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- USSR -

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It has been established through our studies (1) that several species of insects can withstand freezing at the very low temperatures -55° and -78°. The pupae of Saturnia pyri consume oxygen for an extended period, but several eggs of the Asiatic locust Locusta migratoria preserved their normal appearance for many days following exposure of the eggs to a temperature of -183°. However, not all the caterpillars of the corn borer (Pyrausta nubilalis) following cooling down to -78° proved to be alive, and those which preserved their mobility gradually perished. The existence of respiration following chilling is not necessarily indicated by the fact that several signs of life were maintained in the organism. Only recently (2, 3) was it shown for several species of insects and other invertebrates that a certain percentage of the animals show motor reactions following chilling in liquid oxygen or nitrogen. The condition for survival at such temperatures is preliminary cooling at -30°.

Our investigations of 1961-1962, mainly on corn borer caterpillars have shown that preliminary cooling at -30° is not the necessary and only condition for survival at ultra-low temperatures. The highest cold-resistance is shown only by diapaused caterpillars (beginning from November). However, they also cannot withstand chilling down to -183° or -196°, if they are frozen during the initial period of the diapause or at its conclusion, when the period of reactivation has not yet set in. In these cases the preliminary cooling down to -30° does not promote survival.

In our experiments we succeeded in preserving full viability of the corn borer caterpillars following chilling at -78° and obtained a high percentage of live specimens following chilling

TABLE 1

Resistance of Caterpillars to -196°: at -30° for 60 min; at -79° for 1 hr, at -196° for 1 day.

(a) №№ опытов	(b) Дата опыта	(c) Число гусениц	(d) Температура содержания в °C	(e) Реакция после оттаивания: (f) число подвижных в %	(e) Реакция после оттаивания: (g) число реагирующих на эл. ток в %*	(h) Продолжительность жизни в сутках
(i) Охлаждение: —30° 1 (j) час, —79° 1 час, —196° 1 сутки (k)						
19	15 XI	20	22	0	0	0
20	15 XI	20	0	0	0	
26	8 XII	20	0	85	5	2—33
27	11 XII	20	22	5	25	1—10
29	11 XII	20	10	26	45	1—34
28	11 XII	20	0	70	10	3—23
51	18 I	20	10	0	0	0
52	18 I	20	4	0	0	0
53	18 I	20	0	0	0	0
(i) Охлаждение: —30° 1 сутки, —196° 1 сутки						
39	10 I	20	22	0	0	0
40	10 I	20	10	10	10	3—4
41	10 I	20	4	60	5	3—26(10%)
42	10 I	20	0	70	5	3—36
43	10 I	22	—9	6,5	0	3
44	10 I	22	—30	27,8	18,7	4—36(14%)
46	16 I	20	—9	5	0	0—6
47	16 I	20	—9	5	40	4—20(5%)
48	16 I	20	—9	5	30	4—20(10%)

LEGEND: a) No of experiment; b) Date of experiment; c) Number of caterpillars; d) Captivity temperature in oC; e) Reaction following thawing; f) number of active animals in %; number of animals reacting to elec. shock in %*; h) Lifespan in days; i) Freezing; j) hour; k) day

* Immobile caterpillars.

down to -196° under the following conditions. Diapaused corn borer caterpillars, caught in the Stavropol'skiy Kray, were placed under various temperature conditions for long periods (Table 1). Upon keeping the caterpillars at 0° and 4° for 3-4 weeks, their resistance to deep chilling rose sharply, the number of active caterpillars chilled first to -30° and then to -196°, following thawing was 60-70%, and those chilled down to -78° -- 100%. Caterpillars not previously cooled down to -30° survived at -78°, but perished at -183° and -196°. Without preliminary cold hardening at a temperature of around 0°, only a small percentage of the caterpillars survived following exposure to -78° and only individual caterpillars reacted to the electric stimulus after undergoing exposure to -196°. Temperatures below 0° do not promote hardening. The caterpillars, following preliminary deep chilling, were kept at a temperature of 22°, which promoted reactivation. However, as further experiments showed, the highest survival rate of the caterpillars chilled down to -78° was reached when they were kept for a month at 0° (Table 2). In two experiments, 11 out of 30 chilled caterpillars chrysallized, resulting in eight butterflies -- males and females, some of which deposited eggs. Thus far, following such chilling only 50% proved to be active caterpillars, which after the period of one to one and a half months perished.

