Für die Verteilung der Granula im Cytoplasma ist keine bevorzugte Lokalisation zu erkennen; sie liegen in grösseren oder kleineren Gruppen beisammen; Anhäufungen kann man ebenso in der Nähe des Zellkerns wie in der Zellperipherie finden. Deutlich von den Granula verschieden sind die Mitochondrien: diese besitzen auch in den Nebennierenmarkzellen von Maus, Meerschweinchen und Katze die von SJÖSTRAND[14], SJÖSTRAND und RHODIN[15] und anderen beschriebene Struktur (Abb. 3); allerdings sind sie hier nur in verhältnismässig geringer Zahl anzutreffen. Die biochemische Untersuchung der die Granula enthaltenden Fraktionen (daneben auch das histochemische Verhalten der Granula) haben, abgesehen von ihrem Gehalt an Brenzcatechinaminen, eine gewisse Analogie mit Befunden an Mitochondrien anderer Organe ergeben (HILLARP und Mitarbeiter[16]). Aus diesen Ergebnissen jetzt schon weiterreichende Folgerungen zu ziehen, halten wir für bedenklich, denn bei den ihnen zugrunde liegenden Zentrifugierungsversuchen konnte innerhalb der Fraktionen nicht zwischen Granula und anderen Zellbestandteilen (zum Beispiel möglicherweise auch Mitochondrien) unterschieden werden. Der auf diesem Weg erhaltene hohe Wert des angeblichen Anteils der Granula am Zellgewicht (siehe oben) spricht zugunsten der Vermutung, dass in den Fraktionen ausser den Granula noch weitere Zellkomponenten enthalten waren. Nach unseren morphologischen Befunden ist jedenfalls für die untersuchten Tierarten an einer grundsätzlichen Unterscheidung zwischen den beiden so verschieden gestalteten Zellorganellen – phäochromen Granula und Mitochondrien – in den Markzellen unbedingt festzuhalten.

Im Vorausgehenden wurden die bisherigen Beobachtungen an den phäochromen Granula in den Markzellen der Maus mitgeteilt. Bei Meerschweinchen und Katze zeigen die Granula ein sehr ähnliches Bild. Allerdings sind in den Markzellen des Meerschweinchens die manche Granula umgebenden «Höfe» sehr viel kleiner. Die oben gegebene Deutung der «Höfe» – als Stellen herausgelöster Lipide – findet hier eine weitere Stütze in dem Befund BACHMANNS[17], nach dem gerade beim Meerschweinchen sudanophile Substanz in den Markzellen nur selten nachzuweisen war.

Schliesslich ist noch zu bemerken, dass die beschriebenen phäochromen Granula bei den untersuchten Tierarten zwar in den meisten, aber *nicht* in *allen* Markzellen anzutreffen sind. Über die Beobachtung zweier *differenter* Arten von Parenchymzellen des Nebennierenmarks wird demnächst an anderer Stelle[18] berichtet werden.

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Summary

The catechol-containing granules in the adrenal medullary cells (mouse, guinea pig, cat) are true cell organells, clearly distinguished from mitochondria; their size varies over a fairly wide range, the mean diameter being calculated at about 175 mμ in the mouse (osmium-tetroxide-fixation). They have a surrounding membrane of 100 Å thickness; their internal structure is finely granular. On the thin sections, many granules are surrounded by spaces which appear empty under the electron microscope and which are limited by double-membranes; it is most likely that *intra vitam* these spaces contain lipids.

[14] F. S. SJÖSTRAND, Nature *171*, 30 (1953).

[15] F. S. SJÖSTRAND und J. RHODIN, Exper. Cell Res. *4*, 426 (1953).

[16] N.-Å. HILLARP, B. HÖKFELT und B. NILSON, Acta anat. *21*, 155 (1954). – N.-Å. HILLARP und B. NILSON, Acta physiol. Scand. *31*, Suppl. *113*, 79 (1954); *32*, 11 (1954).

[17] R. BACHMANN, Z. mikrosk.-anat. Forsch. *45*, 157 (1939).

[18] R. WETZSTEIN (in Vorbereitung).

