ACTIVATION OF SERUM COMPLEMENT BY ORGANOCHLORINE INSECTICIDES, DDT AND ENDOSULPHAN

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ABSTRACT

A baseline information is provided on the mode of interaction of DDT and endosulphan with serum complement. Both the insecticides activated C_3 (the third component of the complement system) to C_3b when incubated with normal human serum, C_3 activation occurred through alternative pathway as evidenced by the insignificant alteration of total haemolytic complement (CH_{50}) *in vitro*, maintenance of activation despite the removal of Ca^{2+} by ethylene glycol tetra acetic acid and blockade of activation due to the chelation of both Ca^{2+} and Mg^{2+} by ethylene diamine tetraacetic acid.

INTRODUCTION

RECENT developments on complement research have placed this group of serum proteins at the key position of immunopathology and immunoregulation[1-3]. Complement activation has been detected in association with many pathological conditions including several allergic manifestations[4-6]. In a variety of such situations, the etiology of complement activation remains unknown, especially when the activation occurs through antibody independent alternative pathway. At this juncture, it appears appropriate to study the interaction of environmental chemicals, particularly, the persistent ones with the complement system. This communication reports our findings on the mode of interaction of two chlorinated insecticides, DDT and endosulphan with complement system.

MATERIALS AND METHODS

DDT (1,1-bis-(*p*-chlorophenyl) 2,2,2-trichloroethane, technical grade and endosulphan [α,β-1,2,3,4,7,7-hexachlorobycyclo (2,2,1)-heptene (2)-bis-hydroxymethylene(5,6) sulphite) Thiodan, Hoechst] were procured from the Hindustan Insecticide Limited, India. Antisera to human C_3 were purchased from Immunodiagnostics, India.

Serum samples from normal human subjects were incubated with varying concentrations of DDT and endosulphan for 30 min at 37°C.

Activation of C_3 was monitored by crossed immunoelectrophoresis[7]. During the first dimension run, 5 μl of the insecticide treated and untreated serum samples were applied in the well, punched on a 1% agarose gel, coated on a microscopic slide (3 ml). Electrophoresis was run for 2 h at 200 V. Bromophenol blue was applied along with the sample (or in a parallel well) to detect 5 cm run. After the first dimension run, the gel was cut into two longitudinal slabs from the middle and the individual slab was transferred at the edge of another plate (5 × 7.5 cm) horizontally. The plate was coated with 5 ml of 1% agarose containing 2% anti C_3 and the second dimension run was performed at 100 V for 6 h in a perpendicular dimension. At the end of the electrophoresis, the gels were washed, dried and stained by coomassie brilliant blue. EDTA of 0.01 M concentration was added in gel and tank buffer to prevent C_3 activation during electrophoresis.

In separate experiments, serum samples were incubated for 30 min at 37°C with 10 mM ethylene glycol tetra acetic acid (EGTA) or ethylene diamine tetra acetic acid (EDTA) respectively to chelate Ca^{2+} or (Ca^{2+} and Mg^{2+}) both prior to insecticide treatment. Parallel controls remained untreated with DDT or endosulphan. C_3 activation was followed by crossed immunoelectrophoresis.

Total haemolytic complement in the treated and the untreated sera was determined[8] by estimating CH_{50} levels.

RESULTS

Effect of DDT and endosulphan on C_3 activation

Figures 1 and 2 represent the crossed immunoelectrophoretic pattern of insecticide treated and untreated normal human sera (NHS). Appearance of a higher C_3b peak served as the index of C_3 activation. As is evident from the figures, both the insecticides activated C_3 to C_3b at all the concentra-

