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Influence of Low Voltage Electrical Stimulation and Rate of Chilling on Post-mortem Glucolysis in Lamb

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ABSTRACT

The effect of electrical stimulation (ES) using 56 V effective voltage and reversing polarity and the effect of the rate of chilling on post-mortem biochemical processes in lamb carcasses were studied.

Temperature, pH, lactic acid and adenine nucleotide (ATP, ADP, AMP–IMP, and HxR–Hx) content, and R value for the Longissimus dorsi *muscle were analyzed during 48 h* post mortem *for each of three treatments.*

The values of the above-mentioned parameters were dependent upon both the rate of chilling and electrical stimulation. Rapid chilling led to the critical pH and temperature conditions that can bring about cold-shortening; these conditions did not occur when ES was applied or the lamb carcasses were chilled slowly.

ES resulted in an immediate decrease of 0·72 in the pH, an increase in lactic acid of 30 μM g^{-1}, and degradation of 45% of the initial ATP. With ES, the ATP level fell to 50% after 30 min. With slow chilling this level was attained after 5 h and with quick-chilling after 7 h. A linear relationship between the decrease in the ATP concentration and the increase in the R *value was established.*

INTRODUCTION

Treating carcasses with low temperature after dressing yields a series of economic (lower weight loss, reduced cold storage facilities, etc.) and sanitary and hygienic (retarded microbial growth) advantages. However, the application of low temperatures can also result in cold-shortening,

characterized by toughening of the meat, when the temperature decreases to below 11°C before the pH has fallen to under 6·2 (Bendall, 1972).

Acceleration of post-mortem glucolysis by means of electrical stimulation in order to prevent the occurrence of this phenomenon was first suggested by Harsham & Deatherage (1951). However, the method has only been put into practice by the meat industry in recent years.

The application of electrical stimulation using such high voltages as 3600 V (Chrystall & Hagyard, 1976; Gilbert & Davey, 1976) and such low voltages as 5 V (Jiménez-Colmenero *et al.*, 1985; Moral *et al.*, 1986) has been studied. Most of the studies on electrical stimulation have dealt with the application of high voltages. However, certain authors have demonstrated similar decreases in pH with low voltages (Nilsson *et al.*, 1979; Bouton *et al.*, 1980; Eikelenboom *et al.*, 1985). The use of low voltages is preferable to the use of high voltages in view of such factors as safety, lower cost, and simpler stimulator design.

Certain researchers have studied the effect of electrical stimulation on post-mortem glucolysis through the drop in the pH (Eikelenboom *et al.*, 1985; Newbold & Small, 1985; Smulders *et al.*, 1986). They have detected two separate stages: first, during stimulation, the rate of decrease can be increased by up to 150-fold, resulting in a dramatic fall in pH (ΔpH), and, secondly, after cessation of stimulation (dpH/dt), the rate is much lower but is still almost twice as fast as in non-stimulated muscle (Chrystall & Devine, 1978).

Degradation of the adenosine triphosphate (Bendall *et al.*, 1976; Morton & Newbold, 1982; Pëtäja *et al.*, 1985) and adenine nucleotides (Calkins *et al.*, 1983; Fabiansson & Laser Reuterswärd, 1985), and the accumulation of lactate (Fabiansson & Laser Reuterswärd, 1985), have also been studied. However, most such studies have either been carried out without subjecting the muscle to sufficiently rapid cooling to bring about cold-shortening or have dealt with beef and/or high voltages.

Therefore, in view of the effectiveness of electrical stimulation at 56 V while reversing polarity in preventing cold-shortening in lamb (Carballo *et al.*, 1987), the present experiment was designed to study the effect of the conditions of stimulation on post-mortem biochemical processes in lamb carcasses in conjunction with different rates of chilling.

