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THE RELATIONSHIP OF MUSCLE ELECTRICAL ACTIVITY, TREMOR AND HEAT PRODUCTION TO SHIVERING THERMOGENESIS IN JAPANESE QUAIL

BY ESA HOHTOLA AND E. DON STEVENS

University of Oulu, Department of Zoology, SF-90570 Oulu, Finland and *University of Guelph, Department of Zoology, Guelph, Ontario, Canada NIG 2W1*

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SUMMARY

1. We measured the electrical activity and the tremor of the pectoral muscle and total body heat production in control and cold-acclimated Japanese quail at +26°C, +12°C and +2°C before and after 3 weeks of acclimation, using electromyography, accelerometer recordings, and indirect calorimetry.

2. Japanese quail shiver in 0·2- to 3-s bursts that occur in groups. An increase in both the frequency and the duration of bursts and burst groups contributes to the increase in heat production by shivering at low temperatures. A compilation of shivering patterns in birds is given and its implications for the neural control and phylogeny of shivering are discussed.

3. A rather non-specific increase in electromyographic (EMG) activity and heat production was observed after cold acclimation at all experimental temperatures, although many of the normal signs of cold acclimation (e.g. decrease in gonad mass, increase in heart mass and serum triiodothyronine) were seen. The increase in muscle electrical activity was greater than the increase in oxygen uptake, which resulted in a lower $\dot{V}_{O_2}$/EMG ratio.

4. The amplitude distribution of muscle electrical activity remained normal, but a shift towards higher frequencies occurred in the EMG spectra of cold-acclimated birds.

5. Despite the increase in muscle electrical activity, power spectra of accelerometer recordings indicated that the amplitude of the muscle tremor was lower in cold-acclimated birds. The increase in the high-frequency components of the EMG indicates that decreased synchronization of motor unit firing may account for the lower tremor amplitude. We suggest that this change is adaptive because it reduces heat loss and/or because more fatigue-resistant motor units are recruited.

6. These results show that temperature acclimation modifies the neural control of shivering in skeletal muscle.

INTRODUCTION

Heat production by muscular shivering is an autonomic function of skeletal muscles which are normally under voluntary control (Hemingway, 1963). True forms of shivering occur only in endothermic vertebrates, i.e. birds and mammals

Key words: shivering, tremor, heat production, EMG, acclimation, quail.

(Heath, 1968). In both groups, it is an important and phylogenetically the oldest form of extra heat production during cold exposure (Calder & King, 1973; Hulbert, 1980).

Although the electrical, mechanical and energetic correlates of voluntary muscle contraction are now relatively well understood (for a comparative treatise, see Alexander & Goldspink, 1977), much less is known about these correlates in shivering muscles (Hohtola, 1982). The best known relationship is the increase in the electrical activity of the muscles along with the increase in heat production at low ambient temperatures (e.g. West, 1965).

Three additional points related to muscle function during shivering seem especially relevant. First, the temporal pattern of muscle activity both at the level of whole muscles and at the level of individual motor units is a useful indicator of the neuronal control mechanisms of shivering. In comparative studies, the activity patterns also have phylogenetic implications (Aulie, 1976*b*). Second, the mechanical tremor that is always associated with shivering has been recorded only in a few cases (Odum, 1942; Stuart, Ott, Ishikawa & Eldred, 1966). Quantitative measurements of shivering tremor have not been made, although other forms of physiological tremor have been studied in great detail (e.g. Burne, Lippold & Pryor, 1984). Furthermore, the relationship between tremor and actual heat production in the muscles during shivering has been a matter of much confusion. Because shivering is essentially an isometric contraction of skeletal muscles, tremor is not a prerequisite for heat production. In fact, a high tremor amplitude may be maladaptive because of an increase in convective heat loss. Third, changes in the amount of heat liberated per unit of muscle activity, which have been suggested to occur during temperature acclimation (Ivanov, 1980), imply that the efficiency of the chemomechanical coupling in the muscle is subject to adaptive variations.

In this study, we combine controlled electromyographic measurements of shivering with indirect calorimetry and tremor recordings in Japanese quail to elucidate the relationships of these indices of thermogenesis in more detail. We also report the effects of temperature acclimation on these variables in order to test their adaptive significance.

MATERIALS AND METHODS

Birds

Adult, male Japanese quail (*Coturnix coturnix japonica*, mass 0·120–0·185 kg) were used. They were maintained singly in metal wire cages at +24°C with a 16 h:8 h light:dark cycle for at least 3 weeks prior to experiments. Food (Game Bird Breeder Lagena, Ralston Purina of Canada Ltd) and tap water were given *ad libitum*.

Experimental procedure

All 16 birds were tested twice: once before and once after 3 weeks of acclimation. After the first test the birds were randomly divided into two groups. Eight control

birds were maintained in the animal room as before, while the other eight were cold acclimated. To this end, the birds were kept singly in metal wire cages in a cold chamber maintained at +6°C with a 6 h:18 h light:dark cycle. Otherwise their care was unchanged. By testing all birds twice and using non-acclimated birds as controls, non-specific changes due to habituation etc. could be accounted for.

