HCM and to develop preventive measures or therapies suitable to cope with functional impairments and risks associated with HCM.

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Activation of the human serum complement cascade by insecticides

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In view of the importance of complement system in the initiation, regulation and end effects of immune responses and understanding of immune system as one of the targets for the toxic effects of insecticides, we tested benzene hexachloride (BHC) and malathion for their effects on human serum complement using C3 activation as a test parameter. The methodology used was cross-immunoelectrophoresis. Both the insecticides used at different doses (1 to 100 ppm) activated C3, the third component of the complement. This is a finding pertaining to interaction of these insecticides with the immune system. The activation was through alternative pathway. This was confirmed by blocking the classical pathway by EDTA and EGTA. The validity of this strategy was confirmed by subjecting serum to similar treatments using aggregated IgG and zymosan, the activators of classical and alternative pathways, respectively. This study also suggests that C3 activation may serve as an effective test parameter to assess immunointeractions of insecticides.

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INSECTICIDE poisoning is an important cause of worldwide morbidity and mortality. Ninety-five per cent of fatal insecticide poisoning occurs in developing countries[1]. Among the third-world countries, India is the biggest consumer of insecticides[2]. The residual nature of organochlorine and neurotoxicity of organophosphate is well known. The toxic effects in the biological system are due to homicidal, accidental and chronic exposure to humans[3,4]. Toxicological research has indicated that the immune system is a potential 'target organ' for toxic damage by insecticides[5]. There are recommendations for a range of immune function tests, but if segregated individually, they may not lead to any concrete evidence. Many of these chemicals appear to cause immunosuppression[6]. Further studies are required to understand the interaction of insecticides with the immune system and to narrow down the test parameter[7].

Complement proteins are increasingly being recognized as the key components of the immune response network[8–10]. This system consists of proteolytic zymogens and regulatory proteins. The zymogens get activated as a cascade, either through calcium and antibody-dependent classical pathway or through antibody-independent and Mg^{2+}-dependent alternative pathway. C3 is central to the system, which initiates the alternative pathway and is the merging point for the classical and alternative pathways. On activation, chemotactic anaphylatoxins C3a and C5a along with opsonins C3b and C4b are generated. These peptides may bring injury to self, if generated in excessive

