| ANNAL. BIOL. TIHANY | 32 | 83—92 | HUNGARIA 1965 |
|---|---|---|---|

# EFFECT OF AGENTS INFLUENCING THE PERIODIC ACTIVITY OF MUSSELS ON THE *IN VITRO* RESPIRATION OF SIPHON TISSUE OF *ANODONTA CYGNEA* L.

JÁNOS SALÁNKI and FERENC LUKACSOVICS

Received: March 15th 1965

The periodic motor activity of mussels (MARCEAU 1906 and 1909) *i.e.* the regularly occurring rhythmic openings and closures of adductors may be influenced by giving different concentrations of $K^+$ and $Cd^{++}$ ions into the medium (KOSHTOYANTS and SALÁNKI 1958, SALÁNKI 1960). The area of the siphons is especially sensitive to K ions (HOPKINS 1932).

Results of recent investigations show (SALÁNKI 1961) that the periodic activity changes even if KCl is applied to the syphons only of the intact animal.

All these facts suggest that the region of the syphon (together with the pallial margin and the gills may probably play an important role in the formation of nervous activity that coordinates the physiological processes of the animal. This region is able by virtue of its special function respiratory and filtrational mechanism to "scan" with its many receptors (HERBERS 1914, LUCAS 1931, ORLOV 1930) the water flowing in and to obtain information about its physical and chemical properties.

These informations may transported through the numerous nerves in the form of stimuli into the ganglia and are thus involved in the formation of adequate physiological condition.

Because it is possible to change the frequency of the periodic activity also by applying anaërobe conditions and in a way similar to the influence of the above ions (SALÁNKI 1964) it seemed to be important to examine the effect of the above salts (KCl and $CdCl_2$) on the respiration of siphon tissue.

## Material and method

*Anodonta cygnea* specimens of 14—18 cm length originating from the back-water of river Rábca (West Hungary) were used in the experiments. Before experimental use the animals were kept for at least three days in tanks in running Balaton-water.

Because in the majority of cases the experiments were performed in summer (May—September) adaptation to heat in thermostate before experiment was not considered important (PEDERSEN 1947, KORRINGA 1952).

$O_2$ consumption was measured with the usual WARBURG method at 25° C temperature. In the natural biotope of mussels this is the general temperature in summer period, and is in accordance with the temperature used

by HIGASHI and KAWAI (1959) in the measurements of oxygen-consumption of various tissues of mussels originating from similar biotope, and with that used during previous studies on the respiration of gill (LUKACSOVICS and SALÁNKI 1964).

In the excision of siphons special care was taken that the proportion of the various tissues constituting the siphon (tissues with different metabolic rate in agreement with their structure and functions) should be the same in the samples measured (HIGASHI and KAWAI 1959).

Both parts of the siphon the exhalant and the inhalant one was cut out all in one piece taking care that only a minimum quantity of the softer pallial tissue should remain on them. In this way only the strongly pigmented portion supplied with sensory tentacles together with a thicker connective tissue was used for measurements. The whole portion was cut into two symmetrical halves and washed thoroughly to remove the superficial mucuous substance. Thereafter the half portions of siphons were intermingled. Two halfs originating from two different animals were put into one WARBURG vessel (living matter 600—800 mg, dry matter 100—150 mg).

The reason for using total siphon and not homogenizated one was that a considerable decrease in $O_2$ consumption was resulted by the cutting up the tissues and the results obtained were greatly varying.

The measurements show, similar to those performed on the gill of mussels (LUKACSOVICS and SALÁNKI 1964) that respiration measurements should not be performed necessarily in physiological solution, as the values of $O_2$-consumption obtained on the application of both filtrated Balaton-water and physiological solution were similar. Moreover, the sensitivity of excized tissue towards tactic stimuli lasts for a coupe of days at room temperature and in Balaton-water. Therefore the experiments were carried out in Balaton-water.

In every experimental run 3 ml medium was placed into the main compartment and 0.2 ml 10 per cent KOH solution into the center wessel. The measurements were performed at a shaking velocity of 90—100 cycles/minute in air. The results obtained are the averages of 10—120 parallels and are expressed in units of consumed $O_2$ $\mu$l/1 g dry matter/hour. The measurements were continued at least for 2 hours. The results in the Figures are given at the end of the 1st and 2nd hours.

