Dit proefschrift met stellingen van

GERARDUS BENARDUS STAAL,

landbouwkundig ingenieur, geboren te Assen, 19 augustus 1925, is goedgekeurd door de promotor, Dr. J. DE WILDE, hoogleraar in het dierkundige deel van de plantenziektenkunde.

De Rector Magnificus der Landbouwhogeschool,
W. F. EIJSVOOGEL

Wageningen, 8 november 1961

STUDIES ON THE PHYSIOLOGY OF PHASE INDUCTION IN *LOCUSTA MIGRATORIA MIGRATORIOIDES* R. & F.

STUDIES ON THE PHYSIOLOGY OF PHASE INDUCTION IN *LOCUSTA MIGRATORIA MIGRATORIOIDES* R. & F.

(MET EEN SAMENVATTING IN HET NEDERLANDS)

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD
VAN DOCTOR IN DE LANDBOUWKUNDE
OP GEZAG VAN DE RECTOR MAGNIFICUS IR. W. F. EIJSVOOGEL,
HOOGLERAAR IN DE HYDRAULICA, DE BEVLOEIING,
DE WEG- EN WATERBOUWKUNDE EN DE BOSBOUWARCHITECTUUR,
TE VERDEDIGEN TEGEN DE BEDENKINGEN
VAN EEN COMMISSIE UIT DE SENAAT
VAN DE LANDBOUWHOGESCHOOL TE WAGENINGEN
OP VRIJDAG 8 DECEMBER 1961 TE 16 UUR

DOOR

G. B. STAAL

H. VEENMAN & ZONEN N.V. - WAGENINGEN - 1961

STELLINGEN

I

De opvatting van KENNEDY dat de solitaire fase van sprinkhanen moet worden beschouwd als meer juveniel dan de gregaire fase is niet geheel in overeenstemming met de feiten.

J. S. Kennedy, Biol. Rev. **31** (1956): 349–370.

II

Het intreden van de metamorfose bij insekten wordt niet bepaald door het aantal doorlopen larvestadia maar door onafhankelijk hiervan voortschrijdende ontwikkelingsprocessen.

III

De overlevingskansen van populaties van treksprinkhanen in sterk fluctuerende biotopen worden beheerst door de mogelijkheid van snelle fenotypische fasentransformaties. Deze worden enigszins gestabiliseerd door matrokliene vererving en op langere termijn voor enkele kenmerken waarschijnlijk ook door genetische veranderingen in de populatie.

IV

In de aantalsregulatie van zwermende treksprinkhanen spelen abiotische factoren een grotere rol dan biotische factoren.

V

Het is onwaarschijnlijk dat het juveniel hormoon van insekten betekenis zal krijgen als bestrijdingsmiddel.

VI

Bij het beoordelen van het effect van bestrijdingsmiddelen ten aanzien van ziekten en plagen van landbouwgewassen dient de fysiologische toestand van de plant in aanmerking te worden genomen.

VII

Micro-organismen worden ten onrechte beschouwd als verwekkers van de takgallen van *Forsythia* × *intermedia*.

W. M. Docters van Leeuwen: Gallenboek 2e druk 1957
J. McLean Thompson: J. R. Hort. Soc. **71** (1946): 166–172.

VIII

Een afdoende bestrijding van de roest *Coleosporium senecionis* in *Cineraria* dient behalve het vermijden van het gebruik van dennestrooisel ook het opruimen van wilde *Senecio* soorten in de naaste omgeving te omvatten.

Tuinbouwgids 1961.

Diss. G. B. Staal
Wageningen, december 1961

IX

In vele gevallen worden organismen die planteziekten of plagen veroorzaken ten onrechte als de primaire factor beschouwd.

X

De bestrijding van wilde rijstsoorten met rode zaadhuid in commerciële rijstaanplantingen kan beter geschieden door het kiezen van rijstvariëteiten met kortere ontwikkelingsduur dan door cultuurmaatregelen.

XI

De belangrijke functie van kamerplanten in de huidige samenleving wettigt een betere voorlichting ten aanzien van de bestrijding van ziekten en plagen binnenshuis.

Aan mijn Moeder en de
nagedachtenis van mijn Vader
Voor Miep en Rein

Dit proefschrift verschijnt tevens als nr. 40 in de reeks publikaties van het Fonds Landbouw Export Bureau 1916–1918 en als mededeling nr. 72 van het Laboratorium voor Entomologie van de Landbouwhogeschool te Wageningen.

VOORWOORD

Bij het afsluiten van mijn academische studie betuig ik gaarne mijn dank aan U, Hoogleraren en Lectoren aan de Landbouwhogeschool voor Uw bijdragen tot mijn wetenschappelijke vorming.

Zeer in het bijzonder richt ik hierbij een woord van dank tot U, Hooggeleerde de Wilde, Hooggeachte Promotor. Uw stimulerende lessen in de insektenfysiologie waren voor mij richtinggevend. Uw leiding, belangstelling en critiek gedurende het onderzoek droegen in hoge mate bij tot vorm en inhoud van dit proefschrift alsmede tot de verdieping en verrijking van mijn kennis.

De leden van de wetenschappelijke staf en collegae van het Laboratorium voor Entomologie dank ik voor de door hen geboden hulp en de belangstelling bij mijn onderzoek getoond.

Veel dank ben ik verschuldigd aan een groot aantal personeelsleden van het laboratorium voor hulp in velerlei vorm. Van hen noem ik hier in het bijzonder de Heer M. P. van der Schelde die snelle en deskundige hulp verleende bij het vervaardigen van tekeningen en grafieken.

Bijzonder verplicht gevoel ik mij aan Prof. Dr. J. H. Freitag en de Heren N. G. Uylenburg en M. Martin die bereid werden gevonden gedeelten van de Engelse tekst te corrigeren, vaak op zeer korte termijn. Mevrouw G. de Wilde-van Buul verleende taalkundige hulp voor de Nederlandse samenvatting.

Mijn erkentelijkheid gaat uit naar de Stichting „Fonds Landbouw Export Bureau 1916–'18" die het onderzoek en de publicatie financieel mogelijk maakte onder een grote mate van vrijheid en die toestond dat de resultaten als proefschrift werden bewerkt.

Dank is ook verschuldigd aan de Nationale Raad voor het Landbouwkundig Onderzoek T.N.O. voor de vrijheid die zij mij heeft gelaten gedurende de laatste fase van voorbereiding van het manuscript.

Miep, jou dank ik voor de vele opofferingen die jij je hebt willen getroosten en voor je actieve hulp bij het gereedmaken van het manuscript.

GLOSSARY OF SOME TERMS AND ABBREVIATIONS

L 1, L 2, etc.	larval instars
CA	corpus allatum, corpora allata
CC	corpus cardiacum, corpora cardiaca
CG	cerebral ganglion, brain
VG	ventral gland(s)
NCA	nervus corporis allati
NCC	nervus corporis cardiaci
JH	juvenile hormone (from CA)
MH	moulting hormone (from VG)
AH	activating hormone (from CG)
Allatectomy	extirpation of CA
D	number of days of development
CA	volume of single or both CA
E	length of right elytron
F	mean length of posterior femora
C	maximum width of the head
V	minimum distance between the eyes
L	hairlength of the sternal hair covering
K_1, K_2	fresh and dry weight respectively of head
KCs_1, KCs_2	fresh and dry weight respectively of total body
F_1, F_2	fresh and dry weight respectively of posterior legs
Ps	surface of the pronotum
E/F, F/C, F/V	phase discriminating ratios
(n)	number of individuals used for calculation of means
prothetely	anticipated occurrence of adult characters
metathetely	delayed occurrence of adult characters

FIGURE 1. The neuro-endocrine system in the head of *Locusta*, semi-diagrammatic lateral view.

FIGURE 2. The neuro-endocrine system in the head of *Locusta*. Semi-diagrammatic dorsal view.

Abbreviations: CG = cerebral ganglion; FG = frontal ganglion; NR = nervus recurrens; NCC = nervus corporis cardiacum; CC = corpus cardiacum; HCG = hypocerebral ganglion; SOG = suboesophageal ganglion; CA = corpus allatum; NCA = nervus corporis allatum; VG = ventral gland; NOE = nervus oesophagealis externus ; OES = oesophagus; NM = neck membrane; MM = mandibular muscles.

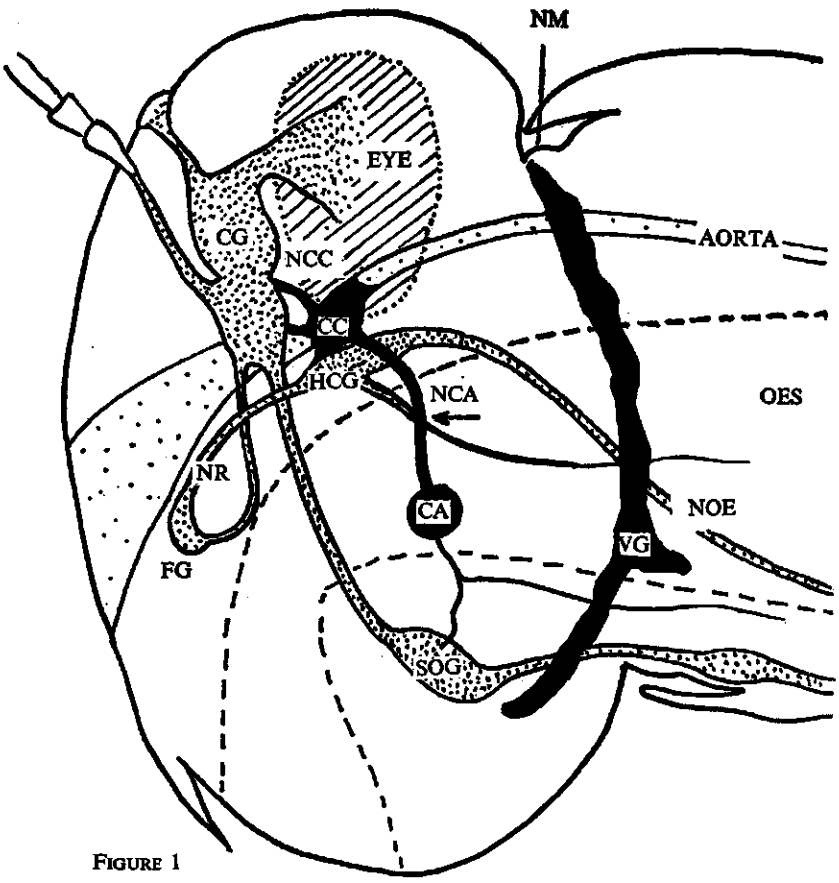


FIGURE 1

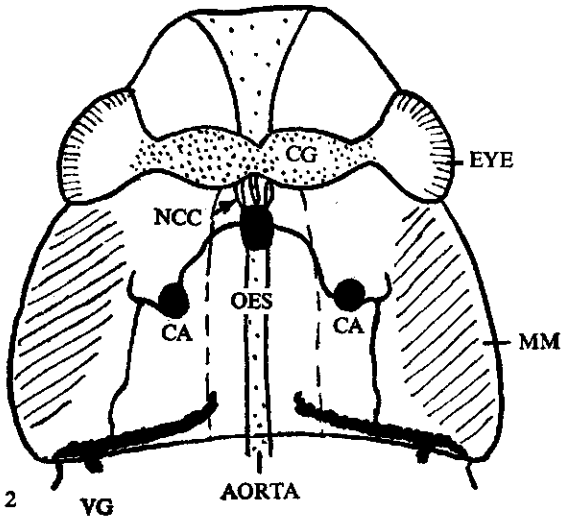


FIGURE 2

Glossary continued

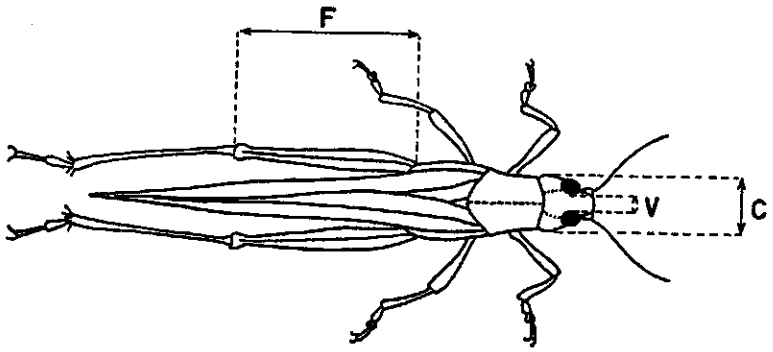


FIGURE 3. Dorsal view on adult locust with some morphometric characters.

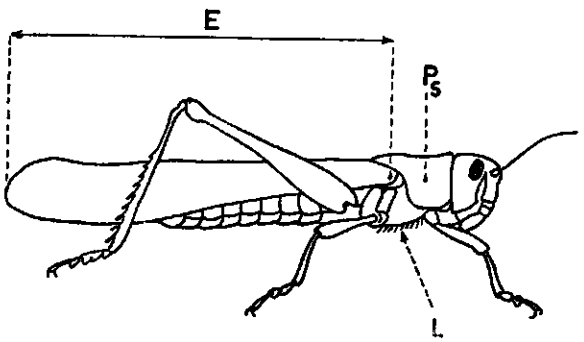


FIGURE 4. Lateral view on adult locust with some morphometric characters. For explanation of abbreviations see glossary.

CONTENTS

1. INTRODUCTION 1

1.1. General 1
1.2. The phase concept 2
1.3. Further analysis of phase phenomena 4
1.3.1. Behaviour 4
1.3.2. Pigmentation 4
1.3.3. Morphometrics 8
1.3.4. Physiological phase differences 11
1.4. The phase inducing mechanisms 12
1.4.1. The corpus allatum 12
1.4.2. The ventral gland 14
1.4.3. Undetermined humoral factors 14
1.5. General endocrine functions probably related to the phase inducing mechanism 14
1.5.1. The corpora allata 14
1.5.2. The ventral gland 15
1.5.3. The activating hormone 16
1.5.4. Myotropic hormones 17
1.5.5. The suboesophageal ganglion 17

2. MATERIAL AND METHODS 17

2.1. Source, breeding and care of experimental animals 17
2.2. Observations, sampling and preservation of experimental animals 19
2.3. Operational techniques 19
2.4. Determination of the volume of corpora allata 22
2.5. Morphometrical determinations 23

3. PHASE BREEDING EXPERIMENTS 25

3.1. The effect of differences in breeding density and humidity throughout larval development on the resulting adults 25
3.2. The effect of a combined difference in density and humidity during the fourth larval instar on the resulting fifth instar larvae 31

4. IMPLANTATION OF EXTRA CORPORA ALLATA IN LARVAE 35

4.1. Implantation of extra CA in second instar larvae (L 2)
4.2. Implantation of extra CA in fifth instar larvae (L 5)

5. ARTIFICIALLY INDUCED CORPUS ALLATUM DEFICIENCY 41

5.1. Total extirpation 41
5.2. Reextirpation of previously extra implanted adult CA in larvae 42
5.3. Total extirpation followed by reimplantation of adult CA at varying numbers of days thereafter 44
5.4. Various operations on the innervation of the CA and CC 47
5.4.1. Experiment 5.4.1 48
5.4.2. Experiment 5.4.2 51
5.4.3. Experiment 5.4.3 57
5.4.4. Experiment 5.4.4 62
5.4.5. Experiment 5.4.5 65

5.5. Comparison of adults and larvae resulting from different treatments in a bivariate phase graph for F and C 68
5.6. Hetero-transplantation of CA 72
5.6.1. Substitution of CA in *Locusta* larvae by CA of *Romalea* 72
5.6.2. Substitution of CA in *Locusta* larvae by CA of *Schistocerca* 73
5.6.3. Substitution of CA in *Schistocerca* larvae 73
5.7. Internal and external administration of extracts of adult male *Hyalophora* silkworms 74

6. THE VENTRAL GLAND 75
6.1. Extirpation 76
6.2. Extra implantation of VG 76
6.2.1. VG implantation in early second instar larvae (L 2) 78
6.2.2. Implantation of extra VG in larvae of varying age and instar 78
6.2.3. Variation of the quantity of VG implanted 79
6.2.4. Hetero-transplantation 79
6.2.5. Implantation of "degenerated" VG of mature adults 79
6.3. Combined implantation of CA and VG 80
6.4. Morphogenetic effects in adults implanted with VG not showing precocious metamorphosis and wing development 81
6.5. Summary of effects of VG implantation 84
6.6. Discussion on the results of VG implantations 85

7. THE CORPUS CARDIACUM 89
7.1. Extirpation 90
7.2. Extra implantation of CC 90

8. GENERAL DISCUSSION 91

SUMMARY AND CONCLUSIONS 97

SAMENVATTING 101

ACKNOWLEDGEMENTS 105

APPENDICES 106

REFERENCES 117

1. INTRODUCTION

1.1. General

Since Uvarov (1921) presented this theory of phenotypical, morphological phase dimorphism for species of the genus *Locusta,* many studies have been devoted to the practical consequences of this theory as well as to the backgrounds of the phenomenon. The theory proved to hold good for many species of plague locusts and has aided a great deal in clearing up the taxonomy of these species.

As is generally recognized now, the phase mechanism did not provide a key for the solution of problems concerning mass multiplication (Key 1950, Kennedy 1956). The conspicuous polymorphism, however, remained a problem of physiological interest, the degree of mutual stimulation as a consequence of population density being recognized as the responsible factor (Plotnikov 1927, Faure 1932).

Uvarov & Zolotarevsky (1929) proposed the terms *phase gregaria* and *phase solitaria* for the extreme morphologic types as they occur in either large migrating swarms or isolated in an abundant vegetation. The term *transiens* was created to describe a type in transition from one phase to another as a result of a change in density, the direction of the change being indicated by the additional suffix *congregans* or *dissocians.*

The terminology is difficult to apply for anything other than the very extremes since the delimitation of the classes always remains arbitrary. It may depend on local conditions, genetical differences between populations and a different degree of quantitative cumulation of a phase transformation trend during some generations (matrocline, non genetical inheritance). Moreover, as the phase syndrome has many – not too closely related – different aspects, the transition does not take place simultaneously for all characters at the same rate. For this reason separate evaluation of characters is often indicated, especially in the laboratory where most aspects of phase transformation can be studied satisfactorily but where the original phase definitions clearly do not apply. Thus the conceptions *solitaria* and *gregaria* could have only a limited application in the laboratory. Perhaps the splitting up into terms describing different aspects is preferable, e.g. the terms solitarispecte, solitaricolore and solitariforme etc. of Joly & Joly (1953).

Phase phenomena include behaviour, pigmentation, morphometric values and a number of probably more fundamental physiological processes expressed in biochemical and morphogenetical differences. The interrelation of characters belonging to the different groups is not known to be direct, all of them being external aspects of little known basic processes. Moreover, the rate of change for the different aspects with density changes is not the same and decreases in the sequence: behaviour – pigmentation – morphometrics.

Since phase transformation affects a number of characters involved in metamorphosis as well as in development, e.g. pigmentation and wing length, the

possibility should be envisaged that both phenomena may have in common a control by neuroendocrine processes (the influence of which on metamorphosis is rather well understood). In the present study further attempts were made to investigate this role of endocrine processes in phase biology by means of experimental methods. In the evaluation of the effects of interferences the use of relative changes of separate characters is stressed rather than the absolute values. Comparisons of absolute values with those obtained by other authors introduces the risk of systematic errors (due, for instance, to genetical population differences, breeding and measuring techniques). The species used for this study, *Locusta migratoria ssp migratorioides* R & F, the African Migratory Locust, is one of the most polymorphic plague locusts. It shows a number of very distinct phase phenomena together with a striking colour adaptation mechanism which is limited to isolated individuals.

1.2. The phase concept

Phase transformation in a population of animals is the result of the action of negative or positive changes in the quantity of group stimuli, exerted directly by one or more congeneres on the individuals concerned. Group stimuli in this sense are always direct stimuli in contrast to the mass effect in which the environment is changed by high population densities, competition for food being one of the final results. Pheromones, which may produce pure group and phase effects but may be transmitted by elements of the habitat indirectly, occupy a special position in this respect (Loher 1958, 1960). Apart from this qualitative difference, group effects may occur with much lower population densities. Some effects may even be observed when only two individuals live together. The typical group effect is a positive influence on the growth rate, resulting in many other phenomena such as a different final weight, a lower mortality, restricted variability in many morphologic characters and a smaller number of instars.

The additional morphologic effects are called *phase phenomena* (Chauvin 1957). Pure group-effects are not rare in insects. Extreme cases are present in the social insects such as bees and termites where isolated individuals soon die in the absence of group stimuli. Group stimuli often induce macropterism in species plastic with respect to this character e.g. *Psocoptera* (Badonnel 1948) and *Aphidae* (Bonnemaison 1949).

Phase dimorphism is by no means restricted to locusts, but in this group the morphologic aspects are the most conspicuous. The division of the family *Acrididae* into locusts and grasshoppers – see Uvarov 1928 – is a purely practical one, the former category comprising the migrating plague locusts, the latter all sedentary species. Thus this division is based on a character which is a part of the phase syndrome and therefore almost, though not entirely, parallels the division in species which do or do not exhibit a phase dimorphism. Most grasshopper species are monomorphic, but a number of them exhibit a definite colour polymorphism independent of phase status. Rubtzov (1935)

describes some grasshopper species in which an incomplete phase-syndrome is found involving some melanisation and changes in morphometric ratios as a result of crowding.

The most destructive locust species show a definite dimorphism. Under high density conditions a very uniform "gregarious" pigmentation (with great interspecific similarity) appears during the larval development. This pattern may be called *aposematic* and always consists of black pigmented areas and contrasting red, orange or yellow areas. In isolation the individuals of these species (e.g. *Locusta migratoria, Schistocerca gregaria, Nomadacris septemfasciata, Locustana pardalina*) acquire a more or less cryptic uniform green colour with the restriction that in *Locusta* and *Locustana,* on isolation and under dry conditions, a very distinct homochromical colour adapation occurs except for the green colour. This colour adaptation also occurs in a number of grasshopper species e.g. *Acrida turrita, Oedalus decorus, Tylopsis liliifolia.* (ERGENE 1955, 1956) and thus may be considered as a phenomenon independent of phase status.

A dimorphism, more correctly indicated as a dichroism, which results in a green and a brown form, is rather common in *Orthoptera,* e.g. in *Mantidae, Phasmidae* and some *Acrididae* as *Anacridium aegyptium.*

In the plague locust *Dociostaurus maroccanus* a dualism in behaviour is accompanied only by indistinct morphological changes (JANNONE 1938). Probably the same applies for *Melanoplus spp* in the New World. The rather brachypterous grasshopper *Zonocerus variegatus* shows true gregarious behaviour as to interattraction and short range migrations, a uniform aposematic colour pattern and even some plasticity in wing length (VUILLAUME 1954, 1955). Since in this species no density dependent changes are noted, it has no phases. Nor is it a true plague locust, as flying swarms do not occur.

KEY (1957) described a pigmentation dimorphism and accompanying morphometric changes typically resembling those in locusts in three Australian species of *Phasmidae.* This density dependent dimorphism did not include any appreciable changes in behaviour under the low activity levels exhibited by these species.

Finally group and phase effects, most often including some degree of diffuse melanisation as a result of crowding have been described for a large number of insects belonging to different families.

Caste-differentiation in bees, ants and termites is another type of polymorphism, partly depending also on very specific group-stimuli.

Apparently the different phase- and group phenomena are rather independent, at least phylogenetically. To what extent this applies too for the ontogeny of phase characters in individuals needs further investigation. This may be carried out most fruitfully in species exhibiting a well developed phase syndrome. In the present study the phenomena in locusts are placed at the centre of interest. Evidence from other sources will occasionally be adduced for reasons of comparison.

1.3. Further analysis of phase phenomena

1.3.1. *Behaviour*

Changes in behaviour are the first to be observed in the process of gregarisation in *Locusta* and *Schistocerca*. They may be evaluated by the degree of social interattraction, which can be measured objectively (Ellis 1953, 1956, 1958, 1959). A few hours of crowding suffice for a complete interattraction behaviour, the reverse taking place much more slowly. This interattraction is a feature of locusts but it is also present in some monomorphic grasshoppers as *Zonocerus*. The interattraction behaviour may be acquired by social contact with non-gregarious grasshoppers. Analysis brought to light that the learning process must take place through frequent mechanical contact with small, even unanimated, objects and not by activity only. For some days after hatching the degree of interattraction is correlated with parental density, a little understood hereditary but non-genetic transfer of phase status obviously taking place. The amount of black pigment and the morphometric characters show a similar transfer as well. Marching behaviour is also more developed in crowded individuals though not fundamentally absent under isolated conditions. Coordination of marching movements into hopper bands is dependent on visual contact. (Ellis 1953).

1.3.2. *Pigmentation*

Pigmentation is certainly one of the most striking phase characters in locusts because of the completeness of the pigment change within the rather short time of one of two larval instars. Intermediate types, in transition from one phase to another, may be referred to as *phase transiens*. It should be clearly understood that non-transient intermediates can also be produced in the laboratory under suitable conditions of intermediate density (Gunn & Hunter Jones 1952, own observations). This is not without importance, for from this it follows that a colour change in either direction is the result of a quantitative control by one or more basic processes.

The number of pigments involved is limited, each of them is restricted to definite parts and layers of the integument, the blood and, for some pigments, other tissues as well. The pigments of *Locusta* and *Schistocerca* were studied in detail by Goodwin (1949, 1950) and Goodwin & Srisukh (1949—1951) and reviewed by Goodwin (1952).

a) melanin occurs only in the exocuticle and is responsible for the black pattern. The brownish colour in certain areas of the cuticle of *Locusta* larvae is ascribed to tanned proteins (scleroproteins) responsible too for the hardening of the cuticle.

b) ommochromes, associated with brownish and orange colours are present in the epidermis as intracellular granules.

c) mesobiliverdine as the blue bile pigment, responsible for the green colour when combined with yellow carotene in a number of insects, may invade the epidermis and the endocuticle in *Locusta* and a number of other insects.

d) carotenes, present in the blood and sometimes also in the epidermis and the endocuticle.

Many of these pigments form compounds with proteins (chromoproteids). The pigments mentioned above all play a role in the phase transformation of locusts. In addition to these another group of pigments, the pterines, although present in *Locusta* (GOODWIN & SRISUKH 1951) play only a minor role in the colour pattern and probably do not vary according to the phase status. They are very interesting, however, in another respect, viz. the supposed antagonism to the synthesis of black melanin. Both pigments appear to exclude each other locally (FUZEAU-BRAESCH 1960). Whether this antagonism plays a role in pattern formation in general remains open to speculation (THOMSON 1960).

The attaining of an aposematic colour pattern most often entails an intensifying and extension of melanin pigmented areas. The statement that gregarisation is invariably accompanied by an increase in melanin pigment obviously is not true with respect to the cryptic, very dark, colour type of isolated *Locusta* hoppers on a black background. According to FUZEAU BRAESCH the seeming decrease of black melanin pigments in *Gryllus bimaculatus* on gregarisation turns out to be a substitution by a yellow melanin pigment (not occuring in *Acrididae*. This author reviewed the whole subject of cuticular and hypodermal insect pigments and their genesis.

Ommochrome pigments in the hypodermis of larvae often accompany the melanin in the adjacent exocuticle. Thus they too may show quantitative differences dependent on phase status. This fact was used by CHAUVIN (1943) in developing a quantitative estimation of phase status in *Schistocerca gregaria* by determining the amount of the ommochrome pigment *acridioxanthine* (insectorubine). This method did not come into general use because of difficulties in standardisation (the pigment decomposes rapidly) and apparent variability in the quantity per individual. Presumably ommochromes contribute much to the cryptic colours as well.

Melanin cannot be analysed quantitatively or qualitatively, this pigment being a chemically very stable polymer bound to protein and evenly divided in the chitin skeleton in contrast to the granulate form present in the hairs and the skin of vertebrates. In the case of *Gryllus,* FUZEAU BRAESCH (1960) was able to prove by means of radioactive labelled tyrosine and tryptophane that the black and yellow cuticular melanins in this species are derived from tyrosine exclusively, the differentiation occurring at the hypodermal level by monosubstitutions at the N atom.

Before melanin synthesis takes place, another tyrosine fraction is generally used in the sclerotizing (tanning) process of the cuticular proteins leading to the hardening of the cuticle (PRYOR 1940). Whether or not the tanning is accompanied by the appearance of brownish colour is not known with certainty, for it may be either the colour of the sclerified protein or a dispersed melanin pigment.

Definite proof is difficult to produce because of the inert nature of both com-

pounds. Hardening without brownish colours occurs in *Schistocerca* (MALEK 1957). Tryptophane derivates were found in the cuticle also, but no definite pattern-bound distribution could be detected. FUZEAU BRAESCH (1960) advances arguments for the formation of a melanin intermediary (presumably DOPA) in the epidermis, its formation being probably more or less bound to the ommochrome redox system (the melanin formation is inhibited by some reducing compounds). The presence of pteridines (yellowish pigments) locally excludes the formation of black melanin also in areas were ommochromes are present at the same time. The formation of yellow melanin, however, is not interfered with. Other possibilities for pattern formation are:

1. a localized distribution of the chromogen,
2. a localized distribution of the enzymes involved.

The first possibility can exist only at the epidermal level, as the blood tyrosine content affords a constant surplus of the chromogen.
The second possibility can be excluded as well, for *in vitro* experiments have shown a permanent enzyme activity in the entire cuticle.
Larval melanin patterns are most often changed and reduced considerably in the final moult, often in favour of ommochrome pigments, which for instance in the immature adults and mature females.
The green pigment in insect blood is identified as a combination of the blue bile pigment mesobiliverdin and yellow carotenes. According to GOODWIN & SRISUKH (1949) the yellow component is β-carotene in the blood; in the integument astaxanthine, a reddish carotene probably derived from β-carotene, is also involved. The carotenes are all derived from the food but they may be changed chemically within the body.
The origin of the mesobiliverdin is still obscure (THOMSON 1960). OKAY (1953) proved the pigment not to be a breakdown product of chlorophyll or haemoglobin. Green blood colour may be seen externally only when the integument is sufficiently opaque. The appearance of green colour in *Locusta* as well as in *Schistocerca* as a result of isolation is accompanied by a gradual decrease in the amount of other cuticular and integument pigments, particularly melanin (JOLY & JOLY 1953 for *Locusta*, STOWER 1959 for *Schistocerca*). This would suggest a direct antagonism between green and other pigments, but evidence against any direct relation or antagonism between green pigment and melanin pigment is found in the fact that both pigments may be present side by side indefinitely under intermediate density conditions.
The conditions favouring the green colour in di- or polymorphic *Orthoptera* species are not always the same. The observed possibilities are:

a) green background colour (c.f. vegetation) in *Acrida turrita* (ERGENE 1955).

b) combination of certain conditions of light, humidity and temperature: in *Mantidae* (JOVANÇIC 1953) and *Phasmidae* (GIERSBERG 1928)

c) absence of gregarious stimuli in *Schistocerca gregaria* (CHAUVIN 1941, STOWER 1959).

d) absence of gregarious stimuli combined with high humidity in *Locusta migratoria* (FAURE 1932)

e) genetical determination leading to races with very well defined partly green colour patterns in a large number of grasshopper species (RUBTZOV 1935, CLARK 1943, RAMME 1950, BLACKITH & ROBERTS 1958).

In the phase opposition gregarious against solitary pattern, the green type can be considered as typical for the latter, with the restriction that a certain minimum humidity is required in *Locusta* and *Locustana*. In its extreme form the insects are entirely and uniformly green dorsally, the integument being completely transparent and without any black patterns. Ventrally a brownish colour persists. The fate of the green pigment during metamorphosis differs according to the species, in some it persists, in others it may dissappear completely.

In *Schistocerca* no green colour can be traced in adults whatever the hopper colour; in *Locusta* the solitary hopper colours persist, including green, although some increase in black pattern most often appears on the pronotum. The gregarious larval pattern, however, changes into a very different gregarious adult pattern. In general the differences between various adult pigmentation types in this species are somewhat less pronounced than are the larval pattern differences.

The yellow colour shown by maturing male locusts was found to be due to a redistribution of yellow carotenes, present already in the other tissues of the animal and now becoming externally visible by invading the epidermis and endocuticle (GOODWIN 1952). In *Schistocerca,* this redistribution is accompanied by very distinct changes in the epidermis at the cytological level and by the secretion of a pheromone which provokes characteristic responses and hastens maturity in other individuals (LOHER 1958, 1960; NORRIS 1954). Females as a rule do not exhibit this yellow colour with the exception of a few small spots. This colour change in males occurs irrespective of their actual colour type and antecedents and depends strongly on actual population density (NORRIS 1954).

Pseudo-heredity ("matrocline" heredity) also governs the melanin pattern. In *Locusta* as well as in *Schistocerca,* the intensity of the black pattern in hatchlings and the percentage of dark individuals from one egg-pod is strictly related to the actual parental density during oogenesis (FAURE 1932, CHAUVIN 1941, ALBRECHT 1955, HUNTER-JONES 1958, PAPILLON 1960). This density is most often the prolongation of the conditions in the preceding larval development and thus related to the pigmentation. Formerly this pseudo-heredity was explained as the effect of a transmission of a pigment precursor, a hormone or other substance from the mother through the egg-stage as this suited very well in the "locustine theory" of FAURE (1932). The phenomenon proved to be more complex, however. When VERDIER (1957) ligated *Locusta* eggs (which were destined to produce stout, black hatchlings) in a very young stage he observed that the resulting, much smaller hatchlings were light-coloured as is the case normally with the offspring of isolated parents. This fact cannot yet

be explained. On the other hand, the transmitted black hatchling colour is a very real thing, especially where it occurs in albino hoppers which occasionally are in the offspring of seemingly normal parents. They are presumably homozygotic for this recessive character and cannot synthesize any melanin themselves. Nevertheless such first instar hoppers are usually entirely black, the pigment does not dissolve before the second instar, and is disappearing entirely still later (own observations in *Locusta*).

Besides density, other environmental factors may influence pigmentogenesis. High temperatures inhibit melanin synthesis, low temperatures favour this process, the same applies for the ommochromes (e.g. STOWER 1959, HUSAIN & TASHKIR AHMAD 1936, FOX & VEVERS 1960, GOODWIN 1952). Artificially induced activity causes the melanin pattern to intensify in *Schistocerca* hoppers (HUSAIN & MATHUR 1936a), as does a high CO_2 content of the ambiant air (HUSAIN & MATHUR 1936b). Humidity influences melanin formation in a negative sense in special cases (*Locusta*), which is in contrast to its influence on physiological colour change in *Dixippus* (DUPONT-RAABE 1957). Finally, light conditions such as intensity and wave length of incident light and background colours influence pigmentogenesis, but only as a part of the colour adaptation reactions in certain species (HERTZ & IMMS 1937, FAURE 1932).

1.3.3. *Morphometrics*

From the very start of phase research, morphometric indices have been used to distinguish phases. UVAROV (1921) was the first to propose the use of ratios of pronotal width to pronotal length, and length of hind femur (*F*) to length of the Elytron (*E*), to discriminate phases. Although his methods were not correct from the mathematical point of view, the use of biometrical ratios proved to be of great value in discriminating between phases and in the classification of populations according to an assumed phase scale. Their use became a wide spread practice in field surveys as well as in more fundamental investigations. The number of measurements and derived ratios was increased by several authors, e.g. MAXWELL-DARLING (1934), THOMAS (1941), RAO (1942), ROONWAL (1946). Most of these extensions had a more or less descriptive significance only, at least their use did not become general practice. An exception to this rule was the ratio F/C proposed by DIRSH (1951, 1953), which in general proved to discriminate even more effectively than the ratio E/F (*C* is the abbreviation for the maximum width of the head). Moreover this ratio can also be used for larvae. The use of morphometrics is based on the observation that certain body parts change distinctly in shape or proportions – as was observed by UVAROV (1921) for the pronotum – or that certain body dimensions show allometric deviations as compared with other body parts during phase transformation. Simple morphometric values are not considered to discriminate sufficiently because they generally overlap for both phases. The reason for this is the variability which exists between individuals with respect to general characters such as total body weight, volume, and size. This variability tends to obscure the smaller allometric deviations completely and should be

eliminated so that allometry becomes apparent. A method which may successfully achieve this is that of dividing the value of the character assumed to show allometry by the value of a character assumed to be isometric with the general size variability. Better yet is the use as denominator of a character showing allometry in the opposite direction as compared with the character in the numerator (DIRSH 1953). What is measured in this way obviously is not the allometry itself but only the outcome of allometric growth during larval development (allomorphosis), which is most often measured in the adult.

One of the most selective phase characters is probably that of sexual dimorphism, whereby the morphometric ratio ♀♀ : ♂♂ for several characters is tending in many species to diminish on gregarisation. In most cases this does mean that the males are larger when crowded and the females smaller. In the case of the phasmid *Podacanthus,* however, crowding causes both sexes to be smaller, but the sex ratios diminish nevertheless (KEY 1957).

It should be emphasized that there is a lack of quantitative knowledge as to the sources of variability of morphometric characters other than phase status. In a few investigations density dependent factors only were taken into account e.g. STOWER et al (1960), ZOLOTAREVSKY (1933), ZOLOTAREVSKY & MURAT (1938), HUSAIN & MATHUR (1944), BRETT (1947), CHAUVIN (1941), LEAN (1936). In general low humidities and high temperature induced more gregarious ratios.

STOWER et al (1960) used modern statistical methods (multivariate analysis, canonical variates and geometrical representation) following ALBRECHT & BLACKITH (1957) and BLACKITH (1957), to separate the density effect from the effect of temperature acting during development. All these authors claim the superiority of these methods in evaluating the phase status of different populations. Multivariate analysis usually appreciably reduces residual variability. Nevertheless, classic methods must be called on for the opposite procedure, i.e. evaluation of certain definite density dependent or density independent influences on separate morphometric characters in order to study the causal mechanisms involved. Residual variability may also be reduced in laboratory experiments by using genetically homogeneous material and standardized methods.

Field studies suffer fundamentally from the uncertainty of several factors originating in the animal populations as well as the external conditions, of the constancy of which one should be sure before making statements about the effect of density. According to GUNN & HUNTER JONES (1952) selection for morphometric characters rapidly leads to genetically different lines in this respect. This indicates a significant variability and renders probable a control of morphometric characters by several genes simultaneously. Matrocline, non-genetic, heredity also may influence actual field results. In the present state of research in this field only laboratory experiments, keeping constant every condition except the factor(s) studied, can be a basis for physiological interpretation of phase mechanisms. To what extent the data of DIRSH (1953) are confounded by uncertified factors is not known. One may only hope that den-

sity is the more important factor in his material on which many important conclusions are based.

In the use of ratios one fact (stressed by STOWER et al, 1960) tends to be neglected: the possible influence of phase status on the general body size. This influence is very obvious but is certainly also confounded to a large extent by environmental factors such as food, temperature and water supply, especially in field investigations. Illustrative in this respect is the case of *Dociostaurus*. In this species general body size is a much more selective phase character than *E/F* (JANNONE 1938). Here again laboratory experiments ought to give the basic information.

In *Schistocerca* as well as in *Locusta* the effect of gregarisation on general size is opposite in the two sexes and is very significant in the laboratory as well as under field conditions (DIRSH 1953, own observations). This could mean that gregarisation does not affect physiological processes in the same way in both sexes. Furthermore it is not a priori certain that the morphological characters or ratios discrimating the phases most perfectly must necessarily be the same for both sexes, or vary in the same direction in both sexes, as was assumed by DIRSH. Another biometric character often used in phase discrimination is the number of eye-stripes (in *Schistocerca* and *Nomadacris*). This character is strictly connected to the number of instars in larval development, 6 being normal, 7 occuring in a certain percentage of the solitary populations of *Schistocerca*. ROONWAL (1947) and ALBRECHT & BLACKITH (1957) found that the number of larval instars was a much less important factor in the final adult morphometric status than actual density. Obviously the two different types of larval development are convergent in the resulting adults.

