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

IV. EGG FORMATION, OVIPOSITION AND STERILITY

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INTRODUCTION

It has long been known that the physical factors of the environment exert profound effects on the various physiological activities of insects, and that temperature is one of the most important of these factors (Uvarov, 1931; Wigglesworth, 1953; Bursell, 1964). Within a biological range the reproductive activity of insects, like any other biological process, is enhanced by rise in temperature, but at a certain point, characteristic of the species, this activity ceases and sterility is induced by high temperature, although other processes seem to function normally (Bursell, 1964). The mechanism by which this sterility is brought about is little studied in insects.

In a series of publications the effects of exposing *Rhodnius* larvae to sub-lethal high temperature have been reported (Okasha, 1968*a–d*). It was found that exposing these larvae either before or after feeding results in a delay during the subsequent moulting under normal temperature conditions. These studies also led to the formulation of the mechanism underlying the inhibition of growth and moulting caused by high temperature.

The present paper describes experiments designed to study the effects of exposing *Rhodnius* adults to sub-lethal high temperature, in an attempt to discover how the reproductive activity of both sexes is affected by heat-treatment following transfer to normal temperature conditions.

MATERIALS AND METHODS

The method of maintaining a culture of *Rhodnius* and the details of exposing the experimental insects to different temperatures are similar to those described by Okasha (1968*a*). The sub-lethal high temperature used in this work is 34 °C, combined with 60% relative humidity, and this is referred to as 'high temperature' unless otherwise stated. This is the threshold temperature at which egg production is completely inhibited in females exposed to it immediately after feeding (Okasha, 1970). The control experiments were carried out in a humid incubator (*c.* 60% r.h.), adjusted to 28 °C, and this is termed 'normal temperature'.

Since starvation affects the reproductive activity in *Rhodnius* adults (Wigglesworth 1936), all the insects used in the present work were of known, and almost of the same,

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age (10–14 days after emergence), and when they were allowed a blood meal they were left to feed to repletion.

The reproductive activity of control insects was studied over a period covering four reproductive cycles which were spaced at intervals of 15 days between successive blood meals. The same was carried out on heat-treated insects after transferring them to normal temperature.

To study egg production during a given cycle, the number of eggs deposited by each female was counted daily and the eggs were removed to separate specimen tubes and kept under the same conditions. The fertility of the eggs produced, as determined by their hatchability, was also recorded. Towards the end of some cycles there were very few eggs in the reproductive tracts of some females. These few eggs were deposited and are included in the calculations and diagrammatic representation of the next cycle. This naturally results in a slightly lower figure representing the average egg production in the first cycle. However, it can be safely stated that this does not appreciably alter the figures representing the average egg output of the second cycle, since such figures are compensated for by the third cycle, and so on. Corrected values, however, are also mentioned where relevant.

Since copulation affects the rate of egg deposition (Coles, 1965; Davey, 1965, 1967), the females were allowed access to males. Thus in normal insects and in those heat-treated after feeding, each female was put together with a male from the same batch in a specimen tube to allow copulation. In the case of insects heat-treated before feeding, the insects were kept individually during exposure to high temperature, then two insects, one of each sex, were put in one tube after feeding and transferring them to normal temperature. In those experiments where a freshly fed normal male was introduced to heat-treated insects, each tube contained after transfer a heat-treated male, a heat-treated female and a normal male. In each experiment an equal number of males from the same batch as that of the female was used.

Although there is some uniformity in the commencement of egg laying, the females did not start ovipositing on the same day. Thus the day on which 50% or more of the females oviposited was considered to be the day of the commencement of egg laying of the whole group of insects.

RESULTS

Pattern of egg laying in normal insects

The pattern of egg laying of normal insects kept permanently at 28 °C is represented in Fig. 1.

During the first cycle egg laying did not start until 6 days after feeding when 60% of the females oviposited, and a day later this figure rose to 90%. During the second cycle only 20% of the females laid one egg each 5 days after feeding, but a day later 70% of the females deposited quite a number of eggs (Fig. 1). The same applies also in the case of the third cycle; 20 and 50% of the females started oviposition 5 and 6 days after feeding respectively; the corresponding figures during the fourth cycle are 10 and 60%. The day of commencement of egg laying is shown in Table 1, together with the average egg production per female in each cycle.

It is important to note that the eggs produced over the four reproductive cycles covered by the experiment are invariably fertile.

