as well as of secondary males. They are located between triangular, interglobular spaces and mainly characterized by a rather large nucleus, well developed SER and many free ribosomes, as well as mitochondria with tubular cristae. A high heterochromatin content probably charactizes the cells in the quiescent phase and the high euchromatin concentration high-lights hormone synthesis. Some Leydig cells contain a few lipid droplets and poorly developed SER. These can be interpreted as being developing but not yet fully differentiated cells.

During sex change, the inactive germ cells are located peripherally in the ovary and develop into male germ cells in the newly organized testes. These germ cells are stimulated to grow and develop into mature spermatocytes. Probably before this, the other male gonadal specific cells, such as Leydig and sertoli cells, develop from the remnants of the ovary, and the external appearance changes; the fish develops the characteristic color and fin pattern.

The effect of androgenous hormones on the color and shape of the dorsal fin (elongated fin rays), as an accompanying phenomenon of the sex change from female to secondary male, would suggest that these are secondary sex characteristics. But the fact that there are no exterior differences between females and primary males seems to deny this suggestion. Influence of testosterone on the external morphology of the males may not be a direct one. Additional factors are probably involved in the development of the 'secondary' characteristics in secondary males.

1 This study was supported in part by grant 3.751.80 from Schweizerischer Nationalfonds.
2 Acknowledgment. The authors are indebted to Mr Naseem Malik for helping with the preparation of the manuscript.
3 Smith, C.L., in: Intersexuality in the Animal Kingdom, p.295. Ed. R. Reinboth. Springer, Heidelberg *1974*.
4 Reinboth, R., Verh. dt. Zool. Ges. (1967) 316.
5 Reinboth, R., Biol. Reprod. *22* (1980) 49.
6 Chan, S.T.H., Phil. Tras. R. Soc. Lond. B *259* (1970) 59.
7 Chan, S.T.H., in: Handbook of Sexology, p.91. Eds J. Money and H. Musaph. Elsevier/North-Holland, Biomedical Press, Amsterdam 1977.
8 Choat, J.H., and Robertson, D.R., in: Intersexuality in the Animal Kingdom, p.263. Ed. R. Reinboth. Springer, Heidelberg 1974.
9 Fishelson, L., Nature, Lond. *224* (1970) 90.
10 Fishelson, L., in: Intersexuality in the Animal Kingdom, p.284. Ed. R. Reinboth. Springer, Heidelberg 1974.
11 Kawaguchi, K., and Marumo, R., Coll. Repr., vol.6, p.253. The Oceanic. Res. Inst. Univ. Tokyo 1968.
12 Lowe, T., and Larkin, J.R., J. exp. Zool. *191* (1975) 25.
13 Smith, C.L., and Atz, E.H., Copeia *1* (1973) 41.
14 Reinboth, R., Zool. Anz., suppl. *24* (1961) 259.
15 Reinboth, R., Zool. Jb., Physiol. *69* (1962) 405.
16 Reinboth, R., Mem. Soc. Endocr. *18* (1970) 515.
17 Roede, M.J., Vie Milieu *17* (1966) 1317.
18 Roede, M.J., Pubbl. Staz. zool. Napoli *39* (1975) 595.
19 Duchac, B., Inaugural-Dissertation. Bibliothek der Universität, Basel 1981.
20 Duchac, B., Rapp. Commun. int. Mer médit. *27* (1981) 187.
21 Duchac, B., Huber, F., Müller, Hj., and Senn, D.G., Experientia *38* (1982) 809.
22 Duchac, B., and Bühler, E.M., Experientia *39* (1983) 767.
23 Chan, S.T.H., O, W.S., and Hui, W.B., in Intersexuality in the Animal Kingdom, p.201. Ed. R. Reinboth. Springer, Heidelberg 1974.
24 Gresik, E.W., Gen. comp. Endocr. *21* (1973) 210.
25 Gresik, E.W., Quirk, J.G., and Hamilton, J.B., Gen. comp. Endocr. *20* (1973) 86.
26 Gresik, E.W., Quirk, J.G., and Hamilton, J.B., Gen. comp. Endocr. *21* (1973) 341.
27 Hoar, W.S., and Nagahama, Y., Annls Biol. anim. Biochem. Biophys. *18* (1978) 893.
28 O'Halloran, M.J., and Idler, D.R., Gen. comp. Endocr. *15* (1970) 361.
29 Nicholls, T.J., and Graham, C.P., Gen. comp. Endocr. *19* (1972) 133.
30 Van den Horst, G., Zool. africana *13* (1978) 233.
31 Van den Hurk, R., Proc. K. Ned. Akad. Wet. Ser.C (1973) 270.
32 Van den Hurk, R., Meek, J., and Peute, J., Proc. K. Ned. Akad. Wet. Ser.C *76* (1974) 470.
33 Van den Hurk, R., Vermei, J.A., Stegengar, J., Peute, J., and Van, P.G., Annls Biol. anim. Bioch. Biophys. *18* (1978) 899.
34 Chan, S.T.H., Tang, F., and Lotts, B., Excerpta med. int. Congr. *256* (1972) 348.
35 Reinboth, R., Gen. comp. Endocr. *2* (1962) Abstr. No.39.
36 Reinboth, R., Verh. dt. zool. Ges. München (1963) 67.
37 Reinboth, R., Verh. dt. zool. Ges. Würzburg (1969) 548.
38 Reinboth, R., Pubbl. Staz. zool. Napoli *39* (1975) 550.
39 Tang, F., Chan, S.T.H., and Lotts, B., Gen. comp. Endocr. *24* (1974) 227.

