

The effect of adaptation temperature on the properties of nerve muscle preparations and on the performance of the frog *Rana temporaria* L.

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KURZFASSUNG: Die Wirkung der Adaptationstemperatur auf die Eigenschaften von Nerv-Muskel-Präparaten und auf die Leistungsfähigkeit des Frosches *Rana temporaria* L. Ziel der vorliegenden Untersuchung war die Beantwortung der Frage, ob sich bei kaltadaptierten und warmadaptierten Fröschen Unterschiede an Nerv-Muskel-Präparationen nachweisen lassen. Die Versuchstiere wurden an 10° bzw. 22° C adaptiert. Danach wurden dann bei 20° C *in vitro* Messungen am Gastrocnemius-Muskel und den ihn versorgenden Nerven durchgeführt. Weder in den Charakteristika der Einzelkontraktion noch des Tetanus konnten Unterschiede festgestellt werden. Auch hinsichtlich des Springvermögens intakter Individuen ließen sich keinerlei Unterschiede nachweisen. Die Bedeutung dieser Ergebnisse wird diskutiert.

INTRODUCTION

Numerous investigations have been carried out on the problem of temperature adaptation in the frog *Rana temporaria*. Many of the adjustments that take place at the adaptation temperature can be seen when frogs adapted to two or more adaptation temperatures are compared at another temperature. These changes involve for instance the basal metabolic rate (GRAINGER 1960), changes in the activities of some enzymes (see PRECHT 1958), hormonal effects (JANKOWSKY 1960), alterations in water balance (GRAINGER 1960, 1963), etc. Maintenance of vital processes and survival of the animal at extreme temperatures are also affected by the adjustments at the adaptation temperatures (PRECHT 1960). The present work was aimed at finding out whether any difference in properties of nerve-muscle preparations could be detected using 10° C and 22° C adapted animals. The *in vitro* measurements were made on the gastrocnemius muscle and the nerve supplying it using isometric twitches at 20° C.

MATERIAL AND METHODS

Male frogs, *Rana temporaria*, were obtained locally during spring and summer from a supplier. They were kept in a little water in covered basins in the dark. Two constant temperatures were used: 10.0° ± 0.1° C and 22.0° ± 0.1° C. The cold adapted frogs were placed at 22° C for 5 days and then at 10° C for 2 weeks. The

warm adapted were placed at 10° C for 2 weeks and then at 22° C for 5 days. This ensured that both groups were equally starved.

The nerve-muscle measurements were made on the gastrocnemius muscle and the nerve supplying it. Firstly a warm or cold-frog was placed in water at 20° C for 10 minutes before being killed (so as to bring all the animals to a common body temperature). It was then weighed and killed by cutting off the head. The nerve-muscle preparation was removed and pinned down in a bath of Ringer's solution without glucose which was thermostatically controlled at $20^{\circ} \pm 0.2^{\circ}$ C. In a few experiments CONWAY'S Ringer solution without glucose was used (BOYLE & CONWAY 1941). This gave identical results. The solution was gently stirred by bubbling a slow stream of oxygen through it. The free end of the muscle was attached to a light chain which in turn was attached to a piece of spring steel which was firmly held at one end. The free end of the spring impinged against a spigot which was soldered on to the end of a RCA 5734 mechanoelectronic transducer. Tension on the muscle could easily be altered by screwing up the hook. The output of the valve was fed into the upper trace of a twin gun Telequipment oscilloscope via the circuitry given by DONALDSON (1958 p. 491). The lower trace was a 50 cycle time trace with the stimulator pulses superimposed on it. In order to reduce pickup the transducer was supplied by a 12 v wet and a 300 v dry battery. A square wave stimulator was used and the output of this was placed across a variable resistance, the other side of which fed the electrode and also the time trace of the oscilloscope. The electrodes were of silver and the nerve was clamped against them and both immersed in the Ringer's solution.

