THE OCCURRENCE OF VITELLOGENIN IN WORKER BEES OF APIS MELLIFICA AND THE POSSIBILITY OF ITS TRANSMISSION TO THE QUEEN

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Almost fifty years ago Nolan (1925) found that the total weight of the eggs laid by a queen of Apis mellifica during 24 hours is often equal to her body weight. An adult queen in oviposition weighs, on average, about 180 mg; the weight of an egg is 0.13 mg. This indicates that the number of eggs laid daily, when oviposition is at its maximum, is approximately 1500. The question arises whether the queen herself synthesises the enormous amount of protein necessary for egg production or, alternatively, if she receives yolk components by means of trophallaxis from the workers. If worker bees produce a female specific protein, then it might possibly have a social function.

The bees used in our experiments were reared at the Apiary of the Swiss Federal Dairy Research Station, Apicultural Section, Liebefeld near Bern. For all experiments cell-free haemolymph from bees of known age was used. In order to test the identity of proteins we have prepared an antiserum against haemolymph of 6-12 days old workers, an antiserum against royal jelly and an antiserum against homogenate of ovaries of queens in oviposition. All antisera were prepared in rabbits.

Double diffusion tests (method of Ouchterlony 1958) show that worker bees contain one antigen in their haemolymph which is missing in drone haemolymph. The presence of such a protein has already been demonstrated by Engels (1972). It is a female specific protein which also occurs in the haemolymph of young and old queens. By means of the double diffusion technique it can be shown that this female specific protein is identical with the main soluble protein fraction in the ovaries of queens during oviposition and in ovaries of egg laying workers. It migrates slowly to the cathode in immuno-electrophoresis at pH 8.2 (method of Grabar and Williams 1955). The Rf value of the female specific protein of worker haemolymph is identical with that of the main soluble protein in ovaries of queens and egg laying workers. It is therefore a vitellogenin and is identical in queens and worker bees.

The vitellogenin titre in the haemolymph of workers of known age was determined by means of rocket electrophoresis against queen ovary antiserum (procedure of Laurell 1965, 1966). A low titre already exists on the day of emergence. On day 3 it begins to increase and reaches a first maximum on day 6. On the following day the titre drops slightly but increases enormously from day 8 to 12. From day 14 to 28 it is low again and stays approximately at the level on day 6.

During the first 12 days of adult life the vitellogenin titre in the worker haemolymph is positively correlated with the corpora allata volume (Gast 1965) or with the relative surface of the corpora allata (Van der Laere 1971), as can be seen in Figure 1. In queenless workers between day 8 and 14 the vitellogenin titre is 2 or 3 times

as high as that of 12 days old queenright workers.

In 2 days old queens, with undeveloped ovaries, vitellogenin can be detected by immunodiffusion analysis. By means of rocket immunoelectrophoresis we determined the titre in the haemolymph of queens aged 6 weeks and one year, both in oviposition. They were nearly the same and about twice as high as that in the haemolymph of 12 day old queenright workers.

We have examined the possibility of the transmission of worker vitellogenin to the queen. According to Rembold (1969) the proteins of worker and royal jelly originate from the hypopharyngeal glands. These glands were therefore thoroughly examined and random samples were taken from mandibular glands, salivary glands and honey stomachs. We could not detect vitellogenin in any of these organs nor in worker or royal jelly. Thus vitellogenin is not passed over to the queen. However in the rocket immunoelectrophoresis of homogenate of hypopharyngeal glands against queen ovary antiserum several peaks appeared, indicating identical proteins or peptides in worker hypopharyngeal glands and in queen ovaries. It seems possible therefore that these proteins or peptides of the worker hypopharyngeal glands are transmitted and contribute to yolk formation in the queen.

This view is supported by our tracer experiments: in 4 colonies 5 day old queenright workers received ^{14}C-amino acid injections into their abdomen*. In 4 other experiments the queens in oviposition were themselves injected into the abdomen. After various periods the proteins of ovaries and thorax of the 8 queens were precipitated, washed and their radioactivity measured (tables 1 and 2).

