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The Influence of Temperature on Shortening and Rigor Onset in Beef Muscle

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SUMMARY

At sufficient ATP concentration and temperatures below about 15°C, pre-rigor beef muscles (neck muscles) contract; this phenomenon is known as cold shortening. There is also a contracture at higher temperatures occurring just before rigor onset which is called rigor shortening. While rigor shortening starts in neck muscles at pH around 6·3–6·0 and at about 2 μMol ATP/g muscle, cold shortening can begin at pH around 7·0 and the full ATP concentration (4 μMol ATP/g) in the muscle. Shortening can take place as long as there is no irreversible formation of the actomyosin complex in the muscle, i.e. before rigor onset occurs, which can be measured by intermittent loading of the muscle. The degree of extensibility which follows starts to decrease at the moment of rigor onset. This irreversible loss of extensibility at temperatures between the freezing point (−1°C) and physiological temperatures (38°C) starts at various pH values and ATP concentrations in the muscle. At 38°C the rigor onset occurs at pH 6·25 and about 2 μMol ATP/g muscle, dropping at 15°C to pH 5·75 and 1 μMol ATP/g muscle. At 0°C, as at all temperatures below 10°C, the loss of extensibility at medium loads (about 250 g/cm²) begins shortly after cold shortening. This loss of extensibility is reversible by increasing the load or raising the temperature. The irreversible loss, or rigor onset, however, occurs at 0°C with pH of 6·1–6·2 and 1·8–2·0 μMol ATP/g muscle. Thus, the onset of rigor is influenced by more than one factor. Temperature, pH and ATP concentration each play a rôle.

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Maximum loss of extensibility or completion of rigor is reached between 10°C and 38°C at pH 5·5–5·6 and less than 0·5 μMol ATP/g muscle. At 0°C the completion of rigor takes place at pH 6·0, but still at 0·5 μMol ATP/g muscle. The latter fact shows that the completion of rigor is solely dependent on the ATP concentration in the muscle; nevertheless, the pH of rigor completion is higher in the extreme cold shortening range. This is apparently due to a different pH/ATP relationship in muscles at low temperatures.

The results are discussed in terms of changes in the concentration of Ca^{2+} ions and ATP.

The results are of particular interest for the handling of hot-boned meat; that is, for both the cooling of pre-rigor muscle and the use of hot-boned meat for processing.

INTRODUCTION

At room temperature the onset of rigor mortis in a muscle is defined as the beginning of the loss of extensibility (Bendall, 1973*a*). At 20°C, the rigor in normal bovine muscle sets in at pH values around pH 5·9 (Currie & Wolfe, 1980; Honikel *et al.*, 1981*a*). This development of rigor is a slow process and it takes some time for the maximum loss of extensibility (completion of rigor) to be reached (Bendall, 1973*a*; Currie & Wolfe, 1979). Besides the loss of extensibility and the development of rigor at room temperature there is also a limited shortening in unrestrained muscles occurring within approximately the same pH range as the rigor onset (Honikel *et al.*, 1980). This shortening is called 'rigor shortening'. At chilling temperatures cold contracture occurs. Shortening of a muscle in general and the development of rigor influence the tenderness and the water retention of beef. Locker (1960), Marsh & Leet (1966) and Locker & Daines (1975) reported greater toughness in shortened muscle. Powell (1978) and Honikel *et al.* (1980) found higher drip losses in contracted than in unshortened muscles. The contracture of a muscle increases the drip loss whereas the onset of rigor decreases the water-holding capacity (WHC) of salted homogenates and meat batters (Jolley *et al.*, 1980/81; Honikel *et al.*, 1980, 1981*b*). Both facts are of paramount importance for the handling and processing of freshly slaughtered (hot-boned) meat. Knowledge of the influence of time and conditioning temperature on the onset of rigor mortis will help to reduce drip loss in meat and to improve the WHC of salted meat products.

MATERIAL AND METHODS

Beef neck muscles (*M. sternomandibularis* and *M. mastoideus*) were excised from the carcass within 30–40 min after slaughter and trimmed of visible fat and connective tissue. Two corresponding muscles of both sides of an animal were cut to the same length and shape, wrapped separately in plastic pouches and incubated at temperatures between $-1\,^{\circ}$ and $+38\,^{\circ}C$ in a thermostat. For each temperature another pair of muscles was taken. The trimmed muscles were about 25 cm long and had a cross-sectional area of 7–11 cm^2. One muscle of the pair was used for extensibility and shortening experiments, the other for the measurement of pH fall and *R*-value determination during the experiment.

Measurement of pH

About 5 g of muscle were cut into small pieces and homogenised with 15 ml distilled water for 10 s in a Bühler homogeniser (Bühler, Tübingen, Germany). The pH measurements were carried out immediately afterwards with a WTW pH meter (WTW 40, Weilheim, Germany) combined with a glass electrode.

Determination of *R* value

The determination of the *R* value is a fast spectrophotometric method for the estimation of the degree of transformation of ATP to IMP and serves, therefore, as an indicator of the ATP breakdown in post-mortem muscle (Honikel & Fischer, 1977). Furthermore, it has recently been shown that the increase in *R*-value parallels the decrease in ATP levels in post-mortem metabolism (Jolley *et al.*, 1980/81). The relationship of the *R*-value and the approximate ATP concentration in *M. sternomandibularis* is shown in Table 1.

