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# DISTINGUISHING METABOLIC HEAT FROM CONDENSATION HEAT DURING MUSCLE RECOVERY

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## Summary

**When a thermopile is used to measure the heat production of isolated muscle, the muscle is surrounded by gas saturated with water vapour, initially in equilibrium with the muscle. After contraction, the osmolarity of the muscle is raised so that it is no longer in equilibrium with the gas around it, and condensation will occur. When artificial muscles of known osmolarity were placed on a thermopile surrounded by gas in equilibrium with a solution of lower osmolarity, their temperature was found to be raised (by 102.7 mK osmol$^{-1}$ l). This temperature increase was greatly reduced by covering the artificial muscle with a Teflon film. Experiments on living muscle from the dogfish *Scyliorhinus canicula* showed that muscle temperature was higher 2 min after a series of 20 twitches at 3 Hz if the muscle was not covered by Teflon than if it was covered. The Teflon covering did not diminish the muscle's contractile performance. We conclude that the condensation of water does contribute to the heat measured during the recovery period, but that when the muscle is covered by Teflon film condensation heat can largely be prevented so that only genuine metabolic recovery heat is produced.**

Key words: muscle, contraction, heat, recovery, condensation heat, metabolism, dogfish, *Scyliorhinus canicula*.

## Introduction

During muscle contraction, ATP is hydrolyzed to ADP and inorganic phosphate by the cross-bridge cycle and by the $Ca^{2+}$ pump of the sarcoplasmic reticulum. However, the ADP is immediately rephosphorylated to ATP by the creatine kinase reaction that transfers inorganic phosphate from phosphocreatine (PCr) to ADP (McFarland *et al.* 1994). Thus, the net reaction is:

$$\text{PCr} \rightarrow \text{creatine} + \text{inorganic phosphate}. \quad (1)$$

One of the consequences of this reaction is an increase in the intracellular osmolarity of the muscle fibres during contraction. In the period following contraction, metabolic recovery occurs and this reaction is reversed. Complete metabolic recovery restores both the PCr concentration and muscle osmolarity to their pre-contraction values.

The changes in osmolarity that occur during contraction and recovery were recognized some 60 years ago, well before the chemical processes that produce them had been identified (Hill, 1929). Hill detected an unexpectedly large heat production after the end of muscle contraction under anaerobic conditions, which he knew would not support heat-producing oxidative recovery reactions. He found that the amount of 'extra heat' varied with the vapour pressure of the gas around the muscle and concluded that the condensation of water on the muscle was the source of the heat.

Hill's (1929) experiments showed that during anaerobic recovery the heat from condensation was large enough to be an important artefact compared with the small amount of metabolic heat produced under these conditions. It was less important under aerobic conditions, where it amounted to only a few per cent of the much larger amount of heat produced during aerobic recovery (Hill, 1965).

The size of the muscle preparation should influence this condensation artefact. Condensation occurs at the surface of the muscle preparation, and thus surface area determines the rate of production of condensation heat. In contrast, the rate of production of metabolic heat depends on the volume of the muscle preparation. So condensation heat should be proportional to the surface-to-volume ratio. Thus, the condensation artefact is likely to be larger in relation to the metabolic recovery heat in experiments on muscle preparations consisting of only a few fibres than it was in Hill's experiments on whole muscle consisting of thousands of fibres. Preliminary experiments on single fibres from *Xenopus laevis* support this view (G. Elzinga, personal communication).

Here we report experiments designed to test whether

detectable condensation heating occurs in the experimental apparatus we use for measuring the heat production of small bundles of white muscle fibres from dogfish. Having found that it does, we have devised a method to prevent the artefact by using Teflon (PTFE, polytetrafluoroethylene) film to shield the muscle from condensation.

## Materials and methods

### *Thermopile*

The thermopile measured the difference between the temperature of the muscle or the artificial muscle and the thermopile frame, which was kept at 19 °C, the reference temperature (Woledge *et al.* 1985). The thermopile was made by deposition of constantan and chromel to form a series of 48 thermocouples on a Kapton substratum. The active region of the thermopile was 12 mm long and contained four thermocouples per millimetre along its length. Recordings were made of the output from a 3 or 4 mm length of thermopile (12 or 16 thermocouples). Each thermocouple produced 34.2 $\mu V\,K^{-1}$.