The birds were fasted for 16 h before the measurements. Each test was begun by weighing the bird after which appropriate probes were fixed with adhesive tape. The bird was then placed, unrestrained, in a cylindrical aluminium metabolic chamber maintained at +26°C. The temperature of the chamber (T_a) was regulated by adjusting the temperature of a water jacket surrounding the chamber with a thermostatted water bath. After 1·5 h of equilibration, readings for oxygen uptake and shivering were taken. Identical measurements were made at +12°C and at +2°C by changing the temperature quickly (10–15 min) and allowing 1 h to adjust to the new temperature.

Measurements

Shivering was measured electromyographically from the right pectoral muscle. The electrode consisted of uninsulated stainless steel pins (diameter 0·25 mm; shafts 5 mm) fixed triangularly 3 mm apart in an epoxy resin plate (Hohtola, Rintamäki & Hissa, 1980). Electrode geometry was identical throughout the experiments. The electrode was inserted into the pectoral muscle 1 cm caudally from the furcular pit, 1 cm laterally to the keel, and fixed with adhesive tape. The EMG signal was differentially amplified with a Tektronix Type 122 preamplifier (60 dB, bandpass 80–1000 Hz). For quantification, the EMG was full-wave rectified with a Grass 7P10D integrator and then fed for smoothing to an r.c.-circuit (time constant 1 s). An 8-kbyte record of the smoothed EMG was taken on a Nicolet 4094 digital oscilloscope using a 15 bit a.d.-converter (Nicolet 4562) and a sampling rate of 10 Hz. A time average (mean rectified value) of the resulting 13·3 min record was obtained using the software of the CRO and was used as a measure of shivering intensity at any specified temperature.

'Raw' EMG was continuously monitored on a second oscilloscope to detect spurious signals and movement artifacts. Measurements were taken only when the EMG indicated that the bird was not moving.

For closer analysis in the frequency and time domains, 16-kbyte records of raw EMG were stored on disks using a sampling rate of 2 kHz.

Oxygen consumption was measured in an open system. Dry, CO_2-free outdoor air was pulled through the chamber at a flow rate of approx. 950 ml min^{-1}. After removal of CO_2 and drying, the outlet airflow was measured with a rotameter (calibrated against a Fisher Mark III flowmeter) and directed to an S-3A oxygen analyser (Applied Electrochemistry Inc.). The system was tested for leaks with nitrogen. The time constant of the system, calculated on the basis of the nitrogen test, was approximately 10 min. Oxygen consumption ($\dot{V}_{O_2}$) was calculated (STPD)

from the formula (see Hill, 1972):

$$\dot{V}_{O_2}(\text{ml min}^{-1}) = \dot{V}_o \times \frac{F_i - F_o}{1 - F_i},$$

where $\dot{V}_o$ is outlet flow of dry CO_2-free air (ml min^{-1}), F_i is fractional oxygen content of dry, CO_2-free inlet air (0·20953) and F_i is fractional oxygen content of dry, CO_2-free outlet air.

All temperatures were measured using copper-constantan thermocouples connected to type BAT-12 thermometers (Bailey Instruments Inc.). Body temperature (T_b) was measured from the cloaca within 30 s of removing the bird from the chamber. Continuous recording of T_b was not done because the probe often made the birds restless, which interfered with shivering measurements.

The tremor associated with shivering was measured with an accelerometer (Model EGBL-125-30D, Entran Devices Inc.) using a bridge circuit. The accelerometer was glued on top of the electrode plate and the whole array was fixed to the breast muscle with adhesive tape. The output from the bridge circuit was amplified by 40 dB (DAM-6 preamplifier, WP-Instruments Inc.) using a bandpass of 1–100 Hz and fed to the digital oscilloscope for data acquisition.

Chemical assays

Serum triiodothyronine (T_3) was assayed using a radioimmunoassay kit (T_3 ^{125}I, Farmos Diagnostica). Serum chemistry tests were carried out by the Ontario Veterinary College clinical chemical laboratory using a computer-controlled analyser and standard assay techniques.

RESULTS

The shivering pattern

Japanese quail shiver in 0·2- to 3-s bursts (Fig. 1). The duration of successive bursts may vary considerably, while the amplitude remains more or less constant at any specified T_a. Both an increase in the frequency of the bursts and an increase in their duration contribute to the elevation in the intensity of shivering at low ambient temperatures.

The bursts tend to occur in groups. This is clearly seen in Fig. 2, where representative samples of rectified and smoothed EMG are shown. Each peak in the records corresponds to a group of bursts. The amplitudes of the bursts are roughly the same throughout any single group of bursts. An increase in the number of burst groups occurs when T_a decreases (Fig. 2).

We found no changes in the pattern of shivering during the 3 weeks of temperature acclimation.

