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STUDIES ON THE ELECTRIC EXCITABILITY OF THE ADDUCTOR MUSCLE OF GLOCHIDIA

ELEMÉR LÁBOS

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In previous works (LÁBOS, SALÁNKI 1963, LÁBOS, SALÁNKI and S.-RÓZSA 1964) the rhythmic motor activity of fresh-water mussels (*Anodonta cygnea* L.) being in their early ontogenetic stage was investigated. The animals were in the veliger phase of individual development preceding their release into open water. Their rhythmic motor activity consists of contraction and relaxing of larval adductor muscle. The fibres of adductor are parallel to the longitudinal axis of the animal and adhere to the middle fields of the two half valves. Their length is about 200 μ, and their thickness 5—15 μ *(Fig. 1)*. It was found that the rate of rhythmic action of the adductor may be increased both considerably and lastingly by potassium chloride and tryptamine. It is a much debated question if these effects and the spontaneous rhythmic activity itself are indicative of the properties of the muscle fibres or they are due to the rhythmic action of nerve elements. The problem of motor innervation has not as yet been solved morphologically, and the only thing we are in knowledge of is that the ganglions are in the initial stage of differentiation (HERBERS 1914).

This present work deals with the electric excitability of glochidia. The findings of RUSHTON and LAPICQUE (RUSHTON 1930, LAPICQUE 1931) show that the parameters of excitability as threshold voltage and chronaxic are mainly dependent on the conditions of excitation. Namely, as it was pointed out by RUSHTON the direction of the electric field is not indifferent with regard to the efficacy of excitation current (RUSHTON 1930). RUSHTON performed his studies on the sartorius of adult specimens of *Rana temporaria*. He discovered that an electric current passing at right angles to the direction of fibres was considerably less efficacious than those passing parallel with it. His findings confirm that similar regularities exist also in connection with the nerves (RUSHTON 1927). His stimulatory experiments were made in liquid medium. This method was successfully employed in investigating the excitability of glochidia. In these present studies an attempt is made to investigate if the parameters of excitability of embryonal nerve fibres of mussel and their dependency on the direction of electric field follow similar regularities as the sartorius and the nerves of onto- and phylogenetically more developed adult specimens of frog.

Nerve and muscle fibres of different excitability and direction were successfully distinguished by RUSHTON by the usual method of excitation in the nerve-muscle complex of frog. Possibility for this distinction was afforded by the observation that the responses of tissues of two different types to stimuli of perpendicular direction was small, whereas optimum excitation was produced by stimuli of longitudinal direction. Thus tissues of diverse direction may get optimally excited by electric fields of different direction. The relation between duration of stimulus and threshold potential of nerve-muscle complex may be illustrated graphically by a composite curve.

The direction of the fibres of adductor in the glochidia is longitudinal. The existence of motor nerve fibres has not been established as yet. Nevertheless if there are such fibres their direction should differ from that of the muscle fibres, because the primitive colonies of ganglia are situated laterally from the muscle fibres.

Material and methods

The experiments were performed on the glochidia of fresh-water mussel (*Anodonta cygnea* L.). The glochidia were removed from the lamellae of the gill of the parent and were rinsed in twice filtered fresh Balaton-water. Thereafter the glochidia were put into a PETRI-dish containing likewise Balaton-water. The PETRI-dish was placed on the objective table of a ZEISS profile-projector. The stimulating electrodes were dipped into the fluid and their position was fixed.

Ag·AgCl electrodes were also used in these investigations. Except for preventing polarization and limiting the degree of excitation by the insulating effect of AgCl layer, these electrodes had no distoring influence on the effects produced.

The tips of the two electrodes were at a 5 mm distance from each other. The diameter of the connectives were 0.2 mm, with spherical ends of 1 mm diameter. Only one half of the sphere was left free, its other hemipshere which was dipped into the fluid was insulated. Under these conditions the resistance of the fluid measured between the electrodes was 20—40 kΩ. Square impulses were applied for excitation.