### *Increased temperature due to condensation*

Experiments were carried out to measure the change in temperature of an artificial muscle due to condensation of water. The condensation occurred because the vapour pressure of the artificial muscle was less than that of the saline in equilibrium with the gas phase. The gas phase in the thermopile chamber was in equilibrium with the 1.00× normal saline saturating the filter paper (8 mm×50 mm) covering the inner surface of the lid of the chamber (Fig. 1). The composition of this saline is shown in Table 1A. The artificial muscle consisted of two layers of filter paper (total thickness 0.32 mm) cut to a width and length similar to those of a muscle preparation (2 mm×8 mm). The layers of filter paper were clipped together (one on top of the other) by the same platinum clips that were used to attach to myosepta in the experiments on muscle. The distance between the two clips was 6 mm, close to the usual muscle fibre length. The artificial muscle was soaked in saline of known composition. Three saline solutions were used: 1.00× normal, 1.13× normal and 1.26× normal osmolarity (see Table 1A for compositions).

After placing the artificial muscle on the thermopile and closing the chamber, thermopile output was recorded for 30–60 min. In some experiments, the artificial muscle was covered with a Teflon film to reduce condensation, as will be described in the Results section. The Teflon film was 12 μm thick and was cut to cover the surface of the artificial muscle. Vaseline filled the gaps between the film and the thermopile surface.

### *Experiments on muscle*

Bundles of 16–20 fibres were dissected from thin slices of white myotomal muscle of dogfish *Scyliorhinus canicula* (L.). The muscles were dissected in dogfish saline containing (in $mmol\,l^{-1}$): NaCl, 292; KCl, 3.2; $CaCl_2$, 5.0; $MgSO_4$, 1.0; $Na_2SO_4$, 1.6; $NaHCO_3$, 5.9; urea, 483; and tubocurarine, 1.5 $mg\,l^{-1}$. The same solution was used during the muscle experiments. The temperature during the experiment was approximately 19 °C. A piece of myoseptum at each end of the muscle fibres was held in a T-shaped platinum clip and mounted in contact with a thermopile between a force transducer (Cambridge Technology, Inc., model 400A) and a motor (Cambridge Technology, Inc., model 300B). In these experiments, all contractions were isometric, so the motor arm remained in a fixed position. A small amount of dogfish saline was left on the thermopile with the muscle preparation, and a piece of filter paper soaked with this saline covered the inner surface of the lid of the muscle chamber as explained above. The vapour pressure in the chamber was therefore in equilibrium with the saline.

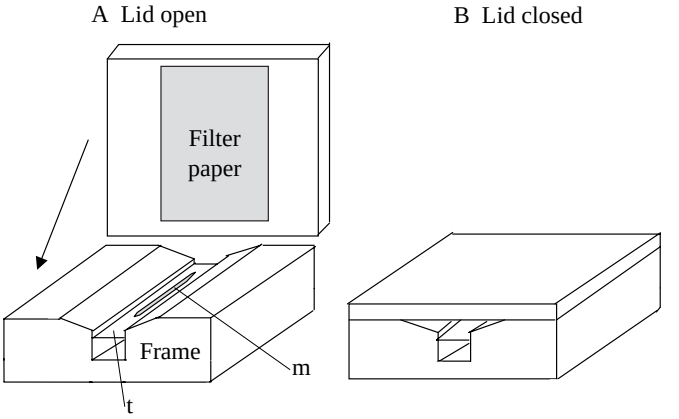


Fig. 1. Diagram (not to scale) of part of the experimental apparatus for heat measurement with the lid open (A) and closed (B). The thermopile (t), which is a thin sheet, has its reference junctions in contact with the aluminium frame, which is thermostatted to the reference temperature. A bundle of muscle fibres or an artificial muscle (m) is shown in place over the 'hot' junctions of the thermopile. The piece of filter paper on the inner surface of the lid is soaked with 1.00× normal saline. When the lid is closed (B), the 1.00× normal saline establishes vapour equilibrium with the gas phase in the chamber.

