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Histopathological Effects of the Insecticides, Heptachlor and Nicotine, on the Gills of the catfish, *Heteropneustes fossilis*

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Abstract The histopathological effects of the two insecticides, heptachlor and nicotine were studied on the gills of the freshwater catfish, *Heteropneustes fossilis*. The fishes exposed to 1.0 ppm of the former and 3.2 ppm of the latter at room temperature (18.89—23.89°C), died within 19 to 44 hours. The fishes exhibited acute respiratory distress with both the insecticides. The gills of fishes exposed to heptachlor were found coated with a layer of mucus, but no such covering was found on the gills of those died in nicotine. The surface cells of the gill filaments of fishes killed by heptachlor showed disintegration, rupture, vacuolation, karyorrhexis, and extrusion and degeneration of nuclei and cytoplasm. The number of mucous cells in these gills was increased which resulted in the secretion of mucus over them. With nicotine, only the basal parts of a few gill filaments were damaged. It appears that injury with heptachlor was due to surface irritation rather than absorption of the poison through the gills. The lesions caused by nicotine appear to be the indirect action of the poison. Due to paralytic action induced by this poison, the fishes failed to renew water of their opercular chamber which resulted asphyxiation and finally death. These findings strongly support the hypothesis put forth by Herr, Greselin and Chappel (1967) rejecting the views of Carpenter (1927), Jones (1935), Westfall (1945) and Derse and Strong (1963).

INTRODUCTION

While discussing the cause of death of fishes in toxicants, many workers maintained that it was the coagulation of mucus over the gills that would impede respiration of fishes to death (Carpenter, 1927; Jones, 1935; Westfall, 1945). Derse and Strong (1963), on the other hand, suggested that the toxicant itself was absorbed through the gills and interfered with the respiratory apparatus of the animal and thus the latter was killed. Recent studies made with Antimycin by Herr, Greselin and Chappel (1967), however, disapproved this view and pointed out that a mechanism involving absorption would not appear plausible since this poison was much less toxic when given intraperitoneally than when they were exposed by immersion. But these workers left the problem unsolved by saying that "perhaps an interference of oxygen transfer directly at the surface cells of the gill might be the explanation of the high immersion toxicity" of the toxicant.

The present author found that highly toxic poisons generally damaged the surface cells of the gills of fishes, the consequences of which strongly support the hypothesis put forth by Herr, Greselin and Chappel (1967). The following report which deals with the histopathological effects of two highly toxic insecticides, heptachlor, a chlorinated hydrocarbon, and nicotine, a plant alkaloid, on the gills of the freshwater catfish, *Heteropneustes fossilis*, is intended to throw more light on the problem.

MATERIALS AND METHODS

The catfishes (average length, 114 mm; average weight, 7.9 g), fully acclimatized under laboratory conditions, were exposed to 1.0 ppm of heptachlor and 3.2 ppm of nicotine. The experiments were conducted at room temperature (18.89—23.89°C) in battery glass

jars each with 8 litres of unchlorinated tap water. Two sets of such jars, each consisting of five jars, were arranged and into each of which was added a batch of five catfishes. Then each of the first set of jars was treated with 0.04 ml of 20% emulsifiable heptachlor (a MICO, India product) and that of the second set with 0.064 ml of water-soluble nicotine sulphate containing 40% pure nicotine (a Chemphar T.M.P.H., West Germany product) to provide the aforesaid concentrations in terms of active ingredients.

Each set of jars was also accompanied with a control in which the same number of fishes were added but without any insecticide. No fish died in any control within seven days. All the treated fishes died in the first set of jars within 44 hours and in the second set of jars within 19 hours due respectively to the acute toxicity of heptachlor and nicotine.

The dead fishes were at once removed from the treated containers, their gills were examined for mucus, and then they were immediately dipped into the fixatives, Bouin's solution, 10% neutral formalin, Zenker-Helly's fluid and Ortho's fluid, to prevent any post-mortem changes. Then the gills were taken out in immersed condition and were transfered to fresh fixatives. The gills from alive control fishes were also removed and fixed in the same fixatives. The tissues, after fixation, were decalcified in 5% formic acid, washed overnight in running tap water, processed and embedded in paraffin as usual, and the sections, cut 6 to 10 μ thick, were stained with haematoxylin-eosin, Mallory's triple and toluidine blue.

