Rate of Transfer of DDT from the Blood Compartment[1,2]

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A number of workers have analyzed blood for pesticides (1-10) and some have correlated the level of pesticides in blood with that in body fat (2, 5). These correlations were obtained with the intention of utilizing blood samples in lieu of adipose tissue biopsy samples for the purpose of determining pesticide storage levels in various segments of the population. Basic to any interpretation of such a correlation is an understanding of the rate of transfer of a pesticide from the blood following a discrete accumulation. This rate of loss must be known in order to interpret whether the pesticides found in the blood are a reflection of an immediately prior overt intake or a reflection of an equilibrium with pesticides stored in adipose tissue.

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Workers who have reported results of the analysis of various kinds of blood for pesticide residues have dealt with the level of pesticide in blood in equilibrium with a certain pesticide exposure history rather than the rate of change of level of pesticide in blood following exposure. Basic to the detection of pesticides in blood is the complete extraction of the pesticide from the blood. Since several authors have used different extraction methods, but offer little evidence as to whether the extraction was complete, it is important to establish that the methods used are adequate. Methods which have been used include KOH hydrolysis followed by pentane extraction or extraction with ethyl ether/ethyl alcohol, ethyl ether/acetone, ethyl ether only, n-hexane only, and petroleum ether only. Crosby and associates (1,5) extracted blood for DDT by direct hydrolysis of the blood with KOH and extraction of the hydrolysate with pentane. This method dehydrochlorinates DDT to DDE and therefore prevents the simultaneous detection of the two products and also precludes a simple detection of the amount of lipid present. These authors generally found good correlation (0.95) between the amount of pesticide in the blood and the amount in the adipose tissue for cows dosed at 30 to 600 ppm with DDT in the diet. If one assumes that the blood contained 400 mgm of lipid per 100 g. of blood, there was about 1/3 the amount of DDT and DDE in the blood as in the adipose tissue when compared on an extractable lipid basis. Dale et al. (2)

extracted blood plasma only using ethyl alcohol and ethyl ether (Bloor (11)), which has long been accepted as sufficient for complete extraction of lipid. Since the chlorinated hydrocarbon insecticides are fat soluble compounds, the complete extraction of lipid material is probably an important criterion for the complete extraction of the insecticide. These authors found about 10 times more DDT in the plasma lipid than that found in adipose tissue lipid from rats receiving 200 ppm of DDT in their diet. Jain et al. (4) used 3 successive extractions with an acetone-ether mixture to extract insecticides from the blood of rats which had received an LD_{50} dose of the insecticide. They showed that the recoveries were not enhanced by use of a greater number of extractions. They analyzed the extract directly without cleanup. This was probably successful because very small samples could be used since the levels of pesticide in the blood were quite high. Dale et al. (3) extracted blood with n-hexane only and analyzed the extract without cleanup. They noted that successive extractions did not increase the yield of insecticide, but also noted that they apparently experienced low recoveries which may have been due to binding with some constituent of the serum. They showed levels of 0.01 ppm of DDE and 0.006 ppm of DDT in the whole blood of persons without occupational exposure. Earlier authors (7,9,10) used ether or ether and acetone solvent systems to extract DDT from blood. They failed to detect any DDT, probably due to the limits of sensitivity

of their detection methods. Stiff (8), using an ether/acetone solvent system and a total chloride detection method, reported rather large values (6.8 to 9.8 ppm) for DDT residues in whole blood from rats receiving a 550 mg/kg. oral dose. Stiff (8) reported on the appearance of DDT in the blood in relation to an oral dose, but no authors have reported on the rate of decline of an insecticide from blood when the decline rate was not compounded by an input rate.

It was the purpose of this study to determine the rate of transfer of DDT from the blood compartment following the establishment of a unique maximum level of pesticide in the blood by a single intravenous injection. A second purpose was to compare the amount of pesticide extracted using either an apolar solvent only or a polar solvent in combination with an apolar solvent and to relate this to the total amount of lipid extracted.

Materials and Methods

Two mature lactating Holstein cows were dosed with a preparation of DDT calculated to be approximately 4 ppm on the basis of consumed feed. The preparation was a mixture of two fractions: 1) 150 ml. of peanut oil, 150 ml. Atlox 1054-A and 15 g. p,p'DDT; 2) 1 g. sodium cholate, 12 g. lecithin, (soy refined) 8 g. NaCl and 685 ml. distilled water. Just before dosing 16 ml. of 1) and 34 ml. of 2) were combined and shaken vigorously. The appropriate amount was then injected directly into the jugular vein. Blood was collected into previously

heparinized bottles before injection and at 1-10 min. and 1,2,4, 8,16 and 24 hours post injection from the subcutaneous abdominal vein. The samples were frozen until time of analysis.