This apparatus permits the muscle to contract isometrically. The muscles were in nearly every case loaded with a tension of 11 grams. Most of the physical characteristics of the preparation were determined from camera film of the oscilloscope trace. The characteristics of a maximum single twitch were measured following a single pulse of 10 v lasting 1 millisecond. Rheobase determinations were made by measuring the minimum voltage necessary for a contraction of the muscle with single pulses of length varying from 0.1 to 5 milliseconds. The muscle was put into tetanus by stimulating with 100 10 v pulses per second, each of length 0.1 millisecond, and this was maintained until the preparation was completely fatigued and the trace returned again to the resting level.

The performance of frogs was determined in the following way. Frogs adapted to either 10° or 22° C were transferred to water at 18° C and kept at this temperature for at least 15 minutes and then weighed. They were then released individually in the center of a grass tennis court and made to hop (encouraged by gentle prodding) until exhausted. This point was reached when no further hops were made after repeated prodding with a sharp point for 10 secs. The actual distance travelled, the time taken and the number of hops were all recorded.

RESULTS

The results of the experiments on the nerve-muscle preparations are given in Tables 1 and 2 and Figure 1. The conclusions to be drawn from these is that there is

no marked difference in properties between the preparations from cold and warm adapted frogs. It will be seen that the *P* value in Tables 1 and 2 shows no significant differences between the two groups with the possible exception of the tension developed in a maximum single twitch. Here the *P* value is only just 0.05, and it is extremely doubtful if any weight can be attached to this. We believe that if a larger number of frogs had been used no significant difference would have been found. The variation between individual frogs in Figure 1 is due to the extent to which the electrodes pressed against the nerve. This greatly affects the true voltage to which the nerve cells are actually exposed. In some cases there may be a small amount of fibrous or fatty tissue close to the nerve which also influence the result. The main point to note is that the pulse length at which the curves flatten out is very similar in the two groups of frogs, and there is no clear and consistent difference in shape between the curves in the two groups.

Table 1

Comparison of maximum single twitch characteristics of preparations from warm and cold adapted frogs measured at 20° C. The standard error is in brackets. The number of animals is in brackets in the first column. This convention is also used in Tables 2 and 3

adaptation temperature	latent period m secs	contraction time m secs	relaxation time m secs	tension g/g of muscle	muscle weight g
10° C (7)	7.8 (0.47)	45.2 (3.4)	68.2 (7.6)	195.8 (4.8)	0.24 (0.017)
22° C (7)	8.6 (0.19)	44.0 (2.0)	56.7 (4.3)	252.0 (23.6)	0.26 (0.018)
<i>P</i>	0.1	0.7	0.2	0.05	0.4

Table 2

Comparison of tetanus characteristics of preparations from warm and cold adapted frogs measured at 20° C

adaptation temperature	tension g/g of muscle	time until fatigued secs
10° C (7)	294 (41.0)	31.1 (3.6)
22° C (7)	370 (16.3)	31.6 (3.3)
<i>P</i>	0.1	0.9

Table 3

Jumping performances of warm and cold adapted frogs measured at 18° C

temperature adaptation	distance m	time taken secs	no. of hops	speed m/sec	mean body wt. g
10° C (18)	36.0 (2.6)	169.7 (13.6)	97.6 (8.9)	0.212	27.8
22° C (18)	29.4 (1.9)	136.1 (13.3)	78.8 (6.2)	0.216	25.9
<i>P</i>	0.1	0.1	0.1		

