

CILIARY ACTIVITY EXAMINATIONS AFTER ANOXYBIOSIS ON THE ISOLATED GILLS OF *ANODONTA CYGNEA* L.

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Beside the recent comprehensive studies which appeared after the monograph of GRAY "Ciliary movement" (1928) (RIVERA 1962, SLEIGH 1962) a number of papers dealt with the ciliary activity of Molluscs. Of these the investigation of the gill cilia of Lamellibranchiata emerges quantitatively. The epithelium of comparatively large surface and intensive ciliary activity of the clams which can be conceived also as bioindicator marks with the change of velocity of movement the changes of physiological and biochemical processes taking place in tissue and cells spontaneously or induced by agents. Thus e. g. the effect of the agents influencing the intermediary metabolism (USUKI 1956 a, b, c, d) and the heat adaptation conditions (VERNBERG, SCHLIEPER and SCHNEIDER 1963, PRECHT and CHRISTOPHERSEN 1965, LAGERSPETZ and DUBITSCHER 1966) were recorded with the motion activity of gill cilia. Valuable knowledge was published also on the mode of action of osmoregulation, anoxymbiosis and other environmental factors (GRAY 1928, SCHLIEPER, KOWALSKI and ERMAN 1958, SCHLIEPER and KOWALSKI 1958).

It is a well known fact that after anoxybiotic effects (= AOB SCHLIEPER et al. 1958) in the tissues an O₂ debt of such dimensions arises which under oxybiotic conditions is compensated by the organism with an oxygen consumption surpassing the normal level. In the course of this process also the activity of the gill cilia is intensified (SCHLIEPER and KOWALSKI 1958). Since in our AOB examinations conducted during the winter semester, in the cold water period (LUKACSOVICS 1966), the onset of the O₂ restitution of isolated gills exhibited a delay, and started 6—24 hours after the AOB, we examined still in this season the behaviour of the ciliary movement of gills to establish whether they also show a similar phenomenon in their intensity.

Material and method

For these experiments specimens of 14 cm shell length of *Anodonta cygnea* were used. During the winter the animals were stored in Lake Balaton, under natural conditions. The temperature of the lake in the experimental period (8th February — 21st March 1966) was +4—+7° C. The temperature of the water of the animals brought into the aquarium was gradually raised —

— in 5 days — to 25° C and at the same time ciliary activity was measured per 5° C. At the time of heat adaptation the water of the mussels was abundantly aerated.

Oxygen-free water was produced in a vacuum cabinet when the initial 8—8.5 mg/l oxygen content was reduced to a value of 1—1.5 mg/l and then shortly after placing in the mussels to an amount which could not be demonstrated. The oxygen content determinations were conducted with the semimicro method of MAUCHA (1947). The evacuated water was closed up with neutral paraffin oil. Accumulation of metabolic products was diminished with the daily exchange of freshly evacuated water. 1 liter water was calculated for 1 mussel.

The mussels were kept for 1—3 days under AOB conditions, then taken out and on the 0., 1st and 2nd day the velocity of the gill-cilia was measured, but on the 1st and 2nd day after AOB the animals were kept under OB conditions, at 25° C temperature.

Ciliary activity was measured according to SCHLIEPER and KOWALSKI (1958) on the transport performance of the gill-cilia. The excised gills were laid in PETRI dishes cast with paraffin, fixed with pins, then Balaton water rich in O₂ was stratified over them and a celluloid ruler standing on 40 mm long legs laid across so that it did not touch the gill. Subsequently small 1 sq.mm aluminium foil pieces were dropped on the lateral surface of the gill near the line of intersection and the time that passed during the 15 mm distance was covered has been measured with a stopper. The transport performance was calculated over into min/mm value. Each average result was obtained from 12—30 individual measurements.

Since in the experimental period the external gill of several mussels may be full of developing glochidium, the measurements were always carried out on the interior side of the interior gills.

Results

The experimental temperature of 25° C was obtained from the temperature of storage (+4 — +7° C) with a daily rise of 5° C. During heat adaptation ciliary activity of the isolated gills was measured at the intermediary temperatures (*Fig. 1*). At 10, 15 and 20° C with rising temperature the transport performance of the cilia increased. When taking the value performed at 10° C as a basis, then at 15° C the measured activity was 21 per cent and at 20° C 74 per cent higher. At 25° C we found a 35 per cent inhibition as compared with the previous temperature grade that is the value of heat stimulation was no more than 39 per cent. Upon AOB effects lasting for various periods (2—3 days) on the gill of the fresh water mussel after isolation the following transport performances were measured (*Fig. 2*). As compared with the control values after one day of AOB the intensity of the movement of the gill cilia increased by 25 per cent, after 2 days by 39 and after 3 days of AOB by 88 per cent. When after AOB the animals were placed for one day in aerated water, the measure of hyperventilation did not change in the case of animals kept in AOB for one or two days, that is they showed the motion velocity that could be measured immediately after AOB. Only the transport performance of the gill-cilia of animals that passed 3 days in AOB decreased after 24 hours aeration