MATERIALS AND METHODS

Twelve merino–entrefino lambs weighing 10–12 kg per carcass were randomly assigned to one of three treatment groups: four lambs were electrically stimulated and quick-chilled (EE); four lambs were quick-chilled

without undergoing electrical stimulation (RR), and four lambs were chilled at a slower rate (MN).

Electrical stimulation was applied for 90 s, immediately after bleeding (1–3 min *post mortem*), using an electrical stimulator designed at the Instituto del Frío (CSIC, Spain), with the following characteristics: square-wave, pulses duration, 5·2 ms; 14·3 pulses per second, peak voltage, 80 V; mean voltage, 40 V; effective voltage, 56 V; polarity reversed every 10 s. The current was applied through electrodes inserted in the nose and the Achilles tendon through a cut made in the skin so that it could not act as an electrical insulator.

Batches RR and EE were quick-chilled for 6 h in a cooling tunnel with an air speed of $2\,m\,s^{-1}$ and an air temperature of $-5 \pm 1°C$. Batch MN was chilled more slowly, for 24 h at $6 \pm 1°C$ and an air speed of $0{\cdot}5\,m\,s^{-1}$. After chilling, the samples were stored in a cold store for 72 h at $1 \pm 1°C$ and an air speed of $0{\cdot}2\,m\,s^{-1}$.

The pH and temperature (T) of the *Gluteus biceps* muscle were determined immediately before (BS) and after (AS) electrical stimulation and at 0·5, 2, 4, 6, 8, 12, 24 and 48 h *post mortem*. The pH measurement at the slaughterhouse (BS, AS and 0·5 h) was carried out using a Crison-506 portable digital pH meter and an Ingold P 6531 electrode. At the Instituto del Frío laboratory, samples for pH determination were homogenized in neutralized iodoacetate-KCl solution as per Bendall (1978), and the pH was measured using a Radiometer PHM 63 and a Radiometer type K 401 electrode.

Temperature was measured using a Crison T-637 portable digital thermometer equipped with a platinum probe.

Samples for the lactate and adenine nucleotide determinations were removed from the *Longissimus dorsi* muscle immediately before (BS) and after (AS) electrical stimulation and at 0·5, 2, 4, 6, 8, 12, 24 and 48 h after slaughter. The samples were immediately frozen in liquid nitrogen and stored for a maximum of four days at $-24°C$ for further processing.

Lactate was measured using a test combination from Boehringer & Mannheim (Cat. No. 139.084).

The adenine nucleotides, adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosine monophosphate (AMP), inosine monophosphate (IMP), inosine (HxR), and hypoxanthine (Hx), were determined by high performance liquid chromatography (HPLC). The HPLC system used was an LKB liquid chromatograph equipped with an LKB model 2150 pump, an LKB model 2152 variable wavelength detector, and a Shimadzu C-R3A integrator, with an LKB μBondapak-NH_2 column. Aqueous solvents were prepared from $PO_4H_2NH_4$ and water from a Millipore Milli Q system in the proportion of 85% 0·25M $PO_4H_2NH_4$ (pH 5·0) and 15% 0·01M $PO_4H_2NH_4$ (pH 3·0). The flow rate was $1\,ml\,min^{-1}$. Sample

preparation was as described by Alique & Carballo (1985). Identification was performed by comparing the retention times for the samples with those of the standards used. The concentration of ATP degradation products was calculated from the integrated peak areas for material absorbing at 254 nm eluting from the column, on the basis of the response factor obtained using external standards at two different concentrations.

The *R* value determined according to Honikel & Fischer (1977) using the same extraction as used for the HPLC determination of the adenine nucleotides.

Four replications of each lactic acid, adenine nucleotide, and *R* value determination were performed.

The degree of significance of the means was determined by analysis of variance using a Tukey test. The linear regressions and the coefficient of determination between parameters were carried out using the 'Paired' program by Hewlett–Packard.

