Transport of DDT, DDE, and Dieldrin in Human Blood[1]

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Through a series of studies, Moss and Hathway (1) showed that telodrin and dieldrin in rat and rabbit blood appear in approximately the same concentrations in erythrocytes and plasma. They further demonstrated an association of these pesticides with the albumin and α-globulin constituents of these animal sera.

Our studies undertake principally to identify the components of human blood with which pp'DDT and pp'DDE are associated. We have studied blood specimens from workers long exposed to chlorinated hydrocarbon pesticides, and also subjects who had ingested measured quantities of technical DDT about 2 years prior to these tests (2).

RBC transport of pp'DDT, pp'DDE, and dieldrin.

Blood samples from 10 workers long exposed to DDT, dieldrin, and other pesticides were collected in heparinized tubes. Erythrocytes separated by centrifugation were washed 3 times in saline. Samples of packed red cells were extracted by a 1:1:1 mixture of ethyl alcohol, ethyl ether, and hexane, the extract then being "cleaned up" by the Mills procedure (3). These extracts, alongside hexane extracts of plasma, were then analyzed by gas-liquid chromotography (electron capture detection) for pp'DDT, pp'DDE, and dieldrin. Blood hematocrit values permitted calculation of the proportions of pesticide carried in the two major blood compartments. (Table 1).

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[1]From the Department of Entomology, Arizona Agricultural Experiment Station. This research was supported under Contract No. EPA 68-03-0091 of the Pesticide Program, Environmental Protection Agency.

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Bulletin of Environmental Contamination & Toxicology,
Vol. 8, No. 6, 1972 © by Springer-Verlag New York Inc.

TABLE 1

Proportion of blood-borne pesticide found in washed erythrocytes from occupationally exposed workers.

Pesticide	No. of workers sampled	Plasma pesticide concentration, ppb, mean and range	Percent of pesticide in erythrocytes*	
			Mean	Range
pp'DDT	10	74 (24-114)	12.5%	7-18%
pp'DDE	10	87 (50-145)	13.4%	9-16%
Dieldrin	6	9 (2-18)	39.8%	17-48%

$$* \quad \frac{Hct \cdot (P)_e}{Hct \cdot (P)e + (100 - Hct) \cdot (P)_p} \times 100$$

The proportion of blood-borne dieldrin measured in the RBC of 5 of the 6 workers heavily exposed to this chemical was essentially the same as the hematocrit (between 40% and 50%). This agrees with the thesis of Moss and Hathway that the red cell membrane is freely permeable to dieldrin. pp'DDT and pp' DDE, by contrast, were restricted almost entirely to the plasmas of 10 workers with elevated DDT-DDE levels. It is possible that the low red cell contents observed in these bloods were, in large part, associated with plasma still adherent to the washed cells.

Pesticide-bearing plasma constituents.

The high solubility of DDT in neutral fat suggests that free triglyceride in the blood (chylomicra) might be a major transport medium. Sera from 2 subjects previously dosed with DDT (current serum DDT 145 ppb and 100 ppb, respectively, serum DDE 110 ppb and 65 ppb, respectively) were centrifuged at 35,000 g for 16 hours, at 15° C, to bring chylomicra to the surface. Sometimes a tiny button of fat was visible on the sample surface, in other samples no visible separation of neutral fat from these non-lactescent sera was apparent. Removal of the uppermost layer was accomplished either by careful aspiration into a Pasteur pipette, or by repeated application of hexane to the surface, followed by aspiration. In no case did these surface extracts yield more than 1% of the pp'DDT or pp'DDE contained in the whole plasma.

Further attempts to analyze chylomicra were made using the additions of sodium phosphotungstate and magnesium chloride to plasma that were found by Burstein (3), to enhance flotation of chylomicra during centrifugation. Again, hexane extraction of surfaces of centrifuged samples failed to yield significant proportions of the DDT or DDE in the plasma. We have therefore been unable to confirm that the chylomicra are an important pesticide-transport medium, at least in non-lactescent sera.

Amounts of DDT and DDE in plasma are reduced to less than detection limits by acid-tungstate precipitation of plasma protein.

We therefore directed our attention to fractions of the macromolecular lipoprotein complexes of blood plasma.

Ultracentrifugal separation of plasma lipoprotein constituents.

To samples of the two high-level DDT sera were added volumes of concentrated NaCl that raised the measured density of the 10.1 ml mixture to 1.072 (5). These were centrifuged at 80,000 g for 16 hours at 10° C., causing separation into 3 (and sometimes 4) layers. These were pipetted off in serial fashion as cleanly as possible, then analyzed for pesticide and for lipid composition by thin-layer chromotography. Results are shown in Table 2.

TABLE 2

Volumes and relative pesticide contents of the top-to-bottom fractions of ultracentrifuged plasmas from two previously DDT - dosed subjects.

	Percent of Total						
	Volume		pp'DDT		pp'DDE		Lipid
	In subject:		In subject:		In subject:		constituents
Layer	#1	#2	#1	#2	#1	#2	identified
1	19%	17%	56%	49%	42%	34%	Ch,ChE,TG,MG
2	77%	55%	37%	31%	46%	36%	Ch,ChE,MG
3		21%		13%		18%	ChE,MG
4	4%	7%	7%	7%	12%	12%	ChE,MG,DG

Ch = cholesterol; ChE = cholesterol esters; TG = triglycerides; MG = monoglycerides; DG = diglycerides.

It is first apparent that pp'DDT and pp'DDE are not restricted to any one density-determined layer. Secondly, concentration of pesticide is highest in the uppermost stratum of low-density and very-low-density lipoproteins. Thin layer chromotographic study of the fractions revealed a strong representation of triglyceride in this upper layer that was not evident in the lower layers.

