Chapter 5

The conversion of muscle to meat

In considering how meat animals grow and how their muscles develop and are differentiated, the distinction between the terms 'muscle' and 'meat' has not been emphasized. Meat, although largely reflecting the chemical and structural nature of the muscles of which it is the post-mortem aspect, differs from them because a series of biochemical and biophysical changes are initiated in muscle at the death of the animal. Some details of the conversion of muscle to meat will now be given.

5.1 Preslaughter handling

Although, at the most, only a few days elapse between the time when meat animals have attained the weight desired by the producer and the actual moment of slaughter, their condition may change appreciably in this period. This will happen to some extent irrespective of whether the animals are driven on the hoof or transported to the abattoir. There may be loss of weight, bruising and, if the animals are in road or rail trucks, suffocation due to inadequate ventilation. The lairage itself can have an appreciable effect on the level of bruising and it is recommended that those animals most vulnerable to bruising should occupy the quietest yards at the abattoir (Eldridge, 1988). In bruising, blood from damaged blood vessels visibly accumulates. (It can also occur in pigs through the use of markers to tattoo identification numbers on the skin). Both in pigs and cattle, such bruising can be caused through the animals slipping and falling especially when attempts are made to speed up their transit by using goads (Warriss, 2000). In 1971 bruising, oedema and emaciation accounted for 4 per cent, 65 per cent and 77 per cent of the total condemnations of pigs, sheep and cattle respectively, in Northern Ireland (Melrose and Gracey, 1975). In most cases of extensive bruising damage to muscles causes the release of enzymes into the blood stream; and the relative concentrations of creatine phosphokinase and aspartate transaminase permits an assessment of how long before slaughter bruising occurred (Shaw, 1973). Cows suffer more from bruising in transit than bullocks (Yeh *et al.*, 1978). Nevertheless, although the incorpora-

tion of bruised beef in meat products is condemned by hygiene regulations in various countries, a comparison of its microbiological and technological properties with those of non-bruised tissue revealed no differences (Rogers and co-workers, 1992, 1993).

Death of pigs in transport increased greatly in the decade 1960–70 (Thornton, 1973). There was a marked seasonal effect, deaths both in transit and lairage being correlated with the environmental temperature (Allen *et al.*, 1974). Above *ca.* 18 °C there is a very rapid increase in mortality. It was reported that the transport of pigs in double-decker road vehicles was particularly liable to affect the musculature adversely as meat (Williams, 1968). In unloading pigs from road vehicles, excessive steepness of the ramps and the absence of foot supports can be significant factors in increasing bruising (Warriss *et al.*, 1991a).

During autumnal conditions (ambient temperature 16–24 °C), stocking densities of between 0.35 and 0.50 m^2/100 kg liveweight (the usual range of values in Europe) had little effect on the blood profile or meat quality of pigs when transported for up to three hours, although there was evidence of an increase of skin bruising, due to trampling and fighting with the lower allocation of space (Barton Gade and Christensen, 1998). Even more restriction of space, viz. less than 0.30 m^2/100 kg liveweight, is not acceptable, especially in journeys above three hours, since it is essential that the pigs have room to lie down (Warriss, *et al.*, 1998a).

Sophisticated analytical techniques have revealed that there are differences in the overall pattern of proteins (the proteome: cf. §3.3.1) in pork meat from pigs slaughtered immediately and that in pork from pigs held for 12 hours before slaughter (Morzel *et al.*, 2004).

It is increasingly recognized that thoughtless or rough handling of animals in the immediate preslaughter period will adversely affect the meat, quite apart from being inhumane. Recommendations on ante-mortem care (Thornton, 1973; Houthius, 1957; Grandin, 1993) will not be discussed at length in the present volume: but some aspects of the question should be considered.

It would appear that only recently have attempts been made to study the behaviour pattern of meat animals with a view to improving preslaughter handling. With pigs, for example, acknowledgement that these animals tend to fight when awaiting slaughter, especially if they come from different farms, led Danish workers to employ a halter which prevents biting and damage to the flesh (Jørgensen, 1963) and droving is so arranged that the more cautious animals follow the bolder ones. With particular reference to pigs, it is clear that responses to transport and handling depend not only on the stress susceptibility of the animal as a whole, but also on which muscles are being considered. Both the metabolic capability of individual muscles and the duration and severity of transport determine whether the PSE condition will develop or whether glycogen reserves will be depleted sufficiently to produce dark meat (Anon., 1971). Much of the stress sustained in transport and handling arises during loading and unloading.

In comparing various pig slaughtering premises, Warriss *et al.* (1994) detected a tendency for increased stress to be associated with the larger operations in which throughput was faster, restraining devices more prevalent and noise levels greater. Subjective assessments of stress correlated well with such objective measures as elevated levels of blood lactate and creatine phosphokinase. High levels of stress were associated with poorer quality meat. An analysis of pig meat quality in Denmark, Italy, the Netherlands, Portugal and the UK (Warriss *et al.*, 1998b) indicated that

preslaughter stress was more closely reflected by a high ultimate pH and dry, dark meat than by that of PSE characteristics. There is a greater potential for achieving optimal welfare in 'organic' systems of pig rearing than in intensive systems, but this requires more complex management (Barton Gade, 2002).

In reviewing the problems of preslaughter handling of meat animals, Gregory (1994) again emphasized the greater susceptibility of pigs to present difficulties than sheep or cattle. Over prolonged periods, however, sheep also suffer stress. Thus, in the seaboard transport of sheep from Australia to the Middle East, up to 6 per cent of the animals may die on the voyage, from lack of nourishment, salmonellosis or stress. Holding the sheep on board before unloading, as when there are no quayside lairage facilities available, also causes losses (Higgs *et al.*, 1991). Tranquillizing drugs (when permitted) may be effective in preventing fighting and struggling, and may reduce the incidence of exudative meat, injury and death.

Transport of animals under stressful conditions, especially when these are prolonged, is clearly inhumane and, indeed, can be lethal. It is now widely recognized that stressed animals are likely to have a subnormal content of glycogen in their muscles, whereby, post-mortem, the pH of their flesh fails to attain acidic values (cf. § 5.1.2 below); and the attributes of eating quality in the meat will be adversely affected (cf. Chapter 10). Both humane and organoleptic considerations may thus be expected to promote increasing legislation designed to ensure that the conditions to which animals are subjected in transport will be strictly controlled.

5.1.1 Moisture loss

The moisture content of pork muscle is especially liable to change because of even moderate fatigue or hunger in the immediate pre-slaughter period (Callow, 1938a, b). When fasted during transit, cattle lose weight less readily than sheep, and sheep less readily than pigs. With the latter species, the wasting can be about 3 lb/24 h (1.35 kg/24 h) in an animal weighing about 200 lb (90 kg) (Callow and Boaz, 1937). In one study it was found that pigs which had travelled for 8 h before slaughter yielded carcasses averaging 0.9 per cent less than corresponding animals which had travelled for only ½ h pre-slaughter. This was regardless of whether they had been fed or not (Cuthbertson and Pomeroy, 1970). Moreover, pigs may lose further weight if they are given water on journeys over 36 h in duration (Callow, 1954). A loss of weight of this order cannot be accounted for entirely by the breakdown of fatty and muscular tissues to produce energy and heat for the fasting pig and may be due, in part, to a loss of water-holding capacity in the muscular tissues (Callow, 1938b). An animal killed immediately on arrival at a slaughterhouse, after a short journey, may provide both a heavier carcass and heavier offal than an animal which has been sent on a prolonged journey, then rested and fed for some days in lairage (Callow, 1955b). Although it is more difficult to cause carcass wastage in cattle, the extreme conditions in Australia, in which cattle may travel up to 1000 miles from feeding areas to abattoir, can cause losses of *ca.* 12 per cent of initial live weight. Dehydration is minimized when cattle have access to water for 3.5–7 h prior to a preslaughter fast (Wythes *et al.*, 1980). In a comparison of the effects of holding cattle for varying periods without feed or water before slaughter (Jones *et al.*, 1990), shrinkage varied from 31 g kg^{-1} in beef cattle slaughtered immediately to 106 g kg^{-1} for those held for 48 hours without feed or water. The weight of the liver and other offal, as a percentage of live weight, decreased progressively as the period of inanition

increased; and there was some suggestion that the ultimate pH of the musculature was elevated (cf. § 5.1.2).

5.1.2 Glycogen loss

The influence of fasting in depleting the glycogen reserve of muscle has been known since the work of Bernard in 1877. Recognition of the importance of this fact in relation to the meat from domestic species is more recent. Callow (1936, 1938b) indicated that inadequate feeding in the period before slaughter could lower reserves of glycogen in the muscles of pigs. Bate-Smith and Bendall (1949) showed that fasting for only 48–72 h lowered the glycogen content of rabbit *psoas* muscle sufficiently to raise the ultimate pH from the normal (for the rabbit) value of 5.9 to 6.5. In contrast, when steers were fasted at normal ambient temperatures for periods up to 28 days, the ultimate pH was unaffected (Howard and Lawrie, 1956).

The importance of exhausting exercise as a factor in depleting glycogen reserves in muscle has also been recognized for a considerable period. The poor keeping quality of the meat from hunted wild cattle was referred to by Daniel Defoe in 1720. Mitchell and Hamilton (1933) showed that exhausting exercise immediately preslaughter could cause a high ultimate pH in the muscles of cattle; but Howard and Lawrie (1956) found it most difficult to deplete the glycogen reserves in this species, even when preslaughter exercise and fasting for 14 days were combined (Table 5.1). Yet such depletion occurred, without fasting, if enforced exercise took place immediately after train travel.

The glycogen reserve of pig muscle, however, is especially susceptible to depletion by even mild activity immediately preslaughter (Callow, 1938b, 1939); a walk of only a quarter of a mile may cause a small but significant elevation of ultimate pH. Bate-Smith (1937a) suggested that if an easily assimilable sugar were fed before death the reserves of muscle glycogen might be restored to a level high enough to permit the attainment of a normal, low ultimate pH – the latter being desirable to avoid microbial spoilage (Callow, 1935; Ingram, 1948). This principle was confirmed in commercial practice by Madsen (1942) and Wismer-Pedersen (1959b) in Denmark and by Gibbons and Rose (1950) in Canada (Table 5.2).

If pigs are rested for *prolonged* periods before slaughter, in an attempt to restore glycogen reserves naturally, there is danger that animals carrying undesirable

Table 5.1 Glycogen concentrations and ultimate pH in *psoas* and *l. dorsi* muscles of steers after enforced exercise and fasting

Treatment	*L. dorsi*		*Psoas*	
	Glycogen (mg %)	**Ultimate pH**	**Glycogen (mg %)**	**Ultimate pH**
Controls (fed and rested 14 days after train travel)	957	5.49	1017	5.48
Exercise (after train travel and 14 days fasting)	1028	5.55	508	5.55
Exercise 1½ hr (immediately after train travel)	628	5.72	352	6.15

Table 5.2 Effect of feeding sugar preslaughter on the ultimate pH of pig muscles

Group treatment[a]	Muscle	Ultimate pH
(a) Held overnight without food	*psoas*	6.00
(b) Fed 3 lb (1.35 kg) sucrose at 22 h and 6 h preslaughter	*psoas*	5.54
(c) No food preslaughter	*psoas*	5.75
	biceps femoris	5.74
(d) 2 lb (0.9 kg) sugar fed 3–4 h preslaughter	*psoas*	5.56
	biceps femoris	5.57

[a] Groups (a) and (b), Gibbons and Rose (1950); groups (c) and (d), Wismer-Pedersen (1959b).

bacteria may infect initially unaffected animals, e.g. with *Salmonella*, which can endanger subsequent human consumers. This fact is reflected in current legislation in the United Kingdom which does not permit holding for more than 48 h, since the most recent outbreak of foot and mouth disease.

