Adminstration of Thiamine and Thiochrome
Enhanced Reproduction of Chlorella,
Drosophila melanogaster, and Danio

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Summary

Thiochrome, a natural metabolite of thiamine, has scarcely attracted the attention of researchers, since many of them considered it to be a biologically inactive substance. We examined a possible effect of thiochrome upon the reproduction of the organisms of Chlorella, Drosophila, and Danio. We added thiamine or thiochrome to the culture medium or to the aquaria. Our data showed that the number of cells and organisms were increased in the presence of thiamine and thiochrome. We suggest possible effect(s) of thiamine and thiochrome on the reproduction of these organisms.

Key Words

thiamine, thiochrome, reproduction

The research performed during the last decades testifies that the functions of thiamine in organisms are not limited to its turning into thiamin diphosphate and participation in the performance of thiamine-dependent enzymes (1). Involvement of thiamine and some of its catabolites is established in the regulation of NAD-dependant enzymes (2–4), proteases (5–7), and thiamine-dependant enzymes (8) and regulation of the expression of genes responsible for the synthesis of the thiazole and pyrimidine (9) components of thiamine, as well as genes coding the synthesis of thiamine diphosphate-dependant enzymes (10).

We initiated research into the possibility of an effect of thiamine and its catabolite—thiochrome—on the reproduction of organisms dissimilar in terms of systematic location: one-celled animals—Chlorella vulgaris, insects—Drosophila melanogaster and fishes—Danio margaritatus.

MATERIALS AND METHODS

The studies were implemented in the following organisms: Chlorella vulgaris (wild type), Drosophila melanogaster, Danio margaritatus, Cambarellus puer, and Enchytraeus buchhoisi. All international standards of “Guide for the Care and Use of Laboratory Animals” were met in the course of the animal studies.

The following medium was used in the course of research in Chlorella vulgaris algae: KNO\textsubscript{3} 0.1 g/L, K\textsubscript{2}HPO\textsubscript{4} 0.01 g/L, MgSO\textsubscript{4}·7H\textsubscript{2}O 0.01 g/L, FeCl\textsubscript{3}·6H\textsubscript{2}O 0.001 g/L.

The algae were subcultured under sterile conditions into the fresh medium and in 2 d thiamine and thiochrome were added to the experiment vessels. The quantity of cells was calculated for 3 d on a daily basis.

The standard culture medium was used for investigations in Drosophila melanogaster. Thiamine or thiochrome was added to the experiment vessels and the quantity of pupa was determined over 4 d. Thereafter, on day four the quantity of adult insects was counted. The calculations were made for F\textsubscript{1} and F\textsubscript{2}.

Twenty-seven groups consisting of 2 males and 1 female of mature fish were selected for experiments for Danio margaritatus. There were nine test groups. The aquariums of the following 9 groups were supplemented with thiamine at the concentration of 15 nmol/L. The same concentration of thiochrome was added to the aquariums of the last nine groups. In the aquarium, the pH of water was 7.8, GH 14˚, and temperature 25˚C.

By the beginning of the experiment, the females were at the 3rd stage of gonad maturity. In 20 d the fish were relocated to the breeding tanks with the following parameters of the water: pH 7.0, GH 6˚, and temperature 27˚C. Upon completion of the spawning, the fish were separated to other aquaria and the quantity of hard roe was calculated.

For research on the worm Enchytraeus buchhoisi, 12 groups of worms were selected for each variant—control, adding the thiamine and thiochrome. Culture of the worms was in containers, wherein foam plastic sheets with a thickness of 1 cm were added. The worms were fed once per day with dry milk powder. Before the start of the experiments, the contents of the containers were moistened with water. Then thiamine and thio-
Thiamine and Thiochrome Enhanced Reproduction

Thiochrome were added to corresponding containers and the contents of the containers were moistened with water for 15 d. On day 15, the worms were taken out and counted.

Experiments on Cambarellus puer were implemented in 30 groups of animals, with 10 groups per each variant—control, adding the thiamine and thiochrome. The crawfish were kept in aquaria with a capacity of 10 L, with the following water parameters: pH 7.8, GH 14˚ and temperature 25˚C.

