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EFFICIENCY OF ENERGY CONVERSION DURING SHORTENING OF MUSCLE FIBRES FROM THE DOGFISH *SCYLIORHINUS CANICULA*

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Summary

Force and heat production were measured during isovelocity shortening of tetanized white myotomal muscle fibres from the dogfish at 12°C. For each fibre preparation a range of velocities was used. Mechanical power was calculated from force×velocity of shortening. The rate of total energy output during shortening was evaluated as the sum of mechanical power and the rate of heat production. The ratio of mechanical power to total energy rate was taken as a measure of efficiency of energy conversion to mechanical power during shortening. Efficiency was maximal and varied little in the range of shortening velocities 0.42–0.89 fibres lengths s^{-1} (0.11–0.23 V_{max}); maximal efficiency was 0.33±0.01 (±S.E.M., N=23 measurements on seven fibre bundles). The efficiency of the white fibres from dogfish was less than that measured in the same way in earlier experiments on frog muscle and tortoise muscle.

Introduction

Fish have efficient locomotion compared with land-living vertebrates (Schmidt-Nielsen, 1984), the energy (oxygen used per kilogram body mass) required to travel 1 km being about 10 times less than for land animals. Locomotion is, from an energetic point of view, a multistage process. The production of mechanical energy in muscle from the splitting of ATP is one of those stages. Among others are the resynthesis of the ATP by oxidative phosphorylation and the transfer of mechanical energy from the muscle to the water through which the fish swims. The efficiency of a multistage process is the product of the efficiencies of all the stages, each having a value less than 1. Therefore, the overall efficiency, which is the biologically relevant quantity, cannot be greater than the efficiency of any one

Key words: muscle, fish, dogfish, efficiency, mechanical power, heat, *Scyliorhinus canicula*.

stage. The aim of the experiments reported here is to measure the efficiency of energy conversion in the muscle.

We have focused on energy changes during the production of work, that is, during shortening, and we have excluded the processes that precede and follow shortening. Therefore, shortening was imposed after the muscle fibres had been stimulated for long enough to produce full isometric force. The idea was that the fibres should be fully active and in a steady state of force production and Ca^{2+} pumping before shortening, so that the only changes we would detect during shortening would be those due directly to the performance of work.

These measurements of efficiency of energy conversion in muscle can be compared with corresponding values for the muscle of other animals. This comparison should reveal whether the efficiency of locomotion of fish is due in part to the properties of their muscles.

Some of the results have been reported in preliminary forms (Curtin and Woledge, 1988*b*, 1989).

Materials and methods

Dogfish, *Scyliorhinus canicula* (L.), from the holding tanks in the Marine Biological Association Laboratory in Plymouth were killed by decapitation followed by pithing. Large fish, ranging in length from 570 to 700 mm, were selected. Bundles of three or four fibres were dissected under saline from thin slices of the white myotomal muscle taken from the immediate post-anal region. A piece of myoseptum at each end of the bundle was held in a platinum foil clip. The saline solution contained (mmol l^{-1}): NaCl 292; KCl 3.2; $CaCl_2$ 1.8; $MgCl_2$ 2.2; Na_2SO_4 3.5; $NaHCO_3$ 5.9; urea 483; and tubocurarine 1.5 mg l^{-1}.

The experiments were carried out at 12°C with the fibre bundle mounted horizontally between a force transducer and a combined motor and length transducer (Cambridge Technology, Inc., models 401 and 300H, respectively). The bundle was in contact with a thermopile containing constantan–chromel thermocouples that measured fibre temperature.

The preparation was electrically stimulated end-to-end with 0.5 or 1.0 ms pulses. In each experiment the stimulus strength–twitch tension relationship was carefully investigated to establish the number of living fibres in the preparation and the optimal stimulus strength. The fibre length–twitch tension relationship was also investigated so that appropriate initial and final lengths could be chosen for the tetani with shortening.

In the main experiment the fibres were stimulated tetanically at a frequency close to the fusion frequency. The frequency was kept low, about 25 pulses s^{-1}, to minimize the stimulus artefacts on the records. The tetanus duration varied between 0.35 and 1.00 s for different preparations, but was kept constant for an individual preparation.