Heat production and body temperatures

A summary of the effects of acclimation on thermogenic indices ($\dot{V}_{O_2}$ and shivering) at the three experimental temperatures is shown in Fig. 3. An initial double-logarithmic plot of $\dot{V}_{O_2}$ against body mass at +26 °C using all the data (i.e. 32

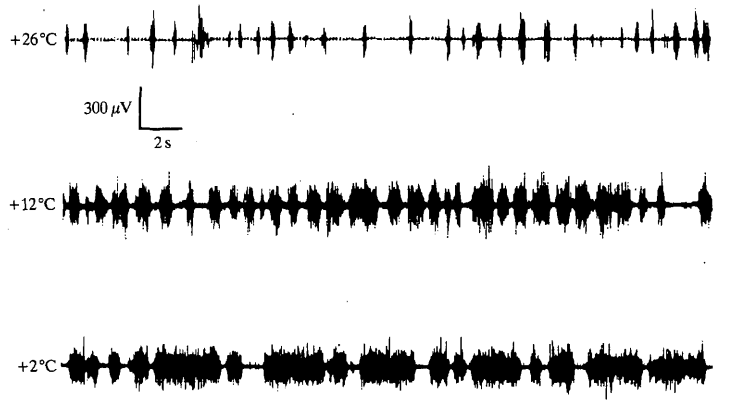

Fig. 1. Representative samples of raw EMG showing the bursting pattern of shivering in Japanese quail at the three experimental temperatures. Voltage scale only approximate.

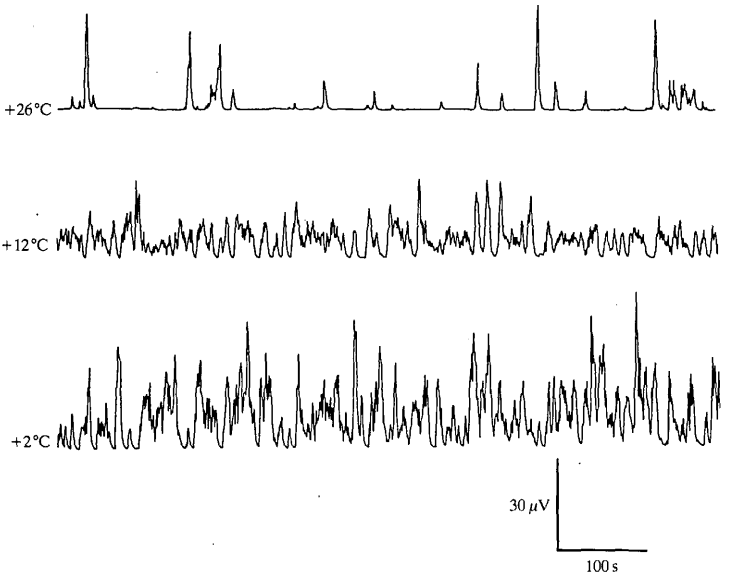

Fig. 2. Representative samples of rectified and smoothed EMG in Japanese quail at the three experimental temperatures showing the groups of shivering bursts as peaks in mean intensity.

measurements on 16 birds) gave a slope of 0·77. As there was considerable variation in body mass initially, and because acclimation had an effect on body mass, all $\dot{V}_{O_2}$ readings are expressed as $ml\,min^{-1}\,kg^{-0.75}$.

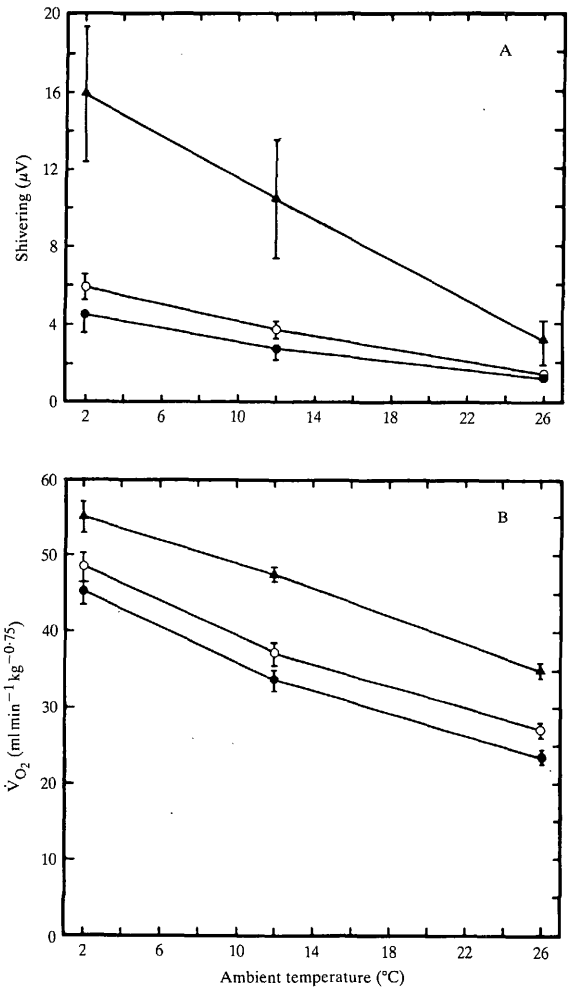

Fig. 3. Shivering (A) and oxygen consumption (B) of Japanese quail (mean ± S.E.M.) at the three experimental temperatures (open circles, pretest values of all 16 birds; filled circles, control birds, no acclimation; triangles, cold-acclimated birds).