RESULTS AND DISCUSSION

The temperatures and pH values recorded during the first 48 h *post mortem* are presented in Table 1. The decrease in temperature during the study period was, obviously, dependent upon the chilling procedure applied, such

TABLE 1
Determination of Temperature and pH during 48 h Post Mortem

Post-mortem period (*h*)	*Temperature*			*pH*		
	MN	*RR*	*EE*	*MN*	*RR*	*EE*
BS			38·7			7·03
AS			39·0			6·31
0·5	$36·8^{a}$	$36·1^{a}$	$36·9^{a}$	$6·81^{x}$	$6·72^{y}$	$6·03^{z}$
2	$27·8^{a}$	$23·2^{b}$	$23·6^{b}$	$6·53^{x}$	$6·47^{x}$	$5·94^{y}$
4	$19·0^{a}$	$10·4^{b}$	$10·7^{b}$	$6·21^{x}$	$6·31^{y}$	$5·83^{z}$
6	$13·2^{a}$	$3·7^{b}$	$3·5^{b}$	$5·92^{x}$	$6·13^{y}$	$5·80^{x}$
8	$9·7^{a}$	$1·5^{b}$	$2·1^{b}$	$5·87^{x}$	$5·99^{y}$	$5·77^{z}$
12	$8·3^{a}$	$1·6^{b}$	$1·7^{b}$	$5·71^{x}$	$5·93^{y}$	$5·82^{x}$
24	$6·1^{a}$	$1·3^{b}$	$1·5^{b}$	$5·82^{x}$	$5·74^{x}$	$5·80^{x}$
48	$2·2^{a}$	$1·8^{a}$	$1·6^{a}$	$5·76^{x}$	$5·82^{x}$	$5·70^{x}$

BS: Determination carried out before electrical stimulation.
AS: Determination carried out after electrical stimulation.
Different letters in each row indicate significant differences ($P < 0·05$); each *T* and pH value is the mean of four replications.

that the drop in temperature in the quick-chilled batches (RR and EE) was significantly faster ($P < 0.05$). No significant differences due to electrical stimulation were found between the two quick-chilled batches, which agrees with the findings reported by other authors using low voltage (Eikelenboom *et al.*, 1985; Smulders *et al.*, 1986).

The pH over the study period was found to be dependent upon both the chilling procedure and the electrical stimulation. The decrease in pH was significantly faster in the stimulated carcasses ($P < 0.05$). The effectiveness of stimulation was demonstrated by the decline in pH when it was applied, $\Delta pH = 0.72$, similar to the results obtained by Eikelenboom *et al.* (1985), Eikelenboom & Smulders (1986), and Carballo *et al.* (1987) at similar voltages. The drop in pH was, however, greater than that reported by authors who employed lower voltages (Chrystall & Devine, 1980; Taylor & Marshall, 1980) or longer times between slaughter and electrical stimulation (Morton & Newbold, 1982). The different rate of decrease in the pH in the unstimulated carcasses can be explained in terms of the different chilling procedures applied, such that the decrease was higher in batch MN ($dpH/dt = 0.09$) than in batch RR ($dpH/dt = 0.04$), although these were both lower than the decrease in batch EE ($dpH/dt = 0.12$). This coincides with the findings reported by other authors for lamb (Chrystall & Devine, 1980; Newbold & Small, 1985). The final pH values in the three batches were not significantly different.

Batch MN quickly attained a pH level below 6·0 at 5 h *post mortem*, at a temperature higher than 13°C, and hence the conditions for the onset of cold-shortening described by Bendall (1972) ($T < 11$°C; pH > 6·2) were not fulfilled. Batch RR attained this critical temperature at 4 h post mortem at a pH of 6·31, while batch EE reached this temperature when its pH was below 6·0 (pH < 6·0; $T > 20$°C). Consequently, in the conditions of the experiment, the application of electrical stimulation successfully prevented the conditions that give rise to cold-shortening.

