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Endocrine influence on protein synthesis in the fatbodies of female red cotton bug, *Dysdercus cingulatus* Fabr

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Abstract. Extirpation of pars intercerebralis neurosecretory cells (PINSC) of female *D. cingulatus* significantly brought down the level of protein synthesis in the fatbodies 48, 72, and 96 hr after the operation, while implantation of active PINSC into both normal and PINSC-ablated females elevated substantially the protein content in the fatbodies. Additional supply of JHa (FME) by topical application activated protein synthesis in the fatbodies both in the allatectomised and normal females. Histochemical studies to demonstrate the protein content in the fatbodies of the above experimental insects also corroborated these findings. Probable regulatory mechanism of protein synthesis in the fatbodies of female *D. cingulatus* by the hormonal principles from PINSC and corpus allatum are discussed in the light of the above findings.

Keywords. *Dysdercus cingulatus*; protein; fatbody; neurosecretory cells; juvenile hormone.

1. Introduction

Fatbodies are the site where most of the haemolymph proteins and vitellogenin are synthesised and released in adult insects and thus it fulfils a variety of functions similar to the hepatopancreas of molluscs and crustaceans or the liver in mammals (Telfer 1965; Chen 1978; Keeley 1978). So neurohormonally dependent changes in the protein should reflect changes in the protein synthetic capacity of the fatbodies as well. As Engelmann (1979) suggests, one of the most exciting aspects and one which attracted increasing attention during the last few years, is the mechanism of control of vitellogenin biosynthesis. The synthesis of haemolymph proteins seems to be controlled, at least in part, by hormones of corpus allatum (Coles 1965a; Engelmann and Penney 1966; Lüscher 1968) and factors from the neurosecretory system (Hill 1962, 1965; Wyss-Huber and Lüscher 1966). The Ca-hormones are intimately involved in various phases of protein metabolism (Gilbert and Schneiderman 1961; Thomas and Nation 1966). Juvenile hormone is demonstrated to influence the synthesis of storage-proteins in the fatbody of *Bombyx mori* (Tojo *et al* 1981). In the American cockroach the RNA content of the fatbodies is cyclic in nature and the protein level is contributed by the fatbody (Mills *et al* 1966). In *Leucophaea maderae* fatbodies seem to react with an increased release of proteins to the changed hormonal environment during oocyte maturation (Wyss-Huber and Lüscher 1972). The brain hormone could be acting directly on the fatbody or through some other organ as the ovary itself (Hagedorn and Fallon 1973).

In the present paper, results of our experiments performed to elucidate the influence of hormonal principles from the pars intercerebralis neurosecretory cells (PINSC) and

that from corpus allatum (JH) on the synthesis of fatbody proteins in the female red cotton bug, *Dysdercus cingulatus* are presented.

2. Material and methods

2.1 *The animal*

The red cotton bug, *Dysdercus cingulatus* (Heteroptera: Pyrrhocoridae), was reared in the laboratory at 29 ∓ 3°C, r.h. 90 ∓ 3% and 12:12 LD regime. The insects were fed *ad libitum* on soaked cotton seeds. The newly emerged adults of both sexes were separated within an hour after emergence from the stock colony and fed as described earlier by Muraleedharan and Prabhu (1979) and adult females of appropriate age groups were selected from among them for experimentation.

2.2 *Surgical techniques*

All the instruments used for microsurgery were washed well in distilled water and sterilised in 70% ethyl alcohol. Surgical procedures for extirpation and implantation of PINSC and allatectomy were followed after Muraleedharan and Prabhu (1979, 1981). Adult donor females within 3 hr after emergence were used for extirpation of PINSC and newly emerged adults served as hosts. Sham-operated insects of corresponding age groups served as controls for each category. Pieces of gut tissue were implanted into the control instead of PINSC. Operated insects were disengaged from plasticine ribbons and after mopping off the Ringer solution sticking to them, a thin film of anti-septic powder consisting of penicillin, streptomycin and phenylthiourea in the ratio 1:1:2 was applied on the wound. Adult females, 24 hr after their emergence, were used for allatectomy. Twenty four hr after the operation such females were allowed to mix with young adult males for free mating.

