had ultimate pH values which were equal to, or above, 6.0. This figure is compatible to data on the prevalence of DFD meat in beef previously reported (Munns and Burrell, 1966; Puolanne and Aalto, 1980; Tarrant, 1981).

The frequency distributions of ultimate pH values of the LD in lambs during the four different sampling periods in this study were all skewed to the right. A similar pattern of the distribution of ultimate pH of the LD from large samples of animals has been reported in beef (Tarrant and Sherington, 1980) and in pigs (Gallwey and Tarrant, 1979). Both the distribution in beef reported by the former workers and the distribution of the total number of lambs in the present study would appear to peak at a value around 5.5 to 5.6. If the portions above 5.8 were excluded both of these distributions would more closely resemble normal distributions.

The data from the present studies were further analysed for normality by graphic methods indicating that the distribution of the 1310 values below 5.80 (representing 85.2 percent of all values) approaches normality. If it is considered that ultimate pH, like many other biological variables, will attain a normal distribution when animals are physiologically normal, then the 'critical' value of 6.00 is too high in view of the present findings. It is therefore suggested that physiologically normal values of ultimate pH of the LD in lambs can be described by a normal distribution curve with a mean of approximately 5.50 and a standard deviation of approximately 0.10 corresponding to a range of 5.20 to 5.80.

Although the 'critical' value of 6.00 may still be applicable in relation to shelflife of meat, the present findings indicate that values above 5.80 (representing 14.7 percent of all values in the present study) should be considered abnormally high and may be indicative of some adverse treatment of the animals prior to slaughter.

Analysis of factors affecting ultimate pH

Two different approaches have been used in the past to investigate the causes of high ultimate pH in meat animals. The use of experimental studies has the advantage that both treatments and group sizes can be controlled making statistical analysis a relative straightforward matter but extrapolation to the general population is more difficult and theoretical. On the other hand, the results of observational studies are usually applicable to the general population but statistical analysis and interpretation of statistics can be difficult.

The statistical packages now available for computers have made it easier to produce large amounts of information from observational studies merely by passing the data files through the system and request all possible statistical associations. However, as a direct consequence of the laws of probability, some statistically significant findings in a large set of tabulations may be caused by coincidental grouping of data rather than by biological differences between groups. The interpretation of a mass of statistical information from these studies can therefore be difficult.

The present studies were designed so that only variables with some theoretical association with ultimate pH of meat were included. Furthermore, during the initial screening of the data, some of the variables for which adequate numbers were not available, were excluded from the study e.g. washing of lambs prior to slaughter and some of the carcase grades. This approach simplified the statistical analysis and the significant statistical associations between ultimate pH and some preslaughter factors reported in this study are therefore more likely to be true causal associations.

Breed

There is a lack of information in all species concerning the effect of breed on ultimate pH. It was reported by Carr *et al*.(1973) that the carcases of British beef-crossbred cattle had lower ultimate pH values than those from Hereford and Angus. Tyler *et al*. (1982) in a comparison of Zebu crossbred cattle and British breed steers found that the former group had carcases with a lower muscle pH at 24 hours. This latter study was conducted in Queensland and the authors suggested that the higher pH values in British breed carcases may have been due to their poorer adaption to the hot tropical environment inducing a greater degree of fatigue during travel.

The highly significant differences in ultimate pH between lambs in the Perendale group and lambs from other breed groups demonstrated in these studies would appear to be the first report on breed differences in lambs. The Perendale group comprised both lambs of the Perendale breed as well as crosses between Perendales and other breeds. However, nearly half of the group were considered to be purebred Perendales and the mean ultimate pH of this purebred group was 5.85 which is considerably higher than that of the total group of Perendales and Perendale crosses (5.76). It is therefore suggested that the breed effect is related to the Perendales and not to any of the unidentified crossbreeds in that group.

The Perendale breed was developed 20-30 years ago by interbreeding of the Cheviot and Romney breeds (Anon, 1980b) and would appear to be a particularly popular breed on hill country. It is thus possible that lambs of this breed have been handled differently on the farms prior to slaughter and that this could account for some of the differences between this group and the other three groups. Examination of the

information about farm of origin of the different breed groups did not indicate that such farm factors were of importance. However, this problem will be further discussed in Chapter Four.

Season

Seasonal effects on ultimate pH have previously been reported in beef (Tarrant and Sherington,1980) and in pigs (Gallway and Tarrant, 1979). It was concluded by Tarrant (1981) that DFD beef is more common in autumn and winter and the reasons put forward to explain such seasonal effects included temperature fluctuations and changes in feeding.

Because the majority of lambs in New Zealand are born the same time of the year (September), the animals sampled during the four different sampling periods would increase in age from the first to the last sampling period. It has previously been shown that older lambs tend to have higher ultimate pH (Corbett *et al.*,1973). In the present studies, the highest mean ultimate pH was observed during the summer period (the second sampling period) whereas there were only small differences between mean ultimate pH of lambs during the three other sampling periods. It would thus appear that the seasonal effect in this study could not be related to age of the animals.

It has been reported by Furnival *et al.* (1977) that lambs subjected to low temperatures during the night before slaughter tend to have higher ultimate pH of muscles when minimum overnight temperatures ranged from -1 to 13^{o}C. The variation in air temperatures was very small during the first three sampling periods of this study and it therefore seems unlikely that the seasonal effect observed is directly related to changes in climatic conditions. However, it was found that the mean rainfall during the summer period was much lower as compared to the three other sampling periods. This is likely to have affected pasture growth and hence the amount of nutrients available for the lambs. The higher ultimate pH found in lambs during the summer period may therefore be related to their state of nutrition during this period.

Grading

Grading of carcases is based on carcase weights and fat cover and the latter has been shown to be influenced by plane of nutrition (Berg and Butterfield, 1975). A statistical association between ultimate pH and

grading could therefore be expected if the state of nutrition is an important factor in the development of high ultimate pH. Such an association has been reported in beef (Munns and Burrell, 1966; Puolanne and Aalto, 1980) although, as suggested in the previous chapter, these results may have been confounded by the fact that pH measurements were obtained after only a 24 hour chilling period which may have resulted in falsely high values in the leaner carcases.

The present studies did not reveal any direct significant association between grading and ultimate pH. However, only carcases in the four major grading groups were included and this may have excluded animals which had been subjected to extreme differences in levels of nutrition for prolonged periods prior to slaughter. There was a highly significant interaction between season and grade in the present studies related to the smaller difference of mean ultimate pH between the two grading groups during the summer period as compared to the other three periods. If the higher mean ultimate pH recorded during the summer period is associated with a reduction in availability of nutrients, this latter factor could have overshadowed the much smaller effect of differences between grading groups and these would then appear more pronounced during the seasons when nutrition was adequate.

Wool score

The highly significant inverse correlation between ultimate pH and wool score could be an indication that handling associated with the shearing of lambs had affected muscle glycogen reserves. However, lambs are not permitted to be slaughtered for three weeks after shearing because of the difficulties associated with the dressing of closely shorn animals. Factors associated with shearing would thus have to be of a long-term nature to have any effect on ultimate pH. It has been reported by Sumner *et al.* (1982) that shearing is associated with an increase in feed requirements lasting for about one month and thus the effect of wool score could be another nutritional factor.

It has been reported that sheep with unshorn fleeces have wider thermo neutral zones as compared to closely shorn sheep (Blaxter *et al.*, 1959; Graham *et al.*, 1959) and it has also been found that the insulation properties of the fleece are directly related to fleece length (Joyce *et al.*, 1966). The lambs with the higher wool scores would therefore

have been better protected against extreme weather conditions and would generally require less energy for heat production as compared to recently shorn lambs. However, it is doubtful that there would have been any occasions during this study when weather conditions were extreme enough to cause a significant increase in heat production since it has been reported that heat production is constant throughout the range of 15 - 35°C environmental temperature in sheep with a 2.5 cm fleece (Blaxter *et al.*, 1959).

The highly significant differences in mean wool scores between the four different sampling periods can be related to shearing practices in New Zealand. The majority of lambs delivered to the works during the early part of the seasons are not shorn. As many farmers shear their lambs during the summer period, the second sampling period comprised many groups of recently shorn lambs and the lowest mean wool score was therefore recorded during this period. Since the highest mean ultimate pH was also recorded during this period, the association between wool score and ultimate pH may either be of an indirect nature or the previously mentioned additional feed requirements for shorn lambs may have further depleted glycogen reserves during a period of low dietary intake of the animals. It is suggested that the latter explanation is the most likely as it has been suggested that feeding level after shearing is critical and if restricted, body growth may actually decrease (Sumner *et al.*, 1982).

Nutrition

Studies have been carried out previously on the effect on ultimate pH of level of feeding in both beef (Lewis *et al.*, 1962; Carr *et al.*, 1973) and in sheep (Shorthose, 1978) but the results have been inconclusive, probably because the number of animals in these studies were small and in some cases the period of reduction in dietary intake was short. The present studies were not specifically designed to assess the effect of the level of feeding of animals prior to slaughter on the ultimate pH of meat but a number of observations made during the course of this investigation are of interest to this particular problem.

The comparison between the two groups of sheep carcases condemned during regulatory meat inspection provides some data on the effects of low levels of nutrition on ultimate pH of meat. Although the two samples were not randomly selected, it is believed that the very large

difference between ultimate pH values may be indicative of the difference in body condition between the two groups of animals. The most common neoplasm found in sheep in New Zealand during regulatory meat inspection is the small intestinal carcinoma (Webster, 1966; 1967). However, this condition does not appear to affect the carcase grade or the condition of the animal prior to slaughter (personal observation). On the other hand, carcases condemned for emaciation are always in very poor condition with little or no body fat and therefore likely to have been subjected to dietary deficiency for some time either as a result of unavailability of food or because of a disease process. It would thus appear that a severe reduction in dietary intake can have a dramatic effect on ultimate pH.

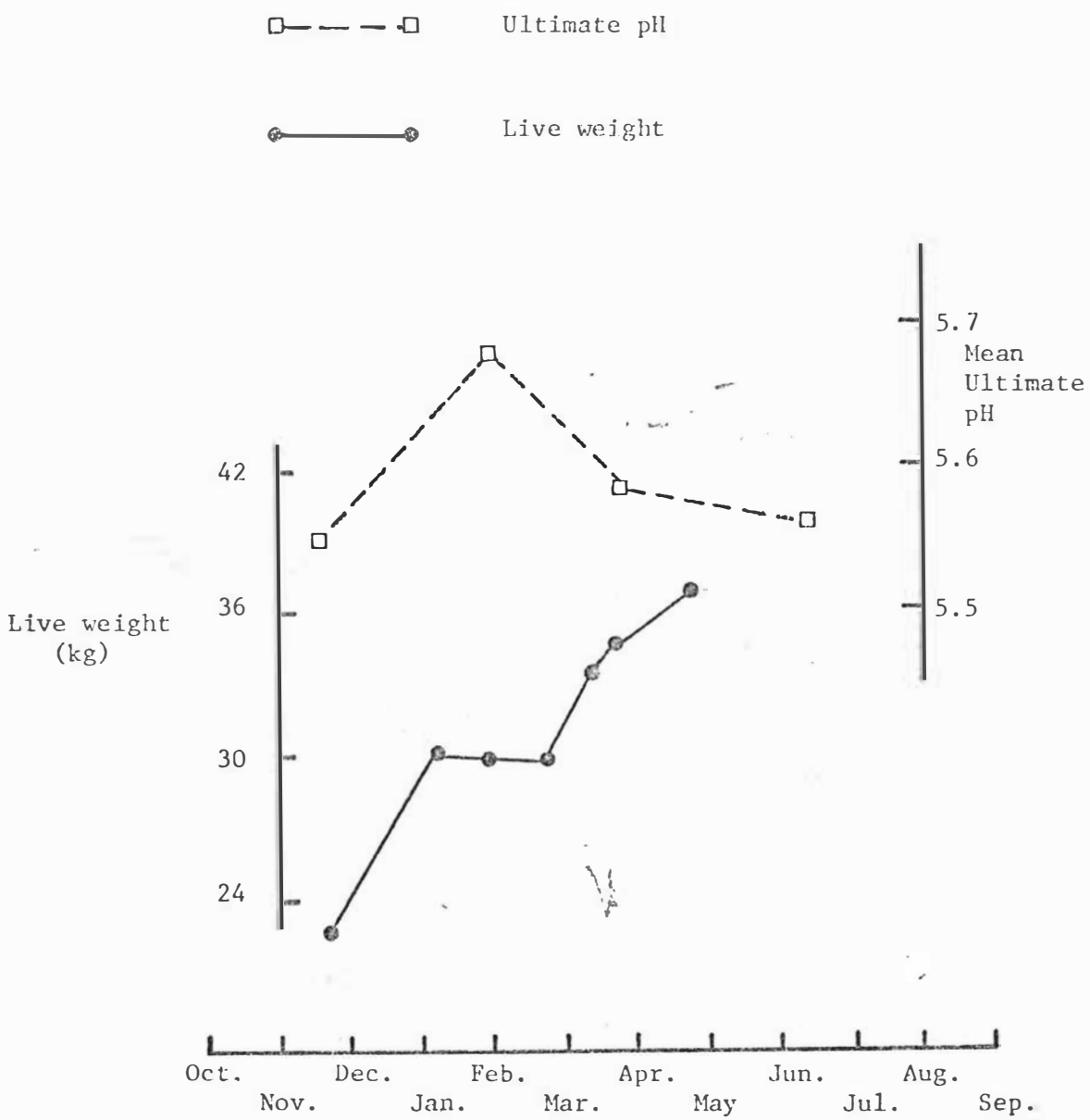
The very low mean ultimate pH values recorded in the four groups of animals used in the last part of these studies are also of interest in relation to the effect of feeding levels on ultimate pH. These lambs had been kept on a high level of pasture feeding for two months prior to slaughter and this might explain both the relative high mean carcase weights as well as the low mean ultimate pH values as compared to lambs used in the earlier part of these studies.

It is interesting to note that the group of lambs with the highest mean ultimate pH also had the lowest mean carcase weight. It would thus appear that the type of pasture (perennial ryegrass) fed to these lambs not only had a significant effect on their weight gains but also on the ultimate pH of the meat. Such an effect may be either directly related to the quality of the pasture or it may be related to the palatibility of the pasture and hence to the quantity ingested.

Both the quantity and the quality of feed available for meat and wool production is likely to be affected considerably by seasonal and climatic conditions in New Zealand. The effect of seasonal changes on liveweight gains of lambs has previously been investigated (Clarke, 1959; Scott *et al.*, 1976) and it was reported that weight gains are often reduced for some time during the summer/autumn period. Such reductions in liveweight gains have been associated with quantitative feed shortages, decline in nutritive value of pastures and subclinical diseases such as parasitism and facial eczema, although the latter factor is thought to be of less importance (Scott *et al.*, 1976). In Figure 3.4 some of the previous

FIGURE 3.4 RELATIONSHIP BETWEEN SEASONAL PATTERN OF ULTIMATE pH VALUES AND LIVE WEIGHT GAINS IN LAMBS

(Data from Scott *et al.*, 1976 and Table 3.8)



results of liveweight gains studies in lambs are shown in relation to the sampling periods used in the present studies and it can be seen that the sampling period with the highest mean ultimate pH coincides with a period of negligible weight gains in lambs.

It has been shown in the United Kingdom that blood glucose, an essential percursor of muscle glyogen, declines progressively in grass-fed cattle during late summer and autumn (Manston *et al.*, 1977). Although similar studies have not been reported in lambs, it would seem likely that the high ultimate pH values in the summer period in the lambs in this study are related to a reduction in dietary intake. Other factors which could have affected the ultimate pH such as environmental temperatures and methods of handling of the animals in the yards appears to have been subjected to lesser variation during the four sampling periods.

Travel distance

It has previously been shown that the occurrence of DFD meat in beef increases with increased distance and duration of transport (Puolanne and Aalto, 1980; 1981). It has also been shown that lambs transported for four hours have increased ultimate pH of some muscles but not of the LD (Gire and Monin, 1979; Monin and Gire, 1980) and more recent studies in New Zealand indicated that lambs transported approximately 400 km for nearly ten hours had only slightly elevated ultimate pH values of the LD (Chrystall *et al.*, 1981a). These latter findings are in close agreement with the findings in the present studies indicating that the distance of transport is not an important factor in the development of high ultimate pH in lambs in New Zealand. However, it is possible that in countries where lambs are frequently subjected to much longer journeys prior to slaughter, the travel distance may have an effect on ultimate pH. This has been indicated by recent studies in Australia (Shorthose, 1977).

Holding periods

The effect of holding periods of cattle prior to slaughter has been investigated in meat works studies during the last few years and it would appear that generally the ultimate pH of carcases increases with increasing holding periods (Puolanne and Aalto, 1980; 1981; Augustini, 1981). However, recent work in Australia indicated that cattle subjected to long journeys had lower ultimate pH of the LD when allowed

to rest for two days prior to slaughter as compared to one days rest only (Wythes, *et al.* 1980). It has also been reported by Shorthose (1977) that sheep allowed to rest for 120 hours after a long journey had lower ultimate pH than sheep rested for 18 hours only and Chrystall *et al.* (1981a) found that ultimate pH of the LD in lambs declined during a 24 hour resting period following severe exercise.

The results of the present studies indicate that ultimate pH of lambs increases with increased holding periods in the yards similar to the previously mentioned meat works studies of beef. It would thus appear that holding periods in both beef and lambs have a different effect on ultimate pH of the LD depending on whether the information is obtained through observational studies at the works or through experimental studies. As the latter are usually carried out in laboratories, such differences may either be related to the different environments or to the different degrees of glycogen depletion of the animal prior to the actual holding periods being investigated.

General

Both meat and wool production in New Zealand depends entirely on pasture growth and it is therefore not surprising that there are considerable variations in production figures both within years and between years. Seasonal variations are frequently associated with a down-turn in production during the summer/autumn period related to changes in quantity and quality of pasture growth. Furthermore, many lambs are shorn prior to this period and the additional food requirements associated with shearing may also contribute to a reduction in liveweight gains in lambs during this period.

The present studies indicate that similar factors also affect the ultimate pH, an important quality factor of meat products. The statistical association between ultimate pH and both season and wool score in these studies can be explained by insufficient availability of pasture and it would thus appear that quantity and quality of pasture may have a major effect on ultimate pH. The seasonal effect can be exaggerated by the effect of breed and the highest ultimate pH values were therefore recorded in Perendale lambs during the summer period.

Two of the major factors associated with the preslaughter handling of

animals at the meat works are the washing of lambs and the subsequent holding periods in the stockyards prior to slaughter. Both these factors may have some effect on ultimate pH but the data obtained in these observational studies were insufficient to reach definite conclusions. However, these factors are well suited for experimental studies at the meat works and it was therefore decided to carry out further work in relation to these variables. This work is reported in the following chapter.

CONCLUSIONS

1. It was found that 85.2% of 1536 lamb carcases had ultimate pH values below 5.80 and the distribution of these values approximates a normal distribution curve with a mean of 5.5 and a standard deviation of 0.1 corresponding to a range of 5.2 to 5.8.

2. The ultimate pH values of the LD from lambs slaughtered during the summer period are significantly higher than those obtained during the three other seasonal sampling periods.

3. The carcases of Perendale lambs have significantly higher ultimate pH values as compared to carcases from other breeds.

4. Carcase grade has no significant effect on the ultimate pH but there is a significant interaction between grade and season.

5. The mean ultimate pH of sheep carcases condemned for emaciation is significantly higher than the mean ultimate pH of carcases condemned for neoplasia.

6. There is no statistical association between travel distance and the ultimate pH in lambs.

7. There is a highly significant direct correlation between the duration of holding periods of lambs and the ultimate pH of meat.

8. There is a highly significant inverse correlation between wool score and ultimate pH.

9. These studies indicate that nutrition plays an important role in the development of high ultimate pH values in the LD of lambs.

CHAPTER FOUR

THE EFFECT OF WASHING AND SUBSEQUENT RESTING ON MEAT QUALITY

INTRODUCTION

One special feature of the preslaughter handling of stock at New Zealand meat works, is the extensive washing of animals prior to slaughter. The importance of contaminated hides as a source of carcase contamination has been emphasised in previous studies in New Zealand (Nottingham *et al.*, 1974; Newton *et al.*, 1978) and as discussed in Chapter One, the washing of stock has been considered necessary to achieve satisfactory hygienic standards of carcase dressing.

A number of different washing techniques for both cattle and sheep have been employed in the past. Cattle are usually washed by hosing or spray washing whereas the washing of sheep and lambs is most commonly achieved by swimming the animals through a bath. This latter method requires considerable extra muscular activity by the animals because they are usually required to swim a length of at least 10 metres. It was therefore considered that the exercise associated with washing of lambs prior to slaughter may have an effect on depletion of muscle glycogen stores and hence have an effect on the ultimate pH of their carcases.

The effect of preslaughter exercise on muscle glycogen and the ultimate pH of meat has previously been studied in sheep and lambs. Forrest *et al.* (1964) reported no increase in the ultimate pH of the LD of lambs which had been exercised to near exhaustion on a tread mill prior to slaughter but some increase in the ultimate pH in lambs which had been exercised by dogs. The effects of a long journey (1100 km) followed by resting periods of either 18 or 120 hours were investigated by Shorthose (1977) who concluded that the ultimate pH of the LD and the *M.semitendinosus* of animals rested for 18 hours was greater than those of the same muscles of similar animals which had been rested and fed for 120 hours before slaughter. Recently, Chrystall *et al.*(1981a) reported that transport (400 km) and light exercise of lambs had little effect on the mean ultimate pH, but the carcases from lambs exhausted by a 5 km rapid walk had an elevated ultimate pH which did not completely decline to normal levels when rested for 24 hours before slaughter.

The various preslaughter procedures referred to in these reports are not often associated with normal practice and extrapolation of results to the meat industry is therefore difficult. Although an attempt was made to include washing as a factor in the observational studies reported upon in Chapter Three it was not possible to reach definite conclusions from the results obtained. This was due to the lack of information on the different degrees of washing to which groups of lambs had been exposed. It was therefore considered important to study this problem further in investigations carried out at the meat works and thus keeping the experimental conditions as close to normality as possible.

The objective of the present studies was primarily to study the effect of washing and subsequent resting periods of lambs on the ultimate pH of their carcases. However, because washing of lambs at the meat works was introduced to achieve a reduction in the extent of carcase conta mination, some preliminary investigations of the relationship between degree of fleece contamination, washing and subsequent carcase contamination were also carried out.

MATERIALS AND METHODS

Animals and their handling

All experiments were carried out at a local meat export works using lambs from different farms located within a radius of 200 km from the meat works. The period between drafting the animals on the farm and their arrival at the works varied from 12 to 24 hours and washing of the lambs was achieved by swimming them through a rectangular bath (115 x 1560 cm and approximately 70 cm deep). The animals were driven with the aid of dogs to the entrance of the baths and discharged into the water by means of a conveyor belt (Plate 4.1). The time taken to swim the length of the wash was observed to be between 30 and 40 seconds. This time did not appear to change appreciably when the animals were washed more than once.

Estimation of removal of solids from the fleece during washing

The wash water was sampled before and after washing of some groups of lambs. Samples of one litre were taken at three different points and the composite sample taken to the laboratory where 100 ml was poured into a dish of known weight which was left in a drying oven ($105^{o}C$) overnight. The dried dish was reweighed and the total solids per litre of water calculated. The difference in total solids between the two

PLATE 4.1 WASHING OF LAMBS IN A SWIM-THROUGH BATH

samples represented the gain in solid content and by multiplying this figure by the calculated total volume of water in the wash, the weight of solids removed from the animals was obtained.

The total amount of solids was related to the number of animals washed and the mean weight of fleeces (wool score) and the amount of solids removed per kilogram of wool was calculated for each group of lambs.

Recording of contamination and bruising of carcases

In some of the groups of experimental lambs, all carcases identified by the M.A.F. inspectors as being contaminated were recorded. The carcases in these groups were also examined at the same time by the author for evidences of bruising.

Ultimate pH measurements

Within one hour of slaughter, approximately two gram of muscle tissue was obtained from the LD of each animal using the plug sampling technique. Samples were overlaid with liquid paraffin, incubated at room temperature (approximately 20^{o}C) for 24 hours and the pH measured by the method previously described.

Experiment 1

This experiment was designed to evaluate the effect of overnight resting on ultimate pH in lambs having been washed at least once.

Lambs from four farms were washed once or twice after arrival in the stockyards and immediately afterwards two sample groups of 24 lambs were taken from each of the farm groups. Animals in the first sample group were slaughtered within four hours of washing whereas lambs in the second sample group were rested for 24 hours prior to slaughter.

Data on ultimate pH were examined by analysis of variance to determine the significance of differences of means between the two treatment groups.

Experiment 2

The objective of the second experiment was to determine the effect on ultimate pH of the usual methods of washing and resting of lambs at the meat works.

From each of two farms (Farm 5 and 6) 160 lambs were obtained and divided into eight treatment groups with 20 animals per group. These eight groups were used in a 4 x 2 factorial experiment where groups were either unwashed or washed, one, two or three times and rested for either one or 24 hours after washing and before slaughter.

The data from lambs from Farms 5 and 6 were combined in a three-way analysis of variance evaluating the effects of farm of origin, washing and resting. The effect of washing was further tested by examining the significance of contrasts for individual degrees of freedom, based on orthogonal coefficients taken from the tables of Rohlf and Sokal (1969).

Experiment 3

This experiment was designed to evaluate whether excessive washing (more than three washings) would further increase the ultimate pH and whether extended resting periods (beyond 24 hours) could counteract such effects.

In this study, 150 lambs originating from the same farm (Farm 7) were divided into nine treatment groups in a 3 x 3 factorial design. Groups were washed one, three and five times and allowed to rest for one, 24 or 48 hours. The three groups washed once, comprised only ten animals per group whereas there were 20 lambs in each of the other six groups.

Data on ultimate pH were examined in a two-way analysis of variance using washing and resting as the two factors but because of unequal sample size in this experiment, the coefficients were multiplied by the number of animals in each group.

Experiment 4

The objective of this experiment was to determine whether breed has an effect on the animal's response to washing in relation to depletion of glycogen stores and subsequent ultimate pH values of the LD.

A group of approximately 50 Romney lambs and 100 Perendale lambs which had been grazing on the same farm, were transported a distance of approximately 20 kilometres to a paddock situated at the meat works. After one week, the animals were walked for a few hundred metres to the covered

stockyards and penned separately from other animals overnight. The following morning one group of 24 animals from each of the two breeds was subjected to three washings and within one hour all animals were slaughtered.

Data on ultimate pH were obtained in the two groups of animals which had been washed and in similar sized groups from both breeds of the unwashed lambs. The data were examined in a two way analysis of variance using breed and washing as the two factors.

