Immunological and Detoxication Interaction in p,p-DDT Fed Rabbits

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The phenomenon of organochlorine insecticides (OCI) as an environmental component has stimulated the interest of biologists and laymen. Many studies have investigated the biological implications of OCI in the internal milieu of living beings.

Our field observations in people occupationally and non-occupationally exposed to OCI and in laboratory animals has led us to the opinion that although no clinical findings or subjective complaints may be attributed to OCI exposure, many subtle, sub-clinical biological findings indicate the influence of OCI on physiological processes.

The adaptation of the animal body to the presence of OCI requires a sustained effort of homeostatic processes. (1,2)

In our studies concerning the relation between detoxication of xenobiotics and immunization processes, we found a statistically significant decrease of gamma globulins and a reduction of antibody titer of ovalbumin in p,p'-DDT-receiving rats on the one hand (3), and activation of the detoxication process of DDT with a consequent reduction of adipose tissue OCI storage, on the other. (4)

This paper reports on the same bi-directional relationship between OCI plasma level and immunological response to particulate antigens in rabbits.

This investigation was supported by a grant from the World Health Organization, International Agency for Research on Cancer, Lyon.

Bulletin of Environmental Contamination & Toxicology,
Vol. 6, No. 5, 1971, published by Springer-Verlag New York Inc.

Materials and Method

Thirty-one young, local strain, male rabbits (body weight 2-2 1/2 kg.) were used. The animals were housed two in a cage and fed an ordinary diet ad libitum. Recrystallized p,p'-DDT was dissolved in ethyl alcohol (200 mg/5cc) and diluted in 1 liter tap water. A 5‰ solution of alcohol in tap water was given to the control groups. p,p'-DDT feeding began 18 days before antigen administration and was discontinued 1 week after the third antigen injection (a total of 38 days).

The titer of agglutinating antibodies to Salmonella was assessed prior to the beginning of the experiment in order to identify those rabbits having the lowest previously acquired antibody titer against Salmonella ($\leq$ 1/8 dilution of rabbit sera) for vaccination with Salmonella. Those rabbits having a higher Salmonella antibody titer ($\geq$ 1/8) were used in the Sheep Red Blood Cell (SRBC) immunization.

Heat killed Salmonella typhi (standard antigen) or SRBC were administered in three weekly injections beginning 18 days after starting the p,p'-DDT exposure.

Salmonella were washed with saline and brought to a concentration of 1×10^8 and 5×10^8 bacteria per ml. 1×10^8 bcteria were given intravenously in the first injection, 5×10^8 intravenously in the second, and 5×10^8 intraperitoneally in the third injection. SRBC were washed three times with sterile saline and brought to a 10% suspension in saline. 0.5 ml were given intravenously, 1.0 ml intravenously, and 1.0 intraperitoneally in the first, second and third injections respectively.

The animals were distributed according to the above mentioned criterion in four groups:

Group 1 (7 rabbits) received Salmonella typhi injections.
Group 2 (10 rabbits) received 200 ppm p,p'-DDT in their drinking water and Salmonella typhi injections.
Group 3 (6 rabbits) received SRBC injections.
Group 4 (8 rabbits) received p,p'-DDT and SRBC injections.

During the week after the third antigen administration three rabbits died, one in the DDT-Salmonella group (Group 2) and two in the DDT-SRBC group (Group 4).

The animals were bled one week after the last injection. Seven weeks later an additional bleeding was performed in the Salmonella groups. The sera were separated, inactivated at 56° C. for 30 minutes and kept at -20° C. for immunological determinations. Plasma was separated and kept at the same temperature for OCI determinations.

Titration of sera by Agglutination Test. Two-fold serially diluted sera were prepared in phosphate buffered saline 0.15 M,pH 7.4. 0.5 ml of the diluted samples were incubated with 0.1 ml of 2% suspension of SRBC or with 0.05 ml of Salmonella standard antigen, for thirty minutes at 37° C. followed by 24 hours at 4° C. The last tube which showed clear cut agglutination was considered as the end point.