The blood was extracted by two methods. The first consisted of weighing out 25 or 50 g. (dependant on expected pesticide level) into a 250 ml. beaker. The blood was then poured into a 500 ml. separatory funnel which contained 3 ml. of 5% potassium oxalate. The beaker was rinsed into the funnel with two 10 ml. washings of distilled water. Fifty ml. of absolute ethanol were then added following which the separatory funnel was inverted 4 times. One hundred ml. of ethyl ether were then added followed by 1 min. of shaking after which 50 ml. of pentane were added and it was again shaken for 1 min. Distilled water was then added until the funnel was ca 3/4 full. The funnel was then inverted 6 times and allowed to stand 10 min. after which the lower layer was discarded. This step of adding water and discarding was repeated twice. The solvent layer was then poured through a funnel containing sodium sulfate and a glass wool plug into a 250 ml. beaker. The separatory funnel was rinsed through the funnel with 4 washings of 10 ml. of pentane each. The solvent was then evaporated to near dryness and transfered from the beaker to a 15 ml. centrifuge tube with pentane. An aliquot representing 5 g. of blood was then removed for fat determination. The contents of the centrifuge tube were then poured onto a column pre-washed with the eluant containing 4 in. of activated

(130°C,16 hr.) florisil and 1/2 in. of sodium sulfate. The centrifuge tube was washed twice with pentane. The pesticide was eluted with 200 ml. of 10% ether in pentane. The eluant was then evaporated to less than 5 ml. and transferred to a 5 ml. centrifuge tube. The analysis for DDT was then carried out by electron capture gas chromatography as previously described (12). The second method of extraction from the blood was identical with the first except that the alcohol and potassium oxalate steps were omitted.

The aliquot for quantitative blood fat determination was evaporated to near dryness at room temperature and then the balance of the solvent was removed by leaving it in an oven for 1 hr. at 50-60°C. Blood lipid was then determined by direct weighing.

Results and Discussion

The results are shown in Table 1. It is important to note that when no alcohol is used in the solvent system approximately 10% as much lipid is extracted as when the alcohol is included. If fat soluble pesticides are uniformly distributed in body lipids, then only 10% of the pesticide should also have been recovered, and the concentration when expressed in terms of ppm in lipid should have remained constant. However, the total pesticide recovered increased by a factor of about 1.6 instead of a factor of 10. This implies that about two-thirds of the pesticide is associated with the easily extractable lipid and the other one-third is associated with that

90% which is more difficult to extract. It is not clear whether these two situations are distinct because of location, i.e. the 90% fraction is in the interior of the red blood cells, or whether they are distinct because of binding or the chemical nature of the lipids, i.e. they are phospholipids, sterol esters, or other alcohol soluble lipids. The choice of extraction method would depend on whether one wished to simplify the cleanup problem by failure to extract all the lipid, or wished to determine the total pesticide content of the blood.

TABLE 1

Comparison of the Extractable Lipid and DDT from Blood Using Two Solvent Systems

Blood Sample	Pentane + Ethyl Ether			Pentane + Ethyl Ether + Ethyl Alcohol		
	Mg fat /100 g. blood	PPM DDT blood lipid	PPM DDT whole blood	Mg fat /100 g. blood	PPM DDT blood lipid	PPM DDT whole blood
511-1	28	180	0.05	416	26.6	0.11
511-2	26	27	0.007	400	4.4	0.018
511-3	32	19	0.006	398	2.9	0.012
511-4	32	16	0.005	388	1.8	0.007
511-5	42	6.0	0.0025	426	0.55	0.0023
511-6	30	2.3	0.0007	396	0.092	0.0003

The results of the rate of decline study are shown in Figure 1. The data do not cover a sufficient period of time to clearly demonstrate whether this situation represents the classical two compartment process. The rapid rate of decline in the early part of the process has a half-life of 60-80 min. and continues for 6 hr., the slower rate of decline in the later part of the process has a half-life of about 8 hr. It appears that equilibrium is nearly re-established 24 hr. after