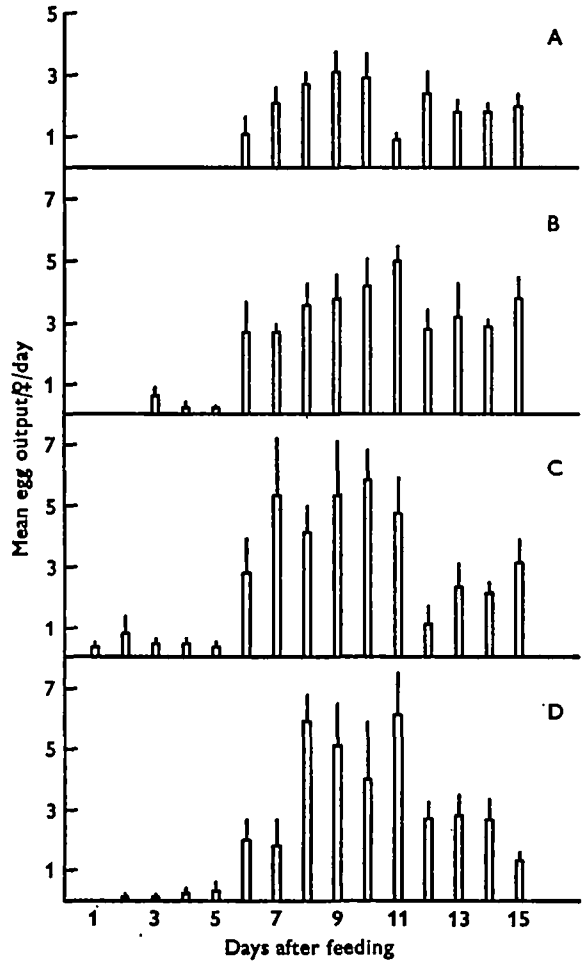

Fig. 1. Pattern of egg laying of normal females at 28 °C. Diagrams A, B, C and D represent the first, second, third and fourth reproductive cycles respectively. Each column is the mean of ten females. Vertical lines on top of columns represent the standard error.

Pattern of egg laying in heat-treated insects

Since unfed insects are physiologically different from fed ones, it was necessary to differentiate between any possible differences in the reproductive activity of heat-treated *Rhodnius* adults, depending on whether exposure to 34 °C was performed prior to or following feeding. This possibility was tested in the following way.

Exposure before feeding

Adults of both sexes were exposed to 34 °C for 15 days, then they were fed and kept permanently at 28 °C, and the data were recorded as described above. The results of this experiment are shown in Fig. 2 and Table 1.

It is quite clear from these results that the reproductive activity of the females during the first cycle after transfer to 28 °C is adversely affected by the previous heat-treatment. Egg laying is greatly delayed. It starts 13 days after feeding (77·8 % of the

Table 1. *Effect of exposure of adults to high temperature on subsequent oviposition, egg production and sterility at normal temperature*

Treatment	No. of females	Reproductive cycle	Average egg output ±S.E. per female	Range of egg production per female	% of sterility of eggs produced	Commencement of egg laying (days after feeding)
Normal temperature	10	First	20·9±1·9	11–29	0·0	6
		Second	35·7±1·5	31–46	0·0	6
		Third	38·8±2·0	29–50	0·0	6
		Fourth	35·1±1·8	28–48	0·0	6
Heat-treated before feeding	9	First	4·4±0·6	3–8	75·0	13
		Second	38·4±3·0	22–53	63·0	5
		Third	36·1±1·9	27–46	75·1	4
		Fourth	40·3±1·8	31–51	73·0	5
Heat-treated after feeding	14	First	18·1±2·3	4–31	79·9	9
		Second	48·3±2·9	37–60	93·0	5
		Third	38·2±1·9	28–50	95·0	5
	10♀ or 1	Fourth	36·8±2·7	28–58	97·3	5
Heat-treated before feeding; normal males present	7	First	0·0	—	—	—
		Second	31·0±1·9	25–37	10·1	5
		Third	36·7±1·7	31–43	8·2	5
		Fourth	36·4±2·3	30–45	5·5	5
Heat-treated after feeding; normal males present	11	First	26·5±1·9	19–37	14·8	9
		Second	43·2±2·9	32–68	9·3	5
		Third	40·3±1·8	32–51	5·0	6