Records were made of force during isometric tetani at the initial (long) and final (short) length reached during tetani with shortening, as well as during tetani tha

included a period of shortening. Shortening started after 0.15 s of stimulation under isometric conditions (see Fig. 1). After full relaxation of force, the fibres were stretched to the starting length at the same velocity as the preceding shortening. The movement was controlled by the motor and occurred at a constant rate (ramp or isovelocity). The amplitude of movement was constant for each bundle of fibres. The mean amplitude (expressed as a percentage of the long length) was 12.8±2.2 % (S.E.M., N=7).

Measurements of energy output

The recorded force was multiplied by velocity to give the mechanical power.

Heat output was determined from temperature changes detected by the thermopile, which was made by J. V. Howarth. It contained eight constantan–chromel thermocouples per millimetre length and was insulated with polyimide film (Kapton, Dupont). The output of each couple was 59.4 μV per degree at 12 °C. Thermopile output was sent to a chopper amplifier (Ancom C3A modified to chop at 1 kHz) and recorded, along with the force and length signal, on a digital oscilloscope. Heat was recorded from a segment, between 1 and 3 mm in length, of the fibre bundle. All fibre lengths were within the range 5.6–7.7 mm.

Correction for stimulus artefact was based on recordings made at the end of the experiment after the fibre bundle had stopped producing force when stimulated.

Heat records were corrected for heat loss on the basis of heat flow characteristics observed in a period of control heating and cooling of the fibre bundle on the thermopile. In these controls, a known current was passed through the thermopile for a known time; the Peltier effect caused temperature to change. Control records could be described as the sum of two exponential functions. The time constants were determined for each bundle and measurements were repeated after procedures that may have changed the heat capacity on the thermopile, such as changing the saline. Average values for 14 determinations of the two time constants for seven bundles were 4.5±0.39 s (±S.E.M., N=14) and 0.161±0.026 s (±S.E.M., N=14).

After correction for heat loss, the rate of heat production by the fibre bundle during stimulation was measured as the slope of the heat *versus* time record (see Fig. 1). In this example for shortening, the rate of heat production increased almost as soon as shortening started; in some of the other experiments it increased more gradually but reached a steady value by the middle of the period of shortening. Therefore, we measured the rate during the second half of shortening. The isometric records were measured over the same period as the records with the slowest velocity of shortening. As can be seen from the example in Fig. 1, the isometric rate of heat production was relatively constant during this time.

Fibre size

At the end of each experiment the length of the fibres was measured under the stereomicroscope and the bundle was removed from the thermopile. Myosepta and any other non-fibre material were carefully removed, and the fibres were dried

at room temperature. Dried fibre bundles were weighed on a Cahn electrobalance. The dry mass and length of active fibres were used to normalize the measured values of force, heat and mechanical power for fibre bundle size.

Results

Fig. 1 shows sample records of force and heat production during a tetanus under isometric conditions at the long muscle length and a tetanus with shortening. The record for shortening is typical in that force was not constant during shortening, even though the velocity was constant. The rapid fall of force at the beginning of shortening is largely due to the change in length of the series elasticity. As described by Curtin and Woledge (1988*a*), this part of the force decline responds to a small step release of suitable size, as expected for a passive, series-elastic element. During the rest of shortening, force declines more slowly. This part of the record is unaffected by an earlier step release, and thus appears to be due to the properties of the crossbridges rather than to the series elasticity. This decline is likely to be caused by 'deactivation' due to shortening that is more marked at lower frequencies of stimulation (Edman, 1975, 1980). In the experiment reported here, the stimulus frequency was kept low to minimize stimulus artefacts on the heat record.

