EFFECTS OF SUB-LETHAL HIGH TEMPERATURE ON AN INSECT, *RHODNIUS PROLIXUS* (STÅL.)

V. A POSSIBLE MECHANISM OF THE INHIBITION OF REPRODUCTION

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INTRODUCTION

Since the basic discovery by Wigglesworth (1936) of the regulation of reproduction in *Rhodnius* adults by the corpus allatum hormone, a great deal of attention has been paid to the relationship between the endocrine system and reproductive activity in insects. These researches led to the formulation of some general ideas of how reproduction is hormonally controlled in a wide variety of insects, and detailed accounts of the subject have been published. Thus a wealth of information is available concerning the histochemical, physiological and biochemical changes in the different tissues during the reproductive cycles of insects and the inter-relationships between these changes and the neuroendocrine system (for reviews see Wigglesworth, 1964; De Wilde, 1964; Highnam, 1964; Davey, 1965; Engelmann, 1968). It is, however, surprising how very little is known about the effects on these changes of one of the most important factors of the environment, namely, temperature.

In the previous paper (Okasha, Hassanein & Farahat, 1970) it has been shown how the reproductive activity of *Rhodnius* adults of both sexes is affected by the different temperature regimes. The present paper is an attempt to elucidate how sterility is caused by exposure to sub-lethal high temperatures in the light of current ideas concerning the control of reproduction.

MATERIALS AND METHODS

The method of keeping a culture of *Rhodnius* has been previously described (Okasha, 1968 a). The experimental insects kept at different temperatures were placed in desiccators in which the relative humidity was maintained at 60% using NaOH solutions according to Madge (1961). The desired temperature for a given experiment was controlled by keeping the insects in an electrically heated incubator.

Decapitation was carried out by cutting through the neck, thus removing the corpus allatum, corpus cardiacum and the brain, and sealing the wound with a mixture of wax and resin just above melting point.

Histological studies were conducted according to conventional histological techniques as given in Pantin (1948), and using agar-ester wax for embedding.

The method used for the determination of the respiratory rate was that described

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in Okasha (1968*d*). Respiration of individual insects was measured and nine insects of each sex were used in each treatment. The insects were fed at 3 weeks after emergence and the same insects were used throughout the experiment. To express the respiratory rate on a fresh-weight basis each insect was weighed to the nearest 0·1 mg just before being placed in the respirometer.

<div align="center">RESULTS</div>

<div align="center">*Critical temperature for the inhibition of reproduction*</div>

To determine the threshold temperature at which reproduction is completely inhibited, adult insects (4 weeks after emergence) of both sexes were exposed immediately after feeding to a series of different temperatures: 32, 33, 34 and 35 °C, each combined with 60% r.h. The females were trans-illuminated before feeding to ascertain that those chosen for the experiment did not contain ripe eggs. Very few eggs were produced at 33 °C, and no ripe eggs were either formed (as tested by trans-illumination) or deposited at 34 °C. All the experiments described here where thermal treatment is involved were therefore performed at the latter temperature. Although this critical temperature applies in the case of the female insect, it is not to be considered the threshold temperature below which the male is fertile. From the results reported in the previous paper (Okasha *et al.* 1970) it is quite obvious that the male is more heat-sensitive than the female.

<div align="center">*Changes in the reproductive system during exposure to high temperature*</div>

Adults of both sexes were exposed to 34 °C for 7 days immediately after feeding, and control experiments were performed at 28 °C using insects from the same batch. When insects of the first group were dissected, the ovaries were found to be very small, and there were no ripe eggs in the genital tracts of the females; however, in the males, the testes were not much reduced in size compared with those of normal insects. Histological examination revealed that in the female the oocytes were developing normally until the stage of yolk deposition, but were then resorbed and the follicular epithelium was disorganized. In the males, the testes appeared normal and packed with sperm in different developmental stages. Gross anatomy showed that the vesiculae seminalis seem to be normal, and when they were teased out in Ringer and examined microscopically the spermatozoa were motile. The accessory glands, however, were not filled with secretion and were thin and attenuated. The results of this experiment are exactly similar to those described by Wigglesworth (1936) for both sexes of *Rhodnius* adults either when deprived of the corpus allatum or when starved.

