J. Exp. Biol. (1968), **48**, 475–486
With 4 text-figures
Printed in Great Britain

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

III. METABOLIC CHANGES AND THEIR BEARING ON THE CESSATION AND DELAY OF MOULTING

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(*Received* 9 *October* 1967)

INTRODUCTION

It has been shown that exposure of *Rhodnius* larvae to a high temperature which inhibits moulting, either before or after feeding, results in subsequent delayed development under favourable temperature conditions (Okasha, 1964, 1968*a*). During the period of exposure to high temperature, the moulting hormone is not secreted (Wigglesworth, 1952, 1955), most probably due to the failure of the secretion of the brain hormone (Okasha, 1968*b*). In insects which exhibit delayed development after transfer to normal temperature, the changes brought about by the brain hormone and consequently by the moulting hormone, are also delayed. However, *Rhodnius* larvae fail to moult at high temperature even in the presence of the moulting hormone. This has been attributed to the inability of the epidermal cells to divide under such conditions (Okasha, 1968*b*).

It is a well-known fact that during the period preceding cell division vast metabolic processes take place. Whether high temperature is rendering the raw materials necessary for the different synthetic processes inaccessible, or producing a substance(s) inhibiting mitosis, or having some other effect, is not known. The present work is an attempt to investigate the factors underlying the inhibition of both cell division and moulting under high temperature conditions.

MATERIAL AND METHODS

The details of breeding a culture of *Rhodnius* and of exposing experimental insects to different temperatures have already been described (Okasha, 1968*a*). Insects used in each experiment were from the same batch and were fed at about 2 weeks after moulting.

Determination of protease activity in the mid-gut

The method of Charney & Tomarelli (1947) for preparing azocasein as a synthetic substrate was modified by replacing casein by haemoglobin (Technical B.D.H.). The method used can be summarized as follows.

Sixty grammes of haemoglobin were dissolved in 1 l. of Ringer's solution and coupled with diazotized sulphanilamide according to the Charney and Tomarelli

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method. The azoprotein was centrifuged, supernatant decanted, the precipitate washed with water and then alcohol, centrifuged and dried under vacuum. Thirty grammes of the dried azohaemoglobin were dissolved in 500 ml. of 1 % $NaHCO_3$. It was found that it dissolves readily in an alkaline or acidic medium. When first dissolved it was alkaline, then it was made acidic until a clear solution was obtained by adding HCl. The pH was measured and found to be 3·4. This stock solution was kept in a refrigerator.

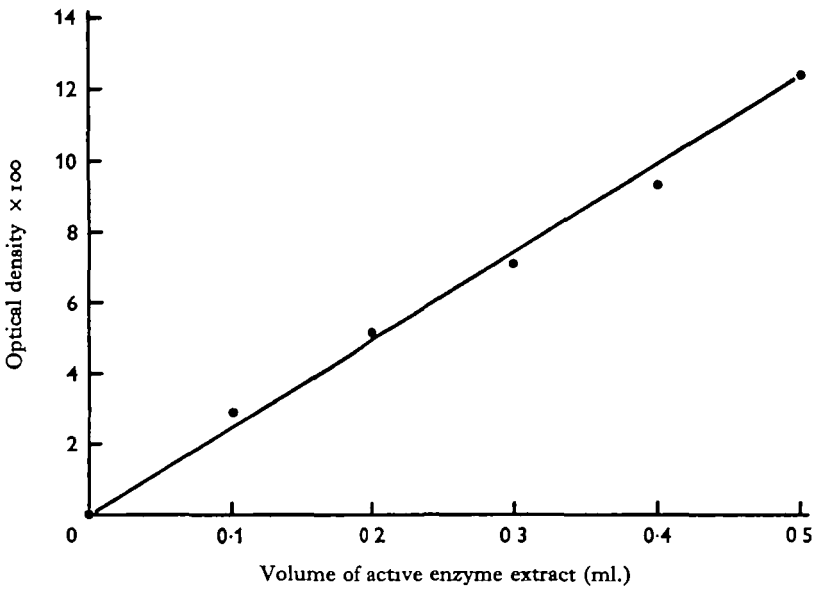

Fig. 1. Relation between the concentration of proteolytic enzymes of the mid-gut and their activity.

The glycerine-phosphate buffer used by Thomsen & Møller (1963) was first tried but proved to be very weak, and citric acid-Na_2HPO_4 buffer was used. The part of the mid-gut between the crop and the rectum was dissected out, put immediately in 1 ml. of ice cold buffer, and stored in the freezer of a refrigerator until used a few hours later. For convenience, this part will be referred to as the mid-gut, three mid-guts being used for each determination. The mid-guts were homogenized in 1 ml. of buffer, and 0·5 ml. of the homogenate was added to 0·5 ml. of the substrate. Two drops of toluene were added to each tube to prevent bacterial activity.