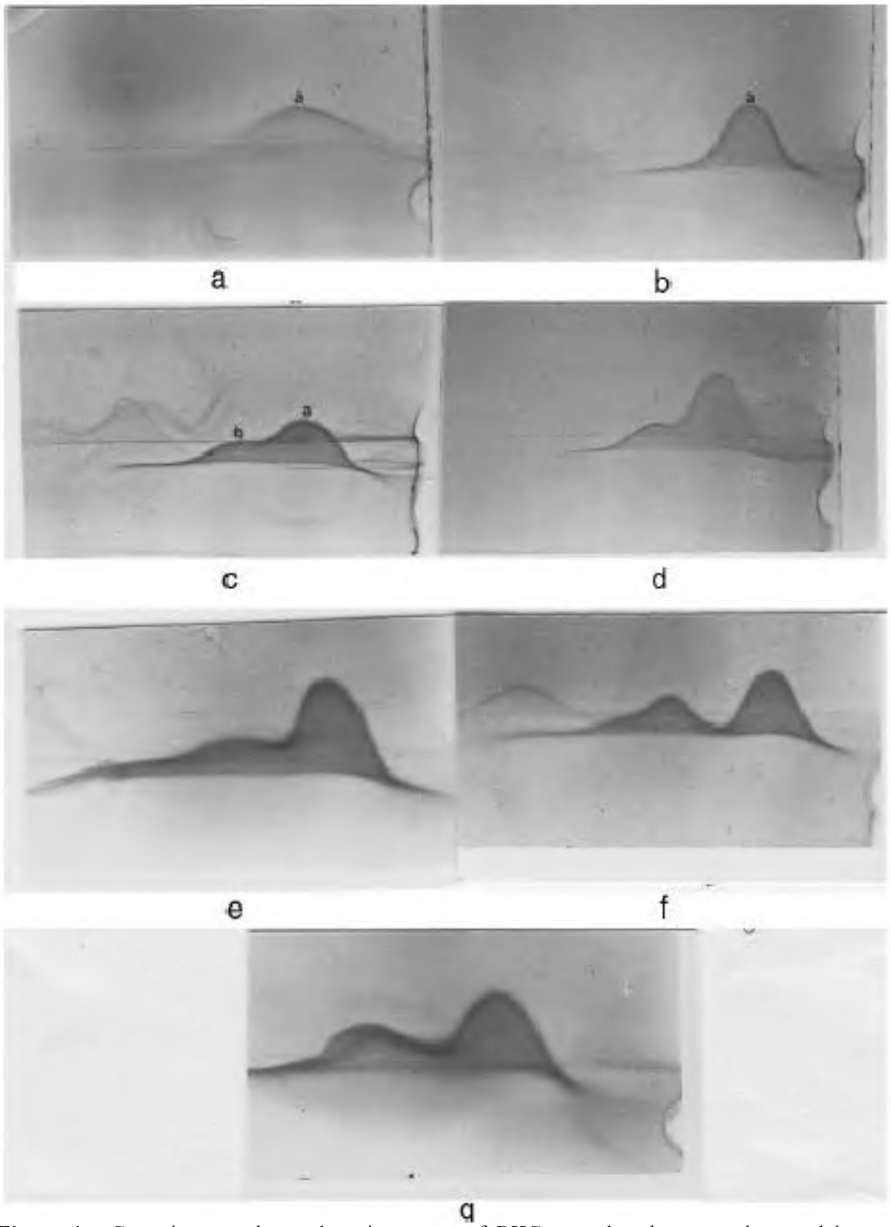

Figure 1. Cross-immunoelectrophoretic pattern of BHC-treated and untreated normal human serum (NHS). Peak 'a' represents C3 and peak 'b' C3b. ***a***, Pure C3; ***b***, NHS untreated; ***c***, NHS + 1 ppm BHC; ***d***, NHS + 5 ppm BHC; ***e***, NHS + 10 ppm BHC; ***f***, NHS + 50 ppm BHC; and ***g***, NHS + 100 ppm BHC.

amounts due to inappropriate activation. We are reporting our preliminary findings on the interaction of benzene hexachloride (BHC) and malathion with human serum complement using third component of the system (C3) activation as our test parameter.

100 µl of normal human serum samples was incubated with different doses of BHC and malathion at 37°C. The reaction was stopped by the addition of 0.01 M ethylene diamine tetra acetic acid (EDTA) after 30 min. All treated samples were stored at –20°C and were analysed within 15 days of storage. C3 activation was monitored by cross-immunoelectrophoresis of insecticide-treated or untreated sera using anti-human C3 raised in rabbit[11]. Experiments were carried out in duplicate for each dose of insecticides. Three different serum samples were studied for each dose to confirm the findings. BHC was a gift from Industrial Toxicology Research Centre, Lucknow. Malathion was procured from Indian Agricultural Research Institute, New Delhi. Both insecticides were of technical grade. Anti-human C3 antibodies were raised in rabbit in our laboratory by using zymosan (Sigma) with incomplete Freund's adjuvant (Sigma). Aggregated IgG (10–20) was prepared in our laboratory[12].