The fibre bundle was stimulated end-to-end with 0.2 ms electrical pulses. The stimulus strength was supramaximal, and muscle length was adjusted to $L_0$, the length at which tetanic force was greatest. Force and heat production were recorded during 20 isometric twitches at a frequency of 3 Hz (this is referred to as the initial period) and then for 2 h without stimulation (the recovery period). This pattern was repeated between two and six times on each muscle preparation under two conditions: (1) the muscle and adhering saline covered with a piece of Teflon film (12 μm thickness) and sealed at the edges with Vaseline, and (2) without this covering.

## Results

### *Temperature and condensation*

Fig. 2 shows examples of the thermopile output during experiments designed to measure the temperature change

Table 1. *Composition and vapour pressure calculations for the saline solutions*

| Solution | Osmolarity (mosmol $l^{-1}$) | [NaCl] (mmol $l^{-1}$) | [Urea] (mmol $l^{-1}$) |
|---|---|---|---|
| A | | | |
| 1.00× normal | 1116 | 316.3 | 483.0 |
| 1.13× normal | 1261 | 357.4 | 545.8 |
| 1.26× normal | 1406 | 398.5 | 608.6 |
| | Water (osmol fraction) | Δosmol fraction | Calculated Δ*T* (mK) |
| B | | | |
| 1.00× normal | 0.98025 | 0 | |
| 1.13× normal | 0.97774 | –0.00251 | 40.1 |
| 1.26× normal | 0.97525 | –0.00500 | 79.8 |

Water osmol fraction, mol water/(mol water plus osmol solute).

Δosmol fraction water, water (osmol fraction) minus value for 1.00× normal saline = Δrelative vapour pressure (see text).

Calculated Δ*T* = Δrelative vapour pressure/0.0626. This is because, at 19°C, the vapour pressure of water increases by 6.26 % $°C^{-1}$ (Kaye and Laby, 1973).

caused by condensation. The thermopile measured the difference (Δ*T*) between the reference temperature and that of an artificial muscle (made from filter paper) saturated with one of the saline solutions described in Table 1A. In all cases, the gas phase in the thermopile chamber was close to equilibrium with the 1.00× normal saline because of the much larger area of the filter paper which covered the lid and was soaked with that saline. The recording started when the thermopile chamber was closed. At this time, the temperature of the artificial muscle was far from the thermopile reference temperature and the thermopile amplifier was saturated. The temperature changes rapidly at the beginning of the recording period because heat flows down the large temperature gradient between the artificial muscle and the thermopile frame, which is thermostatted and acts as a heat sink. The rate of change of temperature diminishes with time as the temperature gradient decreases.

The broken lines in Fig. 2 show recordings made with the artificial muscle saturated with 1.00× normal saline, that is, no difference from the saline with which the gas phase was equilibrated. The thermopile output reaches a final steady value close to zero temperature difference between the artificial muscle and the reference temperature, i.e. the temperature of the thermopile frame. Heat flow has produced a steady temperature, which is uniform. The fact that the temperature is uniform (Δ*T* approaches 0) indicates that no heat is being produced or absorbed in the artificial muscle.

The solid lines in Fig. 2 show the recordings made with the artificial muscle saturated with 1.13× or 1.26× normal saline. In these cases, the saline of the artificial muscle has a higher osmolarity and thus a lower vapour pressure than the saline with which the gas phase was equilibrated. The thermopile output reaches a steady value, indicating that the temperature of the artificial muscle was greater than the reference

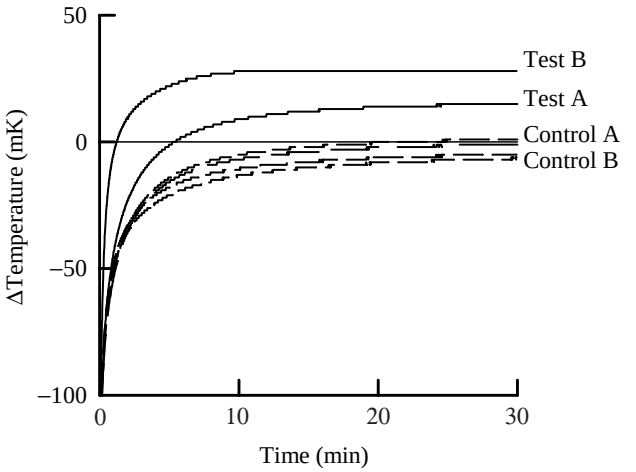


Fig. 2. Examples of recordings of the time course of change in temperature (ΔTemperature) of an artificial muscle (measured with respect to the reference temperature) recorded by the thermopile. In the case of test A, the artificial muscle was soaked in 1.13× normal saline (solid line). In the case of test B, the artificial muscle was soaked in 1.26× normal saline (solid line). In control A and control B, the artificial muscle was soaked in 1.00× normal saline (broken lines). Controls were done before and after tests. A value of zero on the ordinate indicates that the temperature of the artificial muscle was the same as the reference temperature.