Incubation lasted for 4 hr. in a water bath adjusted to 37° C. and the tubes were shaken during the incubation period. The reaction was stopped by adding 4 ml. of 5 % trichloroacetic acid. Then the contents were shaken, filtered, and 2 ml. of the filtrate were added to 2 ml. of 0·5 N-NaOH. The colour was read using a Unicam spectrophotometer at 400 mμ, at which the maximum absorption takes place, against water as a reference. The optimum pH was determined using a series of citric acid phosphate buffers and was found to be 3·55. All subsequent determinations were performed at the optimum pH and with boiled enzyme extract as a blank.

A linear relationship between enzyme concentration and the corresponding proteo-

lytic activity was found (Fig. 1), the straight line passing through the origin. The variation in the readings obtained from insects in the same physiological state was very small; in most cases the readings were almost identical. This method also worked satisfactorily using the cockroach *Periplaneta americana*.

Determination of amino acid concentration in the haemolymph

The α-amino nitrogen was determined in samples of haemolymph ranging from 2 to 2·5 μl. The haemolymph was collected by cutting at the base of one of the hind legs. The sample was measured by using standardized micropipettes. Haemolymph proteins were precipitated by adding the haemolymph to 4 volumes of absolute ethanol. The pipette was washed twice with distilled water and the washings added to the sample. This was followed by centrifugation; then supernatant was collected and sediment washed twice with 80% ethanol, and after centrifugation the washings were added to the sample. The ninhydrin method of Rosen (1957) was followed for the determination of the amino nitrogen in the sample. The blank contained no haemolymph and leucine–isoleucine solution was run as a standard. The colour was read at 570 mμ against water as a reference. The results are expressed as mg. amino nitrogen/100 ml. haemolymph.

Determination of protein concentration in the haemolymph

The haemolymph protein concentration was estimated by the biuret reagent of Gornall, Bardawill & David (1949). Thirty microlitres of haemolymph (collected from two insects, 15 μl. from each, except in unfed insects when it was collected from five insects) were pipetted into 2 ml. of the reagent + 2 ml. of distilled water. The colour was allowed to develop for 1 hr., during which the samples were frequently shaken. The blank was run without adding haemolymph and the colour was read at 540 mμ. The results are expressed as optical density of the sample.

In the experiments carried out on *Rhodnius*, the terms 'high temperature' and 'normal temperature' refer to 36·5° C. and 28° C. respectively.

RESULTS

Effects of high temperature and of decapitation on digestion

Fifth-stage larvae were fed and directly afterwards some of the insects were placed at 36·5° C. while the rest were kept at 28° C. The proteolytic activity of the mid-gut was measured in both groups at different intervals after feeding. Seven days after feeding, the remaining insects at 36·5° C. were transferred to 28° C., and the protease activity was then determined at different intervals. The effect of decapitation on protease activity was also studied by measuring it in 5th-stage larvae decapitated 4 hr after feeding and kept permanently at 28° C. The changes in the activity were followed in the decapitated insects until 12 days after feeding when the experiment was terminated.

The protease activity of insects kept at 28° C. is shown in Fig. 2, which clearly indicates that at normal temperature there is no detectable activity in the unfed larva. At 1 day after feeding the activity increases considerably and then continues to rise until 7 days after feeding, whereupon there is a slight decrease. This is followed by

a further rise to a maximum just before moulting. The inability to detect protease activity in *Rhodnius* before feeding agrees with the finding of Fisk (1950) in adults of *Aedes aegypti*. It has been known for a long time that feeding stimulates insects to secrete digestive enzymes, for example in *Blatella* (Day & Powning, 1949), *Aedes aegypti* (Shambaugh, 1954), *Tenebrio molitor* (Dadd, 1956) and *Calliphora erythrocephala* (Thomsen & Møller, 1963). (For review, see Waterhouse, 1957.)

The curve for the proteolytic activity of insects kept at 36·5° C. directly after feeding is also included in Fig. 2, from which it is evident that at 2 days after feeding the activity is the same as that of normal insects. Thereafter it decreases, reaching a very low level by 7 days after feeding when the insects were transferred to 28° C. This

