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Effect of Lead on Pigment Pattern Formation in Zebrafish (*Brachydanio rerio*)

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Specificity of pigment pattern formation is one of the diagnostic features in identification and classification of species. The adult zebrafish, Brachydanio rerio body pigment pattern consists of five alternating blue-black and silvery -yellow strips aligned parallel to the long axis of the body. Early melanocytes of 3-day old pigmentation consists of a double row of dorsal band,one row of lateral band situated in the horizontal skeletogenous septum, the ventral band and the yolk sac band (MILOS and DINGLE 1978). It is possible that black pigments shield sensitive tissues from harmful radiation. Permanent damage to these melanophores might have far reaching biological and ecological consequences, particularly for the species which possess them. GOODRICH et al. (1954) hypothesized that tissue or cellular antagonisms play a part in the delineation of pigment patterns in zebrafish and Phallichthys amates. MILOS and DINGLE (1978) introduced the theory of "exclusion principle" in accounting for the pigment pattern formation in zebrafish.

Heavy metals interfer with pigmentation. DIAL (1978) observed that methylmercury caused reduced pigmentation in Japanese Medaka, Oryzia latipes, while OZOH (1979a) observed that intraperitoneal injection of lead acetate to the males and females of Cichlasoma nigrofasciatum caused interference in pigmentation in some offspring. These disturbances may be due to physicochemical modifications of the environments of the pigment cells or to direct interference with the developmental cues in the germ plasma.

This communication reports the effect of lead on the pigment pattern formation in zebrafish. Zebrafish eggs with and without shell membranes were exposed to lead.

MATERIALS AND METHODS

Brachydanio rerio eggs obtained from natural spawns were used. The eggs were incubated in media of 50 ppb and 72 ppb of lead for 24 hours at 26 $\pm$ 0.5 °C. The control eggs were incubated for 24 hours in only dis-

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tilled water. Lead concentration of 50 ppb housed 500 eggs,72 ppb housed another 500 and the control housed 500 eggs. Dead eggs were removed from the control and the experimental after 24 hours. Incubating vessels were also cleaned. The eggs incubated in 50 ppb of lead were dechorionated with sharp scalpels under stereo-dissecting microscope. Embryos in 72 ppb lead were transferred to a mixture of distilled and tap water. The control eggs were also dechorionated. Dechorionated embryos from 50 ppb lead concentration were transferred into cleaned conical flasks and incubated for further 24 hours in lead concentration of 50 ppb. Half batch of the dechorionated control embryos were incubated for 24 hours in 72 ppb of lead and the other half in a mixture of tap and distilled water. After 24 hours in lead media and water the embryos were separately transferred to tap water and incubated until they were 22 days old. The embryos were fed fish foods. Many embryos briefly exposed to 72 ppb lead did not hatch due to inhibitory tendency of lead to hatching (OZOH 1979b). The embryos were freed from their egg shells with scalpels. The pigment pattern formation of the embryos exposed to lead intoxicated media and the control were intensively studied. The larvae were photographed with Halogen lamp at 3,400 ^{o}K with CB 12 filter and with or without water immersion objectives using Leitz microscope. To photograph the lateral parts of the larvae,MS 222 solutions were added to immobilise the larvae.

RESULTS

Effect of dechorionation

The embryos dechorionated after 24 hours of incubation survived. Those that failed to survive might have suffered mechanical injuries during dechorionation.

Melanophore patterns

At 4 days of age, the characteristic double rows of dorsal melanophores have been positioned(Fig.1). They appear linearly arranged with the notochordal process separating the left from the right half. The yellow pigment cells (chromatophores) first appearing in the head region at about 49 hours have migrated to the trunk and tail regions at 96 hours. Each melanophore was surrounded by chromatophore.

The lateral band melanophores were being formed around the horizontal skeletogenous septum, the ventral band and the yolk sac band have been formed(Fig.2).