The position and direction of the animal investigated could be adjusted by two manipulators situated at right angles to each other. These manipulators could be turned round above the objective table of the profileprojector. The animal was placed at 1.5 mm distance beneath the plane of the electrodes. (In the followings this will be dismissed). The factors determining the spatial relation between electrodes and glochidia are illustrated in *Fig. 2*. The center of the rectangular system of coordinates drawn in the plane of a glochidium coincides with the middle point of the distance between the two electrodes. The line connecting the two electrodes is the ordinate and the abscissa is set at right angles to its middle point. The glochidium has a central position, if its middle point coincides with the center of the system of coordinates. The bisecting point of transverse axis the contact line of the two valves and longitudinal axis (the line passing through the tips of shell) constitutes the center of the glochidium. The φ-angle between the longitudinal axis of the animal and the ordinate is also an important factor. The glochidium is asymmetric to its longitudinal axis, thus at $\varphi = 90°$ its narrower part always faces to positive direction.

In the course of excitation sufficient time was allowed for restitution. Threshold excitation was determined by increasing stimuli gradually. The value of φ-angle was adjusted after every measurement with an accuracy of 0.5°.

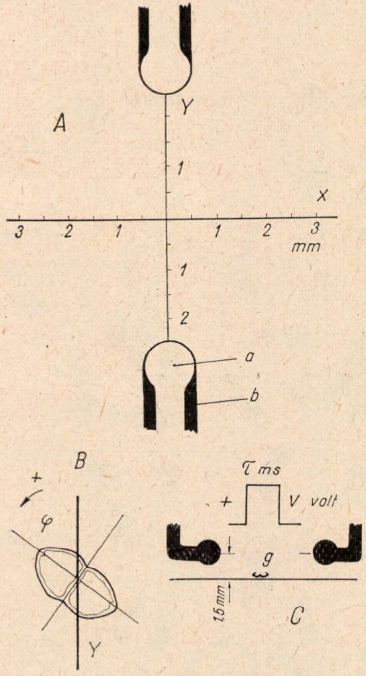

Fig. 2. The position of glochidium on a horizontal plain, and the spatial relation between glochidia and the stimulating electrodes
A — the axes of the horizontal system of coordinates, the position and the dimensions of electrodes; a — Ag or Ag.AgCl, b — insulation
B — spatial position of glochidia: at 1,5 mm beneath the plane of electrodes; square-impulses of τ msec duration and V voltage were taken on the electrodes; g — glochidium

2. ábra. A glochidium helyzete a vízszintes síkon és a glochidiumok és ingerlő elektródok térbeli viszonya
A — a vízszintes koordináta rendszer tengelyei az elektródok helyzete és méretei; a — Ag vagy Ag·AgCl, b — szigetelés
B — a glochidium térbeli helyzete: az elektródok síkja alatt 1,5 mm-re; az elektródokra τ msec időtartamú és V feszültségű négyszögimpulzusokat viszünk; g — glochidium

Results

When placing an unarranged group of glochidia into the electric field and single impulses of constant duration are taken on the electrodes, divergent values of threshold voltages will be obtained. Threshold voltage is greatly influenced not only by the spatial relation between animal and electrodes but also by the orientation of the animal itself (φ-angle). In *Fig. 3* an unarranged group of glochidia is illustrated. The electrodes are indicated by semicircular arches. Values of threshold voltages at impulses of 5 msec duration are indicated by the figures beside the animals. Threshold voltages varied between

2—24 V. The minimum voltage necessary to produce contraction in the adductor of glochidium at an impulse of given duration may greatly vary depending on the position of the glochidium. The purposes of subsequent measurements is to examine methodically the excitability of individual glochidia of central position in the function of φ-angle, further to determine the threshold of the single animals situated in longitudinal direction ($\varphi = 0°$) in various places of the electric field.

Fig. 3. Unarranged group of glochidia between the electrodes. The figures beside the glochidia indicate threshold voltages produced by impulses of 5 msec duration. The values of threshold voltages varied between 3—24 V

3. ábra. Rendezetlen glochidiumhalmaz az elektródák között. A glochidiumok mellé írt számok 5 msec-es impulzusokkal történő ingerlés esetén a küszöbfeszültséget jelentik. Látható, hogy 3 V-tól 24 V-ot meghaladó küszöb egyaránt előfordul

1. Threshold voltage of glochidia of right and longitudinal direction in central position.

Threshold voltages of glochidia of central position and of orientations of $\varphi = 0°$ and $\varphi = 90°$ were studied at impulses of 5 msec duration. Threshold values were much greater in transversal than longitudinal direction. In longitudinal direction, *i.e.* at $\varphi — 0°$ the value of threshold voltage was $3{,}51 \pm 0.2$ V under the given experimental conditions. The data refer to 50 specimens of animals originating from five populations.