Quantitation of serum immunoglobulins by single immunodiffusion method. Quantitation of rabbit 7 S and 19 S fractions of gamma globulins and total gamma globulins was done by the method of Fahey. (5). Four µl of each serum, undiluted or diluted (1/5) were inserted into wells punched out in 1.5% agar plates containing one of the following specific antisera (diluted 1/10): Goat anti-rabbit IgG* (3 mg Ab/ml), goat anti-rabbit IgM* (1 mg Ab/ml), and goat anti-rabbit gamma globulin serum (3 mg/ml Ab), prepared by us against 33% ammonium sulphate fraction of rabbit serum. Purified rabbit 7 S and 19 S (fraction I from sephadex G-200 of rabbit serum prepared by us) and total gamma globulins* were used for standard curves. The agar plates were incubated in a humid chamber at room temperature. Results were read after 24 hours for the 7 S fraction of gamma globulins and the total gamma globulins and after 48-72 hours for the 19 S fraction of gamma globulins.

Electrophoresis in Polyacrylamide gels. The techniques of Ornstein and Davis (6,7) were followed with two modifications: only small pore gel was used, and the protein sample was prepared in 10% sucrose solution in Tris glycine buffer. 0.1 ml of each serum (diluted 1/35) was run in

*Miles

analytical gel (7.5% acrylamide pH 8.3). Following electrophoresis, the gels were stained for 30 minutes in 1% Amido Black in Acetic Acid. Densitometic reading of the protein bands in the gel was monitored by a Gilford Spectophotometer at 550 mμ.

Gas Chromatographic determination of plasma OCI levels. One to two ml of plasma were extracted by shaking followed by centrifugation three times with a total of 20 ml n-hexane. The 20 ml extract was evaporated to 2 ml and cleaned on a florisil column. The volume of the extract was then concentrated to 0.5 ml. 5 μl were injected into a Packard dual electron capture gas chromatograph model A-7420-99, having a QF 5% and a SE 3% chromosorb column.

Results and Comments

Table 1 summarizes the data obtained for total gamma globulins, 7 S and 19 S fractions of gamma globulins and Table 2, the antibody titers to Salmonella typhi bacteria and SRBC.

Table 1

Serum globulins (mg/ml)

Group T.	globulins	7 S Fraction	19 S Fraction
1. Salmonella	17.1*	15.4*	2.47**
2. DDT+Salmonella	12.4	9.7	2.51
3. SRBC	19.1	17.7	1.76
4. DDT+SRBC	15.9	14.1	1.71

Statistical Evaluation
* gr. 1 vs 2 $p < 0.01$
** gr. 1+2 vs 3+4 $p < 0.01$

The antibody titer against Salmonella and SRBC were lower in the DDT receiving rabbits (groups 2 and 4 as compared with groups 1 and 2). The decrease of the antibody titer was statistically significant only for the DDT-Salmonella receiving rabbits (group 2 vs. group 1). Seven weeks later these antibodies decreased in both groups but the difference between the two groups

remained statistically significant. In the DDT-Salmonella and DDT-SRBC receiving groups (groups 2 and 4) the total gamma globulin and the 7 S fraction were lower than in the groups of rabbits receiving only Salmonella or SRBC. These differences were statistically significant only for the DDT-Salmonella receiving rabbits (group 1 vs. group 1).

