Lawsone and the other constituents of the assay mixture were as usual. Nitrite production was estimated as before. The nitrogen content, both ethanol-soluble and ethanol-insoluble (protein) was determined using the method described earlier[13]. A PR-2 international refrigerated centrifuge and a DU_2 spectrophotometer were used as and when needed.

In the control the fungus showed a gradual increase in the enzyme activity up to only the 6th–7th day of growth. Lawsone at different sampling times inhibited in vivo enzyme activity by 42–50%. As shown in figure 1, inhibition of in vitro nitrate reductase activity increased with increasing concentrations of lawsone; the maximum inhibition (70%) was obtained at 1500 ppm. The lowest concentration of lawsone (62.5 ppm) used stimulated nitrate reductase activity by 13%. It is obvious from figure 1 that lawsone action on the enzyme in the presence of L-cysteine showed a similar pattern to that in experiments without L-cysteine (figure 1) and that the inhibition of enzyme activity at higher concentrations of lawsone was not prevented by cysteine. The table shows the effect of lawsone on ethanol-soluble and protein nitrogen content. While the amount of soluble nitrogen was adversely affected from the beginning of the growth period, the protein content was affected only after the 4th day of growth.

Discussion. The present study showed that on day 4 lawsone inhibited in vivo enzyme activity by 42.3% and at the same time soluble nitrogen content was also reduced by 40% approximately. Over the 5-day-periods (day 4–day 8) of growth there was a parallel decrease in the inhibition of nitrate reductase activity and in soluble nitrogen content (figure 2). This suggests that there exists a correlation between nitrate reductase activity and soluble nitrogen. No such correlation, however, was observed between inhibition of nitrate reductase and reduction of protein nitrogen content (figure 2). It would, therefore, appear that the effect of lawsone on protein synthesis was not entirely through its action on nitrate reductase. This indicates that lawsone affects the protein synthesis, at least partly, through its action on some points other than nitrate reductase (an alternative route). Although our data are not sufficient to indicate a definite mechanism of inhibition, it might be similar to the inhibition of papain, an SH containing enzyme, caused by 2-methyl-1,4-naphthoquinone[14,15].

Vitamin K_3 – a structural analogue of lawsone – is known to act as the cofactor for nitrate reductase[16,17]. Slight stimulation observed at lower concentrations in vitro (figure 1), though not very well understood, could possibly be due to lawsone functioning as a cofactor for nitrate reductase. Lawsone thus appears to be a very effective fungicide, for it kills the pathogen[4,10] on the one hand, and stimulates the nitrate reductase activity of the crop plants on the other[18]. A direct correlation between nitrate reductase activity and crop yield has recently been established[19].

1 The present study forms a part of the Ph.D. Thesis of R.D.T., University of Gorakhpur 1979.
2 Present address: Department of Life Sciences, Vigyan Bhawan, University of Indore, Khandawa Road, Indore (M.P.).
3 Acknowledgment. Authors are thankful to Prof. S.N. Mathur for providing necessary facilities, Dr D. Mukerjee and K.M. Mukerjee for help in the preparation of the manuscript. R.D.T. is thankful to CSIR, New Delhi for a post doctoral fellowship.
4 R.D. Tripathi, H.S. Srivastava and S.N. Dixit, Experientia *34*, 51 (1978).
5 M.W. Foote, E.J. Little and T.J. Sporston, J. biol. Chem. *181*, 481 (1949).
6 W.H. Schopfer and E.C. Grob, Helv. chim. Acta *32*, 829 (1949).
7 R.G. Owens, Contr. Boyce Thompson Inst. Pl. Res. *17*, 273 (1953b).
8 Y. Mihashi and M. Tatsumi, J. pharm. Soc. Japan *75*, 146 (1955).
9 L.R. Scherbanovskii, L.N. Blagonravova, I.G. Kovaleva, T.A. Burdeinaya and A.G. Reva, Prikl. Biokhim. Mikrobiol. *21*, 54 (1976).
10 R.D. Tripathi, Thesis, University of Gorakhpur 1979.
11 H.S. Srivastava, Indian J. Biochem. Biophys. *11*, 230 (1974).
12 V.P. Choudary and G.R. Rao, Can. J. Microbiol. *22*, 35 (1976).
13 H.S. Srivastava, Indian J. Pl. Physiol *16*, 57 (1973).
14 W.B. Geiger, Archs Biochem. *11*, 23 (1946).
15 N. Nakai and J. Hase, Chem. pharm. Bull., Tokyo *16*, 2339 (1968).
16 S.D. Wainright, Biochem. biophys. Acta *18*, 583 (1955).
17 E. Itagaki, T. Fujita and R. Sato, J. Biochem. *53*, 389 (1963).
18 S.N. Dixit, H.S. Srivastava and R.D. Tripathi, Ann. Meet. Indian Soc. Pl. Physiol. *80* (1978).
19 C.B. Johnson, W.J. Whittington and G.C. Blackwood, Nature *262*, 133 (1976).

Giant collagen fibres in the gonopodium of the mosquitofish *Heterandria formosa* Agassiz, 1853 (Pisces, Poeciliidae)[1]

R. Riehl

Institut für Allgemeine und Spezielle Zoologie der Justus-Liebig-Universität, Stephanstr. 24, D-6300 Giessen (Federal Republic of Germany), 27 November 1979

Summary. The gonopodium of the mosquitofish *Heterandria formosa* was studied by transmission electron microscopy. In cross-section the gonopodium shows the following structure from the outside inwards: mutilayered epidermis, basal lamina and central supporting tissue, in which vessels and nerves are embedded. At the top of the gonopodium giant collagen fibres are found, which measure up to 150 µm in length and 6 µm in diameter. These fibres reinforce the gonopodium.