In the belief that the musculature of cattle might spare glycogen reserves during activity, by metabolizing lipid stores more readily than monogastric animals, Howard and Lawrie (1957a) injected cattle, after fasting, with neopyrithiamin to inhibit thiamin pyrophosphate and, thereby, fat oxidation. Whereas fasting *per se* had little effect in lowering the level of muscle glycogen in cattle, its combination with neopyrithiamin lowered it markedly, leading to an average ultimate pH level of above 6.1 throughout the musculature. Using similar reasoning, Lister and Spencer (1981) induced 'dark-cutting' characteristics in the meat of sheep and cattle by administering antilipolytic agents such as nicotinic acid and methyl pyrazole carboxylic acid, following exposure of the animals for some hours to isoprenaline (a β-adrenergic agonist, which promotes lipolysis and glycolysis in muscle). The same effect was induced in pigs (Spencer *et al.*, 1983) and annulled by the simultaneous administration of caffeine which stimulates lipolysis (and spares glycogen). They suggested that caffeine administration could be used prophylactically to conserve muscle glycogen (and thus ensure a normal ultimate pH) in animals exposed to stress.

The existence of some influence controlling the level of muscle glycogen other than fatigue or inanition was suggested by the finding that certain steers which had been well fed and rested, and would therefore have been expected to have ample glycogen in their muscles, yielded meat of high ultimate pH (Howard and Lawrie, 1956). It appeared that these steers were of an excitable temperament. In such animals, short range muscular tension, not manifested by external movement, reduced glycogen reserves to a chronically low equilibrium level. Those animals in a group that had shown the greatest resistance to handling, produced muscles with the highest ultimate pH (Howard and Lawrie, 1956). Similar findings with reindeer were reported by Petaja (1983).

Muscles differ in their susceptibility to preslaughter glycogen depletion by stress. Thus, in the ox, Howard and Lawrie (1956) found that the ultimate pH of *psoas*

major tended to be elevated more than that of *l. dorsi*, whereas Warriss *et al.* (1984) reported the reverse. Insofar as red muscles are responsible for shivering, their glycogen stores are more readily lowered by severe cold than that of white muscles (Lupandin and Poleshchuk, 1979). Even individual fibres differ in their response to stress, and to its nature according to whether they are of 'fast' or 'slow' type (Lacourt and Tarrant, 1985; cf. § 4.3.5). The time required to restore glycogen levels in the muscles of young bulls, after their depletion by the stress of mixing animals of different origin, was shown to be not less than 48 h (Warriss *et al.,* 1984). Drugs given pre-slaughter to induce tremor considerably depleted glycogen reserves, causing a high ultimate pH, and confirmed the view that fear was an important factor in this context (Howard and Lawrie, 1957a). (The muscles of the cattle killed after excitement of train travel (Table 5.1) had a high ultimate pH, whereas those of cattle rested for 14 days after travel had normal values.)

A high ultimate pH, in the muscles of the cattle, causes the aesthetically unpleasant phenomenon of dark-cutting beef – known at least since 1774 (Kidwell, 1952) – and in those of pigs that of 'glazy' bacon (Callow, 1935). Apart from its poor appearance, the high pH of 'dark-cutting' beef enhances the growth of bacteria (Ingram, 1948).

Tarrant (1981) reported the results of a survey in which meat scientists in 20 countries were asked their views on 'dark-cutting' beef. The incidence was high in young bulls (reflecting the importance of excitability of temperament in causing the condition) and in cold, damp weather. Preslaughter stress generally was regarded as the prerequisite. Similar conclusions were reached by Brown *et al.* (1990) in a survey of 5000 cattle in the UK.

There is evidence that the relative susceptibility of individual cattle to develop 'dark-cutting' characteristics in their meat post-mortem is positively correlated with the number of 'slow' oxidative fibres in their muscles (Zerouala and Stickland, 1991).

Recognition that stress susceptibility is a factor in determining the condition of animals generally, and thereby the glycogen status of their muscles, has grown as the result of Selye's (1936) concept of the general adaptation syndrome. He noted that animals exposed to a variety of stress-producing factors such as emotional excitement, cold, fatigue, anoxia, etc., reacted by discharge of the same hormones from the adrenal gland irrespective of the nature of the stress – adrenaline from the adrenal medulla, 17-hydroxy- and 11-deoxycorticosterones from the adrenal cortex. These substances elicit a variety of typical responses in the animal. Adrenaline depletes muscle glycogen and potassium; 17-hydroxy-corticosterone and 11-deoxy-corticosterone, respectively, restore the equilibrium level of these substances in normal animals. The release of the latter two hormones is controlled by the secretion of ACTH by the pituitary; and ACTH production is controlled by a releasing factor produced in the hypothalamus (Harris *et al.*, 1966), the part of the brain which is reactive to external stimuli. As mentioned in § 2.5.2.1, an imbalance at various points in this complicated system can cause so-called diseases of adaptation (Selye, 1944, 1946). Such would be expected in individual animals which were stress-susceptible, and the imbalance could be manifested by low equilibrium levels of glycogen, disturbances in the rates of glycogen breakdown and so on (§ 3.4.3). In the plasma of pigs which yield pale, exudative flesh, for example, there is a deficiency of 17-hydroxy-corticosteroids (Topel *et al.*, 1967). Stress-susceptible pigs react to certain anaesthetics by a rise in body temperature

of 1–4 C° (malignant hyperthermia: Gronert, 1980) and limb rigidity, and the development of rigor during light anaesthesia induced by halothane has been used to identify pigs which are prone to stress (Lister *et al.*, 1981). The glycolytic and oxidative pathways in the muscles of pigs of the genotype susceptible to hyperthermia under the influence of halothane, are significantly different from those of pigs which are insensitive to the anaesthetic: the former have a higher concentration of lactate and lower concentration of CP and ATP (Lundström *et al.*, 1989; cf. §§ 2.2 and 2.3).

Differences have been observed between species in their sensitivity to various stressors. Thus, pigs are more affected by sound than are sheep (Lister *et al.*, 1981). In humans it has been shown that the ratio of adrenaline to noradrenaline in the circulation, following stimulation of the hypothalamus, varies according to the nature of the stressor (e.g. heat, exercise, emotion) (Taggert *et al.*, 1972), and, since these two hormones affect different receptors, it may well be that the concept of the general adaptation syndrome, however useful, is an oversimplification of the response of animals to stress.

Tranquillizers known to offset stress susceptibility have been given to calm stock in transit, but they are not without danger as they may induce a state of relaxation so profound that the animals cannot stand and may be suffocated. The metabolic stresses which affect muscle were reviewed by Lawrie (1966).

At the cellular level, stress apparently induces the production of a group of proteins/polypeptides which possess cryoprotective activity (Lindquist and Craig, 1988). They exhibit a very high degree of conservation between species, suggesting their importance throughout evolution. Of these the polypeptide, ubiquitin, is an important member. It is a component of the filamentous inclusions which characterize neurodegenerative diseases, against which it appears to be mobilized as an aspect of immunological reactivity (Lowe and Mayer, 1990; cf. § 11.3).

5.2 Death of the animal

A major requirement for desirable eating and keeping qualities in meat is the removal of as much blood as possible from the carcass, since it can cause an unpleasant appearance and is an excellent medium for the growth of microorganisms. Despite reports that *delayed* bleeding has little effect on the eating quality of meat (Williams *et al.*, 1983), there is no suggestion that carcasses should remain completely unbled, of course.

Except in ritual slaughter, animals are anaesthetized before bleeding. The procedure at both stunning and bleeding is important. When special precautions are taken to ensure sterility, there is some evidence that unbled muscles undergo the tenderizing changes of conditioning to a greater extent than do those which are bled (Shestakov, 1962), but this could scarcely be regarded as a generally valid reason against bleeding.

5.2.1 Stunning and bleeding

Generally, cattle are stunned by a captive bolt pistol or by a blow from a pole-axe. In recent years the dressing of beef carcasses has been carried out more frequently as they hang vertically rather than when supine on the abattoir floor. These chang-

ing circumstances make it rather less important to ensure that the heart is still functioning as blood can drain quite effectively from the carcass even when heart action has ceased. Indeed it has been suggested that the vasoconstrictive effect of the stress of stunning will expel most of the blood from the musculature and that drainage is only necessary to remove blood from major blood vessels (Warriss, 1978; Warriss and Wotton, 1981). In certain countries cattle are stunned electrically.

Sheep and pigs are stunned electrically or anaesthetized by carbon dioxide. It has been observed that in sheep killed by a captive bolt pistol the epithelial lining of the intestines is shed, whereas it remains intact in anaesthetized animals (Badaway *et al.*, 1957); this could have microbial implications which will be referred to later. It is important to emphasize that drugs may not be used to induce unconsciousness in animals which are intended for human consumption since residue could remain in the meat.

In electrical stunning, the characteristics of the current must be carefully controlled, otherwise complete anaesthesia may not be attained and there may be convulsive muscular contractions. The siting of the electrodes is also important, since the current must pass through the brain. Variation in electrical resistance because of differing thicknesses in the skull can cause ineffective stunning. There are three phases in the animal's reaction: (i) as soon as the current is switched on there is violent contraction of all voluntary muscles and the animal falls over; respiration is arrested; (ii) after 10 s (the current being discontinued) the muscles relax and the animal lies flaccid; (iii) after a further 45–60 s the animal starts to make walking movements with its legs and respiration starts again. Usually, alternating current at 70–90 V and 0.3 A is used for 2–10 s (Cruft, 1957). Better relaxation and less internal bleeding is said to result if a high frequency current (2400–3000 Hz; Koledin, 1963) and a square wave form, instead of a sine wave (Blomquist, 1958), are employed.

There is some suggestion that electrical stunning may lower the glycogen reserves of the muscle slightly. The mean ultimate pH of *quadriceps femoris* from 518 electrically stunned pigs was 5.78; that in non-stunned controls was 5.67 (Blomquist, 1959). If the period between electrical stunning and bleeding is prolonged, the rather high pH may foster microbial spoilage (Warrington, 1974). In comparison with captive bolt stunning, electrical stunning has been shown to cause an elevation of amino acids in the plasma (especially of valine; the concentration of isoleucine falls somewhat) (Lynch *et al.*, 1966). It has been found that the level of corticosteroids in the blood of electrically stunned pigs is higher than that of those anaesthetized by carbon dioxide (Luyerink and Van Baal, 1969). There are said to be benefits in a diminished incidence of blood splash if a high-pressure water jet is combined with electrical immobilization in the stunning of pigs (Lambooij and Schatzmann, 1994). Repeated application of electrical stunning to pigs appears to cause no welfare problem but, of course, should normally be avoided (McKinstry and Anil, 2004).