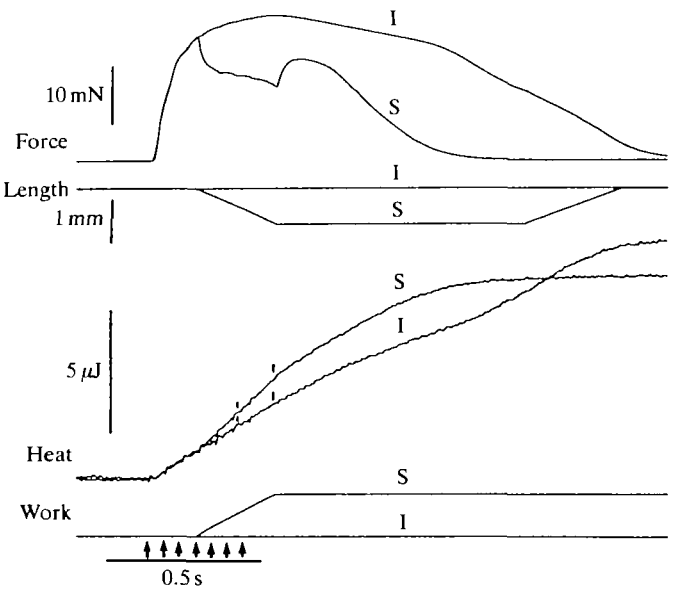

Fig. 1. Sample records of force, length, heat and work during (I) a tetanus under isometric conditions at the long length and (S) a tetanus that includes a period of shortening at constant velocity. The amplitude of shortening was 0.115 fibre length, velocity was 0.46 fibre lengths s^{-1}, fibre length at long length was 7.5 mm and dry mass of fibres was 198.9 μg. The rate of heat production was measured between the vertical bars on the heat record (see Materials and methods). Arrows indicate the stimuli.

The heat was recorded over a long enough period to observe that there was no heat change associated with stretching the relaxed muscle back to its initial length.

The time courses of the heat production under isometric conditions at the long and the short muscle lengths were similar. Under isometric conditions the rate of heat production and force were not influenced much by muscle length in the range used here. In experiments on five different fibre preparations records were made at both lengths; the rate of heat production at the short length was 100.2±2.7 % (±S.E.M.) of that at the long length, and the force was 95.3±3.1 % (±S.E.M.).

The rate of heat production during shortening is clearly greater than that under isometric conditions, even though the force was lower. During shortening, energy was also produced as work, which is shown in Fig. 1 on the same scale as the heat. Values for the mechanical power, and rates of heat production and total energy production (mechanical power+heat rate) are shown as a function of the velocity of shortening in Fig. 2 for six of the seven individual experiments; in the seventh experiment only a narrow range of velocities was used. Fig. 3 shows the mean values based on all seven experiments.

The mechanical power, which is zero under isometric conditions, increased as velocity increased to about 1 fibre length s^{-1}. In some experiments a high enough velocity was used for the mechanical power to decrease from its maximum value (Fig. 2). Mechanical power would, of course, reach zero at V_{max} (the maximum rate of shortening), which is about 3.8 fibre lengths s^{-1} for dogfish fibres under the conditions used here (Curtin and Woledge, 1988*a*).

Fig. 2 shows that the rate of total energy output (mechanical power+rate of heat production) during shortening exceeded that under isometric conditions in every experiment; this is the Fenn effect (Fenn, 1923). Figs 2 and 3 show that shortening heat (Hill, 1938) was produced; that is, the rate of heat production was higher during shortening than in an isometric contraction. From the mean values in Fig. 3 it is clear that the rate of heat production increased as velocity increased up to about 1 fibre length s^{-1}. There is no evidence for a statistically significant increase in the rate of heat production at higher velocities. The shortening heat (heat per unit distance shortened) was evaluated from the slopes of the plots in Fig. 2 of heat rate *versus* velocity, for velocities lower than 1 fibre length s^{-1}; the mean value was 0.276±0.051 J (fibre length shortened)$^{-1}$ g^{-1} dry mass (±S.E.M., N=7).

As shown in Fig. 3 and Table 1, the maximum mechanical power was 0.308±0.019 W g^{-1} dry mass (±S.E.M., N=6) and was produced during shortening at 1.07±0.03 fibre length s^{-1} (±S.E.M., N=6). The rates of heat and energy production also reached their highest values at this velocity of shortening.

