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THE ROLE OF ENVIRONMENTAL TEMPERATURE AND pH ON THE VARIATIONS IN K$^+$- AND TRYPTAMINE- SENSITIVITY OF GLOCHIDIA OF *ANODONTA CYGNEA* L.

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Having investigated for several years the K$^+$- and tryptamine-sensitivity of adductor-response of glochidia (LÁBOS and SALÁNKI 1963, LÁBOS et al. 1964, LÁBOS 1966, 1967) we could state that the intensity of responses show considerable variations. These variations exist under apparently identical circumstances, thus necessitating a more thorough study of some laboratory conditions (temperature, properties of the solvent, as pH, ion composition etc.).

The degree of rhythmic-tonic adductor-response is of importance also from the point of view of the development of individuals. For instance the responding capacity of a glochidium and its changes—getting from the gills of the parent animal into the water—may be of decisive importance as regards the possibility to get into a parasite-state on the gills and fins of fishes (HARMS 1908, 1909, AREY 1921, HEARD and HENDRIX 1964, LUKACSOVICS and LÁBOS 1965). Furthermore it is open to question whether there exist any endogenous sensitivity variations connected with seasonal changes or with the ontogenesis, independent from the non-specific seasonal changes of the environment, e.g. Balaton-water.

Methods

We have investigated under different circumstances rhythmic and tonic muscular responses triggered by KCl and tryptamine in glochidia of *Anodonta*-species.

The animals were observed in groups consisting of 25—100 individuals in the presence of the given agent and the number of rhythmic contractions performed in a minute (a/min) respectively the ratio of the larvae in closed state (c%) was noted.

In the course of investigations lasting for several years (1960—1967) we used ordinary tap-water, Balaton-water as well as distillated water as solvent for the KCl and tryptamine. The use of Balaton-water is justified because it is the natural medium of the animal during the time when getting out of the gills and reaching the parasitic state. Nevertheless in this case we have to deal with a substance which is very variable from physico-chemical viewpoint thus being difficult to be characterized satisfactorily. The use of distillated water is reasonable owing to its rather stable character; nevertheless it is no "physiological" medium.

The agents used were: tryptamine HCl (Fluka), serotonine-creatinine sulphate (5-HT) and KCl of high purity.

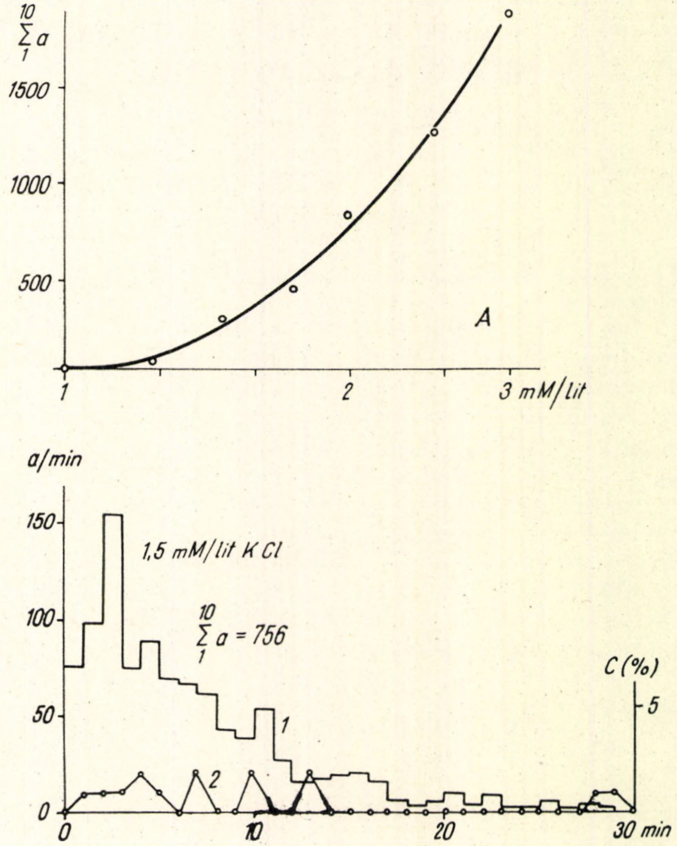

Fig. 1A. — Dosage-effect curve of a population unsensitive against K^+. Balaton-water, April, 24—27 °C. Abscissa: KCl - concentration in mM; Ordinate: Number of contractions of 100 glochidia in 10 min