Experiment 5

The last of these studies was designed to determine the effect of washing of lambs on contamination and bruising of carcases.

Six lines of lambs, comprising between 150 and 200 animals each, were used for these studies. From each of these lines, five animals were selected at random for appraisal of fleece contamination with dust and sand. The animals were held on their backs and the crutch region was closely inspected. The skin in this area has only scattered fine hairs but contains well developed sebaceous glands and apocrine tubular glands (Habermehl, 1981). The secretion of a fatty substance from these glands (the *sebum cutaneum*) forms a brownish greasy layer on the skin in this region and it has been observed that in animals exposed to dusty and sandy conditions, the colour changes to black, presumably because of adherence of black particles to the greasy surface. On the basis of this examination, the six lines were classed as either consisting of lambs with black crutches or lambs with clean crutches.

Each line was divided into two approximately equal sized groups and one group was washed while the other group was left unwashed. Solids removed from the fleece during washing were measured and both carcase contamination and bruising were recorded in all groups. However samples for determination of ultimate pH values were not obtained from any of the groups in this part of the study.

RESULTS

The majority of lambs slaughtered at the meat works where these experiments were carried out are washed once or twice followed by overnight resting

prior to slaughter. Table 4.1 indicates that the mean ultimate pH for the four groups of animals having received such treatment varied from 5.42 to 5.84. It will also be noted that there was a small but non-significant decrease in mean ultimate pH of lambs from three of the farms following the 24 hour resting periods. On the other hand, overnight resting apparently caused a highly significant increase in ultimate pH of lambs from Farm 2 where mean values increased from 5.65 to 5.84.

It can be seen in Figure 4.1 that when lambs were subjected to an increased number of washings the mean ultimate pH increased regardless of farm of origin. When the data from Farms 5 and 6 were combined in an analysis of variance (Table 4.2) it was found that mean pH of animals from Farm 5 (Perendale breed) were highly significantly greater than those from Farm 6 (Romney breed). It can also be seen that washing had a highly significant effect on mean ultimate pH of lambs from these farms and that there is a linear relationship between number of washings and mean pH. However, in this part of the study there was no statistical association between resting periods and ultimate pH and there were no significant effects of any of the interactions between the three variables (farm, washing and resting).

After lambs from Farm 7 had been washed five times, the mean ultimate pH of the group slaughtered after one hour's rest was 6.03 with a range of 5.55 to 6.86 as compared to a group of animals from the same farm having been washed only once with a mean of 5.52 and a range of 5.39 to 5.74 (Figure 4.2). It can be seen in Table 4.3 that there was also a highly significant linear effect of washing on mean ultimate pH in these lambs and that there were no statistical associations between resting periods and ultimate pH.

It can be seen in Figure 4.3 that all groups of lambs from Farm 8 had very low mean pH values as compared to mean values from the previous experiments. Table 4.4 indicates that the differences between breeds were non significant but there was a highly significant effect of washing. This effect would appear to be more pronounced in the Perendale lambs as compared to the Romney lambs. Although the interaction between breeds and washing was non significant, the value of the variance ratio for this statistic was approaching significant levels ($0.05 < P < 0.10$).

TABLE 4.1 EFFECT OF RESTING PERIODS ON ULTIMATE pH IN LAMBS

	No. of Washings	Mean ultimate pH ± S.E. 1 - 4 hrs resting period	24 hrs resting period	Variance Ratios
Farm 1	1	5.60 ± 0.022	5.57 ± 0.025	0.87
Farm 2	1	5.65 ± 0.026	5.84 ± 0.042	14.15**
Farm 3	1	5.44 ± 0.020	5.42 ± 0.020	0.62
Farm 4	2	5.65 ± 0.042	5.57 ± 0.023	2.54

** P <0.01

FIGURE 4.1 THE EFFECT OF FARM OF ORIGIN, WASHING AND RESTING OF LAMBS ON ULTIMATE pH OF THE LD

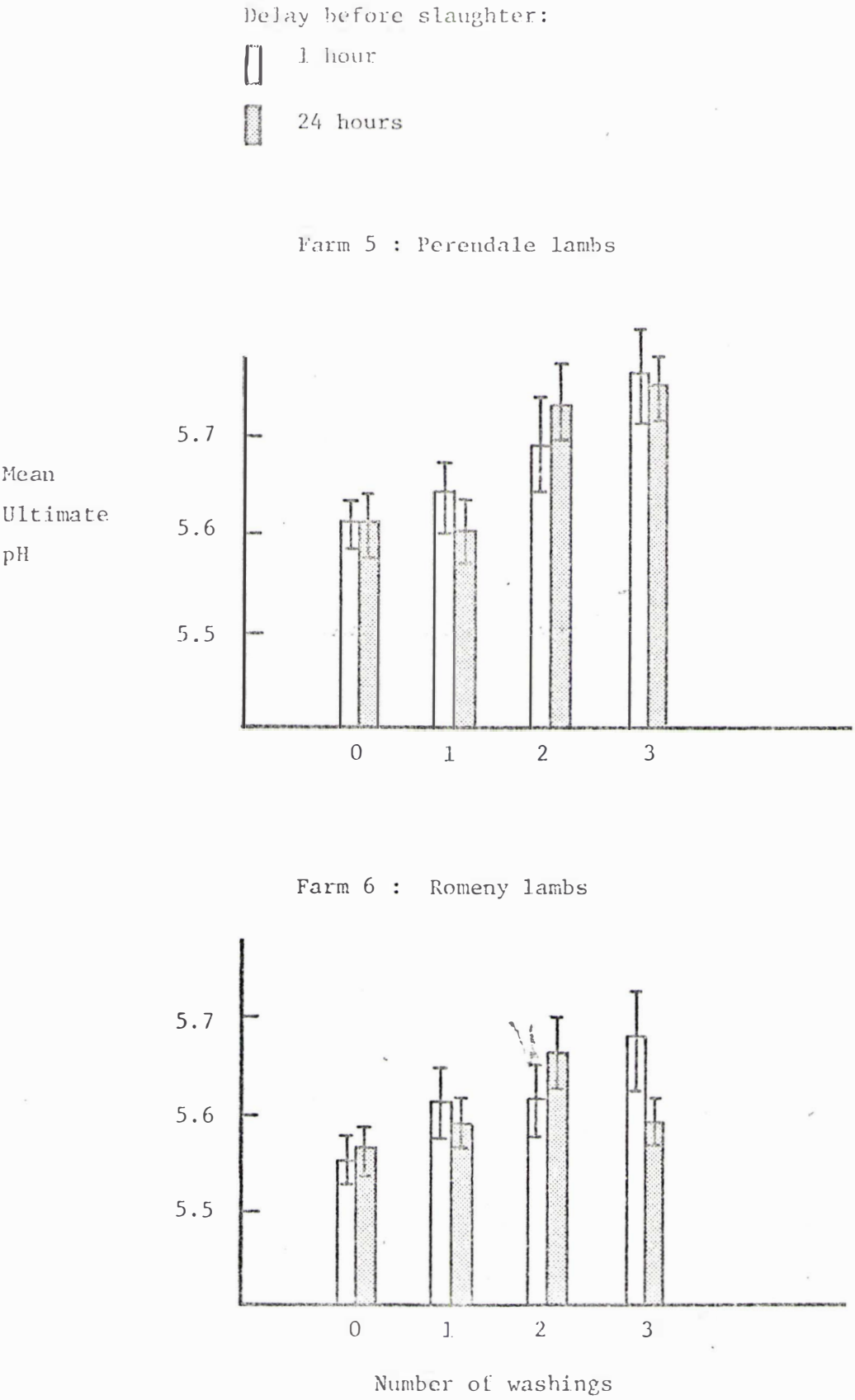


TABLE 4.2 SUMMARY OF A THREE WAY ANALYSIS OF VARIANCE OF DATA FROM FARMS 5 AND 6

Source of Variation	DF		Variance Ratios	
A. Farm	1		17.79**	
B. Washing	3		7.77**	
(i) linear		1		20.61**
(ii) quadratic		1		0.00
(iii) cubic		1		1.60
C. Resting	1		0.30	
A x B	3		1.20	
A x C	1		0.26	
B x C	3		1.45	
A x B x C	3		0.37	
Residual mean square	304	259.33		

** P <0.01

FIGURE 4.2 THE EFFECT OF WASHING AND RESTING OF LAMBS ON ULTIMATE pH OF THE LD

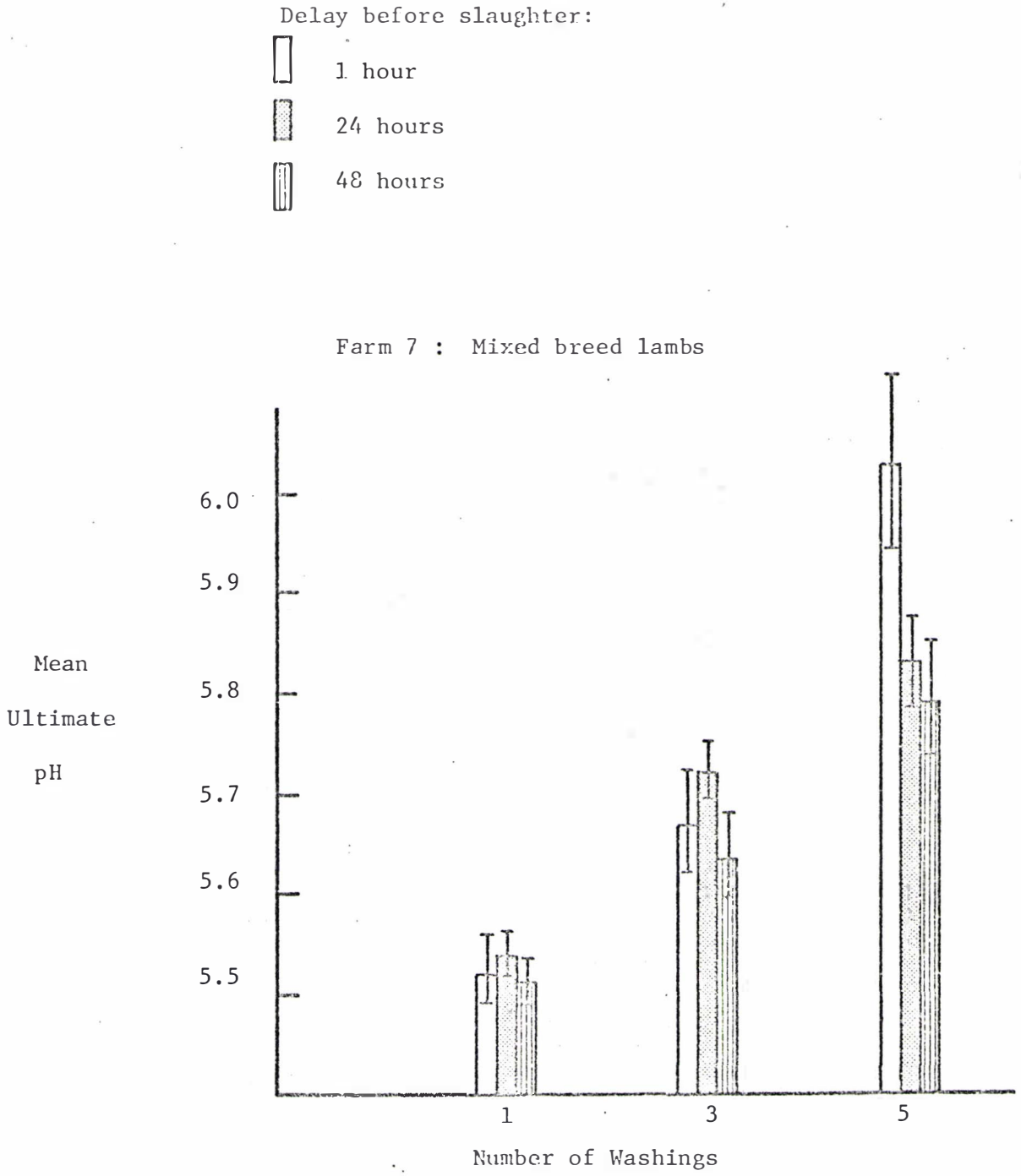


TABLE 4.3 SUMMARY OF A TWO-WAY ANALYSIS OF VARIANCE OF DATA FROM FARM 7

Source of Variation	DF		Variance Ratios
A. Washing	2		
L. Linear		1	27.80**
Q. Quadratic		1	0.51
B. Resting	2		
L. Linear		1	2.55
Q. Quadratic		1	0.00
Interactions			
L x L		1	0.51
L x Q		1	0.21
Q x Q		1	0.35
Q x L		1	0.23
Residual Mean Square	141	570.81	

* P <0.01

FIGURE 4.3 THE EFFECT OF BREED AND WASHING OF LAMBS ON ULTIMATE pH OF THE LD

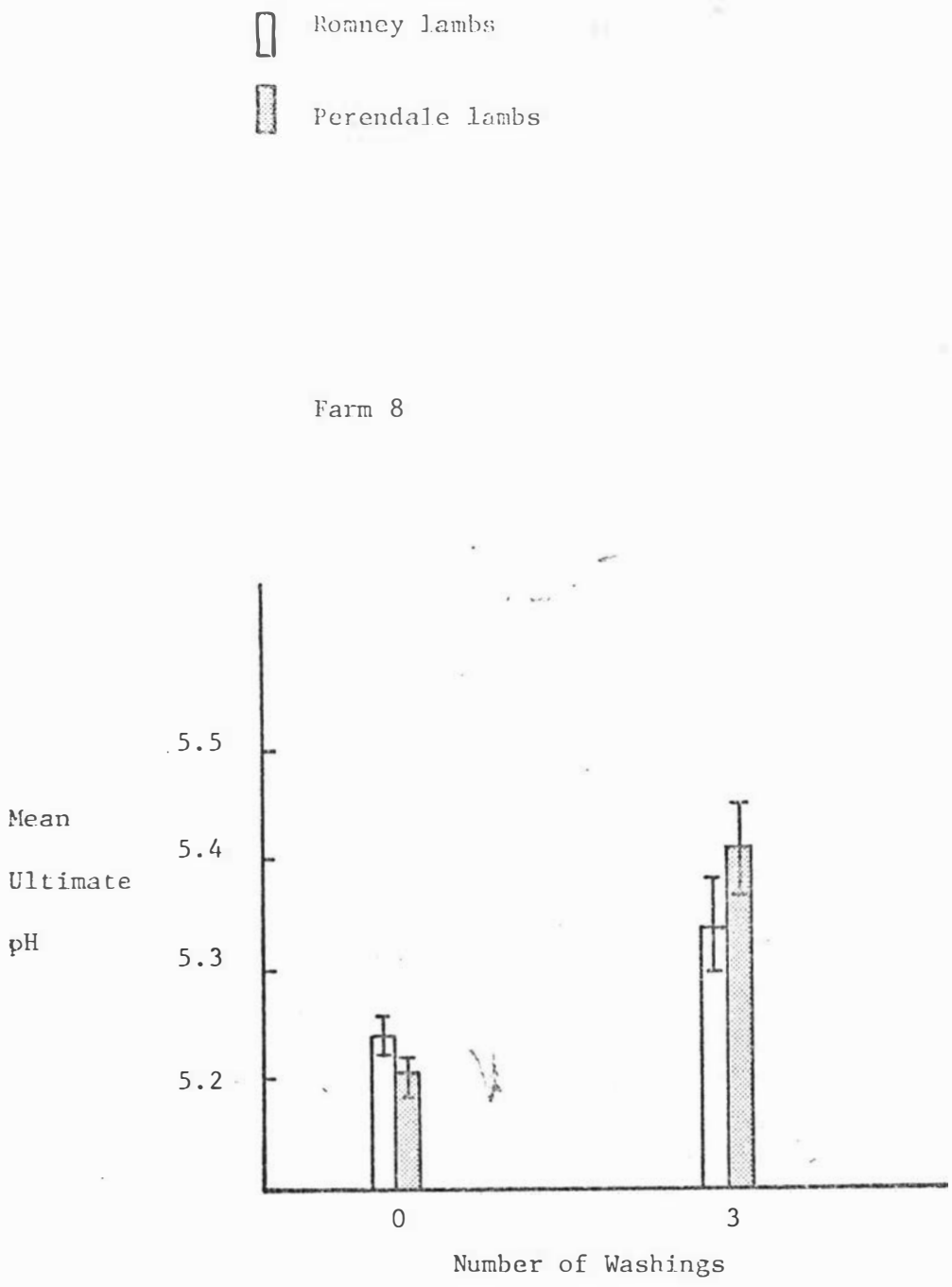


TABLE 4.4 SUMMARY OF A TWO-WAY ANALYSIS OF VARIANCE OF DATA FROM FARM 8

Source of Variation	DF		Variance Ratios
A. Breed	1		0.21
B. Washing	1		24.84**
Interactions	1		3.42#
Residual mean square	92	234.04	

** P <0.01
# 0.05<P<0.10

TABLE 4.5 THE EFFECT OF WASHING OF LAMBS ON CONTAMINATION OF CARCASES

	Unwashed		Washed		Solids removed per kilogram wool (kg)
	Number	% Affected	Number	% Affected	
Lambs with black crutches	76	48.7	78	28.2*	0.30
	97	37.1	90	18.9*	0.28
Lambs with clean crutches	99	21.2	91	23.1	0.15
	72	16.7	87	23.0	0.19
	96	19.8	96	15.6	0.24
	74	20.3	73	16.4	0.16

* Significant reduction in contamination rates ($P < 0.05$)

TABLE 4.6 THE EFFECT OF WASHING OF LAMBS ON BRUISING OF CARCASES

	Unwashed		Washed	
	Number	% Affected	Number	% Affected
Lambs with black crutches	173	9.8	168	32.1**
Lambs with clean crutches	341	16.4	347	28.0**

** Highly significant increase in rate of bruising ($P < 0.01$)

Two of the six lines in the last part of these studies were classed as having black crutches and it will be noticed in Table 4.5 that there was a significant reduction in contamination rates associated with washing in both of these lines. On the other hand washing had no significant effect on contamination rates in the four lines classed as having clean crutches. The mean amount of solids removed from the fleeces varied from 0.15 to 0.30 kilogram per kilogram of wool and the largest amounts of solids were recorded in lambs with black crutches. It was noticed during slaughter and dressing of lambs in this study that the crutches of washed animals were clean, regardless of the cleanliness of the animals prior to washing.

It can be seen in Table 4.6 that washing caused a highly significant increase in bruising in both groups of lambs in this study.

DISCUSSION

Effect of washing on ultimate pH

A high rate of depletion of muscle glycogen associated with severe exercise has been observed in man and it has been reported that one quarter of the muscle glycogen can be depleted within two to four minutes during maximal exercise (Rosell and Saltin, 1973). It has also been shown by Terjung *et al.* (1973) that 90-95% of the glycogen stores in the leg muscles of rats can be depleted by running in a treadmill whereas less glycogen depletion occurs in the leg muscles of rats exhausted by swimming. It would appear that glycogen depletion of muscles induced by exercise has been more difficult to achieve in ruminants than in other animals although Forrest *et al.* (1964) showed that lambs exercised by a dog had a high ultimate pH of some muscles and more recently Chrystall *et al.* (1981a) reported that lambs subjected to a rapid five kilometre walk had increased ultimate pH values of the LD as compared to control animals.

The results of the present experiments indicate that washing lambs in a swim-through bath has a severe and predictable effect on ultimate pH of meat. In one of the experiments of the present studies, the mean ultimate pH increased from 5.52 to 6.03 with several animals having pH values above 6.50 when the lambs were washed five times. These animals were only forced to swim for a total period of approximately

three minutes. It would thus appear that in comparison to other methods of exercise, swimming is a very effective method of inducing muscle glycogen depletion in lambs. This method of glycogen depletion might be applicable for other workers interested in this area of research.

Although lambs are usually washed only once or twice prior to slaughter, the effect of multiple washings on ultimate pH is of some interest. Evaluation of the data from these experiments indicated that there was a linear relationship between washing and ultimate pH regardless of the initial mean ultimate pH and the number of washings to which the animals were subjected. It seems likely that some groups of lambs arriving at the meat works may already have been subjected to treatments resulting in some depletion of glycogen. In such cases the additional effect of one or two washings may be enough to induce high ultimate pH in the muscles of some of the animals.

The highly significant difference in ultimate pH values between Farm 5 and Farm 6 in the second experiment is an interesting finding. As far as it could be ascertained, animals from these farms were subjected to the same treatment prior to arrival at the works but they were of different breeds (Perendale lambs from Farm 5 and Romney lambs from Farm 6). The results recorded in Chapter Three also indicated that Perendale lambs generally had higher ultimate pH values as compared to other breeds.

Although lambs from Farm 5 and Farm 6 responded in a similar manner to washing it will be noted that there was a slightly higher (but non significant) increase in ultimate pH associated with washing Perendale lambs as compared to the increases in similar groups of Romney lambs. A similar trend is apparent in the comparison between the same two breeds of lambs from Farm 8. All the lambs in this latter experiment were in very good condition and care was taken to ensure that they were handled gently prior to their arrival at the works. Both of these factors may have contributed to the low mean ultimate pH values obtained in this experiment. However, the increase in mean ultimate pH values after washing of the Perendale lambs (0.21 pH units) was approximately double of the increase associated with washing of the Romney lambs (0.10 pH units). There was also some indication of an interaction between breed and washing in this experiment and although this was not significant ($0.10 < P < 0.05$) it is believed that in view of the breed tendency

for high ultimate pH reported in Chapter Three it is very likely that Perendale lambs are more susceptible to the effects of washing as compared to Romney lambs.

Effect of resting on ultimate pH

The possible repletion of muscle glycogen stores during resting periods prior to slaughter is an important factor when determining preslaughter holding periods at the meat works. It would appear that muscle glycogen repletion following exercise induced muscle glycolysis, is rapid in rats and is usually complete within two to four hours (Terjung *et al.*,1974; Armstrong and Ianuzzo, 1977). On the other hand, McVeigh and Tarrant (1981) found that young bulls did not recover completely from muscle glycogen depletion within three days and Shorthose (1977) reported that muscle glycogen repletion took up to five days in sheep exhausted by a long journey.

In contrast to previous studies, the animals in the present studies were rested without access to food and water and this may have some effect on their ability to restore glycogen reserves, although it has been suggested (Chrystall *et al.*,1981a) that for relatively short periods of fasting, the considerable amount of food in the gastrointestinal tract should be sufficient to provide energy for replenishing muscle glycogen. However, the present results indicate that there was no significant repletion of muscle glycogen regardless of the degree of depletion and the length of subsequent resting periods. These findings are not in complete agreement with those of Chrystall *et al.*(1981a) who reported a decrease in ultimate pH values after a resting period of 24 hours following exercise. However, their studies were carried out at a small experimental abattoir where the conditions might have been more conducive to adequate resting of the animals than in a large meat export works.

The effect of resting periods prior to slaughter on ultimate pH of meat has been investigated in cattle under commercial conditions. Augustini (1981) reported that bulls kept in lairage at the abattoir were more likely to exhibit DFD meat than those which were not rested. In the first experiment of the present studies, there was also a highly significant increase in ultimate pH after 24 hours resting in animals from Farm 2. These lambs had been treated in a similar manner to

other animals used in the studies and it is therefore difficult to explain their apparent different response to the stockyard environment. However, this finding indicates that under certain conditions, increased holding periods in the stockyards may also cause high pH in lamb carcases.

The aetiology of high ultimate pH

The objective of the studies presented in this thesis was to investigate the effect of preslaughter and slaughter factors on meat quality and it was the intention to study mainly factors which could be investigated at the meat works in order to be able to use such findings directly to alleviate problems affecting the meat industry. However, future research in meat science must obviously also be directed towards studies of some of the fundamental physiological and biochemical events causing the changes in meat quality which can be detected at the meat works. In this context, the causes of meat with high ultimate pH are of particular interest and in the following paragraphs a few comparisons are made between previous work related to metabolism of muscle glycogen and some of the findings in the present studies.

It has been suggested by Rosell and Saltin (1973) that the metabolism of muscle glycogen during exercise may be mainly activated by the so-called "contractile factor". The major effect of this factor is probably related to the release of calcium ions into the myofibril which induces a rapid conversion of phosphorylase b to phosphorylase a (Drummond *et al.*, 1969). The latter enzyme is capable of hydrolysing glycogen to glucose-1-phosphate in the absence of adenosine monophosphate (AMP) whereas phosphrylase b is normally inactive except in the presence of elevated levels of AMP. Although blood levels of catecholamines are increased during exercise (Rosell and Saltin, 1973) and it has been shown that these hormones are potent activators of glycolysis (Drummond *et al.*, 1969), the relative importance of this method of activation of muscle glycogen breakdown during exercise is not clear. It has been shown in humans that blockage of beta receptors does not markedly influence the breakdwon of muscle glycogen during exercise (Harris *et al.*, 1971) and McVeigh and Tarrant (1981) found that injection of beta blockers only had a small protective effect against muscle glycogen metabolism in bulls subjected to stress and exercise. On the other hand, Monin and Gire (1980) reported that the beta effects of catecholamines played a predominant role in muscle glycogen mobilisation during transport stress in lambs.

It is suggested that in the present experiment, both the "contractile factor" and the release of catecholamines may have played a role in activation of glycolysis. It was noted that the animals became more reluctant to move after having been through the bath a few times and the shepherds, aided by their dogs, had to force the lambs into the water. It would seem likely that such handling methods could result in an increase in secretion of catecholamines as it has been shown that blood levels of both adrenaline and noradrenaline are elevated when lambs are subjected to the environment of stockyards in large meat works as compared to animals slaughtered in small plants (Pearson *et al.*, 1977). One of the features of the ultimate pH data from groups of lambs subjected to several washings, was that not only was there an increase in the mean ultimate pH but the range of values was also greatly increased as indicated by the increase in the standard errors of the means. This apparent variation in the reponse of animals to washing may in part be due to differences in their physical condition and partly due to behavioural differences which could be expected to reflect the level of secretion of catecholamines.

Although heritability has not yet been shown to have played an important role in the development of DFD meat, it has been suggested by Lister and Spencer (1981) that the excessive metabolism of glycogen, in animals developing this condition, can be attributed to inadequate aerobic metabolism and to stimulation of anaerobic glycolysis, under the influence of a beta adrenergic mechanism. It was further suggested by Lister and Spencer (1981) that the releasing mechanism for this chain of events may have some heritability component similar to that found in porcine malignant hyperthermia. The primary defect in this latter condition is assumed to be an abnormality of calcium regulation (Hall *et al.*, 1980) and it is associated with a massive increase in plasma catecholamine concentrations (Lister *et al.*, 1974).