Extensibility of muscle

After different times of post-mortem storage in one half of a muscle pair from a carcass the length of the unrestrained, unloaded muscle was measured (unloaded length) simultaneously with pH and *R*-value determination in the other half; then the muscle strip was hung up and stretched by loading with 2·3 kg (210–330 g/cm^2 at a cross-section of

TABLE 1
Conversion of *R* Value into Approximate ATP Concentrations in Bovine Neck Muscles

R *value*	*Equivalent to approximate ATP concentration* (*μMol/g*)	*Per cent of initial concentration*
0·86	4·0	100
0·90	3·0	75
0·95	2·5	62·5
1·00	1·9	47·5
1·05	1·2	30
1·10	0·8	20
1·15	0·5	12·5
1·20	0·3	7·5

7–11 cm^2, i.e. about 250 $g/cm^2 \triangleq 24{\cdot}5$ kPa, assuming a mean cross-sectional area of 9 cm^2) for a short time and the increase in length was measured (loaded length). After taking off the load, the muscle returned to its initial length with a slight hysteresis, indicating that the muscle structure had not been disrupted by the load. The muscle was left for further 1–2 h without a load. Its length without a load was then measured again and it was loaded as described above. This procedure was repeated until the muscle had lost all extensibility. The extensibility is the difference in length of loaded, minus the length of unloaded, muscle in centimetres at each time. This definition of extensibility is necessary as we found (Fig. 1 at −1 °C, Tables 2 and 3) that the length of the unloaded muscle changed during some of the experiments. Thus, the difference between the loaded minus the unloaded muscle length increased before again decreasing. We therefore define the loss of extensibility as the moment when the difference value of length becomes smaller than the previous measured value. Details are discussed in the 'Results' section. In the literature the load for isotonic extensibility and contraction experiments is usually smaller. Bendall (1973*a*, *b*) used about 40–70 g/cm^2, Currie & Wolfe (1979) used 5–25 g/cm^2. We used this high load in order to overcome the tension in cold shortened muscle at temperatures below 10 °C, as we intended to measure the loss of extensibility post mortem, as well as the cold shortening. It transpired, however, that with a load of 250 g/cm^2 at temperatures at 5 °C and below, cold contracture conceals the moment of the loss of extensibility. Higher loads up to 750 g/cm^2

TABLE 2
Influence of the Load on the Extensibility[a] of Bovine Neck Muscle at 5 °C
(Derived from Fig. 2)

Time of incubation (h)	*pH*	R	*Extensibility (cm) under an applied load of (g/cm²)*						
			250	*345*	*435*	*526*	*616*	*674*	*764*
0	7·0	0·86	9	13	13				
1	6·9	0·86	12	15	16	17			
3	6·7	0·86	11	15	15	16	17		
5	6·6	0·88	12	16	17	17	18		
7	6·45	0·91	12	17	17	18	19		
9	6·30	0·92	10	18	19	20	20	21	21
11	6·2	0·98	5	15	17	19	20	21	22
13	6·1	1·00	1	11	13	15	17	17	18

[a] Extensibility ≙ muscle length of loaded muscle minus length of unloaded muscle in centimetres. The increasing loads were applied to the same muscle at each time of measurement. Between the measurements the muscle was incubated at 5 °C without load. All measurements were carried out with the same muscle. Due to the disruption of the muscle structure after 14 h and later, which is indicated by an increase in the length of the unloaded muscle after loading (cf. Fig. 2), the values after 13 h in Fig. 2 are not considered here.

TABLE 3
Per Cent Shortening of Beef Muscles at Various Temperatures Relative to the Unrestrained Muscle at pH 7·0

Temperature of incubation (°C)	*Per cent shortening*
−1	67
±0	57
+2	40
+5	40
+10	29
+15	28
+21	12
+25	15
+30	14
+35	20
+38	25

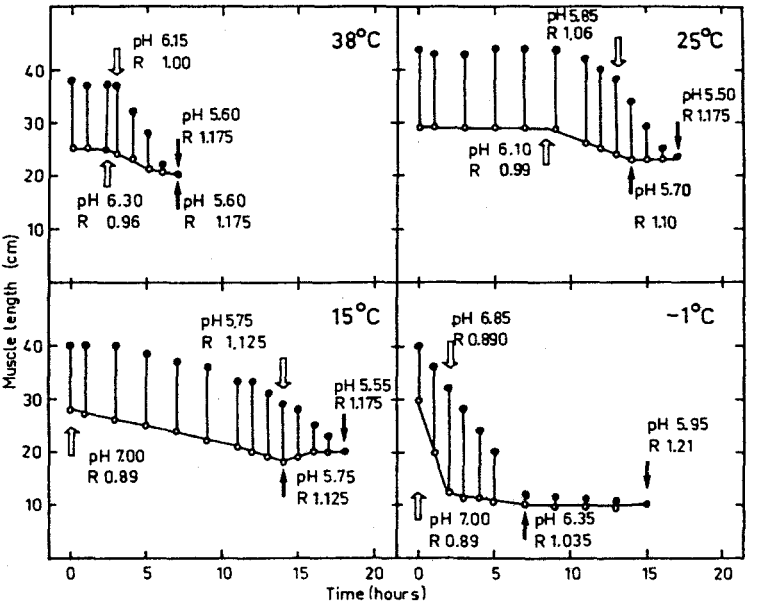

Fig. 1. Changes in extensibility and muscle length of beef neck muscles at four different storage temperatures related to time post mortem. (○) Length of unloaded muscle. (●) Length of briefly loaded muscle. Arrows indicate time, together with pH and *R* value at which shortening (⇧) and loss of extensibility (⇩) begin and are completed, respectively (⇩⇧). Applied load, 250 g/cm^2.

applied to muscles incubated at +1 ° and −1 °C did not overcome the high tension of cold shortened muscle. When we increased the load in these muscles above about 750 g/cm^2, we found that the loads disrupted the muscle structure, a fact shown by the inability of the muscle to shorten fully and to return to its initial length after the removal of the load. As the stress of 250 g/cm^2 is not sufficient to overcome the strong contraction due to cold shortening at low temperatures (at 5 °C and below), two parallel stretch experiments were carried out at 5 °C, one with an unchanged load of 250 g/cm^2 as described above. The other muscle was loaded with increasing loads up to 750 g/cm^2 in order to reach the same absolute maximum extensibility as at zero time. The length of the muscle was first measured without a load. The muscle was then hung up and loaded for 20 s with 345 g/cm^2 and the length was recorded. Next, additional loads were applied up to 425, 526, 616 and 764 g/cm^2 and at each load the length of the muscle was measured after 20 s of loading.

