In vivo Binding of p,p'-DDE to Human Serum Proteins

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Although it is convenient to estimate chlorinated hydrocarbon pesticide levels in man by determining the amount present in serum, few investigators have questioned possible interactions of these compounds with serum proteins and the resulting consequences. DALE et al. (1965) suggested that the binding of pesticides to serum protein was the cause of incomplete pesticide recovery from human serum by hexane extraction, and GUNTHER et al. (1954) proposed molecules were held by proteins with consequent inhibition of the normal function of these proteins. Binding of dieldrin and telodrin to serum proteins has been demonstrated by MOSS and HATHWAY (1964), but the concentrations of these pesticides were considerably greater than those normally encountered in man and pH ranges during the separation deviated too far from the physiological norm to allow much speculation on the results. HATANAKE et al. (1967) attempted to recover pesticides from protein fractions after Sephadex G-50 treatment, but met with inconsistent results. Careful review of these reports emphasizes that direct evidence of serum protein binding under physiological conditions should be obtained.

METHODS

Serum was prepared from the author's blood by allowing it to clot for 2 hr at 0°C, then removing the liquid portion. Two-ml samples were kept at -15°C for not more than 10 days.

Ten g of Sephadex G-200 was soaked for 3 days in 1.0 liter of 0.9% NaCl solution at pH 7.35. This material was placed in a 2.5 cm x 46 cm glass column and allowed to settle under flow. Two ml of serum were diluted to 10 ml with 0.9% NaCl (pH 7.35), placed on the column, and eluted with the same solution. Ten-ml fractions were collected at a flow rate of 2-3 ml/min at constant

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pressure. Protein concentrations were determined with a Zeiss Model PMQ-II spectrophotometer at 280 mμ.

The pooled protein fractions, usually about 150 ml, were extracted with 200-ml portions of hexane. When a 2.0-ml serum sample was diluted to 150 ml and extracted in this way, more p,p'-DDE was recovered and reproducibility was greater than when the serum was extracted directly by the method of DALE et al. (1965, 1970). Since further experimentation showed that a reduction in the volume of the diluted serum from 150 ml to 50 ml caused no significant reduction in p,p'-DDE recovery, the following procedure was adopted for the routine serum extractions:

Two ml of serum, diluted to 50 ml with 0.9% NaCl solution (pH 7.35), and 75 ml hexane were shaken vigorously in a 250-ml separatory flask for 10 sec and the flask inverted and vented. The shaking and venting process was continued for 1 min, after which the phases were allowed to separate. The process was repeated three times. Any interphase emulsion was broken by centrifugation. The extract was reduced to an appropriate volume by evaporation under a gentle stream of air at 35°C. The p,p'-DDE was detected by means of a Microtek MBT-220 gas chromatograph, using an OV-17/QF-1 column for quantitation and a SE-30/QF-1 column for confirmation at 195°C and a tritium-type electron-capture detector at 205°C. Further confirmation was achieved with thinlayer chromatography on silic acid with heptane as solvent.

RESULTS

Only p,p'-DDE was quantitated because it was present in large enough amounts to be confirmed by thin-layer chromatography. The p,p'-DDT present was in such low amounts that reproducibility between chromatograms was very poor. No polychlorinated biphenyl derivatives (PCB's) were present. Table 1 shows that compared with the method of DALE et al. (1965, 1970) the extraction method gives almost a twofold increase in p,p'-DDE recovered as well as less variability (F-test; $p<0.01$). Protein recovery after Sephadex G-200 chromatographic treatment was 95%, and the protein fractions contained approximately 80% of the original amount of p,p'-DDE. When p,p'-DDE in hexane was placed on the column and the hexane was allowed to evaporate, elution under the conditions described for serum chromatography did not yield any p,p'-DDE in the effluent in the range of the protein fractions. Preliminary data indicate that very little p,p'-DDE is found in the gamma globulin fraction. Precipitation of serum proteins with ammonium sulfate and subsequent hexane extraction of the supernatant and the precipitate yielded no p,p'-DDE in the former and only trace amounts in the latter. Serum samples kept at -15 C for more than 10 days showed a decrease in recoverable p,p'-DDE (e.g., 20% less was recovered from a sample stored for 21 days). Both

observations indicate that the degree of binding of p,p'-DDE to the proteins changes as the conformation of the protein changes.

DISCUSSION

The importance of the binding of drugs to serum proteins and the concomitant effects on their pharmacological activity cannot be overemphasized (SELLERS and KOCH-WESER 1969, MEYER and GUTTMAN 1968, CONN and LUCHI 1961, DOLLERY et al. 1961, MACREGOR 1965, BRODIE 1965, CUCINELL et al. 1965). BRODIE (1965) states, "Actually, almost all drugs are reversibly bound to proteins in plasma or tissue. The bound drug, often a high percentage of the total, acts as a reservoir, preventing wild fluctuations between ineffective and toxic levels of the biologically active unbound fraction." It is believed that the same type of mechanism can explain the behavior of p,p'-DDE and, very likely, all chlorinated hydrocarbons, including the PCB's, in the blood.

Although microsomal enzyme induction is usually cited as cause for the reduced serum levels of pesticides, the following interpretation should be considered: (1) Serum concentrations of chlorinated hydrocarbon pesticides normally encountered in human beings reflect "bound" levels that are relatively inert. (2) Any compound that can interfere with the binding of the pesticide may free it for adsorption at a site of toxic action, metabolic breakdown, or storage, depending on the distribution constant. (Large amounts of inertly bound pesticides upon liberation could in this fashion become available for binding at the site of toxic action). (3) The enzyme system responsible for the breakdown of the pesticides is always present, but cannot function because the substrate is tightly bound to the serum proteins. Pesticide metabolism is strictly governed by the difference in the distribution constants between the two sites.

This hypothesis would explain why aldrin enhanced the retention of p,p'-DDT and p,p'-DDE in the blood of dogs (DEICHMANN et al. 1969), the reduced paroxon binding capacity in rat plasma on oral pretreatment with tri-o-tolyl phosphate (LAUWERYS and MURPHY 1969), and the lowering of serum-bound iodine by o,p-DDD in humans (MARSHALL and TOMPKINS 1968). It would also explain the low levels of chlorinated hydrocarbons found in persons treated with anticonvulsant drugs by DAVIES et al. (1969) and SCHOOR (1970).

TABLE 1

Comparison of recovery of p,p'-DDE in human serum by different analytical methods.[a]

Method of DALE et al. (1965)	Present method	
	Combined protein fraction after G-200 treatment	
Serum	Serum	Serum
ppm	ppm	ppm
0.019	0.036	0.025
0.022	0.037	0.028
0.024	0.037	0.026
0.012	0.034	0.029
0.012	0.030	
0.017	0.034	
0.019	0.035	
0.015	0.033	
0.019	0.034	
0.019	0.034	
	0.034	
	0.036	

[a] Two ml of serum were extracted in each analysis

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