temperature. The size of the temperature difference depended on the composition of the saline soaking the artificial muscle: 1.13× normal saline corresponded to Δ*T*=15 mK and 1.26× normal saline corresponded to Δ*T*=33 mK. This is consistent with the temperature difference being due to heat produced by the condensation of water on the artificial muscle.

To test this idea further, we performed additional experiments with the artificial muscle covered by a Teflon film. Teflon was chosen because it is relatively impermeable to water, but relatively permeable to oxygen. (Oxygen permeability is not important in these tests with artificial muscles, but is in other experiments with living muscle fibres.) In these experiments, thermopile output was recorded from artificial muscles with and without Teflon film. Tests were carried out with the three saline solutions shown in Table 1A. Fig. 3 summarizes the results. The steady temperature differences recorded from artificial muscles without a Teflon cover (open symbols) are proportional to the difference in osmolarity between the artificial muscle and the saline with which the gas phase was in equilibrium. A much smaller temperature difference was recorded with Teflon-covered artificial muscle (filled symbols), as would be expected if the Teflon film effectively separates the regions with different vapour pressures and thus largely prevents condensation. The small remaining temperature difference might arise because the sealing of the Teflon around the edge of the artificial muscle is not perfect.

### *Muscle temperature during recovery*

Experiments were performed to record muscle temperature during a 2 h recovery period following a series of 20 isometric

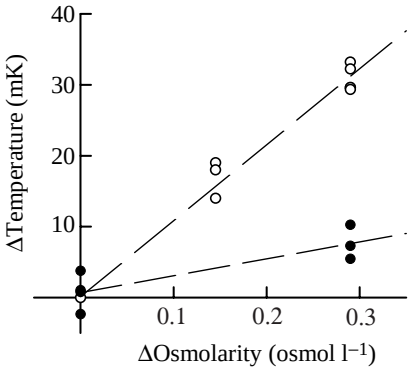


Fig. 3. A summary of measurements of steady temperature from experiments such as those shown in Fig. 2. ΔTemperature is the temperature of the artificial muscle with respect to the reference temperature, as measured by the thermopile. The symbols are values measured for artificial muscle with (filled symbols) and without (open symbols) a covering of Teflon film. The broken lines are the linear regressions of temperature difference on osmolarity (ΔOsmolarity) for the two conditions. The slopes are 23.9 mK osmol$^{-1}$ l ($r^2$=0.725, $P$=0.015) with the Teflon film and 102.7 mK osmol$^{-1}$ l ($r^2$=0.968, $P$<0.001) without the Teflon film.