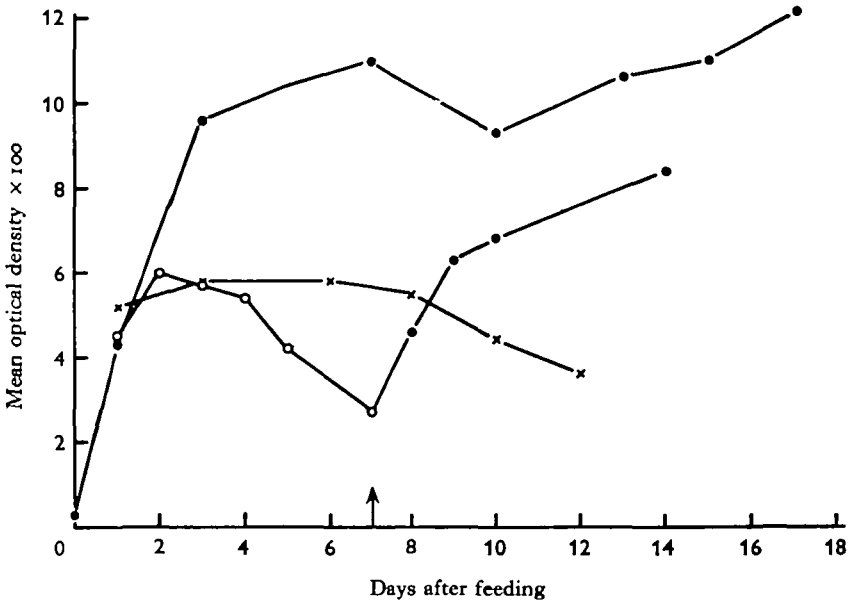

Fig. 2. Proteolytic activity of the mid-gut of normal 5th-stage larvae, of larvae decapitated 4 hr. after feeding and placed at 28° C., and of larvae exposed for 7 days after feeding to 36·5° C. and then transferred to 28° C. The arrow indicates transfer to 28° C.: each point represents the mean of three measurements except at 7 days after feeding in normal insects which represents 1 measurement. ●—●, 28° C.; ○—○, 36·5° C.; × — ×, decapitated.

coincides with the results of Applebaum, Janković, Grozdanović & Marinković (1964), who reported that in the larvae of *Tenebrio molitor*, mid-gut protease activity sharply decreased at high temperature (31° C.) and no activity could be detected after a week, whereas at 13° C. it increased after a lag period. More extreme effects were noted at 34° C., which these authors quoted as being above the limit for larval development, and at that temperature protease activity fell sharply from the first day.

It is interesting to note that the protease activity starts to increase after transfer to 28° C., though to a lower level and at a slower rate than normal (Fig. 2). For comparison, the protease activity of 5th-stage larvae decapitated 4 hr. after feeding and kept at 28° C. is also shown in Fig. 2. This indicates that the activity is normal at 1 day after feeding and almost normal a day later. Protease activity remains constant at this level until 8 days after feeding when it starts to decline gradually. Similarly, it was shown

by Dadd (1961) that protease activity is reduced in adults of *Tenebrio molitor* decapitated either before, or shortly after, emergence.

In conclusion, high temperature and decapitation before the critical period result in a drastic decrease of proteolytic activity in *Rhodnius*. It is also concluded that in heat-treated insects the proteolytic activity of the mid-gut starts to increase after transfer to normal temperature.

Changes in the haemolymph

The haemolymph in insects bathes the tissues and is the medium through which all the biochemical exchanges between the different tissues are effected. In many growing and metamorphosing insects, changes in the haemolymph composition reflect the morphogenetic and biochemical transformations taking place in the tissues (Buck, 1953). As materials necessary for growth and development, amino acids and proteins might be expected to be the most important substances present in the haemolymph. The fact that the haemolymph plays an important part in the metabolism of insects necessitated the investigation of this tissue in *Rhodnius* under different temperature conditions.

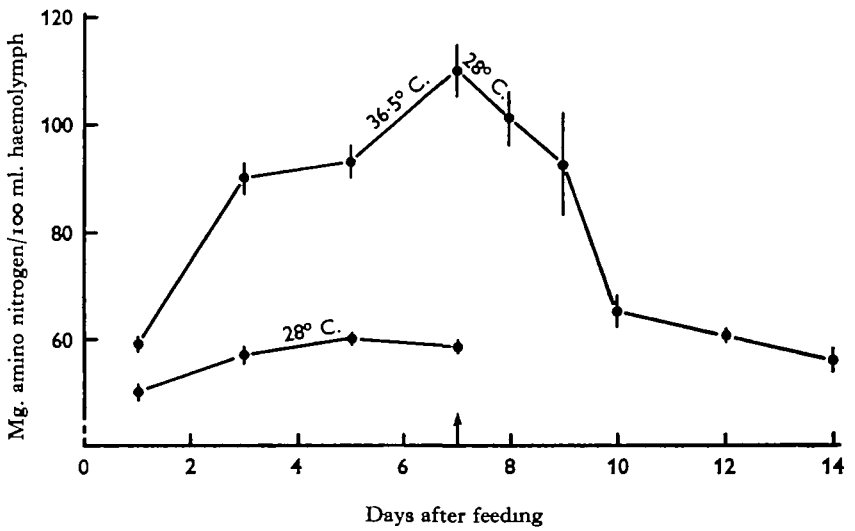

Fig. 3. Concentration of the free amino acids in the haemolymph of normal 5th-stage larvae and of larvae exposed to 36·5° C. for 7 days after feeding and then transferred to 28° C. The arrow indicates transfer to 28° C. Each point represents the mean of ten measurements, and the vertical lines on the curves represent the standard error.