The $\dot{V}_{O_2}$ and shivering data were subjected to an analysis of covariance (ANCOVA, Dixon, 1983) using a repeated measures design. Ambient temperature was used as the covariate to reveal significant changes around the general regression against temperature.

There were no significant differences between the two groups before acclimation with respect to $\dot{V}_{O_2}$ ($t = 0{\cdot}46$) or shivering ($t = 1{\cdot}40$). Thus, pretest values from all 16 birds were combined in Fig. 3.

Cold acclimation resulted in an increased level of both $\dot{V}_{O_2}$ and shivering, while opposite effects were observed in the control birds. In terms of the ANCOVA performed, T_a as a covariate had the strongest effect both on $\dot{V}_{O_2}$ and shivering ($F_{45,1} = 216{\cdot}7$, $P < 0{\cdot}0001$ and $F_{45,1} = 31{\cdot}5$, $P < 0{\cdot}0001$, respectively). With respect to $\dot{V}_{O_2}$, the effect of acclimation *per se* was significant ($F_{46,1} = 6{\cdot}58$, $P = 0{\cdot}014$). For shivering, too, acclimation had a significant effect ($F_{46,1} = 11{\cdot}8$, $P = 0{\cdot}0013$).

The increase in $\dot{V}_{O_2}$ in the cold-acclimated birds was of the same magnitude at all test temperatures. Thus, the basal metabolic rate (i.e. when tested at +26°C) of the cold-acclimated birds was elevated by 31%. A corresponding 21% decrease in the basal metabolic rate was observed in the control birds. Both of these changes were highly significant when tested alone ($P < 0{\cdot}001$, paired t-test). The slope of the curve relating $\dot{V}_{O_2}$ to T_a did not change during acclimation. Thus, there is no indication of a change in insulation.

The relationship of total body $\dot{V}_{O_2}$ to the electrical activity of the pectoral muscle was studied by calculating the ratio of $\dot{V}_{O_2}$ to EMG for measurements made at +2°C. This was done by subtracting the basal values obtained at +26°C from each reading and by using the increment of each variable for the calculations. Because the increase in shivering intensity was greater than the corresponding increase in $\dot{V}_{O_2}$ after cold acclimation (Fig. 3), the mean $\dot{V}_{O_2}$/EMG ratio decreased in these birds. Individually, this was true in seven out of eight birds. The change is statistically significant ($P = 0{\cdot}035$, sign-test). In control birds, positive and negative changes were equally common so the mean change was close to zero ($P = 0{\cdot}363$, sign-test).

Body temperatures in the two groups were identical initially ($40{\cdot}5 \pm 0{\cdot}11$°C and $40{\cdot}6 \pm 0{\cdot}10$°C, mean ± S.E.M., in control and cold-acclimated birds, respectively) and did not change during the course of acclimation ($40{\cdot}5 \pm 0{\cdot}07$°C and $40{\cdot}5 \pm 0{\cdot}28$°C, respectively).

The EMG signal

In order to detect the possible changes in the electrical activity of the muscles resulting from the acclimation, a partial signal analysis on the 16-kbyte EMG records was done using the software packages of the Nicolet oscilloscope. The normality of the amplitude distribution of EMG potentials in individual bursts was studied by plotting the root mean square and the mean rectified values for 20 experiments against each other (Fig. 4). The theoretical form factor (slope) for a Gaussian process is 1·253. The values obtained occur slightly, but significantly, above this ratio ($1{\cdot}384 \pm 0{\cdot}013$, mean ± S.E.M.; $t = 10{\cdot}1$, $P < 0{\cdot}001$), indicating slight leptokurtosis. Acclimation had no effect on the form factor.

The frequency distribution of the EMG recordings was studied from power density spectra obtained by an FFT algorithm. Each spectrum was calculated from 2048 points sampled at 0·5-ms intervals. Most (>95%) of the electrical activity occurred below 700 Hz. The mean peak frequency varied between 225 and 290 Hz. Cold acclimation increased the peak frequency slightly, but the change was not statistically significant. As a more reliable measure of spectral shifts, an index giving the ratio of power between 0 and 250 Hz to total power was calculated for experiments done at +2°C (Table 1). In cold-acclimated birds the ratio decreased significantly, indicating a shift in the EMG towards higher frequencies as a result of acclimation.