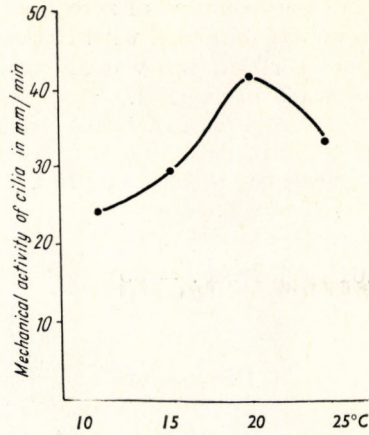


Fig. 1. Ciliary activity of the isolated gills of *Anodonta cygnea* L. specimens kept at different temperatures

1. ábra Különböző hőmérsékleten tartott *Anodonta cygnea* L. példányok izolált kopolyúinak a csillóaktivitása.

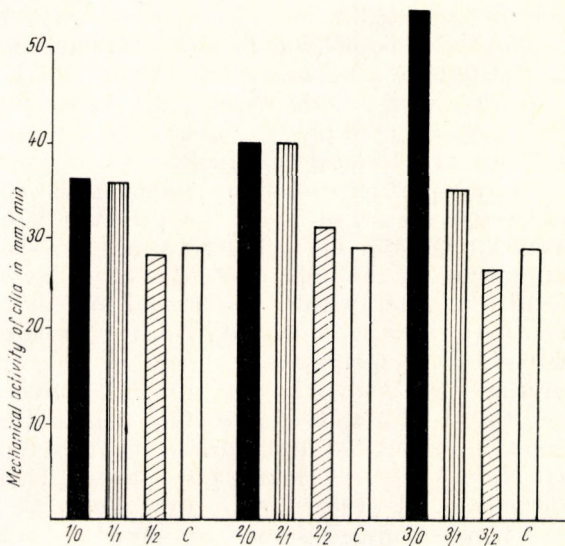


Fig. 2. Mechanical activity of the gill cilia of *Anodonta cygnea* L. specimens kept for various periods in oxygen deficient and subsequently in water with high oxygen content

Signs: Figure above the fraction-line: time spent in an oxygen-deficient medium, in days

Figure below the fraction-line: time spent in water abundant in oxygen, in days.

C = control

2. ábra Különböző ideig oxigén-szegény, majd oxigén-dús vízben tartott *Anodonta cygnea* L. példányok kopolyúcsillóinak a mechanikus aktivitása.

Jelzések: Törtjel fölötti szám: oxigén-szegény közegben eltöltött idő napokban.

Törtjel alatti szám: oxigén-dús vízben eltöltött idő napokban.

C = kontrol

was substantially below the performance of zero day, but it still exhibited a surplus value of 22 per cent as compared with the control. After 48 hours of OB conditions the transport performance was already practically in all three groups equal with the value of the control.

When the transport velocity values measured immediately after AOB were compared not with their control but with each other, the statistical calculations showed the differences to be real (*Fig. 2*):

1/0—2/0	P <	0.01	D. F. = 35	t = 4.53
2/0—3/0	P <	0.01	D. F. = 35	t = 8.94

Discussion

The motion activity of the gill-cilia of Lamellibranchiata that is the flow of the water necessary for respiration and with it the filtration activity is influenced by many factors. In a natural environment the most general active agent according to GRAY (1928, cit. ap. BUDDENBROCK 1939) is temperature, but they are also effectively influenced by the pH conditions and by the amount of dissolved gases, cations and anions.

With the rise of temperature according to van't HOFF's law the O₂ consumption of the isolated gills of *Unio tumidus* ZELEBOR increased (LUKACSOVICS and SALÁNKI 1964) and as measured on the gill of *Mytilus edulis* upon heat effect parallel with the increasing O₂ consumption curve rose also the ciliary activity (GRAY 1928 pp 88, *Fig 68*).

From the viewpoint of ecological physiology the fact is not contradictory that on account of its adaptation to the environment the ciliary motorics of the gills of the fresh water mussel of higher temperature tolerance rose exponentially only until 20° C (*Fig. 1*) and at 25° C already showed decreasing activity. At the same time on the gills of *Mytilus edulis* living, as it is well known, in brack and sea water with lower annual and daily temperature-fluctuation, exponentially rising ciliary activity can be measured up to 35° C. These can be explained by two factors:

1. The temperature effect above the optimum has an inverse time dependence that is during the short action of pessimum temperature (10 minutes) the ciliary movement is stimulated, but during a longer high temperature (e. g. 1 hour) "heat shock" that is blocking effect arises. These results agree with those of SCHLIEPER et. al. (1958) obtained on *Modiolus*.