(*a*) *Concentration of amino acids*

Fifth-stage larvae were fed about 2 weeks after moulting and some of the insects were placed directly after feeding at 36·5° C., while the rest were used as controls and placed at 28° C. The concentration of the free amino acids in the haemolymph was determined in both groups at different times after feeding. In the high-temperature group after a week's exposure, the remaining insects were transferred to 28° C. and their haemolymph was assayed at different periods after transfer. The results of this experiment are represented in Fig. 3, which shows that there is a profound difference

in the concentration of the free amino acids between insects of both groups. In insects kept at 28° C. there is an initial rise until 3 days after feeding, after which the concentration remains at a steady level until 7 days after feeding, when the experiment was terminated. However, at high temperature the concentration sharply increases until 3 days after feeding, reaching a level 1·5 times that of the control insects. The concentration continues to rise between the third and seventh day; at 7 days after feeding it is twice the corresponding value of the control insects.

Pronounced changes occur in the concentration of the free amino acids after returning the insects from high to normal temperature. This is shown in Fig. 3, which indicates that the concentration decreases after transfer to 28° C., until it reaches the normal level at about 5–7 days after transfer (12–14 days after feeding). In this connexion it should be mentioned that delayed moulting occurs after transferring such insects from high to normal temperature; however, moulting is inhibited if the insects are decapitated directly after transfer (Okasha, 1968*b*). The following experiment was carried out to determine whether or not decapitation affects the adjustment of the concentration of free amino acids in heat-treated insects.

Table 1. *Concentration of free amino acids in the haemolymph of 5th-stage larvae exposed to 36·5° C. for 7 days after feeding, transferred to 28° C. and decapitated immediately after transfer*

(Mg. amino nitrogen/100 ml. haemolymph ± standard error.)

Time after transfer	Decapitated	Intact
5 days	118·8 ± 10·9 (10)	68·2 ± 2·1 (10)
13 days	110·6 ± 17·7 (5)	—

Figures in parentheses indicate number of insects.

Fifth-stage larvae were placed at 36·5° C. immediately after feeding, and 7 days later they were transferred to 28° C. Some of the insects were decapitated soon after transfer, while the rest were left intact to serve as controls. The haemolymph was assayed 5 and 13 days after transfer. The results of this experiment are shown in Table 1. From the data represented graphically in Fig. 3, 5th-stage larvae exposed to 36·5° C. for 7 days after feeding have 110 ± 4·9 mg. amino nitrogen/100 ml. of haemolymph. It is evident from Table 1 that, when such larvae are decapitated directly after transfer to 28° C., this high level of amino acids is retained, the concentration being 118·8 ± 10·9 mg. by 5 days after transfer. This high concentration is still sustained when measured as late as 13 days after both transfer and decapitation. The time of decapitation is an important factor in this respect. If it is performed in heat-treated insects after the critical period (7–8 days after transfer to 28° C. following 10 days' exposure to 36·5° C.) they will moult (Okasha, 1968*b*). By that time, the concentration of the amino acids in the haemolymph has returned to the normal level (Table 1). It was on these grounds that it was suggested that the brain, after transfer to normal temperature, may be necessary for repairing damage caused by high temperature during the period of exposure (Okasha, 1968*b*).

It is therefore concluded that exposure to high temperature directly after feeding results in an elevated concentration of free amino acids in the haemolymph and that

this effect is readily reversible when the insects are transferred to normal temperature. This elevated concentration, however, cannot be lowered after transfer except in the presence of the head.

If *Rhodnius* larvae are given a sufficiently long heat-treatment prior to feeding, moulting is delayed, and micturition which normally follows a blood meal is inhibited (Okasha, 1968*a*). This is associated with an enormous increase in the volume of the haemolymph. The concentration of free amino acids in the haemolymph of such treated insects was determined. Unfed 5th-stage larvae were exposed to 36·5° C. for 7 days, then fed and placed at 28° C. The haemolymph of ten insects was assayed 3 days after feeding, and that of ten more insects 4 days later. The results were 48·2 ± 4·7 and 40·3 ± 2·5 mg. amino nitrogen/100 ml. of haemolymph at 3 and 7 days after feeding respectively. The corresponding figures for normal insects were 57·2 ± 1·5 and 58·5 ± 0·9 respectively (Fig. 3). These results indicate that the concentration in heat-treated insects is somewhat below the normal level, despite the great increase in blood volume. Therefore it seems that the insect can compensate for changes in blood volume, as far as the amino acid concentration is concerned.

