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Pesticide Effects on the Immune Response and Metabolic Activity of Chicken Lymphocytes[1] (39595)

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The use of DDT (1,1,bis-[*p*-chlorophenyl]2,2,2-trichloroethane) and mirex (dodecachlorooctahydro-1,3,3,-metheno-2H-cyclobuta[*cd*]pentaline) have caused considerable discussion in recent years because of their potential effects on biological systems. As a result of this, prodigious amounts of research have been generated to study various aspects of physiological systems. Yet a paucity of information exists on the effects of pesticides on the immune response and relatively few publications have appeared over the years (1–5).

Glick (3) administered DDT and mirex during the neonatal period in order to determine their potential for disrupting bursal development and future antibody production, since chemical interference with bursa during neonatal development (6–11) will result in elimination or reduction in immunoglobulin G, antibody production, and maturation of plasma cells. Glick's (3) findings showed that these pesticides were not capable of initiating bursal involution during the early weeks of life. However, DDT treatment did cause a reduction in immunoglobulins G and M. This interesting observation was unexplainable on the basis of bursal response and directed us to other avenues of study.

In the present study, metabolic activity of lymphocytes from different lymphoid compartments and the immune response of chicks treated with DDT and mirex were evaluated. The splenic and thymic lymphocytes demonstrated a marked reduction in their metabolic activity upon pesticide treatment. This observation is further supported by the ratio of 7 and 19 S antibody levels.

Materials and methods. Pesticide treatment. Technical grade mirex (M) and 99.0+% pure *p,p*-DDT (D) were obtained from Allied Chemical and Aldrich Chemical Companies, respectively. Weighed samples of each pesticide were added to soybean oil premix and then combined with basal feed (12) to yield 100 ppm (M-100 or D-100) level. The chickens were from a strain of New Hampshire, an American breed, developed by Professor L. J. Dreesen of our Poultry Department. All eggs were hatched in Jamesway incubators. Feed and water were supplied *ad libitum* from the day of hatch.

Immunological data. Sheep red-blood cells (SRBC) stored in Alsever's solution was used as the antigen. The cells were washed 3 times in physiological saline (0.9% NaCl) prior to use. Twelve 4-week-old birds from each of the three experimental groups were immunized intravenously (iv) with 1 ml of a 5% suspension. A microtiter procedure was followed for antibody titrations (13, 14). The birds were bled from the brachial vein on the fourth, seventh, and tenth day after the antigen injection to collect serum for the micromethod. Serum samples were titrated individually (15). Titers were expressed as the logs of the reciprocal of the highest dilution giving visible agglutination.

Equal volumes of serum and 0.2 *M* mercaptoethanol (ME) in phosphate-buffered saline (pH 7.4) were mixed and incubated at 37° for 30 min prior to serial dilution (16). Agglutination tests were conducted and the titer was recorded as ME-resistant antibody.

Metabolic activity measurement. The method of preparation of thymus, bursal, and splenic lymphocytes was similar to the one used previously in our laboratory (17, 18). The bursa, spleen, and thymus from each bird were rinsed individually in Hank's balanced salt solution (HBSS) and cut into small fragments. They were aspirated

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through a disposable-needleless syringe (5 ml) and were filtered through a lens paper. The cells were washed twice with Ringer's solution and resuspended in Ringer's solution after the final washing. The cell counts were volumetrically adjusted to 25×10^6 cells/ml. A cell viability count was also performed for each cell suspension using eosin Y exclusion technique (19). There were 10 birds per treatment.

Oxygen uptake studies were conducted with a YSI Model 53 biological oxygen monitor at 37°. The test samples contained 2 ml of cell suspension (25×10^6 cells/ml), 0.3 ml of fresh chicken serum from the same bird, and 0.1 ml of glucose (30 *M*). The Ringer's solution was saturated with air at 37° and contained 4.95 μl O_2/ml of solution. Oxygen consumption was determined by calculating the loss of oxygen from the solution during a 15-min period.

Statistical analysis. The agglutinin titers and oxygen consumption rates were analyzed by the analysis of variance (20). Significant mean differences were determined by Duncan's new multiple range test (21).

