cycles vaginal smears were taken daily beween 9 and 11.00 h and only those rats manifesting 2 consecutive 4-day oestrous cycles were included in the study. Each day vaginal smears were taken from 6 to 8 rats at each stage of the oestrous cycle and examined. The animals were then sacrificed by neck fracture at approximately 11.00 h, weighed, their pineal glands removed, rinsed and 2 glands (from rats at the same oestrous stage) homogenized under cooling in 1 ml distilled water. An 0.2 ml aliquot of the suspension was withdrawn for determination of protein and the remainder used for nucleic acids. Protein was determined by the method of LOWRY et al.[9]. The homogenate was washed in order to eliminate any free nucleotids and sugars. RNA was separated from DNA as described by SCHMIDT and TANNHAUSER[10]. RNA in the supernate was determined directly by reading its absorption at 260 nm and again by CERIOTTI's orcinol method[11]. DNA was eluated from the sediment by incubation with 1 *M* perchloric acid at 80°C for 30 min and estimated by the indole procedure of CERIOTTI[12].

*Results.* As can be seen in the Table, there were no significant changes in pineal levels of protein and nucleic acids during the entire oestrous cycle. However, a clear and consistent tendency to high protein and RNA values at dioestrus and low ones at oestrus is evident, the levels at prooestrus and metoestrus being intermediate. Correlation between RNA values obtained by direct reading and those by CERIOTTI's colorimetric method was good, although those obtained by the latter procedure were slightly lower. They were also lower than the values obtained by us in the pineal on previous occasions, as here an additional treatment of the homogenate has been introduced to remove any free nucleotids. DNA levels remained fairly constant throughout the oestrous cycle.

*Discussion.* Our results indicate possible fluctuations in the metabolism of pineal protein linked to the oestrous cycle. However, though consistent throughout the study, the differences between the levels of pineal RNA and protein encountered at the various phases of oestrus were not sufficiently drastic to reach significance and should be confirmed by a more sensitive radioactive method of determination.

Increased metabolic activity of protein at dioestrus is in agreement with the findings of ZWEENS[1] and WURTMAN[2] who reported higher phospholipid and HIOMT contents of the pineal and a gain, although an insignificant one, in its weight during the dioestrous phase of the cycle.

It is interesting that the specific activity of RNA in hypothalamus and pituitary was low at dioestrus and high at oestrus[13, 14], the opposite of that in the pineal gland. These converse hypothalamic-hypophysial and pineal undulations may be related to the cycles of gonadotrophins and gonadal steroids, increased circulating levels of which could be stimulating pituitary and inhibiting pineal metabolism. The link between oestrus and changes in pineal metabolism may be either through primary hypophysial influence[2, 6], or primary gonadal effect mediated by the hypothalamic-hypophysial axis. The possibility also exists that gonadal function regulates independently both pituitary and pineal activity, since hypophysectomy had no influence on oestradiol-induced reduction of pineal adenyl cyclase activity[15]. Moreover, administration of oestradiol abolishes the increased HIOMT activity produced by oophorectomy[6]. Thus, it can also be supposed that ovarian steroids, which are released cyclically and control vaginal cytology, act directly on the pineal and decrease its metabolic activity. It appears, therefore, that both ovary and anterior pituitary could be involved in modifying pineal cyclic activity during the oestrous cycle within the framework of a complicated feedback system.

*Zusammenfassung.* Gesamteiweiss und RNS der Zirbeldrüse steigen während der Dioestrusphase bei Ratten an. DNS bleibt während des ganzen Cyclus konstant.

