THE METABOLIC EFFECTS OF THE CORPUS ALLATUM HORMONE IN THE MALE DESERT LOCUST

I. LIPID METABOLISM

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INTRODUCTION

Pfeiffer (1945) posed an important problem concerning the metabolic effects of the corpus allatum hormone (CAH) when she demonstrated that allatectomy of the grasshopper *Melanoplus* leads to the massive accumulation of lipids and glycogen in the fat body. Other workers (e.g. Vogt, 1949; Thomsen, 1952; Bodenstein, 1953; Orr, 1964a, b) have since found comparable effects after extirpating the corpora allata of various insects (*Drosophila hydei, Calliphora, Periplaneta,* and *Phormia* respectively). Doane's (1960, 1961) experiments of a similar accumulation of lipids in the fat body were of a different kind. She studied female-sterile mutants of *Drosophila melanogaster,* and she was able to show that such genetically determined sterile females have greatly hypertrophied fat bodies accompanied by equally inactive corpora allata. It seemed, therefore, that what would explain allalectomy experiments would also explain her findings (Doane, 1961, 1962).

So far, this 20-year-old problem has not been satisfactorily settled. Pfeiffer (1945) herself, and some others who followed her (e.g. Vogt, 1949; Thomsen, 1952; Bodenstein, 1953), maintained that the corpus allatum acts directly on the fat body by releasing a 'metabolic hormone' that in some way regulates the mobilization of precursor materials synthesized therein. At the same time, the corpus allatum was supposed to release a gonadotropic hormone which acts directly on the ovary, and in this manner stimulating vitellogenic activity in the developing oocytes. Doane (1960, 1961, 1962) suggested the existence of an 'ovarian hormone' that acts directly on the fat body and regulates its lipid metabolism; but she did not exclude the possibility of the CAH acting indirectly on the fat body by its regulatory action upon the maturation processes of the ovary. Orr (1964a, b) has gone further than Pfeiffer in proposing that the sole site of action of the CAH is the fat body, other effects (including that on the gonad) being secondary to this.

There are certain aspects of the metabolic situation of allatectomized adult insects that suggest that other, probably more adequate, explanations should be tested. First, observation of locusts which have undergone careful allatectomy has strongly indicated that they are lethargic—a fact already reported in a preliminary paper (Odhiambo, 1965). Secondly, there is conflicting evidence on the participation of the gonads in the regulation of fat-body metabolism. While Pfeiffer (1945) eliminated the ovary as a factor in the hypertrophy of the fat body after the allatectomy of *Melanoplus,* Thomsen

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& Hamburger (1955) found that the fat body hypertrophied after ovariectomy in *Calliphora*, and Orr (1964a) maintained that the fat body of *Phormia* hypertrophied only if the extirpation of the corpus allatum was performed before vitellogenesis. Thirdly, Pfeiffer (1945) made quantitative estimations of the fatty acids (also water and dry matter) during the development of adult grasshoppers; and she showed that, following an early phase of the accumulation of these materials, a metabolic change occurred involving the utilisation of these reserves, and that this occurred equally well in the ovariectomized females—thus indicating extra-ovarian utilization of lipids. This finding has so far found no satisfactory explanation. Fourthly, wounding of female-sterile mutants of *Drosophila* leads to the further accumulation of fat, rather than the expected utilization of the material for the purposes of repair (Doane, 1961). Fifthly, consideration of the sexual maturation processes in the male desert locust has suggested that the CAH may control these processes by acting on the central nervous system (Odhiambo, 1966a). If this be so, a new avenue opens up for investigation of the accumulation of metabolic materials in the fat body following the removal of the corpora allata.