It appears, therefore, that the corpus allatum is not active in adults kept immediately after feeding at 34 °C; or, if active, then either the juvenile hormone secreted is broken down or the target organs are not responsive. But it must be pointed out here that the state of the reproductive system in both sexes before feeding and exposure to 34 °C was exactly the same as that observed in heat-treated insects (Wigglesworth, 1936). In another experiment it was found that adult females exposed for 6 days directly after feeding to 34 °C will start producing ripe eggs a week after transfer to 28 °C (although about 10% will not produce eggs unless fed again). Twenty such females were decapitated directly after transfer to 28 °C and were kept for 16 days at the latter

temperature; none produced or formed ripe eggs. This clearly shows that the presence of the head is necessary for the resumption of egg maturation after transfer to normal temperature, and this is considered as evidence for the involvement of the corpus allatum in this process. It seems as though sterility induced by heat-treatment is comparable with naturally occurring imaginal diapause (Lees, 1955; see also Wigglesworth, 1964; De Wilde, 1964).

Metabolic changes during exposure to high temperature

The general metabolic rate as determined by the course of oxygen consumption during the reproductive cycle was studied, both in normal insects and also in insects in which reproduction was inhibited by heat-treatment.

The course of oxygen consumption of normal insects is shown in Fig. 1. This figure indicates that in the female insect the respiratory rate rises for the first 5 days after feeding, when it reaches its maximum. Then the rate gradually decreases, almost in a linear manner. By 9 days after feeding the rate of respiration returns approximately to that of 1 day after feeding.
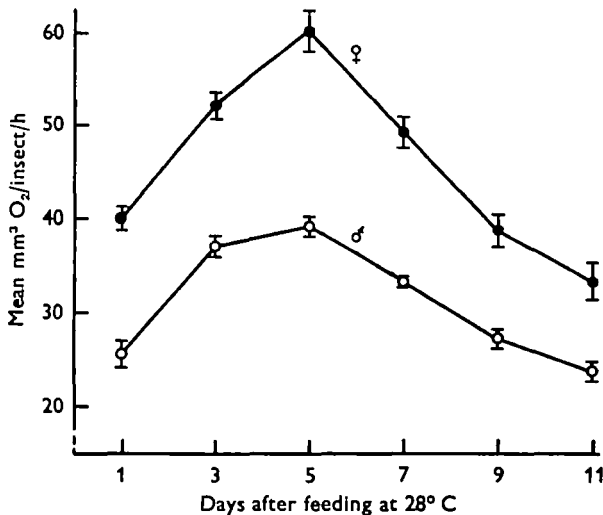

Fig. 1. The course of oxygen consumption of adults kept permanently at 28 °C and their respiration measured at the same temperature. Each point represents the mean of nine insects which were used throughout the experiment. The vertical lines represent the standard error.

In the male insect, however, the peak of oxygen consumption at 5 days after feeding is not far from the level at 3 days, but is definitely higher than that of the same insects 1 day after feeding. By 9 days after feeding, as in the female, the rate of oxygen uptake returns to a level approximately the same as that characteristic of 1 day after feeding.

Fig. 2 shows the course of oxygen consumption of adults placed immediately after feeding at 34 °C, respiration being measured at the same temperature. From this figure it can be seen that high temperature has a differential action on the pattern of the respiratory rate of *Rhodnius* adults. In all the nine females used, the rate of oxygen consumption increased for the first 3 days after feeding. Thereafter, a sharp decrease

