PHYSIOLOGICAL EFFECT OF MALATHION ON THE BRAIN OF *SAROTHERODON MOSSAMBICUS*

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THE sublethal effect of malathion on the metabolism of phospholipids in brain tissue, with which cerebral activity is quantitatively linked, was studied. Malathion toxicity depends on physicochemical properties, such as lipid solubility, which alters the permeability of the lipoprotein cell membrane of neurons and thus affects bioelectrical stimulation.

Sprague[1] considered the biochemical effects of pollution as basic. These can then be related to the efficiency of tissues and organs. According to Mason[2], sublethal effects may be observed at physiological, biochemical and behavioural levels. Investigation of the sublethal effects of malathion on brain physiology of *Sarotherodon mossambicus* should provide useful information.

In the present study, synthetic, biodegradable malathion (50 EC, manufactured by Bangalore Pesticide Ltd), used in agriculture and malaria control operations in the area, was chosen and bioassay tests were conducted. *S. mossambicus* (Peters) of average size 10 ± 1 cm was used. The fish, brought from local ponds, were acclimated in aquaria containing tap-water (pH 7.1). The medium was maintained at 85–100% oxygen saturation.

The static bioassay procedure outlined by Doudoroff *et al.*[3], with some modifications with regard to the laboratory techniques, was employed. Tests were conducted at room temperature, 29 ± 1°C. The specific period of exposure was 96 h. For carrying out the 10-day exposure tests, a batch of 10 fish was kept in 0.01 ml/l EC, which is two-thirds of the 96-h LC_{50} value. Another lot of 10 fish was kept in tap-water as control. The fish (treated and controls) were sacrificed after 5 and 10 days' exposure and the brains dissected out for colorimetric analysis.

The physical reactions of the fish to sublethal concentration of malathion are manifested in erratic swimming, convulsions, commencing with rapid, jerky movements of the body and fins, insensitivity to external stimuli, and weakening of respiration.

According to Corbett[4] the site of action of organophosphates is the enzyme acetylcholinesterase, which hydrolyses the synaptic transmitter acetylcholine. In the presence of an organosphosphate inhibitor, the enzyme is phosphorylated at its anion and esterase centres and cannot react with acetylcholine[5]. The likely consequence of acetylcholinesterase inhibition is the accumulation of acetylcholine, which is likely to cause prolonged excitatory postsynaptic potential. It might first lead to stimulation and later cause a total block in the cholinergic system. It might be due to this that fish initially show hyperactivity, followed by convulsive and uncoordinated movements, ending in paralysis. Table 1 shows that phospholipid content of brain tissue decreased in exposed fish while in controls it increased. The reduction was significant after 10 days of exposure.

Table 1 *Total phospholipid (µg/mg wet wt) in brain tissue of control and malathion-exposed S. mossambicus*

	5 Days	10 Days
Control	8.65 ± 0.24	9.31 ± 0.85
Malathion-exposed	7.79 ± 0.62	6.94 ± 0.27

Each value is mean ± SD of 5 individual observations.

Various workers[6–9] have reported that synthesis of lipids and proteins in brain cells is probably dependent on stimulation of neural tissue. Malathion being fat-soluble[4] might be binding to the nerve cell membrane. Its lipid solubility may be an active factor in altering the membrane permeability of neurons for the conduction of Na^+ and K^+ ions and thus interfering with bioelectrical stimulation. Grundfest[10] found that the bioelectric phenomenon results almost entirely from the permselective properties of the cell membrane, because, due to increased conductance, resistance decreases.

The reduction in phospholipids would retard the physiological activity of the central nervous system. Ramachandran *et al.*[11] observed that phospholipids play a vital role as constituents of neuron cell membrane and in regulating membrane permeability upon which bioelectrical stimulation depends. Phospholipids are also present in the myelin sheath of nerve cells and in electron transport particles. Therefore reduction of phospholipid synthesis in neural tissue might further inhibit neurotransmission. Balasundaram[12] observed that inhibition in neurotransmission can be taken as an indicator of dysfunction in other organ systems whose integration in metabolism is vital for survival. Thus the weakening of respiration might also be due to neurotransmitter failure.

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ACCUMULATION OF PROLINE AND POLY-β-HYDROXYBUTYRATE IN HIGH TEMPERATURE-RESISTANT STRAINS OF *AZOSPIRILLUM*

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WE have recently described the occurrence of high temperature-resistant strains (HTR) of the nitrogen-fixing associative symbiotic bacterium, *Azospirillum*[1,2], and suggested their use as biofertilizer for summer crops. In the tropics high soil temperature appears to limit biological nitrogen fixation (often soil temperature exceeds 50°C). Although the mechanism of thermophilism in bacteria has been fairly well understood[3], no information is available for *Azospirillum*, which is a typical mesophilic bacterium. Proline and poly-β-hydroxybutyrate (PBHP) have been implicated in response to temperature and moisture stresses in organisms[4,5]. We are reporting here the possible mechanism of high-temperature resistance in *Azospirillum*.

Of fifteen HTR strains from 70 isolates of *Azospirillum*, AZ.Ht.1 and AZ.Ht.2 were chosen for the study as these isolates registered fairly high nitrogen-fixing potential. A standard mesophilic strain, *A. brasilense* Sp. 7(ATCC 29145) (supplied by Dr J. Dobereiner, Brazil), was also included in the study for comparison.

To determine PBHB, the method of Zevenhuizen[6] was followed. *Azospirillum* strains were grown in 100 ml quantities of yeast extract glucose broth in 250 ml Erlenmeyer flasks at 30 and 50°C over a temperature-controlled environmental shaker. After five and ten days of incubation cells were harvested by centrifugation at 10,000 *g* for 15 min at 4°C. The cells were suspended in sterile water and one ml of the cell suspension was digested in one ml of 2 N hydrochloric acid at 100°C for 2 h in a steam chamber. After cooling, the digest was extracted with 2.5 ml of chloroform. Samples, containing lipids, were transferred to test tubes after evaporating the chloroform over a water bath. Five ml of 96% sulphuric acid was added and the samples were heated at 100°C for ten min in a steam jacket. The absorbance of the clear solution was recorded at 235 nm in a double-beam spectrophotometer. The PBHB content was calculated from the formula

Extinction coefficient $E_{235} = 0.35$ per 100 μg of PBHB.

To determine proline, the cells were similarly grown in yeast extract glucose broth at 30 and 50°C as described earlier and the procedure of Bates *et al.*[7] was followed for the determination. Colour development was achieved by the addition of acid–ninhydrin mixture (a mixture of 1.25 g of ninhydrin dissolved in 30 ml of glacial acetic acid and 20 ml of 6 M phosphoric acid).

The results are presented in tables 1 and 2. PBHB granules in *Azospirillum* are considered to be a storehouse of energy-rich material usable by the

Table 1 *Poly-β-hydroxybutyrate in Azospirillum isolates*

	5 Days' growth		10 Days' growth	
Isolate	30°C	50°C	30°C	50°C
AZ.Ht.1	0.387	1.908	0.990	3.085
AZ.Ht.2	0.789	2.163	1.209	5.835
Sp.7	0.859	0.834	0.886	0.709
Mean for 15 HTR isolates	0.944	1.654	1.107	3.421

PBHB in mg per g dry weight of cell. Each value is mean of three determinations.