Lactic acid levels over the study period (Fig. 1) were dependent upon both the chilling procedure applied and the use of electrical stimulation. The faster the rate of chilling, the slower the formation of lactic acid. In the stimulated batch there was an increase of close to $30\,\mu M\,g^{-1}$ during stimulation, with final concentrations of around $90\,\mu M\,g^{-1}$ after 6 h *post mortem*. A linear relationship was established between the decrease in pH and lactic acid formation (μM lactate $g^{-1} = 453.29 - 63.31$ pH; $R^2 = 0.73$). The buffering capacity in the present study was calculated to be 63 μM of lactate per pH unit per gram of muscle. This was in accordance with the values of 67 reported by Bendall (1973), 68 given by Fabiansson & Laser Reuterswärd (1985), and 60 μM of lactate per pH unit per gram found by Bendall (1979).

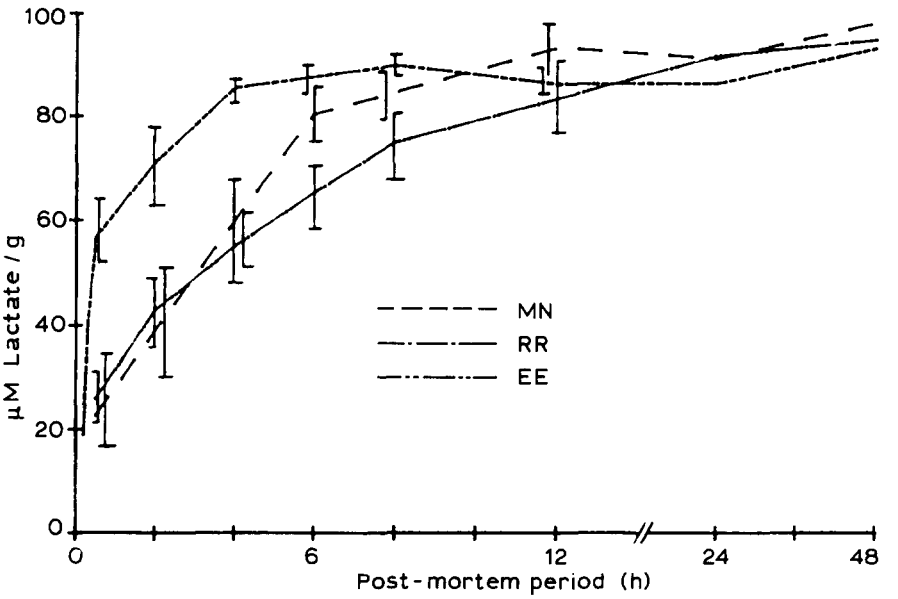

Fig. 1. Lactic acid content during the 48 h *post mortem*: vertical bars indicate standard deviation.

The concentration of adenine nucleotides at different times *post mortem* is represented in Figs 2 and 3. The initial ATP concentrations were around 5·3 μM g^{-1}, similar to those found by Whiting *et al.* (1981) and by Morton & Newbold (1982) in lamb muscle, but lower than those recorded by Pëtäja *et al.* (1985) in beef muscle. The decrease in this compound over the post-mortem period varied in function of the processing applied. The effect of temperature was reflected in a higher rate of degradation in batch MN, where the initial ATP concentration had fallen to 50% by 5 h *post mortem*. This level was reached after 7 h *post mortem* in batch RR. Similar phenomena were described by Nuss & Wolfe (1981). In batch EE, 45% of the initial ATP was degraded during electrical stimulation, and the 50% level was reached after 30 min *post mortem*. Similar periods were reported by Whiting *et al.* (1981) using higher voltages (420 V) applied 45 min *post mortem*, whereas Morton & Newbold (1982) required longer periods using lower voltages (32 V). Thus, the effect of electrical stimulation on ATP degradation depends upon both the voltage employed and the time elapsing between slaughter and ES application.