ALBRECHT (1955) reviewed the known cases of plasticity in the number of larval instars in *Acrididae* and demonstrated convincingly in his material of *Nomadacris* and *Schistocerca* that there is no question of plasticity in the larval development leading to intercalated or duplicated instars but of two quite distinct types in the larval development involving a different rate of development in each instar and a different number of instars in the complete larval cycle. The alternative type of development is determined already at the moment of hatching and thus related to parental density. There was a significant correlation of weight and pigmentation of the hatchlings to the type of development. In a few other cases, genetical determination of the number of instars has been assumed (KEY 1936, RAO 1936) but has never definitely been proven. ALBRECHT is inclined to recognize only phase influences. He considers the supplementary instar (the "mue d'ajustement") as a compensation for the smaller initial size. JOLY (1956) analysed the growth of *E, F, C* and some pronotal values and their mutual relations during larval growth and metamorphosis in *Locusta*. The growth in *F* and the total body length was isometric throughout development, thus *F* was considered to be a reliable general body size parameter. The growth of the wing buds (pterotheca) was positively allometric from the third moult on (compared with *F*). *C*, however, showed strict allometry during larval development and a negative allometric behaviour

during metamorphosis. Some pronotal characters showed a lasting negative allometry from the third moult on. JOLY tried to prove discontinuities in the growth curves by comparing the linear regressions of a pair of characters in the separate instars. This method is probably based on an incorrect assumption, for the regressions within actual instar samples could very well be something other than growth regressions of one or more individuals. His data were concerned only with a gregarious population. Comparable data for isolated individuals were not given, except for some adult ratios in order to allow comparison with individuals implanted with extra CA. The disturbances caused by this interference had a general larval rather than a solitary character. DUARTE (1938) compared growth rates in isolated and crowded *Locusta* populations throughout development. A very conspicuous negative allometry for *C* (compared with *F*) occured during metamorphosis only in the solitary individuals of both sexes, the gregarious figures indicating approximate isometry. The analysis of morphometric characters certainly is one of the best tools in phase research since such characters can be evaluated exactly and may be analyzed statistically. They moreover show a rather gradual transition, often extending even beyond one generation.

1.3.4. *Physiological phase differences*

Changes in behaviour, pigmentation and morphometry are the expressions of changes in more fundamental physiological processes. Knowledge of the character of the mechanisms involved is often lacking. As regards the anatomy and histology of phase differences much work in still needed. CARLISLE & ELLIS (1959) have observed a longer persistence of the ventral glands in solitary adults of *Locusta*. LOHER (1960) has described changes in the epidermal cells accompanying maturation and the appearance of yellow coloration in adult males of *Schistocerca*. ALBRECHT, VERDIER & BLACKITH (1958) have described very significant negative correlations between the weight of female hatchlings in *Locusta* and the total number of ovarioles already present. This weight was related to the degree of crowding of the parents even when this crowding took place only during larval development.

A second group of facts concerns metabolism and respiration of individuals of both phases. BODENHEIMER (1929) found the rate of O_2 uptake to be increased by 100% in gregarious larvae of *Schistocerca*. CHAUVIN (1941) also found a higher rate of O_2 uptake whereas the CO_2 output remained the same. In addition, it was found that gregarious larvae consume much more food without showing a proportionally higher ultimate weight. BUTLER & INNES (1936) observed an almost doubled rate of basal metabolism measured by O_2 consumption in crowded *Locusta* larvae as compared with isolated individuals. Certain relations between metabolism and activity could be expected to exist but they have never been properly investigated. Marching behaviour is not a good expression of spontaneous activity as it depends on stimuli in dense crowds (ELLIS 1951). Most of the earlier authors take a greater spontaneous activity of gregarious locusts for granted although much confusion has

always existed about the question whether activity was the causal factor in phase transformation or only the result of it. CHAUVIN (1941) doubted the higher activity in gregarious *Schistocerca* and concluded that pigmentation changes must be effected by nervous and/or endocrine reactions to sensory stimuli. KEY (1957) supports this view for the already described case of the Australian *Phasmidae* in which no activity change was observed.
Moreover, stimuli induced artificially by mechanical apparatus can never be a complete substitute for group stimuli, although a certain effect cannot be denied (FAURE 1932, HUSAIN & MATHUR 1936a). HODGSON & GELDIA (1959) were able to show a release of neurosecretory material from the corpora cardiaca as a result of stress conditions and also an invasion of the brain by blood cells in cockroaches. Corresponding experiments in locusts are urgently needed.

1.4. THE PHASE INDUCING MECHANISMS

UVAROV & THOMAS (1942) suggested a direct relation between pronotal shape and phase differences in behaviour. Although this has never been refuted, the mechanism for most of the other phase changes must be more complex.
The group stimuli include tactile, visual and in certain cases olfactory stimuli, but most often combinations of the former two, both being necessary for a full reaction to crowding. The receptors for tactile stimuli are probably not very specific. Olfactory perception of pheromones takes place mainly by the antennae (LOHER 1958, 1960). That phase induction consists of a chain of events in which the brain is an important link is not subject to serious doubt, but since the effectors are hardly known, the connecting pathways remain obscure. The most probably intermediary mechanism is the neuro-endocrine system. Much controversy exists concerning the role of the nervous connections between the different parts of this system even with regard to the more general functions since the same nerves probably transmit impulses in some fibres and neurosecretory material in other elements. The evidence for endocrine glands and active humoral factors possibly involved in the phase inducing mechanism will be reviewed here briefly.

1.4.1. *The corpus allatum*

An indication of a possible mechanism in phase induction was suggested by the work of PFEIFFER (1945). She found that implantation of extra corpora allata induced the grasshopper *Melanoplus mexicanus* to develop a green blood colour not unknown in other *Acrididae* but, as she states, uncommon in *Melanoplus* nymphs. *Melanoplus* probably reacts well to isolation by forming green pigment, but PFEIFFER was apparently unaware of this fact. She noticed, however, the green blood colour as a normal feature in adult females during yolk deposition, at which time the corpora allata are assumed to show maximum activity. Moreover, she studied the effects of the corpus allatum on development, metamorphosis and reproduction by extirpation and extra implantation.

Her results were in harmony with the classical concept of the functions of the corpus allatum.

JOLY started the same type of research in 1949 and obtained more detailed data concerning the corresponding corpus allatum effects in *Locusta*. He made an attempt to relate his results to phase phenomena. In general the effect of extra implantation of CA in larvae was the appearence of green pigment, at first in the blood and after the following moult in the integument as well. The black pattern gradually disappeared in this and following moults until an entirely green type of hopper resulted, the pigmentation of which could not be distinguished from that of solitary hoppers under humid conditions. This effect appeared even when the hosts were kept crowded after the operation, indicating that the corpus allatum effect could override the gregarious induction as far as pigmentation is concerned (JOLY 1952). In the same paper similar effects are ascribed to the corpora cardiaca. This, however, has never been confirmed.

The same CA effect was found to develop in *Acrida turrita* (JOLY 1952), which species does not have a phase dimorphism but only a well-developed cryptic polymorphism, the formation of green pigment being dependent on green background colour only. Apparently the intermediary mechanism (via the CA) has much in common with that in *Locusta*, and only the determining external stimuli are different.

Of importance was the observation that extra implantations in *Locusta* larvae very early in L 5 did not produce green colour, but definitely disturbed metamorphosis by the abnormal retaining of larval characters after the "adult" moult. By implantation in earlier instars or late in L 5 the effect was reversed. A satisfactory explanation for this phenomenon has never been given (JOLY 1958).

Wing development in resulting "nymphoid", metathetelic, adults showed all kinds of intermediates between pterotheca of larvae and wings of normal adults. These abnormal individuals occasionally showed signs of sexual maturation and sometimes tried to moult once more. The consequences of the effect upon the phase ratio E/F were discussed, the ratio F/C was unfortunately ignored. Failure to state experimental conditions, the sex of the insects used and the standard errors of the means makes it difficult to draw definite conclusions from most of the papers. The relative unimportance of the age and status of the donors of the implanted CA with respect to the colour change was emphasized by JOLY (1954).

With certain precautions, a local effect resulting in a green spot in the integument could be obtained as well, suggesting a direct influence of the CA on pigment metabolism within the epidermal cells (JOLY 1954).

In later papers (JOLY 1956, 1958) allometry of wing growth as related to excess or deficiency of the CA factor was discussed. A direct proof of the identity of JH with the factor causing the formation of green pigment has never been given. The possibility of more than one CA hormone should be seriously envisaged. LÜSCHER & SPRINGHETTI (1960) and SÄGESSER (1960) provide supporting evidence for this idea.

1.4.2. *The ventral gland*

The anatomical, histological and physiological aspects of the ventral glands in *Locusta* were investigated by STRICH HALBWACHS (1953, 1954, 1959). Successful extirpations resulted in permanent larvae and confirmed the moult inducing function of the ventral gland. Extra implantations in L 3, L 4 and L 5 in general fixed the next moult at an earlier time than normal. Morphometric consequences were explained as a result of the earlier termination of growth processes in the cycle concerned.

No evidence was found for any direct influence upon the differentation of adult structures or upon the pigmentation. A function of this gland in the determination of phase dimorphism apparently could not be established.

ELLIS & CARLISLE (1961) found larger VG in solitary larvae of *Locusta* and *Schistocerca* and claim a positive, quantitative relation between these glands and the green colour for *Schistocerca*.

1.4.3. *Undetermined humoral factors*

Other evidence in favour of humoral control of some phase features are found in the papers of NICKERSON (1954, 1956). In transfusion experiments he collected evidence for a humoral factor stimulating the formation of the black pattern in *Schistocerca* hoppers. This factor was found only in crowded hoppers and proved to be a rather stable compound. About the relation and possible antagonism of this "pattern factor" and the CA factor inducing the green colour little is known hitherto.

1.5. GENERAL ENDOCRINE FUNCTIONS PROBABLY RELATED TO THE PHASE INDUCING MECHANISM

Scarcely any other experimental work concerning phases has been done. Numerous publications however deal with the classic general functions of the endocrine system. Resemblances between those functions and the possible phase functions may be envisaged.

1.5.1. *The corpora allata*

The CA, the hormone of which is often referred to as the juvenile hormone (JH), are known to influence a number of physiological processes, of which the following are of interest here.

a) Basal metabolism is stimulated by implanting active CA in diapausing insects and derived homogenised tissues (DE WILDE & STEGWEE 1958). Similar increases in non-diapausing insects have been claimed repeatedly (e.g. SÄGESSER 1960), but according to NOVÁK (1959) this effect is often only transitory and doubtful. Such a stimulating effect would not accord with the higher basal metabolism in the gregarious phase of locusts, in which the CA are supposed to be less active.

b) Several essential biochemical processes are changed through interferences with the function of the CA, inflicting among other things changes in almost

every type of pigment metabolism (L' HELIAS 1957, 1959). The significance of these processes in the integrated physiological mechanisms of the insect is hardly known. The identification of the CA and CC factors controlling these processes with derivates of folic acid has found little support, at least in as far as these factors are to be identified with the activating hormone (AH) and the juvenile hormone (JH).

c) Water excretion may be under the control of the CA, as has been proven in bees by ALTMANN (1956). A relation of this function to the supposed dependance of CA activity on environmental humidity in *Locusta* should be envisaged.

d) Behaviour is possible governed by a CA factor in cases where adults live under conditions very different from those acting on the larvae. The action on behaviour is not exerted directly but via the central nervous system, in which morphogenetic influences may release latent instincts.

e) Reproduction in many insects depends on CA activity, the crucial point being yolk deposition in the eggs (WEED 1936, WIGGLESWORTH 1936).

f) Metamorphosis is the more spectacular process, resulting from an obvious deficiency of CA hormone in the last larval instar. A great many papers describe the influence of disturbances in the level of CA hormone on development and metamorphosis. Insects showing phase phenomena are by no means an exception to these general rules. Still to be considered is the possibility that minor changes in the general processes are responsible for the morphometric phase changes, since metamorphosis in general affects the same characters used to discriminate between phases in an even much more pronounced way. Many possible ways of bringing about these minor changes may be conceived, ranging from either a simple, continuous higher or lower level of one or more hormones, or a different level only at certain distinct moments in the larval cycles, to every type of interaction between quantity and timing effects of hormone secretions. It must be emphasized that the common cyclic activity of an endocrine gland leads to continuous changes in the hormone level concerned and we do not know on what aspect of this changing hormone titer the physiological mechanisms involved are reacting.

The *a priory* assumption of threshold values probably is an unjustified oversimplification. Experimental interferences with endocrine processes may produce effects described as *prothetely* or *metathetely* depending on a disturbance of the balance between larval and adult growth (NOVÁK 1959). The terms prothetely and metathetely are mostly used to indicate conditions in which adult structures are respectively hyperdeveloped or underdeveloped compared with the normal condition in congeners of the same age or instar. Similar effects are sometimes produced by external conditions apparently acting on the endocrine system (e.g. WIGGLESWORTH 1952).

1.5.2. *The ventral gland*

The known effects exerted by the moulting hormone or ecdyson, (MH) secreted by the ventral glands (VG) are:

a) Its absolute necessity for the induction of moulting. Experimental removal invariably results in the disappearance of moulting activity. This implies of course an influence on growth and differentiation, these phenomena becoming manifest almost exclusively in connection with the process of moulting.
Besides the indirect influence on growth, a direct influence also possibly exists. The hormone has been thought to promote the synthesis of enzyme systems breaking diapause.
b) A direct effect on differentiation is often claimed. This can be demonstrated in the larval development only in connection with the moulting proces. The separation of the effects would be difficult were it not that many cases are known in which moulting occurs without any obvious differentiation. The difficulty is that this often depends on the simultaneous activity of the JH which inhibits the development of adult characters. WILLIAMS (1952) found that the MH in vitro promoted differentiation in the spermatogenesis of *Hyalophora.* HALBWACHS, JOLY & JOLY (1957), however, claim an exclusive effect on moulting, with indirect consequences on growth and differentiation.
c) The special effect of MH on colour change accompanying metamorphosis in *Cerura vinula* (BÜCKMANN, 1959) is well established. This effect is due to a turnover of the redox status of an ommochrome pigment.
effect is due to a turnover of the redox status of an ommochrome pigment. Although similar pigments are present in *Locusta,* they occur mainly in a reduced state according to GOODWIN & SRISUKH (1950). BÜCKMANN claims the functional significance of two different ecdyson levels, a low critical level for the colour change, which operates only in the absence of JH, and a high level for the induction of moulting. When only the latter is applied, the former effect does not appear. NOVÁK (1959) opposes the two level concept and attaches importance only to the high hormone level responsible for the induction of moulting.
d) Some experiments suggest an inhibiting effect of implanted ventral glands upon the activity of the adult corpus allatum. This inhibition can in its turn be overcome by simultaneous implantation of a suboesophageal ganglion (ENGELMANN & LÜSCHER 1957, ENGELMANN 1959).

1.5.3. *The activating hormone*

The source of AH, presumed to be the factor inducing the ventral gland to start activity and probably also the CA, is the brain (cerebral ganglion, CG). The mode of transmission, assumed to be connected somehow to the stainable neurosecretory substance along the axons connecting the brain with the corpus cardiacum can be preponderantly humoral under certain conditions as well. The functions of the nervous connections of the endocrine organs are by no means clear in the light of these phenomena. There are hardly any indications of direct effects of AH on morphogenetic processes. Indirect effects may be connected with the termination of diapause (SELLIER 1949). An important function of the AH with regard to the endocrine coordination is certainly present (NOVÁK 1959). It remains possible that the AH exerts some

direct influences on physiological processess, especially because a precursor status would implicate some chemical resemblance in structure.

1.5.4. *Myotropic hormones*

These hormones, also referred to as "kinetic hormones" in the terminology of CARLISLE & JENKIN (1959) are a group of very active substances exerting a direct influence on effector systems normally innervated exclusively by involuntary nerves (heart, intestine, chromatophores etc.). "Hormones" of this type also regulate the physiological colour change in some insects and crustacea, e.g. the displacement of pigments granules within the epidermal cells in *Dixippus* (GERSCH & MOTHES 1956) and the contraction of melanophore cells on the tracheal air sacs of the *Corethra* larvae (GERSCH 1958). These substances most often originate from the nervous centres, corpus cardiacum included, (see also CAMERON 1953) although some effective principles are found in the CA as well.

No relationship of these principles to morphological colour changes and other phase phenomena has ever been demonstrated. This should not be excluded *a priori,* however, for it has been observed that conditions inducing a certain physiological colour adaptation most often finally lead to a corresponding morphological adaptation in animals possessing both adaptive mechanisms (PARKER 1948).

1.5.5. *The suboesophageal ganglion*

Another centre from which humoral or nervous stimuli may originate is the suboesophageal ganglion, which in some species is demonstrated to exert more functions than those of a simple motor centre only. Some authors claim the secretion of a diapause hormone (FUKUDA 1951). Other known functions include the control of diurnal rhythms in activity or colour change (HARKER 1960, DUPONT RAABE 1957) and the control of CA activity (ENGELMANN & LÜSCHER 1957). The latter authors were the first to ascribe a functional significance to the nervous connection of the suboesophageal ganglion with the CA.

The study of the nature and mechanism of the coordination and quantitative regulation of endocrine processes is only at its beginning. Progress is this domain is retarded by the lack of sufficient quantities of pure hormones and the lack of methods for direct determination of hormone levels within the animal. Much of the bulk of experimental work done on the endocrine glands awaits confirmation using purified hormones. With these the normal or abnormal levels of hormones supposed to exert specific influences may probably be imitated.

2. MATERIAL AND METHODS

2.1. SOURCE, BREEDING AND CARE OF EXPERIMENTAL ANIMALS

The breeding material of *Locusta migratoria ssp migratorioides, Schistocerca*

gregaria and *Romalea microptera* was originally supplied by the Anti Locust Research Centre in London and afterwards continuously bred for some years in our laboratory. Stock breeding cages were kept in a glasshouse and provided with a continuously burning electric bulb, either below or inside the cages, in order to maintain a temperature gradient ranging from 25—40 °C within each cage. Food was supplied daily and consisted of grasses for *Locusta* (e.g. *Glyceria*), leaves of several corn species, *Bambusa japonica* and in addition most often also a few leaves of kale, endive, rapeseed, lettuce or cauliflower. The uptake of bran proved to be neglegible so this was discontinued. *Schistocerca* was fed the same range of food plants except for the coarse grasses. *Romalea* was fed on the same plants as *Schistocerca* except that no grasses were given.

Oviposition took place in jars, screwed under the bottom of a special large cage. Care was taken to maintain a high population density in this cage in order to obtain the most viable hatchlings. Occasionally occurring high post-operative mortalities were imputed to neglect of this condition. The eggs were stored in the jars in an incubator at 30 °C and hatched after ± 11 days. Insects used in experiments were always put in separate cages within 24 hours after the moult to the second instar. The standard procedure used for handling the operated second instar larvae and the controls was to put 10 of them in an ordinary glass jar of 450 ml, equipped with a lid of wire gauze, a bottom layer of absorbing filter paper cuttings and a small roll of very rough paper for support. Daily a compact bundle of grass, approximately 9 cm long and 1.5 to 2.5 cm in diameter, tied up by two thin iron wires, was supplied. These pots and cages were kept in a constant temperature room at 30 °C and approximately 35% RH or in an incubator at the same temperature.

Food was changed and soiled filter paper and paper rolls were renewed daily or when needed. This system was developed gradually and proved finally to be a considerable success with respect to space needed, high survival, easy performance of daily observations and especially also the standardisation of crowded breeding conditions. The bundling of the grass proved to be particularly important in keeping the humidity in the pots as low as possible and yet providing the best possible supply of succulent food during the 24 hour interval for in early experiments an important cause of high post operative mortality proved to be free moisture in the cage or pots. Under these conditions hardly any cannibalism took place and mutual disturbance during moulting was rare except for the adult moult. Experimental larvae were therefore transferred to larger cages (15 × 18 × 30 cm) during the fifth instar in groups of not less than 30 individuals. Each treatment was labelled by an amputation of certain parts of the antennae. This amputation had no appreciable influence on development. For densities in the jars ranging from 5—10 and in the cages from 30—80 the influence of differences in density on phase characters was neglected.

Experimental larvae were examined once per day for pigmentation, morphological instar, casualties etc. For these groups the mean duration of every instar

could be evaluated, but no individual values for this property could be assigned and consequently no error could be calculated for the duration of separate instars.

2.2. Observations, sampling and preservation of experimental animals

In checking pigmentation changes special attention was given to the use of qualitative changes, e.g. the appearance or disappearance of green pigment and the opposition of larval to adult colours, as these were considered to be of greater value than semi-quantitative values assigned to an arbitrary number of pigmentation type groups. Morphometric measurements were almost exclusively performed on the resulting adults. In order to permit a reasonable degree of cuticle hardening the adults were sampled 24—48 hours after the adult moult and measured either in this fresh condition or – most often – transferred to jars with ethanol (70%) and measured after some weeks of preservation. For measurements the animals were taken from the jars after at least some weeks storage and dried only externally. Errors caused by differences in alcohol percentage were not assumed to play a role because all individuals in an experiment were treated alike. In a number of experiments "fresh" weights of these alcohol individuals as well as dry weights after forced drying for at least a day at 60 °C were determined. Although subject to errors caused by differences in the fat going into solution, some relative value may be assigned to these figures nevertheless.

2.3. Operational techniques

The performance of microsurgical operations on the endocrine system made inevitable the development of suitable operation techniques in order to obtain a sufficiently high percentage of survival even in the case of young larvae. The methods described in literature (Pfeiffer 1939, Joly 1949)[1]) were not quite adequate in being too elaborate and resulting in unduly high mortalities, either post-operative or during the next moult. A method was therefore sought which would avoid damage to both the mandibular muscles, which tend to obstruct the operation field seriously when damaged, and the median suture line of the head, damage to which disturbs moulting. These difficulties were overcome by operating through the neck membrane exclusively, following a similar method developed and practised on the Colorado beetle by De Wilde (1958). Our method was essentially the same but owing to the larger size of the animals some special devices were necessary to get the maximum access to and visibility of the organs in the head while holding the insect immobile without exerting any harmful pressure. The apparatus used (figure 5) consists primarily of a clamp in which the head is held in such a manner that the distal

[1]) During the preparation of this paper the paper of L. Joly (1960) came to our disposal. The method of allatectomy described in it turnes out to be improved compared with earlier methods, but does not show some of the essential advantages of our method.

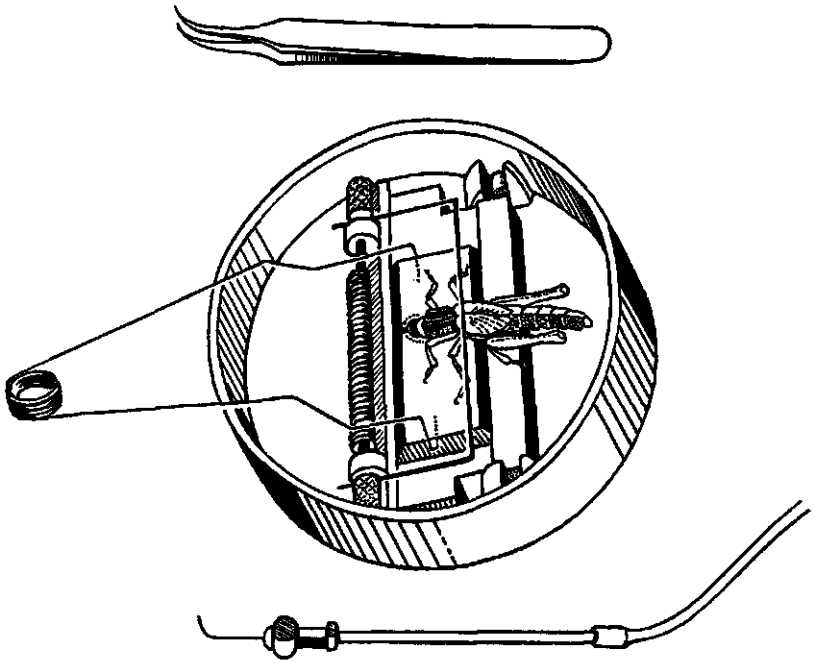


FIGURE 5. Apparatus and tools used for operations in the head cavity.

margin of connection with the intersegmental membrane is parallel to the ultimate level of saline solution in which the whole apparatus is immersed. The abdomen is bent backwards by a spring which immobilizes the posterior legs as well. Care was taken to avoid any part of the body or the clamps disturbing the plain level of the saline solution as this is a prerequisite for good visibility. Adapted interchangeable pieces for the clamp were made separately for every instar. Some difficulties were experienced in sterilizing the apparatus consisting of plexiglass and stainless steel parts. Finally a *Desogen* solution of Geigy, Switzerland, was found satisfactory for keeping the apparatus sterilized when not in use. The visibility of the organs in the head is a function of the transparancy of he blood and this property ranges from quite transparent immediately after a moult gradually to quite opaque immediately before a moult. Hence the best moment for the operation is as soon as the exoskeleton is sufficiently hardened after a moult. To improve visibility use was made of a special device. It consisted of a curved hypodermic needle connected with a bottle delivering a steady flow of saline solution rinsing away the blood in the head and clearing the field of operation (fig. 6). The rate of flow of the saline was controlled by the height of the bottle. The needle was manipulated by one hand and in addition served simultaneously as an expedient to keep the wound open. The other hand carried the microsurgical tools for the operations to be performed. The two most important tools used were very fine

grounded (stainless steel) watchmakers forceps with a beak, curved 45° and a fine needle. Instruments and saline were always carefully sterilized when starting a series of operations. The wound was made by simply piercing the neck membrane with the forceps. The median cephalic air sac, which obstructs the view, could be removed without any appreciable consequences. After the operation the neck membrane was closed automatically by the retraction of the head within the pronotum, no muscles being damaged.
No artificial closing was ever needed, healing taking place rapidly, and no disturbance of moulting was ever noticed provided the operations were properly carried out. The use of antibiotics, disinfectants etc. never improved the results. Occasional failures were ascribed to two factors:

1) larvae less vital than normal. This was thought to be correlated to low

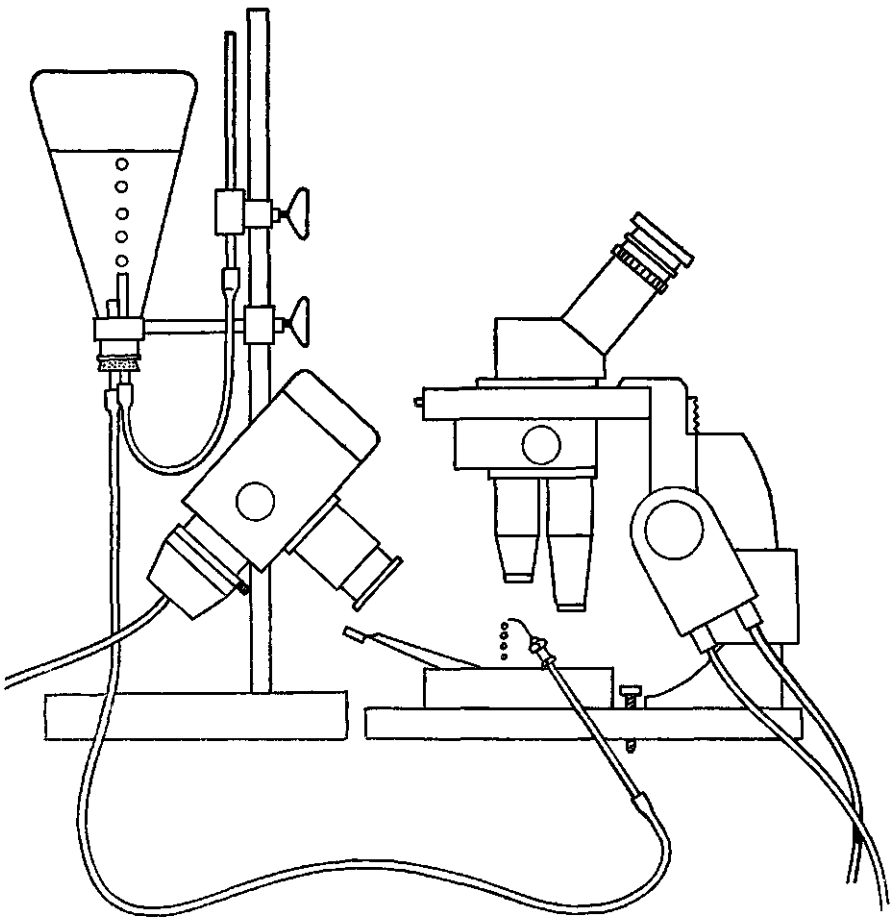


FIGURE 6. Arrangement of apparatus used for operations: foot-controlled microscope, clamps for fixing the locusts, perfusion system and low voltage Monla lamp.

weight and light colour of hatchlings, characters probably indicating a (too) low degree of crowding in the oviposition cages.
2) contact of the operated larvae with free moisture during the first days following the interference. When both sources of difficulties are removed it is possible to have 95% survival or more even in operating on the second instar. The treatment of one individual took not more than 1—2 minutes on the average.
The method described needed no anaesthetics at all for the animals were quite sufficiently immobilized, so mortality from this source was avoided. Much help was experienced by the use of a footcontrolled device in combination with a Leitz Greenough binocular. A low power (8 V) Monla lamp was used as a light source. The light bundle was carefully focussed on the subject, the saline solution providing an ideal spread of light in every direction inside the head.
The saline solution contained 7.5 gr NaCL and 0.375 gr KCL in 1000 ml of distilled water. This composition resulted in less mortality than the LEVY solution which has a slightly higher osmotic value. Obviously, substitution of a great part of the blood by saline is not at all lethal, the only effect being a delay of one day in the next moult at most.
Mortality in the first instar always proved to be very high, in contrast to that in the second instar. For this reason second instar larvae (less than 24 hours old) were adopted as the standard subjects. Allatectomy was carried out by seizing the nervus corporis allatum (NCA) with forceps and tearing it apart from the corpus cardiacum (CC). As the nerves, originating on the other side of the CA and connecting this organ with the suboesophageal ganglion[1]) are much smaller in diameter than the NCA, they are more easily torn off. No separate physiological effect resulting from pulling on the CC could be observed. Occasionally the CA was torn in two pieces, one piece remaining in the head. In order to be quite sure of the complete removal a modification of the described technique was often practised in which the nerves at both sides of the CA were seized simultaneously by the forceps. Extirpation of the CC eventually took place by the use of forceps as well but difficulty was experienced in separating the CC from the hypocerebral ganglion. With some experience, the extirpation of CA of CC could be carried out without damage to other organs. Care was taken to avoid cutting the outer oesophageal nerves, since this resulted in a most harmful and usually delayed lethal effect.

2.4. DETERMINATION OF THE VOLUME OF CORPORA ALLATA

Since these organs are known to go through distinct cycles of mitosis, cellular growth and probably of secretion (MENDES VANUCCI 1948; SCHARRER & VON HARNACK 1958; LÜSCHER & ENGELMANN 1960), the volume determination according to the classical histological methods, involving fixing, is perhaps

[1]) According to a verbal communication by E. J. CLARKE and own observations this connection is an anatomical fact too in *Locusta*, although ALBRECHT (1956) does not mention it.

subject to a systematic error, because the shrinkage due to this treatment need not be *a priory* equal at different moments in the cycle. To eliminate this possible error the volume of the CA was determined in fresh condition using the apparatus shown in fig. 7. It consisted of two thick plates of mirror-glass, the one overlying the other, but separated from it at one end by a coverglass slide glued to the lower plate by means of Canada balsam. In this way, a wedge was formed from zero to the thickness of a cover glass.

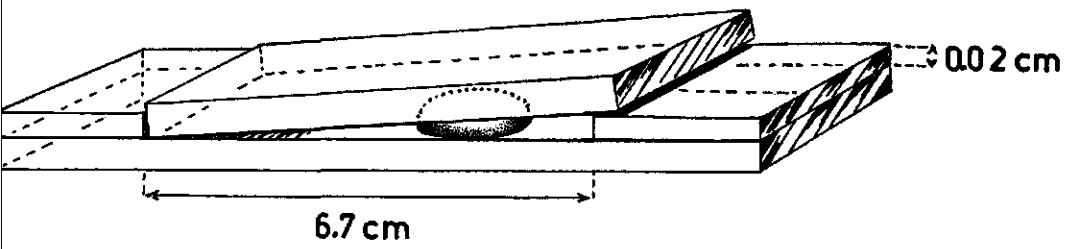


FIGURE 7. Apparatus for measurement of the fresh corpus allatum volume consisting of two glass plates leaving a wedge between them. The corpora allata are compressed between the plates on a fixed spot. The circumference is then drawn and measured.

The CA were always placed in a droplet of saline solution on a marked spot upon the lower plate and then compressed by the upper plate to a diameter several times greater than their original diameter. Thus this apparatus substituted a volume measurement by a surface measurement, both being approximately proportionate for CA which do not differ too much in size, and it corrected for differences in shape. The resulting surfaces were drawn by the use of a Leitz Greenough binocular microscope provided with a Zeiss drawing apparatus and finally measured by means of an OTT planimeter. All the volume data are given in planimeter units obtained by this standard procedure, and are not meant as absolute measurements. A check on the accuracy of the method is the fact that the volume of both CA of one individual, measured consecutively, differs only insignificantly compared with the variability between individuals. The justification for the use of a wedge instead of a parallel distance between the glass plates is found in:

a) a considerably less important risk of errors caused by unperceived dust- and other particles between the plates in the lines of contact.

b) a variable capacity of the apparatus, because different parts of the wedge may be used for CA of very different sizes.

2.5. MORPHOMETRICAL DETERMINATIONS

Measurements were carried out exclusively with a special measuring microscope with a fixed stage and a horizontally running optical system with a range of 80 mm and an accuracy in reading of 0.01 mm. The advantages of this method compared with the usual calliper method are:

a. it is more accurate
b. no compression of parts to be measured by the calliper spring takes place.

Parts to be measured must be carefully fixed in a position parallel to the direction of running of the optical system. This was done with the aid of plasticine. Measurements were taken according to the reference points of DIRSH (1953), the only exception being that a somewhat different point at the base of the elytron was used. This was done because this point could be recognized better in the microscope in normal as well as in abnormal experimental animals. The choice of measurements was limited to a few, the significance of which for phase, size, or metamorphosis discrimination could be expected from the literature.

The characters used and their abbreviations were:

D = the duration of the development from the start of the experiment to the adult moult in days.
CA = the total volume of both corpora allata, or when especially mentioned sometimes the volume of single corpora allata
E = length of the right elytron
F = mean of lengths of the femora of the posterior pair of legs
C = maximum width of the head
V = width of vertex between the eyes
$K_{1,2}$ = weight of the head. K_1 = the weight in fresh or wet condition, most often after storage in alcohol; K_2 = the weight after drying for at least 24 hours at 60 °C
$F_{1,2}$ = fresh and dry weight respectively of posterior femora (total of both)
$KCs_{1,2}$ = fresh and dry weight respectively of entire insect

Some new measurements were introduced:

L = the length of hairs forming part of the velvet hair covering on the sternum. This parameter was introduced as it appeared to undergo a considerable change during metamorphosis, hairs being short in larvae and metathetelic adults compared with normal adults. This length was determined by cutting out a standardised rectangular piece of integument from the sternum, scraping off the epidermal layers from this piece and folding the remaining cuticle along a line midway left to right. The hairs on the ridge of te folded piece of cuticle were stretched between thumb and forefinger in a direction perpendicular to the fold. The piece of cuticle was then mounted between an object glass slide and a cover slip in Euparal. This medium favoured the stretching of the hairs more than any other medium we have tried. Measurements were made under a binocular microscope provided with an eye piece micrometer enlarging 32 times. In adults these hairs are very dense, individual hairs being difficult to distinguish. The longest hair present in a preparation was disregarded to avoid the use of fortuitous extreme values, this longest hair bearing also the risk of being one detached at its base. Thus the longest hair but one was measured and considered as a reliable measurement of the general ventral pilosity. Values are expressed in eye piece micrometer units. The density of the hair

covering certainly is another important aspect but the evaluation of this character presented practical difficulties and has not been carried out.
Ps = the surface of the pronotum. This was introduced in order to obtain a reliable general body size parameter. Pronota were prepared free, mounted dry or in Euparal between a glass object slide and a cover slip. The circumference was drawn on paper by means of a binoculair microscope provided with an ABBE drawing apparatus, and finally the surface was measured by an OTT planimeter. The figures obtained are in planimeter units.

3. PHASE BREEDING EXPERIMENTS

3.1. THE EFFECT OF DIFFERENCES IN BREEDING DENSITY AND HUMIDITY THROUGHOUT LARVAL DEVELOPMENT ON THE RESULTING ADULTS

3.1.1. *Experimental conditions*

In order to have a comparison for further experiments and to obtain some general information, a simple experiment was carried out in which the normal density effect under the prevailing conditions was determined for both sexes. Humidity turned out to be difficult to control in all experiments carried out in jars as it depends on food condition, number of animals in a jar, etc. Different humidities were also introduced as a factor in the experiment bringing the number of treatments to eight for both sexes together. For all of these eight objects 25 (or more in the crowded objects) hatchlings from the gregarious stock breeding were reared under the conditions stated as follows.

Humid and isolated (HI);

450 ml jars, equipped as already described on page 18 but with a plate glass cover instead of a wire gauze lid were used in order to confine the water evaporated by the animal and its food in the jar. A minimum of ventilation was secured however by inserting one blade of grass between the rim of the jar and the glass-lid. The humidities obtained in this manner have not been determined exactly but they were assumed to be above 90%.

Dry and isolated (DI);

Wire gauze cylinders 4 cm in diameter and 10 cm high, which allowed a good circulation of ambient air (RH ± 35%), were used. The small bundles of grass provided as food in these cages rapidly dried out as a result of the low RH. The food was therefore air-dry during a large part of the 24 hour feeding period.

Dry and crowded (DC);

Approximately 170 hatchlings, the sex of which had not yet been determined, were reared in a cage 15 × 18 × 30 cm which had two sides covered with wire gauze, and two sides of glass. About the same conditions prevailed in these cages as in treatment DI above. The number of insects in the cages was re-

duced somewhat by the removal, at every instar, of a small number of larvae showing a delay in moulting compared with the main group. Any physiological abnormalities such as diseases and mutilations are likely to result in a delay in development. It goes without saying that such abnormal individuals should not be present in the final samples. Selection to eliminate these abnormalities is very desirable and may not be considered to cause the final samples to be biased for other characters. Thus the cause of the different numbers of individuals in the final treatment samples are found in the described selection, mortality and mutilations such as lack of both posterior legs or crumpled wings.

Humid and crowded (HC);

30 hatchlings of each sex were reared together in 450 ml jars. In the lid a few holes (approximately 10% of the total surface of the lid) provided limited ventilation. The humidity in the jars was considered to be comparable with that in treatment HI. The 30 larvae in each jar were split up into 2 batches of 15 in the L 3 and divided once more in groups of 5 to 7 in the L 5. They were finally combined in the middle of the L 5 in a closed standard cage to prevent mechanical difficulties during the adult moult.

For the statistical analysis of the differences two possibilities were envisaged:

a) unifying the number of individuals in each treatment to 13, this being the lowest number occurring. This would imply a further random selection in most treatments leading to a loss of data.

b) using the method for variance analysis adapted to disproportionate numbers described in SNEDECOR (1957). This procedure was preferred. The analysis had to be carried out separately for each sex, which is not a disadvantage as sex differences do appear and any interactions may be traced when they are obviously important. When for any character the interaction of RH and density was not important, the effects were not separated. However, when a definite interaction resulted, the four separate effects were calculated and their significance determined by carrying out a variance analysis for the pairs of objects within each treatment as a somewhat less laborious alternative to STUDENT'S t test.

The effects are represented in Appendix 1 as a percentage of the means of the crowded or the dry treatment for comparison with other experiments. In comparing effects on different characters assumed to be not in the same power (e.g. volume or weight data with linear measurements) one must realize that these figures should undergo some correction to be comparable. This can be done approximately by dividing the percentages for second power characters (surfaces, e.g. *Ps*) by two and those for third-power characters (weights and volumes) by three. In case of absence of a significant interaction the main effects were calculated according to a formula in SNEDECOR (1957) in order to be unbiased by the disproportionate numbers.

3.1.2. *Morphometrical results*

The duration of each larval instar (table 1) was not analysed statistically but the conformity between the effect of treatments in the duration of separate instars is evident, they are proportional to the total developmental period.