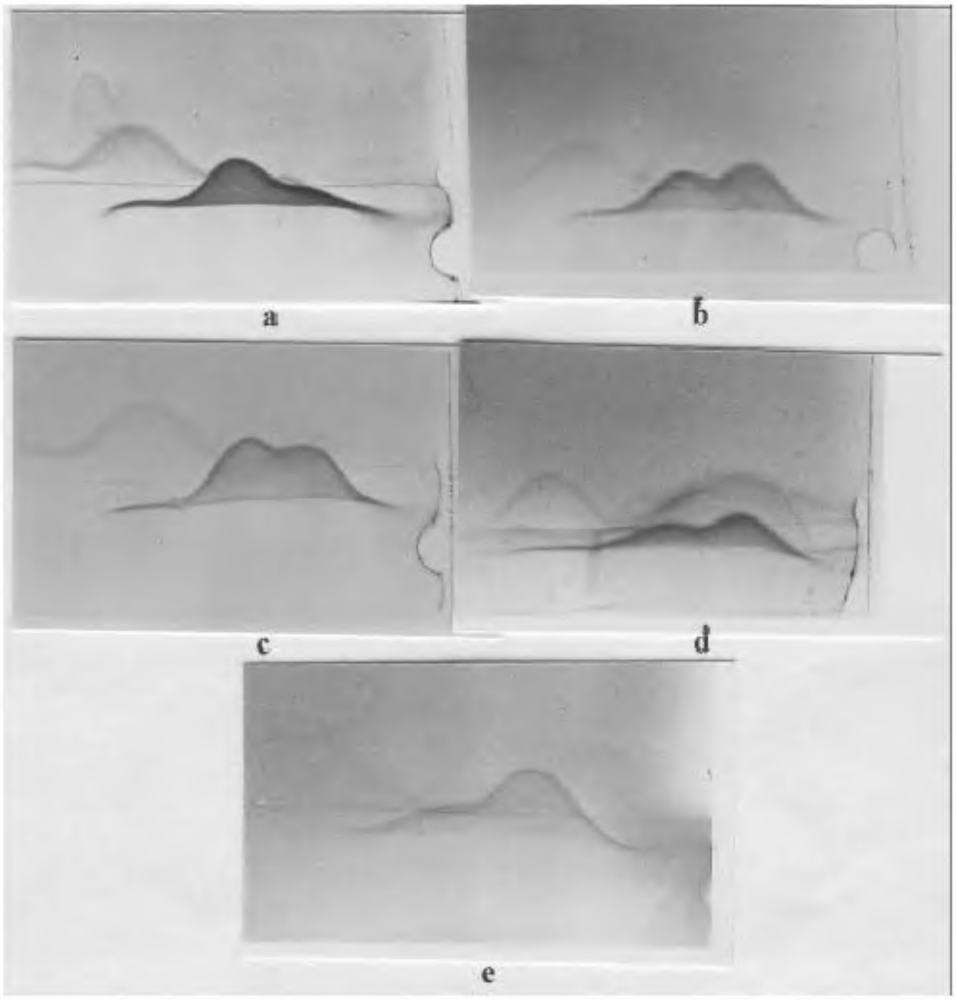

Figure 2. Cross-immunoelectrophoretic pattern of NHS treated with malathion. ***a***, NHS + 1 ppm malathion; ***b***, NHS + 5 ppm malathion; ***c***, NHS + 10 ppm malathion; ***d***, NHS + 50 ppm malathion; and ***e***, NHS + 100 ppm malathion.

The mode of activation was studied by incubating serum samples with 0.01 M ethylene glycol tetra acetic acid (EGTA) or EDTA to chelate Ca^{2+} or Mg^{2+} and Ca^{2+}, respectively, prior to insecticide treatment. Serum samples were also treated with aggregated IgG or zymosan to serve as positive control.

BHC and malathion at different doses (1–100 ppm) activated the complement cascade. This was evident from the appearance of two different peaks on cross-immunoelectrophoresis (Figures 1 and 2). The peaks represented C3 and C3b[11]. Figure 3 *c* and *e* shows that the activation of C3 was maintained on calcium chelation by EGTA. This indicated that activation was not through classical pathway which is non-operative in absence of calcium. Figure 3 *d* and *f* shows that on chelation of calcium and magnesium from the system, the activation of C3 was abolished. This indicated that these insecticides were activating the complement cascade through alternative pathway which needs Mg^{2+}, but not Ca^{2+}. Figure 3 *a* and *b* shows the complement activation by positive controls, aggregated IgG and zymosan.

