NN8201 no 409

BIOCHEMICAL ASPECTS OF JUVENILE HORMONE ACTION IN THE ADULT LOCUSTA MIGRATORIA

A. K. MINKS

NN08201.409

BIBLIOTHEEK
DER
LANDBOUWHOGESCHOOL
WAGENINGEN

LANDBOUWHOGESCHOOL TE WAGENINGEN

BIOCHEMICAL ASPECTS OF JUVENILE HORMONE ACTION IN THE ADULT LOCUSTA MIGRATORIA

(MET EEN SAMENVATTING IN HET NEDERLANDS)

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD VAN DOCTOR

IN DE LANDBOUWKUNDE

OP GEZAG VAN DE RECTOR MAGNIFICUS, IR. F. HELLINGA,

HOOGLERAAR IN DE CULTUURTECHNIEK,

TE VERDEDIGEN TEGEN BEDENKINGEN VAN EEN COMMISSIE

UIT DE SENAAT VAN DE LANDBOUWHOGESCHOOL

TE WAGENINGEN

OP VRIJDAG 16 JUNI 1967 TE 16.00 UUR

DOOR

ALBERT KLAAS MINKS

J. B. WOLTERS GRONINGEN
1967

Dit proefschrift met stellingen van Albert Klaas Minks, landbouwkundig ingenieur, geboren te Leeuwarden op 1 mei 1934, is goedgekeurd door de promotoren, Dr. J. de Wilde, hoogleraar in het dierkundige deel van de plantenziektenkunde en Dr. D. Stegwee, hoogleraar in de algemene plantkunde, plantenfysiologie en farmacognosie aan de Universiteit van Amsterdam.

De Rector Magnificus der Landbouwhogeschool,

F. HELLINGA

Wageningen, 8 mei 1967

STELLINGEN

I

Het juveniel hormoon kan in *Locusta* naast de vorming van vitellogene eiwitten door het vetlichaam, ook die van minstens één niet-vitellogeen eiwit beïnvloeden.

Dit proefschrift.

II

Het is verspilde moeite om door middel van *in vivo* experimenten te willen vaststellen of bepaalde insektenhormonen een effect hebben op de weefseladem haling.

ROUSSEL, J. P., 1963. J. Insect Physiol. 9, 721–725.
SÄGESSER, H., 1960. J. Insect Physiol. 5, 264–285.
SLÁMA, K., 1964. J. Insect Physiol. 10, 283–303.
Dit proefschrift.

III

De toevallig aanwezige hoeveelheid hormoon in de geïmplanteerde corpora allata bepaalt het resultaat van deze implantaties in allatectomie-dieren.

Dit proefschrift.

IV

Een relatie tussen het histologisch beeld van hormoonproducerende cellen en de chemische structuur van hun hormonen is vooralsnog moeilijk vast te stellen.

SCHARRER, B., 1965. Arch. Anat. micr. 54, 331–342.

V

De veronderstellingen, ten aanzien van de regulatie der eiwitsynthese door GRUBER en CAMPAGNE naar voren gebracht, zijn niet goed te rijmen met de gedachtengangen omtrent de synthese van het boodschapper-RNA zoals die naar voren zijn gebracht door BEERMANN, c.s.

BEERMANN, W., 1965. In: Cell Differentiation and Morphogenesis, pp. 24–54. North Holland Publ. Cy., Amsterdam.
GRUBER, M. en R. N. CAMPAGNE, 1965. Proc. Kon. Ned. Akad. Wetensch. A'dam (C) 68, 270–276.

VI

De ontwikkeling van R-rassen van aardappel heeft in het algemeen de resistentie van de aardappel tegen *Phytophthora infestans* verminderd.

VII

Het antwoord op de vraag of de vorming van fytoalexinen onafhankelijk van nekrobiotische verschijnselen verloopt, hangt samen met de definitie van het begrip nekrobiose.

MÜLLER, K.O. en H. BÖRGER, 1939. Landwirtsch. Jahrb. **87**, 609.
OORT, A.J.P., 1967. Vakbl. v. Biol. Ter perse.

VIII

De efficiëntie van de roofmijt, *Typhlodromus pyri*, als predator van de fytofage mijt, *Panonychus ulmi*, hoeft niet nadelig te worden beïnvloed door een verschil in verspreiding op het blad van beide soorten.

CHANT, D.A., 1959. Canad. Ent. **91**, Suppl. 13, 1–164.

IX

Het zou te betreuren zijn wanneer de algemene dierkunde niet als verplicht vak in de nieuwe propaedeuse voor de natuurwetenschappelijke richtingen aan de Landbouwhogeschool zou worden opgenomen.

X

De plannen tot inpoldering van de Waddenzee komen voort uit diezelfde drang naar expansie, welke in het verleden zo vaak aanleiding heeft gegeven tot het voeren van agressie-oorlogen.

Proefschrift A. K. Minks.
16–6–1967.

VOORWOORD

Bij het verschijnen van dit proefschrift wil ik gaarne mijn grote dankbaarheid tot uitdrukking brengen jegens hen die aan mijn vorming en in het bijzonder aan dit werkstuk hebben bijgedragen.

Het volgen van een akademische opleiding is niet een voor de hand liggende zaak in onze familie. Des te meer bewonder ik mijn ouders, die onder veel opofferingen mijn studie mogelijk hebben gemaakt.

Veel dank ben ik verschuldigd aan de hoogleraren en lectoren van de Landbouwhogeschool voor het aandeel dat zij hebben gehad in mijn opleiding.

Hooggeleerde DE WILDE, hooggeachte Promotor, het is mij destijds niet moeilijk gevallen om de richting plantenziektenkunde en vervolgens om als hoofdvak de entomologie te kiezen. Naast het feit dat dit vak zich goed liet combineren met mijn biochemische interesse, maakte uw aantrekkelijk „uitstallen" van de entomologie veel indruk op de Wageningse studenten. Reeds tijdens het bewerken van het ingenieurs-onderwerp kreeg ik het gevoel op het Laboratorium voor Entomologie mee te kunnen werken „in de voorste linies" bij het attakeren van de problemen rondom de insektenhormonen. Uw stimulerend enthousiasme en grote kennis zijn voor mij van groot nut geweest. Voor uw kritiek tijdens het gereed maken van het manuskript ben ik u zeer erkentelijk. Het verblijf op uw laboratorium is voor mij van essentiële waarde geweest.

Hooggeleerde STEGWEE, hooggeachte mede-Promotor, het verheugt mij zeer dat U als zodanig bij de afsluiting van deze studie aanwezig bent. Ik besef, dat ik geluk heb gehad te kunnen werken onder Uw supervisie. In aanraking komende met een zo grote kundigheid, veelzijdige interesse en originaliteit voelde ik mezelf nietig worden, maar werd toch zo geïnspireerd dat ik me in vrij korte tijd thuis voelde in het onderwerp. Met enige heimwee denk ik terug aan de tijd samen op „biochemie" doorgebracht. Veel dank ook voor het doornemen van het manuskript in een zo overladen drukke tijd!

Velen van de staf en het personeel van het laboratorium zijn mij zeer behulpzaam geweest bij het tot stand komen van dit onderzoek. Gesprekken met dr.ir. G. B. STAAL, drs. C. A. D. DE KORT en drs. H. SCHOONEVELD hebben mijn inzicht in de problemen van het insektenhormoon-onderzoek vaak zeer verhelderd. Eerstgenoemde ben ik bovendien dankbaar omdat dank zij zijn eerdere aktiviteiten het kweken van de benodigde grote aantallen sprinkhanen nooit moeilijkheden heeft gegeven. Hier moet uiteraard ook de heer G. LUBOUT met ere worden genoemd.

Mevr. H.J. PIEK-KRAMER verdient veel lof voor de snelheid, akkuratesse en toewijding, waarmee zij bij het grootste deel van het onderzoek assisteerde. Dank ook voor de assistentie van Mej. C.J. RAAK.

De medewerking van de heer J.P.W. NOORDINK (I.P.O.) bij het vervaardigen van de autoradiogrammen was een succes. De heer S. HENSTRA (L.F.T.D.) maakte de elektronen-mikroskopische foto's. De adviezen van de heer C.A. VAN DEN ANKER (I.P.O.) voor het statistisch bewerken van het cijfermateriaal heb ik op hoge prijs gesteld.

Dr. J.D. LATTIN en dr. M.R. HONER ben ik zeer erkentelijk voor hun hulp bij het korrigeren van het Engels, Mevr. G. DE WILDE-VAN BUUL voor het doornemen van de Nederlandse samenvatting, de heren M.P. VAN DER SCHELDE en M. JANSEN voor het tekenen der figuren en Mej. M.C. ALBERS voor het tikken van de definitieve tekst van het manuskript.

De bibliothekaris van het Centrum, de heer G. DE BRUYN feliciteer ik met de moed, waarmee hij mijn huisvesting in de bibliotheek heeft verdragen en zijn charmante assistentes voor de vlotte en deskundige manier, waarop zij steeds de gevraagde literatuur aan de man weten te brengen.

Tenslotte ben ik veel dank verschuldigd aan de Nederlandse Organisatie voor Zuiver-Wetenschappelijk Onderzoek (Z.W.O.) die deze studie mogelijk heeft gemaakt dank zij subsidie R 942–29.

SAMENVATTING

Dit proefschrift heeft betrekking op een studie over de biochemische en fysiologische werking van het juveniel hormoon (JH) in de imago van de Afrikaanse Treksprinkhaan, *Locusta migratoria*. Het behandelt in hoofdzaak twee aspekten van deze werking:

A. Het effekt op de respiratoire stofwisseling.

B. Het effekt op de synthese van bloedproteinen.

A. In Hoofdstuk 3 wordt de vraag besproken, of het JH al dan niet een direkt effekt op de respiratoire stofwisseling uitoefent. Vele onderzoekers hebben getracht deze vraag te beantwoorden door middel van extirpatie en/of implantatie van de corpora allata (CA) tijdens proefnemingen *in vivo*. Experimenteren *in vitro* is echter noodzakelijk om een inzicht te verschaffen in het genoemde probleem. In ons onderzoek werd daarom gebruik gemaakt van mitochondriën, geïsoleerd uit de vliegspieren en uit het vetlichaam van *Locusta* (paragraaf 3.1). Er werden vliegspier-mitochondriën geïsoleerd, afkomstig enerzijds van normale *Locusta*'s en anderzijds van dieren, waarbij de CA waren weggenomen. Ten aanzien van het zuurstofverbruik en de oxydatieve fosforylering – met α-glycerolfosfaat of een mengsel van pyruvaat/malaat als substraat – verschilden beide categoriën onderling niet. Verder bleek, dat gehomogeniseerde aktieve CA geen effekt hadden op het zuurstofverbruik van de mitochondriën, afkomstig van normale of geopereerde sprinkhanen. De CA hadden wel een stimulerende werking op de mate van koppeling tussen het zuurstofverbruik en de fosforylering van vliegspier-mitochondriën met α-glycerolfosfaat als substraat en geen effekt bij gebruik van pyruvaat/malaat als substraat. De gegevens uit deze proeven steunden de veronderstelling, dat de fasen van *Locusta* verschillen in het niveau van hun JH-aktiviteit. De solitaire fase is meer „juveniel". CA, geïsoleerd uit solitaire sprinkhanen, stimuleerden de oxydatieve fosforylering in mitochondriën, afkomstig van sprinkhanen in de gregaire fase en zulke, waarbij allatectomie was toegepast; CA, geïsoleerd uit sprinkhanen in de gregaire fase, stimuleerden alleen de mitochondriën van allatectomie-dieren. Dit was het geval zowel met vliegspier- als met vetlichaam-mitochondriën, beide geïncubeerd met α-glycerolfosfaat. Deze gegevens vormen eveneens een belangrijk argument tegen de veronderstelling, dat het bedoelde effekt zou worden teweeg gebracht door andere bestanddelen van het CA dan het JH (3.2.1).

De ontkoppelaar 2.4-dinitrofenol (DNP) stimuleerde het zuurstof-

verbruik van de mitochondriën in niet-fosforylerende omstandigheden, maar remde dit proces in fosforylerende mitochondriën en ontkoppelde tevens de oxydatieve fosforylering (vliegspier-mitochondriën in beide genoemde substraten). DNP-concentraties beneden 10^{-6} M hadden geen remming tot gevolg. Bij intermediaire DNP-concentraties (10^{-4} à 10^{-5}M) werd de oxydatieve fosforylering niet volledig geremd en juist daar was het mogelijk vast te stellen, dat de CA het ontkoppelend effekt van DNP verminderde. De toestand van de geïsoleerde mitochondriën werd gecontroleerd door vergelijking van de door Mg^{++} en DNP geïnduceerde adenosine trifosfatase aktiviteit. Het leek er op, dat de CA de door DNP geïnduceerde ATP-ase aktiviteit remden, zowel van mitochondriën uit de vliegspieren als van die, welke uit het vetlichaam afkomstig waren (3.2.2).

Cecropia-olie, farnesol en farnesyl methyl-aether hadden, wanneer ze werden toegediend aan vliegspier-mitochondriën, geen effekt op het zuurstofverbruik. Cecropia-extract in concentraties van 10^{-6} – 10^{-8} vergrootte wèl de efficientie van de oxydatieve fosforylering, dit in tegenstelling tot genoemde farnesolverbindingen (3.2.3).

Wij kwamen tot de slotsom, dat er *in vitro* geen enkele betrekking bestond tussen de werking van het JH en het zuurstofverbruik. Er was echter een duidelijk positief effekt op de oxydatieve fosforylering. Het was niet mogelijk de hier verkregen resultaten te verklaren op grond van gegevens, ontleend aan de insekten-endocrinologie. De *in vitro* JH-effekten werden besproken in de volgende punten:

a. Een vergelijking met effekten van thyroxine in ratten;

b. De mogelijke stimulering van de zogenaamde koppelingsfaktoren;

c. Het mogelijke effekt – via de ATP-ase aktiviteit – op de Na^+/K^+ verhouding in de mitochondria (3.3).

B. Vervolgens zijn verschillende aspekten van de stofwisseling, verband houdende met de reproduktie van normale *Locusta*-imagines en die waarvan de CA waren verwijderd, met elkaar vergeleken (Hoofdstuk 4). De betrekkingen tussen de verschillende delen van het neuro-endocriene systeem, dat de reproduktie en de daaraan ten grondslag liggende stofwisselingsprocessen regelt, zijn ingewikkeld. Daarom is het moeilijk vast te stellen, welke rol in de hormonale besturing der reproduktie door een bepaald orgaan wordt gespeeld. De funktie van de CA is in het bijzonder het onderwerp van veel discussie. Een studie van de dooier-eiwit synthese buiten het ovarium, en wel in het vetlichaam, leverde verdere belangrijke gegevens op. Hierbij werd o.m. gebruik gemaakt van elektroforetische scheiding van de hemolymfe-eiwitten (4.1).

Eerst werden quantitatieve analyses van vetlichaam, hemolymfe en ovarium uitgevoerd gedurende de eerste 24 levensdagen van de volwassen sprinkhaan. Het drooggewicht en de gehalten aan eiwit, RNA-fosfaat, vrije aminozuren, vet en glycogeen (alleen in vetlichaam en ovarium) en trehalose (alleen in de hemolymfe) werden bepaald in normale en allatectomie-*Locusta*. Deze gehalten vertoonden een duidelijk cyclisch verloop dat bij vrouwelijke dieren coïncideert met de eerste ovariale cyclus. Na allatectomie trad geen aktiviteit van de ovaria op. Bovendien verdween het cyclisch verloop van de genoemde bestanddelen. Het eiwitgehalte in de hemolymfe, het vet en het glycogeen in het vetlichaam stegen boven het normale peil (4.2.5).

De incorporatie *in vivo* van met ^{14}C gemerkte aminozuren (bepaald 24 uur na injektie) in de eiwitten van het vetlichaam, de hemolymfe en de ovariën werd gedurende de eerste 24 dagen van het imaginale stadium gevolgd. De normale *Locusta* vertoonde een periodieke aktiviteit in deze incorporatie, verband houdende met de geslachtelijke cycli. Deze periodiciteit verdween in insekten, waarvan de CA waren verwijderd. De eiwitsynthese was op de 9e dag aan het begin van de eerste cyclus maximaal (4.2.6).

Vervolgens werd de incorporatie-aktiviteit *in vitro* van gehomogeniseerde vetlichamen van enerzijds normale en anderzijds allatectomie-sprinkhanen met elkaar vergeleken. Alleen op het tijdstip, waarop de ontwikkeling der oöcyten moest beginnen, traden duidelijke verschillen op. Hetzelfde gold voor de incorporatie in mitochondriën, geïsoleerd uit het vetlichaam van beide categorieën dieren, met uitzondering van oudere exemplaren (4.2.7). Na scheiding van de hemolymfe-eiwitten door middel van agar-gel elektroforese konden 7–8 banden worden onderscheiden. Twee negatieve eiwitten 6 en 7 waren gecorreleerd met de geslachtelijke ontwikkeling van de sprinkhaan. Na allatectomie werd de positieve eiwitfraktie 3 het belangrijkst (4.2.8).

Implantatie van aktieve CA in de wijfjes van *Locusta* die tevoren aan allatectomie waren onderworpen stelde in meer dan 95 % van de gevallen onmiddellijk de voortplantingsprocessen in werking, maar slechts voor de duur van één sexuele cyclus. Dit wijst er op, dat de innervatie van de CA vanuit het cerebrale ganglion noodzakelijk is voor de cyclische aktiviteit van deze klieren. Vier uur na injektie van met ^{14}C gemerkt eiwithydrolysaat in deze sprinkhanen werd de hemolymfe verzameld en werden de eiwitfrakties gescheiden. Door autoradiografie van de frakties werd de radioaktiviteit van deze frakties bepaald en vergeleken met het totaal eiwit per fraktie. Hierdoor kon worden vastgesteld dat na implantatie een snelle verandering van de eiwitsynthese optrad.

Door middel van autoradiografie van met ^{14}C gemerkte hemolymfe-eiwitten kon een snelle verandering van de eiwitsynthese worden vast-

gesteld (4.2.9). Eiwitfraktie c van het vetlichaam was waarschijnlijk identiek met hemolymfe-eiwit 3; fraktie f met band 7 (4.2.10).

De eiwitpatronen in de hemolymfe van vrouwelijke *Locusta*, waarvan de CA zijn verwijderd en van wijfjes waarvan de neurosecretorische cellen zijn uitgeschakeld door diathermische cauterisatie, vertoonden een grote onderlinge gelijkenis (4.2.11).

De eiwitpatronen in de hemolymfe van wijfjes, waarvan het ovarium was verwijderd, geleken op de normale patronen, behalve wat de accumulatie van fraktie 4 betreft. Het eiwitgehalte en ook het volume van de hemolymfe vertoonden beide een belangrijke toename in wijfjes, waarvan het ovarium was weggenomen en die samen met aktieve mannetjes waren opgekweekt (4.2.12).

Deze resultaten zijn in overeenstemming met de opvatting, dat in het vetlichaam een aanzienlijk gedeelte van de eiwitten wordt gesynthetiseerd, welke nodig zijn voor de oögenese. Onze gegevens zijn niet in strijd met de ideeën van HIGHNAM c.s. (1963) volgens welke de neurosecretorische cellen de eiwitsynthese in het vetlichaam aktiveren. Ten aanzien van deze eiwitsynthese menen we echter te hebben vastgesteld, dat het CA een meer onafhankelijke rol speelt, dan tot nu toe werd verondersteld. Onze gegevens vormen een steun voor de gedachte dat het JH zijn aktiviteit uitoefent tijdens een aktieve eiwitsynthese en dat het de vorming van specifieke vitellogene eiwitten induceert. Mogelijk kunnen we de verandering van vet- en glycogeen synthese op dezelfde wijze verklaren (4.3).

BIOCHEMICAL ASPECTS OF JUVENILE HORMONE ACTION IN THE ADULT LOCUSTA MIGRATORIA

by

A. K. MINKS
(Agricultural University, Department of Entomology, Wageningen, The Netherlands)

CONTENTS

1. GENERAL INTRODUCTION

The corpora allata are globular organs—already in 1913 described as glands with an internal and periodic activity by NABERT—which are located in the head just behind the insect brain. The control of ovarian activity by the corpora allata in the bug *Rhodnius prolixus* and in the grasshopper *Melanoplus differentialis* was revealed by WIGGLESWORTH (1936) and WEED PFEIFFER (1936), respectively.

Since that time an almost endless series of investigations has been devoted to the corpus allatum and its product, the juvenile hormone. During the symposium on "Insect Endocrines", which was held in Brno, Czechoslovakia, in August, 1966, nearly 65% of the lectures presented dealt with this subject. Several groups of scientists have been working during last years on the isolation and identification of the juvenile hormone, and recently RÖLLER, BJERKE and co-workers (1965a, b) have isolated traces of the substance, in a chromatographically pure state from extracts obtained from the male adult Cecropia silkworm, *Hyalophora cecropia*. Very recently RÖLLER *et al.* published the structure of the juvenile hormone. They did not yet present a method of synthesis of the active substance[1]).

The present study deals with the mode of action of the juvenile hormone in the control of metabolism in the adult *Locusta migratoria*. The question, whether the hormone exerts a direct or an indirect effect on metabolism, is subject to much controversy in literature. Besides this, there is much discussion concerning the rôle of the juvenile hormone in the complicated interactions prevailing within the neuro-endocrine system.

This study consists of two parts. Firstly, the action of the juvenile hormone will be considered in relation to respiratory metabolism. Effects on oxygen consumption and energy metabolism will be described and discussed. In the second part, several metabolic processes will be compared in normal and allatectomized *Locusta*. Especially the changes of protein and lipid metabolism demand our attention.

Availability of the hormone in a more purified form would have facilitated the procedure of the experiments. Under the present circumstances we have to rely upon homogenized corpora allata and a juvenile hormone-concentrate prepared from abdomina of male *Hyalophora cecropia*. In some *in vitro* experiments allatomimetic substances were used such as farnesol derivatives, emulsified in water. Extirpation and implantation of corpora allata and cauterization of neurosecretory cells were among the microsurgical operations carried out to separate the effects of parts of the neuro-endocrine system within the integrated totality of the organism.

[1]) RÖLLER, H., K. H. DAHM, C. C. SWEELY and B. M. TROST, 1967: Angew. Chemie 79, 190.

ACKNOWLEDGEMENTS

The author is much indebted to Prof. Dr. J. DE WILDE for his supervision and for his valuable criticism during the investigations and the preparation of the manuscript. He is further very grateful to Prof. Dr. D. STEGWEE, whose inspiring ideas and continuous encouragement were essential for the successful realization of the present study. This work has been made possible by grant R 942–29 of the Netherlands Organization for the Advancement of Pure Research (Z.W.O.).

The following abbreviations are used:

JH	juvenile hormone
CA	corpus allatum/corpora allata
CC	corpus cardiacum/corpora cardiaca
NSC	neurosecretory cells
AMP	adenosine monophosphate
ADP	adenosine diphosphate
ATP	adenosine triphosphate
ATP-ase	adenosine triphosphatase
NAD	nicotinamide-adenin-dinucleotide
EDTA	ethylene diamine tetraacetic acid
DNP	2.4-dinitrophenol

2. MATERIALS AND METHODS

2.1. EXPERIMENTAL ANIMALS

2.1.1. *The breeding of adult* Locusta

Adults of *Locusta migratoria migratorioides* Reiche and Fairmaire, 1850, the African Migratory Locust, were used throughout the experiments. The breeding material of this locust was supplied by the Anti-Locust Research Centre in London about 10 years ago and since that time it has been reared continuously in our laboratory.

The breeding stock was kept in a climate room with constant temperatures of 30°–32° C and a relative humidity of 35–40 %. Inside the cages containing the adults continuously burning red incandescent bulbs maintained a temperature gradient ranging from 30°–45° C, thus allowing the insects to select their favourite temperature (DUDLEY, 1964). Daylength was maintained at 14 hours, all the year round. Compact bundles of food were supplied daily; this consisted mainly of grasses and young plants of several species of cereals, and some leaves of lettuce, endive, cauliflower or curled kale.

Jars containing a mixture of humid sand and peat-dust were screwed to the bottom of cages in which sexually mature adults were present, to provide a good ovipository site that prevented desiccation of the eggs. These jars were emptied every second day and the mixture of humid sand and eggs was kept in closed boxes at 30° C.

About the time of hatching these boxes were transferred to normal breeding cages, four to six boxes per cage, and there left open. In all cages (40 × 40 × 30 cm) a high population-density (75–100 animals) was maintained and, judging by their pigmentation and behaviour, the locusts were obviously in the gregarious phase. As it was usually important to know the adult age of the experimental animals, groups of 15–20 insects which had just completed their final moult were confined to smaller cages (30 × 18 × 15 cm). The population-density in these cages was also high enough to keep the animals in the gregarious phase. Under the conditions mentioned, the eggs hatch after 12 days approximately. Development of newly hatched insects into adults requires about six weeks; these adults live for another two months on the average.

To obtain experimental locusts in the solitary phase, it was necessary to rear them individually from the egg. We preferred to use green individuals since it is suspected that they have the highest level of corpus allatum hormone activity (STAAL and DE WILDE, 1962). For this reason, the relative humidity had to be at least 85 per cent (temperature 30°–32° C). This condition was achieved by breeding the isolated insects in small, closed, glass jars, in which the supplied food provided the high humidity. A minimum of ventilation was secured through the lid. To avoid the formation of water droplets caused by condensation, the bottom and a part of the walls of the jar were covered with a layer of filter-paper. In spite of the special care taken in breeding, about 40 per cent of the larvae died, presumably because of high humidity. By contrast, in breeding the gregarious locusts, mortality amounted to less than 5 percent.

2.1.2. *Surgical Techniques*

Extirpation and implantation of corpora allata

Extirpation of the corpora allata was always carried out in young adults, 1–2 days after the final moult. By that time the exoskeleton was sufficiently sclerotized and the insect could endure the operation without much harm. Moreover, the haemolymph is a clear green liquid, not obstructing the view of the more deeply situated parts of the post-cerebral complex. A detailed description of microsurgical techniques on the endocrine system of locust larvae is given by STAAL (1961). There is one slight modification when these techniques are used for adults; anaesthetizing the animals (with CO_2) appeared preferable to stop strong contractions of the oesophagus and surrounding muscles after fixing the locust with the clamp. The damage caused by the operation inside the head cavity is minimal and the rate of survival is generally over 95 per cent. The sham-operated animals were treated in the same manner but without removal of the corpora allata. The operated and control animals were kept separately in different groups depending on age and time of operation, but males and females within the groups were put together.

The above-mentioned method of allatectomy is simple and quick. It is also well suited to collect within reasonable time corpora allata required for implantation experiments and for preparation of the homogenates.

For the implantation experiments performed in allatectomized, six to eight days-old insects, only corpora allata isolated from yellow mature males were used. Equipment and conditions were exactly the same as in the allatectomy experiments. As quickly as possible after isolation, the corpora allata were introduced into the head cavity through a slit in the neck membrane and carefully attached between the mandibular muscles. In the control animals, the neck membrane was merely perforated. Implanted females were kept in cages with normal males, implanted males with normal females. Implanted locusts of both sexes were also kept together.

Ovariectomy

The ovaries of 5–7 day-old females were easily removed by pulling them carefully with a pair of forceps through a dorso-lateral slit in the third segment of the abdomen (HILL, 1962). Control animals were only given an incision at the same place. The slit was sealed with a mixture of equal amounts of paraffin wax and colophonium resin. The animals were kept both with and without males.

Cautery of the neurosecretory cells

For some experiments, locusts were needed with non-functioning median neurosecretory cells of the pars intercerebralis. These cells are located superficially in the cerebral tissue at the median dorsal site. This situation corresponds exactly with the one described by HIGHNAM (1961) for the closely related *Schistocerca gregaria* and is in accordance with the description by GIRARDIE (1962). A suitable technique is heat coagulation of the tissue surface cells with a diathermic cautery needle (Elther, frequency 3.10^6 per.). A flap of the cuticle was cut away from the frons of the head capsule. After removal of some cephalic air-sacks the surface of the brain was exposed. Haemolymph was blown away by a small stream of air to keep the brain-surface as dry as possible. After using the cautery needle, the flap of cuticle was closed and sealed with the paraffin-resin mixture. The control animals underwent the same operation, but were not cauterized. This operation was performed on female locusts two to three days after the final moult. Anaesthetizing was done with CO_2. Instruments and saline were always sterilized. In spite of all precautions the percentage of animals that could be used for the experiments was low: 25 per cent. Both control and cauterized females were kept with males.

2.2. ADMINISTRATION OF JUVENILE HORMONE IN VITRO

2.2.1. *Corpora allata homogenates*

The isolated glands were put in 1.5 ml of ice-cold Ringer's solution as modified by EPHRUSSI and BEADLE, and could be kept there for at least three hours without losing their activity. This was concluded from several successful implantations of these corpora allata into allatectomized female locusts, of which more than 95 per cent began to lay eggs 10–14 days after the implantation. The isolated corpora allata, however, were mostly used for experiments immediately after being collected. In the *in vitro* experiments concerned, the corpora allata proved to be most active in a homogenized condition; homogenization of this small quantity was carried out with a hand-driven all-glass homogenizer.

2.2.2. *Preparation of allatomimetica and JH-concentrates*

In some experiments cecropia oil, farnesol and farnesyl-methylether were used. At first these oily liquids were diluted in 10% dioxan, to promote their dispersion in the aqueous media. Later on, very fine homogeneous emulsions were prepared in a MSE sonic desintegrator (output 60 W; frequency 60 KHz). During preparation the mixture was immersed in an ice-bath. The crude cecropia oil was prepared by STAAL according to the method described by WILLIAMS (1956). Its activity was tested by the Galleria test (GILBERT and SCHNEIDERMAN, 1960).

2.3. PREPARATIONS FOR THE IN VITRO EXPERIMENTS

2.3.1. *Isolation of sarcosomes*

Sarcosomes were always isolated from the thoracic flight muscles of nine to ten day-old locusts, following the directions of KLINGENBERG and BÜCHER (1959) in order to obtain a sufficient degree of respiratory control. The locusts were immobilized by cooling at 0°–4° C. All further manipulations were carried out at this temperature. The head, wings, legs, abdomen, gut and sternal part of the thorax were removed. The remaining dorsal thorax (with chitin) was placed into an ice-cooled mortar. Eight thoraces were needed for each preparation and they were gently pounded with a pestle after addition of 5 ml of isolation medium (0.32 M sucrose–0.01 M EDTA, pH 7.5). The resulting mass was filtered by suction through two layers of muslin, which had been previously saturated with isolation medium, and the residue was washed several times with small portions of the medium.

This filtrate (approximately 40 ml) was centrifuged for 4 minutes at 150 *g*. The sediment was discarded and the supernatant again centrifuged for 20 minutes at 2000 *g* to precipitate the sarcosomes. The supernatant was decanted and the sarcosomal pellet was rinsed by resuspending it in 5 ml of 0.32 M sucrose solution, pH 7.5, with the aid of a hand-driven Potter-Elvehjem (teflon and glass) homogenizer. Then the procedure of low and high speed centrifugation was repeated. Again the supernatant was decanted and the pellet was suspended in 4 ml of 0.32 M sucrose solution. The whole operation took place in a cold room at 0°–4° C.