Fig. 4 shows efficiency, the ratio of the mechanical power to the rate of total energy output during shortening, as a function of shortening velocity. For three velocities in the range 0.42–0.89 fibre lengths s^{-1} the value of this ratio remains relatively constant at its peak value; the mean ratio for these three velocities is 0.33±0.01 (±S.E.M., N=23 values from seven fibre bundles). At higher velocities, the efficiency must eventually decrease and reach zero at V_{max}. During shortening

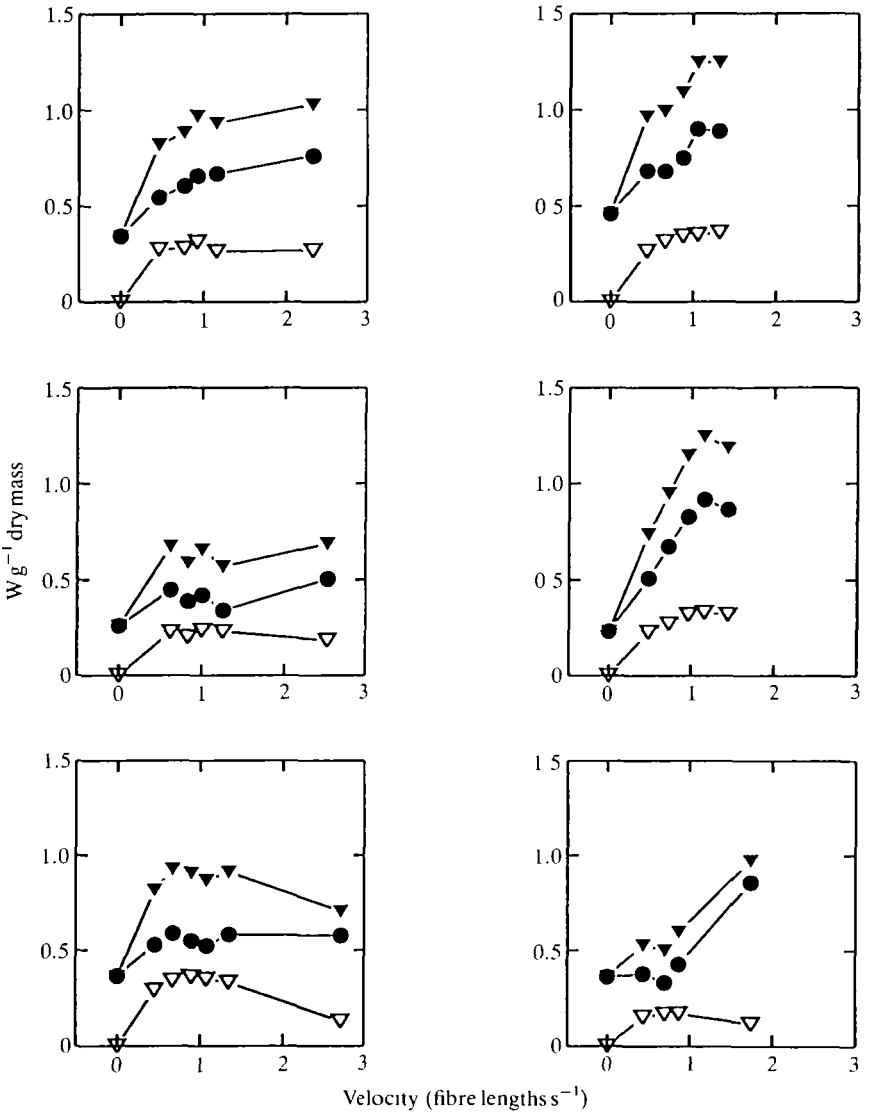

Fig. 2. Mechanical power (▽), rate of heat production (●) and total rate of energy production (▼, =mechanical power+heat rate) during different velocities of shortening and under isometric conditions. Results for six bundles of fibres.

at 1.07 fibre lengths s^{-1}, the velocity giving maximum power, the efficiency is 0.31±0.02 (±S.E.M., N=6), which is slightly less than its peak value of 0.33. This decrease in efficiency is probably due to its decline with velocity.

