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A DEVICE FOR STATIONARY AND NON-STATIONARY MEASUREMENT OF HEAT PRODUCTION IN THE CONTRACTING MUSCLE*

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ABSTRACT

An experimental arrangement is described to measure the heat production in the contracting muscle after a single stimulation (non-stationary) as well as after periodic stimulation (stationary). The variation of temperature is registered by a thermistor, a. c. bridge, and phase-sensitive rectifier, and is stored digitally.

For fresh M. *gastrocnemius* at 0°C, one finds a heat production per cm^3 of about 3.3 mcal/contraction. In order to explain the systematic difference in the results of both methods, the heat conduction equation is solved for a model system in the non-stationary case. This allows one to estimate the tickness d of the necrotic zone which, during the measurement, arises around the thermistor stuck in the muscle. The calculated values are in the range of $d = 0.1$–0.6 mm.

Finally, the possibility of measuring non-stationary heat production in situ is critically discussed.

INTRODUCTION

In classical experiments by Hill and coworkers[1–9], the heat production in a contracting isolated frog muscle was investigated by measuring the surface temperature of the muscle. From these measurements and the experiments of other authors like Carlson et al.[10, 11] and Gibbs et al.[12], also the time dependence of heat production was derived for isometric and isotonic contractions under varying experimental conditions. Recently, works by Edwards et al.[13, 14] were published where temperature measurements of contracting muscles in situ with stuck-in thermistors were described. The authors mention a time delay of temperature registration which they assume to be due to damaged tissue or fluid at the probe tip.

The aim of the present work is to compare stationary and non-stationary measurements of heat production with thermistors stuck in the muscle. Of particular interest was the critical interpretation of the second type of experiment and the

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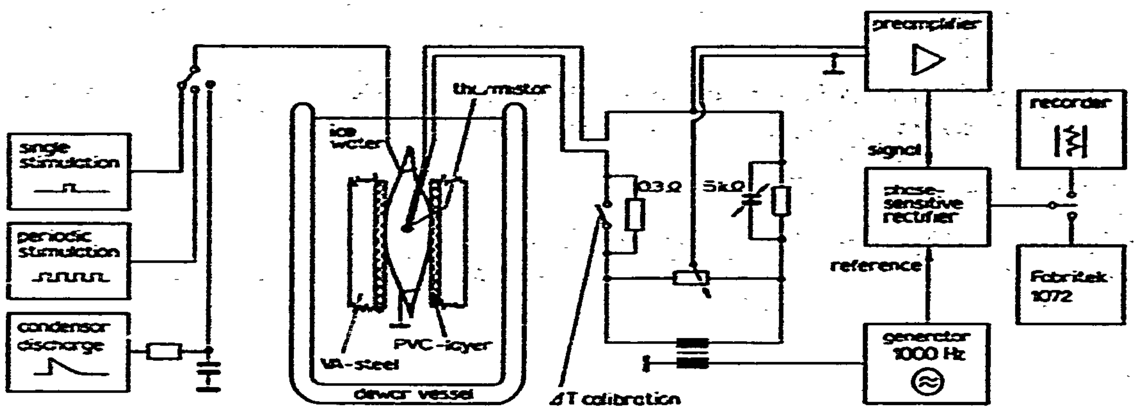

Fig. 1. Block diagram of the measuring device.

question, to which extend one can derive kinetic statements from measurements in situ with stuck-in thermistors.

METHODS

I. Experimental

Figure 1 shows the block diagram of the device used. Single pulses of variable length (4–8 msec) and amplitude (5–30 V) as well as pulse sequences (frequency 0.01–0.1 Hz) are taken from a pulse generator. For the rapid Joulean heating of an inactive muscle (exhausted by repeated contractions) a condenser discharge (3.3 μF, 300 V) was used. The measuring cell lies in a Dewar vessel and is thermostated by an ice–water mixture, piled up vertically. The temperature change of the muscle is registered by the thermistor (type YSI 511, diameter 0.7 mm) as a change in resistance which is measured by means of a 1000 Hz a. c. bridge. The heat production of the thermistor itself is negligible. The detuning of the bridge is measured by a low-noise pre-amplifier (Brockdeal 451) and a phase-sensitive exchangeable lock-in amplifier (home-built)*, and registered either as analogue or as digital information (Fabritek 1072). A combination of resistors in series with the thermistor (here 0.3 Ω) is used for the Δ T-calibration.