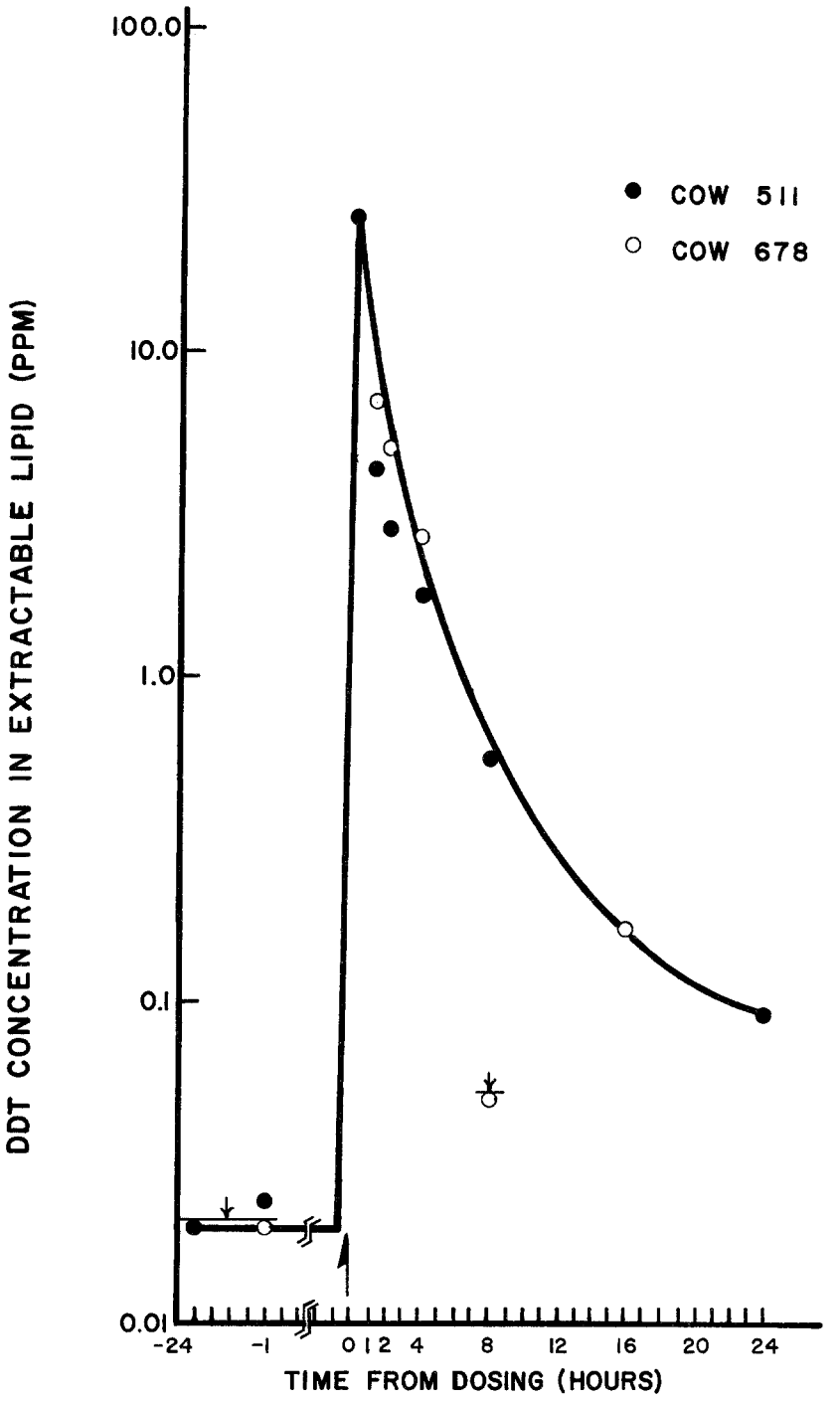

FIG I. RATE OF DECLINE OF DDT FROM BLOOD AFTER INTRAVENOUS DOSING

cessation of pesticide absorption into the blood. It is interesting to note that the level of DDT in the blood lipid of the cow which has not received any overt exposure to DDT is 0.02 ppm or less (0.00008 ppm in whole blood) which is considerably less than the 1.5 ppm normally found in human blood from persons with no occupational exposure. This is probably due to the fact that the diet of the cow contained less than 0.1 ppm of DDT and was storing less than 0.5 ppm of DDT and DDE in its milk fat. This low level in the normal cow's blood is near the reagent blank response and makes the monitoring of normal cows by this method much more difficult than monitoring occupationally unexposed people. The recovery standards for this study ranged from 71 to 137% with a mean value of 99.6%.

Calculation of the level of pesticide expected to be found in the blood can be based on the weight of the cow, blood volume, percent lipid, and the amount of DDT injected. On this basis, it was calculated that only 10.4% of the dose of DDT was accounted for in the "zero time" sample. However, the "zero time" sample was taken from 1 to 5 minutes after injection, so it is not clear whether this low value is due either to poor recovery or an extremely rapid transfer from the blood to other compartments.

Extremely rapid disappearance from the blood following i.v. injection is frequently found in the case of electrolytes. Such behavior is especially striking in the case of manganous

ion where, in the human subjects, only about 2% of the dose per liter of blood (near 10% of the dose for the entire blood supply) is found within 5 min. after injection, and by one hour, about 0.15%/liter (13). In the case of manganese, it was suggested that rapid movement into the intracellular compartment accounted for this behavior.

Conclusions

The rate of decline of DDT from the blood has been shown to be quite rapid and recovery of pesticide from the blood has been related to the recovery of lipid from the blood.

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