

sponse in PH than GGT activity during increased collagen biosynthesis⁷⁻⁹. The hydroxyproline concentration and total hydroxyproline content of the cardiac muscle were also unchanged in the present study. The mean cardiac collagen concentration usually increases only during extensive myocardial hypertrophy, e.g.^{6,10,24}. It should be noted that the hydroxyproline content reflects long-term events of collagen synthesis and degradation. Moreover, the turnover in cardiac collagen is slow, 0.56% per day⁶. It is also possible that alterations in cardiac muscle collagen concentration may be nonuniformly distributed transmurally during hypertrophy^{24,25}. Thus, the relatively small increase in the

rate of collagen synthesis need not result in a measurable increase in total collagen content in a non-hypertrophic adaptation model such as the present one.

It has been suggested that myocardial collagen may be composed of collagen of types I, III, IV and V at least²⁶, and the possible differential effects of exercise on the turnover or net synthesis of different collagen types remain to be established. The present results indicate that, although not reflected in the tissue collagen content during the present observation time, collagen synthesis is involved in non-hypertrophic physiological adaptation of the heart to volume overload induced by physical exercise.

- 1 This study was supported by the Academy of Finland and the Finnish Ministry of Education.
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- 3 Borg, T.K., and Caulfield, J.B., *Tex. Rep. Biol. Med.* 39 (1979) 321.
- 4 Skosey, J.L., Zak, R., Martin, A.F., Aschenbrenner, V., and Rabinowitz, M., *Circulation Res.* 31 (1972) 145.
- 5 Waterlow, J.C., Garlick, P.J., and Millward, D.J., *Protein turnover in mammalian tissues and in the whole body*. Elsevier/North-Holland, Amsterdam 1978.
- 6 Bonnin, C.M., Sparrow, M.P., and Taylor, R.R., *Am. J. Physiol.* 241 (1981) H708.
- 7 Kivirikko, K.I., and Myllylä, R., *Int. Rev. Connect. Tissue Res.* 8 (1979) 23.
- 8 Kivirikko, K.I., Myllylä, R., in: *The enzymology of post-translational modifications of proteins*, p.53. Eds R.B. Freedman and H.C. Hawkins. Academic Press, London 1980.
- 9 Kivirikko, K.I., Myllylä, R., in: *Collagen in health and disease*. Eds M.J.W. Jayson and J.B. Weiss. Churchill Livingstone, Edinburgh 1983, in press.
- 10 Lindy, S., Turto, H., and Uitto, J., *Circulation Res.* 30 (1972) 205.
- 11 Turto, H., *Cardiovasc. Res.* 11 (1977) 358.
- 12 Kiiskinen, A., and Heikkinen, E., *Eur. J. appl. Physiol.* 35 (1976) 167.
- 13 Hickson, R.C., Hammons, G.P., and Holloszy, J.O., *Am. J. Physiol.* 236 (1979) H268.
- 14 Kainulainen, H., Saari, P., and Vihko, V., *IRCS med. Sci.* 10 (1982) 143.
- 15 Vihko, V., Salminen, A., and Rantamäki, J., *Acta physiol. scand.* 104 (1978) 74.
- 16 Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J., *J. biol. Chem.* 193 (1951) 265.
- 17 Kivirikko, K., Laitinen, O., and Prockop, D.J., *Analyt. Biochem.* 19 (1967) 249.
- 18 Kivirikko, K., and Myllylä, R., *Meth. Enzym.* 82A (1982) 245.
- 19 Juva, K., and Prockop, D.W., *Analyt. Biochem.* 15 (1966) 77.
- 20 Myllylä, R., Risteli, L., and Kivirikko, K.I., *Eur. J. Biochem.* 52 (1975) 401.
- 21 Risteli, J., Tuderman, L., and Kivirikko, K.I., *Biochem. J.* 158 (1976) 369.
- 22 Myllylä, R., Risteli, L., and Kivirikko, K.I., *Eur. J. Biochem.* 58 (1975) 517.
- 23 Takala, T., Myllylä, R., Salminen, A., Anttinen, H., and Vihko, V., unpublished observation.
- 24 Medugorac, I., *Cardiovasc. Res.* 14 (1980) 551.
- 25 Medugorac, I., *Res. exp. Med.* 177 (1980) 201.
- 26 Borg, T.K., Gay, R.E., and Johnson, L.D., *Coll. Rel. Res.* 2 (1982) 211.

0014-4754/83/101094-02\$1.50 + 0.20/0
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Occurrence of a pheromone-producing gland in female tobacco beetles

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Summary. A pheromone-producing gland was discovered in the second abdominal segment of virgin female tobacco beetles, *Lasioderma serricorne* (Fabricius). The gland duct extends to an orifice below the genital pore and is supported by a rigid invagination of the integument. Hexane extracts of intact pheromone glands were found attractive to male tobacco beetles and also induced high receptor potentials in the olfactory sensilla of the antennae of male *L. serricorne*. A surface extract of virgin females proved to be significantly more attractive than an extract of pheromone glands.