Carbon dioxide anaesthesia is an effective alternative to electrical stunning provided the concentration of the gas is between 65–70 per cent. If the latter concentration is not exceeded, the musculature of the pigs is relaxed and the ultimate pH is slightly lower, and less variable, than with electrical stunning (Blomquist, 1957). One disadvantage of using a carbon dioxide chamber is that pigs differ somewhat in their susceptibility to anaesthesia by the gas, and that individual control of the animals is not feasible. Moreover, there is evidence that, prior to anaesthesia,

animals suffer considerable stress; and, indeed, it has been suggested that carbon dioxide anaesthesia does not comply with the generally accepted definition of pre-slaughter stunning.

Von Mickwitz and Leach (1977) surveyed the various methods of stunning employed in the EEC (now EU). They rated concussion stunning of cattle as the most effective, followed by captive bolt stunning of sheep and electrical stunning of pigs. Electrical stunning of sheep and captive bolt stunning of calves were deemed ineffective procedures. They concluded that any attempt to standardize stunning methods must specify proper preslaughter treatment of animals as an integral part of the overall procedure. In a comparison of various modes of stunning in pigs, using ^{31}PNMR spectroscopy, Bertram *et al.* (2004a) found that captive bolt stunning caused the most rapid rate of post-mortem glycolysis and the greatest loss of fluid from the meat subsequently. CO_2 anaesthesia was associated with the slowest rate of post-mortem glycolysis and least loss of fluid; whereas electrical stunning was intermediate in these two respects. All three stunning procedures, however, were more stressful than general anaesthesia.

Insensibility has been associated with an electroencephalographic voltage of less than 10 μV. It has been demonstrated, however, that with electrical stunning before bleeding, the voltage takes longer to fall below 10 μV than when throats are cut without prior stunning, suggesting that the electrical stunning of sheep and calves causes a prolonged increase in the electroencephalic voltage. The latter criterion is thus not a reliable index of insensibility with animals which have been electrically stunned (Devine *et al.*, 1986).

Newhook and Blackmore (1982a, b) studied the efficacy of electrical stunning when applied to calves and lambs, using electroencephalography and electrocardiography to indicate the state of the animals. They defined 'death' as 'irreversible insensibility due to cardiac anoxia caused by complete severance of both common carotid arteries and jugular veins'. According to this criterion, lambs were technically dead by 10 s after exsanguination. Calves did not become insensible however until 90 s; and there were indications of recurrent sensibility for up to 5 min after bleeding. It was postulated that delayed death in calves was due to their brains being supplied more lavishly with blood via the vertebral arteries. In calves, following neck sticking, a considerable proportion of animals suffer carotid occlusion (ballooning), whereby large clots impede bleeding and may lead to sustained brain function (Anil *et al.*, 1995). Anil *et al.* (2000) demonstrated that a relatively long cut in the thorax (chest sticking) in pigs (anaesthetized by the head-only procedure) was more humane insofar as it quickly stopped brain responsiveness. This problem does not appear to arise with chest sticking.

These findings related to conditions when the current was applied via two electrodes placed on the head; but when it was delivered via one electrode on the head and one on the back there was immediate cardiac dysfunction and permanent insensibility ensued. This mode of current application was thus regarded as more humane for the electrical stunning of calves (Blackmore and Newhook, 1982; Cook *et al.*, 1995). Nevertheless Channon *et al.* (2002) reported that the latter procedure was associated with greater drip, paler muscle and a faster rate of post-mortem glycolysis than when the current was applied by electrodes on head and back; or with carbon dioxide anaesthesia.

Eike *et al.* (2005) used a computer model to study details of the current density in the head of electrically stunned pigs. The model confirmed that the placing of

electrodes from eye to eye or from eye to ear was associated with high current density across the brain and effective stunning.

In cattle and sheep, bleeding is effected by severing the carotid artery and the jugular vein, and in pigs by severing the anterior vena cave. If the knife penetrates too far, blood may collect beneath the scapula and cause taint by early decomposition (Thornton, 1973). To avoid entry of micro-organisms, the cut made is minimal, especially with bacon pigs which are subsequently placed in a scalding tank. It has been said that bleeding after electrical stunning is more effective than after the use of the captive bolt pistol, but that it is less so than with carbon dioxide anaesthesia (Blomquist, 1957). Chrystall *et al.* (1980–81) could find no difference in the amounts of residual blood in lamb muscles (or in their microbiological status) whether the lambs had been electrically stunned by several procedures, bleeding had or had not been delayed or whether bleeding had been carried out without electrical stunning. Similarly, with head-only stunning of cattle (as required by halal slaughter), although the heart remains active, and its pumping action assists bleeding out, there appear to be no differences in residual blood in comparison with other stunning procedures (Anon., 1987–88). Even with effective bleeding only about 50 per cent of the total blood is removed (Thornton, 1973), different muscles retaining more or less blood according to their nature. In the horse, for example, 50 per cent of the total pigment left in the heart after bleeding is haemoglobin from the blood, whereas in *psoas* and *l. dorsi* the corresponding values are about 25 and 10 per cent respectively (Lawrie, 1950). It has been shown that electrical stimulation (§ 7.1.1.2) of the carcass after severance of the neck vessels increases the weight of blood which drains from the main veins and arteries and the organs, but does not affect its removal from the musculature (Graham and Husband, 1976).

Following the introduction of electrical stunning there was an increased frequency of 'blood splash', i.e. the appearance of numbers of small dark red areas in the muscles. These had previously been noted when pigs or lambs were shot. Blood splash is certainly more prevalent in electrically stunned lambs than in those stunned by captive bolt or percussion cap (Kirton *et al*., 1981a). It is more frequently observed in *l. dorsi* and in various muscles of the hind limb. Microscopic examination has shown that blood splash arises where capillaries have ruptured through over-filling with blood (Anon., 1957a). When the current is applied there is a considerable rise in blood pressure, muscles are contracted and their capillaries are almost empty of blood. Subsequently the muscles relax and if the blood pressure is not released by external cutting, blood is forced into the capillaries again with sufficient force to rupture many of them and enter the muscle itself (Leet *et al.*, 1977). As indicated above, the positioning of the electrodes in stunning lambs is important. Thus, if both are placed on the head, blood pressure increases markedly and blood splash can arise. If, however, one electrode is placed on the head and the other on the back or leg, the blood pressure rises only transiently and there is little blood splash, but minute haemorrhages ('speckle') appear in the fat and connective tissue. These reflect the rupture of small vessels caused by the muscular spasms which occur (Gilbert and Devine, 1982). Emotional stress causes vasodilation of the blood vessels in skeletal muscle, and may enhance fibrinolytic activity in entrained blood. These effects, especially if combined with electrical stunning, could perhaps explain the higher incidence of 'blood splash' reported in excitable animals (Jansen, 1966). The remedy appears to be to bleed the pigs within 5 s of administering the anaesthetizing current (Blomquist, 1959).

Kirton *et al.* (1978) came to similar conclusions in respect of the electrical stunning of lambs. Bleeding should be performed as soon as possible after stunning whatever the method of stunning employed.

The extent of exsanguination is enhanced by vasoconstriction of the blood vessels (Warriss, 1978) which is induced by angiotensins produced from the plasma proteins by the enzyme rennin (Miller *et al.*, 1979). The latter is released by the kidneys when their blood supply is interrupted.

5.2.2 Dressing and cutting

Following bleeding, carcasses are 'dressed', i.e. the head, feet, hides (in the case of sheep and cattle), excess fat, viscera and offal (edible and inedible) are separated from the bones and edible muscular tissue. Cattle and pig carcasses, but not those of sheep, are split along the mid ventral axis into two sides. It is not appropriate here to detail dressing procedures; these are fully considered in other texts (Gerrard, 1951; Swatland, 1994; Warris, 2000). Nevertheless, recent developments in this field should be noted. As has been pointed out by Longdill (1989), the labour required to produce a dressed carcass is greater with small species such as sheep. Whereas beef requires *ca.* 22 man hours to produce 10,000 kg of carcasses, sheep requires *ca.* 80 man hours. In New Zealand, considerable advances have been made in the use of mechanical devices in slaughtering and dressing operations with sheep.

In the automated dressing line a series of mechanical devices stun the animal, remove the pelt (first from the brisket, then completely), eviscerate the carcass and process the head (Longdill, 1994). Other devices debone the loin and thoracic regions and have increased the yield of recovered lean meat. Overall hygiene is also improved. Automated slaughterhouses are also being developed for cattle in Australia and for pigs in the Netherlands (Longdill, 1994). These have reduced man hours by 40 per cent.

Considerable progress has been made in automating the entire dressing and cutting sequence, using portable television facilities to analyse these operations in detail; and the applicability to control these of the robotic equipment already available in industry is being assessed. In respect of dressed meat, video image analysis has been successfully applied to grading for speedy online determination of the fat/lean ratio (Newman, 1984) and fibre optic probes permit objective prediction of such textural defects in the meat as excessive paleness or darkness (MacDougall, 1984). In Denmark, a self-correcting computer system has been developed which determines the positioning of fibre optic probes in carcasses and has proved more accurate than grading by experts in calculating the percentage of lean in pigs.

Until recently it was commercial practice to chill dressed carcasses prior to preservation or processing (cf. Chapters 7, 8 and 9), and, after chilling (which signified after rigor mortis), to prepare primal, wholesale cuts (cf. Fig. 3.1; and Gerrard, 1951) from them. Traditionally, skeletal reference points and straight cutting lines have been used. These have contributed to variability in retail joints since the boneless, primal cuts are aggregates of several muscles rather than muscles isolated individually by 'seaming out' along the muscle fascia – as in certain continental practices (Strother, 1975).

Prior to the introduction of vacuum packaging, wholesale cuts were sold with bone intact to avoid evaporative losses and minimize contamination. Now, however, a considerable proportion of home killed beef in Britain is deboned centrally and

delivered to retailers as boneless primal cuts. Not only is it likely that deboning of beef carcasses will become a standard abattoir operation in the future, such will be effected on hot carcasses immediately after slaughter.

It has been demonstrated that losses due to evaporation and exudation in vacuum-packed, hot deboned beef are markedly reduced. Moreover, it was reported that there was no significant difference between hot and conventionally deboned vacuum-packed beef in respect of the number of aerobic bacteria on the products, either initially or after storage at 0 °C (Sheridan and Sherrington, 1982), although the number of facultative anaerobes was significantly higher with hot deboned packs. Control of eating quality is greater since muscles can be 'seamed out' as anatomical entities. In the absence of bone, and because the meat cuts are less bulky, chilling and freezing can be more rapidly and economically effected. 'Cold-shortening' can be avoided by deboning in rooms at 5–15 °C and holding the vacuum packed cuts for at least 10 h at these temperatures (Schmidt and Gilbert, 1970; Follet *et al.*, 1974).