B — Frequency- (1) and time course of tone ratio (2) of a less sensitive population. Distilled water - 100 animals, Mid-October, 25 °C

(With this and all further figures we give mean values of 100 animals, except for *Fig. 7*)

1. ábra. K^+-érzéketlen feltételek, illetve populációk

A — KCl-érzéketlen populáció dózishatás-görbéje

Balatonvíz, áprilisi populáció, 24—27 C°; Abscissa: KCl-koncentráció mM-ben; Ordinata: 10 perc alatt 100 glochidium által teljesített kontrakciók száma

B — Érzéketlenebb októberi populáció frekvencia (1) és tónusarány időgörbéje (2). Desztillált víz — 100 állat, október közepe, 25° C

(Ennek és a további ábráknak minden egyes pontja 100 db glochidiumon végzett mérés átlagértéke. Ez alól csak a *7. ábra* kivétel. Lásd ott.)

Results

1. *Variations of* the K$^+$*-sensitivity*

The maximum of KCl-sensitivity is represented by a concentration of about 100 μM, while its minimum is around 2.5—3 mM, anyway without an artificial complement (r.g. ion-addition; Lábos 1967). Thus if we use the various solutions randomly in different seasons, we may reckon 25—30 fold variations in the sensitivity.

In Balaton-water (see *Fig. 1/A* resp. Curve 2., *Fig. 2*) the concentration needed for a few hundred contractions and that for a tone of 50% in 10 min may vary between 1—3 mM. In distillated water *(Fig. 2* Curve 1 resp. *Fig. 1/B*) during the whole glochidium-season — except the beginning (October)—a nearly constant sensitivity could be observed. At the beginning of October a small degree of sensitivity can be met both in Balaton water and in distillated water *(Fig. 1/B)*. Solution and seasonal dependences are shown in *Table I*.

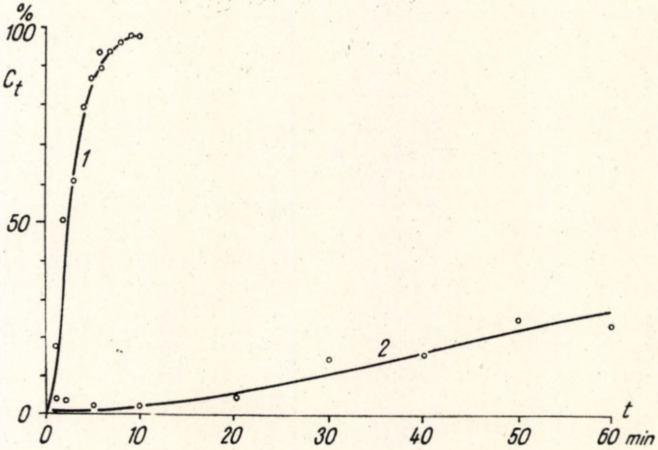

Fig. 2. KCl-response and tone-ratio — time diagrams
Abscissa: time, ordinate: ratio of individuals in tonic contraction at the given time for 100 animals (in %)
1. = 1 mM KCl, distillated water, December,
2. = 1 mM KCl, Balaton-water, April

2. ábra. KCl-válasz és tónusarány-idő diagramok
Abscissa: idő; ordinata: 100 db állat közül az adott időpontban tónusos kontrakcióban levő egyedek aránya (%)
1. = 1 mM KCl; oldószer: desztillált víz, hónap: december,
2. = 1 mM KCl; oldószer: Balatonvíz, hónap: április

2. *Dependence of* K$^+$*-response on the temperature of environment*

In the course of the experiments we collected the glochidia — as a rule — from source-animal living in Balaton-water which was generally cooler than the room temperature. Observations were made in a medium of room tempera-

Table I
Dependence of the sensitivity of glochidia on the solution and the season

Month	Balaton-water	Distillated water	Suppositions	
			Balaton water	Distillated water
Mid-October	Low sensitivity	Low sensitivity*	osmotic immaturity	
November-March	Moderate sensitivity	High sensitivity**	Balaton water undergoes changes	No antagonistic ions are present
March-May	Decreased sensitivity	High sensitivity		

* = Fig. 1/3
** = Fig. 2/1

ture. Some times the populations were kept a day or more — with a small density of animals — at room temperature or at 4—10 °C in refrigerator. Thus it is obvious that temperature changes might play a part among the causes of sensitivity variations.

