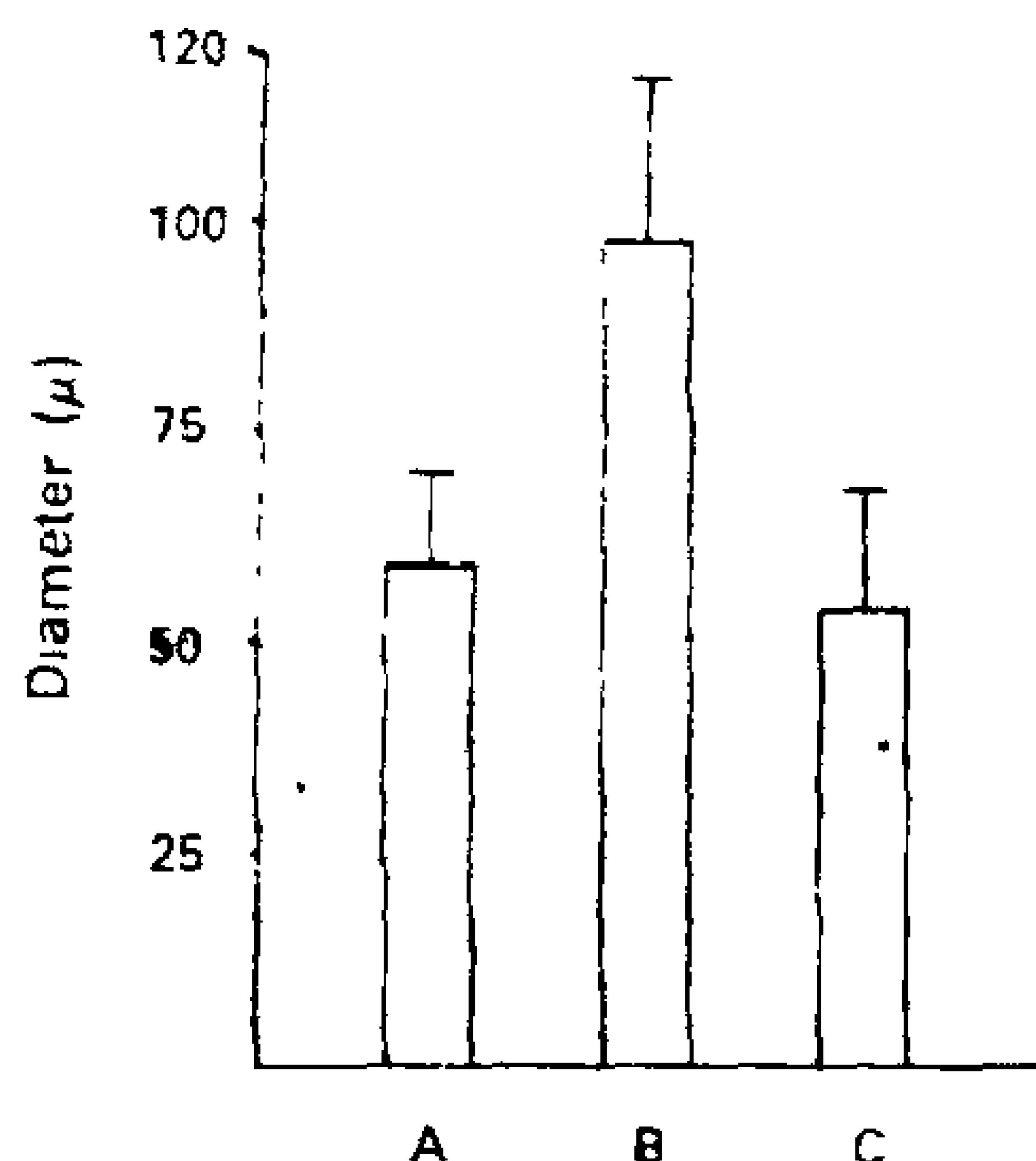

Figure 5A-C. Haemocyte diameter of *Culex quinquefasciatus* mosquitoes. **A.** Normal virgin, **B.** mf positive blood fed (2nd day), and **C.** Control blood fed (2nd day). Vertical bars represent S. D.

circular cells in the infected sample clearly showed proliferation. Figure 5 shows that there is only marginal or insignificant difference between virgin mosquito and control blood fed samples, while distinct differences were observed between control and infected blood fed samples. The difference was proportional to the mf count.