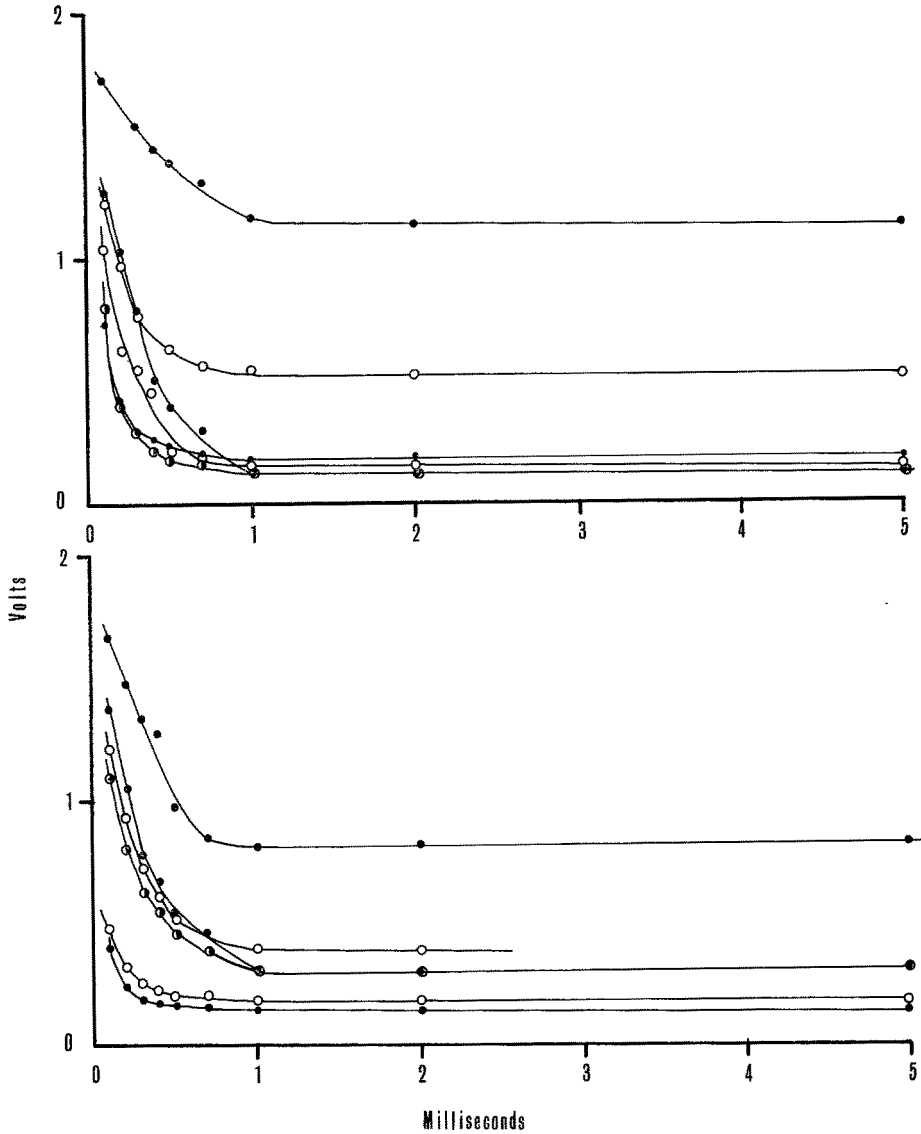


Fig. 1: Curves showing the minimum voltage necessary to produce a minute contraction following single shocks of differing pulse lengths. Each curve is for a single preparation. The upper curves are for 8° C and the lower for 22° C adapted frogs. All measurements were made at 20° C

The performance results on intact frogs are given in Table 3 and Figures 2 and 3. Here again there is clearly no significant difference between the two groups of frogs. It is clear from Figures 2 and 3 that there is no clear separation of the points into two groups. If there had been a marked difference in activity between the two groups of frogs there would have been an obvious separation of the groups of points on the graphs.