Isolation under humid conditions and increasing humidity under isolated conditions considerably shortened the duration of every instar, in males as well as in females.

Isolation under dry conditions however did not clearly affect the development of males but gave an appreciable increase in the duration of larval development in females.

Increasing humidity under gregarious conditions in the males, however, gave somewhat indistinct results, probably because the differences were rather small. In the total number of days, however, the result is significant nevertheless. This condition in females resulted in an irregular trend in the L 3 and L 4 comparable to that of the males in the L 2 and L 3.

In both sexes the individuals isolated under humid conditions starting only in L 3 did develop intermediate values for most characters.

Number of days for complete larval development (*D*).

The figures in Appendix 1 show appreciably lower values for the combination isolated-humid in both sexes compared with any other treatment, indicating that neither isolation nor humidity alone produces the entire effect. It is only this very combination which induces the animal to turn green. The influence of humidity on *D* is even greater than the influence of density, but since the dry treatments may have resulted in a poorer food condition, possible this effect is not a pure humidity effect. The effect may be described also as follows: Humidity variations have an important effect on *D* only in isolated individuals and density variations have an appreciable effect only under humid conditions.

Volume of fresh corpora allata 24-28 hours after the final moult (*CA*).

The same general statement concerning *D* made above applies here also for

TABLE 1. Experiment 3.1. Duration of separate instars in days.

	Treatment	L 1	L 2	L 3	L 4	L 5	Total
♀♀	Dry-isolated DI	5.65	4.60	5.10	6.35	9.45	31.16
	Dry-crowded DC	5.50	4.25	4.61	6.02	9.25	29.63
	DC → HI in L3				5.52	8.58	28.21
	Humid-isolated HI	4.62	3.77	4.08	5.23	8.08	25.77
	Humid-crowded HC	5.26	4.05	5.01	6.27	8.41	29.00
♂♂	Dry-isolated DI	5.47	4.53	4.63	5.84	8.84	29.32
	Dry-crowded DC	5.50	4.25	4.61	5.92	9.27	29.55
	DC → HI in L 3				5.24	8.41	28.00
	Humid-isolated HI	4.75	3.50	4.06	4.75	7.81	24.88
	Humid-crowded HC	5.12	4.24	5.09	5.69	8.03	28.17

both sexes, but in an opposite direction. A maximal CA volume was attained only by isolation under humid conditions, thus strongly suggesting a relation to the green pigmentation. In detail, however, obvious differences are present. Under humid conditions the effect of isolation is comparable in both sexes. Under dry conditions, however, only the females react in this way. A reason for this may be found in the general body size reaction to the density factor c.f. the pronotal surface (*Ps*) which probably counteracts the effect on the CA. The influence of humidity under crowded conditions is insignificant in both sexes. It is extreme in isolated males and less extreme, though still very important in isolated females.

Length of elytron (*E*).

In the males no interaction is obvious. Rearing under isolation results in adults with shorter wings. The effect of humidity variations is much less important, it is much smaller than the density effect in the males. In the females the picture is complicated by an interaction. Here too, changes in general size probably counteract relative changes due to density or humidity variations.

Mean length of both femora of the posterior pair of legs (*F*).

This shows the same general response as *E*. Density variation affect *F* in both sexes in the same direction as general body size or the pronotal surface. Humidity effects are somewhat more evident in the isolated than in the crowded treatment.

Maximal width of the head (*C*).

In the males a density effect is evident and relatively more pronounced than the effect on general body size. In the females a density effect is present only in isolated individuals.

Width of the vertex between the eyes (*V*).

This character shows, as could be expected, more or less the same characteristics as *C* in the males. In the females density variations have no effect, whilst humidity variations have the same effect as in the males.

The sternal hair covering (*L*).

In general *L* is longer in locusts reared under dry conditions as also under crowded conditions. In "dry" males crowding has no effect, whereas in "crowded" males humidity variations have no effect. In females no interaction occurs.

The pronotal surface (*Ps*).

Ps is assumed to be a good general size character. In the females it is clearly reduced by crowding but in the males the effect is opposite. Humidity increases size in both sexes in isolation, but has no appreciable effect under crowded conditions.

Fresh and dry weights (KCs_1, K_2, KCs_2).

Most of the weight characters have a tendency to react similarly. No interaction occurs in the males. Isolation in males always leads to a decrease in

weight, though this is not always significant. The same is true of high humidity. Since in the males development was slowed down in high humidities, D and weight characters vary inversely. In females interaction is evident for every character. This is especially caused by the inverse reaction to humidity in both density groups. In crowded females the "dry" individuals show the higher values by way of exception. Attention is drawn to the fact that the weight of the posterior femora (F_2) varies in the same manner as the length (F), and that K_2 in females varies inversely with F_2 on crowding but parallel under increasing humidity. Crowding in females has somewhat unpredictable, though often significant, results.

Morphometrical ratios.

The ratios E/F, F/C, and F/V show a pure density dependent reaction and are in most cases independent of humidity (except for F/C and F/V in the females). The ratio KCs_2/K_2 was calculated tentatively and appeared to show a density dependent effect, stronger under humid than under dry conditions. As both characters decrease on isolation (with one exception) it may be concluded that K_2 decreases relatively more.

The group isolated in L 3 showed in general characters which were inter-

TABLE 2. Experiment 3.1. Comparison of the adult morphometrics of a group of larvae transferred from dry-crowded conditions to humid-isolated conditions in the third larval instar (L 3) with larvae reared under either combination of conditions throughout larval development.

Character	Females			Males		
	Dry crowded	DC → HI in L 3	Humid isolated	Dry crowded	DC → HI in L 3	Humid isolated
D	30.4 ⟨⟨⟨—	28.2 ⟨⟨⟨—	25.8	29.8 ⟨—	27.7 ⟨⟨⟨—	24.8
CA	180.2 —⟩⟩⟩	277.4 ——	306.2	174.9 —⟩⟩	208.7 —⟩⟩⟩	270.3
E	5424.8 ——	5476.1 ——	5485.8	4808.1 ——	4716.6 ⟨⟨⟨—	4470.1
F	2759.0 —⟩⟩⟩	2866.7 ——	2886.5	2528.5 ——	2554.7 ⟨⟨⟨—	2411.0
C	865.0 —⟩⟩⟩	889.3 ——	877.7	772.9 ——	759.9 ⟨⟨⟨—	693.8
V	325.4 —⟩⟩⟩	337.2 ——	331.8	296.6 ——	297.6 ⟨⟨⟨—	267.7
L	53.2 ——	51.1 ⟨⟨—	47.8	53.3 ——	52.2 ——	50.2
Ps	364.9 —⟩⟩⟩	393.7 —⟩⟩⟩	424.3	286.8 ——	294.2 ⟨⟨⟨—	276.9
KCs_1	154.1 —⟩⟩⟩	175.5 ——	168.0	121.5 ——	127.8 ⟨⟨⟨—	101.6
K_2	58.8 ——	56.9 ⟨—	53.6	44.4 ——	41.7 ⟨⟨⟨—	30.6
F_2	56.5 —⟩⟩⟩	66.3 ——	68.2	46.6 —⟩	51.4 ⟨⟨⟨—	41.6
KCs_2	335.8 ——	349.3 ——	339.6	261.9 ——	257.1 ⟨⟨⟨—	203.1
E/F	196.6 ⟨⟨⟨—	191.2 ——	190.2	190.3 ⟨—	184.7 ——	185.4
F/C	319.2 ——	322.5 ——	329.0	327.4 —⟩⟩⟩	336.5 —⟩⟩⟩	347.7
F/V	848.8 ——	851.5 ——	869.8	853.9 ——	859.8 —⟩	889.5
KCs_2/K_2	571.1 —⟩⟩⟩	614.5 ——	635.0	591.3 —⟩⟩	618.9 —⟩⟩⟩	664.4
KCs_1/KCs_2	460.5 —⟩⟩⟩	503.1 ——	496.6	465.5 —⟩⟩⟩	498.2 ——	503.4

⟨— —⟩ Difference significant at $P < 0.05$.
⟨⟨— —⟩⟩ Difference significant at $P < 0.01$.
⟨⟨⟨— —⟩⟩⟩ Difference significant at $P < 0.001$.
—— —— Difference not significant ($P > 0.05$).

mediate between the extreme treatments (i.e. dry, crowded and humid, isoleted), indicating for most characters a gradual change during larval development due to phase transformation (table 2). Apparently the effects accumulate throughout the entire developmental period. A difference in reaction in males and females is obvious. In the males the individuals isolated later were very different from those isolated since hatching and not significantly different from the "dry crowded" individuals in the population from which they originated. In the females, however, the later isolated individuals resembled much more closely the individuals isolated from the beginning. In some of their characters the later isolated individuals were not between the means of both extremes. In all but one case (F_2 in males), however, the difference with at least one extreme was not significant, and thus could be due to random variation.

3.1.3 *Pigmentation.*

Isolated, humid: only 3 out of 25 larvae became green during L 2. The other larvae were brownish or greyish with only traces of pattern and most of them did not start to become green before L 3. The 4th and 5th instar larvae showed a very uniform green colour without any pattern. No sex difference was observed.

Isolated, dry: all larvae were uniformly greyish-brown from the second instar on, 2 females only becoming green during the L 5.

Crowded, dry and crowded humid: under both conditions the larvae had a uniform gregarious pattern throughout their development.

In the series isolated in the end of L 3, three individuals already showed slight traces of green on the day of moulting, 10 did so on the next day. After the moult to L 5 the green pigment was generally present but parts of the black gregarious pattern remained.

3.1.4. *Discussion of the results.*

The shortening in the larval development time combined with a much higher CA volume in the treatment humid, isolated is correlated with the occurrence of the green pigmentation type. All these characters indicate a process which only takes place when both conditions of isolation and high humidity are fulfilled. We may assume that the high CA volume indicates a higher secretion of CA factor(s), which probably induces the other differences to develop.

When we compare the phase indicating ratios E/F, F/C and F/V with the differences mentioned we observe that the values of these ratios are highly independent of humidity as an environmental factor whereas they are very significantly dependent on the density factor. Thus these ratios are really pure phase indicators. Hardly any relation appears to exists between them and the CA volume. The relation between pigmentation and phase ratios thus is not a strict one. The significance of the CA as far as the pigmentation is concerned is clear, but this does not apply for the phase ratios.

3.2. The effect of a combined difference in density and humidity during the fourth larval instar on the resulting fifth instar larvae

The evidence from the first experiment and observations of responses to extra implantations of CA (Joly 1951, Pfeiffer 1945) suggests a higher, or at least altered, CA activity under isolated, humid conditions. This difference could be brough about in several ways:

a) a higher production of the CA factor either continuously or only during definite periods in a larval cycle.

b) a production of CA factor(s) differing with regard to timing from that produced under normal conditions.

c) a combination of both a) and b).

d) a relatively different production of more than one factor by the CA.

Another fact which also requires some elucidation is that of the retention of the green colour from the L 5 to the adult stage. A contradiction is present here for, whilst a deficiency of CA hormone should be responsible for the metamorphosis, the green colour is only sustained in the presence of excess CA factor. A way out can be found by assuming the existence of one of the possibilities b, c or d.

3.2.1. *Experimental conditions*

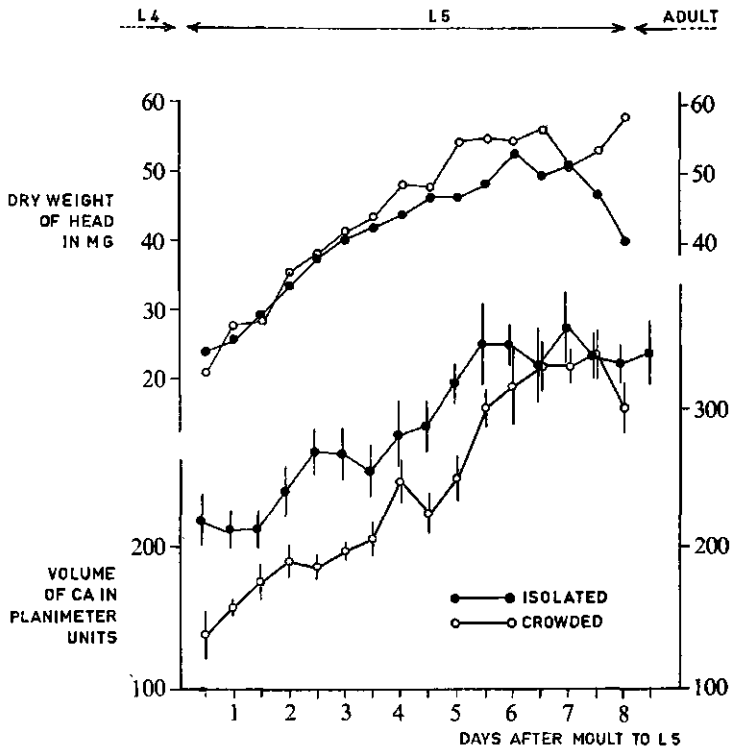
In order to get more evidence about this an experiment was conducted in which a group of isolated larvae was compared with a group of crowded larvae throughout the last larval instar with a view to observing differences in the CA volume during the cycle. Both groups were taken out of the same stock of L 3 just before moulting to the fourth instar. All larvae (120 in each treatment) were reared in standard jars at 30 °C under similar conditions as in the last experiment. In the isolated treatment the jars were provided with paper screens to exclude visual communication between the larvae. Care was taken to choose groups of larvae as homogeneous as possible with respect to size, pigmentation and moment of moulting, for it was very desirable to use animals with the same developmental rate. The animals were reared throughout L 4 and sampled during L 5 at 12 hour intervals in batches of 6 (in which all the individuals had the same age with respect to the preceding moult), starting at the moment of moulting. This moment was also determined with an interval of less than 12 hours. Selection of individuals for the samples was carried out at random according to a fixed scheme. For the crowded treatments separate cages were used for the different 12 hour groups. From the sampled animals the volume of the CA was measured in the way already described, and some morphometric characters were determined as well as the dry weight of the head. The total body weight was not considered because of expected differences in the food quantity present in the gut.

3.2.2. *Results*

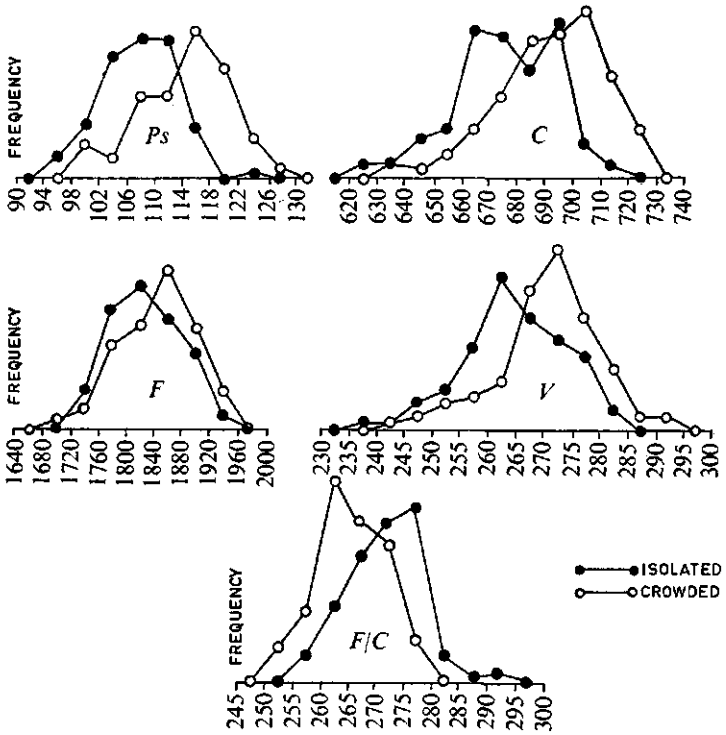
The results are presented in graph no 1 & 2. In graph 1 each point represents a mean of six individuals of one sample, the standard error of this mean being indicated by the length of a vertical line starting at the points. This standard error in the CA volume was surprisingly large, thus any significance of differences in the finer trends of the graphs could not be proven. The graphs, nevertheless, allow for several conclusions:

a. The CA showed rather steady increase in volume only during the first 6 days of the fifth instar in both densities. Thereafter, increase in volume appeared to be arrested.

b. The CA of the isolated males were significantly larger then the CA of crowded males when analysed over the whole instar simultaneously in a variance analysis (Appendix 2). This must be even more true for the first 6 days.



GRAPH 1 (experiment 3.2). The influence of different breeding conditions during the fourth larval instar on the volume of corpora allata and the dry weight of heads in the fifth larval instar, when sampled with twelve hour intervals.



GRAPH 2 (experiment 3.2). The influence of different breeding conditions during the fourth larval instar on various morphometric characters in the fifth instar. The frequence polygons comprise all data in both treatments except for those of the first and the last samples. The larvae in the excluded samples had a soft cuticle and presented difficulties in measuring.

c. The phase difference in CA volume tended to disappear after 6 days. This suggested an exclusive action of density on the secretory activity of the CA which occurs mainly during the first half of the instar, and not on the size and number of inactive cells.

d. The greater CA volume in the isolated group did not correspond completely with larger head weight, for whilst the latter shows a tendency to increase corresponding to that of the CA, it remained definitely smaller as a result of isolation.

e. The ratio *F/C* differed very significantly in both groups of males (table 3) which is a noteworthy fact as this was the result of only 5 days difference in breeding density (the duration of L 4). The same applies to a general size parameter, i.c. the pronotal surface *Ps,* and the pigmentation which became almost green and without pattern in the isolated individuals.

f. The standard error of the means of CA volume in general is very large, par-

ticularly in the isolated males, suggesting a variability in the reaction to the isolated condition.

3.2.3. *Discussion of the results.*

It may be deduced from experiment 3.1, that a relation of CA volume to changes in morphometric ratios is absent. The seeming inverse relation exhibited in experiment 3.2 is brought about probably also by the change in humidity conditions between the two treatments, which affects particularly the CA volume as we have seen. The ratio F/C is probably only influenced by the density conditions. Nevertheless we do not have conclusive proof that the greater CA volume means indeed a higher secretion of CA factor(s); it is only strongly suggested by the relation to the green pigmentation and results of implantation experiments. The presence of two or more CA factors is an alternative possibility that should be considered as well. The greater volume of CA after humid isolation of male larvae in spite of an opposite reaction in general body size indicates either a greater number of cells or a larger cell size and possibly both. This change very probably corresponds to a higher level of CA factor in the blood, leading to the observed morphological differences. In our case this higher level must have been present long before the moult to L 5, otherwise the morphometric differences are hard to understand. A significant difference in CA volume is in fact apparent already at the beginning of the L 5 and lasts throughout the first 6 days. The tendency of this difference to disappear thereafter, at a moment when CA are in fact expected to become inactive (MENDES 1948, LÜSCHER & ENGELMANN 1960) points to the second possibility rather than the first. Speaking in the terms of LÜSCHER & ENGELMANN, a difference in "activity volume", i.e. the difference between actual volume of the gland and the calculated minimum volume based on the number of cells present, between the phases is very probable. The reappearance of the difference before the moment of the final moult, as would be concordant with the former experiment, is probable. Thus a phase or humidity difference in activity of the CA during L 5 seems to exist *during two separate periods*. To test the differences in the finer characteristics of the curves was no possible with this material and shall not be attempted. Attempts to reduce the variability in the CA

TABLE 3 (experiment 3.2). Morphometric changes in fifth instar larvae (L 5) as a result of a combined difference in density and humidity conditions in the preceding instar.

Characters	Crowded	Isolated	Difference	Relative change	Significance
CA	245.9	291.6	45.7	+18.6%	***
Ps	113.8	102.2	6.6	- 5.8%	***
F	1839.9	1828.4	11.5	- 0.6%	
C	691.8	675.5	16.3	- 2.4%	***
K	438.9	414.4	24.5	- 5.7%	**
F/C	267.2	272.2	5.0	+ 1.9%	***
V	270.5	264.3	6.2	- 2.3%	**

volume figures by correlating these volumes to other morphometric values did not prove very successful. Nor could this not have been anticipated in view of the broad range of variability in the CA compared with the much smaller variability of other values. When the result is a smoothening in some parts of the curve by any correction it is always accompanied by a stressing of other irregularities and a determination of the significance of the resulting features of the curves cannot be undertaken. From a comparison of the changes in morphometric values induced by the differences in conditions during L 4 it is apparent that the change in the phase ratio F/C on isolation is brought about by a relatively greater decrease of C. The femur length (F) did not change significantly in spite of the change in general size. In point of fact this means a relative increase.

4. IMPLANTATION OF EXTRA CORPORA ALLATA IN LARVAE

Literature

In a number of experiments extra CA were implanted in L 2 in order to compare morphometrically this interference with the treatment "isolation". Similar experiments were done by Joly (1949) etc. but his results were rather contradictory. In his first report this author stated that he had obtained more gregarious characters after the implantation but conclusive statistical evidence was not given. Apparently it was a misstatement; at least his opinion gradually changed in later papers (in 1952 Joly claims that the adult biometric ratios change in the direction solitaria after the implantation in crowded larvae, "sans qu'il nous ait été possible d'obtenir des copies exactes de cette phase"). These conclusions were derived from the evaluation of the ratio E/F and 3 pronotal ratios (errors were not mentioned). Unfortunately the values of the ratio F/C were not given.

Joly distinguished several categories amongst the adults resulting from the extra implantation, ranging from individuals with very pronounced metathetely ("adultoides") and slight metathetely ("pseudoadultes") to individuals showing no obvious morphometric changes and retaining larval characters. Distinct metathetely, as it usually occurs after extra implantation of CA in any instar, is not a condition ever met with under natural conditions. It involves considerable deformations of the wings which are most often crumpled and shorter than normal and in extreme cases, even larval, i.e. not membranous but having the function of pterotheca. Moreover accompanying deformations in the pronotum, which are probably associated with the wing development, a larval pigmentation and changes in the sternal hair covering are observed.

Joly (1955) described the different effects of implantation on the first and the fifth day of L 5. The former resulted in morphometric changes only whilst gregarious pigmentation remained. The latter gave rise to green pigmentation

with hardly any morphometric changes. Implantations during the last days of L 4 resulted in even more larval pterotheca than did implantations on the first day of L 4 or L 5. This was explained by the influence on the main cellular multiplication in the wings, which takes place mainly between the fourth day of L 4 until the fifth day of L 5 according to JOLY (1955). The absence of pigmentation reactions to implantations on the first day of L 5 was sought in an "inactivation of the implanted CA" in these first days during the period of their influence on wing growth. In the way that it was stated by JOLY, a two hormone concept is implied, but other explanations could be envisaged as well: either an inhibition of green pigment formation during wing growth or a quantitative preferential use of CA factor in the wing development itself. The first possibility would indicate a qualitative control, the second a rather quantitative one, to be overcome by higher amounts of CA factor. This quantity proves difficult to control, however, in implantation experiments, for the results are always very heterogenous. The implantations in the case of JOLY (2 per larva, derived from 18 days old adults) can be considered to have provided an ample surplus of CA factor.

JOLY & JOLY (1953) expressed changed views once more in stating that implantation must be carried out early (in L 2 or L 3) in order to obtain maximal inhibition of wing growth and they claimed that individuals with solitary morphometrics were obtained in this way. The pronotal shape, however, never became truly solitary as represented by UVAROV (1921). JOLY (1954) found that adult CA as well as larval CA are able to produce the mentioned effects and that the quantitative differences present have an influence on the number of larvae reacting positively and the persistance of the pigmentation effect during the subsequent larval development. JOLY (1958) once more specified the influence of the CA on wing development and metamorphosis. Metamorphosis in the course of normal development appears to take place only if the secretory activity of the CA is relatively low during the last days of L 4 and the first days of L 5. The cause of inactivation of the implanted CA during the first days of L 5 with regard to pigmentation remains obscure. There is now sufficient evidence of a release of CA factor during the latter half of L 5 with regard to the influence in pigmentation. It was proven that the induction of the formation of green pigment needs the presence of excess CA factor only at the time of moulting, when growth is assumed to be minimal. Apparently, however, something inhibited this release in the CA implanted by JOLY. Such inactivation cannot be ascribed to a general exhaustion of implanted CA since a difference of 1 day in the moment of implantation (the instar lasting 9 days) may produce the opposite effect, i.e. no inactivation and the formation of green pigment. Since the inhibition factor could not have been transmitted into the implanted CA via the nerves a humoral pathway is the only possible alternative. This opposes the views of SCHARRER (1952) and ENGELMANN & LÜSCHER (1957) who claim an inhibition through nervous pathways.

EXPERIMENTAL CONDITIONS (Exp. 4.1 and 4.2)

Extra implantations of CA were carried out in two groups of experiments. In each group two CA obtained from immature adults were inserted through the neck membrane. In control samples the neck membrane was also perforated. The resulting adults were measured and the data were analyzed in a scheme for disproportionate numbers. For each of the two sexes and group 4 or 5 different samples were available. In case of no interaction the unbiased mean treatment effect was calculated, in case of significant interaction the effects were calculated and analyzed per experiment. The effects were also expressed as a percentage of the control samples. In the first group (4.1.), CA were implanted in the L 2 less than 24 hours after the preceding moult. In the second group (4.2) implantation was carried out in the L 5 after varying time intervals from the last moult in order to detect possible critical periods.

MORPHOMETRIC RESULTS (exp. 4.1. and 4.2.).

The number of days between the operation and the final moult (*D*) was not analysed in the classical way because of deviations from a normal distribution. The test of WILCOXON (independent of distribution) applied separately for the samples assigns significance to a few treatment differences only (Appendices 5 & 6). From a comparison of the data in the Appendices 3, 4, 5 & 6 it is apparent that the general trend in both sexes is a decrease in *D* following implantation (in 16 cases with 2 exceptions). When these differences are tested according to the simplest method (sign test) a probability smaller than 0.01 of this distribution occurring by chance may be assigned to this conclusion.

The lengh of the elytron (*E*).

The wide variability in the animals of the treated groups compared with the controls in fact should prohibit an analysis of variance. This procedure tends to underestimate the differences as can be seen in the separate analyses in cases of a significant interaction. However, the wings were distinctly shorter. Individual variation in reaction probably depended on the actual activity of the implantate.

It is obvious furthermore that only implantations carried out before the third day of L 5 had a clearly shortening effect. Later implantations failed to modify *E*.

The femoral length (*F*) fails to show any effect of implantation.

The maximal width of the head (*C*).

Implantation in L 2 had a decreasing effect in both sexes. This is surprisingly different in L 5, during which instar implantation most often has an opposite effect. No explanation for this difference can be given as yet.

The width of the vertex. For *V* the same remarks apply as were made in relation to *C*.

The mean length of the hair covering (*L*). The effect uniformly is a distinct decrease, especially after implantation in the first days of L 5.

E/F. The remarks made in regard to E apply to a certain extent for this ratio as well. This hardly affects our conclusion that a general decrease is taking place.

F/C. The general increase following implantation in L 2 is obvious, the decrease after implantation in L 5 could be expected according to the behaviour of C. In the L 5 females it is clear that the effect is strongest the earlier the operation is performed, though the interaction is not statistically significant. A more detailed analysis will be carried out in paragraph 5.5.

F/V. This ratio varies in the same way as F/C. In general the significance of differences is lower, so it may be concluded that the ratio F/V discriminates less than F/C.

K_2 and KCs_2. A decrease after implantation in L 2, and an increase in early L 5 (for both characters) are again contradictory results.

KCs_2/K_2. Changes in this ratio were strongest in females, but only significant after implantation in L 2.

PIGMENTATION.

Experiment 4.1, group implanted in L 2. The hoppers selected for these experiments showed a uniform gregarious pigmentation at the moment of treatment. In the controls this character remained unaltered throughout the experimental period. Operated larvae, however, turned green, most often within one day after the next moult and they remained so throughout larval development, with a few exceptions only. The black pattern disappeared to a large extent already in the moult to L 3, and in most cases completely during the moult to L 4, the larvae closely resembling solitary ones at that time. The resulting adults were a little more variable; a number given in table 4 lost the green shade and showed a more or less gregarious pigmentation. This adult pigmentation was always preceded by a return of some pattern and of some orange colour in L 5, but the green colour usually did not disappear entirely in these

TABLE 4. Conspicuous changes in pigmentation and metamorphosis in adults following extra implantations of CA in L 2 (experiment 4.1). Data represent numbers of individuals.

Sample Nr.		Age in instar in days	Wing metathetely		Wings normal		Moulting casualties	Total number
			not green	green	not green	green		
A♂♂	1	1	–	1	–	3	–	4
	2	1	–	2	2	11	1	16
	3	1	–	2	3	6	–	11
	4	1	–	4	3	6	–	13
	5	1	1	–	4	11	–	16
B♀♀	1	1	–	1	–	2	–	3
	2	1	–	–	1	9	–	10
	3	1	–	1	3	5	–	9
	4	1	–	1	2	7	–	10

TABLE 5. Conspicuous changes in pigmentation and metamorphosis in adults following extra-implantation of CA in L 5 of different ages (experiment 4.2). Numbers of individuals.

Sample Nr.		Age in instar in days	Wing metathetely		Wings normal		Moulting casualties	Total number
			not green	green	not green	green		
A♂♂	1	0–1	–	8	–	–	–	8
	2	1–3	–	10	–	–	–	10
	3	3	–	6	2	–	–	8
	4	3–8	–	–	7*)	1**)	–	8
	5	7	–	–	4	2	5	11
B♀♀	1	0–1	3	5	1	–	–	12
	2	1–3	4	3	3	–	–	10
	3	3	–	3	3	–	1	6
	4	3–8	–	–	6	1	2	9
	5	7	–	–	–	10**)	2	12

*) The pattern was distinctly larval.
**) The green colour was atypical, more yellowish or olive-green. The pattern in these individuals was adult, but as in the cases of metathetely the femoral bands were often only slightly or not developed.

cases until at the final moult. As is seen in table 4 & 5, obvious metathetely (short crumpled wings and short sternal hair covering) is almost always accompanied by a more or less green pigmentation. These metathetelic individuals were characterized furthermore by absence of the typically transversal adult black femoral melanin stripes, a character not exhibiting distinct phase-dimorphism in *Locusta* in opposition to the longitudinal stripe in *Schistocerca* (NICKERSON 1956). It is important to note that the persistence of the green colour into the adult in many cases is not accompanied by a clearly disturbed metamorphosis. This is the case in *Locusta* under isolated breeding conditions as well (but not, as was already pointed out, in *Schistocerca* which looses all green pigment in the final moult).

Experiment 4.2, the group implanted in L 5.
Some new aspects concerning pigmentation are observed on implantation of CA at different moments during the last larval cycle (table 5).

1. Implantations from the third to the eighth day do not induce distinct metathetely but do most often influence pigmentation in adults. The result is a greenish yellow or olive green colour unlike the normal green larval shade, which most often continues intensifying during the first days after the moult. Often the patterns in these cases show only slight larval tendencies in the absence of femoral bands (most often not accompanied by larval pronotal bands). The origin of the more yellowish colour is uncertain but one is inclined to connect it with the yellow maturation colour common in male *Acrididae*, which is due to a redistribution of carotenes in the integument. As shall be argued later this colour is undoubtedly related to the CA factor (LOHER 1960).

2. Amongst female larvae only, a number of individuals react to an early implantation by showing metathetely *without green pigment*, a result already reported by JOLY (1955) who unfortunately did not state the sex of the larvae in the experiments concerned.

DISCUSSION OF THE RESULTS OF EXPERIMENT 4.1 AND 4.2.

A comparison of the effects of implantation of extra CA with the effects of solitary breeding (experiment 3.1) shows that certain similarities exist, though not always quantitatively, even when we take into account the somewhat shorter effective period (from L 2 on) in the implantation experiment. *D* certainly reacts similarly but quantitatively less, *E* reacts much more strongly in the cases of apparent metathetely leading to extreme *E/F* ratios. The absence of reaction in *F* is somewhat surprising and probably points to the negligible change in general size. A comparison of *F* and *Ps* in experiment 3.2 makes it clear that changes in *F* are in fact closely related to changes in *Ps*. The implantation effects on *C* are rather uniform for both sexes. They are smaller than by breeding in the males and probably also in the females, in which *C* even increases relatively. The hair length *L* shows more important changes after implantation than by breeding in isolation. This probably reflects metathetely, but the tendencies are comparable. The ratio *E/F* is similarly a good parameter for metathetely. It presumably obscures the proper phase tendencies in implantation experiments. The ratio *F/C* probably is more suitable as a phase character. Here it is obvious that implantation effects are rather similar to low density effects. Unexplained anomalies occur after implantation in L 5 as a consequence of the anomalies in *C* and *V*. The ratio *F/V* adds no new evidence.

Body weight reacts to implantation in the L 2 with a decrease in both sexes, but with an increase when the operation is performed early in L 5. The effect on either of the two sexes is different in isolated breeding.

Summarizing it becomes clear that the effect of implantation of extra CA does parallel the effect of isolated breeding as regards pigmentation (when compared with breeding under humid conditions only) and some morphometric phase characters, particularly *F/C* (table 6).

TABLE 6. Relative changes in F/C as a result of isolation or extra-implantation of CA.

Exp. nr.	Instar in which the operation took place or in which isolated conditions started	Instar in which F/C was measured	Change in F/C as a result of isolation or extra implantation of CA	
			males	females
4.2	Implantation in L 5	adult	max. −4.5%	−2.9%
4.1	Implantation in L 2	adult	+2.7%	+4.5%
3.1	Isolation in L 1	adult	+5.6%	+4.4%
3.1	Isolation in L 3	adult	+2.8%	+1.1%
3.2	Isolation in L 3	L 5	+1.9%	

A distinct difference exists between the general body size reaction which is almost absent after implantation and does not show distinct sex differences either and the occurrence of metathetely which is sometimes claimed to be a more extreme form of solitary morphometrics. This, however, is not proven when we compare the change in F/C following implantation in the adults with the effect of isolated breeding during various periods (table 6).
A certain accumulation of the changes in F/C in successive larval instars is very probable, especially in the males. The effect of CA implantation does not turn out to be quite so effective as the effect of isolation in the male sex, though the ratio E/F tends to be much more extreme. This supports the idea that solitary characters are virtually something different from expressions of a slight metathetely. In the females the effect of implantation equals the effect of prolonged isolated breeding, or perhaps rather the effect of an early solitary induction, a later induction being much less effective on F/C. The effect of implantation in the L 5 on adult C and F/C is anomalous and cannot be explained as yet. The biometric changes are not closely related to pigmentation changes, and under certain conditions either of the two may be produced. Even different elements of the pigmentation are not related: green pigment may accompany larval as well as adult pattern and may exist temporarily in the presence of some gregarious pattern as well.
The femoral bands are another independent character of the metamorphosis. The critical moment for the determination of these bands seems to be different from that for the other aspects, and is presumably situated in the last days of the L 5. Probably a distinct critical moment for the formation of the green pigment is absent. Green colour developing in larvae not previously green most often does so within a day after a moult but may also do so later in the course of the instar. In the first case the induction probably takes place just before or during the moult, for green colour induction during the entire period of L 5 would not agree with the outcome of experiments which point to a low level of CA factor somewhere in this instar. More evidence pertaining to this point will be given in further experiments.

5. ARTIFICIALLY INDUCED CORPUS ALLATUM DEFICIENCY

5.1. TOTAL EXTIRPATIONS

The most drastic way to produce a permanent deficiency of CA factor is of course the total extirpation of these glands (allatectomy). This was carried out by means of methods already described on page 22. The results of this operation agreed with current views on the functions of the juvenile hormone. Depending on the moment within the instar, at which allatectomy is carried out adult characters are developed either during the next moult or one moult later. The most obvious features discriminating adults and precocious adults from larvae are:

a) a thick cuticle

b) an adult pigmentation (e.g. absence of orange pigmentation, different patterns on the pronotum, presence of femoral stripes)

c) a long sternal hair covering

d) true wings, though crumpled, provided with flight muscles which may produce wing vibrations even in prothetelic L 3

e) the performance of copulation attempts by males.

A characteristic phenomenon accompanying the experimentally advanced development of adult characters is a prolongation of the duration of the instar. This may be compared with the longer duration of the L 5 in normal development.

A fraction of the thus produced prothetelic individuals tries to moult once more. This, however is invariably fatal, because these larvae obviously are incapable of shedding the already adult cuticle and consequently perish. The remaining fraction may live for weeks and months and in both sexes develop the characteristic reddish brown shade normal to mature females. The males never turn yellow at all as do normal males under crowded conditions. The proportion of prothetelic individuals attempting to moult once more varies, one of the basic sources of variation being the moment of allatectomy within the instar. An important part of the variation, however, cannot be explained as yet.

The effects of allatectomy on the pigmentation are manifest. They do not need a detailed description of the experiments concerned. The following points may be briefly mentioned:

a) allatectomy on L 2 with gregarious pigmentation induces the corresponding gregarious adult pigmentation in the L 3 or L 4.

b) allatectomy in the L 2 adapted in colour to the background induces metamorphosis with retention of the characteristic adapted colours, as is the case in the normal metamorphosis in such larvae.

c) allatectomy in green solitary larvae is a somewhat difficult procedure because these larvae are much less resistent to the stress of the operation than normal larvae. The laborious breeding of these isolated larvae is another point to be considered. Both factors together make a good experiment almost impossible.

Thus no serious attempts were made to operate upon a large number of solitary individuals. An alternative method is the reextirpation of earlier implanted CA which already have caused the hosts to become green. This procedure gives better results and has been followed in some experiments.

5.2. Reextirpation of previously extra implanted adult CA in larvae

Experiment A

A number of L 2 were subjected to allatectomy within 48 hours after the moult. In each of these larvae one CA obtained from immature adults was implanted and attached to the mandibular muscles in the head capsule by in-

serting one of its nerve stumps between the muscle fibers by means of a very thin needle. These larvae all turned green after the moult to the L 3 (2—4 days later). On the 6th day the implanted CA was reextirpated in 7 selected uniformly green larvae, a group of 7 green larvae acting as a control series. Mortality finally reduced both batches to 3 in the course of the following days. The moult to L 4 took place 2—3 days after the reallatectomy, at that time the green colour in the first group was reduced to a greyish or yellowish green with only slight traces of pattern. Two days later, however, all green had disappeared, a greyish yellow colour remaining. The control group was still very green. Strong adult prothetelic characters did not develop before the moult to L 5 (16–17 days after reallatectomy). The control group produced some slight adultoid characters in this moult as well (hypertrophied wings, femoral pattern) but the green colour remained present for at least a week afterwards. The reextirpated CA were reimplanted in some gregarious L 2. They all became green in L 3. Consequently the CA retain their activity even after passage through another host.

Experiment B

In this experiment CA from young mature adults, implanted in the first half of the L 2, were reextirpated 7 days later, when all ten hosts were L 3 and had an almost solitary green pattern. The complete loss of green colour after reextirpation occurred in the middle of L 4. The reappearence of some pronotal pattern (a broad pale band) accompanied the loss of green pigment. In L 5 the normal gregarious patterns were present again. In the male group a rather normal gregarious pattern returned, but in the females an unmistakable tendency to develop a grey, black, more or less solitary pigmentation type with much less orange was observed, despite a somewhat higher breeding density in this group. In the control groups without reextirpation the green colour was preserved throughout further development. Faint traces of pattern came back in a few cases in the L 5. The resulting adults sometimes showed metathetelic characters. In a group of male larvae the CA were extirpated together with the implanted CA. The green colour disappeared at about the same rate as after reextirpation of the implanted CA only. Adult characters did not develop before the moult to L 5. The most important biometric data for the adults are given in table 7.

TABLE 7 (experiment 5.2). Some morphometric values for the treatments.

Treatment		(n)	*D*	*E/F*	*F/C*	*L*
♂♂ Implanted CA reextirpated	a	10	23.3	182.6 ± 1.5	350.8 ± 1.7	51.9 ± 0.8
♂♂ Controls (no implantation)	d	3	23.7	180.7 ± 3.8	350.3 ± 7.3	54.0 ± 1.5
♂♂ Controls with CA implantation	c	4	23.5	171.3 ±11.4	355.3 ± 4.5	43.0 ± 3.2
♀♀ Implanted CA reextirpated	e	9	24.8	186.6 ± 1.0	337.6 ± 1.7	47.0 ± 1.2
♀♀ Controls (no implantation)	f	8	24.0	188.1 ± 1.6	326.9 ± 2.6	49.3 ± 0.8
♀♀ Controls with CA implantation	g	3	23.3	179.0 ± 3.0	352.5 ± 5.5	41.0 ± 2.3

It may be concluded that the green pigment is strictly dependent on the CA factor. When the source of this factor disappears, the green colour is sustained only for a few days.