(*b*) *Protein concentration*

The haemolymph protein concentration was measured in 5th-stage larvae, some of which were placed immediately after feeding at 36·5° C. and the rest at 28° C. The results of this experiment are represented in Fig. 4. This figure shows that in insects

Table 2. *Haemolymph protein concentration of 5th-stage larvae exposed to 36·5° C. for 7 days after feeding, then transferred to 28° C. (values for normal insects are included for comparison)*

(Optical density × 100 ± standard error)

Time	Heat-treated (time after transfer)	Normal (time after feeding)
1 day	44 ± 3	43 ± 3
2 days	53 ± 1	—
3 days	51 ± 3	43 ± 2
5 days	57 ± 2	69 ± 4

Means are from five determinations (two insects for each determination).

of both groups, the protein concentration drops drastically after feeding and remains at a steady low level until 3 days after feeding. After this the concentration increases in insects kept at normal temperature. However, in insects kept at high temperature there is almost no rise in concentration after the initial decrease. When the insects are transferred to 28° C. after a week's exposure to 36·5° C. there is a very slow rate of increase in the protein concentration (Table 2).

Similarly, in 5th-stage larvae exposed to 36·5° C. for 7 days prior to feeding, the normal rise in protein concentration is almost abolished (Fig. 4). In this case, the concentrations are approximately half those of insects exposed to the same temperature after feeding; this is correlated with a large blood volume.

Thus it is concluded that in insects exposed to high temperature either before or after feeding, there is no increase in the haemolymph protein concentration which normally follows the initial decrease.

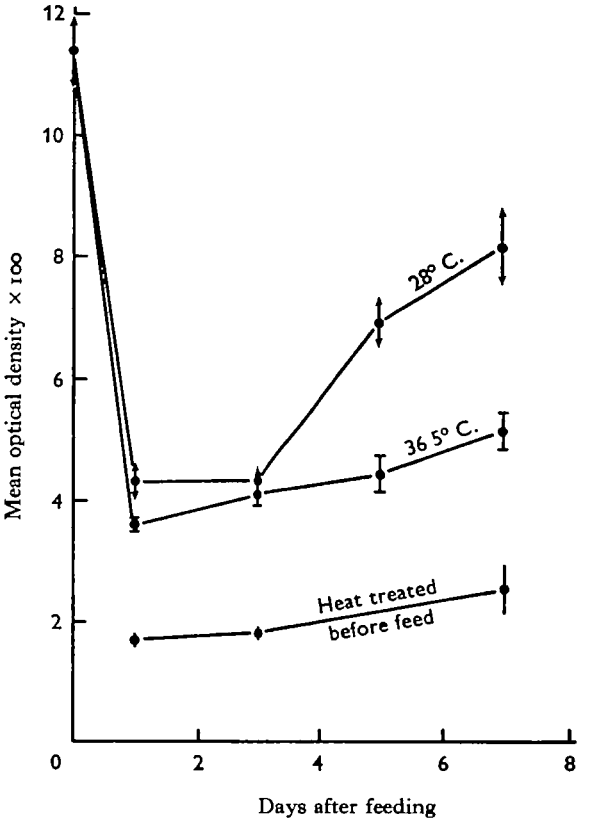

Fig. 4. Haemolymph protein concentration in normal 5th-stage larvae and in larvae placed at 36·5° C. immediately after feeding. The concentration at 0 day is that of unfed insects. Each point represents the mean of five measurements, and the vertical lines on the curves represent the standard error.

DISCUSSION

It was pointed out by Thomsen & Møller (1963) that the first indication of a possible hormonal control of digestion was the observation of Wigglesworth (1936, 1948, 1957) that in the decapitated adult female of *Rhodnius* and in the 'resting' larva, where no corpus allatum hormone and no moulting hormone are being secreted respectively, there may be plenty of undigested blood in the stomach. The suggestion was put forward by Day & Powning (1949) that hormonal regulation of digestion might occur in some insects, and later Dadd (1961) found that a factor originating in the head and carried in the blood regulates protease activity in *Tenebrio molitor*. Working on *Calliphora erythrocephala* adults, Thomsen & Møller (1959, 1963) showed conclusively that protease activity is controlled by the median neurosecretory cells of the brain. The present results strongly suggest that digestion in *Rhodnius* larvae is under hormonal control. This is supported by the finding that decapitation results in a great reduction of the protease activity (Fig. 2). It is not possible to classify with certainty the hormone(s) which might be responsible for the regulation of protease activity in *Rhodnius*. It may be the brain hormone, the corpus allatum hormone, the moulting

hormone, or a combination of these. However, Thomsen & Møller (1963) stated that it does not seem likely that the influence of the hormone from the median neurosecretory cells on the initial development of protease activity should go via the thoracic gland, since the latter usually degenerates during the first 2 days after emergence. Moreover, these authors showed that the corpus allatum has only a minor effect on the protease activity. Following the same line, it seems likely that the brain hormone is the hormone responsible for the regulation of protease activity in *Rhodnius*.

