
KONGRESS- DOKUMENTATION

WISSENSCHAFTLICHE BEITRÄGE
PROCEEDINGS

II

HELSINGIN YLIOPISTO
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LAITOS



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PROCEEDINGS

II

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Zusammengestellt von
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E

1. SYMPOSIUM

**DER EINFLUSS VON CALCIUM IONEN AUF POSTMORTALE
VERÄNDERUNGEN IM FLEISCH**

**INFLUENCE OF CALCIUM IONS ON POST MORTEM
CHANGES IN MEAT**

Die elektrische Stimulierung von Schlachttierkörpern als Methode cold-shortening und Zähwerden von Fleisch zu verhindern

JAMES R. BENDALL

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Die elektrische Stimulierung von Schlachttierkörpern unmittelbar nach dem Schlachten wurde als Mittel gegen das cold-shortening und Zähwerden von Fleisch vorgeschlagen. Diese Erscheinungen sind zu beobachten, wenn Tierkörper nach dem Schlachten schnell abgekühlt werden. Eine kurze Beschreibung der Biochemie des cold-shortening sowie eine Hypothese zur Erklärung für den Effekt des Zähwerdens werden vorgetragen.

Die Veränderungen im Muster der Säugetiermuskulatur bei Eintritt des rigor mortis von stimulierten Schlachttierkörpern werden diskutiert und eine geeignete Methode der Stimulierung von Rinder- und Lammschlachttierkörpern wird vorgetragen werden.

Electrical Stimulation of Carcasses as a method of avoiding the Cold-shortening and Toughening of Meat.

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The electrical stimulation of animal carcasses shortly after slaughter has been suggested as means of avoiding the cold-shortening and toughening of meat which occurs if carcasses are chilled quickly. The biochemistry of cold-shortening will be briefly described and a hypothesis to explain its toughening effect will be presented.

The changes in the pattern of rigor mortis in mammalian muscles from stimulated carcasses will be discussed, and a suitable method of stimulation of beef and lamb carcasses will be presented.

E 1:2

La stimulation électrique des carcasses comme une méthode à empêcher cold-shortening et le durcissement de la viande

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La stimulation électrique des carcasses immédiatement après l'abattage a été proposée comme moyen contre le cold-shortening et le durcissement de la viande. Ces apparations sont à observer si les carcasses sont refroidis rapidement après l'abattage. Une courte description de la biochimie du cold-shortening ainsi qu'une hypothèse à expliquer l'effet du durcissement seront présentées.

Les changements du dessin de la musculature des mammifères au commencement du rigor mortis des carcasses stimulés seront discutés et une méthode propre à stimuler des bovins et des agneaux sera présentée.

Электрическая стимуляция туш как метод избежания сокращения мышц из-за холода и жесткости мяса

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Электрическая стимуляция туш вскоре после убоя предложилась для избежания сокращения мышц из-за холода и жесткости мяса; эти явления встречаются в быстро охлажденных тушах. Дается короткое описание биохимии сокращения мышц из-за холода, и представляется гипотеза с целью объяснения его действия в развитии жесткости.

Обсуждаются изменения характера посмертного окоченения мышц из-за стимулированных туш млекопитающих, и представляется подходящий метод стимуляции туш крупного рогатого скота и ягнят.

Electrical stimulation as a means of obviating cold-shortening of muscles during rapid chilling of carcasses

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Introduction

Cold-shortening or cold-shock (Locker and Hagyard, 1963) has for several years been recognised as the main cause of the toughening which develops on cooking meat, chilled or frozen rapidly within a few hours of slaughter (Marsh and Leet, 1966). As originally described by Locker and Hagyard (1963), the phenomenon was defined as the shortening which occurs spontaneously when muscles, particularly of the slow red type, are cooled below about 10° in the early pre-rigor period during which the ATP content of the muscle is high and the pH is above about 6.6. This type of shortening is reversed by merely raising the temperature above 10° during this pre-rigor period, and is also to some extent spontaneously reversible, if the muscle is under load or tension (Bendall, 1975). In many muscles however, this slow relaxation phase is succeeded by a phase of very slow re-shortening as the ATP level begins to fall during the rigor period proper. Because of this second phase of slow but extensive re-shortening, it is unsafe to begin to cool lamb or beef carcasses rapidly until a pH of 6 or below has been reached; in beef muscles more than 50% of the initial ATP content of the muscles has been destroyed at this pH. This means in normal abattoir practice that the carcasses must be hung at an ambient temperature of 12° or above for at least 12h before rapid cooling can be safely begun.