5.3. Total extirpations followed by reimplantations of adult CA a varying number of days thereafter

In order to determine critical periods for the influence of CA factors on morphology and pigmentation, allatectomy was performed in young larvae and reimplantation of an adult CA was effected at different intervals after the operation. In certain respects the results should resemble those of extra implantations of CA in the L 5, if it is true that the CA factor is at a very low level in this instar during normal development.

5.3. (A). *Allatectomy followed by reimplantation after the next moult.*

Extirpation of CA was carried out in a number of L 2 of both sexes and less than 24 hours after the preceding moult. For reimplantation the manifestation of clear signs of CA deficiency was awaited, i.e. the appearance of adult characters at the next moult. In this case all reimplantations were carried out within 24 hours after the moult to L 3.

Results.

Males. Of the prothetelic L 3, twelve out of the 17 turned more or less yellow some days later (3 after 4 days, 4 after 5 days, 1 after 6 days, 2 after 7 days, 1 after 8 days and 1 after 9 days). This yellow colour most often intensified further during next few days and finally resembled to a considerable degree the yellow maturation colour of mature male adults. Amongst the other 5 larvae there were two which died before the 4th day after the operation. These two died on the 12th and 15th day. Three of the yellow individuals attempted to moult again (one after 6 and two after 11 days) but did not survive. In the individual moulting again after 6 days it was observed that the new pronotal cuticle was coloured green although in the L 3 only yellow was seen and no green whatsoever.

Females. Of 12 females treated in the same way 3 became slightly yellow, particularly on the frontal side of the head, after 5, 6 and 8 days respectively. These three all attempted to moult again after 10, 15 and 17 days. Two other larvae died after 2 days and 7 never became yellow at all in 15 days. Two out of these 7 did attempt to moult again after 15—17 days. The 4 others died at the age of 10—17 days through other causes.

The difference in reaction of the sexes is in agreement with the situation during maturation. Crowded males turn almost entirely yellow – even the formerly isolated and consequently green ones –, whereas females under the same conditions show only traces of yellow, particularly on the head.[1])

[1]) In *Schistocerca*, females turn yellow almost to the same extent as males.

From this experiment it may be concluded that:

a) The yellow maturation colour is dependent on a CA factor.

b) The yellow maturation colour appears only when some adult differentiation has taken place. Thus the presence of the CA factor must be preceded by a period of deficiency.

c) A low level of the CA factor and subsequent adult differentiation are not the only elements in the breakdown of the activity of the VG, not even when the CA factor is introduced again afterwards. According to BODENSTEIN (1953) breakdown of the prothoracic glands in *Periplaneta* is possible only in adults with active CA and not in allatectomized adults and prothetelic larvae. In the present experiment, however, we see that the contingent presence of CA is without influence on the induction of moulting and presumably also on the state of the VG. Generalization is thus obviously not justified.

d) It remains a peculiar fact that isolation in normal larvae and crowding in adult males both result in stimulation of the CA with subsequent pigmentation effects. The possibility cannot be excluded yet that crowding in the adult males in particular prepares the substrate so that reaction to the CA factor becomes possible.

e) The difference in the yellow coloration between the sexes probably has other causes than a difference in quantity of the CA factor.

f) The transition from yellow to green in the supernumary moult of a prothetelic L 3 means that some degree of regression in the differentiation must have taken place. Without this the green colour could not have established itself in the integument. In fact, a turnover to green colour later than directly after the final moult has never been observed in adults.

5.3 (B) *Allatectomy followed by reimplantation at various intervals thereafter*

In this experiment a comparable complete extirpation of CA in males was followed by reimplantations in the same L 2, a variable number of days after the first operation. The results are taken together with a complete account of the experiment in table 8.

It may be concluded that reimplantation within the first 2 days after allatectomy in most cases prevents the appearance of adult characters. The larvae turned green and developed quite normally at about the same rate. Reimplantation after 3 days cannot prevent the appearance of slightly adult characters in a few L 3. The prothetelic abnormality observed in these green larvae was a hypertrophy of the wings without other distinctly adult characters. A few only attained the adult stage. With a 4 days interval distinct adult characters appeared in most of the L 3 which were nevertheless green. Consequently the L 3 lasted longer than normal and was followed by an abnormal moult during which most larvae concerned perished. After a 5-day interval the effect was still somewhat stronger and in this series one completely green prothetelic larvae appeared which turned somewhat yellow after a few days. This effect was present more strongly in the group which was reimplanted after 6 days.

TABLE 8 (experiment 5.3.B). Males. The effect of an increasing interval between allatectomy and the reimplantation of one adult corpus allatum. In the upper row in each treatment the mean duration of each instar (in the first column the number of days between reimplantation and next moult) is given. In the second row the numbers of individuals showing distinct adult characters, green colour and absence of green colour are given. The decrease of the numbers during development is mainly due to the perishing of larvae with a more or less adult cuticle during subsequent moulting. These perishing individuals could not be used in a classification of the pigmentation in most cases.

Days between allatectomy and reimplantation	Duration of instar	Adult characters	± green	not green	Duration of L 3	Adult characters	± green	not green	Duration of L 4	Adult characters	± green	not green	Duration of L 5	± green	not green
	4.3				*4.8*				*6.2*				*9.8*		
0	12	0	11	0	9	0	9	0	9	2	9	0	9	⩾4	
+1	*3.7*				*4.8*				*7.8*				*10.0*		
	10	0	9	0	8	0	8	0	8	5	6	1	3		
+2	*2.6*				*5.0*				*7.8*				*17*		
	10	0	8	0	8	2	8	0	5	2	3	1	1		
+3	*2.4*				*6.9*				*6.8*				*10.3*		
	11	2	9	1	10	6[1])	8	1	6	2	5	1	3	3	
+4	*1.1*				*10.5*				-				-		
	10	7	6	2	8										
+5	*2.3*				*13.0*				-						
	10	7	6	1	4	[2])									
+6 (L 2)	*3.3*				-										
	4	4	3			[3])									
+6 (L 3)					*7.9*										
	17	17	0	17	7	[4])									
Controls	*3.1*				*4.7*				*6.5*				*9.3*		
	10	0	0	10	10	0	0	10	10	0	0	10	10	0	10
L 2		L 3				L 4				L 5				Adult	

[1]) Only a slight wing hypertrophy was observed.
[2]) One green L 3 did not moult again and turned somewhat yellow.
[3]) The three remaining larvae did not moult again and turned distinctly yellow.
[4]) The ten remaining larvae did not moult again and turned distinctly yellow.

The 3 surviving larvae all turned green after the moult. Yellow coloration was observed in these larvae 8 days after the moult and continued to intensify during the next day. As the duration of the L 2 of these allatectomized larvae was not longer than 5 to 6 days, one more batch of them was treated after the moult ot L 3. (The interval without CA of these 17 larvae all being more or

less prothetelic was also 6 days). Fourteen of them became yellow 3 to 7 days after the reimplantation, 3 did not respond. Of this group of seventeen 7 moulted again and perished in their exuvium. The others died up to 23 days following the reimplantation without any sign of moulting.

Since we do not know either the values of the decreasing hormone level after allatectomy in the body or the amount of secretion of the CA after implantation, definite conclusions about critical periods cannot be drawn. Nevertheless it may be concluded that an interval of 0 to 2 days between allatectomy and reimplantation has no irreversible results. With an interval of 3 days slight prothelic effects are produced, which are probably partly reversible. With intervals of 4 days and longer distinct adult characters appear and the subsequent moult is inhibited or is abnormal. With intervals of 5 and 6 days preponderantly permanent L 3 occur. A fraction of these yellow larvae, however, attempts to moult once more.

Under these experimental conditions the course of events in green solitary adults which turn yellow on crowding was imitated by the timed reimplantation. Induction of green pigment formation in *Locusta* apparently may take place in larvae with any degree of adult differentiation.

A relation between the occurrence of further moulting attempts and the appearance of yellow and green colour is obviously absent. This already renders it improbable that the *ventral gland* plays a direct role in the control of the pigments concerned. Attention is drawn to the fact that the reimplanted larvae were reared under gregarious conditions, so that the possibility that gregarious stimuli were still a factor in the induction of yellow colour cannot be excluded.

Another direct proof of the CA function with regard to the yellow coloration is found in experiments in which allatectomy was performed either in L 5 or in young adults. In the first case quite normal adults are produced suggesting once more that normal development in the last larval instar is not dependent on a high activity of the CA. The resulting adults in neither case turn yellow under conditions suitable to produce this colour in untreated males.

5.4. Various operations on the innervation of the CA and CC

In order to obtain more quantitative details about the CA functions, methods were attempted in which the quantity of CA factor produced could be influenced by surgical methods other than extra or replacing implantations, since these always tend to produce rather variable results. The following procedures were envisaged:

a) extirpation of one or more CA but leaving at least one or a part of one CA intact

b) severance of the nervi corporis allati (NCA), the nervi corporis cardiaci (NCC) or the nerves between the CA and the suboesophageal ganglion. The CA are retained in situ in these operations by strands of connective tissue or by the nerves connecting it with the suboesophageal ganglion. The results of

severance of the nervous pathways to the brain could not be predicted beforehand, as this operation may produce:

a) a release of inhibition by the brain, particularly in the L 5, as was proven by ENGELMANN & LÜSCHER (1957) and SCHARRER (1952) in cockroaches.

b) the opposite effect, i.e. a decrease in activity by cutting off the neurosecretory supply or by severing of activating nerve fibers. Moreover the possibility exists that a total severing of the supplying nerves has a double result i.e. both a and b, resulting in an unpredictable change in activity. When in reality both functions are exerted by one nerve trunk simultaneously it should not *a priori* be considered impossible to separate them by refined surgical methods, since both are probably conducted by different nerve fibres.
A controlling function of the nerve connecting the CA with the suboesophageal ganglion was proved by ENGELMANN (1959) for adult *Leucophaea.*

5.4.1. *Experiment 5.4.1.*

In a group of female L 2 less than 24 hours after the preceding moult the following operations were carried out:

a) Severing of the right nervus corporis allatum (NCA) only
b) The same, but with also the left CA removed
c) The right CA slightly bruised with forceps
d) Severing of both nervi corporis cardiaci (NCC)
e) Severing of the nervi corporis cardiaci on the right side only.

The volumes of the CA were determined 24 hours after the final moult in the adults and simultaneously in the prothetelic L 5. The values for both groups were pooled in some cases when no significant difference between the groups could be detected. For the calculation of the means only those individuals were considered in which dissection in the adult showed that the previous operation had been carried out in agreement with the intention. Morphometric data were determined in adults only.

From table 9 we learn that severing of the nerve connecting the CA with the CC seriously inhibits the growth of the CA. When the other CA was left undisturbed no influence on development was observed (with one exception) but when it was removed simultaneously, 5 out of 10 treated larvae developed prothetely in the L 3 or the L 5. The remaining 5 larvae, however, developed normally in spite of the very small total CA volume. From this it may be concluded that these small CA show at least a minimum of activity, which apparently is just above the minimum necessary for a normal development. Obviously we should distinguish between two processes:

1) growth of the gland, by an increase either in cell volume or in the number of cells
2) secretion.

By intersecting the NCA (a, b) we have arrested the increase in volume of the gland but not its secretory activity. In the present experiment we are not able

TABLE 9 (experiment 5.4.1). Influence of severing the nervus corporis allatum (NCA) or the nervus corporis cardiacum (NCC) on the duration of the pre-adult development, the growth of the corpora allata and the occurrence of prothetely. The CA volumes are given in planimeter units and accompanied by their standard errors (of means). The numbers between brackets denote the numbers of individuals or individual observations used for the calculation of means.
The sign ≠ means "severing".
I = number of individuals completing a normal development.
II = number of prothetelic larvae and instar concerned.
III = number of individuals dying or disappearing during the development.

	Operation	Mean volume of single CA one day after adult moult	Mean volume of single CA 3 weeks after adult moult	Mean number of days until adult moult	I	II	III
a							
	Left undisturbed	259.6 ± 32.0	972.5 ± 94.0	28.3 ± 0.4			
	Right ≠ NCA	46.6 ± 3.2	51.8 ± 8.8				
	(n) (31)	(10)	(6)	(26)[1])	26	–	5
b							
	Left CA removed	–	–	28.4 ± 0.9			
	Right ≠ NCA	46.8 ± 3.9				1×L 3	
	(n) (15)	(5) [2])		(5)	5	4×L 5	5
c							
	Left CA removed	–	–	29.7 ± 0.4			
	Right Ca bruised	163.9 ± 13.6	–			1×L 3	
	(n) (15)	(7) [3])		(6)	7	4×L 5	4
d							
	Left ≠ NCC	126.6 ± 24.4	–	27.1 ± 0.9			
	Right ≠ NCC		–				
	(n) (15)	(12) [4])		(7)	7	1×L 5	7
e							
	Left undisturbed	227.3 ± 34.5	1073.6 ± 109.1	27.4 ± 0.5			
	Right ≠ NCC	89.5 ± 17.7	151.0 ± 64.5				
	(n) (15)	(8)	(3)	(13)	13	0	2

[1]) Including one metathetelic adult.
[2]) 2 adults and 3 prothetelic L 5.
[3]) 5 adults and 2 prothetelic L 5.
[4]) Only adults.

to discern whether the secretion per volume unit is higher in the denervated small CA than in normal CA. This possibility should be envisaged, for it may not be excluded that hormone production by the CA is regulated partly by the quantity of a precursor delivered by the brain. In this case as well as in the

case of extra implanted CA the activity continues, so that it is certain that stimuli from the brain can reach the CA without any nervous connection.
In the series in which one CA was bruised with forceps (c) the final CA volume was definitely larger than in b but less than in undisturbed CA (a, e); 5 larvae showed prothetely (1 in L 3, 4 in L 5). The final CA volume of the four L 5 was not different from the adults in the same treatment. The interpretation of this result when compared with the results of denervation is not easy. As we will see in further experiments, growth without secretory activity is not impossible and may apply in these cases.
Severance of the NCC on both sides (d) resulted in a growth inhibition in the CA which was less severe than after intersecting the NCA. The final size of the CA was less than in group e (though not significant) but nevertheless only one larva developed into a prothetelic L 5. We should envisage at least three possibilities:

a) the intact connection with the hypocerebral ganglion still provides for some transmission of neurosecretion. Supporting this possibility is the histologically observed neurosecretion in this nervous connection (HIGHNAM 1961).

b) the remaining parts of the CC have a function in the collection and passing on of humorally transmitted neurosecretion and/or activating hormone. The described functions must also be present in detached and implanted CA, but this is hardly detrimental to the hypothesis as these implanted CA are likely to show some decrease in activity as compared with the normal condition.

c) special stimuli originating in this part of the CC are transmitted by activating nerve fibres to the CA.

Severing the NCC on the right side only (e) had a comparable result. The difference with (d) is not significant at a 5% level. Because of the remaining intact CA no obvious signs of shortage of CA factor appear in treatment (a). Though not significant, an increase in the volume of the abnormal CA during adult life is observed.

The occurrence in treatment (a) of one metathetelic adult which had a gregarious larval pigmentation was of special interest. On dissection, the volumes of the CA of this individual turned out to be 141 and 170, differing considerably from the normal response to this treatment. But the volume of these CA was still less than the means of untreated CA. This means that the observed volume of the CA could not provide an explanation for the assumed abnormal activity during the last instar. It rather suggests a release of inhibition without disturbing the supply of precursor or activating stimulus. Presumably a very special local injury to the NCA or an abnormal regeneration of the nervous connection had occurred. It must, however, be stressed that this individual was the only case of apparent metathetely produced in several experiments by a treatment other than extra implantation. For the calculation of the volume means and the mean of biometric ratios this individual was left out of consideration as being an apparent abnormality. The mean morphometric values and their ratios for the different treatments most often did not

show significant differences. This is certainly partly due to the low numbers of individuals in some treatments. For this reason, these figures will not be analysed more intensively.

5.4.2. *Experiment 5.4.2.*

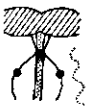
A comparable experiment was carried out on gregarious male L 4, less than 48 hours from the preceding moult. The treatments applied were:

a) Severing of the NCA on both sides.
b) Severing of the NCA on the right side only.
c) Controls (all the manipulations of a and b except for the severance).
d) Controls (non-operated).
e) Allatectomy on both sides.

All larvae were reared together in a cage at 30 °C. Dissection of a proportion of the larvae was carried out after the final moult. All the larvae moulting on one day situated at about the peak of the moulting activity for the whole group were retained for one more month in order to observe the changes in CA volume. This group comprised adults from all treatments except e.

Direct results of the treatments: The larvae of group e all became fifth instar

TABLE 10 (experiment 5.4.2). The influence of severing the nervus corporis allatum on the appearance of yellow maturation colour in crowded male adults.

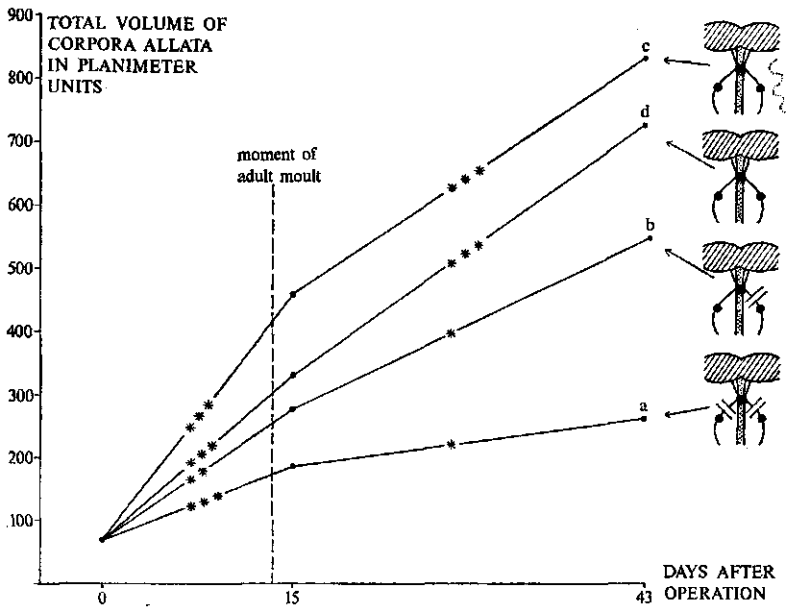
Operation	Number of adults in which the yellow colour was observed for the first time										Not yellow after 4 weeks of observation	Total
	10	11	12	13	14	15	16	17	18	19....		
a 		1									12	13
b 		1	2	2	4		1	1			1	12
c 	1	4	2	4							0	11
d 				2	4	1					0	7
											28	Number of days after adult moult

adults. In the other treatments no deviations from the normal development were observed. The resulting adults always had a normal appearance.

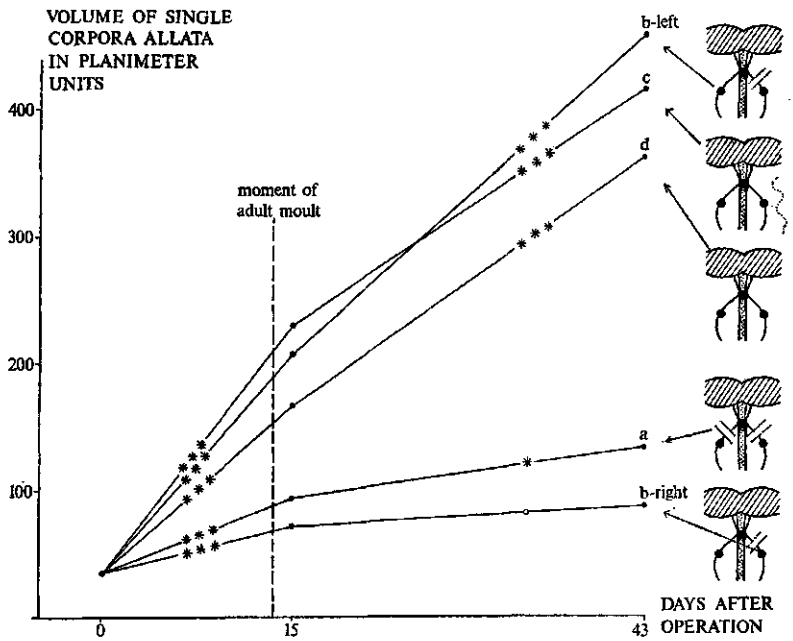
A difference between the treatments became manifest in the retained group with respect to the yellow maturation colour (table 10). In order to prevent a mutual influence on the velocity of maturation of adults reacting more slowly (NORRIS 1954), the individuals were moved to another cage at the first signs of a yellow colour. In table 10 we observe marked differences between treatments for this character. Both control groups c and d and the group b with one normal CA turned almost entirely yellow between the 10th and the 17th day after the final moult, but in group a), only one male turned yellow during the 28 days of observation.

A difference between group c and d is evident and this is in agreement with observations of NORRIS (1954) who found that mutilation accelerated the onset of maturation in *Schistocerca*. It remains remarkable however that this has still an influence after two moults in a group in which all the individuals performed their last moult on the same day. The means of the volumes of the CA together with the significance of differences are presented in a graph. For comparison the mean CA volume of a group of 10 gregarious L 4 of the same origin as the operated groups was introduced in the graphs 3 and 4.

Severance of both NCA a) again inhibited the growth of the CA compared



GRAPH 3 (experiment 5.4.2). The influence of severing the nervus corporis allatum on subsequent growth of the involved corpora allata. The significance of the differences between the time samples is indicated by asterisks (*: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$). The differences between the treatments were analyzed in table 11A and 11B.

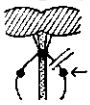
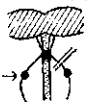
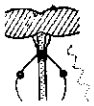


GRAPH 4 (experiment 5.4.2.). The influence of severing the nervus corporis allatum on the total volume of both corpora allata during subsequent development. See remarks in graph 3.

with the controls. Nevertheless, very significant growth takes place after the operation especially during the first few days. A further increase during adult life was evident as well. On severing the right NCA only the subsequent increase of the gland concerned is less important. As a compensation the left undisturbed CA seems to grow faster than in the controls, at least during adult life, but this was not statistically significant.
The different volume of the CA of the operated and the non-operated control groups at the moment of the last moult was surprising (table 11). This difference tended to disappear later.

From a comparison of the morphometric data (table 11) we learn that in general the important treatment differences in total CA volumes observed in the adults are not accompanied by significant morphometric differences (table 12). The most important difference a) – c) was hardly perceptible in the morphometric data. Only *E/F* and KCs_2 showed significant differences between these groups. In the case of *E/F* this turned out instead to be some accidental abnormality for the treatment c. Thus only the dry weight KCs_2 in the group retained until 4 weeks after the adult moult tends to be higher. An explanation of this result may be found in the fact that the material used for these experiments and the breeding methods do not allow for any shift to the gregarious direction as the maximum of gregarious expression is probably already attained. In fact the larvae used were bred for years under gregarious condi-

TABLE 11A (experiment 5.4.2). Mean volume of single corpora allata, standard errors and significance of differences (STUDENT's t test). The differences between the means of group *1* and *2* are indicated in graph 4.

Treatment means	**b** right (5)  70.5 ± 3.0	**a** (26)  94.2 ± 5.2	**d** (18)  166.1 ± 4.8	**b** left (6)  207.2 ± 39.5
c (16)  229.9 ± 9.7	t = 15.627***	t = 12.336***	t = 5.853***	t < 2
b left (5)  207.2 ± 39.5	t = 3.452*	t = 2.839*	t < 2	
d (18)  166.1 = 4.8	t = 16.772***	t = 10.127***		
a (26)  94.2 ± 5.2	t = 3.950*	*1. At the moment of the adult moult.*		

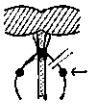
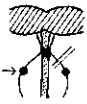
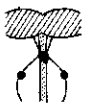
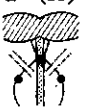
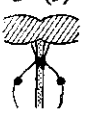
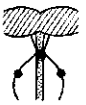
Treatment means	**b** right (5)  86.6 ± 21.8	**a** (14)  132.7 ± 12.2	**d** (20)  364.0 ± 21.1	**c** (22)  417.2 ± 20.2
b left (5)  458.6 ± 77.8	t = 4.604**	t = 4.136*	t < 2	t < 2
c (22)  417.2 ± 20.2	t = 11.131***	t = 12.055***	t < 2	
d (20)  364.0 ± 21.1	t = 9.155***	t = 9.480***		
a (14)  132.7 ± 12.2	t < 2	*2. Four weeks after the adult moult.*		

TABLE 11B (experiment 5.4.2). Mean total volume of corpora allata, standard errors and significance of differences (STUDENT's t test) The differences between means of groups *1* and *2* are indicated in graph 3.

Treatment means	a (13)  188.4 ± 13.4	b (9)  277.7 ± 40.3	d (9)  332.2 ± 12.2
c (8)  459.9 ± 27.7	t = 8.815***	t = 3.726*	t = 4.215**
d (9)  332.2 ± 12.2	t = 7.560***	t < 2	
b (6)  277.7 ± 40.3	t = 2.101**	*1. At the moment of the adult moult*	

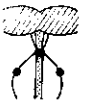
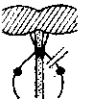
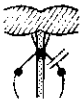
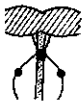
Treatment means	a (7)  265.4 ± 34.9	b (5)  545.2 ± 84.6	d (10)  727.9 ± 53.1
c (11)  834.5 ± 57.3	t = 8.469***	t = 2.828*	t < 2
d (10)  727.9 ± 53.1	t = 7.283***	t < 2	
b (5)  545.2 ± 84.6	t = 3.055**	*2. Four weeks after the adult moult*	

TABLE 12 (experiment 5.4.2). Means of morphometric characters for the treatments. For each mean the difference with the mean of the untreated controls (d) is given as a percentage of the latter mean. The significance of the differences was calculated according to Students t test and denoted by asterisks. For groups 1 and 2 see table 11.

Character \ Treatment		a 	b 	c 	d 
CA total	*1*	−43.3%*** 188.4 ± 13.4	−16.4% 277.7 ± 40.3	+38.4%** 459.9 ± 27.7	332.2 ± 12.2
CA total	*2*	−63.5%*** 265.4 ± 34.9	−25.1% 545.2 ± 84.6	+14.6% 834.5 ± 57.3	727.9 ± 53.1
E	*1*	−1.7% 4790.8 ± 35.5	−3.2%* 4718.5 ± 63.6	−2.7%* 4741.3 ± 44.6	4874.1 ± 39.3
F	*1 + 2*	−1.0% 2428.2 ± 17.5	−3.1%* 2377.9 ± 20.1	−1.2% 2422.2 ± 17.0	2452.8 ± 17.2
C	*1 + 2*	−2.9%** 755.6 ± 5.8	−2.8%** 756.4 ± 6.5	−2.0%* 762.6 ± 4.3	778.5 ± 4.1
V	*1 + 2*	−3.6%** 285.7 ± 2.3	−2.9%* 287.8 ± 3.4	−1.9% 290.8 ± 2.6	296.5 ± 2.3
L	*1 + 2*	−9.3%*** 50.0 ± 0.9	−6.5%** 51.5 ± 0.9	−8.2%*** 50.6 ± 0.7	55.1 ± 0.7
E/F	*1 + 2*	−1.5% 197.6 ± 0.7	−0.8% 199.0 ± 1.6	−2.8%* 194.9 ± 0.7	200.6 ± 1.6
F/C	*1 + 2*	+1.8% 320.9 ± 1.6	+0.1% 315.6 ± 2.5	+0.8% 317.7 ± 1.8	315.2 ± 2.4
F/V	*1 + 2*	+2.5%* 848.5 ± 5.0	+0.2% 830.1 ± 9.0	+0.6% 833.7 ± 6.4	828.5 ± 6.7
KCs_1	*1*	−10.4% 158.2 ± 6.4	−15.3%* 149.5 ± 12.9	−3.9% 169.6 ± 8.4	176.5 ± 4.9
KCs_1	*2*	−9.8%* 192.0 ± 4.3	−5.9%* 200.2 ± 2.5	−7.4%* 197.0 ± 4.4	212.8 ± 4.3
KCs_2	*1*	−15.5%* 278.4 ± 13.1	−17.6%* 271.5 ± 26.1	−6.3% 308.5 ± 17.5	329.3 ± 11.6
KCs_2	*2*	+5.5% 607.7 ± 13.0	−12.9%* 501.6 ± 13.0	−13.7%*** 497.2 ± 14.6	575.8 ± 16.7
n (1 + 2) =		20	11	19	19

tions. In this respect more information could be expected from experiments using solitary larvae, bred under isolation at least during some preceding instars. This experiment has not yet been carried out because of practical difficulties (laborious breeding and high postoperative and spontaneous mortality).

The general difference over many characters between all treatments and the non-operated controls was also surprising. All evidence from this experiment suggests that the operation itself exerts an influence in the solitary direction, on the CA as well as on many morphometric values. F/C and F/V did not show this tendency. We have seen that for these characters only prolonged exposure to suitable conditions produces extreme changes. The character L, in large measure independent of other characters, especially of "general size", showed the "solitary" or perhaps rather the metathetelic influence of any treatment in very clear way. If this solitary tendency following the operation is real, we should expect that perhaps treatment a) would restore the normal condition of maximum gregarisation. In fact, for many characters treatment a) takes an intermediate position between the non-operated controls d) and the operated groups. Exception are L, F/C, F/V, V and some weight values.

5.4.3. *Experiment 5.4.3*

The results of the foregoing experiments suggest that growth in the CA during development depends on an undisturbed nervous connection with the CC. To obtain more information about the character of the stimuli involved, the severing of the NCA in female L 2 was followed in some experiments by reimplantation of the CA concerned in the CC. For comparison reimplantation of such a CA in the mandibular muscles was carried out. In the latter case certainly no direct contact could take place between the CA and the CC. The extirpations were as usually carried out by means of forceps. The CA obtained were examined outside the body in order to be sure that every nervous connection was disrupted. The reimplantation took place with the aid of a very fine needle. Only the nervous stumps remaining on the CA were actually fixed. In every case the CA remained in free contact with the blood circulation. The treatments applied were:

a) The right CA was removed

b) Both CA were severed; the CA were detached and reimplanted in the mandibular muscles

c) The right NCA was severed; this CA was detached and reimplanted in the CC. The left CA remained undisturbed

d) As in c, but left CA removed

e) A large part of the CC comprising all parts intimately connected with the aorta was removed, leaving intact the anterior unpaired lobe with its nervous connections to the CA (the NCA) and the hypocerebral ganglion. Thus the posterior glandular parts and the anterior paired lobes were removed almost

TABLE 13 (experiment 5.4.3). Influence of various operations on the endocrine system on growth and activity of the corpora allata.

ad. = normal adults. pr. = prothetelic larvae.
For other abbreviations see table 9.

		Operation	Mean volume of single CA	Mean total CA volume	Mean number of days until adult moult	I	II	III
a 	Left	undisturbed	244.4 ± 9.7	244.4 ± 9.7	26.3 ± 0.4			
	Right	CA removed	–					
	(n)	(15)	(8)	(8)	(8)	8	0	7
b 	Left	≠ NCA	23.9 ± 2.4		27.4 ± 0.7			
	Right	≠ NCA						
	(n)	(16)	(8) pr. L 5		(5) ad	5	9×V	2
c 	Left	undisturbed	204.5 ± 12.7	299.3 ± 12.9	26.5 ± 0.3			
	Right	≠ NCA [1]	94.8 ± 10.0					
	(n)	(16)	(11)	(11)	(11)	13	0	3
d 	Left	removed	–	202.2 ± 51.1	27.0 ± 0.6			
	Right	≠ NCA [1]	202.2 ± 51.1					
	(n)	(32)	(5) ad.	(5) ad.		7	18×V	7
d′			–	244.6 ± 46.7				
			244.6 ± 46.7					
			(9) pr. L 5	(9) pr. L 5				
e 	Left	dorsal part of CC removed	112.5 ± 11.0	225.1 ± 20.9	26.6 ± 0.5			
	Right							
	(n)	(16)	(22)	(11)	(11)	12	1×V	2
f 	Left	operated controls	195.2 ± 5.4	390.5 ± 15.0	26.1 ± 0.2			
	Right							
	(n)	(45)	(74)	(37)	(37)	43	0	2

[1]) CA reimplanted in CC.

entirely. This was confirmed later during the dissection of the adult (the terminology of HIGHNAM 1961 is used)

f) Operated controls.

Results (table 13, 14)

Prothetelic larvae occurred only when both NCA were severed; apparently it made hardly any difference whether the CA concerned were reimplanted in the CC or in the mandibular muscles. A surprise came with the analysis of the values obtained by determination of the CA volumes. The CA reimplanted in the mandibular muscles were even smaller than those from earlier experiments, e.g. experiment 5.4.1. However, the CA of treatment d had a size comparable with the size of the CA in the control larvae. This size was even greater in the prothetelic L 5 dissected simultaneously with the adults in the same treatment but this difference was not significant owing to the rather large standard errors. On dissection the implanted CA appeared to have fused together with the CC, most often by a thick strand of tissue obviously originating in the nervous stumps on the CA. In some other cases the CA and the CC were found closely fused together. Thus the reestablishment of a connection between the CA and the CC had restored normal growth but not the normal secretion of the juvenile hormone.

In earlier experiments (nr 5.1) we have seen that a temporary shortage of CA factor decidedly does not lead to a delay in the appearance of prothetelic features in the L 5, but only to immediate prothetely in the next stage. The results of the present experiment suggest that a shortage of CA factor has only become apparent later in the development (L 4 or L 5) despite the normal size. With respect to the development of prothetely in L 5, the majority of individual larvae in which the detached CA were reimplanted in the CC reacted in the same way as individuals in which the CA were reimplanted in the muscles. This result is difficult to explain. On the one hand it is clear that a nervous connection with the CC is not an absolute prerequisite for a sufficient secretion of the CA factor in the case of arbitrarily implanted CA (when they are at least sufficiently large), on the other the size must be a prerequisite to a certain extent. The best explanation available at present is the assumption that a maximum of secretory activity in a CA may be attained only when the proper nervous connection with the CA is undisturbed, but that a minimum of secretion occurs anyhow. Detached CA are capable of some activity but this probably never exceeds the activity at the moment of disconnection.

Some of the larvae with restored CC–CA connection showed normal development without prothetely. In these cases apparently some increase in secretory activity took place parallel with the increase in size. The difference in reaction resulting from the operation concerned was possibly caused by the different character of the restored connection, which might of course depend also on the localization of the implanted CA on the CC. This was difficult to control during the operation and suggests a subject for histological study.

Treatment means	b (8)  23.9 ± 2.4	c right (11)  94.8 ± 10.0	e (22)  112.5 ± 11.0	f (74)  195.2 ± 5.4	d (adults, 5)  202.2 ± 51.1	c left (11)  204.5 ± 12.7	a (8)  244.4 ± 9.7
d (proth. 9)  244.6 ± 46.7	t = 4.716**	t = 3.134*	t = 2.752*	t < 2	t < 2	t < 2	t < 2
a (8)  244.4 ± 9.7	t = 22.050***	t = 10.739***	t = 8.997***	t = 4.432**	t < 2	t = 2.494*	
c left (11)  204.5 ± 12.7	t = 14.032***	t = 6.801***	t = 5.434***	t < 2	t < 2		
d (adults, 5)  202.2 ± 51.1	t = 3.482*	t < 2	t < 2	t < 2			
f (74)  195.2 ± 5.4	t = 29.033***	t = 8.807***	t = 6.779***				
e (22)  112.5 ± 11.0	t = 7.883**	t < 2					
c right (11)  94.8 – 10.0	t = 6.904						

TABLE 14 (experiment 5.4.3). Mean volumes of single corpora allata, standard errors and significance of differences (STUDENT's t test).
For abbreviations see table 13.

A comparison of the treatments (c) and (d) allows for the conclusion that the presence of an intact CA inhibited the restoration of growth in an implanted one. This suggests that either a quantitative growth stimulus, originating in the CC, might be transported to the CA or that an endocrine feedback mechanism is involved.
An estimation of the activity of the implanted CA in this case was not possible as it was obscured by the intact CA.

A comparison of treatment a) with the controls f) shows that after the removal of one CA some compensatory growth in the undisturbed CA may occur. This also could point to a more or less quantitative growth stimulus exerted by or through the CC or to the above "feedback mechanism".

The extirpation of the dorsal part of the CC (f) severed the dorsal NCC, whereas the NCA were not damaged. This had a less severe effect upon the growth of the CA than the severing of the NCA. Nor was the secretion of CA factor inhibited so severely considering the only rare occurrence of prothetely. According to HIGHNAM (1961) the unpaired anterior lobe of the CC and the short nervous connections with the hypocerebral ganglion beneath it contain neurosecretory material. In this case therefore the CA were not deprived of their entire neurosecretory supplies. They seem to secrete at least the minimum amount of hormones required for a normal development in spite of an ultimate size which is distinctly less than the size of the CA in treatment d) (prothetelic L 5). Two more possibilities for explaining the continuation of the activity in the intact system CA-ventral part of CC were considered on page 50. The second hypothesis mentioned there is rendered improbable by the similarity of results in both experiments. The third possibility remains open.

Thus the principal conclusion derived from this experiment is that the growth of the CA in normal individuals and the gradual increase in secretory activity during development are two different processes. The growth promoting factor probably originates from the CC. The factor promoting secretion, originating probably from the brain, is considered to be transmitted mainly or exclusively via the NCA under normal conditions. It has been proved to be transmitted also by the blood, at least under experimental conditions. This would exclude the third possibility, i.e. activation by nervous impulses. Additional evidence may point to secretion stimuli originating from the hypocerebral ganglion. This organ might supply, at least under abnormal experimental conditions, stimuli for a level of secretion sufficient to allow for a normal development in addition to the blood transmitted stimuli.

The comparison of morphometrical values for the treatments, though carried out in detail, revealed hardly any significant differences and therefore needs no further discussion. It should be taken into account however that these comparisons only applied to the adults, thus involving a measure of selection in some treatment groups in which prothetely in L 5 occurred. The few significant differences noted were (compared with the controls f):

a) markedly lower E and derived E/F, not accompanied by other metathetelic or solitary features in treatment d.

b) a peculiarity in the dry weight distribution as a result of partial removal of CC. K_2 was significantly lower than in the controls. KCs_2, however, was significantly higher. As a result KCs_2: K_2 also changed considerably: from 455.5 in the controls to 538.1.

Thus, the enormous differences in CA volume do not have much influence on the morphometric characters of the resulting adults. In this case too the possibility exists that the gregarious population lacks plasticity in the gregarious direction. The fact that no important changes in the solitary or methathetelic direction have occurred again supports the hypothesis that in *Locusta* no inhibition of the secretion during the last larval instar occurs. The absence of an effect in treatment (e) is especially illuminating in this respect.

5.4.4. *Experiment 5.4.4*

Comparable implantations were performed in young male L 2, but now the CA of L 5 donors about half way through their last instar were used. The treatments applied were:

a) Right CA removed only, CA of L 5 reimplanted in the CC

b) The same, but left CA also removed 6 days later when the larvae were in the third instar. This treatment was added in order to check whether late prothetely was induced in the period directly after the operation or later

c) Both CA removed, with the same reimplantation as in a)

d) Both CA removed, one CA from L 5 reimplanted in one of the mandibular muscles

e) The right CA removed

f) The same operations as in (c) but carried out 6 days later

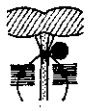
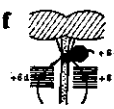
g) Non operated controls.

Results.

In the experiments 5.4.4 and 5.4.3, different sexes were used. This probably had a systematic influence upon the size of the CA, but the results are certainly comparable. As to the pigmentation a marked difference was observed. In the treatments (a), (b), (c) and (f) a small proportion of individuals turned slightly greenish in L 3 and L 4, while retaining most of their pattern. This effect had almost disappeared in L 5, and no traces of green were detected in any adult. This means that in a number of larvae a surplus of CA factor had been present during some time after the implantation.

