

lymph node alone were also performed and no obvious angiogenesis was observed in both the cases (data not presented).

Incidences of growth of the tumor piece graft into a bigger tumor which often bulged out of the limit of eye, have been registered in the table as percentages of occurrence of tumors. This index was lower in group B than in 2 other groups. Angiogenesis and tumor growth have been thought to depend on each other<sup>7</sup>. Possibly, this phenomenon may be due to lesser vasoproliferation causing inhibition of growth of the tumor in the case of grafting of the tumor plus a stimulated lymph node piece.

Several suggestions may be put forward to explain the inhibition of angiogenesis and then tumor growth. It has been shown that the phenomenon of angiogenesis is mediated by tumor-angiogenesis factor (TAF), released from the tumor graft<sup>2</sup>. Thus it seems possible the factor(s) released by the activated lymphocytes might neutralize TAF or affect the production of it. It is also conceivable that Con A stimulated lymphocytes mount a cell-mediated cytolytic reaction against the tumor cells and, in consequence, hinder the production of TAF and growth of the tumor graft. The later possibility might be supported with some evidences from previous work. Several authors have demonstrated the activation of cytotoxic functions in mouse thymocytes by lectins, particularly Con A. Although this substance is a polyclonal stimulator, it has been shown that Con A-mediated cytotoxicity is expressed through antigen-specific membrane receptors in unprimed<sup>8-10</sup> and primed lymphocytes<sup>11,12</sup> against a variety of targets, including tumor cells.

Activated lymphocytes were obtained from lymph node pieces from an animal injected earlier with Con A. We have shown elsewhere<sup>6</sup> that Con A is capable of inducing

blast transformation in lymphocytes *in vivo*; differentiation of lymphocytes into blasts is considered as an index of activation. Preliminary experiments indicate that these *in vivo* transformed cells are capable of mounting cytotoxic reactions (to be published elsewhere). Modulation of tumor induced angiogenesis and growth of a tumor graft in the presence of polyclonally-activated lymphocytes suggests an interesting model for probing immune responses against neoplasms.

- 1 Acknowledgment. The authors are thankful to ICMR, New Delhi, for financial assistance and to Mr N.C. Ghosh for illustration.
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0014-4754/83/050542-03\$1.50 + 0.20/0  
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### Triiodothyronine and tetraiodothyronine in human semen prior to, and following, treatment with thyroid extracts

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**Summary.** The levels of triiodothyronine and tetraiodothyronine were measured in blood and semen of normozoospermic, oligozoospermic, and azoospermic men, prior to, and following, treatment with thyroid extracts. The semen, hormones and sperm quality were not affected by treatment.

Some evidence indicates that in some animal species normal blood levels of thyroid hormones are essential for reproduction<sup>1-3</sup> and during a considerable period thyroid hormones were occasionally used in the treatment of infertile men and women<sup>4-6</sup>. Although this therapeutic approach has recently been abandoned we decided to carry out a study to determine whether the administration of thyroid extracts affects the levels of triiodothyronine (T<sub>3</sub>) and tetraiodothyronine (T<sub>4</sub>) in the semen and/or the quality of the sperm.

The subjects studied were 40 clinically euthyroid patients aged from 25 to 45 years. 5 were azoospermic, 24 oligozoospermic (sperm density,  $1.0-50 \times 10^6/\text{ml}$ ; % motility,  $45.6 \pm 2.6$  (SE); motility grade,  $1.9 \pm 0.1$ ; % viability,  $58.1 \pm 2.6$ ; % normal morphology,  $25.9 \pm 1.5$ ) and 11 normozoospermic (sperm density,  $> 50 \times 10^6/\text{ml}$ ; % motility,  $58.5 \pm 3.1$ ; motility grade,  $2.6 \pm 0.01$ ; % viability,  $64.5 \pm 2.4$ ; % normal morphology,  $46.6 \pm 3.3$ ). The sperm count was made with a Neubauer camera. Motility was assessed according to Cockett et al.<sup>7</sup> and viability by the eosin-

nigrosin test. Morphology was determined from smears stained by the Papanicolaou technique. Levels of T<sub>4</sub> and T<sub>3</sub> in blood and seminal plasma (obtained by centrifugation of semen at 15,000 rpm for 30 min) were determined by radioimmunoassay techniques.

In 8 volunteers (3 of them azoospermic and 5 of them oligozoospermic) treatment was instituted with thyroindin (0.1 g thyroindin (USP) has a biological activity of 0.04 mg T<sub>3</sub> and 0.16 mg T<sub>4</sub>), given at a daily dose of 0.1 g for 4 weeks and then 0.2 g for an additional 2 weeks. Blood and semen samples were obtained for repeated determination of T<sub>3</sub> and T<sub>4</sub> levels and assessment of the andrological parameters after 4 weeks and again 2 weeks later.

The table presents the blood and semen levels of thyroid hormones in the untreated subjects. Although blood levels of T<sub>3</sub> and T<sub>4</sub> were within the normal range the values of T<sub>3</sub> tended to be lower in specimens from azoospermic subjects and those of T<sub>4</sub> were somewhat lower in specimens from both azoospermic and oligozoospermic subjects than they were in specimens from normozoospermic subjects. The

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

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

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 study<sup>8</sup>, 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  $2.0 \pm 0.18$  ng/ml and of  $11.7 \mu$ 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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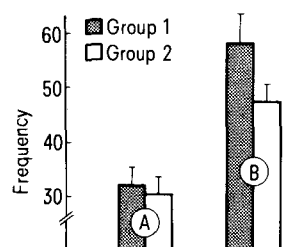
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**Summary.** Juvenile hormone (JH) administration increases the sensitivity of labellar chemosensilla in *Phormia*. It is suggested that this hormone plays a role in controlling both chemosensillar sensitivity and ovarian cycles.

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<sup>1,2</sup>. Since vitellogenesis is known to be triggered by the release of juvenile hormone (JH) from the corpus allatum<sup>3</sup>, 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 hairs<sup>4</sup> 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).