The sex pheromone emitted by female tobacco beetles (*Lasioderma serricorne* F.) has been investigated mostly from a chemical viewpoint¹⁻⁸, whereas the biological aspects of this subject have received less attention⁹⁻¹³. Since studies on the site of pheromone production have been neglected so far, a detailed investigation of the exocrine gland producing the sex attractant was undertaken.

The tobacco beetles used were reared under constant climatic and nutritional conditions¹⁰; unmated females and males were frozen at the age of 1-2 weeks after pupal-imaginal ecdysis, decapitated and subjected to fixation and embedding, subsequently cut into thin sections (average thickness 10 µm) and stained by Ehrlich's hematoxylin¹⁴.

The apodeme (integumental invagination) was removed together with the adjoining gland (fig. 1a) from the abdomen of virgin females of *L. serricorne* (1-2 weeks of age) which had been frozen previously (-20°C for 24 h). The 5th abdominal segment was gently compressed, ovipositor and ovaries removed, whereupon the apodeme and adjoining gland were pulled out with pointed forceps and cleaned of adhering tissues. Batches of 200 extirpated glands, either homogenized or intact, were immersed in 2-ml portions of n-hexane for 48 h (20±1°C) and the resulting extracts diluted to provide gland equivalents ranging from 10⁻⁴ to 10⁰. The gland and surface extracts of virgin females and males were used for studies on the olfactory responses¹⁵

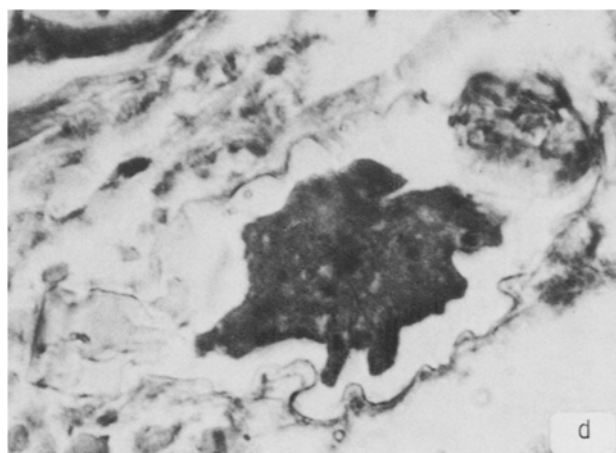
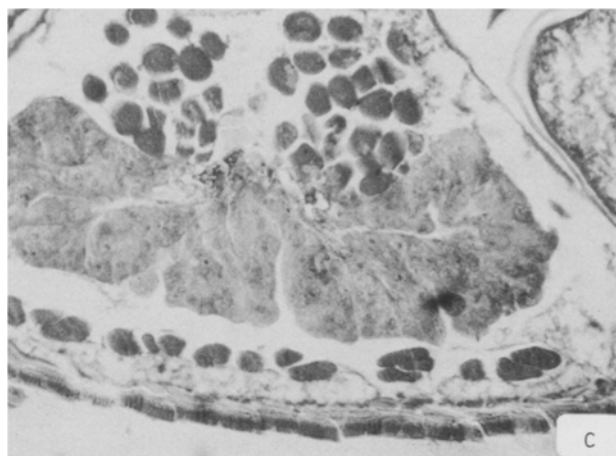
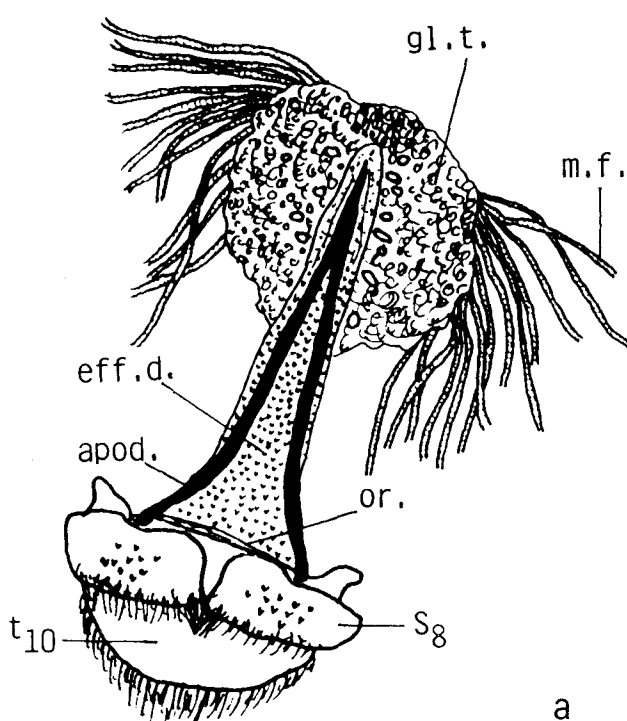