This was not observed in (d) but it is not known whether the difference is significant. In comparing the effects on the subsequent development (table 15) we observe that any reimplantation of a CA from L 5 into an L 2 deprived of its CA does not prevent the appearance of prothetely in L 5 (c). This was the case on reimplantation of its own CA as well (experiment 5.4.3-d). Nevertheless it is obvious that these implanted CA had grown to a

TABLE 15 (experiment 5.4.4). Influence of various operations on the endocrine system on growth and activity of the corpora allata. The CA volumes were determined after the adult moult, except for treatment c). The CA which were reimplanted in the CC or in the mandibular muscles (MM) were obtained from gregarious L 5. The non-cursivated treatments were carried out in young L 2, the cursivated 6 days later, in young L 3. For abbreviations see also table 9.

		Operation	Mean volume of single CA	Mean total CA volume	Mean number of days until adult moult	I	II	III
a 	Left	undisturbed	169.6 ± 10.8	347.1 ± 30.8	27.4 ± 0.4			
	Right	CA removed, +CA in CC	177.5 ± 23.7					
	(n)	(15)	(8)	(8)	(8)	11	0	4
b 	Left	*CA removed*	-	287.7 ± 44.6	29.2 ± 0.9			
	Right	CA removed, +CA in CC	287.7 ± 44.6					
	(n)	(15)	(6)	(6)	(6)	8	5×V	2
c 	Left	CA removed	240.8 ± 7.6	240.8 ± 7.6	-			
	Right	CA removed, +CA in CC						
	(n)	(15)	(8) pr.	(8) pr.		2	12×V	1
d 	Left	undisturbed	180.9 ± 15.1	408.9 ± 17.3	27.9 ± 0.4			
	Right	CA removed, +CA in MM	228.0 ± 18.8					
	(n)	(15)	(8)	(8)	(8)	15	0	0
e 	Left	undisturbed	197.2 ± 8.7	197.2 ± 8.7	28.5 ± 0.2			
	Right	CA removed	-					
	(n)	(15)	(15)	(15)	(15)	15	0	0
f 	Left	*CA removed*						
	Right	*CA removed, +CA in CC*						
	(n)	(14)				4	6×V	4
g 	Left	undisturbed	197.3 ± 6.2	394.6 ± 12.8				
	Right	undisturbed						
	(n)	(12)	(16)	(8)		9	0	3

size significantly larger than the size of any undisturbed CA. This increase in size did not take place when an undisturbed CA was left in the individual. Prothetely in L 5 occurred also when the remaining intact CA was removed only 6 days later (b). This is an indication that the induction of adult charac-

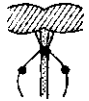
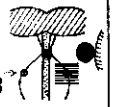
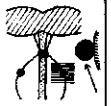
Treatment means	Fifth instar larvae on moment of operation (20)  149.3 ± 10.8	a left (8)  169.6 ± 10.8	a right (8)  177.5 ± 23.7	d left (8)  180.9 ± 15.1	e (15)  197.2 ± 8.7	g (16)  197.3 ± 6.2	d right (8)  228.0 ± 18.8	c (6)  240.8 ± 7.6
b (6)  287.7 ± 44.6	t = 3.080**	t = 2.573*	t = 2.349*	t = 2.268	t < 2	t = 2.008	t < 2	t < 2
c (8)  240.8 ± 7.6	t = 9.242***	t = 5.394***	t = 2.542*	t = 3.544**	t = 3.303**	t = 4.223***	t < 2	
d right (8)  228.0 ± 18.8	t = 3.924***	t = 2.691*	t < 2	t < 2	t < 2	t < 2		
g (16)  197.3 ± 6.2	t = 5.393***	t = 2.387*	t < 2	t < 2	t < 2			
e (15)  197.2 ± 8.7	t = 4.435***	t < 2	t < 2	t < 2				
d left (8)  180.9 ± 15.1	t < 2	t < 2	t < 2					
a right (8)  177.5 ± 23.7	t < 2	t < 2						
a left (8) 	t < 2							

TABLE 16 (experiment 5.4.4). Mean volume of single corpora allata, standard errors and significance of differences (STUDENT's t test).
For abbreviations see table 15.

ters did not take place during the period immediately following the operation but much later.

The differences in size between the CA implanted in the CC and those implanted in the mandibular muscles (table 16) are insignificant (b and c compared with d right). Thus the operation produced a result differing considerably from that of experiment 5.4.3. To explain this we must assume either that in L 2 hosts CA of L 5 behave otherwise than CA of L 2, or that the growth promoting CC effect is only acting on the CA of the individual itself (homo-transplantation) and not on CA of other individuals (allo-transplantation). In both cases, however, a close fusion of CA with CC was observed. Though it was clear that these alloplastic transplantations led to a considerable loss of activity, complete inactivation was probably not achieved. The ultimate total CA volume therefore bore no relation to the onset of precocious metamorphosis.

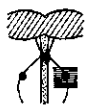
The removal of one CA, in contrast to the former experiment, did not induce a hypertrophy of the other CA (e). Whether the sex-difference might be responsible for this is as yet unknown.

A comparison of the morphometric data for adults (table 17) shows that in all the treatments the resulting adults were smaller and had a lower dry body weight (KCs_2) and dry head weight (K_2) than the unoperated controls (g). This was not merely the effect of damage due to the operation since the differences between each treatment and the operated controls (e) for every character not only showed the same tendency but also the same significance for every difference compared with the corresponding differences in the table. This means that we may consider these effects as true treatment effects. Thus a general shift to a solitary direction was obvious. This was confirmed by the effects on the values of the ratios E/F and F/C which are rather independent of general body size, and showed a solitary tendency.
It is not conceivable that this influence had been exerted preponderantly during the last part of development. This follows from a comparison between the ultimate size of the implanted CA and the normal size at that time, and from the common occurrence of prothetely.

5.4.5. *Experiment 5.4.5*

The experiments described suggested that the green colour is provoked either by a continuous excess of CA factor or by a relative excess only at certain definite moments in the course of a larval instar. In the last experiments we have seen that a reduction of the activity of the CA may be brought about experimentally by the severing of the NCA at an early stage.
Further contributions to this argument may be found in the observation of changes in individuals operated on in this way and brought afterwards under conditions normally inducing the formation of green pigment. This was done with gregarious male L 2 together with some other operations. Four days after

TABLE 17 (experiment 5.4.4). Means of morphometric characters for the treatments a, b, d, e and g. For each mean the difference with the untreated controls (g) is given as a percentage of the latter mean. The significance of these differences was calculated by means of STUDENT's t test.

	a (8) 	b (6) 	d (8) 	e (15) 	g (8) 
D	−0.4% 27.4 ± 0.4	+6.2% 29.2 ± 0.9	+1.5% 27.9 ± 0.4	+3.6% 28.5 ± 0.2	27.5 ± 0.3
CA total	−12.0% 347.1 ± 30.8	−27.1% 287.7 ± 44.6	+3.6% 408.9 ± 17.3	−50.0% 197.2 ± 8.7	394.6 ± 12.6
E	−4.8%** 4671.9 ± 33.7	−9.8%** 4425.7 ± 97.9	−6.5%** 4589.1 ± 49.6	−1.6% 4829.4 ± 33.8	4906.4 ± 65.7
F	−0.7% 2507.8 ± 21.7	−7.9%* 2325.8 ± 40.2	−3.1% 2446.8 ± 22.8	−1.2% 2495.0 ± 19.1	2526.3 ± 27.2
C	−3.8% 760.3 ± 10.7	−10.0%** 711.3 ± 10.8	−9.3%** 735.8 ± 7.2	−1.7% 776.9 ± 4.4	790.5 ± 14.3
V	−7.2%** 286.6 ± 3.9	−10.1%*** 277.8 ± 3.9	−8.0%** 284.2 ± 3.8	+3.2%* 299.1 ± 1.8	308.9 ± 5.3
L	+7.3%* 52.9 ± 1.2	−0.6% 49.0 ± 1.9	+3.0% 50.8 ± 1.0	+7.5%** 53.0 ± 0.8	49.3 ± 0.5
E/F	−4.1%** 186.3 ± 1.8	−2.1% 190.2 ± 3.3	−3.5%* 187.5 ± 2.2	−0.4% 193.6 ± 1.7	194.3 ± 1.3
F/C	+3.1% 330.0 ± 3.6	+2.3% 327.2 ± 4.2	+4.0%* 332.7 ± 3.3	+0.4% 321.4 ± 2.5	320.0 ± 3.7
K_2	−20.3%** 37.4 ± 0.7	−26.9%** 34.3 ± 2.4	−20.9%** 37.1 ± 0.9	−11.1%* 41.7 ± 0.9	46.9 ± 2.2
KCs_2	−11.3%* 236.8 ± 4.5	−18.4%* 218.0 ± 11.1	−12.2%* 234.4 ± 3.4	−5.1% 253.5 ± 3.8	267.0 ± 9.2

the operation 8 larvae of every treatment were isolated in standerd jars under humid conditions and examined every day. The treatments applied were:

a) Left: CA removed
Right: nervous connections with the suboesophageal ganglion severed only. This treatment would show whether any special stimulation takes place through these nerves on solitary breeding

b) Left: CA removed
Right: undisturbed

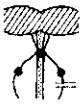
c) Left: CA removed
Right: severing of the NCA
d) non-operated controls.

In treatments (a) and (b) the total CA volume was at least halved. According to the preceding experiments, it might have been expected that the activity of the CA would stay behind during further development.

Results (table 18)

The time interval between the operation and the second moult was not very different between treatments. The resulting L 3 all showed a more or less gregarious pigmentation, the black pattern however was not strongly developed in some of the larvae (table 18). Only one individual in treatment (a) was slightly greenish as L 3. The duration of L 3 (the instar in which the external conditons were changed) was not determined. The pigmentation of each individual was examined daily after the moult to L 4. In the L 4 the first differences due to operations were observed. The pigmentation of (a), (b) and (d)

TABLE 18 (experiment 5.4.5). The influence of various operations involving the endocrine system on the appearance of green colour and prothetely in larvae reared under isolated-humid conditions after the operation.
For abbreviations see table 9.

		Operation	Post Operative mortality	Number turning green during L4	Number turning green only in L5	Number not turning green	Number showing prothetely in L5	Duration of L4 in days
a 	Left Right (n)	CA removed ≠ nerve to suboesophageal ganglion	(2)	(6)	(0)	(0)	(0)	5.5 (6)
b 	Left Right (n)	CA removed undisturbed	(0)	(6)	(1)	(1)	(0)	5.3 (8)
c 	Left Right (n)	CA removed ≠ NCA	(1)	(2)[1]	(0)	(5)	(4)	6.9 (7)
d 	Left Right (n)	non-operated controls	(0)	(5)	(3)	(0)	(0)	(8)

[1]) The green colour of these individuals was very pale when compared with green individuals in other treatments.

larvae showed no marked differences: in most individuals a green colour was observed for the first time the day after the moult. This green colour gradually intensified during the remaining part of L 4. The black pattern was already greatly reduced after moulting and disappeared almost entirely during the next moult. The resulting L 5 were classified as almost solitary green types but for traces of pattern in some of them.

No prothetelic abnormalities were noted in any of these three treatments. Quite normal, preponderantly green adults finally developed from them. In treatment (c) in L 3 only two individuals developed some green colour and this was distinctly less intense than in the other treatments. In one of the individuals concerned the green shade did not persist into the prothetelic L 5, whilst in the other the green colour disappeared gradually during a normal fifth instar. Five individuals did not develop any green colour. Three of these in addition to one of the slightly greenish types ultimately developed into normal adults, the others including one of the greenish types mentioned, became prothetelic L 5. The mean duration of the L 4 was distinctly longer in c, probably due to the development of prothetely.

It may be concluded that the reduction of the total CA volume by 50% had no influence on the development of green colour. Severing of the NCA combined with the reduction mentioned clearly inhibits the appearance of green colour. The isolated case of one larva turning green before developing prothetely in L 5 demonstrated, however, that a certain increase in the secretion of the CA in the second half of the instar is not always rendered immediately impossible by the operation. The further history of the individual concerned left no doubt as to the effectiveness of the operation. The nervous connection between the CA and the suboesophageal ganglion is not involved in the formation of the green pigment.

5.5. Comparison of adults and larvae resulting from different treatments in a bivariate phase graph for F and C

To answer the question whether phase differences are the result of some degree of metathetely of prothetely, we should compare populations exhibiting an extreme phase status with populations in which we have experimentally interfered with metamorphosis. Such a comparison could be made for instance between the experiments 3.1 and 3.2 on the one hand and the experiments on implantations on the other. The trends rather than the absolute values are important.

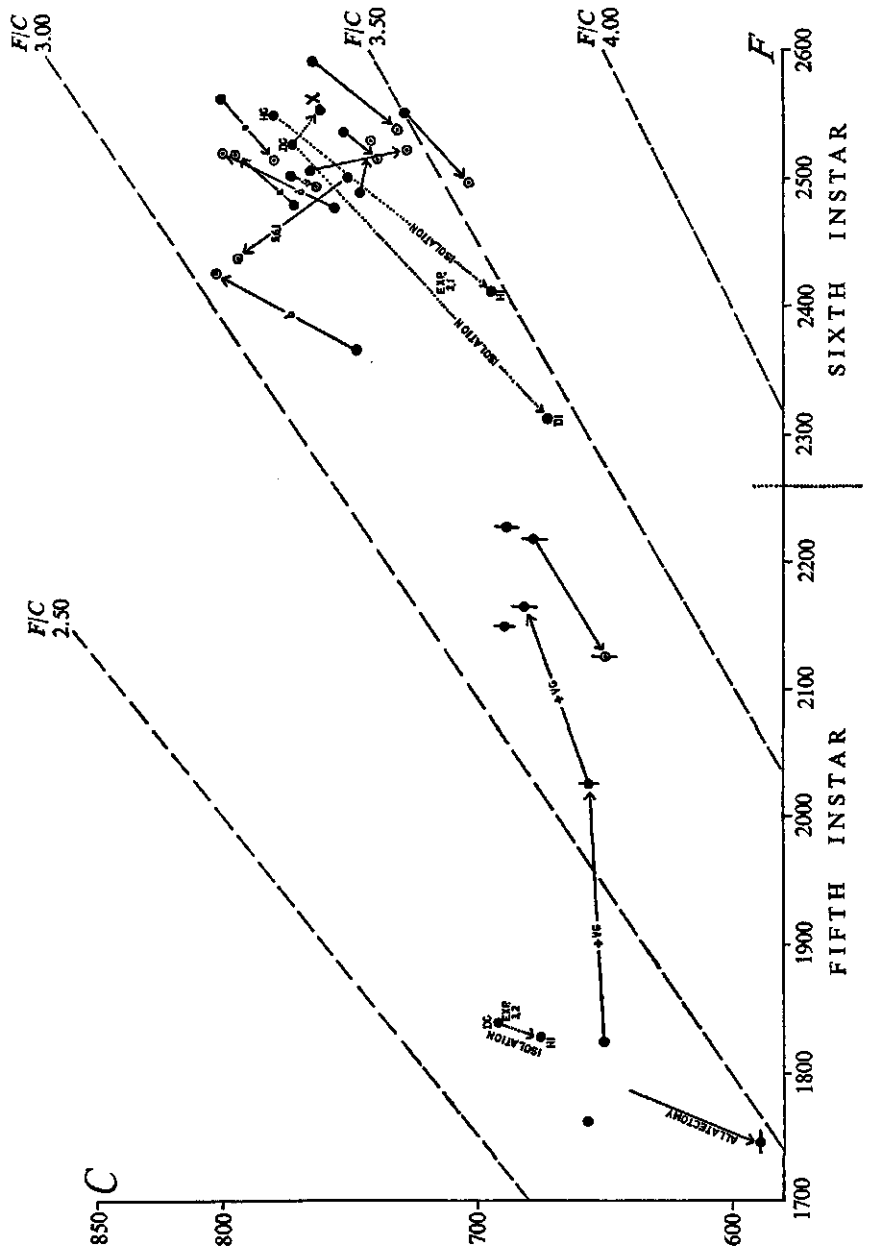
For the ratio E/F statistical analysis is not even necessary. Excess CA factor during certain larval periods has an influence which undoubtedly shows the same tendency as the effect of isolation. The reason why the ratio E/F has the capacity to change experimentally much more than any other character lies in the fact that the elytron (E) is the body part quantitatively most involved in metamorphosis. Nevertheless isolation produces only minor changes in this

character as compared with experimentally induced metathetely. So far as we know the latter abnormal condition does not occur under field conditions. In spite of the arguments mentioned, the ratio F/C obviously discriminates better than the ratio E/F (DIRSH 1953).

As we have seen, the change in F after implantation of CA is insignificant. The same applies for experiment 3.2. In experiment 3.1, however, F was changed on isolation. Presumably the longer duration of this experiment caused the "general body size" to modify, the femur length being strongly related to this character. In general the solitary phase differs from the gregarious phase with the same tendencies as show individuals in which extra CA were implanted, at least in as far as the principal phase characters are concerned. It would be of interest to compare the influence of an experimentally induced CA shortage with the influence of crowding. As we have seen in the experiments there is presumably no point in taking an established gregarious population as the starting point since for most characters the limit of plasticity in the gregarious direction will already have been attained.
A more promising procedure would be to start from a group of stabilized solitary individuals. Although this seems possible from the theoretical point of view, practical difficulties have prevented us from applying this method. The high numbers of solitary individuals necessary for morphometriacl analysis are not easy to produce because of a greater mortality and variability in this material and the laborious breeding involved. Analysis of the adults does not, however, quite exhaust all the possibilities for we may also resort to the larvae. They as well may show phase differences (vide experiment 3.2) and have the advantage of a high degree of plasticity in the direction of adult differentiation. They may exhibit various degrees of prothetely, sometimes even when development is not interfered with experimentally (own observations of *Schistocerca* in the laboratory). On the other hand, larvae are not known to be able to show any apparent metathetely, for this change presupposes a minimum degree of adult differentiation. Not even the implantation of a large number of extra CA in a larva is able to prevent normal larval wing development.
Apparently metamorphosis induces a change which causes the adult wings emerging from the fleshy larval pterotheca to become membranous. These changes may be inhibited by the CA factor, but the positive allometry of larval wing growth remains undisturbed.

The evaluation of the length of larval pterotheca presents some difficulties owing to the lack of reliable reference points. Probably for this reason the ratio E/F has never been extensively used in the study of larval morphometrics. These objections do not apply, however, for the ratio F/C the evaluation of which is not more difficult in the larvae than in the adults. It was used in experiment 3.2 and we have seen that solitary breeding induces a change in this ratio as easily in larvae as in adults. We now have the opportunity to discern whether the effect of prothetely is comparable with that of crowding. Such a comparison has been carried out for males in the bivariate graph no 5. In this

graph the characters F and C were represented simultaneously. This offers the advantage over the simple use of the values of the ratios F/C (a univariate) that changes in this ratio may easily be explained by changes in either one of the two characters. The possibility remains open to use general body size as



an additional character. We may assume that larger general body size may have consequences for both *F* and *C* without implications for the ratio *F/C*. This is exactly what happens when a point moves along the straight lines of equal ratio.

The value of the ratio *F/C* increases during the adult moult in normal development. The contradiction is that metamorphosis brings about a change in the ratio *F/C* in the same direction as does isolated breeding, i.e. an increase. A different reaction is to be observed in general body size which increases during metamorphosis but relatively decreases as a result of isolated breeding in L 5 as well as in adults (experiment 3.2 and 3.1). In adults the decrease in size is even the more important aspect of the change.

When we compare the effects of implantation of extra CA with those of isolated breeding we may conclude that the operation, when carried out in L 2 (experiment 4.1), produces similar effects in three out of 5 samples. The other two reactions were somewhat atypical in producing somewhat longer femora (*F*) but the resulting changes in the *F/C* values were still in agreement with those in the other samples.

The results of extra implantations of CA are inverse when carried out early in L 5 (experiment 4.2) with respect to the changes in general size as well as to the value of *F/C*. In the subsamples 3 and 5, however, the trends were in agreement again. The divergent results produced by extra implantations of CA early in L 5 presumably agree with the observations of JOLY, whose earlier results also point in this direction. We may seek the explanation in the apparent metathetely induced by early implantations, resulting in a larval *F/C* ratio. In harmony with this conception is the change brought about by implan-

---

GRAPH 5 (several experiments compared), male adults and fifth instar larvae. The influence of various operations and different breeding conditions on the characters F and C and the ratio F/C.

The arrows represent the effects of the treatments or the effect of isolated breeding compared with controls.

- means of samples fifth instar adults resulting from extra implantation of ventral glands.
- means of samples of normal fifth instar larvae and normal adults.
- mean of a sample of prothetelic fifth instar larvae resulting from allatectomy in a preceding instar.
- means of samples sixth instar adults implanted with extra corpora allata in L 2 or L 5, often exhibiting metathetely.

a), b), c), d), e); effect of implantation of extra corpora allata in L 5 (experiment 4.1). unmarked arrows: effect of implantation of extra corpora allata in L 2 (experiment 4.2).

DI, DC, HI, HC: combinations of breeding conditions (D = dry, H = humid, I = isolated, C = crowded). These conditions were applied throughout the larval development in experiment 3.1. and during the fourth larval instar only in experiment 3.2.

X = HI applied from L 3 on in experiment 3.1.

5.6.1: the effect of implantation of extra corpora allata obtained from adult *Romalea* in second instar larvae of *Locusta*.

tation of extra CA of adult *Romalea* in L 2, producing a larger surplus than do *Locusta* CA and inducing strong metathetely in the metamorphic moult (mean E/F ratio 148.6). The more larval F/C ratio in this case is very obvious, whereas size is not modified, probably by a balance of solitary and larval tendencies. Larval growth in *Locusta* is probably always accompanied by considerable increase in body size.

A consideration of the relative changes involved in the development of prothetely in the graph provides additional evidence. A number of 12 prothetelic L 5, resulting from intersections on the NCA in L 2 were compared with some batches of normal gregarious L 5, from different breeding sources. Although the L 5 were not of the same source the differences are sufficiently distinct to be reliable. The most important modification is the higher value of F/C, again not pointing to an identification of solitary with neotenous tendencies. The difference appears to be brought about by a relative decrease in general size involving a more important decrease for C than for F. The direction of the change is exactly the opposite of the change involved in the development of metathetely after extra implantation of CA in L 5. When we compare the observed changes due to various conditions, it may be concluded that the development of solitary morphometric characters in adults does differ essentially from strong metathetely as far as F and C concerned. Yet both effects are probably induced by the same organ i.e. the CA, but presumably during different periods. In the development of solitary characters the long lasting CA influence during the early instars seems to be determining, metathetely on the contrary depends mainly upon a strong CA influence just before the metamorphic moult. Thus, although both phenomena are result of an equal stimulus, the effect is essentially different as far as the relation F/C is concerned, whereas the effect on E/F is only quantitatively strongly different and the correspondance is probably only superficial.

5.6. Hetero-transplantation of CA

All the effects produced by the allo-transplantation of adult CA in L 2 of *Locusta* as far as the pigmentation and morphometrics are concerned, may be obtained after hetero-implantations as well.
CA from adult *Schistocerca gregaria* and *Romalea microptera* were used in these experiments. Conversely, implantations of *Locusta* CA in *Schistocerca* larvae were also successful in this respect.
Differences in the persistence of the reactions to allo- and hetero-implantations during subsequent larval development were however evident.

5.6.1. *Substitution of CA in Locusta larvae by CA of Romalea*

a) Substitution of both CA in each of ten L 2 of *Locusta* by one CA of adult male *Romalea*. The remaining 8 larvae turned green already in L 3. They lost almost any trace of pattern in L 4. The resulting adults were all green and

showed strong metathetelic characters. Thus the effect was even stronger than that of allo-transplantations.

b) Substitution of both CA in eight L 2 of *Locusta* by one CA of adult male *Romalea* obtained via dissection on *Locusta* adults resulting from the same treatment as described in a) in a similar experiment carried out some weeks earlier. The 6 surviving larvae all turned either solitary green (2 out of the 6) or yellowish green (4) in L 3. Particularly in L 4 the green coloration was considered to be much less intense then in treatment a. In L 5 in 4 out of 6 larvae the green colour was replaced by a more or less gregarious pigmentation without green.

Amongst the resulting adults only one was classified as slightly metathetelic and 4 as quite normal (gregarious). This result demonstrated that heterotransplanted CA may remain active during a long time, extending even beyond one larval generation of *Locusta*. However, a gradual decrease in activity is manifest.

From the morphometric data we learn that the strong metathetely in the adults from treatment a is accompanied by significantly lower values of F/C. This is interesting since it means that the CA were still very active during the beginning of L 5, so that the effect could be compared with implantations in early L 5. Again it is clear that metathetely is a condition essentially different from solitary morphometrics.

The result is interesting in yet another respect. The CA of *Romalea* induce the green colour in *Locusta*, although this colour does not occur in *Romalea*, being a monomorphic grasshopper. This suggests the identity of the factors inducing changes in pigmentation and morphometrics as far as the effect of CA is concerned.

5.6.2. *Substitution of CA in Locusta larvae by CA of Schistocerca*

a) 10 *Locusta* larvae were allatectomized in L 2. Immediately thereafter each larva was reimplanted with one CA from immature *Schistocerca* adults.

b) As a control a group of 10 larvae treated in the same way but with CA obtained from immature female *Locusta* adults was used. The results were as follows:

The 7 surviving larvae in (b) were all green in L 3, L 4 and L 5. Out of the 7 resulting adults 3 were classified as distinctly metathetelic and the remaining 4 as normal. Five of them were more or less green, including all cases of metathetely. In treatment (a) on the contrary, 5 survived but none of them became green. Four were developing prothetely as early as in L 4.

It may be concluded that the CA of *Schistocerca* are less active quantitatively than those of *Locusta*.

5.6.3. *Substitution of CA in Schistocerca larvae*

The treatments were:

a) allatectomy and subsequent implantation of 1 CA from mature *Schistocerca*

b) the same but with CA obtained from mature male *Locusta.*

The result was that in treatment (a) from the 15 surviving larvae only 3 turned green and remained so until the end of L 5. No green colour was observed in the adults. This pigment is never produced in normal adults of *Schistocerca.* Prothetely developed in six L 4 and 3 more L 5. Six individuals developed to normal adults.

When however CA of *Locusta* were used, all 9 larvae surviving from 10 implantations turned green in L 3. Three larvae developed prothethely in L 5 only. Five apparently normal adults were still green. Thus hetero-transplanted CA of *Locusta* proved to be even more active than allo-transplanted CA of *Schistocerca.*

An important difference with *Locusta* was noted with regard to the fate of the black pattern when green colour appeared as a result of extra implantations. In *Schistocerca* this black pattern decreased somewhat but never disappeared completely in contrast to the situation obtained in solitary breeding of *Schistocerca* and *Locusta* larvae or *Locusta* larvae implanted with extra CA. This is probably due to the continuation of crowded conditions in these experiments. In *Schistocerca* the green pigment apparently does not override the pattern factor, which probably is governed rather by density factors independently. Thus the already postulated independence of both pigment mechanisms is even more evident in *Schistocerca* than in *Locusta.*
When we compare these experiments it may be concluded that the activity of the CA of *Romalea, Locusta* and *Schistocerca* quantitatively decreases in this sequence independent of the contingent difference in species between donor and host.

5.7. Internal and external administration of extracts of adult male *Hyalophora* silkworms

The oily elements in the abdomens of male adults of *Hyalophora cecropia* are so far the best source of juvenile hormone (JH). Principles in this ether extracted oil are known to perform functions equal to those exerted by the CA in most insects. The effects associated with these extracts were:

a) stimulation of basal metabolism in homogenized tissues of diapausing adults of *Leptinotarsa* (de Wilde 1960).
b) inhibition of metamorphosis. This inhibition may take place locally in the cuticle when the active extracts are applied either externally on the intact cuticle after abrading the epicuticular layers by means of fine cristalline powders (Wigglesworth 1958), by the wax test of Schneiderman & Gilbert (1958) or by injections of diluted extracts. In the case of *Locusta,* local external applications as well as injections were attempted. Local applications of the oily extract were carried out on different parts of the cuticle of *Locusta* larvae in various instars according to the method described by Wigglesworth (1958). These experiments failed to produce any effect. No green colour ap-

peared although this colour is known to develop as a reaction to locally present CA factor after implantations of CA just under the epidermis (JOLY 1954). In addition no signs of metathetely were observed, not even when the extract was applied during the first days of L 5. For injections the oily extract was either used pure or diluted with two parts of water and one part of lecithin. The mixture was stirred up until a homogenous emulsion was obtained. This was done because in earlier experiments of WIGGLESWORTH (1958) injected pure oil tended to collect in certain parts of the animals.

Injections, especially in young larvae, always resulted in very high mortalities, probably due to toxic principles in the extracts. Of larvae surviving the injection of any quantity of pure or emulsified extract none produced green colour or apparent metathetelic abnormalites. Because of this lack of success with the various types of administration of the extracts the experiments were discontinued. The absence of a response did not prove of course the non identity of the factor(s) in the extract and the factor necessary for the induction of green pigment. It should be taken into consideration that we have no reason to expect the JH effect of the extract to be different as between *Locusta* and other insects, provided the quantities applied and the mode of administration are adequate.

The purification of the crude *Hyalophora* extract is now in progress. When the pure principles, freed from toxic elements become available, further attempts to determine the identity of the CA factors will be worth undertaking.

6. THE VENTRAL GLAND

The ventral gland (VG), the known effects of which have been reviewed already in the introduction, may be supposed to influence phase characters as well. The effects on pigmentation obtained by BÜCKMANN (1959) and ELLIS & CARLISLE (1961) and the morphometric results of STRICH HALBWACHS (1959) give rise to this presumption. Deficiency studies following complete extirpation of ventral glands are of little value since all development comes to a standstill. The converse operation – the implantation of extra ventral glands – was carried out in L 3 and older larvae of *Locusta* by STRICH HALBWACHS (1959). It resulted in anticipated moulting and its accompanying morphometric implications. The differences obtained could be satisfactorily explained by this author by assuming an anticipated arrest of growth in the instar. This means that, when the normal body proportions of an insect depend on allometric growth processes in the preceding instar, this anticipation brings about a relative change in the proportions of the resulting insect. In the case described this means an E/F ratio which is more larval and more solitary.

The pigmentation changes in *Cerura* were shown to be brought about by the change in the redox status of the ommochrome pigments. Since *Locusta* also possesses such pigments, the formation of which is probably connected with redox systems in the epidermis, an effect in *Locusta* might be expected.

Experiments on ventral glands.

The operations carried out on the ventral gland comprised:

a) Attempts to extirpate the gland partly or entirely
b) Extra implantations
c) Combined extra implantations of VG and CA.

6.1. Extirpation of the ventral gland

In some experiments extirpation of the VG was attempted in gregarious L 2. The dorsal parts of the VG were removed by seizing them through lateral perforations in the neck membrane. As they extend ventrally far into the head, access to these parts cannot be obtained through the dorso-lateral perforations. An additional incision was therefore made also in the ventral side of the membrane, through which these ventral parts were extracted with forceps. Although no certainly existed about the completeness of the removal, it was considered to have been complete or almost complete. Nevertheless no abnormalities in the subsequent development and appearance of the treated larvae was noted and normal gregarious adults resulted. The reason was found upon dissection of the adults. It turned out that the removal had not been complete in any case, fragments of the glands were found again, particularly in the most ventral region. In no case were these fragments estimated to be more than 5% of the VG volume that would have been present in normal adults.
Thus, although the main purpose of the experiment was not fulfilled, it nevertheless allowed for the conclusion that either the effects exerted by the VG are qualitative rather than quantitative, or that the secretory function of the whole gland may be taken over by the small parts remaining after the operation. The present experiments does not allow one to conclude which of the two possibilities in fact operates.

6.2. Extra implantation of ventral glands

These operations were as a rule carried out in young L 2 and occasionally in older larvae. The VG were obtained from gregarious L 5 of both sexes and often also from immature adults within a few days after the adult moult. No difference in the reaction to implantations of VG from either of the two sources was ever observed. The results of this operation were not what was expected when compared with the results of Strich Halbwachs, who operated on L 3 or older larvae. Initially there was no reason to assume that implantations in L 2 would have other effects than those described by Strich Halbwachs. The effects she found may be briefly summarized as an anticipation of the next moult by one day at most. As a consequence, slight differences in morphometrics, explained as a result of the early conclusion of developmental periods without changes in the rate of development were demonstrated.
It was therefore surprising to find a very specific and entirely new reaction to

implantation of VG in L 2. The abnormal reaction in a varying fraction of the insects treated consisted of the omission of the next (third) morphological instar as far as wing development is concerned. All the larvae showing this abnormality underwent a delay in moulting from 1–4 days compared with the treated controls. During this delayed moult they turned into L 3 with a wing posture and shape normally occurring in L 4. The distinction between the wing development of L 3 and L 4 in normal development happens to be very conspicuous. Whereas the pterothecae in L 3 are stretched downwards along the tergites, during the normal moult to L 4 a reversion takes place causing the wings to take an upward position with the posterior wings outside. It was especially this anticipated reversion which was initially noted as a result of implantation.

Apart from a varying degree of wing hypertrophy accompanying the reversion, no changes in the larval gregarious pigmentation ever occurred as a result of the operation. The only difference which remained between those abnormal L 3 and normal L 4 was the size, which in the first case was mostly intermediate between normal L 3 and L 4.

That in reality an omission of a morphologic instar took place became very obvious during the further development of the abnormal larvae. They moulted again, this time to L 4 with wings of the appearance of normal L 5, but otherwise normal. At the next moult fifth instar adults appeared which most often differed from normal adults only in a smaller body size. The wings were most often in relatively good proportion to the rest of the body and never crumpled as in L 5 resulting from allatectomy.

In some experiments series of larvae sometimes responded to the implantation by developing fifth instar adults with wings ranging from normally proportionate to very short, resembling more closely those of prothetelic L 5 and not protruding beyond the abdomen. Even in the latter case, however, the wings were always well stretched. The abnormal fifth instar adults might be referred to as prothetelic larvae. For the preceding instars however this term makes little sense.

In order to obtain a better insight into the nature of the phenomenon several series of experiments were designed in which certain factors were varied:

The instar in which the implantation was carried out;
The number of days after the preceding moult on the moment of implantation;
The sex of the receiving larvae;
The quantity of implanted VG;
The instar and age of the donors;
The species of the donor (hetero-transplantation versus allo-transplantation);
Simultaneous implantations of CA and VG.

Survey of the experimental results. The percentages given below represent the number of individuals showing adult differentiation in L 5 and therefore the individuals in which a morphologic instar was omitted. Evaluation of the

effects in L 3 proved to be not quite satisfactory since occasionally somewhat less distinct wing abnormalities occurred in this instar.

6.2.1. *Implantations of extra V.G. in early L 2*

The standard treatment, i.e. extra implantation in L 2 less than 24 hours after the moult of one almost complete VG, obtained from normal L 5 or very young adults. The responding percentages were:

males 60% (of a total surviving number of 67 out of 72)
females 15% (of a total surviving number of 13 out of 20)

These figures differ somewhat, but not essentially, from figures given in a preliminary paper (STAAL 1960).
The difference in reaction between sexes is very evident.

6.2.2. *Implantation of extra V.G in larvae of varying age and instar*

Variation of the age of the receiving larvae leads to considerable differences as to the reacting percentages. In all groups one almost entire VG from a L 5 or young adult was implanted.

a). Implantation in L 1, less than 24 hours old

males 90% (of a total surviving number of 19 out of 25)
females 25% (of a total surviving number of 8 out of 10)

In the case of L 1 the implantation did not produce morphological effects in the L 2, but in the resulting L 3 the usual abnormalities were observed regarding hypertrophy and the reversion of the pterotheca.

b). Implantation in L 2, less than 24 hours old

males 60% (see above)
females 15% (see above)

c). Implantation in L 2, 24–48 hours old

males 31% (of a total surviving number of 45 out of 60)
females 15% (of a total surviving number of 18 out of 20)

d). Implantation in L 2, 48–72 hours old

males 0% (of a total surviving number of 18 out of 20)

e). Implantation in L 2, 72–96 hours old

males 0% (of a total surviving number of 10 out of 10)

f). Implantation in L 3, les than 24 hours old

males 0% (of a total surviving number of 18 out of 20)
females 0% (of a total surviving number of 12 out of 12)

g). Implantation in L 4, less than 24 hours old

males 0% (of a total surviving number of 15 out of 15)
females 0% (of a total surviving number of 15 out of 15)

It is clear that implantation of VG should be carried out in L 1 or during the

first two days of L 2 to be effective in producing the special effect. Later implantations only very rarely do so (one case has been observed in L 3). The different reaction in either of the two sexes is again manifest.

6.2.3. *Variation of the quantity of VG implanted*

Only male L 2 less than 24 hours after the preceding moult were implanted with a varying number of VG from L 5 or young adults.

a). Implantation of one half VG

30% (of a total surviving number of 10 out of 10)

b). Implantation of one entire VG

60% (of a total surviving number of 67 out of 72)

c). Implantation of one entire VG

86% (of a total surviving number of 21 out of 22)

d). Implantation of four entire VG

71% (of a total surviving number of 7 out of 12)

The results of implanting greater quantities of VG became visible only in a somewhat higher percentage of larvae showing the abnormal reaction. The resulting prothetelic L 5 on the other hand did not differ from those in the groups in which smaller quantities were implanted.

6.2.4. *Hetero-implantation*

In some experiments VG of *Schistocerca* L 5 were implanted in male *Locusta* L 2 less than 24 hours after the preceding moult.

44% (of a total surviving number of 18 out of 22)

The difference with allo-transplantation is only slight and probably not significant.

6.2.5. *Implantation of "degenerated" VG of mature adults*

The degeneration of the ventral glands in adults of almost any insect species after the adult moult is taken for granted and spontaneous regenerations are not reported in literature. When dissecting mature adults of *Locusta* and *Schistocerca* the conspicuous whitish or milky appearance of the glands in L 5 or young adults is absent, the remnants of the glands are almost glassy and are difficult to find. When such glands of *Locusta* adults are implanted in L 2 less than 24 hours after the preceding moult, it is not surprising that they do not have much effect:

25% (of a total surviving number of 8 out of 12)

The fact that nevertheless 2 out of 8 larvae reacted positively should be noticed. This percentage is of the same magnitude as the percentage of similar L 5 adults occasionally resulting from implantation of extra CA.

Implantation of VG of mature *Schistocerca* remained without reaction in L 2 larvae.

From this result we might conclude that the degenerated VG are not active any more apart from the two positive reactions. The results of the next series e) however show us that this can be only partly true.

6.3. Combined implantations of VG and CA

The VG were obtained from L 5 or young adults, the CA from mature adults. The CA implantations secured an ample surplus of CA factor, so that in all cases the implanted larvae became green after the next moult. The immediate morphologic effect of the combined implantation in L 2 was not different from that obtained by implantation of either VG or CA, except for the percentages responding. It was therefore already possible to draw the important conclusion that the effect of the combination was merely an addition of the effects of either of the two glands separately with regard to pigmentation and immediate influence on wing development.

In the L 5, as a result of implantation of VG only, rather extreme prothetely developed, i.e. an almost complete metamorphosis. When CA were implanted simultaneously in L 2 the effect on these abnormal L 5 was the same as the effect of implantation of CA only in L 2 on the resulting sixth stage adults, i.e. a more or less complete inhibition of metamorphosis. In our case this meant that these L 5 were almost larval. The pterothecae, however, were distinctly larger than normal and not membraneous. These individuals most often attempted to moult again but they appeared to encounter difficulties in moulting. Consequently no sound 6th instar individuals from these abnormal L 5 were obtained. These individuals would undoubtedly have shown a wing length appreciably greater than that of normal adults, had metamorphosis not been seriously disturbed again during this moult.

In the description of the condition of the resulting animals the application of the normal terminology meets with difficulties. A precocious metamorphosis in L 5 is a case of pure prothetely. The anticipation in the wing development in the preceding instar is already more difficult to classify since this concerns only a minor detail in the process of metamorphosis. This, however, is only superficially so since the future metamorphosis will appear to be anticipated by one instar as well. We may assume that this anticipation is induced already in the L 2, otherwise implantations in L 3 or L 4 would certainly have had considerable success as well.