Strip electrophoresis of the serial layers bore out a striking increase in all protein components from top to bottom. Detectable amounts of albumin and the lighter globulins remained in the top layer, which was essentially devoid of γ-globulin.

Separation of plasma proteins by continuous electrophoresis.

Electrophoretic separation of the proteins contained in high-level DDT serum (DDT = 145 ppb, DDE = 110 ppb) was accomplished with the Beckman Continuous-flow Paper Electrophoresis apparatus, employing Veronal buffer at pH 8.6, 93 ma current, and a heavy-duty cooling system (6). In our best separation, the faint yellow and brown streaks on the paper (natural serum chromogen marking the albumin and principal globulin constituents) descended in a constant pattern for approximately 18 hours, permitting clean separation of proteins in approximately 3.6 ml of serum. Once separation was accomplished, the individual collecting tubes were analyzed for DDT and DDE, and by strip electrophoresis for the principal plasma protein fractions represented. Results are shown in Figure 1, the symbols at the bottom indicating the visible prominence of protein bands on electrophoretic strips. Most of the pesticide appeared to be associated with serum albumin, smaller amounts with the β and α_2 globulins. Interestingly, the pesticide was accurately associated with the plasma protein constituents bearing natural plasma chromogen. Separations of plasmas having lower pesticide contents have confirmed the association with albumin, but the globulin-bearing component has been more difficult to demonstrate than it was in this one separation.

Conclusions:

1. Less than 18% of the pp'DDT and pp'DDE found in human blood is carried in the erythrocytes, while dieldrin is distributed between RBC and plasma roughly in proportion to volume.

2. In non-lactescent sera, chylomicra carry less than 1% of the total pesticide. Protein-free serum is virtually devoid of pesticide.

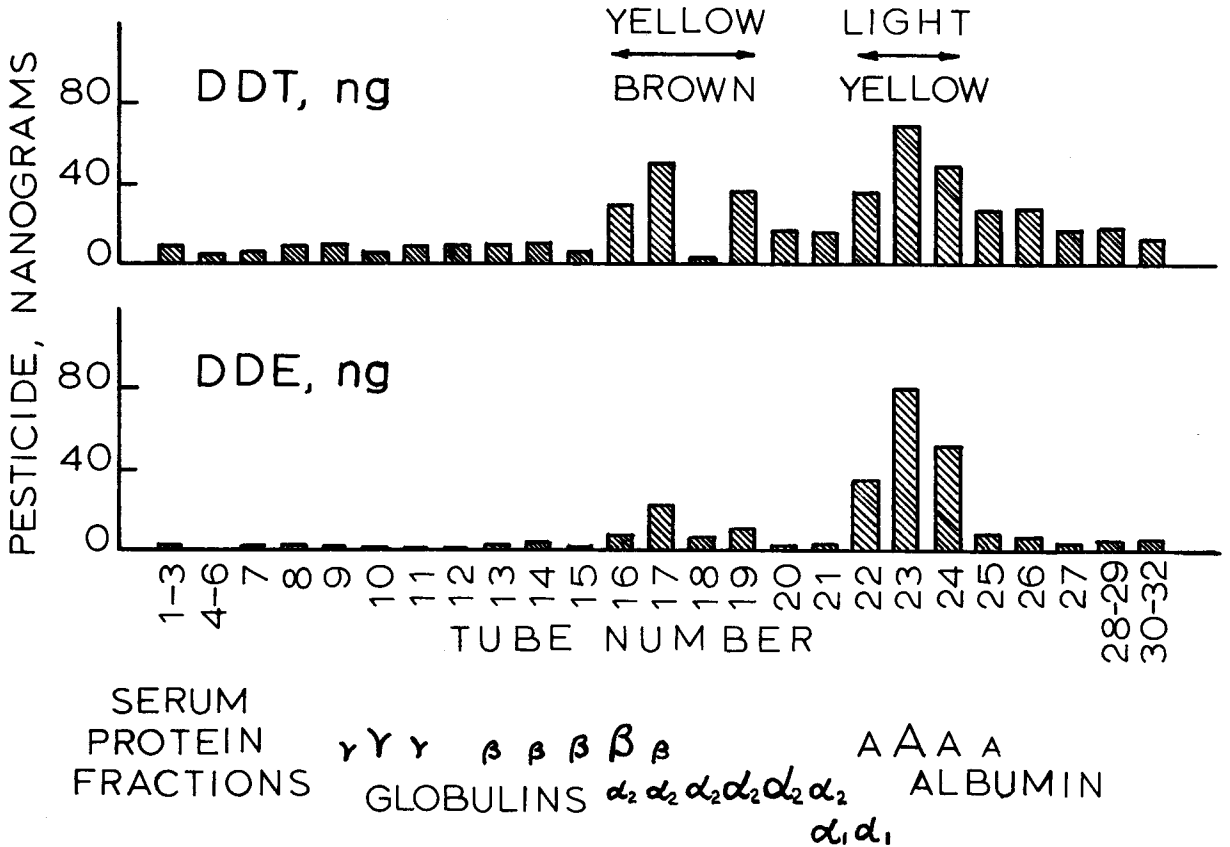

Figure 1. pp'DDT and pp'DDE in electrophoretically separated fractions of human serum.

3. pp'DDT and pp'DDE are found in relation to lipoproteins of various densities, but principally in the triglyceride-rich low-density and very-low-density lipoproteins.

4. The plasma albumin, and secondarily the smaller globulins are the principal plasma protein constituents associated with blood-borne pp'DDT and pp'DDE.

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