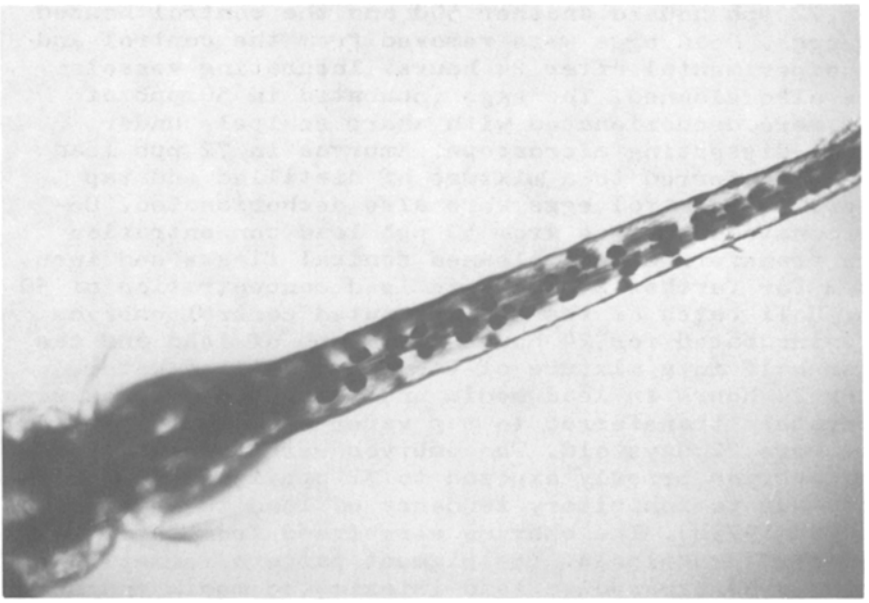

Fig. 1 . 4 days old larva with dorsal row of melanophores. Mag. x 40.

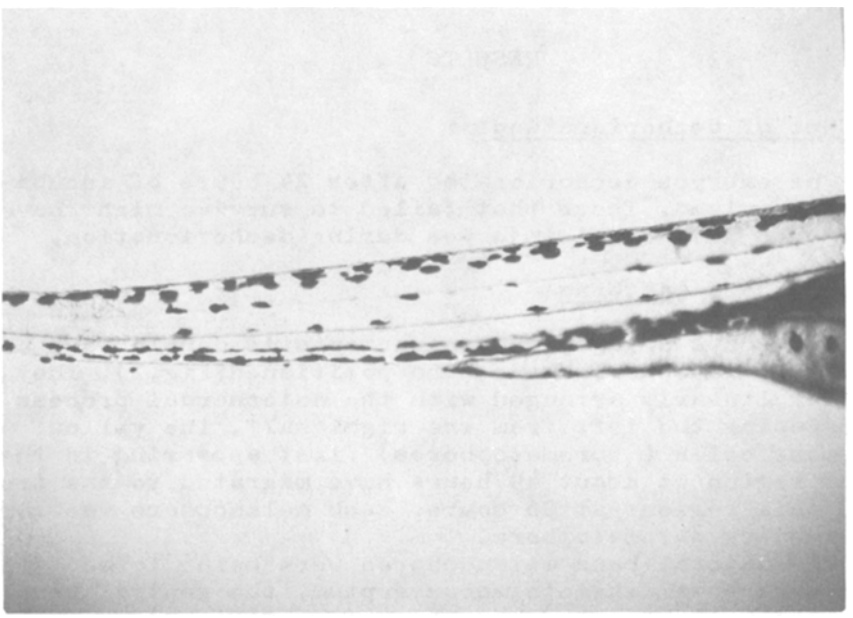

Fig.2 . Lateral band melanophores being formed. Mag. x 120.