Discussion

Comparisons of contractile properties

Comparable measurements of mechanical properties and energy output as

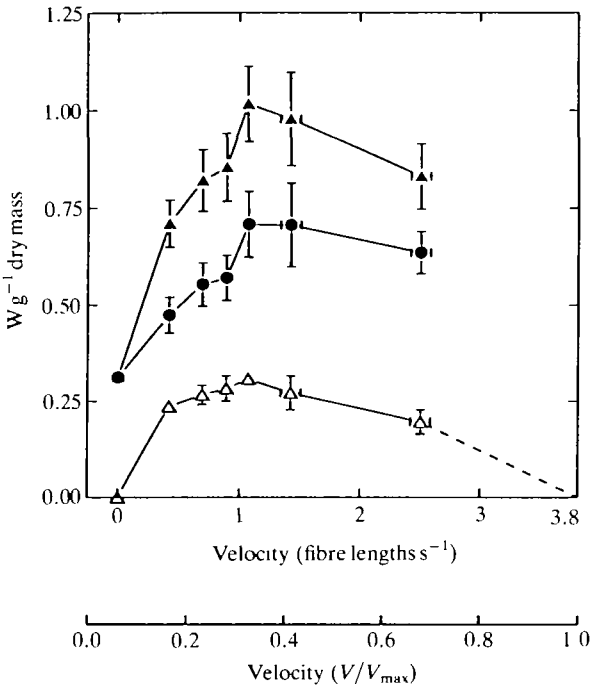

Fig. 3. Mean values (±1 S.E.M.) of mechanical power (△), rate of heat production (●) and rate of total energy output (▲, =mechanical power+rate of heat production) during different velocities of shortening and under isometric conditions. The broken line is an extrapolation of the mechanical power to zero, the value it would have at V_{max}, the maximum velocity of shortening. The velocity scale, V/V_{max}, was constructed by using the V_{max} value of 3.8 fibre lengths s^{-1} measured under the same conditions by Curtin and Woledge (1988*a*). Results are for seven bundles of fibres.

mechanical power and heat have been made on frog and tortoise muscle; the results are summarized in Table 1. The tortoise experiments were made at 0°C whereas the dogfish and frog experiments were at 12°C and 14°C, respectively. Therefore, to make the comparisons between species easier, published Q_{10} values have been used to adjust the tortoise data to 12°C. The values reported here for dogfish V_{max}, isometric rate of heat production and maximum mechanical power are in each case about the same as for the frog. All are considerably greater than the values for the tortoise. For conditions giving maximum mechanical power, the ratio of the maximum mechanical power to the total rate of energy production is less in the dogfish muscle than in the frog and very much less than in the tortoise.

Efficiency

Efficiency can be defined in a number of different ways. Here we are focusing on energy conversion while mechanical power is being produced, that is during shortening. Thus, efficiency is taken as the ratio of mechanical power to the rate of total energy output during shortening. (This is not numerically equal to the

Table 1. *Comparisons of contractile properties*

	Dogfish	Frog (b)	Tortoise (c)
Temperature (°C)	12	14	12
P_0 (N m g^{-1} dry mass)	0.873 (0.078, 7)	1.07 (0.06, 4)	1.126
V_{max} (fl s^{-1})	3.8 (a) (0.2, 13)	4.05 (0.75, 4)	0.75
Isometric rate of heat production (W g^{-1} dry mass)	0.313 (0.037, 7)	0.333 (0.090, 4)	0.007
Maximum mechanical power (W g^{-1} dry mass)	0.308 (0.019, 6)	0.384 (0.056, 4)	0.038
Velocity at maximum mechanical power (fl s^{-1})	1.07 (0.03, 6)	1.20 (0.18, 4)	0.16
Total rate of energy production at maximum mechanical power (W g^{-1} dry mass)	1.016 (0.097, 6)	1.002 (0.160, 4)	0.053
Mechanical power/total rate of energy production at maximum mechanical power	0.31 (0.02, 6)	0.38 (0.019, 4)	0.72

Values are means and, in parentheses, S.E.M. and the number of values; see also Figs 3 and 4. Fibre length is abbreviated fl.

The letter in parentheses indicates a source other than this report. (a) Curtin and Woledge (1988*a*), (b) Hill (1938), and (c) Woledge (1968) adjusted to 12 °C from measured values at 0 °C using Q_{10} values from Edman (1979), Cecchi *et al.* (1978) and Hill and Woledge (1962). P_0, maximum isometric stress; V_{max}, maximum rate of shortening.

thermodynamic efficiency, mechanical power/rate of free energy change of the driving reaction.) A few points should be noted. (1) The use of total energy rate (power+rate of heat production) is based on the fact that all the energy is ultimately derived from ATP splitting. It is important to realize that not all of this ATP splitting necessarily occurs simultaneously with shortening or even during the period of stimulation, because some processes are energetically 'primed' before stimulation starts, release energy during stimulation, and are not 'reprimed', by coupled ATP splitting, until after contraction (for references see Woledge *et al.* 1985). (2) The efficiency value calculated here is not identical to the efficiency of energy conversion by the filaments because the total energy output during shortening includes the heat output from other processes in addition to the filament interaction, which produces mechanical power. Of the processes not producing mechanical work, the one that is likely to have the largest quantitative impact on the efficiency we calculate here is Ca^{2+} pumping by the sarcoplasmic reticulum. This point is considered further below.