It has been established, by cytological means, that glycogen accumulates in the fat body of allatectomized male desert locusts (Odhiambo, 1966b). Although a comparable accumulation of lipids is found in the fat body after allatectomy, cytological observations alone are not entirely convincing. In the following pages the effects of allatectomy and gonadectomy on total lipid accumulation by a chloroform-extraction method will be explored. In the second paper in this series the effects of these two operations on the general level of locomotor activity will be examined. This line of investigation will suggest that the key to the problem of the accumulation of metabolic materials (such as fat and glycogen) after allatectomy lies in its relation to general activity—in other words, in the probable effects of the CAH on the central nervous system.

MATERIALS AND METHODS

Male adult desert locusts (*Schistocerca gregaria* Forskål) were used throughout the experiments. The locusts were maintained under crowded conditions throughout their development, and thus the acceleration and synchronization of sexual maturation was assured (Norris, 1954; Loher, 1960). The breeding method adopted has already been described in a previous report (Odhiambo, 1966a). Under these breeding conditions the male locusts became sexually mature within 6–10 days after adult emergence (Odhiambo, 1966a).

Fat was extracted from whole locusts with chloroform by the usual Soxhlet apparatus. The guts of locusts to be chloroform-extracted were pre-emptied by imposing a short (2–4 hr.) starvation period before the animals were killed. Cheu (1952) has shown that each *Schistocerca* adult expels three faecal pellets every hour irrespective of the amount of food eaten. The partially starved locusts were then paralysed and killed by sectioning the ventral nerve cord in the neck region and by further making a longitudinal cut down the thoracic ventral nerve cord. They were immediately placed in fat-extracted thimbles (15 × 100 mm., of double thickness), previously oven-dried and weighed. They were dried in a 60° C. oven until they attained a constant weight, which usually took 2–4 days. The locusts were then chloroform-extracted for

5 hr. They were again oven-dried to a constant weight. The difference between the two dry weights of locusts gave the amount of chloroform-soluble lipids.

Surgical procedures for allatectomy, gonadectomy, implantation of corpora allata, and sham-operation have been described previously (Odhiambo, 1966 a). The term 'gonadectomy' has been used to denote the extirpation of both the testes and the accessory reproductive glands. In all cases, operations were performed on the day of adult emergence.

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

Since the major portion of lipid deposits are located within the fat body, fat extraction from whole locusts may be taken as an adequate estimation of fat in the fat body (Kilby, 1963). The utilization and accumulation of lipids was followed by extracting with chloroform whole adult male locusts of known age, from the time of adult emergence up to the time that the locusts were approaching senescence (18–25 days at a temperature of $30 \pm 1°$ C. under the breeding conditions employed).

Fig. 1 shows that, in unoperated controls, the first 2 days are characterized by a reduction in total body lipids, perhaps a consequence of the little feeding taking place during this period and the latter days of the last larval instar (Odhiambo, 1966a). The next 5–7 days, however, show a rapid rise in the total body lipids to approximately 300 mg. of chloroform-extracted lipids per g. of dry matter. Then ensues a progressive depletion of fat reserves during the rest of adult life. It should be recalled that this pattern of accumulation and then depletion of lipids resembles, in a general way, similar patterns of feeding activity and body growth in adult male desert locusts (Odhiambo, 1966a).

The lipid patterns for sham-operated controls is similar to that for unoperated locusts (Fig. 3), except that there is no reduction in lipid deposits during the first 2 days of adult life.

Gonadectomy does not seem to result in a different pattern of the build-up and depletion of fat (Fig. 4). Since the male gonads comprise about one-fifth of the total weight of a mature locust, it is significant that they have no marked effect on the utilization of lipids. It is conceivable therefore that any effect the corpus allatum might have on lipid metabolism is not grossly affected by the possible utilization of precursor materials in the fat body (and elsewhere) by the male gonads. Consequently, the effects of the gonads upon lipid stores can clearly be distinguished from any possible effects of the corpus allatum on the same stores.