These investigations do not furnish exact data on the average value of transversal threshold, because transversal threshold of great many animals surpassed the upper limit of the voltage (24 V) of the stimulator.

2. Dependency of threshold voltage on the direction of the longitudinal axis of glochidium of central position.

In this run threshold excitation was determined as follows. The glochidium was removed from its longitudinal position by turning it round at equal arches. The duration of excitation impulses was 5 msec. At a turn from 0° to 60—65° threshold voltage became approximately doubled. It increased more rapidly by increasing the angle and at about 70—80° it was higher than 24 V. The changes of threshold of a single glochidium at a complete turn is given in *Fig. 4.*

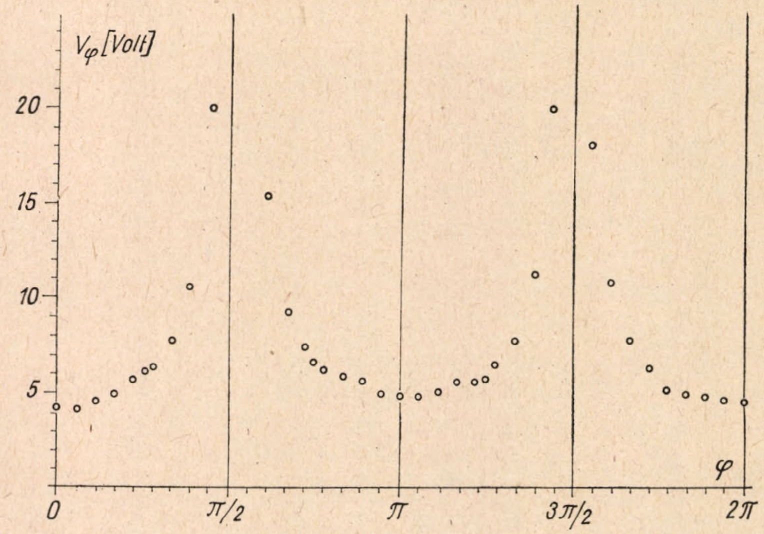

Fig. 4. Dependency of threshold voltage on the φ-angle at impulses of constant duration (5 ms). On the abscissa the φ angle, on the ordinate the $V\varphi$ threshold voltage is given. The points indicate the data of a single glochidium

4. ábra. A küszöbfeszültség függése a φ-szög nagyságától állandó (5 ms) impulzusszélesség esetén. Abszcisszán orientációs szög, ordinátán $V\varphi$ küszöbfeszültség. A pontok egyetlen glochidium adatait jelentik

Because lateral parts of the animal during this turn round get to regions where the strength of electric field is smaller, it is suggested that this difference in electric field might be responsible for the above changes in threshold. For elucidating this problem threshold voltage determinations were made when moving the animals either in the direction of abscissa or of the ordinate. During these investigations the direction of glochidium was longitudinal, *i.e.* $\varphi = 0°$.

3. Changes in threshold voltage by changing the position of a glochidium of longitudinal direction.

Threshold voltage measurements were performed on glochidium of longitudinal direction at different points (at 1.5 mm distances) of the abscissa. The averages of ten serial measurements are presented in *Fig. 5.* The increase in threshold voltage may be represented graphically by a parabole. At a 5 mm distance from the center threshold voltage was about 10 V. The suggestion that perhaps the decrease in strength of field in lateral direction may be responsible for the increases in threshold voltage during rotation has to be rejected namely, as it is also evidenced by the graphs, every portion of the animal located centrally was nearer to the center than 0.5 mm.

When moving the glochidium of longitudinal direction along the ordinate the changes registered in the critical zone of rotation are similarly negligible.