set in and the rate reached a relatively low level by 9 days after feeding, when the experiment was terminated. The males, on the other hand, showed a steady decrease in their rate of oxygen uptake during the whole period of the experiment. At 3 days after feeding, six males out of nine had a lower rate of respiration than on the first day, the rates of two remained unchanged, and one male showed a very slight increase.
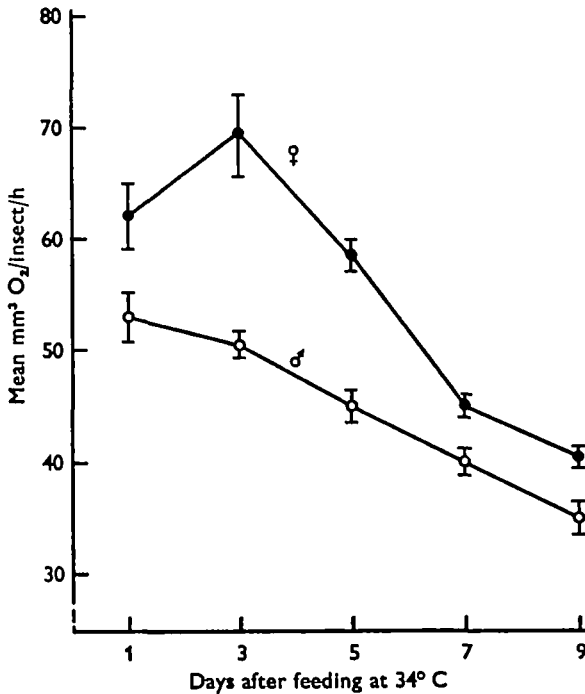

Fig. 2. The course of oxygen consumption of adults kept at 34 °C immediately after feeding and their respiration measured at the same temperature. Each point represents the mean of nine insects which were used throughout the experiment. The vertical lines represent the standard error.

It is of some interest to note the differences in the rate of respiration of the females compared with the males under the two different temperature regimes used here. Sexual differences have been noted in other insects. In *Periplaneta americana* adults the oxygen consumption/g fresh weight is *c.* 30% higher in the females than in the males (Richards, 1963). Similar results have been recorded in the respiration of *Drosophila melanogaster* adults (Kucera, 1934), and in *Galleria mellonella* (Burkett, 1962). In *Calliphora erythrocephala* adults, however, there is no difference in the rate of respiration between the sexes (Thomsen, 1949).

If the results are expressed as mm^3 O_2/insect/h, it will be found that at any given time after feeding, the female has invariably a higher respiratory rate than that of the male, both at 28 and 34 °C (cf. Figs. 1, 2). However, if the rate of oxygen consumption is expressed on a fresh weight basis, the sexual differences become less pronounced at 28 °C especially during the early part of the reproductive cycle (Fig. 3). This can be attributed, at least in part, to the fact that females are always heavier than males.

But it must be pointed out that the expected increase in body weight of the female insect due to egg formation and the weight loss due to oviposition complicate the situation, besides any possible differences in the rate of water loss due to differences in size of the sexes.
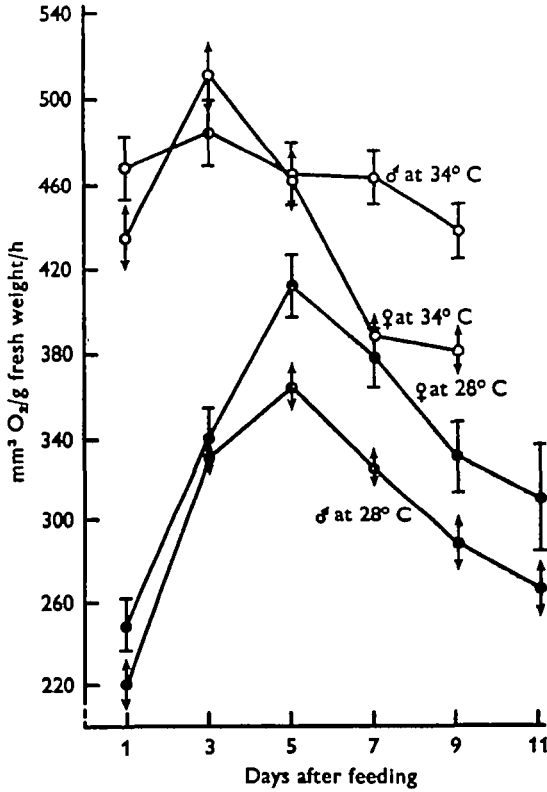

Fig. 3. The course of oxygen consumption of adults at both 28 and 34 °C calculated on a fresh weight basis. Data are originally those documented in Fig. 1 and Fig. 2.