RESULTS

The catfishes exposed to both the insecticides exhibited acute respiratory distress till they died. When autopsy was performed, it was revealed that a layer of mucus was deposited over the gill surfaces of the fishes killed with heptachlor but not over the same structures of those killed with nicotine.

A histopathological examination of the gills indicated that the surface tissues of these structures were severely damaged in fishes killed with heptachlor (Fig. 1, 2). The epithelial cells of the gill filaments showed disintegration, rupture, vacuolation, karyorrhexis, and extrusion and degeneration of the nuclei and cytoplasm. Although a count of mucous cells was not made, their number apparently increased. A number of these cells swelled up while some others burst out. The blood capillaries were also ruptured and dislocated.

The histopathological lesions of the gills of fishes killed with nicotine were limited only to the basal parts of a few secondary gill filaments (Fig. 1, 3). The epithelial cells of these regions of the filaments showed eruption, rupture, atrophy and nuclear pycnosis. But the surface cells of the distal ends of the gill filaments remained almost unaltered. Apparently no increase in mucous cells was observed.

DISCUSSION

Evidently, the damage with heptachlor to the surface cells and blood capillaries of the gill filaments were so intense that there was little doubt about the definite interference in oxygen intake and transfer in these fishes. This fact is corroborated from the increased respiratory rate and subsequent suffocation of the fishes exposed to heptachlor. But the symptoms indicating respiratory distress by those exposed to nicotine were largely due to the cessation of the opercular movement resulting from the paralysis of the opercular muscles.

The cause of injury to the gills of fishes killed with heptachlor appears to be due to surface irritation by the poison rather than the absorption of it through them. Though it is conceivable that at least some amount of the toxicant must enter the body of the fishes by diffusion through gills. But sooner the absorptive surfaces of the gills of these fishes are disrupted, there would naturally be little or no further absorption of the toxicant through these structures.