The technique described above was principally the same as has been followed in the experiments by VAN DEN BERGH and SLATER (1962), and STEGWEE and VAN KAMMEN-WERTHEIM (1962), respectively with the housefly and the Colorado potato beetle.

2.3.2. *Homogenates and subcellular fractions of the fat body*

Fat bodies were collected in the following manner: the chilled locusts were decapitated and the body was opened along the mid-ventral line by cutting from the prosternum to the tip of the abdomen. The gut was pulled backwards and the gonads were removed. The yellow-coloured tissue of the fat body was brought together with a forceps and immediately transferred to an ice-cold teflon-and-glass Potter-Elvehjem homogenizer containing 4 ml of 0.32 M sucrose, pH 7.5. For each experiment four to five fat bodies were required. The yellow homogenate was kept in the ice-cold sucrose medium until the moment of use for experiments, but never longer than 15 minutes.

For the isolation of fat body mitochondria the same procedure of isolation, collection and homogenization was followed. Fifteen to twenty fat bodies were homogenized in a mechanically driven Potter-Elvehjem for each experiment, using two times ten passes at a speed of 1500 rpm, in 5 ml of 0.32 M sucrose-0.01 M EDTA, pH 7.5 solution. The homogenate was further diluted to a volume of 45 ml ready for centrifugation. A preliminary centrifugation was carried out at low speed: 10 minutes at 150 *g*. After discarding the residue of cell debris the supernatant with much fatty material was again centrifuged for 20 minutes at 12.000 *g*. All operations were performed at 0°–4° C and it was easy now to decant the cloudy supernatant and to separate completely the fatty material as a compact layer. The mitochondrial pellet was then washed in fresh 0.32 M sucrose solution, pH 7.5, by resuspension, and again centrifuged. The final pellet was resuspended in 4 ml of ice-cold sucrose and used immediately. Plate I gives an impression of the composition and condition of the mitochondrial fraction thus isolated.

2.4. RESPIRATION EXPERIMENTS

2.4.1. *Measurement of respiration and oxidative phosphorylation*

Oxygen consumption was measured with the conventional Warburg technique. The standard incubation medium given by VAN DEN BERGH and SLATER (1962) containing 15 mM KCl, 2mM EDTA, 5 mM $MgCl_2$, 50 mM Tris (hydroxylmethyl) aminomethane, 30 mM potassium phosphate buffer (pH 7.5), supplied with bovine serum albumin-0.12%, was used in all experiments. As substrate was provided 30 mM α-glycerophosphate or a mixture of 15 mM sodiumpyruvate and malic acid each. For phosphorylating conditions 1.5 mM ATP, 150–180 K.M. units of hexokinase and 5 mM glucose were needed. For the measurement of phosphorylation the procedure of STEGWEE and VAN KAMMEN-WERTHEIM (1962) was followed. At first, oxygen consumption in a non-phosphorylating system was determined and after addition of ATP from the side-arm of the Warburg flasks both respiration and phosphorylation were measured. The phosphorylation was determined from the disappearance of inorganic phosphate in the reaction medium, deproteinized with trichloroacetic acid (TCA) according to the method of FISKE and SUBBAROW (1925), modified by SUMNER (1944).

2.4.2. *Determination of ATP-ase activity of mitochondria*

ATP-ase activity was measured in both thoracic flight muscle- and in fat body mitochondria. The method described by STEGWEE and VAN KAMMEN-WERTHEIM (1962) was used.

2.5. EXPERIMENTS WITH RADIOACTIVE COMPOUNDS

2.5.1. *In vivo experiments*

A volume of 0.015 ml of aqueous solution containing 0.75 μc of ^{14}C-protein hydrolysate, universally labelled, was injected into the abdomen of each insect, using a Hamilton microsyringe. After that, the locusts were kept under normal breeding conditions, but without food, for 24 hours. They were then decapitated and the fat body, ovary, and haemolymph were gathered and put into a cold 5% solution of TCA. The tissue samples were homogenized in a teflon-and-glass homogenizer and the proteins were isolated from the homogenate according to the method of SIMKIN and WORK (1957), removing all radioactivity not incorporated into the proteins. Pentane instead of ether was used finally before drying the protein precipitates at 40° C.

For the isolation of haemolymph protein the more simple method of TELFER and WILLIAMS (1960) was followed. Again, the final drying of the protein powder was achieved via pentane instead of ether.

The isolated samples were put on pre-weighed counting planchets, weighed, and dissolved in one to two droplets of a 95% formic acid solution to achieve a regular distribution of the radioactive materials all over the counting disc. All counting operations were performed with a Philips Geiger-Müller counter tube, number 18505, with thin end-window. The background was 15–17 counts per minute. In all cases specific activities were expressed as counts per minute per milligram of protein at infinite thickness.

2.5.2. *In vitro experiments*

Tissue homogenates and mitochondrial fractions of the fat body were isolated in exactly the same manner as described above for the experiments on respiratory metabolism. The isolation medium, however, was different: 0.32 M sucrose, 2 mM EDTA, 0.03 M nicotinamide, pH 7.5. (Roodyn *et al.*, 1961, pH adapted to insect tissues). Care was taken to resuspend between the sedimentations as gently as possible by hand with the teflon-and-glass homogenizer with 5–10 slow strokes of the pestle.

In all *in vitro* experiments the incubation medium B of Roodyn *et al.* (1961) was used, containing 0.1 M sucrose, 0.04 M KCl, 1.3 mM EDTA, 0.02 M nicotinamide, 0.01 M sodium pyruvate, 0.01 M malic acid, 0.016 M K_3PO_4, 4 mM AMP, 0.5 mM NAD, 8 mM $MgSO_4$ and 50 µg of a synthetic amino acid mixture per ml, composed by Truman and Korner (1962). The pH of the medium was adjusted to 7.5.

Two ml of the reaction medium was pipetted into small conical flasks, which were shaken – to assure aeration – at 30° C. Tissue homogenate was added, 0.5 ml containing 30–40 mg protein; or a suspension of mitochondria, 0.5 ml = 5–7 mg protein.

After 10 minutes equilibration time the incubation was started by the addition of 1 µc ^{14}C-protein hydrolysate, U-labelled.

The reaction time was four hours for homogenates and two hours for the mitochondrial suspensions, and was finished by the addition of a cold solution of unlabelled carrier amino acids, followed by cold TCA to a final concentration of 5 %. Subsequently, the protein was isolated for plating by the method of Simkin and Work (1957).

2.6. AGAR-GEL ELECTROPHORESIS OF PROTEINS

2.6.1. *Apparatus, electrophoretic run*

The electrophoresis was performed on microscope slides according to the description by Wieme (1959), with some small modifications.

The electrophoretic tank was provided with a central compartment completely open to the air. This promoted an intensive cooling by evaporation of the low-boiling liquid, mostly pentane (b.p. ± 36° C), contained in this compartment.

The slides, with the agar-gel layer facing downward, were placed in the central compartment as a bridge, supported by agar blocks in the side-compartments, which in their turn were connected with the electrodes via a veronal buffer (pH 8.6, μ = 0.05). The composition of the agar-gel applied in the electrophoretic tank and on the slides was identical.

A direct current stabilizer (Philips PE-series) supplied a potential gradient of c. 20 V/cm during all operations. This constant voltage resulted in an electric current of 50–70 mA under our temperature conditions. Every electrophoretic run took 15 minutes. After each run the polarity of the electrodes was reversed, so that the same agar block could be used for approximately one week.

2.6.2. *Preparation of the agar-gel slides*

The microscope slides used in these experiments were of standard dimensions (76 × 26 mm) and were covered with a 1.5 mm layer of agar-gel (0.9 %), made up in veronal buffer (pH 8.6 μ = 0.05).

The cleaning of the slides was found to be of great influence on the reproducibility of the results (Koch *et al.*, 1964). The slides were subjected to a thorough washing with

hot water and detergent, rinsed with several changes of redestilled water and, one day before use, degreased by a wash in reagent grade iso-propanol. The slides were then placed in an oven at 60° C and left overnight. Next morning the slides were placed on a horizontal agar-layer in a box with a known volume and immediately afterwards suffused with a known quantity of agar to obtain the 1.5 mm thick agar cover needed. It is necessary to keep the slides for at least 24 hours in a refrigerator at 4° C before using them in experiments.

The material to be analysed was introduced into a 1 cm long slit cut in the agar layer. A small amount of the sample was brought on a clean coverglass (1 × 1 cm) and spread out along one of the edges. After partial evaporation of the aqueous fraction of the sample, the glass was pressed carefully in one movement into the agar. After one minute the coverglass was removed, again carefully, avoiding the formation of ruptures along the slit.

2.6.3. *Preparing of the samples for electrophoresis*

The haemolymph was collected by centrifugation of whole locusts at low speed: 100 *g* for one minute. No special precautions were taken to avoid contamination of the haemolymph by the intestinal content. Samples with this contamination could easily be distinguished, the clear yellow green haemolymph turning brownish, and were discarded. Legs and antennae of the locusts were cut off and a small hole was made in the front of the head capsule. During centrifugation the haemolymph emerged through the holes and deposited at the bottom of the tube. The haemolymph was brought at pH 8.6 and centrifuged again for three minutes at 100 *g* before use.

In order to obtain a sufficiently strong solution of soluble proteins, five to seven fat bodies were homogenized in a minimum amount of Ringer's solution (1.0–1.5 ml), using a mechanically driven teflon-and-glass homogenizer for several minutes at 2000 rpm. The resulting homogenate was centrifuged for 30 minutes at 2000 *g*. The clear zone between the fatty top-layer and the sediment was collected, brought at pH 8.6 and centrifuged once more.

2.6.4. *Fixation, staining and preservation of the agar-gel films*

The procedure described by WIEME (1959) was followed: fixation for 30 minutes in a mixture of 5% (v/v) acetic acid in 70% (v/v) ethanol, immediately after the electrophoretic run; drying of the agar films covered with filter paper (SCHLEICHER and SCHULL 2043 aMgl) at 37° C until the film is entirely dry and transparent; staining for one hour in a mixture of amidoblack 0.5 g, mercurichloride 5.0 g, acetic acid 5.0 ml in 100 ml water and filtered before use; rinsing with a 5% (v/v) acetic acid solution until complete decoloration of the background, changing the rinsing solutions several times; and finally drying at room temperature. The dried agar-films are firmly attached to the slides and can be preserved for an unlimited time.

2.6.5. *Quantitative estimation of the protein bands*

The relative amounts of the different protein fractions could be scanned in a Beckman DV spectrophotometer with an optical slit of 0.5 mm. The standard cuvette house was modified and provided with a holder, which lead the agar slides vertically through the lightbeam, driven by a slow-speed electromotor (0.7 mm/minute). The spectrophotometer was connected with a linear/log Varicord 43 recorder (Photovolt Corp., New York).

2.6.6. *Autoradiography of electrophoretic slides*

The rate of amino acid incorporation into the different haemolymph protein bands could be detected in the following manner. The experimental animals were injected intra-abdominally with 0.5 μc of ^{14}C-protein hydrolysate, U-labelled. After four hours of incubation—unless otherwise stated—the blood was collected and subjected to electrophoresis, as already described above. At least two electropherograms of each sample were made. One served for the normal measurement of the total proteins of the bands by colouring with amidoblack after staining. In the other slide the radioactivity of the different protein bands was determined by exposing the slide to a Kodak Medical X-Ray Film for four weeks.

2.7. FURTHER ANALYTICAL PROCEDURES

2.7.1. *Estimation of soluble and mitochondrial protein*

The colorimetric method with the Folin-Ciocalteu phenol reagent of LOWRY *et al.* (1951) was used. It proved to be recommendable to remove lipoid material before estimation of the protein content of the fat body homogenates and mitochondria by the method of CLELAND and SLATER (1953). With the aid of a range of known concentrations of bovine serum albumin solutions a standard curve was prepared on the basis of Kjeldahl nitrogen determinations.

2.7.2. *Determination of dry weight*

All following quantitative analyses of fat bodies and ovaries were carried out after homogenizing the tissues in small amounts of Ringer's solution. The haemolymph was used directly after collecting by centrifugation.

The homogenates were placed into an oven and gently dried at 45° C for 48 hours. The samples were cooled to room temperature in a desiccator over P_2O_5 for ten minutes and weighed immediately afterwards.

2.7.3. *Fat content*

The fat content of the dried homogenates was determined by extraction with an ether-ethanol mixture (3:1, v/v) in a Soxhlet apparatus for eight hours. The ether-ethanol was evaporated and the residue was weighed as lipid.

2.7.4. *RNA – content*

RNA was estimated by the orcinol method described by SCHNEIDER (1957). It was necessary before the colorimetric determination to remove the acid-soluble and lipoidal compounds by treatment with cold 10% (w/v) TCA and 95% (v/v) ethanol, respectively. The extinction of the green colour was read at 660 mμ. A standard curve was prepared with purified yeast-RNA, relating μg of RNA-phosphorus to optical density at 660 mμ.

2.7.5. *Free amino acids*

The total content of the free amino acids was determined by the ninhydrin method according to MOORE and STEIN (1948). Before analysis the haemolymph needed no special care, but for reliable determinations in the fat body and ovary it was preferable to liberate the homogenates from lipoidal and protein-like materials by centrifuging for 15 minutes at 3000 *g*. A standard curve was prepared with leucin, expressed as amino-N.

2.7.6. *Glycogen*

The glycogen content of fat body and ovary was estimated in a $HClO_4$-extract of the homogenate according to the method of BADE and WYATT (1961). There were some slight modifications: the samples, each from one insect, were mixed with 5 ml of 96 % ethanol to precipitate the glycogen and kept for 12 hours at 38° C. The flocculated glycogen was washed several times with 70 % ethanol and eventually determined using the anthrone reagent described by MOKRASCH (1954). The period of heating for colour development was extended from 15 to 30 minutes. The recovery of known pure glycogen samples, added to tissue homogenates and treated in this manner, was 88–94 %.

2.7.7. *Trehalose*

The trehalose content in the haemolymph was determined with the aid of the anthrone reagent following the indications of WYATT and KALF (1957). The recovery of pure trehalose, added to tissue homogenates, was 90–95 %.

2.8. ELECTRON MICROGRAPHS

For the preparation of electron micrographs the procedure was as follows. The fat body was exposed *in situ* to a 6.25 % solution of glutar-aldehyde for one minute; then small pieces were cut and treated for another 1–2 hours in glutar-aldehyde at 4° C. After removal of the aldehyde the specimens were rinsed with several changes of 0.2 M sucrose in 0.1 M Na-cacodylate pH 7.4 at 4° C for at least one hour, while the rinsing mixture should be refreshed several times. An extra fixation followed in 2 % osmium tetroxide for 1½ hour at 4° C. The fixed fragments of fat body were rinsed and dehydrated at room temperature in graded ethanols and finally in propylene-oxide. Before embedding in Epon-resin 812 they passed a mixture of propylene oxide—Epon 1:1 (v/v) for 30 minutes. The embedded material was kept at 35° C for 12 hours, then at 45° C for 24 hours and at last for some days at room temperature.

Ultrathin sections were cut with the aid of a L.K.B. microtome, type "Ultratome I", provided with a glass knife. The sections were examined with a Siemens Elmiskop I electron microscope.

The mitochondrial pellet from the fat body, obtained after the final centrifugation was stained and fixed through a treatment in a 1 % osmium-tetroxide solution in 0.32 M sucrose at pH 7.5 for 60 minutes at 4° C (STEGWEE, 1964). After washing and dehydrating, embedding in methacrylate followed. The procedure of cutting and examination was the same as mentioned above.

2.9. CHEMICALS

All chemicals used were reagent grade. In all experiments redistilled water was used.

Radioactive compounds. — Protein hydrolysate-^{14}C (U), universally labelled, was purchased from The Radiochemical Centre, Amersham, Bucks, England.

Reagents for electronmicroscopy. — Osmium-tetroxide was obtained from Brocades, Arnhem, The Netherlands; Epon 812 from Struyck, Zutphen, The Netherlands; glutar-aldehyde 25% from Koch-Light Laboratory Ltd., England; Na-cacodylate and propylene-oxide from the British Drug House, England, and methyl- and butylmethacrylate from Th. Schuchardt, GMBH, Munich, German Federal Republic. The agar films used in the electrophoretic experiments were prepared from Difco Bacto-Agar Special (Noble), Difco Laboratories, Detroit, Mich., U.S.A.; Amidoschwarz 10 B was purchased from Bayer, Leverkusen, German Federal Republic.

Other reagents were obtained from the following sources: AMP, ADP-sodium salt, ATP-disodium salt, NAD, hexokinase Type III, bovine serum albumin, α-glycerophosphate, glycogen and trehalose from Sigma Chemical Co, St. Louis, Mo., U.S.A.

EDTA-disodium salt, sodium pyruvate, DL-malic acid, and trichloroacetic acid (TCA) from E. Merck A. G., Darmstadt, German Federal Republic.

Farnesol from Koch-Light Laboratory Ltd., England; farnesylmethyl-ether from Hoffmann-La Roche, Basel, Switzerland; actinomycin D was a gift to Dr. STEGWEE from Merck, Sharpe and Dohme, U.S.A.

3. JUVENILE HORMONE AND RESPIRATORY METABOLISM

3.1. INTRODUCTION

A number of activities of juvenile hormone (JH) during development and reproduction of insects, has been studied in detail. The diversity of opinion as to the metabolic effects of the JH is remarkable. So far as the respiratory rate is concerned, most authors agree on the stimulating effect of JH. This effect has been inferred for the most part from experiments with extirpation or implantation of corpora allata (CA). The problem is how to differentiate between a direct and an indirect effect of JH on metabolism. Direct action means interference with enzymatic processes, indirect action affects growth of target organs, which in their turn influence the rate of metabolism.

THOMSEN (1949) found a stimulating effect of JH on oxygen consumption of the blowfly, *Calliphora erythrocephala*, but it is possible that differences in oxygen requirement between normal and allatectomized females were due to differences in development of the ovaries. Moreover, the males showed practically no effect. The sexual maturation of the males does not involve energy-consuming activities of the gonads, so that it is difficult to separate the direct and indirect effects of JH in the

in vivo experiments. However, THOMSEN and HAMBURGER (1955) remained convinced of a direct effect, since they found that castrated females of *Calliphora* have a normal oxygen consumption.

At first PFLUGFELDER (1952), working with the phasmid *Carausius morosus*, favoured the idea of an indirect effect of JH on oxygen consumption, but later he found only temporary reactions following allatectomy or implantation (PFLUGFELDER, 1958), and no longer believed in any effect. One of his pupils (NEUGEBAUER, 1961) confirmed these results; earlier L'HÉLIAS (1957) had come to the same conclusion. In contrast to other insects, the rate of oviposition was unaffected in 66 per cent of the allatectomized *Carausius* (NEUGEBAUER, 1961).

NOVÁK and his co-workers strongly support the idea of indirect action. Implanted CA did not increase the oxygen consumption of castrated females of the bug *Pyrrhocoris apterus*, whereas they did so in normal females (NOVÁK *et al.*, 1959). The increase in the amount of "respiring tissues" in larvae as well as in adults were under the control of JH and the stimulating effect could thus be explained (NOVÁK and SLÁMA, 1962). JH produced by larvae and adults had the same effect (SLÁMA and HRUBEŠOVÁ, 1963). More recently, SLÁMA (1964, 1965a, 1965b) distinguished 3 types of respiration in the female *Pyrrhocoris*. The first type is "reproduction metabolism", characterized by a high rate and a cyclic course. This fraction of total metabolism is under the control of the median neurosecretory cells of the protocerebrum (NSC) and the CA. It is closely connected with ovarian growth. The second type is "digestive metabolism", showing a steady intermediate rate, in which cycles are lacking. It can be found in allatectomized and/or castrated females, is under control of the NSC and in connection with nutrition and digestion. The third type, the "basal cell metabolism", is the hormone-independent fraction of metabolism, found in cardiac-allatectomized or in diapausing females. Such females are characterized by suppressed growth and a decreased ingestion and digestion. JH indirectly affects total body metabolism, reflecting the metabolic activity in special "target" organs or cells in adults (SLÁMA, 1964, 1965a) as well as in larvae (SLÁMA, 1965b). SLÁMA (1965c) did not observe any effect on respiratory metabolism after injection of farnesol, a JH-mimetic substance.

SÄGESSER (1960) described a direct positive effect of JH, after implantation of active CA into castrated females of the cockroach *Leucophaea maderae*. But recently LÜSCHER and LEUTHOLD (1965) repeated his experiments and could not confirm this conclusion. They and other authors reported that the metabolism of isolated fat bodies of *Leucophaea* is stimulated in the presence of active corpora cardiaca (CC) (LÜSCHER, 1965; LÜSCHER and LEUTHOLD, 1965; WIENS and GILBERT, 1965). The CA and the CC are difficult to separate in *Leucophaea* and the possibility

that this circumstance has influenced SÄGESSER's results cannot be excluded.

The results of ROUSSEL (1963a, b) indicate a direct action of the JH. In *Locusta*, there was no distinct positive effect after implantation of one pair of CA. However, a significant difference in oxygen consumption between normal and allatectomized females up to the 25th day of adult life was noticed. There was no relation to sexual development.

In our laboratory DE WILDE and STEGWEE (1958) reported a decrease in the oxygen consumption following allatectomy in the Colorado potato beetle, *Leptinotarsa decemlineata*. But in this insect the action of JH is also closely related to the occurrence of diapause and cause and effect are again difficult to separate. Possibly this relation is a decisive element. EL-IBRASHY (1965) found a rise of the Respiratory Quotient (R.Q.) above unity after allatectomy in *Leptinotarsa*, whereas he did not observe such an effect in the non-diapausing yellow mealworm, *Tenebrio molitor*. The elegant electron microscopic studies of STEGWEE *et al.* (1963) suggest that the reversible flight muscle degeneration in "normally" and "artificially" diapausing *Leptinotarsa* is under control of JH. Probably flight muscle degeneration is the principal cause of the change in metabolism during diapause. These experiments do not make clear, however, whether or not we may speak of a direct action of JH on metabolism.

For the solution of the problems mentioned, experiments *in vitro* may be more suitable. In this respect, however, the desirability of measuring the oxygen consumption of fragments of thoracic muscles of *Leucophaea* (SAMUELS, 1956), of crude tissue homogenates of *Leptinotarsa* (DE WILDE and STEGWEE, 1958), and of partly purified homogenates of muscle and fat body of *Leucophaea* (RALPH and MATTA, 1965), is also dubious. The chemical and cytological composition of the reacting suspension is completely undefined. Which component will be activated by JH? Simplifying the experimental conditions is necessary.

A much better understanding of the nature of metabolic effects is obtained by the use of isolated mitochondria, the cell particles which are mainly responsible for respiratory activities. Our knowledge of the metabolic properties of insect mitochondria, especially of flight muscle sarcosomes, has much increased during the last 15 years. A comprehensive review has been given by SACKTOR (1961). Recently extensive biochemical studies on *Locusta* flight muscle mitochondria have been made by KLINGENBERG and BÜCHER (1959, 1961) and BROSEMER *et al.* (1963). Housefly (*Musca domestica*) sarcosomes have been studied to a great extent by VAN DEN BERGH and SLATER (1962), ROCKSTEIN and BRANDT (1963), VAN DEN BERGH (1964), and GREGG *et al.* (1964); sarcosomes of active and diapausing *Leptinotarsa* by STEGWEE and VAN KAMMEN-WERTHEIM (1962) and STEGWEE (1964); muscle mitochondria of the

American cockroach *Periplaneta americana*, by COCHRAN (1963); of flight muscle sarcosomes in the silkworm *Hyalophora cecropia*, by MICHEJDA (1964), and finally flight muscle sarcosomes of the honey bee, *Apis mellifica*, by BALBONI (1965).

To date, isolated mitochondria have been used in only two studies dealing with insect endocrines. In both cases a direct relation of JH and respiratory metabolism has been reported. STEGWEE (1960), using mitochondria of diapausing *Leptinotarsa* found a slight stimulation of oxygen consumption and a pronounced positive effect on oxidative phosphorylation after addition of low concentrations of cecropia extract, known to contain JH (WILLIAMS, 1956). CLARKE and BALDWIN (1960) reported, on the basis of preliminary experiments, an increase in oxygen consumption of fat body and thoracic muscle mitochondria of *Locusta* after addition of homogenized CA. The positive effects were even stronger when both CA and DNP were added. They suggested that JH controls the formation of ATP by acting on the mitochondrial cytochrome system. Unfortunately no further studies by these authors on this subject have followed to support this suggestion.

The results published in the last-mentioned papers, obtained six years ago, have been a stimulus to undertake the present study. It has been our aim to follow the subcellular effect of JH on respiratory metabolism by means of *in vitro* experiments with isolated mitochondria.

3.2. EXPERIMENTAL RESULTS

3.2.1. *Juvenile hormone and oxidative phosphorylation*

In these experiments a standard procedure was followed (see section 2.3 and 2.4.1). As quickly as possible after finishing the isolation, samples of mitochondrial suspension were pipetted into Warburg flasks. Generally, the amount of mitochondrial protein was 1–2.5 mg, which was equivalent to 2 individuals. The total volume of the reaction mixture in the flasks was 2.2 ml. After an equilibration time of 10 minutes, oxygen consumption was measured during 9 minutes, prior to the addition of ATP from the side-arm, and followed by reading during 18 minutes after this addition. Immediately afterwards the reaction mixture was used for determination of the rate of phosphorylation.

The freshly prepared mitochondria oxidized the two substrates used in the present series of experiments (α-glycerophosphate and pyruvate/malate) at a constant rate for several hours. A well-coupled oxidative phosphorylation, however, could not be maintained for longer than about one hour, in spite of the presence of bovine serum albumin and

EDTA in the medium. This is a common feature of ageing insect mitochondria, which, by producing fatty acids, appear to inhibit the over-all phosphorylation (see CHEFURKA, 1965).

A continuous supply of ADP in the reaction medium was ensured by the hexokinase/glucose "trapping" system for ATP. This system was put into operation after tipping ATP from the side-arm into the reaction medium.

TABLE I

The respiration rate and phosphorylating efficiency of flight muscle mitochondria of normal and allatectomized *Locusta*.

a. Substrate: α-glycerophosphate.

	Normal		*Allatectomy*		*Comparison of means*
$Q'o_2$	65.6 ± 3.4	(5)	63.7 ± 2.3	(5)	not significant
$Q''o_2$	130.7 ± 4.6	(5)	137.5 ± 4.5	(5)	not significant
R_C	2.02	(5)	2.17	(5)	
P:O	1.06 ± 0.06	(5)	1.01 ± 0.07	(5)	not significant

b. Substrate: Pyruvate/malate.

	Normal		*Allatectomy*		*Comparison of means*
$Q'o_2$	34.4 ± 2.4	(5)	35.7 ± 2.7	(5)	not significant
$Q''o_2$	165.6 ± 6.3	(5)	166.5 ± 6.5	(5)	not significant
R_C	4.68	(5)	4.75	(5)	
P:O	2.40 ± 0.05	(5)	2.35 ± 0.04	(5)	not significant

Oxygen consumption measured in shaking Warburg flasks with mitochondrial suspension + reaction medium (see also 2.4.1) + KOH absorbens = 2.2 ml. T = 25° C.

Incubation medium: 15 mM KCl, 2 mM EDTA, 5 mM $MgCl_2$, 50 mM tris, 30 mM potassium phosphate buffer (pH 7.5), serum albumin 0.12 %.

Substrate: 30 mM α-glycerophosphate or 15 mM Na-pyruvate + malic acid. For phosphorylation: 15 mM ATP, 5 mM glucose + 180 K.M. hexokinase.

The number of experiments is given in brackets.

Qo_2 = µl O_2 uptake per mg protein per hour.
$Q'o_2$ = Qo_2 before addition of the ATP from the side-arm.
$Q''o_2$ = Qo_2 after addition of the ATP from the side-arm.
R_C = The ratio of $Q'o_2$ and $Q''o_2$.
P:O = µmoles inorganic phosphate taken up per µatom oxygen consumed.

In this and the following tables of chapter 3 the values of $Q'o_2$, $Q''o_2$ and P:O are given as $\bar{x} \pm$ S.E. (standard error). Student's t-test is used for the comparison of means, assuming that the underlying distribution should be at least approximately normal and that unknown variances should be equal (BAILEY, 1959).

Generally, the Q_{O_2}-values (explanation of symbols, see legend Table I) showed little variation in comparable experiments. The Q'_{O_2}-values with α-glycerophosphate as a substrate were always higher than those with pyruvate/malate, in contrast with the Q''_{O_2}-values, which were higher with pyruvate/malate. So the R_C was within the range of 1–2.5 for α-glycerophosphate and about 5 for pyruvate/malate. The P:O ratios of mitochondria of the normal individuals were rather low, approximately 50% of the theoretical maximum with α-glycerophosphate, and somewhat higher (± 70%) with pyruvate/malate.

In studying the effect of JH on these parameters, it was obviously of interest to compare its activity in mitochondria obtained from normal and allatectomized locusts. If observed with the naked eye, the thoracic flight muscles of both categories showed no differences, which was in contrast to the situation in normal and allatectomized (diapausing) *Leptinotarsa* (STEGWEE *et al.*, 1963). It remained possible, however, that mitochondria from allatectomized locusts would be more "unsaturated" with respect to JH and that therefore this hormone would be more a limiting factor with regard to the processes studied. The data presented in Table I, showing *in vitro* no differences in oxygen consumption nor in oxidative phosphorylation between the mitochondria obtained from normal (= sham operated) and allatectomized locusts, seem to refute this supposition.

The design of the next experiment was different. Mitochondrial suspensions obtained from groups of differently treated insects were compared in several respects. The variability in the preparation of the mitochondria from day to day was an uncertain factor. This might affect the results of the comparison. The variability in the preparation was eliminated in the other experiments by dividing the mitochondrial suspension in two parts, the treated and the control part. During treatment of the mitochondria with crushed CA in Ringer's solution, an equal amount of Ringer's solution was added to the control mitochondria. To each flask-content 4 pairs of CA, homogenized in 0.3 ml Ringer's solution, were added. The quantity of protein, added by the CA themselves, 300–400 μg at most, was negligible in comparison with the amount of serum albumin present in the reaction mixture (0.12%).

Looking at the data of Table II, again no significant effect of the CA oxygen consumption can be detected. There was only a slight tendency towards higher Q_{O_2}-values after CA administration. In mitochondria without added substrate, which were still able to respire at a low rate, this tendency was relatively more pronounced, but still not significant.

Esterification of inorganic phosphate could not be detected, but there was a slight reaction when ADP became available in the reaction system, considering the fact that the R_C was above 1.

TABLE II

The effect of CA on the respiration rate and phosphorylating efficiency of flight muscle mitochondria of *Locusta*.

a. Substrate: α-glycerophosphate.