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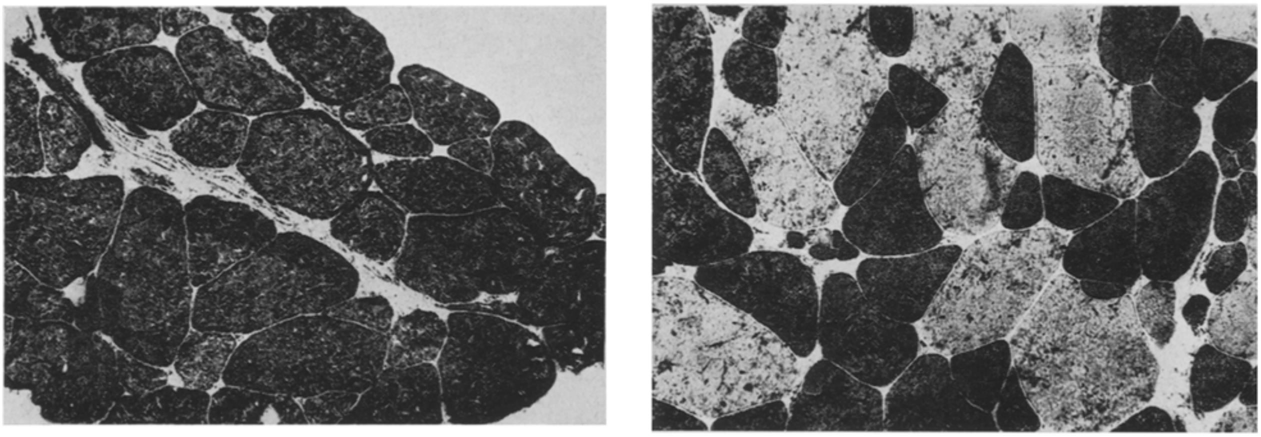
## Sexual Dimorphism in Contraction Properties and Fibre Pattern of the Flexor Carpi Radialis Muscle of the Frog (*Rana temporaria* L.)

Sex hormones play a paramount role in the regulation of size and function of secondary sexual organs and sexual behaviour, involving presumably genetically controlled formation of specific receptor mechanisms[1] in target organs, including cross striated muscles. The resulting androgen-dependency differs, however, in different muscles and varying degrees of sexual dimorphism of muscles may be observed[2]. Some are absolutely endrogen-dependent, e.g. the levator ani muscle of the rat, which undergoes complete perinatal involution in the female rat[3], others are relatively androgen-dependent, e.g. the temporal muscle of the guinea-pig, which exists in both sexes but differs in weight, fibre size[4] and enzyme pattern[5].

A further example of a relatively androgen-dependent muscle is the flexor carpi radialis of the frog, associated with the 'clasping reflex' of the male during the mating act. The sexual dimorphism of this muscle has been known for a long time[6], the 'male' muscle being larger and consisting of larger diameter fibres than the 'female'[7]. Muscle fibre size shows seasonal variations, it is largest at the mating time (March–April) and smallest in summer, when progressive decrease of androgen levels can be assumed. Correspondingly, there is a decrease of muscle fibre size after castration and an increase after testosterone administration[7].

It appeared of interest to study the sexual dimorphism of this muscle also with respect to its contraction properties and histochemical fibre patternt, especially that of ATPase activity, which is closely related to speed of contraction[8].



ATPase activity of flexor carpi radialis muscle of frog (*Rana temporaria* L.) determined histochemically during the time of mating (March): a) Female animal. b) Male animal.

*Material and methods.* Mature male and female frogs (*Rana temporaria* L.) were used for the experiments. The muscles were removed after decapitation of the animals and dissected free leaving intact the bony attachment at the proximal end of the muscle and the tendon at the distal end. Ligatures were tied to both ends and the muscles were set up in a chamber containing oxygenated (95% $O_2$ + 5% $CO_2$) Frog-Ringer solution and Pt electrodes for massive stimulation[9] at a temperature of 20 °C. Isometric twitch responses of the muscles under optimal tension, i.e. resting tension giving maximal tension output were monitored on an oscilloscope and recordings of contraction time (time to peak), half contraction time, latency period, maximal rate of twitch tension development and half relaxation time were made by an automatic analyzer of muscle contraction properties[10]. The maximal rise of tension development is expressed as a time constant, which is independent of amplitude of contraction and concerns speed of tension development in msec in the middle (25%–75%) part of the ascending contraction curve where the slope may be considered linear. For histochemical analysis, the muscles were removed, quickly frozen in $CO_2$ and transverse sections were cut in a cryostat at a temperature of –20 °C and stained for ATPase activity[11].