The effect of the following agents was examined: KCl, $CdCl_2$, L-cysteine, and the influence of L-cysteine produced on the effect of $CdCl_2$. In the latter case the organ was incubated for 30 minutes in $CdCl_2$ solution of different concentration and washed four times with filtrated Balaton-water.

## Results

1. *Influence of KCl on respiration*

KCl was applied in the following concentrations:

$$10^{-4},\ 2 \cdot 10^{-3} \text{ and } 10^{-2}\ \text{M}.$$

Results are illustrated in *figure 1*. Though tissue respiration was apparently stimulated by the lowest concentration ($10^{-4}$ M) of KCl solution, but if checked by statistical computations this cannot be considered as real stimulation ($t = 1.763$ and $0.10 > P > 0.05$) (HETÉNYI 1954). At the other two con-

centrations ($2 \cdot 10^{-3}$ M and $10^{-2}$ M) there were also no statistical differences between the mean values. It must be noted, that whereas only minimum differences are observable between the averages of respiration values of control and that of siphons treated with KCl the latter shows always higher

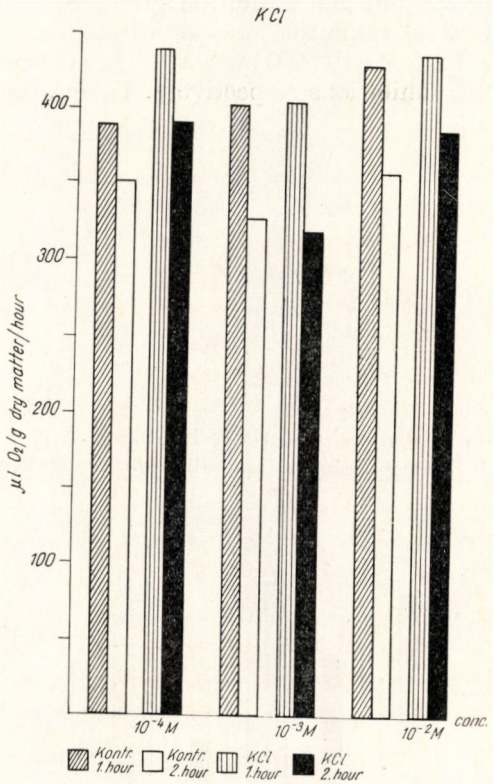


*Fig. 1.* The effect of various concentrations of KCl on the in vitro respiration of siphon tissue

*1. ábra.* KCl különböző koncentrációinak hatása a szifo-szövet in vitro légzésére

respiration rates. Nevertheless, it may be concluded that KCl at the above concentrations and under the circumstances applied does not produce essential changes in the respiration of the organ investigated.

2. *Influence of $CdCl_2$ on respiration*

By determining the time curve of $10^{-2}$ M $CdCl_2$ solution and the amount of consumed $O_2$ *(Fig. 2)* in an experimental run of 6 hours duration it was found that in the case of control the respiration rates of siphon decreased gradually and not vigorously and there was a parallel decrease also in the partial pressure of $O_2$. This decrease is at the end of the sixth hour about only 17% of that observed in the first hour of exposition. In the presence of $10^{-2}$ M

$CdCl_2$ solution the decrease is more considerable, namely in the 6th hour there is a 47% inhibition.

Examining the effect produced by various concentrations of $Cd^{++}$ ion *(Fig. 3)* it becomes evident that inhibition increases not only with time but also with the increase in concentration. Though all concentrations applied proved to be inhibitory, but the inhibition produced by $10^{-4}$ concentration was not significant. Real inhibition was produced only by the two higher concentrations. The $10^{-4}$ M, $10^{-3}$ M and $10^{-2}$ M concentrations resulted in 4.4% 16.4% and 28% inhibitions respectively. Taking the greatest inhibition

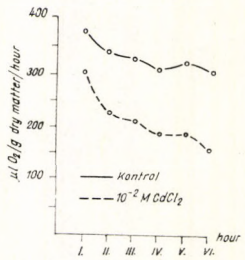


*Fig. 2.* $O_2$-consumption values of siphon tissue incubated in $10^{-2}$ M $CdCl_2$ for six hours
*2. ábra.* $10^{-2}$ M $CdCl_2$-ben 6 órán át inkubált szifo-szövet $O_2$-fogyasztási értékei