2. The value of temperature resistance, optimum and tolerance changes seasonally as changes the vital activity, chemical composition etc. (GALTSOFF 1964, KRÜGER 1960, OKAZAKI and KOBAYASHI 1929, MITCHELL 1915, RUSSEL 1923 and HENSCHER 1952). We conducted the experiments in the winter, so-called "Cold water" period when the Lamellibranchs, as the poikilothermic organisms generally, exercise a vital activity of reduced metabolic level.

Although we carried out heat adaptation before the experiments, the "plafond value" of the heat resistance of *Anodonta* is lower in the winter season, that is its temperature tolerance is lesser. Under the conditions of our experiments the "plafond" referred to evaluated on ciliary activity may be at a temperature of about 20° C.

As mentioned in the Introduction, the examination of ciliary activity after AOB was induced by the experimental result gained earlier on *Anodonta* gills that on the isolated gill of mussels kept under AOB conditions there was no restitution demonstrable within a few hours. The consumption of the gill of mussels kept hypoxially for one day surpassing the oxygen consumption value of the control could not be measured even after 4 days of aeration. In the case of 2 days of hypoxia restitution manifested itself only after 24 hours of aeration and after 3 days of hypoxia the restitution could be measured only in 48 hours (LUKACSOVICS 1966). In contrast to the above "time shift" on AOB effect the gill cilia immediately responded to the cessation of AOB with intensive mechanical activity. On the single days of 3 consecutive hypoxial days the increase of the ciliary movement was 25, 39 and 88 per cent. The order of increased activity agrees with that published by SCHLIEPER and KOWALSKI (1958) on *Mytilus edulis* of the North Sea, but the percentual growth values are much higher in *Anodonta*. Hyperventilation subsisted for about 24 hours on specimens which suffered AOB for 1–2 days. In the specimens kept under AOB conditions for 3 days, after 24 hours the transport performance diminished as compared with the 0 hour performance, but even so a substantial hyperventilation (22 per cent) could be measured as compared with the control.

The contradiction of this kind between respiration and ciliary activity which arose upon AOB effect allows several assumptions of which the following seems to be most obvious:

The members of the class Lamellibranchiata, as it is well known, have a higher capacity of adaptation as compared with several classes of the phylum Mollusca (e. g. Gastropoda, Cephalopoda) since in their natural biotope the change of the oxygen content can be substantial even daily, either as regards the conditions of ebb-tide + temperature (sea water) or of the dissimilating or O₂-consuming organisms + temperature (fresh-water). In the course of the oxygen impoverishment of the natural water the mussel as a consequence of the given conditions of its movement is unable to escape and therefore closing its shells it carries out only movements requiring less energy (BRAND 1946, SALÁNKI 1964) shifting to anaerobic energy production (anaerobic glycolysis). The water enriching itself with oxygen by assimilative activity of the phytoplankton or by the phenomenon of high tide exciting the receptors of siphonic or pallial edge (HERBERS 1914, LUCAS 1931, ORLOV 1930) opens the carapaces of the animal and the epithelial cilia of the gill begin to ventilate. It is possible that the energy necessary for ciliary activity originates still from the energy of the earlier anaerobic glycolysis. The oxydative enzyme activity of the tissues of the gill and the tissues of the body start only in the second stage. This process is ensured by the ciliary activity set on when the O₂ diffusion of the flowing water rich in oxygen reaches a considerable value. This may be the explanation of the "time contrast" between the respiratory and ciliary activity after AOB. The above considerations should be of course verified by further ecological-physiological and biochemical analyses.

Summary

Anodonta cygnea specimens were kept for 1–3 days under anoxybiotic (AOB) conditions and then after AOB immediately or in 1–2 days, during which the mussels were kept in water rich in oxygen, the mechanical activity

(transport performance) of the lateral cilia on the isolated gills was measured, in February-March 1966 with the metal plate method of SCHLIEPER et al. (1958). While raising the temperature from that of the natural environment (+4 — +7° C) gradually to 25° C the activity of the gill cilia was measured also at every 5° C. The following results were obtained:

1. At temperatures of 10, 15 and 20° C the ciliary activity increased together with the temperature, but at 25° C already a decreasing activity could be measured (*Fig. 1*) the cause of which may be "heat shock" which occurs also in other, marine Lamellibranchiata species.

