protein content and the hæmolymph protein concentration are much less at high temperature. Directly after transfer to the lower temperature, the amino-acid concentration decreases, while the fat body and hæmolymph proteins and the protease activity in the midgut start to increase. If the insects are decapitated immediately after transfer, the hæmolymph amino-acid concentration does not decrease to the normal level, even 13 days after transfer. However, even after transfer to normal temperature, the levels of these metabolites and of oxygen consumption do not revert to normal; this is correlated with the longer period required for development under these conditions.

It is concluded that high temperature interferes mainly with protein synthesis. This is reflected in the inhibition of cell division, the impairment of differentiation, the reduction in midgut protease activity and the cessation of secretion of the brain hormone which may prove to be a protein or a proteinaceous compound. This compares with Wigglesworth's suggestion that the characteristic defect in the resting or dormant insect, where no moulting hormone is being secreted, is a failure of protein synthesis[6].

Preliminary investigations on the inhibition of reproduction in both sexes at high temperature revealed signs of inactivity of the corpus allatum.

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## A Large Amount of Trehalose in a Frost-resistant Insect

With a few exceptions[1], almost all the remarkably frost-resistant insects have been shown to contain glycerol or sorbitol[2]. The overwintering pre-pupal larvæ of a sawfly, *Trichiocampus populi* Okamoto, however, were found to survive freezing at a liquid gas temperature without any polyhydric alcohol. An extraordinarily large amount of a sugar, trehalose. was detected in this insect.

*T. populi* is one of the common posts of poplar trees. The full grown larvæ of this insect leave the food-plant in autumn and pass the winter as prepupæ in withered plants. The body weights of the prepupæ were more than 30 and 80 mg in male and in female respectively. Since some of them are found in small holes or slits in the twigs of some trees or shrubs, and remain uncovered by snow during the coldest season, they are exposed to severe cold. In fact the overwintering prepupæ were found to survive freezing at −30° C for many days. As the supercooling point in the prepupæ is −8·6° ± 0·4° C, and the highest freezing temperature is about −3° C, they conceivably freeze and thaw many times during the winter.

Our earlier work showed that an animal could survive freezing even at liquid gas temperatures provided it could withstand prefreezing at −30° C (refs. 3 and 4). Our prefreezing method was also applied to the overwintering prepupa of *T. populi*. Four groups of ten prepupæ were subjected to freezing in air at −30°, −25°, −20° and −15° C respectively for 1·5 h. One other group was also frozen at −30° C as control. All groups of the insects, except the control, were then immersed directly in liquid nitrogen. After being kept there for 2 h they were rewarmed in air at −30° C for 30 min, and transferred into air at room temperature. As shown in Table 1, the prefreezing treatment at temperatures below −20° C were sufficiently effective to enable the insect to withstand a very low temperature. It is of interest that pre-freezing proved to be as effective at −20° in the sawfly prepupa as at −30° C, because in extremely frost-resistant insects so far known, pre-freezing has been found to be far less effective at a temperature above −25° C than at −30° C. After re-warming from the liquid nitrogen temperature, most of the prepupæ survived for more than 100 days. Many of them were able to resume development even up to the formation of the imago, but could not shed their pupal skins. This was also the case in other insects in which our pre-freezing method had been successfully applied.

Table 1. Prefreezing Temperature and Survival in the Sawfly Larvæ after Cooling to and Thawing from the Liquid Nitrogen Temperature

| | Prefreezing at (° C) | Prefreezing for (h) | No. insects used | No. surviving insects |
|---|---|---|---|---|
| Control* | −30 | 4·0 | 10 | 9 |
| 1 | −30 | 1·5 | 10 | 9 |
| 2 | −25 | 1·5 | 10 | 10 |
| 3 | −20 | 1·5 | 10 | 10 |
| 4 | −15 | 1·5 | 10 | 0 |

* Prefreezing only.

The possession of small-molecule neutral substances has been thought to favour survival during freezing[2,5]. The protective substance has been shown to be sugar, mainly sucrose, in higher plants, and polyhydric alcohol, mainly glycerol, in insects. No glycerol was found in the prepupa of *T. populi*, in which a remarkably large amount of sugar was detected. The total sugar content of the overwintering prepupæ ranged from 5 to about 9 per cent of the fresh body-weight (average more than 6·3 per cent). It was nearly constant throughout the 4 months cold season. The termination of diapause, therefore, seems to have no direct effect on the high sugar content in this insect, because the prepupæ were generally released from diapause by the beginning of January.

The sugar in the prepupæ was identified by a chromatographic method[6]. In midwinter about 97 per cent of the sugar content was the non-reducing disaccharide, trehalose, which has recently been shown to be the major blood sugar in a variety of insects[7]. As the water content of the prepupa of *T. populi* was found to be about 65 per cent, the concentration of sugar in solution in the insect must be about 9 per cent. So far as we are aware, this is a higher concentration of trehalose than has previously been recorded in any insect. The concentration of sugar in these sawfly larvæ is comparable with that of the sum of polyhydric alcohol and sugar in very frost-hardy plants[8].

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# MICROBIOLOGY

## Lysogenic Conversion in *Staphylococcus aureus*, to a Change in the Production of Extracellular 'Tween'-splitting Enzyme

Observations on death or survival of 554 patients with staphylococcal bacteræmia[1,2] indicated a correlation between virulence in this situation and the lack of diffusible extracellular lipase as investigated on 'Tween'/calcium agar plates. Therefore an attempt was made to discover whether the negative lipase reaction was a