0014-4754/83/121376-05$1.50 + 0.20/0

Effect of sublethal concentrations of sumithion on limb regeneration of fresh water field crab *Oziotelphusa senex senex*

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Summary. The initiation and progress of regeneration following the removal of the left 4th walking leg were altered in the crab *(Oziotelphusa senex senex)* by exposure to sumithion. Depending on the concentration used, sumithion caused a complete inhibition of regeneration, a delay of initiation of limb bud development or a reduction of limb bud growth rate. Crustacean limb regenration can also be used as a sensitive bioassay for studying the effects of environmental pollutants.

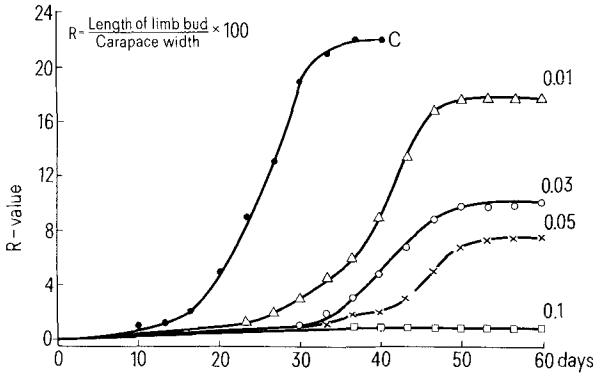
Sumithion, an organophosphorus pesticide, which is widely used in this area to control the rice stem borer *Tryporhyza sps.* is known to pollute the aquatic environment. Not much attention has been given until now to the consequences of sumithion in non-pest aspects of the ecosystem. Determination of acute toxicity levels has little relevance in the estimation of ecologic consequences. There is little data available on toxic effects of sublethal concentrations, possibly because no standardized tests exist. A sensitive parameter would be crustacean limb regeneration. This has already been applied for measuring sublethal effects on fiddler crabs[2-4] and shrimps[5].

We have tested the effects of sumithion (fenitrothion; O-O-dimethyl-O-(3 methyl-4-nitrophenyl) phosphorothioate) on limb regeneration of *Oziotelphusa senex senex* at concentrations of 0.01-0.1 mg/l.

Materials and methods. Sumithion (Technical grade 99% W/V) obtained from Rallis India Ltd was used as a test chemical. Only adult, healthy male specimens were used. The crabs were intermolt individuals, having a body wt 30–32 g; carapace width 32–35 mm. The animals were kept singly each in 1000 ml medium at 28 ± 1 °C. 300 crabs were divided into 5 equal groups. The left 4th walking leg was removed from all the crabs. One of the groups served as control while the others were exposed to 0.01, 0.03, 0.05 and 0.1 ppm sumithion from the day of limb removal until the animals completed at least 1 ecdysis or until the termination of the experiment (60 days). The media for control and experimental crabs were replaced with fresh solutions daily. The crabs were fed with frog muscle on alternate days for the duration of the experiment. The limb bud growth rate (R-value) of animals were determined after[6].