Blood feeding results in the formation of egg clusters. However control blood does not appear to cause any proliferation of the haemocytes. But infective blood causes proliferation. Thus the results clearly show that infection leads to proliferation of haemocytes. This differential effect in the proliferation by the control and infected blood fed is very interesting. It is possible that proliferation is a response on the part of the vector against the development of microfilariae to infective larvae through different moulting stages. The nature of this is currently being worked out.

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EFFECT OF IODOPHOR TREATMENT ON THE HATCHING OF *ARTEMIA* CYSTS

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THE dormant eggs or 'cysts' of the brine shrimp *Artemia* form an important source of live feed for a variety of finfishes and shellfishes. Earlier studies have either reported contamination of the cysts with bacterial/fungal spores[1,2] or heavy bacterial load in canned cysts[3,4]. Thus, there is a possibility of predator organisms getting infected. It is therefore necessary to disinfect *Artemia* cysts before their use. Use of 'Antiformin'[5], hypochlorite solution[6], decapsulation technique[7] and antibiotics[4,8] has been found to be effective in suppressing bacterial growth and increased hatchability of cysts. Iodophor (POLYSAN—Polypharm Pvt. Ltd, Bombay) is a non-selective germicide with a surface-active agent (alkyl phenoxy polyoxyethylene ethanol) and provides a minimum of 1.6% titratable iodine. Iodophor is known to be an effective disinfectant in aquaculture practices[9,10]. The present work was undertaken to study the effect of iodophor treatment at different concentrations and exposure time on the hatchability of *Artemia* cysts and survival of associated bacteria.

Iodophor solutions with different concentrations of active ingredient were prepared in sterile distilled water containing 0.05% $NaHCO_3$. To weighed amounts of *Artemia* cysts (5 mg), different concentrations of iodophor (25, 50, 75 and 150 ppm) were added and exposed for 30, 60, 120 and 300 s. At the

end of the exposure time, the cysts were washed thoroughly with sterile seawater. From the washed samples, a loopful of cysts were inoculated in duplicate into tubes containing seawater complete broth (SWC). SWC was prepared with the following composition: 750 ml aged seawater, 250 ml tap-water, 5 g proteose peptone and 3 g of yeast extract/l. Similarly, cysts from all the treatments were inoculated into nutrient broth (NB) containing 20% NaCl to check whether any of the extremely halophilic bacteria survive these treatments. All tubes were incubated at room temperature ($28 \pm 2°C$) for 10 days and observed for microbial growth. The remaining cysts were incubated in natural seawater (salinity 35×10^{-3}; temp. $-28 \pm 1°C$) and hatching experiments were carried out[11]. For each concentration and exposure time, three replicates were maintained. At the end of 24 h, 1 ml samples were drawn from each replicate. The hatched nauplii were killed by adding 1–2 drops of Lugol's solution and counted.

Iodophor tested at different concentrations for its bacteriostatic/bactericidal activity and the per cent of hatching of *Artemia* cysts are given in table 1. Presence of bacteria was detected in the samples exposed for 30 and 60 s at 25 ppm and for 30 s at 50 ppm. In all the remaining samples including those with NB containing 20% NaCl, bacterial growth was not detected during the 10-day observation period. From the results it is evident that the cyst-associated bacteria and spores did not survive in the iodophor concentrations of 50 ppm and above exposed for more than 30 s. Iodophor also appears to be effective on the extremely halophilic bacteria.

Table 1 *Bactericidal activity of iodophor and hatching percentage of Artemia cyst at different concentrations and exposure time*

Exposure time (s)		Iodophor concentration (ppm) 0	25	50	75	150
30	SWC	+	+	+	–	–
	NB	+	–	–	–	–
	Hatching	36	19.74	24.84	69.97	63.11
60	SWC	+	+	–	–	–
	NB	+	–	+	–	–
	Hatching	36	26.22	39.47	63.36	62.82
120	SWC	+	–	–	–	–
	NB	+	–	–	–	–
	Hatching	36	31.02	44.22	65.60	65.49
300	SWC	+	–	–	–	–
	NB	+	–	–	–	–
	Hatching	36	35.82	56.79	70.83	69.89
	Average		28.20	41.33	67.44	65.33

SWC, Seawater complete broth; NB, Nutrient broth with 20% NaCl; +, Presence of bacterial growth; –, No bacterial growth.