About 5 years ago, workers in New Zealand (Carse, 1973; Chrystall and Hagyard, 1976; Davey et al., 1976) began to develop a method of electrically stimulating lamb and beef carcasses shortly after slaughter with the aim of accelerating the post-mortem fall of muscle pH and destruction of ATP, and thus shortening drastically the waiting period necessary before rapid chilling could be safely begun without danger of cold-shortening. Electrical stimulation as a practical technique has now been fully developed and is currently to be introduced into New Zealand abattoirs and hopefully into British abattoirs also.

In the present paper we shall confine our attention mainly to beef carcasses.

Materials and methods. The beef carcasses used in this work are those reported on by Bendall et al., (1976) and the analytical methods also follow the procedure of these authors and those of Bendall (1975, 1976). Cold-shortening was studied by the methods of Bendall (1973a and b, 1975). The electrical stimulation method we recommend at present is as follows: a pair of electrodes of one polarity is attached to the Achilles tendons of a beef carcass as soon as possible after slaughter; another pair of electrodes of opposite polarity is attached to the severed neck muscles. A pulsed current of about 4 amps (peak) is then applied at 600-700V (peak) and 25 pulses per sec (25 pps) for 2 min, the polarity of the electrodes being reversed each 30s. The form of the pulses can be either a square wave or the upper half-sine-wave derived from a 50Hz AC mains supply. Stringent precautions must be taken to isolate the apparatus and also the carcass from earth by hanging it from a nylon rope on the metal abattoir rails.

Results and discussion

1. Outline of the cold-shortening phenomenon. Cold-shortening occurs in all muscles of the red slow type, so that most beef muscles cold-shorten. In some animals (for example the pig) very pale muscles such as the *M. longissimus dorsi* (LD), also cold-shorten, but this is exceptional (Bendall, 1975). Here we shall confine our attention mainly to the LD muscles of beef animals.

Fig.1 illustrates a typical experiment with 3 strips of beef LD muscles (40mm long; 8mm diameter) plunged into a bath of paraffin at 2°C at $t=0$. The strips were loaded with weights, the loads being 7, 36 and 77 gcm^{-2} , respectively. In each case we see two clearly defined phases of shortening, one beginning immediately the strips were placed in paraffin at 2° and reaching a maximum in 0.5 to 0.75h, after which a phase of partial relaxation began, and a second phase beginning at about 3h and continuing until 24h. The second phase coincided with the slow decline in the ATP content.