Results and discussion. No mortalities occurred at either of the 4 sumithion concentrations or in the control group during the experiment. The crabs in normal water lacking a left 4th walking leg that served as controls regenerated limbs at a rapid rate (fig.). However, the crabs that were in sumithion regenerate limbs at a much slower rate than the control group. The degree of inhibition increased with the concentration of sumithion exposed. Depending on the concentratiaon used, sumithion caused a complete inhibition of regeneration, a delay of initiation of limb bud development, or a reduction of limb bud growth (fig.). Similar results in limb bud growth rate was also recorded in fiddler crab[2-4] and shrimp[5] after exposing a sublethal concentrations of mercury, DDT, pentachlorophenol.

The results in this investigation suggests, that both delay in crustacean limb bud initiation and inhibition of growth rate are sensitive parameters and can also be used for monitoring toxic responces of chemical pollutants in aquatic ecosystem without sacrificing the animals.



Effect of sumithion on limb regeneration of *Oziotelphusa senex senex* Fabricius. Number on each curve indicates concentration of sumithion in mg/l.

1 Acknowledgments. We are thankful to Rallis India Ltd (Bangalore) for providing us technical grade sumithion. We wish to express our gratitude to CSIR, New Delhi for providing financial support to PSR. Reprint requests should be addressed to RR.

2 Weis, J.S., U.S. Fish. Wild Serv. Fish Bull. *74* (1976) 464.
3 Weis, J.S., Biol. Bull. *152* (1977) 263.
4 Weis, J.S., and Mantel, L.H., Estuarine Costal Mar. Sci. *4* (1976) 461.
5 Ranga Rao, K., Conklin, P.J., and Brannon, A.C., in: Pentachlorophenol, p.193. Ed. K. Ranga Rao. Plenum Press, New York and London 1978.
6 Bliss, D.G., in: Bartil Hanstrom, zoological papers in honor of his 65th birthday, p.56. Ed. K.G. Wingstrand. Zool. Inst., London/Sweden 1956.

0014-4754/83/121380-02$1.50 + 0.20/0

Protamine inhibits adenylate cyclase activity: a possible reason for the toxicity of protamine

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Summary: Protamine is an effective inhibitor of the various activated forms of adenylate cyclase of liver plasma membranes. Inhibition of adenylate cyclase may account for its toxic but not its antitumor effects.

Protamine is an inhibitor of the growth of certain tumors[2-5]. This property is probably related to its tumor angiogenesis-inhibitory activity[6], the mechanism of which is unclear. Unfortunately, systemic administration of protamine is limited by its toxicity[6]. The reason for the toxicity of protamine is not known. Clearly, knowledge of the mechanism(s) which is (are) responsible for either of the action of protamine may help in the development of more specific antitumor agents.

We report here that protamine strongly inhibits the adenylate cyclase system of liver plasma membranes.

Materials and methods. ATP, GPP(NH)P(guanyl-5′-yl imidodiphosphate), glucagon, NaF, protamine (free base; prepared from salmon), creatine phophate and creatine phosphokinase were purchased from Sigma (St. Louis, Mo., USA). (α-^{32}P) ATP (500 Ci/mmole) was prepared by the Isotope Institute of the Biological Research Center, Szeged. Preparation of liver plasma membranes and the assay of adenylate cyclase activity was performed as described earlier[7] with the only difference that 10μg plasma membrane proteins were used.

For the solubilization of adenylate cyclase, 1.7 mg plasma membrane proteins were incubated at 4 °C for 30 min in the presence of 5.1 mg Lubrol PX, 20 mM Tris/HCl pH 7.5 and 2 mM $MgCl_2$ (volume 1.5 ml). In some cases the solubilization mixture also contained 10 mM NaF ± 0.5 mM ATP. The solubilized cyclase was recovered in the high speed supernatant (centrifugation at 4 °C for 1 h at 105,000 × g in a Beckman L 50 ultracentrifuge).

Protein was determined according to the method of Lowry et al.[8] using bovine serum albumin as standard.

Each documented experiment was repeated with 3 different plasma membrane preparations with similar results.

Results. Protamine, tested up to 20 μM, did not inhibit the basal cyclase activity of liver plasma membranes (fig.). It did, however, strongly supress the activatory effect of GPP(NH)P, a nonhydrolyzable analog of GTP. The GPP(NH)P plus glucagon as well as the fluoride activated