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Effect of Phenobarbital and Hexobarbital Treatment on *Lepidocephalichthys thermalis*, a Fresh Water Fish

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BRODIE and MAICKEL (1962) have reported the absence of TPNH linked mixed function oxidase activity in fish liver microsomes. However, recently KACHOLE et al. (1977) observed the protective effect of phenobarbital and hexobarbital pretreatment on endrin toxicity in four fresh water fishes, indicating the possibility of presence of the system in fishes. Very recently, the presence of cytochrome P 450 and drug metabolizing activity in fish liver microsomes has been reported (STANTON and KHAN 1975, SCALES and YARBROUGH 1975, ASHOKAS et al. 1976, LIDMAN et al. 1976). Data pertaining to the effect of phenobarbital and hexobarbital on the glycogen uptake of the fish liver lipids, glycogen and lipase activity and brain cholinesterase activity is still inadequate. The present studies were therefore planned to investigate these parameters in Lepidocephalichthys thermalis.

MATERIALS AND METHODS

The species L. thermalis was chosen for present study as it showed higher resistance and response to pretreatment induced protection against endrin (KACHOLE et al. 1977). The fishes were collected from River Kham, 3 km from the laboratory and were acclimatized to laboratory conditions of 12L 12D rhythm. The containers were wide mouth gallon jars and had fresh water changed twice a day. Temperature of the water was within the range of 20-24 °C around the day which was always a degree or two lower than the extremes outside the laboratory and no attempt was made to control it.

Treatment: The fishes measuring 4-6 cm in length were divided into the following three groups of about 150 fishes in each group.

Group A	Control
Group B	Phenobarbital treated
Group C	Hexobarbital treated

Phenobarbital sodium and hexobarbital sodium concentrates in distilled water were added to the surrounding water of fishes in Group B and C respectively. The final concentration of the drugs was 1 ppm. The treatment was carried out for seven days and on the eighth day water in the aquaria was kept devoid of drugs to allow depletion of the compounds or their metabolites (if any) inside the fish body. On the ninth day the fishes were used for different determinations.

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Oxygen uptake: Apparatus used for determination of oxygen uptake was similar to that used by GALTSOFF and WHIPPLE (1930) and the procedure used was as reported by MANE (1972). The determinations were carried out in duplicate using 5-7 fishes per estimation.

Liver glycogen, lipid and protein: Fishes from each group were taken out and killed by cold shock treatment. Livers were dissected out, blotted dry and weighed. Glycogen content was determined from few pieces of the livers following the procedure of MONTGOMERY (1957). Similarly, lipids were extracted from another portion of liver by the procedure of DOLE (1956). The remaining liver was homogenized in a motor driven Teflon pestle glass homogenizer and the protein content of the 9,000 g supernatant fraction was determined by biuret method (GORNALL et al. 1949) using bovine serum albumin as the standard protein.

Liver lipase: A part of the liver homogenate was used for determining lipase activity as described by PAWAR and TIDWELL (1968) in a buffered (Tris-HCl) media containing 5 % olive oil as substrate and 5 % albumin at pH 8.5. The aliquot was extracted with Dole's extraction mixture and titrated with 0.02 M NaOH electrometrically to pH 10.0 with nitrogen streaming. The amount of fatty acid liberated per hour per gram tissue was calculated using palmitic acid as the standard.

Brain acetylcholinesterase: Brains dissected out from the animals killed by cold stress were homogenized in 0.25 M icecold sucrose using a Teflon pestle glass homogenizer. The homogenate was immediately used to determine acetylcholinesterase activity using acetyl choline chloride as substrate in phosphate buffer at pH 7.2. The activity was determined from the unhydrolysed substrate concentration.

RESULTS AND DISCUSSION

Figure 1 summarizes the changes in various parameters due to phenobarbital and hexobarbital treatment in the fresh water fish L. thermalis. Relative liver weights and liver 9,000 g protein levels were found to be concomitantly decreased. Body weight, oxygen uptake and liver lipase activity did not show any considerable differences in treated and untreated fishes. Brain cholinesterase activity was found to be decreased in fishes treated with phenobarbital and hexobarbital.

The decrease in glycogen content parallel with the increase in 9,000 g protein and gain in liver weight suggests its consumption as energy during liver growth and increase in endoplasmic reticulum. The glycogen might also have been consumed for reducing equivalents during the detoxication of the drugs administered.

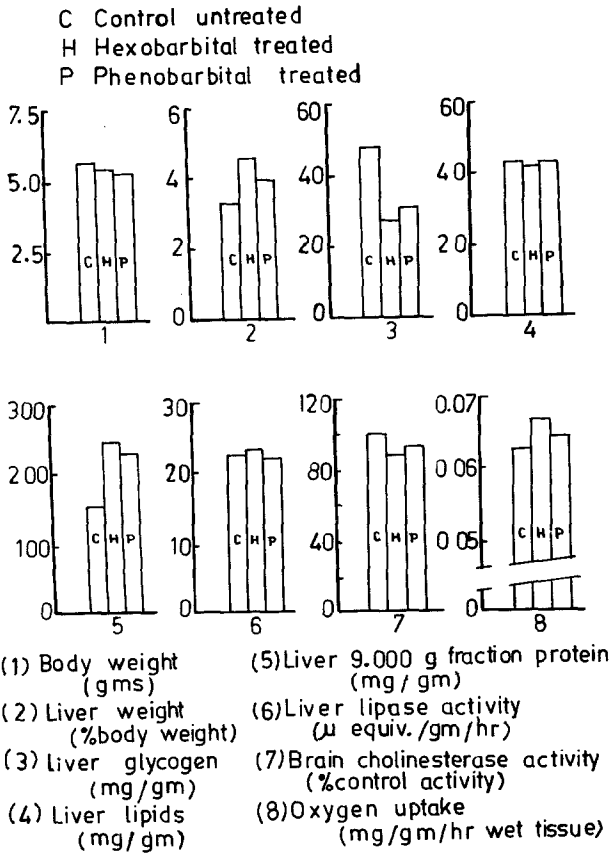

Fig. 1. Effect of phenobarbital and hexobarbital on L. thermalis.

The possibility of protection observed in earlier study (KACHOLE et al. 1977) is further supported by the present observations. The significantly changing parameter, 9,000 g supernatant fraction protein level is an indicative of increased endoplasmic reticulum. Higher microsomal detoxication due to increased drug metabolizing protein can be an influencing factor in protection. Recently, the presence of cytochrome P 450 and drug metabolizing activity in fish liver microsomes has been reported by several workers. The induction of this system appears to be the major factor affording protection by increased detoxication, a fully documented phenomenon in mammals and aves.

The possibility of protection due to altered mobilization of lipids effecting changes in compartmentalization of liposoluble substances does not seem to be valid in this case as there is no change in total liver lipid as well as liver lipase activity seen in treated fishes.

Slightly low cholinesterase values in treated fishes might be due to the presence of small amounts of the barbiturates or their metabolites at the 'site'. The possibility of CNS protection by masking effect of the barbiturates by binding reversibly at the 'site' cannot be ruled out.

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