10. J. C. H. MAO and P. L. TARDREW, *Biochem. Pharmac.* **14**, 1049 (1965).
11. J. R. GILLETTE, B. B. BRODIE and B. N. LADU, *J. Pharmac. exp. Ther.* **119**, 532 (1957).
12. D. M. ZIEGLER, F. H. PETTIT and W. H. O. JOHNSON, *Fedn. Proc.* **24**, 604 (1965).
13. L. SHUSTER and R. V. HANNAM, *Can. J. Biochem. Physiol.* **43**, 899 (1965).
14. H. GOLDENBERG and V. FISHMAN, *Biochem. biophys. Res. Commun.* **14**, 404 (1964).
15. A. A. KURLAND, C. L. HUANG, K. J. HALLAM and T. E. HANLON, *J. psychiat. Res.* **3**, 27 (1965).
16. T. NASH, *Biochem. J.* **55**, 416 (1953).
17. K. N. MURRAY and S. CHAYKIN, *J. biol. Chem.* **241**, 3468 (1966).

Biochemical Pharmacology, Vol. 17, pp. 171–174. Pergamon Press. 1968. Printed in Great Britain

The sub-cellular partition and metabolism of orally administered 1,1,1-trichloro-2, 2-*bis* (*p*-chlorophenyl) ethane by rat liver cells*

(*Received* 6 *April* 1967; *accepted* 5 *July* 1967)

Abstract—Uptake of orally administered DDT† by rat liver cells has displayed the following characteristics:

1. The pesticide was not exclusively segregated from any one cell fraction.
2. Pesticide was depleted with time from all fractions.
3. A redistribution of pesticide occurred with time. A passive DDT distribution either occurs at some point in time or at low levels of exposure.
4. Non-lipid binding of pesticide appeared to occur.
5. Liver cells appeared tc be detoxifying DDT to DDE and DDD by 16 hr post treatment.

THE ABILITY of DDT to influence specific metabolic processes of cellular constituents has been of interest to investigators for some time. Rat liver has been the most universal material studied.

DDT has been shown to interfere with oxidative metabolism in rat liver mitochondria.[1] The soluble cell fraction contains enzymes which are inhibited by DDT.[2] The important role played by rat liver microsomes in the detoxification of DDT[3] and also the stimulation of microsomal drug-metabolizing enzymes by DDT have been demonstrated.[4]

Since DDT is capable of interactions of a very diverse nature depending upon its cellular location, it is important to characterize the uptake of orally administered DDT and subsequent intracellular involvement. This work involved analysis of the various subcellular components of rat liver cells from dosed and control rats for DDT and its analogs. Blood samples and studies *in vitro* were included to aid in the characterization.

EXPERIMENTAL

University of Arizona strain female rats of Sprague–Dawley origin, weighing 200–300 g and maintained on a Wayne Lab Blox diet, were used as experimental animals. The rats were dosed via a stomach tube with 10 mg of 99·3% *p,p'*-DDT in 1 ml peanut oil. The dosed rats and control rats

* Arizona Agricultural Experiment Station, Technical Paper No. 1219.

† Abbreviations: DDT, 1,1,1-trichloro-2,2-*bis*(*p*-chlorophenyl)ethane; DDE, 1,1-dichloro-2,2-*bis*(*p*-chlorophenyl)ethylene; DDD, 1,1-dichloro-2,2-*bis*(*p*-chlorophenyl)ethane.

were then fasted for 16 hr. After sacrificing and decapitation, blood was collected (*ca.* 2 ml) and the livers were rapidly excised and washed in ice-cold 0·25 M sucrose.

Components were separated by a modification of the method of Schneider and Hogeboom.[5] The microsomal, mitochondrial, and nuclear fractions were resuspended in 50% saturated sodium sulfate. These suspensions along with the serum and soluble fractions were placed in separatory funnels and extracted additively with 25 ml ethanol, 50 ml diethyl ether, and 25 ml pentane, followed by several water washes. The organic phase was then filtered through sodium sulfate into a 250-ml beaker, evaporated to *ca.* 2 ml volume, and diluted to 5 ml with pentane. A 2·5 ml aliquot was removed for a total lipid determination by evaporation to dryness in tared 150-ml beakers. The remaining 2·5-ml sample was subjected to a florisil column cleanup, as employed by Witt *et al.*[6] The purified samples were subsequently analyzed for DDT, DDE, and DDD by electron-capture gas chromatography by the method Witt *et al.*[7] Recovery standards for the three compounds ranged from 75–82 per cent.