Results. Feeding mirex and DDT from hatch to 40 days of age did not influence weights of body, bursa, spleen, thymus, or liver during the entire period of the experiment. There were no significant differences in total antibody production in normal and pesticide fed birds when measured by agglutinin titers (Fig. 1). However, DDT and mirex treatment significantly suppressed the ME-resistant antibody (IgG) and markedly increased ME-sensitive antibody (IgM) production at 5 and 7 days postantigen injection (Fig. 1).

The oxygen consumption for thymic and splenic cells was significantly lowered in the presence of both mirex and DDT (Table I). On the other hand, oxygen consumption of bursal cells was not consistently influenced by the pesticides.

Discussion. The agglutinin titers to a primary response of SRBC were normal in pesticide-treated birds. However, the ME-resistant (IgG) antibody was significantly lower and the ME-susceptible (IgM) antibody significantly higher in the pesticide-fed birds. These perturbations in the class of antibody of pesticide-treated birds cannot be attributed to a toxic effect of the pesticide on the bird since body weight was normal, nor can they be attributed to an overt catabolic influence on central peripheral lymphoid tissue since the weight of the bursa, thymus, and spleen in the DDT and mirex birds did not differ from the control birds.

The subtle influence of the pesticides on T-cells was reflected in the reduced oxygen consumption of thymic lymphocytes subsequent to DDT and mirex feeding. Failure to obtain a consistent change in O_2 uptake by bursal lymphocytes would suggest that the significant reduction in O_2 uptake of splenic cells from pesticide-fed birds was a reflection of a reduced T-cell function.

The reduced metabolism of T-cells would, in part, account for the depressed IgG antibody titers of DDT and mirex birds since this class of antibody is known to be dependent of T-cell function (22–24), and interaction of T- and B-cells is a prerequisite for normal antibody response to a variety of antigens (22, 25–27). The similarity in total

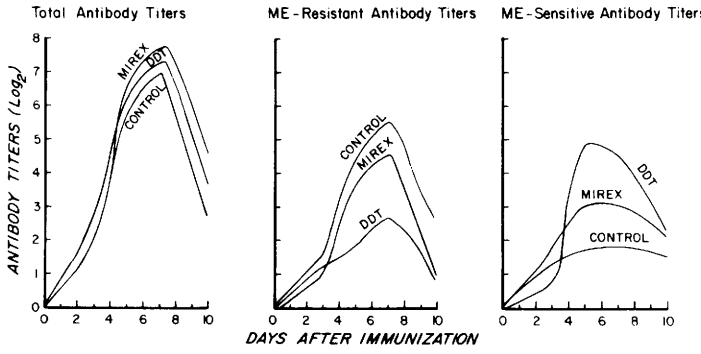

FIG. 1. Effect of pesticides on the antibody response to sheep red-blood cells.

TABLE I. OXYGEN CONSUMPTION (MICROLITERS PER HOUR) OF THYMUS, BURSAL, AND SPLENIC LYMPHOCYTES FROM CONTROL CHICKENS AND DDT- AND MIREX-FED CHICKENS.[a]

Treatment	Thymus	Bursa	Spleen
3 Weeks			
Control	9.75 ± 0.33[b]	8.48 ± 0.35[b]	14.43 ± 0.48[b]
Mirex	6.28 ± 0.38[c]	7.59 ± 0.43[b,c]	10.81 ± 0.41[c]
DDT	5.64 ± 0.29[c]	6.87 ± 0.23[c]	8.44 ± 0.61[d]
5 Weeks			
Control	12.74 ± 0.92[b]	9.02 ± 0.85[c]	14.89 ± 0.79[b]
Mirex	9.12 ± 0.73[c]	11.35 ± 0.81[b]	12.74 ± 0.92[c]
DDT	8.23 ± 0.82[c]	10.14 ± 0.51[b,c]	8.83 ± 0.82[d]

[a] Means and standard errors for 10 observations. Means not possessing the same superscript within each column are significantly different at 5% level (21).

agglutinin titers for the three groups suggests a normally functioning B-cell. Further evidence that the B-cell in pesticide-treated birds is normal could be obtained by utilizing a thymic-independent antigen.

Summary. Immune response and metabolic activity of bursal, splenic, and thymic lymphocytes from chickens treated with DDT and Mirex were evaluated. The results indicated a marked reduction in the metabolic activity upon pesticide treatment. There was no significant difference in total antibody production, but IgG levels were significantly reduced and IgM levels were markedly elevated. The significance of reduced metabolic activity and IgG production were discussed in relation to T-cell activity.

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