	Control		*+ CA*		*Comparison of means*
$Q'O_2$	66.1 ± 5.4	(8)	67.1 ± 5.6	(8)	not significant
$Q''O_2$	123.5 ± 9.6	(8)	129.8 ± 6.5	(8)	not significant
Rc	1.87	(8)	1.93	(8)	
P:O	0.79 ± 0.16	(8)	1.37 ± 0.15	(8)	$0.02 > P > 0.01$

b. Substrate: pyruvate/malate.

	Control		*+ CA*		*Comparison of means*
$Q'O_2$	33.0 ± 3.7	(9)	34.3 ± 4.2	(9)	not significant
$Q''O_2$	166.9 ± 12.1	(9)	174.3 ± 11.6	(9)	not significant
Rc	5.06	(9)	5.08	(9)	
P:O	2.37 ± 0.08	(9)	2.30 ± 0.09	(9)	not significant

c. No Substrate.

	Control		*+ CA*		*Comparison of means*
$Q'O_2$	11.2 ± 3.1	(4)	13.5 ± 0.9	(4)	not significant
$Q''O_2$	11.8 ± 3.5	(4)	16.2 ± 2.2	(4)	not significant
Rc	1.05	(4)	1.20	(4)	
P:O	–	(4)	–	(4)	

In each of these experiments 2 pairs of corpora allata were administered to a mitochondrial suspension equivalent to 1 individual.

The salient feature of this experiment (Table II) was the distinct stimulation—almost a redoubling—of the oxidative phosphorylation by added CA in mitochondria with α-glycerophosphate as a substrate. With pyruvate/malate such a stimulation was not found. These facts contrast curiously with the data represented in Table III where the oxidative phosphorylation of the mitochondria in pyruvate/malate reacted signifi-

TABLE III

The effect of CA on the respiration rate and phosphorylating efficiency of flight muscle mitochondria of *Locusta*.

No addition of ATP; hexokinase/glucose is present in the medium.

a. Substrate: α-glycerophosphate.

	Control		*+ CA*		*Comparison of means*
Q_{O_2}	83.6 ± 5.0	(9)	83.7 ± 4.7	(9)	not significant
P:O	0.51 ± 0.29	(5)	0.79 ± 0.22	(4)	not significant

b. Substrate: pyruvate/malate.

	Control		*+ CA*		*Comparison of means*
Q_{O_2}	34.1 ± 1.1	(8)	34.8 ± 3.3	(8)	not significant
P:O	0.32 ± 0.15	(6)	1.35 ± 0.18	(8)	$0.01 > P > 0.001$

c. No Substrate.

	Control		*+ CA*		*Comparison of means*
Q_{O_2}	8.3 ± 1.1	(4)	11.8 ± 0.9	(4)	not significant
P:O	–	(4)	–	(4)	

In each of these experiments 2 pairs of corpora allata were administered to a mitochondrial suspension equivalent to 1 individual.

cantly with more than twice the intensity. A stimulation was also present in mitochondria in α-glycerophosphate, but far less clear. Unfortunately in this case the number of experiments was not sufficient for significantly different results, because the data showed quite large standard deviations. The rate of oxidative phosphorylation was surprisingly high considering the experimental conditions, where only ADP (and ATP) present within the mitochondria could provide for it. Of course, the Q_{O_2} here was the same as the Q'_{O_2} in the other experiments, before the ADP produced by the reaction of added ATP with hexokinase/glucose became present in the medium. The initial rate of both Q_{O_2}-values

and the phosphorylation, however, could not be maintained longer than approximately half an hour; after that period the Q_{O_2} decreased gradually during several hours and the phosphorylation diminished very sharply to zero. Without substrate there was again a slight but not significant increase in Q_{O_2} and a complete absence of phosphorylation.

Adult *Locusta migratoria* provide favourable material for an extra controlling test of the stimulation of oxidative phosphorylation, established in the previously described experiments. It has long been assumed that phases of *Locusta* differ in their level of JH-activity, the solitary phase being more "juvenile" (KENNEDY, 1961; STAAL and DE WILDE, 1962). Accordingly, one can produce locusts with three different grades of JH-titer:

1. The green solitary phase with a high JH-titer.
2. The gregarious phase with an intermediate titer, and
3. Allatectomized individuals presumably without JH.

This division in categories could be confirmed in a series of experiments. In addition, the results were a strong argument for the specific action of JH on oxidative phosphorylation. However, the assumption must be taken into account, that JH exhibits a concentration effect. There also seems to be a certain "conditioning" of the mitochondria for high hormone-levels.

With flight muscle mitochondria the experiments were carried out in the four possible ways (Table IV). Only α-glycerophosphate was used as a substrate because of the results of previous experiments. As usual there were no differences in oxygen consumption between these treatments and their controls. The effects of "solitary" CA on the oxidative phosphorylation of "gregarious" and "allatectomized" mitochondria were very clear and also the action of "gregarious" CA on "allatectomized" mitochondria was statistically significant. However, "gregarious" CA did not stimulate the oxidative phosphorylation of "solitary" mitochondria. In view of these results the above-mentioned classification seems to be a very reasonable one.

The last-mentioned experiments have been repeated, using isolated mitochondria from the fat body (Table V). The same distinctly significant results concerning oxidative phosphorylation were obtained. The "solitary" CA stimulated the "gregarious" mitochondria, the "gregarious" CA the "allatectomized" mitochondria, whereas the "gregarious" CA did not do so with the "solitary" mitochondria. In this case the Q'_{O_2}- and Q''_{O_2}-values did not differ more than in the above-men-

TABLE IV

The effect of CA on aspects of the respiratory metabolism of flight muscle mitochondria of *Locusta*.

Substrate: α-glycerophosphate.

a. CA from solitary locusts added to "gregarious" mitochondria.

	Control		*+ CA*		*Comparison of means*
$Q'O_2$	98.5 ± 6.8	(4)	87.0 ± 2.2	(3)	not significant
$Q''O_2$	156.0 ± 10.6	(4)	142.7 ± 7.7	(3)	not significant
R_C	1.58	(4)	1.64	(3)	
P:O	0.88 ± 0.13	(4)	1.52 ± 0.08	(4)	$0.02 > P > 0.01$

b. CA from solitary locusts added to "allatectomized" mitochondria.

	Control		*+ CA*		*Comparison of means*
$Q'O_2$	76.3 ± 2.2	(4)	76.3 ± 2.9	(4)	not significant
$Q''O_2$	120.3 ± 3.5	(4)	119.3 ± 3.5	(4)	not significant
R_C	1.58	(4)	1.56	(4)	
P:O	0.86 ± 0.03	(4)	1.45 ± 0.04	(4)	$P < 0.001$

c. CA from gregarious locusts added to "solitary" mitochondria.

	Control		*+ CA*		*Comparison of means*
$Q'O_2$	79.3 ± 11.4	(4)	77.2 ± 15.5	(4)	not significant
$Q''O_2$	126.0 ± 17.1	(4)	117.8 ± 21.1	(4)	not significant
R_C	1.59	(4)	1.52	(4)	
P:O	1.26 ± 0.14	(4)	1.27 ± 0.16	(4)	not significant

d. CA from gregarious locusts added to "allatectomized" mitochondria.

	Control		*+ CA*		*Comparison of means*
$Q'O_2$	79.5 ± 6.1	(4)	84.0 ± 4.6	(4)	not significant
$Q''O_2$	142.5 ± 3.4	(4)	144.8 ± 5.2	(4)	not significant
R_C	1.79	(4)	1.72	(4)	
P:O	0.92 ± 0.03	(4)	1.24 ± 0.10	(4)	$0.05 > P > 0.02$

In each of these experiments 2 pairs of corpora allata were administered to a mitochondrial suspension equivalent to 1 individual.

tioned experiments. The rate of both Q'_{O_2} and especially Q''_{O_2} was much lower. It was clear that the giant flight muscle mitochondria (in longitudinal section 1–10 μ, in transverse section 0.25–1 μ) have a greater respiratory capacity than the somewhat smaller fat body mitochondria, (mostly spherical, 0.2–1 μ, sometimes oval to 2.0 μ), *in vivo* as well as *in vitro*. The R_C was still present and more pronounced in the CA-treated category than in the controls.

TABLE V

The effect of CA on aspects of the respiratory metabolism of fat body mitochondria of *Locusta*.

Substrate: α-glycerophosphate.

a. CA from solitary locusts added to "gregarious" mitochondria.

	Control		*+ CA*		*Comparison of means*
Q'_{O_2}	21.0 ± 1.2	(4)	18.3 ± 1.6	(4)	not significant
Q''_{O_2}	26.7 ± 1.9	(4)	23.0 ± 1.7	(4)	not significant
R_C	1.27	(4)	1.26	(4)	
P:O	0.89 ± 0.02	(4)	1.36 ± 0.02	(4)	$0.05 > P > 0.02$

b. CA from gregarious locusts added to "solitary" mitochondria.

	Control		*+ CA*		*Comparison of means*
Q'_{O_2}	27.0 ± 1.7	(3)	19.7 ± 3.2	(4)	not significant
Q''_{O_2}	27.7 ± 2.8	(3)	28.3 ± 5.5	(4)	not significant
R_C	1.03	(3)	1.44	(4)	
P:O	1.28 ± 0.04	(3)	1.26 ± 0.03	(4)	not significant

c. CA from gregarious locusts added to "allatectomized" mitochondria.

	Control		*+ CA*		*Comparison of means*
Q'_{O_2}	21.0 ± 1.8	(4)	18.3 ± 2.2	(3)	not significant
Q''_{O_2}	31.3 ± 1.5	(4)	31.7 ± 2.6	(3)	not significant
R_C	1.49	(4)	1.64	(3)	
P:O	0.86 ± 0.05	(4)	1.08 ± 0.02	(3)	$0.05 > P > 0.02$

In each of these experiments 2 pairs of corpora allata were administered to a mitochondrial suspension equivalent to 1 individual.

3.2.2. *The effect of CA in a reaction medium containing DNP*

In the experiments dealt with in the present section, DNP —2.4-dinitrophenol—is introduced into the reaction media. DNP has been described in the reviews by RACKER (1961) and WADKINS and LEHNINGER (1962) as a true uncoupler. This means that DNP, at appropriate concentrations, inhibits phosphorylation and either stimulates or does not affect respiration. It is generally known that DNP stimulates ATP-ase, which enzyme cleaves the terminal phosphate-group from ATP. The ATP-ase is also, but less extensively, activated by Mg^{++}-ions, and not by Ca^{++}-ions (SACKTOR, 1953); it has a strict specificity and is inhibited by ADP. Several types of mitochondrial ATP-ases can be distinguished: the DNP-induced ATP-ase, the Mg^{++}-stimulated ATP-ase, the Na^{+}-K^{+}- de pendent ATP-ase and also the above-mentioned ATP-ase, induced by the ageing of isolated mitochondria.

When DNP at a concentration of 10^{-3} M was applied to freshly isolated *Locusta* flight muscle mitochondria, a total inhibition of the oxidative phosphorylation could be observed in both substrates (Table VI, a and b). The course of the P:O ratios was nearly the same with either substrate.

TABLE VI

Effect of DNP on the oxygen consumption and oxidative phosphorylation of flight muscle mitochondria.

a. Substrate: α-glycerophosphate.

Addition		*Control*	*+ DNP*	
+ DNP 10^{-3} M	$Q'O_2$	145.5	155.5	(2)
	$Q''O_2$	160.5	125.5	(2)
	RC	1.10	0.81	(2)
	P:O	1.39	–	(2)
+ DNP 10^{-4} M	$Q'O_2$	123.5	142.5	(2)
	$Q''O_2$	131.0	122.0	(2)
	RC	1.06	0.86	(2)
	P:O	1.52	0.23	(2)
+ DNP 10^{-5} M	$Q'O_2$	138.0	135.0	(2)
	$Q''O_2$	148.0	123.0	(2)
	RC	1.08	0.91	(2)
	P:O	1.35	1.02	(2)
+ DNP 10^{-6} M	$Q'O_2$	109.5	113.0	(2)
	$Q''O_2$	135.5	132.0	(2)
	RC	1.22	1.17	(2)
	P:O	1.33	1.39	(2)

b. Substrate: pyruvate/malate.

Addition		*Control*	*+ DNP*	
+ DNP	$Q'O_2$	31.5	30.0	(2)
10^{-3} M	$Q''O_2$	139.0	36.0	(2)
	R_C	4.41	1.20	(2)
	P:O	2.33	–	(2)
+ DNP	$Q'O_2$	41.0	79.0	(2)
10^{-4} M	$Q''O_2$	87.0	88.5	(2)
	R_C	2.12	1.12	(2)
	P:O	2.38	0.50	(2)
+ DNP	$Q'O_2$	28.0	35.5	(2)
10^{-5} M	$Q''O_2$	164.5	179.0	(2)
	R_C	5.88	5.04	(2)
	P:O	2.57	2.30	(2)
+ DNP	$Q'O_2$	44.5	44.0	(2)
10^{-6} M	$Q''O_2$	160.5	158.0	(2)
	R_C	3.61	3.59	(2)
	P:O	2.43	2.41	(2)

The figures in this table represent average values. The number of experiments is given in brackets.

Oxygen consumption measured in shaking Warburg flasks with mitochondrial suspension + reaction medium + DNP + KOH absorbens = 2.2 ml. T = 25° C.

Incubation medium: 15 mM KCl, 2 mM EDTA, 5 mM $MgCl_2$, 50 mM Tris, 30 mM potassium phosphate buffer (pH = 7.5), serumalbumin 0.12 %.

Substrate: 30 mM α-glycerophosphate or 15 mM Na-pyruvate + malic acid. For phosphorylation: 15 mM ATP, 5 mM glucose + 180 K.M. hexokinase.

At a DNP concentration of 10^{-4} M the uncoupling effect was still clear, at 10^{-5} M it was only slightly present and when 10^{-6} M was administered to the medium the oxidative phosphorylation attained the same level as in the control medium and was then quite recovered. To test the other part of the above-mentioned characteristics of a true uncoupling agent was rather difficult. At 10^{-3} M DNP there was a distinct inhibition of the Q''_{O_2} in both substrates, but perhaps this concentration of DNP was not "appropriate". With α-glycerophosphate a tendency for inhibition of the Q''_{O_2}-value remained at the lower DNP concentrations. The general picture, however, agreed with the characterization; there was either a more or less distinct stimulation of the Q_{O_2}-values or the differences were not important. The R_C appeared to be a good criterion for the DNP-effect. With α-glycerophosphate the R_C was below unity and reached a normal level only at 10^{-6} M DNP, with pyruvate/malate the R_C reached

its normal value earlier, at 10^{-5} M DNP. In considering these arguments the very restricted number of experiments must be kept in view.

It was stated in the preceding paragraph that DNP and CA had opposite effects on the oxidative phosphorylation of fresh *Locusta* mitochondria. Now the question arose as to whether it would be possible to detect an antagonism between these agents by means of this type of *in vitro* experiments. Consulting Table VII, a and b, one can see that the supposed antagonistic action of DNP could indeed be verified. With both substrates the effect of 10^{-3} M DNP was apparently dominating over the action of CA, in view of the complete lack of phosphorylation. The mitochondria gradually regained their oxidative phosphorylation ability when passing through the DNP concentration-range from 10^{-4} M to 10^{-6} M, at which concentration the situation was quite normal and comparable with the data represented in Table VI, a and b.

In the transition phase of partly restored phosphorylating conditions,

TABLE VII

Effects of CA on aspects of the respiratory metabolism of flight muscle mitochondria in a DNP containing medium.

a. Substrate: α-glycerophosphate.

Medium		*Control*	*+ CA*		*Comparison of means*
DNP	$Q'O_2$	172.5	159.5	(2)	
10^{-3} M	$Q''O_2$	110.5	126.5	(2)	
	R_C	0.64	0.79	(2)	
	P:O	–	–	(2)	
DNP	$Q'O_2$	128.7 ± 28.3	130.3 ± 36.1	(3)	not significant
10^{-4} M	$Q''O_2$	128.3 ± 24.5	100.7 ± 9.3	(3)	not significant
	R_C	1.00	0.77	(3)	
	P:O	0.19 ± 0.05	0.40 ± 0.13	(3)	not significant
DNP	$Q'O_2$	127.3 ± 16.3	127.0 ± 11.9	(3)	not significant
10^{-5} M	$Q''O_2$	118.0 ± 2.1	109.7 ± 11.5	(3)	not significant
	R_C	0.93	0.86	(3)	
	P:O	1.12 ± 0.10	1.38 ± 0.09	(3)	P = 0.05
DNP	$Q'O_2$	101.0	104.0	(2)	
10^{-6} M	$Q''O_2$	121.0	109.5	(2)	
	R_C	1.20	1.05	(2)	
	P:O	1.32	1.37	(2)	

Average values are represented in experiments with only 2 treatments (number in brackets). The t-test has been applied to compare the mean differences of the P:O ratios when 3 experiments have been carried out.

b. Substrate: pyruvate/malate.

Medium		*Control*	*+ CA*		*Comparison of means*
DNP	Q'_{O_2}	27.5	21.0	(2)	
10^{-3} M	Q''_{O_2}	22.5	21.5	(2)	
	R_C	0.81	1.02	(2)	
	P:O	–	–	(2)	
DNP	Q'_{O_2}	77.5 ± 5.6	78.3 ± 4.2	(4)	not significant
10^{-4} M	Q''_{O_2}	111.0 ± 6.5	109.8 ± 7.9	(4)	not significant
	R_C	1.43	1.40	(4)	
	P:O	0.44 ± 0.09	0.89 ± 0.08	(4)	not significant
DNP	Q'_{O_2}	74.3 ± 3.6	81.0 ± 3.8	(3)	not significant
5.10^{-5} M	Q''_{O_2}	148.3 ± 4.2	143.3 ± 2.3	(3)	not significant
	R_C	2.00	1.77	(3)	
	P:O	1.20 ± 0.05	2.07 ± 0.06	(3)	$0.05 > P > 0.02$
DNP	Q'_{O_2}	48.5	47.5	(2)	
10^{-5} M	Q''_{O_2}	146.5	153.5	(2)	
	R_C	3.02	3.23	(2)	
	P:O	2.19	2.28	(2)	

Average values are given in series with no more than 2 experiments. The t-test has been applied to compare the P:O ratios, when 3 or 4 experiments have been carried out.

In each of these experiments 2-3 pairs of corpora allata were applied to a mitochondrial suspension equivalent to 1 individual.

the stimulating influence of the CA became noticeable. The CA antagonized DNP, but only when the DNP concentration was not too high (10^{-4}–10^{-5} M). At the lowest DNP concentrations, mentioned in Table VII, the differences had disappeared for the greater part. It could be expected that here the CA had the same stimulating effect on the oxidative phosphorylation as was described earlier (Table II, a and b) but the number of experiments was too small to draw any definite conclusion.

The Q_{O_2}-values in this reaction system were not subject to any stronger changes than in the case described before. The R_C was at 10^{-3} M DNP below unity, except in one case, but rose step by step to its normal level when the concentration of DNP was lowered. The further data for the R_C were rather confusing: CA gave positive as well as negative effects.

When the ATP-ase activity was measured in *Locusta* flight muscle mitochondria, some of the previously mentioned properties of this enzyme could soon be recognized (Table VIII). There was, under the usual conditions, a considerable Mg^{++}-activated ATP-ase, whereas it was clear that Ca^{++} did not have any effect on ATP-ase activity. DNP, given in

addition to Mg^{++} to the reaction medium, resulted in extra stimulation of ATP-ase activity. This extra stimulation, however, was far less pronounced than STEGWEE and VAN KAMMEN-WERTHEIM (1962) found with sarcosomes of the Colorado potato beetle. No such extra stimulation was found in the presence of Ca^{++}.

The difference between Mg^{++}-induced and DNP-induced ATP-ase

TABLE VIII

ATP-ase activity of flight muscle mitochondria of *Locusta*.

Additions	*Inorganic phosphate released over control (μM/mg protein/hr.)*	
	Experiment 1	*Experiment 2*
None	1.70	1.88
+ $MgCl_2$	2.74	2.95
+ $CaCl_2$	1.72	1.90
+ $MgCl_2$ + DNP	3.83	3.90
+ $CaCl_2$ + DNP	1.68	1.91

The preparations are suspensions obtained after one cycle of low and high speed centrifugation.

$MgCl_2$ and $CaCl_2$ were added so as to give a final concentration of 2.5×10^{-3} M. The final concentration of DNP was 10^{-4} M.

is used as a criterion for the quality of the isolated mitochondria. In a preparation principally consisting of damaged mitochondria there is much less "latent" ATP-ase, which shows up only in the presence of DNP, so that in that case the differences between the ATP-ase activities are negligible (see also Table IXb). But when the quality is good the differences become significant (Table IXc).

Table IXa shows a series of ATP-ase activities in flight muscle mitochondria after different additions. DNP addition resulted in a significant extra stimulation of the ATP-ase (beside the Mg^{++}-induced ATP-ase). CA also had a stimulatory effect. However, in a DNP containing medium addition of CA tended to depress the ATP-ase. In fat body mitochondria, even though the quality was good (Table IXc, see also electron micrographs Plate I), the difference between the Mg^{++}- and DNP-induced ATP-ase activity was much smaller than in flight muscle mitochondria. Yet this difference was significant. CA addition depressed both the Mg^{++}- and the DNP-activated ATP-ase. In this series of experiments CA appeared to be antagonistic in its action to DNP.

TABLE IX

Effect of CA on ATP-ase activity of flight muscle and fat body mitochondria of *Locusta*.

a. Flight muscle mitochondria.

Addition	*Inorganic phosphate released over control (μM/mg protein/hr.)*			$\bar{x} \pm S.E$	*Comparison of means*
	Expt. 1	*Expt. 2*	*Expt. 3*		
a. $MgCl_2$	2.94	2.85	2.78	2.86 ± 0.06	a → b $0.05 > P > 0.02$
b. $MgCl_2$ + DNP	6.13	3.65	4.84	4.87 ± 0.55	a → c $0.05 > P > 0.02$
c. $MgCl_2$ + CA	3.25	3.01	3.11	3.12 ± 0.05	b → d not significant
d. $MgCl_2$ + DNP + CA	4.16	3.62	3.74	3.84 ± 0.13	

b. Fat body mitochondria (inferior quality).

Addition	*Inorganic phosphate released over control (μM/mg protein/hr.)*			$\bar{x} \pm S.E$	*Comparison of means*
	Expt. 1	*Expt. 2*	*Expt. 3*		
a. $MgCl_2$	0.92	1.28	1.16	1.12 ± 0.08	a → b not significant
b. $MgCl_2$ + DNP	0.91	1.20	1.23	1.11 ± 0.08	a → c ,, ,,
c. $MgCl_2$ + CA	1.03	0.96	1.25	1.08 ± 0.07	b → d ,, ,,
d. $MgCl_2$ + DNP + CA	1.31	0.96	1.31	1.20 ± 0.10	

c. Fat body mitochondria (good quality).

Addition	*Inorganic phosphate released over control (μM/mg protein/hr.)*				$\bar{x} \pm S.E$	*Comparison of means*
	Expt. 1	*Expt. 2*	*Expt. 3*	*Expt. 4*		
a. $MgCl_2$	1.08	1.08	1.21	1.17	1.14 ± 0.03	a → b $P < 0.001$
b. $MgCl_2$ + DNP	1.87	1.59	1.77	1.92	1.79 ± 0.06	a → c $0.01 > P > 0.001$
c. $MgCl_2$ + CA	0.99	1.01	0.98	1.00	1.00 ± 0.01	b → d $P < 0.001$
d. $MgCl_2$ + DNA + CA	1.66	1.24	1.05	1.03	1.24 ± 0.11	

The preparations are suspensions obtained after one cycle of low and high speed centrifugation.

In each experiment 3 pairs of CA from the solitary *Locusta* were administered to a mitochondrial suspension equivalent to 1 individual.

3.2.3. *Effects of cecropia oil and farnesol*

It was now of interest, in view of the above described relationship between CA and some aspects of the respiratory metabolism, to determine whether similar phenomena occurred when some of the allatomimetic substances or concentrates were applied in this type of *in vitro* experiments. All three agents which were used, *viz.*, cecropia oil, farnesol and farnesylmethyl-ether, were insoluble in the aqueous reaction media.

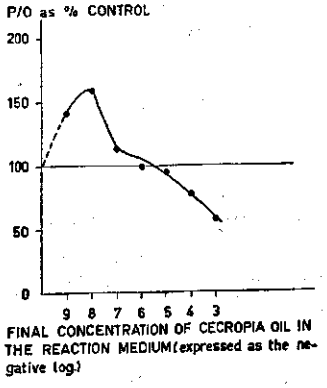


Fig. 1. Effect of cecropia oil on the oxidative phosphorylation of sarcosomes of adult *Locusta*.

Initially, the cecropia oil was dissolved in 10% (v/v) aqueous dioxan. Dioxan was also added to the controls and, apparently, did not disturb the respiratory metabolism at all. Later on, it was possible to use the oily substances emulsified in water by ultrasonic vibration.

Samples of cecropia oil, in concentrations ranging from 10^{-2} to 10^{-8} (v/v), were applied to the *Locusta* flight muscle mitochondria. The experimental procedure was exactly the same as described before (see section 3.2.1.). At the higher concentrations (10^{-2} and 10^{-3}) the Q'_{O_2}-and Q''_{O_2}-values were lowered by approximately 20%, probably because of toxic effects. Lowering the concentration first did not produce any effects, while still higher dilutions ($10^{-6} - 10^{-8}$) caused a slight stimulation of the oxygen consumption of sarcosomes of diapausing *Leptinotarsa* with succinate as a substrate.

Oxidative phosphorylation was also inhibited at high concentrations

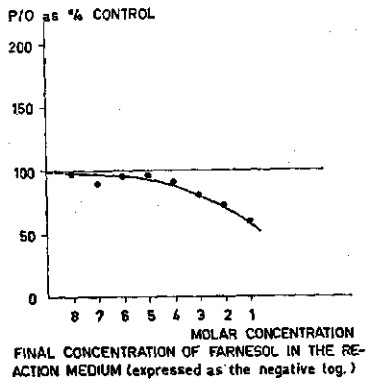


Fig. 2. Effect of farnesol on the oxidative phosphorylation of sarcosomes of adult *Locusta*.

(fig. 1), was normal at 10^{-5} and 10^{-6} and even stimulated up to 60%, when the concentration of the cecropia oil was lowered from 10^{-6} to 10^{-8}. The latter results agree with those of STEGWEE (1960), who described a still more pronounced stimulation in diapausing *Leptinotarsa*.

In the same manner the activity of farnesol and farnesyl methyl-ether was tested (figs. 2 and 3). At high concentrations a similar inhibition of oxygen consumption and oxidative phosphorylation (both 15–20%) was obtained. At lower concentrations, however, these substances did not

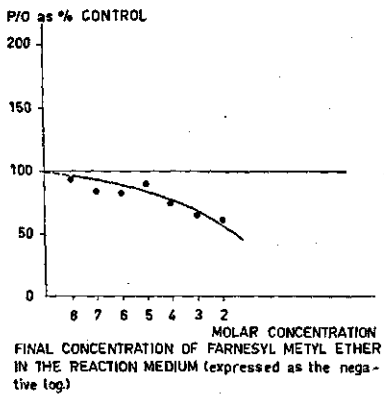


Fig. 3. Effect of farnesyl methyl-ether on the oxidative phosphorylation of sarcosomes of adult *Locusta* (for metyl read methyl).

increase the P:O ratio in the same way as did cecropia oil. The respiration rates as well as the phosphorylating efficiency seemed to be completely unaffected by both farnesol and its derivative farnesyl methyl-ether, which, as far as inhibition of insect metamorphosis is concerned, is even more effective than farnesol (WIGGLESWORTH, 1963).

3.3. DISCUSSION

The flight muscle mitochondria of *Locusta*, used in the present series of experiments, exhibited a quite uniform rate of respiration. The Q_{O_2}-values reached maxima of 175 and 156, respectively, with pyruvate/malate and α-glycerophosphate as substrates. These values contrast sharply with the high Q_{O_2}-values obtained with housefly mitochondria, reported to oxidize α-glycerophosphate at a maximum of 750 by BIRT (1961), at an average of 673 by VAN DEN BERGH and SLATER (1962) and at over 900 by HESLOP and RAY (1963). Housefly sarcosomes, however, seemed to be an exception in this respect. Our Q_{O_2}-values were in good agreement with data given by STEGWEE and VAN KAMMEN-WERTHEIM (1962). With flight muscle mitochondria isolated from a good flyer like the honeybee the Q_{O_2} also did not exceed 200 (BALBONI, 1965).

The fact that in a phosphorylating medium the *Locusta* mitochondria oxidized pyruvate/malate at a higher rate than α-glycerophosphate is outstanding. α-Glycerophosphate almost without exception has been found to be oxidized most rapidly, *e.g.*, by housefly sarcosomes at a rate trice that of pyruvate/malate (VAN DEN BERGH and SLATER, 1962) and by blowfly sarcosomes, according to BIRT (1961) even at a tenfold rate. SACKTOR (1965) described the equal rates of oxidation of succinate and α-glycerophosphate found by STEGWEE and VAN KAMMEN-WERTHEIM (1962) in mitochondria of *Leptinotarsa* as an exception.

In the non-phosphorylating system the Q'_{O_2} with α-glycerophosphate was always higher than with pyruvate/malate. Consequently the availability of ADP gave the pyruvate/malate oxidation an extra stimulus, as was also clearly reflected by the differences between the R_C values.

VAN DEN BERGH and SLATER (1962) offered the idea that housefly mitochondria were not readily permeable for all substrates. They supported this view with experiments, in which the mitochondria were treated in an ultrasonic desintegrator, whereupon the rate of oxidation increased considerably.

Fat body mitochondria of *Locusta* had a much more limited respiratory capacity with Q_{O_2}-values ranging from 20 to 35. Differences in oxidizing

capacity between the above-mentioned two substrates, as just described for the flight muscle mitochondria, were more difficult to determine.

Concerning the relation between oxygen consumption and CA one conclusion can be drawn: JH apparently exerts neither a positive nor a negative effect. There is no relation at all, as was recently ascertained by LÜSCHER and LEUTHOLD (1965) with the roach *Leucophaea*. The present experimental results also show that with cecropia oil, farnesol and farnesylmethyl-ether again no effects were obtained on oxygen consumption of mitochondria.

The present experiments, however, do furnish good evidence that JH exerts a stimulatory effect upon oxidative phosphorylation. As a matter of fact, they constitute the first unequivocal proof of an interrelation between JH and oxidative phosphorylation. There is only the report of STEGWEE (1960) describing a stimulatory effect of cecropia oil (conc. 10^{-5} to 10^{-8} v/v) on oxidative phosphorylation. His results are fully confirmed in the present study. Surprisingly, farnesol and farnesylmethyl-ether proved to be ineffective.

STEGWEE (1960) pointed to the resemblance between the quoted *in vitro* action of cecropia oil and that of thyroxine, as described by DALLAM and HOWARD (1960) for isolated rat liver mitochondria. Both hormones uncoupled oxidative phosphorylation at high- and stimulated at low concentrations. Moreover, no effect on oxygen consumption was detected in either case. KARLSON and SCHULZ-ENDERS (1963) studied the action of thyroxine on oxidative phosphorylation of mitochondria of *Schistocerca gregaria*. Uncoupling occurred either after injection of thyroxine into the intact animals, or after pre-incubation with 10^{-5} thyroxine. This concentration, however, must be considered as unphysiological and its action on oxidative phosphorylation was in accordance with the results of DALLAM and HOWARD (1960).