In addition to the influence of pH and temperature in bringing on cold-shortening, a certain ATP level is also required, placed by some authors at 25% of the initial ATP (Penny, 1980) or between 0·3 and 1·5 μM g^{-1} (Bendall, 1973), both of which are higher than those found in batches EE and MN at

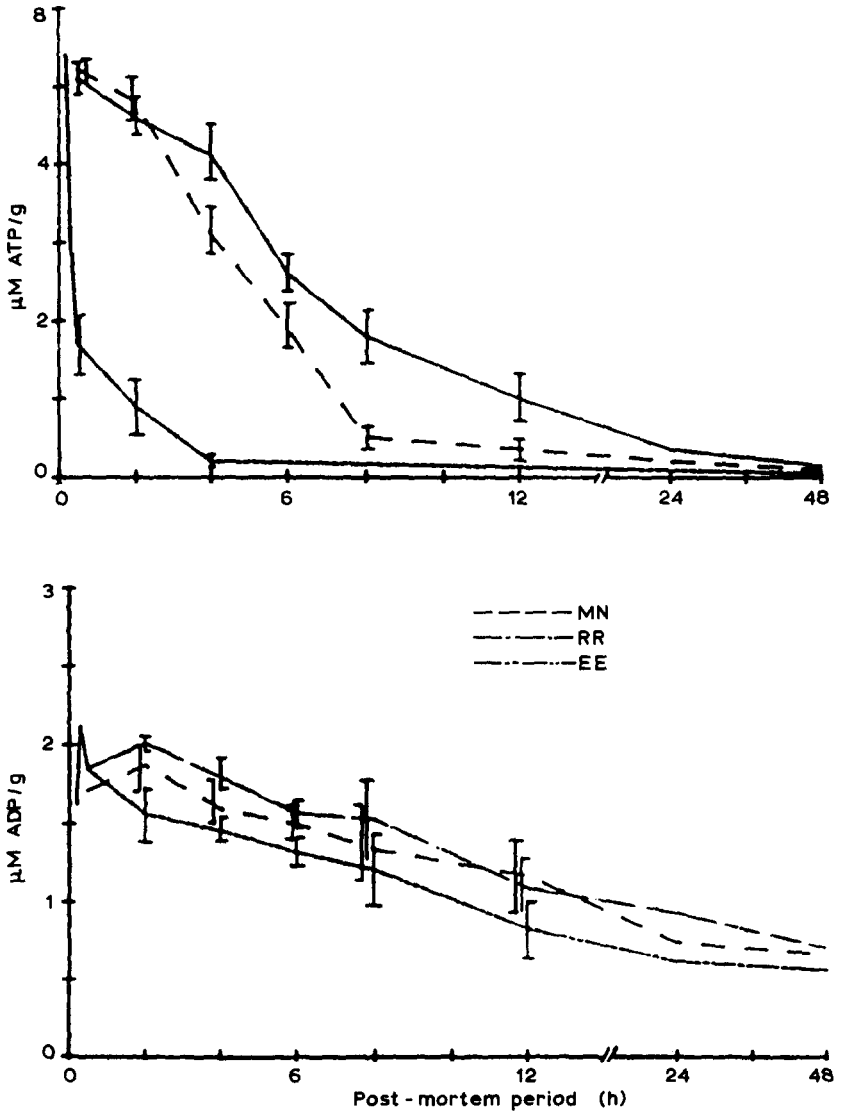

Fig. 2. ATP and ADP content in the muscle during the 48 h *post mortem*; vertical bars indicate standard deviation.

the critical point for pH and T, thereby preventing the conditions that might trigger cold-shortening.