Figure 2 shows the experimental set-up with the thermostated measuring chamber, where the measuring cell containing the muscle is inserted. The cell itself consists of a VA cylinder, coated inside with a PVC layer of known thickness (gradient principle).

The experiments have been performed with the M. *gastrocnemius* of rana temporaria.

The muscle is prepared so, that on the one hand part of the tendon, on the other hand part of the bones femur and os cruris were kept to fasten the muscle. It is

* Construction details are obtainable on request.

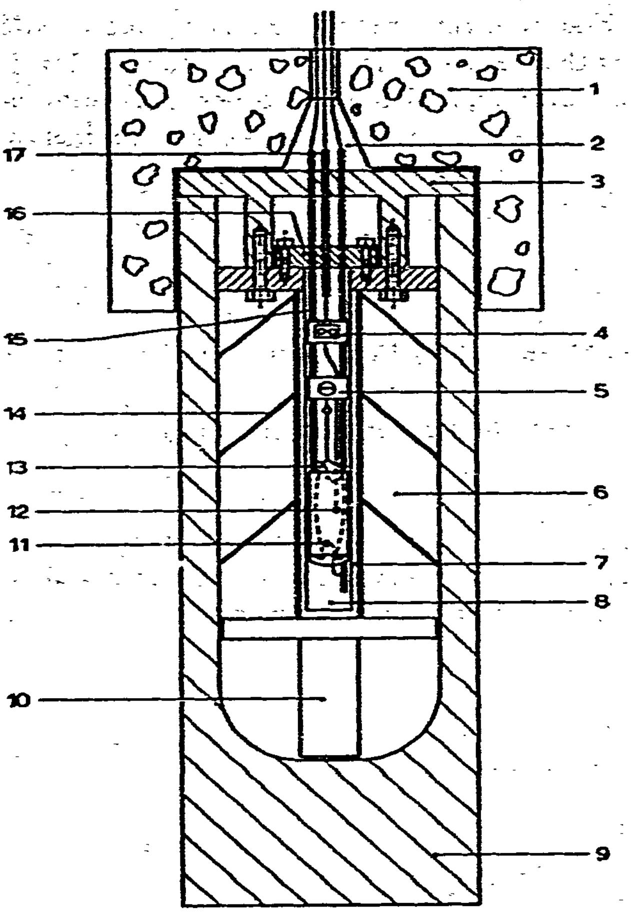

Fig. 2. Thermostated measuring chamber. 1 = thermal isolation (foamed plastics); 2 = cable of electrodes and thermistor; 3 = supporting frame of measuring chamber (plexiglas); 4 = clamping bolt for needle and thermistor; 5 = clamping bolt for tendon; 6 = interspace of ice chamber; 7 = cylindrical steel tube (VA) of measuring chamber; 8 = inside of measuring chamber; 9 = dewar vessel; 10 = pedestal of ice chamber (plexiglas); 11, 13 = stimulation electrodes; 12 = muscle in measuring cell with thermistor tip inside; 14 = wire netting; 15 = steel tube, holding device for clamping bolts and measuring cell; 16 = cap of measuring chamber; 17 = thermistor tube.

stimulated with voltage pulses (5–30 V) fed to the isometrically stretched muscle by annular electrodes of Pt wire. The thermistor is inserted axially with the help of a cannula, which is removed after that.

In preliminary experiments, the response function of the thermistor to a temperature jump ΔT in aqueous environment was determined. The temperature jump was produced by condenser discharge in a suitable measuring cell (0.1 M NaCl), the response function was digitally stored (Fabritek 1072). Within the experimental error, it turned out to be a pure exponential for $\Delta T = 3\,^{\circ}C$, with a time constant $\tau =$ 160 ms.

