Acute toxicity of copper and its probable effect on thyroid of the fry of *Ophiocephalus punctatus* (Bloch)

D.K. Srivastava and R.K. Tyagi

Department of Zoology, K.A.D. College, Allahabad-211 001 (India), and CIFR Sub-station, 24, Pannalal Road, Allahabad-211 002 (India), 10 March 1981

Summary. LC_{50} values for 24, 48, 72, 96 and 120 h were estimated at 1.507; 1.065; 0.785; 0.607; 0.559 mg/l copper respectively for the fry of *Ophiocephalus punctatus.* Copper induced hyperthyroidism resulting in exopthalmos, has been reported for the first time.

Copper is known to be toxic to animals. The effluents of smelters, paper mills and chemical industries contain large quantities of heavy metals including copper. The evaluation of the toxicological effects of copper on the aquatic ecosystem is therefore essential. Though considerable literature is available on the toxicology of copper for adult fishes[1], much less work has been done on their more susceptible early stages[2-5]. The available information on the effect of copper on endocrine glands is meagre[10]. The present study has been attempted with the belief that the median tolerance (TLm) values for developmental stages like fry and fingerlings would be better criteria for establishing the maximum acceptable concentration of copper.

The advanced fry, mean size 27 (26-28)mm and weight 205 (200-210)mg, were obtained by natural breeding of *Ophiocephalus punctatus.* The fry were acclimatized in tap water for 7 days and were fed on live plankton. The feeding was discontinued 48 h before and during biotesting. Stock solutions of reagent grade $CuSO_4 \cdot 5\ H_2O$ was prepared in distilled water and a few drops of acetic acid were added to prevent precipitation. Exploratory tests were carried out for determining the concentrations for the final experiment and the data were analysed using nomograms[6]. Based on the preparatory tests, 10 copper (Cu^{++}) concentrations, viz. 0.375; 0.500; 0.575; 0.625; 0.750; 1.000; 1.250; 1.500; 1.750 and 2.000 mg/l were taken. Each of these concentrations was replicated. For the control and all concentrations, 10 test animals were used in 5 l dechlorinated tap water in round glass jars. The control and test media were changed every day. Mortalities were monitored at 24-h intervals for 120 h, and LC_{50}'s were determined by probit analyses[7]. Chi-square tests were applied to test the homogeneity of the experimental material and mutual independence of the subjects, and in all cases this was found to be not significant at 5% level of significance.

Water analyses were carried out at 24-h intervals, using standard methods[8]. The values of different parameters for the water were found to be: temperature, 22.5(21.7-24.3) °C; pH, 7.1(6.8-7.4); DO, 6.2(5.3-7.2) mg/l; alkalinity, 108.7(87.6-140.0) mg/l $CaCO_3$; hardness, 71.7(60.0-85.4) mg/l $CaCO_3$.

During the course of the experiment, up to 60% mortality occurred below 0.750 mg/l concentration. In 0.750 and 1.000 mg/l, the mortalities observed were 20% in 24 h and 90% within 120 h. Above these a steep rise in the rate of mortality was seen and 90% of the test animals died within 72 h. No mortality occurred in the control. The 24-, 48-, 72-, 96- and 120-h LC_{50}'s were estimated at 1.507; 1.065; 0.785; 0.607 and 0.559 mg/l respectively. The LC_{50}'s and their 5% fiducial limit are shown in figure 1.

Nearly all test animals exhibited initial erratic swimming and frequent surfacing. In higher concentrations, 1.250 mg/l and above, the surfacing lasted only 3-5 h and subsequently the test fry became dull and settled down on the bottom showing occasional movements. The fry exposed to 0.750 and 1.000 mg/l showed signs of hyperexcitability, as manifested by a tendency to jump out of the jars, coming about 10-13 cm above the water surface. From these concentrations, 50% of the surviving fry exhibited enormously bulging eyeballs (fig. 2) after 24-h exposure. All these fry ultimately died before 96 h. The surviving fry from these and all other concentrations, as well as the controls, however, had normal eyeballs.

Prompted by the appearance of the aforesaid symptoms, histological observations were carried out on the fry with bulging eyeballs, to study the probable effect of copper on thyroid follicles. It revealed an average 20% increase in the height of thyroid follicles over that of the control.