Figure 1. Structure and location of the pheromone-producing gland, apodeme and efferent duct in the abdomen of female tobacco beetles. *a* Ventral view of the exocrine gland with adjoining apodeme (integumental invagination) and abdominal tip, length 1.26 mm. *b* Transverse section through the 2nd abdominal segment exposing the exocrine gland vertically below the alimentary canal and close to the ventral epidermis, $\sim 63\times$ natural size. *c* Lobate exocrine gland near proximal part of the apodeme in cross section, $\sim 220\times$ natural size. *d* Cuticular sheath around the sclerotized rods of the apodeme displaying an intensely stained secretion in the efferent duct, $\sim 380\times$ natural size. gl.t., glandular tissue; m.f., muscle fibers; eff.d., efferent duct; apod., apodeme; or., orifice; t_{10} , tenth tergite; s_8 , eight sternite (terminology according to Dailey and Happ¹⁶).

and behavior¹¹ of unmated male tobacco beetles (13–16 days after pupal-imaginal ecdysis).

The pheromone gland. Using the above methods, we have demonstrated the occurrence in virgin females of a lobate exocrine gland (figs 1a, 1c) which is lacking in unmated males of *L. serricorne*. This gland has a length of $\sim 400\ \mu\text{m}$, a width of $\sim 250\ \mu\text{m}$ and a depth of $\sim 135\ \mu\text{m}$; it is situated in the 2nd abdominal segment vertically below the alimentary canal and close to the ventral epidermis (fig. 1b). The gland is ventrally attached to the narrower part of an inverted V-shaped apodeme consisting of 2 sclerotized rods (length 700–800 μm , diameter $\sim 20\ \mu\text{m}$) which extend from the terminal to the 2nd abdominal segment (fig. 1a). The apodeme is enveloped by a wrinkled cuticular sheath (fig. 1d) appearing as a conical duct which extends from the gland tissue to a horizontal orifice (length $\sim 300\ \mu\text{m}$) below the genital opening (fig. 1a). The internal surface of the

above sheath is ventrally covered by many denticles (length 5–8 μm) which point towards the orifice. The gland tissue comprises numerous secretory cells with large nuclei, opaque droplets and many tubuli leading to the conical duct which frequently contains an intensely stained secretion (fig. 1d).

Pheromone perception. Olfactory perception of extracts of exocrine glands obtained from virgin females was demonstrated by recording receptor potentials from the antennae of male tobacco beetles¹⁵. Relatively high receptor potentials were induced in olfactory sensilla upon stimulation of the latter by a hexane extract of intact glands applied in graded dosages of 10^{-4} up to 10^0 gland equivalents (fig. 2). The threshold dosage eliciting a receptor potential was found to be approximately 10^{-5} of a gland equivalent, and the response increased, dose-dependently, to 2.4 mV with one gland equivalent. On the other hand, 10^{-1} and 10^0

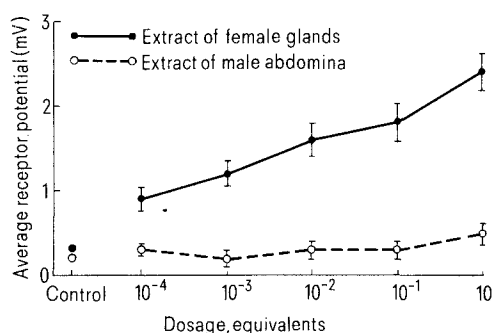


Figure 2. Receptor potentials recorded from olfactory sensilla on the terminal antennal segment of male *Lasioderma serricorne* F. evoked by surface extracts (hexane) of intact female glands and male abdomina.

Stimulation was carried out by passing air puffs (20 ml/0.5 sec) over graded levels of the above extracts. Extracellular DC potentials were recorded by glass electrodes (microcapillaries) containing Beadle-Ephrussi's Ringer solution. Each point on the curves represents the average receptor potential recorded from eight antennae and the relevant standard deviation (vertical line).

equivalents of a hexane extract of abdomina excised from unmated male *L. serricorne* caused receptor potentials of 0.3 and 0.5 mV respectively, which failed to differ significantly from the control values (stimulation by air puffs).