Alternatively, 'cold-shortening' can be avoided by electrical stimulation of the carcass or side immediately after slaughter (Carse, 1973; Bendall, 1980) (cf. § 7.1.1.1). Clearly this procedure would be especially useful with relatively small, hot-deboned portions of meat. On the other hand, although the swift lowering of temperature which would thereafter be permissible would assist in controlling microbial spoilage, it would prevent the early accelerated conditioning to which the tenderness achieved by electrical stimulation can be partially attributed (Savell *et al.*, 1977a,b; George *et al.*, 1980). The principles have been extended to the hot cutting of lamb and mutton carcasses (McLeod *et al.*, 1973), cuts being shrink-wrapped and held at 10 °C for 24 h before freezing. Tenderness, far from being diminished, was enhanced in some cuts over that of cuts from carcasses which had been chilled intact before cutting. Shrink wrapping, by moulding the warm fat and musculature, produces pleasing cuts from the initially untidy portions (Locker *et al.*, 1975). It also eliminated weight losses. Similarly, the development of a hot-boning system for pork could increase processing efficiency and improve shelf-life (Reagan, 1983).

Weight losses, and in particular, exudation in deeper muscles where the combination of body temperature and low pH denature proteins, have been much reduced also by partial excision of beef muscles on the warm sides immediately after slaughter and exposing the 'seamed out' muscles to air at 0 °C (Follet, 1974). The bulk of the musculature, in these circumstances, prevents temperatures falling fast enough to cause 'cold-shortening'.

More detailed consideration will be given to 'cold-shortening', electrical stimulation and conditioning below (§§ 5.4, 7.1.1.2, 10.3.3.1 and 10.3.3.2).

5.3 General consequences of circulatory failure

Stoppage of the circulation of the blood at death initiates a complex series of changes in muscular tissue. The more important of these are outlined in Fig. 5.1. It will be appreciated, from what has been indicated in Chapter 4, that the speed and extent of these changes may be expected to differ in different muscles.

At the moment of death of the animal as a whole, its various tissues are continuing their particular types of metabolism under local control. Although muscle is not actively contracting at such a time, energy is being used to maintain its

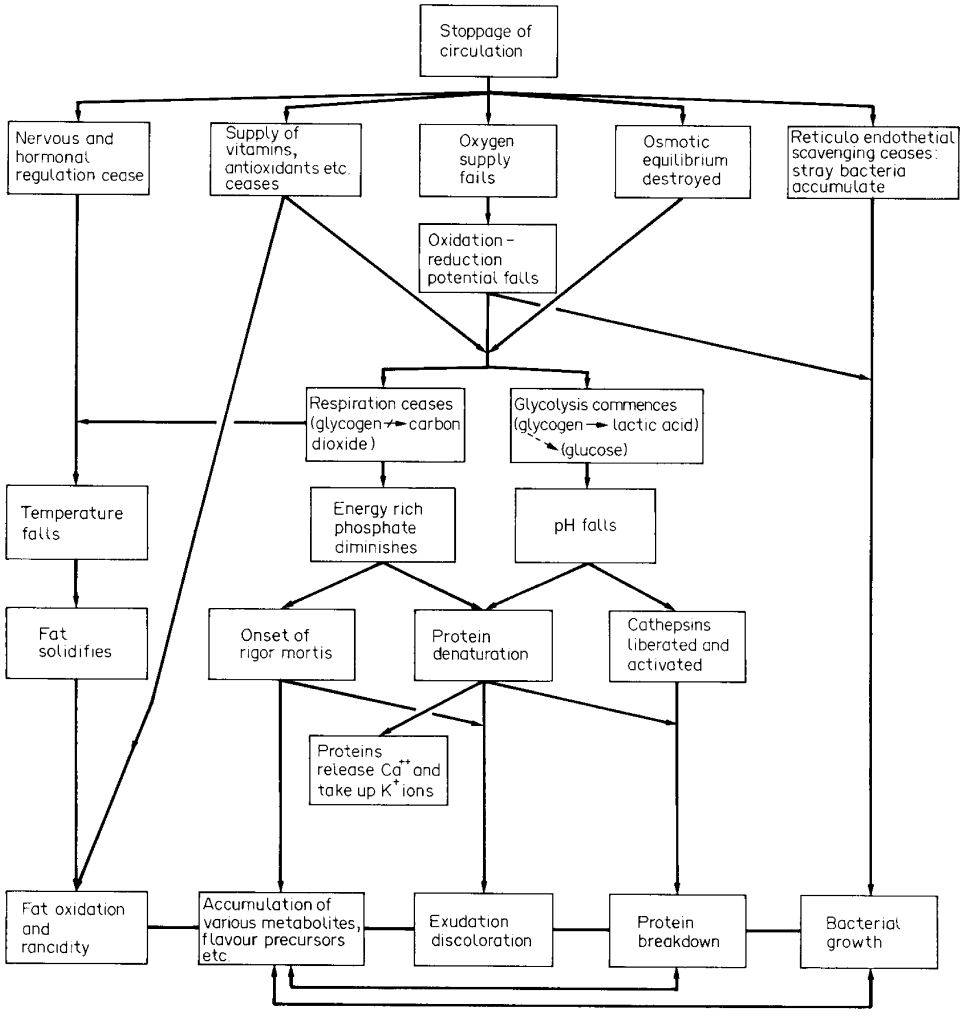

Fig. 5.1 The consequences of stoppage of the circulation in muscular tissue.

temperature and the organizational integrity of its cells against their spontaneous tendency to break down. The non-contractile ATP-ase of myosin, and not the contractile ATP-ase of actomyosin, is one of the enzymes involved in this context (Bendall, 1951). The most immediate change caused by bleeding is the elimination of the blood-borne oxygen supply to the muscles and the consequent fall in oxidation reduction potential. As a result the cytochrome enzyme system cannot operate, and the resynthesis of ATP from this source becomes impossible. The continuing operation of the noncontractile ATP-ase of myosin depletes the ATP level, simultaneously producing inorganic phosphate which stimulates the breakdown of glycogen to lactic acid. The ineffectual resynthesis of ATP by anaerobic glycolysis cannot maintain the ATP level and, as it drops, actomyosin forms and the inextensibility of rigor mortis ensues (as detailed in § 4.2.3). The lowered availability of ATP also increases the difficulty of maintaining the structural integrity of proteins. The

lowered pH, caused by the accumulation of lactic acid, also makes them liable to denature. Denaturation is frequently accompanied by loss of the power to bind water and the falling pH causes the myofibrillar proteins to approach their isoelectric point. Both events cause exudation. Denaturation of the sarcoplasmic proteins also makes them liable to attack by the proteases or cathepsins of muscle, which are probably held inactive *in vivo* within particles known as lysosomes (De Duve and Beaufay, 1959) but are liberated and activated when the particle membranes are weakened by the falling pH.

The breakdown of proteins to peptides and amino acids, and the accumulation of various metabolises from the glycolytic process and from other sources, affords a rich medium for bacteria. Although growth of the latter is somewhat discouraged by the extent to which the pH falls, they are no longer subject to the scavenging action of 'white' blood corpuscles (since blood circulation has stopped).

A further aspect of the stoppage of the circulation is the cessation of long-term hormonal control of tissue metabolism. As it fails, the temperature falls and fat solidifies. The tendency for the fat to oxidize and become rancid is facilitated by failure of the blood to renew the supply of anti-oxidants, and by the accumulation of pro-oxidant molecules in the tissues.

5.4 Conditioning (ageing)

Although muscle is increasingly liable to suffer microbial spoilage in direct proportion to the time and temperature of holding post-mortem, hygienic abattoir operations will generally ensure satisfactory storage for a few days at room temperature and for about 6 weeks if the meat is held just above its freezing point (–1.5 °C). Various processes applied to the commodity, such as curing, freezing, dehydration and irradiation, will vastly extend storage life, but, in so far as they are artificial, they are not relevant in this chapter. In the absence of microbial spoilage, the holding of unprocessed meat above the freezing point is known as 'conditioning' or 'ageing', and it has long been associated with an increase in tenderness and flavour (cf. Bouley, 1874). During the first 24–36 h post-mortem, the dominant circumstance is post-mortem glycolysis. This has already been considered in some detail in §§ 4.2.2 and 4.2.3. Even before the ultimate pH has been reached, however, other degradative changes have commenced. These continue until bacterial spoilage or gross denaturation and desiccation of the proteins have made the meat inedible. The extent of these changes, which affect the nature and amount of both proteins and small molecules, is generally limited, however, by the cooking and consumption of the meat.

5.4.1 Protein denaturation

Muscle, like all living tissues, represents a complexity of organization among molecules which is too improbable to have arisen from, or to be maintained, by, their random orientation. The structure of the proteins which characterize contractile tissue can only be preserved against the tendency of the component atoms and molecules to become disorientated by the provision of energy (as ATP). Such energy is not available after death and the proteins will tend to denature. Denaturation may be defined as a physical or intramolecular rearrangement which does not involve

hydrolysis of the chemical bonds linking the constituent amino acids of the proteins' polypeptide chains (Putnam, 1953). It is generally accompanied by an increase in the reactivity of various chemical groups, a loss of biological activity (in those proteins which are enzymes or hormones), a change in molecular shape or size and a decrease in solubility. Proteins are liable to denature if subjected, during post-mortem conditioning, to pH levels below those *in vivo*, to temperatures above 25 °C or below 0 °C, to desiccation and to non-physiological salt concentrations.

Of the proteins in muscle, it has been generally accepted that the collagen and elastin of connective tissue do not denature during conditioning (Ramsbottom and Strandine, 1949; Wierbicki *et al.*, 1954). This view was supported by the concomitant absence of soluble, hydroxyproline-containing molecules, indicating that neither collagen nor elastin were proteolysed (Sharp, 1959); proteolysis would not normally precede denaturation. When, however, collagen is denatured, for example, by heating beef muscles to 60–70 °C for 20–25 min, there is a continuous nonenzymic, breakdown to hydroxyproline-containing derivatives (Sharp, 1963).*

During post-mortem conditioning, the proteins of the myofibril and of the sarcoplasm denature in varying degree. Immediately after death and before the onset of rigor mortis, muscles are pliable and tender when cooked. The principal proteins of the myofibril, actin and myosin, are dissociated and myosin is extractable at high ionic strength (Weber and Meyer, 1933; Bailey, 1954). With the onset of rigor mortis, as we have considered above, the muscle becomes inextensible and is tough when cooked (Marsh, 1964). As conditioning proceeds, the muscle becomes pliable once more (and increasingly tender on cooking); but this is *not* due to dissociation of actomyosin (Marsh, 1954); inextensibility remains. Although it was suggested (dos Remedios and Moens, 1995) that there are different levels of association between actin and myosin in rigor mortis which could change during conditioning, Hopkins and Thompson, (2001a, b) found no evidence that dissociation of actomyosin contributed to tenderness increments at this time. Increased lengthening under applied stress is observed, however, in post rigor muscle. This phenomenon appears to depend on changes in extracellular components (e.g. the mucopolysaccharide of the ground substance, the sarcolemma or collagen) which commence at the onset of rigor mortis (Dransfield *et al.*, 1986). Busch *et al.* (1972b) followed the onset of rigor mortis by observing changes in isometric tension. This increases as extensibility decreases; but, whereas extensibility remains low, isometric tension diminishes again during the so-called resolution of rigor. Increase in isometric tension post-mortem is more marked in 'red' than in 'white' muscles. This difference appears to be related to dissimilarities in the sarcolemma of these two types of muscle.