Subsequently, the whole load was taken off and the unrestrained muscle was incubated for another 1 or 2 h, after which the procedure was repeated. At the times of loading, pH and *R*-value were measured in separated muscle pieces of the same muscle.

RESULTS

Effect of storage temperature on muscle shortening and extensibility

The behaviour of muscles stored shortly after death at different temperatures within the temperature range $-1°$ to $+38°C$ differs considerably. The differences are observed in the patterns of shortening and loss of extensibility when regularly loaded with 250 g/cm^2 and related to time of storage or to the rate of pH decrease and ATP depletion. According to these variations we consider four distinct temperature ranges: (1) the range near physiological temperatures (34–38 °C); (2) the range between the latter and room temperature (18–33 °C); (3) the range between 5–17 °C and (4) the pre-freeze chilling temperatures (-1 to $+4$ °C). For each of these groups a typical example of muscle shortening and extensibility behaviour is presented in Fig. 1. The absolute lengths before and after intermittent loading of muscle at 1- or 2-h intervals are shown in the Figure; muscle shortening is given by the decrease in length of the unloaded muscle and muscle extensibility is the difference in centimetres between loaded and unloaded muscle length. The beginning and end of shortening, as well as the beginning and end of loss of extensibility, are indicated in Fig. 1 by arrows in terms of time, pH and ATP concentration (*R*-value). The beginning of decrease in extensibility is, according to Bendall (1973*a*), the definition of the onset of rigor mortis. If the muscle was incubated at 38 °C, the post-mortem changes occurred rather fast due to the high temperature. The loss of extensibility or the onset of rigor started at pH 6·15 and *R* 1·00 (about 1·9 μMol ATP/g or 50 % of the initial ATP concentration); the maximum loss of extensibility was reached within 7 h post mortem at pH 5·60 and an *R* value of 1·17 (i.e. less than 0·5 μMol ATP/g or 10 % of the initial ATP concentration, see Table 1). The shortening of the muscle started at pH 6·3 and $R = 0{\cdot}96$ (about 2·4 μMol ATP/g muscle or 60 % of the initial ATP concentration), proceeding slowly until the full extent of rigor was observed. Rigor shortening begins, therefore, just before the onset of rigor.

At 25 °C it took 17 h before rigor was complete and post-mortem changes ceased. The loss of extensibility started at lower pH (5·85) and ATP concentration ($R = 1{\cdot}06$, i.e. 1·2 μMol ATP/g muscle) than at 38 °C. The completion of rigor took place, however, at a similar pH and the same ATP level as at 38 °C. There was again a shortening starting at pH 6·1 and $R = 0{\cdot}99$ (about 2 μMol ATP/g or 50 % of the initial ATP concentration). The pH and ATP concentration when rigor shortening occurred were lower than at 38 °C. The shortening stopped at pH 5·70.

At 15 °C rigor was complete after 18 h. As the length of the loaded muscle decreased parallel to shortening, the onset of rigor, as defined above, occurred at pH 5·75 and $R = 1{\cdot}12$; that is, at lower values than at 25° and 38 °C. The shortening of the muscle, however, started right from the beginning at pH 7·0 and maximum ATP concentration, ending at pH 5·75 and less than 0·7 μMol ATP/g or 20 % of the initial ATP concentration. The muscle shortened from 29 to 19 cm, i.e. much further than at 25° and 38 °C, at which temperatures it shortened by only about 5 cm.

At −1 °C, i.e. under extreme cold shortening conditions, the muscle shortened by 20 cm within 2 h of incubation. Full contraction of the unloaded muscle was reached at pH 6·35 and $R = 1{\cdot}03$ (i.e. 1·5 μMol ATP/g or about 40 % of the initial ATP concentration). On loading the muscle one also observed a reduced lengthening of the muscle, but this occurred at a lower rate than with the unloaded muscle. After 2 h the loaded muscle was only 10 cm less extensible whereas the unrestrained muscle had shortened by 17 cm. After 7 h of incubation extensibility in cold shortened muscle had decreased to the point where a load of 250 g/cm^2 could not stretch the muscle by more than 2 cm. According to the definition of onset of rigor mortis given above, at −1 °C the onset of rigor seemed to occur at pH 6·85 and full ATP concentration. This seems to be unreasonable. As long as a muscle can contract in the reversible, physiological manner it should also be extensible. But the extensibility depends on the load applied (Bendall, 1973*b*). Using a load of 526 g/cm^2 instead of 250 g/cm^2 we found, under the extreme conditions of −1 °C, no measurable difference in extensibility. Using 764 g/cm^2 the muscle could be stretched below pH 6·3 but it did not return fully to its unloaded length, indicating a disruption of the muscle cell structure by the heavy load. As we obtained a similar, but less extreme, behaviour of the muscle on incubation at 5 °C we carried out experiments at this temperature, loading the muscle from the beginning with increasing weights and