females), while in normal females it starts 6 days after feeding (cf. Fig. 1, 2; Table 1), i.e. there is a period of delay approximately equivalent to half the period of exposure to high temperature. Another deleterious effect of exposure is the excessive reduction in the number of eggs deposited by such treated females during the first cycle at 28 °C, egg production being only 21 % of that of the corresponding cycle in normal insects. Even after correction, that is, after allowing for the number of eggs belonging to the first cycle which were oviposited after the insects were given the next blood meal (those deposited before 5 and 6 days after feeding in heat-treated and normal insects respectively), egg production of heat-treated females is only 23 % of that of normal insects. In fact, the reduction in egg output during the first cycle could be greater than this, since in another experiment (to be mentioned later) egg production during the first cycle was completely inhibited when the insects were exposed to 34 °C for 2 weeks before feeding.

During the second, third and fourth cycles, however, both the total egg output (Table 1) and the frequency of egg laying (Fig. 2) are apparently normal. Also it should be noted that the commencement of oviposition during these cycles is no longer delayed; it occurs a day or two earlier than in normal insects (Table 1).

Another point of interest is the reduced fertility of the eggs produced during the period covering all the cycles studied. The percentages of sterile eggs produced during the different cycles are given in Table 1. This sterility is attributed to harmful and irreversible effects exerted by the exposure to high temperature on the males as discussed below.

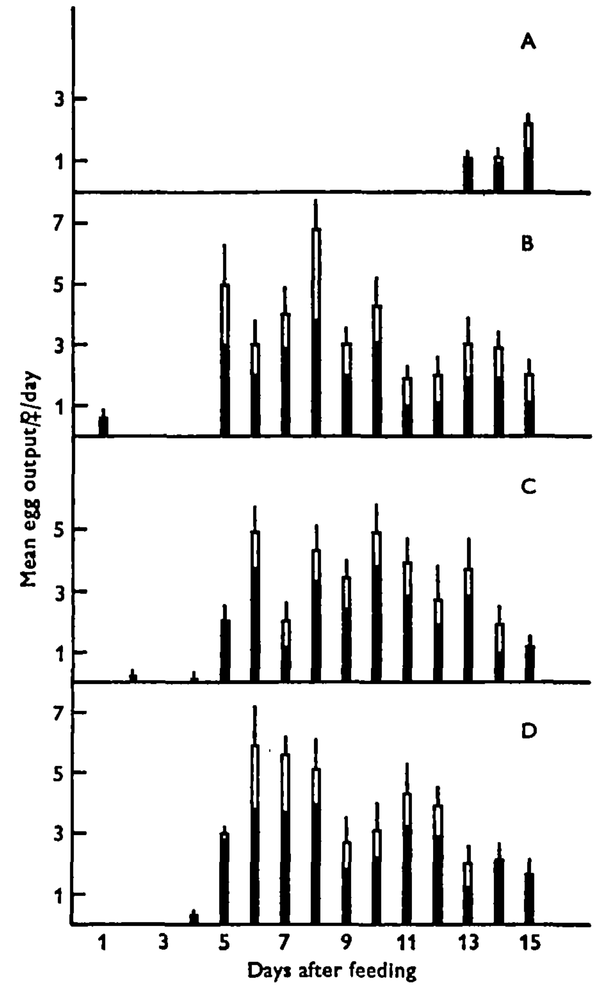

Fig. 2. Pattern of egg laying at 28° C of females exposed to 34 °C for 15 days before feeding. Diagrams A, B, C and D represent the first, second, third and fourth reproductive cycles respectively. Each column is the mean of nine females. White columns represent fertile eggs, black columns sterile eggs, and vertical lines on top of the columns the standard error.

From this experiment it can therefore be concluded that heat-treatment before feeding results in a different pattern of egg formation and deposition under normal temperature conditions during the first reproductive cycle; egg formation is greatly reduced and egg deposition is greatly delayed, probably due to a delay in egg formation. It can also be concluded that this effect on the female is temporary, persisting only for a limited period (which presumably depends upon the length of the period of exposure to high temperature), after which the reproductive activity of the female reverts back to normal in so far as egg formation and oviposition are concerned.

Exposure after feeding

In this experiment the insects were exposed to 34 °C for 15 days immediately after feeding, were fed again and transferred to 28 °C, and the following four reproductive cycles were studied at 28 °C as previously described. As expected, ripe eggs were neither formed nor deposited during the exposure period. The results of this experiment are shown in Fig. 3 and Table 1.