To clear this problem we divided the glochidia coming from the same mother-animal in two groups. One group was kept at 20—26 °C for some days, while the other remained in Balaton water at 6—10 °C. On both groups we observed daily the effect of a KCl concentration of 1—1.5 and 2 mM. The above temperatures were kept unchanged during the observations.

In the warmer medium K^+-response was observed for 4 days, while in the cooler — one for 11 days, till the animals died.

We observed the rate of the tone in the case of animals kept in warmth to increase from day to day when using the same concentration of KCl. That was characteristic for all three concentrations *(Fig. 3*, upper row of graphs, curves marked 1). The response of animals kept in cold appeared for 1—4—5 days practically without tone so that the responses of the two groups kept at different temperatures deviated more and more. But later—beginning from the 4th—5th days — we found a gradually increasing tone even in the group adapted to low temperature *(Fig. 3*, upper row, curves marked 2).

The frequency of rhythmic activity shows also characteristic variations in the two groups. At all three concentrations in the warm-group the frequency was increasing on the 2nd day, then it decreased *(Fig. 3*, lower curves). The response of the group with cold adaptation, on the other hand, became less frequent from day to day and the variations were larger. When applying the 2 mM KCl concentration we got a frequency-maximum parallel to the tone-minimum *(Fig. 3*, lower row, last graph, curve 2). Parameters characterizing the rhythm and tone ($\sum^{10}$ = number of contractions performed by 100 animals in 10 min and, c(%) is the percentual ratio of animals found in tonic state at the 10th min) are not independent. *Fig. 3* shows that the cases of higher frequency in the cold-adapted group are connected in general with a lower level of tone. On the other hand, we can observe a deviation in the correlation of the frequency-tone ratio of the two groups, so that at low temperature a higher frequency is to be expected together with the same lower tone-ratio.

The effects of sudden and significant temperature changes are shown in the experiments where groups consisting of 5 × 100 larvae were used:

1. group: the mother animal was collected from Balaton-water and the glochidia were put in distilled water of 22 °C (control),
2. group: stored for 1 hour at 6 °C,
3. group: stored for 1 hour at 37 °C,
4. group: stored for 1 hour at 37 °C, then immediately after it for 1 hour at 6 °C,
5. group: stored for 1 hour at 6 °C, then immediately after it for 1 hour at 37 °C.

The tone ratios observed at 1.5 mM KCl concentration after 120 min in the five groups were *(Fig. 5)*: 94 — 76 — 92 — 34 — 34%.

We can state that the sudden temperature change of significant value decreases the duration of tone, i.e. increases the degree of the so-called late-relaxation. The latter follows the tone and not a phasic contraction (LÁBOS 1967).