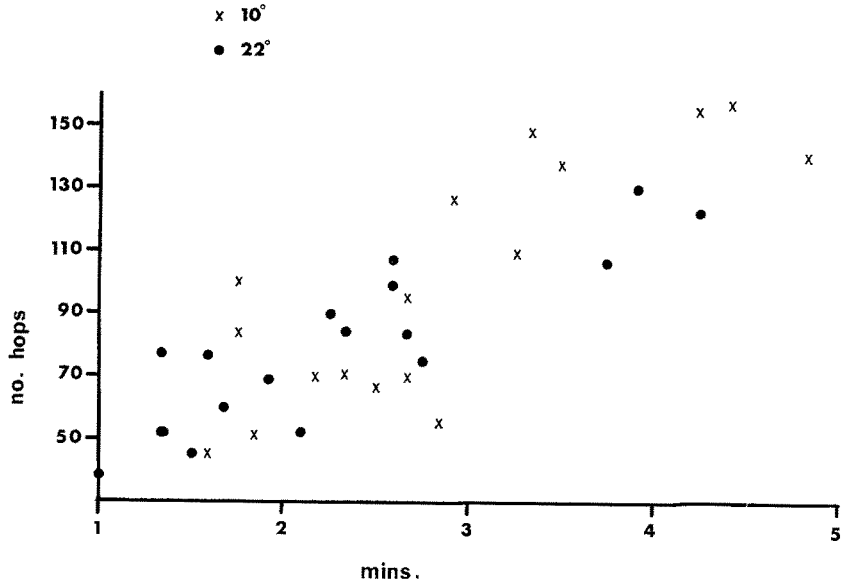


Fig. 2: The number of hops graphed against the time taken to become exhausted for 10° and 22° C adapted frogs measured at 18° C

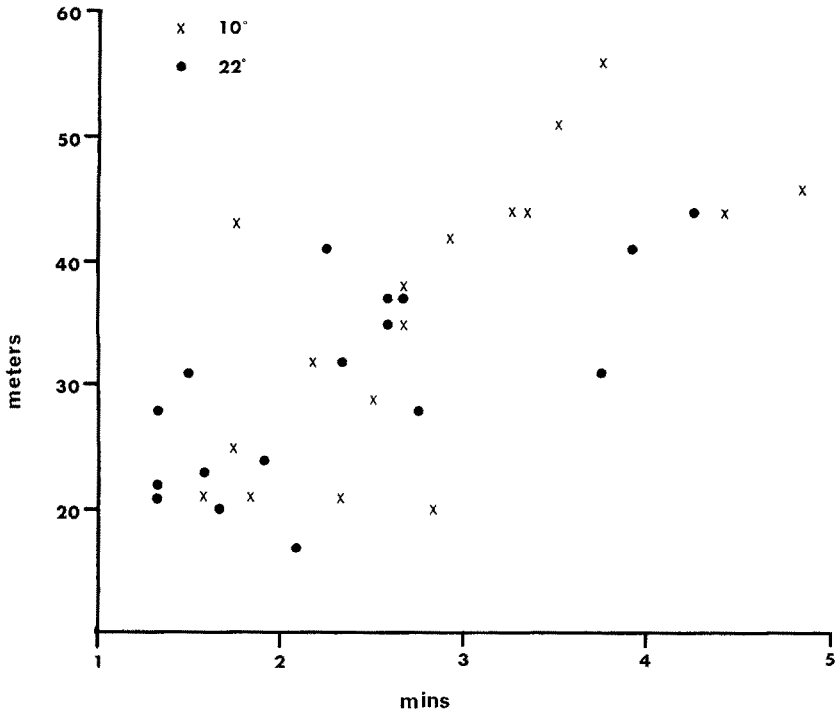


Fig. 3: The distance travelled before 10° and 22° C adapted frogs became exhausted graphed against the time taken. All measurements were made at 18° C

DISCUSSION

Like many cold blooded animals the normal *Rana temporaria* shows an adaptation in its rate of oxygen consumption to temperature belonging to PRECHT's type 3 (JANKOWSKY 1960). *Rana esculenta* shows the same response (STANGENBERG 1955). This means that when the resting oxygen consumption rates of cold and warm adapted frogs are compared at an intermediate temperature the former show a higher rate, even when sufficient time elapses at the intermediate temperature for any overshoot effects due to the temperature change to pass off (GRAINGER 1960). A wide range of cold blooded animals behave in the same way (PRECHT 1958). For this reason it was completely unexpected to find no difference between the performances of warm and cold adapted frogs in the present work (Table 3, Figs. 2 and 3). One can only conclude that adaptation to temperature is of no significance as far as this type of locomotory activity is concerned. This is strange because such an adaptation might be of great survival value. There is the possibility of course that an adjustment is made, but is carried out so quickly that the animal is fully adjusted to the intermediate temperature before the measurements are made. In the case of the present experiments this was about 15 minutes. It is most unlikely that this is the case since adjustments in oxygen consumption rate, carbon dioxide output, enzyme activities etc take several days to be completed (PRECHT et al. 1955).