II. Theoretical

The evaluation of the stationary experiments was based on a model of two concentric cylinders with radii r_i and heat conductivities λ_i, one being the muscle ($i = 1$), the other being the PVC coat of the measuring cell ($i = 2$). The heat flow is assumed to be only radial. When, by periodic stimulation with frequency f, a stationary state with mean temperature ΔT_{stat} is reached, the heat production per contraction becomes

$$\Delta Q_{stat} = \frac{1}{\dfrac{r_1^2}{4\lambda_1} + \dfrac{r_2^2}{2\lambda_2} \ln \dfrac{r_2}{r_1}} \frac{1}{f} \Delta T_{stat} \tag{1}$$

The constants are $r_1 = 0.35$ cm, $r_2 = 0.425$ cm, $\lambda_1 = 1.18$ mcal cm^{-1} s^{-1} K^{-1}, $\lambda_2 =$ 0.38 mcal cm^{-1} s^{-1} K^{-1}.

The heat production in the single contraction experiment is simply

$$\Delta Q_{single} = c \cdot \rho \cdot \Delta T \tag{2}$$

with the specific heat of muscle tissue $c = 0.86$ cal g^{-1} K^{-1} and the mass density $\rho = 1.06$ g cm^{-3}. ΔT should be the exact excess temperature caused by contraction. For a simple evaluation, the measured maximum excess temperature ΔT_{max} was taken.

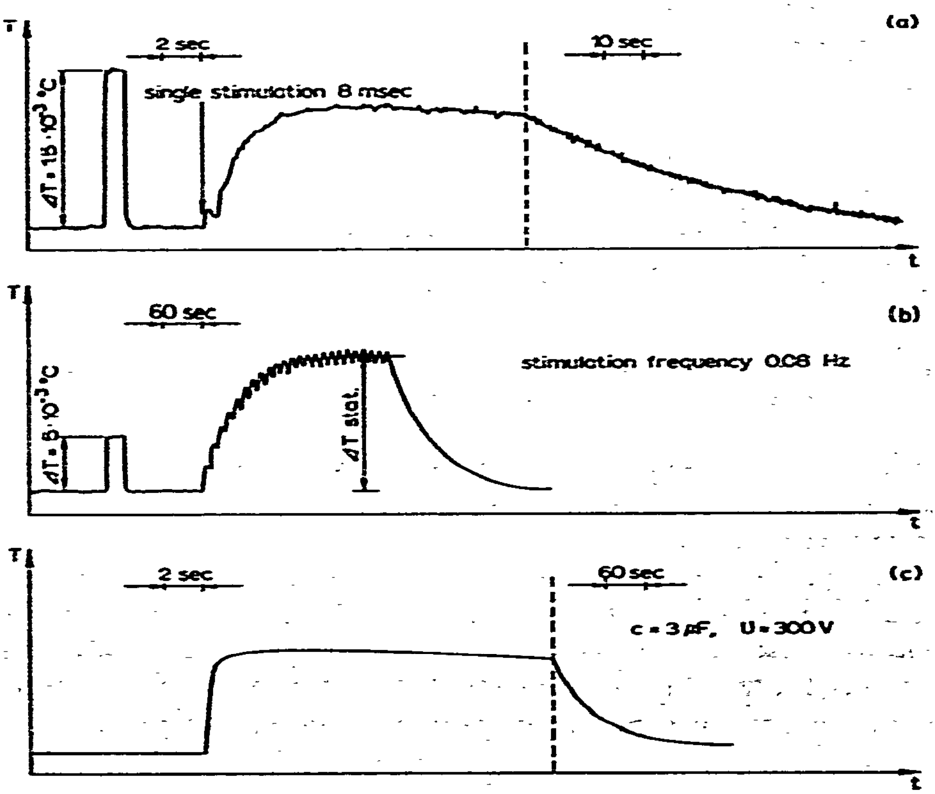

Fig. 3. Temperature measured for (a) single contraction; (b) periodic contractions; (c) Joulean heating of frog's muscle.

A better approach is to consider in more detail the heat diffusion process after the heat production. The calculation of the temperature as a function of time was based on a model consisting of five concentric circular cylinders (cf. Fig. 3):

(1) thermistor core (radius $r < r_1$)

(2) thermistor envelope ($r_1 < r < r_2$)

(3) necrotic muscle–tissue zone around the inserted thermistor, not giving rise to heat production ($r_2 < r < r_3 = r_2 + d$)

(4) muscle tissue ($r_3 < r < r_4$)

(5) inside coat of the measuring cell, PVC ($r_4 < r < R$).

