

Blood and semen levels of thyroid hormones in untreated normozoospermic, oligozoospermic and azoospermic patients

Group	No. of samples	Sperms/ml ($\times 10^6$)	Blood T_4 (μ g/100 ml)	T_3 (ng/ml)	Semen T_4 (μ g/100 ml)	T_3 (ng/ml)
1	5	Azoospermia	7.70 ± 2.12	1.30 ± 0.45	1.32 ± 1.0	0.25 ± 0.07
2	24	1-50	7.85 ± 1.95	1.75 ± 0.03	1.32 ± 0.68	0.26 ± 0.15
3	11	> 50	9.07 ± 1.14	1.80 ± 0.16	1.54 ± 0.42	0.42 ± 0.25

Values are mean \pm SE. The differences between groups were statistically nonsignificant.

differences between the groups were, however, statistically non-significant. As found in a previous study⁸, the levels of these hormones were very low in semen, with the normozoospermic specimens showing a non-significant tendency towards higher values.

Following the administration of 0.1 g thyroindin daily for 4 weeks neither blood nor semen showed an increase in levels of T_3 and T_4 . After the additional 2-week period of treatment with 0.2 g thyroindin daily, however, there was an increase in the blood levels of T_3 and T_4 to a mean value of 2.0 ± 0.18 ng/ml and of 11.7μ g/100 ml respectively. There

was no change in the levels of thyroid hormones in the semen nor in the quality of the semen.

Although the thyroid hormones may play a role in the metabolic and enzymatic activities of spermatozoa, as they do in other cells, in our study the increased levels of T_3 and T_4 found in the blood following treatment were not associated with an improvement in sperm quality nor with an increase in their levels in the semen. The thyroid hormones were evidently unable to influence the spermatozoa probably due to the inability of even the higher dosage of thyroindin to cross the barrier of the male urogenital tract.

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0014-4754/83/050544-02\$1.50 + 0.20/0
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An endocrine control mechanism for chemosensillar activity in the blowfly

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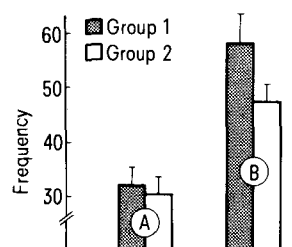
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Summary. Juvenile hormone (JH) administration increases the sensitivity of labellar chemosensilla in *Phormia*. It is suggested that this hormone plays a role in controlling both chemosensillar sensitivity and ovarian cycles.

Previous observations showing that cyclic variations of the chemosensory function in the blowfly were time-related to ovarian cycles have led to the suggestion that a single endocrine mechanism controls both the ovarian and the chemosensory function. An increase in sensitivity of the labellar chemosensilla occurs at the beginning of vitellogenesis^{1,2}. Since vitellogenesis is known to be triggered by the release of juvenile hormone (JH) from the corpus allatum³, we planned a study aimed at verifying whether JH could be the endocrine factor also influencing the chemosensillar sensitivity.

Experiments were performed on the 'largest' labellar hairs⁴ of 2-day-old adult females *Phormia regina* (Meig.). Sensitivity of the chemosensilla was evaluated by measuring their response frequency to a test stimulus (0.150 M NaCl) in the 1st sec after stimulation onset. The active electrode was a stimulating-recording glass micropipette that was slipped over the tip of each chemosensillum, whereas the reference electrode was a chronically implanted platinum wire. The insertion of this electrode was carried out as follows. At the beginning of the experiment each insect was restrained by means of a parafilm envelope. A small opening was made in the parafilm, thus allowing just a small portion of the lateral thoracic surface anterior to the

wing to be exposed. A segment of platinum wire (0.1 mm in diameter, 2 mm in length) was inserted into the thorax (approximately 0.5–1 mm in depth), 0.1–0.2 mm cranially to the wing root. The inserted electrode was thereafter glued to the cuticle by means of a fast drying cyanoacrylic glue and earthed through a glass micropipette containing



Response frequency (imp/sec) from labellar chemosensilla in *Phormia*. Group 1, before (A, 1st measurement) and 24 h after JHA treatment (B, 2nd measurement); group 2, control, untreated insects. 24 h are interposed between the 1st and the 2nd measurement (A and B respectively). Each datum is the mean value \pm SEM of 50 measurements in 10 flies (5 chemosensilla tested in each fly).

3 M KCl. Previous experiments demonstrated that this surgical treatment did not modify the survival capabilities of the insect in a significant way. The experimental set up used allowed us to test sensitivity of the same chemosensilla of the same flies before (1st measurement) and 24 h after JH administration (2nd measurement).