2.3 *Protein estimation*

Fatbodies from different experimental insects were dissected out 48, 72 and 96 hr after each experimental manipulation. Pre-weighed specimen tubes containing 0·5 ml of isotonic potassium chloride solution were again weighed along with the fatbodies and the weight of fatbodies used for protein estimations were determined from the weight difference. Protein extract of fatbodies was prepared in isotonic KCl solution after homogenisation, precipitation with 10% TCA solution and subsequent centrifugation at 5000 g for 20 min. The residue dissolved in 1 ml of 0·1N NaOH served as the protein extract. Total proteins in the fatbodies were estimated according to the method of Lowry *et al* (1951), using phenol reagent of Folin-Ciocalteau. Bovine serum albumin (Sigma chemical Company, USA) was used as standard. Concentration of protein was expressed in μg protein/mg tissue. Mean values of 8 different determinations were adopted as the protein concentration in each group. Significance of the data were analysed employing student's t test.

2.4 *Histochemistry*

For histochemical demonstration of proteins, the mercury bromophenol blue method (Pearse 1968) was followed using formalin-fixed fatbodies from different categories of experimental insects along with their respective controls.

2.5 *JHa treatment*

Farnesyl methyl ether (FME) (Econ. Control Inc., USA) was the juvenile hormone analogue (JHa) used. FME was dissolved in acetone for topical application; the concentration being 0·5 μg/μl and 4 μl (containing 2 μg) was applied to each animal (effective dose was determined in the preliminary experiments) with the aid of a calibrated microcapillary. FME dissolved in acetone was topically applied underneath the wings to mildly anaesthetised females. Controls were treated similarly with the same quantity of acetone.

3. Results

Protein concentration in the fatbodies was significantly lower in the PINSC-ablated females 48, 72 and 96 hr respectively after the operation than in the respective stages of the sham-operated control groups (figure 1). Implantation of active PINSC into normal females elevated the protein concentration to a significant level when compared with that of the operated controls ($P \leqslant 0{\cdot}01$). Substantial increase in the fatbody protein concentration was noticed in PINSC-ablated insects when they were implanted with active PINSC (figure 1). Histochemical observations corroborate these findings (figure 3; 1 to 4). Significant reduction in fatbody protein concentration was noticed in the allatectomised females as well when these were estimated 48, 72 and 96 hr after the operation ($P \leqslant 0{\cdot}05$). Topical application of FME (JHa) on the normal as well as allatectomised females (figure 2) enhanced protein concentrations to a significant level ($P \leqslant 0{\cdot}05$). However, the rise in protein concentration in allatectomised females was less than that in normal females supplied with additional JHa (figure 2). Histochemical investigations also corroborate these findings (figure 3; 5 to 8).

4. Discussion

Present studies show that in the female *D. cingulatus*, synthesis of protein is inhibited in the absence of PINSC while implantation of active PINSC into PINSC-ablated insects restores the level significantly; implantation into normal females enhances the protein level well above the normal level. The protocerebral neurosecretory cells have been reported to be indispensable for oogenesis in many insect species like *Calliphora erythrocephala* (Thomsen 1948, 1952; Possompes 1956), *Schistocerca gregaria* (Highnam 1962a, b, c; Hill 1962), *Schistocerca pararensis* (Strong 1965a, b), *Locusta migratoria* (Girardie 1966), *Anacardium aegyptium* (Geldiay 1967), *Dysdercus cingulatus* (Jalaja *et al* 1973). However, this does not necessarily mean that PINSC control egg maturation directly. In many insects, MNSC ablation from the brain results in a lowered