Table 1 compares values of the efficiency in different species during shortening at the velocity that gives maximum power. The values for dogfish muscle are from this study and those for muscles from frog and tortoise muscle are from earlier

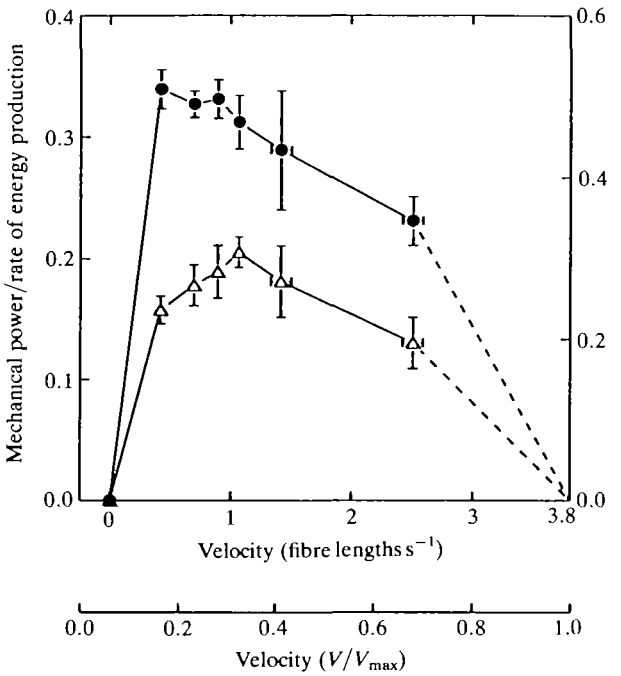

Fig. 4. Mean values (±1 S.E.M.) of the mechanical power (△) and the efficiency (●, =ratio of mechanical power to total rate of energy production) during different velocities of shortening and under isometric conditions. The broken lines are extrapolations of the mechanical power and efficiency to zero, the value they would have at V_{max}, the maximum shortening velocity. The velocity scale, V/V_{max}, was constructed by using the V_{max} value of 3.8 fibre lengths s^{-1} measured under the same conditions by Curtin and Woledge (1988*a*). Results are for seven bundles of fibres.

studies. The value of 0.31 for dogfish muscle at 12°C is less than the value of 0.39 for frog muscle and 0.72 for tortoise muscle (Table 1).

Relevance to locomotion

The results show that the efficiency of energy conversion in the muscles of this one species of fish is lower, rather than greater than, that of some land-living animals. This contrasts with the greater efficiency of locomotion by the whole animal. Therefore, the source of the difference in locomotor efficiency must be sought in some other stage of the overall, multistage process.

The lower efficiency of the dogfish muscle could arise because of less efficient conversion of chemical energy into mechanical power by the crossbridges themselves. Alternatively, a larger proportion of the energy output may be used for processes other than the conversion to mechanical power, such as Ca^{2+} pumping in the active muscle fibre. The cost of Ca^{2+} pumping has been measured in frog muscle (Smith, 1972; Homsher *et al.* 1972; Rall, 1979; Curtin and Woledge, 1981), but not in dogfish muscle, so we do not have the information to distinguish between these possibilities.

However, the comparison of frog and tortoise muscle is of particular interest in this context. Ca^{2+} pumping in the frog is responsible for about 10% of the rate of energy production during maximum mechanical power production. Thus, even if it was reduced to zero, the efficiency would only increase from about 0.40 to about 0.44, which is far less than that of tortoise muscle, 0.72. So it seems that Ca^{2+} pumping cannot explain completely the difference in efficiency; there must be a real difference between these two species in the efficiency of conversion of chemical energy into mechanical power by the crossbridges. Therefore, it is quite likely that the difference in efficiency between frog and dogfish muscle will be found to have a similar explanation.

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