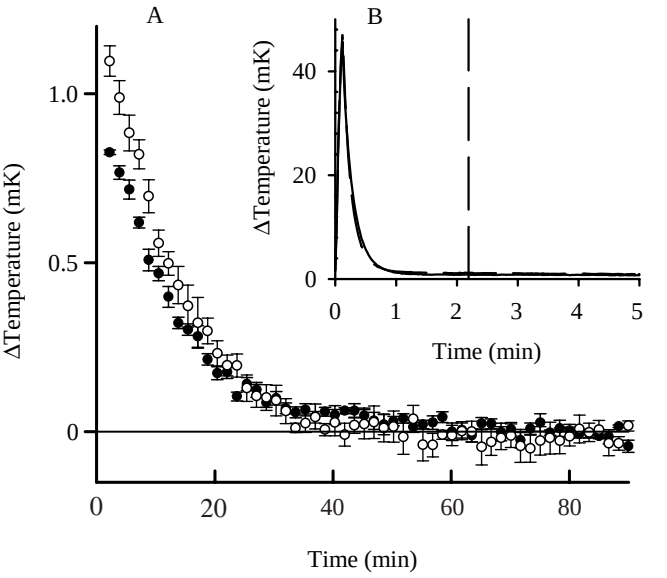


Fig. 4. (A) An example of the time course of the change in temperature (ΔTemperature) of a muscle preparation recorded by the thermopile during 90 min of recovery after a series of 20 isometric twitches at 3 Hz. Each data point is the mean value over a period of 100 s and the bars show ± S.E.M.; $N$=4 for results with Teflon (filled symbols) and $N$=5 for results without Teflon (open symbols). The values of the first points are 0.827±0.007 mK (mean ± S.E.M., $N$=4) with Teflon and 1.096±0.045 mK ($N$=5) without Teflon. The inset (B) shows the temperature change during the first 5 min after the beginning of stimulation. The same muscle preparation as in A. Note the differences in both temperature and time scales between A and B. Unbroken line with Teflon; broken line without Teflon. The vertical broken line shows the time of the first point in A.

twitches at 3 Hz. Experiments were carried out on four muscle preparations, with recordings made both with and without a Teflon film covering the muscle fibres. Up to six repeat recordings were made for each condition.

We used the tension–time integral as a measure of contractile performance and calculated the ratio for each set of twitches to the value for the first set of twitches performed by that muscle preparation (in all cases this was with Teflon). The fourth set had a tension–time integral of 0.95±0.09 (mean ± S.E.M., $N$=4). After the Teflon had been removed, the tension time integral was 0.93±0.10 ($N$=3) in the second set of twitches without Teflon. The extent of decline under both conditions was small, approximately 1 % decline for each set of twitches and its 2 h recovery period. It was similar with and without Teflon. These results suggest that Teflon did not prevent or seriously impair metabolic recovery between the sets of twitches by, for example, preventing adequate amounts of oxygen from reaching the muscle fibres. It seems likely that, if recovery had not occurred between the sets of twitches, the decline in the tension–time integral would have been greater than 1 % per set of twitches.

Fig. 4A shows the means of four temperature recordings with Teflon (filled circles) and five without Teflon (open circles) on the same muscle preparation during recovery. (These recordings start after the end of a set of twitches; see Fig. 4B). The recordings of temperature during recovery show that muscle temperature is initially above the reference temperature and that muscle temperature decreases towards the reference temperature with time. These general trends in muscle temperature reflect the heat produced by metabolic recovery processes resynthesizing the ATP and phosphocreatine that had been used during the series of twitches. The relevant points here are as follows. (1) The temperature of the muscle was significantly higher when not covered with Teflon film than when covered; $P$<0.01 (Student's $t$-test) for the first point shown in Fig. 4A, results for one muscle preparation. Experiments were performed on four muscle preparations. Combining all of the results to give a best estimate (Curtin and Woledge, 1979) of the temperature difference near the start of recovery gives a value 0.182±0.046 mK (mean ± S.E.M., $P$=0.029). (2) The size of the difference between the two recordings decreases with time, and by 30 min in all experiments the difference was not significant. As the metabolic recovery progresses, phosphocreatine and ATP are resynthesized and the osmolarity of the muscle decreases. Thus, the difference between the two recordings has the time course and sign expected for extra heat produced by water condensing on the muscle while it is not covered by Teflon.

## Discussion

The results of the experiments with artificial muscle and solutions of known composition are qualitatively consistent with the hypothesis that heat from the condensation of water is responsible for increasing the temperature of the artificial muscle by a detectable amount. Figs 2 and 3 show that the temperature of the artificial muscle is high under conditions

where the vapour pressure of the artificial muscle is low because its osmolarity is high.

This hypothesis can be examined more quantitatively by considering whether condensation could produce a large enough temperature change to account for the observed effect. The temperature change that can be produced by condensation is limited because an increase in temperature of the muscle increases its vapour pressure. Condensation stops when the vapour pressure of the muscle matches that of the gas phase. To calculate this limiting temperature, we have calculated the temperature at which the vapour pressure of the artificial muscle would reach that of the gas phase. For temperatures near 19 °C, the vapour pressure of water increases with an increase in temperature by 6.26 % °C$^{-1}$ (Kaye and Laby, 1973). The vapour pressure of the gas phase can be calculated from the osmolarity of the solution in equilibrium with it (1.00× saline in our experiments) using Raoult's Law:

$$P/P_o = 1 - X_s, \quad (2)$$

where $P$ is the vapour pressure of the water in the solution, $P_o$ is the vapour pressure of pure water, $X_s$ is the osmol fraction of solute in the solution and $1–X_s$ is the osmol fraction of solvent in the solution. We have assumed that NaCl is fully ionized in the solutions. Table 1B shows the values calculated from these relationships for the saline solutions we used. These calculations show that the temperature of the artificial muscle containing 1.13× normal saline would have to be approximately 40.1 mK above that of 1.00× normal saline for their vapour pressures to be equal. For artificial muscle containing 1.26× normal saline, the temperature difference would have to be 79.8 mK. In other words, these are the maximum temperature changes that could be produced by condensation. The temperature differences that we measured, 15 and 33 mK (see Figs 2, 3), were only approximately half these maximum values and thus could be fully accounted for by condensation.

We can suggest two reasons for our observed temperature difference being less than the full amount that could be produced by condensation. First, we assumed fully ionized solutes and ideal behaviour in our calculations. If the solutes are not fully ionized and the solution behaviour is not ideal, then increasing osmolarity would have less effect on the vapour pressure than predicted and a smaller temperature difference would suffice to make vapour pressures match. Second, and probably more importantly, some of the heat produced by condensation on the artificial muscle is 'lost' by conduction along the thermocouples into the thermopile frame, which acts as a heat sink and is thermostatted. The final, steady temperature of the artificial muscle in Fig. 2 represents the balance between the competing processes of heat gain from condensation and heat loss by conduction. This balance will be established when the rate of heat loss is equal to the rate of heat gain. The rate of heat gain is limited by the diffusion of water vapour through the gas phase to the surface of the muscle and/or by the rate of the condensation process itself.

## *Muscle heat*

### *Condensation artefact and recovery heat*

In a similar way, we can calculate how much PCr splitting would have to occur in the muscle experiments to account for temperature differences such as those shown in Fig. 4. For this calculation, we used the value of 102.7 mK osmol$^{-1}$ l from our observed relationship between $\Delta T$ and osmolarity (Fig. 3) and the maximum observed temperature difference between recordings with and without Teflon (first point in Fig. 4A). At this time, very little metabolic recovery has occurred and the effect of PCr splitting on osmolarity would be near maximal. The largest temperature difference observed in the muscle experiments was 0.27 mK. This temperature change could be explained by the osmotic effect of the splitting of a small fraction (approximately 13 %) of the PCr in resting muscle (100 %=20 mmol l$^{-1}$ intracellular water). The stimulus pattern used here would cause about this amount of PCr splitting (Curtin *et al.* 1997) and is thus sufficient to account for the observed heating.

To assess whether condensation heating would have a significant effect on the measurement of metabolic heat during the recovery period, we have evaluated the recovery heat (1) from the recordings made with the muscles covered with Teflon, which would prevent condensation heating, and (2) from the recordings made with the muscles not covered, in which case the condensation heating causes some of the observed heat production. We also evaluated the initial heat (the heat produced during stimulation) for each recording and expressed the recovery heat as a multiple of the initial heat (the recovery ratio) to take account of the differences in muscle size. The mean value of the recovery ratio was 1.36 ($N$=19) when the muscles were covered and 3.49 ($N$=16) when they were not covered. The difference was statistically significant ($P$<0.001, two-way analysis of variance).

### *Condensation artefact and initial heat*

Fig. 4B shows recordings of temperature change for 5 min from the start of stimulation. The temperature rises rapidly and reaches its maximum value at the end of the period of stimulation. During the next minute, the temperature falls to approximately 2 % of its peak value, as heat is lost to the thermopile frame. The temperature then remains slightly above the baseline because of heat production due to metabolic recovery processes and due to condensation. The vertical broken line marks the time of the first point shown in Fig. 4A. Although condensation has a considerable effect on the temperature during recovery, it has a negligible effect on the temperature during stimulation because the temperature rise due to metabolic processes during this time is so much greater than that during recovery.

We conclude that contraction can cause muscle osmolarity to increase enough for water condensation on the muscle to produce a detectable amount of heat during recovery. The condensation heating is not negligible compared with metabolic recovery heat in a preparation consisting of a small number of muscle fibres. Most of the heat artefact is eliminated

when condensation is prevented by covering the muscle with Teflon film.

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