It would appear that breed differences have not previously been reported in relation to DFD meat. The present findings indicate that Perendale lambs generally have higher ultimate pH values and they are probably more susceptible to the effects of washing on ultimate pH as compared to lambs of other breeds. It is suggested that the enforced swimming of lambs could be used in future studies of the protective effect of beta blockers on muscle glycogen metabolism in different breeds of lambs. Unfortunately

such studies could not be carried out at the meat works because of the problems associated with residues in meat. However, it might be possible to use a percutaneous needle biopsy technique similar to that used in cattle by Tarrant and McVeigh (1979). If this technique was used, sequential glycogen estimations could be obtained and this would reduce the number of animals required for such an investigation.

Application of results

Most sheep and lambs are washed at least once prior to slaughter and this is usually achieved by swimming the animals through a bath. The results of the present studies indicate that such methods of washing of lambs is a contributing factor to the induction of high ultimate pH of meat. It was also shown that there is a significant increase in bruising of carcases associated with washing of lambs prior to slaughter. This latter finding is in agreement with some previous work where a different method of washing of lambs was used (Petersen, 1978). It may thus be concluded that washing of lambs prior to slaughter is not only time consuming and expensive but can also have serious deleterious effects on meat quality.

Washing of lambs in the stockyards was introduced by the meat industry to achieve a reduction in carcase contamination. However, it would appear that no studies have been carried out to evaluate the effect of washing of stock on contamination of carcases. This is probably because of the difficulties in finding some objective methods for measuring contamination on meat surfaces. In the present studies data from the regulatory meat inspection service were used to determine the contamination of carcases. Although this is a relatively subjective measure, it was considered to be the most accurate method available for measuring carcase contamination. It is also directly related to the economic importance of this factor as carcases are detained and condemned on such judgments. The results of these studies clearly indicate that it is possible by relatively simple methods to make a distinction in the stockyards between lines of lambs requiring washing and those lines where washing is unlikely to reduce the risk of carcase contamination. It is suggested that by using these or similar methods for assessment of cleanliness of animals in the stockyards, it would be possible for the meat industry to avoid unnecessary washing of animals and thereby reduce the risks of associated effects on meat quality.

The results of the present studies and those reported in the previous chapter clearly indicate that resting of animals prior to slaughter has no effect on repletion of glycogen reserves and may in some cases be contraindicated. It would thus be advisable to keep holding periods in the stockyards to a minimum. However, this factor may also have other effects on meat quality and these will be discussed in the following chapter.

CONCLUSIONS

1. There is a highly significant linear relationship between the number of times lambs are washed prior to slaughter and the ultimate pH of the LD.

2. Resting of animals prior to slaughter has no apparent effect on repletion of glycogen stores after washing and may in some cases exacerbate the condition.

3. The increase in ultimate pH values following washing is generally greater in Perendale lambs as compared to Romney lambs.

4. There is a highly significant increase in bruising of carcases associated with washing of lambs prior to slaughter.

5. Reduction of carcase contamination by washing lambs prior to slaughter can only be achieved when dirt is visible on the skin of the inside aspects of the thighs.

CHAPTER FIVE

THE EFFECT OF FASTING ON CARCASE WEIGHTS AND NATURE AND WEIGHTS OF RUMINORETICULAR CONTENTS

INTRODUCTION

Differences in times between animals leaving the farm and being slaughtered could theoretically have an effect on several aspects of meat quality. During this time animals are usually deprived of food and water, subjected to exercise and changes in environment and contaminated by faeces and other extraneous material. Some of these factors could affect muscle glycogen reserves, body weight (including both carcase weight and the weight and nature of gastrointestinal contents) and potential carcase contamination.

The time spent in transport from the farm to the meat works is dependant largely on the distance to be travelled. However, the time the animals spend in the stockyards at the meat works (holding period) is dependant on both legislative requirements and logistics of inspection and production. The animals must be in the yards for sufficient time to allow for adequate ante mortem inspection. There must also be time to wash excessively dirty animals to ensure a regular supply of clean and inspected animals at the point of slaughter.

The duration of time for which meat animals must be held in stockyards before being slaughtered is subject to statutory regulations (Anon, 1969). The general requirements are that stock must not be slaughtered on the day of arrival at the meat works and the Ministry of Agriculture and Fisheries (M.A.F.) can issue instructions with regard to any class of stock specifying required holding periods prior to slaughter. At present, the specified holding time at the works for adult sheep is 24 hours, while lambs are required to be received at the works by midnight on the day prior to slaughter. The regulations also allow for dispensations from these requirements and stock can under certain conditions be slaughtered earlier than specified. However, it is stipulated that such dispensations can only be given when cleanliness, emptiness and provision for adequate resting of the animals have been taken into account. It would thus appear that emptiness (which presumably refer to gastrointestinal fill) and

resting of the animals prior to slaughter is considered important by regulatory authorities (Bennett,1967; Watt, 1968).

There is little doubt that the nature and weights of gastrointestinal contents can have an effect on meat quality as it is generally accepted that hygienic evisceration without contamination of meat and meat products is easier to achieve when gastrointestinal contents are reduced in weights and are of a firm consistency. However, apart from some earlier work by Kirton *et al.*(1968) which was mainly related to the effect of fasting on carcase weights of lambs, this particular problem appears to have received little attention in the past in New Zealand.

The work reported in this chapter was undertaken to investigate the effect of different holding periods before slaughter and the provision of drinking water in the stockyards at meat works on the nature and weights of ruminoreticular contents of sheep and lambs. At the same time an attempt was made to measure the effect of these various factors on the weights of dressed carcases.

MATERIALS AND METHODS

Part 1 : Effect of holding times in stockyards

This part of the investigation was carried out during the later part of the killing season (March to June). The estimated age of lambs was six to eight months and the adult sheep were discarded breeding ewes, most of which would be over five years of age. Two separate lines of adult sheep and lambs from farms within a 50 km radius of the works were investigated. Both groups of animals had been drafted on the farms late in the day, were held in the farm yard overnight and transported to the works the following morning. After arrival at the works, the animals were divided into five approximately equal treatment groups as shown below.

Group A Slaughtered the same day
Group B Held for 24 hours without food and water
Group C Held for 24 hours without food, with access to water
Group D Held for 48 hours without food and water
Group E Held for 48 hours without food, with access to water.

The animals were slaughtered and processed at the times indicated. Each carcase was individually identified and final carcase weights recorded. Paunches were opened by a 10-15 cm incision in the ventral aspect of the rumen and a smaller one in the reticulum, and the contents were emptied into buckets of known weight.

The contents were well mixed and weighed and a 125 ml sample taken from at least 10 animals from each group for subsequent estimation of dry matter content.

The nature of the ruminoreticular contents were assessed using the following scoring system adapted from that used by Ross (1934) :

I	Solid	=	moulded to the shape of the rumen
II	Semisolid	=	not moulded but not flowing out of the rumen
III	Semifluid	=	slowly flowing out of the rumen
IV	Fluid	=	freely flowing containing a mixture of finely divided particles
V	Watery	=	practically no solid material.

In the laboratory, the samples were transferred to dry 500 ml beakers of known weight. The beakers and contents were reweighed and placed in an oven for drying overnight (135^{o}C). After reweighing, the dry matter was calculated and expressed in grams per 100g ruminal contents. A preliminary study showed that no further decrease in weight occurred after this time and temperature treatment.

Part 2 : Further investigations of the nature of ruminoreticular contents

The objective of these studies was to measure the dry matter content of the ruminoreticular contents in a cross section of sheep and lambs which had been subjected to the usual overnight holding period prior to slaughter.

Five lines of sheep and 12 lines of lambs slaughtered after a holding period of approximately 24 hours without access to water were selected for this study. From each of these lines, samples of ruminoreticular contents were obtained from five randomly selected animals. These samples were transferred to the laboratory and dry matter contents were estimated as previously described.

Part 3 : Water consumption of sheep and lambs

Shortly after arrival at the works, groups of approximately 25 sheep or lambs were held separately in identical wire mesh pens and water was made available from a plastic bucket fastened to the middle of the back wall of the pen. The quantity of water consumed was measured by refilling the bucket from a one litre measuring cylinder to a premarked level. The water was changed between groups of animals and a control bucket of identical dimensions was set up just outside the pen, out of reach of other animals. On each day of the study, one group of adult sheep and one group of lambs were selected for investigation. These groups usually originated from different farms. The animals were left undisturbed in the pens until just prior to slaughter the following day. This was usually a period of approximately 24 hours but on some occasions the animals were left in the pens for periods of up to 48 hours. In such cases, the water consumption during the first and second day of the holding period was measured separately.

Other details concerning the study groups of animals such as breed, wool score and transport times were also recorded and records of the daily temperature and humidity were obtained from a local meteorological office.

RESULTS

The mean weights of the dressed carcases from the different treatment groups of sheep and lambs are shown in Table 5.1. It will be seen that the differences between groups are small and analysis of variances indicated that none of these differences were significant. However, in the groups of lambs, there was a trend in reduction of weights with increasing holding periods and in some groups this weight reduction amounted to approximately 0.5kg per animal daily.

In Table 5.2, the mean weights of ruminoreticular contents are recorded and it will be noted that these appear to be reduced with increased holding periods. It was found that in sheep slaughtered after a 24 hour holding period, the weights of ruminoreticular contents were significantly higher in sheep having access to water, as compared with the group without water. The analysis of variances also indicated that lambs slaughtered after a 48 hour holding period showed a significant decrease in weights of

TABLE 5.1 WEIGHTS OF CARCASES (kg)

		Slaughtered on day of arrival	In yards 24 hours		In yards 48 hours	
			No water	Water available	No Water	Water available
SHEEP	No.	20	18	19	19	20
	Mean	16.5	17.4	18.1	16.8	16.7
	S.E.	0.78	1.13	1.00	0.67	0.89
LAMBS	No.	18	19	20	19	20
	Mean	16.5	16.4	16.0	15.9	15.6
	S.E.	0.39	0.49	0.43	0.41	0.41

TABLE 5.2 WEIGHTS OF RUMINORETICULAR CONTENTS(kg)

		Slaughtered on day of arrival	In yards 24 hours		In yards 48 hours	
			No water	Water available	No water	Water available
SHEEP	No.	20	18	19	19	20
	Mean	4.49	4.03	4.81	4.15	3.79
	S.E.	0.245	0.215	0.213	0.235	0.412
LAMBS	No.	18	19	20	19	20
	Mean	2.74	2.80	2.50	2.53	2.38
	S.E.	0.123	0.105	0.107	0.104	0.080

ruminoreticular contents as compared to those slaughtered on day of arrival. The differences between other treatment groups were not found to be statistically significant.

The physical nature of ruminoreticular contents is indicated in Table 5.3 and 5.4 for sheep and lambs respectively. It will be noted that the ruminoreticular contents of animals slaughtered soon after arrival are generally of a semisolid nature and that fluidity appears to increase with increased holding periods. There is also an apparent increase in fluidity in those groups of adult sheep having access to water as compared to groups without water. However, there appears to be no difference between groups of lambs in this respect.

The subjective nature of the scoring system is liable to errors and rather unsuitable for statistical analysis. However, Figure 5.1 indicates that there is a good correlation between these rather subjective scores of the physical nature and the more objective measurement of dry matter contents in sheep. The dry matter of ruminoreticular contents for all treatment groups of sheep and lambs are shown in Table 5.5 and these results are summarised in Figure 5.2. The fluidity of the ruminoreticular contents of all groups of animals increased in relation to the time they were held in the yards prior to slaughter. All groups showed a highly significant ($P<0.01$) decrease in dry matter content during the first 24 hours and this trend continued for the second 24 hours of the experiment but was only statistically significant in the lambs ($P<0.01$). Although the access to water during the holding period had no detectable effect on the nature or dry matter content of the ruminoreticular contents in lambs, it had a significant effect in adult sheep on reducing the dry matter content during holding periods in the yards ($P<0.05$).

The mean and range of dry matter estimations of ruminoreticular contents of the five lines of adult sheep and 12 lines of lambs included in the second part of these studies have been recorded in Table 5.6. It will be noted that these values are close to those obtained after a 24 hour holding period in the earlier experiments as reported in Table 5.5.

The hourly water consumption per animal was calculated for each group of animals used in this part of the investigation and the mean values of these are recorded in Table 5.7. The evaporation from water in the control

TABLE 5.3 PHYSICAL NATURE OF RUMINORETICULAR CONTENTS OF SHEEP

	Slaughtered on day of arrival	In Yards 24 hours		In Yards 48 hours	
		No water	Water available	No water	Water available
I Solid	2	1			
II Semisolid	15	4		2	
III Semifluid	3	7	8	6	3
IV Fluid		6	11	7	7
V Watery				4	10
Total in group	20	18	19	19	20

TABLE 5.4 PHYSICAL NATURE OF RUMINORETICULAR CONTENTS OF LAMBS

Description		Slaughtered on day of arrival	In Yards 24 hours		In Yards 48 hours	
			No water	Water available	No water	Water available
I	Solid					
II	Semisolid	12				
III	Semifluid	5	1	1		
IV	Fluid	1	12	14		
V	Watery		6	5	19	20
Total in Group		18	19	20	19	20

FIGURE 5.1 : RELATIONSHIP BETWEEN SCORING OF THE NATURE OF RUMINORETICULAR CONTENTS AND ESTIMATION OF DRY MATTER IN SHEEP

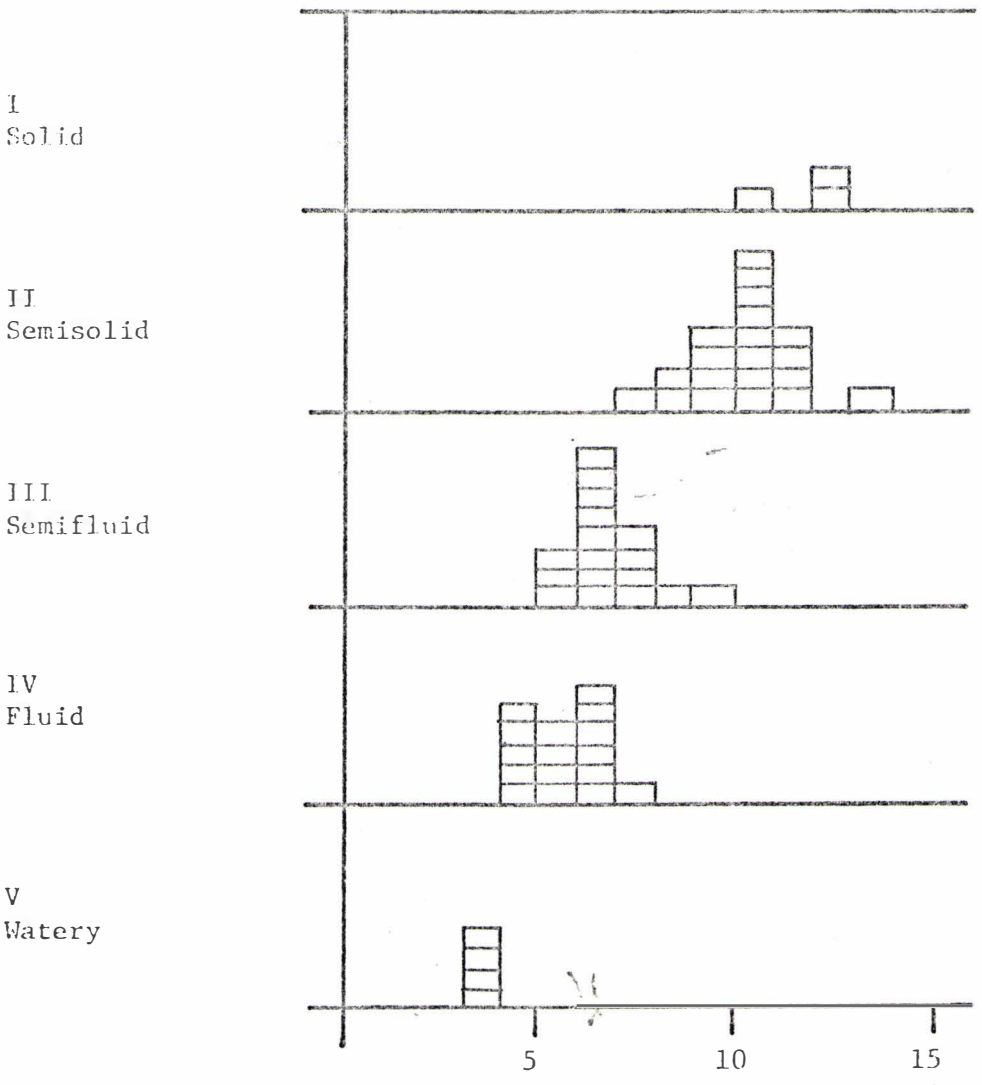


TABLE 5.5 DRY MATTER (g/100g) OF RUMINORETICULAR CONTENTS

		Slaughtered on day of arrival	In yards 24 hours		In yards 48 hours	
			No water	Water available	No water	Water available
SHEEP	No.	20	10	10	10	10
	Mean	10.32	7.73	5.95	6.47	5.10
	S.E.	0.297	0.661	0.405	0.645	0.436
LAMBS	No.	18	19	20	19	20
	Mean	8.30	3.77	3.73	2.48	2.58
	S.E.	0.271	0.138	0.210	0.080	0.094

FIGURE 5.2 : THE EFFECT OF HOLDING PERIOD AND ACCESS TO WATER ON DRY MATTER OF RUMINORETICULAR CONTENTS

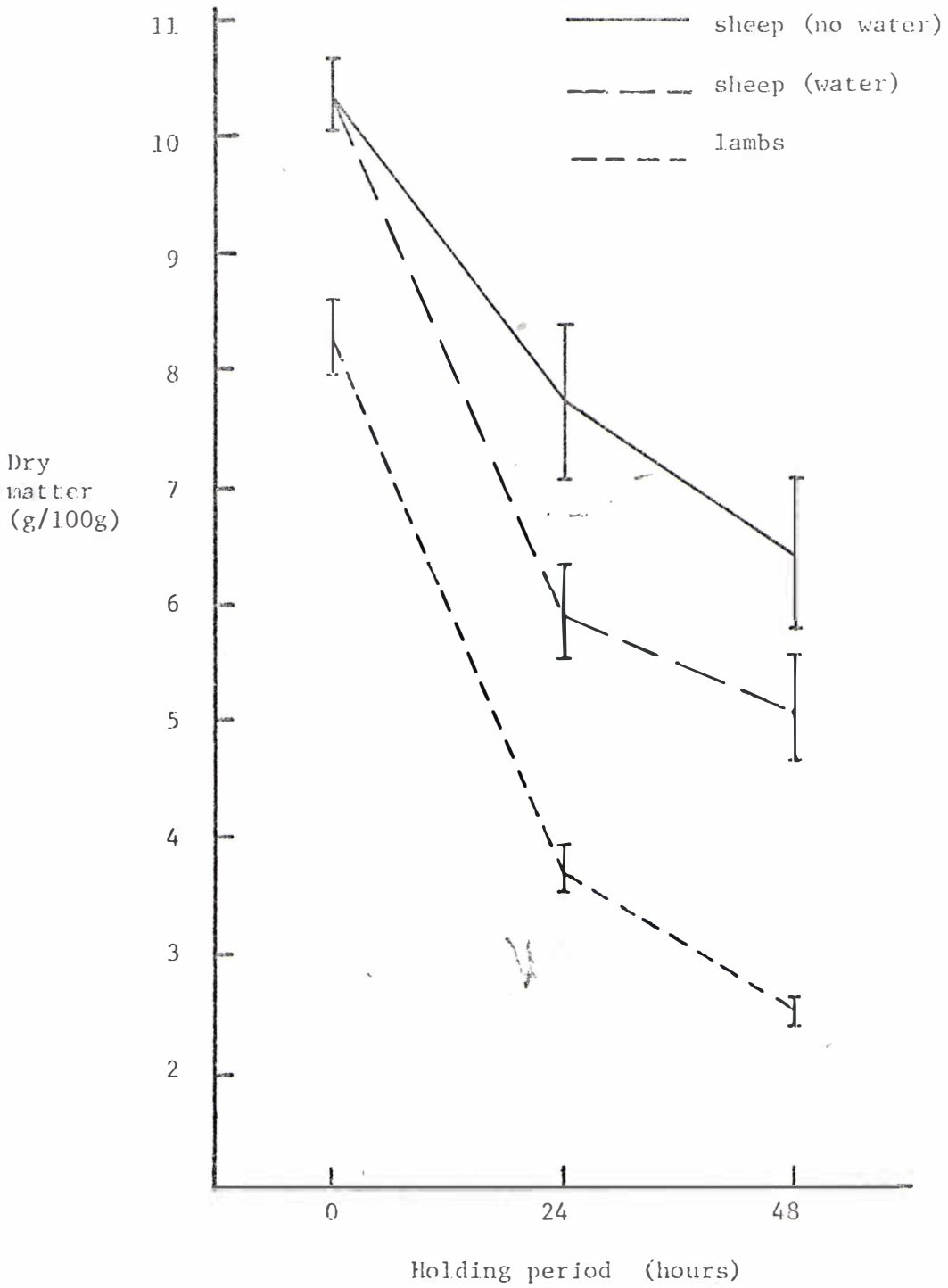


TABLE 5.6 DRY MATTER (g/100g) OF RUMINORETICULAR CONTENTS OF ANIMALS HELD WITHOUT WATER FOR ONE DAY

	No. of times sampled	Mean of lines	Range
Sheep	5	6.07	4.11 - 7.94
Lambs	12	4.31	2.78 - 6.20

TABLE 5.7 WATER CONSUMPTION ml/hour/animal

	SHEEP		LAMBS	
	No. of groups	Mean of groups	No. of groups	Mean of groups
Day 1	10	2.98	10	1.06
Day 2	4	10.42	4	2.58

bucket was found to be negligible. There was a highly significant increase in water consumption in the groups of sheep as compared to the groups of lambs ($P<0.01$). There was also an apparent increase in water consumption of both sheep and lambs during the second day of holding in the yards.

Although no significant correlations were found between water consumption and weather conditions, there appeared to be a trend towards higher water consumptions on days with higher ambient temperatures. This effect has also been suggested by other authors (Kirton *et al.*, 1971) but further investigations over a wider range of climatic conditions, would be required to clarify this question.

DISCUSSION

The potential loss of carcase weight during preslaughter fasting periods of animals has always been of concern to the meat producer and a number of investigations have been carried out to evaluate this problem in lambs. The results of these studies have been reviewed by Kirton *et al.* (1968) and Hughes (1976) who concluded that the main components of live weight loss during the first 24 hours of fasting of lambs are associated with reduction in the contents of the gastrointestinal tract and weight loss of some internal organs. They also reported a 33% reduction in the weights of stomach contents during the first 24 hours of the fasting of lambs, compared to a 6% reduction over the following day. On the other hand, loss of carcase weight was small during the first day of fasting but increased to nearly 0.5kg per animal during the next 24 hours. Although similar studies have not been carried out in adult sheep in New Zealand, it has been reported from the United Kingdom (Boyne *et al.*, 1956) that a significant decrease in weights of ruminoreticular contents of sheep occurs during the first 12 hours of fasting. However, these results are not directly comparable with the present findings as these animals were housed inside and fed a diet of hay.

The present studies were not specifically designed to measure the effect of fasting on carcase weights but it would appear that the results are in general agreement with the findings of others which have been described. It was estimated that the animals in this investigation had been subjected to 12-24 hours fasting prior to arrival at the works and this may explain the relatively small decrease in the weights of ruminoreticular contents

during the holding periods at the works. The decrease in carcase weights in the groups of lambs are also of the same magnitude as those reported by other workers.

It would thus appear that fasting of lambs for up to 24 hours does not affect carcase weights appreciably, but further periods of fasting may result in weight losses of approximately 0.5 kg per animal per day. On the basis of these figures, one additional day of holding of all lambs in New Zealand could cost the Industry as much as $5 million annually(based on schedule prices for the 1981/82 season).

The dry matter contents of ruminoreticular contents are of importance in relation to the hygienic evisceration of the animals. When the ruminoreticular contents are very fluid, seepage past an oesophageal clip or ligature is more likely to occur and accidental incision of the paunch may also result in more widespread contamination of the carcase with ingesta. Some earlier work in Australia (Ross, 1934) had indicated that in sheep, the ruminal contents become watery after withholding of food for 24-48 hours. More recent work in New Zealand (Kirton *et al.*,1968) indicates that two days of fasting more than halved the percentage of dry matter of stomach contents of lambs. The present investigations have shown that in both sheep and lambs which have been subjected to routine drafting and transportation methods, and therefore been deprived of food for 12-24 hours prior to arrival at the works, there is a dramatic increase in fluidity of ruminoreticular contents during the first 24 hours of holding at the works. This is followed by a further decrease in dry matter content over the next 24 hours and an apparent reduction in particle size of the ruminoreticular contents so that the physical nature becomes even more fluid.

Some variation of the nature of ruminoreticular contents could be expected between different lines of animals as this factor is affected by the type of pasture grazed by the animals (Nicol and McLean, 1970). Animals are also likely to have been subjected to varying periods of fasting prior to arrival at the works. However, the present results indicate that the two lines used in the first part of the survey were typical of stock received at the works. This view is supported by personnel within the Industry who have indicated that the majority of sheep and lambs are subjected to 12-24 hours fasting before arriving at the works.

It would appear from the results obtained in this study that the availability of water during holding periods has a pronounced effect on the fluidity of ruminoreticular contents in adult sheep, whereas there seems to be no effect on lambs. This difference may be due to differences in actual water consumption as indicated in the last part of these studies. It has been noticed previously that lambs drink little during the first 24 hours of holding (Anon, 1943) and it was suggested that the lambs require this period to settle down in their new surroundings.

The difference in water consumption between sheep and lambs is difficult to explain but it has been observed that dairy cows drink considerably more following the first calving (R.E.Munford, personal communication). This increased water consumption in cows apparently continues even after the time when there is no physiological requirement for increased water consumption and it has been suggested that some ruminants may become habitual drinkers. A similar phenomenon may occur in sheep following their first lactation.

The present findings, together with previously published reports, makes it possible to gain a better appreciation of some of the important changes taking place during preslaughter holding periods of sheep and lambs. It would appear that if these animals are removed from pasture immediately after a period of grazing and slaughtered within a short time, they are likely to have a completely full rumen and reticulum making evisceration difficult. However, during the initial fasting period of 18-24 hours, frequently associated with drafting and transportation to the works, there appears to be a dramatic reduction in weights of stomach contents but no appreciable decrease in carcase weights. Further holding period at the works are not likely to affect the weights of ruminoreticular contents but may reduce the carcase weights of lambs. It would also appear that an extended holding period at the works will increase the fluidity of ruminoreticular contents and that this effect is exacerbated in adult sheep if water is available.