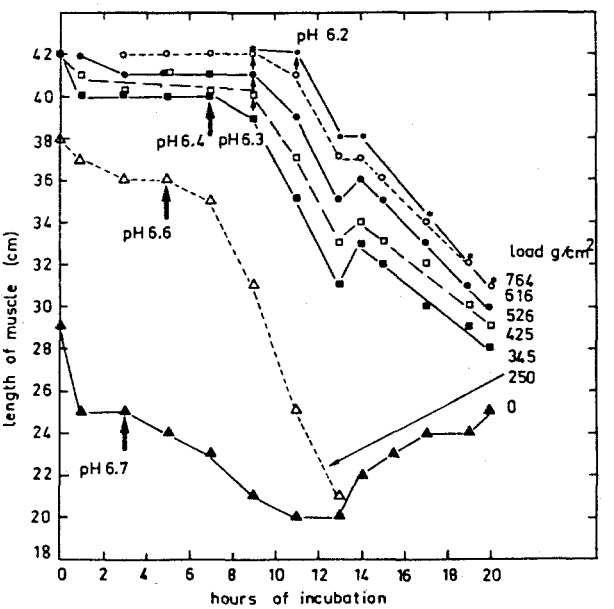

Fig. 2. Shortening and changes in extensibility with increasing loads of pre-rigor beef muscle at 5 °C. The slaughter fresh *M. mastoideus* (neck muscle) (cross section, 9 cm^2) was incubated without load at 5 °C. The muscle was loaded every 1–2 h with increasing load (250–764 g/cm^2) for a short period. (↑) Beginning of final shortening.

unloading it as described in the 'Materials and Methods' section. The results are presented in Fig. 2 and Table 2.

Muscle shortening at 5 °C and the effect of load on the extensibility of cold shortened muscles

Muscles were incubated unloaded, unrestrained (zero load) at 5 °C. At different times post mortem the strips were loaded with different loads from 250 up to 764 g/cm^2 for a short period. The lengths of the muscles are shown for one representative experiment in Fig. 2. The unloaded muscle shortened within the first hour of incubation, followed by a period where no further shortening could be observed. After 3 h a further contracture was recorded. This observation has been described previously by Bendall (1973*b*) who even found, in some cases, a relaxation of a cold shortened muscle after an initial shortening. The second phase of shortening started at pH below 6·7, ending after 13 h at pH 6·1. At all

times indicated the incubated unloaded muscle was loaded briefly with increasing loads from 250 to 764 g/cm^2. With a load of 250 g/cm^2 the muscle length also decreased immediately, followed by a plateau phase. The second decrease of length with 250 g/cm^2 started below pH 6·6, 0·1 pH unit less than in the muscle with zero load. After 13 h the extensibility was largely lost. With a load of 345 g/cm^2 the immediate loss of length was small, followed by an extended plateau phase. Below pH 6·45 the length started to decrease for the second time. A further increase of the load led to the disappearance of the immediate phase of decreased length and the second phase of shortening started at decreasing pH values. With 764 g/cm^2 the muscle length was reduced only below pH 6·2. This experiment shows that the cold shortening of muscles can be overcome to some extent by increasing loads. Parallel to the experiment with increasing loads the other muscle of the pair from a carcass was loaded briefly with only 250 g/cm^2. Its length with and without a load was identical with the sample shown in the Fig. 2 (increasing loads) up to 13 h, indicating that the increasing load did not alter the muscle structure below 13 h or above pH 6·2.

As mentioned above, the onset of rigor is defined as the beginning of the loss of extensibility of the muscle (length with load minus length without load). These difference values derived from Fig. 2 are presented in Table 2. With a load of 250 g/cm^2 the extensibility decreased at pH 6·3, with 345–526 g/cm^2 at pH 6·2 and with 616–764 g/cm^2 at pH 6·1. Taking these results into consideration, the question arises as to whether onset of rigor mortis should be defined as the moment when a muscle under largest load sustained without damage loses extensibility. The increase of load, however, is limited, because exceeding a certain load disrupted the muscle structure. We had already observed this with a load of 764 g/cm^2, the maximum load in our experiments shown in Fig. 2. Below pH 6·1 (after 13 h) the muscle strip does not return fully to its unloaded length, a fact indicated by the increase of the muscle length with zero load. This increase in unloaded muscle length was not observed in the parallel experiments with 250 g/cm^2 only. The muscle's unloaded length remained constant between 13 and 20 h of incubation. From the results presented in Fig. 1 (the experiment at -1 °C), Fig. 2 and Table 2 it becomes evident that at cold shortening temperatures the loss of extensibility at a load of 250 g/cm^2 may not coincide with the real onset of rigor.

The same experimental set up as that used for the experiment of Fig. 2, applied to muscle strips incubated at 20 °C and 38 °C, showed that the

beginning of the loss of extensibility was not shifted with increasing load to lower pH as was observed at 5 °C. At higher temperatures and between 250 and 600 g/cm^2 the loss of extensibility always occurred at the same pH and R values. The behaviour of muscle at 5 °C seems to be due to the tension developing during cold shortening.

We tried to solve the problem of the determination of the real onset of rigor mortis in extremely cold shortened muscle with a different set of experiments. This kind of experiment at 5 °C is not applicable to muscles at −1 °C, as already mentioned above, due to the limitations in the permissible load.