If the heat conduction is assumed to be only radial, the heat conduction equation is simplified to:

$$\frac{\partial T}{\partial t} = a\left(\frac{\partial^2}{\partial r^2} + \frac{1}{r}\frac{\partial}{\partial r}\right)T \equiv aDT \tag{3}$$

where $a = \lambda/\rho c$ is the temperature conductivity.

The initial and boundary conditions for the solution read:

$$T(r,0) = \begin{cases} \Delta T_{\text{jump}} \text{ for } r_3 < r < r_4 \\ 0 \text{ elsewhere} \end{cases} \tag{4}$$

$$T(R,t) = 0\,^\circ\mathrm{C} = \text{const.}$$

The radial heat conduction equation is solved numerically with the "implicit method" which will be described here briefly for the case of equally spaced radial points $r_i = (i-1)\cdot\Delta r$.

The temperature values $T_{1i} = T(r_i, t_1)$ at time t_1 are given, the values $T_{2i} = T(r_i, t_2 = t_1 + \Delta t)$ of the temperature distribution at a later time t_2 are to be determined. Replacing the differential quotient by the quotient of finite differences, one obtains approximately:

$$\frac{T_{2i} - T_{1i}}{\Delta t} = a\,(DT_2)_i \tag{5}$$

Corresponding approximations for the spatial derivatives on the right-hand side give:

$$a\,(DT_2)_i = a\left(\frac{T_{2i-1} - 2T_{2i} + T_{2i+1}}{(\Delta r)^2} + \frac{1}{r_i}\,\frac{T_{2i+1} - T_{2i-1}}{2\Delta r}\right) \tag{6}$$

Both equations are combined to result in a system of linear equations:

$$a\,\frac{\Delta t}{(\Delta r)^2}\left\{T_{2i-1} - 2T_{2i} + T_{2i+1} + \frac{\Delta r}{2r_i}(T_{2i+1} - T_{2i-1})\right\} - T_{2i} = -T_{1i} \tag{7}$$

These are implicit equations for T_2.

Iterative application to a given temperature distribution yields the function at any later time. For the spatial derivative, it is important to see that a discontinuity in heat conductivity at a radius r causes a jump in the first derivative:

$$\lambda(r-0)\frac{\partial}{\partial r}T(r-0,t)=\lambda(r+0)\frac{\partial}{\partial r}T(r+0,t) \quad (8)$$

For the numerical calculation of $T(r, t)$ a Fortran program was written which, following the procedure described above, permits to calculate the temperature distribution at times $t > 0$ iteratively from that at $t = 0$.

RESULTS AND DISCUSSION

Figure 3b shows an example of the temperature as a function of time measured for periodic contractions of the muscle with $f = 0.08$ Hz. The stationary state appears after a few minutes where an average excess temperature ΔT_{stat} of about $1.25 \cdot 10^{-2}$°C is measured. The corresponding heat ΔQ is calculated with eqn (1).

A typical temperature curve after a single stimulation (length 8 ms) is shown in Fig. 3a. Here, a maximum excess temperature $\Delta T_{max} = 10^{-3}$ C is reached after 8 s. For this experiment, ΔQ can be calculated from eqn (2) with ΔT_{max}.

To be consistent, of course, results obtained at the same muscle with either method should be compared. This is done in Table 1 where both values of ΔQ are listed in columns a and b. They decrease with the number of contractions as they should. There is, however, a discrepancy growing from 10 to 30%, ΔQ_{stat} always being larger. This, together with the fact that the temperature increase, see Fig. 3a, is remarkably slow, leads to the assumption of an increasing zone of inactive tissue around the thermistor. As a check, the muscle was exposed to Joulean heating by a fast condenser discharge. The resulting curve Fig. 3c shows a much faster temperature increase which was to be expected because the whole tissue was now uniformly heated.

To get a deeper understanding, the time behaviour of the local temperature distribution was calculated with the model system described above for single contraction. In this model, it was assumed, that there is a zone of necrotic tissue cylindrically symmetric around the stuck-in thermistor. It does not produce any heat, but should influence the heat conduction and its thickness d is used as a parameter for the calculation.