STUDIORUM PROGRESSUS

The Influence of a Queen on the Ovary Development in Worker Bees

By STIEN VOOGD, Utrecht[1]

During the last few years experiments have been carried out on the relation between queen and worker bees in a colony of honeybees (*Apis mellifica* L.). This problem has been approached in two different manners with regard to the testing method used in establishing the absence or presence of a queen: BUTLER[2] used *building of emergency queen cells* by worker bees as criterion, whereas DE GROOT and VOOGD[3] and VOOGD[4] used *ovary development* in worker bees. The experiments of BUTLER strongly suggest that bees obtain some substance from their queen which inhibits them from building emergency queen cells. The experiments of DE GROOT and VOOGD, and VOOGD have demonstrated the existence of a substance present on the body of a queen which is responsible for the inhibition of ovary development in worker bees.

Moreover, experiments on the ovary development in worker bees were set up by Miss PAIN[5]. Most of these experiments are open to criticism in different respects. Several theories concerning the factors regulating the development of the ovaries were put forward by Miss PAIN[6].

According to one of these theories, ovary development would depend on the presence of a "fertility hormone" in worker bees, normally *transmitted to the queen*, but accumulating in the case of her absence[7]. At the same time a substance on the body of the queen *transmitted to the worker bees* and inhibiting ovary development is assumed to be proven[8] by experiments which, in a subsequent paper, were considered as inconclusive by the author herself[9]. Slightly more convincing are the results of Miss PAIN's experiments with pieces of elder-pith impregnated with chloroform extracts of queens. The scanty data given are quite insufficient to justify the assumption of attractiveness and inhibitory effect with regard to these impregnated objects[10]. In order to

[1] Laboratory of Comparative Physiology, University of Utrecht.

[2] C. G. BUTLER, Trans. Roy. entomol. Soc. London *105*, 11 (1954).

[3] A. P. DE GROOT and C. VOOGD, Exper. *10*, 384 (1954).

[4] C. VOOGD, Exper. *11*, 181 (1955).

[5] J. PAIN, Arch. int. Physiol. *59*, 203 (1951); C. r. Acad. Sci. *240*, 670 (1955).

[6] J. PAIN, C. r. Soc. Biol. *145*, 1505 (1951).

[7] J. PAIN, Insectes Sociaux *1*, 59 (1954).

[8] J. PAIN, C. r. Acad. Sci. *239*, 1869 (1954).

[9] J. PAIN, Insectes Sociaux *2*, 35 (1955).

[10] J. PAIN, C. r. Acad. Sci. *239*, 1869 (1954); *240*, 670 (1955).

Table I.—Influence of extraction fluid of queens (q.) and worker bees (w.b.) mixed with food *when offered in the feeder(s)*.—The experimental bees were observed at regular intervals and those individuals were marked which consumed food. In experiment 3 different marks were used at each feeder.

Experiment	Feeder with	Number of bees marked during food consumption	Percentage developed ovaries in marked bees	Number of unmarked bees	Percentage developed ovaries in unmarked bees	Average percentage developed ovaries
1	Food + w.b.	24	54	21	48	51
2	Food + q.	31	35	11	18	31
3	Food + w.b. and Food + q. . . . . (offered in two feeders; see fig. 1).	6 consuming food + q.	67	28	64	71
		9 consuming food + w.b.	83			
		3 consuming food + q. and food + w.b.	100			

draw justified conclusions, many further experiments would be needed.

In our own experiments we have experienced difficulties due to the considerable variability of ovary development, even though all precautions were taken to reduce this variability to minor proportions. We started each series of experiments with groups of worker bees equally composed with regard to moment of emergence and origin of colony. During the experiment the environmental conditions were rigidly controlled. Nevertheless, variability remained. Especially in the controls, belonging to one and the same series of experiments, ovary development was sometimes extremely variable. This leads us to presume that one or more uncontrolled factors interfered with our experiments. Therefore each experiment had to be repeated several times in order to obtain reliable data. For this reason too we are inclined to question the reliability of the experimental basis of Miss PAIN's assumptions.

In our previous paper (VOOGD[4]), a substance present on the body of a queen is supposed to be only a link in the mechanism which results in inhibition of the ovary development in worker bees. We have made experiments in an attempt to analyze this inhibitory mechanism more closely.