The absence of any significant difference between the properties of the nerve-muscle preparations (Tables 1 and 2, Fig. 1) is also curious, but perhaps not so astonishing as the absence of an effect in the intact animals. Several authors have investigated muscles from frogs kept at different temperatures, and have found that the adaptation temperature has in many cases little or no effect. For instance no effect of adaptation temperature is found on the activities of succinic dehydrogenase, aldolase (PRECHT 1958, p. 59), catalase (GRAINGER 1960) when these are measured under standard conditions. The same is also true of the rate of oxygen consumption of minced muscle (GRAINGER 1960). So it is perhaps not surprising that no difference in the properties of isolated muscle were found in the present work.

When one turns to high temperatures the effects of the adaptation temperature are very clear. PRECHT (1960) has clearly shown that muscle from warm adapted frogs still contracts, when it is stimulated directly, at a temperature at which muscle from a cold adapted frog will no longer respond. The end plate and nerve however show the reverse effect. PASHKOVA (1962, 1963), SCHLACHTER (1961) and ZHIRMUNSKY & SCHLACHTER (1963) point out that there are marked seasonal variations in response. BENTHE (1954) found that the temperature at which the muscle membrane becomes depolarised is higher in the case of warm adapted animals. In the entire frog a difference in survival is seen between warm and cold adapted individuals – the warm adapted will survive at a higher temperature (e. g. PRECHT 1960). At low temperatures the cold adapted survive better (PRECHT 1960). There is, thus, obvious survival value in adaptive changes in so far as extreme temperatures are concerned. The present work has failed to show any significance in the adaptation adjustments at normal temperatures. It remains to be seen whether future research will reveal any significance at normal temperatures in the adaptation adjustments and whether their

only significance is improved survival under extreme conditions. This does not mean of course that the adaptation changes which have been observed in frogs are of no significance at all to the animal at normal temperatures. It is known that many adjustments are made and these may serve to make the animal function more efficiently. This however has not yet been proved for any cold blooded animal.

SUMMARY

1. Comparisons were made between the characteristics of nerve muscle preparations measured at 20° C from 22° and 10° C adapted frogs. In the maximum single isometric twitches no significant difference was found in latent period, contraction time, or relaxation time between the two groups. There was a barely significant difference ($P = 0.05$) in tension developed/gram muscle but it was thought that with a larger number of experiments no true difference would be found.
2. With isometric tetanus again no difference was found between the 10° and 22° C adapted frogs, either in tension developed or in fatigue time.
3. The jumping performances were measured in intact 10° and 22° C adapted frogs brought to 18° C for 10 minutes and then measured at this temperature. No significant difference could be found between the two groups. The significance of the results is discussed.

LITERATURE CITED

- BENTHE, H. F., 1954. Über die Temperaturabhängigkeit neuromuskulärer Vorgänge. *Z. vergl. Physiol.* **36**, 327–351.
- BOYLE, P. J. & CONWAY, E. J., 1941. Potassium accumulation in muscle and associated changes. *J. Physiol.* **100**, 1–63.
- DONALDSON, P. E. K., 1958. Electronic apparatus for biological research. Butterworth, London, 718 pp.
- GRAINGER, J. N. R., 1960. The early stages in the adaptation of the frog *Rana temporaria* to higher temperatures. *Verh. dtsh. zool. Ges.*, 60–72.
- 1963. The effect of temperature on weight changes and water fluxes in the common frog *Rana temporaria*. (In press.)
- JANKOWSKY, D., 1960. Über die hormonale Beeinflussung der Temperaturadaptation beim Grasfrosch *Rana temporaria* L. *Z. vergl. Physiol.* **43**, 392–410.
- PASHKOVA, I. M., 1962. Kanalizu sezonnykh izemenenii kletok u travyanykh lyagushek. (The analysis of seasonal changes in common frog cells.) *J. gen. Biol. Moscow* **23**, 313–317.
- 1963. In: Problemy tsitoeologii zhivotnykh. (Problems of the cytoecology of animals.) 62–68. *Collected Articles No. 6 Academy of Sciences of USSR, Institute of Cytology, Moscow & Leningrad*, 232 pp.
- PRECHT, H., CHRISTOPHERSEN, J. & HENSEL, H., 1955. *Temperatur und Leben*. Springer Verlag, Heidelberg, 514 pp.
- PRECHT, H., 1958. Concepts of the temperature adaptation of unchanging reaction systems of cold-blooded animals. In: *Physiological adaptation* (C. L. PROSSER, Editor). American Physiological Society, Washington 185 pp.
- 1960. Über die Resistenzadaptation gegenüber extremen Temperaturen bei einigen Organfunktionen des Grasfrosches (*Rana temporaria* L.) *Z. wiss. Zool.* **164**, 336–353.