The ADP concentrations fell in all three batches as from 2 h *post mortem*, remaining nearly constant after 24 h *post mortem*. Similar behaviour was reported by Calkins *et al.* (1983) and Fabiansson & Laser Reuterswärd (1985). The increase observed during electrical stimulation was related to the rapid breakdown of ATP, but no differences were found between the various

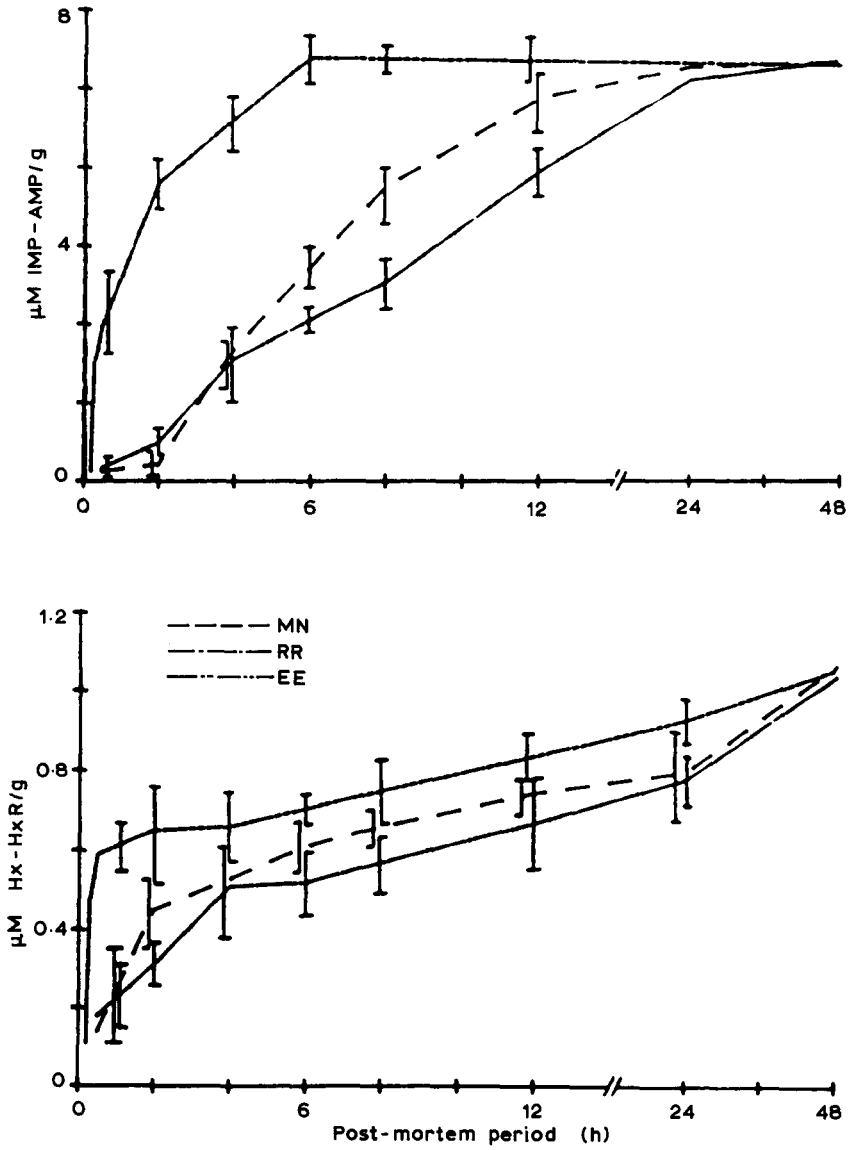

Fig. 3. AMP–IMP and HxR–Hx content in the muscle during the 48 h *post mortem*; vertical bars indicate standard deviation.

processing treatments, despite the different rates of ATP degradation. The AMP and IMP contents (Fig. 3) were measured together, because the AMP concentrations were less than 0·3 μM g^{-1} and practically constant over the 48 h *post mortem* (Calkins *et al.*, 1983; Fabiansson & Laser Reuterswärd, 1985) and because the deamination of IMP is practically instantaneous (Honikel & Fischer, 1977). It was therefore felt that changes in the

AMP–IMP value were mainly due to the variations in IMP. IMP formation was dependent upon both the chilling procedure applied and electrical stimulation. The ATP and IMP concentrations were inversely related over the study period (μM IMP $g^{-1} = 6{\cdot}37 - 1{\cdot}25$ μM ATP g^{-1}; $R^2 = 0{\cdot}83$). A slight increase in the Hx and HxR concentrations was observed during the 48 h post-mortem period (Fig. 3), and this was somewhat higher in the stimulated batch.