So far, the extraction fluid of queens was offered to the worker bees by applying this fluid to the body of a dead worker bee, which was glued to the comb in the experimental cage. The presence of this impregnated body led to a typical behaviour of the worker bees. They touched the body with their antennae and licked it with their tongue. In this situation their ovaries did not develop. These observations suggest that intake of the extraction fluid by licking with the tongue inhibits ovary development.

To investigate this intake separately, extraction fluid of queens (q.) mixed with the food was offered *in the feeder* in the experimental cage. In control experiments, extraction fluid of worker bees (w.b.) was offered in the same way. The striking fact that emerged was that in both cases the ovaries of the worker bees developed to the same extent. These mixtures, however, led to a different extent of ovary development when offered *applied to the body of a dead worker bee*, which was glued to the comb. In the latter case only the presence of a body impregnated or perhaps only covered with food mixed with extraction fluid of queens—and not of worker bees—led to the typical behaviour pattern and failure to develop ovaries.

The results of only one series of these experiments are given in the Tables and the Photographs.

In the controls (experiments 8 and 9), only bees were present in the experimental cage and food without extraction fluid was offered. The average percentages which developed ovaries in these experiments were 38 and 48.

In addition, experiments 1, 4 and 6 are control experiments, as extraction fluid of worker bees was used instead of extraction fluid of queens.

It is worth mentioning that the food mixed with w.b. and the food mixed with q. used in the experiments 1–5[c]

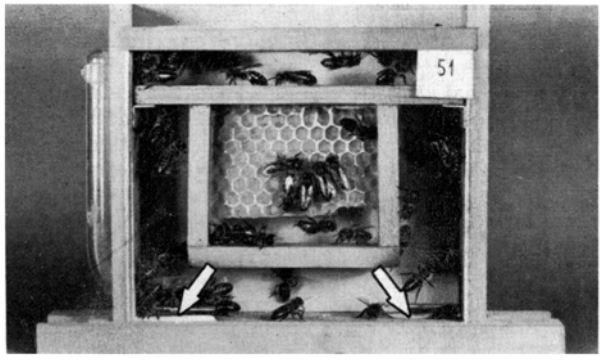

Fig. 1.—Two feeders are present in the experimental cage; food with extraction fluid of queens is offered in the left feeder, food with extraction fluid of worker bees in the right feeder (arrows indicate feeders).

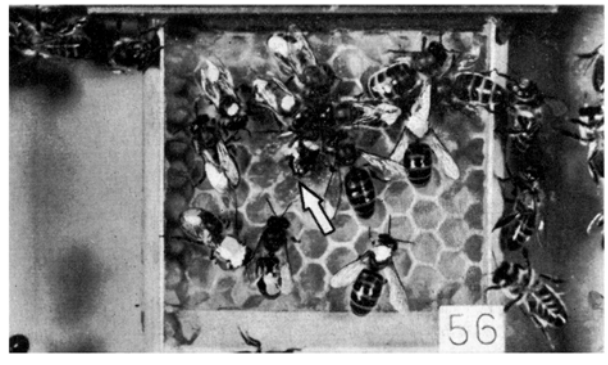

Fig. 2.—A dead worker bee, impregnated with food + extraction fluid of queens, is glued to the comb (arrow indicates the impregnated object).

Table II.—Influence of extraction fluid of queens (q.) and worker bees (w.b.) purely or mixed with food *when offered on the body of a dead worker bee glued to the comb.* Food without extraction fluid was offered in the feeders. The experimental bees were observed at regular intervals and those individuals were marked which touched and licked the impregnated object under observation.

Experiment	Dead worker bee with	Number of bees marked when touching and licking the impregnated object under observation	Percentage developed ovaries in marked bees	Number of unmarked bees	Percentage developed ovaries in unmarked bees	Average percentage developed ovaries
4	Food + w.b.	0		all		10
5[a]	Food + q.	31	0	14	21	7
5[b]	Food + q.	36	0	14	43	12
5[c]	Food + q.	26	4	19	32	16
6	w.b.	0		all		44
7	q.	35	9	13	38	17

were all taken from their respective fresh prepared stock mixtures.