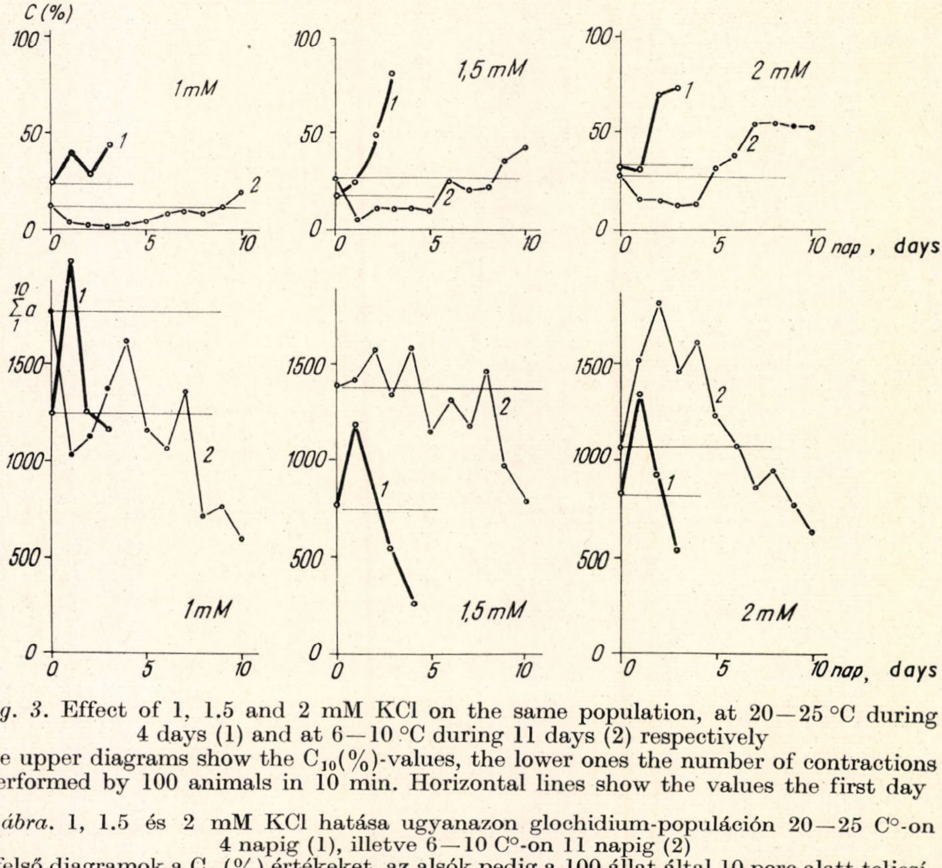

Fig. 3. Effect of 1, 1.5 and 2 mM KCl on the same population, at 20—25 °C during 4 days (1) and at 6—10 °C during 11 days (2) respectively

The upper diagrams show the C_{10}(%)-values, the lower ones the number of contractions performed by 100 animals in 10 min. Horizontal lines show the values the first day

3. ábra. 1, 1.5 és 2 mM KCl hatása ugyanazon glochidium-populáción 20—25 C°-on 4 napig (1), illetve 6—10 C°-on 11 napig (2)

A felső diagramok a C_{10}(%) értékeket, az alsók pedig a 100 állat által 10 perc alatt teljesített kontrakciók számát mutatják. A vízszintes vonalak az első napi értékeket jelölik

3. K^+*-response and pH of the medium*

The pH-dependence of the K^+-response was investigated by observing the tone ratio at the 5th, 10th and 20th min with pH-values between 5—9. The investigation was carried out in November, with distilled water as solution. Temperature of the medium was 22—25 °C and only freshly collected animals were used. On *Fig. 6* we can see the pH-dependence of responses against a concentration of 2 mM KCl (curves A-2 and B-1-2-3). We can state in general that the tonicity increases towards the direction of alkalinity. So the tone ratio values of 5 min follow the regression: $c_5(\%) \sim 7.1\,(\mathrm{pH}) - 27$ with a correlation coefficient of $r > 0.9$. It is characteristic that the values of 10—20 min show a decrease in the case of pH < 7 (acidic solutions) up to the pH-value of about 5.5, while they increase again with the decrease of pH about pH ~ 5.

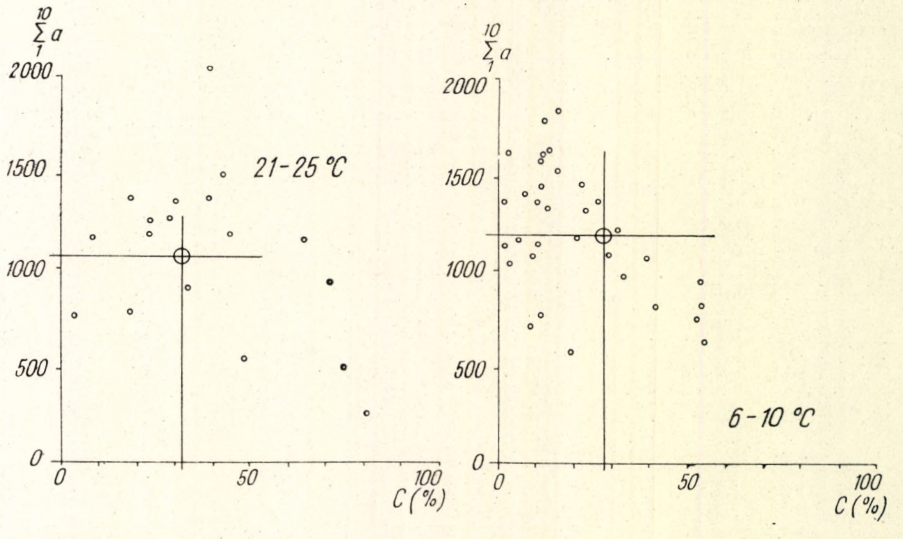

Fig. 4. Connection between corresponding values of tone-ratio (C%) and number of contractions performed by 100 animals in 10 min at two different temperature levels