The 48-h LC_{50} value for copper for adult specimens of *O. punctatus* has been reported[9] to be 70 mg $CuSO_4$/l. If the concentration of the toxicant in the present study is expressed in terms of copper sulphate, the value of the 48-h LC_{50} would be only about 4 mg/l for the fry. The water qualities in both cases are within reasonable limits. Thus, with the increase in size, from fry to adult, of this fish, nearly 18-fold rise in tolerance level of this metal occurs.

Heavy metals have been suspected to act upon the endocrine glands[10], though any direct proof is still lacking. The height of thyroid follicles has been considered as measure

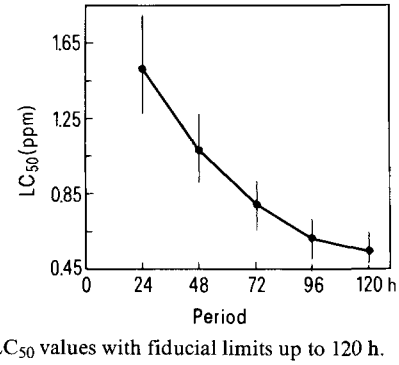

Figure 1. LC_{50} values with fiducial limits up to 120 h.

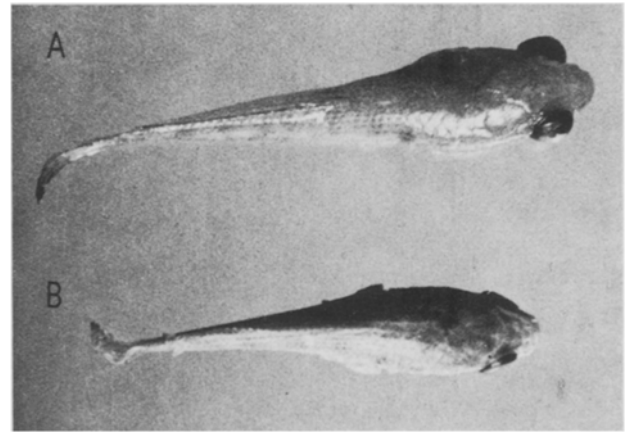

Figure 2. *A* Fry with exopthalmos, exposed to 1.000 mg/l copper for 48 h, *B* control fry.

of thyroid activity[11]. The 20% increase in the height of the follicles, in the present study, indicates hyper thyroid activity. Hyperthyroidism results in a syndrome known as 'exopthalmos', the symptoms being bulging eyeballs with signs of hyperexcitability[12]. However, exopthalmos has also been reported in the trout infected with a virus known as VHS[13]. Apart from exopthalmos, no symptom associated with viral infection could be detected in the present study. Therefore, the exopthalmos observed here may be attributed to hyperthyroidism. Copper-induced hyperthyroidism, resulting in exopthalmos, has not hitherto been reported in any fish.

The appearance of exopthalmos in only 2 out of 10 copper concentrations could only be explained on the presumption that, perhaps, the lower concentrations are too weak to act on the nervous system to activate the thyroid through the hypophysis, and that the higher concentrations are strong enough to cause quick and intensive brain damage, thereby preventing activation of the thyroid. It is well known that the brain exerts control over many functions of the hypophysis, including production and release of TSH, which in turn activates production of thyroid hormones[11]. Consequently, any damage to the nervous system may possibly stop thyroid stimulation. The neurotoxic effects of copper on fishes are already well documented[14,15].

Thus it appears that 0.750 and 1.000 mg/l of copper are optimum doses for the fry of *Ophiocephalus punctatus*, for triggering the hypothalamo-hypophysial-thyroid mechanism, resulting in hyperthyroidism. However, further studies employing sophisticated techniques, for which facilities do not exist with us, are needed in order to understand fully the process of thyroid activation by copper.