At 34 °C the rate of oxygen consumption of the males, calculated on fresh weight basis, remains more or less constant, perhaps tending to decrease slightly; that of the females, however, rises during the first 3 days after feeding and then declines steeply (Fig. 3). The sexual differences are not manifested during the early period of exposure to 34 °C, but at 7 and 9 days after feeding the females exhibit a lower rate of respiration than that of the males. This state of affairs is not manifested under normal temperature conditions. It is relevant to remember that in this case complications in changes of body weight of the female due to egg maturation and oviposition do not arise. The significance of these results is difficult to perceive, but it may possibly be related to differential effects of high temperature on the reproductive activity of each sex (see Okasha *et al.* 1970).

DISCUSSION

The results which emerge from the histological and decapitation studies described in this paper provide only cursory information about the mechanism of the inhibition of reproduction by high temperature. It has been shown that in the immature stages of *Rhodnius* the endocrine system responsible for moulting is interfered with by exposure to high temperature (36·5 °C) immediately after feeding, and almost certainly the brain fails to secrete its hormone (Okasha, 1968b). It is tempting to suggest that in the adults high temperature (34 °C) inhibits the secretion of the corpus allatum hormone, just as a slightly higher temperature (36·5 °C) inhibits the secretion of the brain hormone and consequently of the moulting hormone in the larval stages (Wigglesworth, 1952, 1955; Okasha, 1968b).

The secretion of the corpus allatum hormone might be inhibited by heat-treatment directly or indirectly. Indirect inhibition could be ascribed either to inhibition of the secretion of the brain hormone, or to interference with the action of the latter on the corpus allatum. This is based on the assumption that the neurosecretory cells of the brain are involved in egg-production in the female of *Rhodnius*, as appears to have been demonstrated by the work of Davey (1967). At present it is impossible to decide with certainty which of these factors is operating in the case of heat-treated adults. Although the decapitation experiment described in this work demonstrates the necessity of the head for the subsequent resumption of egg formation after transfer to normal temperature, yet it does not differentiate between the various possibilities mentioned above. Detailed studies of the histochemical changes in the neuro-endocrine system coupled with extirpation and implantation experiments would be necessary before this problem can be solved. It may be relevant here to mention that Wigglesworth (1964) could not observe any distinct histological changes in the neuro-secretory cells of the brain or in the corpus cardiacum in *Rhodnius*, but he did not exclude the possibility that such changes could be demonstrated by closer observation.

Another point of interest needs discussion. It has been shown that protein synthesis is impaired in *Rhodnius* larvae exposed to 36·5 °C directly after feeding, that this is the primary reason underlying the arrest of growth and development, and that after transfer to normal temperature conditions growth and development are resumed again, albeit more slowly, through the activation of the endocrine system, protein synthesis and all the other metabolic responses associated with moulting (Okasha, 1968c). By analogy, it is probable that protein synthesis is impaired when reproduction is in-hibited by high temperature, and that after transfer to normal temperature, this activity is resumed in the female, again more slowly than usual, provided the head (corpus allatum?) is left intact. This suggestion might seem to contradict what has been found in the larval instars, since some 90% of *Rhodnius* larvae placed at 34 °C immediately after feeding are able to grow and develop to the next instar (Wiggles-worth, 1952; A. Y. K. Okasha, unpublished); a temperature of 34 °C inhibits the reproductive activity. But it may well be that it is the synthesis of specific adult proteins which is inhibited or impaired at 34 °C. Thus the enzyme systems necessary for the synthesis of the specific adult proteins have a lower heat-inactivation threshold than those necessary for the synthesis of proteins required for growth and develop-ment of the larvae.