4. ábra. Az összetartozó tónusarány (C%) és 100 állat által 10 perc alatt teljesített-kontrakciószám értékek összefüggése két különböző hőmérsékleten

Table II
Dependence of sensitivity against tryptamine and solvent in glochidia

Season	Balaton-water	Distilled water	Tap-water
Beginning of October	low activity		
Winter	High activity	low activity or lack of activity	High activity
Spring	Decreased activity		Decreased activity

In one of our experiments with a KCl concentration of 1 mM at the 10th min (*Fig. 6/A*, curve marked 1) we could observe a tone decrease when progressing towards the alkalinity instead of the decrease as mentioned above.

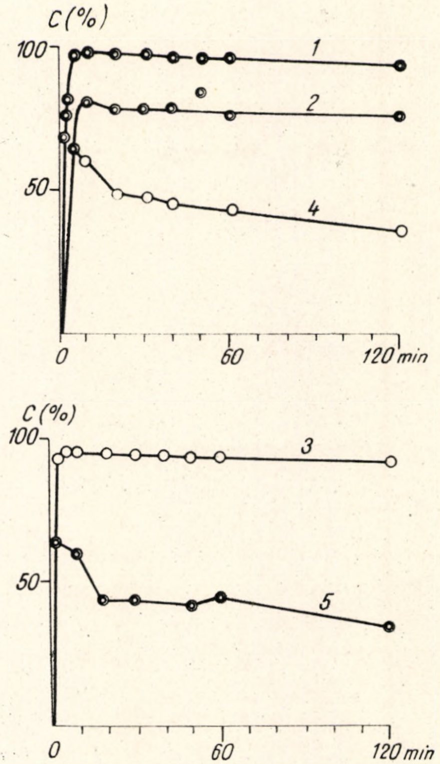

Fig. 5. Effect of a temperature jump on the K^+-tone
Tone-ratio-time diagrams (explanation in the text)

5. ábra. A hőmérsékletugrás hatása a K-tónusra
Tónusarány—idő diagramok (Magyarázat a szövegben)

4. *Examination on tryptamine sensitivity*

Very early populations (October) show, as a rule, hardly any sensitivity (see *Table II*). In winter we find a high sensitivity in Balaton-water and a low one in distilled water. In spring sensitivity decreases in general (LÁBOS et al. 1964, LÁBOS 1965). Tryptamine solved in distilled water has little or no effect at all, but when alkalinizing the medium we obtained a considerable tryptamine response (see under 5).

5. *Relation between tryptamine-response and pH of the medium*

Fig. 7 shows the dependence of the number of rhythmic contractions on the pH. We can see that the effect is considerable mainly in the alka-

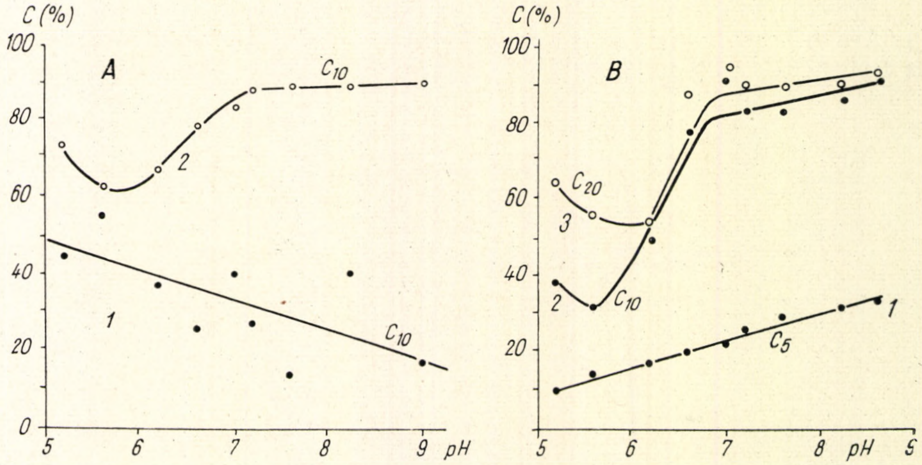

Fig. 6. Dependence of KCl-response on pH
The values of C_5, C_{10} and C_{20} represent the tone-ratio-values in % found at the 5th, 10th and 20th min; November; distilled water; tris-maleate buffer of 3 mM concentration; temperature 22—25 °C