Influence of oscillating temperatures on extensibility and shortening

Fresh pre-rigor muscles were loaded for 20 s at 20 °C immediately before incubation at 0 °C for 2 h,/then stretched shortly with a load of 250 g/cm^2 at 0 °C and incubated for 1 h at 20 °C without a load. After loading the muscle at 20 °C, the temperature of incubation was again lowered to 0 °C for 1 h, and so on (Fig. 3). Chilling to 0 °C induced cold shortening, accompanied by a reduced length of the loaded muscles. Continuing the incubation at 20 °C caused a relaxation of the unloaded and loaded muscle. This relaxation–contraction cycle proceeded in the unloaded muscle down to pH 6·3. The irreversible loss of extensibility in the muscle starts at about pH 6·1/6·0 and $R = 1{\cdot}03$. Figure 3 presents one of five similar experiments. The data are collected in Fig. 4. Under these conditions the irreversible loss of extensibility starts at pH 6·15 and $R = 1{\cdot}02$ (ATP level about 1·5 μMol/g). These experiments reveal two facts. (1) The cold contracture is reversible. This has already been pointed out by Bendall (1973*b*). (2) The loss of extensibility in cold shortened muscle is also reversible by raising the temperature. At 0 °C the irreversible loss of extensibility starts at around pH 6·1–6·0 in agreement with the results at 5 °C in Table 2 where the extensibility of the muscle became irreversible at pH 6·1 (applying increasing loads). Therefore, we define the onset of rigor in cold shortened muscle as the moment when the loss of extensibility becomes irreversible, as measured by increasing loads or raising the temperature. At temperatures above the cold shortening range the irreversible loss of extensibility occurs at loads as low as 250 g/cm^2. At 20 °C and above, greater loads did not alter the moment of loss of extensibility.

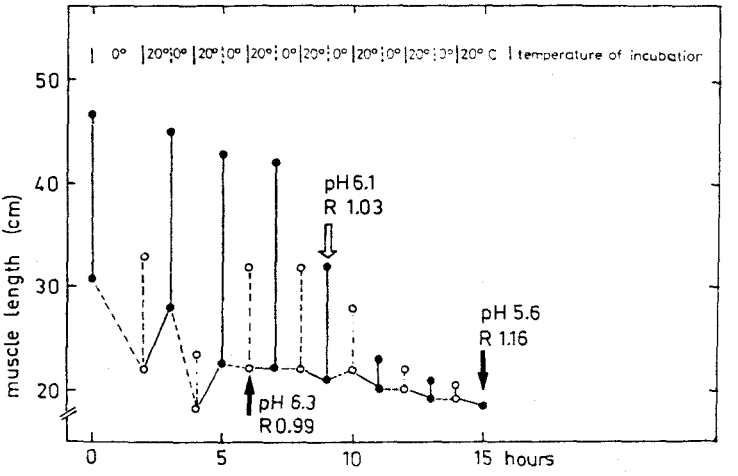

Fig. 3. Shortening and changes in extensibility of a pre-rigor beef muscle at temperatures ranging between 0 °C and 20 °C. The muscle (*M. mastoideus*) was incubated at 0 °C and 20 °C at the time intervals indicated at the top of the Figure and by continuous (20 °C) and broken lines (0 °C). The points of measurement of length, with and without load, are also indicated: (●) measurement at 20 °C, (○) measurement at 0 °C. The straight upward lines give, in their end point, the length of the loaded muscle. The load was 250 g/cm^2 in all cases applied for a short period of time. (ǂ) Moment after which no further shortening in the unloaded muscle occurred. (⇩) Beginning of loss of extensibility. (ǂ) Full loss of extensibility.

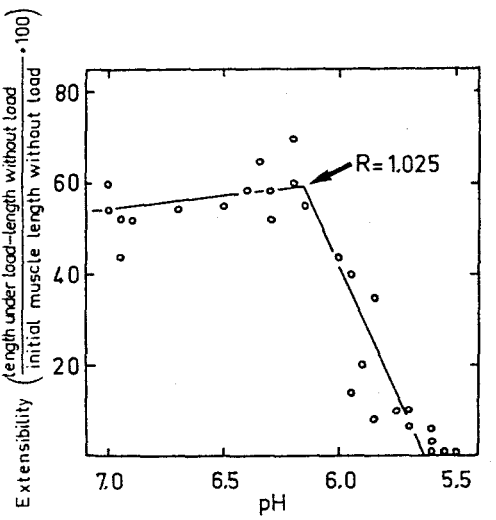

Fig. 4. Loss of extensibility of beef neck muscles when stored at 0 °C and periodically (1–2 h) warmed up to 20 °C, related to pH decrease. Only the recovery of extensibility (loaded–unloaded muscle length, expressed as a percentage of initial muscle length) at 20 °C is given. Values of five experiments are shown.

The influence of temperature on the onset and completion of rigor

The results presented in Fig. 1 for four different temperatures indicate that the times of onset and of maximum development of inextensibility in a muscle depend on the temperature of incubation. In Fig. 5 the influence of temperature on the onset and full development of rigor in all experiments is shown with regard to pH and *R* value (ATP concentration). With regard to pH (Fig. 5(A)), the onset of rigor at 38 °C occurs at pH 6·25, exhibits a pH minimum (5·75) at 12–15 °C, and at 0 °C rigor again occurs at pH 6·1–6·2. The maximum loss of extensibility (completion of rigor) is reached between 8 °C and 38 °C at pH 5·5–5·6 and at 0 °C at pH 5·9–6·0. With regard to *R* value (Fig. 5(B)), the onset of rigor at 38 °C starts at about 0·95 (2·5 μMol ATP/g muscle or about 60 % of the initial ATP concentration), reaching a minimum at about 15 °C at

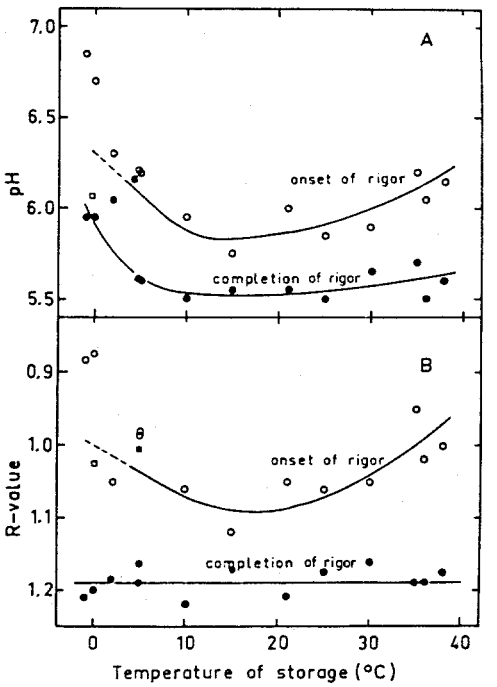

Fig. 5. pH (A) and *R* value (B) at which loss of extensibility begins (○) or is completed (●) in beef neck muscles stored post mortem at various temperatures (see arrows in Fig. 1). (□) Beginning of loss of extensibility of muscles stored at 0 °C and periodically (1–2 h) warmed up to 20 °C derived from Fig. 3. (■) Value at 5 °C derived from Table 2.