If this interpretation is correct, it would explain the low protease activity in intact insects kept at high temperature after feeding, and also in the decapitated insects (Fig. 2), since the brain hormone is absent in both (Okasha, 1968*b*). The initial rise of protease during the first 2 days following feeding is believed to be due to the effect of the brain hormone which is released during feeding from the corpus cardiacum (Wigglesworth, 1957). On the other hand, this might be due to direct action; the passage of food from the crop may serve as a stimulus acting directly on the mid-gut secretory cells and causing them to secrete the corresponding enzymes. It is worth mentioning here that at high temperature blood is very slowly released from the crop, and consequently the rest of the mid-gut is much thinner and has less contents in the rumen compared with that of normal insects. It was also noticed that in such insects the peristaltic movements of both the crop and the rest of the mid-gut are not as frequent as normal. As was pointed out by Waterhouse (1957), it is probable that direct stimulation in response to feeding coexists with endocrine mechanisms.

The rise in the concentration of free amino acids in the haemolymph in insects kept at high temperature (Fig. 3) suggests that the latter does not adversely affect the absorptive mechanisms from the mid-gut to the haemolymph. This is based on the assumption that these amino acids are mainly products of the breakdown of the blood meal. It has been shown that proteolytic activity under high temperature conditions is normal until 2 days after feeding (Fig. 2), but nothing is known about possible catabolic metabolism of any synthesized proteins during this period. Another assumption upon which this conclusion depends is that the observed rise in the concentration of amino acids in the haemolymph is not a direct result of evaporation and decreased blood volume caused by high temperature. Although no measurements of blood volume were made, 30 μl. of haemolymph were collected with equal facility from both two normal and two heat-treated insects. It is possible, however, that heat-treatment results in an unobservable decrease in the blood volume.

The finding that protein concentration in the haemolymph remains at a very low level in insects kept at high temperature (Fig. 4) is very interesting. There seems no reason to assume that such insects have a blood volume higher than normal. Conversely, were the blood volume normal or subnormal, this would mean that the protein content of the haemolymph would be very small compared with that of normal insects.

Taken together, the changes in amino acid and protein concentration in the haemolymph clearly indicate that protein metabolism is adversely affected at high temperature. The accumulation of amino acids in the haemolymph under these conditions suggests that *Rhodnius* larvae are not suffering from a lack of raw materials; it is rather the synthetic mechanisms which are affected. If, as seems likely, protein synthesis is impaired by exposure to high temperature, the sequence of events caused by this exposure is visualised as follows: (1) digestion proceeds as normal until 2 days after

feeding; (2) amino acids accumulate in the haemolymph, since they are not utilized for protein synthesis; (3) the rate of digestion slows down; (4) and, consequently, protein concentration remains at a low level.

The metabolic changes that occur in the heat-treated insect after being returned to normal temperature support this view. After transfer, protein synthesis proceeds and sets in motion the other changes consequent upon this synthesis. Thus, the concentration of amino acids in the haemolymph decreases (Fig. 3), while the proteolytic activity in the mid-gut increases (Fig. 2). However, the haemolymph protein concentration does not show a corresponding rapid increase. This is difficult to explain, though it is possible that the rate of release of proteins in the haemolymph is paralleled by an increased turnover of these proteins, so that the pool size changes slowly.

Evidently, if high temperature impairs protein synthesis, growth and development cannot occur. This compares with the suggestion of Wigglesworth (1957, 1959) that in the 'resting' or dormant insect, where no moulting hormone is being secreted, growth and development are arrested due to the failure of protein synthesis. Indeed, Wigglesworth (1957) suggested that the primary action of the moulting hormone is to restore protein synthesis which is necessary for growth and development. The first detectable changes that take place in response to the moulting hormone in some of the tissues of *Rhodnius* support this view. A few hours after feeding or after the injection of ecdyson, the nucleolus of the epidermal cell becomes enlarged, the mitochondria increase in number and become rounded, cytoplasmic RNA increases and protein synthesis begins (Wigglesworth, 1957, 1963).

The role of insect hormones in controlling protein synthesis is gaining support from the results of various authors working on different insect material. Thus, the haemolymph proteins were shown to be under the control of the neurosecretory cells of the brain in *Schistocerca gregaria* (Hill, 1962, 1963). When the neurosecretory cells are cauterized, the concentration of the free amino acids in the haemolymph increases, whereas the haemolymph protein concentration remains constant (Hill, 1962). In *Tenebrio molitor*, the haemolymph protein concentration is also significantly reduced by cautery of the neurosecretory cells, either before or 2–3 days after adult emergence (Mordue, 1965). It is of interest to recall here that in the same species there is a reduction of the mid-gut protease activity following decapitation before or shortly after adult emergence (Dadd, 1956, 1961), and that there is a sharp decrease in this activity at high temperature (Applebaum *et al.* 1964). The hormonal control of protease activity in *Calliphora* has been regarded as hormonal control of specific protein synthesis, since enzymes themselves are proteins (Thomsen & Møller, 1963). By analogy, it would appear that high temperature is also impairing protein synthesis in *Tenebrio*.