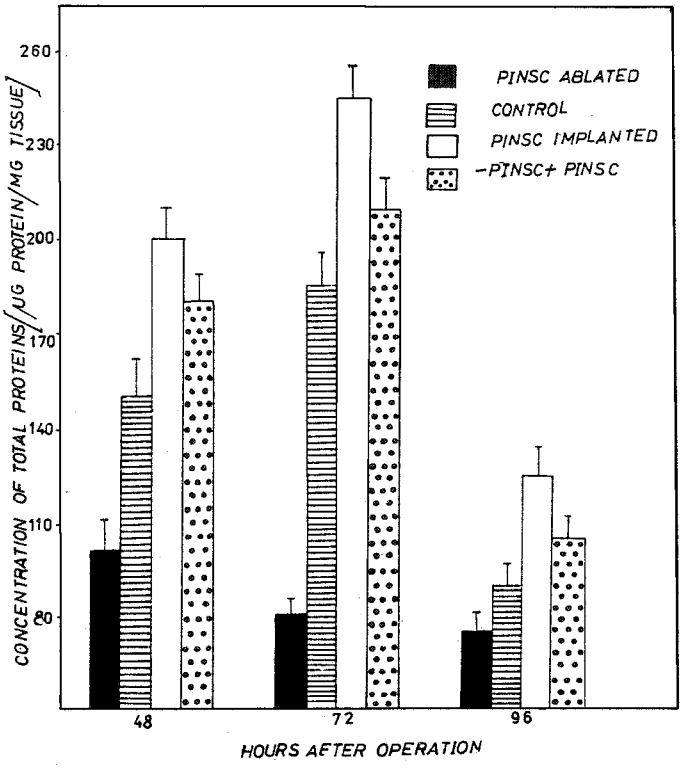

Figure 1. Histogram showing the effect of extirpation and implantation of PINSC on the protein content in the fatbodies of female *D. cingulatus*. Each column represents mean of 8 values and the bars denote ∓ SEM.

food intake as in *Calliphora erythrocephala* (Thomsen and Moller 1963), *D. cingulatus* (Muraleedharan and Prabhu 1979), *Hyblaea puera* (Muraleedharan and Prabhu 1981) resulting in a low protein concentration and the subsequent cessation of oocyte maturation. Therefore, the relationship between MNSC and oogenesis can only be studied properly if MNSC cauterisation has been carried out in such a way that it does not affect food intake. In the experiments performed on *Schistocerca gregaria* (Hill 1965) and *Leptinotarsa decemlineata* (de Loof and de Wilde 1970; de Loof and Lagasse 1970) this condition was fulfilled and the effect of MNSC on oogenesis was established. In *Schistocerca* this is explained by a direct effect on the synthesis of vitellogenic proteins and in *Leptinotarsa* by an effect partly on the fatbody in conjunction with the corpus allatum (vitellogenic protein synthesis) and partly *via* the corpus allatum on the terminal oocyte. Thomsen (1952) has shown that in *C. erythrocephala* females the extirpation of MNSC causes total exhaustion of the cells of fatbodies. The rate of protein synthesis in the fatbodies as well as the concentration of haemolymph proteins are reduced in pars intercerebralis-cauterised female locusts (Hill 1962, 1965). These findings by Hill corroborated a suggestion made earlier by Thomsen (1952) that a principle liberated from pars intercerebralis regulates protein metabolism. Supporting data for this may be found in the observation of low protein concentration in the haemolymph of brain operated females of *Gomphocerus rufus* (Loher 1965), *L. maderae* (Engelmann 1966; Engelmann and Penney 1966) and in young females of *T. molitor*