These observations and the findings reported in the two previous chapters in relation to the effect of holding periods on ultimate pH, indicate that the present regulatory requirements for holding of stock prior to slaughter at the meat works may in some cases be detrimental to the quality of meat products. It is suggested that as an alternative, arrangements could be

made for sheep and lambs to arrive at the works after a fasting period of 18-24 hours and for the holding periods at the works to be kept to a minimum. Such a procedure would reduce the prevalence of those factors which are potentially detrimental to meat quality which have been described in this chapter.

CONCLUSIONS

1. Lambs lose little body weight during the first 24 hours of fasting but further holding periods in the stockyards without food result in weight losses of approximately 0.5 kg per animal per day.

2. The weights of ruminoreticular contents are reduced by as much as one third during the initial 24 hours of fasting. Further reduction occurs with increased fasting periods but at a reduced rate.

3. The fluidity of ruminoreticular contents increases with increasing periods of fasting in both sheep and lambs. This effect is exacerbated by availability of water in sheep but not in lambs.

4. The water consumption of lambs held in the stockyards is negligible as compared to sheep. However, both lambs and sheep drink more during the second day in the stockyards as compared to the first day of holding.

5. These results indicate that the ideal time to slaughter lambs is 18 to 24 hours after removal from pasture.

CHAPTER SIX

THE EFFECT OF DIFFERENT METHODS OF SLAUGHTER ON THE RATE AND EXTENT OF POST MORTEM GLYCOLYSIS

INTRODUCTION

The methods of slaughter of stock (i.e. stunning and exsanguination) have undergone many changes during the last few years. Some of these have been initiated by new legislation, whereas other developments have been associated with attempts to improve the commercial efficiency of the process, particularly in relation to the immobilisation of animals during exsanguination. Irrespective of the method of stunning, the slaughter process results in varying degrees of spasmodic muscular movements. It was shown in Chapter Four that excessive muscular exercise immediately prior to slaughter can deplete muscle glycogen reserves and result in higher than normal ultimate pH values of meat. It was therefore considered that differences in the degree of muscular activity of animals during slaughter might also have an effect on the rate and decline of the pH of muscle post mortem.

It has been reported by Pearson *et al.* (1973) that carbon dioxide narcosis of ewes increases post mortem glycolysis as compared to that of unstunned animals and these authors suggested that this method of inducing insensibility could be utilised to reduce the time required for conditioning and ageing lamb carcases. Shorthose (1978) reported a decreased rate of post mortem glycolysis in aged wethers stunned by captive bolt as compared to unstunned animals and attributed this effect to the lesser degree of kicking observed in stunned sheep. More recently, Chrystall *et al.* (1981b) found no differences in post mortem pH values of muscles in lambs slaughtered without prior stunning and those slaughtered after stunning by either an electrical 'head-only' stun or an electrical 'head-to-back' stun. However, all these studies were carried out on small groups of experimental animals and may not have completely reflected circumstances which usually occur in a meat works.

The studies presented in this chapter were carried out to gain more precise information on the type and amount of muscular activity associated with different methods of slaughter and effects of such activity on the rate and decline of the pH of muscle post mortem.

MATERIALS AND METHODS

All the studies were carried out at meat export works in the lower part of the North Island. Groups of lambs from within the same lines were subjected to different methods of stunning and exsanguination during normal operating procedures at the meat works at rates varying from four to eight per minute. Although selection of the animals into different treatment groups could not always be achieved on a strictly random basis, care was exercised to assure that groups within the same line were comparable with respect to carcase weights and grades.

Part 1 : Effect of different methods of slaughter on pH decline

The following methods of slaughter were used :

(a) Exsanguination without prior stunning by a transverse incision of the ventral neck region with severance of the major vessels, the trachea, the oesophagus and the spinal cord at the occipito-atlantal junction, i.e. the "gash-cut" method of exsanguination traditionally used in New Zealand up to 1977.

(b) Stunning by a penetrating captive bolt followed by severance of the major vessels within the thoracic inlet, i.e. the thoracic stick described by Blackmore and Newhook (1976).

(c) 'Head-only' electrical stunning (0.75-1.25 Amp. 400V, 50Hz for 2-3 s) with two electrodes on the head using a "Paralec" stunner* followed by exsanguination as in (a).

(d) Electrical 'head-to-leg' stunning using the same electrical parameters as in (c) but with a third electrode connected to metal rails in contact with the lower part of the legs ("Paralec Split-Stun System")*. This was followed by thoracic stick as in (b).

The muscular activity of animals following stunning and subsequent exsanguination was recorded by two observers and, where possible, the duration of specific events were timed. Heart activity was also monitored during the same period by auscultation and palpation of the chest.

*McKenzie and Holland (NZ) Ltd., Rata St., Naenae, New Zealand.

After dressing, inspection and grading, all carcases were transferred to holding areas where the ambient temperatures were approximately 20°C. Muscle tissue samples (2-3 g) were obtained from the LD and overlaid with liquid paraffin. The samples were kept at room temperature until processed in the laboratory where one gram of muscle tissue was homogenised in 10 ml of 5mM neutral iodoacetate in water. The first sample from each carcase was processed approximately three hours after slaughter and a second sample was processed the following day. The pH of the solutions were measured immediately following homogenisation of samples.

Part 2 : Comparison between stunning by a captive bolt and an electrical 'head-to-leg' method.

In this study, the pH values of carcases of lambs stunned by a captive bolt and by an electrical 'head-to-leg' method were compared at intervals during a 24 hour period.

The carcases were transferred from the holding area to a chiller approximately four hours after slaughter. The temperature in the chiller was approximately 15°C at the time of loading and declined to 12-13°C during the next two to three hours and remained at this temperature for the rest of the study period. The pH values were obtained by direct probe methods using a combined type Lot 406-M4 Ingold pH electrode[+]. The probe was inserted at a depth of approximately 15mm in the lumbar region of the right LD. Subsequent measurements were taken from a different site in close proximity to the first. Temperature measurements were obtained in the similar region of the LD using a Koch Rechargeable High-Low C/F Thermometer[++] with the probe positioned at the same depth as the pH probe.

Part 3 : Effect of low voltage stimulation on pH decline and tenderness

Three different methods of slaughter were used in these studies. One of these was Method (a) as described in the first part of these investigations, i.e. transverse incision of the ventral neck region without prior stunning. The second method used was electrical 'head-to-leg' stunning followed by thoracic stick (Method (d) in Part 1) and the third method was similar to the second but immediately after stunning the lambs were subjected to low voltage stimulation (50-150mA peaking at 50V for approximately 90 seconds). This was achieved by suspending the animals by all four legs by metal hooks

[+]Dr. W. Ingold AG, CH-8902 Urdorf, Zurich, Switzerland.

[++]Koch Supplies Inc., Kansas City, Montana, U.S.A.

from two rails connected to the electrical supply. The current thus passed through the carcase from fore- to hindlegs. While suspended and being stimulated, exsanguination of the animals was carried out.

Muscle samples (approximately 3 g) were obtained from the LD within 45 minutes of slaughter and overlaid with liquid paraffin. The pH of one gram samples homogenised in 10 ml of an iodoacetate/water solution were measured approximately one hour, two hours and 24 hours after slaughter.

The tenderness of the LD after frozen storage was also measured in two groups of carcases in this investigation. These carcases were transferred to freezer rooms approximately two hours after slaughter. They were subjected to temperatures between $-20^{o}C$ and $-25^{o}C$ during a 24 hour period and the following day, two transverse sections (4 to 5 cm in length) were obtained from the lumbar part of the LD. All samples were immediately transferred on ice to the laboratory where they were stored at temperatures below $-18^{o}C$ until measurements of tenderness were carried out.

One sample from each carcase was taken directly from frozen storage to a water bath at $80^{o}C \pm 1^{o}C$, cooked for one hour, chilled on ice and stored at approximately $2^{o}C$. Tenderness was measured with a "MIRINZ Tenderometer" within 12 hours of cooking as described by Macfarlane and Marer (1966) and two or three 'Force Score' values were obtained from each sample.

The other samples were removed from frozen storage and kept at approximately $2^{o}C$ for 16-18 hours prior to cooking and tenderness assessment.

Part 4 : Comparison between low voltage and high voltage stimulation

All lambs in these studies were stunned by the 'head-only' electrical method followed by transverse incision of the ventral neck region (Method (c) in Part 1).

Four groups of 12 lambs from five different lines were used in a 2 x 2 factorial experiment where groups were either stimulated or not by a low voltage method and either stimulated or not by a high voltage method. The low voltage method was similar to that described in Part 3 but using a period of stimulation of only approximately 45 seconds applied immediately following initiation of bleeding. The high voltage stimulation (peaking at 1100 volts, 14.3 pulses per second for 90 seconds) was applied to the carcases after dressing (20-25 minutes after slaughter).

The pre rigor pH values of the LD were obtained at least twice in all carcases within two hours of slaughter by the direct probe method described in Part 2 of these investigations. Plug samples were also obtained from six carcases in each group for later measurement of the ultimate pH values as previously described.

RESULTS

Muscular reactions associated with slaughter

The reactions of the animals to different methods of slaughter are recorded in Table 6.1. Both methods of electrical stunning caused tonic contraction of all skeletal muscles which lasted for approximately 25 seconds. This is the characteristic tetany seen as the animals emerge from the conveyor and is followed by a two to four minute period of clonic spasms characterised by intermittent leg and body movements. The extent and duration of such movements appeared to be slightly increased in those animals stunned by the 'head-only' method as compared to those stunned by the 'head-to-leg' technique.

Animals stunned by a captive bolt also exhibited tonic spasms but of shorter duration (approximately 15 seconds). This was followed by intermittent leg and body movements for up to four minutes which appeared to be of a more vigorous nature than those seen in animals which had been electrically stunned. Tonic spasms did not occur in lambs exsanguinated withour prior stunning but these animals exhibited the most vigorous leg and body movements.

No rhythmical heart activity could be detected in any of the animals stunned by the 'head-to-leg' method but in all the other groups, heart activity could be detected by auscultation and palpation until after bleeding had commenced.

Effect of method of slaughter on pH decline

In Table 6.2, a comparison is shown of the pH of the LD between lambs stunned by the 'head-to-leg' method and those slaughtered by the three other methods. There were no significant inter group differences of mean ultimate pH values and these have therefore been excluded from this table. It can be seen that pH values at three hours after slaughter were constantly lower in the animals stunned by the 'head-to-leg' method as compared to

TABLE 6.1 MUSCULAR REACTIONS OF LAMBS SUBJECTED TO DIFFERENT METHODS OF SLAUGHTER

Method of slaughter	No. of animals observed	Delay between stunning and slaughter (s)	Detectable heartbeat at slaughter	Duration of tonic spasms Mean ± S.E. (s)	Severity of clonic body movement *
(a) No stun, transverse cut	12	n.a.	+	0	very vigorous movements
(b) Captive bolt, thoracic stick	24	60 - 90	+	14.6 ± 0.84	vigorous movements
(c) Electric 'head-only' transverse cut	12	5 - 10	+	25.0 ± 0.92	some intermittent movements
(d) Electric 'head-to-leg' thoracic stick	23	60 - 90	-	26.3 ± 0.75	very little movement

* some intermittent leg movements were noted in all animals for up to three minutes after slaughter.

n.a. = not applicable

TABLE 6.2 COMPARISON OF ELECTRIC 'HEAD-TO-LEG' STUNNING WITH THREE OTHER METHODS OF SLAUGHTER

(pH values three hours post mortem)

	Alternative Method		Electric 'Head-to-leg'		
	No. in group	Mean ± S.E.	No. in group	Mean ± S.E.	Sample Variance Ratio (F_s)
(a) No stun					
Group A	12	6.28 ± 0.022	12	6.19 ± 0.027	5.77 *
Group B	12	6.28 ± 0.020	12	6.19 ± 0.031	5.40 *
(b) Captive bolt					
Group C	21	6.35 ± 0.021	24	6.27 ± 0.015	9.01 **
Group C	14	6.28 ± 0.016	14	6.15 ± 0.032	11.68 **
(c) Electric 'head-only'					
Group E	12	6.28 ± 0.023	12	6.19 ± 0.038	4.01
Group F	15	6.36 ± 0.032	15	6.27 ± 0.026	4.54 *

* = P <0.05
** = P <0.01

other groups. These differences were significant in all groups apart from one comparison between a group stunned by a 'head-only' method and a group stunned by a 'head-to-leg' method.

The rate of decline of pH of the LD during a 24 hour period in lambs stunned with a captive bolt and by an electrical 'head-to-leg' method is recorded in Table 6.3 and in Figure 6.1. It can be seen that at the time of the first measurement (90m), the pH of the LD of the electrically stunned animals (6.42) was significantly less than those stunned with a captive bolt (6.66). This difference, of approximately 0.2 of a pH unit, remained the same for the next seven and a half hours, but by 15 hours after slaughter the pH measurements of both groups were similar. Table 6.3 also indicates that the LD from animals stunned by a captive bolt took three hours longer to reach a pH of 6.0 as compared to those electrically stunned.

However, the increased rate of glycolysis in the electrically stunned animals responsible for this more rapid decline, had occurred during the early part of the pre rigor period and was not due to an increased rate of glycolysis throughout the period as this rate was approximately the same in the two groups from 1½ hours to nine hours after slaughter (see Table 6.3). Although there were at times some differences in temperatures in the two study groups, they were generally very small (less than $1^{o}C$) whereas there were marked differences within the two groups depending on the position of carcases in the chiller. The mean temperature of all carcases are also recorded in Figure 6.1 and it can be seen that there was a steady drop in temperature during the 15 hours after slaughter by which time the meat had equilibriated at a temperature of approximately $13^{o}C$.

Effect of electrical stimulation

The effect of low voltage stimulation for 90 seconds during exsanguination on rate and decline of muscle pH is recorded in Table 6.4 and Figure 6.2. It can be seen that in three separate comparative trials there was a highly significant difference in the pre rigor pH values of stimulated and non stimulated carcases. On average the pH of stunned and stimulated carcases from the three study groups had declined to a value of 6.0 within two hours whereas the pH of stunned, non stimulated carcases had only declined to approximately 6.3 and non stunned, non stimulated carcases (one group only) had declined to 6.4. There were no significant differences between the ultimate pH values in any of these groups.

TABLE 6.3 EFFECT OF METHOD OF STUNNING ON pH DECLINE IN LAMBS

(16 animals per group)

		Captive Bolt	Electric 'head-to-leg'
Mean pH ± S.E. 1.5h after slaughter		6.66 ± 0.046	6.42 ± 0.028
Mean pH ± S.E. 24h after slaughter		5.59 ± 0.009	5.60 ± 0.018
Time to reach pH 6.0 (h)		8.5	5.5
Rate of pH decline pH units/h	1.5 - 9h after slaughter	0.092	0.088
	9 - 15h after slaughter	0.057	0.025

FIGURE 6.1 MEAN pH VALUES AND TEMPERATURES OF THE LD OF LAMBS STUNNED BY EITHER A CAPTIVE BOLT OR AN ELECTRIC 'HEAD-TO-LEG' METHOD

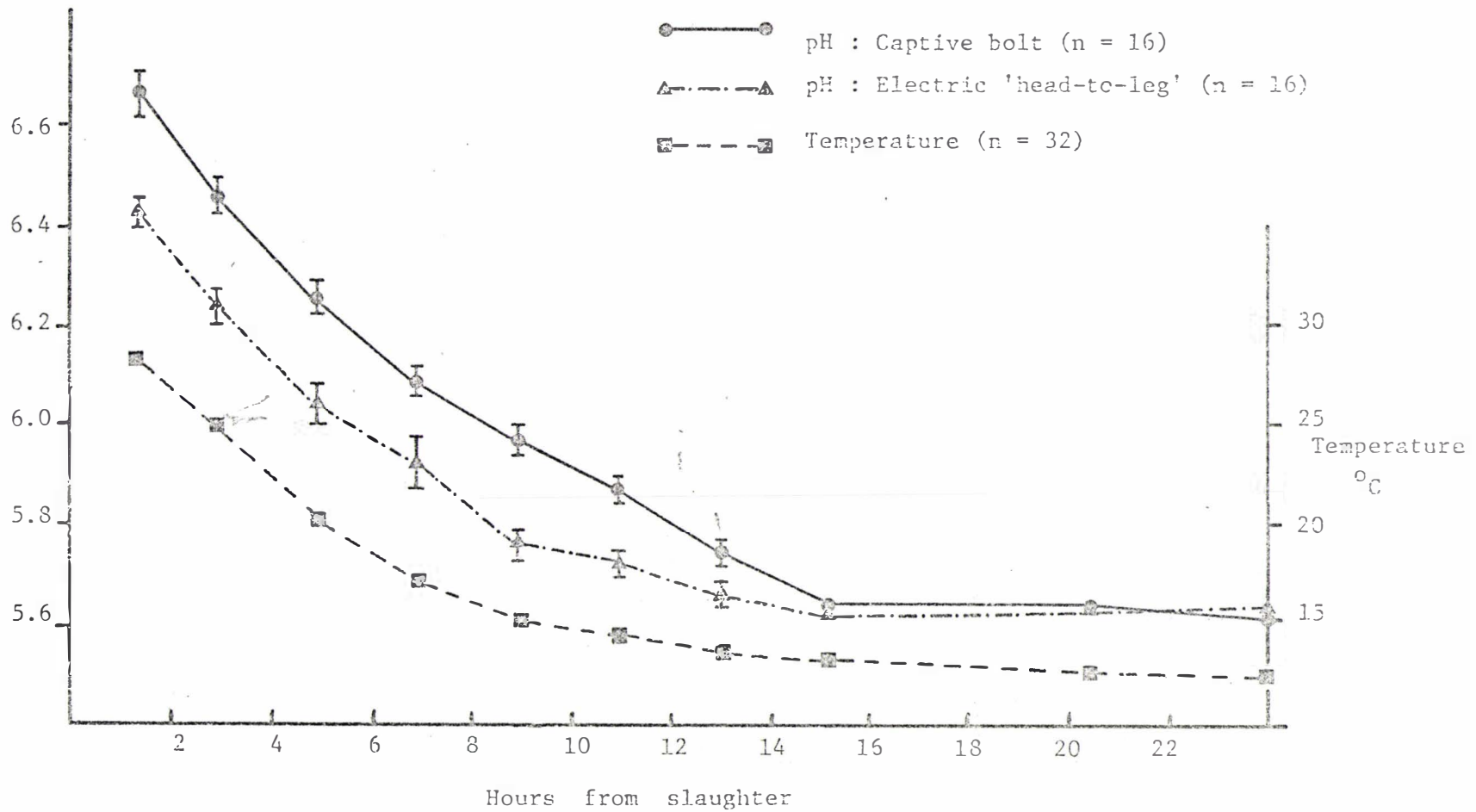
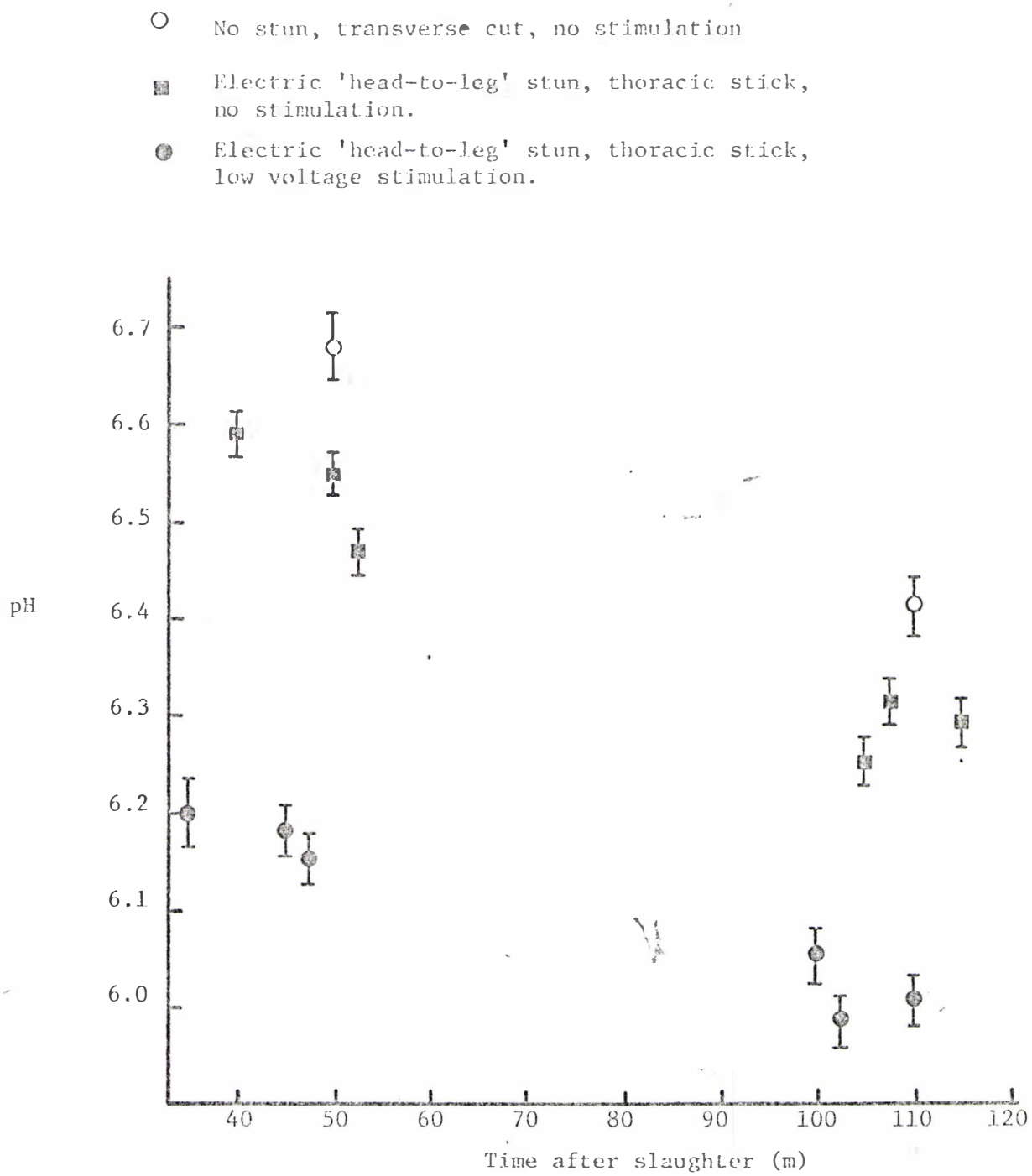


TABLE 6.4 THE EFFECTS OF ELECTRICAL STUNNING AND LOW VOLTAGE STIMULATION ON DECLINE OF pH

(Mean ± S.E. of 12 lambs per group)

Line No.	Stunned by electric 'head-to-leg' method	Low voltage stimulation	Approximate time from slaughter 1h	2h	24h
1	-	-	6.68 ± 0.034	6.41 ± 0.030	5.69 ± 0.032
	+	-	6.55 ± 0.022	6.29 ± 0.024	5.74 ± 0.062
	+	+	6.18 ± 0.029	6.05 ± 0.030	5.74 ± 0.044
2	+	-	6.47 ± 0.025	6.25 ± 0.026	5.61 ± 0.063
	+	+	6.15 ± 0.029	5.98 ± 0.029	5.48 ± 0.044
3	+	-	6.59 ± 0.023	6.31 ± 0.022	5.62 ± 0.024
	+	+	6.20 ± 0.037	6.00 ± 0.028	5.65 ± 0.032

FIGURE 6.2 THE EFFECTS OF ELECTRICAL STUNNING AND LOW VOLTAGE STIMULATION ON DECLINE OF pH OF THE LD



The results of the tenderness measurements of samples from the LD from either frozen or thawed samples are recorded in Table 6.5. It can be seen that the mean 'Force Score' values from stimulated carcases were lower than those from non stimulated carcases but this difference was only significant when the samples were cooked while still frozen. It will also be noted that samples cooked from thawed meat generally had lower 'Force Score' values as compared to those cooked from frozen meat.

The effects of low voltage and high voltage electrical stimulation on the decline of pH in five different lines of lambs are recorded in Table 6.6 and Figure 6.3. In two of these lines (Line No 4 and Line No 5), the first pH measurements were obtained approximately half an hour after stunning and the high voltage stimulation was therefore delayed for nearly one hour after stunning. It will be noted that in these lines, there were only small differences between mean pH values at two hours post mortem of the groups subjected to high voltage stimulation as compared to the groups treated by low voltage stimulation only. High voltage electrical stimulation had a relative greater effect when the carcases were subjected to this treatment within 30 minutes of slaughter (Lines 6,7 and 8).

The effect of low voltage stimulation appeared to vary considerably both between lines and within lines. It will be noted in Table 6.6 that in two lines, the mean pH values of groups subjected to low voltage stimulation only, declined to below 6.0 within two hours of slaughter whereas the mean pH of a similar group from another line was 6.64. It is also apparent from Figure 6.3 that the standard errors of the means of those groups subjected to low voltage stimulation only, were greater as compared to all other groups.

The results of two-way analysis of variance tests on the data obtained one and two hours after slaughter have been recorded in Table 6.7. It will be noted that low voltage stimulation had a relative greater effect in the two lines where high voltage stimulation was delayed. On the other hand, high voltage stimulation had the greatest effect in the three lines where this treatment was performed within 30 minutes of slaughter.