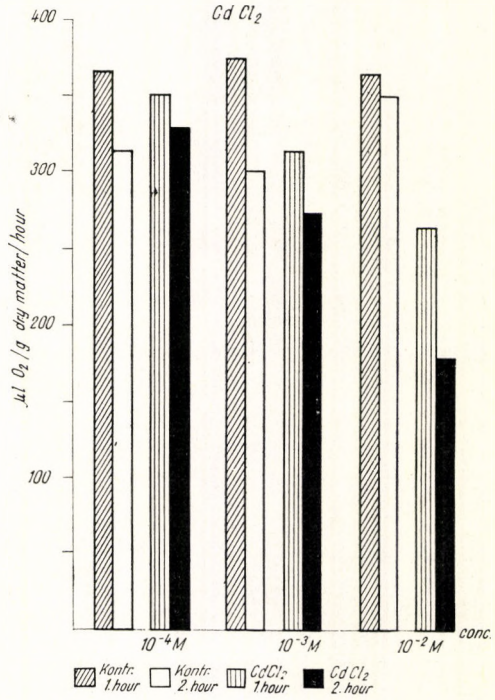


*Fig. 3.* The effect of various concentrations of $CdCl_2$ on the in vitro respiration of siphon
*3. ábra.* $CdCl_2$ különböző koncentrációinak hatása a szifo in vitro légzésére

as 100, as compared to the average of control, and expressing the other inhibition values in its percentage a linear relationship is obtained between the concentration of the effective material and the increase of inhibition.

3. *Effect of cysteine on the respiration of siphon and on the effect produced by* $Cd^{++}$

The following concentrations of cysteine were used:

$10^{-3}$ M, $10^{-2}$ M and $2 \cdot 10^{-2}$ M.

It is well-known that cysteine is easily oxidized also in a non enzimatic way it was attempted to determine the degree of this oxidation by incubating various concentrations of cystein in WARBURG vessels. The degree of spont-

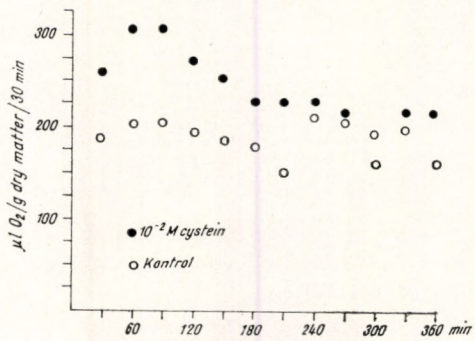


*Fig. 4.* $O_2$-consumption values of siphon incubated in $10^{-2}$ M cystein solution. Readings obtained at every thirty minutes

*4. ábra.* $10^{-2}$ M cysteinben inkubált szifo $O_2$-fogyasztási értékei félórás leolvasásokkal

aneous oxidation was negligible (in the case of 2·5 ml $10^{-1}$ M cystein 12 µl $O_2$/hour) notwithstanding, in all experimental run in which oxygen consumption was measured in the presence of cysteine, the cysteine solution used on the special occasion was pipetted into one of the thermobarometers and its readings were taken into correction in the computation of experimental results. The time curve of $O_2$-consumption was plotted on basis of tissue incubated in $10^{-2}$ M cysteine *(Fig. 4)*. The single points of graph represent readings performed at 30 minute intervals. In this series of measurements an increase in respiration intensity of both groups is observable in the first and second hour of exposition. Further on this decreases by 100—150 µl gradually until the fourth hour of exposition. Latter a considerable spread in the single readings is observable, the control also included, and the values obtained are nearly on the same level. The differences are significant until the 210 minute of exposition.

In contrary to $CdCl_2$ cysteine increases considerably the oxygen consumption of siphon tissue *(Fig. 5)*. Increases of $O_2$-consumption expressed in percentages are:

at $10^{-3}$ M cysteine solution 34%
at $10^{-2}$ M cysteine solution 59%
at $2 \cdot 10^{-2}$ M cysteine solution 102% .