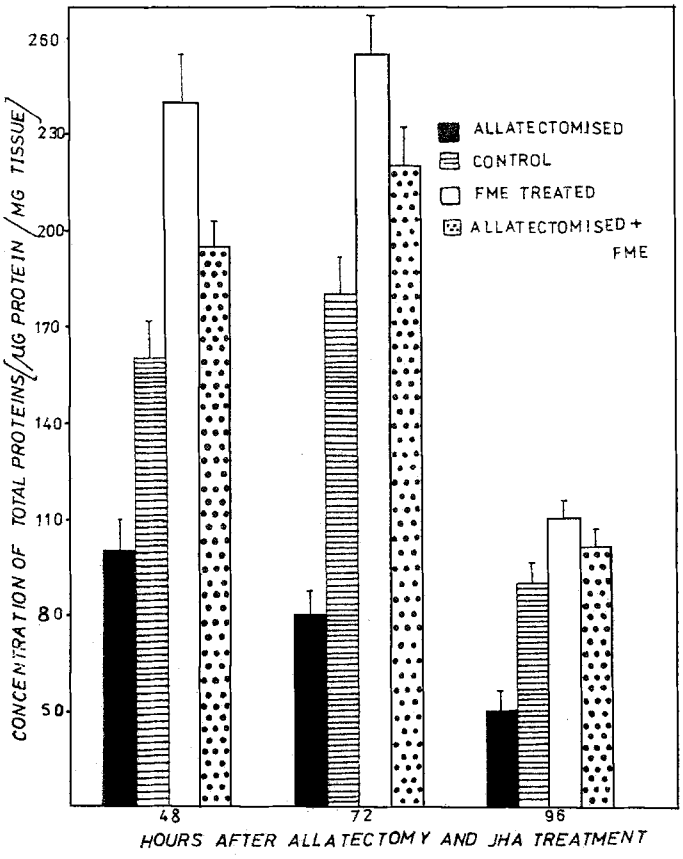

Figure 2. Histogram showing the effect of allatectomy and topical application of FME (JHa) on the protein content in the fatbodies of female *D. cingulatus*. Each column represents mean of 8 values and the bars denote ∓SEM.

(Mordue 1965). MNSC are known to stimulate protein synthesis in the fatbodies in many species of insects such as *S. gregaria* (Highnam *et al* 1963; Hill 1963), *M. sanguinipes* (Elliot and Gillot 1979). The content of the neurosecretory material in the MNSC of *D. cingulatus* increases steadily during the early days of the first gonotrophic cycle when active vitellogenesis is taking place and the protein build up in the haemolymph is under the control of neurosecretion (Jalaja and Prabhu 1977). A sudden decline in the haemolymph proteins is reported in the female *D. cingulatus* 72 hr after emergence which was suggested to be related to heavy yolk protein deposition in the ovaries (Jalaja and Prabhu 1971). The present studies also demonstrate a sudden decline in the protein concentration of fatbodies after 72 hr in the sham-operated controls of the PINSC-ablated insects. This indicates that the decline found in the control is due to vitellogenesis. So it is suggested that in *D. cingulatus* females hormones from PINSC stimulate protein synthesis in the fatbodies during vitellogenesis.

A highly reduced level of protein synthesis as noticed in the allatectomised females and its substantial increase when these insects are supplied with additional JHa and also the increase noticed in protein synthesis when normal females are supplied with extra titre of JH in the form of JHa, demonstrate that JH stimulates protein synthesis in the