The trend in the *R* value over the study period is shown in Fig. 4 and was dependent upon the processing treatments applied. The increase was faster at the slower chilling rate and when ES was applied. In batch EE there was an increase of 0·16 during electrical stimulation, corresponding to the rapid breakdown of ATP at that time. There was a linear relationship between the decrease in the ATP concentration and the increase in the *R* value (*R* value $= 1{\cdot}24 - 0{\cdot}1$ μM ATP g^{-1}; $R^2 = 0{\cdot}86$), similar to that found using the data reported by Honikel *et al.* (1983) (*R* value $= 1{\cdot}19 - 0{\cdot}09$ μM ATP g^{-1}; $R^2 = 0{\cdot}92$). Enzymatic or chromatographic ATP determination can thus be replaced by the easier and faster *R* value measurement.

On the basis of the loss of muscle extensibility, Honikel *et al.* (1981) indicated that rigor mortis set in at the following values: pH = 5·9; ATP = 1 μM g^{-1}; *R* value = 1·10. Applying these criteria, rigor occurred at about 8 h *post mortem* in the slow-chilled batch, at 12 h *post mortem* in the quick-chilled but unstimulated batch, and at 2–4 h *post mortem* in the stimulated batch, which highlights the acceleration of post-mortem glucolysis brought about by ES.

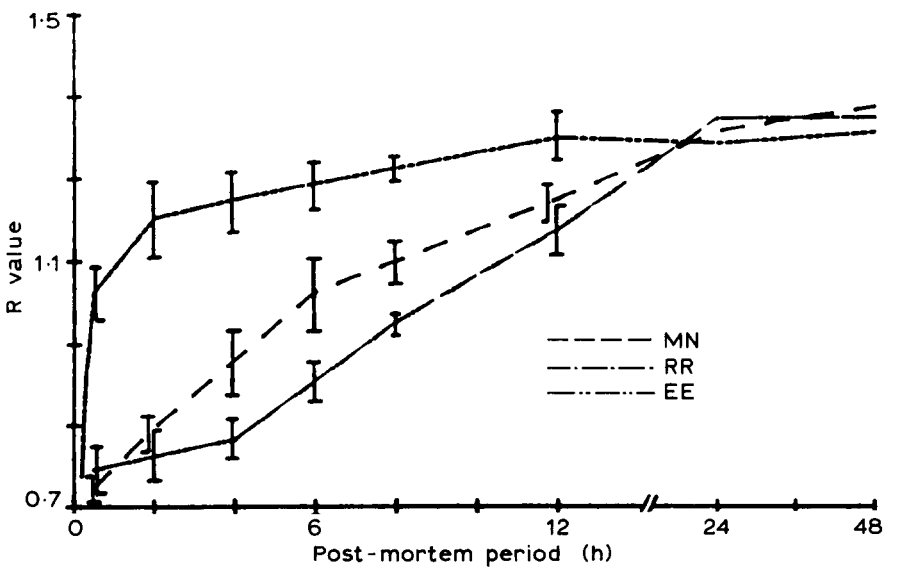

Fig. 4. *R* value during the 48 h *post mortem*; vertical bars indicate standard deviation.

In conclusion, applying a current at 56 V of effective voltage while reversing polarity every 10 s to lamb carcasses accelerated post-mortem glucolysis and prevented cold-shortening in conjunction with quick-chilling immediately after dressing.

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