On the influence of $10^{-2}$ M $CdCl_2$ solution the $O_2$-consumption of tissues shows a decrease of 76% as referred to the control as 100 *i.e.* an inhibition of 24%. A $10^{-3}$ M cysteine solution cannot eliminate this inhibition. At higher cysteine concentration ($10^{-2}$ M), however, an $O_2$-consumption greater by about

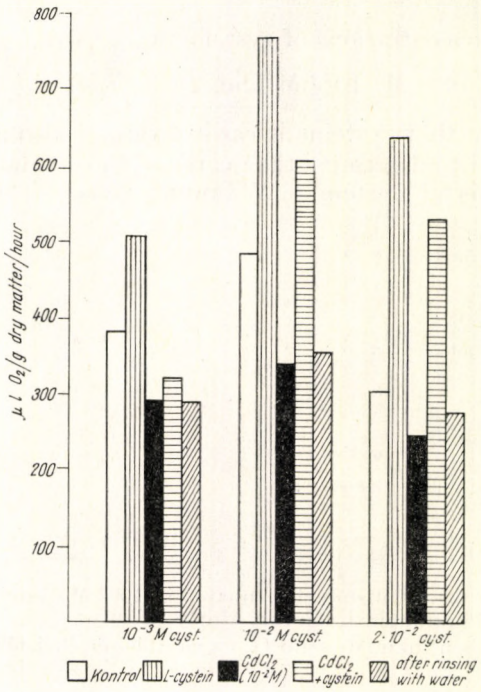


*Fig. 5.* Respiration stimulating influence of various cysteine concentrations, and their reactivating effect on the respiration of tissues blocked by $Cd^{++}$ on basis of measurements made in the first hour

*5. ábra.* Különböző cystein-koncentrációk légzést stimuláló, valamint $Cd^{++}$-al blokkolt szövet légzését reaktiváló hatása az első óra alapján

26% than that of control, and by 75% than that blocked by $CdCl_2$ is observable. The $10^{-2}$ M cysteine solution produces not only reactivation but also an increase in respiration amounting to about 102% and 151% in comparison to control and blocked sample respectively.

In the second hour of exposition these values change as follows *(Fig. 6)*.

Increases of $O_2$ consumption in percentages of the central after the application of various concentrations of cysteine:

| | | | |
|---|---|---|---|
| $10^{-3}$ M | cysteine | solution | 16% |
| $10^{-2}$ M | ,, | ,, | 100% |
| $2 \cdot 10^{-2}$ M | ,, | ,, | 155% . |

$O_2$-consumption of tissues incubated previously in $10^{-2}$ M $CdCl_2$ solution and washed subsequently are not activated by $10^{-3}$ M cysteine solution even in the second hour of exposition. On the influence of $10^{-2}$ M and $2 \cdot 10^{-2}$ M

cysteine, however, respiration increases more considerably after the first hour in comparison to both control and tissues incubated in $CdCl_2$.

Finally, reference is due to experiments, in the course of which tissues respiring in $CdCl_2$ medium and those washed from it and incubated in Balaton-

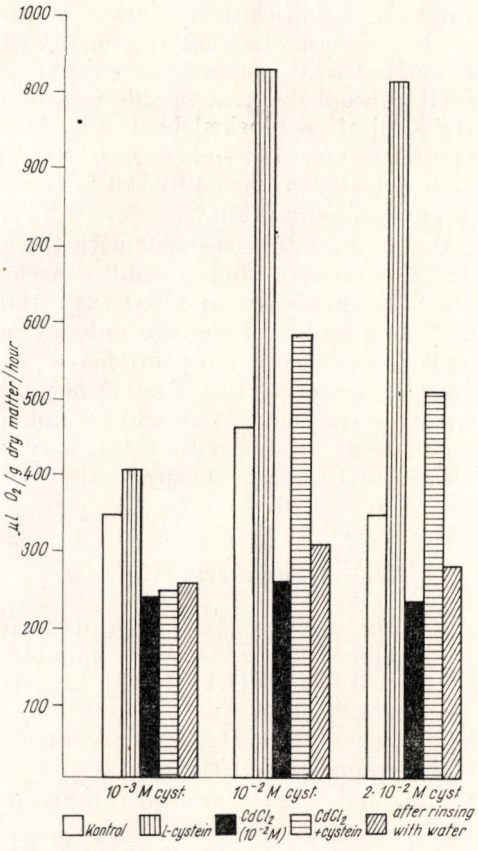


*Fig. 6.* Respiration stimulating influence of various cysteine concentrations and their reactivating effect on the respiration of tissues blocked by $Cd^{++}$ at the end of the second hour

*6. ábra.* Különböző cystein-koncentrációk légzést stimuláló, valamint $Cd^{++}$-al blokkolt szövet légzését reaktiváló hatása a második óra végén

water were compared. The results show that the $O_2$-consumption of washed tissue is only by about 5% and 16% higher than that of unwashed ones in the first and second hours of exposition respectively.