The females which recently bore larvae were used for the experiment. At the beginning of the experiment, the females were not impregnated. The test aquaria were supplemented with thiamine or thiochrome to a final concentration of 30 nmol/L. After the spawning, the deposited eggs were counted and the time interval from mating to egg laying was established.

In the studies in one-cell organisms, the quantity of cells was calculated in the test variant and after adding the thiamine and thiochrome to the culture medium. In the experiments in Drosophila melanogaster, the quantity of pupae and adult insects was calculated in the first and second generation in the presence of thiochrome in the culture medium. Total contents of nucleic acids, as well as DNA and RNA were determined by the spectrophotometric method (11). When dealing with the fish, the quantity of hard roe was calculated after introduction of the studied preparations into the water.

Statistical analysis. Values are reported as means ± SE. All data were analyzed using one-way ANOVA (Biostat). Statistical significance was considered to be \( p \leq 0.05 \).

RESULTS

Effect of thiamine and thiochrome upon the reproduction processes

Data obtained in the course of study of the intensity of Chlorella vulgaris cell reproduction are given in Table 1.

In this experiment it was determined that thiochrome can stimulate the reproduction of cells of Chlorella vulgaris. Such stimulation didn’t depend on the dose of thiochrome within a range of concentrations from 1.5 to 15 nmol/mL.

At the same time, adding thiamine into the medium at the same concentrations had no effect upon the reproduction of such algae.

In the next series of experiments we studied the effect of thiamine and thiochrome upon the reproduction of a representative of insects—Drosophila melanogaster. These data are represented in Table 2.

Introduction of thiochrome into the culture medium in the amount of 0.03 μmol had no significant effect upon the quantity of formed pupae in F1. At the same

### Table 1. Dynamics of the number of Chlorella vulgaris in the presence of various concentrations of thiamine and thiochrome (10^3 cell/mL of medium) (n=12) in the medium.

| Variant   | Concentrations of the metabolites, nmol/mL | Day 1        | Day 2        | Day 3        |
|-----------|--------------------------------------------|--------------|--------------|--------------|
| Control   | —                                          | 258±23       | 366±32       | 590±51       |
| +Thiochrome | 1.5                                       | 361±35*      | 421±40*      | 1.040±98*   |
|           | 3.0                                        | 360±32*      | 469±43*      | 1.178±101*  |
|           | 15                                         | 384±35*      | 452±41*      | 1.221±110*  |
| +Thiamine | 1.5                                        | 222±20       | 335±31       | 496±45      |
|           | 3.0                                        | 219±19       | 350±32       | 512±48      |
|           | 15                                         | 234±21       | 348±30       | 524±50      |

* \( p \leq 0.05 \) in comparison with the control.

### Table 2. Effect of thiamine and thiochrome upon the fecundity of Drosophila melanogaster (n=14).

| Generations | Stage | Thiochrome | Thiamine |
|-------------|-------|------------|----------|
|             |       | 0.03 μmol  | 3.0 μmol | 0.03 μmol | 3.0 μmol |
| F1          | Pupa  | 120±14     | 146±15   | 315±32*   | 120±13   | 240±21*   | 309±32*   |
|             | Imago | 110±10     | 130±12   | 307±30*   | 110±14   | 227±23    | 295±30*   |
| F2          | Pupa  | 174±18     | 168±18   | 302±29*   | 174±16   | 165±15    | 297±31*   |
|             | Imago | 170±16     | 160±14   | 297±28*   | 170±19   | 159±17    | 287±30*   |

* \( p \leq 0.05 \) in comparison with the control.
time, the same quantity of thiamine increased the quantity of pupae by 2 times. A similar picture was noticed for imago in F1. Thiamine in the quantity of 30 nmol increased their quantity by 2 times, while thiochrome had no effect upon such a parameter.

Another picture was observed when adding the studied preparations in the amount of $3 \mu$mol into the culture medium. In such a case, both thiamine and thiochrome caused a sharp increase in the number of both pupae and imagoes. Concurrently, the effect caused by thiochrome was scarcely different from the effect when using thiamine.