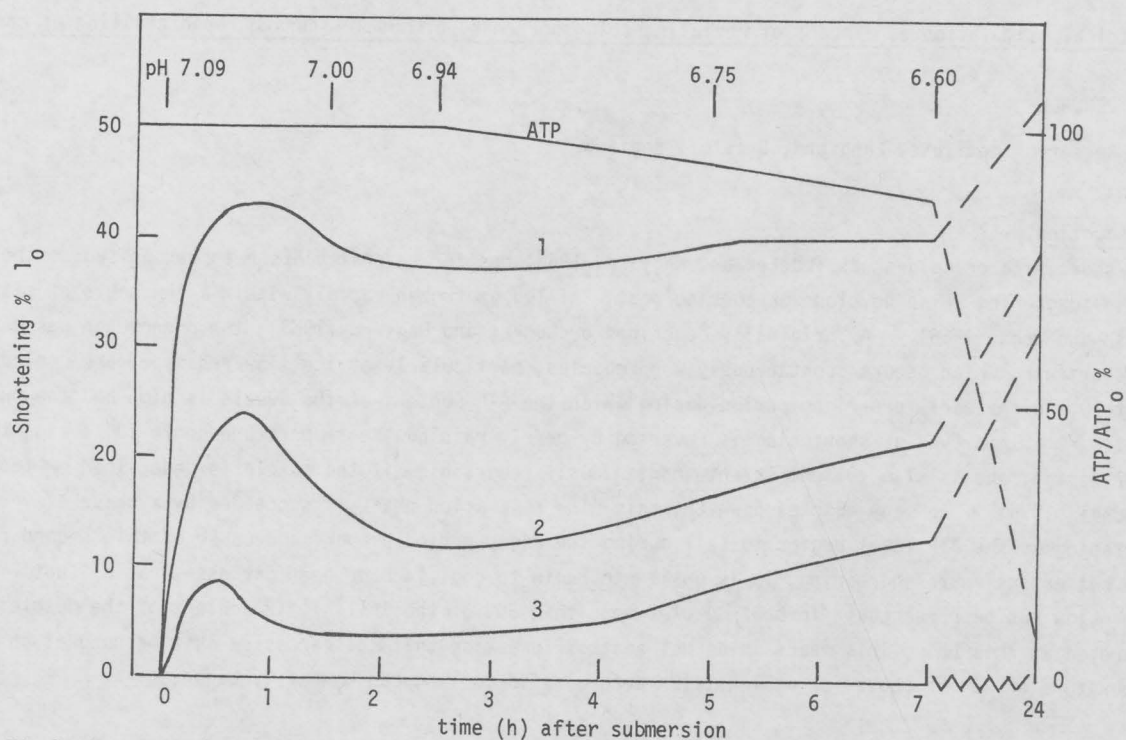


Fig.1. The shortening of pre-rigor strips of beef LD muscle under various loads, after submersion in a paraffin bath at 2°C. The changes in the ATP content of the strips and their pH are also shown.

Curve 1 : load = 7 gcm⁻²
 2 : " = 36 "
 3 : " = 77 "

Both shortening phases are load dependent, the first much more so than the second. In phase 1, increasing loads restrict not only the extent of shortening but also its velocity. Thus the initial velocities of shortening in this phase, in increasing order of loads are 4.5, 1.2 and 0.6% l_0 /min. The velocities of lengthening in the succeeding relaxation phase are also load dependent, but are much lower at each load than those of the shortening. The velocities of relaxation as % of the velocities of shortening, in increasing order of loads, are 4.0, 17 and 27%.

The velocity of reshortening after relaxation phase is over is far less than that in phase 1, the initial velocities for the two higher loads being only 0.05% l_0 /min. The extent of shortening in this second phase is also much less load dependent than that of phase 1, the total reshortening at 24h being, in increasing order of loads, 15.5, 27.0 and 28.0% l_0 . These features of phase 2 shortening are what would be expected for rigor-shortening which, at higher temperatures, always accompanies the decline of the ATP-level in the so-called fast phase of rigor (Bendall, 1951). Whatever the cause of this reshortening, it is clearly of great importance in muscle cooling under tension on a carcass, particularly because it can develop more force than phase 1 shortening.

The reshortening in phase 2 starts at about pH 6.8 (Fig.1) and continues until the final pH of 5.5 is reached. This is why it is advisable to wait until a pH of 6.0 has been reached in the major muscles before beginning rapid chilling or blast-freezing of lamb or beef carcasses.

2. The biochemical mechanism of cold-shortening.

The mechanism of cold-shortening in phase 1 is not yet fully understood, but in common with all other known contractile responses in muscle (Ashley and Moisescu, 1972), the trigger must almost certainly be release of Ca²⁺ ions, either from the Ca-pump of the sarcoplasmic reticulum (SR) (Bendall, 1973b) or from the mitochondria (Buege and Marsh, 1975), into the intrafibrillar spaces and their subsequent activation of the contractile actomyosin ATP-ase.

According to Buege and Marsh (1975), mitochondria would release the calcium they contain as soon as they became

anaerobic after death (Lehninger et al. 1967; Cheah and Cheah, 1976). If the muscle were cooled rapidly below 12° at this stage, it might be expected that the released Ca^{2+} ions would be present at sufficiently high concentration for long enough to activate the contractile system, particularly as the Ca-pump of the SR works slowly at these low temperatures. Eventually, however, the pump would catch up and remove the activating Ca^{2+} ions, so that relaxation would ensue, as indeed is observed (Fig.1). In support of this hypothesis, Buege and Marsh point out that admission of oxygen to a cold-shortening muscle inhibits and partially reverses the shortening, because under restored aerobic conditions the mitochondria can pump Ca^{2+} ions back into themselves. A further attractive feature of this hypothesis is that it might explain the increasing intensity of cold-shortening in redder muscles because they contain more mitochondria.

Unfortunately Buege and Marsh's hypothesis runs into three serious objections. First, the particular muscle they chose to study (beef M.sternomandibularis) can be left at 15° under anaerobic conditions for an hour or more after excision without in any way impairing the cold-shortening which will set in if it is then cooled below 12° (Bendall, unpublished observations; Scopes, 1971). During this hour it would be expected that the mitochondria would have lost all their store of Ca^{2+} ions and that these would have been pumped into the SR, and would thus become unavailable for release during the subsequent cooling.