Hatching experiments showed that at 25 ppm, hatching was low (av: 28.2) and increased to 41.33% at 50 ppm concentration. Maximum hatching was recorded with the cysts exposed to 75 and 150 ppm (av: 66.4%) respectively. Cysts taken from the same batch before the treatment with iodophor when incubated in natural seawater resulted in 36% hatchability. Hatching percentage increased in cysts exposed to iodophor concentrations of 50 ppm and above. The results when subjected to two-way analysis of variance indicated that both exposure time and iodophor concentrations significantly enhanced hatching. This may probably be due to the suppression of bacterial growth as well as decapsulation effect of iodophor at higher concentrations. It is known that hatchability of *Artemia* cysts increases significantly after decapsulation[12].

For the use of *Artemia* cysts on a large scale, Coleman *et al*[4] suggested use of UV light, chlorination or washings to suppress bacterial growth. In unprocessed wet cysts development of moulds such as *Penicillium* spp. and *Aspergillus* spp. has been detected and application of calcium hypochlorite and treatment with 2% formalin before drying has been suggested[13]. Iodophors are non-selective and have low level of toxicity to fish, hence, could be safely used in aquaculture. It has been shown that some selected strains of bacteria known to cause shell diseases in shrimps and fin-rot in fishes respond well to iodophor treatment[10]. Methods to overcome microbes associated with *Artemia* cysts ensure healthy live feed for cultivable organisms and in this context iodophor offers good scope.

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CYTOGENETIC EFFECTS OF MALATHION ON BUFFALO BLOOD CULTURES

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THE problem of body pests and external parasites in buffalo and other livestock is acute in India and several types of organophosphorus and organochlorine pesticides are used. Direct applications of these compounds on animals and their dwellings, lead to their entry into the body. Malathion and other related compounds replace in part the chlorinated hydrocarbons in use, as these are degraded relatively quickly. They do, however, have acute toxic effects that may be fatal.

Pesticides, besides their toxic effects, have also been assumed to cause mutagenic and carcinogenic effects. Petropolis and Kamra[1] observed a dose-dependent increase in mitotic and DNA labelling indices in peripheral lymphocytes exposed to organophosphorus pesticides and chromosomal breaks were found even at 10 μg/ml dose. Several types of structural and numerical chromosomal anomalies were observed in peripheral blood cultures treated with malathion and other related organophosphates[2-4]. The cytogenetic effects of malathion on peripheral blood cultures of Murrah buffalo have been described in this paper.

Commercial grade malathion 50% (O,O-dimethyl phosphodithioate of diethyl mercaptic succinate) was obtained from the Pesticide India Ltd and dissolved in Hank's basal salt solution to get concentrations of 125, 250, 375 and 500 μg/ml. Culture medium TC 199 (Bios Bombay, India) was reconstituted and supplemented with 1-Glutamine, Phytohemagglutinin (PHA Sigma, USA), sodium benzyl penicillin, streptomycin and sterile cattle serum. The reconstituted medium was filtered through millipore filters (0.2 μ) into sterile culture vials (5 ml in each). Forty culture vials were divided into five groups and 0.5 ml whole blood was added to each culture. They were incubated at 37°C for 72 h and 0.1 ml of malathion solution was added at the above mentioned concentrations to each vial of treatment groups I, II, III and IV respectively, 24 h before harvesting the cultures. Group V cultures (negative control) were not treated with pesticide.

The chromosomal preparations were made using colchicine (250 μg/ml), hypotonic (0.075 M KCl), acetic : methanol (1 : 3) fixation and air drying protocol[5]. The slides were stained in 2% Giemsa (phosphate buffer, pH 6.8) for 30 min and mounted in DPX.

Under the influence of alkylation of chemicals including the pesticides, both normally dividing (bone marrow) and neoplastic (cell cycle induced tissues) tissues, suffer a reduction in mitotic activity of cells probably due to delay in the synthetic

Table 1 *Mitotic index of peripheral blood lymphocytes treated with malathion*

Dose level (μg/ml)	Cells scored	Blast cells (%)	Cells at metaphase (%)	Mitotic index
12.5	2250	16.98	0.98	17.96
25.0	2392	16 47	0 88	17.35
37.5	2169	16.50	0 80	17.30
50 0	2039	15 60	0.70	16 30*
Control	2192	17.29	1.00	18.09

*Significant ($P < 0.05$).