The ultimate pH of the LD was also measured in these studies and it can be

TABLE 6.5 'FORCE SCORE' VALUES OF THE LD FROM STIMULATED AND NON-STIMULATED CARCASES

(12 animals per group)

		Cooked from frozen samples	Cooked from thawed samples
Stimulated Carcases	Mean ± S.E.	35.1 ± 2.19	34.3 ± 2.38
	Range	22.0 - 52.5	21.3 - 46.0
Non-stimulated Carcases	Mean ± S.E.	44.2 ± 1.68	38.8 ± 2.54
	Range	33.0 - 53.0	22.7 - 54.5
Significance of differences between means of groups		$P < 0.01$	$P > 0.10$

TABLE 6.6 THE EFFECT OF THE LOW VOLTAGE AND HIGH VOLTAGE STIMULATION ON pH DECLINE OF THE LD IN LAMBS

(Mean values of 12 animals per group)

Line No.	Low voltage stimulation	High voltage stimulation	Delay between stunning and high voltage stimulation (m)	Time from stunning (h) 0.5	1	2	24
4	+	+	50 - 55	6.67	-	6.24	5.28
	-	+		6.77	-	6.12	5.28
	+	-	n.a.	6.27	-	6.20	5.38
	-	-		6.84	-	6.56	5.31
5	+	+	41 - 45	6.17	5.94	5.77	5.51
	-	+		6.90	6.23	5.99	5.41
	+	-	n.a.	6.16	5.94	5.86	5.37
	-	-		6.92	6.69	6.46	5.46
6	+	+	25 - 30	-	5.81	5.72	5.42
	-	+		-	5.85	5.73	5.39
	+	-	n.a.	-	6.07	5.94	5.29
	-	-		-	6.77	6.73	5.33
7	+	+	20 - 25	-	5.93	5.78	5.40
	-	+		-	5.92	5.78	5.46
	+	-	n.a.	-	6.73	6.64	5.41
	-	-		-	6.87	6.84	5.41
8	+	+	22 - 26	-	5.94	5.77	5.56
	-	+		-	5.98	5.81	5.56
	+	-	n.a.	-	6.39	6.33	5.54
	-	-		-	6.90	6.79	5.57

FIGURE 6.3 THE EFFECT OF LOW VOLTAGE AND HIGH VOLTAGE STIMULATION ON pH DECLINE OF THE LD IN LAMBS

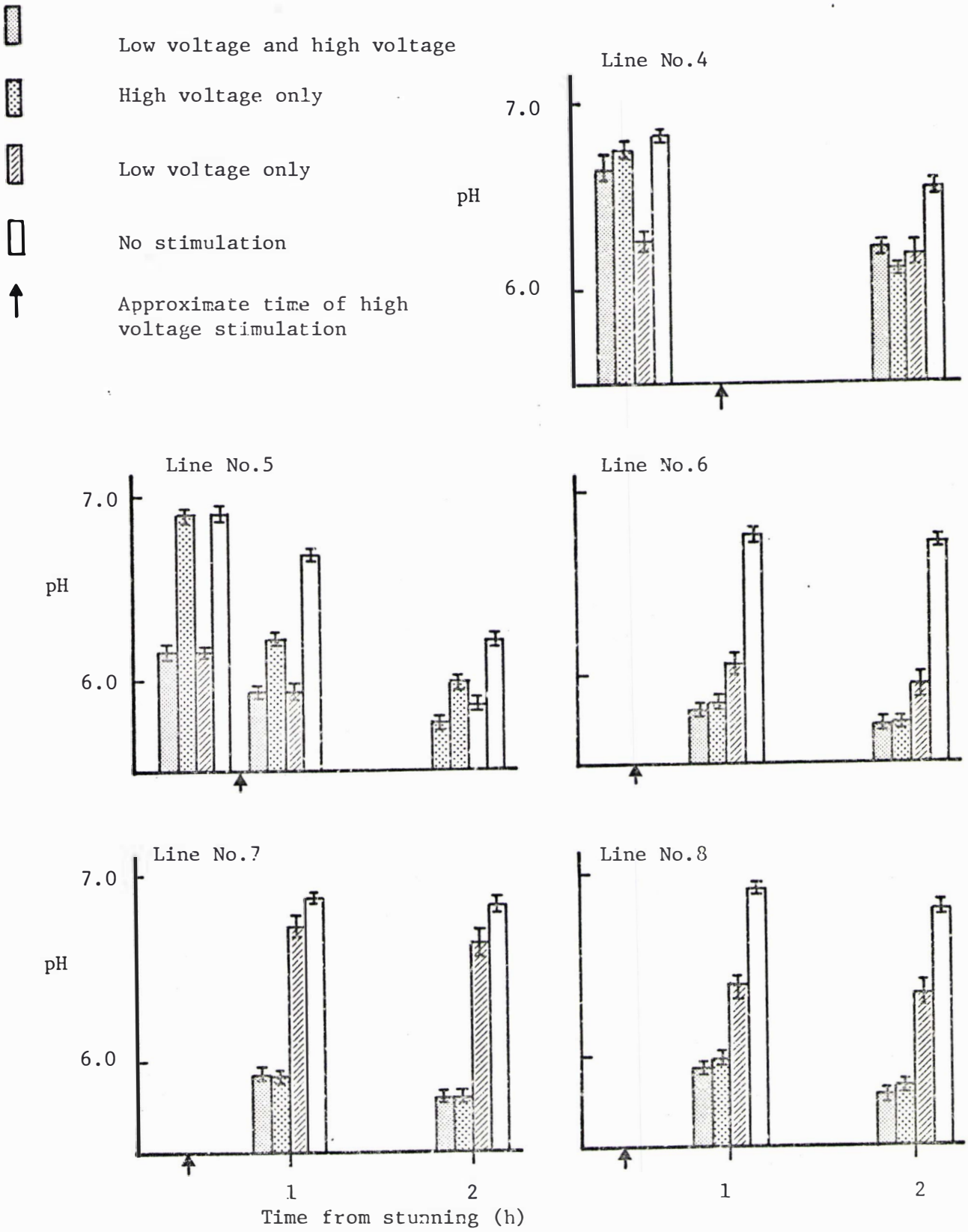


TABLE 6.7 SUMMARY OF TWO-WAY ANALYSIS OF VARIANCE OF THE EFFECTS ON pH DECLINE OF LOW VOLTAGE AND HIGH VOLTAGE STIMULATION IN THE LD OF LAMBS

(Variance ratios and their significance levels)

	TIME FROM STUNNING					
	1 hour			2 hours		
Line No.	Low voltage effect	High voltage effect	inter-action	Low voltage effect	High voltage effect	inter-action
4				6.71	19.21	26.56
5	209.26	41.38	38.76	197.57	91.03	43.03
6	62.11	161.87	49.79	85.65	208.17	84.20
7	1.52(n.s.)	504.25	2.42(n.s.)	7.85	586.54	9.06
8	52.81	328.62	369.05	35.76	337.69	25.06

n.s. = $P < 0.05$
All other groups: $P < 0.01$

seen in Table 6.6 that these values were not affected by method of slaughter or subsequent stimulation.

DISCUSSION

Muscular activity and pH decline during slaughter

It was not possible in this study to compare all four methods of stunning and slaughter at the same time. However, it would appear that 'head-to-leg' stunning increases the rate of post mortem glycolysis as compared to other methods but this method of stunning had no significant effect on ultimate pH values.

Animals slaughtered by the two different methods of electrical stunning appeared to react similarly, although those stunned by the 'head-only' method seemed to exhibit slightly more leg and body movements after the initial phase of tetany. However, as expected the 'head-to-leg' stunned animals all had an immediate cessation of normal cardiac activity at the time of stunning, whereas those stunned by the 'head-only' method had a detectable heart beat until after commencement of bleeding. Thus the increased duration of a functional circulating system in the lambs stunned by the 'head-only' method may remove some of the lactic acid formed during the initial post stunning glycolysis. Such a removal of lactic acid would however only be of a very short duration as the delay between stunning and exsanguination was only five to ten seconds. Furthermore if lactic acid was removed to any appreciable extent from the muscles of animals stunned by the 'head-only' method, this should have resulted in higher ultimate pH values in these groups as compared to the groups stunned by the 'head-to-leg' method but such differences were not observed in any of the studies.

The lower pH values in the LD of animals shortly after slaughter by a 'head-to-leg' method are more likely to be caused by an increase in the rate of glycolysis rather than decreased removal of lactic acid from the muscles. It has been shown by Devine *et al.* (1979) that increases in glycolysis can be produced both indirectly through intact nervous pathways and by direct stimulation of muscles. Both methods of electrical stunning investigated would presumably induce a degree of both direct and indirect stimulation of muscles. However it would seem likely that there would be more direct stimulation of muscles when the 'head-to-leg' method is used

as the distance between the electrodes applied to the animal's body is greater. This effect is likely to be exacerbated under the commercial conditions studied where the animals were sprayed with water and restrained in a stainless steel conveyor which also acted as a body electrode. Thus the direct stimulation of muscles by the 'head-to-leg' method is probably the major cause of the increased rate of post mortem glycolysis in animals stunned by this method. This hypothesis is also supported by further unpublished studies by the author which indicate that muscles from shorn and very damp lambs have significantly lower pH values shortly after slaughter than muscles from woolly, dry lambs stunned by the 'head-to-leg method. This finding could be explained by the reduced conductivity of the fleeces of the latter group and therefore less direct stimulation of muscle.

The muscles of animals stunned by a captive bolt or slaughtered without prior stunning are only subjected to indirect stimulation through nervous pathways. It is therefore not surprising that the pH values, shortly after slaughter, were always significantly higher than the corresponding groups stunned by the 'head-to-leg' method. Previous reports by Chrystall *et al.* (1981b) indicated that there were no differences in glycolysis post mortem between non stunned lambs and electrically stunned lambs but these studies were carried out using 'head-only' or 'head-to-back' methods and presumably with lambs with less wet fleeces than those routinely slaughtered at a meat works. Such differences may account for apparent discrepancies between their findings and those reported here.

Extent and effect of pH decline during slaughter

The present studies indicate that 'head-to-leg' stunning increases the pH decline by the order of 0.1 - 0.2 pH unit. It would also appear that this effect occurs immediately following stunning and that there is no increase in the rate of glycolysis during the remainder of the pre rigor period. However, the decline in pH associated with stunning by the 'head-to-leg' method is sufficient to reduce the time during which carcase muscles are susceptible to cold shortening by two to three hours. This phenomenon could be of value in smaller abattoirs where electrical stimulation is usually not available and carcases have to be held in chillers for several hours before being frozen.

Low voltage stimulation was introduced by the meat industry in New Zealand

to immobilise animals during slaughter and subjective assessment indicated that this was achieved in most cases. Lambs subjected to low voltage stimulation usually exhibited no leg and body movements during exsanguination. Most animals appeared completely immobilised and only continuous slight tremors of carcase muscles were noted. The present studies also indicated that this procedure is associated with an immediate decline in pH of the LD which is apparently additive to the pH decline associated with 'head-to-leg' stunning.

The effect of electrical stimulation on pH decline of lamb muscles has previously been investigated in New Zealand. Carse (1973) reported that when carcases were subjected to square wave pulses (2-13.5 ms duration) at 0-250 volts, delivered at rates from 3-17.5 per second, only variations in voltage appeared to affect the rate of pH decline. In some further studies by Chrystall and Hagyard (1976) the carcases were subjected to much higher voltages (3600 volts) resulting in both an immediate decline of pH as well as an increased rate of decline during the remainder of the pre rigor period. It was subsequently suggested by Hagyard *et al.* (1980) that an alternating waveform, of 1130 volts, pulsed at 14.3 pulses/second, 10 ms duration pulses, applied for 90 seconds within 30 minutes of slaughter represents optimal stimulation condition.

The extent of the pH decline associated with low voltage stimulation varied considerably both between lines (mean decline from 0.32 to 0.75 pH units) as well as within lines as indicated by the wide confidence limits in the groups subjected to low voltage stimulation only (see Figure 6.3). These investigations were carried out at two different meat works shortly after the equipment had been installed and a variety of problems related to design of equipment were encountered. These included consistency of electrical output and difficulties in maintaining adequate contact between the legs of the animals and the hooks from which they were suspended. In spite of these problems,it would appear that the immediate pH decline following the low voltage stimulation was of the same magnitude as that reported in relation to high voltage stimulation of lambs (Davey and Chrystall, 1980). However, this effect appeared to be of short duration as the differences between stimulated and non stimulated groups were of the same magnitude one and two hours after slaughter. This is in contrast to high voltage stimulation which has been reported to cause an increase in the rate of glycolysis during the remainder of the pre rigor period (Davey and Chrystall, 1980).

The most appropriate method for assessing the effect of low voltage stimulation on meat quality is an objective measurement of tenderness of meat subjected to the treatment compared to that from unstimulated carcases. It has been shown that there is a good correlation between 'Force Score' values and the tenderness values obtained by a trained panel of tasters (McCrae *et al.*, 1971; Davey and Gilbert, 1975) and the following relationship has been suggested by Davey and Winger (1979).

'Force Score' values	Meat tenderness
< 25	very tender
25-40	tender
> 40	undesirably tough

The relative high 'Force Score' values recorded in this study of both stimulated and unstimulated carcases are probably associated with the rapid method of freezing used in the investigation. It has been suggested by Chrystall (1980) that acceptable tenderness following electrical stimulation can only be achieved if the temperature of deep muscles is not reduced to below $-4^{o}C$ within 14 hours of slaughter. Although meat temperatures were not measured in this study, it was estimated from the environmental temperatures that the deep muscle temperatures were below $-4^{o}C$ within eight to ten hours of slaughter.

The highly significant difference in mean 'Force Score' values between stimulated and non stimulated carcases indicates that low voltage stimulation can improve the tenderness of lamb. However, it is suggested that more studies would be required for a complete assessment of this technique and in this context, it would be particularly valuable to attempt to reduce the variability recorded in the present studies.

Combined effects of low voltage and high voltage stimulation

Previous work has shown that electrical stimulation of carcases must take place within 30 minutes of slaughter to have maximum effect on muscle glycolysis (Chrystall *et al.* 1980; Davey and Chrystall, 1980). This is supported by the findings in this study which indicated that the pH decline in carcases subjected to high voltage stimulation later than 30 minutes post mortem was considerably less than the pH decline of carcases stimulated earlier by high voltage. This difference is probably related to a decrease in the effect of indirect nervous

stimulation of muscles since it has been suggested that when stimulation is delayed for more than 30 minutes post mortem, the neuromuscular junction can no longer be excited to induce muscle depolarisation (Swatland, 1977; Devine *et al.*, 1979; Chrystall *et al.*, 1980).

The present studies also indicate that when high voltage stimulation is carried out within 30 minutes of slaughter, the earlier low voltage stimulation has very little effect on the mean pH values one and two hours after slaughter. It would thus appear that at meat works using high voltage stimulation in accordance with the guidelines described by Davey and Chrystall (1980) the addition of a period of low voltage stimulation will make little difference to the time during which carcases are susceptible to cold shortening. The reasons for the effects of the two methods of stimulation to be non-additive in the present studies are not clear but it has previously been suggested that the decline in pH associated with stimulation is reduced at lower pH values and approaches zero when the muscle pH has reached approximately 6.2 (Davey and Winger, 1979). In view of the present widespread use of the combination of low voltage and high voltage stimulation in New Zealand,it would appear to be important that these problems are investigated further.

The present studies indicate that the ultimate pH values of the LD of lambs are not affected by methods of slaughter and electrical stimulation. This is not surprising because cardiac induced circulatory failure occurs either at, or very near, the time when muscular activity is induced by the various methods of slaughter. Thus lactic acid can no longer be removed from the muscle and the ultimate pH value is therefore only dependent on the amount of glycogen present in the muscle at the time of slaughter.

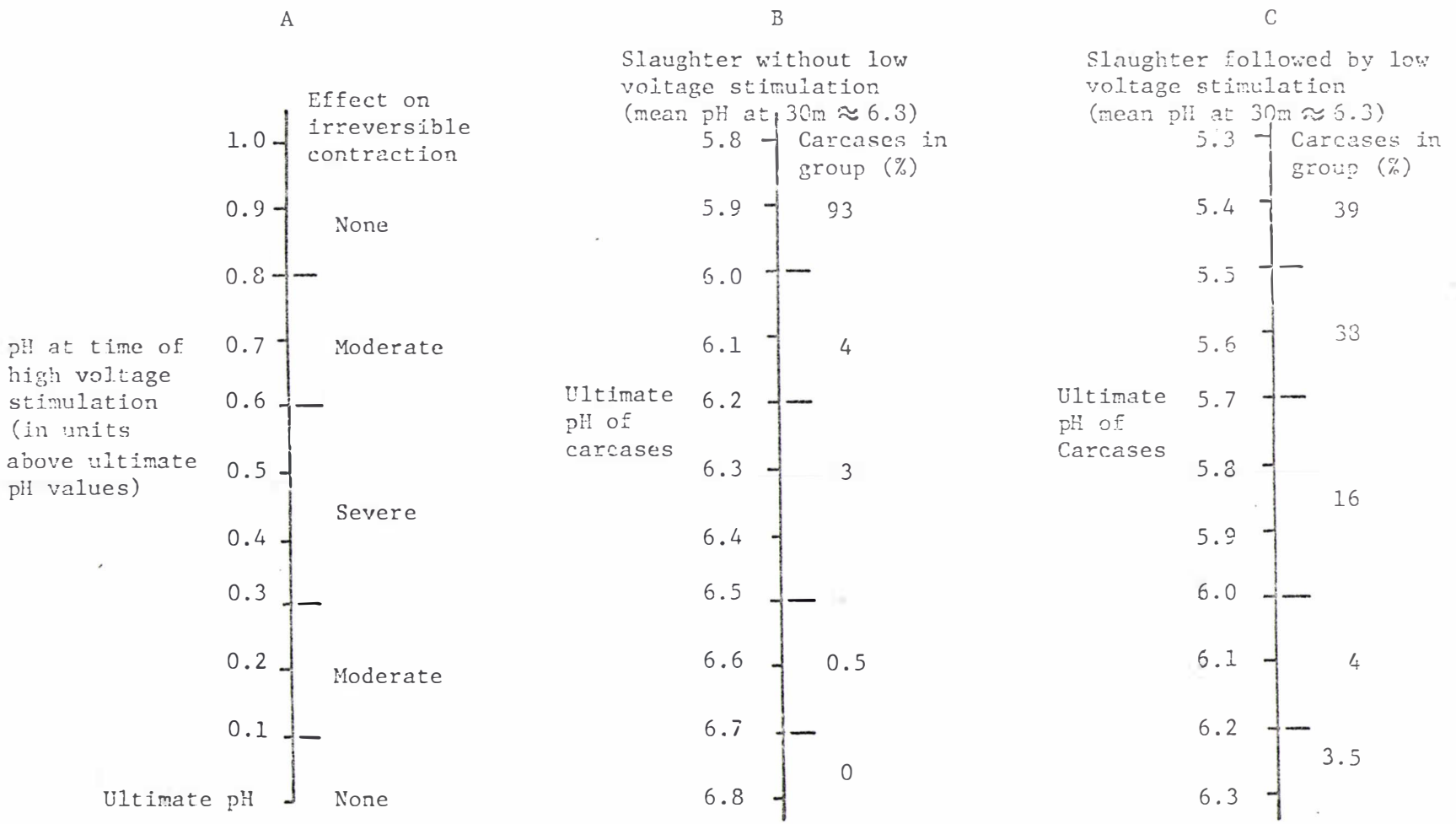
Although the methods of slaughter and associated electrical stimulation have no effect on ultimate pH, recent studies indicate that the effectiveness of electrical stimulation in relation to tenderness of muscles may be affected by the ultimate pH value attained by the muscle. It was reported by Dutson *et al.* (1981) that electrical stimulation of beef with high ultimate pH values (above 6.5) had no effect on tenderness and Chrystall *et al.* (1982) reported that electrical stimulation of "exercise-stressed" lambs (mean ultimate pH above 6.3) decreased tenderness as compared to non stimulated "exercise-stressed" lambs. It was suggested by the latter

authors that muscles with very high ultimate pH values (above 6.5) enter rigor mortis sufficiently early to ensure that they are insensitive to low temperatures and the tenderness is therefore not affected by early chilling. The toughness, associated with stimulation of muscles with pH values in the range of 6.0 - 6.5, was attributed to the rapidity with which they entered rigor mortis and it was suggested that no relaxation occurred in these muscles after completion of the stimulation period. Such muscles would therefore remain in a partially contracted state (irreversible contraction) and hence be less tender. It was further suggested that meat from animals having very high ultimate pH values will always be tender regardless of methods of slaughter and stimulation because the muscles from such animals are insensible to cold shortening within a very short period after slaughter. These findings may explain the reasons for some of the variability which has previously been associated with tenderness of meat from electrically stimulated lamb carcases (Hagyard, 1980) It is also interesting to speculate on the effect the combination of low voltage and high voltage stimulation may have on irreversible contraction of muscles with different ultimate pH values.

According to the hypothesis put forward by Chrystall *et al.* (1982), muscles exposed to high voltage stimulation at a time when the muscle pH is in the range of 0.3 - 0.6 pH units above its ultimate value, would be subjected to a severe degree of irreversible contraction. This concept is depicted in Figure 6.4.A, which also indicates that there are probably some muscles with pH values on either side of this range, which may also be affected, but to a lesser extent.

Prior to the 1982/83 killing season, virtually all lambs were slaughtered without being subjected to low voltage stimulation but 90 percent of export lambs were subjected to high voltage stimulation (Chrystall, 1980). This treatment usually takes place about 30 minutes after slaughter at which time the pH of the muscles would have declined to approximately 6.8. It can therefore be estimated that muscles with ultimate pH values in the range 6.2 - 6.5 are likely to be subjected to severe irreversible contraction. If the data on the distribution of ultimate pH values reported in Chapter Three are used, it can be seen in Figure 6.4.B that three percent of carcases would have been subjected to such severe irreversible contraction and would theoretically yield very tough meat. It can also be seen that a further 4.5 percent of carcases may be

FIGURE 6.4 THE EFFECT OF LOW VOLTAGE STIMULATION AND ULTIMATE pH ON IRREVERSIBLE CONTRACTION DURING HIGH VOLTAGE STIMULATION



affected to a lesser extent by irreversible contraction. These figures would appear to be compatible with the previous reports on tenderness of export lambs (Hagyard, 1980) and could thus explain the reasons for a small but significant proportion of lambs being less tender than desirable.

During the 1982-83 season many meat works installed low voltage stimulation to immobilise carcases during exsanguination. The present studies indicate that such treatment may result in an almost immediate pH decline of approximately 0.5 pH unit. The great majority of these lambs are also subjected to high voltage stimulation within 30 minutes of slaughter and at this time, the pH would have declined to approximately 6.3. Under these conditions, muscles with ultimate pH values in the range 5.7 - 6.0 would theoretically be exposed to severe irreversible contraction and Figure 6.4.C indicates that 16 percent of all lamb carcases might be in this category while a further 42 percent might be affected to some extent.

Although the evidence presented in the foregoing discussion is of a somewhat speculative nature, it does highlight the serious implications associated with the implementation of new techniques in the meat industry without first having evaluated all the effects of such techniques on meat quality. If the theory of electrical stimulation inducing irreversible contraction during the latter part of the pre rigor period is correct, the associated effects on meat tenderness could be a far more serious marketing problem than those related to the cold shortening of some 20 years ago. Further research into this area of meat science is therefore urgently required.

CONCLUSIONS

1. Stunning by electrical methods causes tonic contraction of skeletal muscles lasting for approximately 25 seconds. This is followed by a two to four minute period of clonic spasms characterised by intermittent leg and body movements.

2. There is a significant increase in the rate of pH decline following stunning by a 'head-to-leg' electrical method as compared to other methods of slaughter.

3. Low voltage stimulation, at the time of slaughter, is associated with an immediate decline in pH of the LD (Approximately 0.5 units) but there

is no increase in the rate of pH decline during the remainder of the pre rigor period.

4. There are no significant differences between pH values, two hours post mortem, of groups of lamb carcases subjected to low voltage and high voltage stimulation as compared to groups subjected to high voltage stimulation only.

5. It is concluded that the combined effects of low voltage and high voltage stimulation can cause irreversible contraction and associated toughness in a significant proportion of carcases.

6. The method of slaughter has no effect on ultimate pH.

CHAPTER SEVEN

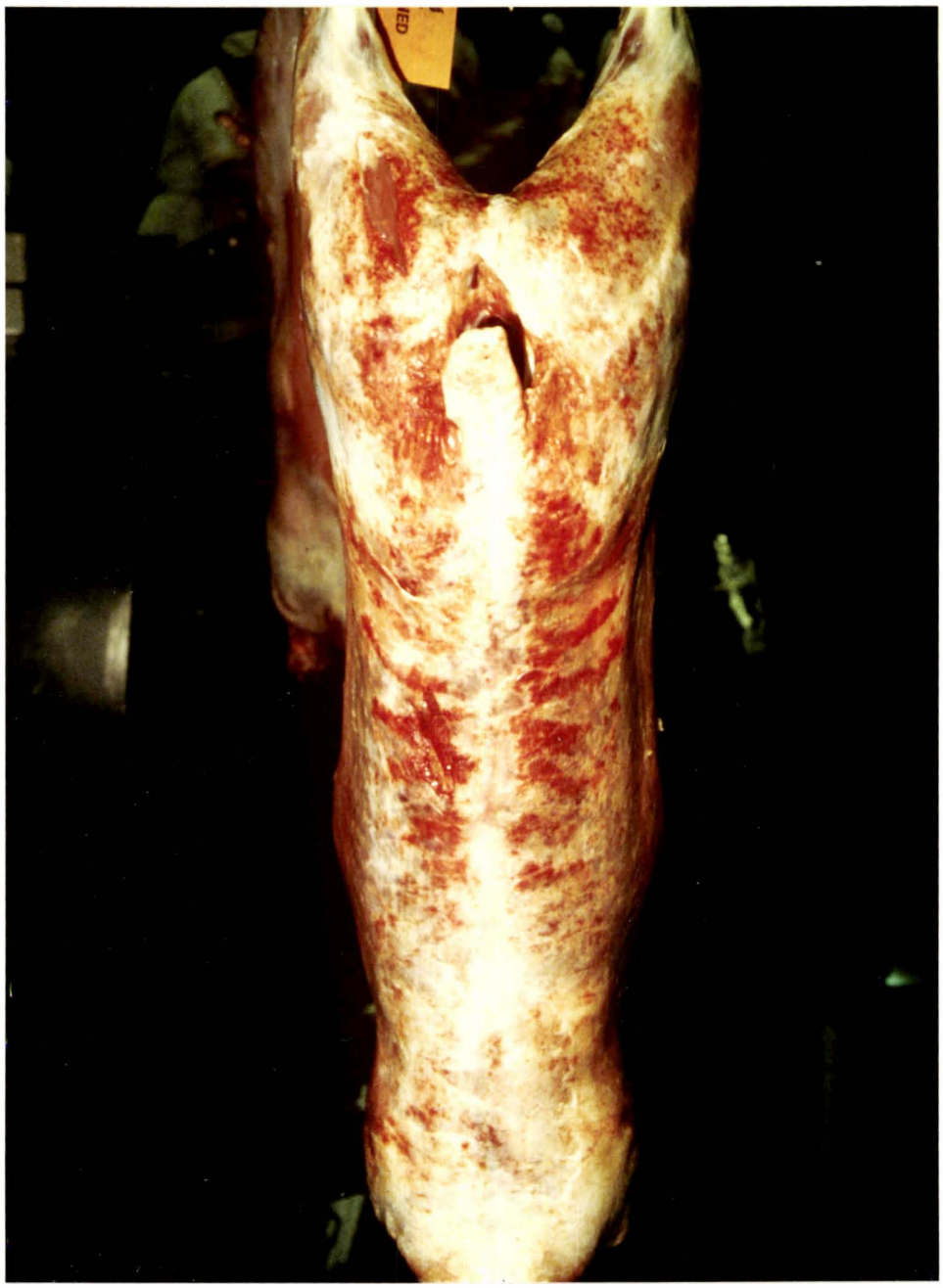
OBSERVATIONAL STUDIES OF HAEMORRHAGES ASSOCIATED WITH SLAUGHTER OF LAMBS

INTRODUCTION

Two different haemorrhagic syndromes appear to be associated with stunning by electrical methods. One of these syndromes is usually described as exhibiting oval shaped ecchymotic haemorrhages from a few millimetres to a few centimetres in size frequently occurring in the carcase muscles of animals and to a lesser extent in organs such as liver, spleen, gallbladder and lungs (Ducksbury and Anthony, 1929; Tweed *et al.*, 1931; Jorgensen, 1959; Charles, 1960). These lesions have for many years been referred to as "blood splash" and this term will be used in this thesis. The other haemorrhagic syndrome associated with electrical stunning occurs in the subcutaneous fat of lambs and has been described as being characterised by the presence of multiple discrete petechial haemorrhages with a diameter of 0.5 to 3.0 mm (Thornton *et al.*, 1979). The distribution of these haemorrhages varies considerably but in extreme cases the major part of the subcutaneous carcase fat may be involved (Plate 7.1). When these lesions were first reported, they were referred to as "speckling" of the carcase fat (Anon, 1978) and this term has been retained in the present studies.