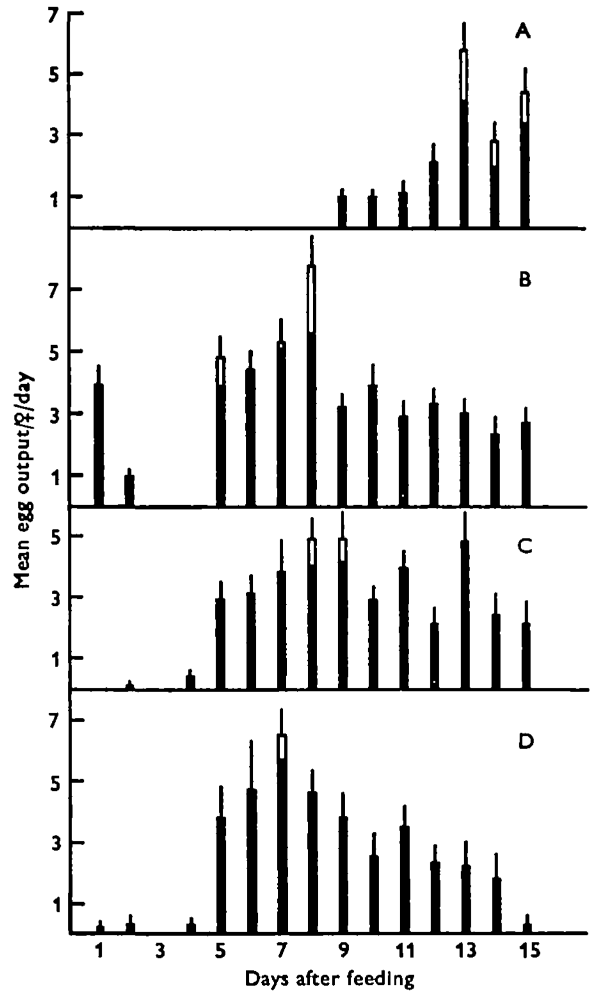

Fig. 3. Pattern of egg laying at 28 °C of females exposed to 34 °C immediately after feeding for 15 days. Diagrams A, B, C and D represent the first, second, third and fourth reproductive cycles respectively. Each column is the mean of 14 females except in D where each column represents the mean of ten females only, due to the death of four females. White columns represent fertile eggs, black columns sterile eggs, and vertical lines on top of the columns the standard error.

During the first reproductive cycle egg laying was delayed until 9 days after feeding (Table 1), when 57% of the females started oviposition; it was not until 13 days after feeding that all the insects were ovipositing. Thus the period of delay in oviposition is equivalent to one-fifth of the period of exposure to high temperature. The average egg production per female during this cycle is maintained at 87% of that of normal insects during the corresponding cycle. However, after correction the heat-treated insects show an average egg output per female which is 5% higher than in normal insects. If the average egg production per female during the first cycle of heat-treated insects is compared with that of the second cycle of normal females, it will be seen that the former is only 51% of the latter; the corresponding figure after correction is 62%. The basis for such a comparison is that egg production during the first cycle in heat-treated insects and that during the second cycle in normal insects are both the consequence of the second blood meal during the adult life of the insect.

During the second, third and fourth reproductive cycles the commencement of egg laying and the average egg output per female are not markedly different from normal (Table 1), although oviposition starts a day earlier in all of the three cycles. During the second cycle the heat-treated insects produce 18% more eggs than normal insects (after correction).

The progressive increase in sterility of eggs deposited during all the four reproductive cycles (Table 1) is comparable to the results obtained in the previous experiment. The reason for this sterility can be attributed to the irreversible effects of high temperature on the males.

From these results it is concluded that exposure of *Rhodnius* adults to 34 °C immediately after feeding results in a delay in oviposition only during the first reproductive cycle that follows feeding and transfer to normal temperature conditions, but that the average egg production per female is not seriously affected. This is also a temporary reaction in the female insect, since the commencement of oviposition reverts back to the normal pattern.