Thus it is extremely likely that the deviations produced by rotation are related primarily to changes in angle between muscle fibres of glochidium and strength of field.

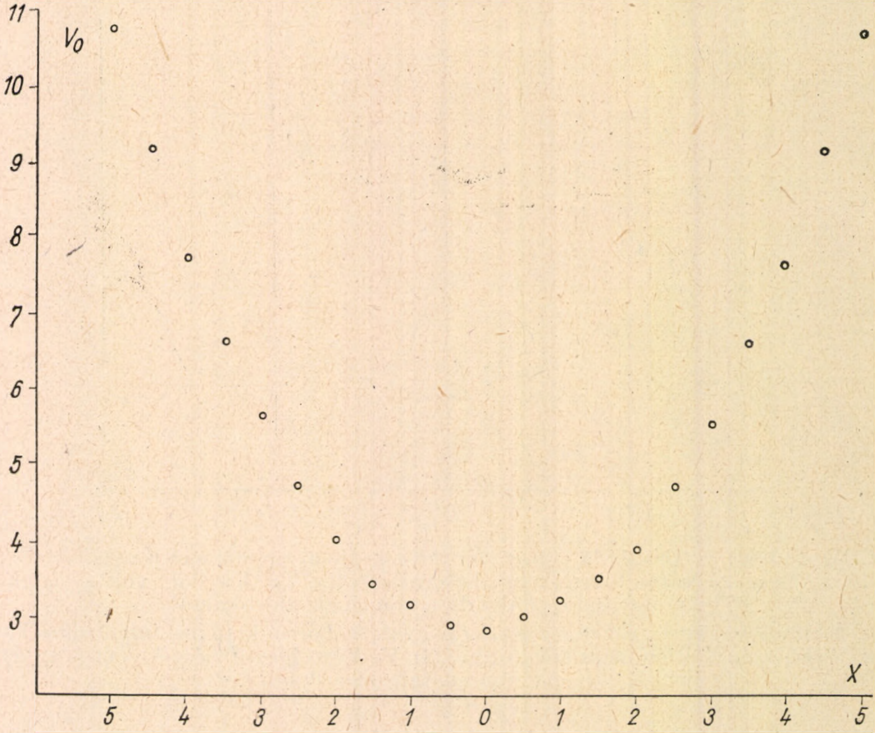

Fig. 5. Differences in threshold voltage when moving the glochidium along the abscissa; $\varphi = 0°$ (glochidium of longitudinal direction). On the abscissa the points of X-axis, on the ordinate threshold voltage are illustrated; $\tau = 5$ msec, distance between the electrodes is 5 mm. The graph illustrates the averages of results obtained on ten animals separately

5. ábra. A glochidium küszöbfeszültségének változása az X-tengely mentén elmozdítva; $\varphi = 0°$ (hosszanti helyzetű glochidium). Abszcisszán az X-tengely pontjai, ordinátán a küszöbfeszültség; $\varphi = 5$ msec, az elektródok közötti távolság 5 mm. A görbe 10 állat átlagát mutatja

4. Deviations in threshold voltage of glochidium of central position induced by impulses of different durations.

It has been established in the foregoings that the thresholds of glochidia at impulses of constant duration are subject to changes depending on the transversal or longitudinal direction of the animals. In the next experimental run the relationship between threshold voltage and duration of impulses is investigated at three different directions ($\varphi = 0°$, $45°$ and $65°$). Duration of impulses varied between 200 μsec and 120 msec. The results of ten animals obtained are presented in *Fig. 6*. At every direction investigated a fast and a slow decrease in threshold voltage was observable between 200 μsec—1 msec and 1—120 msec respectively. The slope of curves obtained by increasing the φ-angle passes along gradually higher values. The curve is more off centered between 0°—45° than between 45°—65°, though the difference in the case of the later is only 20°. Secondary changes in the slope of curves may also occur which is indicative of their composite character.