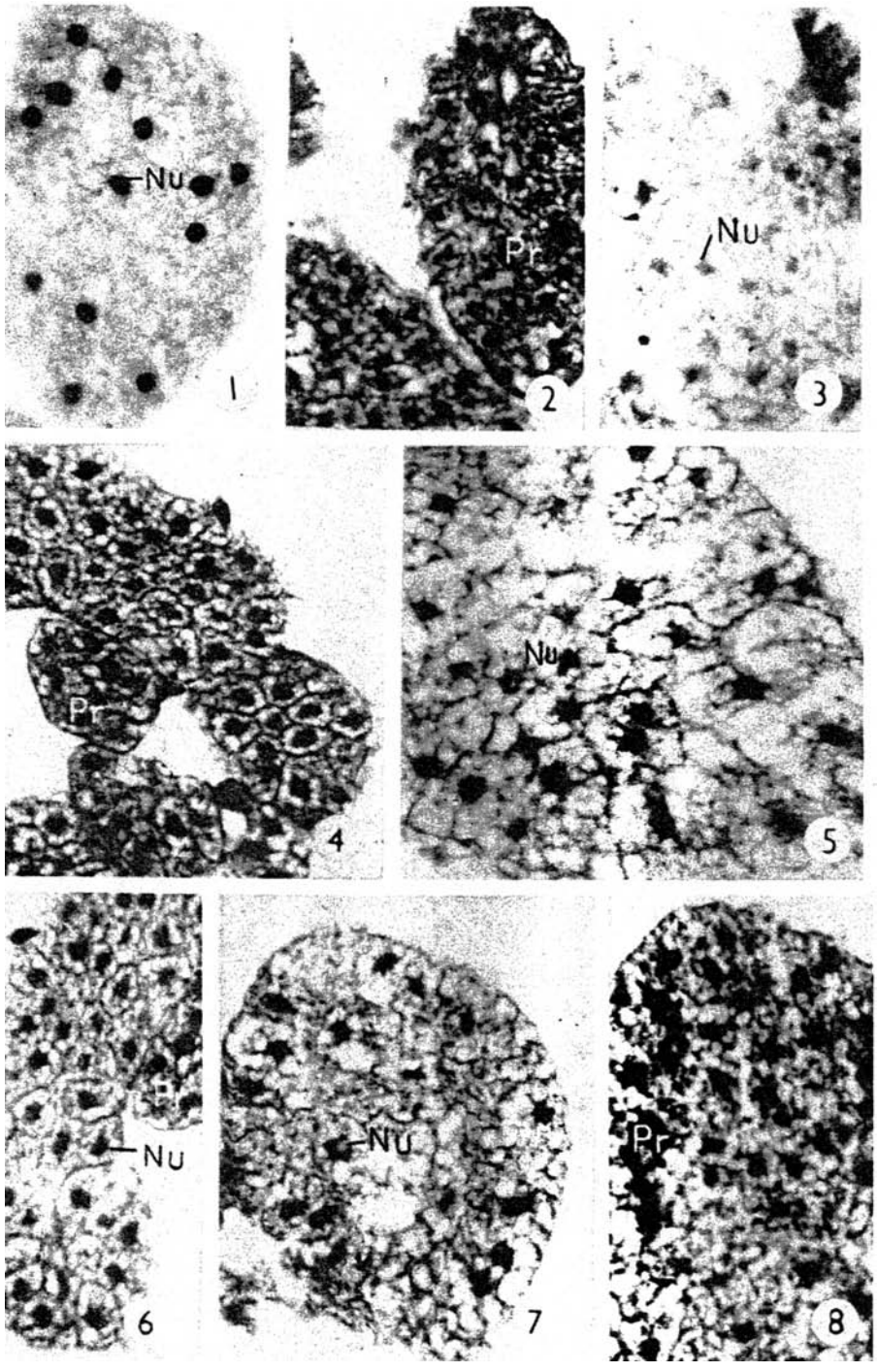

Figure 3. Sections of fatbodies fixed in formalin and stained for protein using mercury bromophenol blue technique (Pearse 1968). **1.** and **3.** 48 and 72 hr after PINSC ablation, **2.** and **4.** their respective controls. **5.** and **7.** 48 and 72 hr after allatectomy, **6.** and **8.** their respective controls. (Nu-Nucleus, Pr-protein) Magnifications of all figures are × 400.