Allatectomy has a profound effect on lipid metabolism (Figs. 1 and 2). Instead of the lipid stores being depleted after the time the male locust should have normally become mature (10 days), fat continues to accumulate. This high level of lipid accumulation (up to 450 mg. of lipid per g. of dry matter) is more or less maintained for the remainder of adult life.

The implantation of active corpora allata into previously allatectomized male locusts similarly has a profound effect on lipid accumulation. Table 1 summarizes the results of experiments in which some newly emerged locusts were allatectomized and then implanted, 5 days later, with three pairs of corpora allata from 9-day-old adult locusts. Previous experiments have shown that such corpora allata are at the peak of their hormonal activity during the adult stage (Odhiambo, 1966c). When locusts treated

in this way were chloroform-extracted 20 days after the implantation of corpora allata, it was clearly demonstrated that the body lipid content of the locusts had been reduced to a level comparable with that existing in normal unoperated or sham-operated locusts.
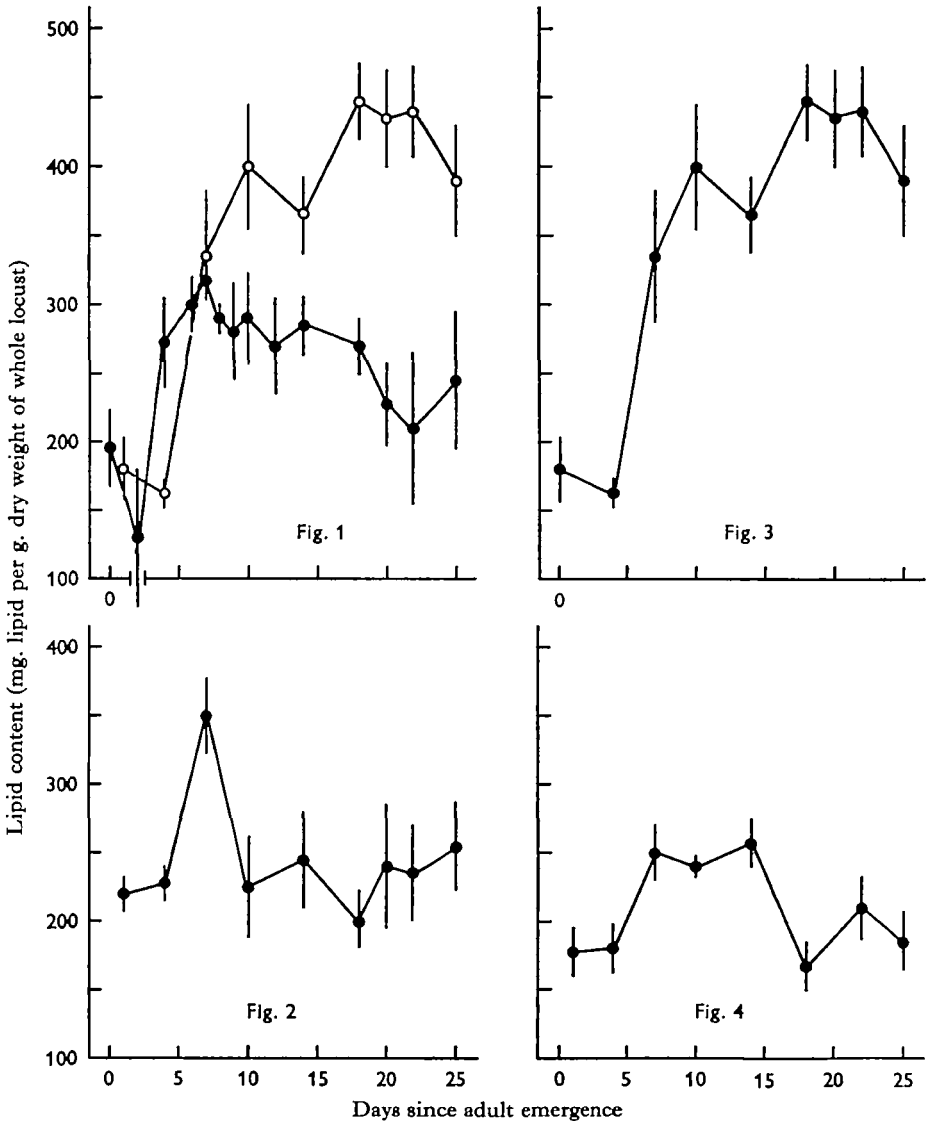
Figs. 1–4. The amount of lipids extracted with chloroform from adult male locusts of known age. The points in the graphs are each a mean of five to eight individual extractions; the vertical lines represent twice the standard error for each mean. Fig. 1 depicts a graph for unoperated controls (solid circles), and a graph for locusts allatectomized at adult emergence (open circles). Fig. 2, a graph for sham-operated controls. Fig. 3, a graph for allatectomized locusts. Fig. 4, a graph for locusts gonadectomized soon after emergence.