The extractability at high ionic strength of total myofibrillar proteins decreases by about 75 per cent with the onset of rigor mortis, from the value immediately post-mortem, but on subsequent storage at 2 °C the extractability again rises – up to and even beyond the initial level (Locker, 1960a). It is significant that, in addition to a predominance of actomyosin, the myofibrillar proteins extractable at high ionic strength now include α-actinin, tropomyosin, and the troponin with which the latter is associated *in vivo* (Ebashi and Ebashi, 1964; Valin, 1968). This suggests that the

* It has become evident that collagen, together with an increasingly recognized number of other proteins, can exist in an unfolded form (i.e. not uniquely folded) under physiological conditions. Moreover, it seems likely that the unfolding of collagen induced by heat may well occur at a markedly lower temperature than has hitherto been believed (Gross, 2002).

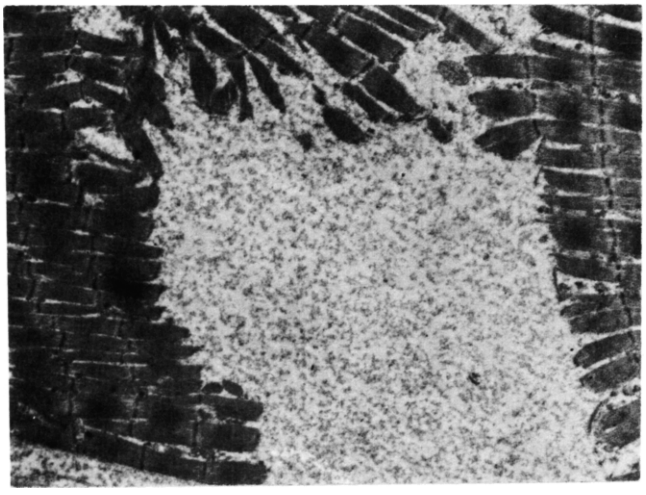

Fig. 5.2 Electron micrograph of a break across an aged, stretched muscle fibre. Each broken fibre has parted at the *Z*-line (×5000). (Courtesy Dr M. R. Dickson.)

process of conditioning detaches the actin filaments from the *Z*-line with which their union, probably via tropomyosin (Huxley, 1963), and zeugmatin (cf. Fig. 3.9) is weaker than with myosin. Figure 5.2 demonstrates that aged myofibrils break at the *Z*-lines on mild homogenizing (Davey and Dickson, 1970). The actin filaments collapse on to those of myosin, leading to lengthening of the *A*-bands (Davey and Gilbert, 1967), and there is increased weakness at the *A–I* junction, as shown by an increased gap between the *A* and *I* bands of the sarcomere (Davey and Graafhuis, 1976b).

During conditioning or ageing, the *Z*-lines in 'white' muscles appear to be more labile than those in the 'red' variety (Goll, 1970). Thus, they alter more rapidly in rabbit and porcine muscles than in those of the bovine (Henderson *et al.*, 1970), and more in bovine *semitendinosus* than in *psoas* (Goll *et al.*, 1974). Tenderness changes little in bovine *psoas* over 4 days ageing at 2 °C, whereas in *semitendinosus* it increases markedly during this period. It is significant that the latter has about three times the activity of CASF (calpains see below) as bovine *psoas*. It may be noted that 'white' muscles are less susceptible to 'cold shortening' than 'red', and that this has been attributed to their greater ability to control intramuscular concentrations of calcium ions because of a more effective sarcotubular system (§§ 4.3.5 and 10.3.3).

The extractability of myofibrillar proteins is affected by the ultimate pH of the muscle, a high ultimate pH tending towards greater extractability (cf. Table 4.29). The temperature post-mortem is also important, a high temperature being associated with lower extractability (Wierbicki *et al.*, 1956). This is partly due to the precipitation of sarcoplasmic proteins on to those of the myofibril (Bendall and Wismer-Pedersen, 1962). Some denaturation of the latter also occurs, however (cf. Table 5.3). This is implied by the greater difficulty of splitting muscle fibres into myofibrils after aseptic storage for 30 days at 37 °C than at 5 °C (Sharp, 1963), but in this case changes in the sarcoplasmic reticulum between each myofibril may be responsible (Lawrie and Voyle, 1962). Even at 35 °C denaturation of *isolated* myosin

Table 5.3 Percentage of sarcoplasmic protein precipitating from extracts of post-rigor beef *l. dorsi* (after Scopes, 1964)

Temp.:	0 °C	10 °C	15 °C	20 °C	25 °C	30 °C	37 °C	45 °C
pH								
4.5	4.4	3.8	4.2	4.0	4.4	4.7	5.1	8.1
4.8	6.6	5.7	4.8	6.4	6.6	7.4	5.3	15.0
5.2	5.0	4.9	5.5	6.2	7.8	10.5	18.5	35.0
5.7	3.1	2.9	3.2	3.1	4.2	6.2	12.2	34.0
6.0	2.1	1.9	2.2	2.5	3.2	6.3	8.5	29.0
6.5	0.6	0.8	0.7	2.1	2.8	3.4	6.6	24.5
7.1	0.4	0.4	0.6	0.5	1.2	2.1	5.2	22.0

Extracts exposed to temperature/pH conditions for 4 h.

is relatively speedy (Penny, 1967), and it can be presumed that some denaturation of actomyosin *in situ* occurs during post-mortem glycolysis.

An important aspect of changes in the myofibrillar proteins post-mortem, which is reflected in their extractability and tenderness, is the degree of shortening which occurs during the onset of rigor mortis (Locker, 1960a; Locker and Hagyard, 1963; Marsh, 1964). In muscles which go into rigor mortis in an extended condition, the filaments of actin and myosin overlap and cross-bond at fewer points, and the amount of actomyosin formed is small. Such meat is tender on cooking. On the other hand, when muscles go into rigor mortis in a contracted condition, there is considerable shortening since the actin and the myosin filaments interpenetrate extensively. There is much cross-bonding and the meat is relatively tough on cooking. Normally, muscle goes into rigor mortis in an intermediate condition wherein the overlapping of actin and myosin, the degree of cross-bonding and the toughness are somewhere between the two extremes (cf. Fig. 4.2). It has been shown that the rate of tenderizing during conditioning is minimal in muscles which have shortened substantially at onset of rigor mortis (Davey *et al.*, 1967). Lower activity of μ-calpain and increased calpastatin levels have been reported in cold-shortened muscles (Zanora *et al.*, 1998), and these circumstances must contribute to their toughness. The degree of shortening during rigor mortis is temperature dependent (cf. §§ 7.1.1 and 10.3.3).

By far the most labile proteins of muscle post-mortem are those of the sarcoplasm, the diversity of which is represented in Fig. 4.1 (p. 77). It has been realized for many years that proteins precipitate when muscle extracts of low ionic strength are allowed to stand at room temperature, the process being accelerated by raising the temperature and by the addition of salt and acid (Finn, 1932; Bate-Smith, 1937b). It has been shown, for example, that as the pH falls to acid levels during post-mortem glycolysis, glyceraldehyde phosphate dehydrogenase (cf. Fig. 4.3), which is a basic protein, denatures and combines with the more acidic myosin and actin, further promoting actomyosin formation and the predominance of actomyosin ATP-ase (rather than that of myosin) during subsequent conditioning (Matsuishi and Okitani, 2000). The behaviour of sarcoplasmic proteins in extracts from beef *l. dorsi* muscle under various temperature–pH combinations is shown in Table 5.3. It will be seen that an increase of temperature causes increasing precipitation of sarcoplasmic proteins at all pH values studied; that at all temperatures maximum

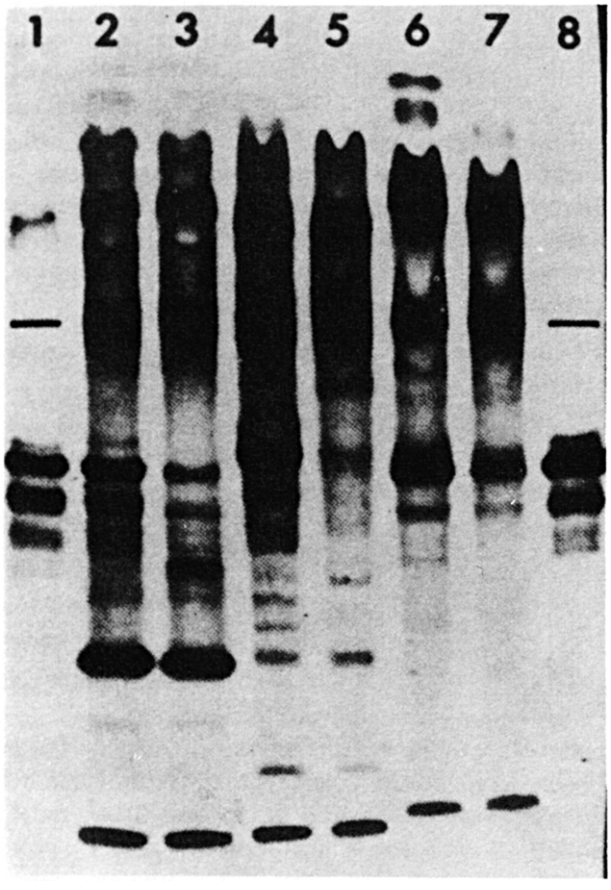

Fig. 5.3 Starch gel electrophoretograms showing relative stability of sarcoplasmic proteins in extracts from *l. dorsi* muscles of various species undergoing rigor mortis at 0 or 37 °C. (1) Purified beef creatine kinase. (2) Beef, 0 °C. (3) Beef, 37 °C. (4) Rabbit, 0 °C. (5) Rabbit, 37 °C. (6) Pig, 0 °C. (7) Pig, 37 °C. (8) Purified pig creatine kinase. (Courtesy Dr R. K. Scopes.)

precipitation occurs at a pH of 4.8–5.2; but that at some temperatures between 37 and 45 °C, a high ultimate pH no longer protects sarcoplasmic proteins against precipitation (Scopes, 1964). Even after heating at 60 °C for 10 h a proportion of the sarcoplasmic proteins is still soluble and will separate electrophoretically; but after 2 h at 80 °C almost all sarcoplasmic proteins except myoglobin have become insoluble (Laakkonen *et al.*, 1970). Obviously, even the attainment of a normal ultimate pH (about 5.5) during post-mortem glycolysis must be associated with the precipitation of some of the sarcoplasmic proteins (on the presumption that *in vivo* behaviour reflects that *in situ*). A high temperature during post-mortem glycolysis causes additional precipitation. This is exemplified in Fig. 5.3, from which it will be clear that one of the most labile of the sarcoplasmic proteins in the muscles of beef, rabbit and pig is the enzyme creatine kinase (Scopes, 1964). Particularly severe precipitation of sarcoplasmic proteins occurs during post-mortem glycolysis in the muscles of pigs which appear pale and are exudative post-mortem (cf. § 3.4.3; Scopes and Lawrie, 1963). In these there is a combination of low pH and high temperature

(Bendall and Wismer-Pedersen, 1962), and sarcoplasmic proteins precipitate on to those of the myofibril lowering their extractability and water-holding capacity. As observed, histologically, the precipitated sarcoplasmic proteins form bands across the muscle fibre (Fig. 3.11) and lower the extractability of the myofibrillar proteins, even although the latter may not be denatured themselves (Bendall and Wismer-Pedersen, 1962). The greater prevalence of such bands in affected musculature of ultimate pH 5.4 than in those of ultimate pH 4.7 and their presence in muscle of high ultimate pH (Lawrie *et al.*, 1963a) follows from the behaviour indicated in Table 5.3. There is clearly a critical temperature, between 37 and 45 °C, above which a high ultimate pH fails to keep sarcoplasmic proteins in solution. That pigs affected by the so-called PSE condition seem to have a higher temperature than normal immediately post-mortem (Bendall and Wismer-Pedersen, 1962) and that the condition is said to be induced artificially by holding pigs at 45 °C for a period before slaughter (Sayre *et al.*, 1963b) substantiates this view. It is interesting that, as already indicated, some adverse change should also occur in the myofibrillar proteins at about this temperature (Marsh, 1962).