As an example, Fig. 4 shows for $d = 0.2$ mm the calculated local temperature after time intervals of 0.5 s. For $t = 0$ (time of stimulation), the distribution is rectangular according to the initial conditions. The temperature at $r = 0$ (thermistor core) is shown as a function of time in Fig. 5. The maximum temperature ΔT_{max} is reached at the time t_{max}, which is here ($d = 0.2$ mm) 6 s. If one denotes by ΔT_{jump} the (excess) temperature of the active muscle at $t = 0$, i.e., immediately after stimulation ΔT_{max} of the thermistor is here only 90% of ΔT_{jump}. This quotient as well as t_{max} are shown in Fig. 6 as functions of d. Thus, one must conclude that an inactive zone around the thermistor, even if it is very thin, leads to a considerable time delay, and consequently, a reduction of the temperature measured.

TABLE 1

EXPERIMENTAL RESULTS FROM MEASUREMENTS AT ONE MUSCLE, TEMPERATURE 0°C

Explanations in the text

Stimulation	*No.*	$\Delta T_{max.}$ (10^{-3} °C)	ΔQ_{jump} (mcal cm^{-3})	ΔQ_{stat} (mcal cm^{-3})	$t_{max.}$ (sec)	d (mm)	$\frac{\Delta T_{max.}}{\Delta T_{jump}}$	$\Delta Q_{jump}{}^{0}$ (mcal cm^{-3})	$\frac{\Delta Q_{stat}}{\Delta Q_{jump}{}^{0}}$
single	3	2.2	2.01		5	0.09	0.94	2.14	
periodic									1.10
0.03 Hz	4–16			2.24					
0.06 Hz	17–36			2.32					
single	37	1.85	1.69		7	0.38	0.84	2.01	
periodic									1.04
0.04 Hz	38–53			2.05					
single	54	1.76	1.61		7	0.38	0.84	1.92	
single	55	1.74	1.60		7	0.38	0.84	1.90	
periodic									1.08
0.05 Hz	56–72			2.06					
single	73	1.68	1.54		7.5	0.46	0.81	1.90	
			a	b				c	d