Table 1. *Effect of the implantation of active corpora allata upon the accumulation of lipids in allatectomized male adult locusts*

(All the locusts were chloroform-extracted when 25 days old. Allatectomy was performed on the day of emergence; 5 days later, some of these received 3 pairs of corpora allata from 9-day-old adult locusts. Six lipid determinations were made for each treatment.)

Treatment	Mean lipid content (mg./g. of dry matter) ± S.E.
Unoperated	227 ± 36·5
Sham-operated	258 ± 46·6
Allatectomized	409 ± 24·8
Allatectomized, then implanted with active corpora allata	259 ± 27·0

DISCUSSION

Studies on the hormonal regulation of fat metabolism have generally been carried out using female adult insects, perhaps because a considerable proportion of yolk is lipid in nature. In our investigations on the lipid metabolism of the male desert locusts, therefore, it was essential to confirm that there was a significant accumulation of fat following allatectomy.

A simple lipid-extraction method has shown that there was considerable accumulation of fat following allatectomy of male locusts soon after adult emergence, and this high level of fat content was more or less maintained throughout the remainder of adult life. After gonadectomy, on the other hand, there was a progressive depletion of fat reserves during the period following the attainment of sexual maturity. Such depletion of fat reserves was normal in unoperated locusts. Implantation of active corpora allata reversed the tendency to fat accumulation. Cytological methods have also revealed that allatectomy leads to accumulation of both glycogen and lipid in the locust fat body (Odhiambo, 1966b). There seems to be little doubt, therefore, that the CAH is closely involved in the regulation of lipid (and possibly also glycogen) metabolism in the male desert locust.

Odhiambo (1966a) has determined the feeding activity of male desert locusts from the time of adult emergence till the beginning of senescence. In unoperated locusts, feeding activity reaches a peak on or about the sixth adult day, remains more or less steady for the next 3 days or so, and then declines very steeply. This low level of feeding is continued for the rest of the insect's life. The same pattern of feeding activity is maintained by sham-operated, gonadectomized, and allatectomized locusts. The amount of digestive enzymes, as indicated by proteinase activity in the midgut, was not a limiting factor for digestion at any time—even immediately after adult emergence. Consequently, it is clear that feeding and digestion can be discounted as factors in the control of the metabolism of the fat body.

A new hypothesis to explain the accumulation of fat following allatectomy will be put forward in the next paper in this series.

SUMMARY

1. An account is given of the normal pattern of total lipid content of male desert locusts from the time of adult emergence to the time the locusts are approaching senescence (18–25 days). Up to the seventh adult day, there is a rapid increase in the amount of body lipids; thereafter, there is a progressive depletion of lipid reserves.

2. Allatectomy of the locusts soon after adult emergence leads to considerable accumulation of lipids which becomes apparent when the locusts attain sexual maturity and persists thereafter. Gonadectomy or mere wounding has no such effects.

3. Implantation of active corpora allata in previously allatectomized locusts reverses the tendency to lipid accumulation.

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