The occurrence of blood splash in carcase muscles has been extensively studied and earlier investigations indicated that different stunning procedures were associated with both the occurrence of the lesion and an increase in arterial blood pressure (Tweed *et al.*, 1931; Clark and Tweed, 1932; Roos and Koopmans, 1934). Such an increase in blood pressure was thought to cause rupture of capillaries and hence blood splash but these earlier investigations also indicated that the increased blood pressure was not the sole cause of the lesion although the prevalence of blood splash was apparently affected by different methods of stunning (Anthony, 1932). Several studies of pigs have been carried out in Denmark (Jorgensen, 1959; Mandrup, 1964) and it was suggested by workers in that country that blood splash was not caused by rupture of the capillaries but was a result of diapedesis which may be exacerbated by increased blood pressure. Later work by Shaw *et al.* (1971) indicated that changes in blood pressure may have some affect on the occurrence of

PLATE 7.1 LAMB CARCASE AFFECTED BY SPECKLING



blood splash in rats and these workers concluded that an increased mean capillary blood pressure was an important factor in the aetiology of the condition. This view was supported by van der Wal (1978) who suggested that increased levels of catecholamines during stunning of pigs resulted in an increase in blood pressure and subsequent rupture of capillaries.

Blood splash in carcases of lambs has also been investigated in New Zealand and Kirton *et al.* (1978) found that a delay between stunning and bleeding increased the prevalence of the defect. It has also been shown that both captive bolt and percussion stunning of lambs cause less blood splash than electrical stunning (Blackmore,1979; Kirton *et al.*, 1981a) and Kirton *et al.* (1981b) found that electrical stunning by 'head-only' methods increased the rate of blood splash as compared to the electrical 'head-to-back' method.

It would appear that fewer studies have been carried out in relation to the aetiology of speckling as compared to the work reported on the aetiology of blood splash. When speckling was first reported in New Zealand, it was suggested that it was caused by animal movements in the restraining conveyor during stunning (Anon,1978). Thornton *et al.* (1979) reported that the causal factor was electrical stunning and that the method of restraint during stunning or subsequent method of exsanguination had no effect on the prevalence of speckling. It was suggested by Petersen and Wright (1979) that the primary factor related to speckling was associated with electrical stunning by a 'head-to-body' method and that the prevalence could be reduced by reducing the distance between the head electrodes and the body electrode. More recently Gilbert and Devine (1982) suggested that speckling is caused by muscle movements during stunning resulting in rupture of blood vessels in fat and connective tissue.

In view of the uncertainty of the cause of speckling and the lack of information about predisposing factors which may contribute to the occurrence or severity of the condition in lambs, some preliminary studies were carried out to investigate this problem and the results of these studies are reported in this chapter.

MATERIALS AND METHODS

Part 1:Effect of stunning on the prevalence of haemorrhages

This part of the study was carried out at two meat export works where different methods of electrical stunning were being introduced and it was therefore possible to make a comparative assessment of their effect on haemorrhages of carcases. At one of the meat works, a comparison was made between 50 lambs stunned by the electrical 'head-only' method and 50 lambs stunned by the electrical 'head-to-back' method. All lambs originated from the same farm and were slaughtered in four groups of 25 animals, using alternate methods of stunning. At another meat works, the prevalence of haemorrhages was compared in the same manner, between 50 lambs stunned by electrical 'head-only' method and 50 lambs stunned by the electrical 'head-to-leg' method.

After dressing, the surfaces of the carcase muscles, hearts and gallbladders were examined for evidence of blood splash. As it had previously been observed that the majority of speckling was confined to the hind quarters of lambs, only speckling of subcutaneous fat in this area was recorded.

Part 2 : Severity of speckling and rate of post mortem glycolysis

Five lambs affected by speckling were selected for this study. Details concerning the extent and degree of speckling were recorded and temperatures and pH values of two muscles were measured at approximately 1.5 hours and 2.5 hours after slaughter of the animals. These recordings were made on each side of the carcase in the LD (lumbar part) and the *M.biceps femoris* (proximal part). At each interval, only one recording of the temperature was made for each muscle whereas two pH values were obtained (1 to 2 cm apart). The temperature was measured at a depth of 15 mm using a Koch thermometer* and pH values were measured at the same depth using a combination glass electrode attached to a Triac pH meter as described in the previous chapter. After completion of the pH recordings a plug sample was obtained from the LD of one side of the carcase for later estimation of the ultimate pH as previously described.

Part 3 : One stage prothrombin times and speckling

Blood samples were collected from 100 lambs originating from ten different farms. The samples (4.5 ml) were obtained by intracardiac puncture within

* Koch rechargeable high-low C/F Thermometer.Koch Supplies Inc.,Kansas City,USA

five to ten seconds after stunning using siliconised Venoject** tubes containing 0.5 ml of 3.8% tri-sodium citrate. Previous studies had indicated that it was not possible to obtain whole blood samples in either glass beakers or plastic cups at the time of bleeding (40-80 s after stunning) as the blood clotted almost immediately and before it could be transferred to tubes containing anticoagulant. All samples were kept on ice and transferred to the laboratory where they were centrifuged at 1400 g for 15 minutes and the plasma removed. The plasma samples were frozen and stored at -20°C for no more than four weeks. The one stage prothrombin times were estimated for each sample by the method developed by Quick (19) using human brain thromboplastin. For each batch of samples, a control test was performed on pooled human plasma from six or more healthy males (20-40 years old). The test results of the study samples were only regarded as valid when the one stage prothrombin time of the control was 15-16 seconds. All animals from which samples had been obtained were identified and assessed for speckling after dressing of the carcases. The prevalence of speckling was also recorded in a further 50-100 carcases from each line of lambs used in the study. The examination was carried out in a similar manner to that used in the first part of these studies, except that carcases were also graded according to the extent and severity of lesions on a scale from 0 to 5. This allowed the mean score of speckling for each of the ten lines in the study to be calculated.

RESULTS

There was a significant increase in the prevalence of speckling of carcase fat in lambs stunned by either the 'head-to-back' method (Table 7.1) or the 'head-to-leg' method (Table 7.2) as compared to the prevalence of speckling of lambs stunned by the 'head-only' method. On the other hand there was very little blood splash in the carcases, hearts and gallbladders of animals stunned by electrical 'head-to-body' methods, whereas both groups of lambs stunned by the 'head-only' method had a high prevalence of blood splash. These differences in prevalence rates of blood splash were highly significant in all groups in this study.

The carcases used for measurement of post mortem temperatures and pH

** Teramo Corporation, Tokyo, Japan.

TABLE 7.1 COMPARISON OF THE PREVALENCE OF HAEMORRHAGES BETWEEN 'HEAD-ONLY' AND 'HEAD-TO-BACK' ELECTRICAL STUNNING

(50 animals per group)

	No. of lambs affected			
	'Head-only' stunned	'Head-to-back' stunned	Chi Square	P
Speckling	6	19	9.01	P <0.01
Blood Splash				
Carcase	12	0	13.64	P <0.01
Heart	11	2	7.16	P <0.01
Gall Bladder	16	0	19.05	P <0.01

TABLE 7.2 COMPARISON OF THE PREVALENCE OF HAEMORRHAGES BETWEEN 'HEAD-ONLY' AND 'HEAD-TO-LEG' ELECTRICAL STUNNING

(50 animals per group)

	No. of lambs affected			
	'Head-only' stunned	'Head-to-' stunned	Chi Square	P
Speckling	3	10	4.33	P <0.05
Blood Splash				
Carcase	25	2	26.84	P <0.01
Heart	18	0	21.95	P <0.01
Gall Bladder	19	0	23.46	P <0.01

decline, all had bilateral speckling of the subcutaneous fat of the hind quarters, but the severity of lesions varied both between carcases and between sides. The temperatures of the *M.biceps femoris* and the LD, measured 1.5 hours and 2.5 hours after slaughter are recorded in Table 7.3. Measurements were not obtained for three of the carcases at the time of the first temperature recording. It can be seen that the temperatures of the muscles were very similar regardless of the extent of speckling in the subcutaneous fat overlaying the muscles. The small differences between mean temperatures were also found to be non significant.

The mean pH values of the same muscles have been recorded in Table 7.4 and it can be seen that in all cases these values are lower from the most severely affected sides as compared to the similar muscles from the least affected sides. This difference was greatest in the *M.biceps femoris* measured 1.5 hours after slaughter and the paired t-test indicated that the difference was significant at the 5% level. All other differences were non significant.

Table 7.4 also indicates that there were considerable differences between carcases. Two of these (Case No 4 and Case No 5) appeared to have a much slower rate of glycolysis as compared to the three other animals. These two carcases were also recorded as having smaller and less severe lesions as compared to the three animals with faster rates of glycolysis.

The ultimate pH values measured on plug samples from the LD are recorded in the last column of Table 7.4 and it will be noted that there were only small differences between carcases.

It can be seen in Table 7.5 that 37% of the 775 carcases included in the last part of the study were affected by speckling and 2.5% of the carcases had major lesions (graded 4 or 5). It will also be noted that there were considerable differences between lines of lambs in the degree of speckling regardless of whether this was measured by the grading system used in the study (as expressed by the mean score) or whether it was measured by the total number of carcases affected.

The one stage prothrombin time measured on individual samples from 100 lambs varied from 13 to 24 seconds with a mean of 16.6 seconds (Table 7.5). The means of the one stage prothrombin time from each line are also shown in

TABLE 7.3 MUSCLE TEMPERATURES OF LAMB CARCASES AFFECTED BY SPECKLING

Case No.	Description of Speckling	1.5 h post mortem				2.5 h post mortem			
		Most severely affected side		Least affected side		Most severely affected side		Least affected side	
		BF	LD	BF	LD	BF	LD	BF	LD
1	very severe	30.0	32.0	30.0	31.0	25.0	25.5	26.0	25.0
2	medium	32.0	34.0	30.0	33.5	27.5	29.0	26.5	29.5
3	medium	-	-	-	-	27.0	25.5	25.5	26.0
4	medium-light	-	-	-	-	25.5	26.5	26.0	27.0
5	very light	-	-	-	-	27.0	26.0	25.5	26.0
Mean		31.0	33.0	30.0	32.3	26.4	26.5	25.9	26.7
± S.E.		±0.07	±0.07	-	±0.07	±0.04	±0.06	±0.02	±0.07

BF = *M. biceps femoris*

TABLE 7.4 pH VALUES OF MUSCLES OF LAMB CARCASES AFFECTED BY SPECKLING (MEAN VALUES OF TWO READINGS PER SITE)

Case No.	Description of Speckling	1.5 h post mortem: Most severely affected side BF	1.5 h post mortem: Most severely affected side LD	1.5 h post mortem: Least affected side BF	1.5 h post mortem: Least affected side LD	2.5 h post mortem: Most severely affected side BF	2.5 h post mortem: Most severely affected side LD	2.5 h post mortem: Least affected side BF	2.5 h post mortem: Least affected side LD	24 h post mortem LD*
1	very severe	6.13	5.99	6.19	6.00	5.93	5.85	5.80	5.85	5.68
2	medium	6.19	6.08	6.30	6.13	5.91	5.92	6.03	5.86	5.53
3	medium	6.07	6.11	6.07	6.07	5.91	5.86	6.03	5.91	5.72
4	medium-light	6.26	6.34	6.34	6.50	6.22	6.10	6.11	6.45	5.58
5	very light	6.31	6.24	6.39	6.25	6.17	6.19	6.22	6.11	5.61
Mean		6.19	6.15	6.26	6.19	6.03	5.98	6.04	6.04	
± S.E.		±0.02	±0.06	±0.05	±0.08	±0.06	±0.06	±0.06	±0.10	

BF = *M.biceps femoris*

* One reading only

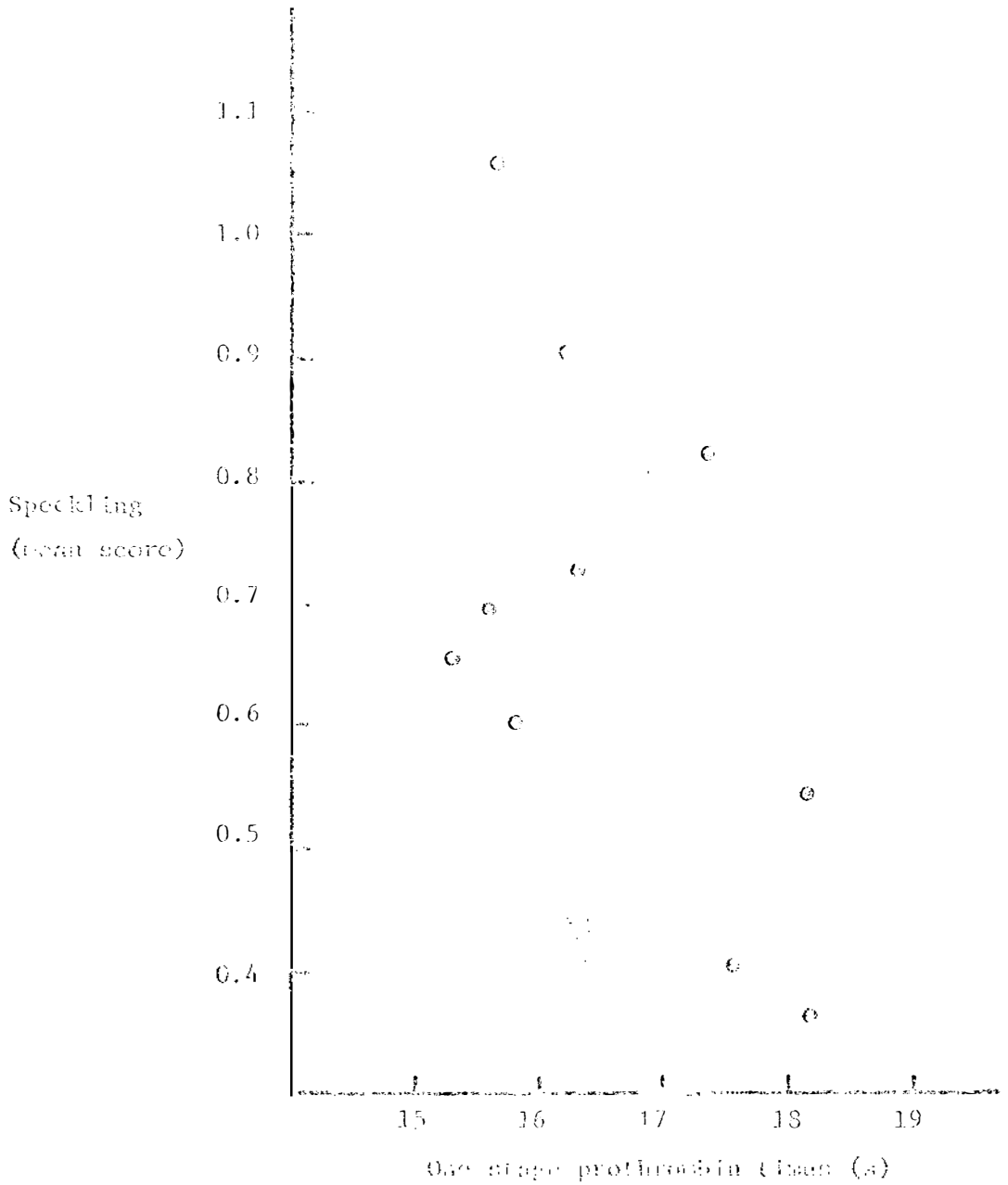
TABLE 7.5 ASSOCIATION BETWEEN DEGREE OF SPECKLING AND ONE STAGE PROTHROMBIN TIMES MEASURED IN TEN LINES OF LAMBS

Line Code	Speckling				One stage prothrombin times	
	No. Exam.	Mean Score	Percentage carcases affected with Major Lesions (graded 4 & 5)	Any Lesions (graded 1-5)	No. tested	Mean ±S.E. (sec)
1	50	0.90	2.00	46.00	5	16.2 ± 0.66
2	50	0.40	4.00	18.00	5	17.6 ± 0.54
3	75	0.69	5.33	32.00	11	15.6 ± 0.30
4	100	1.05	8.00	48.00	10	15.6 ± 0.38
5	50	0.82	2.00	50.00	11	17.3 ± 0.67
6	100	0.54	0	36.00	10	18.2 ± 0.73
7	75	0.36	0	26.67	12	18.2 ± 0.65
8	75	0.60	0	38.67	12	15.8 ± 0.54
9	100	0.72	0	42.00	11	16.3 ± 0.78
10	100	0.65	3.00	36.00	13	15.3 ± 0.33
	775	0.68	2.45	37.68	100	16.6
r*				-0.60		
r**				-0.11		

r* = correlation coefficient between mean scores of speckling and mean one stage prothrombin times (n = 10)

r** = correlation coefficient between grades of speckling and one stage prothrombin times of individual animals (n = 100)

FIGURE 7.1 [illegible]


1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
Speckling
(mean score)
15
16
17
18
19
Gas stage profundia (mm) (a)

the table and analysis of variances indicates that there are significant differences between these means. Further examination of the results indicate that such differences are not related to day of sampling (five days) or day of testing (three days) and it can thus be assumed that there is a true variation in one stage prothrombin times between animals from different farms.

The correlation coefficient between mean scores of speckling and mean one stage prothrombin time is -0.69 (Table 7.5 and Figure 7.1). Although this is a rather high value, statistical analysis indicates that the observed inverse correlation between the two means is not significantly different from zero. There is also an inverse correlation of smaller magnitude (-0.11) between grades of speckling and one stage prothrombin times of individual animals.

DISCUSSION

It has been difficult in the past to carry out investigations at the meat works to compare the effects of different methods of stunning on the prevalence of haemorrhages in carcases and organs using lambs from the same line. The first part of the studies presented in this chapter were carried out at a time when many meat works were changing from 'head-to-body' methods to 'head-only' methods of electrical stunning and it was thus possible to compare the effects of the two methods during commercial operations.

It has previously been shown that 'head-to-back' stunning decreases the prevalence of blood splash as compared to stunning by the 'head-only' method (Kirton *et al.*, 1981b). The present findings support this view and also indicate that blood splash is of little importance when the 'head-to-leg' method of stunning is used. It would also appear that during the years when most meat works were using electrical 'head-to-body' methods, there were few reports of problems associated with blood splash of carcase muscles. It would thus appear that blood splash is a problem mainly associated with the electrical stunning of lambs without the use of a third body electrode.

The reduction in speckling associated with the 'head-only' method as compared to other methods of electrical stunning, is in agreement with

some previous findings (Petersen and Wright, 1979; Blackmore and Petersen, 1981). However, more recently Gilbert and Devine (1980) reported that 'head to-leg' stunning resulted in less speckling, as compared to 'head-only' and 'head-to-back' stunning but these workers measured the degree of speckling after incision of fat and connective tissue. In the present studies, speckling was measured without incisions of tissues and it is believed that this method reflected more closely the commercial importance of the condition.

The latter part of the present studies was carried out during the early part of the 1981/82 killing season at a meat works using the electrical 'head-to-leg' method of stunning. The results indicated that 37% of the carcases had some degree of speckling and 2.5% had major lesions (graded 4 or 5). A similar study was carried out at the same meat works during the early part of the 1977/78 season when the electrical 'head-to-back' method of stunning was used (Petersen and Wright, 1979). The results of this study indicated that 29% of 3058 carcases examined were affected by speckling and 2.6% had major lesions. As both of these studies were carried out at the same time of the year, the results are comparable and indicate that the prevalence of speckling is similar in animals stunned by 'head-to-leg' methods as compared to animals stunned by 'head-to-back' method. It may thus be concluded that speckling is a problem associated mainly with electrical 'head-to-body' methods of stunning, whereas blood splash is usually associated with 'head-only' methods of stunning.

The increased rate of pH decline in muscles underlaying subcutaneous fat affected by severe speckling is an interesting finding. It was reported in Chapter Six that 'head-to-leg' stunning causes an increased rate of pH decline of the LD as compared to 'head-only' stunning. It was further suggested that the apparent increase in post mortem glycolysis associated with 'head-to-leg' stunning is caused by direct stimulation of the muscles when a third electrode is placed on the body of the animal. It would thus appear that there may be a direct or indirect association between the degree of exposure to electrical current and the appearance of speckling in subcutaneous fat.

At the meat works the lambs are placed in stainless steel conveyers while being stunned and the pelts are usually sprayed with water at the points of contact of the stunning electrodes. The amount of water used differs not only between the works but also within works depending on operator

preference and it would also appear that in some cases, the amount of water sprayed onto the animals is so liberal that the sides of the conveyor may act as auxiliary body electrodes. It is therefore likely that there is considerable variability between animals with respect to conductivity of the fleece and skin and this may explain some of the variability of speckling found in these studies.

The prevalence of blood splash also appears to be subject to considerable variation between different lines of lambs and with season of the year. It is considered that if such differences were associated with variation in diet, both temporal and geographical variations in prevalence of blood splash and speckling could be partially explained. Recently blood splash has been linked to consumption of plants such as sweet clovers containing coumarin and a statistical association was established between extended one stage prothrombin times and the occurrence of blood splash in lambs (Restall, 1981). The one stage prothrombin time measures the extrinsic system of blood coagulation (Coles, 1974) and any increase above normal values indicates a deficiency of one or more of the factors associated with this system, i.e. factors I, II (prothrombin), V, VII and X. Of these prothrombin has attracted most interest, as a deficiency of this factor can occur as a result of inadequate absorption of vitamin K or from an impaired synthesis of prothrombin by the liver. Such impaired synthesis occurs after ingestion of anticoagulant substances such as dicoumarol which can be formed from coumarin in sweet clovers (Clarke and Clarke, 1975). The haemorrhagic disorders associated with exposure to anticoagulants are characterised by increased capillary permeability and increased blood clotting times (Hatch, 1977). The cause of the change in capillaries is not known but it would be logical to consider such a condition to predispose to haemorrhages in carcases.

The one stage prothrombin times recorded in this study are in close agreement with those previously reported in sheep. Tillman *et al.* (1981) recorded a mean one stage prothrombin time of 19.3 seconds (range 16.4 to 23.9) in ten mature wethers using rabbit brain thromboplastin and it has previously been shown that the one stage prothrombin times in sheep can be expected to be greater (by approximately 16%) when using rabbit rather than human brain thromboplastin (Didisheim *et al.*, 1959).

Although there were significant differences in the mean one stage

prothrombin times between lines, such differences did not appear to be related to speckling. These results indicate that slightly extended one stage prothrombin times do not predispose to speckling. On the contrary, it would appear from the comparison of group means that, within the range studied, extended one stage prothrombin times may be inversely correlated with speckling. However, such a hypothesis is not supported by comparisons of the 100 individual measurements and there would thus appear to be no statistical association between one stage prothrombin times and speckling. This adds further evidence to the hypothesis that the aetiology of blood splash and speckling are fundamentally different. It is believed that such differences may be related to the different effects of 'head-only' stunning and 'head-to-body' stunning may have on blood pressure and/or muscular activity. Further investigations of these problems are described in the following chapter.

CONCLUSIONS

1. The prevalence of speckling is significantly higher in carcases from lambs stunned by 'head-to-body' methods as compared to carcases from animals stunned by 'head-only' methods.

2. There is a highly significant increase in the prevalence of blood splash in carcases and organs from animals stunned by a 'head-only' method as compared to that from animals stunned by 'head-to-body' methods.

3. There is an increased rate of pH decline in some muscles underlying subcutaneous fat with severe speckling as compared to the pH decline of similar muscles associated with less extensive speckling of the subcutaneous fat.

4. The one stage prothrombin time measured on individual blood samples from 100 lambs varied from 13 to 24 seconds with a mean of 16.6 seconds.

5. Extended one stage prothrombin times are not associated with an increase in the prevalence of speckling.

CHAPTER EIGHT

THE EFFECT OF DIFFERENT METHODS OF STUNNING ON BLOOD HAEMORRHAGE AND MUSCULAR ACTIVITY

INTRODUCTION

The work presented in Chapter Seven, as well as the work of others, indicates that blood splash is mainly related to 'head-only' stunning whereas speckling is usually associated with 'head-to-body' stunning. It is thus appropriate to consider whether or not differences between the two methods of stunning can be related to differences in the aetiology of the two types of haemorrhage.

It has been shown that 'head-only' stunning of sheep is associated with a rapid and prolonged increase in arterial blood pressure (Clark and Tweed, 1932; Kirton *et al.*, 1978; Blackmore and Newhook, 1982; Gilbert and Devine, 1982) but although it has been suggested that an increase in arterial pressure may exacerbate blood splash, it is not believed to be the sole cause of such haemorrhages. On the other hand, 'head-to-back' stunning apparently results in an immediate but brief increase in arterial pressure followed by a rapid decline to levels below those recorded prior to stunning (Gilbert and Devine, 1982). It was therefore suggested by these workers that speckling is not related to an increase in blood pressure during stunning but is associated with muscular activity immediately following stunning. However, it would appear that the duration and intensity of muscular activity following different methods of electrical stunning has not previously been investigated apart from the work reported in Chapter Six of this thesis.

One of the main features of 'head-to-body' stunning is that it causes immediate cardiac dysfunction (Blackmore and Petersen, 1981) and subsequent cessation of blood flow. It has been shown by Pearson *et al.* (1977) that electrical stunning of lambs causes a secretion of noradrenaline and adrenaline into the bloodstream resulting in levels respectively 20 and 14 times higher than in non-stunned lambs. In the case of 'head-to-body' stunning, the adrenaline would not be circulated whereas noradrenaline could still be secreted at the sympathetic nerve endings as a result of the electrical stimulation. Such a hormonal imbalance would theoretically favour a stimulatory action at the alpha-receptor

after resulting in contraction of smooth muscles in the walls of blood vessels, particularly those in the skin and mucosa (Tietz and Hall, 1977). As there is no longer any blood flow, contraction of small vessels could cause a significant increase in pressure in localised areas and this could contribute to haemorrhages in such sites.