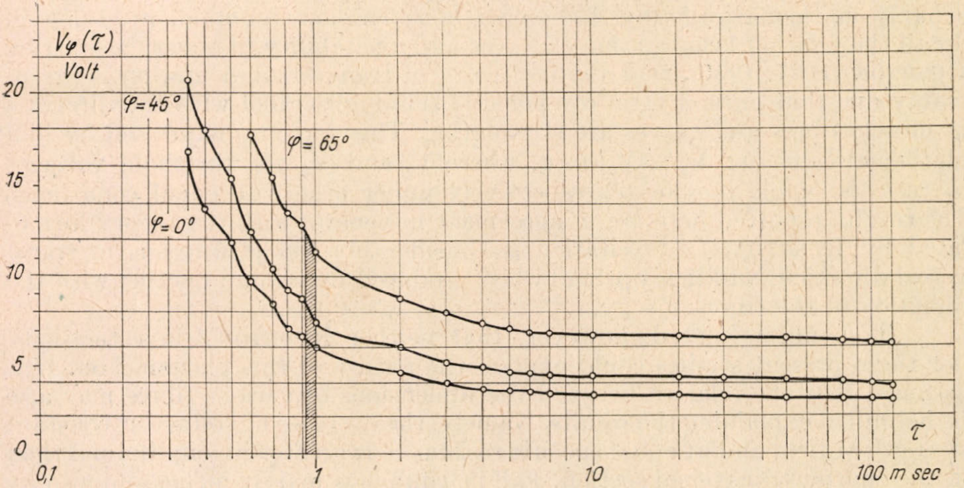

Fig. 6. Threshold voltage-duration relation. Abscissa: duration of stimulating impulses in msec. Ordinate: values of threshold voltages at different φ-angles and at impulses of different durations ($V\varphi(\tau)$) expressed in V. Semilogarithmic illustration. The investigated directions were: $\varphi = 0°, 45°, 65°$
The experimental points indicate averages of measurements performed on 10 different animals

6. ábra. Küszöbfeszültség—időtartam összefüggése. Abszcissza: az ingerimpulzus tartama msec-ban. Ordináta: a küszöbfeszültség értékei különböző φ-szögek és impulzusszélesség esetében ($V\varphi(\tau)$) Voltban. Féllogaritmikus ábrázolás. A vizsgált irányok $\varphi = 0°, 45°, 65°$. Minden pont 10 állaton végzett mérések átlagát jelenti

The value of chronaxie is 0.7—1.5 msec (1 msec in average).

Discussion

It may be established on basis of foregoings that the degree of excitation produced with a pair of electrodes in liquid medium is mainly influenced by the spatial relations between glochidia and electrodes. Especially two factors proved to be responsible for the changes in threshold voltage in excitation experiments run under constant conditions and at impulses of equal duration.

The one is the position of the animal in the electric field, the other the angle between electric strength of field and muscle fibres.

The importance of the first factor is indicated by increases in threshold values obtained when moving the glochidium of given direction off from the electrodes along the abscissa. This is obviously due to external causes. Namely, there is a decrease in the density of line of forces in lateral direction.

The importance of the other factor was detected when the glochidium placed in a central region of relatively constant strength of field was turned round. The dependency of threshold voltage on φ-angle seems to be approachable mathematically by a discontinuous circular function. Studying in detail the relation between line of forces and excitability, it was suggested by **Rushton** (1927, 1930), that the inefficacy of transversal excitation is presumably due that only electric components running parallel with the direction of muscle fibres and nerves are stimulating. This was also expressed by him mathematically as: $V_\varphi = V_0/\cos\varphi$, where V_φ and V_0 are threshold voltages in case the angle is φ or 0° respectively under otherwise equal conditions. The results reported here are in agreement in general with the theory formulated by **Rushton**, *i.e.* excitation is considerably less efficaceous in transversal direction, and it is optimal when produced by stimuli parallel with the direction of the fibres.

It should be noted, however, that neither **Rushton**'s investigations, nor these present studies could explain the nature of this phenomenon. It is assumed that the relation between the dimensions of various fibres may also be helpful in explaining the results. Namely, the decrease in voltage at constant strength of field and at equal specific resistance is greater in longitudinal than in the shorter transversal section. Fall in voltage is mainly influenced also by the value of specific impedance, which may be direction dependent. Thus, the actual fall in voltage in a given section of muscle fibre is determined mainly by these two factors. The changes of specific resistance, however, may originate from the fine differences existing between longitunidal and transversal structures.