After the ultimate pH has been reached, further changes occur in the sarcoplasmic proteins, there being a general alteration in the nature of the components (Deatherage and Fujimaki, 1964).

Denaturation of the principal muscle pigment, myoglobin, which is another of the sarcoplasmic proteins, accelerates the oxidation of its iron to the ferric form, the pigment turning brown (metmyoglobin). Although, considering the muscle as a whole, this is not an extensive process, it is, nevertheless, a very important one for it occurs preferentially near exposed surfaces or where the oxygen tension is about 4 mm (Brooks, 1935, 1938). Such factors as desiccation can initiate the denaturation and discoloration, especially where the ultimate pH is relatively low. It is also linked with still surviving activity in the oxygen utilizing enzymes (succinic dehydrogenase and cytochrome oxidase) which persists for some time at 0 °C. This matter will be referred to again below.

As far as meat quality is concerned, perhaps the most important manifestation of the post-mortem denaturation of the muscle proteins is their loss of water-holding capacity, because in practice it is a more universal phenomenon than discoloration. The point of minimum water-holding capacity of the principal proteins in muscle (i.e. the isoelectric point) is 5.4–5.5 (Weber and Meyer, 1933). Since, as we have seen in Chapter 4, the production of lactic acid from glycogen, at any given temperature and rate, will *generally* cause the pH to reach 5.5, normal meat will lose some fluid ('weep'). This will, obviously, be less if the ultimate pH is high, however (Empey, 1933).

The contribution by the sarcoplasmic proteins to overall water-holding capacity, once lost by precipitation during the attainment of even a normal ultimate pH, cannot be regained by applying a buffer of high ultimate pH to the muscle. Thus, the relatively low water-holding capacity of fibres prepared from muscle of low ultimate pH remains lower than that of fibres prepared from muscle of an intrinsically high ultimate pH, even when placed in a medium having the latter pH value (Penny *et al.*, 1963).

For a given muscle, water-holding capacity is at a minimum at the ultimate pH; thereafter, on subsequent conditioning of the meat, it tends to increase (Cook *et al.*, 1926). This may be due to an increased osmotic pressure, caused by the breakdown of protein molecules to smaller units (proteolysis will be discussed below); but much

intramolecular rearrangement, not involving splitting but causing changes in the electrical charges on the protein, may also be responsible (Bendall, 1946). Insofar as Kristensen and Purslow (2001) found that vinculin, desmin and talin were proteolysed during conditioning, they suggested that the break-up of the cytoskeleton destroyed the force expressing water to the cell exterior; and its reabsorption could be responsible for the observed increase in water-holding capacity on ageing. There is concomitantly an increase in the pH of meat when it is held above the freezing point (Sair and Cook, 1938; Wierbicki *et al.*, 1954; Bouton *et al.*, 1958). The pH rise is more marked when the temperature of holding is high and is greater in pork than in beef (Lawrie *et al.*, 1961).

These changes in pH are accompanied by changes in ion–protein relationships. Arnold *et al.* (1956) found that sodium and calcium ions are continuously released into the sarcoplasm by the muscle proteins, and potassium ions are absorbed after the first 24 h. Because of the large excess of potassium ions absorbed on to the muscle proteins, the net charge on the latter increases, and, thereby, the water-holding capacity.

5.4.2 Proteolysis

Denatured proteins are particularly liable to attack by proteolytic enzymes (Anson and Mirsky, 1932–1933; Lineweaver and Hoover, 1941). The increase in tenderness, observed on conditioning, was found many years ago to be associated with an increase in water-soluble nitrogen (Hoagland *et al.*, 1917; Fearson and Foster, 1922), due to the production of peptides and amino acids from protein. There has been much controversy as to which proteins undergo proteolysis during the holding of meat at temperatures above the freezing point.

Although *extensive* proteolysis of the collagen and elastin of connective tissue might appear to be the most likely change causing increased tenderness, the proteins of connective tissue are not normally changed in this way during conditioning in skeletal muscle. This was conclusively shown by Sharp (1959). There is no increase in water-soluble hydroxyproline-containing derivatives, even after storage of sterile, fresh meat for one year at 37 °C. Despite the absence of massive proteolysis of native collagen during conditioning, such as would require the action of a true collagenase capable of cleaving all three chains in the helical region of tropocollagen (Gross, 1970), there are lysosomal enzymes (Valin, 1970) which can attack the cross-links in the non-helical telopeptide region of collagen. In 1974 Etherington isolated two collagenolytic cathepsins from bovine spleen which cleaved the non-helical, telopeptide region of native tropocollagen between the lysine-derived cross-links and the triple helix of the main body of the molecule. This resulted in longitudinal splitting and dissociation of the protofibrils. The enzymes operated at pH 4–5 (28 °C). Suzuki *et al.* (1985) isolated a collagenolytic enzyme from rabbit muscle which is bound to collagen; and Stanton and Light (1988) provided direct biochemical evidence for the action of proteolytic enzymes on the perimysial collagen during conditioning.

Collagen fibres appear to swell during conditioning.

The collagen of the endomysium, which opposes the swelling of the muscle fibre initially, is weakened during conditioning (Wilding *et al.*, 1986). Electron micrography reveals that the sheaths of connective tissue become diffuse (Fig. 5.4; Nishimura *et al.*, 1995). Stanton and Light (1990) demonstrated that it is the type III collagen which is preferentially attacked in the endomysium during conditioning rather than

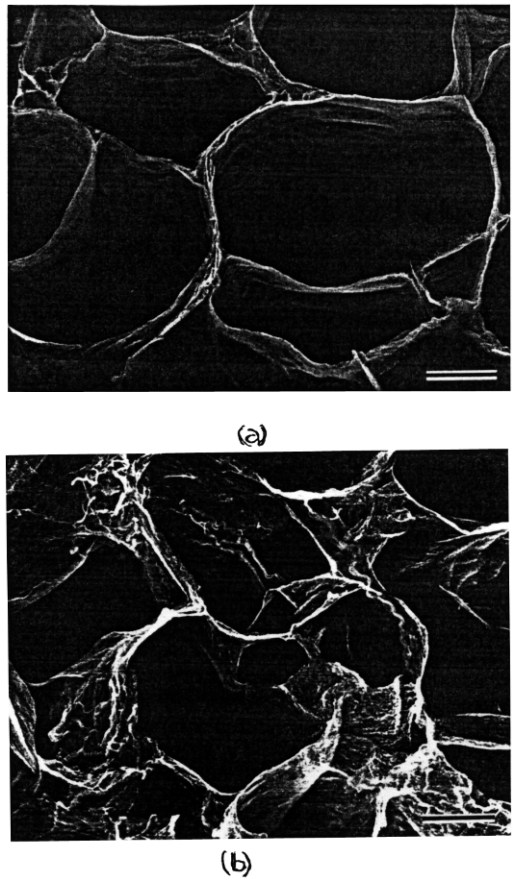

Fig. 5.4 Scanning electron micrographs of the endomysial sheaths of bovine *semitendinosus* muscle from which muscle fibres have been removed (a) immediately post-mortem and (b) after conditioning for 28 days at 4 °C, when the endomysial sheaths have become diffuse. The bar represents 25 μm. (Reprinted from Nishimura *et al.*, 1995, with kind permission from Elsevier Science Ltd. and courtesy of Prof. K. Takahashi.)

the type I component. Changes in the links between muscle fibres and their endomysial sheaths, whereby the latter are more readily removed as conditioning proceeds, may also be invoked in accounting for the swelling which is observed.

As tenderness increases, there is a concomitant increase in the titre of free β-glucuronidase (Dutson and Lawrie, 1974). This enzyme can attack the mucopolysaccharide of the ground substance or carbohydrate moieties in collagen itself. One of the points of attachment of carbohydrate to collagen is the ε-amino group of lysine; and the ε-aminoglycosylamines are probably involved in binding collagen to the ground substance (Robins and Bailey, 1972). Nishimura *et al.* (1996) clearly demonstrated that proteoglycans in both the basement membrane and the perimysium are degraded during conditioning. It may be, therefore, that splitting of both carbohydrate and peptide links contribute to increased tenderness in conditioning.

It is clear, however, that under some circumstances connective tissue proteins are much more labile than they appear to be post-mortem. Preceding the repair of

damaged muscles, collagen and elastin are evidently removed *in vivo* (Partridge, 1962). There is a general increase in phagocytic (Rickenbacher, 1959) and in proteolytic activities – the latter due to the liberation of catheptic enzymes from lysosomes (Hamdy *et al.*, 1961). Anti-inflammatory (anti-rheumatic) drugs, such as cortisone, inhibit the formation of the acid mucopolysaccharides of the ground substance of connective tissue by suppressing sulphation (Whitehouse and Lash, 1961) and decrease the amount of free hydroxyproline (Kivirikko, 1963). Vitamin C deficiency interferes with collagen formation by inhibiting the hydroxylation of soluble proline (Stone and Meister, 1962) Again, during post-partum involution of the uterus, enzymes are elaborated which are capable of breaking down connective tissue proteins to their constituent amino acids (Woessner and Brewer, 1963). It has been postulated that the collagen fibril is first attacked extracellularly by a secreted neutral collagenase and that its subsequent digestion is intracellular (Etherington, 1973), by macrophages (Parakkal, 1969; Eisen *et al.*, 1971). These reactions imply that *in vivo* muscle is capable of elaborating enzymes which proteolyse connective tissue proteins, in abnormal circumstances, even if they are not present, or are inactive, during conditioning.