As regards the hormone administration, 0.25 µl of a JH analogue (JHA, Altosid, Zoecon Corporation, Palo Alto, Ca., USA) were applied topically on the ventral abdomen of each insect. The oily consistency of this chemical allowed easy application. Its ability to reproduce the JH biological effect on ovary maturation in *Phormia* when administered using the above procedure has already been demonstrated³. The same experiments (without JH treatment) were also performed on a group of control flies.

Results obtained in the 1st measurement (A in the fig.) showed no significant differences in response frequency ($0.6 \leq p \leq 0.7$ with the Student t-test) between control flies and those in the group to be treated with JHA. The electrophysiological activity proved to be quite changed in both groups of flies at the 2nd measurement (B in the fig., 24 h later) as compared to the 1st one, the firing frequency in each group being remarkably increased. Differences were statistically significant ($p \leq 0.001$ in both cases). In other words, responsiveness of chemosensilla in both groups was higher in 3-day-old than in 2-day-old flies; we

recall at this point that age-related changes in chemosensillar sensitivity (depending – at least in part – on ovarian cycles) seem to be normal physiological events in blowflies^{1,2}. In addition, spike firing frequency was significantly higher in the JHA treated flies than in the control flies ($p \leq 0.001$). In short, JHA administration enhanced chemosensillar sensitivity. This latter observation leads us to suggest that physiologically-occurring increases in chemosensillar sensitivity during ovarian cycles^{1,2} – a sample of which is provided in this research by the increased response in control flies at the 2nd measurement as compared to the 1st one – may also be due to JH influence. Thus, this hormone could play a role in controlling both ovarian and chemosensory function.

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0014-4754/83/050545-02\$1.50 + 0.20/0
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Cyclic sensitivity variations in the labellar chemosensilla of *Calliphora*

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Summary. Cyclic variations in the sensitivity of labellar chemosensilla are time-related to ovarian development in *Calliphora*. It is assumed that a single endocrine mechanism controls both sensitivity of chemosensilla and ovarian function.

Electrical resistance of labellar chemosensilla in the female blowfly varies with age according to a pattern that has been supposed to be related in some way to ovarian cycles¹. Since previous research strongly suggests that variations in the electrical resistance of chemosensilla may account for variations in their sensitivity², it seemed to be of interest to investigate directly whether some ovarian cycle-related variations in sensitivity of chemosensilla do indeed exist. 500 adult female blowflies, *Calliphora vomitoria* L., were used. The insects, kept at 26 °C and 70% relative humidity were divided on emergence into 2 groups. The 1st group ('M' flies) was fed on a standard, protein-containing diet (minced beef, sucrose, and water); the 2nd group ('WM' flies) was provided with a protein-free diet (sucrose and water) that hindered full ovarian development³. 10–12 insects were taken from each group daily from day 3 to 9 after their emergence. Ovarian development was evaluated by measuring transverse as well as longitudinal ovarian diameters, and assessing the appearance of yolk (i.e., the beginning of vitellogenesis) in the follicles, according to the method described by Stoffolano⁴. Since these diameters showed very similar variation patterns (correlation coefficient $r = +0.995$; $p < 0.001$), only the values of the longitudinal diameter will be reported here. Electrophysiological recordings were taken from the 'largest' labellar chemosensilla⁵ by using the tip-recording technique⁶. A 0.5 M NaCl solution was adopted as a stimulus. Spike firing frequency (in the 1st sec. after stimulation onset) and electrical resistance (according to the method used by Stürckow⁷) were measured on 4–5 chemosensilla in each insect used.

Results on the 'M' flies are reported in figure 1. Two increasing phases in ovarian length were present. That means (considering the ovarian length as an index of follicle maturation⁴) that 2 subsequent ovarian cycles took place. This observation is in full agreement with previous results, according to which ovarian cycles in this insect follow each other without delay⁸. The sensitivity of the labellar chemosensilla attained maximum levels (maximum and minimum peaks in frequency and resistance respectively; these parameters were inversely correlated at $r = -0.840$; $p < 0.001$) at the beginning of the vitellogenic period of the 1st as well as of the 2nd ovarian cycle (on days 4 and 7 respectively). Except in these peaks, sensitivity of chemosensilla showed a tendency to decrease toward the low level observed at the beginning of the experiments (or, in other words, to the level observed before vitellogenesis took place). The firing frequency and the electrical resistance (i.e. chemosensitivity) varied with time in a very similar way in the 'WM' insects also, despite their incomplete ovarian development (fig. 2). Still, the 2 parameters were inversely correlated ($r = -0.800$; $p < 0.001$).

In short, chemosensitivity showed a similar and cyclic variation pattern in flies either with regular or with incomplete ovarian function. On the basis of the time-related changes in both chemosensory and ovarian function in the 'M' flies, we suggest that a single control mechanism may act on both functions. This suggestion is further endorsed by the cyclic chemosensitivity changes we observed in the 'WM' flies, in which ovarian function was not achieved owing to the lack of protein in the diet. Our results do not