Thus on basis of foregoings it is regarded unnecessary to suggest the presence of motor nerve fibres of special direction in order to explain the direction-dependency of excitability.

Nevertheless, the question arises wether further inferences may be drawn on basis of these results concerning the presence of motor innervation. In *Fig. 6* the graphs representing threshold voltage show secondary changes in slope. These graphs were drawn on the basis of measurements performed on ten animals. Similar properties were reflected by measurements on the single individual. It was assumed by W. A. H. **Rushton** (1930) on basis of similar differences that there may motor nerves be present in the muscle fibres. This assumption is, however, confronted by the fact, that the average value of chronaxie is approximately constant: 1, that is 1—0.92 at angles of 0°, 45° and 65°. It was observed by **Rushton** that nerve elements were highly excitable (γ-excitability), whereas muscle elements displayed low excitability (α-excitability), which corresponds to small and great chronaxie respectively. On the present occasion, however, no considerable differences in chronaxie were observed by changing the angle. It is suggested that if the secondary changes in slope are due to the presence of nerve elements, then they would be isochronous with the excited muscle fibres at $\varphi = 0°$. In that case the differences in chronaxie at the above angles would be negligible, and the differences in the direction of hypothetical nerve elements should also be small or strongly deviating respectively, for the secondary changes of slope are unimportant. It is also presumably that only muscle fibres were excited at every angle applied. In that latter case the secondary changes in slope, as illustrated in *Fig. 6*, may be considered only as experimental errors.

The reciprocal cosinus relation between transversal and longitudina excitability as established by Rushton applies, apart from certain little deviations, also for the relation presented in *Fig. 4*. *Fig. 7/A* illustrates the results of threshold voltage measurements performed on ten glochidia each. The glochidia of central position were turned round and were excited at impulses of 5 msec duration. The results are illustrated in a polar system of coordinates. On the side of φ angle the corresponding value of threshold voltage ($V\varphi$) is marked off. The values should have taken place along the *f*-line in agreement