$R = 1{\cdot}08$–$1{\cdot}10$ (about $0{\cdot}9\,\mu$Mol ATP/g or 25% at the initial ATP concentration). At 0 °C the irreversible loss of extensibility again starts at $R = 1{\cdot}0$.

The development of rigor is completed at $R = 1{\cdot}17$ (less than $0{\cdot}5\,\mu$Mol ATP/g or about 10% of the initial ATP concentration) at all incubation temperatures. It is noticeable that rigor completion occurs at the same ATP concentration for any given temperature but not at the same low pH. The reason for this becomes evident from Fig. 6. The R value–pH relationship during the time post mortem is clearly separated into two

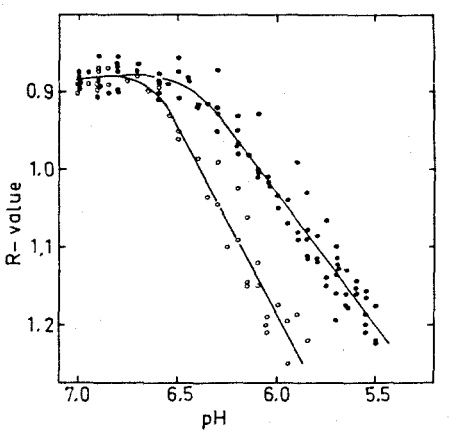

Fig. 6. R value changes related to pH fall of beef neck muscles stored post mortem at various temperatures. (○) −1 °; 0 °; 2 °C. (●) 5; 10; 15; 21; 25; 30; 35; 36; 38 °C.

curves. At temperatures between −1 °C and +2 °C the delay phase, where, with falling pH no change in R value occurs, is short; the R value starts to increase at pH 6·75. At temperatures within the range 5 °–38 °C, as already described by Jolley *et al.* (1980/81), ATP levels are maintained during a delay phase until about pH 6·45 is reached. Then the R value begins to increase at a linear rate since creatine phosphate and glycolysis are no longer able to resynthesise ATP in the amounts used by muscle cell ATPases. When rigor is completed (Fig. 5) ATP has almost totally disappeared ($R = 1{\cdot}17$) and muscle pH has decreased below 5·6. On the other hand, when muscle is stored at low chilling temperatures (−1 ° to +2 °C) ATP is depleted in relation to the pH fall at a higher rate. Therefore, $R = 1{\cdot}17$ is reached even at pH 6·0.

Influence of temperature on the shortening of muscle

The shortening of unloaded muscles incubated at various temperatures shows a behaviour different from the loss of extensibility. All results obtained are presented in Fig. 7. At temperatures above 15 °C shortening does not start at pH values above 6·25 (Fig. 7(A)). Below 15 °C the muscle starts shortening immediately after incubation at pH 7·0. Above 15 °C

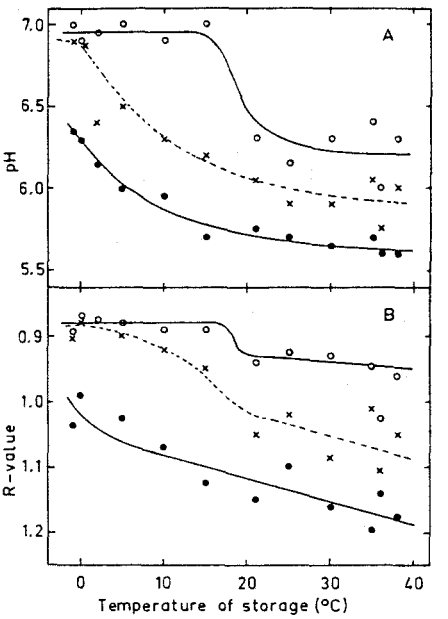

Fig. 7. pH (A) and *R* value (B), at which shortening begins (○), is completed (●) or reaches 50 % of maximum final shortening (×), in beef neck muscles stored post mortem at various temperatures (see arrows in Fig. 1).

50 % shortening is reached at pH 5·9–6·0 and full shortening at pH 5·6–5·7. Below 15 °C 50 % shortening is obtained at increasing pH values as the temperature of incubation falls, reaching pH 6·9 at −1 °C. The full shortening occurs at increasing pH values in a similar manner to the 50 % shortening value. It occurs at pH 5·6 at 38 °C and at pH 6·35 at −1 °C. Shortening starts at fairly low *R* values (0·89–0·96, equivalent to about 2·5–3·0 μMol ATP/g muscle) over the whole temperature range studied (Fig. 7(B)). Above 15 °C, *R* values for 50 % shortening and full shortening run nearly parallel with those for the beginning of shortening.

Below 15 °C, in the temperature range of cold shortening, the 50 % shortening value is reached at high ATP concentrations; below 5 °C full shortening is reached at about 2 μMol ATP/g muscle.