Research recently carried out in Sweden has presented details concerning the *in vivo* action of thyroxine. TATA *et al.* (1962, 1963) injected rats with physiological doses of thyroid hormones. These hormones stimulated both mitochondrial respiration and phosphorylation by selectively increasing the number of respiration and phosphorylation units at the cost of the formation of other proteins. So the P:O ratio did not change in most cases, though the overall capacity of both electron-transport chain and phosphorylation altered. An electron-microscopic study carried out by GUSTAFSSON *et al.* (1965) showed in fact an enlargement of the total amount of mitochondria in rat skeletal muscle after thyroidectomy. Besides an increase of the mitochondrial population a change of the profile ratio of cristae: matrix was also clearly established.

Electron micrographs (Plates I and II), prepared, respectively, from the flight muscle of normal and allatectomized *Locusta*, exhibit a similar

phenomenon as described by GUSTAFSSON *et al.* In allatectomized insects the total amount of mitochondria increased, although this is rather difficult to estimate. Perhaps it is better to speak of a considerable rise of the ratio mitochondrial clusters to muscle fibrils after allatectomy, as can easily be seen in the transverse as well as in the longitudinal sections of the flight muscles. Some preliminary data can be presented: the ratio mitochondria: fibrils varies from 0.75 to 0.81 in the transverse sections and from 0.63 to 0.75 in the longitudinal sections of normal females. The ratios are 0.92–0.97 and 0.91–1.15, respectively, in allatectomized females. Further experiments on this phenomenon are planned.

The biochemical properties of thyroid hormones and their relation to oxidative phosphorylation cannot very well be brought into line with the described JH-effects. In the latter case there is an improvement of the coupling of phosphorylation, with respiration remaining at a constant rate. It may be important that within the dense mitochondria no changes can be discovered. The respiratory capacity of the mitochondria does not seem to change. The effect of the relatively higher content of mitochondria in the muscle cannot be clearly expressed *in vitro*. A comparison of the rate of respiration of tissue fragments of normal and allatectomized locusts should be very interesting.

Another fact, which adds to the confusion in this respect is the identical effect of thyroxine injections and thyroidectomy reported in the afore-mentioned article of GUSTAFSSON *et al.* (1965). LINDBERG (1965), one of the members of the Swedish research team was also surprised: "Erstaunlich ist vor allen Dingen der Umstand, daß eine grössere Population von Mitochondrien sowohl durch erhöhtes wie auch durch herabgesenktes Thyroxin-Niveau verursacht werden kann".

In which manner can we further explain the action of JH? In view of the above-described data obtained with vertebrates, a discussion of *in vitro* effects on oxidative phosphorylation in *Locusta* seems to be based on rather scarce information. An extra difficulty is created by the fact that despite the great importance of oxidative phosphorylation, enzymological details are still essentially unknown. It has been discovered that the mitochondrial phosphorylating system can be resolved into a particulate and a soluble fraction. PULLMAN *et al.* (1960) and RACKER (1962) described a series of soluble "F-" factors, which were required for a significantly coupled, succinate-linked phosphorylation. Also LINNANE and TITCHENER (1960) and SANADI *et al.* (1964) identified coupling enzymes, which restored phosphorylation, dependent upon a recombination of the soluble factor and the particle, in the presence of Mg^{++}. All these factors exhibited a rather high ATP-ase activity. Without entering further into this very complicated field of research, we only wish to suggest the possibility that JH in some way

facilitates the action of one or more of these soluble coupling factors.

This suggestion might be based upon the observed antagonism between JH and ATP-ase. The antagonistic relation can be concluded from the experiments described in section 3.2.2. JH exerted an inhibitory effect on Mg^{++}- and DNP-stimulated ATP-ase. Mitochondrial ATP-ase is principally concentrated in the membrane and is of special interest for its permeability (SIEKEVITZ *et al.* 1958). Virtually, the mitochondrial membranes contain enzyme activities which can bring about changes in the conformation of the membrane, resulting in stretching, shrinking and puckering, with concomitant changes in permeability. ULRICH (1963) reported a correlation between changes in the mitochondrial ATP-ase activity and an accumulation of Na^+- and K^+-ions. Isolated mitochondria from mammalian tissues are able to maintain concentration gradients of Na^+- and K^+-ions, when ATP is available. ATP-ase seems to be inhibited in a non-specific manner by alkali-metal ions and it presumably also plays a rôle in the transport of these cations across cell membranes. ULRICH suggested that one of the functions of the alkali-metal ions may be to keep ATP-ase activity at a low level for maximum efficiency of oxidative phosphorylation. Already in 1955 PRESSMAN and LARDY wrote, that K^+-ions are necessary for maximum rates of respiration and phosphorylation by rat-liver mitochondria. BLOND and WHITTAM (1965) confirmed that K^+-ions facilitate the oxidative phosphorylation and the respiratory response to ADP in kidney mitochondria, Na^+-ions have an antagonistic effect to K^+-ions on mitochondria, but a chemically well defined medium must be present to secure an efficient phosphorylation. Not the absolute K^+-ion concentration is the determining factor, but rather the relative concentrations of K^+- and Na^+-ions. BLOND and WHITTAM suggested that Na^+ might be competing with K^+ for a site controlling a reaction that is stimulated by K^+ only.

Thus stressing the importance of the K^+/Na^+ ratio in mitochondria, we indicate the possibility that JH has an effect on the K^+—Na^+ "pump", which results in a decrease of ATP-ase activity. The following sequence of events can be represented: arrival of JH molecules at the cell surface in a certain concentration → stimulation of the K^+— Na^+ pump → decrease of intracellular Na^+, increase of intracellular K^+ → repression of ATP-ase activity and increase of phosphorylating efficiency. The first part of this scheme, as far as the action of the hormone on the K^+/Na^+ balance is concerned, exhibits a striking resemblance with that concerning the action of ecdysone as proposed by KROEGER and LEZZI (1965). KROEGER (1963) stated that both ecdysone and changes in the K^+/Na^+ balance within a cell may result in similar puffing patterns.

Until now, only JH prepared from Lepidoptera has been applied and

it is possible that these preparations have no effect in the species of Diptera used for this type of experiments. Consequently we have no information because the crucial experiments are still lacking.

When JH has been purified so far that it can be applied in "puffing" experiments, the results will be decisive for the validity of the above proposed mode of action of JH. Decisive only if we assume that KROEGER's hypothesis is correct! It may also be that JH exerts its effect by another route than by the activation of genes. Moreover, it is still difficult to assume that JH and ecdysone both have an effect on the K^+/Na^+ ratio, whereas they greatly differ in morphogenetic effects during the larval development of the insect.

4. A COMPARATIVE STUDY OF THE EFFECTS OF ALLATECTOMY AND SOME OTHER OPERATIONS

4.1. DISCUSSION OF LITERATURE

4.1.1. *Corpora allata and reproduction*

The gonadotropic action of the juvenile hormone (JH) — according to NOVÁK (1965) the original part of its functions acquired in the course of insect evolution — has only partially been elucidated. WIGGLESWORTH (1936) and WEED PFEIFFER (1939) were the first to establish, in *Rhodnius prolixus* and *Melanoplus differentialis*, respectively, that the CA control oogenesis from the beginning of proteid yolk deposition (fig. 13, "e"). The activity of follicle cells and the continued growth of terminal oocytes was always inhibited after allatectomy performed in young adults or during the last larval stages. These findings were confirmed by the outcome of a long range of similar studies in many insects of different orders. In many cases allatectomy not only causes an arrest of ovarian activity, but also a resorption of already deposited yolk, as was shown *e.g.*, by DE WILDE and DE BOER (1961) in *Leptinotarsa*. The effect is reversible as is obvious when active CA of either sex are implanted. In *Locusta* the CA are also indispensable for yolk deposition (JOLY, 1960). On the other hand in the phasmid *Dixippus morosus* (PFLUGFELDER, 1937), in the dipteran *Sypiloidea sypilus* (POSSOMPÈS, 1956) and in the lepidopterans *Bombyx mori* (BOUNHIOL, 1942) and *Hyalophora cecropia* (WILLIAMS, 1952) allatectomy does not inhibit yolk formation, not even when the operation is performed one or two instars prior to the adult stage. This exception might be due to a different mechanism of yolk formation. Until now no data are available to support this view. It is a remarkable fact,

however, that this mechanism is so uniform in the most divergent species of the animal kingdom (RAVEN, 1961). The presence of a small quantity of JH in the young adults is more probable. In the above-mentioned Lepidoptera oogenesis is practically completed during the pupal stage.

The study of the relation between JH and reproduction became more complex since THOMSEN (1952) reported that in *Calliphora* yolk formation was also arrested when the median neurosecretory cells of the brain (NSC) were put out of action. HIGHNAM (1962a) and GIRARDIE (1962, 1964a) found a similar relation in *Schistocerca* and in *Locusta*. It is now a well-established fact that the NSC can stimulate the activity of the CA via their neurohaemal organs, the corpora cardiaca (CC). On the other hand, there are some indications that the activity of the CA can promote the release of neurosecretory material from the CC (fig. 13, "g"; for more extensive reviews, see DE WILDE, 1964; WIGGLESWORTH, 1964). These interrelationships tend to complicate the study of the separate functions of the NSC and the CA.

4.1.2. *Extra-ovarian yolk protein synthesis*

Recently it has become evident that the extra-ovarian synthesis of proteins as a contribution to vitellogenesis is very important. These proteins are transported from the site of formation to the ovaries and are then taken up by the oocytes (*cf.* the review by TELFER, 1965). The haemolymph, the transporting medium of many metabolites, shows a clear reflection of the developmental and reproductive activity of the insect. TELFER and RUTBERG (1960) have correlated the level of two "prospective" yolk proteins in the haemolymph with the growth rate of successive oocytes in the ovariole. An impressive series of other studies dealing with this relation followed. Electrophoretic techniques were often used, *e.g.*, by LAUFER (1960, 1963) with the giant silkmoths *Hyalophora cecropia* and *Samia cynthia*, by HILL (1962) with *Schistocerca*, by LOUGHTON and WEST (1965) with the Lepidoptera *Malacosoma americanum* and *Rothschildia orizaba*, by LIU and DIXON (1965) in relation to caste development in the honeybee and finally by DUKE (1966) and by MCGORMICK and SCOTT (1966), both with *Locusta*. Blood proteins were found to be absorbed by the growing oocytes.

4.1.3. *The rôle of the fat body in yolk protein synthesis*

The extra-ovarian synthesis of yolk proteins probably takes place in the fat body. CLEMENTS (1959) reported a considerable *in vitro* incorporation

of ^{14}C-labelled glycine into proteins of the fat body in *Schistocerca*. Experimental evidence that the fat body synthesizes haemolymph proteins was provided by SHIGEMATSU (1958, 1960) in *Bombyx*. FAULKNER and BHEEMESWAR (1960) found in *Bombyx* after injection of ^{14}C-glycine a more rapid labelling of fat body proteins than of haemolymph proteins, the difference being dependent on the phase of the reproductive cycle of the insect. Similar results were reported by HILL (1965) in *Schistocerca*, where a cyclic rate of incorporation into the proteins of the fat body corresponded with the cycles of oocyte growth. *In vitro* incorporation of ^{14}C-L-valine into the fat body of larvae of *Calliphora* was studied by PRICE (1966). The proteins synthesized appeared to be released in the surrounding incubation medium and they were therefore compared with haemolymph proteins. The electrophoretic patterns of both were almost identical (PRICE and BOSMAN, 1966). COLES (1964, 1965a, b) also concluded that haemolymph protein synthesis occurred in the fat body of larvae as well as of female adults in *Rhodnius*. He demonstrated by electrophoresis the presence of two identical proteins in the fat body, haemolymph, and oocytes.

4.1.4. *Midgut and yolk synthesis*

ROTH and PORTER (1964) suggested that the midgut of the mosquito *Aedes aegypti*, is the principal site of yolk formation. The midgut in this respect is also important in *Periplaneta*. MILLS *et al.* (1966) measured the RNA-content, and also examined electron micrographs of a series of organs. They concluded that both midgut and fat body synthesize yolk proteins.

4.1.5. *Other properties of the fat body*

The metabolic activities of the fat body are many-sided. The ability of the fat body to synthesize fatty acids has been demonstrated in *Locusta* by TIETZ (1961, 1962). CHINO and GILBERT (1965) described a rapid incorporation of ^{14}C-labelled fatty acids *in vitro* by the fat body of three different insect species. They also showed that the diglyceride fraction was released into the haemolymph and was transported as a diglyceride-protein complex.

In carbohydrate metabolism the fat body also plays an important rôle, *e.g.*, as the site of trehalose formation (CANDY and KILBY, 1961), and as a reservoir for reserve material deposited as glycogen. Reference is made to the review by KILBY (1963).

4.1.6. *CA and fat body metabolism*

A possible relationship between protein and fat metabolism in the fat body can very well be studied by means of allatectomy and ovariectomy. Various authors reported hypertrophy of the fat body in insects deprived of their CA, mainly caused by an extra deposition of lipid materials in the fat body. This was found in Orthoptera (WEED PFEIFFER, 1945; BODENSTEIN, 1953) and in Diptera (THOMSEN, 1952; ORR, 1964a, b). The protein content of the fat body was close to normal. However, an accumulation of proteins was observed in the haemolymph, a fact which easily led to the interpretation that these proteins would have been deposited in the oocytes, had the hormonal situation been normal. The picture, however, is more complex because after ovariectomy no hypertrophy of the fat body occurred (WEED PFEIFFER, ORR), although the protein content of haemolymph did increase as was reported from many sides *e.g.*, TELFER (1954) in *Hyalophora*, HILL (1962) in *Schistocerca*, MENON (1963) and THOMAS and NATION (1966) in *Periplaneta*, ORR (1964b) in *Phormia*, COLES in *Rhodnius* (1965b) and MINKS (1965) in *Locusta*.

These data suggest that no direct relation between CA and protein synthesis exists. ORR concluded that the primary action of CA is to regulate lipid metabolism of the fat body. It is not yet clear whether or not there occurs a partial shift from protein to fat metabolism after allatectomy. The fact that after allatectomy the turnover of both triglycerides and phospholipids is slowed down, as reported for *Periplaneta* by VROMAN *et al.* (1965), is of interest in view of the afore-mentioned hypertrophy and indicates a control by CA of lipid biosynthesis and turnover. ODHIAMBO (1965, 1966), in an electron microscopic study, detected large deposits of glycogen and lipid material in the fat body of the male allatectomized *Schistocerca*. So the CA also seems, directly or indirectly, to be connected with carbohydrate metabolism.

4.1.7. *Some other effects of allatectomy*

L'HÉLIAS (1953) reported that adults of *Dixippus*, that contained less protein after allatectomy also had less RNA. BERREUR (1961) provided evidence that RNA metabolism is under control of the CA in *Calliphora*. VANDERBERG (1963) showed in an autoradiographic study that ovary, fat body and midgut synthesized less protein in allatectomized adult *Rhodnius* than in control insects. On the contrary, allatectomy has no effect on the rate of RNA synthesis of tissues of adult *Samia*, where the CA does not have a gonadotropic function, according to KRISHNAKUMARAN and SCHNEIDERMAN (1964). Mention can also be made here of the

report by RÖLLER (1962) who showed in *Galleria* larvae that after allatectomy the synthesis of some tissue proteins and specific enzymes was inhibited. WANG and DIXON (1960) established a diminished activity of the muscle transaminases in allatectomized female adults of *Periplaneta.*

4.1.8. *The neurosecretory cells*

THOMSEN (1952) was the first to show that the median neurosecretory cells (NSC) of the pars intercerebralis of the brain are also essential for ovarian development and normal reproduction in *Calliphora.* HIGHNAM *et al.* (1963) further elaborated this finding and reported that the haemolymph protein concentration in allatectomized mature females of *Schistocerca* exceeded that in normal females. After cauterization of the NSC the blood protein concentration was lower than in normal locusts (HILL, 1962). Similar results were described by MILLS *et al.* (1966) for *Periplaneta.* HIGHNAM *et al.* concluded that the NSC are involved in the control of protein synthesis (Fig. 13"a") and that JH enables the oocytes, or at least the follicle cells, to take up proteins from haemolymph. It has to be assumed that the haemolymph proteins accumulating after allatectomy are vitellogenic for this conclusion to be valid (see sections 4.1.2. and 4.1.3.).

In *Tenebrio* MORDUE (1965a, b) also demonstrated the vital rôle of the NSC in the regulation of oocyte development. The NSC control the production of protein and their secretion acts "synergistically" with JH to bring about normal oocyte growth. A direct relationship exists between the brain and the CA, as was shown by cutting the nervus corporis cardiaci I, the nerve between the NSC and the CC, which operation also caused the prevention of oocyte growth.

Remarkable is the finding of STRONG (1965a, b) that in *Schistocerca paranensis,* the Central American Locust, the lateral instead of the median neurosecretory cells are involved in the regulation of oocyte growth and activation of the CA. Other recent studies dealing with the relation between the neuro-endocrine system and oocyte development were carried out by LOHER (1965) with the grasshopper *Gomphocerus rufus*; by SIEW (1965a, b) with the beetle *Galeruca tanaceti,* and by SAINI (1966) with the beetle *Aulacophora foveicollis.*

4.1.9. *The "diagnostic" use of the haemolymph*

When yolk protein formation is inhibited by extirpation of NSC or CA, or when ovariectomy has been carried out, the consequences of this interference will immediately be observable in the haemolymph, as we have seen before. The importance of the extra-ovarian yolk protein syn-

thesis is evident (see sections 4.1.2. and 4.1.3.). Changes in the total protein content or of some protein fractions in the haemolymph reflect the controlling activity of the neuro-endocrine system.

HILL (1962) and SLÁMA (1964b) showed, in *Schistocerca* and in *Pyrrhocoris*, respectively, that an "active" neuro-endocrine system and developing ovaries were correlated with a high protein concentration in the haemolymph. Some protein fractions in the haemolymph of *Periplaneta* (MENON, 1963; THOMAS and NATION, 1966) and of *Locusta* (MINKS, 1965) seem to be influenced by the CA. COLES (1965a–c) did not find that egg-production of *Rhodnius* was reflected in changes of the total protein content and of four protein fractions of the haemolymph. In this case, however, an explanation can be provided by the assumption that the CA control both yolk protein synthesis and oocyte absorption, thus depressing all abnormal changes in the haemolymph.

4.1.10. *Influence of the diet on ovarian development*

It has to be borne in mind that, in addition to the hormonal system, the state of nutrition may also have an influence on ovarian development. A number of insects require no food during adult life (autogenous), while others do (anautogenous). In the latter category no egg maturation ever occurs when the adult females are kept on a diet of sugar and water only, *i.e.*, without protein (see review by HOUSE, 1962). A recent study of BODNARYK and MORRISON (1966) showed that the diet in *Musca* has profound effects on the ratios of different haemolymph protein fractions. It has become evident that in certain female insects the activity of the neuro-endocrine system is correlated with protein intake. This holds for the NSC in *Calliphora* (STRANGWAYS-DIXON, 1961), where a reduction of protein intake can be seen after removal of the NSC. THOMSEN and MØLLER (1959, 1963) reported a reduced activity of the intestinal proteases in the midgut after cautery of the NSC in *Calliphora*, whereas DADD (1961) found the same phenomenon in allatectomized *Tenebrio*. It is interesting to note that the contrary can also be seen. In *Phormia* (ORR, 1964a, b) evidence indicates that ingestion of a specific amount of dietary protein brings about the activation of the CA.

ENGELMANN (1965) remarked that the consequences of the severe operation of cautery of the NSC and of allatectomy are not purely hormonal, but are effects of a "general deficiency syndrome" principally caused by a disturbed process of feeding. If this were true, identical effects should be obtained by simple starvation. HIGHNAM *et al.* (1966) found, however, that destruction of the NSC or allatectomy had effects upon oocyte growth that were distinctly different from those of starvation. The effects of removal of the frontal ganglion can roughly be compared with star-

vation (*e.g.*, protein starvation in *Locusta*, CLARKE and GILLOT, 1965). It should also be mentioned here that CLARKE and LANGLEY (1961, 1963) thought that the removal of the frontal ganglion in the young adult *Locusta*, which operation prevented sexual maturation, interfered with the activity of the neurosecretory system. Moreover, STRONG (1966) reported a negative effect on CA-volume.

4.1.11. *Effects of JH-concentrate and allatomimetics on reproduction*

Finally, some remarks should be devoted to the action of the JH-concentrate, cecropia oil, and the allatomimetic substance, farnesol, on reproduction.

WIGGLESWORTH (1961, 1963) found that farnesol and farnesyl methyl-ether dissolved in linseed oil, induces yolk formation, when smeared over the surface of the cuticle as well as after injection, in allatectomized *Rhodnius*. CHEN *et al.* (1962), injecting crude cecropia oil, reported a stimulation of oocyte growth of *Periplaneta*. Negative results were obtained by SLÁMA (1965b) on the reproduction of allatectomized female *Pyrrhocoris*. JOLY (1965) mentioned a more rapid development of the ovaries of *Locusta* after injection of cecropia oil or farnesol which had been dissolved in peanut oil.

4.2. EXPERIMENTAL RESULTS

4.2.1. *Some introductory remarks*

It is clear that we are still far from a generalized picture of the function of the various elements of the neuro-endocrine system in the control of reproduction. Especially the part played by the CA within this system apparently varies in different species of insects. We should like to stress that the activity of the CA is indispensable in the normal course of reproduction. We will now discuss their position in the neuro-endocrine system of *Locusta* against the background of some experimental results. The effect of CA on protein synthesis in the fat body is very important in this respect.

The inhibition of yolk deposition in the oocytes of allatectomized female *Locusta* must undoubtedly cause profound effects on metabolism as a whole. The colour of the operated females becomes a deeper brown. The allatectomized male *Locusta* exhibits the same colour, in contrast to the normal mature males that are a bright yellow. The CA are essential for maturation of the gregarious adult male, for their sexual behaviour and probably for the production of a sex pheromone which accelerates

maturation in surrounding young males, as is stated for *Schistocerca* by LOHER (1960). It is generally accepted that JH does not directly affect spermatogenesis, but stimulates the activity of the male accessory glands (DE WILDE, 1964). Quantitatively, the biochemical activities concerned are a relatively small fraction of the total metabolism. It can therefore be expected that allatectomy in males does not produce the same profound effects on metabolism as in females.

4.2.2. *Sexual development in* Locusta

In the present chapter several aspects of the reproductive metabolism of normal and allatectomized *Locusta* will be compared. It is useful to describe here the sexual development of the normal insect. In all experiments, sham-operated insects were used in order to eliminate possible secondary effects (*e.g.*, by wounding) caused by the operation. The sham-operation consisted in simply piercing the neck-membrane and thus is a very small injury. Perhaps this precaution was superfluous, because we never found differences between normal and sham-operated individuals. Under our breeding conditions (see section 2.1.1., locusts in gregarious phase) the adult locusts generally live for 2–3 months. During the first 8–9 days after the final moult in both female and male locusts, there are no signs indicating the coming reproductive phase. Subsequently, in the female, yolk deposition is initiated and the oocytes start growing considerably. From the 16th day of adult life onward oogenesis is completed, provided that a copulation has occurred. So the first gonotrophic cycle lasts from the 9th to 17th day, the second from the 21st to 28th day and thereafter still more cycles can follow. In a group of locusts of the same adult age, however, only the first gonotrophic cycle exhibits reasonable synchrony; during the next cycles the inevitable individual differences confuse the general picture. So possible effects of the sexual development on the aspects of metabolism, as will be described in this chapter for representative numbers of *Locusta*, can be most clearly demonstrated during the first cycle.

The males do not exhibit such a distinct cyclic activity of sexual development. From the 11th to 12th day of adult life they become sexually active, by showing mating behaviour, concomitant with the development of the yellow pigmentation.

4.2.3. *Allatectomy and feeding activity*

The allatectomized *Locusta* does not show a decreased food intake (own observations, by comparing the weight of faeces of normal and allatectomized locusts). This is in accordance with data given by HILL *et al.* (1966).

4.2.4. *Quantitative analyses*

During the gonotrophic cycles mentioned above, changes take place in a number of body constituents closely related to oogenesis. These changes find their expression in the dry weight of some organs actively concerned in yolk formation: fat body and ovary. In the following lines, an account will be given of some quantitative estimations. They concern:

Dry weight (fat body ♀ and ♂, ovary). Figure 4*a*.
Proteins (fat body ♀ and ♂, ovary). Figure 4*b*.
Proteins (haemolymph ♀ and ♂). Figure 4*c*.
RNA-phosphorus (fat body ♀ and ♂, ovary). Figure 4*d*.
RNA-phosphorus (haemolymph ♀ and ♂). Figure 4*e*.
Free amino acids (fat body ♀ and ♂, ovary). Figure 4*f*.
Free amino acids (haemolymph ♀ and ♂). Figure 4*g*.
Lipids (fat body ♀ and ♂, ovary). Figure 4*h*.
Glycogen (fat body ♀ and ♂, ovary). Figure 4*i*.
Trehalose (haemolymph ♀ and ♂). Figure 4*j*.

Care was taken to ensure that the experimental animals were of approximately the same size and weight and that they were in a good condition. Insects, which somehow differed from the others of the group were discarded. The analyses were performed on animals up to 24 days after the imaginal moult, with an interval of 3 days. Some exceptions to this rule are duly indicated.

The results of the quantitative analyses are plotted against the adult lifetime of the locusts (fig. 4*a–j*). They are presented as the mean values of 5–10 observations. The standard deviations are only indicated in the figures when they exceeded 5 %.

Dry weight of the fat body and ovaries (fig. 4*a*)

The dry weight of the fat body of normal females gradually increases till the 18th day, then falls sharply and rises again. In allatectomized females the dry weight is initially 40 % lower than in normal ones, yet rises constantly and is twice as high as in normal females at the 24th day. The differences between the ovaries of both categories of insects are enormous, obviously as a result of the inhibition of yolk deposition in the allatectomized female. In the male fat body — normal as well as allatectomized — the dry weight curves follow a cyclic pattern. The "normal" fat body weighs 5–25 mg more than the "allatectomized" fat body.

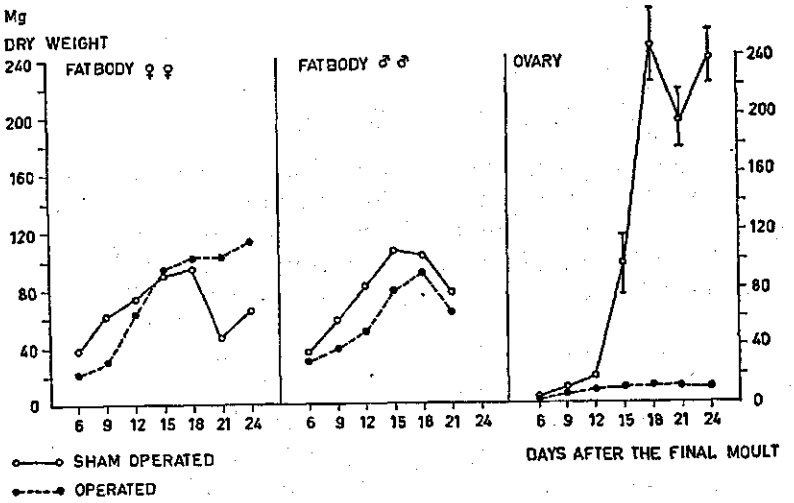


Fig. 4*a*. For explanation see figure 4*j*.

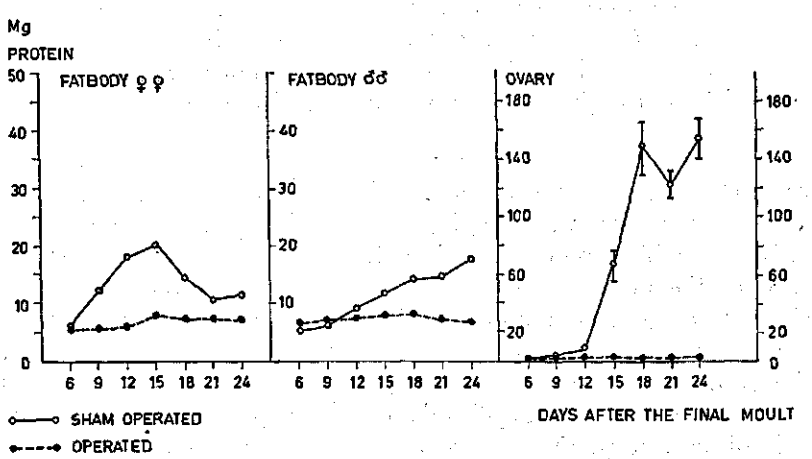


Fig. 4*b*. For explanation see figure 4*j*.

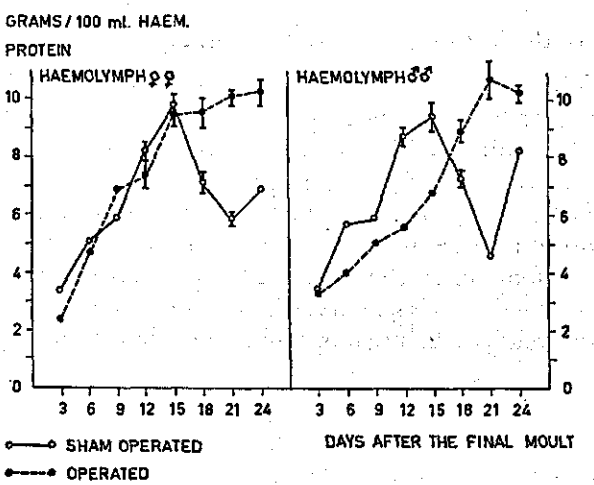


Fig. 4*c*. For explanation see figure 4*j*.

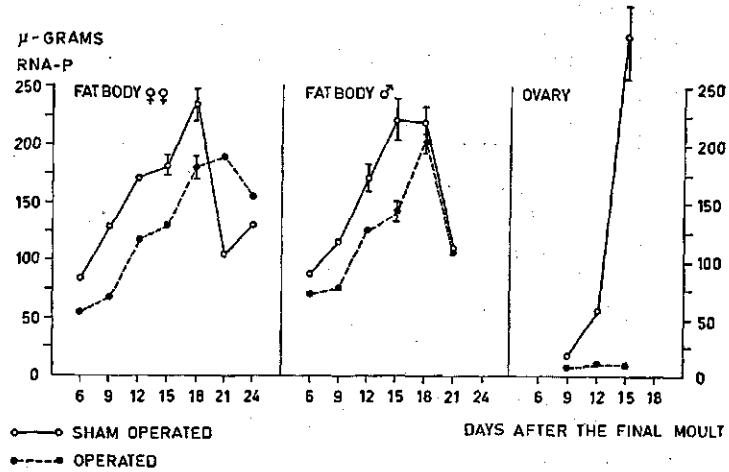


Fig. 4*d*. For explanation see figure 4*j*.