From autoradiographic studies Vanderberg (1963) states that in allatectomized *Rhodnius* females there is a drastic inhibition of RNA and protein synthesis in the ovaries, fat body and mid-gut. Working on the same species Coles (1965a) reported that there are two adult-specific proteins in the haemolymph. In addition, Coles (1965b) found that in females decapitated 24 h after feeding, no 'yolk proteins' accumulated in the haemolymph or in the fat body (the site of synthesis), whereas ovariectomy resulted in the accumulation of 'yolk proteins' in the haemolymph. Both authors concluded that the corpus allatum hormone controls the synthesis of 'yolk proteins'. The possibility that high temperature is directly inhibiting the synthesis of specific adult proteins, or indirectly inhibiting it by somehow inactivating the corpus allatum, remains to be investigated.

Working on the larvae of *Rhodnius*, Zwicky & Wigglesworth (1956) correlated the cytological changes that take place in the epidermis at various times after feeding during the moulting cycle with the rate of oxygen consumption and concluded that the latter largely represents the rate of protein synthesis during growth and development. Other studies on *Rhodnius* larvae in which moulting has either been inhibited or temporarily arrested by heat-treatment lend support to this theory (Okasha, 1968c, d). The curve of oxygen consumption during the reproductive cycle (Fig. 1), is an inverted U-shaped curve, thus being similar to that of endopterygotes during the pupal stage, and that of *Rhodnius* larvae when the descending part of the curve during moulting is combined with the ascending part of the curve during the next moulting cycle (Zwicky & Wigglesworth, 1956). Following the same line of thought, the course of oxygen consumption during the reproductive cycle could be regarded as a curve representing protein synthesis. Indeed, histochemical studies concerning changes that occur in the follicular epithelium of the ovary in *Rhodnius* during the reproductive cycle as described by Patchin & Davey (1968) support this view. It has been found, moreover, that the rate of oxygen consumption mirrors the proteolytic activity in the mid-gut of *Rhodnius* adults at various times after feeding (A. Y. K. Okasha, unpublished) and proteases are themselves proteins (see Thomsen & Møller. 1963). Thus, the reproductive activity in *Rhodnius*, which goes through cycles whose frequency is governed by the availability of a large blood-meal, is paralleled by cyclical changes in the rate of oxygen consumption, mid-gut protease activity and protein synthesis. If the above-mentioned interpretation is correct, then the different effects of high temperature upon males and upon females observed in the course of oxygen consumption may be considered as reflexions of the rates of syntheses of the specific adult proteins.

The chain of events after transferring previously heat-treated adults to normal temperature (see Okasha *et al.* 1970) can be visualized as follows:

(i) *In the case of the female.* The corpus allatum would be activated, perhaps more slowly than normally, during the first reproductive cycle that follows transfer, and in a normal manner during the subsequent cycles. Consequently, the female would be able to resume egg formation, 'yolk protein' synthesis, egg maturation and oviposition.

(ii) *In the case of the male.* There is no reason to believe that there would be a sexual difference in the heat-sensitivity of the corpus allatum, although such a possibility cannot be entirely excluded. If it is assumed that the corpus allatum hormone would be secreted in the male and yet the latter is permanently sterile, then the main reason

for sterility may be due to an irreversible impairment of spermatogenesis. In *Ephestia kühniella*, Norris (1933) obtained evidence that interference with spermatogenesis is the reason underlying the sterility of 100% of males exposed to 30 °C. It is possible that in *Rhodnius* this might be coupled with lack of secretion in the accessory glands in case of the corpus allatum remaining inactive after transfer or its hormone being ineffective.

SUMMARY

1. The reproductive activity in both sexes is completely inhibited during the exposure of adults to 34 °C immediately after feeding. The changes that take place in the reproductive system in males and females during exposure parallel those brought about by starvation or allatectomy.

2. Females exposed for 6 days to 34 °C immediately after feeding, then decapitated and placed at 28 °C, fail to form eggs, while those left with the head intact form mature eggs.

3. The course of oxygen consumption of normal males and females is compared with that of insects placed immediately after feeding at 34 °C and their respiration measured at the same temperature.

4. It seems that the corpus allatum is not active at high temperature, or if active its hormone is ineffective. It is suggested that it is the synthesis of specific adult proteins which is interfered with by high temperature, hence the inhibition of the reproductive activity.

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