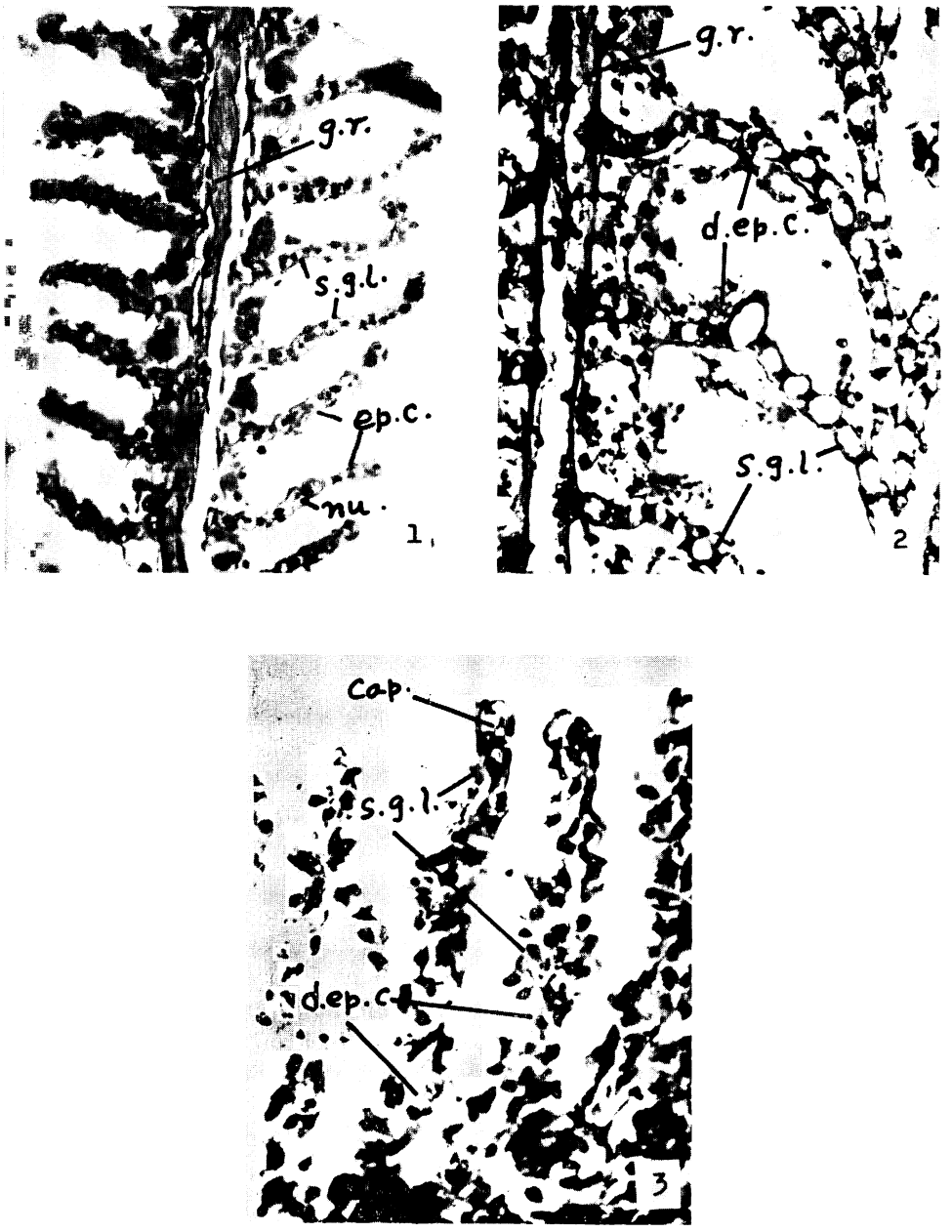

Fig. 1. Longitudinal section of the gill of catfish, *Heteropneustes fossilis* experimented. 1. Control fish untread ×200; 2. Fish killed by heptachlor showing severe injury to gill epithelium ×600; 3. Fish killed by nicotine showing rupture and atrophy of the basal epitherial cells ×600. Symbols: cap . . . blood capillary; d. ep. c . . . degenerateing epitherial cells; ep. c . . . epitherial cells; g. r. . . gill ray; nu . . . nucleus; s.g.l. . . secondary gill lamella.

Moreover, due to enormous growth of the mucous cells of the gill filaments, a layer of mucus is secreted over them. There is reason to believe that this mucus coating would prevent further penetration of the toxicant through the gills. Obviously, the mucus secretion seems to be the sign of injury to the gills as well as it acts as a defence mechanism, as suggested by Neuhold and Sigler (1960), against further diffusion of the toxicant into these structures.

The histopathological lesions in the gills of fishes killed with nicotine do not appear to be the result of direct action of the poison. As noted above, due to paralytic action of the poison, the fishes failed to renew water of their opercular chamber containing gills. This resulted asphyxiation and finally death of fishes.

But what appears most convincing is that the death of fishes in a toxicant is caused not by damage to an organ or system in particular but due to cumulative effect induced as a result of irreversible disorder brought about in the histological and physiological framework of the fishes. The results of the exhaustive investigations made on this line would be published soon.

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殺虫剤ヘプタクロールとニコチンのインド産ナマズ *Heteropneustes fossilis* の鰓に及ぼす組織病理的影響 S. K. コナール

ヘプタクロール (1.0 ppm), ニコチン (3.2 ppm) に投ぜられた魚は，室温 19～24°C において，激しい呼吸困難をおこし，19～44 時間で死亡した．前者の場合，鰓は粘液で包まれ，鰓瓣の表面細胞の核と原形質には崩壊，分裂，空胞化，核切断，突出，退化がみられ，粘液細胞は増加した．ニコチンの場合，粘液は認められず，少数の鰓瓣の基部のみが害されていた．よって，ヘプタクロールによる害は毒物が鰓を通じて吸収されるのではなく，鰓は表面の刺激に起因するものであり，ニコチンによる障害は毒物の間接的影響と解され，その麻痺作用によって鰓腔への水の転換が抑圧され仮死より死に至るものと解される．本実験により，Herr, Greselin and Chappel (1967) の説が支持され，Carpenter (1927), Jones (1935), Westfall (1945), Derseand Strong (1963) の説は退けられた．

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