TABLE 2

Effect of Captivity Conditions Before and After Freezing on Caterpillar Resistance to -78°

№№ опытов	Даты опытов	Число гусениц	Температура содержания в °C: до охлаждения	Температура содержания в °C: после охлаждения	Реакция после оттаивания	Выживаемость и развитие
23	30 XI	20	22	22	5% реагируют на эл. ток	Погибли через 1 сут.
22	30 XI	20	10	22	25% двигаются, 15% реагируют на эл. ток	Жили от 2 до 34 сут.
24	30 XI	21	4	22	76.2% двигаются, 14,3% реагируют на эл. ток	Жили от 5 до 55 сут.
[illegible]	30 XI	20	0	До 28 XII 0, с 28 XII 22	100% двигаются	40% окуклилось, 30% бабочек
[illegible]	30 XI	10	–2	То же	100% двигаются	30% окуклилось, 30% бабочек
21а	30 XI	10	–2	22	100% двигаются	Жили от 2 до 28 сут.

TABLE 3

Caterpillar Resistance to Deep Freezing for Various Sequences, Rates, and Lengths of Freezing. Captivity Temperature = 4°.

№№ опытов	Даты опытов	Число гусениц	Последовательность охлаждения	Реакция после оттаивания	Продолжительность жизни в сут.
22	15 XII	20	30 мин. –30° 2 сут. –78° 120 мин. –196°	55% двигаются, 15% реагируют на эл. ток	4–41
24	23 XII	20	60 мин. –30° 60 мин. –78° 120 мин. –196°	50% двигаются, 30% реагируют на эл. ток	6,7–30
53	26 XII	20	30 мин. –30° 30 мин. –60° 60 мин. –100° 130 мин. –196°	80% двигаются, 10% реагируют на эл. ток	7–36
25	26 XII	20	60 мин. –30° 60 мин. –78° 120 мин. –196°	0% подвижных, 5% реагируют на эл. ток	4–6
27	27 XII	20	60 мин. –30°	0% подвижных, от 15 до 25% реагируют на эл. ток	до 10 6–10
26	28 XII	20	60 мин. –78°	53,3% двигаются, 33,3% реагируют на эл. ток	

[LEGENDS to Tables 2 and 3 on page 5]

The chilling conditions (rate, succession of temperature changes) is of much importance in testing for deep freezing. From the data in Table 3 it is clear that a temperature of -50° results in the death of the caterpillars, that is, the intermediate temperature of -50° for the corn borer is more damaging than -78° and even -196°. The reasons for this have not yet become clear, but it is probable that they stem from the character of water crystallization in the tissues.

The length of residence of the caterpillars at a temperature of -78° essentially does not affect their survival rate: for chilling from 30 minutes to five days 100% of live caterpillars were obtained following thawing, and after 25 days of chilling -- 79.2%. Consequently, there is a possibility of preserving in a state of anaviosis whole complex animals, containing from 65-75% water, that has been converted into ice.

We can judge the condition of the cells and organs following deep freezing from dissection of the caterpillars after several hours subsequent to thawing. For caterpillars not showing spontaneous movement following the freezing, motor reactions can be observed in response to electrical stimulus, as well as pulsations of the heart and intestines, respiration and the formation of neutral red granules. Therefore, total immobility following freezing is not a criterion of death; movement can be restored in one to two days following thawing.

Conclusions

The resistance of insects to deep freezing results from many conditions both preceding and during the freezing, as well as following thawing. Chief among these is adaptation to below zero temperatures. This stems from the following: 1) phylogenetic adaptation of the species to low temperatures; 2) seasonal change in cold resistance related first of all with diapause; 3) ontogenetic adaptations -- the process of hardening under the influence of ecological factors during the start of hibernation. The adaptation of whole insects to low and ultra-low temperatures is the consequence of those changes occurring in the cells.

Slow chilling increases the survival rate of the insects, but there are dangerous temperature zones which the organism evidently must "traverse" rapidly. The rate of warming is not essentially important for the insects. Of great interest is the study of the conditions under which the repairing of damage arising under the influence of low temperatures takes place.

The resistance of insects to ultra-low temperatures is possible only due to the ability of the tissues and cells with normal water content to survive freezing and the total cessation of metabolism. Vitrification was absent in our experiments. Analysis of the reasons for such stability is reserved for future studies.

The resistance of highly organized animals to deep freezing indicates that diverse cells of various tissues and entire organisms can withstand the environmental conditions which they did not exist under during the process of evolution, and that arising in the cells during the process of adaptation is such a reserve of resistance to extreme influences as would not be necessary for conditions of earthly existence.

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/LEGEND to Table 2/

a) No of experiment; b) Date of experiment; c) Number of caterpillars; d) Captivity temperature in °C; e) before freezing; f) after freezing; g) Reaction following thawing; h) Survival rate and development; i) reacts to elec. current; j) moves about; k) before 28 Dec, 0° C, since 28 Dec, 22°C; l) Perished in 1 day; m) Lived from 3 to 34 days; n) chrysallised; o) butterflies; p) Lived from 2 to 23 days; q) As above.

/LEGEND to Table 3/

a) No of experiment; b) Date of experiment; c) Number of caterpillars; d) Sequence of freezing; e) Reaction following thawing; f) Lifespan in days; g) minutes; h) days; i) move about; j) react to elec. current; k) up to 10.

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