The inadequacy of the terminology becomes apparent when classifying the L 5 resulting from the combined implantation of CA and VG. These individuals are clearly metathetelic when compared with abnormal L 5 resulting from VG implantation only, since metamorphosis is inhibited. The use of the term metathetely is out of place when we compare these L 5 with normal L 5, which are always larval. At the other hand our L 5 show an anticipated wing development and this would justify the use of the term prothetely.

The percentages of larvae reacting to the combined implantations were:

a) As a result of implanting together one VG of L 5 and one CA of mature adults in L 2 less than 24 hours after the preceding moult:

males 93% (of a total surviving number of 14 out of 20).

In these percentages are included all the L 5 individuals which had wings or pterothecae distinctly larger than normal. No account was taken therefore of the degree of metamorphosis in L 5 in order to obtain figures comparable with those of other treatments with respect to the VG effect.

b) The same as in a) but in L 2, 24–48 hours after the preceding moult

males 100% (of a total surviving number of 12 out of 20)
females 10% (of a total surviving number of 10 out of 10).

c) The same as in a) but in L 2, 48–72 hours after the preceding moult

males 43% (of a total surviving number of 7 out of 10).

d) Implantation of one adult CA and one VG of L 5 in L 3 less than 24 hours after the preceding moult.

males 100% (of a total surviving number of 8 out of 10).

The results of treatments a), b), c) and d) show that the simultaneous implantations of CA supports rather than inhibits the immediate effect of the implantation of VG. The period after the preceding moult, over which the implanted VG produced effects is extended by the simultaneous implantation of CA.

e) Implantation of VG and CA, both obtained from yellow mature male adult *Locusta* in L 2 less than 24 hours after the preceding moult.

males 89% (of a total surviving number of 9 out of 10).

This result is highly interesting. It suggests a reaction as a result of transplantation which does not normally take place in the adult donor itself. Compared with treatment 6.2.5-(b), a considerable difference is noted, indicating a synergistic action of CA and VG.

6.4. Morphogenetic effects in adults implanted with VG not showing precocious metamorphosis and wing development

Mention was made above that anticipated wing development already apparent in L 3 is always accompanied by precocious metamorphosis in L 5. The nature of the relationship between these two phenomena has not yet been clarified. It cannot be excluded that the degree of wing development itself is a factor in the determination of the moment of metamorphosis, but both could also be expressions of a more fundamental process as well. If wing development itself actually determines the moment of metamorphosis then we might expect to find intermediary forms, showing slightly hypertrophied pterothecae but still without anticipation of metamorphosis. Such intermediary forms may be expected to show somewhat hypertrophied wings in the adult stage as well. Our methods may be considered suitable for discerning such an abnormality.

More extreme yet in this respect would be the adults resulting from the L 5 having clearly hypertrophied wings obtained by combined implantations of CA and VG. These individuals, however are so obviously abnormal that they cannot survive this moult.

In a number of earlier experiments (STAAL 1960), a relative increase in *E*, expressed as *E/F* was actually evident. Subsequently these experiments were repeated on a larger scale. In general this effect could not be established in these later experiments, unknown external differences probably being responsible for this failure.

The effects of the implantation of extra VG on resulting adults were analized in a large number of experiments in the same way as in section 4 i.e. using a method for disproportionate numbers for both sexes separately. These analyses failed to show results consistent throughout the various experiments and they will not be presented here for this reason. This failure does not weaken our main conclusions since the exceptional individuals occurring in the earlier experiments with *E/F* values of 2.07, 2.12, 2.28 and even 2.35 are well outside the normal range obtained in a large number of controls. Most often these individuals developed from L 5 which were classified as having pterothecae distinctly larger than normal as a result of simultaneous implantation of extra CA and VG.

The L 5 which combine hypertrophied pterothecae with a larval status most often failed to moult properly. The consequence is that the most extreme *E* and *E/F* values fail to appear in the morphometric evaluation.
All the individuals with extreme *E/F* values did show *F/C* values not significantly different from those of the control groups. This once more confirms the dissociation between wing development and other characters.

The anticipation of the wing development is always accompanied by a longer duration of the instar concerned. When a group of well synchronized L 2 is implanted within 24 hours after the preceding moult, the first individuals to moult are those which do not show any visible reaction in wing development. The last larvae to moult show the most hypertrophied pterothecae. This is illustrated from some randomly chosen experiments (table 19). The larvae with hypertrophied pterothecae tend to show a longer duration of every separate instar, so that the moment of their final moult (to "L 5" adults) is located just before the adult moult of the controls (table 19).

Comparison of male anticipated fifth instar adults with normal gregarious L 5 and normal sixth instar adults in the bivariate *F, C* graph allows for the conclusion that these L 5 adults show absolute and relative values for *F* and *C*, that are intermediate between those of normal L 5 and normal adults. The same change in the *F/C* ratio is observed on comparison with normal L 5 occurring during normal metamorphosis. That a range of differences exists also for these characters becomes apparent when the group of abnormal L 5 is divided in two subgroups. A first group comprises the L 5 adults with wings

TABLE 19 (experiments 6.2). Some examples of the changes in the rate of development accompanying the occurrence of anticipated wing development and metamorphosis. Each number represents the individuals in a sample moulting on the day indicated in the upper row. The moults to L 2, L 4 and adult are denoted by a straight line. The moults to L 3 and L 5 are denoted by a dotted line. A point near a figure indicates distinct wing hypertrophy, an asterisk indicates metamorphosed individuals.

	Treatment \ Days after implantation	4 5 6 7 8	9 10 11 12	13 14 15 16 17 18 19	20 21 22 23 24 25 26 27 28	29 30 31 32 33 34 35 36
♂♂	Controls	10 1	9 2	6 5	1 9 1	1* 7* 3*
	L 1 + VG	2 10 2	2 8* 3* 1*	(2/2. 7* 1* 1* 1*	2	1* 4* 4* 1* 1* 1*
♀♀	Controls	10 1	8 2	1 1 6 2 1	2 5 1 2	7* 1* 2*
	L 1 + VG	2 5 1	2 3 (1/2.	1 4 1 2*	1 7	2* 3* 2* 1*
♂♂	Controls	5	3 2	2 3	1 3 1	3* 2*
	L 1 + VG	6 3 1	4* 3*	1* 6*	3* 2* 2*	
♀♀	Controls	p.m.	6 4	8 2	2 4 2 1	2* 2* 2* 1* 1*
	L 2 + VG	p.m.	4 (7/1.	3 3 2* 3* 1*	3 1 1 2* 2*	1* 1* 1* 1* 1* 1*
		L 2	L 3	L 4		L 5

not protruding beyond the abdomen, a second group with wings longer than the abdomen. With respect to the shift towards the adult situation there is a perfect agreement between wing development and the F/C ratio.

Comparison with a group of pure prothetelic L 5 resulting from allatectomy clearly shows that these larvae are different from the L 5 adults. A difference has already been mentioned concerning the condition of the wings, which are sound and well stretched only in the latter group. It is not improbable that the presence of the CA is the determining factor for this difference.

6.5. Summary of effects of VG implantations

a) The VG exert no appreciable influence upon the pigmentation of *Locusta* larvae.

b) Implantation of extra, active, VG in L 1 or L 2 induces the larvae to switch over to a type of development with one instar less than normal, whilst general body size undergoes some compensation in the direction of the size of normal adults. The abnormality in development already becomes apparent in L 3 in which often an anticipated reversion of the wings and some hypertrophy is observed.

c) The abnormal "L 5 adults" often show typically adult values for the ratio E/F and the length of the sternal hair covering (L), and almost adult F/C ratios.

d) In a few cases individuals which show some wing hypertrophy do not exhibit an anticipated metamorphosis and become giant adults. These adults sometimes show very high E/F ratios (up to 2.35) not otherwise occurring.

e) Anticipated metamorphosis may be inhibited by simultaneous implantation of CA, but the effect of the VG on wing development is not affected. This combination at implantation produces larval L 5 provided with hypertrophied wings which however, most often die in the next moult.

f) A much larger percentage of the males responded to extra implantation of VG in L 2 and L 3, whatever the quantity of implanted VG and the moment of implantation.

g) The switch over to another type of development was in most cases an "all or none" effect. Without interference of a distinct surplus of CA factor a complete metamorphosis in the L 5 of individuals with anticipated development took place. This precocious metamorphosis could not be advanced further, whatever was the quantity of VG implanted. Variations in this quantity only affected the fraction of larvae responding with anticipated development of the pterothecae.

h) The simultaneous implantation of CA and VG increased the percentage of responding larvae considerably. This contributed to the enlargement of the period during which implantations are successful.

i) VG obtained from mature yellow males, which are assumed to be inactive

in these adults, only rarely produced a response when implanted in L 2. However, simultaneous implantation of CA and VG from mature adults gave a considerable response. This suggests a reactivation under these experimental conditions which does not occur in the intact insect.

6.6. Discussion on the results of VG implantations

Many of the results mentioned above are contrary to those of Halbwachs, Joly & Joly (1957), Halbwachs (1954), Strich Halbwachs (1959). The principal reason for this undoubtedly may be found in our use of L 2 and L 1 as hosts instead of older larvae. In our experiments distinct and consistent results were not obtained when L 3 or older larvae were used. The results of the authors mentioned above were nevertheless in perfect agreement with the classical concepts, i.e. shortening of the intermoult period with accompanying implications concerning morphometry. Our results throw a new light upon the functions of the VG.

In the mode of action of the moulting hormone the main aspect is certainly the induction of the moulting process. The activity in the cells which prepare the moult may involve various degrees of differentiation and growth. Moulting without any apparent signs of growth or differentiation has been shown to occur in starved larvae in some insect species. Thus, the moult inducing function is well established.

Whether we are justified to ascribe also a direct influence on growth and differentiation to this hormone, as has been done repeatedly, is not entirely clear. The growth of transplanted imaginal discs in *Drosophila* depends quantitatively on the amount of MH available (Vogt 1943, Bodenstein 1943).

Growth in insects is heterogonic, i.e. different body parts grow with different velocities. Among the structures growing faster than most other structures the pterothecae are the most conspicuous. Such a growth rate may also be referred to as being positively allometric.

The stepwise but gradual change in body proportions during larval development of hemimetabola is often considered as a more or less gradual differentiation. In our opinion the term differentiation ought to be reserved for the distinct specialisation in function and shape taking place in cells and tissues during the process of metamorphosis in the strict sense. The positively allometric wing growth may take place without differentiation in the cells involved.

The concept of gradual differentiation entails hypotheses about its induction. The mechanism most often held responsible for it is a growth in each instar cycle during a restricted period in which MH is present already before a level of JH becomes manifest. It has even been claimed that the CA activity is induced by a previous MH level (Wigglesworth 1936). The latter author was able to prevent the supposed small amounts of metamorphosis in *Rhodnius* by means of experiments with parabiosis. In this case the conclusions were based

on minor changes in the external genitalia. During larval development imaginal discs in *Drosophila* however only show growth, differentiation taking place not before the pupal stage (BODENSTEIN 1953).
The same might hold for the development of pterothecae in hemimetabola e.g. *Locusta*. No amount of implanted CA ever turned out to be effective in arresting the allometric growth in these larval pterothecae appreciably.

Adult differentiation on the contrary is always prevented by high levels of JH.
Returning to our experiments we may attempt to find confirmation for any of these theories in the results. Obviously we should distinghuish between two major effects:

a) the immediate wing hypertrophy, accompanied by a longer duration of the instar concerned and anticipation of the normal wing reversion.
b) the anticipation of the ultimate metamorphosis.

We have sufficient reason to presume a relation to exist between these two effects, but the second effect is assumed to be only secondary. It is absent when a high JH level is maintained and in some rare cases even without that. So the effect a) will be considered at first. Of importance is that:

1) Simultaneous implantations of CA have a supporting effect and may even cause a response when implanted alone.
2) Only implantations early in L 2 or even still earlier produce a response.
3) The responsive interval is widened by simultaneous CA implantations.
4) Any wing hypertrophy in an individual is accompanied by a delay in moulting.

The theory of a gradual differentiation spread over short periods in the beginning of each instar seems to find a confirmation in point 2. This conclusion is rendered invalid when considering that the influence of excess CA prolongs instead of shortens the assumed differentiation period.
Another possibility is suggested by WIGGLESWORTH (1952) in the reabsorption of JH by the CA, but this has found no evidence in our other experiments so far.

For a determining influence of the hormone balance JH–MH no evidence was found either. The supporting influence of the CA renders this improbable. Besides, this balance cannot play a role in pigmentation either, since everything points to an independent action of the CA on this feature. It remains a fact, however, that somehow we have brought about a prolongation in the period in which induction of positively allometric wing growth takes place. The CA influence somehow, but not quantitatively, the response of the hosts. This might be effected either by a direct activating influence of the CA on the implanted VG or the VG of the host, or by increasing the responsiveness of the tissues. In view of the results with adult "degenerated" VG we would prefer the first possibility.

It must be clear that never any immediate adult differentiation in the strict sense took place as a result of VG implantations. Thus it does not seem justi-

fied in our case to call the MH a differentiation hormone. It is probable that in our case the moment of moulting is the result of prolonged development rather than its cause. It remains a peculiar effect however when compared with the inverse results of STRICH HALBWACHS.

The fact that only in very young instars (L 1 and L 2) the implantations provoke a clear response must still be explained. The possibility that this depends on quantitative relations between the amount of VG implanted and the body weight of the hosts may be excluded since higher amounts of VG do not result in a response in L 3 either. Another explanation may be sought in wing development itself which perhaps is not yet so strongly allometric in these first instars. This might be due to the observed low mitotic activity in the VG in these instars (STRICH HALBWACHS 1959). Thus, artificially increasing the level of MH might induce a relatively stronger allometric growth, normal for later instars.

The anticipated wing reversion has not been the subject of a more close examinition; we suppose it to be a secondary phenomenon, connected with a certain degree of wing development.

No explanation has been found for the sex difference in the response of L 1 and L 2. Assuming different endocrine cycles or levels in either of the two sexes does not seem to be realistic. Some evidence pertaining to this point is found in the fact that in many *Orthoptera* the males are smaller and count one instar less in their larval development. Thus we may presume that in the case of *Locusta* this genetically determined property is latent and only brought to the surface under the conditions of our experiments. It is also known that extra instars in *Locusta* as well as in other locust species which may occur under solitary conditions do affect a higher percentage of females (ALBRECHT 1955), although the sex difference is by no means so important as in our experiments. In our *Locusta* material the number of instars was invariably 5, whatever the breeding conditions.

The anticipated metamorphosis (effect b) involves a real differentiation in all external parts of the insects which is completely comparable with the changes during a normal metamorphosis. This is demonstrated by the quite normal E/F ratios and values for L which are not significantly different from those in sixth instar adults.

Incomplete or patchy metamorphosis never develops as a result of VG implantations only, the switch over is absolute. This means that we have interfered with the mechanism which "counts the instars" and fixes the moment of the metamorphosis.
According to the literature we may assume that metamorphosis is brought about by a change in the endocrine levels. A relative preponderance of JH in the earlier instars is followed by a dominance of the MH later on. Again no unanimity exists as to the details of this change and its causes (WIGGLESWORTH 1954). It is by no means certain that these processes are quantitati-

vely similar in all insect species. The completeness of the shift to metamorphosis under normal conditions may be due to mechanisms on each of the following levels:

a) The central nervous system
b) A self redressing hormone balance
c) The responsiveness of the tissues

The first possibility suggests that the central nervous system has the power to "count the instars" and to decide the moment of the adult moult. Our experiments suggest that not the number of instars is counted but rather a degree of development in some tissue, organ or superordinated unit.

Possibility b) may not be altogether excluded. It suggests a shift in the hormone balance depending on a relatively decreasing CA activity, brought about by a negatively allometric growth of this organ during the last instars combined with the outcome of a positively allometric growth of the VG. The anticipated metamorphosis in our case may thus be provoked by the artificially increased MH level or by a suppressing influence on the CA of the host. The first suggestion does not hold good, for there would not be any reason for the metamorphosis not to take place earlier, say in L 3 or L 4 when extra VG are implanted. The second suggestion finds some support in results obtained with combined implantations which inhibit anticipated metamorphosis. It is not yet possible to decide with certainty which of the suggested regulating mechanisms is responsible for the normal course of events and the abnormalities produced in experiments.

Possibility c) does not seem to hold good in view of the results of extra implantations of CA which produces all sorts of intermediates.
The same experiments show that it is not the quantity of precursor delivered by the brain which regulates the amount of CA hormone, for in that case extra implantations would be far less active than they actually are.

Our results demonstrate that the VG may actually exert an influence upon the amount of allometric wing growth and thus on the phase E/F in a positive sense. The question of omitted instars is certainly related to the existence of various developmental types differing in the number of larval instars in some locust genera. Of these should be mentioned *Schistocerca, Nomadacris* and also *Locusta.*
ALBRECHT (1955) reviewed the literature on this subject and demonstrated the dependence of the above – mentioned developmental types on hatchling size, thus on parental density and phase status. Each developmental type comprised a fixed number of instars and a definite growth rate. It is possible that the resulting adults are hardly different, even with respect to their response to density factors. In the resulting adult we thus have no possibilities to detect the type of larval development. The same applies to our abnormal fifth instar adults with respect to the E/F ratio and L. However, the F/C ratio and the

general size showed some compensation for the smaller number of instars but did not attain values normal for sixth instar adults.
It should be considered that the short type of development in our case was the result of an induction starting during the development instead of at the very beginning of it. ALBRECHT found the type with fewer instars to be connected to the gregarious phase status of the hatchlings. In our case the development with fewer instars was a result of increased VG activity. When we compare this with the greater volume of the VG in solitary larvae of *Locusta* and *Schistocerca* observed by ELLIS & CARLISLE (1961) there is an unexplained contradiction, when it is indeed so that a greater volume of VG means more secretion.

The phase ratio F/C is hardly or not affected even in individuals with extreme E/F ratios as a result of extra VG implantations. This once more emphasizes the mutual independence of many of the phase characters and the more or less fortuitous simultaneous modifications as a result of density changes in normal individuals. Nevertheless, these experiments indicate that the VG affects the number of larval instars and also the relative wing development, both being phase characters.

7. THE CORPUS CARDIACUM

A number of general functions of the CC is rather well known, but concerning details much remains to be studied. The partly glandular and partly nervous structure of this organ, e.g. HIGHNAM 1961, is obvious.
An obstacle in the experimental work on the CC is the fact that operations on this organ are likely to disturb the nervous connection of the CA with the brain, leading to ultimate deficiency symptoms with respect to the CA hormone(s).
The function of the CC in the temporary storage and release of neurose-cretory material is not a subject of controversy either, although a direct transmission of neurosecretory material to the CA is not yet proven. Some difficulties are presented by the fact that the AH may reach the CA by humoral pathways as well, for otherwise implanted CA would irreversibly loose their activity and this is not the case. The functions of the CC with respect to the activity of the CA have been discussed already in paragraph 5.4.
What remains to be discussed are the specific secretory functions of the glandular parts. According to GERSCH (1957) and CAMERON (1953) the CC are a rich source of myotropic hormones. Their influence on phase phenomena remains to be investigated.
In addition to the experiments already described in paragraph 5.4 (extirpation of dorsal part, intersection of nervous connections) other operations were carried out, including total extirpations and implantations of extra CC, sometimes in combination with implantation of other organs as VG and CA.

7.1. Extirpation

It appears to make a difference whether the organ is extirpated entirely or partly. Total extirpation is a difficult operation, especially with respect to the connection between the CC and the hypocerebral ganglion, it invariably results in a very high mortality. This mortality is different from the normal post-operative mortality occuring in all experiments since the individuals submitted to this treatment do not die immediately but much later and not at all simultaneously. Only rarely do individuals survive into the adult stage after being submitted to this total operation in L 2. The delay in mortality allows for some observations:

a) Whenever individuals survive into the L 5 they exhibit prothetely as they do after severing the NCA or NCC.

b) Surviving individuals show considerable delay in the moment of moulting. Such delay is not encountered as a result of other operations except for those on the recurrent nerves, hypocerebral ganglion and outer oesophageal nerves.

c) There is sometimes a distinct effect of the extirpation on pigmentation. A small fraction of the larvae surviving into L 3 and next instars appears to have lost most black pigment. They resemble solitary individuals reared on a light coloured background in spite of the actual density of larvae in the jars. It is not known in which respect the larvae showing this response differ from the non-responding larvae.

The lethal effect of the operation may be due either to damaging the connected hypocerebral ganglion or to some essential function of the ventral part of the CC (the anterior unpaired lobe) from which originate the NCA. All evidence points rather to the first possibility since preliminary experiments showed that almost any sort of damage to the recurrent nerves, the hypocerebral ganglion or the outer oesophageal nerves resulted in the same type of delayed mortality, even when the CC remained intact.

It goes without saying that the few surviving adults do not allow for a morphometrical analysis.

The removal of the dorsal part of the CC only, involving a removal of the parts intimately connected with the aorta, did not have such harmful effects as total extirpation (experiment 5.4.3.e). In a series of 15 larvae operated in this way 14 survived into the next instar. Of these fourteen L 3 one was almost solitary greyish yellow, 5 showed a somewhat reduced pattern and the rest were almost normal. Twelve normal adults developed ultimately without appreciable delay. In the controls the pattern was fully developed throughout.

Although these results are not very constant, the fact that a few very abnormal individuals loose almost completely their black pattern while being subjected to the normal density stimuli suggests that the extirpation of parts of the CC may affect the formation of black melanin pigments.

7.2. Extra implantation of extra CC

Extra implantations were carried out in L 2, with or without simultaneous im-

plantations of extra CA, both obtained from adults. Compared with the controls, this produced an unmistakable effect on the pigmentation of the resulting L 3 which exhibited an increase in the amount of black pattern present with respect to both intensity and extend. Per individual the effect was slight and difficult to express in figures, but when the batches were compared as a whole it was easy to be observed. In our case we not only relied upon our own probably subjective judgment but the effect was also confirmed by some unbiassed observers and may be considered as well established.
In the experiment concerned, consisting of several samples each of about 10 individuals, the effect of implantation of extra CC developed in the absence as well as in the presence of extra implanted CA. In the former case the increase in melanin pattern established itself in the normal gregarious pattern. In the latter case the reduction in the black pattern accompanying the appearance of a general green colour proved to be less than in the case of implantations of CA only.
In any event the effect proved to be transitory, for in the next instar the difference had disappeared entirely. These results demonstrate that:

a) The formation of black pigment is not closely connected with the formation of green pigment.
b) The implanted CC probably release a certain amount of stimulus only once.

There is a certain analogy between the observed release of stored neurosecretory material in CC of cockroaches as a result of stress conditions, particularly with regard to the assumed role of related phenolic compounds in the formation of black pigments (HODGSON & GELDIA 1959). Moreover the existence of myotropic "hormones" in the CC of several insect species and their influence on physiological colour change in many *Crustacea* and some insect species (*Dixippus*) may be considered as well established (DUPONT RAABE 1957, BROWN & MEGLITSCH 1940, THOMSEN 1943). Stimuli producing physiological colour changes are likely to produce corresponding morphological colour changes in animals possessing both mechanisms (PARKER 1948).
Our results confirm the role of the CC and its principles in the morphological colour change suggested already by PFLUGFELDER (1938), who found a very specific pigmentation in *Dixippus* as a result of eliminating the CC functions by nerve severing.

Extra implantation of CC failed to provoke any appreciable effect on the subsequent development and the morphometrics of resulting adults.
For the time being nothing prohibits identification of the pattern factor in the corpus cardiacum with the humoral pattern factor of NICKERSON (1956).

8. GENERAL DISCUSSION

Several endocrine glands of *Locusta* appear to be involved in the development

of phase transformation as was demonstrated by experimental interferences with their activity.
The role of the CA in the development of green pigment in *Locusta* as well as in other locusts and many non-gregarious *Orthoptera* is no longer questionable.
Extra implantations of CA in larvae induced the formation of this pigment, overriding simultaneous gregarious stimuli which under normal conditions never resulted in green pigmentation. It may be concluded that gregarious stimuli somehow prevent the CA from producing a secretion in excess of the quantity of JH necessary for normal development.
This is confirmed to some extent by the results of volume measurements in the CA in breeding experiments, which show that conditions which turn the animal green also result in a high CA volume.
The green solitary type is only one of the possible solitary morphs and thus cannot be considered as "the" phase extreme in *Locusta*.

A glance at the other modifications in phase characters in experiment 3.1 reveals that the length of development is also more related to the CA volume than to the morphometric phase differences.
The classical phase ratios E/F and F/C, however, are dependent on the density only. The ratios proved to be almost independent of humidity, changes in the volume of CA, the duration of the larval development, and the general body size. The latter characters all show appreciable differences according to the humidity conditions, particularly in the isolated group. In our case it seems to be justified to presume a positive correlation between absolute CA volume and secretory activity. When this correlation actually exists, a direct causal relation between CA activity and the magnitude of phase ratios becomes improbable.
In any case we are not able to confirm the earlier suggestions in the literature (e.g. STOWER et al. 1960) that relative humidity (as well as temperature) would modify phase characters in the same sense as does density, at least not in so far as *Locusta* in concerned.
The combined influence of isolation and humidity becomes manifest already after a few days breeding under these conditions (experiment 3.2). A change in F/C and general body size accompanies a relative change in CA volume. In the experiment concerned, gradual, approximately isometric growth in the CA in L 5 is apparent.

No indications have been found so far for differences in the secretory cycle of the CA, supposing that such differences would be reflected in changes in the actual CA volume.

In order to study causal relations between CA activity and phase characters, extra implantations of CA and various operations resulting in reducing the CA volume were undertaken in larvae. The results strongly suggest that it is desirable to distinguish in the resulting adults between the morphometric

changes produced during larval development and those resulting from partial inhibition of metamorphosis.

It is suggested that the ratio E/F is particularly likely to express metathetelic disturbances. These possibly obscure real phase differences acquired earlier. The ratio F/C, however, is more an expression of the influence of long lasting endocrine stimuli since its normal changes during metamorphosis are smaller. Moreover, the ratio F/C has the very particular property of being plastic in gregarious adults in both directions. Metathetelic influences during the metamorphic moult bring about a decrease in this ratio. However, isolation during larval development exerts an influence comparable to that of early implantations of CA in increasing this ratio, provided that the implantations are not able to disturb the ultimate metamorphosis appreciably. This is obviously the case when for instance the very active and large CA of *Romalea* are used or when the implantation is carried out during or just before the morphogenetic processes in L 5.

In most of the papers of JOLY and in recent reviews of KENNEDY (1956, 1961) the well discriminating F/C ratio is left out of consideration, unfortunately. It is a fact that the changes in this ratio do not quite support KENNEDY's idea that the solitary phase bears a more juvenile character.

Extra implantations of CA in L 2 bring about changes in F/C in the adults comparable with those of isolated breeding, but this is the outcome of rather different reactions in the single characters. Head width (C), for example, shows a decrease corresponding to that of general body size and weight. As we have seen in experiment 3.1 this is not what happens as a result of isolation in females. Nevertheless, the ratio F/C indicates a rather normal phase change in both sexes. The E/F ratio, being very significantly lower in distinctly metathetelic adults, is deliberately left out of consideration in most of the following arguments.

The larvae actually show distinct morphometric phase transformation even in the solitary direction. It is rather difficult to imagine how such larvae could be more "larval".

As far as pigmentation is concerned the fact that implantations of CA in early L 5, while inducing metathetely, sometimes fail to induce formation of green pigment (JOLY 1955 and own observation) is still waiting for an explanation. Although we may suppose that the pigment induction takes place well after the morphogenetic induction, the character of the inactivation is not clear.

Yellow colour in mature males, being the result of a displacement of pigments from underlying tissues into the integument, appears to be dependent on CA activity as well. The change may establish itself only after a period of deficiency in CA factor in which some metamorphic differentiation has taken place.

Green and yellow colours are produced by different pigments, and their localisation in very different. Nevertheless is it peculiar that both depend upon CA activity. Green pigment is normally produced only under isolation, and

yellow colour only under crowded conditions in which the transfer of pheromones is supposed to play a role (LOHER 1960). In the latter case the dependence on an actual excess of CA factor is not yet established; it may be that the CA factor is only a prerequisite in conditioning the tissues of the insect for the response to communication with congeners.
The sex-difference in the response is not due to a quantitative difference in the CA factor but must have a more qualitative cause.

Interference with the innervation of the CA in young instars causes a considerable lagging behind in the growth of the organ compared with the controls. When it is effected in L 2, deficiency symptoms become apparent in L 5 (prothetely).
The influence of such operations on the formation and disappearence of green pigment is in accordance with expectations. A lasting CA activity is necessary for the maintenance of the green colour.
The innervation of the CA probably does not conduct inhibiting impulses, for intersections never result in any increased activity in *Locusta*. This is definitely not so in several other insect species. The example of *Leucophaea* is illuminating in this respect (SCHARRER 1952; ENGELMANN & LÜSCHER 1957).
How the increased CA activity under humid and/or isolated conditions is induced is not yet known. Severance of the NCA sometimes does not prevent a temporary pigmentation effect by subsequent isolated breeding until apparently the CA volume becomes the limiting factor, ultimately leading to prothetely (experiment 5.4.5). Nor does the nervous connection with the suboesophageal ganglion conduct such phase dependent stimuli to the CA for severing remains without result. In our case the regulatory function of this ganglion upon the CA as supposed by ENGELMANN & LÜSCHER (1957) could not be confirmed.

It is peculiar that the effect of isolation on the CA is not strictly related to the comparatively small increases observed in the volume. Extirpation of one CA reduces the CA volume (and surface) by 50%, but does not at all prevent the insect from turning green. Thus, either the remaining CA is induced to a considerable hyperactivity or the response has a more qualitative basis. The latter possibility is difficult to reconcile with the effect of extra implantations of CA and finds no evidence in other experimental work with locusts as yet. It has been proved, however, to be a good working hypothesis in the analysis of caste polymorphism in termites (LÜSCHER & SPRINGHETTI 1960).

It is of interest to draw attention to the observations by ELLIS & CARLISLE 1961. They found a hypertrophy of the ventral gland of *Locusta* and *Schistocerca* larvae under isolated conditions comparable to that in the CA. They demonstrated an effect on the solitary pigmentation following elimination of a small part of this gland, suggesting that pigmentogenesis depends also on the VG and even on minor changes in the volume of these glands. This suggestion does not find any confirmation in our experiments. Although the same type of operation was not carried out by us, it is hardly conceivable that extra implan-

tations of VG would remain without effect when the suggested relation exists. The persisting activity of such implanted glands is not subject to serious doubt. The experiments of ELLIS & CARLISLE were carried out on *Schistocerca,* which possesses a green pigment mechanism comparable with that in *Locusta,* but with many differences in the details of its regulation. Confirmation for *Locusta* seems very necessary before generalisation is permissible.

The absence of a strict relation between growth and volume of the CA on the one hand and activity on the other was very obvious in experiments in which the completely detached CA were reimplanted in the CC. Growth is restored but in most cases activity is not. At least under these abnormal conditions the volume of the CA is not a parameter of activity. Thus the restoration of growth of the CA depends on a regeneration of some connection along the remaining nervous stumps of the CA.
When the same type of operation was carried out in the presence of an undisturbed CA, growth of the reimplanted CA lags behind. The cause of this difference is likely to be found in either a feed back mechanism or in a quantitative relation between the trophic stimulus originating in the CC and the resulting growth of the CA.

Hitherto detailed knowledge of the character of the stimuli regulating secretion in the CA has not been forthcoming. A role is probably played by the activating hormone released by the neurosecretory cells in the brain but the mode of transmission to the CA is not known in detail and may be either humoral or connected with the stainable neurosecretory droplets along the efferent axons. The CC probably have a function in its transport, modification and release in the haemocoel.

A second possible regulatory mechanism might be found in nervous impulses transmitted along other nerve fibres in the same nervous trunks. Experiment 5.4.5 shows us that such a mechanism is not involved in the green colour induction.

A third possible action exerted upon the CA via its innervation is inhibition, demonstrated to be responsible for the breakdown of CA activity in the last larval instar and the cyclic induction of oögenesis in adults of some cockroaches (SCHARRER 1952). Intersections of the NCA in *Leucophaea* release the inhibition and result in abnormalities due to an excess of CA hormone. No effect of this kind was ever observed in *Locusta.*

In addition to this it was found in our experiments that the nervous connection of the CA via the anterior (ventral) part of the CC with the hypocerebral ganglion may be responsible for the maintenance of a sufficient CA secretion for subsequent normal development. Simultaneously, growth was inhibited to a high degree by eliminating the dorsal part of the CC with its nerves to the brain. We feel inclined to suppose a relation with neurosecretion in the nervous connection between the CC and the hypocerebral ganglion, observed by HIGHNAM (1961) in *Locusta.*

Combining all evidence we come to the following conclusion:

1) It is probable that in *Locusta* solitary induction, causing the insect to become green, is exerted by means of a higher CA activity. The stimulus inducing the CA to modify may be transmitted humorally but not exclusively so.

2) The CA do not depend on the nervous connection with the central nervous system for a basic level of secretion, although maximal activity and probably also the integration of the quantity of secretion are likely to depend on an intact innervation.

3) There is no causal relation in the CA between actual volume, growth and secretion. The actual size sets a limit to the maximum of secretion, but below this any correlation is absent as is demonstrated by large but relatively inactive and small but active CA as a result of experimental interferences.

When gregarious larvae were subjected to an operation reducing CA activity the morphometric changes in the resulting adults were not very great. This was ascribed to a lack of plasticity in the material in the gregarious direction as a consequence of permanent gregarious breeding.

An influence of the ventral gland upon the pigmentation of *Locusta* appeared to be absent in all experiments with extra implantation and extirpation. The experiment of ELLIS & CARLISLE demonstrating an opposite effect has already been dealt with.

With regard to the effects in morphometry our results deviate considerably from those described in papers of STRICH HALBWACHS and HALBWACHS on this subject. The effects which STRICH HALBWACHS obtained by extra implantation in L 3, L 4 and L 5 are not contested and are well in agreement with classical concepts concerning their function.
Our opposite results only contest the validity of generalization of her conclusions, the difference being probably due to the younger stages of larvae used.
Our results briefly amount to an abnormal prolongation of positively allometric growth in certain body parts accompanied by a delay in the next moult and as a consequence a decrease by one in the number of larval instars. The effect is qualitative rather than quantitative and is supported by an excess of CA activity in as far as the numbers of responding individuals are concerned.
Our results are difficult to bring in agreement with the classic concept although the observed effects might be ascribed to a prolongation of the period of active growth in the cycle of the instar involving a higher degree of positively allometric growth. Any explanation immediately gives rise to the question why the same effects do not appear in later instars. We therefore ought to look for essential differences between the early and later instars.
A difference in the number of mitoses in the VG, probably related to differences in the quantitative distribution of growth in the earlier instars, has already been indicated by STRICH HALBWACHS (1959). A survey of other differences or "directional changes", part of which refer directly to physiological

processes, is presented by ROONWAL (1940). Any light on the biological background of such differences is lacking, however.

The anticipation of the metamorphic moult is certainly a phenomenon related to differences in the number of instars between phases in some *Locusta* species, *Locusta* included. (This plasticity was not present in our *Locusta* material in which a high parental density was maintained).

It would be logical to suppose the activity of the VG to be responsible for the difference in type of development, but the fact that the alternative type is fixed throughout the larval development imposes some restrictions. Hitherto the evidence is not sufficient to draw definite conclusions in this respect.

Except for modifications in the type of development, sometimes only the wing hypertrophy persisted into the adult after a normal number of instars. This is an indication of a probable role of minor effects of this kind in the development of phase differences, although implantation experiments failed to produce very consistent results. The fact that the extreme E/F values were not accompanied by corresponding aberrant F/C ratios could indicate an exclusive influence on wing growth.

The effect of the CC on a detail of the pigmentation, the black pattern, is indicative of more or less independent functions of the CC in phase development, at least in as far as pigmentation is concerned. On the other hand a certain relation of higher order between pattern and green pigment is probable. Our CC effect might be identified with the humoral pattern factor of NICKERSON (1956).

The effects obtained by experimental interferences with the endocrine functions concern almost every detail of the phase syndrome. In most cases the separate effects were purely additive and without synergistic or opposite interactions. This, of course, does not exclude mutual influence on the secretion of glands or "feedback" mechanisms. Complete phase transformations were not obtained by interference with the glands only. This may be explained by the complexity of the endocrine integration, not only involving quantitative regulation but also a precise timing of the activities. The higher centre effecting the integration presumably is the brain.

SUMMARY AND CONCLUSIONS

(abbreviations are explained in the glossary)

1. INTRODUCTION

The scope and character of phase differences, the position of locust phase polymorphism among related phenomena and some evidence for endocrine and other physiological mechanisms controlling phase induction are briefly reviewed and commented upon.

2. MATERIAL AND METHODS

Breeding methods and post-operative treatment of *Locusta* larvae used in all experiments are described. An apparatus for convenient and rapid performance of operations on the endocrine system of all larval instars was developed, avoiding the use of anaesthetics. Survival percentages as a rule were very high. The determination of CA volumes was carried out in the fresh state by means of a specially designed, simple instrument.

For morphometric body measurements use was made of a measuring microscope with an accuracy of 0.01 mm.

3. PHASE BREEDING EXPERIMENTS

In some breeding experiments the actual scope of phase variations as a result of different breeding densities was determined. The CA volumes were also measured. High humidity as an additional factor proved to be of little influence on the proper phase ratios E/F and F/C, but of major importance (when combined with isolation) on the green pigmentation, the duration of the development and the CA volume. In experiment 3.1 a clear relation between phase ratios and CA volume was absent. In experiment 3.2 larvae were reared for one instar (L 4) under different conditions and analyzed in L 5. Significant changes in CA volume and morphometric characters were the result of this brief lasting influence. No evidence was found for different secretory cycles in the CA of L 5, assuming that differences in secretion would be reflected in differences in volume.

4. IMPLANTATION OF EXTRA CA IN LARVAE

4.1 IMPLANTATION OF EXTRA CA IN L 2. This paralleled the results of isolated breeding in as far as phase ratios were concerned in both sexes. Differences were noted, however, in the sexual dimorphism ratios.

4.2 IMPLANTATION OF EXTRA CA IN L 5. This mainly resulted in a disturbance of the metamorphic moult by inducing metathetely. The accompanying F/C ratios were more larval rather than more solitary.

5. ARTIFICIALLY INDUCED CA DEFICIENCY

5.1 TOTAL EXTIRPATION. This resulted in prothetelic larvae, showing metamorphic changes comparable to those in a normal metamorphic moult. The cryptic colorations in treated isolated larvae persisted after the moult in which prothetely developed.

5.2 REEXTIRPATION OF PREVIOUSLY IMPLANTED ADULT CA IN LARVAE. The result revealed the absolute relation between green pigmentation and the presence of active CA.

5.3 TOTAL EXTIRPATION FOLLOWED BY REIMPLANTATION OF ADULT CA AFTER VARYING NUMBERS OF DAYS. A critical period of 2–3 days was found for the occurrence of irreversible adult differentiation. Later reimplantations nevertheless induced green coloration in the resulting prothetelic larvae. When the

reimplantation was carried out on about the day of the next moult or thereafter, no green but only yellow colour appeared, and this only in the males.

5.4 VARIOUS OPERATIONS ON THE INNERVATION OF THE CA AND CC.

5.4.1 *Experiment 5.4.1.* Severance of the NCA reduces the growth rate in the CA concerned to a very low value. When no intact CA remained the CA activity became deficient after some delay. Most often prothetely developed in L 5. Severance of the NCC provoked a less severe inhibition of growth and activity of the CA. Some hypotheses to explain this are discussed.

5.4.2 *Experiment 5.4.2.* Operations comparable to those in experiment 5.4.1 were carried out on young L 4. Again the growth was inhibited but normal adults resulted, as was to be expected. Severance of both NCA prevented the development of yellow maturation colour. In the control treatments it appeared 10–17 days after the metamorphic moult.

The morphometric consequences of the treatments were negligible. This was ascribed to a lack of plasticity in the adult and a gregarious direction of the gregarious adults.