Secondly, the hypothesis fails completely to explain the well-known reversibility of cold-shortening when the temperature is raised above 12° , a process which can be repeated several times with the same muscle strip (Bendall, 1973b). As in the first objection, this cooling and re-heating of the muscle would be expected to completely denude the mitochondria of Ca^{2+} .

Thirdly, in thaw contracture (Bendall, 1973b), which in many ways resembles cold-shortening but is faster and more forceful, a muscle rapidly frozen in the pre-rigor period and then thawed starts to shorten as soon as any part of it passes through the critical temperature zone for cold-shortening (0 and 12°). As the temperature rises above 12° , relaxation immediately begins (Bendall, 1973b). Now the contradiction which arises in this case is that thaw-contracture is just as vigorous if not more so in very white muscles (rabbit psoas) as it is in red muscles (beef sternomandibularis). Hence in the former case, mitochondria can play no role in the process.

An alternative hypothesis to that of Buege and Marsh can be advanced which explains the differences between red and white muscles. In the first place, isolated SR from the red sternomandibularis of beef animals pumps calcium at about 1/7th of the specific rates characteristic of the white LD muscle of the pig and the white psoas of the rabbit (Dr.R.E. Jeacocke, unpublished observations). This in itself would suggest that red muscle should be much more susceptible to low temperature than white muscle. Secondly, the temperature coefficient of Ca-pumping by the SR of both white and red muscles is much higher below 17° than above (Inesi et al., 1973; Madeira and Antunes-Madeira, 1975); in other words the rate of pumping has fallen by a factor of 4.2x between 32° and 17° , but 14x between 17° and 2° . If one assumes that the steady rate of Ca-leak through the SR membrane actually increases below 12° during sudden cooling, i.e. a cold-shock effect perhaps on the lipids of the SR membrane, then combination of this effect with the low rate of pumping would satisfactorily explain the greater degree of cold-shortening in red than in white muscles. To explain the subsequent spontaneous relaxation, one would have to assume that the postulated membrane changes were slowly reversed, so that eventually the Ca-pump would slowly recover the released Ca^{2+} ions. In addition this hypothesis simply explains the reversibility of cold-shortening on raising the temperature above 15° .

3. The toughening due to cold-shortening.

When a muscle cold-shortens on a carcass before and during rigor, most of the fibres which have actively shortened become fixed at the short length and remain in this state indefinitely thereafter. There is, however, very great variation in the degree of shortening amongst the mass of fibres which make up a large muscle, some being quite long and kinked (Voyle, 1969) and others supercontracted. In the super-contracted fibres, the myosin filaments of the sarcomeres are compressed tightly together, so tightly in fact that contraction nodes of overlapping myosin filaments appear in the Z-disc region of the sarcomere. EM photos of these structures readily suggest that the overlapping is the direct cause of the toughening associated with cold-shortening.

However, this naive view of the toughening effect is directly contradicted by experimental evidence because as Table 1 shows, uncooked cold-shortened meat becomes more tender the greater the shortening, whereas when the same meat is cooked it becomes tougher (Dransfield and Rhodes, 1976). Hence the toughening effect is directly due to some change during cooking.

Two main changes occur during cooking, first the coagulation of the fibrillar and sarcoplasmic proteins, which occurs between 45 and 60^o, and second the denaturation and shrinkage of collagen fibres which begins above 63^o (Davey and Gilbert, 1974). The

second change causes, of course, most of the shrinkage which occurs during cooking to the usual internal temperature of say, 75^o. This shrinkage will cause further compression of the already highly compressed sarcomeres of cold-shortened meat, and may well account for a large part of the extra toughening. However, the fact that the myosin filaments are compressed together in cold-shortened meat suggests that they may well coalesce during cooking to give a tough continuum of coagulated filaments amongst which are entangled the remains of the actin filaments of the I-bands. This is essentially the hypothesis of Marsh and Carse (1974). Dransfield and Rhodes (1976) on the other hand suggest that the shrinkage of collagen during cooking may be the more important factor, and a further hypothesis in terms of the so-called gap-filaments in the sarcomeres has been put forward by Locker et al. (1977). The latter has not as yet been confirmed by other workers.