Table 1. Radioactivity of queen proteins after injection of ^{14}C-amino acids into workers

Hive Nr	Number of injected workers	Injected quantity	Injected activity	Duration of experiment	Radioactivity in queen proteins. CPM per mg protein dry weight		Ratio ovary/thorax
					ovary	thorax	
1	50	5 µl	0.5 µC	48 h	876	118	7.42
2	50	5 µl	0.5 µC	60 h	2'411	420	5.74
3	137	5 µl	0.5 µC	72 h	22'032	2'170	10.15
4	75	10 µl	1.0 µC	120 h	12.775	2.067	6.18

Mean = 7.37 $\pm$ 0.99

* A ^{14}C-amino acid mixture with a specific activity of 57 mCi/mAtom carbon, 50 µCi/ml from Amersham was used.

Table 2. Radioactivity of queen proteins after injection of ^{14}C-amino acids into the queen herself.

Hive Nr	Number of injected queens	Injected quantity	Injected activity	Duration of experiment	Radioactivity in queen proteins. CPM per mg protein dry weight		Ratio ovary/thorax
					ovary	thorax	
5	1	5 µl	0.5 µC	48 h	5'972	1'777	3.26
6	1	5 µl	0.5 µC	60 h	11'720	3'653	3.21
7	1	5 µl	0.5 µC	72 h	14'438	3'428	4.21
8	1	5 µl	0.5 µC	120 h	4.831	1.312	3.68
						Mean =	3.59 $\pm$ 0.23

One of the queens only (No. 3) continued to lay eggs during the experiment. In this experiment we were able to collect radioactive eggs 72 hours after the injection of labelled amino acids into the workers.

The ratios of the radioactivity, ovary/thorax are significantly different in tables 1 and 2 ($P = < 0.01$). The proteins extracted from the thorax of queens which received radioactive food from the workers show a comparatively low activity. This indicates that the food passed on to the queen contained relatively small amounts of free amino acids. A high radioactivity in the ovary proteins, however, seems to indicate that radioactive proteins or peptides were passed, which could be directly incorporated into the yolk material. These results therefore confirm the conclusions drawn from the immunological tests of the worker hypopharyngeal glands. Rather than haemolymph vitellogenin proteins, peptides of the hypopharyngeal glands seem to be passed on from the workers to the queen, and may be involved in building up yolk material in the queen.

Our results may be summarized as follows: a vitellogenin appears in worker bee haemolymph which is identical with the vitellogenin in haemolymph and ovaries of the queen. Its titre is correlated with the corpora allata volume during the first 12 days after emergence. This vitellogenin does not seem to have a social function, since it is not secreted by the worker glands. However several identical antigens occur in worker hypopharyngeal glands and in queen ovaries. Tracer experiments indicate that these substances secreted by worker hypopharyngeal glands play a role in yolk formation in the queen.

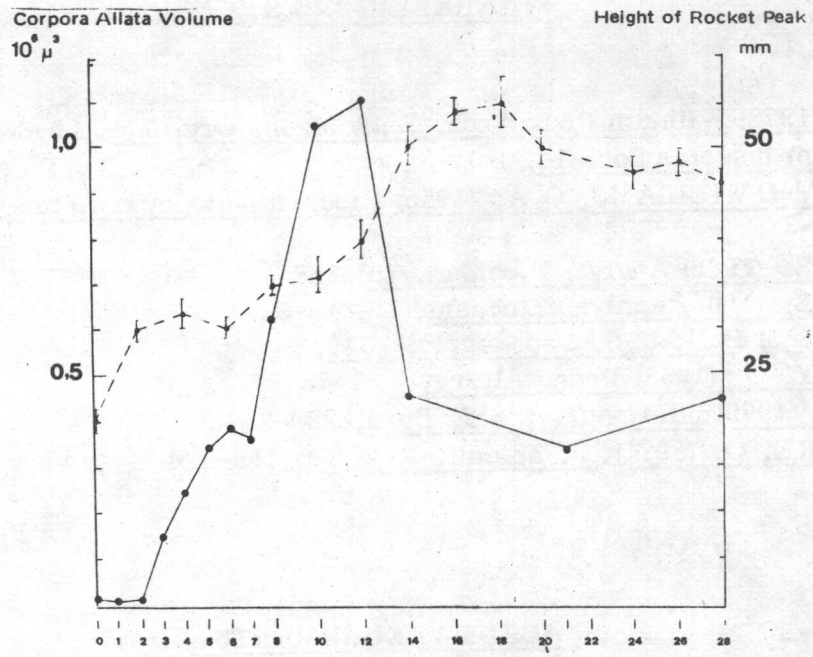

Figure 1. Vitellogenin titre in workers of known age as determined by rocket immunoelectrophoresis (solid line) and corpora allata volume of queenright workers of known age (broken line: from Gast 1965)

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