Muscle tremor

The frequency and amplitude of the tremor associated with shivering was studied from power density spectra of the accelerometer recordings. Each spectrum was computed from 2048 points constituting a 0·41-s record. The samples were selected near the midpoint of individual tremor bursts, which correlated exactly with bursts

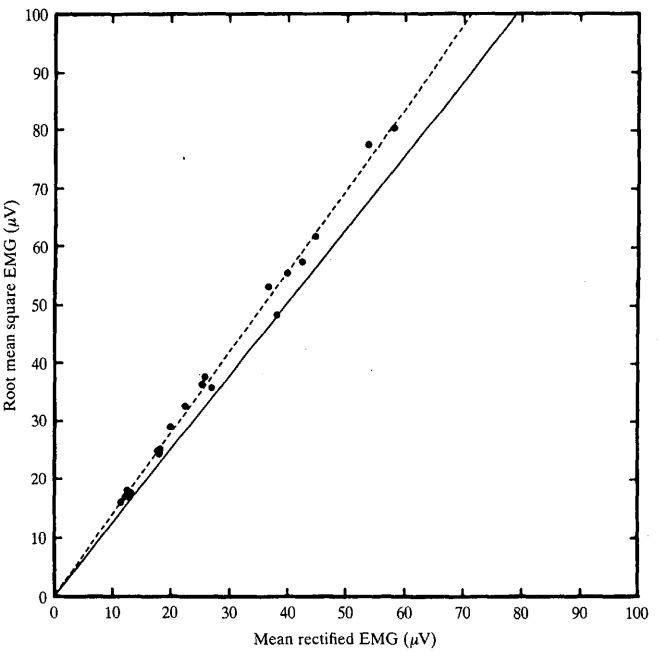

Fig. 4. A plot of mean rectified values against root mean square values, showing the fit of EMG amplitude distributions (dots, broken line obtained by least-squares regression) to the normal distribution (solid line).

of electrical activity (Fig. 5). The tremor measurements were done separately after 4 weeks of acclimation using a moderate cold exposure during the measurements (+6°C).

The peak frequency of tremor was consistent within birds but varied between birds. For example, in one cold-acclimated bird the peak frequency was 30 Hz in seven out of eight records and 32·5 Hz in the other. In the cold-acclimated birds, the peak frequency ranged from 25 Hz (two birds) to 40 Hz (two birds); in the control birds it ranged from 20 Hz (one bird) to 35 Hz (one bird).

The amplitude of the tremor was calculated by measuring the area under power density spectra between 10 and 50 Hz for the control and cold-acclimated birds

Table 1. *Relative power (mean ± S.E.M.) between 0 and 250 Hz in shivering EMG spectra expressed as percentage of total power in Japanese quail before and after acclimation*

	Before	After
Control	33·0 ± 1·5	35·6 ± 2·8
Cold-acclimated	31·8 ± 4·1	26·7 ± 1·6*

*Significantly different ($P < 0·05$) from control value.
$T_a = 2$ °C, $N = 8$.

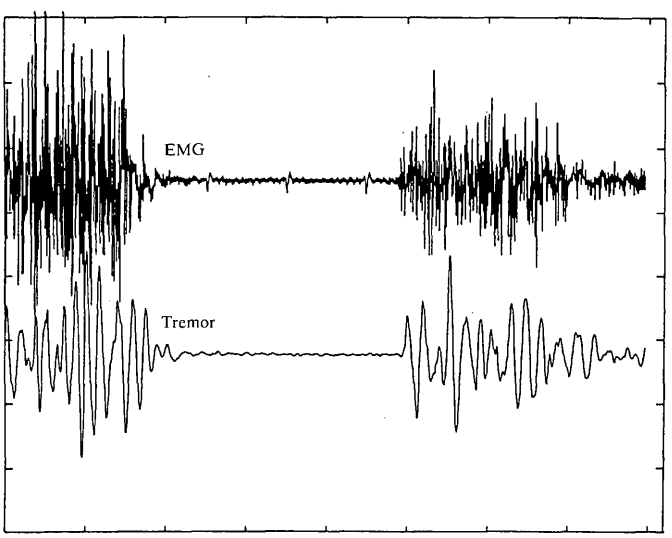

Fig. 5. Representative tracings of EMG (top) and accelerometer recordings during two bursts of shivering in the Japanese quail. Horizontal markings, 200 ms; vertical markings, 100 μV (EMG) and 3·92 m s^{-2} (acceleration).

(Fig. 6). Four spectra were averaged for each bird in these calculations. The power spectrum curves varied considerably between birds, making it necessary to normalize the data so that the total area under each curve from 10 to 50 Hz was equal to the average value for all birds in that group.

Despite their lower level of electromyographic activity, the area of averaged tremor spectrum was 1·8 times larger in control birds ($t = 2{\cdot}1$, $P < 0{\cdot}025$). Furthermore, the tremor in control birds has a single peak at 27·5 Hz, whereas the tremor in cold-acclimated birds shows a broad peak from 27·5 to 40 Hz. This was clearly evident if all spectra were normalized to equal area between 10 and 50 Hz. Then the tremor in cold-acclimated birds was less than that of controls from 10 to 32·5 Hz but greater from 35 to 50 Hz.