1 R.L. Spehar, G.W. Holcombe, R.W. Carlson, R.A. Drummond, J.D. Yount and Q.H. Pickering, J. Wat. Pollut. Control Fed. *51*, 1615 (1979).
2 C.R. Hazel and S.J. Meith, Calif. Fish Game *56*, 121 (1970).
3 C.W. O'Rear, Jr, Proc. 26th a. Conf. SEast Ass. Game Fish Commn, Comm. October 1972; p.484.
4 J.M. Mckim, J.G. Eaton and G.W. Holcombe, Bull. Environm. Contamin. Toxic. *19*, 608 (1978).
5 P.T.E. Ozoh and C. Jacobson, Bull. Environm. Contamin. Toxic. *21*, 782 (1979).
6 A. Granmo and M. Larsstuvold, in: 4th FAO/SIDA Training course on aquatic pollution in relation to protection of living resources, bioassays and toxicity testing, Sweden, 13 October–29 November, 1975.
7 D.J. Finney, Probit Analysis, 2nd edn. Cambridge Univ. Press, London 1964.
8 Standard Methods for the Examination of water and wastewater, 13th edn. American Public Health Association, New York 1971.
9 S. Mukherjee and S. Bhattacharya, Environm. Physiol. Biochem. *4*, 226 (1974).
10 E. Jackim, J.M. Hamlin and S. Sonis, J. Fish. Res. Board Can. *27*, 383 (1970).
11 I. Chester Jones, J.N. Ball, I.W. Henderson, T. Sandor and B.I. Baker, in: Chemical Zoology, vol.8, p.523. Ed. Florkin and Scheer. Academic Press, New York/London 1974.
12 G.H. Bell, J.N. Davidson and H. Scarborough, Textbook of Physiology and Biochemistry, 6th edn. ELBS, Edinburg 1965.
13 R.J. Roberts, Fish Pathology. Bailliere Tindall, London 1978.
14 F.S. Vogel, J. exp. Med. *110*, 801 (1959).
15 G.R. Gardner and G. La Roche, J. Fish. Res. Board Can. *30*, 363 (1973).

Effect of enkephalins in the presence of the antibiotic bacitracin in the longitudinal muscle strip preparation from guinea-pig ileum

Amaya Aleixandre, P. Garcia de Jalón and Isabel Martin

Departamento de Farmacologia, Facultad de Medicina, Universidad Complutense, Madrid 3 (Spain), 23 March 1981

Summary. The antibiotic bacitracin (5×10^{-5}–4×10^{-4} M) increases the inhibition of the contractile response caused by both enkephalin release and direct application of Met-enkephalin 5×10^{-7} M in the longitudinal muscle strip preparation from guinea-pig ileum. This effect is attributed to an inhibition of enkephalin degrading peptidases by bacitracin.

Bacitracin is a peptidase-inhibiting antibiotic which prevents enzymatic peptide degradation. This characteristic of bacitracin was investigated by Schulz et al. in 1977[1], using longitudinal muscle strips from guinea-pig ileum. When electrically stimulated, this preparation releases enkephalins[1-3]. These polypeptidic substances are very quickly enzymatically degraded in vivo as well as in vitro[4]. The addition of bacitracin should increase the enkephalin concentration in the medium[1]. However, the results were not the expected ones; addition of 2×10^{-5} M bacitracin caused a marked decrease of enkephalinergic material released by electrical stimulation. (This material was detected by TLC.) Consequently the authors mentioned suggested a possible interaction of bacitracin with the enkephalin release mechanism or, as a more likely explanation, with the mechanism by which opiate-like material is formed from a larger peptide through enzymic cleavage. This precursor would lack opiate-like activity and was not detected by the methods used.

In the present investigation, we have provoked a massive enkephalinergic release from longitudinal muscle strips from guinea-pig ileum by means of 10-Hz tetanizing shocks, according to the method described by Puig et al. in 1977[2]. Met-enkephalin and morphine have also been administered directly, and the effect of bacitracin on the phenomena observed has been investigated.

Materials and methods. The myenteric plexus-longitudinal muscle strip from guinea-pig ileum was prepared as described by Paton and Zar[5]. Each strip was suspended in a 40-ml organ bath containing Krebs-Henseleit[6] solution at 32 °C bubbled with a mixture of 95% oxygen and 5% carbon dioxide. The isometric contractions of the muscle were recorded by means of a force transducer coupled to a Ugo Basile polygraph. The tension of the strip was maintained at 1 g. The tissue was stimulated by field stimulation through 2 platinum ring electrodes with a basic 0.3 Hz, 2-msec electric stimulus and supramaximal voltage ($\simeq$ 60 V). A Grass SD9 stimulator was used. 5 min after the bacitracin (2.5×10^{-5} M–4×10^{-4} M) administration, a tetanizing shock was delivered by increasing the stimulus frequency up to 10 Hz for 5 min and then returning to the basic stimulus conditions with 0.3 Hz.