## Discussion

These experiments do not present evidence on modifying effect of KCl on the respiration of the siphon tissue. This implies that whereas the changes in the periodic activity of the animal in toto effected by $K^+$-ions is primarily induced from the siphon still it is not directly related to the respiration of

siphon tissue. It is thus presumable that the regulation of interchanges between rest and activity periods by KCl is connected with its stimulating influence on the receptors and connected nerve cells. It is suggested that if the tissue is incubated in $CdCl_2$ the oxidizing enzymes (primarily their SH groups of siphon tissue are blocked (BARRON and SINGER 1945). This produces a considerable decrease in $O_2$-consumption. Significant inhibition is produced even by a $10^{-3}$ M $CdCl_2$ solution. It is assumable that the changes in periodic activity produced by $CdCl_2$ are related to this effect.

By virtue of its SH content cysteine is able to stimulate ground respiration and to reactivate respiration blocked by $CdCl_2$. Analogous results were obtained in *in vivo* experiments on *Anodonta cygnea* (SALÁNKI 1960) inasmuch as the changes in periodic activity produced by $CdCl_2$ was restored by cysteine, which supports the previous assumption.

The results reported here are in agreement with the experimental results obtained previously in the course of similar studies performed on the tissue of gill of *Unio tumidus* (LUKACSOVICS and SALÁNKI 1964). The deviations produced by $K^+$, $Cd^{++}$ ions and cysteine are only of quantitative nature, namely the isolated gill tissue shows more intensive $O_2$-consumption. This may be attributed to causes related to the function of gill tissue, in particular to the quantity of oxidative enzymes. It should be noted that the decrease in $O_2$ consumption of gill tissue produced by $CdCl_2$ may be restored only by $2 \cdot 10^{-2}$ M cysteine, while in the case of siphon tissue even by $1 \cdot 10^{-2}$ M cysteine.

**Summary**

Authors investigated the changes in the *in vitro* respiration of the siphon of *Anodonta cygnea* L. produced by substances capable of modifying the periodic activity of the animal in toto (KCl, $CdCl_2$, L-cysteine).

The followings were established:

1. KCl solutions between $10^{-4}$ M—$10^{-2}$ M concentrations do not produce significant changes in the respiration of tissues.

2. $CdCl_2$ at $10^{-3}$ M and higher concentrations produces significant inhibition.

3. L-cysteine considerably increases the ground respiration of siphon tissue.

4. Respiration blocked by $10^{-2}$ M $CdCl_2$ is restored by cysteine of same molar concentration.

5. The present experiments produced analogous results with those similar ones performed on the gill tissue of *Unio tumidus* with the difference that the ground respiration of siphon tissue was lower and that only $2 \cdot 10^{-2}$ M L-cysteine was able to eliminate the inhibition produced by $10^{-2}$ M $CdCl_2$ on the gill tissue.

REFERENCES

BARRON, E. S. G., T. P. SINGER (1945): Studies on biological oxidations. XIX. Sulfhydryl enzymes in carbo-hydrate metabolism. — *J. Biol. Chem.* **157**, 221—227.

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

HETÉNYI G. (1954): Kísérleti eredmények statisztikai értékelése. In KOVÁCH, A.: A kísérleti orvostudomány vizsgáló módszerei. (Statistical evaluation of experimental results in A. KOVÁCH: The investigation methods of experimental medicine.) — *Akadémiai Kiadó, Bp.* **2**, 655—702.

HIGASHI, S., K. KAWAI (1959): The respiratory metabolism of fresh-water mussels. — *Bull. Jap. Soc. Sci. Fish.* **25**, 222—227.

HOPKINS, A. E. (1932): Chemical stimulation by salts in the oyster, Ostrea virginica. — J. Exp. Zool. 61, 14—27.

KORRINGA, P. (1952): Recent advance in oyster biology. — *Quart. Rev. Biol.* **27**, 266—308.

KOSHTOYANTS, CH. S., J. SALÁNKI (1958): On the physiological principles underlying the periodical activity of Anodonta. — *Acta Biol. Hung.* **8**, 362—366.

LUCAS, A. M. (1931): The distribution of the branchial nerve in Mytilus edulis and its relation to the problem of nervous control of ciliary activity. — *J. Morphol. Physiol.* **51**, 195—203.

LUKACSOVICS, F., J. SALÁNKI (1964): Effect of substances influencing tissue respiration and of the temperature on the $O_2$ consumption of the gill tissue in Unio tumidus. —*Ann. Biol. Tihany* **30**, 55—63.