2. On the zero day after each 1—3 day of AOB effect a substantial increase of transport velocity appeared as compared with the control, in direct proportion with the period spent in AOB.

3. Upon a one day aeration effect after AOB the mechanical activity of the cilia generally did not decrease, only in the mussels kept for 3 days under AOB conditions, but also here the performance was still significantly higher than in the control (*Fig. 2*).

4. Upon the effect of 2 days aeration after AOB the transport performance agreed with the value of the control.

5. It is assumed that the contradiction between the O₂ hunger of the gill tissue appearing with a time shift after AOB and the immediately appearing ciliary transport arises from the more rapid response disposition of the cilia in contrast to the slower start of the mechanism of the oxidatory enzyme activity.

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CSILLÓAKTIVITÁS VIZSGÁLATOK ANOXYBIOZIS UTÁN AZ *ANODONTA CYGNEA* L. IZOLÁLT KOPOLTYÚJÁN

Összefoglalás

Lukacsovics Ferenc

Szerző *Anodonta cygnea* példányokat tartott 1–3 napon keresztül anoxybiotikus (AOB) körülmények között, majd AOB után közvetlen, vagy 1–2 napon keresztül oxigéndús vízben tartott kagylók izolált kopoltyúin mérte a lateralis csillók mechanikus aktivitását (transzportteljesítményét), 1966. febr.–márc. hónapokban, SCHLIEPER et. al. (1948) fémlapos módszerével. A természetes környezet hőmérsékletéről (+4–+7 °C) fokozatosan 25 °C-ra való hőmérséklet emelés közben 5 °C-onkint is megmérte a kopoltyú-csillók aktivitását. A következő eredményeket nyerte:

1. 10, 15 és 20 °C-hőmérsékletnél a csillóaktivitás a hőmérséklettel együtt nőtt, 25 °C-nál azonban már csökkenő aktivitás volt mérhető (1. ábra), amelynek oka — más, tengeri Lamellibranchiata fajoknál is fellelhető — „hősokk” lehet.

2. Minden 1–3 nap AOB hatást követő nulladik napon a kontrollal szembeni jelentős transzportsebesség növekedés jelentkezett éspedig az AOB-ban eltöltött idő tartamával egyenes arányban.

3. AOB utáni egynapos aerációs hatásra általában nem csökkent a csillók mechanikus aktivitása, csupán a 3 napot AOB viszonyok között tartott kagylóknál, azonban itt is még szignifikánsan magasabb volt a teljesítmény mint a kontrollnál. (2. ábra)

4. AOB utáni 2 napos aeráció hatására a transzportteljesítmény a kontroll értékével egyezett.

5. Szerző feltételezi, hogy a kopoltyúszövet AOB utáni időeltolódással jelentkező O₂-éhsége és az azonnal jelentkező csillótranszport közötti ellentét oka a csillók gyorsabb reakcióképességéből adódik, szemben az oxidációs enzimtevékenység lassabban beinduló mechanizmusával.

ИЗУЧЕНИЕ АКТИВНОСТИ РЕСНИЧЕК ИЗОЛИРОВАННЫХ ЖАБЕР
ANODONTA CYGNEA L. ПОСЛЕ АНОКСИБИОЗА

Ференц Лукачович

Взрослых беззубок выдерживали 1—3 дня в условиях отсутствия кислорода. Вслед за этим либо сразу после аноксбиоза, либо после выдерживания беззубок в течении 1—2 дней в воде, насыщенной кислородом, определяли механическую активность латеральных ресничек изолированных жабер. Опыты проводились по методу Шлипера и др. (1958) в феврале — марте 1966. Температуру естественной среды (+4—+7° С) постепенно повышали до 25°С, активность ресничек измеряли через каждые 5°С. Получены следующие результаты.

1. При повышении температуры до 15 и 20°С активность ресничек усиливалась, а при 25°С снижалась. Это, возможно, объясняется тепловым шоком, как это известно для морских двустворчатых.

2. Активность ресничек у беззубок, выдержанных 1—3 дня в условиях отсутствия кислорода, повышена, причем повышение пропорционально времени пребывания в аноксбиотических условиях.

3. Реснички беззубок, выдержанных в течение суток после аноксбиоза в воде, насыщенной кислородом, также проявляли повышенную активность в сравнении с контролем.

4. Реснички беззубок, выдержанных в течение двух суток после аноксбиоза в воде, насыщенной кислородом, совпадает с контрольной.

5. Высказывается предположение, что повышение активности ресничек после кислородного голодания, наступающего вследствие аноксбиоза, объясняется их более высокой реактивностью в сравнении с системой окислительных ферментов.