Table 2

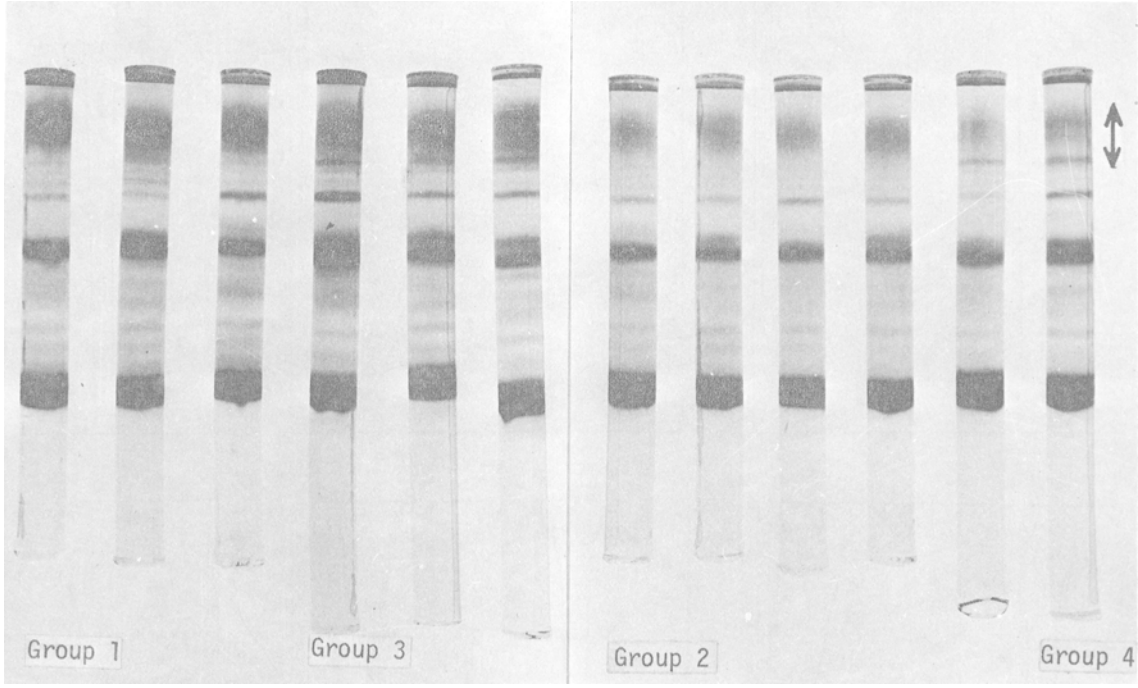
Antibody Titer

Group	After 1 week	After 7 weeks
1. Salmonella	2633*	1705*
2. DDT+Salmonella	939	421
3. SRBC	640	---
4. DDT+SRBC	256	---