Premating behavior. Bioassays¹¹ on circular filter papers (diameter 75 mm) impregnated in the central region (diameter 3 mm) by a hexane extract of intact glands displayed the attractiveness of the latter for unmated male

tobacco beetles. The tests involved responses of 100 insects exposed in groups of 10 to the above experimental conditions and revealed the following average results: 1 gland equivalent of the above extract induced 39.7 (s.d.=1.8) visits of male *L. serricorne* on the impregnated region, while 10 gland equivalents increased this parameter to 51.2 (s.d.=2.4) and also caused several attempts of copulation, within an observation period of 15 min. However, a surface extract (hexane) of virgin females proved to be significantly more attractive than the gland extract inducing 62 (s.d.=2.7) visits and repeated attempts of copulation, when compared at the level of one equivalent. This is probably due to repeated pheromone secretion from the gland resulting in pheromone accumulation in the cuticle lipids of virgin females. Moreover, the mean receptor potentials induced by the surface extract of virgin females were significantly higher than those elicited by the gland extract: one equivalent of the former induced 3.2 ± 0.3 mV, whereas one equivalent of the latter elicited 2.4 ± 0.2 mV in the antennae of male tobacco beetles.

Interestingly, the behavior of male tobacco beetles induced by a hexane extract of homogenized glands was found to differ from that observed in presence of a hexane extract of intact glands: although unmated males rapidly approached the region impregnated with extract of the homogenate (1 or 10 gland equivalents), they left it instantaneously followed by a sharp turning. It appears that the extract of intact glands merely provides the sex pheromone emitted from the orifice (fig. 1a), whereas the extract of homogenized glands also comprises less volatile components and/or metabolites evoking the repulsion of male tobacco beetles.

Acknowledgment. We thank the B.A.T. Cigarettenfabriken GmbH for financial support and Miss M. Neff for technical assistance.

- Chuman, T., Kohno, M., Kato, K., and Noguchi, M., *Tetrahedron Lett.* 25 (1979) 2361.
- Chuman, T., Kato, K., and Noguchi, M., *Agric. biol. Chem.* 43 (1979) 2005.
- Ono, M., Onishi, I., Chuman, T., Kohno, M., and Kato, K., *Agric. biol. Chem.* 44 (1980) 2259.
- Mori, K., and Nomi, H., *Tetrahedron Lett.* 22 (1981) 1127.
- Mori, K., Chuman, T., Kohno, M., Kato, K., and Noguchi, M., *Tetrahedron Lett.* 23 (1982) 667.
- Mori, M., Chuman, T., Kato, K., and Mori, K., *Tetrahedron Lett.* 23 (1982) 4593.
- Chuman, T., Kohno, M., Kato, K., Noguchi, M., Nomi, H., and Mori, K., *Agric. biol. Chem.* 45, (1981) 2019.
- Hoffman, R.W., Helbig, W., and Ladner, W., *Tetrahedron Lett.* 23 (1982) 3479.
- Coffelt, J.A., and Burkholder, W.E., *Ann. ent. Soc. Am.* 65 (1972) 447.

- Levinson, H.Z., Levinson, A.R., Francke, W., Mackenroth, W., and Heemann, V., *Naturwissenschaften* 68 (1981) 148.
- Levinson, H.Z., Levinson, A.R., Francke, W., Mackenroth, W., and Heemann, V., *Naturwissenschaften* 69 (1982) 454.
- Chuman, T., Mochizuki, K., Mori, M., Kohno, M., and Kato, K., *Agric. biol. Chem.* 46 (1982) 593.
- Chuman, T., Mochizuki, K., Mori, M., Kohno, M., Kato, K., Nomi, H., and Mori, K., *Agric. biol. Chem.* 46 (1982) 3109.
- Romeis, B., *Mikroskopische Technik*, 16th edn. Oldenbourg, München/Wien 1968.
- Levinson, A.R., Levinson, H.Z., Schwaiger, H., Cassidy, R.F. Jr, and Silverstein, R.M., *J. Chem. Ecol.* 4 (1978) 95.
- Dailey, R.J., and Happ, G.M., *J. Morph.* 171 (1982) 259.

0014-4754/83/101095-03\$1.50 + 0.20/0
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Fat-supplemented diet protects against activity-stress ulcers in rats¹

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Summary. Rats were exposed to a fat-supplemented (32.5% beef tallow) diet from weaning until they were 200 g in weight. Half the animals were exposed to the activity stress paradigm consisting of housing in standard activity-wheel cages while restricting their food intake to 1 h each day. Control rats were treated in the same fashion but on a fat-free (0.08%) diet. Fat-supplemented activity-stressed rats exhibited high levels of running wheel activity but less mortality and less gastric pathology than fat-free controls which were exposed to the activity-stress procedure. Ulcers were not observed in home cage housed rats in either diet condition.

If young adult male rats are housed in standard laboratory activity wheel cages and fed only 1 h each day, they will die within 7-10 days and reveal massive gastric glandular

ulcers²⁻⁴. Much of this gastric damage is indeed true ulcer, penetrating the muscularis⁵. Home cage housed rats which are also fed only 1 h per day (food 'yoked' to the wheel-