In essence, this report demonstrates that irrespective of their chemical nature, BHC, an organochlorine, and malathion, an organophosphate, activated the complement cascade through alternative pathway. The exact mechanism of interaction of these insecticides with the individual components of this system is yet to be elucidated. Earlier we reported similar findings on DDT and endosulphan[13]. These findings emphasize that complement is a potent target for the interaction of insecticides with the immune system. This notion is important in view of the fact that all the known complement activators like cobra venom[14], endotoxins[15], house dust mites[16] and immune complex are proven phlogistogens. We would also like to suggest C3

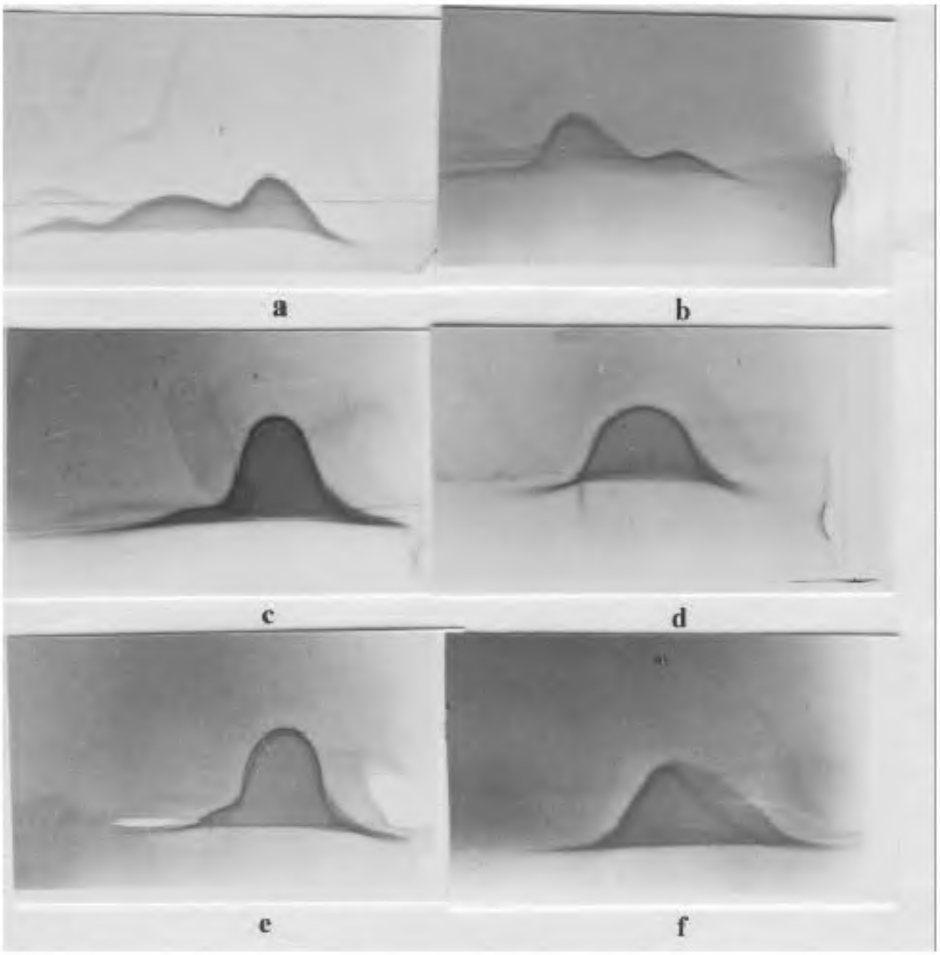

Figure 3. Cross-immunoelectrophoresis pattern of complement activation by positive controls and EDTA or EGTA treated NHS with insecticide. ***a***, NHS + aggregated IgG; ***b***, NHS + zymosan; ***c***, NHS + 0.01 M EGTA + 100 ppm BHC; ***d***, NHS + 0.01 EDTA + 100 ppm BHC; ***e***, NHS + 0.01 M EGTA + 100 ppm malathion; and ***f***, NHS + 0.01 M EDTA + 100 ppm malathion.

activation as a simple test parameter to screen and assess the interaction of insecticides and other xenobiotics with the complement system.

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