5.4.3 *Experiment 5.4.3.* As normal growth of the CA appeared to depend on an intact connection of the CA with the CC, it was attempted to reestablish a contact between entirely detached CA and the CC by reimplantation in L 2. This was successful in so far as the growth of the CA was concerned. These CA finally attained a normal size, but activity was not higher than in the CA which were not reimplanted in the CC after detaching them. When the dorsal part of the CC was removed, leaving intact the NCA and the anterior lobe of the CC connected with the hypocerebral ganglion, growth lagged behind but activity apparently not. Thus, growth and activity are not causally related.

5.4.4 *Experiment 5.4.4.* The operations were comparable with those in experiment 5.4.3 but with allo-transplantations of CA obtained from L 5. No greater activity of these CA was noted than of the CA in 5.4.3. Prothetely occurred in L 5 in the same measure. From the colour change to green could be concluded that the initial activity of these alien CA was not deficient.

5.4.5 *Experiment 5.4.5.* Activity reducing operations and subsequent isolated breeding. Gregarious larvae in which the NCA were severed were brought under isolated humid conditions. The development of green pigment was in most cases prevented and prothetely always developed in L 5. In some cases, however, a change to green coloration preceded later deficiency symptoms. It was concluded that the green pigment inducing stimuli acted via the CA as they would have done in undisturbed CA so that no nervous pathways for these hyperactivity stimuli could be detected.

5.5. COMPARISON OF ADULTS AND LARVAE RESULTING FROM DIFFERENT TREATMENTS IN A BIVARIATE PHASE GRAPH FOR F AND C. It became possible to separate two influences of excess CA activity on the relative changes in F and C. a) those exerted during larval development, b) those exerted during the metamorphic moult. The effects of both a) and b) on the ration F/C appeared to be

opposite. The ratio E/F was not considered to be a good phase indicator in our experimental work as the CA influence during metamorphosis tends to dominate all other influences.

5.6 HETERO-TRANSPLANTATIONS OF CA. The absolute activity as well as the size of the CA tended to decrease in the sequence *Romalea – Locusta – Schistocerca.* Hetero-transplantations were not less successful than homo- or allo-transplantations in the persistence of the changes.

5.7. INTERNAL AND EXTERNAL ADMINISTRATION OF EXTRACTS OF ADULT MALE *Hyalophora* SILKWORMS. The results were decidedly poor. This was attributed to toxic principles in the extract, causing mortality on injection. In the case of external applications failures were possibly due to difficulties encountered in the penetration of the active principle in the thick cuticle of *Locusta.*

6. THE VENTRAL GLAND

6.1 EXTIRPATIONS. Attemps to carry out total extirpations in L 2 were unsuccessful with regard to effect on either character, although at most 5% of the gland appeared to have escaped removal. No explanation is available for the fact that this considerable reduction in volume did not effect development more seriously.

6.2 EXTRA IMPLANTATIONS OF VG. These were mainly carried out in early instars in order to obtain data about a very peculiar implantation result opposite to that obtained by other authors. When carried out during the first day of the L 2 or earlier, an important fraction of the larvae responded with a delayed next moult and an anticipated wing development. The number of larval instars decreased by one. The fraction of the males responding to the implantation was at least 4 or 5 times as large as the fraction responding in the females. Occasionally superadults resulted with a normal number of larval instars but hypertrophied wings.

6.3 COMBINED EXTRA IMPLANTATIONS OF CA AND VG. Simultaneous CA and VG implantations supported the effect mentioned in 6.2. regarding the number of responding larvae. No interaction between CA and VG was noted in the appearance of green pigmentation or the immediate morphological effects.

6.4 DISCUSSION ON THE RESULT OF VG IMPLANTATIONS. The data could not be fitted properly in the classical concepts of the VG functions. All evidence points to a prolongation of the period of positively allometric growth of wings leading to relatively hypertrophied wings. This superdevelopment, being apparent also in general size, is compensated by an earlier metamorphosis. A possible function of the VG in the phase inducing mechanism remains open for discussion.

7. THE CORPUS CARDIACUM

7.1 EXTIRPATIONS. Despite a high mortality a clear result in a few larvae was the loss of black pattern.

7.2 EXTRA IMPLANTATIONS OF CC. This produced a temporary, rather diffuse increase in the black pattern, only to be observed in the next instar. This effect also appeared in gregarious L 3 and in L 3 of the green type resulting from simultaneous implantations of CA and CC.
The described effects were attributed to a release of some hormone or other active, probably myotropic, substance inducing or maintaining a black pattern.

8. GENERAL DISCUSSION

The observed endocrine effects are discussed with regard to their mutual relations. Most of the endocrine effects appeared to be independent but their integration is likely to be effected by an organ or higher order, presumably the brain.
The effects found need confirmation by the use of purified hormones before they may be considered as being sufficiently established. This applies particularly to the quantitative aspects and the mutual relations of endocrine glands.

SAMENVATTING EN CONCLUSIES

(Voor afkortingen zie glossary)

1. INLEIDING

Een overzicht wordt gegeven van de aard en de omvang van faseverschillen bij treksprinkhanen. De plaats van dit fasenpolymorfisme temidden van verwante verschijnselen wordt besproken. Diverse gegeven pleiten ervoor dat endocriene mechanismen bij het ontstaan van fasen een rol spelen.

2. PROEFMATERIAAL EN METHODIEKEN

Vrijwel alle proeven zijn verricht met larven van *Locusta migratoria migratorioides* R. & F. Kweekmethoden en post-operatieve behandeling van de larven worden beschreven. Een operatiebassin voor het snel en gemakkelijk uitvoeren van ingrepen op het endocriene systeem van larven van ieder stadium zonder gebruik te maken van narcotica, is in dit onderzoek ontwikkeld. Het overlevingspercentage was als regel hoog. Volumemetingen zijn uitgevoerd aan verse CA met behulp van een speciaal hiervoor geconstrueerd eenvoudig instrument. De afmetingen der lichaamsdelen zijn bepaald met behulp van een meetmikroskoop met een nauwkeurigheid van 0.01 mm. Voorzover niet anders vermeld wordt hebben de ingrepen plaats gevonden in jonge L 2.

3. FASEKWEEKPROEVEN

In enkele proeven is de omvang van de fasevariaties als resultaat van verschillende kweekdichtheden vastgesteld. Het volume der corpora allata is eveneens bepaald. De mede als proeffactor toegepaste hoge luchtvochtigheid blijkt van weinig invloed te zijn op de klassieke faseverhoudingen E/F en F/C, maar daarentegen van groot belang voor de groene pigmentering, de duur van

de ontwikkeling en het CA volume. In experiment 3.1 is geen duidelijke correlatie aanwezig tussen faseverhoudingen en CA volume. In experiment 3.2 zijn larven gedurende één larvestadium (L 4) onder verschillende omstandigheden gekweekt en vervolgens onderzocht in het volgende stadium (L 5). De veranderingen in het CA volume en de morfometrische kenmerken waren reeds na deze kortdurende behandeling significant. Er zijn geen aanwijzingen gevonden voor verschillen in secretoire werkzaamheid van de CA van deze L 5, aangenomen dat verschillen in secretie hun uitdrukking zouden vinden in verschillen in volume.

4. IMPLANTATIE VAN EXTRA CORPORA ALLATA IN LARVEN

4.1 IMPLANTATIE VAN EXTRA CA IN L 2. Deze ingreep had in vele opzichten hetzelfde resultaat als geïsoleerd kweken ten aanzien van de morphometrische faseverhoudingen.

4.2 IMPLANTATIE VAN EXTRA CA IN L 5. Het voornaamste gevolg van deze implantatie was een verstoring van de metamorfose-vervelling door het veroorzaken van metathetelie. De begeleidende F/C verhoudingen veranderen meer in larvale dan in solitaire richting.

5. KUNSTMATIG OPGEWEKTE CORPUS ALLATUM DEFICIENTIE

5.1 VOLLEDIGE EXTIRPATIE. Deze deed in larven prothetelie ontstaan. Cryptische kleuraanpassingen bij solitaire larven worden door de operatie niet beinvloed.

5.2 EXTIRPATIE VAN EERDER GEIMPLANTEERDE IMAGINALE CA IN LARVEN. Onze proeven gaven een correlatie te zien tussen de groene pigmentatie en de aanwezigheid van actieve CA.

5.3 VOLLEDIGE EXTIRPATIE GEVOLGD DOOR HERIMPLANTATIE VAN IMAGINALE CA NA EEN VERSCHILLEND TIJDSINTERVAL. Indien deze extirpatie onmiddellijk na de vervelling tot L 2 wordt uitgevoerd ligt de critieke periode voor de inductie van irreversibele imaginale differentiatie 2–3 dagen na deze vervelling. Implantatie op een later tijdstip induceerde niettemin toch een groene kleur in de hierbij optredende prothetele larven. Wanneer de herimplantatie werd uitgevoerd onmiddellijk vóór de volgende vervelling of daarna, dan ontstond geen groene maar een gele kleur, doch alleen bij mannelijke individuen.

5.4 DIVERSE OPERATIEVE INGREPEN TEN AANZIEN VAN CA EN CC.

5.4.1. *Experiment 5.4.1.* Doorsnijding van de NCA of de NCC had een sterke vermindering van de groeisnelheid van de betrokken CA tot gevolg. Indien niet tenminste één intact CA aanwezig was ontstond een vertraagde CA deficientie, die tenslotte resulteerde in prothetelie in L 5.
Doorsnijding van de NCC had minder sterke remming van groei en activiteit tot gevolg. Enkele hypothesen die dit zouden kunnen verklaren worden besproken.

5.4.2 *Experiment 5.4.2.* De bovenstaande operaties zijn eveneens uitgevoerd

in jonge L 4. Hierdoor werd de normale ontwikkeling vrijwel niet meer gestoord. Wanneer de imagines werden voortgekweekt trad een gele kleur niet meer op. Individuen met intacte CA werden 10–17 dagen na de laatste vervelling geel van kleur. De morfometrische gevolgen van de ingrepen waren zeer gering. Dit wordt toegeschreven aan een gebrek aan plasticiteit in de imaginale of gregaire richting in deze gregaire imagines.

5.4.3 *Experiment 5.4.3.* Daar de normale groei in CA bleek af te hangen van een intacte verbinding van de CA met de CC is geprobeerd een contact te herstellen tussen volledig losgemaakte CA en het CC door reïmplantatie. Dit had een positief effect op de groei van deze CA. Zij verkregen normale afmetingen, maar hun activiteit was niet groter dan in CA die niet in het CC werden gereïmplanteerd.
Indien alleen het dorsale gedeelte van de CC werd geëlimineerd, waarbij de NCA en de voorste ongepaarde kwab van het CC, die verbonden is met het hypocerebrale ganglion ongemoeid werden gelaten, bleek de groei ook achter te blijven bij de normale, maar deficientiesymptomen traden niet op. Groei en activiteit zijn dus zeker niet causaal verbonden.

5.4.4 *Experiment 5.4.4.* Een dergelijke serie ingrepen werd uitgevoerd in L 2 maar nu werden niet de eigen CA maar die van L 5 gereïmplanteerd. Dezelfde mate van prothetelie trad op in L 5. Uit een aanvankelijke groenkleuring kon worden afgeleid dat althans de initiële activiteit van deze allotransplantaten niet ontoereikend was.

5.4.5 *Experiment 5.4.5.* Activiteit reducerende operaties en daarop volgend geïsoleerd kweken. Gregaire larven, waarin de NCA waren doorgesneden, werden onder geïsoleerd vochtige omstandigheden gekweekt. De ontwikkeling van groen pigment werd hierdoor in de meeste gevallen verhinderd en steeds trad toch prothetelie in L 5 op. Soms echter ging een geringe groene kleur vooraf aan dergelijke latere deficientiesymptomen. Hieruit volgt dat de CA ook langs andere dan nerveuze weg kunnen worden geactiveerd.

5.5. VERGELIJKING VAN IMAGINES EN LARVEN DIE ZICH ONTWIKKELDEN NA DIVERSE INGREPEN IN EEN TWEEDIMENSIONALE GRAFIEK VOOR F EN C. Het bleek mogelijk twee invloeden te onderscheiden van overmaat CA activiteit op de relatieve veranderingen in F en C.

a) de invloeden tijdens de larvale ontwikkeling

b) de invloeden tijdens de metamorfose-vervelling. Het effect van a) en b) is tegengesteld. De verhouding E/F wordt in onze proeven niet als een goede fase-indicator beschouwd, daar de CA invloeden tijdens de metamorfose de neiging hadden over alle andere invloeden te domineren.

5.6 HETERO-TRANSPLANTATIE VAN CA. De absolute activiteit evenals de grootte van de CA hebben de neiging af te nemen in de volgorde *Romalea- Locusta- Schistocerca.* Hetero-transplantaties waren even werkzaam als homo- of allo-transplantaties voor wat betreft het voortduren van geïnduceerde veranderingen.

5.7 INWENDIGE EN UITWENDIGE TOEDIENING VAN EXTRACTEN VAN MANNETJES VAN HYALOPHORA ZIJDERUPSEN. De resultaten zijn vrijwel nihil. Dit is mogelijk toe te schrijven aan toxische factoren in het extract. Bij uitwendige applicatie was waarschijnlijk de penetratie van het actieve bestanddeel in de dikke cuticula de beperkende factor.

6. DE VENTRALE KLIER

6.1 POGINGEN TOT VOLLEDIGE EXTIRPATIE in L 2 zijn niet met succes bekroond. Ofschoon tenminste 95% van de klier werd verwijderd was er nauwelijks enige invloed op ontwikkeling of morfometrie te bespeuren. Dit blijkt een moeilijk te verklaren punt.

6.2 EXTRA IMPLANTATIES VAN VENTRALE KLIEREN. Deze zijn voornamelijk uitgevoerd in jonge stadia om gegevens te verkrijgen omtrent een merkwaardig gevolg van dergelijke implantaties dat tegengesteld was aan de resultaten van andere onderzoekers. Als de ingreep werd uitgevoerd in de eerste 24 uur van de L 2 of vroeger, vertoonde een aanzienlijk deel van de larven een vertraagde volgende vervelling en een vervroegde vleugelontwikkeling. Het totaal aantal larvestadia verminderde dan met één. Het aantal reagerende mannetjes was altijd 4–5 keer zo groot als dat van de wijfjes. Soms ontwikkelden zich "superimagines" uit larven met een normaal aantal larvestadia maar gehypertrofieerde vleugels.

6.3. GECOMBINEERDE EXTRA IMPLANTATIES VAN CA EN VENTRALE KLIEREN. Deze doen nog meer dieren reageren dan implantaties van ventrale klieren alleen. Er werd geen interactie tussen het effect van beide klieren waargenomen ten aanzien van de groene kleur of het directe morfologische gevolg.

6.4 BESPREKING DER RESULTATEN VAN VENTRALE KLIER IMPLANTATIES. De uitkomsten passen niet goed in de klassieke ideeën omtrent de functies van de ventrale klier. Alle aanwijzingen doen een verlenging van de periode van positieve allometrische vleugelgroei vermoeden, welke leidt tot relatief overontwikkelde vleugels. Deze "over" ontwikkeling leidt blijkbaar tot een vervroegde metamorfose als een zekere compensatie. De functie van de ventrale klier in de fase-inductie blijft nog open voor discussie.

7. HET CORPUS CARDIACUM

7.1 VOLLEDIGE EXTIRPATIE. In enkele larven was een verlies van zwart patroon onmiskenbaar. Bovendien was de mortaliteit bijzonder hoog.

7.2 EXTRA IMPLANTATIES VAN CC. Deze induceerden tot een tijdelijke tamelijk diffuse toename in het zwarte patroon, alleen waar te nemen in het volgende larvestadium. Dit effect trad zowel op in L 3 van het gregaire kleurtype als in de groene L 3, na gelijktijdige implantatie van CA en ventrale klieren.
De vermelde effecten worden toegeschreven aan afgifte van een hormoon of andere active, mogelijk myotrope, stof die een zwart patroon induceert of instand houdt.

8. ALGEMENE DISCUSSIE

De waargenomen endocriene effecten worden besproken, waarbij aandacht is geschonken aan hun wederzijdse relaties. De meeste effecten waren geheel onafhankelijk van elkaar, maar hun integratie wordt waarschijnlijk bewerkstelligd door een gesuperordineerd orgaan, vermoedelijk het cerebrale ganglion.
Tot slot zij opgemerkt dat zuivere hormoonpraeparaten waarvan in de toekomst zeker meer ter beschikking zullen komen, grote diensten zouden kunnen bewijzen bij de verdere analyse van de gevonden endocriene relaties.

ACKNOWLEDGEMENTS

The investigations were carried out at the Laboratory of Entomology of the Agricultural University, Wageningen, the Netherlands.
Sincere thanks are due to Prof. Dr. J. DE WILDE for his stimulating guidance, interest and criticism during all stages of the work.
The author is indebted to Dr. J. S. KENNEDY for advice in an early stage of the investigation.
Thanks are due to many members of the personel of the Laboratory of Entomology for help of various natures, especially to the breeding assistants Mrs. A. M. NILWIK–DE RUYTER and Miss F. J. E. VAN REMMEN, to Mr. G. VAN SURKSUM for constructing the apparatus and to Mr. M. P. VAN DER SCHELDE for the drawing of graphs and figures.
Special thanks are due to Prof. Dr. J. H. FREITAG, Mr. N. G. UYLENBURG and Mr. M. MARTIN for correction of parts of the English text, often with the shortest possible delay, and to Mrs. G. DE WILDE–VAN BUUL for correction of the Dutch summary.
Locust material, literature and helpful advice was generously supplied by the Anti Locust Research Centre in London (Gr.Br.) on many occasions.
The author feels much indebted to the Stichting „Fonds Landbouw Export Bureau 1916–'18", which sponsored the investigations and the publication.
The work was finished while in the service of the National Council for Agricultural Research (T.N.O.).

APPENDIX 1 (experiment 3.1). The influence of density and humidity on several morphometric characters. The conditions and the numbers of individuals used for the calculations are given in the first row. The treatment differences are expressed in percentages of the crowded or the dry treatment. Their significance is denoted by asterisks. When no interaction appeared to be significant only the main effects were calculated in the analysis of variance.

Males

	Isolated	Crowded	Effect of isolation		Effect of high humidity	
Dry	n = 20	n = 22				
Humid	n = 17	n = 13	Dry	Humid	Isolated	Crowded
D	29.4 24.8	29.8 29.0	−1.6%*	−14.5%***	−15.6%***	−2.7%**
CA	158.6 270.3	174.9 188.4	−9.3%*	+43.5%***	+70.4%***	+7.8%
E	4276.5 4470.1	4808.1 4852.8	−9.7%***		+2.3%**	
F	2312.9 2411.0	2528.5 2549.8	−8.5%***	−5.4%***	+4.2%***	+0.8%
C	672.0 693.8	772.9 779.3	−12.2%***		+2.0%*	
V	258.4 267.7	296.6 302.9	−12.4%***		+2.8%**	
L	54.4 50.2	53.3 52.6	+2.1%	−4.6%*	−7.7%***	−1.3%
Ps	246.7 276.9	286.8 299.0	−13.9***	−7.4%***	+12.2%***	+4.3%
KCs_1	94.3 101.6	121.5 130.5	−22.3%***		+7.6%**	
K_2	30.7 30.6	44.4 44.9	−31.2%***		+0.6%	
F_2	36.0 41.6	46.6 49.7	−20.0%***		+10.6%***	
KCs_2	187.5 203.1	261.9 265.4	−26.4%***		+4.4%	
E/F	185.1 185.4	190.3 190.3	−2.7%***		−0.1%	
F/C	344.2 347.7	327.4 327.3	+5.6%***		−0.5%	
F/V	895.8 889.5	853.9 842.4	+5.2%***		−1.0%	
KCs_2/K_2	613.1 664.4	591.3 593.7	+3.7%*	+11.9%***	+8.4%***	+0.4%

APPENDIX 1 (continued).

Females

	Isolated	Crowded	Effect of isolation		Effect of high humidity	
Dry	n = 20	n = 22				
Humid	n = 13	n = 21	Dry	Humid	Isolated	Crowded
D	31.2 25.8	30.4 29.8	+2.4%**	−13.4%***	−17.3%***	−2.0%**
CA	246.0 306.2	180.2 189.7	+36.5%***	+61.4%***	+24.5%***	+5.3%
E	5320.4 5485.8	5424.8 5312.7	−1.9%**	+3.3%***	+3.1%***	−2.1%***
F	2802.0 2886.5	2759.0 2692.7	+1.6%*	+7.2%***	+3.0%**	−2.4%**
C	848.1 877.7	865.0 864.6	−2.0%*	+1.5%*	+3.5%***	0%
V	321.3 331.8	325.4 331.7	−0.7%		+2.5%**	
L	50.3 47.8	53.2 51.5	−6.3%**		−3.9%*	
Ps	388.8 424.3	364.9 354.3	+6.5%***	+19.8%***	+9.1%***	−2.9%
KCs_1	158.9 168.0	154.1 152.5	+3.1%	+10.1%***	+5.7%*	−1.0%
K_2	51.0 53.6	58.8 55.0	−13.3%***	−2.5%	+5.1%	−6.5%*
F_2	61.1 68.2	56.5 52.8	+8.2%**	+29.3%***	+11.6%**	−6.5%*
KCs_2	314.2 339.6	335.8 305.7	−6.4%*	+11.1%***	+8.1%*	−9.0%***
E/F	190.0 190.2	196.6 197.5	−3.5%***		+0.3%	
F/C	330.4 329.0	319.2 311.5	+4.4%***		−1.6%*	
F/V	872.8 869.8	848.8 812.1	+2.8%*	+7.1%***	−0.3%	−4.3%
KCs_2/K_2	616.8 635.0	571.1 557.3	+8.0%***	+13.9%***	+3.0%	−2.4%

APPENDIX 2 (experiment 3.2). Statistical analysis of morphometric results.

Character	Source of variation	d.f.	Variance	F	P.
CA	Density groups	1	106906	53.94	***
	Time samples	16	40667	20.52	***
	Density × time samples	16	1822	<1	
	Residual variation	170	1982		
Ps	Density groups	1	2233	63.80	***
	Time samples	16	39	1.11	
	Density × time samples	16	38	1.08	
	Residual variation	170	35		
F	Density groups	1	5112	1.86	
	Time samples	12	3770	1.37	
	Density × time samples	12	5489	2.00	
	Residual variation	130	2750		
C	Density groups	1	10388	26.77	***
	Time samples	12	150	<1	
	Density × time samples	12	684	1.76	
	Residual variation	130	388		
K_2	Density groups	1	26791	13.24	**
	Time samples	14	125838	62.17	***
	Density × time samples	14	3038	1.50	
	Residual variation	150	2024		
F/C	Density groups	1	1001	21.30	***
	Time samples	12	53	1.13	
	Density × time samples	12	16	<1	
	Residual variation	130	47		
V	Density groups	1	1520	19.86	**
	Time samples	12	102	1.33	
	Density × time samples	12	86	1.12	
	Residual variation	130	77		

APPENDIX 3 (experiment 4.1). Male adults. Changes in morphometric characters and ratios as a result of extra implantation of corpora allata in second instar larvae (L 2).

Character	+CA	Contr.	Diff.	%, Sign.	Source of variation	d.f.	Variance	F	Sign.
a	23.5	23.7	− 0.2	− 0.9%					
b	23.8	25.8	− 2.0	− 7.8%					
D c	22.2	24.9	− 2.7	− 10.9%					
d	22.1	24.1	− 2.0	− 8.3%					
e	26.3	27.4	− 1.1	− 4.0%					
	4271.8	4604.3	−332.6	− 7.2%	Treatm.	1	2859196	12.96	***
	4439.8	4813.7	−373.9	− 7.8%−	Exp.	4	825127	3.74	**
E	4612.0	4818.8	−206.7	− 4.3%−	T×E	4	805832	3.65	**
	3868.8	4800.2	−931.4	−19.4%**	Resid.	96	220534		
	4770.8	4739.6	+ 31.3	+ 0.7%−					
	2499.0	2550.6	− 50.9		Treatm.	1	2011	<1	
	2523.7	2507.7	+ 16.0		Exp.	4	8250	<1	
F	2538.0	2593.2	− 55.1	− 0.6	T×E	4	8248	<1	
	2517.5	2537.1	− 19.6	(0.0%)	Resid.	96	12900		
	2529.5	2488.3	+ 41.2						
	703.8	728.3	− 24.6		Treatm.	1	9929	14.35	***
	727.9	765.3	− 37.4		Exp.	4	1474	2.13	
C	731.0	765.0	− 34.0	−19.7	T×E	4	1259	1.82	
	740.1	753.3	− 13.2	(2.6%)	Resid.	96	692		
	742.4	746.3	− 3.9						
	279.3	292.3	− 13.1		Treatm.	1	1493	10.37	**
	277.3	290.9	− 13.6		Exp.	4	245	1.70	
V	282.3	293.6	− 11.3	− 7.6	T×E	4	146	1.01	
	286.8	292.1	− 5.3	(2.6%)	Resid.	96	144		
	281.1	283.3	− 2.3						
	43.0	54.0	− 11.0	−20.4%*	Treatm.	1	2632	46.95	***
	43.1	54.4	− 11.4	−20.9%**	Exp.	4	28	<1	
L	48.7	52.6	− 3.9	− 7.4%−	T×E	4	174	3.11	*
	41.2	59.8	− 18.6	−31.2%***	Resid.	96	56		
	47.8	54.1	− 6.4	−11.9%***					
	171.3	180.7	− 9.4	− 5.2%−	Treatm.	1	5234	17.26	***
	175.3	192.1	− 16.9	− 8.8%**	Exp.	4	1680	5.54	***
E/F	181.9	185.9	− 4.0	− 2.1%−	T×E	4	1371	4.52	**
	149.8	189.4	− 39.6	−20.9%**	Resid.	96	303		
	188.9	190.5	− 1.6	− 0.9%−					
	355.3	350.3	+ 4.9		Treatm.	1	2104	13.14	***
	346.6	327.7	+ 18.4		Exp.	4	410	2.56	*
F/C	347.5	339.0	+ 8.5	+ 9.0	T×E	4	199	1.24	
	340.3	336.9	+ 3.4	(2.7%)	Resid.	96	160		
	340.3	333.6	+ 7.2						

APPENDIX 3 (continued).

Character	+CA	Contr.	Diff.	%, Sign.	Source of variation	d.f.	Variance	F	Sign.
	897.0	872.9	+ 24.0		Treatm.	1	14853	10.82	**
	909.7	862.7	+ 47.0		Exp.	4	1290	<1	
F/V	899.7	884.0	+ 15.7	+ 24.0	T×E	4	1120	<1	
	878.8	868.8	+ 10.0	(2.7%)	Resid.	96	1373		
	900.6	878.8	+ 21.8						
	39.3	46.8	− 7.5	−16.0%**	Treatm.	1	186	6.64	*
K_2	40.1	41.1	− 1.0	−2.6%	Exp.	1	71	2.55	
					T×E	1	141	5.03	*
					Resid.	53	28		
	265.1	315.1	− 50.0	−15.9%*	Treatm.	1	4629	2.68	
KCs_2	266.8	263.6	+ 3.2	+ 1.2%	Exp.	1	7499	4.34	*
					T×E	1	9601	5.50	*
					Resid.	53	1725		
	673.6	671.0	+ 2.7	+17.2	Treatm.	1	4156	1.97	
KCs_2/K_2	666.9	640.4	+ 26.8	(2.6%)	Exp.	1	4327	2.06	
					T×E	1	1972	<1	
					Resid.	53	2105		

APPENDIX 4 (experiment 4.1). Female adults. Changes in morphometric characters and ratios as a result of extra implantation of corpora allata in second instar larvae (L 2).

Character		+CA	Contr.	Diff.	%, Sign.	Source of variation	d.f.	Variance	F	Sign.
D	a	23.3	24.0	− 0.7	− 2.9%					
	b	26.0	26.5	− 0.5	− 1.9%					
	c	23.1	23.9	− 0.8	− 3.4%					
	d	22.6	21.5	+ 1.1	+ 5.1%					
E		4378.6	5321.5	−942.9	−17.7%	Treatm.	1	993242	13.59	***
		5229.4	5342.4	−113.0	− 2.1%	Exp.	3	405950	5.55	**
		5287.6	5483.1	−195.4	− 3.6%	T×E	3	436043	5.96	**
		5358.4	5438.3	− 79.7	− 1.5%	Resid.	64	73109		
F		2804.0	2854.3	− 50.3	+19.0 (0.7%)	Treatm.	1	5847	<1	
		2748.3	2696.5	+ 51.8		Exp.	3	91579	13.43	***
		2857.1	2828.7	+ 28.4		T×E	3	5669	<1	
		2874.7	2884.5	− 9.7		Resid.	62	6816		
C		796.5	869.1	− 72.6	−31.0 (3.5%)	Treatm.	1	15640	22.50	***
		841.3	864.3	− 23.0		Exp.	3	3546	5.10	**
		871.7	887.5	− 15.8		T×E	3	1750	2.52	
		844.0	889.4	− 45.4		Resid.	63	695		
V		317.5	330.9	− 13.4	−7.2 (2.2%)	Treatm.	1	833	5.99	*
		314.9	323.6	− 8.7		Exp.	3	591	4.24	*
		332.3	332.9	− 0.6		T×E	3	130	<1	
		322.0	333.6	− 11.6		Resid.	63	139		
L		41.0	49.3	− 8.3	−5.4 (10.4%)	Treatm.	1	484	18.17	***
		49.0	54.7	− 5.7		Exp.	3	109	4.09	*
		46.1	50.1	− 4.0		T×E	3	11	<1	
		47.8	52.9	− 5.1		Resid.	64	27		
E/F		179.0	188.1	− 9.1	−6.9 (3.6%)	Treatm.	1	777	17.75	***
		190.4	198.2	− 7.8		Exp.	3	262	5.98	**
		185.2	194.0	− 8.8		T×E	3	39	<1	
		186.4	188.7	− 2.3		Resid.	62	44		
F/C		352.5	326.9	+ 25.6	+14.3 (4.5%)	Treatm.	1	3321	29.41	***
		326.6	312.2	+ 14.4		Exp.	3	952	8.43	***
		327.8	318.8	+ 9.0		T×E	3	129	1.14	
		341.3	324.4	+ 16.9		Resid.	62	113		
F/V		883.0	862.3	+ 20.8	+24.8 (2.9%)	Treatm.	1	10015	13.92	***
		872.9	833.3	+ 39.6		Exp.	3	3048	4.24	**
		860.2	849.7	+ 10.5		T×E	3	770	1.07	
		893.5	866.9	+ 26.7		Resid.	62	719		

APPENDIX 4 (continued).

Character	+CA	Contr.	Diff.	%, Sign.	Source of variation	d.f.	Variance	F	Sign.
K_2	50.4	55.4	− 5.0	−7.7	Treatm.	1	548	4.71	*
	54.4	65.9	− 11.4	(13.0%)	Exp.	1	455	4.87	*
					T×E	1	94	<1	
					Resid.	33	116		
KCs_2	334.6	335.1	− 0.5	−13.6	Treatm.	1	1695	<1	
	359.9	391.4	− 31.5	(3.8%)	Exp.	1	14385	4.37	*
					T×E	1	2222	<1	
					Resid.	33	3291		
KCs_2/K_2	664.3	605.3	+ 59.0	+62.1	Treatm.	1	35332	15.05	***
	663.2	597.2	+ 66.1	(10.3%)	Exp.	1	177	<1	
					T×E	1	111	<1	
					Resid.	33	2347		

APPENDIX 5 (experiment 4.2). Male adults. Changes in morphometric characters and ratios as a result of extra implantation of corpora allata on different moments in the last larval instar (L 5).

Sample	Number of indiv. +CA	Number of indiv. controls	Age from preceding moult in days at moment of implantation
4.2. a	8	7	0
b	8	5	1–3
c	5	8	3
d	7	7	3–8
e	5	3	7

Character	+CA	Contr.	Diff.	%, Sign.	Source of variation	d.f.	Variance	F	Sign.
	10.2	11.3	− 1.1	− 9.7%*					
	8.6	9.2	− 0.6	− 6.5%					
D	6.5	7.4	− 0.9	−12.2%*					
	5.9	6.3	− 0.4	− 6.3%					
	5.2	5.0	+ 0.2	+ 4.0%					
	3715.1	4926.3	−1211.2	−24.6%***	Treatm.	1	4430692	111.50	***
	3933.3	4772.8	−839.6	−17.6%***	Exp.	4	1195842	30.09	***
E	4717.2	5006.6	−289.4	− 5.8%*	T×E	4	874809	22.01	***
	4774.9	4858.9	− 84.0	− 1.7	Resid.	53	39739		
	4908.6	4873.3	+ 66.1	+ 0.7					
	2520.0	2482.3	+ 37.7		Treatm.	1	8825	2.55	
	2427.1	2367.1	+ 60.1		Exp.	4	29791	8.61	***
F	2494.0	2502.3	− 8.3	+23.9	T×E	4	4601	1.33	
	2521.0	2479.1	+ 41.9	(1.0%)	Resid.	54	3462		
	2517.6	2564.0	− 46.4						
	796.4	772.4	+ 23.9	+ 3.1%*	Treatm.	1	8946	23.77	***
	802.4	747.0	+ 55.4	+ 7.4%*	Exp.	4	353	<1	
C	761.6	771.4	− 9.8	− 1.3%	T×E	4	2742	7.29	***
	800.1	755.5	+ 44.6	+ 5.9%**	Resid.	53	376		
	780.4	801.0	− 20.6	− 2.6%					
	298.0	289.3	+ 8.7		Treatm.	1	466	3.54	
	280.1	283.0	− 2.9		Exp.	4	330	2.50	
V	291.0	286.5	+ 4.5	+ 5.5	T×E	4	184	1.39	
	293.1	278.5	+ 14.6	(1.9%)	Resid.	54	132		
	288.8	292.7	− 3.9						
	19.6	48.0	− 28.4	−59.1%***	Treatm.	1	3003	145.57	***
	28.5	53.2	− 24.7	−46.4%***	Exp.	4	907	43.98	***
L	45.2	49.3	− 4.1	− 8.2%	T×E	4	563	27.29	***
	48.0	52.9	− 4.9	− 9.2%**	Resid.	63	21		
	49.1	51.4	− 2.3	− 4.5%					

APPENDIX 5 (continued).

Character	+CA	Contr.	Diff.	%, Sign.	Source of variation	d.f.	Variance	F	Sign.
	147.5	198.3	− 50.8	−25.6%***	Treatm.	1	8525	121.85	***
	162.0	201.6	− 39.6	−19.6%***	Exp.	4	1424	20.35	***
E/F	189.4	200.1	− 10.7	− 5.4%*	T×E	4	1632	23.33	***
	195.2	190.0	− 7.4	− 3.8%	Resid.	53	70		
	195.2	190.0	+ 5.2	+ 2.7%					
	316.6	321.3	− 4.7	− 1.5%	Treatm.	1	609	11.90	**
	302.6	316.8	− 14.2	− 4.5%**	Exp.	4	506	9.88	***
F/C	327.6	324.5	+ 3.1	+ 1.0%	T×E	4	190	3.71	*
	315.3	328.3	− 13.0	− 3.9%*	Resid.	53	51		
	322.4	319.5	+ 2.9	+ 0.9%					
	846.4	858.9	− 12.5		Treatm.	1	1586	<1	
	857.3	836.6	+ 20.7		Exp.	4	2060	<1	
F/V	858.4	873.6	− 15.5	−10.1	T×E	4	1149	<1	
	861.4	892.0	− 30.6	(1.2%)	Resid.	54	2466		
	872.2	879.0	− 6.8						
	42.4	39.0	+ 3.4	+3.9	Treatm.	1	103	6.45	*
K_s	42.4	38.1	+ 4.3	(10.1%)	Exp.	1	1	<1	
					T×E	1	1	<1	
					Resid.	24	16		
	262.0	241.6	+ 20.4	+18.5	Treatm.	1	2323	5.77	*
KCs_s	254.0	237.1	+ 16.9	(7.7%)	Exp.	1	280	<1	
					T×E	1	21	<1	
					Resid.	24	402		
	621.3	619.4	+ 1.9	−13.7	Treatm.	1	1307	<1	
KCs_s/K_s	598.3	627.3	− 29.0	(2.2%)	Exp.	1	323	<1	
					T×E	1	1836	<1	
					Resid.	24	2086		

APPENDIX 6 (experiment 4.2). Female adults. Changes in morphometric characters and ratios as a result of extra implantation of corpora allata on different moments in the last larval instar (L 5).

Sample	Number of individ.		Age from preceding moult in days at moment of implantation
	+CA	controls	
4.2. a	8	8	0
b	10	5	1–3
c	4	4	3
d	6	6	3–8
e	6	8	7

Character	+CA	Contr.	Diff.	%, Sign.	Source of variation	d.f.	Variance	F	Sign.
	10.5	11.0	− 0.5	− 4.8%					
	7.8	9.0	− 1.2	−15.4%					
D	7.0	7.0	0	0					
	4.9	6.2	− 1.3	−21.0%					
	5.4	5.6	− 0.2	− 3.7%					
	4611.9	5507.6	−895.8	−16.3%**	Treatm.	1	2573856	23.76	***
	4674.1	5212.8	−538.7	−10.3%*	Exp.	4	689465	6.37	***
E	5278.3	5596.3	−318.0	− 5.7%	T×E	4	459635	4.24	**
	5315.0	5320.7	− 5.7	− 0.1%	Resid.	55	108320		
	5398.1	5496.5	− 98.5	− 1.8%					
	2766.1	2752.2	+ 13.9		Treatm.	1	11870	2.33	
	2581.8	2584.6	− 2.8		Exp.	4	88631	17.36	***
F	2692.3	2731.8	− 39.5	−27.4	T×E	4	5649	1.10	
	2523.8	2617.8	− 94.0	(1.0%)	Resid.	55	5103		
	2657.7	2692.0	− 34.3						
	916.0	868.3	+ 47.8	+ 5.5%***	Treatm.	1	4906	10.83	***
	894.0	847.2	+ 46.8	+ 5.5%**	Exp.	4	3628	8.01	***
C	870.0	868.0	+ 2.0	+ 0.2%	T×E	4	3091	6.82	***
	846.0	855.8	− 9.8	− 1.1%	Resid.	55	453		
	849.9	862.5	− 12.7	− 1.5%					
	335.8	322.3	+ 13.5	+ 4.2%*	Treatm.	1	543	6.09	*
	322.1	304.0	+ 18.1	+ 6.0%**	Exp.	4	2052	23.00	***
V	321.5	320.5	+ 1.0	+ 0.3%	T×E	4	345	3.87	**
	293.8	296.2	− 2.3	− 0.8%	Resid.	55	89		
	311.8	316.8	− 4.9	− 1.6%					
	25.3	44.0	− 18.7	−42.5%***	Treatm.	1	1071	40.43	***
	35.5	48.2	− 12.7	−26.3%*	Exp.	4	608	22.97	***
L	45.3	45.0	− 0.3	− 0.6%	T×E	4	285	10.77	***
	47.0	49.1	− 2.1	− 4.4%	Resid.	67	26		
	47.6	48.2	− 0.6	− 1.3%					

APPENDIX 6 (continued).