Notwithstanding the absence of massive proteolysis in the collagen and elastin of fresh sterile meat, even after 1 year at 37 °C, such breakdown does occur in sterile meat which has been heated. For example, in beef held at 37 °C, after heating for 15 min at 70 °C, soluble hydroxyproline rose from about 2 per cent to about 23 per cent of the total hydroxyproline during 97 days (Sharp, 1964). In corresponding beef which had been heated for 45 min at 100 °C (being thus cooked), the value rose from 12 per cent initially to 55 per cent over the same period of subsequent holding at 37 °C. Histological examination revealed that the connective tissues of the perimysium had been weakened, since fibre bundles were easily separated from one another. In view of the preceding heat treatment, however, the breakdown of collagen (or elastin) in these circumstances can scarcely have been due to enzymic action: progressive physical changes in the connective tissue proteins are probably involved (Gustavsen, 1956).

As mentioned in § 5.4.1, the absence of changes in the extensibility of muscle in conditioning – and subsequent to actomyosin formation during the onset of rigor mortis – despite the concomitant increase in tenderness, indicated that the latter phenomenon did not involve dissociation of actin from myosin (Marsh, 1954). A similar conclusion was reached by Locker (1960b). He applied Sanger's method of N-terminal analysis (1945) to the salt-soluble proteins of beef muscle during conditioning at low and high temperature and failed to detect any significant increase in the number of protein N groups. It must be appreciated, however, that significant changes in muscle proteins, which might alter the tenderness of meat, could occur without extensive proteolysis, if a few key bonds were broken, as indicated above.

At least some of the changes in the myofibrillar proteins during conditioning are apparently initiated by the release of Ca^{++} ions from the sarcoplasmic reticulum post-mortem (the capacity of which to accumulate Ca^{++} ions decreases during conditioning: Newbold and Tume, 1976) and operate through water-soluble enzymes. These enzymes are variously referred to as calcium-activated sarcoplasmic factors (CASF), calcium-activated neutral proteinases (CANP), calcium-dependent proteinases (CDP) or calpains. It is significant that the Ca^{++}-chelating agent, ethylenediamine tetraacetate, should prevent ageing changes (Penny, 1974; Koohmaraie *et al.*, 1988).

The calpains are now known to belong to a complex family of Ca^{++}-dependent proteinases (Sorimachi *et al.*, 1997). Whereas the calpain isoenzymes 1, 2 and 3 have been long recognized, recently other members, numbered 5, 7, 10, 12, 14 and 15 have been discovered in biological tissues (Dear *et al.*, 1997). Of these, Ilian *et al.* (2004) concluded that 10 was strongly correlated with the degree of tenderization of meat during ageing through its action on nebulin and desmin. The calpain proteolytic system in mammalian striated muscle comprises the ubiquitous enzymes, calpains 1, 2 and 10, and a tissue-specific isozyme, calpain 3 (Goll *et al.*, 2003).

The calpains are inhibited by the protein, calpastatin (cf. §4.3.5). The helical sequences of calpastatin prevent calpains from binding to membranes (Mellgren *et al.*, 1989). The calpains degrade desmin (Granger and Lazarides, 1978; Penny, 1980; Slinde and Kryvi, 1986) and weaken the binding of α-actinin to the *Z*-disc. Tropomyosin and *M*-line protein are also degraded (Penny, 1980). Penny and Dransfield (1979) and, later, Nishimura *et al.* (1996) showed that, during conditioning of beef muscles, troponin T is proteolysed, with concomitant production of four peptides, of which the principal member has a MW of 30,000. Earlier it had been demonstrated that CASF degrades the so-called 'gap filaments' (Locker, 1976) (connectin, titin; Maruyama *et al.*, 1979). Thus when *stretched* muscles are aged, subsequent cooking causes the 'gap filaments' to disappear (whilst the *Z*-lines appear intact; Davey and Graafhuis, 1976b) and Young *et al.*, 1980 detected increased solubility of connectin and aged beef muscle. In a detailed investigation of ageing changes in rabbit muscles, Mestre-Prates *et al.* (2002) proposed that tenderness was due to specific cleavage of titin and nebulin in the vicinity of the N_2 line by calpains; and these enzymes have been shown to degrade the integrin complex by which the cell proteins are attached to the cell membrane (Lawson, 2004; cf. §§ 3.2.2. and 10.2).

It is worth noting that the calpain system has no action on actin or myosin *per se* (Penny, 1974; Robson *et al.*, 1974) and that it is, in fact, located at the *Z*-line (Goll *et al.*, 1992). Koohmaraie *et al.* (1987) demonstrated that during storage of *l. dorsi* muscles over 14 days at 0 °C, there was a concomitant increase in myofibrillar fragmentation (as an index of conditioning changes) and a decrease in the activity of that calcium-activated neutral proteinase which depends on a *low* concentration of Ca^{++} ions (calpain I, μ-calpain), whereas the CANP which depends on a relatively high concentration (calpain II m-calpain) remained unchanged in activity. About 50 per cent of the total change in these parameters occurred during the first 12 hours, when the temperature was falling from *ca.* 37 to 10 °C, indicating that the tenderness increment during conditioning may well commence before the ultimate pH has been reached, especially if the temperature is slow to fall.

Because of the difficulty of attributing conditioning changes precisely to individual proteases and their inhibitors *in situ*, models of the system have been studied. Dransfield (1992, 1993) and Dransfield *et al.* (1992) examined extracts of beef muscle *in vitro* when these were stored at a range of temperatures from 0 to 30 °C. They postulated that the low levels of free Ca^{++} ions in the immediate post-mortem period would be insufficient to activate calpain I, but that, when the pH had fallen to *ca.* 6.1, the Ca^{++} level would have become high enough to activate this enzyme (calpain II would act similarly, but at a greater Ca^{++} level). At this pH calpains are bound to the inhibitor, calpastatin, but this inhibitory action falls as the pH drops further, from 6 to 5.5 and the activated calpains proteolyse the calpastatin. Tenderizing is initially due to the action of calpain I. Subsequently calpain II is

responsible: it ceases as the calpains self-destruct by autolysis (Dransfield *et al.*, 1992; Dransfield, 1993). Subsequently, Dransfield (1994), in a detailed assessment of the data provided by modelling, concluded that variability in the post-mortem activity of calpains *per se* can be adduced to account for toughness, irrespective of such factors as sarcomere length. It must be acknowledged, however, that such models omit consideration of the contribution of the lysosomal cathepsins, of their activators and inhibitors and of the 'multicatalytic proteinase complex' (Orlowski, 1990) to post-mortem tenderizing (cf. below). Since the activation energy for the autolysis of calpain I is higher than for its proteolytic activity, less intense tenderizing occurs at higher temperatures. In addition to the solubilizing action of the calpains, however, it has been suggested that Ca^{++} ions *per se* cause non-enzymic 'salting-in' changes in certain myofibrillar proteins (Taylor and Etherington, 1991). Moreover, Tatsumi and Takahashi (1992) and Takahashi (1992) were able to demonstrate that *in vitro* 0.1 mM calcium chloride caused fragmentation of nebulin and the release of paratropomyosin (from the A-I junction region) which then binds to actin filaments. These non-enzymic factors lead to disruption of the latter and to lengthening of the sarcomeres. They thus concluded that the increasing level of Ca^{++} ions, as they are released from the sarcoplasmic reticulum post-mortem, contribute directly to the tenderizing changes during ageing. Although Whipple *et al.* (1994) postulated that most of the increase in tenderness was due to stimulation of the calpains by the Ca^{++} ions investigations by Hopkins and Thompson (2001b) could not confirm that they had a rôle in the process independently of the calpains.

The proteolysis of troponin T and increase of tenderness correlate well when conditioning takes place between 3 and 15 °C (Penny and Dransfield, 1979). At higher temperatures (25–35 °C), when protein denaturation is a contributory factor, and at 0 °C, when 'cold-shortening' may be anticipated, toughness is greater than the degree of proteolysis of troponin T would predict. On the other hand, the tenderness of electrically stimulated muscles is greater, for a given degree of troponin T breakdown, than that of controls (George *et al.*, 1980), suggesting that, although the proteolysis of troponin T is a useful indicator of change during conditioning, other factors must be considered.

There is at least one other enzyme system involved in meat conditioning, namely that of the lysosomes already mentioned. In contrast to the calcium-activated sarcoplasmic factors (calpains) which have pH optima above 6, the cathepsins (B, D, H and L) of the lysosomes represent a series of proteolytic enzymes with pH optima below 6 (Penny and Dransfield, 1979; Etherington, 1984). Of these, cathepsin H cannot degrade native myofibrillar proteins and, although cathepsin D can degrade myofibrillar proteins below pH 5, its action in post-mortem conditioning at the normal ultimate pH of 5.5 is minor (Ouali *et al.*, 1987).* On the other hand, both cathepsins B and L can degrade these proteins in post-mortem muscle (Bird *et al.*, 1977; Matsukura *et al.*, 1981). Cathepsin L is probably the most important lysosomal proteinase in conditioning (Mikami *et al.*, 1987). It degrades troponins T and I, and C-protein rapidly, and titin (connectin), nebulin, α-actinin, tropomyosin, actin and the light and heavy chains of myosin slowly. Its action at pH 5.5 is faster than

* *In vitro* examination has shown that cathepsin D can degrade (bovine) F-actin at numerous locations, bonds containing at least one hydrophobic amino acid residue being preferentially cleaved (Hughes *et al.*, 2000).

at pH 6, but slower than at pH 5 (Mikami *et al.*, 1987). The breakdown of myosin is not marked unless circumstances are exceptional (e.g. pH below 5, over 24 h at 25 °C: Penny and Ferguson-Pryce, 1979), or when meat is stored at 35 °C for some days (Penny and Dransfield, 1979).

The structurally important sites of enzyme actions by calpains and lysosomal enzymes (including cathepsins B, D and L) during conditioning can be summarized:

(a) *CASF (calpains)*
  (i) troponin T (above pH 6)
  (ii) *Z*-lines (desmin)
  (iii) connectin, 'gap filaments'
  (iv) *M*-line proteins and tropomyosin
(b) *Lysosomal enzymes (including cathepsins B, D and L)*
  (i) troponins T and I (below pH 6) and C-protein: relatively rapidly
  (ii) myosin (heavy and light chains), actin, tropomyosin, α-actinin, nebulin and titin ('gap filament'): relatively slowly above pH 5 or below 35 °C
  (iii) cross-links of non-helical telopeptides of collagen
  (iv) mucopolysaccharides of ground substance.

A third source of proteolytic activity has been discovered. This resides in a large complex of molecular weight *ca.* 700 kdalton, which consists of at least three distinct sub-units, each having different proteolytic activity against hydrophobic, basic and acidic amino acid sequences (Wilk *et al.*, 1979; Wilk and Orlowski, 1983). Since there is evidence that these different enzymic components cooperate in their proteolytic action (Wilk and Orlowski, 1983) and that disruption of the complex causes complete loss of proteolytic activity, it appears to be a functional unit and to justify the name 'multicatalytic protease complex' (Orlowski, 1990). Also referred to as the proteasome, its 20S component rapidly hydrolyses myofibrillar proteins *in vitro* (Robert *et al.*, 1999). Proteasome activity persists post-mortem and, after the action of μ and m-calpains, participates in a second wave of proteolysis, attacking denatured proteins (Lamare *et al.*, 2002). The complex has been identified in many tissues. Its presence in muscle suggests that it must be considered in elucidating proteolysis in this tissue.