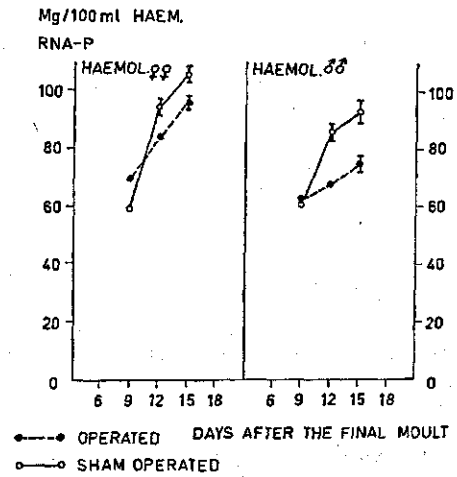


Fig. 4*e*. For explanation see figure 4*j*.

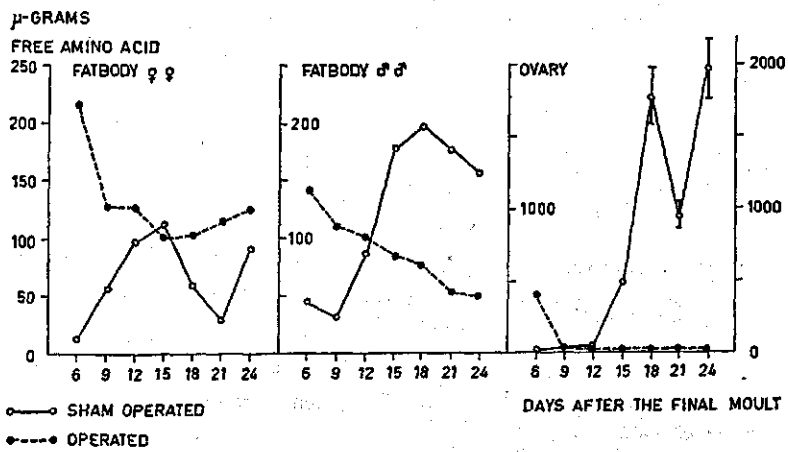


Fig. 4*f*. For explanation see figure 4*j*.

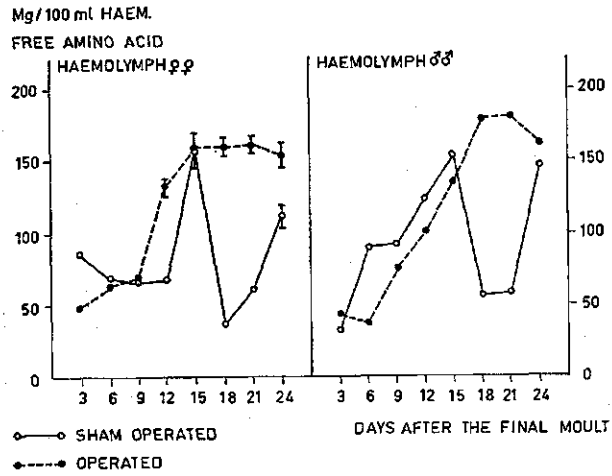


Fig. 4*g*. For explanation see figure 4*j*.

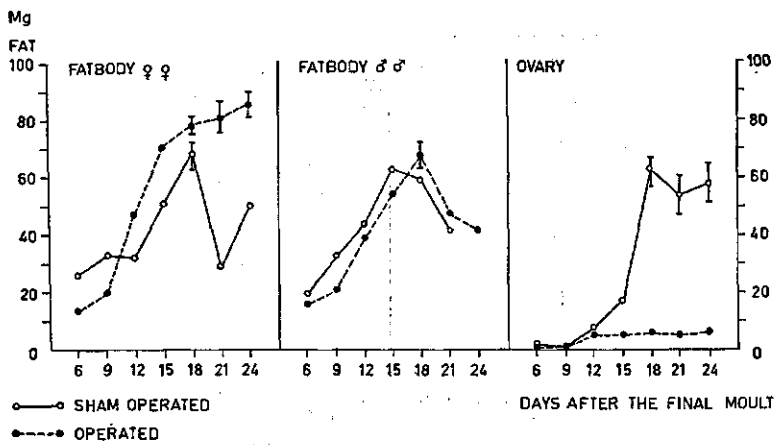


Fig. 4*h*. For explanation see figure 4*j*.

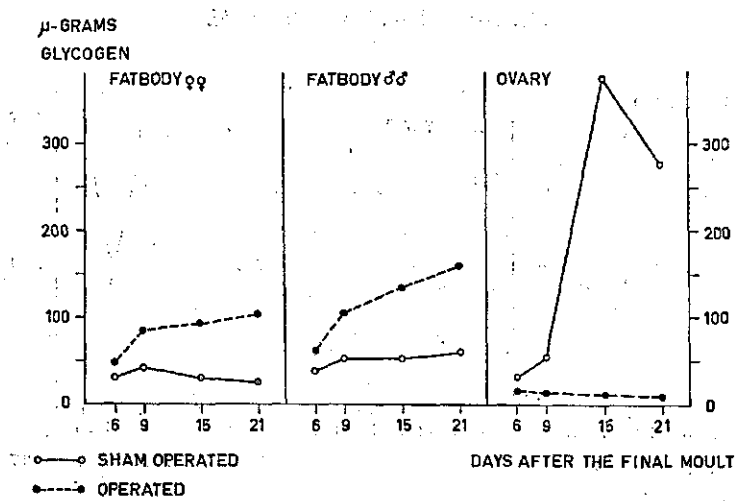


Fig. 4*i*. For explanation see figure 4*j*.

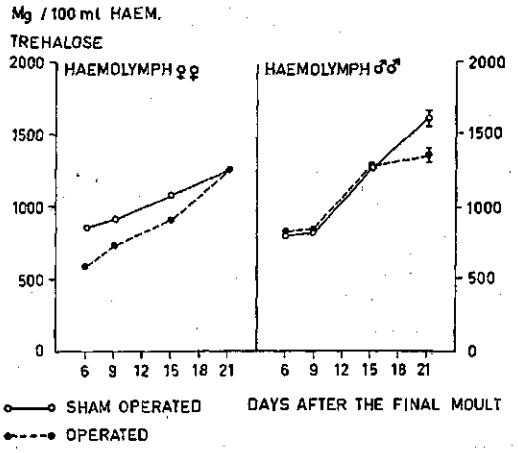


Fig. 4*j*. The figures 4*a* to 4*j* represent the results of quantitative analyses in the fat body, ovaries and haemolymph of normal and allatectomized *Locusta*, carried out at different times after the final moult.

4*a*. Dry weight in mg; 4*b*. Protein content in mg; 4*c*. Proteins in grams per 100 ml; 4*d*. RNA-P in μg; 4*e*. RNA-P in mg per 100 ml; 4*f*. Free amino acids in μg; 4*g*. Free amino acids in mg per 100 ml; 4*h*. Lipid content in mg; 4*i*. Glycogen in μg; 4*j*. Trehalose in mg per 100 ml.

The values in the figures are averages from 4–6 experiments. The standard errors are represented as vertical lines only if they exceed 5 % of the mean values.

The proteins of fat body and ovaries (fig. 4*b*)

In the fat body of normal females the protein content gradually increases until the 15th day, the end of the first gonotrophic cycle, and then decreases. There is no rise of the absolute protein quantity, related with the second sexual cycle, but the relative content, based on the dry weight of the fat body, correlates with it. In the normal males the protein level is initially below that of allatectomized males, but rises constantly and soon exceeds that of operated insects. The proteins of the allatectomized female as well as the male fat body are at a constant low level. In the ovaries the differences between the normal and operated insects are especially striking.

The proteins of the haemolymph (fig. 4*c*)

In haemolymph the protein content generally increases during adult development. Both normal females and males exhibit a cyclic pattern running synchronously with the female gonotrophic cycle. In the allatectomized insects of both sexes the protein content gradually increases and rises above that of the normal locusts.

RNA-phosphorus in fat body, ovary (fig. 4*d*) and haemolymph (fig. 4*e*)

The contents of RNA-P are of course closely related to the protein content. It can easily be seen that in the fat body of normal females the content of RNA-P during the first sexual cycle is higher than in allatectomized females, just as in the ovaries where only a limited number of observations is available. The absolute amount of RNA-P in the fat body of normal and allatectomized males runs almost parallel with the dry weight curve and its percentage is thus rather constant during adult life. The RNA-P content is somewhat higher in the haemolymph of normal locusts of both sexes.

Total content of free amino acids in fat body, ovary (fig. 4*f*) and haemolymph (fig. 4*g*)

As far as the free amino acid content is concerned, in the "normal" fat body and ovary a cyclic curve is shown in females which again is closely related to the gonotrophic cycle; in the males it shows a sharp rise to a level 3–4 times higher. After the 18th day there is only a partial fall. In allatectomized females initially a very high quantity of amino acids is found, but it decreases sharply. After the 15th day, however, it gradually increases and at the 24th day an excess of free amino acids as compared with the normal females is still found. On the contrary, the quantity in allatectomized males gradually decreases during adult life and is finally far below the normal level. The curves of the free amino acids in haemolymph in females as well as in males are very similar to the curves of haemolymph proteins.

Lipids in fat body and ovary (fig. 4*h*)

The deposition of fat in the fat body seems to be more or less reciprocal to the protein synthesis. There is also a cyclic pattern of lipid content in the female fat body of which the cycle passes through a peak at the 18th day, just at the moment that protein synthesis is nearly at its maximum. The quantity of lipids in the fat body of normal females is generally lower than in allatectomized locusts. Comparing the fat bodies of allatectomized and normal males, however, the lipid contents do not differ much. Both exhibit a cyclic pattern; at first the "normal" fat body contains somewhat more lipid, but after the 15th day the situation is quite the reverse. In active ovaries the fat content is much higher than in the small ovaries of the allatectomized locusts.

Glycogen in fat body and ovary (fig. 4*i*)

The glycogen content is higher in fat bodies of allatectomized locusts than in those of the normal individuals. By contrast, the normal ovary contains much more glycogen.

Trehalose in the haemolymph (fig. 4*j*)

The differences in trehalose content in the haemolymph of normal and allatectomized *Locusta* are not clear. There is a gradual increase during the whole adult life of the locusts.

4.2.5. *In vivo incorporation of ^{14}C-labelled amino acids*

HILL (1965) followed the *in vivo* incorporation of radioactive amino acids into the proteins of fat body, haemolymph and ovary from one hour after injection onwards. In the following experiments, just as in the previous section, the first 24 days of adult life of normal and allatectomized *Locusta* were compared.

Radioactive amino acids were used in order to obtain an impression of the rate of protein synthesis in fat body, ovary and haemolymph in normal and allatectomized *Locusta*. An amount of 0.75 μc of ^{14}C-labelled protein hydrolysate, corresponding with ± 48.000 cpm according to our counting procedure, was injected into the body cavity. (see also 2.5.1.). After 24 hours haemolymph was collected and fat bodies and ovaries were isolated. Subsequently, the radioactivity in the respective protein fractions was determined.

TABLE X

Rate of radioactive protein, 24 hours after injection of ^{14}C-protein hydrolysate in fat body, haemolymph and ovary in normal *Locusta*.

Age	*Fat body cpm/mg protein*		*Haemolymph cpm/mg protein*		*Ovary cpm/mg protein*
	♀	♂	♀	♂	
6 days	69.0 ± 3.1	69.1 ± 3.6	53.3 ± 4.7	68.5 ± 8.3	43.6 ± 3.6
9 „	113.0 ± 6.4	113.1 ± 7.6	107.5 ± 7.3	99.6 ± 4.5	190.1 ± 10.4
12 „	84.4 ± 5.8	67.2 ± 4.6	26.1 ± 2.0	30.0 ± 2.0	69.7 ± 5.9
15 „	28.4 ± 1.3	35.8 ± 1.7	35.9 ± 1.7	45.2 ± 1.4	44.9 ± 2.1
18 „	82.1 ± 6.9	83.2 ± 3.0	45.6 ± 2.4	50.6 ± 2.5	35.7 ± 7.5
21 „	42.4 ± 3.1	44.3 ± 4.4	29.4 ± 2.4	46.0 ± 3.3	26.6 ± 9.1
24 „	48.6 ± 3.7	32.1 ± 1.8	26.0 ± 1.0	47.9 ± 4.4	37.4 ± 9.7

TABLE XI

Rate of radioactive protein, 24 hours after injection of ^{14}C-protein hydrolysate in fat body, haemolymph and ovary in allatectomized *Locusta*.

Age	*Fat body cpm/mg protein*		*Haemolymph cpm/mg protein*		*Ovary cpm/mg protein*
	♀	♂	♀	♂	
6 days	45.4 ± 2.3	63.2 ± 3.3	31.8 ± 6.5	34.7 ± 4.7	26.9 ± 3.1
9 „	51.3 ± 3.0	39.8 ± 5.7	27.8 ± 3.0	36.5 ± 2.4	24.0 ± 1.8
12 „	40.6 ± 3.4	35.4 ± 4.3	22.2 ± 1.4	21.9 ± 2.0	13.8 ± 1.5
15 „	30.0 ± 1.7	24.9 ± 2.9	31.0 ± 2.5	30.4 ± 2.3	18.2 ± 1.9
18 „	32.1 ± 2.0	18.0 ± 1.2	32.0 ± 1.4	23.9 ± 1.8	18.3 ± 2.2
21 „	34.5 ± 1.7	24.9 ± 4.2	32.8 ± 3.5	40.7 ± 2.0	15.7 ± 2.1
24 „	38.0 ± 2.5	26.3 ± 2.2	35.6 ± 2.3	36.0 ± 2.3	16.2 ± 1.7

In Tables X and XI the specific activity of *in vivo* labelled protein in fat body, ovary and haemolymph is represented as counts/mg protein/min. (cpm). The differences between normal and allatectomized insects are clear. It is necessary, however, to correct these values on the basis of the free amino acid content of fat body and haemolymph as given in figures 4*f* and 4*g*. The injected amount of 0.75 μc radioactive amino acids is more or less diluted in the amino acid pool present in the above-mentioned organs. Of course, the synthesis of radioactive proteins is dependent on the concentration of ^{14}C-labelled amino acids.

The corrected specific activities are presented in figures 5*a* and 5*b*. The differences between normal and allatectomized *Locusta* have still

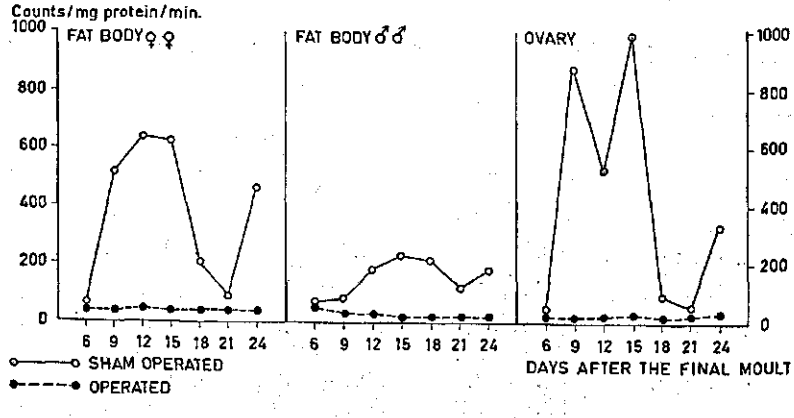


Fig. 5*a*. For explanation see figure 5*b*.

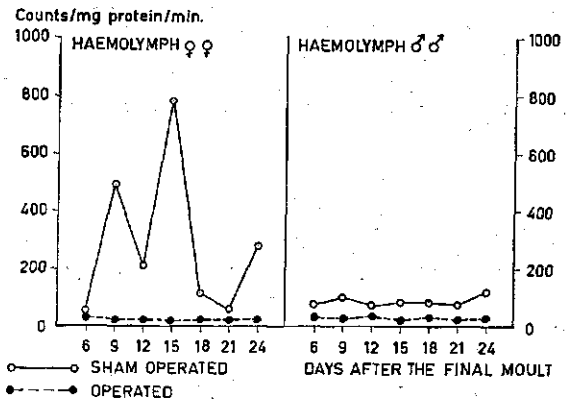


Fig. 5*b*. The figures 5*a* and 5*b* represent the rate of radioactive proteins 24 hours after injection of ^{14}C-protein hydrolysate in fat body and ovary (fig. 5*a*), and haemolymph (fig. 5*b*) in normal and allatectomized *Locusta*. The presented values are corrected for the amino acid content already present in the above-mentioned organs.

increased. The shapes of the curves obtained with the fat body of both normal females and males are similar to those representing their respective protein contents (fig. 4*b*). The curves suggest a strong relation with the sexual activity of the insects. The specific activities of the haemolymph and ovarian proteins of normal females also are high from the 9th to 15th day of adult life. The sharp fall shown in both cases at the 12th day is not clear. The first maximum of specific activity of the ovary is at the 9th day, the second maximum is at the 15th day, 3 days earlier than the maximum protein content of the ovary (fig. 4*b*). The haemolymph of normal males does not exhibit much activity.

The specific activities of normal female fat body reach their maximum in 12 days old locusts, in haemolymph this maximum occurs at the 15th day. The increase of specific activity is a measure for the rate of protein synthesis. Comparing this with the protein content of the fat body (fig. 4*b*) it is clear that at least in the normal female fat body the net synthesis of protein is at its maximum in 9 day-old *Locusta*. After this date the incorporation rate and also the specific activity probably slow down by a quick "turnover".

The general level of the incorporation rate is fairly low in the present experiments. During 24 hours after injection the insect is able to remove an important quantity of radioactive materials as CO_2 and in the faeces. In Table XII a survey of the destination of approximately 65 % of the injected protein hydrolysate is given. More than 50 % has already left the body as excretion products and only 8–10 % of the isotopes has been

incorporated into fat body and ovarian proteins. The most important incorporation within the insect is apparently into the latter organs.

To obtain more distinct results it would be better to stop the incorporation earlier after injection or to inject still more radioactive material.

TABLE XII

The distribution of radioactivity in 9-day-old *Locusta* after 24 hours of *in vivo* incorporation of ^{14}C-labelled protein hydrolysate.

Injection 48,000 cpm.	♀♀ *9 days Normal*		♂♂ *9 days Normal*	
Excretion as CO_2	21,450 cpm	44.7%	20,193 cpm	42.1%
„ „ faeces	3,706 „	7.7%	2,593 „	5.3%
Proteins in the head	609 „	1.4%	661 „	1.4%
„ „ „ abdomen	693 „	1.4%	990 „	2.1%
„ „ „ thorax	902 „	1.9%	1,532 „	3.2%
„ „ „ gut	945 „	2.0%	626 „	1.3%
„ „ „ (testis) + fat body	3,228 „	6.7%	3,026 „	8.0%
„ „ „ ovary	1,603 „	3.3%		
Total amount recovered	33,216 cpm	69.1%	30,375 cpm	63.4%

Values presented as averages of 4 insects.

The insects were kept for 24 hours in respiratory flasks, in the presence of a 1% NaOH solution to absorb CO_2. The locusts had no access to the sodium-hydroxide. A slow current of air was lead through the system. The proteins were collected after homogenization of the above-mentioned parts of the body following SIMKIN and WORK (1957). See section 2.5.1. The faeces were also roughly homogenized and analysed.

Apparently 30–35% of the radioactivity has not been detected and has probably been washed out while present in the amino acids and fatty materials.

4.2.6. *In vitro incorporation of ^{14}C-amino acids in fat body proteins*

After considering the incorporation *in vivo*, it will be useful now to compare the protein synthesis *in vitro* of mitochondria from normal and allatectomized *Locusta*. Fat body mitochondria seem to be most appropriate for this purpose in view of the results described in the previous section.

Before working with these subcellular particles a series of experiments has been carried out with homogenates of the total fat body. The incorporation of ^{14}C-amino acids into proteins of the fat body of normal and allatectomized locusts is represented in Table XIII. For experimental

TABLE XIII

The *in vitro* incorporation of radioactive amino acids into proteins of homogenized fat body of normal and allatectomized *Locusta*.

Age	♀♀ *Normal* *cpm/mg protein*	♀♀ *Allatectomy* *cpm/mg protein*	*Comparison of means*
9 days	50.1 ± 4.3 (10)	35.5 ± 8.4 (7)	$P < 0.001$
15 „	47.0 ± 4.2 (8)	49.7 ± 13.5 (6)	not significant
21 „	51.5 ± 3.4 (7)	46.0 ± 2.7 (6)	not significant
Age	♂♂ *Normal* *cpm/mg protein*	♂♂ *Allatectomy* *cpm/mg protein*	*Comparison of means*
9 days	95.9 ± 20.2 (5)	53.5 ± 5.0 (6)	$P < 0.001$
15 „	62.7 ± 5.1 (6)	51.8 ± 10.9 (7)	not significant
21 „	58.6 ± 4.2 (3)	51.4 ± 10.4 (4)	not significant

The number of experiments is represented within brackets.

Incubation medium: 0.1 M sucrose, 0.04 M KCl, 1.3 mM EDTA, 0.02 M nicotinamide, 0.01 M sodiumpyruvate + 0.01 M malic acid (= substrate), 0.016 M K_3PO_4, 4 mM AMP, 0.5 mM NAD, 8 mM $MgSO_4$. pH = 7.5.

In addition 50 μg of synthetic amino acid mixture per ml (TRUMAN and KORNER, 1962) and 1 μc ^{14}C-algal protein hydrolysate. Tissue homogenate 0.5 ml = 30–40 mg protein. Reaction time 4 hours. Temp. = 30°C. Aeration was ensured by shaking the reaction flasks.

conditions see section 2.5.2. Only in 9 day-old females and males differences in the protein synthesis of the fat body tissue are evident after allatectomy.

An important problem in long-time incubations with radioactive tracers *in vitro* is how to exclude the possibility that contamination with micro-organisms is responsible for the measured activity. ROODYN *et al.* (1961) estimated the amount of bacteria in the incubation medium and were unable to show any correlation with the incorporation activity measured. KROON (1966) gave an enumeration of reasons why an important contribution of bacteria to the incorporation activity can be excluded. In this particular instance mitochondria from beef heart and rat liver are concerned, but we think it reasonable to apply the same considerations to our experiments with insect fat body.

These considerations were the following:

1. No important incorporation was found in incubations in which mitochondria were omitted from the incubation medium.

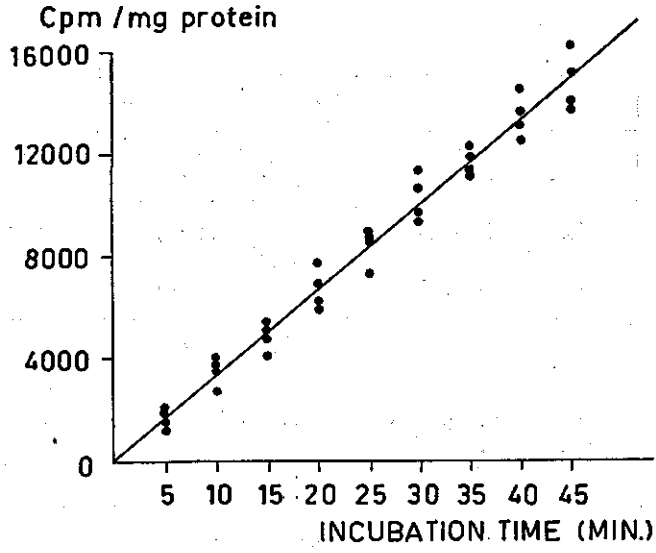


Fig. 6. Rate of *in vitro* incorporation of ^{14}C-protein hydrolysate into proteins of isolated fat body mitochondria of *Locusta*.

Incubation medium as in Table XIII. pH = 7.5. Temp. 30° C. Mitochondrial suspension 0.5 ml = 2–4 mg protein per flask. Incubation radioactivity by 10 μc ^{14}C-protein hydrolysate.

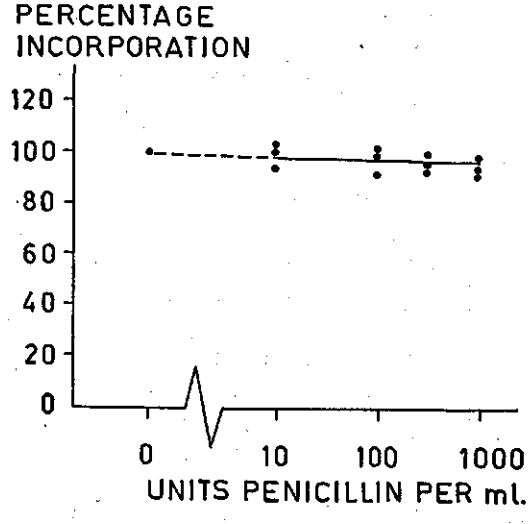


Fig. 7*a*. For explanation see figure 7*c*.

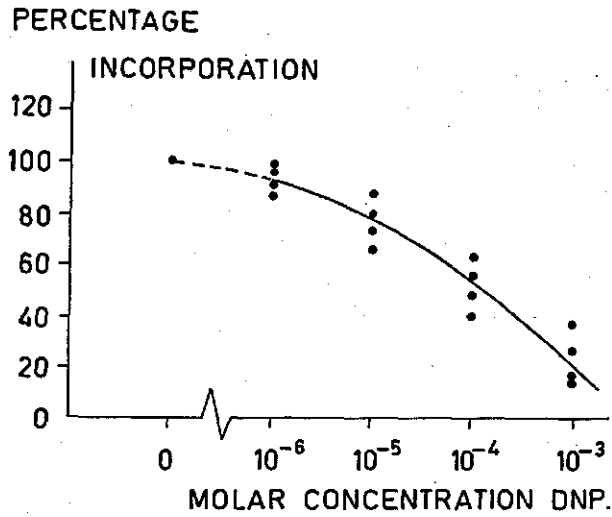


Fig. 7*b*. For explanation see figure 7*c*.

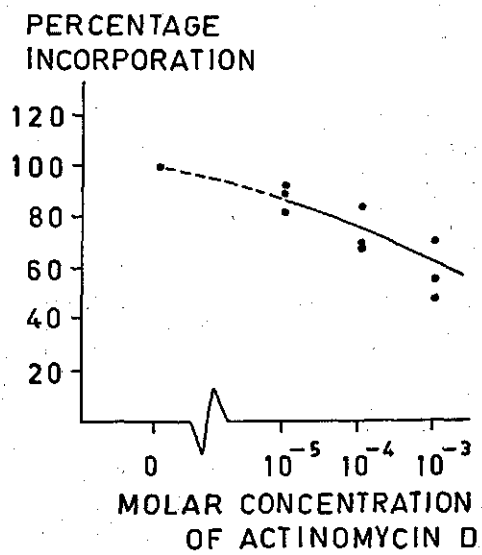


Fig. 7*c*. The figures 7*a* to 7*c* represent the effects of penicillin (fig. 7*a*), DNP (fig. 7*b*) and actinomycin (fig. 7*c*) on the *in vitro* incorporation rate of ^{14}C-protein hydrolysate into proteins of the fat body mitochondria of *Locusta*. Incubation medium as in Table XIII. pH = 7.5. Temp. = 30° C. Mitochondrial suspension 0.5 ml = 2–4 mg protein per flask. Addition 1 μc ^{14}C-protein hydrolysate. Reaction time 2 hours.

2. The time course of the incorporation is linear (fig. 6).
   There is a fair reproducibility in the results, in spite of the high incorporation activity.

3. Penicillin does not influence the incorporation activity (fig. 7*a*).

4. Figure 7*b* shows that in locust fat body mitochondria, DNP inhibits the incorporation activity. The fact that the process of amino acid incorporation in mitochondrial preparations is dependent on oxidative phosphorylation, is generally accepted (RoodyN *et al.* 1961; TRUMAN and KORNER, 1962; KROON, 1964, 1966).
   It is possible to use DNP, an inhibitor of energy conserving reactions of mitochondria (see 3.2.2.), to distinguish between mitochondrial and bacterial processes. DNP inhibits protein synthesis in bacterial systems (*i.e.*, in *Escherichia coli* anyway) only at concentrations of 1 mM and higher (GROS *et al.*, 1963).

5. In addition, actinomycin D also has an inhibitory effect on mitochondrial protein synthesis, as is shown in figure 7*c*. This is in accordance with results described by KROON (1965) with housefly sarcosomes.

From the results of these measurements the conclusion has been drawn that at least in our mitochondrial suspensions the bacterial contamination was negligible.

TABLE XIV

In vitro incorporation of radioactive amino acids into the proteins of mitochondria, isolated from the fat body of normal and allatectomized *Locusta*.

Age	♀♀ *Normal* cpm/mg protein	♀♀ *Allatectomy* cpm/mg protein	*Comparison of means*
9 days	812.7 ± 177.8 (10)	94.0 ± 14.5 (6)	$P < 0.001$
15 ,,	425.4 ± 139.6 (7)	191.2 ± 13.2 (7)	$P < 0.001$
21 ,,	715.4 ± 204.2 (6)	75.3 ± 4.2 (4)	$P < 0.001$
27 ,,	–	164.5 ± 54.3 (4)	–
Age	♂♂ *Normal* cpm/mg protein	♂♂ *Allatectomy* cpm/mg protein	*Comparison of means*
9 days	944.1 ± 219.5 (8)	164.4 ± 37.8 (5)	$P < 0.001$
15 ,,	301.2 ± 46.2 (5)	110.5 ± 3.7 (4)	$0.01 > P > 0.001$
21 ,,	329.4 ± 76.6 (5)	81.2 ± 2.4 (5)	$P < 0.001$
27 ,,	131.7 ± 4.0 (3)	142.8 ± 35.3 (4)	not significant

The number of experiments is given in brackets.

Conditions as in the experiments of Table XIII. Per flask 0.5 ml of mitochondrial suspension (2–4 mg protein) was added. The reaction time was 2 hours.

After these introductory experiments rates of amino acid incorporation in the fat body mitochondria of normal and allatectomized *Locusta* were compared. The isolation has been described in section 2.3., the conditions of incubation in section 2.5.2. In Table XIV the results of experiments with both females and males of four different ages are represented. The differences in incorporation rate, especially of the 9 days-old females and males, and of the 21 days old females are very clear. The standard deviations are quite large, in spite of the considerable number of experiments. The significance of the mean differences, however, remains distinct except in the case of the 27 days old females. These results are not in line with those described in section 3.2.1., in which experiments oxygen consumption and oxidative phosphorylation of flight muscle mitochondria of normal and allatectomized locusts were compared in the same way, without obtaining significant differences.

4.2.7. *Further investigations on the haemolymph proteins*

Yolk proteins, which are mainly synthesized by the fat body, are transferred to the oocytes by way of the haemolymph. The haemolymph is very suitable for a detailed investigation of its proteins by agar-gel electrophoresis. Its use requires a minimum of preparation except some centrifugation of the samples (see section 2.6.).

After electrophoresis, fixing, staining, etc., 7–8 different protein bands can be distinguished. This protein pattern is always present in all treatments, except band 4 which is only present in normal females of 15 days and older and in allatectomized females. The protein fractions of normal and allatectomized *Locusta* of both sexes from 0 to 24 days are compared. A schematic survey of the protein patterns in the females is presented in figures 8*a* (normal) and 8*b* (allatectomized).