fatbodies of female *D. cingulatus*. The rate of incorporation of radioactive amino acids into fatbody proteins is reported to be significantly slow in allatectomised *P. americana* females (Thomas and Nation 1966). Implantation of active corpus allatum into decapitated *N. cinerea* is also reported to elevate the rate of protein synthesis in the fatbody (Lüscher 1968). However, allatectomised queens of *Apis mellifica* contain vitellogenin at a high titre (eventhough some what lower than in operated controls) and did even lay eggs when treated with CO_2 (Engels and Ramamurthy 1976). Repeated application of JH restored vitellogenin titres to those observed in normal queens (Ramamurthy and Engels 1977). JH stimulates the protein content in the fatbodies of a number of insects such as *Musca domestica, Locusta migratoria, Melanoplus sangunipes* and *Diatraea grandiosella* (Adams and Nelson 1969; Lauwerjat 1977; Elliot and Gillot 1978; Turnen and Chippendale 1980). Enlargement of nuclei and abundance of rough endoplasmic reticulum and golgi complexes were noticed in the fatbodies in connection with the progress of vitellogenesis during which proteins were synthesised in abundance in *L. migratoria* (Couble *et al* 1979). JH is involved in the synthesis of vitellogenins in *Rhodnius prolixus* (Coles 1964, 1965 a, b) and in *Sarcophaga bullata* (Wilkens 1969).

Muraleedharan and Prabhu (1981) have shown that in *D. cingulatus* allatectomy does not affect food consumption. So the decrease in the fatbody as noticed in the allatectomised insects cannot be attributed to deficiency of food. It was demonstrated by Jalaja and Prabhu (1977) that in *D. cingulatus* both MNC-hormone and JH are involved in vitellogenesis and MNC-hormone stimulates the process by influencing the production of JH by the corpus allatum. A neurosecretory influence is observed on the protein synthesis while a direct gonotrophic effect is with corpus allatum and a reciprocal relationship between the neurosecretory system and corpus allatum in which interference with one component of the neuroendocrine system results in interference with the other (Hill 1962; Highnam *et al* 1963).

In the light of the present findings and the pertinent available literature, it may be suggested that in adult females of *D. cingulatus* hormonal principles both from PINSC and corpus allatum have a stimulatory effect on the synthesis of proteins in the fatbody. The influence imparted by PINSC seems to be either through a trophic mechanism on the corpus allatum or by its direct effect on the fatbody while hormones from CA seem to stimulate the process directly.

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References

Adams T S and Nelson 1969 Effect of corpus allatum and ovaries on the amount of pupal and adult fatbody in the house fly, *Musca domestica*; *J. Insect Physiol.* **15** 1729–1749

Chen P S 1978 Protein synthesis in relation to cellular activation and deactivation. IV. Regulation of proteolytic digestive enzymes; in *Biochemistry of insects* (ed.) M Rockstein (New York: Academic Press) 162–168

Coles G C 1964 Some aspects of decapitation on metabolism in *Rhodnius* prolixus Stal; *Nature (London)* **203** 323

Coles G C 1965a Haemolymph proteins and yolk formation in *Rhodnius prolixus* Stal; *J. Exp. Biol.* **43** 425–431

Coles G C 1965b Studies on the hormonal control of metabolism in *Rhodnius prolixus* Stal; *J. Insect Physiol.* **11** 1325–1330

Couble P, Chen T T and Wyatt G R 1979 Juvenile hormone controlled vitellogen in synthesis in *Locusta migratoria* fat body; *J. Insect Physiol.* **25** 327–337

de Loof and de Wilde 1970 Hormonal control of synthesis of vitellogenic female protein in the colorado potato beetle, *Leptinotarsa decemlineata*; *J. Insect Physiol.* **16** 1455–1466

de Loof and Lagasse A 1970 Juvenile hormone and the ultrastructural properties of the fatbody of the adult colorado beetle, *Leptinotarsa decemlineata*; *Z. Zellforsch.* **106** 439–450

Elliott R H and Gillot C 1978 The neuroendocrine control of protein metabolism in the migratory grass hopper, *Melanoplus sanguinipes*; *J. Insect Physiol.* **24** 119–126

Elliot R H and Gillot C 1979 Electrophoretic study of proteins of the ovary, fatbody and haemolymph in the migratory grasshopper, *Melanoplus sanguinipes*; *J. Insect Physiol.* **25** 405–410