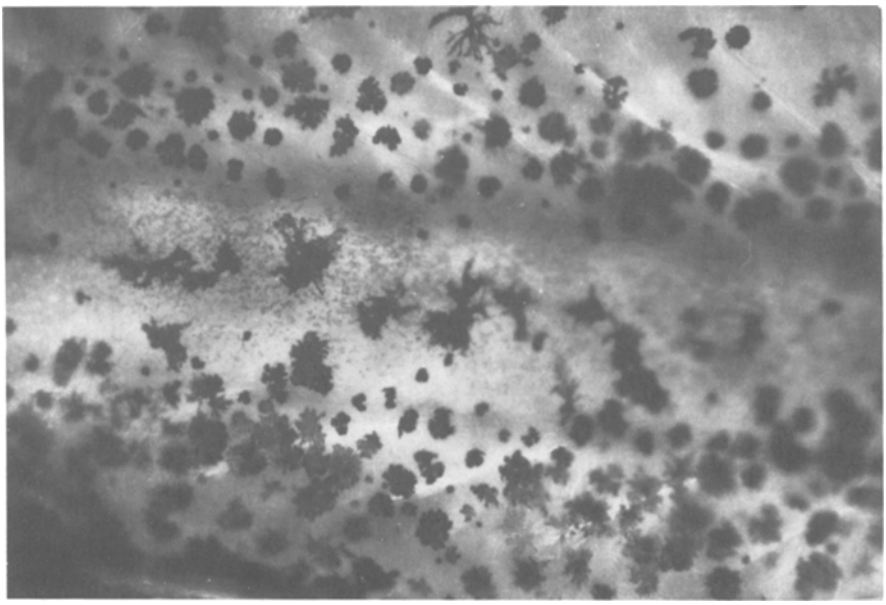

Fig.3. Lateral band melanophores of larva exposed to 50 ppb lead,22 days old. The 5 alternating strips were destroyed. Mag. x 125.

Fig. 4. Clumped and randomised melanophores from larva 22 days old from 50 ppb lead exposition.Mag.x 125.

Effect of incubation in lead media

Zebrafish embryos incubated for 24 hours in 72 ppb lead concentration before transfer to lead free media developed normal melanophore patterns,dorsally,laterally and on the yolk sac region. The fleeting horizontal skeletogenous melanocytes between the furrow delineating epaxial from hypaxial musculature have started to be positioned. The origin and migratory pathways of these melanophores will be published elsewhere. But the pigment pattern formation of dechorionated embryos exposed for 24 hours in 72 ppb lead concentration differed in the lateral bands melanophores. The larvae photographed at 10 days of age lacked ventral and horizontal skeletogenous bands quantitatively.The chromatophores which usually segregate adjacent melanophores from their neighbours were not many.

The larvae exposed to 50 ppb of lead before and after dechorionation never developed proper pigment patterns after 22 days. All the 5 alternating blue-black melanophores and silvery -yellow strips aligned parallel to the long axis of the body were destroyed(Fig.3). The areas where the yellow bands normally appear were badly diluted grey/white colours.Black pigments have invaded these regions. Black pigments not only lacked linearlity but also were clumped together(Fig.4). The linear bands with black strips alternating with yellow strips were destroyed at the tail region also. Clumped melanophores were on the left and right part of the tail region at the anterior end of the caudal fins (Fig.5).

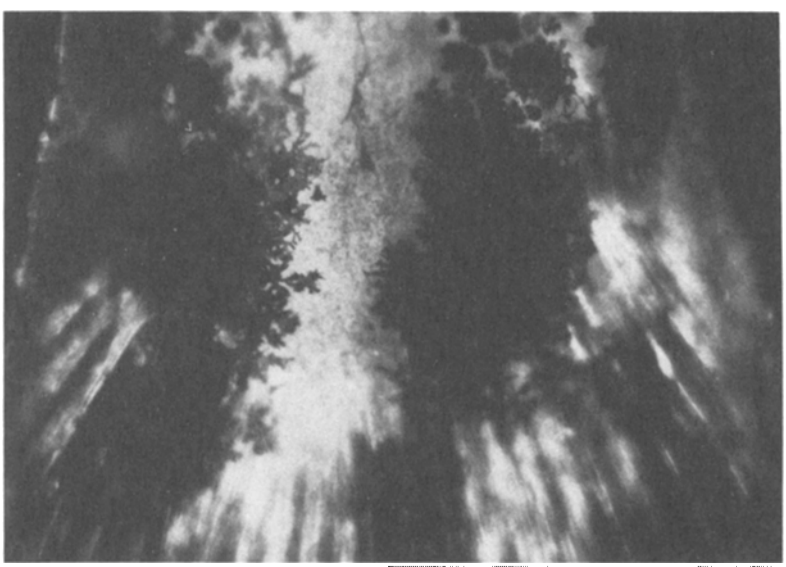

Fig.5. Tail end with clumped melanophores.Mag.x 125.