Cause of sterility of eggs produced by heat-treated insects

The reason(s) underlying the sterility of the eggs produced by either type of thermal treatment described above may be attributed to many factors. It may be due to: (i) the inability of heat-treated males to copulate; (ii) unsuccessful copulation, hence non-fertilization of the ova, e.g. the male passing an empty spermatophore to the female (see Davey, 1958); (iii) the sperm not being released from the spermathecae of the female in an actively motile form (see Davey, 1965) (assuming the male is capable of copulation and of passing into the female a 'normal' spermatophore) or (iv) the presence of some mechanical and/or biochemical changes in the properties of the eggs produced that interfere with the embryonic development, as for example the lack of a necessary metabolite in the eggs or the presence of an inhibitor, or the lack of functional micropyles (Beament, 1946). It should be repeated here that the questions of possible excessive water loss or exposure to unfavourable developmental temperatures in the case of eggs produced by heat-treated insects do not arise, because all the eggs in both types of treatment are deposited and kept permanently under normal temperature conditions.

The following experiments were carried out to test some of the above mentioned hypotheses, by putting freshly fed males, with no thermal treatment, together with previously heat-treated insects.

Normal males with females heat-treated before feeding

Unfed insects were exposed to 34 °C for 14 days after which they were fed and transferred to 28 °C. In each specimen tube containing a heat-treated male and a heat-treated female, an additional normal male which was fed on the same day of transfer was introduced. Data were recorded as previously described, and the results are represented in Fig. 4 and Table 1.

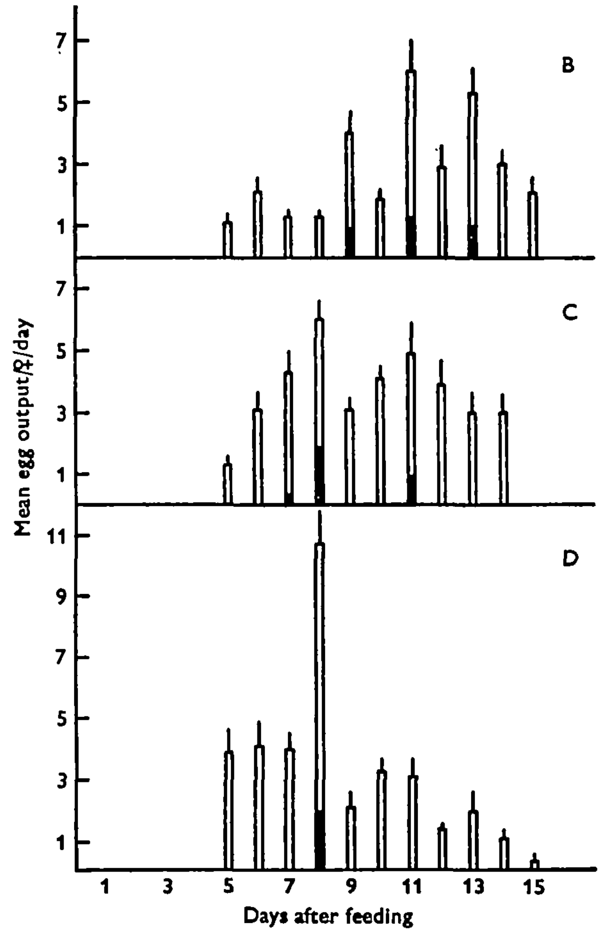

Fig. 4. Pattern of egg laying at 28 °C of females exposed to 34 °C for 14 days before feeding coupled with additional freshly fed normal males after transfer. Diagrams B, C and D represent the second, third and fourth reproductive cycles respectively. Each column is the mean of seven females. White columns represent fertile eggs, black columns sterile eggs, and vertical lines on top of columns the standard error.

No ripe eggs were laid during a period of 15 days, after which the next blood meal was allowed. In a previously described comparable experiment, the average egg output per female was very small and oviposition started after a considerable period of delay. During the second, third and fourth cycles of the present experiment, however, both egg production and the commencement of oviposition seem to be quite normal (Fig. 4, Table 1). There was a great deal of uniformity in oviposition; six out of seven females started oviposition at 5 days after feeding during the second and third cycles, and all seven females at the same time after feeding during the last cycle. This experiment shows that the introduction of freshly fed normal males to previously heat-treated females has no influence on the number of eggs oviposited per female in any cycle. The significance of this finding will be discussed later. It is interesting to note that the sterility of the eggs oviposited dropped to progressively lower levels during cycles 2–4 inclusive (Table 1).

Normal males with females heat-treated after feeding

This experiment was similar to the previous one except that thermal treatment was performed directly after feeding. Exposure to 34 °C was carried out for 14 days after which the insects were fed and permanently kept at 28 °C, together with freshly fed normal males. Recording of the data was carried out as usual and the experiment was terminated after the end of the third reproductive cycle. The results of this experiment are shown in Fig. 5 and Table 1.