Engelmann F 1966 Endocrine activation of protein metabolism during egg maturation in an insect; *Am. Zool.* **5** 673

Engelmann F 1979 Insect vitellogenin: Identification, biosynthesis and role in vitellogenesis; in *Advances in insect physiology* (eds) J E Treherene, M J Berridge and V B Vigglesworth (London, New York and San Fransisco: Academic Press) **14**

Engelmann F and Penney D 1966 Studies on the endocrine control of metabolism in *Leucophaea maderae*. i. The haemolymph proteins during egg maturation; *Gen. Comp. Endocrinol.* **7** 314–325

Engels W and Ramamurthy P S 1976 Initiation of oogenesis in allatectomised virgin honeybee queens by carbon dioxide treatment; *J. Insect Physiol.* **22** 1427–1432

Geldiay S 1967 Hormonal control of adult reproductive diapause in the Egyptian grasshopper, *Anacardium aegyptium* L.; *J. Endocrinol.* **37** 63–71

Gilbert L and Schneiderman H 1961 Some biochemical aspects of insect metamorphosis; *Am. Zool.* **1** 11–51

Girardie A 1966 Controle de l'activite genitale chez *Locusta migratoria* Mise en e'vidence d'un facteur gonadotrope et d'un facteur allatorope dans la pars intercerebralis; *Bull. Soc. Zool. Fr.* **91** 423–439

Hagedorn H H and Fallon A M 1973 Ovarian control of vitellogenin synthesis by fatbody in *Aedes aegypti*; *Nature (London)* **244** 103–105

Highnam K C 1962a Neurosecretory control of ovarian development in *Schistocerca gregaria*; *Q. J. Microsc. Sci.* **103** 57–72

Highnam K C 1962b Neurosecretory control of ovarian development in the desert locust *Schistocerca gregaria*; *Mem. Soc. Endocrinol.* **12** 379–390

Highnam K C 1962c Neurosecretory control of ovarian development in *Schistocerca gregaria* and its relation to phase differences; *Colloq. Int. centrnatl. reserche. Sci. (Paris)*, **114** 1–10

Hill L 1962 Neurosecretory control of haemolymph protein concentration during ovarian development in the desert locust; *J. Insect Physiol.* **8** 609–619

Hill L 1963 Endocrine control of protein synthesis in the female desert locust, *Schistocerca gregaria*; *J. Endocrinol.* **26** 17–18

Hill L 1965 Incorporation of C^{14} glycine into the proteins of the fatbody of the desert locust during ovarian development; *J. Insect Physiol.* **111** 1605–1615

Jalaja M and Prabhu V K K 1971 Blood protein concentration in relation to vitellogenesis in *Dysdercus cingulatus*; *Experientia* **27** 639–640

Jalaja M and Prabhu V K K 1977 Endocrine control of vitellogenesis in the red cotton bug, *Dysdercus cingulatus* Fabr; *Entomon* **2** 17–29

Jalaja M, Muraleedharan D and Prabhu V K K 1973 Effect of extirpation of median neurosecretroy cells in the female red cotton bug, *Dysdercus cingulatus*; *J. Insect Physiol.* **19** 29—36

Keeley L L 1978 Endocrine regulation of fatbody development and function; *Ann. Rev. Entomol.* **23** 329–352

Lauverjat S 1977 Le evolution post imaginale due tissue adipeux femelle de *Locusta migratoria* et son controle endocrine; *Gen. Comp. Endocrinol.* **33** 13–34

Loher W 1965 Hormonale Kontrolle der Oocytentwicklung bei der Heuschreeke *Gomphocerus rufus* L.; *Zool. Jahrb. Abt. Physiol.* **71** 677–684

Lowry D H, Rosenbaum N J, Farr A L and Randall R J 1951 Protein measurement with Folin phenol reagent; *J. Biol. Chem.* **193** 265–268