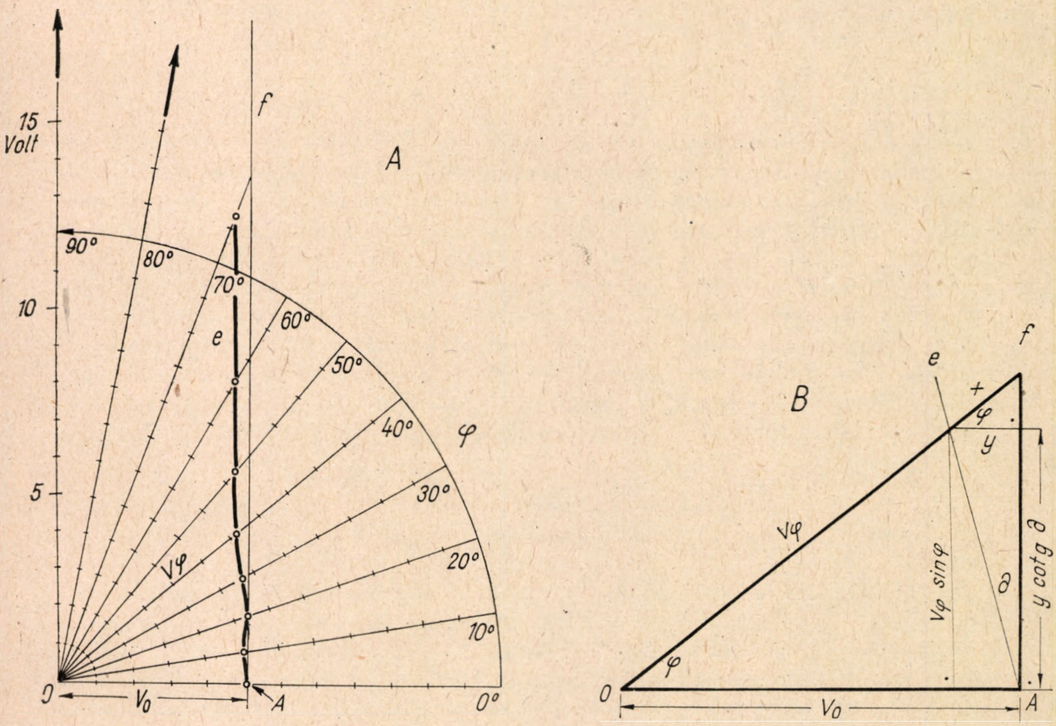

Fig. 7. A — a relation in agreement with figure 4: dependency of V_φ threshold voltage on φ-angle, represented in a polar system of coordinates. The values of V_φ are marked on the sides of φ-angles. The relation is approximately linear. e — curve deviating by φ-angle ($V_\varphi/\cos\varphi$) from f-line set at right angles on the abscissa. B — explanatory figure

7. ábra. A — a *4. ábrá*nak megfelelő összefüggés: a V_φ küszöbfeszültség függése a φ szögtől, polárkoordinátarendszerben ábrázolva. A φ-szögek szárára mértük rá a V_φ értékeket. Az összefüggést közel lineárisnak tekintjük (e), mely ∂-szöggel eltér az abszcisszára merőleges f-egyenestől ($V_0/\cos\varphi$). B — magyarázó ábra to the correlation as determined by **Rushton** ($V_\varphi = V_0/\cos\varphi$). Newertheless on every occasion $V_\varphi < V_0/\cos\varphi$. Consequently the points obtained took place along the *e*-curve, which on basis of simple trigonometric considerations may be expressed as (see *Fig. 7/B)*

$$V_\varphi = \frac{V \cos \partial}{\cos (\varphi - \partial)}$$

where ∂ is the angle between the *f*-line obtained by **Rushton** and the *e*-line obtained in these experiments. The actual value of this angle may be expressed mathematically as

$$\frac{V_{\pi/2}}{V_0} = \operatorname{cotg} \partial$$

In case $\partial = 0°$ then the relation according to **Rushton** is obtained. It is assumable on basis of this ∂ angle that there may be in the glochidium excitable structures present, which are deviating from the direction of the longitudinal axis of the animal (these may be either muscle fibres of deviating direction or nerve elements). It is also presumable that the deviations from **Rushton**'s rule may originate in the differences of electric structures in the external medium and muscle fibres, which produce a break in strength of forces along the contact line of the two substances and further in the fact that the experimental animal was placed under the plane of the electrodes. In view of these and other questions discussed so far it is considered important to investigate further the limits of the validity of **Rushton**'s rule and the problem of a hypothetical motor innervation.

Summary

In this work the electric excitability of the adductor of glochidia of freshwater mussel *(Anodonta cygnea* L.) was investigated. The reciprocal cosinus relation between excitability and direction of electric field as established by **Rushton**, holds true approximately also in these experiments. The threshold obtained at impulses of 5 msec duration running parallel with muscle fibres was 3.5 V. Threshold voltage is approximately doubled and the transversal threshold becomes very high when increasing the angle to 60°—65°. Up to 65° chronaxie was about constant *i.e.* 1 msec. The possibility of a hypothetical motor innervation is discussed on basis of secondary changes in the relation between threshold voltage and duration, and of other results deviating from the findings of **Rushton**.

LITERATURE CITED

Herbers, K. (1914): Entwicklungsgeschichte von Anodonta cellensis Schröt. — *Zeitschr. wiss. Zool.* **108**, 1—174.

Lapicque, L. (1931): On the electric stimulation of muscle through Ringer's solution. — J. *Physiol.* **73**, 219—246.

Lábos, E., J. **Salánki** (1963): The effect of alkali metal ions and alkaline earth metal ions on the rhythmic activity of glochidia of the freshwater mussel. — Anodonta cygnea L. — *Annal. Biol. Tihany* **30**, 45—57.

Lábos, E., J. Salánki, K. S.-Rózsa (1964): Effect of serotonin and other bioactive agents on the rhythmic activity in the glochidia of fresh-water mussel (Anodonta cygnea L.). — *Comp. Biochem. Physiol.* **11**, 161—172.

Rushton, W. A. H. (1927): The effect upon the threshold for nervous excitations of the lenght of nerve exposed and the angle between current and nerve. — J. *Physiol.* **63**, 357—377.