Once more it should be noted that oviposition during the first reproductive cycle was delayed until 9 days after feeding in all the 11 females used in this experiment. In general, this agrees fairly well with the previously mentioned results where insects were heat-treated after feeding, except that in the present experiment there was a striking uniformity in the commencement of egg laying. The same phenomenon was also manifested during the second and third cycles; in all the females without exception egg laying started 5 and 6 days after feeding respectively.

This experiment also shows that the introduction of freshly fed normal males to previously heat-treated females does not seem greatly to influence the mean number of eggs produced per female. Although the figures (before correction) presented in Table 1 might give the impression that it does, closer examination reveals that there are no appreciable differences due to the accessibility of these normal males. After correcting (where applicable) for the eggs which belong to the first cycle but which were deposited during the second cycle, it was found that the average production in normal insects, in insects heat-treated after feeding, and in those which were heat-treated after feeding but had access to normal males is 21·7, 22·9 and 26·5 egg per female respectively. The finding that the presence of normal males does not affect egg production is substantiated if the results are expressed as the number of eggs produced per females per cycle, i.e. taking the overall average during the reproductive cycles studied in a given treatment. In the case of heat-treatment after feeding a female produces 35·4 eggs per cycle and only 36·7 eggs per cycle if freshly fed normal males are made accessible to it. In the case of insects heat-treated before feeding the average is 29·8 eggs per female per cycle, whereas it is only 26·0 eggs per female per cycle when they are with normal freshly fed males.

As in the previous experiment the presence of freshly fed males results also in a substantial and progressive increase in the fertility of the eggs produced (Table 1).

From the last two experiments it is concluded that the reason for the sterility of eggs produced at normal temperature by insects previously heat-treated either before or after feeding is the sterility of the males and that sterility cannot be attributed to the quality of the eggs produced or to the failure of the female's spermathecae to activate the sperm if it is there. This point will be dealt with in the discussion.

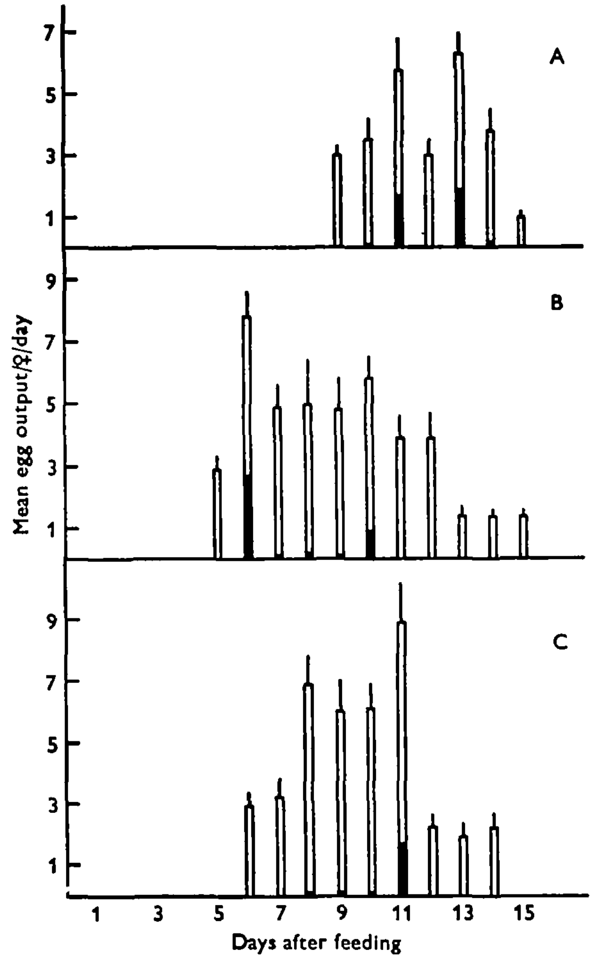

Fig. 5. Pattern of egg laying at 28 °C of females exposed to 34 °C immediately after feeding for 14 days coupled with additional freshly fed normal males after transfer. Diagrams A, B and C represent the first, second and third reproductive cycles respectively. Each column is the mean of 11 females. White columns represent fertile eggs, black columns sterile eggs, and vertical lines on top of the columns the standard error.

