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Tiiu HANSEN, Malle VIIK

EFFECT OF TEMPERATURE ON THE GLYCEROL AND FREE AMINO ACID CONTENT IN THE HIBERNATING LARVAE OF *ARCTIA CAJA* L. (*LEPIDOPTERA*)

The hybernating stages of a number of *Lepidoptera, Coleoptera, Hymenoptera* and *Diptera* species are known to withstand the freezing of tissues. Such insects are called freezing-tolerant. Many of them have been found to contain large quantities of glycerol during the diapause (Sømme, 1964, 1965; Salt, 1959; Baust, Miller, 1970, etc.). These insects have been found to have a great freezing tolerance: they withstood freezing at a temperature below —40 °C. On the other hand, a number of insect species are known to have a great freezing tolerance, but a comparatively small glycerol content (Takehara, Asahina, 1959, 1960; Asahina, 1966; Asahina, Tanno, 1966). There are also freezing-tolerant species whose hibernating stages have been found to contain no glycerol. As a rule, the freezing tolerance of these species is not great, not lower than —15° (Takehara, Asahina, 1960, 1961, etc.). It is well known that glycerol may protect the animal tissues from injuries otherwise caused by ice formation. The opinion has been expressed that glycerol is responsible for the ability of a number of insects to survive freezing.

Data are also available in the literature on the high concentration of some amino acids in diapausing insects and on changes in the amino acids content when insects are kept at a low temperature. Thus, an increase in the amount of alanine has been observed in the larvae of *Anagasta kuehniella* (Sømme, 1966), in the pupae of *Pieris brassicae*, in the larvae of *Nemapogon personellus* (Sømme, 1967), and an increase in the alanine content and a decrease in the glutamic acid content has been stated in the pupae of *Celerio euphorbia* and *Sphinx ligustri* (Florkin, 1959) stored at low temperatures. A high proline, alanine and threonine content has been ascertained in the diapausing larvae of *Antheraea pernyi* (Mansingh, 1967) and a high proline, alanine, threonine and glutamic acid content has been estabiished in the diapausing larvae of *Blastesthia turionella* (Веймер, 1972). The concentrations of the above-mentioned amino acids in the haemolymph decreased rapidly when diapause was broken. It is assumed by some authors that the cold-hardiness of insects may depend on the presence of proline and alanine.

The present paper represents a study of changes in the glycerol and free amino acid content in the hibernating larvae of the tiger moth *Arctia caja* when kept under various temperature conditions.

Material and methods

The larvae of the third and the fourth instar of *Arctia caja* served as objects of study. The material was grown under natural conditions in glass jars where the larvae were kept until the beginning of the experiments. The experiments were carried out in the months of October, November and April 1970—71.

The glycerol and free amino acid content was determined by the paper-chromatographic method (Веймер, Хансен, 1970). Of the amino acids, the alanine content was determined separately. The amounts of the remaining amino acids were estimated in toto, being expressed in alanine.

Glycerol concentrations are given as percentages of fresh weight, and free amino acid contents are expressed in milligrams per gram.

Results and discussion

The effect of the temperatures of 20, 5, 0, —5, —7 and —12° on the metabolism of glycerol and free amino acids was studied. Experiments were started on October 13, when the larvae contained on an average 4 per cent of glycerol. The insects were kept at different temperatures, and their glycerol and free amino acid contents were determined at the end of October. Our studies showed that the diapausing larvae of *Arctia caja* contained relatively much alanine in all treatments, as compared with other amino acids (on account of their small quantities they were determined in toto).

Table 1

Content of glycerol and free amino acids in diapausing larvae of *Arctia caja* following different temperature treatments

Treatment	Glycerol, %	Alanine, mg/g	Other amino acids mg/g	Number of specimens	Instar
20° C, 14 days	2.77±0.03	1.40±0.24	0.60±0.01	11	IV
5° C, 14 days	4.34±0.10	1.96±0.02	0.59±0.01	11	IV
0° C, 17 days	6.02±0.01	1.90±0.02	0.53±0.04	13	IV
−5° C, 17 days	7.03±0.16	2.40±0.03	0.81±0.00	10	III
−7° C, 14 days	4.39±0.18	1.90±0.02	0.61±0.05	10	IV
−12° C, 14 days	4.35±0.02	2.16±0.02	0.62±0.01	11	III, IV

The experimental results are presented in Table 1 and in the Figure. It is evident from the Table that the largest amounts of glycerol accumulated at the temperatures of 0 and —5°, i. e. at the lowest temperatures, at which the larvae had not yet frozen. At —7 and —12° the larvae froze, and the glycerol content practically did not increase in them. A temperature of 20° brought about a decrease in the glycerol content.