Some of the protein bands do not show important changes in their intensity during the above-mentioned period, like bands 1, 2, 5 and 8. The intensity of the other bands shows some interesting features. Band 4 may be called a "female protein", since it is not present in the male haemolymph. In the normal females protein 6 and especially 7 become gradually more important till the 15th day of adult development, then a temporary decrease follows, but its intensity rises again till the 24th day. Protein 6 reaches its maximum about 3 days earlier than protein 7. The concentrations of both proteins 6 and 7 exhibit a cyclicity which in the latter fraction runs exactly parallel to the gonotrophic cycles of the normal female *Locusta*.

This cyclicity is completely lacking in allatectomized females. The amounts of the proteins 6 and 7 decrease during the entire adult development. In addition, in "normal" haemolymph protein 7 is the main frac-

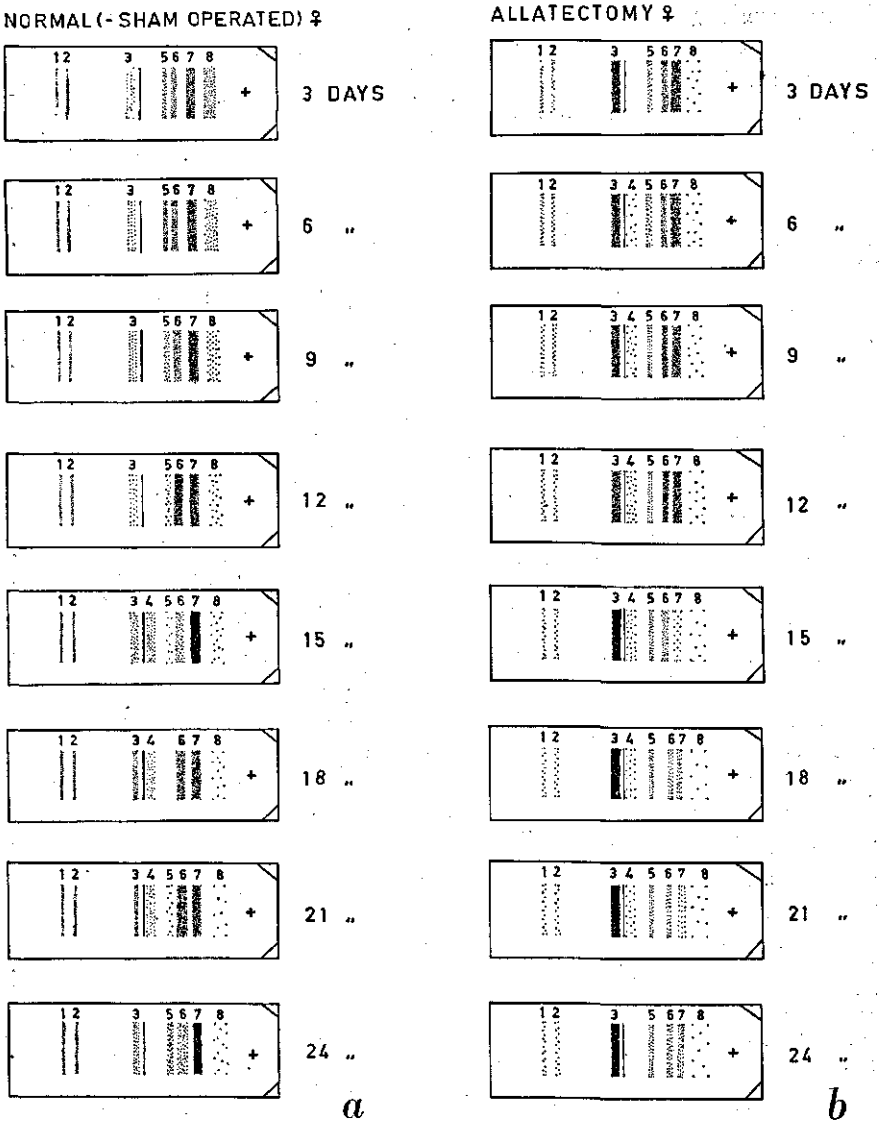


Fig. 8. A general view of the haemolymph protein patterns of the normal female (*a*) and allatectomized female *Locusta* (*b*). Note the cyclicity of the bands 6 and 7 in 8*a* and the differences of band 3 between *a* and *b*.

The haemolymph samples used for electrophoresis were pooled from 3–5 insects. The haemolymph was introduced into a slit in the agar, represented by the drawn line. The anode is indicated by the "+" sign in the right corner.

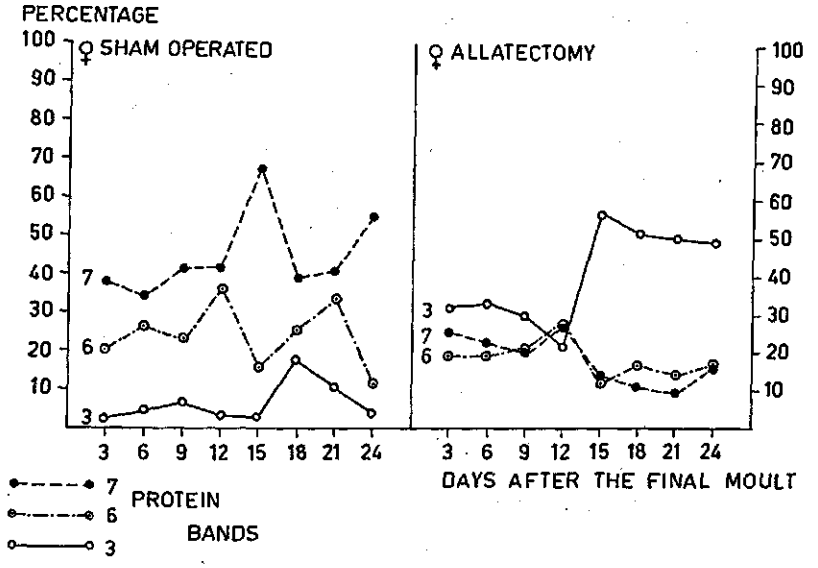


Fig. 9*a*. For explanation see figure 9*b*.

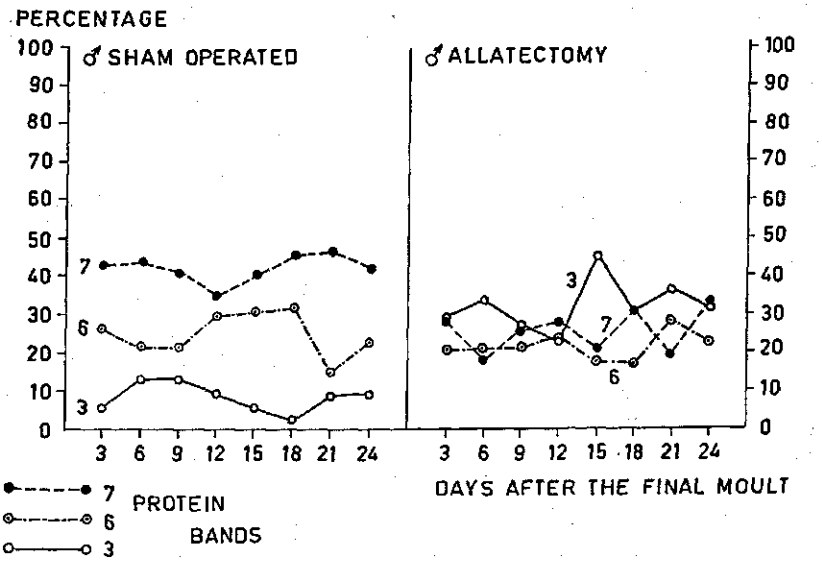


Fig. 9*b*. The figures 9*a* and 9*b* represent the percentages of the three important protein fractions 3, 6, and 7 in the haemolymph of normal and allatectomized females (fig. 9*a*) and males (fig. 9*b*).

tion, but after allatectomy protein 3 is far more important, especially after the 12th day. It thus appears that after allatectomy an interesting shift of the relative synthesis of the different protein fractions takes place!

The ratio of the intensities of the protein bands can easily be scanned in a densitometer connected with a recorder. The percentages of the most important proteins in this respect, numbers 3, 6 and 7, are represented in figure 9*a*.

The above-mentioned cyclic variations in protein band 7 cannot be so easily determined in the normal male *Locusta* (see fig. 9*b*). The shift from the proteins 6 and 7 to 3 is not so clear as in females, but the differences in ratio between the 3 protein fractions are still noteworthy.

4.2.8. *Effects of implantation of corpora allata in allatectomized* Locusta

Implantation of 3–4 pairs of CA obtained from active yellow males into allatectomized females can be easily carried out. In our experiments implantation was always performed on 8–9 days old locusts, which had been allatectomized two days after the final moult. More than 95 % of the female as well as the male *Locusta* react positively and initiate sexual development, which was inhibited before implantation. After 7–8 days the females are able to lay eggs, while their pigmentation becomes lighter during that period. The males take on the yellow colour and they clearly show normal sexual activity. It is remarkable, however, that the females lay only one egg-pod; thereafter they become inactive again. When the mandibular muscles, between which the implanted CA have been deposited, are inspected 18–20 days after implantation, in most cases the CA cannot be found. Apparently they are completely resorbed. So there is only one gonotrophic cycle, which is probably induced by the hormone present in the CA at the moment of implantation. Another possibility is that the implanted CA are still able to produce their hormone, but only for a short time.

On the basis of these implantation experiments, it is possible to obtain a clear impression of the shift in protein synthesis from an "allatectomized" back to a "normal" protein pattern. After labelling the protein fractions with radioactive ^{14}C, the turnover of the different haemolymph proteins can easily be followed. Autoradiography of the electrophoretic patterns of haemolymph proteins has been applied.

During a period of 13 days after implantation, each day 2 or 3 locusts were injected with 0.75 μc of ^{14}C-protein hydrolysate intra-abdominally. After 4 hours the haemolymph was collected and the 2 or 3 blood samples were pooled to eliminate individual differences. The samples were prepared in the usual way for agar-gel electrophoresis. Two slides of each treatment were prepared. One of these was used for determination of the

total protein content in the different fractions, as has been mentioned in section 4.2.7. The other slide, after exposure during a month, was intended for estimation of the level of radioactivity in the protein fractions. The protein bands of both treatments were measured in the densitometer and the relative values of the corresponding fractions were compared as the ratio radioactive protein: total protein.

In case this ratio was above unity, there was a net synthesis of the fraction concerned; values below unity indicated a negative balance. In figure 10 the ratios of proteins 3 and 7 are plotted logarithmically. It can easily be seen that 2 days after implantation already the synthesis of protein number 3 has stopped and that protein 7 is synthesized during the whole period of ovarian growth. After oviposi-

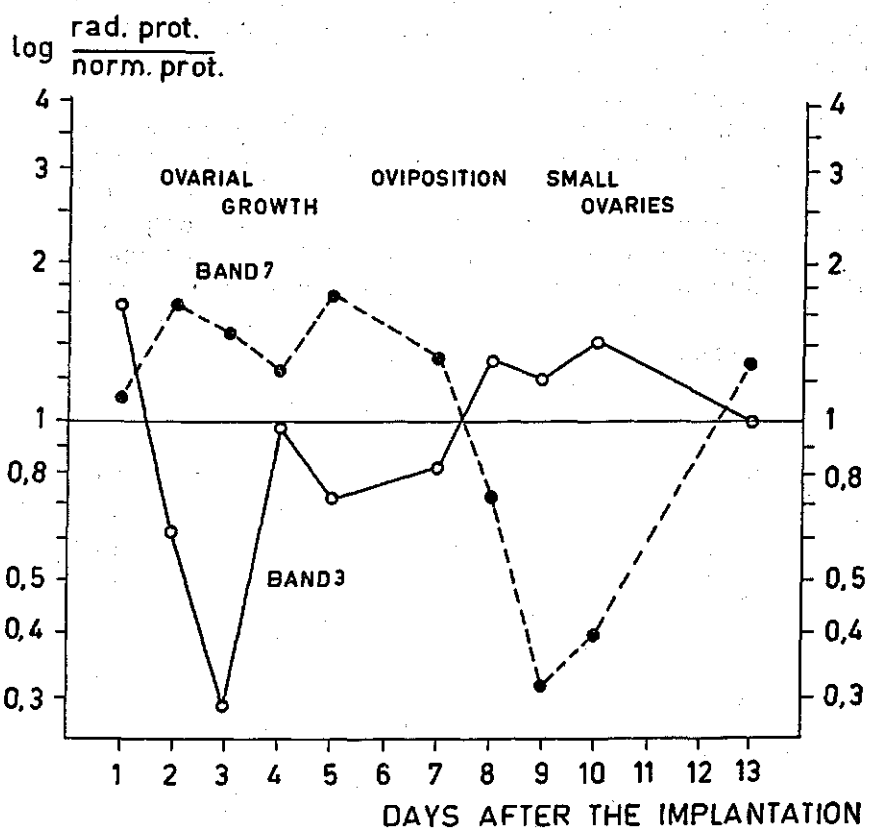


Fig. 10. The effect of implantation of 3 to 4 pairs of CA on the synthesis of proteins 3 and 7 in allatectomized females.

The ordinate represents the logarithm of the ratio radioactive protein: total protein. Above unity means a net synthesis, below unity a negative balance, *i. e.*, the protein fraction concerned is decreasing.

tion, the first gonotrophic cycle is finished and the ovaries remain small. Protein number 7 is no longer synthesized and the synthesis of protein 3 becomes "positive" again.

Unfortunately, we did not follow the proteins longer than 13 days after implantation, but at a certain time a balance between syntheses of the different proteins can be expected. In that case the curves will fluctuate around unity.

4.2.9. *Protein fractions in the fat body*

Apart from the haemolymph proteins the water-soluble proteins of the fat body were also separated by electrophoresis. The fat body proteins must be dissolved in a volume as small as possible to obtain a concentration high enough for sufficient staining of the protein fractions after electrophoresis (see section 2.6.3.). Especially the fatty material must be removed as completely as possible.

The electropherograms of fat body proteins are not of such an excellent quality as those obtained from haemolymph proteins. A fairly complicated pattern of 10–15 bands becomes visible. There are, however, only 3–4 more important bands of which the different concentrations can be measured in the densitometer. In figure 11 the relative proportions of the 2 main protein components c and f in normal and allatectomized

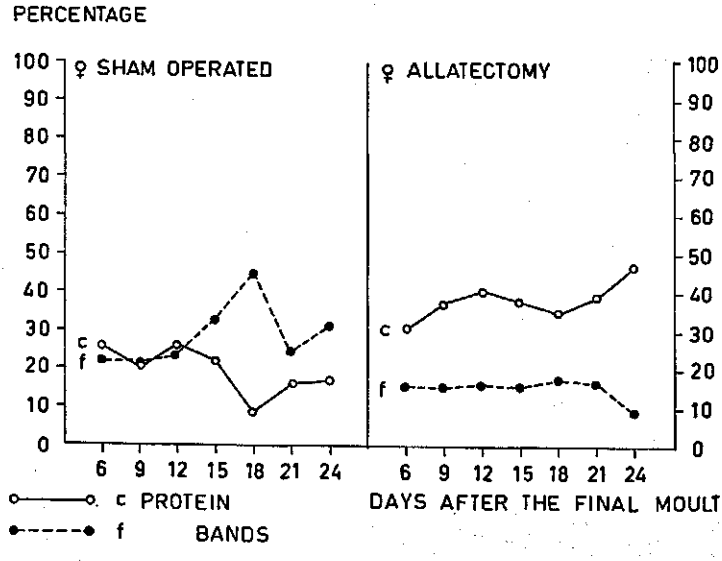


Fig. 11. The percentages of two important protein fractions c and f in the fat body of normal and allatectomized females.

female *Locusta* are compared. In allatectomized insects protein c is more important whereas protein f is the main fraction in normal females of more than 12 days old. As the mobility of proteins c and f in the electric field corresponds exactly with those of the haemolymph protein fractions number 3 and 7, respectively, there are reasons to believe that these bands represent identical proteins. This fits in with the assumption that the fat body synthesizes some proteins which are transported by the haemolymph to the ovaries.

4.2.10. *Influence of cautery of the median neurosecretory cells on the haemolymph proteins*

A fairly low percentage of female *Locusta* with cauterized median neurosecretory cells (see 2.1.2.) could be kept alive for several weeks.

TABLE XV

The relative size of different haemolymph protein fractions in *Locusta* females after cautery of the median neurosecretory cells.

Adult Age	*Dry weight Ovary*	*Protein band number*								
		1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	
6 days control	8.6 mg	1.2	2.0	12.1	–	9.0	9.4	49.7	16.6	(1)
6 „ operated	8.4 „	1.2	1.2	44.0	–	10.0	11.4	29.8	2.4	(1)
9 days control	10.5 mg	1.5	0.8	34.1	–	6.4	17.0	37.6	2.6	(2)
9 „ operated	9.7 „	1.6	1.4	62.3	–	7.3	13.7	9.7	4.0	(2)
15 days control	163.4 mg	1.2	0.6	8.6	–	–	13.4	64.5	11.7	(1)
15 „ operated	9.8 „	2.0	4.4	54.5	–	–	11.1	23.0	5.0	(1)
21 days control	264.8 mg	2.6	1.7	6.5	–	–	20.3	49.8	19.1	(2)
21 „ operated	11.6 „	1.1	1.3	48.4	–	–	8.0	29.3	11.9	(2)

Number of experiments is given in brackets.

In a limited number of experiments the haemolymph proteins of cauterized locusts of 4 different ages were compared with their controls. In Table XV the relative quantity of all protein fractions is represented and also the dry weight of the ovary. In 6 and 9 days-old females no differences in weight are noticeable. The differences in haemolymph pattern between normal and operated locusts are most obvious in 15 and 21 days-old females, which normally are at a high level of their oogenetic

activity. Especially the synthesis of proteins 3 and 7 seems to be affected by the operation. As a whole, the protein pattern does not differ much from that obtained after allatectomy.

The haemolymph of the cauterized animals of 6 and 9 days old is difficult to handle for purposes of electrophoresis, because of its change to a gel-like substance soon after isolation. Its volume, however, gradually increases and is already above normal in 21 days old operated females.

4.2.11. *Ovariectomy and the haemolymph proteins*

After ovariectomy the haemolymph proteins of *Locusta* were compared. In this respect 2 groups of females can be distinguished, *i.e.*, females bred in the presence of active males and those bred without such males.

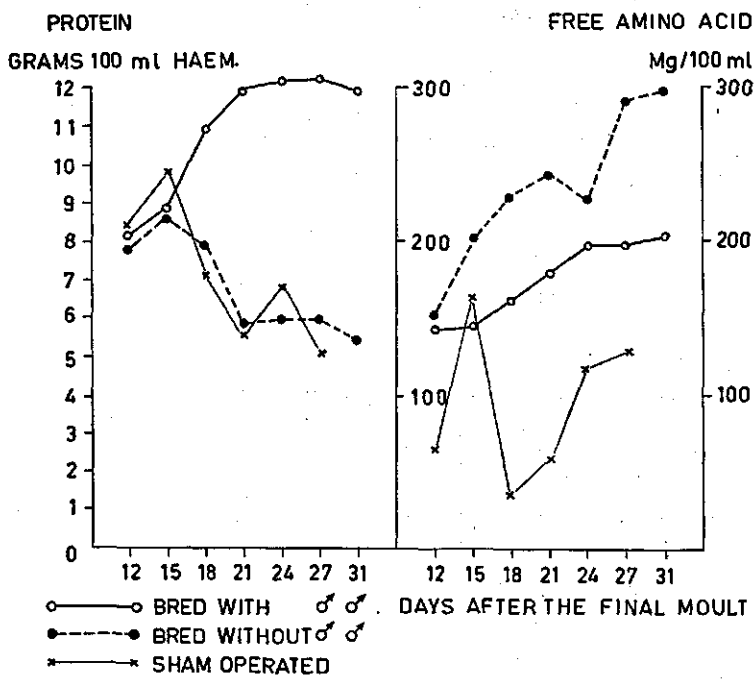


Fig. 12. The protein and amino acid content in the haemolymph of ovariectomized *Locusta*, bred with and without active males.

Standard errors were negligible, the represented values are averages of 4–5 determinations.

From figure 12 one can see that the differences in haemolymph protein content between the 2 categories at different times after the final moult

are very clear. The protein content in ovariectomized females bred with males exceeds the content in normal females, but it decreases below the normal level in females without males. Concerning the amino acid content exactly the opposite results are obtained, with a higher content in females bred without males.

TABLE XVI

The relative size of different haemolymph protein fractions in *Locusta* females after ovariectomy.

a. Reared with males

Adult Age	Protein bands number								
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	
9 days	0.3	0.2	16.6	—	5.8	23.9	50.1	3.1	(2)
15 „	0.8	0.2	24.9	19.0	5.5	8.4	40.0	1.2	(2)
18 „	0.3	0.2	9.9	36.5	5.2	9.4	35.4	3.1	(2)
24 „	0.4	0.2	8.0	39.1	5.6	8.5	34.2	4.0	(2)

b. Reared without males.

Adult Age	Protein bands number								
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	
9 days	0.2	0.1	38.7	—	4.2	18.2	36.9	1.7	(2)
15 „	1.1	0.7	18.1	7.0	8.7	8.6	51.3	4.5	(2)
18 „	0.5	0.4	25.8	8.2	7.3	14.1	39.1	4.6	(2)
24 „	0.7	0.5	22.4	8.6	7.0	16.2	38.8	5.8	(2)

The number of experiments is given in brackets.

Some data on the relative size of the protein fractions obtained by electrophoresis of haemolymph from castrated females are presented in Table XVI. The relative size of the fractions approximates the normal pattern; initially protein number 7 is the most important, while protein 3 decreases during adult life. The accumulation of protein 4 is an outstanding feature; it apparently accounts for the increase of total proteins mentioned above.

The protein pattern observed in castrated females without males is also close to normal, with two exceptions: the band of protein 3 is slightly larger and accumulation of protein 4 is much less pronounced.

Finally, it should be emphasized that the haemolymph volume in

females is greatly influenced by the presence of males. From 24 days-old females with males 1–1.5 ml haemolymph can easily be collected. This volume gradually increases during adult life and finally the insects exhibit a strongly stretched abdomen. Females without males do not show this phenomenon.

4.3. DISCUSSION AND CONCLUSIONS

Discussing the effects of JH on reproduction, we may take as a starting point the assumption that part of the proteins and lipids of the oocytes are synthesized in the fat body, released into the haemolymph and finally taken up from the circulating body fluid by the ovarian follicles and the developing oocytes (TELFER, 1965). This dynamic system is subject to co-ordinating and regulating mechanisms.

In the female *Locusta* distinct sexual cycles occur that become manifest in the pattern of oviposition. The close connection between reproductive activity and fat body metabolism is apparent from the parallelism between *e.g.*, protein content of the fat body (and haemolymph) and oocyte growth, during the first sexual cycle. Fat body and haemolymph attain their maximum protein content at the 15th day after the final moult. Protein in the ovaries is at its maximum somewhat later, *i.e.*, at the 17th or 18th day, the end of the first sexual cycle.

As is to be expected, a similar cyclic pattern is observed with the amino acid concentration in fat body and in haemolymph. The rate of protein synthesis can be studied most conveniently by *in vivo* ^{14}C-amino acid incorporation experiments. Protein synthesis in the fat body reaches its maximum 12 days after the final moult. Radioactive proteins are also found in the haemolymph and here the relative amount of fractions 6 and 7 show a distinct cyclicity in the total quantity of proteins, as is evident from the electropherograms and, especially for protein 7, from autoradiography. The similar mobility in the electrical field and the similar decrease in relative amount after allatectomy of haemolymph protein fraction 7 and fat body protein fraction f suggests that these are identical vitellogenic proteins.

The cyclic nature of protein synthesis in the fat body of normal female *Locusta* is very clearly reflected in the *in vitro* ^{14}C-amino acid incorporation capacity of isolated fat body mitochondria. In 9 days-old locusts this capacity is much greater than in 15 days-old ones. Of course, this is not meant to suggest that the mitochondria are the site of yolk protein synthesis. Actually, ROODYN (1962) and TRUMAN (1964) suggested that mitochondria are only able to synthesize insoluble, presumably structural membrane proteins; the site of synthesis of soluble proteins such as *e.g.*, enzymes, would be the endoplasmic reticulum. This view, based

upon work with rat liver and ox heart mitochondria, was supported by NEUPERT and BÜCHER (quoted by BÜCHER, 1965) who worked with *Locusta* flight muscle mitochondria *in vitro*. If, however, the ^{14}C-amino acids were administered *in vivo*, they found, analogous to ROODYN, that both insoluble and soluble protein fractions appeared equally well labelled. It can be added that KROON (1966) also felt objections against the generalization by TRUMAN. Be this as it may, the mitochondrial activity reported above is strong evidence of a general activity of fat body cells with respect to protein synthesis. The picture of the endoplasmic reticulum of the fat body of a 12 days-old female *Locusta*, shown in Plate III, is also highly suggestive of intensive protein synthesis.

In addition to proteins, the growing oocyte also needs relatively large quantities of lipids; the dry weight of the ovary of a 15 days-old *Locusta* is ± 250 mg, while fat content accounts for 60–70 mg. Ovarian lipid has been found to consist for 70 % of triglycerides in *Periplaneta* (VROMAN *et al.*, 1965). There is a striking parallelism between the content of protein and of lipids in the fat body. Presumably, the ovary also receives part of its lipid material from the fat body. According to results obtained by TIETZ (1961, 1962) with *Locusta*, and CHINO and GILBERT (1965) with *Hyalophora*, *Melanoplus* and *Periplaneta*, diglycerides that become bound to vitellogenic haemolymph proteins are given off by the fat body.

In the following part of the discussion we often shall refer to the studies of HIGHNAM and co-workers, carried out with *Schistocerca gregaria*. The assumption is made that the endocrine relations in *Locusta* are similar to those in the closely related *Schistocerca*. We must be very careful in making this comparison. HIGHNAM and HASKELL (1964) reported striking differences between the two species of locusts with respect to phase differences and oocyte growth.

Reproduction is clearly under influence of the CA. Extirpation of the CA causes a complete arrest of egg formation. It was found that in the absence of JH protein synthesis in the fat body of female *Locusta* was greatly reduced. This follows directly from the measured values of protein content and of ^{14}C-amino acid incorporation, both *in vivo* and *in vitro*. It appeared that especially the synthesis of those proteins was affected, that are related to oogenesis. To such proteins the term "vitellogenic" has been applied in this paper.

We therefore assume that one of the first consequences of allatectomy is a standstill of the synthesis of vitellogenic proteins. The decrease of the content of fat body protein f and haemolymph proteins 6 and 7 confirms this suggestion. It is also apparent from the implantation experiments that the presence of protein 7 is closely connected to ovarian activity.

This protein, therefore, is probably vitellogenic. The gradual rise of total protein content of haemolymph after allatectomy of the female *Locusta* as has previously been found in *Schistocerca* (HIGHNAM *et al.*, 1963), is mainly due to a rise of protein fraction 3. This protein, which is probably identical to protein c of the fat body, is by far the main component of the haemolymph proteins, especially after the 12th day of adult life. Thus, the conception of HIGHNAM *et al.* (1963) that after allatectomy the accumulated haemolymph proteins are mostly vitellogenic, is disputable. Indeed, the gradual accumulation of haemolymph proteins in allatectomized females probably is the consequence of some protein synthesis and of a much reduced demand of the gonads, especially for protein 3.

Obviously, in the absence of JH fat body metabolism as a whole is not simply turned off or at least reduced. It appears that by extirpation of the CA this metabolism is changed to such effect that, instead of proteins, more lipids are synthesized. The ovarian growth is inhibited and the female fat body hypertrophies, which is mainly due to an extra deposition of fatty materials. The storage function of the fat body has become more important. In this connection the distinct rise of the glycogen content of the fat body after allatectomy should also be mentioned. Glycogen is not an important storage material in *Locusta*, in contrast to many other insects. *Locusta* is able to utilize lipids directly in providing energy for sustained flight (BEENAKKERS, 1963, 1964). While the glycogen content of the fat body is basically low, a rise is apparent after allatectomy (see also Plate IV). No relation was found between the accumulation of lipids and glycogen in the fat body and a lack of locomotory activity, as was suggested by ODHIAMBO (1965) with the male *Schistocerca*.

The interrelationship between the parts of the neuro-endocrine system and their specific relations with the subordinated organs involved in reproduction of the female insect, are described in the introduction to the present chapter (section 4.1.). The group of median neurosecretory cells of the pars intercerebralis is considered to be the superordinated organ, the CC is their neurohaemal organ. It is clear that the CA is another essential unit of the neuro-endocrine system. Figure 13 gives a schematic survey, mainly based upon data obtained with locusts: the relations a, d, e, f, and g have already been considered in the introduction (section 4.1.) and in the first part of this discussion. The other relations, especially b, which we should like to introduce as a new element in the endocrine relationships, will be discussed in the following lines.

It has been shown experimentally by GIRARDIE (1962, 1964a, b) that in *Locusta* the pars intercerebralis-corpora cardiaca complex is involved in the control of oocyte growth. Our results agree with these experiments:

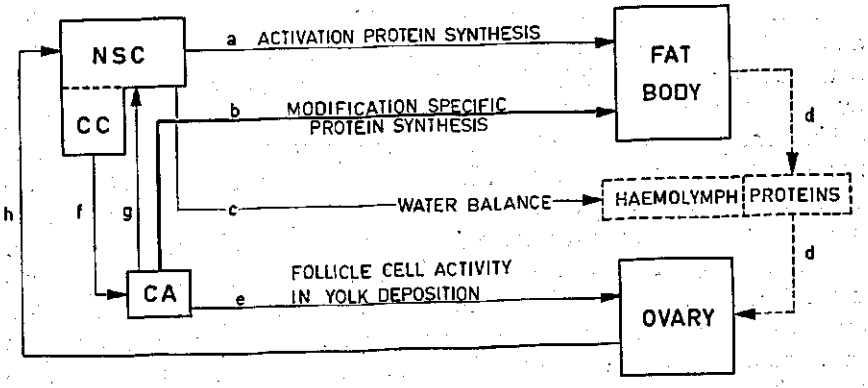


Fig. 13. Endocrine relations in adult locusts.

cautery of the NSC inhibits development of the oocytes. The haemolymph protein pattern in cauterized female *Locusta* corresponds with that obtained after allatectomy. This result is in line with the fact that the CA is the subordinated organ of the NSC and is inhibited in its action when the NSC are non-functioning. In addition, several authors (ENGELMANN and LÜSCHER, 1956; ENGELMANN, 1957, with *Leucophaea maderae*) observed a nervous inhibition of the CA by way of the nervus corporis allati and originating from the brain. JH is apparently produced under influence of both factors with a certain periodicity. In consequence, this periodicity can also be found in the fat body metabolism and the oogenesis, that are under a two-way control by the JH. This, as we have seen, holds particularly for the synthesis of vitellogenic proteins.