A comparison of Figs 5 and 7 shows that at physiological temperatures (35 °–38 °C) the beginning of shortening and loss of extensibility are very close together (at pH 6·2–6·3 and about $R = 0{\cdot}95$). At lower temperatures, down to 15 °C, the loss of extensibility starts at lower pH and higher R values (lower ATP levels) than the shortening which begins at a constant pH of 6·25 but at R values which slightly decrease with falling temperature. Unlike the beginning of loss of extensibility, no temperature-dependent minimum of the beginning of shortening is observed. Below 20 °C, however, a sharp jump is observed in the pH/temperature relationship for the onset of shortening.

As can be seen from Fig. 1, the degree of shortening varies with temperature. Figure 7 does not show the extent of shortening—only the moments of the beginning and end of shortening. Table 3 shows that the degree of cold or rigor shortening varies drastically—a fact also reported by Locker & Hagyard (1963). At −1 °C the muscle shortens by 67 %. This excessive shortening is supported by sarcomere length measurements by the method of Voyle (1971), which showed a shortening of 70 % and 53 % at −1 ° and 0 °C, respectively. At 21 °C shortening is at a minimum of 12 %, increasing at 38 °C to 25 %. Rigor shortening at 38 °C is less extensive than cold shortening at 15 °C (28 %).

DISCUSSION

Between 38 °C and −1 °C all unloaded pre-rigor muscles show shortening (Figs 1 and 7) but to a different extent (Table 3); in addition, time post mortem, pH and ATP concentration at the beginning of shortening are different. In general, shortening of muscles should take place before the onset of rigor since contracture needs a sufficient ATP concentration. Additionally, shortening needs an increase in Ca^{2+} concentration around the myofibrils. The sarcoplasmic reticulum (SR) accumulates Ca^{2+} ions by means of a Ca^{2+} pumping system which is located in the SR membrane and driven by ATP hydrolysis. The question arises why, before the onset of rigor in the presence of an ATP concentration sufficient for contraction, the release of Ca^{2+} ions into the myofibrillar space takes place. We have to distinguish between two different kinds of shortening—'rigor

shortening' above 20 °C and 'cold shortening' below 15 °C. Rigor shortening can be explained by recent studies. Whiting (1980) and Cornforth *et al.* (1980) showed that there is a pH dependence of the SR membrane Ca^{2+} uptake system. Whiting (1980) reports that at a constant ATP concentration and 25 °C the SR–Ca^{2+} uptake has a pH optimum at about pH 6·3, decreasing rapidly when pH falls below pH 6·0. This pH optimum at 6·3 also exists under simulated post-mortem conditions, i.e. as temperature, pH and ATP concentration fall. These observations agree very well with our results that above 20 °C rigor shortening starts at pH 6·25 and about 2·5 μMol ATP/g muscle (Fig. 7). At a high pH around 7·0, ATP concentrations above 1·5 μMol ATP/g muscle are optimal for the Ca^{2+} uptake activity by the SR (Whiting, 1980). These results indicate that there is a high sensitivity of the Ca^{2+} uptake activity of the SR membrane with regard to pH and ATP concentration. On the other hand, Bendall (1969) showed that the myofibrillar Mg/Ca-ATPase is nearly pH independent between pH 7 and 6. After the release of Ca^{2+} ions due to the reduced activity of the Ca^{2+} accumulating system of the SR at an ATP concentration sufficient for contraction, shortening can take place. These observations allow one to explain the observed 'rigor shortening' in muscles at, and above, room temperature at a pH below 6·3.

As well as a pH-dependent increase of the Ca^{2+} concentration in the myofibrillar space during the post-mortem changes in muscle above 20 °C, an increase in Ca^{2+} concentration due to low temperatures (below about 15 °C) is observed at high ATP levels. Buege & Marsh (1975) explained this phenomenon, known as cold shortening, by an anoxic release of Ca^{2+} ions from the muscle mitochondria and a reduced rate of Ca^{2+} uptake by the SR. Pearson *et al.* (1973) and Davey & Gilbert (1974) suggested that cold shortening is primarily due to a leakage of Ca^{2+} from the SR. Cornforth *et al.* (1980) suggested that apparently both explanations might be correct because both processes may contribute to Ca^{2+} release, leading to cold contracture of pre-rigor muscle.

If these assumptions are correct then rigor shortening and cold contracture may both be explained by the release of Ca^{2+} ions into the myofibrillar space at ATP concentrations sufficient for contraction. The Ca^{2+} ions, however, would be released for different reasons. Cold contracture can be induced in pre-rigor muscles at any time post mortem; rigor shortening would be limited to the late pre-rigor phase.

Shortening is due to the release of Ca^{2+} ions in the presence of a sufficient ATP concentration, causing a build-up of tension within the

muscle which is indicated by a loss of extensibility. In the cold shortening range below +15 °C this tension and shortening increase with falling temperature whereas, at temperatures above 20 °C, the degree of shortening (Table 3)—and consequently the build up of tension—is less. Due to the high tension developed, extremely cold shortened muscles show, under medium load, a loss of extensibility at high pH and ATP level which can be overcome by increasing the load or raising the temperature (Figs 2 and 3). These effects show that an early loss of extensibility in cold shortened muscle is not equivalent to the onset of rigor. However, at temperatures above 20 °C the loss of extensibility is irreversible. Increasing loads do not change the pH and *R* value at which loss of extensibility begins. From Figs 2, 3 and 5 it becomes obvious that the irreversible loss of extensibility or the onset of rigor take place at relatively low ATP concentrations. Rigor onset occurs when actin and myosin form a stable actomysin complex in the absence of sufficient ATP. As well as ATP, Ca^{2+} ions play a role in this process. Shortly after the release of Ca^{2+} ions, which causes a shortening in muscles above 20 °C, the onset of rigor occurs (Figs 1, 5 and 7). The level of ATP concentration, which leads to actomyosin formation, is, however, temperature dependent. This has been supposed already by Bendall & Davey (1957) who found that, at 37 °C, the onset of rigor occurred after 50 % of the ATP had disappeared in the muscle whereas, at 20 °C, only 25 % of the initial ATP was left at the moment of rigor onset. We observed a similar temperature dependence. At 38 °C the onset of rigor begins at $R = 0{\cdot}95$ (60 % of the initial ATP concentration), falling to 25 % of the initial ATP concentration at 15 °C (Fig. 5). Below 15 °C, the ATP concentration for rigor onset again increases to values which are at 0 °C in the same range as at 38 °C. With the experimental set-up used in the studies reported in this paper, we cannot give a full explanation of this phenomenon. The results presented in Fig. 7 may indicate that the influence of temperature between 0 °C and 38 °C on the binding of Ca^{2+} ions to myofibrillar regulatory and contractile proteins may be different at different temperatures, pHs and ATP concentrations. In addition, the binding of ATP to myosin, causing the separation of the actomyosin complex, may be temperature dependent. Thus, various factors might be responsible for the decrease of the ATP concentration and pH at which the onset of rigor occurs with a decrease in temperature from 38° to 15 °C. For corresponding changes in the cold shortening range (−1° to 15 °C) similar factors may be at work. Further studies are necessary in order to clarify these problems.