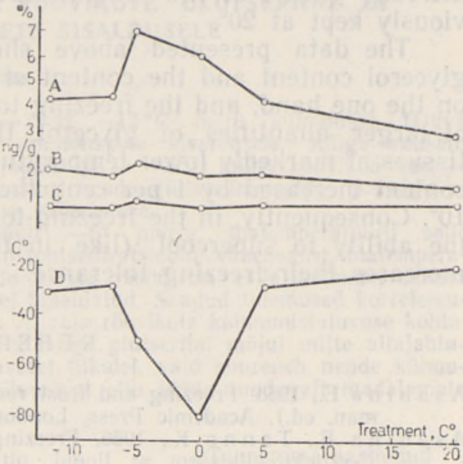
An increase in the quantities of alanine was observed only at a temperature of —5°. The results obtained are in agreement with the results of the investigators mentioned above. At room temperature (20°) the alanine content diminished. Similar results were obtained by Sømme (1967), who established that the alanine content in the pupae of *Pieris brassicae* at 20° diminished during the diapause as well as after its termination.

All the freezing-tolerant species studied by us had their supercooling points within the range of —5 to —10°. Merivee (1971) determined the supercooling point of the larvae of *Arctia caja* within the period of September to December as ranging from —9 to —10°, without practically undergoing any changes, although there occurred substantial changes in the freezing tolerance of the larvae under the action of low temperatures. The temperatures of 0 and —5° proved to be optimal for acclimation, while at —7 and —12° the larvae froze and did not cold-harden. These data correlate well with changes in the glycerol and alanine content at the corresponding temperatures (Figure).

Table 2

Reversibility of the glycerol content of *Arctia caja* larvae under the action of temperature in October and in April

Treatment	Glycerol, %
Outdoors (Oct., 13)	4.00±0.10
20 °C, 14 days	2.77±0.03
0 °C, 20 days	4.63±0.09
Outdoors (April, 1)	4.62±0.27
20 °C, 2 days	0.67±0.02
0 °C, 25 days	0.65±0.02



Content of glycerol and free amino acids and freezing tolerance in diapausing larvae of *Arctia caja* following different temperature treatments.
A — glycerol content, *B* — alanine content, *C* — content of other amino acids, *D* — freezing tolerance (after Merivee, 1971)

The reversibility of the glycerol and alanine content under the action of temperature was also investigated, namely in October and in April (Table 2). It was established that the glycerol content in October, during the diapause, decreased by 1.2 per cent when the larvae were kept at 20° for two weeks, and increased again to 4.6 per cent when the larvae were kept at 0°, i. e. at an optimal temperature for the synthesis of glycerol, for three weeks. The glycerol concentration, however, did not reach the maximum level peculiar to the species.

Likewise, the content of alanine decreased at room temperature and increased again after keeping the larvae at 0°, but the concentration, however, exceeded the previous level.

Similar changes have been observed in the field of the freezing tolerance of larvae: at 0° it increased again, but the larvae did not acquire the maximum freezing tolerance peculiar to the species (Меривээ, 1971).

At the beginning of April the glycerol content at room temperature decreased considerably faster: within two days it fell by 4 per cent, but did not rise any more when the larvae were kept at 0° for 25 days. The behaviour of glycerol was similar to that of the freezing-susceptible species *Petrova resinella* in December and April (Hansen, 1973). Baust and Miller studied the reversibility of glycerol under the action of temperature in the adults of the freezing-tolerant species, *Pterostichus brevicornis*, and found that at 20° glycerol vanished very rapidly, decreasing from 18 per cent almost to zero within 36 hours. This loss of glycerol was irreversible (Baust, Miller, 1970). Unfortunately, data on the dates of the performance of the experiment are lacking in their paper.

As to the freezing tolerance, the species *Pterostichus aethiops* and *Phosphuga atrata* preserved their ability of acclimation also in April, but it was lost irreversibly after the insects had been kept at 20° for a few days (Меривээ, 1971). On the basis of this circumstance it may be assumed that the freezing tolerance of the larvae of *Arctia caja* did not increase either, at low temperatures in April after they had been previously kept at 20°.