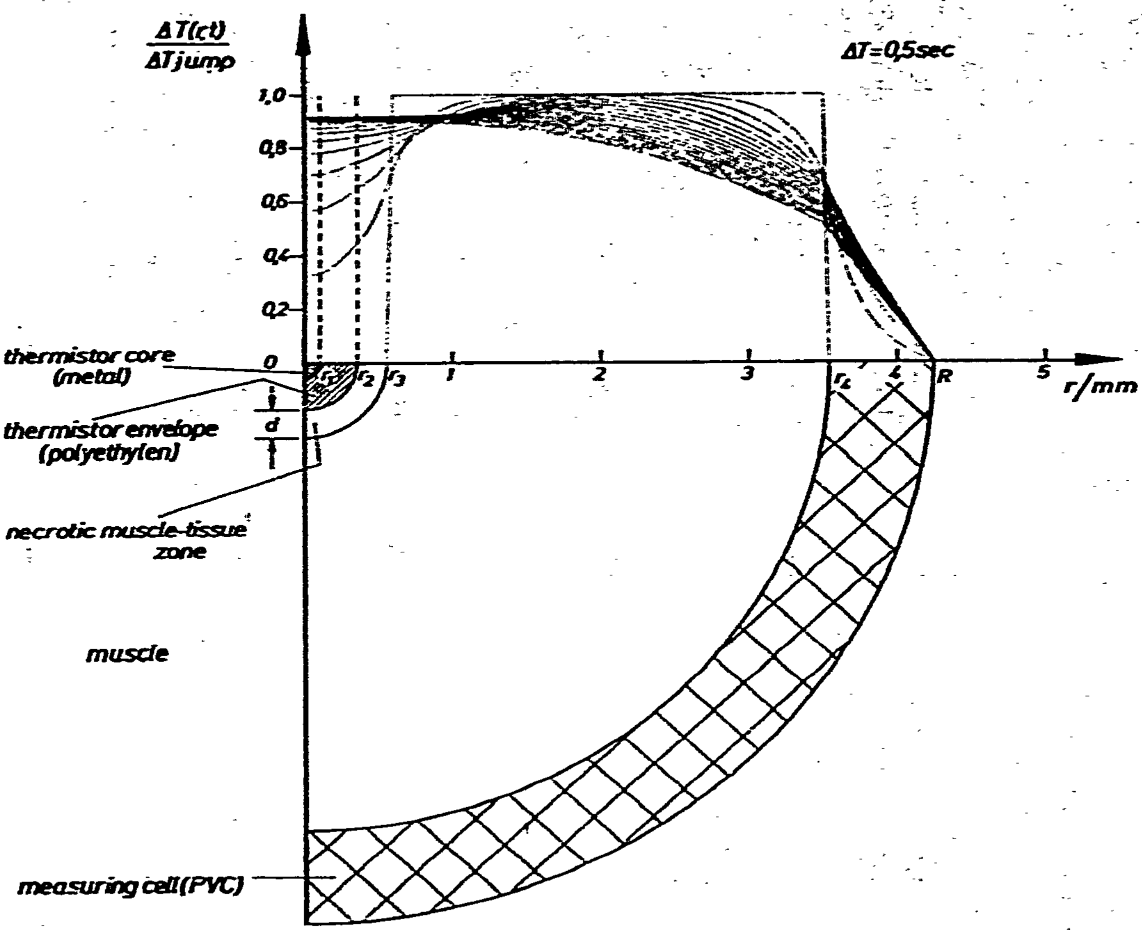

Fig. 4. Calculated local temperature with the geometry of the thermistor–muscle system.

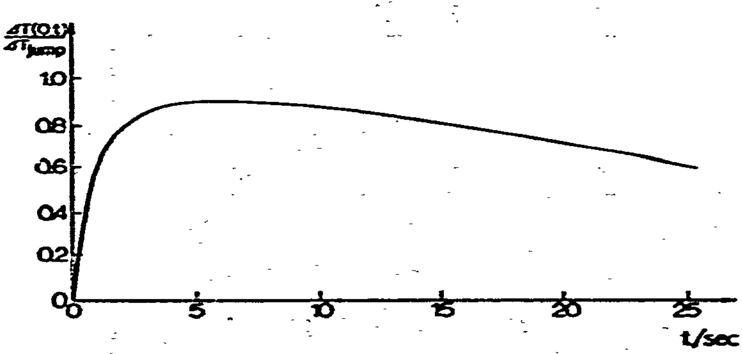

Fig. 5. Time dependence of the core temperature of the thermistor.

Figure 7 gives a comparison of the theoretical and experimental values for the central temperature inside the thermistor for times up to 10 s. The thickness d of the necrotic zone is used as a parameter of the calculation. The curves are normalized to have the maximum value 1, because the jump temperature is not known a priori and

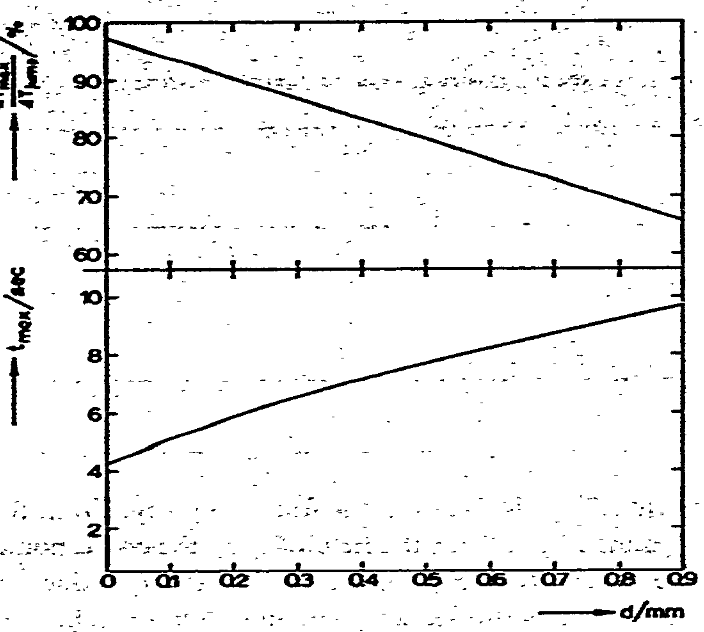

Fig. 6. $\Delta T_{max}/\Delta T_{jump}$ and t_{max} calculated as functions of d.