*Results and discussion.* The table shows the changes in contraction properties of the flexor carpi radialis muscle of male and female frogs during the period of one year. It can be seen that during the mating season there are considerable differences in all parameters of contraction properties between 'male' and 'female' muscle, the 'male' muscle being considerably slower than the 'female' one. The difference appears to be most pronounced in half relaxation time. In the flexor carpi radialis muscle of the male frog the contration time, rate of tension development, the latency period, half relaxation time shortens in the months following the mating period but again shows a gradual decrease of contraction time and prolongation of half relaxation time between July and November, apparently in connection with the gradual rise of andorgen levels during this period[6]. The flexor carpi radialis muscle of the female frog is considerably faster and shows a small shorteining of contraction time during the summer. The Figure shows a cross section of the flexor carpi radialis muscle removed from female (a) and male b) during the mating period, stained for ATPase activity. In the 'female' muscle, all fibres, large and small ones, have a rela-

Seasonal variation in contration characteristics of flexor carpi radialis muscle of frog (*Rana temporaria* L.) at temperature 20 °C
Male animals: contraction characteristics (in msec)

| Month | *n* | CT | HCT | TCC | LT | HRT |
|---|---|---|---|---|---|---|
| March | 18 | 44.2 ± 1.26 | 21.0 ± 0.48 | 16.1 ± 0.69 | 6.7 ± 0.10 | 58.3 ± 2.49 |
| May | 7 | 44.9 ± 1.49 | 19.6 ± 0.48 | 13.9 ± 0.42 | 6.4 ± 0.36 | 50.0 ± 4.62 |
| August | 8 | 37.0 ± 1.60 | 17.3 ± 0.44 | 12.6 ± 0.66 | 6.1 ± 0.10 | 40.8 ± 3.66 |
| November | 10 | 48.0 ± 1.99 | 21.8 ± 0.67 | 18.9 ± 1.00 | 6.6 ± 0.22 | 64.0 ± 0.26 |
| $P_{MA}$ | | <0.005 | | <0.005 | | <0.001 |
| $P_{AN}$ | | <0.001 | | <0.001 | | <0.01 |

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Female animals: contraction characteristics (in msec)

| Month | $n$ | CT | HCT | TCC | LT | HRT |
|---|---|---|---|---|---|---|
| March | 10 | $32.8 \pm 0.71$ | $16.1 \pm 0.53$ | $11.4 \pm 0.32$ | $6.0 \pm 0.32$ | $30.2 \pm 1.84$ |
| May | 9 | $32.7 \pm 1.64$ | $15.0 \pm 0.71$ | $10.3 \pm 0.66$ | $6.1 \pm 0.75$ | $32.8 \pm 3.58$ |
| August | 8 | $28.7 \pm 1.46$ | $15.7 \pm 0.59$ | $8.5 \pm 0.54$ | $6.1 \pm 0.30$ | $21.1 \pm 2.58$ |
| November | 7 | $34.9 \pm 1.05$ | $17.9 \pm 0.60$ | $11.7 \pm 0.45$ | $5.2 \pm 0.22$ | $31.7 \pm 4.20$ |
| $P_{MA}$ | | $<0.02$ | | $<0.001$ | | $<0.01$ |
| $P_{AN}$ | | $<0.005$ | | $<0.001$ | | $<0.05$ |

CT, contraction time; HCT, half contraction time; TCC, maximal rise of tension development; LT, latency period; HRT, half relaxation time. $P_{MA}$, probability values: March–August; $P_{AN}$, probability values: August–November.

tively high ATPase activity, i.e. are type II muscle fibres[12], whereas in the 'male' muscle, both fibres with high ATPase activity and very large fibres with very low ATPase activity (i.e. type I fibres), can be found.