Since one action of the moulting hormone is to stimulate protein synthesis (Wigglesworth, 1964), and since this synthesis is impaired by high temperature in *Rhodnius*, then it is not surprising to find that moulting ceases at high temperature even in the presence of the moulting hormone (Okasha, 1968*b*).

The process of wound healing in *Rhodnius* is inhibited by high temperature at the stage of mitosis (Okasha, 1968*b*). This could be explained according to the present view, that protein synthesis is impaired by high temperature. Indeed, several studies have shown that injury of diapausing insects is associated with an increase in the rate of protein synthesis, which is similar to what occurs after the onset of adult develop-

ment (Telfer & Williams, 1960; Skinner, 1963; Wyatt, 1962; Shappirio & Harvey, 1965). Therefore, if protein synthesis is impaired, cell division will be inhibited.

The impairment of protein synthesis serves also to explain the reduction and malformation of the bristles and plaques of previously heat-treated *Rhodnius* larvae (Okasha, 1968*a*). In addition, it provides an explanation of the 'cessation' of the secretion of the brain hormone in the same insect during exposure to high temperature (Okasha, 1968*b*). This is based on the assumption that the brain hormone is a protein or a proteinaceous compound, and there is some evidence indicating that this is so (Ichikawa & Ishizaki, 1963; for review see Wigglesworth, 1964).

In conclusion, high temperature interferes mainly with protein synthesis. It is not known whether one is dealing with a special case in *Rhodnius*, or whether this may prove to be a general phenomenon in other insects. Neither is it known how high temperature interferes with protein synthesis. Such a complex process could be inhibited by high temperature in many ways. One thing should be emphasized, that this deserves detailed investigation to understand the integrated effects of temperature on insects.

SUMMARY

1. Protease activity of the mid-gut of 5th-stage larvae was studied under different conditions. The activity is not detectable in the unfed insect, but increases after feeding, reaching a maximum just before ecdysis.

2. In insects kept at high temperature immediately after feeding, protease activity is approximately normal for the first 2 days, but thereafter it decreases; after transfer to normal temperature the activity starts to increase.

3. Decapitation reduces protease activity under normal temperature conditions. Possible hormonal control of digestion is discussed, and it is suggested that the brain hormone is involved.

4. The concentration of free amino acids in the haemolymph greatly increases at high temperature, but returns to the normal level after transfer to normal temperature. The adjustment to the normal level does not occur if the insects are decapitated immediately after transfer.

5. The concentration of protein in the haemolymph at high temperature does not recover from the initial decrease. After transfer to normal temperature there is a tendency to a very slow increase.

6. Protein synthesis is impaired at high temperature and thus amino acids accumulate in the haemolymph, since they are not used in protein metabolism.

7. It is concluded that the impairment of protein synthesis results in the cessation of moulting, of cell division in the epidermis during wound healing, and of the secretion of the brain hormone.

This paper forms part of a thesis submitted to the University of Cambridge for the Ph.D. degree. It is a pleasure to record my sincere thanks once again to Professor Sir Vincent B. Wigglesworth, under whose supervision this work has been carried out. His encouragement and valuable guidance throughout the whole work have been of great help. I am indebted to my colleague Dr Graham Pratt for useful discussions. The help given me by my wife Dr Elisabeth Okasha, Department of English, University of Aberdeen, in reading and typing the manuscript is deeply appreciated. My thanks are due to the University of Assuit, U.A.R., for giving me study leave and a grant.

REFERENCES

APPLEBAUM, S. W., JANKOVIĆ, M., GROZDANOVIĆ, J. & MARINKOVIĆ, D. (1964). Compensation for temperature in the digestive metabolism of *Tenebrio molitor* larvae. *Physiol. Zoöl.* **37**, 90–5.

BUCK, J. B. (1953). Physical properties and chemical composition of insect blood. In *Insect Physiology*, ed. Roeder, K. D., pp. 147–90. New York.

CHARNEY, J. & TOMARELLI, R. M. (1947). A colorimetric method for the determination of the proteolytic activity of duodenal juice. *J. biol. Chem.* **171**, 501–5.

DADD, R. H. (1956). Proteolytic activity of the midgut in relation to feeding in the beetles *Tenebrio molitor* L. and *Dytiscus marginalis* L. *J. exp. Biol.* **33**, 311–24.

DADD, R. H. (1961). Evidence for humoral regulation of digestive secretion in the beetle, *Tenebrio molitor*. *J. exp. Biol.* **38**, 259–66.

DAY, M. F. & POWNING, R. F. (1949). A study of the processes of digestion in certain insects. *Aust. J. scient. Res.* B **2**, 175–215.