In summary, the accelerometer-based recordings of the shivering tremor in Japanese quail have a lower amplitude and show a less prominent rhythmicity after cold acclimation.

Body mass

Although the body mass of fasting birds from the two groups was similar initially, a significant difference was observed after the 3-week acclimation. The mass of the

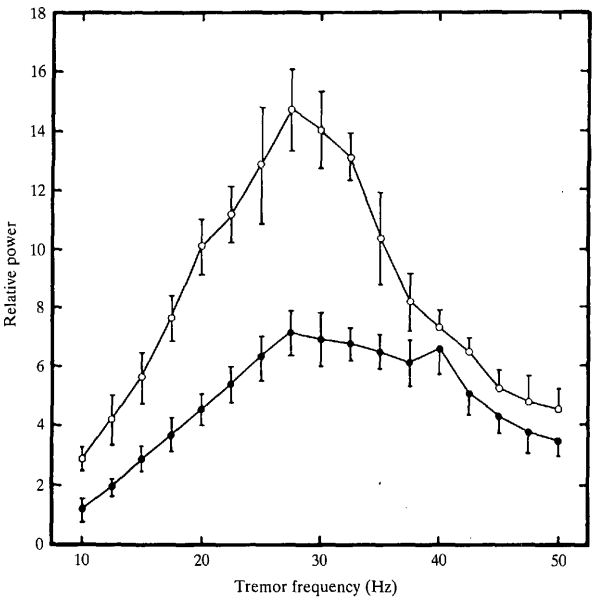

Fig. 6. Power density spectra of accelerometer recordings from control (open circles) and cold-acclimated (filled circles) Japanese quail at +6°C. Means ± S.E.M. for eight birds in each group.

control birds increased 0·20% per day (6·8 ± 1·9 g per 3 weeks, mean ± S.E.M.), while the cold-acclimated birds lost mass at a rate of 0·57% per day (12·7 ± 5·1 g per 3 weeks). Both changes were statistically significant (paired *t*-test). Weighings done during the carcass analyses after 6 weeks of acclimation indicated that the control birds continued to gain mass at a slower rate of 0·04% per day, while cold-acclimated birds regained some of the lost body mass (0·10% per day).

The effects of the 16-h fast itself were tested in a separate experiment where the birds were weighed before and after the fast. During the overnight fast, the birds in the cold chamber lost significantly more mass, expressed as daily values, than the control birds (12·5 *vs* 8·5% of initial body mass).

Carcass and blood analyses

The relative mass of skin plus feathers did not change during acclimation (Table 2). The only significant changes in the carcass components were a decrease in the relative mass of the gonads and an increase in the relative mass of the heart in cold-acclimated birds (Table 2). These birds also had a higher T_3 concentration and alkaline phosphatase activity but less cholesterol in their serum.

DISCUSSION

Shivering pattern

Although all mammalian species that have been studied seem to shiver in bursts, both bursting and continuous patterns of shivering have been reported in birds.

Table 2. *Carcass components (expressed as percentage of total body mass) and the concentrations of selected serum constituents in control and cold-acclimated Japanese quail*

	Control	Cold-acclimated
Carcass analysis		
Skin + feathers	12·1 ± 0·42	13·5 ± 0·60
Heart	0·91 ± 0·03	1·13 ± 0·06**
Liver	1·25 ± 0·07	1·50 ± 0·13
Gut	8·19 ± 1·27	7·37 ± 0·24
Gonads	2·73 ± 0·22	0·029 ± 0·006***
Carcass	65·3 ± 1·54	65·7 ± 0·55
Serum analysis		
Cholesterol (mmol l^{-1})	6·91 ± 0·41	5·30 ± 0·38*
Triglycerides (mmol l^{-1})	1·53 ± 0·19	1·40 ± 0·30
Uric acid (μmol l^{-1})	601 ± 82·4	916 ± 198
Alkaline phosphatase (units l^{-1})	360 ± 33·2	732 ± 56·2**
T_3 (nmol l^{-1})	1·56 ± 0·13	2·89 ± 0·32***

Carcass = total body mass − (head + other components).
* Significantly different from control, $P < 0·05$.
** Significantly different from control, $P < 0·01$.
*** Significantly different from control, $P < 0·001$.
T_3, triiodothyronine.

Table 3 is a compilation of shivering patterns in birds, including only those studies that are based on electromyography (mechanical recording in one case: Odum, 1942). Unfortunately, not all papers show authentic recordings. Thus, the decision between the patterns is in many cases indirect and based on the description given by the authors.