There is histological evidence that in *Schistocerca* allatectomy causes inhibition of the release of neurosecretory material and a consequent accumulation of this material in the NSC and the CC (HIGHNAM *et al.*, 1963). It is clear from haemolymph protein accumulation, still observed in operated insects, that protein synthesis is going on. These two observations would seem rather contradictory.

It is, moreover, questionable whether or not vitellogenic proteins are still produced after allatectomy, as is assumed by HIGHNAM and coworkers (1963). Our own observations (sections 4.2.7. and 4.2.8.) would rather indicate that vitellogenic proteins are not formed in the absence of CA. We do not doubt that HIGHNAM *et al.* are right in claiming that protein synthesis in the fat body is going on under influence of the NSC. Their finding, that after cauterization of the NSC the haemolymph protein level decreases below normal, can only be explained by an inhibition of that protein synthesis (*cf.* fig. 13, "a").

A comparison between the separate effects of cauterization of NSC, allatectomy and ovariectomy could discern between the direct effect of these organs and their indirect action through mutual influence. After ovariectomy, accumulation of neurosecretory substance occurs in *Schistocerca* (HIGHNAM, 1962a, b) and we may assume that the same phenomenon takes place in *Locusta*. This observation may be due to a feedback effect of the ovary on the NSC (fig. 13, "h") and would support the idea that the above-mentioned effect of CA on NSC is an indirect one.

Further arguments are provided by the effects on haemolymph volume. We mentioned an enlargement of the haemolymph volume in both ovariectomized and cauterized locusts (fig. 13, "c") and this can be interpreted as a consequence of inhibited release of neurosecretory material (fig. 13, "h"). Many authors report disturbances of the water balance in insects with cauterized NSC: THOMSEN (1952) in *Calliphora*, GIRARDIE (1964a) in *Locusta*, GIRARDIE (1966) in the cricket *Gryllus bimaculatus* and HIGHNAM *et al.* (1965) in the male *Schistocerca*. In our experiments, water retention is never found after allatectomy and also in the literature this effect has never been mentioned to occur in locusts.

Another difference between effects of allatectomy and ovariectomy is provided by the haemolymph protein patterns. Ovariectomy shows a characteristic accumulation of protein fraction 4, which is apparently bound to many yellow-coloured pigments, while fraction 3 is gradually decreasing in the ageing locust. After ovariectomy, indeed, the accumulation of haemolymph proteins is due to protein 4 and also to the vitellogenic protein 7.

The phenomena produced by allatectomy as well as by ovariectomy can only partly be interpreted via an inhibition of the release of neurosecretory substance. The increase of haemolymph volume does not take place after allatectomy. The accumulation of proteins in the haemolymph after ovariectomy is in contrast to a supposed inhibition of the activities of the NSC.

After allatectomy a fundamental modification of the protein synthesis occurs. We think that the CA *actively* regulates the rate of synthesis of the different proteins in the fat body, a synthesis which can be initiated by the activities of the NSC (fig. 13 "a"). This new element in the endocrine relations of reproduction in locusts is represented in fig. 13 as relation b. Our view is supported by the fact that a change of the haemolymph protein pattern in allatectomized females is clearly noticeable within 24 hours after implantation of active CA. An alternative theory, supported by HIGHNAM *et al.* and based upon the observation that CA can also stimulate the NSC (LEA and THOMSEN, 1962; HIGHNAM, 1962b), is represented by the following scheme: implantation CA → activation NSC → activation CA → activation NSC → etc. This is a step by step mutual

activation of parts of the neuro-endocrine system. We are of the opinion that this type of mutual stimulation, this "positive feedback", is highly improbable; anyway, it has never been described earlier in insect endocrinology.

COLES (1964) attached a more extended function to the CA in *Rhodnius* than we do in *Locusta*: JH acts on the fat body cells and serves to activate those components of the gene system that induce the synthesis of specific proteins; these are discharged into the blood and are taken up by the oocytes. It is an attractive possibility to explain the rôle of JH in insect reproduction following the mechanism proposed by KARLSON (1963) for ecdysone. During larval development ecdysone initiates protein synthesis by a direct effect on the gene system and in the adult stage the NSC possibly do the same thing. JH apparently needs an active protein synthesis to become effective. JH "conditions" the synthesis of specific vitellogenic proteins (and perhaps that of specific lipids) by acting in conjunction with already synthesized allosteric proteins, which in their turn can bring about the synthesis or activation of various types of enzymes (see MONOD *et al.*, 1963).

4.3.1. *Reproductive metabolism in the male* Locusta

It is not easy to explain that in many cases the male *Locusta* also shows a cyclicity very similar to that of the female. Especially the course of specific activities of the *in vivo* incorporation experiments exhibits a great similarity. After allatectomy the cyclicity completely disappears and we have already mentioned the absence of yellow pigmentation characteristic of mature males and the abnormal sexual behaviour. It is known, however, that spermatogenesis is not affected.

Apparently there is a mutual influence between females and males, reflected in the reproductive metabolism of both sexes. In *Schistocerca* the male pheromone plays an important part in accelerating both male and female maturation. In a fraction of the females eggs mature only when the locusts are exposed to the pheromone, in other females the rate of oogenesis is merely increased (LOHER, 1960). HIGHNAM and LUSIS (1962) have demonstrated that the presence of mature males brings about the release of neurosecretory substance in females and consequently stimulates egg growth. NORRIS (1964) showed that in *Locusta* and in *Schistocerca* mature males exert an accelerating influence on the maturation or readiness to copulate of young males. We come to the conclusion that, when female and male locusts are reared together, their reproductive metabolism is strongly interdependent.

5. SUMMARY

The present study deals with the action of the juvenile hormone (JH) in the adult *Locusta migratoria*. It is divided into two parts.

The first problem, discussed in chapter 3, is whether or not JH exerts a direct influence on respiratory metabolism. Many authors have tried to answer this question definitely by means of extirpation and/or implantation of the corpora allata (CA) in experiments *in vivo*. Only *in vitro* experiments can, however, provide a clue to this problem. Therefore, mitochondria isolated from flight muscles and fat body were used (section 3.1.).

No differences exist between the rates of oxygen consumption and oxidative phosphorylation of flight muscle mitochondria – with substrates α-glycerophosphate and pyruvate/malate – from normal and allatectomized *Locusta*. Homogenized active CA never have any effect on the oxygen consumption of both types of mitochondria. The CA have a stimulating effect on the oxidative phosphorylation of flight muscle mitochondria with α-glycerophosphate and no effect with pyruvate/malate as a substrate. More evidence is given in support of the assumption that the phases of *Locusta* differ in their level of JH-activity. The solitary phase is more "juvenile". CA isolated from solitary locusts stimulate the oxidative phosphorylation in mitochondria from the gregarious and also from the allatectomized locusts; CA isolated from gregarious locusts only the mitochondria from allatectomized locusts. This applies to flight muscle- as well as to fat body mitochondria, incubated with α-glycerophosphate. These data at the same time are a strong support for a specific action of the JH in these experiments (3.2.1.).

The uncoupling agent 2.4.-dinitrophenol (DNP) stimulates oxygen consumption under non-phosphorylating conditions, but inhibits this process under phosphorylating conditions and uncouples oxidative phosphorylation of flight muscle mitochondria in both substrates. This effect of DNP has disappeared when it is applied at a concentration of 10^{-6} M. The CA depress the uncoupling effect of DNP at intermediate DNP-concentrations, at which the oxidative phosphorylation is not completely inhibited ($10^{-4} - 10^{-5}$ M). The condition of the isolated mitochondria is controlled by a comparison of the Mg^{++}- and DNP-induced adenosine triphosphatase activity. It appears that CA inhibit the DNP-induced ATP-ase activity of both flight muscle- and fat body mitochondria (3.2.2.).

Cecropia oil, farnesol and farnesyl methyl-ether, added to flight muscle mitochondria, have no effects on oxygen consumption. Cecropia extract at $10^{-6} - 10^{-8}$ v/v enlarges the efficiency of the oxidative phosphorylation in contrast to the farnesol compounds (3.2.3.).

We can conclude that there is no relation whatsoever between JH-action and oxygen consumption *in vitro*. However, a positive effect of JH on oxidative phosphorylation is evident. It is not possible to explain the present results against a background of data derived from insect endocrinology. The *in vitro* JH-effects are discussed in the following points:

a. A comparison with thyroxine effects in rats;

b. The possible stimulation of the so-called coupling factors, and

c. The possible effect, via the ATP-ase activity, on the Na^+/K^+ ratio of the mitochondria (3.3.).

After that, several aspects of the reproductive metabolism of normal and allatectomized adult *Locusta* have been compared (chapter 4). The relations between the different parts of the neuro-endocrine system which regulate reproduction and its underlying metabolic processes are very complicated. It is, therefore, difficult to establish, which part is played by various organs in the hormonal regulation of reproduction. Particularly the function of the CA is subject to much diversity of opinion. A study of extra-ovarian protein synthesis by the fat body yielded further useful information. For this purpose the haemolymph, as transporting medium of the proteins, can be used to convenience (4.1.).

At first quantitative analyses of fat body, haemolymph and ovary have been carried out during the first 24 days of adult life. Dry weight and content of protein, RNA-phosphorus, free amino acids, fat and glycogen (only in the fat body) and trehalose (only in the haemolymph) have been determined and compared in normal and allatectomized *Locusta*. The relation with especially the first sexual cycle is clear in the normal females as well as in the males. There is no ovarian development in the allatectomized insects and the cyclicity of the above-mentioned constituents of fat body, haemolymph and ovary completely disappears. The protein content in haemolymph, fat and glycogen in the fat body rises above normal (4.2.5.).

The *in vivo* incorporation activity of ^{14}C-labelled amino acids (as determined 24 hours after injection) into the proteins of fat body, haemolymph and ovary has been followed throughout the first 24 days of the adult stage. The normal *Locusta* exhibits a periodic activity, related to the sexual cycles, which disappears in the allatectomized insects. The protein synthesis is at its maximum at the 9th day, just at the beginning of the first cycle (4.2.6.).

The *in vitro* incorporation activity of ^{14}C-amino acids into the proteins of homogenized fat body shows only differences between normal and allatectomized *Locusta* at the time oocyte development ought to begin.

In this respect mitochondria isolated from the fat body of both categories significantly differ, except for ageing locusts (4.2.7.).

After separation of haemolymph proteins by agar-gel electrophoresis, 7–8 bands can be distinguished. Two "negative" proteins (6 and 7) are correlated with the sexual development of the locust. After allatectomy a "positive" protein 3 becomes the most important fraction (4.2.8.).

Implantation of active CA in allatectomized female *Locusta* immediately brought the reproductive processes into action in more than 95% of the cases, but only for the duration of a single cycle. By means of autoradiography of ^{14}C-labelled haemolymph proteins a quick shift of the protein synthesis can be established (4.2.9.).

Protein fraction c of the fat body is probably identical to haemolymph protein 3; fraction f to band 7 (4.2.10.).

The haemolymph protein patterns of allatectomized and NSC-cauterized female *Locusta* bear a great similarity (4.2.11.).

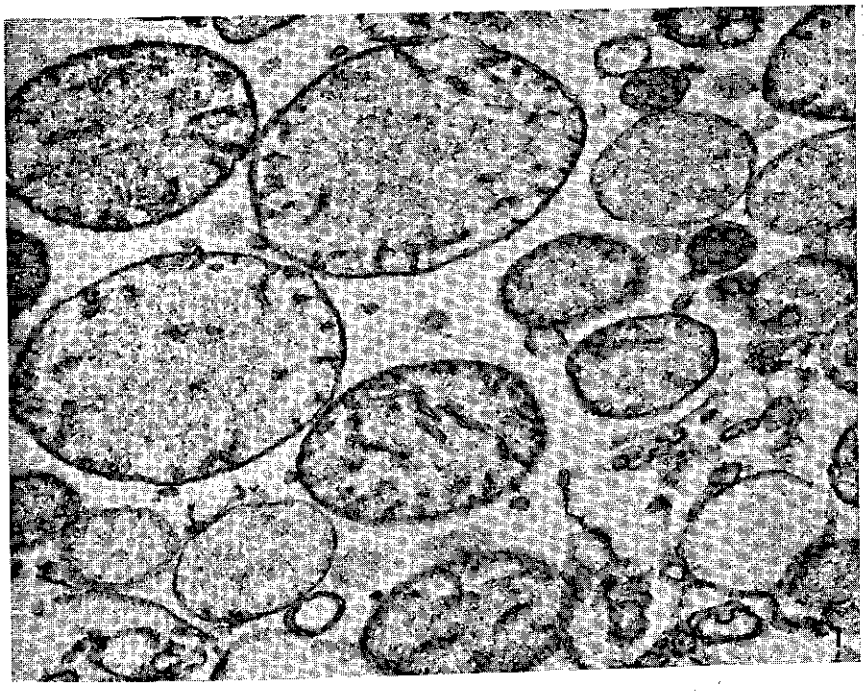
However, the haemolymph protein patterns of ovariectomized females bred in isolation are similar to normal ones, except for the accumulation of fraction 4. Haemolymph volume and protein content show an important increase in ovariectomized females bred with active males (4.2.12.)

These results are in accord with the conception that the fat body produces a considerable fraction of the proteins required for oogenesis. We agree with the ideas of HIGHNAM *et al.* (1963) according to which the NSC activate protein synthesis in the fat body. We attach, however, a more independent rôle to the CA in this process. Our data are more in accord with the idea that JH exerts its activities in the presence of active protein synthesis and induces the formation of specific vitellogenic proteins. We possibly may explain the change of lipid and glycogen metabolism in the fat body in the same way (4.3.).

6. APPENDIX: ELECTRON MICROGRAPHS

The electron micrographs are reproduced on plates I (facing page 248) to IV (facing page 249).

PLATE I



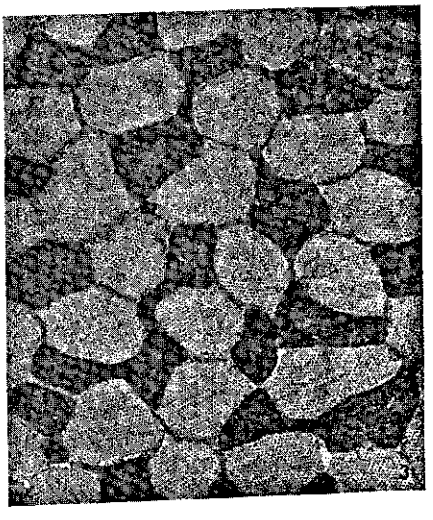
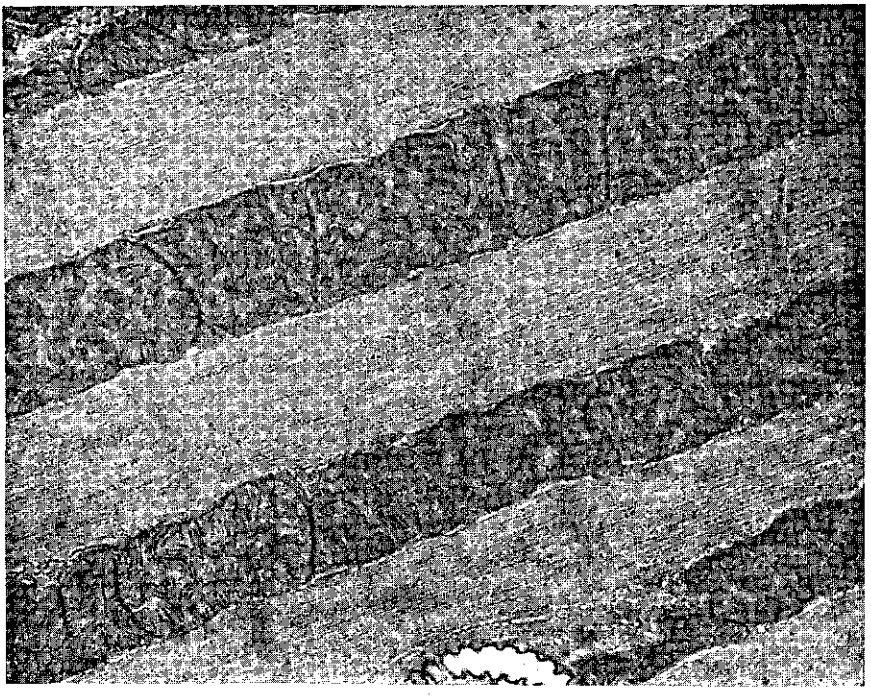


Fig. 1. Sample of fat body mitochondria isolated from 9 days-old female *Locusta*. Somewhat swollen, but not damaged. Note the double membrane. (× 30,000).

Fig. 2. Transverse section through flight muscle of a 12 days-old normal female *Locusta* (× 10,000).

Fig. 3. Transverse section through flight muscle of a 12 days-old allatectomized *Locusta* (× 10,000). Compare the ratio mitochondria : fibrils to that of fig. 2.

PLATE II



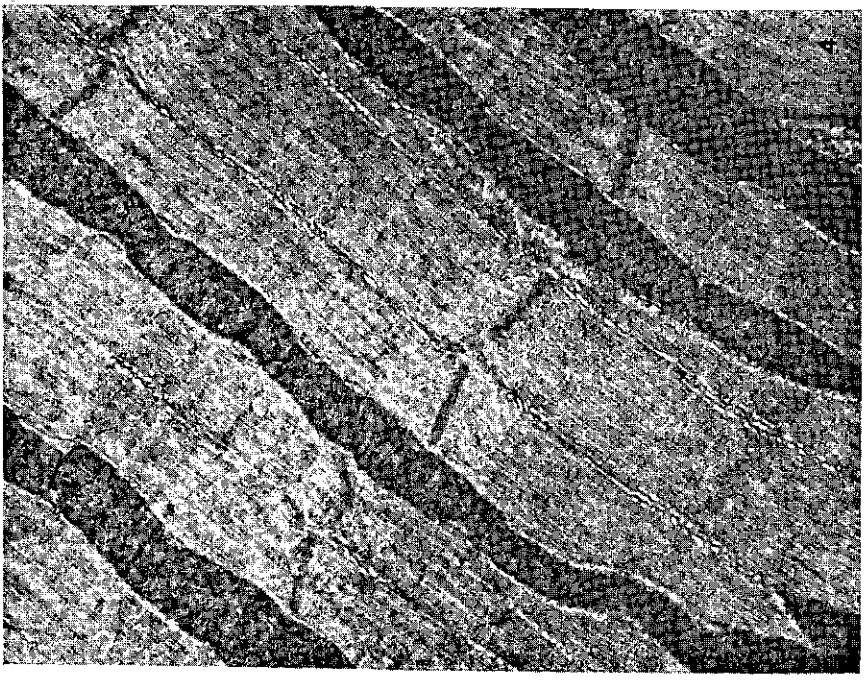


PLATE III

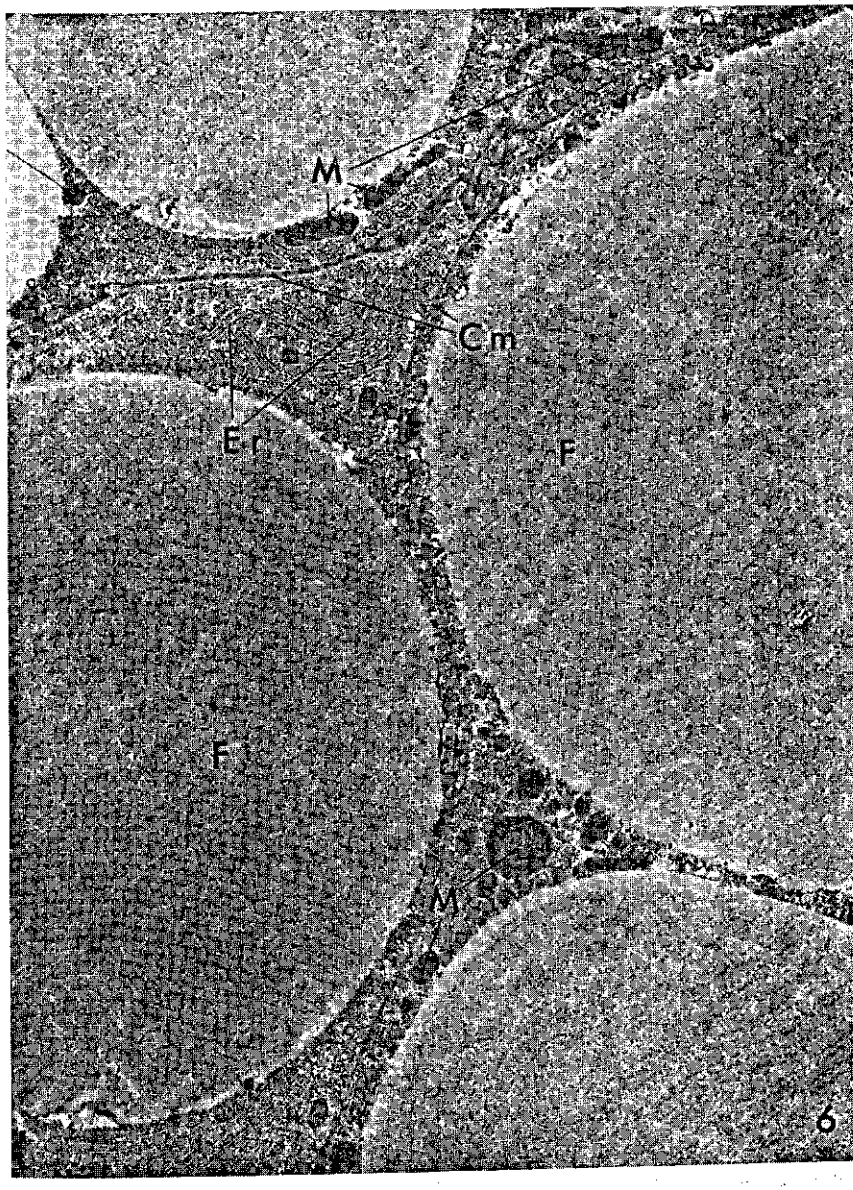


Fig. 4. Longitudinal section through flight muscle of a 12 days-old normal female *Locusta* (× 15,000).

Fig. 5. Longitudinal section through flight muscle of a 12 days-old allatectomized female *Locusta* (× 15,000). Compare the ratio mitochondria: fibrils to that of fig. 4.

Fig. 6. Fat body of a 12 days-old normal female *Locusta* (× 15,000). F = Fat storage; M = Mitochondrion; Cm = Cell membrane; Er = Endoplasmic reticulum; this indicates a very intensive protein synthesis.

PLATE IV

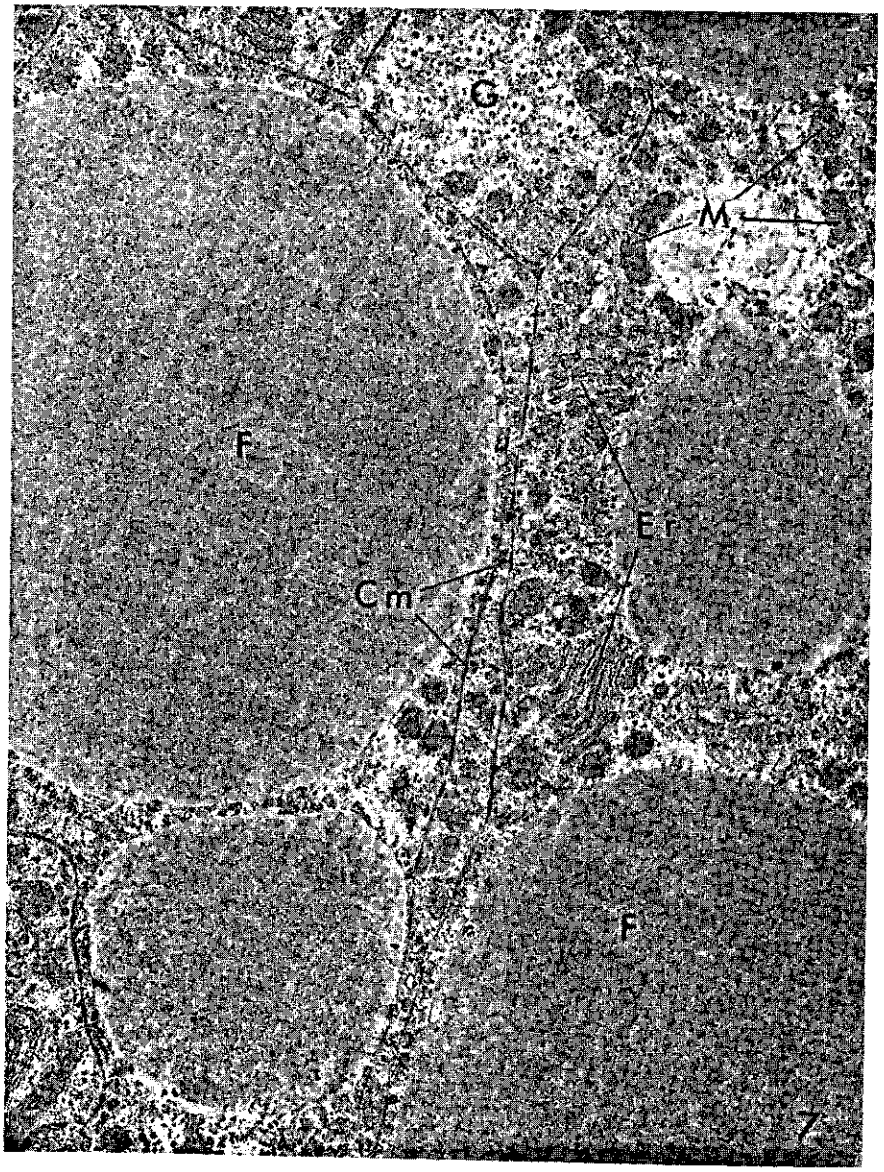


Fig. 7. Fat body of a 12 days-old allatectomized female *Locusta* (× 15,000). Legenda see fig. 6. G = Glycogen. The endoplasmic reticulum is less conspicuous.

REFERENCES

BADE, M. L. and G. R. WYATT, 1961: Metabolic conversions during pupation of the Cecropia silkworm. Biochem. J. **83**, 470–478.

BAILEY, N. T. J., 1959: Statistical methods in biology. The English University Press Ltd, London, pp. 200.

BALBONI, E. R., 1965: Influence of preparative procedure on the oxidative activity of honeybee flight muscle sarcosomes. J. Insect Physiol. **11**, 1559–1572.

BEENAKKERS, A. M. T., 1963: Fatty acid oxidation in insect muscles. Acta physiol. pharm. néerl. **12**, 332–335.

BEENAKKERS, A. M. T., 1964: Vetzuuroxydatie in de vliegspieren van *Locusta migratoria* L. Thesis, Utrecht.

BERGH, S. G. VAN DEN, and E. C. SLATER, 1962: The respiratory activity and permeability of housefly sarcosomes. Biochem. J. **82**, 362–371.

BERREUR, P., 1961: Effects de l'ablation et de l'implantation de l'anneau de Weismann sur l'évolution des acides ribonucléiques au cours de la métamorphose de *Calliphora erythrocephala* Meig. (Diptère). C. R. Acad. Sci., Paris **252**, 4201–4203.

BIRT, L. M., 1961: Flight muscle mitochondria of *Lucilia cuprina* and *Musca domestica*. Biochem. J. **80**, 623–631.

BLOND, D. M. and R. WHITTAM, 1965: Effects of sodium and potassium ions on oxidative phosphorylation in relation to respiratory control by a cell-membrane adenosine triphosphatase. Biochem. J. **97**, 523–531.

BODENSTEIN, D., 1953: Studies on the humoral mechanisms in growth and metamorphosis of the cockroach, *Periplaneta americana*. III. Humoral effects on metabolism. J. exp. Zool. **124**, 105–115.

BODNARYK, R. P. and P. E. MORRISON, 1966: The relationship between nutrition, haemolymph proteins and ovarian development in *Musca domestica* L. J. Insect Physiol. **12**, 963–976.

BOUNHIOL, J. J., 1942: L'ablation des corps allates au dernier âge larvaire n'affecte pas, ultérieurement, la reproduction chez *Bombyx mori*. C. R. Acad. Sci., Paris **215**, 334–336.

BROSEMER, R. W., W. VOGELL and TH. BÜCHER, 1963: Morphologische und enzymatische Muster bei der Entwicklung indirekter Flugmuskeln von Lokusta m. Biochem. Z. **338**, 854–910.

BÜCHER, TH., 1965: Formation of the specific structural and enzymic pattern of the insect flight muscle. In: T. W. Goodwin, Aspects of Insect Biochemistry, 15–28. Acad. Press, London.

CANDY, D. J. and B. A. KILBY, 1961: The biosynthesis of trehalose in the locust fat body. Biochem. J. **78**, 531–536.

CHEFURKA, W., 1965: Some comparative aspects of the metabolism of carbohydrates in insects. Ann. Rev. Entom. **10**, 345–382.

CHEN, D. H., W. E. ROBBINS and R. E. MONROE, 1962: The gonadotropic action of cecropia extracts in allatectomized American cockroaches. Experientia **18**, 577–578.

CHINO, H. and L. I. GILBERT, 1965: Lipid release and transport in insects. Biochim. biophys. Acta **98**, 94–110.

CLARKE, K. U. and R. W. BALDWIN, 1960: The effect of insect hormones and of 2,4-dinitrophenol on the mitochondrion of *Locusta migratoria* L. J. Insect Physiol. **5**, 37–46.

CLARKE, K. U. and G. GILLOT, 1965: Relationship between the removal of the frontal

ganglion and protein starvation in *Locusta migratoria* L. Nature, London **208**, 808–809.

CLARKE, K. U. and P. A. LANGLEY, 1961: Effect of the removal of the frontal ganglion on the development of the gonads in *Locusta migratoria* L. Nature, London **198**, 811–812.

CLARKE, K. U. and P. A. LANGLEY, 1963: Studies on the initiation of growth and moulting in *Locusta migratoria migratorioides* R. and F. III. The role of the frontal ganglion. J. Insect Physiol. **9**, 411–421.

CLELAND, R. W. and E. C. SLATER, 1953: Respiratory granules of heart muscle. Biochem. J. **53**, 547–556.

CLEMENTS, A. N., 1959: Studies on the metabolism of locust fat body. J. exp. Biol. **36**, 665–675.

COCHRAN, D. G., 1963: Respiratory control in cockroach-muscle mitochondria. Biochim. biophys. Acta **78**, 393–403.

COLES, G. C., 1964: Some effets of decapitation on metabolism in *Rhodnius prolixus* Stål. Nature, London **203**, 323.

COLES, G. C., 1965a: The haemolymph and moulting in *Rhodnius prolixus* Stål. J. Insect Physiol. **11**, 1317–1323.

COLES, G. C., 1965b: Haemolymph proteins and yolk formation in *Rhodnius prolixus* Stål. J. exp. Biol. **43**, 425–431.

COLES, G. C., 1965c: Studies on the hormonal control of metabolism in *Rhodnius prolixus* Stål. I. The adult female. J. Insect Physiol. **11**, 1325–1330.

DADD, R. H., 1961: Evidence for humoral regulation of digestive secretion in the beetle, *Tenebrio molitor*. J. exp. Biol. **38**, 259–266.