FISK, F. W. (1950). Studies on proteolytic digestion in adult *Aëdes aegypti* mosquitoes. *Ann. ent. Soc. Am.* **43**, 555–72.

GORNALL, A. G., BARDAWILL, C. J. & DAVID, M. M. (1949). Determination of serum proteins by means of the biuret reaction. *J. biol. Chem.* **177**, 751–66.

HILL, L. (1962). Neurosecretory control of haemolymph protein concentration during ovarian development in the desert locust. *J. Insect Physiol.* **8**, 609–19.

HILL, L. (1963). Endocrine control of protein synthesis in female desert locusts (*Schistocerca gregaria*). *J. Endocr.* **26**, 17–18.

ICHIKAWA, M. & ISHIZAKI, H. (1963). Protein nature of the brain hormone of insects. *Nature, Lond.* **198**, 308–9.

MORDUE, W. (1965). Neuro-endocrine factors in the control of oöcyte production in *Tenebrio molitor* L. *J. Insect Physiol.* **11**, 617–29.

OKASHA, A. Y. K. (1964). Effects of high temperature in *Rhodnius prolixus* (Stål.). *Nature, Lond.* **204**, 1221–2.

OKASHA, A. Y. K. (1968*a*). Effects of sub-lethal high temperature on an insect, *Rhodnius prolixus* (Stål.). I. Induction of delayed moulting and defects. *J. exp. Biol.* **48**, 455–63.

OKASHA, A. Y. K. (1968*b*). Effects of sub-lethal high temperature on an insect, *Rhodnius prolixus* (Stål.). II. Mechanism of cessation and delay of moulting. *J. exp. Biol.* **48**, 465–73.

ROSEN, H. (1957). A modified ninhydrin colorimetric analysis for amino acids. *Archs Biochem. Biophys.* **67**, 10–15.

SHAMBAUGH, G. F. (1954). Protease stimulation by foods in adult *Aedes aegypti* Linn. *Ohio J. Sci.* **54**, 151–60.

SHAPPIRIO, D. G. & HARVEY, W. R. (1965). The injury metabolism of the cecropia silkworm. II. Injury-induced alterations in oxidative enzyme systems and respiratory metabolism of the pupal wing epidermis. *J. Insect Physiol.* **11**, 305–27.

SKINNER, D. M. (1963). Incorporation of labelled valine into the proteins of the cecropia silkworm. *Biol. Bull. mar. biol. Lab., Wood's Hole* **125**, 165–76.

TELFER, W. H. & WILLIAMS, C. M. (1960). The effects of diapause, development, and injury on the incorporation of radio-active glycine into the blood proteins of the cecropia silkworm. *J. Insect Physiol.* **5**, 61–72.

THOMSEN, E. & MØLLER, I. (1959). Neurosecretion and intestinal proteinase activity in an insect, *Calliphora erythrocephala* Meig. *Nature, Lond.* **183**, 1401–2.

THOMSEN, E. & MØLLER, I. (1963). Influence of neurosecretory cells and of corpus allatum on intestinal protease activity in the adult *Calliphora erythrocephala* Meig. *J. exp. Biol.* **40**, 301–21.

WATERHOUSE, D. F. (1957). Digestion in insects. *A. Rev. Ent.* **2**, 1–18.

WIGGLESWORTH, V. B. (1936). The function of the corpus allatum in the growth and reproduction of *Rhodnius prolixus* (Hemiptera). *Q. Jl microsc. Sci.* **79**, 91–121.

WIGGLESWORTH, V. B. (1948). The functions of the corpus allatum in *Rhodnius prolixus* (Hemiptera). *J. exp. Biol.* **25**, 1–14.

WIGGLESWORTH, V. B. (1952). Hormone balance and the control of metamorphosis in *Rhodnius prolixus* (Hemiptera). *J. exp. Biol.* **29**, 620–31.

WIGGLESWORTH, V. B. (1955). The role of the haemocytes in the growth and moulting of an insect, *Rhodnius prolixus* (Hemiptera). *J. exp. Biol.* **32**, 649–63.

WIGGLESWORTH, V. B. (1957). The action of growth hormones in insects. In *The Biological Action of Growth Substances. Symp. Soc. exp. Biol.* XI, 204–27.

WIGGLESWORTH, V. B. (1959). *The Control of Growth and Form.* Ithaca: Cornell University Press.

WIGGLESWORTH, V. B. (1963). The action of moulting hormone and juvenile hormone at the cellular level in *Rhodnius prolixus*. *J. exp. Biol.* **40**, 231–45.

WIGGLESWORTH, V. B. (1964). The hormonal regulation of growth and reproduction in insects. *Adv. Insect Physiol.* **2**, 247–336.

WYATT, G. R. (1961). The biochemistry of insect haemolymph. *A. Rev. Ent.* **6**, 75–102.