The pattern of shivering is probably determined genetically, since it is not affected by acclimation. The bursting and continuous patterns are well exemplified by the Japanese quail and the pigeon, respectively. In both species, the specific pattern is present in all individuals irrespective of age, acclimation or T_a. Some authors report that bursts of shivering fuse into a continuous pattern at low ambient temperatures. However, in most cases there are fluctuations of shivering intensity that diminish at low temperatures. This is sometimes interpreted as a change in the shivering pattern. In a truly bursting pattern of shivering, electrically silent periods alternate with bursts of motor unit activity. Thus, fluctuations in intensity do not represent bursts

Table 3. *The pattern of shivering in various species of birds*

Species, age	Muscle	Pattern	Reference
Passeriformes			
Parus atricapillus, *a*	*b*	bursting	Chaplin, 1976
Parus major, *a*	*b*	continuous	Hissa & Palokangas, 1970
Troglodytes aedon, *n*	*t*	continuous	Odum, 1942
Acanthis flammea, *a*	*b*	continuous	West, 1965
Hesperiphona vespertina, *a*	*b*	continuous	West, 1965
Quiscalus quiscula, *a*	*b*	continuous	West, 1965
Corvus brachyrhynchos, *a*	*b*	continuous	West, 1965
Chloris chloris, *a*	*b*	continuous	S. Saarela, B. Klapper & G. Heldmaier (unpublished data)
Passer domesticus, *a*	*b*	continuous	E. Hohtola (unpublished data)
Galliformes			
Gallus domesticus, *a*	*b*	continuous	Aulie, 1976*b*
Gallus domesticus, *a*	*l*	bursting	El-Halawani, Wilson & Burger, 1970
Lagopus lagopus, *a*	*b*	intermittent	Aulie, 1976*b*
Lagopus lagopus, *n*	*b*	continuous	Aulie, 1976*a*
Lyrurus tetrix, *a*	*b*	bursting	Rintamäki, Saarela, Marjakangas & Hissa, 1983
Tetrao urogallus, *a*, *n*	*b*	bursting	Hissa *et al.* 1983
Coturnix coturnix, *a*	*b*	bursting	S. Saarela & G. Heldmaier (unpublished data)
Coturnix coturnix, *a*	*b*	bursting	This study
Coturnix coturnix, *a*	*l*	bursting	E. D. Stevens, J. Ferguson, V. G. Thomas & E. Hohtola (unpublished data)
Other			
Bubulcus ibis, *n*	*l*	continuous	Hudson, Dawson & Hill, 1974
Podiceps cristatus, *n*	*b*+*l*	bursting	Keskpaik, Onno & Davydov, 1968
Columba livia, *a*	*b*	continuous	Steen & Enger, 1957
Columba livia, *a*	*b*	continuous	Rautenberg, 1969
Columba livia, *a*	*b*	continuous	Hohtola, 1982

a, adult; *n*, nestling; *b*, breast muscle; *l*, leg muscles; *t*, total body recording.

in this sense. On the other hand, the magnitude of interspecific variation in burst duration (0·25–5 s in the quail; 6–20 s in the black-capped chickadee; up to 50 s in adult willow ptarmigan; Table 3) indicates that there may be a continuum of patterns from short bursts to continuous shivering.

One general idea that emerges from Table 3 is that different authors observe the same species-specific patterns, thus confirming their genetic nature. In addition, the patterns are not correlated with the phylogenetic position of the species, although there is a tendency for passerine and columbiform birds to show a continuous pattern in most cases, while galliform species usually have a bursting pattern. This may be related to the level of basal metabolism and aerobic endurance, which are high in passerine and columbiform species and lower in galliform birds (Calder & King, 1973).

A role for gamma-motoneurones in the generation of shivering has been suggested by several authors (von Euler & Söderberg, 1957; Schäfer & Schäfer, 1973; Sato & Hasegawa, 1977). This hypothesis is supported by the fact that birds and mammals are the only vertebrates that have an efferent innervation of muscle spindles (Krishna Murthy, 1978), and also by the fact that the discharge activity of gamma-motoneurones is influenced by changes in hypothalamic and skin temperatures (von Euler & Söderberg, 1957; Sato & Hasegawa, 1977). If the gamma-efferent system has a pivotal role in the generation of shivering, the existence of two distinct patterns of shivering may then indicate that dynamic gamma-motoneurones are preferentially involved in the generation of the bursting pattern, while static gamma-motoneurones are responsible for the continuous pattern.

Heat production

The response of the Japanese quail to experimental cold acclimation is of the metabolic type. Instead of an increase in insulation, which is typical for many species living at high latitudes, a rather unspecific increase in the metabolic rate was observed at all test temperatures. Such responses have been observed in other birds (Calder & King, 1973), and they probably represent a natural reaction in conditions of unlimited food supply (Johnson & West, 1975). On the other hand, changes in the carcass components (decrease in gonad mass, increase in heart mass) and blood constituents (T_3, alkaline phosphatase) indicate that many of the classical acclimatory changes took place. Carcass analysis also supports the lack of insulative adjustments, in that the relative mass of skin and feathers did not change.