In the course of research on the pupae and imagoes in F2, a significant increase in the number of both was detected when using both thiamine and thiochrome. The effect was noticed only in the case of adding 3 $\mu$mol of thiochrome and thiamine into the culture medium.

The next experimental subject was a representative of the fishes, *Danio margaritatus*. In this experiment, thiamine and thiochrome were added to the aquaria water at the concentration of 15 nmol/L. These data are given in Fig. 1.

As may be seen from the data given in Fig. 1, adding thiamine to the water decreased the quantity of hard roe by 24%, but thiochrome increased this parameter by 34%. A special experiment studied the quantity of...
Table 3. Effect of thiamine and thiochrome upon the roe generation of *Cambarellus puer* (*n*=10).

| Variant                  | Control       | Thiamine, 30 nmol | Thiochrome, 30 nmol |
|--------------------------|---------------|-------------------|---------------------|
| Quantity of roe          | 69±5          | 74±7              | 75±5                |
| Incubation period (d)    | 23±1          | 16±1*             | 16±1*               |

* Differences from control are significant, *p*≤0.05.

Table 4. Determination of the thiochrome level when adding thiamine to the medium (30 nmol) in the tissues of crawfish and worms (μg/g of tissue) (*n*=10–12).

| Variant                  | Control       | +Thiamine, 30 nmol |
|--------------------------|---------------|-------------------|
| *Cambarellus puer*       | 0.052±0.006   | 0.116±0.009*      |
| *Enchytraeus buchhoisi*  | 0.660±0.063   | 3.060±0.234*      |

* Differences from control are significant, *p*≤0.05.

![Fig. 4. Total contents of nucleic acids per 10⁴ of *Chlorella vulgaris* cells.](image)

Table 5. Percentage of cells with autospores *Chlorella vulgaris* in the presence of thiamine and thiochrome.

|                 | 1 h     | 2 h     | 4 h     | 24 h    |
|-----------------|---------|---------|---------|---------|
| Control         | 0.32±0.03| 0.34±0.03| 0.37±0.04| 0.32±0.03|
| +Thiamine       | 0.36±0.04| 0.36±0.03| 0.41±0.04| 0.44±0.04*|
| +Thiochrome     | 0.39±0.03| 0.42±0.02*| 0.49±0.03*| 0.50±0.04*|

* *p*≤0.05 in comparison with the control.

Impregnated and unimpregnated roe in the presence of males in the aquaria with thiochrome added. In this experiment thiamine was not used since its addition decreased the total quantity of roe (Fig. 1). These data are given in Fig. 2.

As may be seen from Fig. 2, any increase in the quantity of roe under the effect of thiochrome is determined by a sharp increase in the quantity of unimpregnated roe.

In the next series of experiments we studied the effect of adding thiamine and thiochrome to the culture liquid upon the reproduction of the worm *Enchytraeus buchhoisi*. The duration of the experiment was 15 d. The concentration of thiamine and thiochrome in the medium was 30 nmol.

Results are given in Fig. 3.

These data are testimony to the fact that thiochrome at the concentration of 30 nmol stimulates the reproduction of the worms. Thiamine doesn’t have such an effect.

Similar studies were also implemented in a representative of the crustaceans, *Cambarellus puer*. These data are given in Table 3.

These studies testify that both thiamine and thiochrome significantly accelerate the roe formation in crawfish.

**Thiamine metabolism before thiochrome in crawfish and worms**

It is well known that thiochrome is one of the main catabolites of thiamine in humans and mammals. However, metabolism of this vitamin in the tissues of invertebrates has scarcely been studied. Therefore, we researched the possibility of thiochrome formation from thiamine in the tissues of worms and crawfish. These data are given in Table 4.