The completion of rigor, however, takes place at the same low ATP level over the whole temperature range (Fig. 5). The increasing pH at the completion of rigor at temperatures below 5 °C can be explained by the results presented in Fig. 6. Due to cold shortening, more ATP is apparently split by contraction of the muscle post mortem than at higher temperatures; anaerobic glycolysis is the only source for ATP resynthesis after the depletion of creatine phosphate. The velocity of glycolysis at low temperatures is apparently not able to follow the need for ATP resynthesis; this results in a reduced ATP level in the muscle. Therefore, ATP starts to disappear at a higher pH and rigor is completed (at pH 6·0) before the minimum pH value is reached.

Figures 5 and 6 clearly demonstrate that the ATP depletion is the only factor determining rigor completion. In this context it is interesting to note that rigor shortening of muscles is still taking place after the onset of rigor. Shortening starts before the onset of rigor but continues above 15 °C until the completion of rigor (Figs 5 and 7). Above 15 °C the maximum shortening is reached at the same pH and similar R values as the rigor completion. Within the cold shortening range (below 10 °C), when shortening starts early and is comparatively rapid, the maximum shortening ceases at the same ATP concentration at which the onset of rigor starts (Figs 5 and 7). This can also be shown by measuring the sarcomere length of tiny pieces of the muscles used for the extensibility experiments. Sarcomeres shorten at low temperatures ($-1°$ to $+2$ °C) in a more or less linear fashion until the moment of rigor onset (Honikel *et al.*, unpublished data). The final degree of sarcomere shortening at these low temperatures is approximately the same as the shortening of the muscle as a whole. The fact that rigor shortening proceeds below the measured onset of rigor can be explained by the hypothesis that in a whole muscle the post-mortem changes occur in the different cells at slightly different rates. Some fibres reach the state of rigor onset earlier than others. After some of the fibres have developed a state of rigor, other muscle cells can still contract as they remain in the pre-rigor state. The measured pH and R values are mean values over a number of muscle cells. The shortening muscle cells cause the muscle as a whole to contract whereas the extensibility of the muscle begins to fall once some of the fibres are in rigor and resist stretching.

At chilling temperatures, shortening starts early and intensively and, therefore, a considerable number of actomysin complexes are formed (high tension). At the moment when an irreversible loss of extensibility is

observed (rigor onset as defined) maximal shortening has taken place in most of the muscle cells. Further shortening seems to be impossible.

In rigor contracture at temperatures $\geq 20\,°C$, cells which are already in the state of rigor might, to some extent, hamper the contraction of other cells which are not yet in rigor. This might be the reason why the extent of rigor contracture of a muscle as a whole is smaller than that of cold contracture (Table 3) where the contracture is completed before fibres enter the rigor state. This explanation is supported by the observation of Honikel *et al.* (1981*b*) that fibre fragments of pre-rigor muscle shortened at temperatures $\geq 21\,°C$ at least partially to the same extent as at 0 °C. With this in mind we want to emphasise that the onset of rigor, as defined, is the irreversible loss of extensibility of a bundle of muscle cells as a whole.

CONCLUSIONS FOR THE HANDLING OF MEAT

As mentioned above, shortening of muscles increases the toughness and drip loss of meat. As can be seen from Figs 1 and 7 and from Table 3, shortening occurs in unloaded muscle at all temperatures between freezing and physiological temperatures but to a different extent (Table 3). This was shown earlier by Locker & Hagyard (1963) and Powell (1978). Further, shortening starts and ends at different pH values and ATP concentrations post mortem. At temperatures above 15 °C shortening starts at pH 6·3, being lowest at around 20 °C and ending at pH as low as 5·6. Post-mortem shortening can be kept to a minimum if 20 °C is reached in the muscle as soon as possible. This is important for the handling of hot-boned meat. Also, the drip loss is at a minimum if temperatures of 10–15 °C are reached as soon as possible (Powell, 1978; Honikel *et al.*, 1980).

The onset of rigor is the event which is critical for the salting of 'hot' beef because rigor causes a remarkable drop in the water-holding capacity of salted meat and results in an undesirable increase in the separation of juice and fat in 'emulsion type' sausages (e.g. frankfurters) (Hamm, 1981). Therefore it is also important to know that the onset of rigor is dependent on the muscle temperature. Meat at 35 °C must be salted above pH 6·25 which is reached within 3–4 h post mortem. If the meat can be rapidly cooled to 15 °C, then rigor sets in at pH 5·75 which occurs 10–15 h post mortem. This is important for the handling of hot-boned meat for processing.

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