Statistical Evaluation: *gr. 1 vs 2 : $p < 0.01$

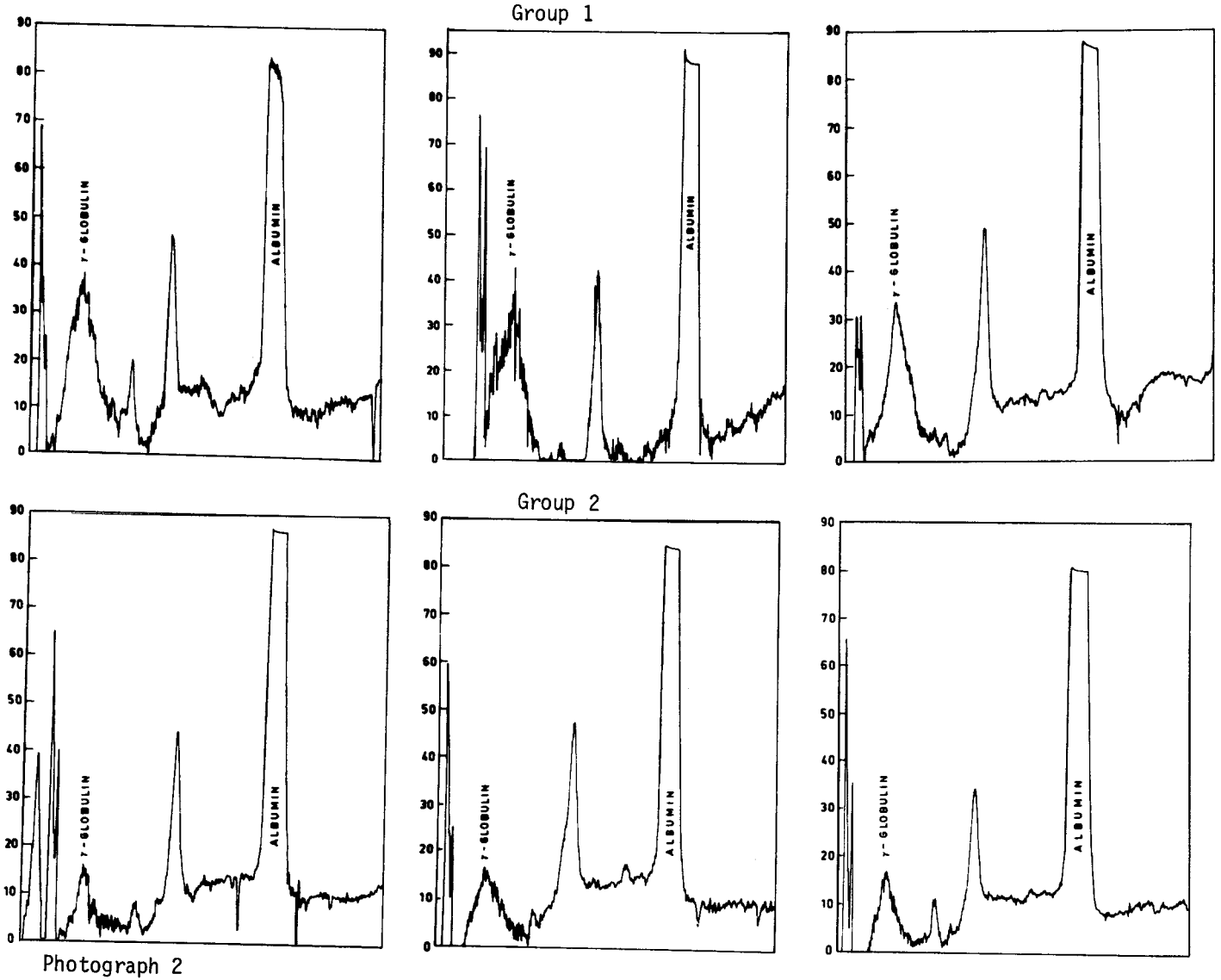
Photographs 1 and 2 show the marked decrease of gamma globulins in polyacrylamide gel disc electrophoresis of serum proteins in DDT receiving rabbits. Photo 3 shows the differences in the 7 S fraction of gamma globulins in the four groups of rabbits as measured by the single radial immunodiffusion method, the DDT-Salmonella receiving rabbits having the smallest amount of 7 S fraction. As we can see, total gamma globulin decreased at the expense of the 7 S fraction while the 19 S fraction remained almost unchanged in the DDT-Antigen receiving rabbits when compared with the rabbits receiving only antigen. It is worth stressing that though p,p'-DDT did not affect the level of 19 S fraction of gamma globulins it differed in rabbits receiving Salmonella and SRBC (groups 1 and 2 vs. groups 3 and 4).

Sampling for DDT and its metabolites in plasma showed a rise in the total DDT in the p,p'-DDT treated groups (groups 2 and 4). There are however, striking differences in the plasma levels of DDT and its metabolites in these two groups, the DDT-Salmonella receiving group having an average of 322.9 ppb total DDT compared with 164.7 ppb in the DDT-SRBC receiving group. This difference is statistically significant.