4. Electrical stimulation as a means of accelerating the post-mortem changes.

Electrical stimulation of carcasses soon after slaughter, either just before or just after degutting and dehiding, has the effect of causing an extremely rapid fall of muscle pH to 6.2-6.4 (Fig.2), thus exhausting about half of the total energy reserves of the muscles during the 2 min duration of stimulation a process which normally occupies some 7h after death. After stimulation the rate of pH fall appears to be much higher than that in muscles from unstimulated carcasses (Fig.2) but both temperature and pH conditions are quite different in the two cases.

In the first place, it is essential to compare the rates of pH fall in the same range of pH, i.e. below pH 6.3, and it is a feature of most pH/time curves at constant temperature that the pH always falls faster in this range (about twofold) than in the range from pH 7 to pH 6.6 (Bendall et al. 1976; Bendall, 1978, in press). Thus if one corrects the pH/time curves in Fig.2 back to a temperature of 38^o, utilising Jeacocke's (1977) extensive data on the energy of activation of the process, there is seen to be virtually no difference between the rates of pH fall (below pH 6.3) in control and stimulated muscles. Although Chrystall and Devine (1978) report an accelerating effect of stimulation on the post-stimulation pH fall in isolated beef neck muscles at constant temperature, we have been quite unable to detect such an effect either in muscles on the carcass or in isolated *M.triceps brachii*.

However, whether or not electrical stimulation has an accelerating effect in the above sense, the fact remains that it effectively reduces the time required for the major muscles on a beef carcass to reach pH 6 from 10-14h to less than 1.5h (Table 2) at ambient temperatures of 16^o, and thus enables rapid cooling or blast-freezing to be begun on most modern beef slaughter lines soon after the completion of dressing and splitting without danger of cold-shortening (Davey et al. 1976).

Additional advantages of the electrical stimulation technique are first that it makes hot de-boning somewhat easier because the muscles become firm as they go quickly into rigor, and secondly that it has some tenderising effect in its own right (Saveall et al., 1977). The tenderisation is probably due to the rapid attainment of a low pH at a high muscle temperature, conditions ideal for tenderisation by proteolytic enzymes.

Table 1

The effect of muscle length, relative to the slack length (l_0), on the toughness of uncooked and cooked samples of beef neck muscles (post-rigor)

Muscle Length	Relative toughness	
	Uncooked	Cooked
1.29	1.00	1.88
1.00	0.63	1.91
0.75	0.32	2.19
0.58	--	2.96

(after Dransfield and Rhodes, 1976)

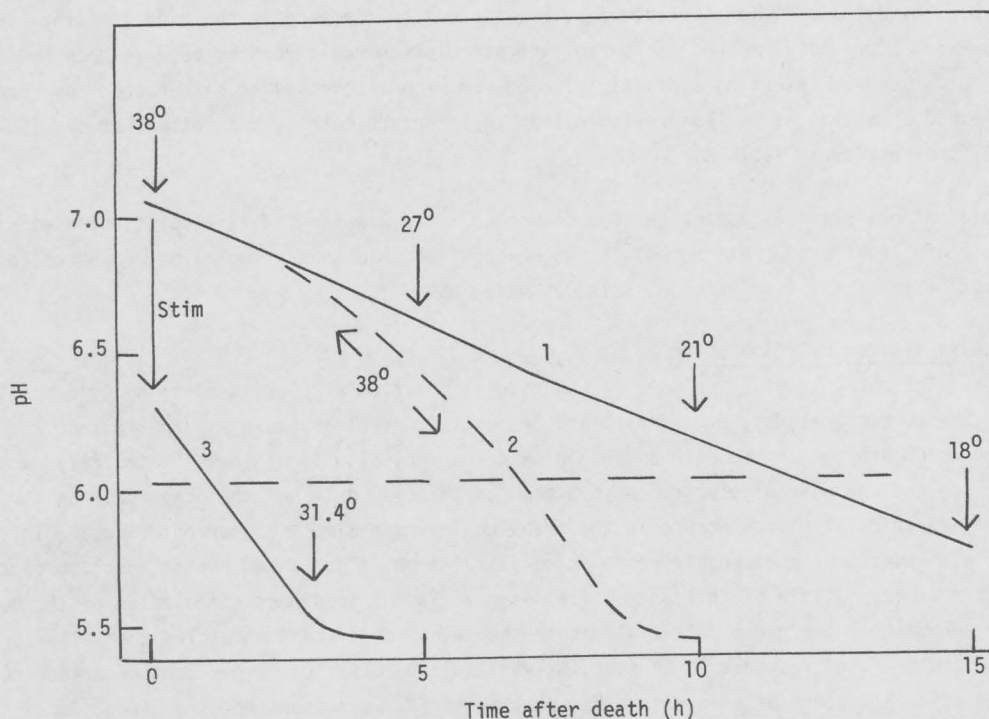


Fig.2. pH/time curves of LD muscles in cooling beef carcasses. The muscle temperatures are given above the curves. The horizontal line indicates the pH (6.02) at which half the initial ATP content disappeared in each case.