Rushton, W. A. H. (1930): Excitable substances in the nerve-muscle complex. — *J. Physiol.* **70**, 317—337.

ELEKTROMOS INGERLÉKENYSÉG VIZSGÁLATA *ANODONTA*-LÁRVÁK (GLOCHIDIUMOK) ZÁRÓIZMÁN

Lábos Elemér

Összefoglalás

Tavi kagyló *(Anodonta cygnea* L.) glochidiumainak záróizmát folyadékon keresztül, elektromosan ingerelve azt találtuk, hogy az ingerlékenység és elektromos tér iránya között a Rushton-féle reciprok cosinus összefüggés közelítőleg fennáll. Az izomrostokkal párhuzamos ingerlés során talált küszöb, 5 msec ingeridőtartam mellett 3,5 V. A küszöbfeszültség 60—65°-ig kb. kétszeresére nő, a harántküszöb igen magas. A chronaxia 65°-ig közel állandó, 1 msec. A küszöbfeszültség-ingeridőtartam összefüggés másodlagos meredekségváltozásai és a Rushton-összefüggéstől való kisfokú eltérések alapján taglaltuk a motoros beidegzés jelenlétének kérdését.

ИССЛЕДОВАНИЕ ЭЛЕКТРИЧЕСКОЙ ВОЗБУДИМОСТИ НА ЗАПИРАТЕЛЬНОЙ МЫШЦЕ ЛИЧИНОК (ГЛОХИДИЕВ) БЕЗЗУБКИ

Э. Ладош

Раздражая электрическим током запирательную мышцу глохидиев беззубки через жидкость, было установлено, что обратная косинусная зависимость Раштона между возбудимость и направлением электрического пространства приблизительно остается в силе. Пороговым напряжением оказалось 3,5 V если раздражение происходит паралеллно мышечными волокнами и при продолжительности импульса в 5 мсек. Пороговое напряжение между 60—65° увеличивается примерно вдвое, и поперечный порог очень высокий. Хронаксия до 65° почти постоянна и равняется 1 мсек. На основе вторичного изменения кривизны в зависимости продолжительности импульса и порогового напряжения и принимая во внимании небольшое отклонение от закона Раштона обсуждается вопрос о наличии моторной иннервации эмбриональной запирательной мышцы.

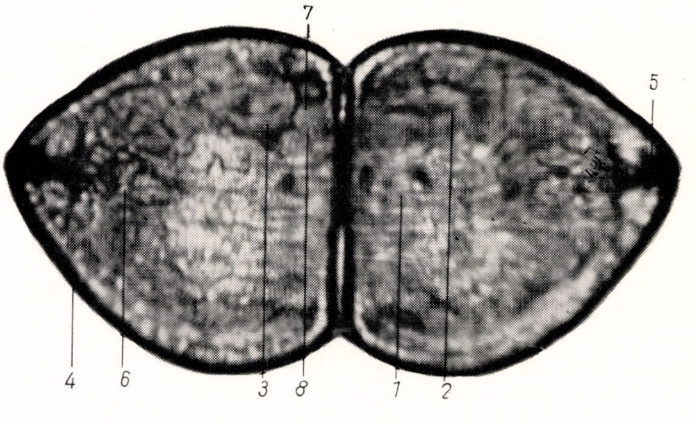

Fig. 1. Photo of the larva of *Anodonta cygnea* (glochidium)
1 = larval adductor; 2 = lateral cavity; 3 = mesoderm band; 4 = shell; 5 = tooth of shell; 6 = larval mantle; 7 = primordium of the foot; 8 = intestinal sac
According to K. Herbers (1913) the primitive ganglion colonies develop in the region of intestinal sac and the primordium of the foot

1. ábra. Anodonta lárva (glochidium) képe
1 = a lárvális adduktor izomrostjai; 2 = oldalsó gödör; 3 = mezoderma csík; 4 = héj; 5 = héjfog; 6 = lárvális köpeny; 7 = lábdudor; 8 = entodermazsák
A primitív ganglionteleplek K. Herbers (1913) szerint az entoderma zsák és lábdudor táján fejlődnek