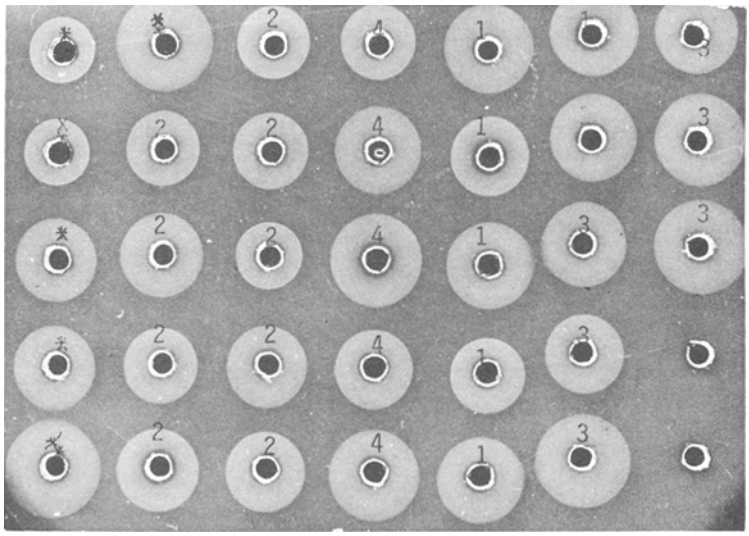


Photograph 1. Marked decrease of 7 S γ-globulins in DDT receiving rabbits

↕ — region of 7 S γ-globulin fraction



Photograph 2



Photograph 3. 7 S fraction of γ globulins in single radial immunodiffusion method.
*standard (1.5 mg/ml, 3 mg/ml, 6 mg/ml)
1: Salmonella 2: DDT+Salmonella
3: SRBC 4: DDT+SRBC

Table 3

Plasma DDT level (ppb)

Group	Total DDT
1. Salmonella	3.9
2. DDT+Salmonella	322.9
3. SRBC	7.5
4. DDT+SRBC	164.7

Statistical Evaluation 2 vs 4 $p < 0.01$

The difference in the impairment of the immunological indices found in the DDT-Salmonella receiving rabbits as compared with the DDT-SRBC receiving rabbits may depend on the different levels of total DDT in the plasma of the two groups of rabbits.

In a previous study (3) we showed that OCI accumulation in the epididymal pad of the white rat was reduced during the immunological process against ovalbumin when compared with control rats. Furtermore, it follows from the findings in the present paper, that the immunological response to different antigens, affects the detoxication of a given xenobiotic (p,p'-DDT) to different a degree.

These data also raise the problem of the relationship between two physiological processes involved in animal body defense against environmental hazards: metabolization of a xenobiotic (DDT) and immunization against soluble (ovalbumin) or particulate antigens (Salmonella typhi, SRBC).

This bi-directional relationship between the degree of detoxication of p,p'-DDT and the degree of immunological response to antigens seems to us to be of tremendous importance when implications in drug detoxication and immunological processes were considered

Summary

Thirty-one rabbits were immunized against Salmonella or SRBC. These rabbits had an impaired immunological response when they received 200 ppm p,p'-DDT in their drinking water during a period of 38 days. The total gamma globulins as reflected by the 7 S fraction were significantly decreased in the p,p'-DDT-Salmonella receiving rabbits when compared with the rabbits receiving only Salmonella. This decrease was not statistically significant in the p,p'-DDT-SRBC receiving rabbits when compared with the rabbits receiving only SRBC.

The antibody titer against Salmonella was significantly decreased in p,p'-DDT-Salmonella receiving rabbits when compared with the rabbits receiving only Salmonella. The decrease of the SRBC antibody titer in the p,p'-DDT-SRBC receiving rabbits was not statistically significant.

The plasma total DDT level differed significantly in the two groups receiving p,p'-DDT (p,p'-DDT-Salmonella and p,p'-DDT-SRBC receiving rabbits). This finding may explain the different degree of impairment of the immunological response, the higher plasma DDT level having a more marked effect. These differences in total DDT plasma level in the two groups of rabbits which received the same amount of p,p'-DDT in their drinking water may be considered as a consequence of the concomitant presence of a different kind of foreign antigen in the internal milieu.

The bi-directional relationship between a detoxication process and an immunological response is emphasized.

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