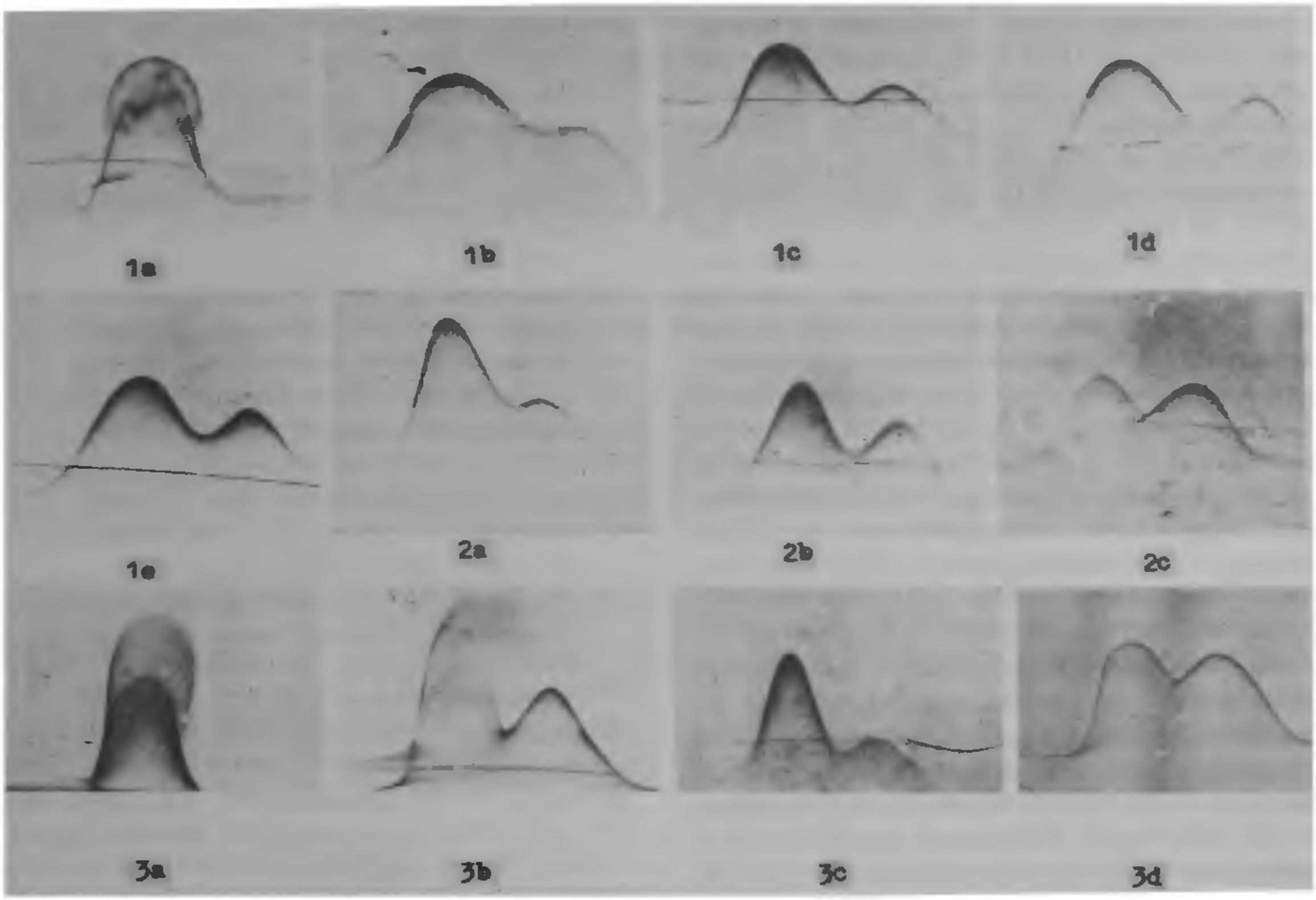

Figure 1a–e. **a.** Normal human serum; **b–e.** Serum samples treated with 20, 50, 100 & 500 μg/ml concentrations of DDT. **Figure 2a–c.** Serum samples treated with 0.1, 1 and 100 μg concentrations of endosulphan. **Figure 3a–d.** **a, b.** Serum samples treated with 500 μg/ml of DDT along with EDTA and EGTA respectively; **c, d.** Serum samples treated with 10 μg/ml concentrations of endosulphan along with EDTA and EGTA respectively.

tions tested. However, strict linearity could not be observed between the extent of activation and concentrations of DDT and endosulphan.

Mode of activation

Table 1 shows the CH_{50} levels in serum samples treated and untreated with DDT and endosulphan. None of these insecticides had any effect on CH_{50} levels which remained the same in controls and serum samples treated with various concentrations of these insecticides.

Table 1 *CH_{50} levels in DDT treated and untreated normal human serum*

DDT μg/ml NHS	CH_{50}	Endosulphan μg/ml NHS	CH_{50}
0	22	0	23
10	22	0.1	22
50	21	1.0	27
100	29	10.0	22

Figure 3 represents the effect of DDT and endosulphan on serum samples pretreated with EDTA or EGTA. DDT and endosulphan could not overcome the blockade of complement cascade caused by EDTA. However, pretreatment of serum with EGTA had no influence on the activation of C_3 by DDT and endosulphan.

DISCUSSION

The present investigation has proved the activation of complement cascade by DDT and endosulphan *in vitro*. The mode of activation was studied by blocking the complement activation by EDTA through the chelation of Ca^{2+} and Mg^{2+}, the essential factors for the operation of classical and alternative pathway respectively[9]. Both the insecticides failed to overcome the inhibition caused by EDTA. However, the selective blockade of classical pathway by EGTA did not have any effect on the C_3 activation induced by DDT and endosulphan. These

observations emphasize the participation of alternative pathway in DDT and endosulphan-induced activation of the cascade. The involvement of classical pathway was ruled out by estimating CH_{50} levels *in vitro* which serve as an index of complement activation through classical pathway[10]. Either of these insecticides did not produce any alteration in CH_{50} levels when incubated with normal human sera. Since these insecticides are likely to gain constant entry in the body through food chain series and other routes and can produce cumulative manifestations, they may play a significant role in many of the pathological situations associated with the antibody independent activation of the complement cascade. Such manifestations may range from immunodeficiency to allergic diseases[10,11]. Immunosuppressive effects of chlorinated insecticides including DDT and histamine release by DDT have earlier been documented[12,13]. Chang *et al*[14] reported a reduction in the Fc and complement receptor bearing leukocytes in the patients suffering from polychlorinated biphenyl poisoning. They speculated the low immunity to infection observed in these patients as the outcome of this impairment. The present investigation provides some additional clue to the mode of interaction of DDT and endosulphan with immune system.

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