6. ábra. A KCl-válasz pH-függése
A C_5, C_{10} és C_{20} értékek az 5., 10., illetve a 20. percben talált tónusarány-értékeket jelentik %-ban. Novemberi populációk; oldószer: desztillált víz; 3 mM trisz-maleát puffer; hőmérséklet: 22—25° C

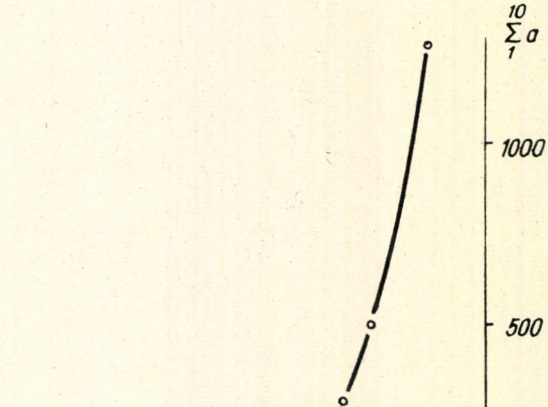

Fig. 7. Dependence of tryptamine effect on pH; distilled water; 10 μg/ml tryptamine, winter; temperature: 22—25 °C, number of glochidia: 25

7. ábra. A triptaminhatás pH-függése
Oldószer: desztillált víz; 100 μg/ml triptamin; téli populáció; 25° C hőmérséklet; 25 glochidium

linic domain $8 < \mathrm{pH} < 9$ and at a neutral pH we get practically no response at all. The investigations were carried out in a tris-maleate buffer of 3 mM. Because of the presence of a given amount of organic material, even

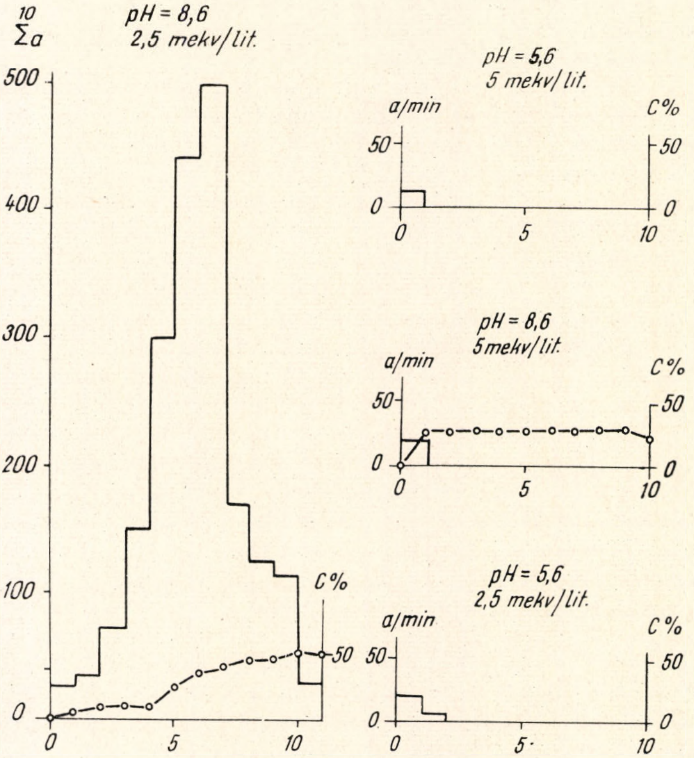

Fig. 8. Rhythmic and tonic muscular response produced by 100 μg/ml tryptamine, in a 2.5 and 5 mekv./lit. tris-maleate buffer. Distilled water, spring, room temperature

8. *ábra.* 100 μg/ml triptaminnal kiváltott ritmikus és tónusos izomválasz, 2, illetve 5 mekv/lit. triszmaleát pufferben. Oldószer: desztillált víz, tavaszi populáció, szobahőmérsékleten

with a use of buffers at low concentration, it is rather difficult to carry out an isolated study of H^+-ion effect. In the following we used tris-maleate buffers of 5 mM and 2,5 concentration at pH 8.6 and 5.6. It is obvious from the figure *(Fig. 8)* that an alkalinic pH value is only a necessary condition of the high sensitivity, since a too high buffer-concentration may cover this effect, i.e. the effect necessitates the absence of different inhibitory factors. Nevertheless this can not be realized by the application of buffers alone. As in earlier experiments the response of the glochidia against 5-HT (LÁBOS et al. 1964, LÁBOS 1966) could not be observed either in this case even by alkalinizing the medium.