Thus the sexual dimorphism of the flexor carpi radialis muscle of the frog concerns not only muscle weight and fibre size[7] but also contraction properties and the related histochemical fibre pattern according to ATPase activity. 'Male' muscles are relatively slow and have a greater proportion of type I fibres, i.e. large muscle fibres with low ATPase activity. This relative 'slowness' of 'male' muscles is apparently due to the relative androgen-dependency of the 'male' muscle, resulting in seasonal variation of contraction properties related to seasonal changes of androgen levels. Androgens affect speed of the fast levator ani muscle, highly sensitive to the male sex hormone in vitro[13] and in vivo[14]. A prolongation of concentration time in this muscle can be observed after castration[5]. On the other hand 'male' and 'female' temporal muscles, serving primarily masticatory function, are both fast muscles, the sexual dimorphism being, however, marked in the enzyme pattern, i.e. especially in the ratio of glycolytic to oxidative enzymes[14]. Thus 3 different androgen-dependent muscles show differences in speed of contraction and in their reaction to changes of andorgen level. In contrast to the fast levator ani of the rat and the fast temporal muscle of the guinea-pig, the flexor carpi radialis of the frog is a slow muscle with a more tonic function operating in the 'clasping reflex' of the male. Enlargement of various muscles of the forelimb, associated with this reflex of the male during the mating act clearly shows seasonal variations[7] and can apparently be interpreted as results of androgen dependence[15]. In this respect the prolongation of contraction and especially relaxation time in the flexor carpi radialis during the mating season, and the following temporary shortening of these parameters, may be explained by changes in level of androgen, seasonally increasing or decreasing the tonic function of the muscle. However, environmental changes may also affect related changes in contraction properties. Rise of environmental temperature in the summer months may participate in the shortening of contraction time, as it affects both 'male' and 'female' muscle. There is also an increase of Na-K activated activity of ATPase[16], increase of $Ca^{2+}$, $Na^{+}$ and decrease of $K^{+}$[17] and respiratory quotient[16] and decrease of contracture response to ACh[18] during the summer months.

The mechanisms by which genetic and environmental factors determine the sexual dimorphism, and the seasonal variations in the flexor carpi radialis muscle of the frog, have still to be elucidated.

*Zusammenfassung.* Es wird ein Sexualdimorphismus beim Daumenbeugemuskel (m. flexor carpi radialis) des Frosches (*Rana temporaria* L.) mit Bezug auf das Kontraktionsverhalten, wie auch in histochemischer Richtung, festgestellt. Kontraktions- und Relaxationszeit sind bei männlichen Muskeln verlangsamt mit entsprechender ATPase-Aktivität. Saisonbedingte, geschlechtstypische Testosteronkonzentrationen stehen damit im Zusammenhang.

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# Wirkung von NIH-LH auf das Ovar unreifer Ratten unter Progesteron-, Norgestrel- und Lynestrenol-Einfluss

Das Luteinisierungshormon LH stimuliert im Ovar das Interstitium, ist für die Follikelreifung und zur Ovulation nötig und bewirkt am reifen Follikel eine Stoffwechselumstellung zur Progesteronsynthese[1]. Ob das Luteinisierungshormon unbedingt zur Oestrogensynthese im Ovar erforderlich ist, wird in Frage gestellt. Im Tierexperiment ist gezeigt worden, dass ein weitgehend LH-freies FSH-Präparat auch Östrogensynthese induzieren kann[2]. Zahlreiche klinische und tierexperimentelle Arbeiten befassen sich mit der Funktion des Ovariums unter der Wirkung von verschiedenen Steroidhormonen. Die oft widersprüchlichen Ergebnisse lassen sich mit dem inhomogenen