Curve 1: unstimulated carcass
 2: ditto corrected to 38°
 3: stimulated carcass

A serious disadvantage of electrical stimulation theoretically arises from the very fact that the muscle temperature is high (>30°) at a time when the pH has already fallen below 6.0 (Fig.2). These conditions are supposedly ideal for the occurrence of PSE meat (Bendall and Wismer-Pedersen, 1962). However, carefully controlled experiments comparing unstimulated with stimulated sides of the same beef carcass and a large number of observations on stimulated whole beef carcasses have failed to reveal any significant differences in drip loss during 48h after slaughter.

Table 2

The time required (h) for the muscle pH to reach 6.0 after stimulation of beef carcasses at varying peak voltages. Undressed carcasses stimulated at 12 min after death, and split sides at 55 min after death. Duration of stimulation = 2 min at 25pps. Ambient temp. = 16°.

N	Type	Volts	Time to reach pH 6			
			LD	TB	BF	SM
11	undressed	0	12.0	12.4	10.6	10.2
			+2.0	+3.0	+3.0	+3.0
7	"	21	4.5	6.3	4.7	5.8
			+2.0	+1.0	+2.2	+1.6
5	"	100	2.1	4.0	3.0	4.7
			+0.6	+2.2	+0.9	+1.7
7	"	300	2.5	2.7	1.3	2.5
			+2.0	+2.0	+0.4	+2.0
7	"	650	1.1	1.4	1.1	1.1
			+0.5	+0.7	+0.5	+0.4
12	sides	650	1.7	2.9	2.5	2.9
			+0.6	+1.5	+1.6	+1.3

LD = M.longissimus dorsi; TB = M.triceps brachii;
 BF = M.gluteo-biceps; SM = M.semimembranosus.

5. Possible modifications of the stimulation technique.

From the point of view of abattoir practice it would clearly be desirable to reduce the stimulating voltage to as low values as possible. However, with electrodes positioned on the neck and Achilles tendons, reduction of the voltage below 650V in beef leads to increasing variability and extent of the time taken for the pH to fall to 6.0 (Table 2), thus making stimulation at reduced voltages very unreliable in practice. The situation

E 1:8

is not improved by using additional electrodes, positioned for instance in the middle of the back (Saveall et al. 1977). However, a practical method of low voltage stimulation could perhaps be developed by stimulating via the spinal cord, e.g. by inserting a plastic pithing rod with electrodes at either end; our preliminary results with this method show that it is less reliable than the standard method, but better, at say 150V (peak) than indicated by the results in Table 2.

Another modification, possibly useful on some slaughter lines, is to stimulate split, dressed sides, preferably within 40 min of slaughter. This method is rather less reliable than stimulation of undressed carcasses (Table 2) but better than stimulation at voltages below 650V.

6. The pathways excited by stimulation

Although very high currents of 4A or more flow during stimulation at 650V, there is no evidence that muscles directly in the current pathway, e.g. the LD and BF muscles, react any more vigorously than the TB muscles in the forelimb which are not in the pathway and in which current flow is minimal. Similarly, when a carcass is hung by one leg and one pair of electrodes is placed on this leg only and the other pair on the neck, vigorous contraction and rapid fall of pH occurs in the free leg in which almost no current flows. This, combined with almost complete absence of contractile response to stimulation of curarised rabbit and lamb carcasses (Bendall, 1976), shows that very little of the contractile response is due to direct stimulation of the muscles but rather that surviving motor nerve pathways in both the spinal cord and the muscles themselves are activated and are the main cause of the response. In fact, Galvani's (1790) classical experiment of attaching two dead frogs together and stimulating one of them with a bi-metallic strip, whereupon the other frog jumped in time with the first, can be repeated with two beef carcasses touching each other.

Indirect evidence in favour of the almost exclusive implication of nervous pathways in the stimulation process comes from the dying away of the contractile response in rabbit, lamb and beef carcasses within an hour or so of death. At this time the muscle pH is usually high (>6.8) and the ATP-level maximal, so that muscles excised from the carcass can still respond fully to direct stimulation (Bendall, 1977).

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