Lüscher M 1968 Hormonal control of respiration and protein synthesis in the fatbody of the cockroach *Nauphoeta cinerea* during oocyte growth; *J. Insect Physiol.* **14** 499–511

Mills R R, Greenslade F C and Cough E F 1966 Studies on vitellogenesis in the American cockroach; *J. Insect Physiol.* **12** 767–779

Mordue W 1965 The endocrine control of oocyte development in *Tenebrio molitor* L.; *J. Insect Physiol.* **11** 505–511

Muraleedharan D and Prabhu V K K 1979 Role of the median neurosecretory cells in secretion of protease and invertase in the red cotton bug *Dysdercus cingulatus*; *J. Insect Physiol.* **25** 237–240

Muraleedharan D and Prabhu V K K 1981 Hormonal influence on feeding and digestion in a plant bug, *Dysdercus cingulatus* and a caterpillar, *Hyblaea puera*; *Physiol. Entomol.* **6** 183–189

Pearse A G E 1968 *Histochemistry: Theoretical and applied* 3rd edn (London: Churchill) **1**

Possompes B 1956 Development ovarian apres ablation due corpus allatum juvenile chez *Calliphora erythrocephala* Meig. (Diptrere) et chez Sipploidea sipylus W (Pharmoptere); *Ann. Sci. Nat. Zool. Ser.* **11** 313–314

Ramamurthy P S and Engels W 1977 Allatektomie- und juvenilhormon-wirkungen auf synthese und einlagetrung von vitellogenin bei der bienenkonigin (*Apis mellifica*); *Zool. Jahrb. Physiol.* **81** 165–176

Strong L 1965a The relationship between the brain, CA and oocyte growth in the Central American locust, Schistocerca-sp. I. The Central neurosecretory system, CA and oocyte growth; *J. Insect Physiol.* **11** 135–146

Strong L 1965b The relationship between the brain, CA and oocyte growth in the Central American locust, *Schistocerca*-sp. II. The innervation of the CA, the lateral neurosecretory complex and oocyte growth; *J. Insect Physiol.* **11** 271–280

Telfer W H 1965 The mechanism and control of yolk formation; *Ann. Rev. Entomol.* **10** 161–184

Thomas K K and Nation J L 1966 RNA, protein and uric acid content of the body tissue of *Periplaneta americana* L. as influenced by CA during ovarian development; *Biol. Bull.* **130** 442–449

Thomsen E 1948 The gonotropic hormones in the Diptera; *Bull. Biol. Fr. Belg. Suppl.* **33** 68–80

Thomsen E 1952 Functional significance of the neurosecretory brain cells and the corpora cardiaca in the blow fly *Calliphora erythrocephala* Meig; *J. Exp. Biol.* **29** 137–172

Thomsen E and Moller I 1963 Influence of neurosecretory cells and CA on intestinal protease activity in the adult *Calliphora erythrocephala* Meig., *J. Exp. Biol.* **40** 301–321

Tojo S, Kiguchi K and Kimura S 1981 Hormonal control of storage protein synthesis and uptake by the fatbody in the silk worm *Bombyx mori*; *J. Insect Physiol.* **27** 491–497

Turnen S and Chippendale G M 1980 Proteins of the fatbody of non-diapausing larvae of the southwestern corn borer, *Diatraea grandiosella*: Effect of JH; *J. Insect Physiol.* **26** 163–169

Wilkens J L 1969 The endocrine control of protein metabolism as related to reproduction in the fleshfly *Sarcophaga bullata*; *J. Insect Physiol.* **15** 1015–1024

Wyss-Huber M and Lüscher M 1966 Uiber die hormonale Bee-influssebarkeit der protein – synthesis in vitro im Fettkorper von *Leucophaea maderae* (Insecta); *Rev. suisse Zool.* **73** 517–521

Wyss-Huber M and Lüscher M 1972 *In vitro* synthesis and release of proteins by the fatbody and ovarian tissue of *Leucophaea maderae* during sexual cycle; *J. Insect Physiol.* **18** 689–710