The present studies were undertaken to investigate the effects of administration of noradrenaline and different methods of electrical stunning on both arterial and venous pressure. At the same time, attempts were made to measure objectively the muscular activity associated with the different methods of stunning.

MATERIALS AND METHODS

Animals and their preparation

Lambs from the Massey University farm were used for these studies. They were approximately six months of age and of different breeds but care was taken to assure thatall lambs within the same experiment were of the same breed. All animals were denied access to food and water for 24 hours prior to the experiments.

The ten lambs used in the first of the studies were prepared by the following surgical procedures :

The lambs were initially anaesthetised by intravenous injection of approximately 12 ml of a 4% solution of sodium thiamylal. This was followed by intubation and the administration of a halothane and oxygen mixture during the operation. The femoral artery and saphenous vein of the right hindleg was exposed and a 60 cm long polyethylene tube with a 3 mm external diameter was inserted in each vessel. The cannulae were gently pushed into the vessels until they reached a point just caudal to the diaphragm in the aorta and the caudal vena cava respectively. Both cannulae were flushed and filled with saline (approximately 2.5 ml) and provided with a two-way tap on their external free ends which were held in position during recovery and the overnigh resting period by gauze netting.

Restraint and slaughter

During the experiments, the lambs were placed in a stationery restrainer of similar shape to the moving conveyors used at the meat works(Plate 8.1).

PLATE 8.1 SHEEP IN RESTRAINING CRATE BEING STUNNED BY THE 'HEAD-TO-BACK' METHOD

The animals were stunned either by the 'head-only' method described in Chapter Six or by the 'head-to-back' method. The latter method utilised the same electrical parameters as the 'head-only' method (0.75 - 1.25 Amp, 400V, 50 Hz for 3 seconds) but was also equipped with a third electrode connected to a metal plate placed over the back of the animal in the region of the last thoracic and the first lumbar vertebrae.

All animals were exsanguinated by transverse incision of the ventral neck region with severance of the major vessels, the trachea, the oesophagus and the spinal cord at the occipito-atlantal junction.

Recording of data

Electrocardiography (ECG) : Needle electrodes, provided with overvoltage protection diodes on the input cables, were inserted subcutaneously in the thoracic wall. The electrical signals were recorded graphically on a Devices 8 Channel Recorder.*

Blood pressure : Both arterial and venous pressures were obtained by attaching a Bell and Howell Type PI pressure transducer** to the taps on the free ends of the cannulae. The transducers were placed at the level of the heart to equilibriate to zero hydrostatic head and pressures were recorded on the Devices 8 Channel Recorder simultaneously with the ECGs.

Electromyography (EMG) : Two needle electrodes, provided with overvoltage protection diodes, were inserted in the muscles at a depth of 15 mm and 60 mm apart. A reference electrode was placed between the two needles and recordings in Experiment 2 were obtained in a similar manner to the ECGs whereas the EMG signals in Experiment 3 were recorded using different equipment which is described later.

Haemorrhages : Following slaughter, dressing and evisceration, the carcases and organs of the animals were carefully examined for speckling and blood splash.

Experiment 1 :

Ten lambs were used for this experiment in which ECGs and arterial and

* Device Sales Ltd., Hertfordshire, United Kingdom.

** C.E.C. Division, Pasadena, California, 91109, U.S.A.

venous pressures were obtained before, during and after stunning. Because of some initial problems with the intravascular cannulae, both arterial and venous blood pressure measurements were not obtained in all cases.

Four of the lambs were injected through the intra-arterial cannula with noradrenaline (Levophed*) before being stunned by the 'head-to-back' method. In three of these animals a dosage of 20µg/kg liveweight was administered from five to fifteen seconds prior to stunning. The fourth lamb, used in this part of the experiment, was dosed four times with varying amounts of noradrenaline and after each dose, the animal was left undisturbed until the arterial pressure had declined to approximately the same level as before the treatment. This animal was stunned and exsanguinated one minute after the last injection with 40µg noradrenaline /kg liveweight.

The remainder of the lambs in this experiment had no treatment prior to stunning and three of these were stunned by the 'head-only' method whereas the last three lambs were stunned by the 'head-to-back' method.

Experiment 2 :

This experiment was designed to measure the duration of muscular activity following stunning and to obtain more information about venous pressures associated with the two different types of stunning used in these studies. The venous pressure was measured through a cannula inserted in the jugular vein just prior to stunning rather than using the saphenous vein as in the first experiment.

Recording of the EMGs were obtained from the middle region of the neck (lateral aspect), the lumbar part of the LD and the proximal part of the hindleg (lateral aspect).

Two animals in this experiment were stunned by the 'head-only' method and three animals were stunned by the 'head-to-back' method.

* Winthrop Laboratories, Sydney, Australia.

Experiment 3 :

The objective of this experiment was to measure the myo-electrical activity in the LD during the initial period of muscular contraction following electrical stunning.

Ten lambs were used in this experiment. Five of these were stunned by the 'head-only' method and five were stunned by the 'head-to-back' method. EMG recordings were obtained from two sites of the right LD of each animal. A cranial site was selected in the region of the fourth to sixth thoracic vertebrae and the caudal site was situated in the area adjacent to the tuber coxae.

The EMG signals from the two sites were recorded for a 20 second period immediately after stunning. After amplification (Tektronix, Type 122 Amplifier*), these records were stored on a DMS-6430 Digital Memoryscope**.

At the completion of a 20 second recording period, selected parts of the information were expanded, using the Memoryscopes zoom facility, and transferred to a chart recorder (Omniscribe recorder ***). The transfer of information was in all cases initiated one, eight, twelve and nineteen seconds after stunning and at these times, the EMG signals were recorded on the chart paper for one second. These action potential recordings were later integrated for a 0.25 second period, randomly selected from each time period by means of a planimeter as described by Lippold (1952). It was thus possible to obtain measurements of action potentials associated with the four different periods of EMG recordings (Figure 8.1). These were measured in mV.s x k,where k is an arbitrary constant.

RESULTS

Experiment 1 :

The main effects of noradrenaline on blood pressure in four lambs are recorded in Table 8.1. The arterial pressure increased, usually within a few seconds of the injection and the extent of the pressure rise appeared to be dependant on the dose given. When one of the animals was subjected to

* Tektronix Inc., Portland Oregon, U.S.A.
** Iwatsu Electric Co., Japan.
***Houston Instruments, Austin, Texas, U.S.A.

FIGURE 8.1 EMG RECORD AFTER EXPANSION AND TRANSFER TO CHART RECORDER. AREA INTEGRATED BY PLANIMETER SHOWN IN BLACK.

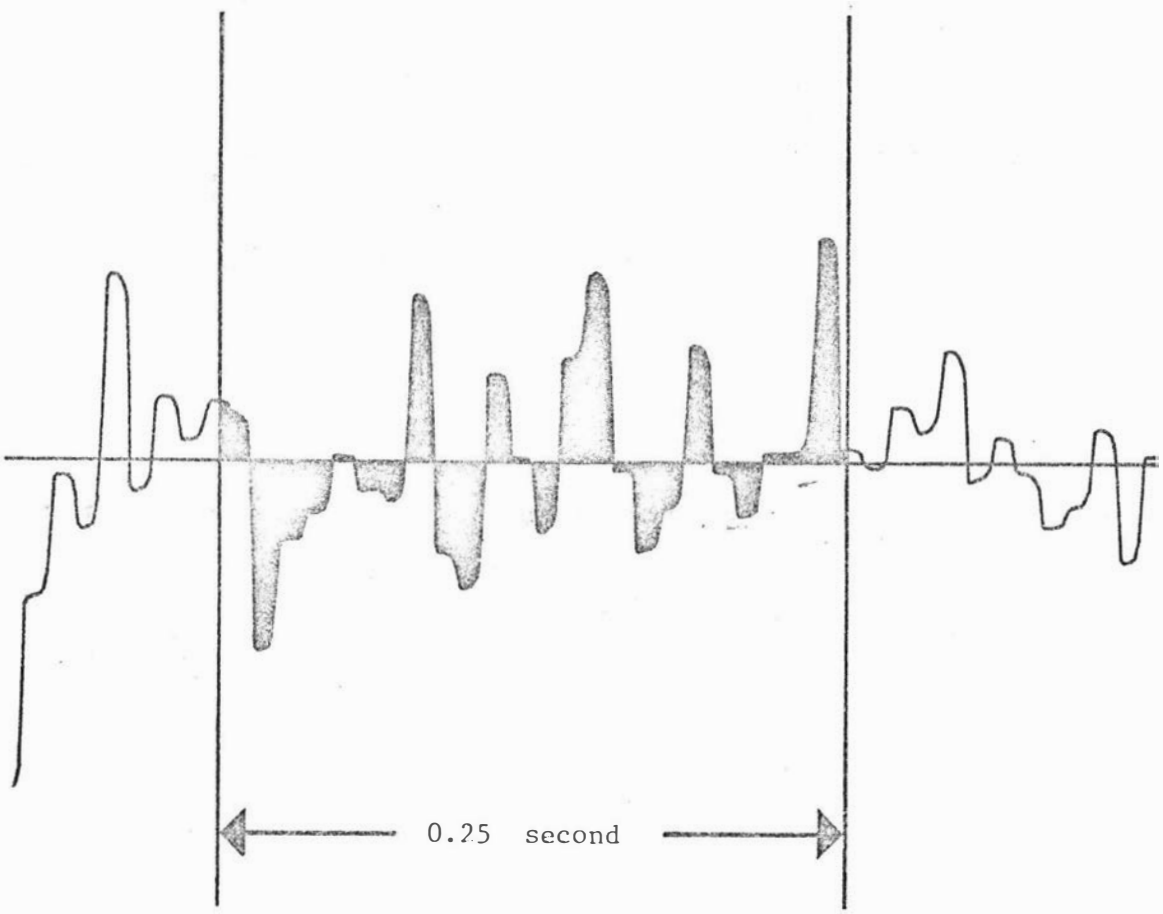


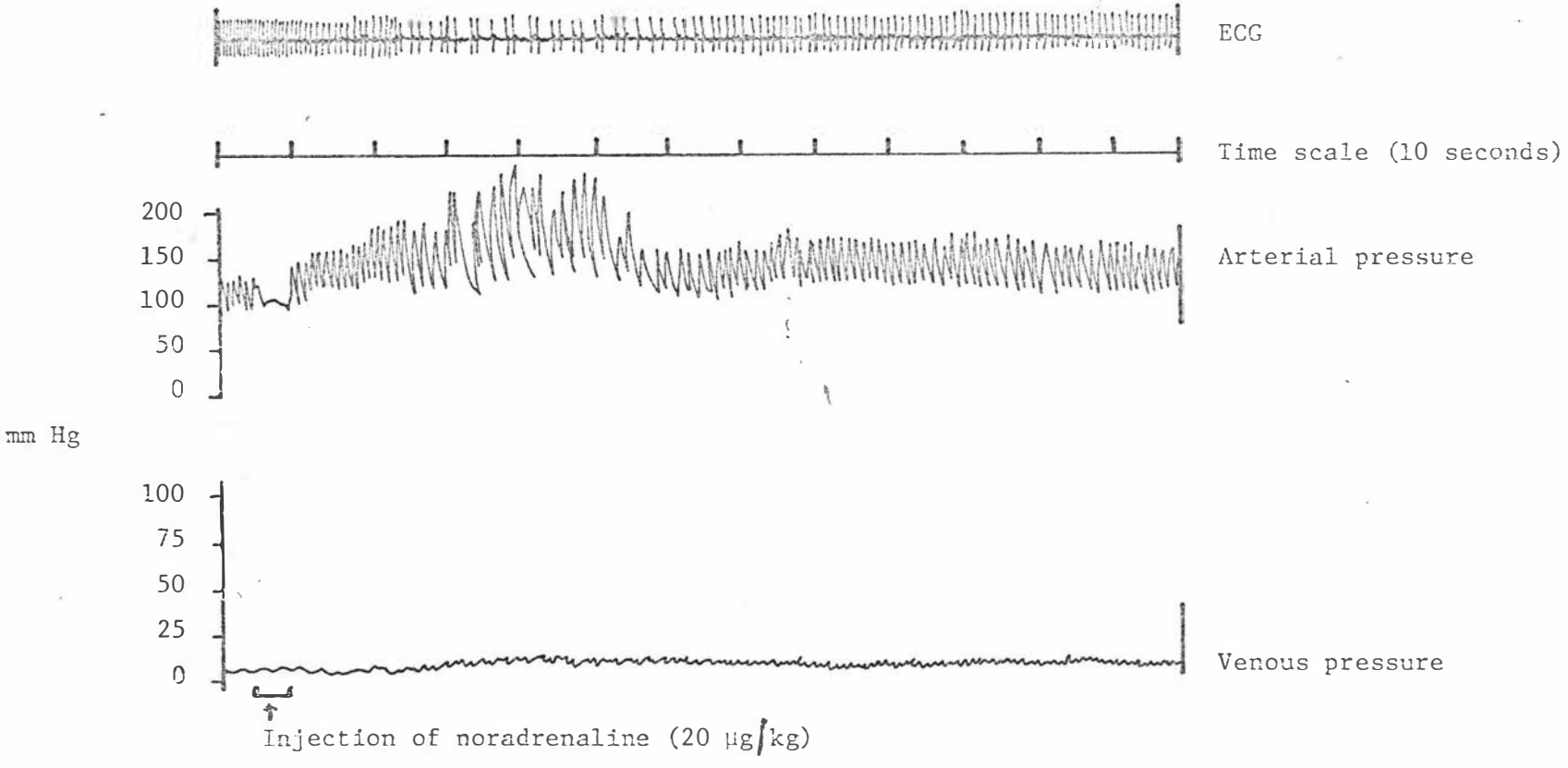
TABLE 8.1 THE EFFECTS OF ADMINISTRATION OF NORADRENALINE ON THE BLOOD PRESSURE (mm Hg) OF LAMBS PRIOR TO STUNNING

Animal Identification	Dosage µg/kg	Maximal arterial pressure Pre injection	Post injection		Maximal venous pressure Pre injection	Post injection	
1	20	120	185	(10)	*	*	*
2	20	130	145	(5)	5	3	(5)
3	20	115	165	(5)	10	10	(7)
4a	2	130	140	(30)	*	*	*
4b	4	120	145	(30)	*	*	*
4c	20	125	245	(30)	5	10	(30)
4d	40	120	250	(30)	5	10	(30)

* not recorded

Figures in parenthesis indicate time in seconds to reach maximal pressure.

FIGURE 8.2 THE EFFECT OF NORADRENALINE ON ECG AND BLOOD PRESSURE



successive injections, the return to preinjection levels varied from one to two minutes and the maximal arterial pressure recorded was 250 mm Hg approximately 20 seconds after injecting 40 µg noradrenaline/kg liveweight. The venous pressure was not obtained in all cases but it can be seen in Table 8.1 that administration of noradrenaline appeared to have only a very small effect on increasing venous pressure. It can also be seen in Figure 8.2, that the noradrenaline effect on arterial pressure was followed by a decrease of the heart rate within a few seconds. This effect appeared to be directly related to the dose of noradrenaline and at 20 µg/kg or more, there was a 50% decrease in heart rate within 30 seconds.

The mean maximal blood pressures for lambs stunned by the two different methods and for animals stunned after noradrenaline treatment is shown in Table 8.2. The three animals stunned by the 'head-only' method all exhibited an immediate drop in arterial pressure when the stunner was applied (Figure 8.3). This was followed by a dramatic increase in arterial pressure during the first 5-10 seconds after stunning and in one animal the pressure rose to 370 mm Hg. This high pressure (above 200 mm Hg) was maintained for 30-60 seconds and then declined rapidly. On the other hand, there was only a moderate increase in venous pressure during the 'head-only' stunning.

Lambs stunned by the 'head-to-back' method exhibited an immediate rise in arterial pressure during stunning followed by a decline within a few seconds (Figure 8.4). This effect was slightly more pronounced in lambs treated with noradrenaline prior to stunning where a maximal pressure of 240 mm Hg was measured in one animal whereas the maximum pressure measured in untreated lambs was 180 mm Hg. There was also a more rapid decline of arterial pressure in noradrenaline treated animals with the pressure dropping to below 100 mm Hg within 10 seconds of stunning whereas this level was not reached in untreated lambs until approximately 30 seconds after stunning.

There was an immediate increase in venous pressure in all animals stunned by the 'head-to-back' method and this increase was slightly greater in the noradrenaline treated lambs as compared to untreated lambs. The maximum venous pressure reached was in a lamb subjected to four injections of noradrenaline. In this case the venous pressure rose to 85mm Hg

TABLE 8.2 THE EFFECT OF METHOD OF STUNNING ON MEAN MAXIMAL BLOOD PRESSURES (mm Hg)

Method of Stunning	Arterial Pressure No.	Pre stunning	During stunning	Post stunning	Venous Pressure No.	Pre stunning	During stunning	Post stunning
'Head-only'	3	140	160	307	3	10	22	37
'Head-to-Back'	2	133	165	115	3	6	68	51
'Head-to-Back' after noradrenaline injection	4	156	209	143	3	8	77	53

FIGURE 8.3 THE EFFECT OF 'HEAD-ONLY' STUNNING ON ECG AND BLOOD PRESSURE

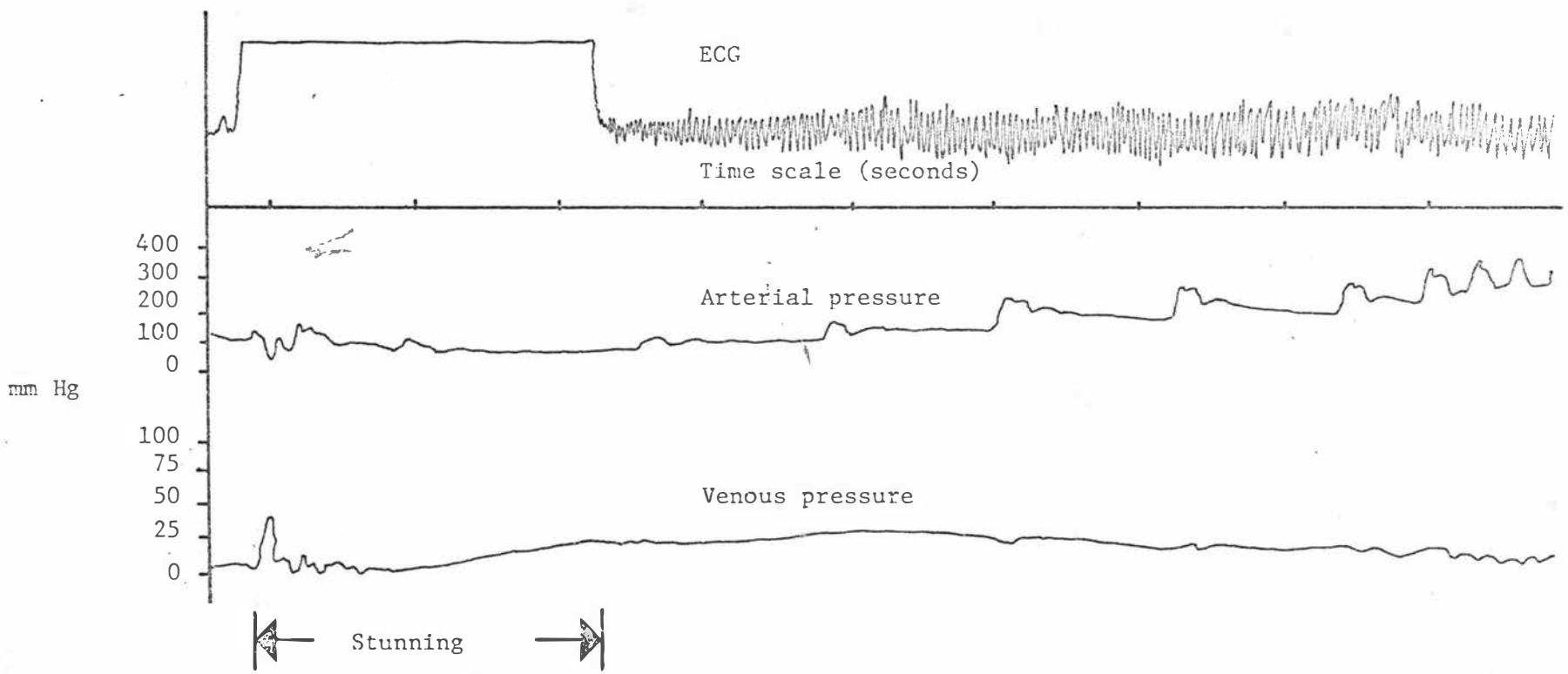
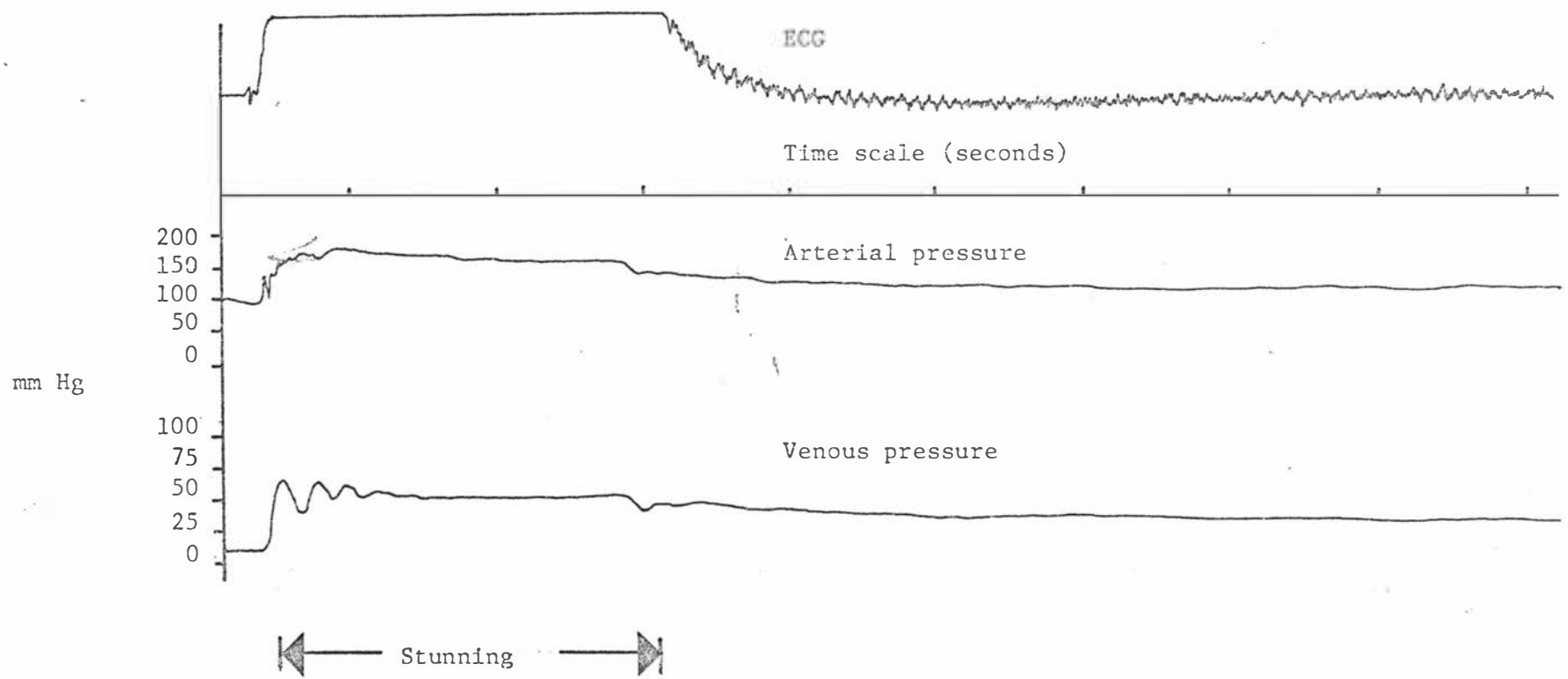


FIGURE 8.4 THE EFFECT OF 'HEAD-TO-BACK' STUNNING ON ECG AND BLOOD PRESSURE



immediately after stunning. A relatively high venous pressure (25-50 mm Hg) was maintained for a period of 20-30 seconds in all lambs stunned by the 'head-to-back' method.

Experiment 2 :

The EMG recordings from the five animals in the second part of these studies indicated that immediately following stunning, there was a period of muscular activity in all three sites from which recordings were obtained (Figure 8.5). It can be seen in Table 8.3 that the duration of this initial period of muscular activity was approximately 25 seconds and appeared to be unaffected by the method of stunning and the site of recording. The intensity of the muscular activity was not measured but the EMG records indicated that muscular activity may have been more intense in animals stunned by the 'head-to-back' method as compared to animals stunned by the 'head-only' method. It was also noted that animals stunned by the 'head-to-back' method exhibited little or no muscular activity following this initial period, whereas several short periods (three to five seconds duration) of muscular activity were recorded from animals stunned by the 'head-only' method.

There was also an increase in venous pressure associated with 'head-to-back' stunning and there appeared to be some correlation between the increase in venous pressure and increases in muscular activity (Figure 8.5).

Experiment 3 :

The EMG recordings of a 20 second period immediately following stunning have been reproduced on Plate 8.2 for four of the ten lambs. It can be seen that there appeared to be more muscular activity associated with 'head-to-back' stunning as compared to 'head-only' stunning and also more activity associated with recordings from the cranial sites as compared to the caudal sites.

The mean integrated action potentials for 0.25 second periods have been recorded in Table 8.4 and it can be seen that these values were higher in the group of animals stunned by the 'head-to-back' method as compared to the group stunned by the 'head-only' method. These mean values were also higher in recordings obtained from the cranial sites as compared to those obtained from the caudal sites. It can be seen in Table 8.5 that these differences between mean values were significant in most cases.