The decrease of $\dot{V}_{O_2}$ in the control birds is either a habituation reaction or indicates that the birds were not fully adapted to the warm pretest conditions at the beginning of the experiments. Both alternatives make the shift observed in cold-acclimated birds even more remarkable.

Ivanov (1980) has suggested that an adaptive increase in the amount of heat liberated per unit of muscle electrical activity occurs during cold acclimation in mammals, and has substantiated his hypothesis by direct temperature measurements in muscle. Similar adaptations have also been reported in birds (Slonim, 1971). The observation that the chemomechanical efficiency of muscles *in situ* is markedly

decreased in hyperthyroid animals (Everts, van Hardeveld, Keurs & Kassenaar, 1983) makes this hypothesis plausible. Assuming that the relative contribution of the pectoral muscles to heat production is constant, such changes should appear as an increase in the $\dot{V}_{O_2}$/EMG ratio in our experiments. We did not find evidence of such shifts. In fact, an opposite change was observed. This may be related to the metabolic type of response to cold exposure in this species. A greater increase in EMG than in $\dot{V}_{O_2}$ has also been observed in pigeons experimentally acclimated to cold (Saarela, Rintamäki & Saarela, 1984). It is questionable whether the adjustments proposed by Ivanov (1980) represent universal responses in endothermic animals exposed to cold.

EMG and tremor

The electromyographic signal arising from bursts of shivering in the Japanese quail is similar to that of other birds studied. Thus, amplitude distributions that are slightly flat have been found in passerines (West, Funke & Hart, 1968), while in the pigeon the distribution shows leptokurtosis (Hohtola, 1982) as it does in the quail. The quasinormal distributions indicate that a reasonable number of randomly spiking motor units has been sampled in each case (see Bendat & Piersol, 1971).

The shift towards higher frequencies that was observed in the EMG spectra after cold acclimation has two possible explanations. It may be related to the overall increase of shivering found in cold-acclimated birds. However, no such shifts occurred during changes in shivering intensity that were induced by ambient cooling. In pigeons, the increase in shivering intensity at low ambient temperature likewise had no effect on the median frequency of EMG spectra (Hohtola, 1982). A more interesting hypothesis is that the shift in the spectrum is related to the changes in muscle tremor observed in the cold-acclimated birds. Theoretical investigations show that a decrease in the level of synchronization between motor units results in a shift to higher frequencies in the EMG spectra (Weytjens & van Steenberghe, 1984), such as was observed in the present study. This lack of synchrony in motor unit contractions curtails the mechanical output of the muscle and renders the ensuing displacements more random. Because acceleration in a mass-invariant system is directly proportional to the applied force, the smaller area of the accelerometer spectra in cold-acclimated quails indeed suggests that the peak contractile force in the muscles is decreased. The displacement caused by the tremor movements can be calculated from the equation (Stuart *et al.* 1966)

$$d = \frac{a}{4\pi^2 f^2},$$

where d is displacement, a is acceleration and f is tremor frequency. Both the decreased acceleration and the increased frequency of the tremor in cold-acclimated birds suppress actual displacements. A calculation of the actual displacement at each frequency clearly shows a marked reduction in displacement, especially at the lower frequencies (Fig. 7). All these facts suggest that cold acclimation influences the

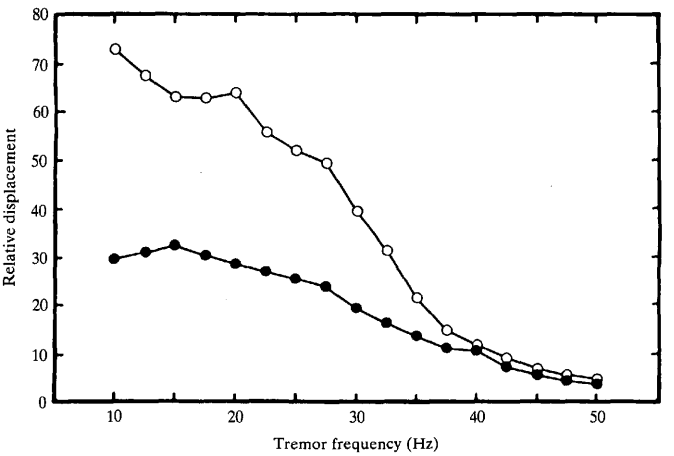

Fig. 7. Power spectra of tremor displacements in control (open circles) and cold-acclimated (filled circles) quails. The values are calculated from the mean accelerations shown in Fig. 6.

mechanism of shivering so that the ensuing tremor has less amplitude and is less rhythmic.

Whether the decrease in tremor amplitude is adaptive as such because of the eventual curtailment of convective heat loss, or whether it is the result of an adaptive recruitment of smaller and fatigue-resistant motor units for shivering in these birds cannot be resolved here. Besides differential recruitment of slow and fast twitching motor units, changes in fibre composition of muscles, which are known to occur in birds during cold acclimation (Ballantyne & George, 1978), may have a similar effect.

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