MARCEAU, F. (1956): Sur l'état des muscles adducteurs pendant la vie chez les Mollusques acéphales. — *C. R. Acad. Sci., Paris* **142**, 1294—1296.

MARCEAU, F. (1909): Recherches sur la morphologie, l'histologie, et la physiologie comparées des muscles adducteurs des Mollusques acéphales. — *Arch. Zool. Exp. gen. (Sér. 5)* **2**, 295—469.

ORLOV, J. (1930): Über die Innervation des Anodontamantels. — *Z. mikr. anat. Forsch.* **20**, 551—556.

PEDERSEN, E. (1947): Ostersens respirasjon. — *Fisk. Skr. Havundersök.* **8**, 1—51.

SALÁNKI, J. (1960): On the slow rhythm of the periodic activity in fresh-water mussel (Anodonta cygnea). — *VII. Conf. Int. Soc. Ritm. Biol.* „ATTI" 1—11.

SALÁNKI, J. (1961): Role of afferentation in the regulation of the rhythm in the periodic activity of fresh-water mussels. — *Acta Biol. Hung.* **12**, 161—167.

SALÁNKI, J. (1965): Oxygen level as a specific regulator in the rhytmic activity of fresh-water mussel (Anodonta cygnea L.). — Acta Biol. Hung. 15, 299—310.

## KAGYLÓK PERIODIKUS AKTIVITÁSÁT BEFOLYÁSOLÓ ÁGENSEK HATÁSÁNAK TANULMÁNYOZÁSA *ANODONTA CYGNEA* L. SZIFO-SZÖVETÉNEK IN VITRO LÉGZÉSÉN

### Összefoglalás

*Salánki János és Lukacsovics Ferenc*

Szerzők olyan anyagok hatását tanulmányozták az *Anodonta cygnea* L. szifojának in vitro légzésén, amelyek módosítják a totál állat periodikus aktivitását (KCl, $CdCl_2$, L. cystein).

Megállapították, hogy:

1. A KCl $10^{-4}$ M — $10^{-2}$ M koncentrációig nem befolyásolja a szöveti légzés nagyságát szignifikánsan.
2. A $CdCl_2$ $10^{-3}$ M koncentrációban és e fölött szignifikáns gátlást eredményez.
3. Az L-cystein jelentősen megnöveli a szifoszövet alaplégzését.
4. A $10^{-2}$ M $CdCl_2$-al blokkolt légzést az ugyanazon molaritású cystein kivédi.
5. Jelen vizsgálatok a korábban *Unio tumidus* kopoltyúszövetén végzett hasonló kísérletekkel analóg eredményt mutattak, azzal a különbséggel, hogy a szifoszövet alaplégzése kisebb és hogy a $10^{-2}$ M koncentrációjú $CdCl_2$ által okozott gátlást a kopoltyúszöveten csak $2 \cdot 10^{-2}$ M L-cystein tudta kivédeni.

# ИЗУЧЕНИЕ ЭФФЕКТА ВЕЩЕСТВ, ВЛИЯЮЩИХ НА ПЕРИОДИЧЕСКУЮ АКТИВНОСТЬ БЕЗЗУБКИ, НА ДЫХАНИЕ СИФОННОЙ ТКАНИ

## Anodonta cygnea L. in vitro

Я. Шаланки, Ф. Лукачевич

Авторы изучали влияние на дыхание сифонной ткани беззубки таких веществ, которые видоизменяют периодическую активность целого животного (KCl, $CdCl_2$, L-цистеин).

1. $10^{-4}$ M — $10^{-2}$ M концентрации хлористого калия не изменяют уровень тканевого дыхания.
2. $CdCl_2$ в концентрации $10^{-3}$ M и выше этой вызывает достоверное торможение.
3. L-цистеин значительно увеличивает основное дыхание сифонной ткани.
4. Блокирование дыхания, вызванное хлористым кадмием в концентрации $10^{-2}$ M, снимается с помощью цистеина в такой же концентрации.
5. В этих экспериментах были получены аналогичные данные полученным раньше данным на жаберной ткани Unio tumidus той только разницей, что основное дыхание сифонной ткани ниже, и что в жабрах торможение, вызванное $CdCl_2$ в концентрации $10^{-2}$ M, снимается только $2.10^{-2}$ M концентрацией L-цистеина.