A distribution *in vitro* of DDT was obtained by adding 1·50 mg DDT in 5 μl of ethanol to the sucrose medium prior to homogenization. This was considered to be sufficient mixing and therefore was followed by only a short incubation period.

RESULTS

The average uptake and distribution of DDT, DDD, and DDE by 2-g samples of rat liver from 2–4 animals are shown in Table 1. Sixteen hr after oral administration of 10 mg DDT, the total uptake

TABLE 1. THE DISTRIBUTION OF DDT, DDD AND DDE AMONG THE CELLULAR FRACTIONS OF RAT LIVER AND TOTAL PESTICIDE PER FRACTION ON A LIPID BASIS

Fraction	DDT (μg/ fraction)	DDD (μg/ fraction)	DDE (μg/ fraction)	Per cent of the total analogs per fraction			DDT and its analogs	
				DDT	DDD	DDE	(μg/ fraction)	(μg/ mg fat)
16 hr post, *in vivo*								
Microsome	1·00	0·37	0·07	69·4	25·7	4·9	1·44	0·16
Mitochondria	1·53	0·76	0·10	64·0	31·8	4·2	2·39	0·18
Crude nuclear	0·95	0·38	0·05	69·1	28·1	3·7	1·38	0·22
Soluble	1·68	0·73	0·11	67·0	28·8	4·2	2·47	0·41
Total	5·16	2·23	0·33				7·68	0·97
Blood	0·42	0·10	0·05	73·7	17·5	8·8	0·55	
1st week post, *in vivo*								
Microsome	0·81	0·36	0·15	61·2	27·6	11·3	1·32	0·13
Mitochondria	0·66	0·34	0·10	59·9	31·0	9·1	1·09	0·11
Crude nuclear	0·20	0·10	0·03	61·0	29·0	10·0	0·33	0·08
Soluble	0·18	0·11	0·05	54·0	30·8	15·2	0·34	0·16
Total	1·85	0·91	0·33				3·06	0·48
Blood	0·08	0·01	0·03	66·7	8·3	25·0	0·12	
Control								
Microsome	0·11	0·03	0·02	71·9	18·3	9·8	0·15	0·01
Mitochondria	0·12	0·02	0·01	80·1	13·2	6·6	0·15	0·01
Crude nuclear	0·19	0·02	0·01	85·6	9·9	4·5	0·22	0·04
Soluble	0·06	0·02	0·01	70·1	20·7	9·2	0·09	0·02
Total	0·48	0·09	0·05				0·61	0·08
Blood	0·01	0·01	0·01	33·3	33·3	33·3	0·02	
In vitro								
Microsome	0·24							0·03
Mitochondria	0·29							0·03
Crude nuclear	0·30							0·06
Soluble	0·17							0·15
Total	1·00							

in vivo was 5·16 μg DDT, 2·23 μg DDD, and 0·33 μg DDE. Approximately 60 per cent of the DDT is divided between the soluble and mitochondrial fractions on a roughly equal basis, the remaining 40 per cent being split between the nuclear and microsomal fractions. The distribution of DDD and DDE is of the same order of magnitude.

It can be seen from the portion of Table 1 labeled "Per cent of the total analogs per fraction" that 25–32 per cent of the pesticide found in the various fractions was DDD. In all fractions, DDT was the most predominant material found.

Liver samples from rats sacrificed 1 week post treatment (Table 1) showed decreases in total DDT, DDD, and DDE contents. The soluble fraction displayed the most marked depletion of DDT; by comparison, the microsomal fraction decreased only slightly. The shift in distribution from the soluble fraction to the microsomal fraction accompanying this depletion is not attended by a variation in the overall distribution of the nuclear and mitochondrial fractions. There was a noticeable increase in the per cent of the total analogs represented by DDE, with a slight increase in DDD.