The gonopodium of the mosquitofish *Heterandria formosa* was studied recently in detail not only morphologically but also electron-microscopically[2,3]. The gonopodium of poeciliid fish represents a considerably modified analis, which is formed by the rays III–V.

In cross-section the gonopodium shows the following structure from the outside inwards: multilayered epithelium, basal lamina and central supporting tissues of varying structure (connective tissue and bony tissue consisting of osteoblasts, osteocytes and osteoclasts). Blood vessels and nerves are embedded in the supporting tissue.

The gonopodia were fixed and treated using methods which have already been published[4]. The specimens were decalcified with EDTA for 3–4 weeks[5]. The gonopodia

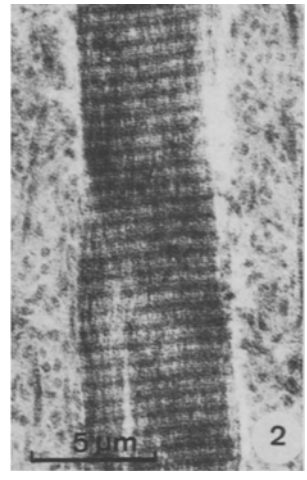

Fig. 1. Cross-section of the gonopodium at the top. The arrow points to a giant collagen fibre.

Fig. 2. High magnification of a giant collagen fibre, which gives an impression of the characteristic period and band pattern of the mature collagen.

were embedded in araldite and vestopal W, cut with an ultratome Om U 3 (Reichert) and later examined in an EM 9S (Zeiss) at 60 kV.

Many filaments and collagen fibres take part in the formation of gonopodium. Filaments occur not only in epidermal cells, but also in the central supporting tissue. At the top of the gonopodium filaments and collagen fibres increase in number and compactness.

In epidermal cells collagen fibres did not occur, but other filaments could be identified there. On the other hand, a thin course of collagen fibres lies below the basal lamina. The collagen fibres are arranged in 20–30 layers. 3–5 layers always cross one another at an angle of 90°. In this way they form a texture. At the top of the gonopodium there are collagen fibres, too, but they do not show any texture.

In the apical part of the gonopodium giant collagen fibres are a peculiarity. They are aligned in the transversal axis of the gonopodium (figure 1). Giant collagen fibres measure up to 150 µm in length and 6 µm in diameter (figures 1+2). In longitudinal section the fibres show the characteristic period and band pattern of mature collagen (figure 2). The giant collagen fibres are embedded in a great number of minute collagen fibrils, which are arranged irregularly. Most of the minute collagen fibrils are synthetized from osteoblasts. These collagen fibrils correspond to the 'preosseous matrix' of light microscopy[6].

Collagen fibrils, giant collagen fibres and cytofilaments reinforce the gonopodium, which is stressed strongly during copulation. The place of synthesis of the giant collagen fibres is still unknown.

1 This research was supported by the Deutsche Forschungsgemeinschaft. Address for reprints: Forschungsgruppe Dermatologie der Universität Heidelberg, Im Neuenheimer Feld 324, D-6900 Heidelberg.

2 R. Riehl, A. Holl and E. Schulte, Zoomorphologie *91*, 113 (1978).
3 R. Riehl, Acta zool. (Stockh.) *59*, 199 (1978).
4 R. Riehl and E. Schulte, Protoplasma *92*, 147 (1977).
5 H. Warshawsky and G. Moore, J. Histochem. Cytochem. *15*, 542 (1967).
6 N.M. Hancox, Biology of Bone. Cambridge University Press, Cambridge 1972.

Electrical resistance and spike activity in tarsal chemosensilla of *Phormia regina* (Meig.)[1]

A.M. Angioy, A. Liscia and P. Pietra

Institute of General Physiology, University of Cagliari, Viale Poetto 1, I-09100 Cagliari (Italy), 15 November 1979

Summary. The relationship between hair electrical resistance and responsiveness to stimulation has been investigated in the tarsal chemosensilla of *Phormia regina*. Results showed that the lower the hair electrical resistance, the higher is the spike-firing frequency.

The electrical resistance of the chemosensory hairs of the blowfly, *Phormia regina*, has been taken into account in previous research studies on the hypothesis that reliable information about the responsiveness of hairs to external stimulants may be gained by measuring it[2–5]. This hypothesis has been based mainly on the following assumptions: 1. the viscous layer that separates the external environment from the chemosensory dendrites at the hair tip may be of a mucopolysaccharide nature; 2. by acting as a barrier, this layer could be the major factor in modulating chemical fluxes from the external environment to the chemosensory dendrites, and consequently could be responsible for setting hair electrical resistance. The effectiveness of external chemicals as stimulants could thus be evaluated by measuring electrical resistance.

As regards assumption 1, preliminary experiments carried out in our laboratory but not yet published, have indeed proved that the apical viscous layer is of a mucopolysaccharide nature. As far as assumption 2 is concerned, we undertook the present investigation in order to make a direct study of the relationship between the electrical resistance and responsiveness of chemosensory hairs.