Since most of the proteins of connective tissue and the myofibrils are not subjected to *extensive* proteolysis during conditioning, the considerable increments in the soluble products of protein breakdown must arise from the sarcoplasmic proteins. As we have seen, these denature in varying degrees during post-mortem glycolysis (§ 5.4.1); and chromatography of extracts prepared from muscle after increasing periods of storage show a gradual diminution of various components (Deatherage and Fujimaki, 1964).

During storage at 37 °C of sterile *l. dorsi* muscles of beef, the total soluble *protein* nitrogen was found to fall from 28 to 29 per cent of the total nitrogen to 13, 11 and 6 per cent after 20, 46 and 172 days respectively (Sharp, 1963). The lowered concentration of sarcoplasmic proteins was due rather to their proteolysis to amino acids and not to precipitation, which could only account for a small amount of the diminution: the nitrogen soluble in trichloroacetic acid rose from 11 per cent of the total protein to 17, 23 and 31.5 per cent respectively in these same periods. Moreover, in terms of a specific amino acid, the percentage of total tyrosine soluble in trichloroacetic acid rose from 11 per cent initially to 13, 17 and 35 per cent over 20, 46 and 172 days respectively (Sharp, 1963). Comparable changes

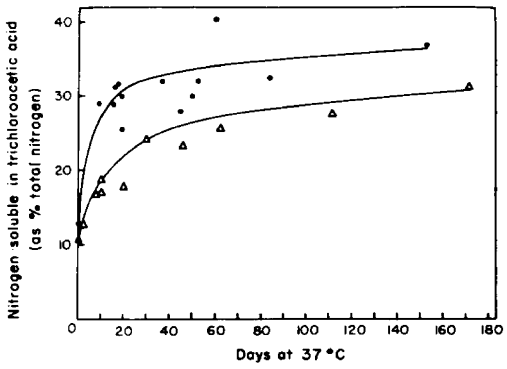

Fig. 5.5 Production of nitrogen soluble in trichloroacetic acid during aseptic storage of muscle at 37 °C. ●–●, rabbit muscle, △–△, beef muscle. (Courtesy of the late Dr J. G. Sharp.)

were found in rabbit *l. dorsi* although the rates of proteolysis are different in the two species (Fig. 5.5), and even between different muscles (J. G. Sharp, personal communication).

Ouali and Talmant (1990) and Monin and Ouali (1991) have confirmed such differences in extensive studies and established the basis for them. Variability in the rate of ageing reflects the contents of calcium-dependent proteinases (calpains I and II and their inhibitor, calpastatin), of lysosomal cathepsins B and L (and their inhibitors), the relative resistance of the muscle proteins to proteolysis and the intramuscular osmotic pressure. In turn, these variations are systematically related to the proportions of 'red', 'white' and intermediate type fibres which characterize the muscles concerned.

According to Radouco-Thomas *et al.* (1959), proteolysis is less marked in the muscles of pigs and sheep than in those of lamb and rabbit under comparable conditions. In a comparative study of conditioning changes in *l. dorsi*, Dransfield *et al.* (1981) confirmed these findings. Rates of tenderness increments in beef, lamb, rabbit and pork were, respectively, 0.17, 0.21, 0.25 and 0.33% per day. Subsequently, Etherington *et al.* (1987) showed that the relative rates were reflected by the relative concentrations of cathepsins B and L in the muscles; and Ouali and Talmant (1990) demonstrated that the rates were also correlated with the ratio of calpain II: calpastatin in the muscles of different species.

It is most important to note that these observations refer to a normal ultimate pH (i.e. about 5.5). At a higher ultimate pH the extent of proteolysis is less (Radouco-Thomas *et al.*, 1959). Thus, in rabbit *l. dorsi* after storage for 16 days at 37 °C, 17 per cent of the total tyrosine was soluble when the ultimate pH was 5.8. The corresponding value was only about 9 per cent, however, when the ultimate pH was 6.8 (Sharp, 1963); and there was a smaller degree of disintegration of muscle fibres during homogenizing.

Dransfield (1993) confirmed that, although the *extent* of proteolysis is relatively less at high ultimate pH, its *rate* is increased. The relationship between the rate of proteolysis and the ultimate pH was shown to be complex by Watanabe *et al.* (1996) in an extensive study of ovine *l. dorsi* of ultimate pH ranging from 5.5 to 7.0, the rate being minimal at *ca.* pH 6.0 and greater below and (especially) above this value. On the other hand myofibrillar fragmentation was least at pH 6.4.

Table 5.4 Dimensions of fibres present in greatest number in low-speed homogenates of sterile stored beef *l. dorsi* muscle of normal ultimate pH

Storage characteristics	Length (μm)	Diameter (μm)
Control (2 days at –20 °C)	650–1300	200–600
30 days at 37 °C	250–430	43–170
30 days at 5 °C	50–170	14–86

The extent of proteolysis is also temperature dependent, being greater at 37 °C than at 5 °C, although the degree of histological breakdown, as shown by the cohesiveness of fibres after homogenizing, is much greater at 5 than at 37 °C. This is, presumably, because there is a greater degree of denaturation of the myofibrillar proteins at the higher temperature (Table 5.4, Sharp, 1963) which would oppose breaking up of the tissue.

Low ultimate pH may enhance proteolytic activity in another way. The lysosomes, which contain enzymes having proteolytic activity and acid pH optima (De Duve, 1959a), have lipoprotein membranes which, whilst intact at *in vivo* pH levels under normal conditions, rupture when the pH falls post-mortem, or when there has been extensive tissue damage (Hamdy *et al.*, 1961), and liberate the proteolytic enzymes. The permeability of these membranes appears to be controlled by the vitamin A status of the tissue, hypervitaminosis A being associated with undue fragility (Fell and Dingle, 1963). It is also lowered following tissue breakdown (De Duve, 1959b) as in the dystrophies due to recessive genes or to vitamin E deficiency (Tappel *et al.*, 1962); in such dystrophies the activity of the lysosomal proteolytic enzymes is increased.

It is evident that some proteolytic activity may be due to residual blood in the muscle (Shestakov, 1962), and in 1974 Bailey and Kim showed that the proteinases from the porcine leucocyte lysosomes can degrade myofibrillar proteins. The question of whether the proteolytic activity observed in muscle post-mortem is a property of lysosomes intrinsic to the tissue or of those belonging to entrained phagocytes was resolved by Canonico and Bird (1970) who demonstrated that the former had relatively greater contents of acid phosphatases than of cathepsins. Venugopal and Bailey (1978) compared the lysosomal proteinases of the muscular tissue and leucocytes of beef and pork. They found that cathepsins D and E,* which had pH optima of 4.0 and 2.5, respectively, were the most active proteolytic enzymes found in both tissues, and that all the enzymes from the lysosomal leucocytes were more active than their counterparts in the lysosomes of the muscular tissue (cf. Table 5.5).

It has always been difficult to accept that proteolytic enzymes have only catabolic functions in muscle. It is now evident, however, that the calpain/calpastatin enzyme system is involved in protein turnover (§ 2.5.2.1), in the control of muscular excitation (§§ 3.2.2 and 4.2.1) and, indeed, in a number of other intracellular processes which are mediated by Ca^{++} ions.

* Terminology of Barrett (1977).

Table 5.5 Specific activity of bovine lysosomal proteinases (after Venugopal and Bailey, 1978) (pH of measurement in brackets)

Source	Enzymes						
	Carboxypeptidases		Cathepsins			Collagenase	Dipeptidyl-aminopeptidase I
	A (5.0)	B (6.0)	B (7.8)	D (4.0)	E (2.5)	(7.0)	(6.8)
Leucocytes	30	23	110	2751	1482	0.75	140
Muscle (diaphragm: 10,000g fraction)	12	8	24	1878	1132	0.15	28

5.4.3 Other chemical changes

By the time the ultimate pH has been reached, ATP has been largely broken down to inosinic acid, inorganic phosphate and ammonia (§ 4.2.3). Although some degradation of inosinic acid to phosphate, ribose and hypoxanthine will have occurred at this stage, the latter process is substantially a function of time, temperature and pH after the attainment of the ultimate pH (Solov'vev, 1952; Lee and Webster, 1963). According to Howard *et al.* (1960b), conditioning is organoleptically at an optimum when the hypoxanthine level has reached 1.5–2.0 μmoles/g. In beef this is attained after 10–13 days at 0 °C, 4–5 days at 10 °C, 30–40 h at 20 °C and 10–11 h at 30 °C (Lee and Webster, 1963). The rate of hypoxanthine formation is increased by a high ultimate pH, however, and this circumstance must be considered when assessing the time–temperature history of meat.

In view of the development of flavour which accompanies conditioning it is of interest that many years ago hypoxanthine, or its precursor inosinic acid, was reported to enhance flavour when added to meats (Kodama, 1913). It has been shown that inosinic acid (or inosine and inorganic phosphate) when heated with a glycoprotein containing alanine and glucose (also isolated from the water-soluble extracts of beef) produces a basic meat flavour and odour (Batzer *et al.*, 1962). The breakdown of protein and fat during conditioning also contributes to flavour by producing hydrogen sulphide, ammonia, acetaldehyde, acetone and diacetyl (Yueh and Strong, 1960); but prolonged conditioning, e.g. 40–80 days at 0 °C is associated with loss of flavour (Hoagland *et al.*, 1917). And, of course, where oxidative rancidity occurs in fat, the products affect flavour in a highly adverse manner (Lea, 1939). Oxidative rancidity in fat is retarded by a high ultimate pH as also is the oxidation of myoglobin (Watts, 1954) with which it is frequently linked. These phenomena will be considered in more detail in a later chapter.

Apart from the increase in free amino acids arising from proteolysis, their concentration is also augmented by the breakdown of various peptides. During conditioning, for example, the dipeptides carnosine and anserine are progressively hydrolysed to β-alanine and histidine (Bouton *et al.*, 1958). The accumulation of free amino acids, and of soluble carbohydrates, such as glucose (by the action of α-amylase on glycogen; Sharp, 1958), glucose–6-phosphate (one of the intermediaries in the glycolytic pathway), ribose (from nucleotide breakdown) and other sugars in traces, is potentially undesirable. During the preparation of dehydrated meat, for example, the carbonyl groups of the carbohydrates will combine with the amino

nitrogen of amino acids non-enzymically to form unsightly brown compounds which are also troublesome in having a bitter taste. The Maillard reaction, as it is known, may also take place between the sugars and intact protein (Lea and Hannan, 1950).

Although conditioning enhances the water-holding capacity of proteins to some extent, the loss due to denaturation changes and to post-mortem pH fall predominates, and meat exudes fluid post-mortem.