The data in Table 4 testify that the addition of thiamine to the medium results in the formation of thiochrome both in the representative of crawfish, and in the representative of worms. Thus, we established that thiochrome is the natural metabolite of thiamine in the representatives of invertebrates and mammals.
Table 6. Effect of thiamine and thiochrome upon the contents of DNA and RNA in *Cambarellus puer* and *Enchytraeus buchhoisi* (µg/g of tissue) (n=8).

| Parameter | RNA          | DNA           |
|-----------|--------------|---------------|
|           | Species      | Control       | Thiamine      | Thiochrome     | Control       | Thiamine      | Thiochrome     |
|           | *Cambarellus puer* | 0.95±0.05    | 1.75±0.19*    | 3.59±0.33*    | 0.15±0.01    | 0.26±0.02*    | 0.46±0.05*    |
|           | *Enchytraeus buchhoisi* | 2.41±0.23    | 2.39±0.12    | 4.08±0.19*    | 0.16±0.01    | 0.19±0.02    | 0.26±0.02*    |

* p<0.05 in comparison with the control.

**Effect of thiamine and thiochrome upon the contents of nucleic acids in Chlorella vulgaris, Cambarellus puer and Enchytraeus buchhoisi**

In order to find out the causes of thiochrome’s stimulating effect on the reproduction processes, we studied the effect of thiamine and thiochrome on total contents of nucleic acids and quantity of cells with the autospores *Chlorella vulgaris*. These data are given in Fig. 4 and Table 5.

The obtained results (Fig. 4) testify that thiochrome added into the medium at the concentration of 15 nmol/mL of medium nearly triples the total contents of the nucleic acids per 10^4 of cells in just 1 h after adding. In 2 h, this effect decreases and is 150% of the control. In 4 h the parameters become even with the control. The effect of adding thiamine to the medium is the opposite. In such a case, the parameters become lower than those of the control within 24 h from the second hour.

The main parameter of *Chlorella vulgaris* readiness to the reproduction is the quantity of cells with autospores. Our tests demonstrated (Table 5) that adding thiochrome to the medium results in an increase in the percentage of the cells with autospores in relation to the total quantity of cells, starting at 2 h after thiochrome addition to the medium and increases for up to 24 h.

Comparison of the data in Fig. 4 and Table 5 leads to the conclusion that adding thiochrome within the first hour activates the synthesis of nucleic acids. Thereafter the process of autospore formation and reproduction starts (Table 5) and the contents of the nucleic acids per cell normalize.

After obtaining the data on the activation of nucleic acid synthesis by thiochrome, we studied the effect of this compound upon the synthesis of DNA and RNA. These data are represented in Table 6.

As may be seen from the data given in Table 6, in research on *Cambarellus puer* it was established that both thiamine and thiochrome increased the contents of both DNA and RNA in the body of this organism. However the effect caused by thiochrome was significantly larger than the effect of thiamine. In *Enchytraeus buchhoisi* the increase in the contents of DNA and RNA was caused only by thiochrome.

**DISCUSSION**

Thiochrome, a natural metabolite of thiamine, has scarcely attracted the attention of researchers, since many specialists considered it to be a biologically inactive substance. Over recent years, a number of studies was implemented in our laboratory, testifying to the existence of specific biological and biochemical functions of thiochrome in organisms.

In this research we set a goal to answer the question about the possible regulatory effect of thiochrome upon reproduction of the organisms of various systematic groups. It is believed that thiochrome in the tissues of mammals cannot turn into thiamine. However such a possibility was not researched in one-cell invertebrates or lower vertebrates. Therefore, in our experiments we compared the effect of thiamine and thiochrome upon the process of reproduction in organisms of various systematic positions.

The results of our studies demonstrated that the thiochrome in concentrations similar to physiological ones, can increase the intensity of the reproduction process of the representatives of one-cell organisms, worms, crustaceans, insects and fishes. In the majority of cases the effect of thiochrome was not identical to the effect of thiamine. In cases when thiamine had no effect upon the generation of new cells, thiochrome significantly intensified this process. The only subject in which the effect of thiamine and thiochrome coincided was *Drosophila melanogaster*. It seems that this effect is attributed to previously established intense turning of thiamine into thiochrome in the organism of *Drosophila melanogaster*.

Thus, our research has demonstrated that thiochrome, as a thiamine metabolite, can intensify the reproduction process in the organisms of various systematic groups.

The fact of the activating effect of thiochrome on the reproduction, established by us for the first time, may be related to the effect of this compound on the transcription process, as was demonstrated for thiamine. However it seems that the effect of such action fundamentally differs from the effect caused by thiamine.

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