DISCUSSION

The foregoing results show quite clearly that exposure of *Rhodnius* adults to sub-lethal high temperature not only inhibits reproduction during the exposure period, but also exerts harmful effects on this activity when the insects are returned to normal temperature conditions. The extent to which these effects are manifested at normal temperature depends on the timing of the application of the thermal treatment. If the thermal dose is applied before feeding, the subsequent ill-effects on the following reproductive cycle of the female insect are more pronounced than if the same treatment is performed after feeding. This, however, does not apply in the case of the male, since the harmful effects are apparently the same whether heat-treatment is carried out prior to or after feeding; sterility sets in and persists permanently or for at least

2 months after returning the insects to normal temperature with regular feeds. The reason for the discrepancy in the rates of recovery of females heat-treated before and after feeding is not clear, but can be regarded simply as a reflexion of the physiological differences that exist between the fed and the unfed insect when heat-treatment is carried out. It is not known whether the fact that the bulk of the volume of a fed insect is in an 'inert' state, consisting mainly of the blood-meal, is an important factor in decreasing the harmful effects during the exposure period. Nor, unfortunately, is anything known about possible differences in the actual body temperature between fed and unfed insects at 34 °C. It is relevant here to note that there are obvious differences in the pattern of the general metabolic rate of the fed and unfed *Rhodnius* larvae in response to exposure to 36·5 °C (Okasha, 1968*d*).

The conclusion that the confinement of normal freshly fed males with females previously heat-treated either before or after feeding did not result in an enhancement of oviposition or in an increase in the average number of eggs produced per female during the reproductive cycles studied, but only caused an increase in the fertility of the eggs produced, is very interesting. It clearly demonstrates that the heat-treated females are not behaving as if they were virgin females as far as egg laying is concerned. It is now established that in *Rhodnius*, as in other insects also, e.g. in *Zeiraphera diniana* (Benz, 1969), mating doubles the rate of oviposition, and that virgin females retain fully formed eggs in their genital tracts (Coles, 1965; Davey, 1965, 1967). It has been suggested that a blood-borne factor from the spermathecae which contain spermatozoa is the primary stimulus for increased egg production in the mated female of *Rhodnius* (Davey, 1965, 1967). The results reported in this paper clearly indicate that whatever factors are involved in increasing egg production by mating they are still operating in the previously heat-treated female. In this connexion it should be pointed out that either the complete or partial failure of females heat-treated before feeding to produce eggs during the first cycle at 28 °C is not a matter of their retaining eggs in the genital tracts; it is certainly due to the failure of the eggs to mature in the ovaries. This is supported by the observation that such females do not start ovipositing during the subsequent reproductive cycle before the normal time lapse necessary for egg formation and oviposition, i.e. 5 days after the second blood meal.

The present results further suggest that males heat-treated either before or after feeding are able to copulate. Unfortunately, copulation was not observed during the course of these experiments, but it may be relevant to mention that in insects kept at 34 °C immediately after feeding, there exist many spermatophores in the container (A. Y. K. Okasha, unpublished observations). If the inference that copulation takes place between heat-treated adults is correct (since the introduction of freshly fed males had no effect on egg production) and yet the eggs produced are mostly sterile over a period of 2 months, then either empty spermatophores are passed into the females or some necessary ingredient in them is lacking. The first possibility does not seem very likely, because according to Davey (1965), *Rhodnius* females into which an empty spermatophore is passed during mating (from males without the vesiculae seminalis or the opaque accessory glands) fail to exhibit the increased egg production which normally follows mating. However, in the present work the possibility that the females used in the various experiments might have had the chance of being mated before feeding and heat-treatment cannot be entirely excluded.

SUMMARY

1. Heat-treatment of *Rhodnius* adults for 14 or 15 days before feeding may either inhibit egg production during the first reproductive cycle at normal temperature, or cause a great reduction in the number of eggs deposited. In the latter case, oviposition is long delayed.

2. During the subsequent cycles commencement of oviposition and egg production seem to be normal.

3. If heat-treatment is performed immediately after feeding for a similar period, subsequent egg production at normal temperature is apparently not affected, while oviposition is slightly delayed. During subsequent cycles, however, the reproductive activity of the females is not markedly different from normal.

4. The majority of eggs produced under either type of treatment show progressive sterility over a period covering four reproductive cycles.

5. Allowing heat-treated females to copulate with freshly fed normal males results in a great increase in the fertility of the eggs produced. Egg-production, however, does not seem to be affected.

6. The significance of these results is discussed.

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