Discussion

The explanation of high K^+-sensitivity perhaps requires the supposition of an unusual mechanism. Without dealing the problem in detail we note that above all the potentials of electrokinetic nature of colloidal systems (see e.g. Buzágh 1958) are sensitive against an ion-concentration of 10^{-4} M. The Nernst-mechanism furnishes a satisfactory explanation only in the case when the critical threshold of K^+-depolarisation is very low.

The intensive variations of the latter can be caused by the presence or absence of K^+-antagonistic ions. Observation of *Table 1* may lead to the hypothesis that the higher sensitivity observed in distilled water could originate in the lack of K^+ antagonistic ions (Na^+, Mg^{2+}, Ca^{2+}) of the other solution i.e. of the Balaton-water. Data on ion-antagonism (Lábos 1967) and on the composition of Lake Balaton-water (Entz, 1953, 1959) make this explanation obvious. But the explanation does not hold for the low sensitivity in October *(Table I., Fig. 1/B)*, found with both solutions. The explanation may be brought in connection with the immaturity of early glochidia. It seems probable, namely, that the larvae more adapted to the maternal lymph and ready for the life in fresh water must pass through certain ripening stages to be able to get adapted to osmotic variations. A further question is the change of sensitivity presenting itself in winter and spring and observable only in Balaton-water. Therefore these variations may origine in the alteration of chemism of the Balaton-water, e.g. the increase of activity and of Ca^{2+} ionization (Entz 1953, 1959).

Thus variations in K^+ sensitivity can not be explained by one reason alone. Among the reasons changes in the animal and those of the ,,natural medium'' are equally probable. Only the early change might be of ontogenetic origin.

For the interpretation for variations of nonseasonal character the thermic past of the larvae seems to be sufficient. On the one hand: the intensity of metabolic activity of oyster-mantle decreases with the change of temperature of any direction (Pedersen 1947), but on the other hand: the cold-adaptation may induce a K^+-lost in other species, e.g. in *Loligo*-nerve (Shanes 1954), or in erythrocytes (Solomon 1952). In the case of glochidia the steadily hypoosmotic environment (fresh-water or Balaton water) may easily lead to ion-loss serving as explanation for the phenomena under consideration.

The variations of tryptamine sensitivity are of a different character. One of them is that the sensitivity decrease of spring could be observed both in tap-water (Lábos et al. 1964) and in Balaton water. Thus this variation can not be explained by the changes of the environment. However it should be noted that the pH of the Balaton-water is 7.9–8.8, therefore a high tryptamine response could sooner be expected here than in neutral or acidic distillated water. Thus the pH-variation would be sufficient to explain the inhibiting effect of distillated water, since we have no activity with a neutral pH, but with a corresponding buffer we may still produce a high rhythm even at spring and in distillated water. The alkalinic pH, of course, may bring about an effective acceleration of both permeation and alkalinic hydrolysis.

We have to point out the fact that the change of solvent: Balaton-water $\rightleftarrows$ distillated water cause opposite changes in K^+- and tryptamine-responses argumenting for a different mechanism.

The dependence of K^+-effect on the pH shows a surprisingly good linearity when measuring at the 5th min which itself could be explained in a satisfying manner by simple kinetic conceptions. Nevertheless, the pH-dependence is connected with K^+-concentration, furthermore the toneincrease observable in an extremely acidic environment (see *Fig. 6* at pH ~ 5) points also to an activation of a further reactive system.

Summary

An experimental analysis on K^+- and tryptamine-sensitivities of rhythmic and tonic adductor-response in *Anodonta*-larvae was made and their dependence from temperature, osmotic and pH-changes was investigated.