The data presented above show a good correlation between the glycerol content and the content of alanine in the larvae of *Arctia caja* on the one hand, and the freezing tolerance, on the other. In the presence of larger quantities of glycerol the larvae withstood the freezing of tissues at markedly lower temperatures than otherwise. When the glycerol content increased by 1 per cent, the freezing tolerance increased by 7 to 10°. Consequently, in the freezing-tolerant insects, glycerol does not raise the ability to supercool (like in the freezing-susceptible insects), but increases their freezing tolerance.

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Academy of Sciences of the Estonian SSR,
Institute of Zoology and Botany

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Tiiu HANSEN, Malle VIIK

TEMPERATUURI MÕJU PÄEVAKOERA *ARCTIA CAJA* L. (*LEPIDOPTERA*) TALVITUVATE RÖÖVIKUTE GLÜTSERIINI- JA VABADE AMINOHAPETE SISALDUSELE

Resümee

Uuriti temperatuuride 20, 5, 0, −5, −7 ja −12 °C mõju külmumist taluva *Arctia caja* röövikute glütseriini- ja vabade aminohapete sisaldusele. Kõige rohkem glütseriini kogunes madalate temperatuuride juures (0 ja −5°), milles röövikud veel ei külmunud. −7 ja −12° juures nad külmusid ning nende glütseriinisisaldus peaaegu ei suurenenud. 20° kutsus esile glütseriinisisalduse vähenemise.

Alaniinihulga suurenemist täheldati ainult −5° juures, toatemperatuuril selle sisaldus vähenes. Peale glütseriini- ja alaniinikontsentratsiooni vähenemist toatemperatuuril oli oktoobris-novembris võimalik nende ainete resünteesi esile kutsuda, hoides putukaid temperatuuris 0°. Aprillis resünteesi ei täheldatud. Saadud tulemused korreleeruvad hästi Merivee (Меривээ, 1971) andmetega *A. caja* röövikute külmumistaluvuse kohta

Järeldati, et külmumist taluvatel liikidel ei lange glütseriini mõjul mitte allajahtumispunktid nagu allajahtumisseisundis talvituvatel liikidel, vaid suureneb nende külmumistaluvus, mis võimaldab putukatel peale külmumist ellu jääda tunduvalt madalamate temperatuuride juures kui muidu.

Eesti NSV Teaduste Akadeemia
Zooloogia ja Botaanika Instituut

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Тийу ХАНСЕН, Малле ВИЙК

ВЛИЯНИЕ ТЕМПЕРАТУРЫ НА СОДЕРЖАНИЕ ГЛИЦЕРИНА И СВОБОДНЫХ АМИНОКИСЛОТ У ЗИМУЮЩИХ ГУСЕНИЦ *ARCTIA CAJA* (*LEPIDOPTERA*)

Резюме

Изучали влияние температуры 20, 5, 0, —5, —7 и —12 °С на метаболизм глицерина и свободных аминокислот у гусениц морозостойкого* вида *Arctia caja*. Наибольшие количества глицерина накоплялись при самых низких температурах, при которых гусеницы еще не замерзали (0 и —5°). При —7 и —12° гусеницы замерзали и содержание глицерина в них почти не повышалось. При 20° концентрация глицерина уменьшалась.

Повышение содержания аланина наблюдалось только при температуре —5°, при комнатной температуре количество аланина уменьшалось. В октябре—ноябре после уменьшения в гусеницах содержания глицерина и аланина при комнатной температуре их концентрации снова повышались при 0°. В апреле же ресинтеза этих веществ не наблюдалось.

Полученные результаты хорошо коррелируют с соответствующими данными Меривээ (1971) о морозостойкости гусениц *A. caja*.

Таким образом, у морозостойких видов** под влиянием глицерина увеличивается не способность к переохлаждению, как у холодостойких видов, а их морозостойкость, способствуя выдерживанию замерзания жидкостей тела при более низких температурах.

Институт зоологии и ботаники
Академии наук Эстонской ССР

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* Морозостойкие насекомые — насекомые, которые выдерживают в течение длительного периода замерзание жидкостей тела.

** Холодостойкие насекомые — насекомые, которые переносят в течение длительного периода переохлаждение жидкостей тела, но не их замерзание.