It follows from the Tables that food with q., offered in the feeder, has no special attractiveness for the bees: when a feeder containing food with q. as well as a feeder containing food with w.b. are present in an experimental cage as many bees consuming food with q. as bees consuming food with w.b. are marked (see Fig. 1). Moreover, the ovaries of the bees that consumed food with q. are developed to the same extent as in the controls. However, food with q. as well as extraction fluid of queens applied to a dead worker bee (furtheron mentioned as q.-impregnated object) glued to the comb are attractive

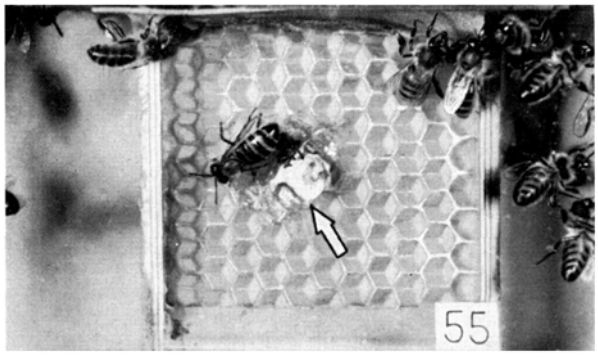

Fig. 3.—A dead worker bee, impregnated with food + extraction fluid of worker bees, is glued to the comb (arrow indicates the impregnated object).

for the bees, whereas food with w.b. as well as extraction fluid of worker bees are not at all attractive in this situation (see Fig. 2 and 3). Moreover, only the ovaries of the bees, attracted to the q.-impregnated objects (marked bees in the experiments 5[a], 5[b], 5[c] and 7) show lack of development, whereas the ovaries in the not attracted bees (unmarked bees in the experiments 5[a], 5[b], 5[c] and 7) develop to the same extent as in the controls. Consequently, the average percentage which developed ovaries in the presence of q-impregnated objects (experiments 5[a], 5[b], 5[c] and 7) is less than the average percentage which developed ovaries in the controls (experiments 1, 4, 6, 8 and 9).

These results suggest that the substance present in extraction fluid of queens, when applied to a dead worker bee glued to the comb, is a substantial factor in releasing the typical pattern of behaviour in worker bees mentioned before. This pattern of behaviour seems to be causally related to failure of ovary development. From the evidence at hand it does not seem justifiable to assume a simple, direct relation between the intake of this substance and failure of ovary development, as we pointed out in a previous paper[11].

We are engaged in further experiments to establish the factors involved in the causation of this behaviour pattern as well as in the resulting failure to develop ovaries.

Zusammenfassung

Unabhängig voneinander haben in den letzten Jahren einerseits BUTLER, andererseits DE GROOT und VOOGD gefunden, dass der Einfluss, den die Anwesenheit einer Königin auf die Arbeiterinnen der Honigbiene ausübt, durch Aufnahme einer Substanz bedingt ist, die die Arbeiterinnen vom Körper der Königin ablecken, und die von BUTLER als «queen substance» bezeichnet wurde. Als Kriterium benützten BUTLER den Bau von Weiselzellen, DE GROOT und VOOGD die Ovarienentwicklung bei den Arbeiterinnen. Den zuletzt genannten Autoren gelang es weiterhin, die wirksame Substanz zu extrahieren und in Extraktform erfolgreich anzuwenden.

Auch PAIN hat sich in mehreren Veröffentlichungen zu diesen Fragen geäussert; ihre Schlussfolgerungen sind aber durchwegs experimentell nicht ausreichend begründet.

In weiteren Versuchen konnte gezeigt werden, dass die *Aufnahme* der extrahierten Substanz durch die Arbeiterinnen *an sich* (mit dem Futter) noch keinen Effekt (Hemmung der Ovarienentwicklung) hervorruft. Dazu musste der Extrakt – mit oder ohne Futter – *in besonderer Weise dargeboten* werden (zum Beispiel auf der Leiche einer Arbeiterin). Nur dann auch wirkte die Substanz anziehend auf die Arbeiterinnen und löste ein charakteristisches Verhalten aus, dessen Bedeutung für die Hemmung der Ovarienentwicklung weitere Experimente näher aufklären sollen.

[11] C. VOOGD, Exper. *11*, 181 (1955). – This is consistent with a supposition put forward only recently by C. G. BUTLER, Amer. Bee Journal *95*, 275 (1955).