- SCHLACHTER, N. A., 1961. Teploustoichivost' myshts lyagushki v raznye sezony goda. (Temperature stability of frog's muscles at different seasons of the year.) *Tsitologia* **3**, 95-99.
- STANGENBERG, G., 1955. Der Temperatureinfluß auf Lebensprozesse und der Cytochrom-c-Gehalt beim Wasserfrosch. *Pflügers Arch. ges. Physiol.* **260**, 320-332.
- ZHIRMUNSKY, A. V., & SCHLACHTER, T. A., 1963. In: Problemy tsitoeologii zhivotnykh. (Problems of the cytoecology of animals.) 78-86 *Collected Articles No. 6, Academy of Sciences of USSR, Institute of Cytology*. Moscow & Leningrad, 232 pp.

Discussion following the paper by GRAINGER & GOLDSPINK

VERNBERG: Some locomotor activity studies involving invertebrates (*Uca*) have been done. At reduced temperatures animals were sluggish but became more active with time.

LOCKER: Did you measure the water content of muscle?

GRAINGER: In this particular series of experiments, I did not measure the water content of the muscle, but in earlier work I have done this (1961; *Zool. Anz. Suppl.* **24**, 60). There is a change in water content of the muscle, but it is small. Furthermore, one can bring about this change by warming or cooling one leg. If one leg is warmed, then the water content of the muscle in this leg is brought to the same level as in warm-adapted animals. The changes are, however, small.

LOCKER: Do you find your result astonishing? In my opinion it would not be peculiar, if we understand adaptation as a mechanism which enables an organisms to maintain its normal activity under altered new conditions.

GRAINGER: Yes, it is astonishing. In these experiments on whole frogs, the 10° and 22° C adapted animals were at 18° C for only 15 minutes before being released. This means that either no adjustment is made or that an adjustment is made but is completed within 15 minutes. Experiments have shown that it takes 15 minutes to bring the body temperature of the frog to the new temperature so one cannot say whether any adjustment is made or not during this period. However, some experiments carried out with isolated nerve-muscle preparations removed from 10° C adapted animals which had only been 8 minutes at 20° C showed no difference from the results given in the present paper. This indicates strongly that there is in fact no adjustment. In other words, prolonged exposure to a given temperature does not appear to bring about any changes in the characteristic of the nerve-muscle system in the frog, which would be seen when animals kept at different temperatures are compared at a common temperature. This is contrary to our general experience with other animals.

ZERBST: Ist die überschießende Wasserpermeabilität der Froschhaut im Temperaturversuch eventuell auf einen Adaptationseffekt der ADH-Produktion zurückzuführen? Es wäre eine quantitative Untersuchung des ADH-Spiegels bei solchen Versuchen vorzuschlagen.

GRAINGER: There is no doubt that the ADH production is of very great importance in the frog. I had hoped to carry out some experiments with hypophysectomized frogs, but for some reason the Irish *Rana temporaria* are not suitable for these experiments because sham operated animals (frogs in which the skull hole is drilled and the incision then sewn up again, but the pituitary not removed) behave as if the pituitary is removed. The effect is so great in the sham operated animals that one cannot satisfactorily investigate this question.