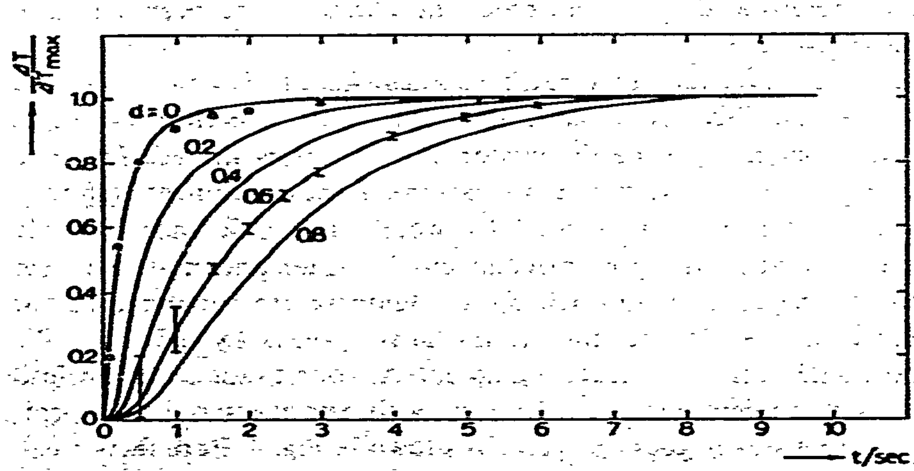

Fig. 7. Time dependence of the core temperature of the thermistor with parameter d. Theoretical (full lines), experimental Joulean heating (circles), and experimental single contraction (bars).

besides, the absolute value does not change the shape of the curves. The time dependence of the thermistor temperature after a single stimulation is reproduced correctly under the assumption of a necrotic zone of thickness $d = 0.6$ mm. For this case, one gets from Fig. 6

$$\frac{\Delta T_{max}}{\Delta T_{jump}} = 0.77$$

which means that the temperature to be determined, ΔT_{jump}, is obtained from the measured temperature ΔT_{max} by multiplication with a factor $1/0.77 = 1.3$.

TABLE 2

HEAT PRODUCTION DETERMINED FROM THE FIRST SINGLE CONTRACTION OF DIFFERENT MUSCLES

Experiment No.	*ΔQ^c single (mcal cm^{-3})*
1	3.6
2	2.9
3	3.1
4	3.5
	3.3 ± 0.33

Generally, the true temperature ΔT_{jump} is found in the following way. From the experiment, one always knows t_{max} and ΔT_{max}. With the help of t_{max}, the corresponding values of $\Delta T_{max}/\Delta T_{jump}$ can be taken from Fig. 6. Correcting the Q values of column a in Table 1 in this way, one obtains the values given in column c. The new values are in good agreement with those of the stationary experiment. Obviously, the true temperature and, therefore, also the heat production would have been seriously underestimated without correction.

The time dependence of the temperature after Joulean heating is well reproduced by the curve with $d = 0$ mm. This is reasonable, since in the latter experiment all parts of the muscle, also the zone next to the thermistor, are uniformly heated. The time constant of this process, however, is larger than measured in the experiment described above, where the thermistor was surrounded by water. The difference is easily explained by the different heat transport mechanisms in water and muscle tissue. Since, in water, convection predominates, the outside temperature remains constant while the thermistor is heated. In the muscle, the heat spreads by diffusion, the velocity being proportional to the temperature gradient. As soon as the heat flow into the thermistor starts, the gradient goes down continuously and the surface temperature first decreases before it slowly goes up again, as shown in Fig. 4. Both facts mean that the time constant must be larger in this case.

Summarizing these arguments one must state that: (a) the time constant of a thermistor measurement depends crucially on the thermal properties of its environment and, (b) this time delay may provide information about this environment as shown here for the thickness of the necrotic zone.

Finally, values of ΔQ measured at the first isometric contraction of different muscles are shown in Table 2.

CONCLUSIONS

Measurements of the heat production in fresh M. *gastrocnemius* of rana temporaria yield for the first isometric single contraction at 0°C $\Delta Q = 3.3 (1 \pm 10\%)$

mcal cm^{-3}. For further contractions the values decrease rapidly. The single contraction experiment can be evaluated only by means of a model calculation. As a result of this calculation, one can state that for measurements in situ with a thermistor probe, a time delay of 5–10 sec is to be expected due to the inactive tissue zone around the thermistor which in our experiments was found to have a thickness of 0.1–0.6 mm. The values taken from the stationary and non-stationary experiment agree well.

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