The low value of K^+-sensitivity in October is supposed to be in connection with the osmotic immaturity of the larvae; the higher sensitivity found in distillated water was attributed to the absence of antagonistic ions while the sensitivity-decrease in spring to the changes of Balaton-water.

The possibility of a change of ontogenetic character can not be excluded in the decrease of tryptamine response in spring. In distillated water no tryptamine response was found while there is a high K^- response.

For the pH-depence of the responses it is characteristic that

1. the K^+ — tone increases in general with pH between 6 and 9; a pH < 6 again — decreases the tone,

2. the tryptamine-rhythm — in case of a corresponding buffer — is pronounced with an alkalinic value of pH, but it is very low with a neutral pH.

In the case of K^+-response the importance of temperature adaptation was demonstrated. The cold-adapted animals give a less tonic response with a rhythm of higher frequency. The animals are capable to response for **11** days at temperatures of 6—10 °C, and for 4 days at temperatures of 20—25 °C. The jumps in temperature are of a sensitivity-decreasing effect.

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A KÖZEG HŐMÉRSÉKLETÉNEK ÉS pH-JÁNAK SZEREPE AZ *ANODONTA CYGNEA L.* GLOCHIDIUMOK K+- ÉS TRIPTAMIN-ÉRZÉKENYSÉGÉNEK ELTÉRÉSEIBEN

Lábos Elemér és *Lukacsovics Ferenc*

Összefoglalás

Anodonta-lárvák ritmikus és tónusos záróizomválaszának K$^+$- és triptaminérzékenységét és ezeknek külsőleg szezonális és egyéb jellegű változásait elemeztük kísérletesen.

Az alacsony októberi K-érzékenységet a lárvák ozmotikus éretlenségével, a desztillált vízben tapasztalt magas érzékenységet az antagonista-ionok hiányával, a tavaszi érzékenységcsökkenést a Balatonvíz változásával hozzuk összefüggésbe.

A ritmikus triptamin-válasz nagyságának tavaszi csökkenésében az ontogenetikus jellegű változás nem zárható ki. Desztillált vízben nincs triptaminválasz, de nagy a K$^+$-érzékenység.

A válaszok pH-függésére az alábbiak jellemzőek:

1. a K$^+$-tónus általában nő a pH-val 6—9 között; pH 6 újra tónusnövelő;
2. a triptaminritmus — megfelelő pufferválasztás mellett — lúgos pH-n kifejezett. Neutrális pH-n igen alacsony.

A K-válasz esetében a hőadaptáció fokozott jelentőségét mutattuk ki. A hidegadaptált állatok kevésbé tónusos és nagyfrekvenciájú ritmussal válaszolnak. 6—10 C°-on 11, 20—25 C°-on 4 napig válasz (élet)képesek. A hőmérsékletugrások érzékenység-csökkentőek.

РОЛЬ ТЕМПЕРАТУРЫ И pH СРЕДЫ В РАЗЛИЧНОЙ ЧУВСТВИТЕЛЬНОСТИ ГЛОХИДИЕВ БЕЗЗУБКИ К ТРИПТАМИНУ И ИОНАМ КАЛИЯ

Э. Лабош и *Ф. Лукачевич*

Были проанализированы причины наблюдающихся сезонных и иных изменений чувствительности ритмических и тонических реакций запирательной мышци глохидиев беззубки к калию и триптамину.

Результаты исследования показывают, что низкая чувствительность к ионам калия в октябре связана с осмотическим недоразвитием личинок, а высокая чувствительность к дестиллированной воде с отсутствием ионов антагонистического действия. Весенние изменения чувствительности ставятся в связь с изменением состава воды Балатона, но в весеннем снижении чувствительности к триптамину могут играть роль и онтогенетические изменения самих личинок. Ответ на триптамин не наблюдается в дестиллированной воде.

Зависимость ответа личинок от pH такова:

1. Калиевый тонус глохидиев увеличивается между pH 6—9.
2. Триптаминовый ритм более выражен при щелочных значениях pH, в нейтральной же среде он очень низок.

Для реакции на калий имеет значение тепловая адаптация: личинки, адаптированные к холоду, реагируют на калий менее тонично и ритмом высокой амплитуды. Они переносят температуры 6—10° в течение 11 дней, а 20—25° в течение 4 дней. Резкие изменения температутры снижают чувствительность личинок.