Character	+CA	Contr.	Diff.	%, Sign.	Source of variation	d.f.	Variance	F	Sign.
	166.6	200.1	− 33.5	−16.7%**	Treatm.	1	2568	18.60	***
	181.0	201.8	− 20.8	−10.3%*	Exp.	4	1322	9.57	***
E/F	196.0	205.0	− 9.0	− 4.4%	T×E	4	926	6.70	***
	210.8	203.2	+ 7.7	+ 3.8%	Resid.	55	138		
	203.2	204.0	− 0.8	− 0.4%					
	302.1	317.1	− 15.0		Treatm.	1	1296	15.43	***
	289.0	305.0	− 16.0		Exp.	4	657	7.82	***
F/C	309.5	314.5	− 5.0	− 9.1	T×E	4	168	2.00	
	298.5	305.8	− 7.3	(2.9%)	Resid.	55	84		
	312.8	312.1	+ 0.7						
	824.6	854.4	− 29.8		Treatm.	1	8864	8.88	**
	802.3	850.6	− 48.3		Exp.	4	4355	4.36	**
F/V	837.5	852.5	− 15.0	−23.7	T×E	4	1163	1.16	
	860.7	884.7	− 24.0	(2.8%)	Resid.	55	999		
	852.7	850.3	+ 2.4						
	63.4	48.4	+ 15.0	31.0%***	Treatm.	1	259	10.12	*
K_2	48.8	52.0	− 3.2	6.1%	Exp.	1	310	12.12	*
					T×E	1	521	20.37	***
					Resid.	23	26		
	351.5	281.2	+ 70.3	25.0%**	Treatm.	1	4059	3.69	
KCs_2	276.2	300.8	− 24.7	8.2%*	Exp.	1	8093	7.36	*
					T×E	1	14240	12.94	*
					Resid.	23	1100		
	553.8	580.2	− 26.4	−20.7	Treatm.	1	2730	3.34	
KCs_2/K_2	565.7	580.2	− 14.5	(3.6%)	Exp.	1	485	<1	
					T×E	1	224	<1	
					Resid.	1	816		

REFERENCES

ALBRECHT, F. O. (1953). The anatomy of the migratory locust. London 118 p.

ALBRECHT, F. O. (1955). La densité des populations et la croissance chez *Schistocerca gregaria* (Forsk.) et *Nomadacris septemfasciata* (Serv.); la mue d'ajustement. J. Agr. trop. Bot. appl. **2**, 109–192.

ALBRECHT, F. O. & R. E. BLACKITH (1957). Phase and moulting polymorphism in locusts. Evolution (Lancaster) **11**, 165–177.

ALBRECHT, F. O. & R. E. BLACKITH (1960). Poids et délai de survie des larves nouveau-nées chez les acridiens migrateurs. Données physiologiques. C. R. Acad. Sci., Paris **250**, 3388–3390.

ALBRECHT, F. O. & M. VERDIER (1956). Le poids et le nombre d'ovarioles chez les larves nouveau-nées de *Locusta migratoria migratorioides* R. & F. C. R. Acad. Sci., Paris **243**, 203–205.

ALBRECHT, F. O., M. VERDIER & R. E. BLACKITH (1958). Détermination de la fertilité par l'effet de groupe chez le criquet migrateur *Locusta migratoria migratorioides* R. & F.). Bull. Biol. Fr. et Belg. **92**, 350–427.

ALBRECHT, F. O., M. VERDIER & R. E. BLACKITH (1959). Maternal control of ovariole number in the progeny of the migratory locust. Nature, Lond. **184**, 103–104.

ALTMANN, G. (1956). Hormonale Steuerung des Wasserhaushaltes der Honingbiene. Zool. Anz. Suppl. **19**, 107–112.

BADONNEL, A. (1948). L'effet de groupe chez *Psyllipsocus ramburi* (Psocoptère). Bull. Soc. zool. Fr. **73**, 80–83.

BLACKITH, R. E. (1957). Polymorphism in some Australian locusts and grasshoppers. Biometrics **13**, 183–196.

BLACKITH, R. E. & F. O. ALBRECHT (1959). Morphometric differences between the eyestripe polymorphs of the red locust. Sci. J. Roy. Coll. Sci. **27**, 13–27.

BLACKITH, R. E. & F. O. ALBRECHT (1960). Poids et délai de survie des larves nouveau-nées chez les acridiens migrateurs. Données chimiques. C. R. Acad. Sci., Paris **250**, 3514–3515.

BLACKITH, R. E. & M. I. ROBERTS (1958). Farbenpolymorphismus bei einigen Feldheuschrecken. Z. Vererbungslehre **89**, 328–337.

BODENHEIMER, F. S. (1929). Studien zur Epidemiologie, Ökologie und Physiologie der Afrikanischen Wanderheuschrecke (*Schistocerca gregaria* Forsk.). Z. Angew. Ent. **15**, 435–557.

BODENSTEIN, D. (1943). Hormones and tissue competence in the development of *Drosophila*. Biol. Bull. Wood's Hole **84**, 34–58.

BODENSTEIN, D. (1953). Postembryonic development. In K. D. ROEDER (1953). Insect physiology, New York: 822–865.

BODENSTEIN, D. (1953). The role of hormones in moulting and metamorphosis. In K. D. ROEDER (1953). Insect physiology, New York: 879–931.

BODENSTEIN, D. (1957). Humoral dependence of growth and differentiation in insects. Rec. Adv. in invert. Physiol., Univ. Oregon Publ. 1957, 197–211.

BONNEMAISON, L. (1949). Action de l'effet de groupe sur la production des ailés chez *Brevicoryne Brassicae*. C. R. Acad. Sci., Paris **229**, 142–143.

BRETT, C. H. (1947). Interrelated effects of food, temperature and humidity on the development of the lesser migratory grasshopper, *Melanoplus mexicanus mexicanus* (Saussure). Tech. Bull. Oklahoma Agr. Exp. Sta. **T 26**, 5–50.

BROWN, A. & A. MEGLITSCH (1940). Comparison of the chromatophorotropic activity of insect corpora cardiaca with that of crustacean sinus glands. Biol. Bull. Wood's Hole **79**, 409–418.

BÜCKMANN, D. (1959). Die Auslösung der Umfarbung durch das Häutungshormon bei *Cerura vinula* L. (Lep. *Notodontidae*). J. Ins. Phys. **3**, 159–189.

BUTENANDT, A. (1959). Wirkstoffe des Insektenreiches. Naturwissenschaften **46**, 461–471.

BUTLER, C. G. & J. M. INNES (1936). A comparison of the rate of metabolic activity in the solitary and migratory phases of *Locusta migratoria* L. Proc. Roy. Soc. Lond. (B) **119**, 296–304.

CAMERON, M. L. (1953). The secretion of an orthodiphenol in the corpus cardiacum of the insect. Nature, Lond. **172**, 349.

CARLISLE, D. B. & P. E. ELLIS (1959). La persistance des glandes ventrales céphaliques chez les criquets solitaires. C. R. Acad. Sci., Paris **249**, 1059–1060.

CARLISLE, D. B. & P. M. JENKIN (1959). Terminology of hormones. Nature, Lond. **183**, 336–337.

CHAUVIN, R. (1941). Contribution a l'étude physiologique du criquet pèlerin et du déterminisme des phénomènes grégaires. Ann. Soc. Ent. Fr. **110**, 133–272.

CHAUVIN, R. (1943). Une nouvelle méthode d'appréciation de l'effet de groupe chez les acridiens migrateurs. Ann. Soc. Nat. Zool. **5**, 79.

CHAUVIN, R. (1957). Reflexions sur l'écologie entomologique. Rev. Zool. Agr. et Appl.

CHAUVIN, R. (1958). L'action du groupement sur la croissance des grillons *Gryllulus domesticus*). J. Ins. Phys. **2**, 235–248.

CLARK, E. J. (1943). Colour variation in British *Acrididae*. Ent. Mon. Mag. **79**, 91–104.

CLARKE, K. U. (1957). On the increase in linear size during growth in *Locusta migratoria* L. Proc. R. ent. Soc. Lond. (A) **32**, 35–39.

CLARKE, K. U. (1957). The relationship of oxygen consumption to age and weight during the post embryonic growth of *Locusta migratoria* L. J. exp. Biol. **34**, 29–41.

CLARKE, K. U. & R. W. BALDWIN (1960). The effect of insect hormones and of 2,4 dinitrophenol on the mitochondrion of *Locusta migratoria* L. J. ins. Phys. **5**, 37–46.

COUSIN, G. (1956). Biometrie et définitions de morphologie quantitative des espèces et de leurs hybrides. Bull. Soc. zool. Fr. **81**, 247–289.

DIRSH, V. M. (1951). A new biometrical phase character in locusts. Nature, Lond. **167**, 281–282.

DIRSH, V. M. (1953). Morphometrical studies on phases of the desert locust Anti-locust Bulletin **16**, 34 p.

DUARTE, A. J. (1938). Problems of growth of the African migratory locust. Bull. ent. Res. **29**, 425–456.

DUPONT-RAABE, M. (1957). Les méchanismes de l'adaptation chromatique chez les insectes. Arch. Zool. exp. gen., Paris **94**, 61–293.

ELLIS, P. E. (1951). The marching behaviour of hoppers of the African migratory locust (*Locusta migratoria migratorioides* R. & F.). Anti-Locust Bull. **7**, 46 p.

ELLIS, P. E. (1953). The gregarious behaviour of marching *Locusta migratoria migratorioides* (R. & F.) hoppers. J. exp. Biol. **30**, 214–234.

ELLIS, P. E. (1953b). Social aggregation and gregarious behaviour in hoppers of *Locusta migratoria migratorioides* (R. & F.). Behaviour **5**, 225–260.

ELLIS, P. E. (1956). Differences in social aggregation in two species of locusts. Nature, Lond. **178**, 1007.

ELLIS, P. E. (1959). Some factors influencing phase characters in the nymphs of the locust *Locusta migratoria migratorioides* (R. & F.). Insectes sociaux **6**, 21–39.

ELLIS, P. E. & D. B. CARLISLE (1961). The prothoracic gland and colour change in locusts. Nature, Lond. **190**, 368–369.

ENGELMANN, F. (1959). Über die Wirkung implantierter Prothoraxdrüsen im adulten Weibchen von *Leucophaea maderae* (*Blattaria*). Z. vergl. Phys. **41**, 456–470.

ENGELMANN, F. & M. LÜSCHER (1957). Die hemmende Wirkung des Gehirns auf die Corpora allata bei *Leucophaea maderae* (Orthoptera). Zool. Anz. Suppl. **20**, 215–220.

ERGENE, S. (1954). Das Verhalten der Hämolymphe im Verlauf des Farbwechsels. Z. vergl. Phys. **36**, 238–240.

ERGENE, S. (1955). Über die Faktoren die grünfarbung bei *Acrida* bedingen. Z. vergl. Phys. **37**, 221–225.

ERGENE, S. (1956). Homochromer Farbwechsel bei geblendeten *Oedalus decorus*. Z. vergl. Phys. **38**, 311–314.

ERGENE, S. (1956). Farbanpassungen bei *Tylopsis liliifolia* Z. vergl. Phys. **38**, 315–316.

FAURE, J. C. (1932). The phases of locusts in South Africa. Bull. ent. Res. **23**, 293–424.

FOX, H. M. & G. VEVERS (1960). The nature of animal colours. London 246 p.
FUKUDA, S. (1951). The production of the diapause eggs by transplanting the suboesophageal ganglion in the silkworm. Proc. Imp. Acad. Japan. **27**, 672–677.
FUZEAU-BRAESCH, S. (1958). Aspect de la biologie de la pigmentation cuticulaire d'un insecte. C. R. Soc. biol., Paris **152**, 755–758.
FUZEAU-BRAESCH, S. (1959). Inhibition de la pigmentation et constitution des pigments cuticulaires d'un insecte. C. R. Soc. biol. Paris **153**, 57–60.
FUZEAU-BRAESCH, S. (1960). Étude biologique et biochimique de la pigmentation chez un insecte: *Gryllus bimaculatus* de Geer (Orthopt., *Gryllidae*). Bull. biol. Fr. et Belg. 1960, 525–627.
GARDINER, B. G. (1958). Some observations on the respiration of young nymphs of *Schistocerca gregaria* (Forsk.) in relation to phase and rearing density. Proc. R. ent. Soc. Lond. (A) **33**, 159–166.
GERSCH, M. (1957). Das Hormonsystem der Insekten auf Grund neuer Forschungsergebnisse. Ber. über die Hundertjahrfeier der D. ent. Ges. 1957.
GERSCH, M. (1957b). Wesen und Wirkungsweise von Neurohormonen im Tierreich. Naturwissenschaften **44**, 525–532.
GERSCH, M. (1958). Neurohormonen bei wirbellosen Tieren. Verh. D. zool. Ges. 1958, 40–76.
GERSCH, M. & G. MOTHES (1956). Neurohormonaler Wirkungsantagonismus beim Farbwechsel von *Dixippus morosus*. Naturwissenschaften **43**, 542.
GIERSBERG, H. (1928). Über die morphologische und physiologische Farbwechsel der Stabheuschrecke *Dixippus morosus*. Z. vergl. Phys. **7**, 567–595.
GILBERT, L. I. & H. A. SCHNEIDERMANN (1958). The inactivation of juvenile hormone extracts by pupal silkworms. Anat. Rec. **131**, 557–558.
GILBERT, L. I. & H. A. SCHNEIDERMANN (1959). Prothoracic gland stimulation by juvenile hormone extracts of insects. Nature, Lond. **184**, 171–173.
GOODWIN, T. W. (1949). Biochemistry of locusts. 2. Carotenoid distribution in solitary and gregarious phases of the African migratory locust (*Locusta migratoria migratorioides* R. & F.) and the desert locust (*Schistocerca gregaria* Forsk.). Biochem. J. **45**, 472.
GOODWIN, T. W. (1950). Biochemistry of locusts. 4. Insectorubin metabolism in the desert locust (*Schistocerca gregaria* Forsk.) and the African migratory locust (*Locusta migratoria migratorioides* R. & F.). Biochem. J. **47**, 554–562.
GOODWIN, T. W. (1952). The biochemistry of locust pigmentation. Biol. Rev. **27**, 439–460.
GOODWIN, T. W. & S. SRISUKH (1949). Biochemistry of locusts. 1. The carotenoids of the integument of two locust species (*Locusta migratoria migratorioides* R. & F. and *Schistocerca gregaria* Forsk.). Biochem. J. **45**, 263.
GOODWIN, T. W. & S. SRISUKH (1950). Biochemistry of locusts. 3. Insectorubin: the redox pigment present in the integument and eyes of the desert locust (*Schistocera gregaria* Forsk.), the African migratory locust (*Locusta migratoria migratorioides* R. & F.) and other insects. Biochem. J. **47**, 549–554.
GOODWIN, T. W. & S. SRISUKH (1951). Biochemistry of locusts. 5. The green pigment of the haemolymph and integument of solitary locusts (*Locusta migratoria migratorioides* R. & F.) and *Schistocerca gregaria* Forsk.). Biochem. J. **48**, 199–203.
GOODWIN, T. W. & S. SRISUKH (1951). Biochemistry of locusts. 6. The occurrence of a flavin in the eggs and of a pterin in the eyes of the African migratory locust (*Locusta migratoria migratorioides* R. & F.) and the desert locust (*Schistocerca gregaria* Forsk.). Biochem. J. **49**, 84.
GRASSÉ, P. P. (1958). L'effet de groupe sur l'animal et sur l'homme. J. Psychol. norm. path. **55**, 129–150.
GUNN, D. L. & P. HUNTER-JONES (1952). Laboratory experiments on phase differences in locusts. Anti-locust Bulletin **12**.
HALBWACHS, M. C., L. JOLY & P. JOLY (1957). Résultats d'implantations de "glandes ventrales" à *Locusta migratoria* L. J. ins. Phys. **1**, 143–149.
HAMILTON, A. G. (1936). The relation of humidity and temperature to the development of

three species of African locusts – *Locusta migratoria migratorioides* (R. & F.), *Schistocerca gregaria* (Forsk.), *Nomadacris septemfasciata* (Serv.). Trans. R. ent. Soc. Lond. **85**, 1–60.

HARKER, J. E. (1960). Internal factors controlling the suboesophageal ganglion neurosecretory cycle in *Periplaneta americana* L. J. exp. Biol. **37**, 164–170.

HERTZ, M. & A. D. IMMS (1937). On the responses of the African migratory locust to different types of background. Proc. Roy. Soc. (B) **122**, 281–297.

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

HODGSON, E. S. & S. GELDIA (1959). Experimentally induced release of neurosecretory materials from roach corpora cardiaca. Biol. Bull. Wood's Hole **117**, 275–283.

HUNTER-JONES, PH. (1958). Laboratory studies on the inheritance of phase characters in locusts. Anti-locust Bulletin **29**, 32 p.

HUSAIN, M. A. & C. B. MATHUR (1936a). Studies on *Schistocerca gregaria* (Forsk.). V. Pigmentation and physical exertion. Ind. J. agr. Sci. **6**, 591–623.

HUSAIN, M. A. & C. B. MATHUR (1936 b). Influence of carbon dioxide on development of black pigmentation in *Schistocerca gregaria* (Forsk.) Ind. J. agr. Sci. **6**, 665–671.

HUSAIN, M. A. & G. TASHKIR AHMAD (1936). Influence of temperature on the intensity and extent of black pattern in the desert locust hoppers bred crowded. Ind. J. agr. Sci. **6**, 624–664.

JANNONE, G. (1938). Aspetti bio-morfologici e somatometrici del problema della fasi nel *Dociostaurus maroccanus* Thunb. in Italia e fuori, con particolare riguarde alla provincia di Napoli (Secondo contributo). Boll. Lab. ent. agr. Portici **1**, 261–328.

JOHANNSON, A. S. (1958). Relation of nutrition to endrocrine-reproductive functions in the milkweed bug *Oncopeltus fasciatus* (Dallas) (Heteroptera; Lygaeidae). Nytt. Mag. Zool. Oslo **7**, 1–132.

JOLY, L. (1954). Résultats d'implantations systématiques de corpora allata à de jeunes larves de *Locusta migratoria* L. C. R. Soc. biol., Paris **148**, 579–583.

JOLY, L. (1955). Analyse du fonctionnement des corpora allata chez la larve de *Locusta migratoria* L. C. R. Soc. biol., Paris **149**, 584–587.

JOLY, L. (1958). Comparaison des divers types d'adultoïdes chez *Locusta migratoria* L. Insectes sociaux **5**, 373–378.

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

JOLY, P. (1949). Le système endocrine rétrocérébral chez les acridiens migrateurs. Ann. Sci. Nat. Zool. 1949, 255–262.

JOLY, P. (1951). Déterminisme endocrine de la pigmentation chez *Locusta migratoria* L. C. R. Soc. biol., Paris **145**, 1362–1366.

JOLY, P. (1952). Production d'adultoïdes chez *Locusta migratoria* L. C. R. Acad. Sci., Paris **235**, 1555–1557.

JOLY, P. (1952b). Déterminisme de la pigmentation chez *Acrida turrita* L. C. R. Acad. Sci., Paris **235**, 1054–1056.

JOLY, P. (1954). Étude de la croissance alaire chez *Locusta migratoria* L. C. R. Soc. biol., Paris **148**, 2082–2087.

JOLY, P. (1955). Chronologie de la mitose chez *Locusta migratoria* L. Arch. Anat. Hist. Embr. **37**, 87–96.

JOLY, P. (1956). Croissance et indices de grégarisation chez *Locusta migratoria* L. Insectes sociaux **3**, 17–24.

JOLY, P. (1958). Les corrélations humorales chez les acridiens. Année biol., Paris **34**, 97–118.

JOLY, P. & JOLY, L. (1953). Résultats de greffes de corpora allata chez *Locusta migratoria* L. Ann. Sci. nat. Zool. **15**, 331–345.

JOLY, P. JOLY, L. & M. C. HALBWACHS (1956). Contrôle humoral du développement chez *Locusta migratoria* L. Ann. Sci. nat. Zool. 1956, 257–261.

JONES, B. M. & W. SINCLAIR (1958). Induction of melanin patterns and of hardening as separate processes in the cuticle of albino locusts with internally absorbed phenol substrates. Nature, Lond. **181**, 926–927.

JOVANÇIC, L. (1953). Génèse des pigments tégumentaires de la mante religieuse et d'autres animaux; leur rôle biologique et physiologique. Bull. biol. Fr. et Belg. **87**, 92–104.

KAISER, P. (1949). Histologische Untersuchungen über die corpora allata und Prothoraxdrüsen der Lepidopteren in Bezug auf ihre Funktion. Arch. Entw. Mech. Org. **144**, 99–131.

KENNEDY, J. S. (1956). Phase transformation in locust biology. Biol. Rev. **31**, 349–370.

KENNEDY, J. S. (1961). Continuous polymorphism in locusts (in press).

KEY, K. H. L. (1936). Observations on rate of growth, coloration and the abnormal six-instar life-cycle in *Locusta migratoria migratorioides* R. & F. Bull. ent. Res. **27**, 77–85.

KEY, K. H. L. (1950). A critique on the phase theory of locusts. Quart. Rev. Biol. **25**, 363–407.

KEY, K. H. L. (1957). Kentromorphic phases in three species of *Phasmatodea*. Austr. J. Zool. **5**, 247–284.

LEA, A. (1959). An appraisal and a proposed extension of KENNEDY's hypothesis of the adult-neotenous nature of the gregarious and solitary phase of locusts. S. Afr. J. agr. Sci. **2**, 419–426.

LEAN, O. B. (1936). *Locusta migratoria migratorioides* R. & F.: an ecological reconnaissance of the suspected Middle Niger outbreak area. Bull. ent. Res. **27**, 105–184.

L'HELIAS, C. (1957). Action du complexe rétrocérebral sur le métabolisme chez le phasme *Carausius morosus*. Bull. biol. Suppl. **44**, 1–96.

L'HELIAS, C. (1959). Biochemie de la métamorphose et rôle du complexe rétrocérebral chez les insectes. Act. Soc. Linn. Bordeaux 97, 103–113.

LOHER, W. (1958). An olfactory response of immature adults of the desert locust. Nature, Lond. **181**, 1280.

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

LÜSCHER, M. & F. ENGELMANN (1960). Über die Steuerung der corpora allata Funktion bei der Schabe *Leucophaea maderae*. Rev. Suisse Zool. **62**, 649–657.

LÜSCHER, M. & F. ENGELMANN (1960). Histologische und experimentelle Untersuchungen über die Auslösung der Metamorphose bei *Leucophaea maderae* (Orthoptera). J. ins. Phys. **5**, 240–258.

LÜSCHER, M. & A. SPRINGHETTI (1960). Untersuchungen über die Bedeutung der corpora allata für die Differenzierung der Kasten bei der Termite *Kalotermes flavicollis*. J. ins. Phys. **5**, 190–212.

MALEK, S. R. A. (1957). Sclerotization and melanization: two independent processes in the cuticle of the desert locust. Nature, Lond. **180**, 237.

MATHÉE, J. J. (1945). Biochemical differences between the solitary and gregarious phases of locusts and noctuids. Bull. ent. Res. **36**, 343–371.

MAXWELL-DARLING, R. C. (1934). The solitary phase of *Schistocerca gregaria* Forsk. in North-Eastern Kordofan (Anglo-Egyptian Sudan). Bull. ent. Res. **25**, 63–83.

MENDES, M. V. (1948). Histology of the corpora allata of *Melanoplus differentialis* (Orthoptera Saltatoria). Biol. Bull. Wood's Hole **94**, 194–207.

MENDES, M. V. (1953). The function of the corpora cardiaca of a grasshopper. Preliminary note. Dusenia Curitiba **4**, 439–442.

NICKERSON, B. (1954). A possible endocrine mechanism controlling locust pigmentation. Nature, Lond. **174**, 357.

NICKERSON, B. (1956). Pigmentation of hoppers of the desert locust in relation to phase coloration. Anti-locust Bulletin **24**, 34 p.

NORRIS, M. J. (1954). Sexual maturation in the desert locust. Anti-locust Bulletin **18**, 44 p.

NOVÁK, V. J. A. (1956). Versuch einer zusammenfassenden Darstellung der postembryonalen Entwicklung der Insekten. Beitr. Ent. **6**, 205–239 & 464–493.

NOVÁK, V. J. A. (1959). Insektenhormone. Praha 1959, 283 p.

OKAY, S. (1953). Formation of green pigment and colour changes in Orthoptera. Bull. ent. Res. **44**, 299–315.

ÖZBAS, S. (1957a). Two kinds of secretions in corpora cardiaca of *Locusta migratoria* L. phase solitaria. Comm. Fac. Sci. Univ. Ankara **8**, 45–57.

ÖZBAS, S. (1957b). Morphological and histological studies on the corpora allata and cardiaca in Orthoptera. Comm. Fac. Sci. Univ. Ankara **8**, 19–44.

PAPILLON, M. (1960). Étude préliminaire de la répercussion du groupement des parents sur les larves nouveau-nées de *Schistocerca gregaria* Forsk. Bull. biol. Fr. et Belg. **93**, 203–263.

PARKER, G. H. (1948). Animal colour changes and their neurohumours. Cambridge 1948, 377 p.

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

PFEIFFER, I. W. (1945). Effect of the corpora allata on the metabolism of adult female grasshoppers. J. exp. Zool. **99**, 183–233.

PFEIFFER, I. W. (1945b). The influence of the corpora allata over the development of nymphal characters in the grasshopper *Melanoplus differentialis*. Trans. Connecticut Acad. Sci. **36**, 489–515.

PFLUGFELDER, O. (1938). Farbveranderungen und Gewebsentartungen nach Nervendurchschneidung und Extirpation der corpora allata von *Dixippus morosus* Br. Verh. D. zool. Ges. 127–137.

PFLUGFELDER, O. (1958). Entwicklungsphysiologie der Insekten. Leipzig 1958, 490 p.

PLOTNIKOV, V. I. (1927). *Locusta* (*Pachytylus*) *migratoria* L. and *Locusta danica* L. as independent forms and their derivates. Rev. appl. Ent. (A) **15**, 162.

POISSON, R. & R. SELLIER (1947). Brachyptérisme et actions endocrines chez *Gryllus campestris* L. C. R. Acad. Sci., Paris **224**, 1074–1075.

PRYOR, M. G. M. (1940). On the hardening of the cuticle of insects. Proc. Roy. Soc. (B) **128**, 378–393 & 394–407.

RAABE, M. (1959). Neurohormones chez les insectes. Bull. Soc. zool. Fr. **84**, 272–316.

RAO, Y. R. (1942). Some results of studies on the desert locust (*Schistocerca gregaria*, Forsk.) in India. Bull. ent. Res. **33**, 241–265.

RAMME, W. (1950). Die parallele Färbungsvariation (Parallelochromie) der *Acrididae*; ihre genetische und phylogenetische Bedeutung. (Orthoptera). EOS. Rev. Esp. Ent. Tomo extraord. 1950, 249–260.

ROONWAL, M. L. (1940). Preliminary note on some directional changes among locusts and other *Acrididae* and the importance of the IIIrd instar. Ind. J. Ent. **2**, 137–144.

ROONWAL, M. L. (1946). On variation in the number of hind tibial spines in the desert locust (*Schistocerca gregaria* Forsk.) Ind. J. Ent. **8**, 71–77.

ROONWAL, M. L. (1947). Body size and biometrical ratios in various types of individuals of the desert locust (*Schistocerca gregaria*, Forsk.). Rec. Ind. Mus. Delhi **45**, 149–165.

ROONWAL, M. L. (1947b). The role of some variations e.g. eye stripes, etc. as „population indicators" in the desert locust and their practical importance. Rec. Ind. Mus. Delhi **45**, 167–180.

ROONWAL, M. L. (1947c). Variation and structure of the eyes in the desert locust (*Schistocerca gregaria*, Forsk.). Proc. Roy. Soc. (B) **134**, 245–272.

RUBTZOV, I. A. (1935). Phase variation in non-swarming grasshoppers. Bull. ent. Res. **26**, 499–520.

SÄGESSER, H. R. (1960). Über die Wirkung der Corpora allata auf den Sauerstoffverbrauch bei der Schabe *Leucophaea maderae* (Fabr.). J. ins. Phys. **5**, 264–285.

SCHARRER, B. (1948). The prothoracic gland of *Leucophaea maderae* (Orthoptera). Biol. Bull. Wood's Hole **95**, 186–198.

SCHARRER, B. (1952). Neurosecretion XI. The effect of nerve-section on the intercerebralis-cardiacum-allatum system of the insect *Leucophaea maderae*. Biol. Bull. Wood's Hole **102**, 261–272.

SCHARRER, B. & M. VON HARNACK (1958). Histophysiological studies on the corpus allatum of *Leucophaea maderae*. I. Normal life cycle in male and female adults. Biol. Bull. Wood's Hole **115**, 508–520.

SCHNEIDERMAN, H. A. & L. I. GILBERT (1958). Substances with juvenile hormone activity in Crustacea and other invertebrates. Biol. Bull. Wood's Hole **115**, 530–535.

SCHNEIDERMAN, H. A. & L. I. GILBERT (1959). The chemistry and physiology of insect growth hormones. In Cell, organism and milieu, edited by D. RUDNICK 1959.

SELLIER, R. (1949). Diapause larvaire et macroptérisme chez *Gryllus campestris* (Orthoptera). C. R. Acad. Sci., Paris **228**, 2055–2056.

SELLIER, R. (1954). Récherches sur la morphogénèse et le polymorphisme alaire chez les Orthoptères gryllides. Ann. Sci. nat. Zool. 1954.

SNEDECOR, G. W. (1956). Statistical methods. Ames (Iowa) 1956, 534 p.

STAAL, G. B. (1960). Endocrine effects in phase development in locusts. In: The ontogeny of insects, edited by I. HRDY, Prague 1959, 142–146.

STOWER, W. J. (1959). The colour patterns of hoppers of the desert locust (*Schistocerca gregaria* Forsk.). Anti-locust Bulletin **32**, 75 p.

STOWER, W. J., D. E. DAVIES, I. B. JONES (1960). Morphometric studies of the desert locust *Schistocerca gregaria* (Forsk.). J. anim. Ecol. **29**, 309–339.

STRICH, M. C. (1953). Étude de la glande ventrale chez *Locusta migratoria migratorioides* L. (Orthoptera *Acridoidea*). Ann. Sci. Nat. 1953, 399–410.

STRICH-HALBWACHS, M. C. (1954). Rôle de la glande ventrale chez *Locusta migratoria* (L.). C. R. Soc. biol., Paris **148**, 2087–2091.

STRICH-HALBWACHS, M. C. (1958). Action de la glande ventrale sur le développement ovarien de *Locusta migratoria* L. (Orthoptera). J. ins. Phys. **1**, 346–351.

STRICH-HALBWACHS, M. C. (1959). Contrôle de la mue chez *Locusta migratoria* L. Ann. Sci. nat. Zool. 1959, 483–570.

THOMAS, J. G. (1941). The relative size of the eye as a phase character in the African migratory locust. Bull. ent. Res. **31**, 431–433.

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

THOMSEN, M. (1943). Effect of corpus cardiacum and other insect organs on the colour change of the shrimp, *Leander adspersus*. Biol. Medd. Kbh. **19**, 1–38.

THOMSON, R. H. (1960). Insect pigments. Verh. XI Int. Congr. Ent. Wien 1960 **3**, 21–43.

UVAROV, B. P. (1921). A revision of the genus *Locusta* L. (= *Pachytylus*, Fieb.) with a new theory as to the periodicity and migrations of locusts. Bull. ent. Res. **12**, 135–163.

UVAROV, B. P. (1928). Locusts and grasshoppers. London, 352 p.

UVAROV, B. P. & B. N. ZOLOTAREVSKY (1929). Phases of locusts and their interrelations. Bull. ent. Res. **20**, 261–265.

UVAROV, B. P. & J. G. THOMAS (1942). The probable mechanism of phase-variation in the pronotum of locusts. Proc. R. ent. Soc. Lond. **17**, 113–118.

VERDIER, M. (1957). Observations sur la transmission par l'oeuf des caractères phasaires chez *Locusta migratoria migratorioides* R. & F. 3 ième Congr. pour l'étude des insectes sociaux, Paris 1957.

VOGT, M. (1943). Zur Produktion und Bedeutung metamorphosefordernder Hormone während der Larvenentwicklung von *Drosophila*. Biol. Zbl. **63**, 395–446.

VUILLAUME, M. (1954). Biologie et comportement en A. O. F. de *Zonocerus variegatus* avec essais de comparaison entre acridiens grands et petits migrateurs. Rev. Path. veg. **33**, 121–198.

VUILLAUME, M. (1955). Étude de l'effet de groupe chez *Zonocerus variegatus*. Vie et milieu **6**, 161–193.

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

WEED, I. G. (1936b). Experimental study of moulting in the grasshopper *Melanoplus differentialis*. Proc. Soc. exp. Biol., N.Y. **34**, 885–886.

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

WIGGLESWORTH, V. B. (1952). Hormone balance and the control of metamorphosis in *Rhodnius prolixus* (Hemiptera). J. exp. Biol. **29**, 620–631.

WIGGLESWORTH, V. B. (1954). The physiology of insect metamorphosis. Cambridge 1954.

WIGGLESWORTH, V. B. (1958). Some methods for assaying extracts of the juvenile hormone in insects. J. ins. Phys. **2**, 73–84.

WILDE, J. DE (1960). Action of the juvenile hormone in the adult Colorado beetle *Leptinotarsa decemlineata* Say. Verh. XI Kongr. F. Entom. Wien 1960 B III, 215–217.

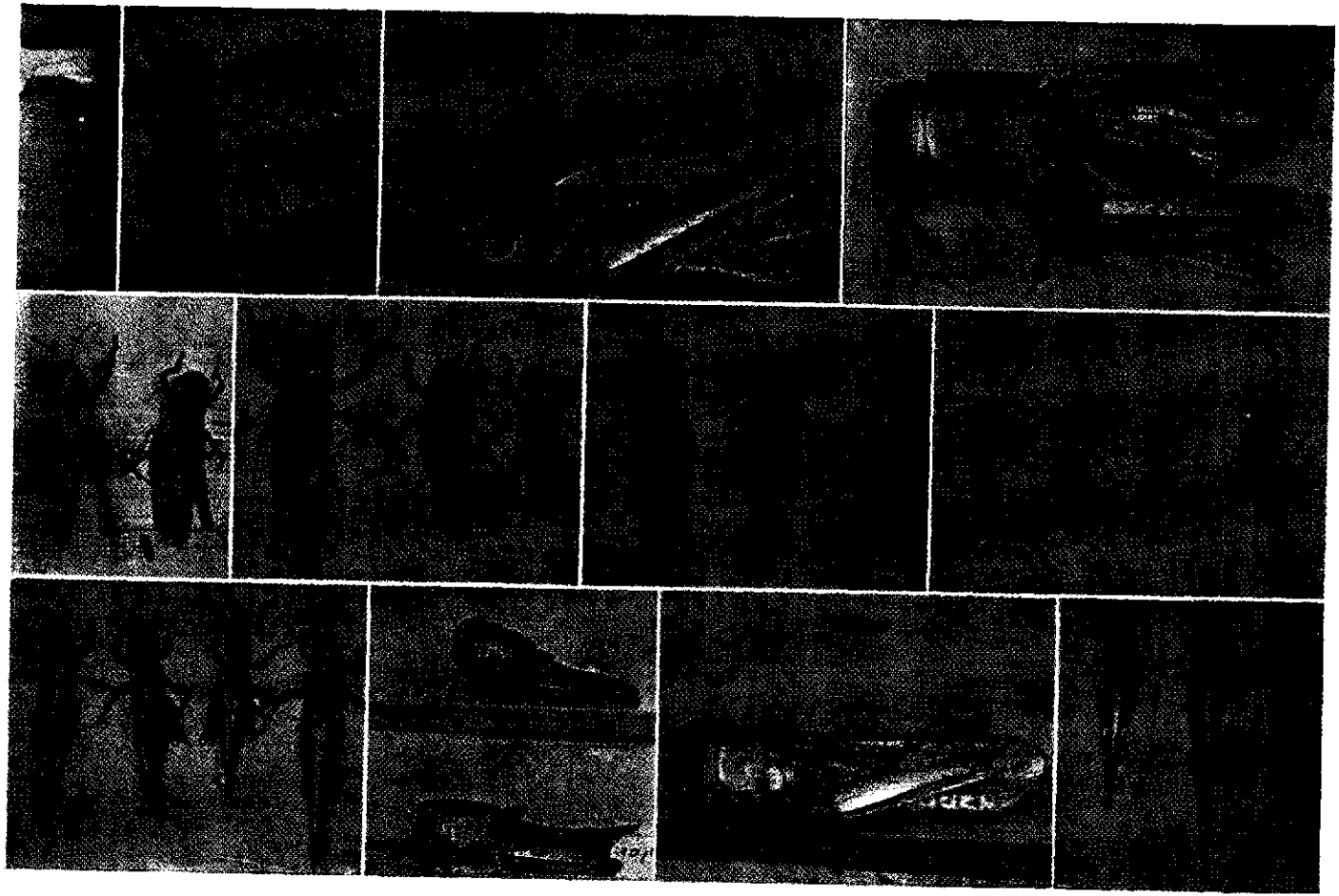
WILDE, J. DE & D. STEGWEE (1958) Two major effects of the corpus allatum in the adult Colorado beetle (*Leptinotarsa decemlineata* Say). Arch. Néerl. Zool. Suppl. **13**, 277–289.

WILLIAMS, C. M. (1952). Morphogenesis and metamorphosis of insects. The Harvey Lectures **47**, 126–155.

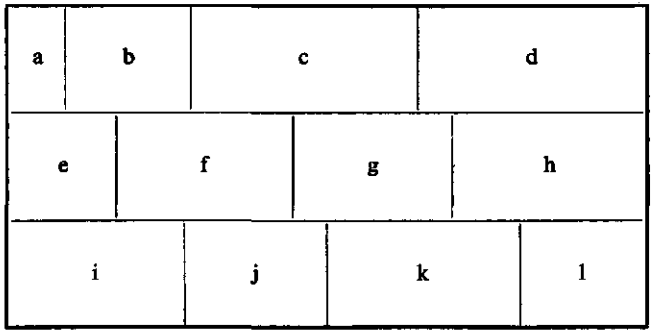
WILLIAMS, C. M. (1952)b). Physiology of insect diapause. IV. The brain and prothoracic glands as an endocrine system in the Cecropia silkworm. Biol. Bull. Wood's Hole **103**, 120–138.

ZOLOTAREVSKY, B. N. (1933). Contribution à l'étude biologique du criquet migrateur (*Locusta migratoria capito*, Sauss.) dans ses foyers permanents. Ann. Epiphyt. **19**, 47–142.

ZOLOTAREVSKY, B. N. & M. MURAT (1938). Rapport scientifique sur les recherches de la Mission d'Études de la Biologie des Acridiens en Mauretanie (A.O.F.). Bull. Soc. Hist. nat. Afr. Nord. **29**, 29–103.



PLATE



a. *Allatectomy early in L 2 followed by reimplantation of one adult CA after the next moult* (paragraph 5.3): male prothetelic L 3 showing the yellow maturation colour.

b. *Allatectomy early in L 4* (paragraph 5.1): prothetelic L 5 (right) compared with normal L 5 (left). The pigmentation is entirely adult including the femoral pattern. The wings are not quite typical for this operation.

c. *Extra implantation of CA early in L 5* (paragraph 4.2): metathetelic almost green adult.

d. *Extra implantation of CA in L 2* (paragraph 4.1): strongly metathetelic not green adult. Only some green at the site of implantation is observed. Note the absence of femoral pattern. However, the absence of green colour combined with metathetely is not a common result of extra implantation of CA.

e. *Combined extra implantation of CA and VG in L 2* (paragraph 6.3): in the next instar a green colour appeared together with an anticipated (fourth instar) wing development in the larva at the left. At the right a larva which only received CA.

f. *Extra implantation of VG in L 2* (paragraph 6.2.1): anticipated fifth instar wing development in L 4 (centre) compared with normal L 5 (left) and normal L 4 (right).

g. *Extra implantation of VG in L 2* (paragraph 6.2.1): anticipated fourth instar wing development in L 3 (centre) compared with normal L 4 (left) and normal L 3 (right).

h. *Breeding some Locusta larvae in a dense crowd of Schistocerca larvae of the same age:* pigmentation type intermediate between gregarious and cryptic grey demonstrating the specificity of group stimuli.

i. *Extra implantation of VG in L 2* (paragraphs 6.2.1 and 6.3): 3 fifth instar adults compared with a normal sixth instar adult (right). A somewhat smaller size and relatively shorter wings are the only differences.

j. *Extra implantation of VG in L 2* (paragraph 6.2.1): slight hypertrophy of wings in L 4, not leading to anticipated metamorphosis but to high E/F values in the resulting adult. Above a normal L 4.

k. *Extra implantation of VG in L 2* (paragraph 6.2.1): fifth instar adult. See remarks under i.

l. *Extra implantation of VG in L 2* (paragraph 6.2.1): Giant sixth instar adult (right) with high E/F value compared with normal adults.