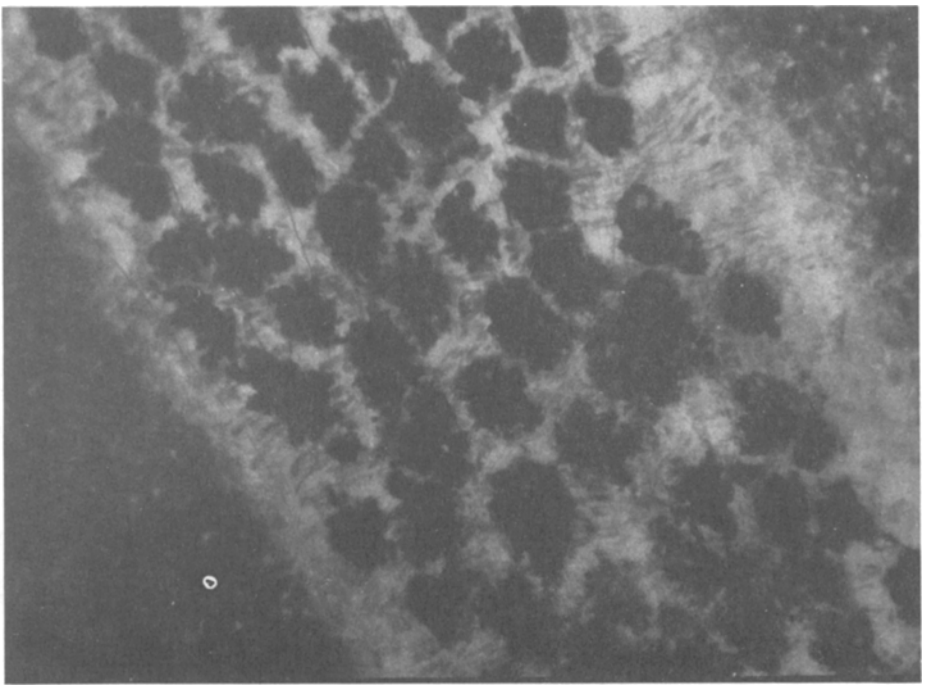

Fig. 6. Ventral band melanophores in adult zebrafish. Mag. x 125.

The linear arrangement of the ventral band melanophores in an adult zebrafish is shown (Fig.6). Melanophores were separated by yellow chromatophores which appear white.

DISCUSSION

Irreversible damage to the pigment pattern formation in dechorionated zebrafish embryos has been caused by lead. Lead appeared not to interfer with the random wandering of early melanocytes. Also, the amoeboid-like spread of melanophores was not affected. Lead affected the waves of chromatophores migration which originated from only the head region and spread to the trunk and tail regions. It would appear that the yellow and black pigment cells have different ontogenetic origin. This difference in ontogeny could explain why chromatophores have special property of limiting the random wandering of melanophores. Lead intoxicated larvae lacked enough quantities of chromatophores which normal-

ly limit the migratory activities of melanophores. Lead not only destroyed the quantities of chromatophores,but also appeared to have limited their regulatory properties.

The cellular-tissue antagonism of GOODRICH et al. (1954) and the"exclusion principle" of MILOS and DINGLE (1978) failed to explain the linearlity of pigment pattern formation. Lead not only destroyed this pattern but also the melanophores were clumped together.The shell membranes appeared to offer some protective action to the embryos in lead intoxication. All the embryos incubated without shell membranes in lead suffered irreversible damage in pigment pattern formation.

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