DALLAM, R. D. and R. B. HOWARD, 1960: Thyroxine-enhanced oxidative phosphorylation of rat-liver mitochondria. Biochim. biophys. Acta **37**, 188–189.

DUDLEY, B. A. C., 1964: The effects of constant and varying conditions on the endocrine system of maturing male desert locusts. Lecture in: Symposium on the endocrine control of metabolism and maturation in insects. Organ. by Dr. D. B. Carlisle. London, 1964.

DUKE, E. J., 1966: Lymph protein patterns in the development of *Locusta migratoria migratorioides*. Comp. Biochem. Physiol. **17**, 661–664.

EL-IBRASHI, M. T., 1965: A comparative study of metabolic effects of the corpus allatum in two adult Coleoptera, in relation to diapause. Meded. Landbouwhogeschool, Wageningen **65** (11), 1–65.

ENGELMANN, F., 1957: Die Steuerung der Ovarfunktion bei der ovoviviparen Schabe *Leucophaea maderae* (Fabr.). J. Insect Physiol. **1**, 257–278.

ENGELMANN, F., 1965: The mode of regulation of the corpus allatum in adult insects. Archs Anat. microsc. **54**, 387–404.

ENGELMANN, F. and M. LÜSCHER, 1956: Die hemmende Wirkung des Gehirns auf die Corpora allata bei *Leucophaea maderae* (Orthoptera). Verh. dtsch. zool. Ges. 1956, 215–220.

FAULKNER, P. and B. BHEEMESWAR, 1960: Studies on the biosynthesis of proteins in the silkworm *Bombyx mori* L. Biochem. J. **76**, 71–78.

FISKE, C. H. and Y. SUBBAROW, 1925: The colorimetric determination of phosphorus. J. biol. Chem. **66**, 375–400.

GILBERT, L. I. and H. A. SCHNEIDERMAN, 1960: The development of a bioassay for the juvenile hormone of insects. Trans. Amer. micr. Soc. **79**, 38–67.

GIRARDIE, A., 1962: Fonctions de la pars intercerebralis chez *Locusta migratoria* L. C. R. Acad. Sci., Paris **254**, 2669–2670.

GIRARDIE, A., 1964a: Action de la pars intercerebralis sur le développement de *Locusta migratoria* L. J. Insect Physiol. **10**, 599–609.

GIRARDIE, A., 1964b: Fonction gonadotrope de la pars intercerebralis chez le mâle de *Locusta migratoria* L. (Orthoptère). C. R. Acad. Sci., Paris 258, 2910–2911.

GIRARDIE, A., 1966: Contribution à l'étude du contrôle du métabolisme de l'eau chez *Gryllus bimaculatus* (Orthoptère). Fonction diurétique de la pars intercerebralis. C. R. Acad. Sci., Paris 262, 1361–1364.

GREGG, C. T., J. R. JOHNSON, C. R. HEISLER and L. F. REMMERT, 1964: Inhibition of oxidative phosphorylation and related reactions in insect mitochondria. Biochim. biophys. Acta 82, 343–349.

GROS, F., J. M. DUBERT, A. TISSIÈRES, S. BOURGEOIS, a.o. 1963: Regulation of metabolic breakdown and synthesis of messenger RNA in bacteria. Cold Spr. Harb. Symp. quant. Biol. 28, 299–313.

GUSTAFSSON, R., J. R. TATA, O. LINDBERG and L. ERNSTER, 1965: The relationship between the structure and activity of rat skeletal muscle mitochondria after thyroidectomy and thyroid hormone treatment. J. Cell Biol. 26, 555-578.

HESLOP, J. P. and J. W. RAY, 1963: The metabolism of glycerol 1-phosphate in resistent and susceptible houseflies (*Musca domestica* L.) and the effect of dieldrin. Biochem. J. 87, 31–34.

HIGHNAM, K. C., 1961: The histology of the neurosecretory system of the adult female desert locust, *Schistocerca gregaria*. Quart. J. micr. Sci. 102, 27–38.

HIGHNAM, K. C., 1962a: Neurosecretory control of ovarian development in *Schistocerca gregaria*. Quart. J. micr. Sci. 103, 57–72.

HIGHNAM, K. C., 1962b: Neurosecretory control of ovarian development in the desert locust. In: H. Heller and R. B. Clark, Neurosecretion, 379–390. Acad. Press, London.

HIGHNAM, K. C. and P. T. HASKELL, 1964: The endocrine systems of isolated and crowded *Locusta* and *Schistocerca* in relation to oöcyte growth, and the effects of flying upon maturation. J. Insect Physiol. 10, 849–864.

HIGHNAM, K. C., L. HILL and D. J. GINGELL, 1965: Neurosecretion and water balance in the male desert locust (*Schistocerca gregaria*). J. Zool. 147, 201–215.

HIGHNAM, K. C., L. HILL and W. MORDUE, 1966: The endocrine system and oöcyte growth in *Schistocerca* in relation to starvation and frontal ganglionectomy. J. Insect Physiol. 12, 977–994.

HIGHNAM, K. C. and O. LUSIS, 1962: The influence of mature males on the neurosecretory control of ovarian development in the desert locust. Quart. J. micr. Sci. 103, 73–83.

HIGHNAM, K. C., O. LUSIS and L. HILL, 1963: The role of the corpora allata during oöcyte growth in the desert locust, *Schistocerca gregaria* Forsk. J. Insect Physiol. 9, 587–596.

HILL, L., 1962: Neurosecretory control of haemolymph protein concentration during ovarian development in the desert locust. J. Insect Physiol. 8, 609–619.

HILL, L., 1965: The incorporation of C^{14}-glycine into the proteins of the fat body of the desert locust during ovarian development. J. Insect Physiol. 11, 1605–1615.

HILL, L., W. MORDUE and K. C. HIGHNAM., 1966. The endocrine system, frontal ganglion and feeding during maturation in the female desert locust. J. Insect Physiol. 12, 1197–1208.

HOUSE, H. L., 1961: Insect nutrition. Ann. Rev. Entom. 6, 13–26.

JOLY, L., 1960: Fonctions des corpora allata chez *Locusta migratoria* (L.). Thèse, Strasbourg, pp. 103.

JOLY, P., 1965: Réactions de *Locusta migratoria* aux substances juvénilisantes. Gen. comp. Endocr. 5, 689–690.

KARLSON, P., 1963: New concepts on the mode of actions of hormones. Perspectives Biol. Med. 6, 203–214.

KARLSON, P. and A. SCHULZ-ENDERS, 1963: Über die Wirkung des Thyroxins auf die oxydative Phosphorylierung in Insektenmitochondrien. Gen. comp. Endocr. **3**, 111–119.

KENNEDY, J. S., 1961: Continuous polymorphism in Locusts. Symposium on Insect Polymorphism, 80–90, London.

KILBY, B. A., 1963: The biochemistry of the insect fat body. Advanc. Insect Physiol. **1**, 111-174.

KLINGENBERG, M. and TH. BÜCHER., 1959: Flugmuskelmitochondrien aus *Locusta migratoria* mit Atmungskontrolle. Biochem. Z. **331**, 312–333.

KOCH, H. J. A., E. BERGSTRÖM and J. C. EVANS., 1964: The microelectrophoretic separation on starch gel of the haemoglobins of *Salmo salar* L. Meded. Vlaamsche Acad. Kl. Wet. **26**, 3-32.

KRISHNAKUMARAN, A. and H. A. SCHNEIDERMAN, 1964: Developmental capacities of the cells of an adult moth. J. exp. Zool. **157**, 293–306.

KROEGER, H., 1963: Chemical nature of the system controlling gene activities in insect cells. Nature, London **200**, 1234–1235.

KROEGER, H. and M. LEZZI, 1965: Regulation of gene action in insect development. Ann. Rev. Entom. **11**, 1–22.

KROON, A. M., 1964: Protein synthesis in mitochondria. II. A comparison of mitochondria from liver and heart with special reference to the role of oxidative phosphorylation. Biochim. biophys. Acta **91**, 145–154.

KROON, A. M., 1965: Protein synthesis in mitochondria. III. On the effects of inhibitors on the incorporation of amino acids into protein by intact mitochondria and digitonin fractions. Biochim. biophys. Acta **108**, 275–284.

KROON, A. M., 1966: Protein synthesis in mitochondria. Thesis, Amsterdam, pp. 48.

LAUFER, H., 1960: Blood proteins in insect development. Ann. N.Y. Acad. Sci. **89**, 490–515.

LAUFER, H., 1963: Antigens in insect development. Ann. N.Y. Acad. Sci. **103**, 1137–1154.

LEA, A. O. and E. THOMSEN, 1962: Cycles in the synthetic activity of the medial neurosecretory cells of *Calliphora erythrocephala* and their regulation. In: H. Heller and R. B. Clarke, Third Symposium on Neurosecretion. Mem. Soc. Endocr. **12**, 345–347,

LEHNINGER, A. L. and C. L. WADKINS, 1962: Oxidative phosphorylation. Ann. Rev. Biochem. **31**, 47–78.

L'HÉLIAS, C., 1953: Étude comparée de l'azote total et de l'azote non protéinique chez le phasme *Dixippus morosus* après ablation des corpora allata. C. R. Acad. Sci., Paris **236**, 2489–2491.

L'HÉLIAS, C., 1957: Action du complexe rétrocérébral sur le metabolisme chez le phasme *Carausius morosus*. Bull. Biol. suppl. **44**, 1–96.

LINDBERG. O., 1965: Morphogenetische und metabolische Wirkungen des Hormons der Schilddrüse. Naturwissenschaften **52**, 379–388.

LINNANE, A. W. and E. B. TITCHENER, 1960: Studies on the mechanism of oxidative phosphorylation. VI. A factor for coupled oxidation in the electron transport particle. Biochim. biophys. Acta **39**, 469–478.

LIU, T. P. and S. E. DIXON, 1965: Studies in the mode of action of royal jelly in honeybee development. VI. Haemolymph protein changes during caste development. Canad. J. Zool. **43**, 873–879.

LOHER, W., 1960: The chemical acceleration of the maturation process and its hormonal control in the male of the desert locust. Proc. R. Soc. (B) **153**, 380–397.

LOHER, W., 1965: Hormonale Kontrolle der Oocytenentwicklung bei der Heuschrecke *Gomphocerus rufus* L. (Acridinae). Zool. Jb., Physiol. **71**, 677–684.

LOUGHTON, B. G. and A. S. West, 1965: The development and distribution of haemolymph proteins in Lepidoptera. J. Insect Physiol. **11**, 919–932.

LOWRY, O. H., N. J. ROSEBROUGH, A. L. FARR and R. J. RANDALL, 1951: Protein measurement with the Folin phenol reagent. J. biol. Chem. **193**, 265–275.

LÜSCHER, M. and R. LEUTHOLD, 1965: Über die hormonale Beeinflussing des respiratorischen Stoffwechsels bei der Schabe *Leucophaea maderae* (F.). Rev. suisse Zool. **72**, 618–623.

MCGORMICK, F. W. and A. SCOTT, 1966: A protein fraction in locust hemolymph associated with the moulting cycle. Experientia **22**, 228–229.

MENON, M., 1963: Endocrine influences on protein and fat in the hemolymph of the cockroach, *Periplaneta americana*. Proc. XVIth Intern. Congr. Zool. **1**, 297.

MICHEJDA, J., 1964: Physiology and structure of flight-muscle sarcosomes in silkworm, *Hyalophora cecropia* L. Bull. Soc. Amis Sci. Lett. Poznan, (D) **4**, 62–102.

MILLS, R. R., F. C. GREENSLADE, and E. F. COUGH, 1966: Studies on vitellogenesis in the American cockroach. J. Insect Physiol. **12**, 767–779.

MINKS, A. K., 1965: Hemolymph protein and amino-acid composition as influenced by the corpus allatum in *Locusta migratoria migratorioides* (R. and F.). Proc. Kon. Ned. Akad. (C) **68**, 320–323.

MOKRASCH, L. C., 1954: Analysis of hexose phosphates and sugar mixtures with the anthrone reagent. J. biol. Chem. **208**, 55–59.

MONOD, J., J. P. CHANGEUX and F. JACOB, 1963: Allosteric proteins and cellular control systems. J. mol. Biol. **6**, 306–329.

MOORE, S. and W. H. STEIN, 1948: Photometric ninhydrin method for use in the chromatography of amino acids. J. biol. Chem. **176**, 367–388.

MORDUE, W., 1965a: The neuro-endocrine control of oöcyte development in *Tenebrio molitor* L. J. Insect Physiol. **11**, 505–511.

MORDUE, W., 1965b: Neuro-endocrine factors in the control of oöcyte production in *Tenebrio molitor* L. J. Insect Physiol. **11**, 617–629.

NABERT, A., 1913: Die Corpora allata der Insekten. Z. wiss. Zool. **104**, 197–359.

NEUGEBAUER, W., 1961: Wirkungen der Exstirpation und Transplantation der corpora allata auf den Sauerstoff verbrauch, die Eibildung und den Fettkörper von *Carausius* (*Dixippus*) *morosus* Br. et Redt. Arch. Entw. Mech. Org. **153**, 314–352.

NORRIS, M. J., 1964: Accelerating and inhibiting effects of crowding on sexual maturation in two species of Locusts. Nature, London **203**, 784–785.

NOVÁK, V. J. A., 1965: The question of evolution of the juvenile hormone. Acta Ent. bohemoslov. **62**, 165–170.

NOVÁK, V. J. A. and K. SLÁMA, 1962: The influence of juvenile hormone on the oxigen consumption of the last larval instar of *Pyrrhocoris apterus* L. J. Insect Physiol. **8**, 145–153.

NOVÁK, V. J. A., K. SLÁMA and K. WENIG, 1959: Influence of implantation of corpus allatum on the oxygen consumption of *Pyrrhocoris apterus*. Acta Symp. Evol. Insects. Prague, 147–151.

ODHIAMBO, T. R., 1965: Metabolic effects of the corpus allatum hormone in the desert locust, *Schistocerca gregaria*. Nature, London **207**, 1314–1315.

ODHIAMBO, T. R., 1966: Growth and hormonal control of sexual maturation in the male desert locust *Schistocerca gregaria*. Trans. R. ent. Soc. Lond. **118**, 393–412.

ORR, C. W. M., 1964a: The influence of nutritional and hormonal factors on egg development in the blowfly *Phormia regina* (Meig.). J. Insect. Physiol. **10**, 53–64.

ORR, C. W. M., 1964b: The influence of nutritional and hormonal factors on the chemistry of the fat body, blood and ovaries of the blowfly *Phormia regina* Meig. J. Insect Physiol. **10**, 103–119.

PFLUGFELDER, O., 1937: Entwicklung und Funktion der Corpora allata und cardiaca von *Dixippus morosus* Br. Z. wiss. Zool. **149**, 477–512.

PFLUGFELDER, O., 1952: Entwicklungsphysiologie der Insekten. Akad. Verl. Gesell. Geest en Portig, Leipzig, pp. 332.

PFLUGFELDER, O., 1958: Entwicklungsphysiologie der Insekten. Ed. 2. Akad. Verl. Gesell. Geest en Portig, Leipzig, pp. 490.

POSSOMPÈS, B., 1956: Corpora allata, croissance post-embryonnaire et ovogénèse chez *Sipyloidea sipylus* W. (Phasmoptère). Trans. 10th Int. Congr. Ent. **2**, 267–268.

PRESSMAN, B. C. and H. A. LARDY, 1955: Further studies on the potassium requirements of mitochondria. Biochim. biophys. Acta **18**, 482–487.

PRICE, G. M., 1966: The *in vitro* incorporation of [U-^{14}C] valine into fat body protein of the larva of the blowfly, *Calliphora erythrocephala*. J. Insect Physiol. **12**, 731–740.

PRICE, G. M. and T. BOSMAN, 1966: The electrophoretic separation of proteins isolated from the larva of the blowfly, *Calliphora erythrocephala*. J. Insect Physiol. **12**, 741–745.

PULLMAN, M. E., H. S. PENEFSKY, A. DATTA and E. RACKER, 1960: Partial resolution of the enzymes catalyzing oxidative phosphorylation I. Purification and properties of soluble dinitrophenol-stimulated adenosine triphosphatase. J. biol. Chem. **235**, 3322–3329.

RACKER, E., 1961: Mechanisms of synthesis of adenosine triphosphate. Advanc. Enzym. **23**, 323–399.

RACKER, E., 1962: Studies of factors involved in oxidative phosphorylation. Proc. natn. Acad. Sci. U.S.A. **48**, 1659–1670.

RALPH, C. L. and R. J. MATTA, 1965: Evidence for hormonal effects on metabolism of cockroach from studies of tissue homogenates. J. Insect Physiol. **11**, 983–991.

RAVEN, C. P., 1961: Oogenesis: the storage of developmental information. Pergamon Press, London, pp. 274.

ROCKSTEIN, M. and K. F. BRANDT, 1963: Enzyme changes in flight muscle correlated with aging and flight ability in the male housefly. Science **139**, 1049.

RÖLLER, H., 1962: Über den Einfluss der Corpora allata auf den Stoffwechsel der Wachsmotte. Naturwissenschaften **49**, 524.

RÖLLER, H. and J. S. BJERKE, 1965: Purification and isolation of juvenile hormone and its action in Lepidopteran larvae. Life Sci. **4**, 1617–1624.

RÖLLER, H., J. S. BJERKE and W. H. MCSHAN, 1965: The juvenile hormone I. Methods of purification and isolation. J. Insect Physiol. **11**, 1185–1197.

ROODYN, D. B., 1962: Protein synthesis in mitochondria. 3. The controlled disruption and subfractionation of mitochondria labelled *in vitro* with radioactive valine. Biochem. J. **85**, 177–189.

ROODYN, D. B., P. J. REIS and T. S. WORK, 1961: Protein synthesis in mitochondria. Requirements for the incorporation of radioactive amino acids into mitochondrial protein. Biochem. J. **80**, 9–21.

ROTH, T. F. and K. R. PORTER, 1964: Yolk protein uptake in the oöcyte of the mosquito *Aedes aegypti* L. J. Cell Biol. **20**, 313–332.

ROUSSEL, J. P., 1963a: Étude de la consommation d'oxygène chez *Locusta migratoria*. L. J. Insect Physiol. **9**, 349–361.

ROUSSEL, J. P., 1963b: Consommation d'oxygène après ablation des corpora allata chez des femelles adultes de *Locusta migratoria* L. J. Insect Physiol. **9**, 721–725.

SACKTOR, B., 1953: Investigations on the mitochondria of the housefly, *Musca domestica*. L. I. Adenosine triphosphatases. J. gen. Physiol. **36**, 371–387.

SACKTOR, B., 1961: The role of mitochondria in respiratory metabolism of flight muscle. Ann. Rev. Entom. **6**, 103–130.

SACKTOR, B., 1965: Energetics and respiratory metabolism of muscular contraction. In: M. Rockstein, The Physiology of Insecta **2**, 483–580. Acad. Press, New York.

SÄGESSER, H., 1960: Über die Wirkung der Corpora allata auf den Sauerstoffverbrauch bei der Schabe *Leucophaea maderae* (F.). J. Insect Physiol. **5**, 264–285.

SAINI, R. S., 1966: Neuro-endocrine control of oöcyte development in the beetle *Aulacophora foveicollis* Luc. J. Insect Physiol. **12**, 1003–1008.

SAMUELS, A., 1956: The effect of sex and allatectomy on the oxygen consumption of the thoracic musculature of the insect, *Leucophaea maderae*. Biol. Bull., Wood's Hole **110**, 179–183.

SANADI, D. R., T. E. ANDREOLI and K. W. LAM, 1964: An oxidative phosphorylation coupling factor activates reversed electron transfer. Biochem. biophys. Res. Comm. **17**, 582–586.

SCHNEIDER, W. C., 1957: Determination on nucleic acids in tissues by pentose analysis. In: Methods in Enzymology **3**, 680–684. Acad. Press, New York.

SHIGEMATSU, H., 1958: Synthesis of blood protein by the fat body in the silkworm, *Bombyx mori* L. Nature, London **182**, 880–882.

SHIGEMATSU, H., 1960: Protein metabolism in the fat body of the silkworm *Bombyx mori* L. Bull. Sericult. exp. Sta. Tokyo **16**, 141–170.

SIEKEVITZ, P., H. LÖW, L. ERNSTER and O. LINDBERG, 1958: On a possible mechanism of the adenosine triphosphatase of liver mitochondria. Biochim. biophys. Acta **29**, 378–391.

SIEW, Y. C., 1965a: The endocrine control of adult reproductive diapause in the chrysomelid beetle *Galeruca tanaceti* (L.) I. J. Insect Physiol. **11**, 1–10.

SIEW, Y. C., 1965b: The endocrine control of adult reproductive diapause in the chrysomelid beetle *Galeruca tanaceti* (L.) II. J. Insect Physiol. **11**, 463–479.

SIMKIN, J. L. and T. S. WORK, 1957: Protein synthesis in Guinea-pig liver. Incorporation of radioactive amino acids into proteins of the microsome fraction *in vivo*. Biochem. J. **65**, 307–315.

SLÁMA, K., 1964a: Hormonal control of respiratory metabolism during oöcyte growth, reproduction and diapause in female adults of *Pyrrhocoris apterus* L. (Hemiptera). J. Insect Physiol. **10**, 283–303.

SLÁMA, K., 1964b: Hormonal control of haemolymph protein concentration in the adults of *Pyrrhocoris apterus* L. (Hemiptera). J. Insect Physiol. **10**, 773–782.

SLÁMA, K., 1965a: Effects of hormones on growth and respiratory metabolism in the larvae of *Pyrrhocoris apterus* L. (Hemiptera). J. Insect Physiol. **11**, 113–122.

SLÁMA, K., 1965b: The effect of hormone mimetic substances on the ovarian development and oxygen consumption in allatectomized adult females of *Pyrrhocoris apterus* L. J. Insect Physiol. **11**, 1121–1129.

SLÁMA, K., 1965c: Effect of hormones on the respiration of body fragments of adult *Pyrrhocoris apterus* L. (Hemiptera). Nature, London **205**, 416–417.

SLÁMA, K. und H. HRUBEŠOVA, 1963: Übereinstimmung in der Einwirkung von larvalen und imaginalen Corpora allata auf den Respirationsmetabolismus und die Reproduktion bei *Pyrrhocoris apterus* L. – Weibchen. Zool. Jb., Physiol. **70**, 291–300.

STAAL, G. B., 1961: Studies on the physiology of phase induction in *Locusta migratoria migratorioides* R. en F. Thesis, Wageningen, pp. 125. Also in: Publ. Fonds Landb. Export Bur. 40.

STAAL, G. B. and J. DE WILDE, 1962: Endocrine influences on the development of phase characters in *Locusta*. Colloq. int. Cent. nat. Rech. sci. **114**, 89–105.

STEGWEE, D., 1960: Metabolic effect of a corpus allatum hormone in diapausing *Leptinotarsa decemlineata* Say. Verh. XI Int. Kongr. Entom., Wien (B) **3**, 218–221.

STEGWEE, D., 1964: Respiratory chain metabolism in the Colorado potato beetle II. Respiration and oxidative phosphorylation in "sarcosomes" from diapausing beetles. J. Insect Physiol. **10**, 97–102.

STEGWEE, D., and A. R. VAN KAMMEN-WERTHEIM, 1962: Respiratory chain metabolism in the Colorado potato beetle I. Respiration and oxidative phosphorylation in sarcosomes from active beetles. J. Insect Physiol. **8**, 117–126.

STEGWEE, D., E. C. KIMMEL, J. A. DE BOER and S. HENSTRA, 1963: Hormonal control of reversible degeneration of flight muscle in the Colorado potato beetle *Leptinotarsa decemlineata* Say (Coleoptera). J. Cell Biol. **19**, 519–527.

STRANGWAYS-DIXON, J., 1961: The relationships between nutrition, hormones and reproduction in the blowfly *Calliphora erythrocephala* (Meig.). II. The effect of removing the ovaries, the corpus allatum and the median neurosecretory cells upon selective feeding, and the demonstration of the c. allatum-cycle. J. exp. Biol. **38**, 637–646.

STRONG, L., 1965a: The relationships between the brain, corpora allata, and oöcyte growth in the Central American Locust *Schistocerca* sp. I. The cerebral neurosecretory system, the corpora allata, and oöcyte growth. J. Insect Physiol. **11**, 135–146.

STRONG, L., 1965b: The relationships between the brain, corpora allata, and oöcyte growth in the Central American Locust, *Schistocerca* sp. II. The innervation of the corpora allata, the lateral neurosecretory complex, and oöcyte growth. J. Insect Physiol. **11**, 271–280.

STRONG, L., 1966: Effect of removal of the frontal ganglion on corpus allatum function in *Locusta migratoria migratorioides* R. & F. Nature, London **210**, 330–331.

SUMNER, J. B., 1944: A method for colorimetric determination of phosphorus. Science **100**, 413–414.

TATA, J. R., L. ERNSTER and O. LINDBERG, 1962: Control of basal metabolic rate by thyroid hormones and cellular function. Nature, London **193**, 1058–1060.

TATA, J. R., L. ERNSTER, O. LINDBERG, E. ARRHENIUS a.o. 1963: The action of thyroid hormones at the cell level. Biochem. J. **86**, 408–428.

TELFER, W. H., 1954: Immunological studies of insect metamorphosis. II. The role of a sex-limited blood protein in egg formation by the Cecropia silkworm. J. gen. Physiol. **37**, 539–558.

TELFER, W. H., 1965: The mechanism and control of yolk formation. Ann. Rev. Entom. **10**, 161–184.

TELFER, W. H. and L. D. RUTBERG, 1960: The effects of blood protein depletion on the growth of the oöcytes in the Cecropia moth. Biol. Bull., Wood's Hole **118**, 352–366.

TELFER, W. H. and C. M. WILLIAMS, 1960: The effects of diapause, development and injury on the incorporation of radioactive glycine into the blood proteins of the Cecropia silkworm. J. Insect Physiol. **5**, 61–72.

THOMAS, K. K. and J. L. NATION, 1966: Control of a sex-limited haemolymph protein by corpora allata during ovarian development in *Periplaneta americana* (L.). Biol. Bull., Wood's Hole **130**, 254–264.

THOMSEN, E., 1949: Influence of the corpus allatum on the oxygen consumption of adult *Calliphora erythrocephala* Meig. J. exp. Biol. **26**, 137–149.

THOMSEN, E., 1952: Functional significance of the neurosecretory brain cells and the corpus cardiacum in the female blowfly, *Calliphora erythrocephala* Meig. J. exp. Biol. **29**, 137–172.

THOMSEN, E. and H. HAMBURGER, 1955: Oxygen consumption of castrated females of the blowfly, *Calliphora erythrocephala* Meig. J. exp. Biol. **32**, 692–699.

THOMSEN, E. and I. MØLLER, 1959: Neurosecretion and intestinal proteinase activity in an insect, *Calliphora erythrocephala* Meig. Nature, London **183**, 1401–1402.

THOMSEN, E. and I. MØLLER, 1963: Influence of neurosecretory cells and of the corpus allatum on intestinal protease activity in the adult *Calliphora erythrocephala* Meig. J. exp. Biol. **40**, 301–321.

TIETZ, A., 1961: Fat synthesis in cell-free preparations of the locust fat body. J. Lipid Res. **2**, 182–187.

TIETZ, A., 1962: Fat transport in the locust. J. Lipid Res. **3**, 421–426.

TRUMAN, D. E. S., 1964: The fractionation of proteins from ox-heart mitochondria labelled *in vitro* with radioactive amino acids. Biochem. J. **91**, 59–64.

TRUMAN, D. E. S. and A. KORNER., 1962: Incorporation of amino acids into the protein of isolated mitochondria. Biochem. J. **83**, 588–596.

ULRICH, F., 1963: The inhibition of mitochondrial ATP-ase. The effects of alkali-metal ions. Biochem. J. **88**, 193–206.

VANDERBERG, J. P., 1963: The role of the gonadotropic hormone in the synthesis of protein and RNA in *Rhodnius prolixus* (Hemiptera). Biol. Bull., Wood's Hole **125**, 576–581.

VROMAN, H. E., J. N. KAPLANIS and W. E. ROBBINS., 1965: Effect of allatectomy on lipid biosynthesis and turnover in the female American cockroach, *Periplaneta americana* (L.). J. Insect Physiol. **11**, 897–904.

WANG, S. and S. E. DIXON., 1960: Studies on the transaminase activity of muscle tissue from allatectomized roaches, *Periplaneta americana*. Canad. J. Zool. **38**, 275–283.

WEED PFEIFFER, I. G., 1936: Removal of corpora allata on egg production in the grasshopper, *Melanoplus differentialis*. Proc. Soc. exp. Biol. N.Y. **34**, 883–885.

WEED PFEIFFER, I. G., 1939: Experimental study of the function of the corpora allata in the grasshopper, *Melanoplus differentialis*. J. exp. Zool. **82**, 439–461.

WEED PFEIFFER, I. G., 1945: Effect of the corpora allata on the metabolism of adult female grasshoppers. J. exp. Zool. **99**, 183–233.

WIEME, R. J., 1959: An improved technique of agar-gel electrophoresis on microscope slides. Clinica chim. Acta **4**, 317–321.

WIENS, A. W. and L. I. GILBERT., 1965: Regulation of cockroach fat body metabolism by the corpus cardiacum *in vitro*. Science **150**, 614–616.

WIGGLESWORTH, V. B., 1936: The function of the corpus allatum in the growth and reproduction of *Rhodnius prolixus* Stål (Hemiptera). Quart. J. micr. Sci. **79**, 91–121.

WIGGLESWORTH, V. B., 1961: Some observations on the juvenile hormone effect of farnesol in *Rhodnius prolixus* Stål (Hemiptera). J. Insect Physiol. **7**, 73–78.

WIGGLESWORTH, V. B., 1963: The juvenile hormone effect of farnesol and some related compounds. Quantitative experiments. J. Insect Physiol. **9**, 105–119.

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

WILDE, J. DE, 1964: Reproduction – Endocrine control. In: M. Rockstein, The Physiology of Insecta **1**, 59–90. Acad. Press, New York.

WILDE, J. DE, 1965: Photoperiodic control of endocrines in insects. Archs Anat. microsc. **54**, 547–564.

WILDE, J. DE and J. A. DE BOER., 1961: Physiology of diapause in the adult Colorado beetle II. Diapause as a case of pseudo-allatectomy. J. Insect Physiol. **6**, 152–161.

WILDE, J. DE and D. STEGWEE, 1958: Two major effects of the Corpus allatum in the adult Colorado beetle. (*Leptinotarsa decemlineata* Say). Arch. néerl. Zool. **13**, suppl., 277–289.

WILLIAMS, C. M., 1952: Physiology of insect diapause. IV. The brain and prothoracic glands as an endocrine system in the Cecropia silkworm. Biol. Bull., Wood's Hole **103**, 120–138.

WILLIAMS, C. M., 1956: The juvenile hormone of insects. Nature, London **178** 212–213.

WYATT, G. R. and G. F. KALF, 1957: The chemistry of insect hemolymph. II. Trehalose and other carbohydrates. J. gen. Physiol. **40**, 833–847.