FIGURE 8.5 THE EFFECT OF 'HEAD-TO-BACK' STUNNING ON VENOUS PRESSURE AND DURATION OF MUSCULAR ACTIVITY

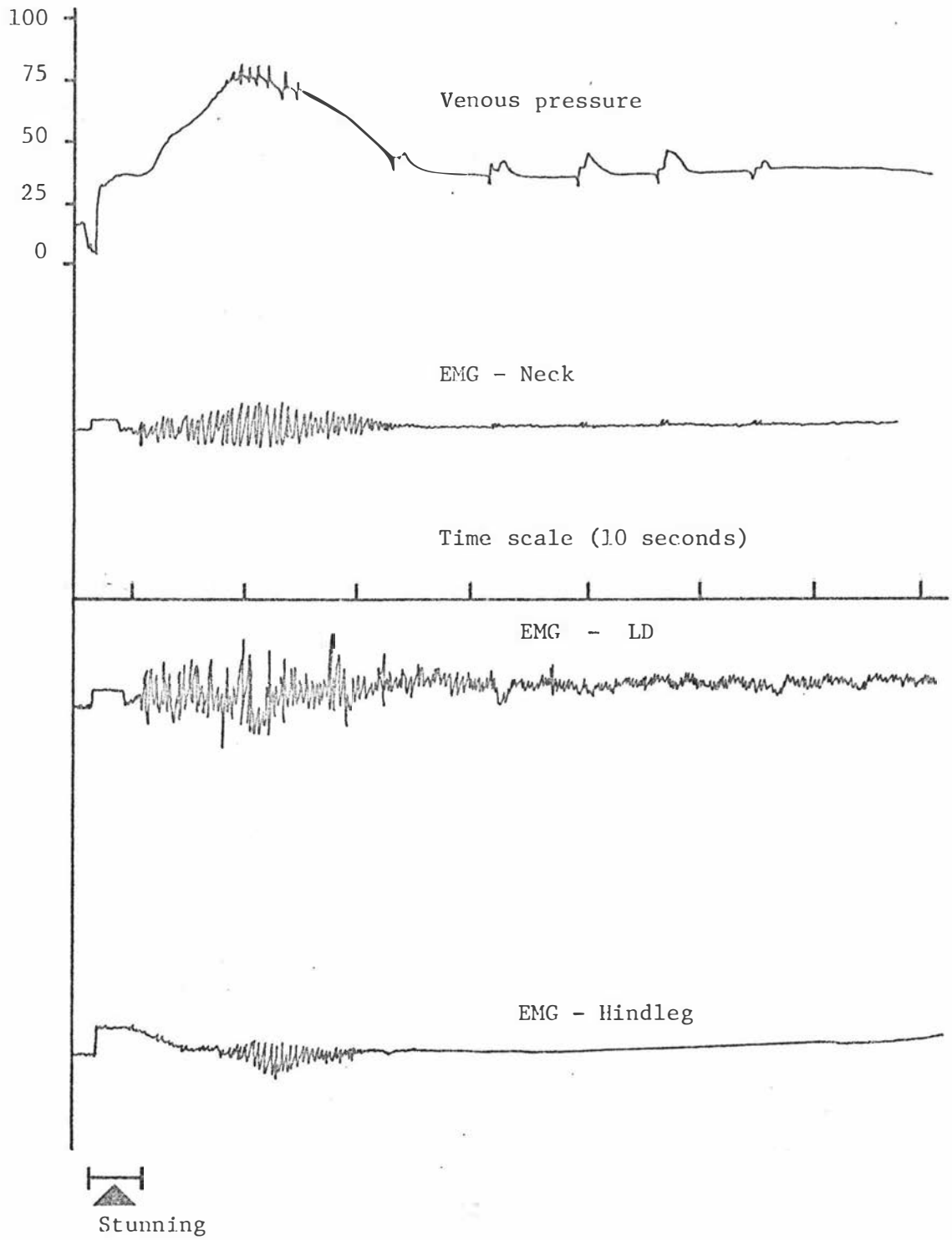


TABLE 8.3 DURATION OF MUSCULAR ACTIVITY FOLLOWING ELECTRICAL STUNNING

Method of Stunning	No. of Animals	Mean duration of muscular activity (s) Neck	Loin	Hind Leg
'Head-only'	2	23.5	25.0	23.5
'Head-to-Back'	3	25.0	26.3	20.3

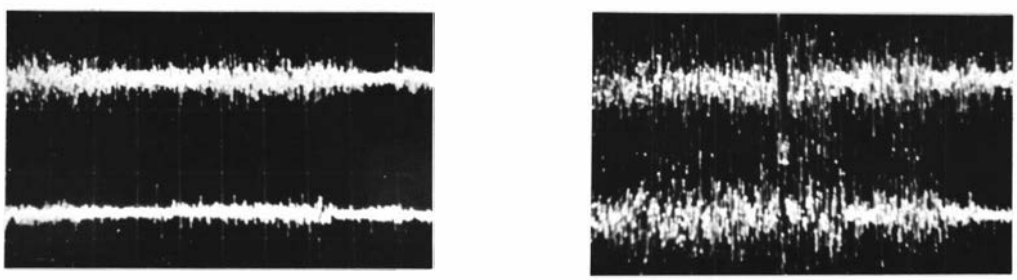
PLATE 8.2 EMG RECORDS FROM THE LD FOLLOWING ELECTRICAL STUNNING

(20 s recordings of four different lambs)

Top record = cranial site

Bottom record = caudal site

'Head-only' stunning



'Head-to-back' stunning

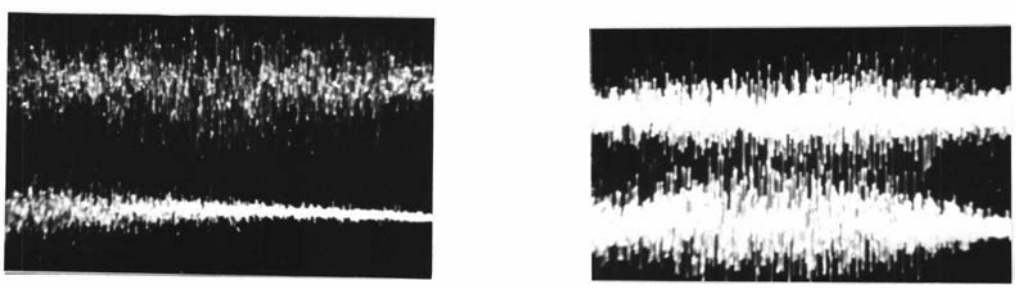


TABLE 8.4 THE EFFECT OF METHOD OF STUNNING ON MUSCULAR ACTIVITY

(Integrated action potentials of 0.25 second periods; means of five animals per group in mV.s x k, where k is an arbitrary constant)

	Time after stunning (seconds)	'Head-only' stunning	'Head-to-Back' stunning
Cranial Site	1	73 ±17	131 ±24
	8	73 ±22	134 ±17
	12	70 ±26	145 ±11
	19	44 ±13	47 ±14
Caudal Site	1	35 ±11	90 ±11
	8	31 ±16	83 ±25
	12	16 ± 4	57 ±17
	19	7 ± 1	18 ± 4

TABLE 8.5 SUMMARY OF TWO-WAY ANALYSIS OF VARIANCE OF THE EFFECTS ON MUSCULAR ACTIVITY OF METHOD OF STUNNING (Variance Ratios)

Time after stunning (seconds)	Effect of method of stunning[1]	Effect of Electrode position[2]	Interaction
1	9.15**	4.53*	0.01
8	6.18*	4.22	0.03
12	9.72**	14.31**	0.86
19	0.38	8.99**	0.10

1 = 'Head-only' stunning vs 'Head-to-Back' stunning

2 = Cranial position vs Caudal position

* = $P < 0.05$

**= $P < 0.01$

Although all carcases and organs were examined for haemorrhages only two cases of speckling were recorded. Both of these were minor, consisting of one to three petechiae (grade 1) and occurred in the subcutaneous fat of the loin of two lambs stunned by the 'head-to-back' method.

DISCUSSION

All of the peripheral arterial system contains alpha-receptors and it is also thought that the adrenergic receptors in the venous system are primarily of the alpha-type (Smith and Hamlin, 1977). An increase in both arterial and venous pressure could therefore have been expected following noradrenaline injections. However, it would appear that the decrease in heart rate, initiated through arterial baroreceptors, is so effective in establishing homeostasis of blood pressure that the major potential effect of noradrenaline on contraction of the veins was masked by a decrease in blood flow. It was therefore not possible to exacerbate, to any significant extent, the conditions which were thought to exist during 'head-to-back' stunning as a result of noradrenaline release.

The decline in arterial blood pressure during electrical stunning by the 'head-only' method can be attributed to the cardioinhibitory effect of vagal stimulation as was already suggested by Roos and Koopmans (1934). Immediately after stunning, there is a two to three fold increase in arterial pressure which is probably associated with the marked increase in heart rate and the effect of sympathetic stimulation of blood vessels. The smaller increase in venous pressure following 'head-only' stunning may also be initiated by sympathetic stimulation. This elevated blood pressure is then sustained until either the heart rate returns to normal or the pressure decreases as a result of exsanguination.

The pressure curves obtained following 'head-to-back' stunning were similar to those previously recorded by Blackmore and Newhook (1982) and Gilbert and Devine (1982) although the former workers did not report the short period of increase in arterial pressure during stunning. It has been suggested that this occurs as a result of thoracic pressure changes during application of the stunner (Gilbert and Devine, 1982) but it may also be associated with sympathetic stimulation of vessels. The decrease in arterial pressure following removal of the stunner from the animal is presumably caused by cardiac dysfunction induced by 'head-to-body' stunning.

It has therefore been suggested that blood pressure is not associated with speckling (Gilbert and Devine,1982). However it is believed that these workers may not have taken into account the changes taking place in venous pressure during and after stunning by a 'head-to-body' method.

If it is considered that speckling is associated with the microcirculatory bed (i.e. vessels less than 100 μ in diameter) then pressure changes within this system are important. It has been reported by Smith and Hamlin (1977) that blood pressure decreases in the microcirculatory bed from 35 mm Hg in the arterial side to 15 mm Hg in the venous side and that normal mean capillary pressure is about 25 mm Hg. This latter value is determined by the ratio of postcapillary to precapillary resistance. The first of these values is small under normal conditions since the difference between mean capillary pressure and venous pressure (approximately 5 mm Hg) is small whereas the second value is much larger because of the larger difference between mean arterial pressure (approximately 100 mm Hg) and mean capillary pressure. Thus a change in postcapillary resistance has a much larger effect upon capillary pressure than the same change in precapillary resistance.

It is of interest to note that although precapillary pressure is greatly increased by 'head-only' stunning, the postcapillary pressure is only moderately increased. On the other hand, there would appear to be a substantial increase in postcapillary pressure following 'head-to-back' stunning with venous pressures increased as much as 10-15 times normal values, possibly as a result of noradrenaline release. It is therefore not unlikely that mean capillary pressure may rise to higher levels following 'head-to-back' stunning as compared to 'head-only' stunning.

The data from this experiment and the previous work of Gilbert and Devine (1982) indicates that following 'head-to-back' stunning, the venous pressure may rise to nearly 100 mm Hg while at the same time, the arterial pressure is still maintained at levels well above 100 mm Hg. It would thus appear that it is possible for the mean capillary pressure to reach a level of three to four times normal values. It is therefore believed that blood pressure changes associated with 'head-to-back' stunning may be implicated in the aetiology of speckling and this will be discussed further later in this chapter.

The EMG findings related to the duration of muscular activity following stunning are in close agreement with the more subjective observations reported in Chapter Six. These studies indicate that both methods of electrical stunning induce tonic spasms lasting for approximately 25 seconds. This is followed by some intermittent movements in lambs stunned by the 'head-only' method whereas lambs stunned by 'head-to-back' methods exhibit little movement after the initial spasms. It is therefore not surprising that the meat industry prefers the 'head-to-back' method of stunning because it results in a greater degree of immobility during exsanguination.

It has been shown in human volunteers that the electrical activity recorded from muscles by means of EMG signals is a good measure of muscular activity (Lippold, 1952; Bigland and Lippold, 1954). This principle has also been used in more recent work (Milner-Brown and Stein, 1975; Brown and Cooke, 1981) and was the basis for the experiments carried out in the last part of these studies.

The increase in muscular activity associated with 'head-to-back' stunning as compared to 'head-only' stunning is probably caused by direct electrical stimulation of muscles. This is supported by the finding that the muscular activity was significantly greater in the cranial part of the LD as compared to the caudal part. The cranial site for the EMG recordings was between the position of the head electrodes and the back electrode of the electrical stunner and this site was therefore more directly associated with the current pathway as compared to the caudal site. It would thus appear that muscles or part thereof, which are located close to current pathways during electrical stunning, exhibit more intense activity immediately following stunning as compared to muscles which are further removed from current pathways and mainly activated through indirect nervous stimulation. The increased activity of these muscles is probably responsible for the increased rate in post mortem pH decline in the LD of animals stunned by 'head-to-body' methods reported in Chapter Six.

These studies indicate that there is a correlation between muscular activity and venous pressure. It is thus likely that the increase in venous pressure associated with 'head-to-back' stunning may partly be caused by the increase in muscular activity associated with this method of stunning.

It has been suggested by Leet *et al.* (1977) that blood splash is caused by 'super contracture' of some muscle fibres, causing severe strain on adjacent blood vessels and consequent rupture of their walls. It has also been suggested recently by Gilbert and Devine (1982) that speckling is caused by muscle movements during stunning. Thus both types of haemorrhages associated with electrical stunning have been linked to the extent of muscular activity during and after stunning. However, if the cause of both types of haemorrhagic lesions was muscular activity, it could be expected that 'head-to-back' stunning would result in the highest prevalence of both speckling and blood splash. This is clearly not the case.

It was not possible in the present studies to identify the causes of either blood splash or speckling and attempts to induce haemorrhages by increasing blood levels of noradrenaline also failed. However, it is believed that some important information in relation to the aetiology of haemorrhages was obtained. On the basis of these findings and some of the previous reports published on these haemorrhagic syndromes, the following hypothesis was formulated.

Electrical stunning by the 'head-only' method is accompanied by a massive release of catecholamines in the circulating blood (Pearson *et al.*, 1977) and this would favour some vasoconstriction in skin and mucosa and partial dilatation of vessels in skeletal muscle according to the distribution of alpha- and beta-receptors (Tietz and Hall, 1977). This method of stunning also causes a considerable increase in heart rate and as a result of this, and the increase in circulating catecholamines, arterial blood pressure is increased to levels two to three times above normal. There is therefore an increase in blood flow under high pressure in the partially dilated vessels of skeletal muscles and it is suggested that this may be the triggering mechanism causing blood splash as a result of the rupture of smaller arteries and veins.

The 'head-to-body' method of stunning causes immediate cessation of blood flow and hence an imbalance of catecholamines released into the blood vessels favouring noradrenaline which is mainly acting on alpha-receptors (stimulatory effect). Because the vessels in skeletal muscles contain mainly beta-receptors, abundance of noradrenaline will have a minimal effect on vascular contraction. There is also a decline in arterial

pressure following 'head-to-body' stunning and therefore less blood flow through the vessels of skeletal muscles. Rupture of small vessels in skeletal muscles, as a result of the combination of high blood pressure and dilated vessels, is therefore not likely to occur under such conditions.

Stunning by 'head-to-body' methods is also associated with an increase in venous pressure which is sustained for 20-30 seconds after stunning. As previously discussed this can have a dramatic effect on the blood pressure in the microcirculatory bed. The preponderance of noradrenaline, as a result of electrical stimulation of sympathetic nerves, may further increase the blood pressure in small vessels, mainly equipped with alpha-receptors, such as those in skin and mucosa but perhaps also in subcutaneous fat although this does not appear to be well investigated. It has been suggested that the basement membranes of capillaries are of a thixotropic nature with the ability to undergo a reduction in viscosity as a result of increase in pressure and thus allowing both fluids and cells to penetrate without permanent damage to the membrane (Simpson, 1980). It is therefore suggested that an increase in blood pressure in the microcirculatory bed is the direct cause of speckling associated with 'head-to-body' stunning.

In view of the importance of both blood splash and speckling to the meat industry, it is believed that it is important to further investigate these problems. It would be of particular interest to measure capillary blood pressure following different methods of stunning and to develop methods of electrical stimulation of sympathetic nerves to localised areas of subcutaneous tissue.

CONCLUSIONS

1. Administration of noradrenaline is followed by an increase in arterial pressure up to 250 mm Hg but there is only a small increase in venous pressure.

2. There is a two to three fold increase in arterial pressure following 'head-only' stunning whereas there is only a moderate increase in venous pressure.

3. Stunning by the 'head-to-back' method is followed by a decrease in arterial pressure but venous pressure increases to levels above 50 mm Hg.

4. There is an initial period of muscular activity following electrical stunning by either of the two methods. The duration (25 s) of this activity is unaffected by the method of stunning and the site of EMG recording.

5. There is a significant increase in the intensity of muscular activity following 'head-to-back' stunning as compared to 'head-only' stunning.

6. There is a correlation between the intensity of muscular activity and the location of the recording site in relation to the position of stunner electrodes.

7. Pressure changes in the microcirculatory bed are likely to be associated with speckling.

CHAPTER NINE

GENERAL DISCUSSION

Although DFD meat has probably been recognised since 1774 (Lawrie, 1974), it is only during the last ten to twenty years that the importance of preslaughter handling of stock, in determining meat quality, has been fully appreciated. The economic importance of high pH meat was apparently of little consequence to the New Zealand meat industry until new methods of processing and marketing were developed. The introduction of packaging of chilled beef meat in gas impermeable films has increased the export value of this product and created new markets. However, it has also resulted in the rejection, at some meat works, of up to 30% of beef carcases destined for these markets because of high ultimate pH values (A.S.Glover, personal communication). A similar trend in the marketing of lamb is now developing and the results, presented in Chapter Three of this thesis, indicate that problems associated with high ultimate pH of meat can also be expected in lamb products.

Studies of ante mortem depletion of muscle glycogen and consequent high ultimate pH values of meat have been particularly extensive in beef. Earlier investigations discounted both fasting and exercise of animals as primary causes of DFD meat (Lawrie, 1958) and attention was then focussed on the role of catecholamines in the development of the condition in both beef and sheep (Hedrick *et al.*, 1961; Hedrick, 1965; Ashmore *et al.*, 1973). It was also suggested that the so called "psychological stressors" such as excitement of animals prior to slaughter were more likely to cause high ultimate pH meat as compared to physiological factors including fasting of the animals (Grandin, 1980). Such statements were supported by the finding that mixing strange animals could cause depletion of muscle glycogen, but it was later shown, by the administration of beta blocking agents, that such a depletion in cattle is not predominantly mediated by adrenalin (McVeigh and Tarrant, 1981a). It should also be noted that there do not appear to be any substantial reports on the adverse affects on ultimate pH of meat from sheep and lambs following mixing of strange groups of animals prior to slaughter. Thus there is so far no scientific evidence to support the view that "psychological stressors' are of major importance in inducing DFD meat in sheep and lambs.

Although experimental studies in ruminants have failed to produce evidence of an association between levels of nutrition and ultimate pH, it has been shown in beef that there is a statistical association between carcase grading and ultimate pH (Munns and Burrell, 1966; Poulanne and Aalto, 1980).

It is surprising that so few studies on factors affecting the ultimate pH of beef have been carried out at the meat works, and apparently no such studies have previously been reported in sheep and lambs. This may be partially associated with previous difficulties in determining ultimate pH of carcase muscles during commercial operations. The plug sampling technique developed during the course of these studies was found to have the same degree of precision as those techniques previously employed in laboratory studies. It therefore overcomes some of the difficulties associated with the measurement of ultimate pH values in situations where carcases are frozen before rigor has occurred.

The seasonal pattern of ultimate pH values found in these studies, as well as the statistical association between wool score of lambs and ultimate pH of meat, indicates that the level of nutrition may be an important factor in the development of DFD meat. This is supported by the findings that extreme levels of dietary intake can signficantly affect ultimate pH values. These results are not in agreement with recent findings by Devine *et al.*, (1983) who reported that low levels of nutrition in lambs did not affect ultimate pH of the LD. However, the latter results were based on experimental studies using small groups of lambs (n = 12) and may reflect the difficulties associated with attempting to reproduce in the laboratory the conditions which prevail in the field.

Recent work by Lister and Spencer (1983) supports the hypothesis that level of nutrition is an important predisposing factor in the aetiology of DFD meat. These workers found that beta-adrenergic stimulation in sheep caused some reduction in muscle glycogen and when this was combined with inhibition of lipolysis, muscle glycogen was severely depleted at slaughter. They concluded that a decrease in the availability of energy substrates from adipose tissue (free fatty acids and glycerol) is an important factor in the development of DFD meat. It was also pointed out, by these workers, that the cattle most at risk to the DFD condition are lean young bulls and cows in relatively poor condition. These observations

are in close agreement with the findings regarding lambs presented in this thesis which indicate that the nutritional state of lambs prior to slaughter may be an important factor in determining the ultimate pH of meat. The extent of muscle glycogen metabolism during preslaughter exercise of animals may be dependant upon both adrenergic stimulation of glycogen metabolism and the availability of other substrates such as free fatty acids. It seems likely that the latter is associated with the nutritional state of animals prior to slaughter, whereas the former is probably related to both handling factors as well as genetic characteristics of the animals.

Work presented in Chapter Four has shown that Perendale lambs are more susceptible to muscle glycogen depletion following washing as compared to Romney lambs. It has also been shown that Perendale lambs have significantly higher ultimate pH values as compared to other breeds. The Perendale breed is very common on hill country (Anon, 1980b) and it is thus possible that lambs of this breed have been handled and fed differently on the farm prior to slaughter. However, further studies indicated that the cause of high ultimate pH values in Perendale lambs is more likely to be directly associated with the breed rather than indirectly with methods of husbandry. This breed associated difference indicates that further comparisons should be carried out on the ultimate pH values of some of the other common breeds in New Zealand (e.g. Coopworth, Corriedale and Merino).

Although the aetiology of high ultimate pH meat is still unclear, it is possible on the basis of the present findings to identify the type of lamb carcase which is most likely to exhibit a high ultimate pH value. The more important animal and farm related factors to be considered in such an evaluation would include breed, level of nutrition and wool score. The major preslaughter handling factors which should be taken into account are washing prior to slaughter and the duration of holding periods at the meat works. These latter two factors must also be evaluated in terms of their effect upon carcase contamination. It is therefore concluded that the holding period at the meat works should be kept to a minimum but there should also be a sufficient delay (18 to 24 hours) between drafting of lambs on the farm and slaughter at the meat works. During this period, animals should be kept under clean conditions to avoid soiling of pelts as this will necessitate washing of the lambs at the meat works. Such washing may result in an increase in the prevalence of bruising of carcases and has now also been shown to increase the ultimate pH of muscles.

With the introduction of new slaughter methods in 1977, the New Zealand meat industry was faced with major technological problems which have not yet been fully overcome. The development of 'head-to-body' methods of electrical stunning provided the Industry with a reliable method of inducing insensibility of animals prior to exsanguination but it apparently also introduced a hither to unknown type of haemorrhage in carcases. In spite of extensive investigations, the causes of either blood splash, or the more recent syndrome of speckling, are still unknown. However, the results of the studies reported in this thesis, indicate that local pressure changes in the microcirculatory bed associated with the different methods of stunning are likely to be associated with the development of haemorrhagic syndromes associated with stunning of animals. These pressure changes were measured by indirect methods in the present studies but it is suggested that the use of micro techniques for measuring intra capillary pressures should be used in future studies.

The high voltage electrical stimulation of lamb carcases, which has been used in New Zealand for the past five to ten years, was developed after extensive studies of rate of pH decline and tenderness in "normal" lambs. Recently Chrystall *et al.* (1982) reported that in some cases, when ultimate pH values of muscles are elevated, electrical stimulation may have a detrimental effect on tenderness of meat. The work presented in this thesis indicates that this adverse effect on tenderness by electrical stimulation may be exacerbated by prior application to the carcases of low voltage stimulation. Thus, the inter relationship between rate of pH decline, ultimate pH and tenderness of meat requires further investigation.

Future studies of the development of irreversible contraction during high voltage stimulation and its affect on tenderness requires experiments utilising groups of animals exhibiting a wide range of ultimate pH values. In the past, such experimental conditions could only be achieved by either using large groups of animals or by artificially inducing high ultimate pH by preslaughter administration of adrenalin (Bouton *et al.*, 1974). It is suggested that the findings presented in this thesis could be used to improve the design of such experiments as it is now possible to identify lines of lambs likely to produce some carcases with elevated ultimate pH values under natural conditions. The preslaughter handling of such lambs, and in particular the washing of animals prior to slaughter, can also be manipulated to obtain a wider range of ultimate pH values. Furthermore, the use of the

plug sampling method in combination with the technique for early identification of DFD meat described by Davey and Graafhuis (1981) makes it possible to measure ultimate pH values of muscles within one hour of slaughter and without mutilation of carcases. It would thus be possible to select relative small study groups with known mean ultimate pH values and subject these to the different methods of processing currently used at the meat works followed by tenderness measurements of meat samples. It is believed that if such a study design was used, conditions could be kept close to normal, minimal number of experimental animals would be required and valuable information for the meat industry could be obtained.

The majority of investigations presented in this thesis were carried out at meat works and it is believed that the use of an industrial setting for research work in meat science can be most successful. Some of the major findings in these studies, such as the effect of breed, season, nutrition and washing of lambs on ultimate pH of meat, can now be directly applied by the meat industry, whereas in the past such investigations have usually been carried out as small scale experiments making extrapolation of results and direct implementation by the Industry difficult. It is therefore suggested that in the future a greater emphasis on this type of approach, in spite of some of the logistical problems of integrating research with a commercial operation, would result in scientific work of merit and the solution of many problems of economic importance.

In conclusion, the ideal circumstances for production of high quality lamb meat is the selection of lambs of suitable genetic composition, on a high level of nutrition, not recently shorn, delivered to the works in a clean condition and slaughtered between 18 and 24 hours after being removed from pastures. The animals should be slaughtered by one of two methods. One option is stunning by an electrical 'head-to-body' method followed by high voltage electrical stimulation of carcases within 30 minutes and chilling and freezing after two hours. The other option is stunning by a 'head-only' method and immediately subjecting carcases to low voltage stimulation followed by a three to four hour delay prior to freezing.

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APPENDIX I

MEASURING THE pH OF MUSCLE SAMPLES

Samples: Two gram samples in tissue culture trays overlaid with liquid paraffin and incubated for 24 hours at room temperature.

Solution: Mix 1 ml of stock reagent* with 20 ml of glass distilled water for each sample.

Homogenisation: Use 20 ml of solution per bag. Place sample in solution and homogenise in Stomacher for two minutes (6 bags at a time.

pH: Measure pH of solution within 30 m of homogenisation after adjustment of the pH meter using two standard buffer solutions.

Quality control: Use two frozen control samples whenever batches of samples of unknown values are tested. All values of controls must be within the range +/- 0.06 pH unit of the mean of the control.

If one or more control values are outside the limits, check all equipment and solutions and retest using additional controls if available. If all controls have the same bias (e.g. using new pH meter or probe) values from unknown samples must be adjusted before comparing with previous results.

* Dissolve 9.3 g iodoacetate acid in 400 ml glass distilled water. Neutralise to pH 7.0 and make up to 500 ml. Store in brown bottle at 0 - 5°C.

APPENDIX II

LIST OF APPENDED SCIENTIFIC PUBLICATIONS

Petersen, G.V. (1982): A plug sampling technique for measuring the pH of carcass muscles. Meat Sci., 7: 37-42.

Bager, F.; Petersen, G.V. (1983): The relative precision of different methods of measuring pH in carcass muscles. Nord. Vet. Med., 35: 86-90.

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