The livers of control rats contained predominantly DDT with only small amounts of DDD and DDE. A salient feature of the control study is the low level of DDD. It is noteworthy that the nuclear fraction contained almost 40 per cent of the DDT present in the control samples but maintained about the same concentration as the first week post-treatment samples (0·19 and 0·20 μg, respectively).

Table 1 contains the correlation between the lipid determination and the pesticide content. The lipid extracted probably contains some lipoprotein. *In vitro*, the nuclear fraction displayed the greatest concentration of DDT on a lipid basis (Table 1). The soluble fraction had a higher value than the approximately equal values of the mitochondrial and microsomal fractions. This relationship is similar to the total analog values obtained for the control livers. In the 16-hr post-treatment and 1-week post-treatment studies *in vivo*, however, the soluble fraction had the highest concentration of analogs per mg of lipid.

DISCUSSION

The detection of DDD and DDE in rat liver is consistent with that reported in the literature, as is the finding of DDD as the principal DDT metabolite.[8] The data show that there is apparently no complete segregation of pesticide from any one fraction. Some sort of shift or redistribution of pesticide with time seems to be taking place, but it is not necessarily uniform. The pesticide content and redistribution seem to be a function of the particular point in time.

A seemingly tenable interpretation of the study *in vitro* would be that this distribution is an expression of the relative DDT binding affinities of the various components of the intracellular milieu. The assumption is made that very little, if any, of the pesticide exists in a free, unbound state. This follows from the fact that DDT has an extremely low solubility in water of 1 ppb,[9] and that any colloidally dispersed DDT would be preferentially bound by hydrophobic groups of the cellular milieu.

If this study is an indication of binding affinities, then the lack of uniformity in the DDT distribution on a lipid basis, as seen in the study *in vitro* (Table 1), leads to the supposition that there is a capability for non-lipid binding. This is easily seen in the control study, and may account for some of the lack of uniformity in the studies *in vivo*, even though the system is flooded with pesticide. This lack of uniformity may also be an indication either of incomplete extraction of different lipid species or of the relative accessibility to binding of the lipids in the various fractions.

The "first week post" pesticide distribution was not parallel with the passive distribution *in vitro*. However, the control distribution of DDT was very similar to the results *in vitro*, indicating that a passive DDT distribution does occur in time or at low levels of dosage. It is of interest to note that the DDT level in the nuclear fraction was approximately equal in the control and first week post samples. This indicates that some sort of unusual binding occurred and is further evidence supporting non-lipid binding.

Morello[3] demonstrated that rat liver microsomes detoxify DDT, although he was unsure of the detoxification products. Peterson and Robison[10] found that rat liver cells metabolize DDT to DDD, and to a lesser extent to DDE. In accordance with these findings, the data in Table 1 show that in the 16 hr post liver cells the primary detoxification product, DDD, represents a greater per cent of the total analogs than it did in the blood, indicating that the liver cells seem to have detoxified DDT. Some detoxification of the ingested DDT to DDD (but not to DDE) has probably been shown to occur in the intestine.[11] The presence of DDE in the blood is also indicative of liver cell detoxification,

as this step is not known to occur elsewhere. Undoubtedly, after 16 hr, a small amount of the DDD and most of the DDE found in the blood are of liver cell origin.

It is important to note at this point that Peterson and Robison[10] found DDE to be a terminal metabolite, whereas DDD is further metabolized to more polar analogs. Thus, once formed, DDE is seen to be efficiently transferred back to the blood for transport to adipose tissue, where it is found as the principal metabolite,[12] or to the kidney for excretion. This accounts for the fact that DDE represents a greater per cent of the total analogs in the blood than in the various cell fractions in both studies *in vivo*. DDT is also metabolized to DDD, and then further metabolized in a seemingly slow step to undetected polar derivatives, which are then probably excreted. This follows from the high levels of DDD in the first week post liver fractions and the low levels in the corresponding blood sample.

It is also noteworthy that the detoxification of DDT is either more efficient or more complete by the first week post treatment. This is consistent with the work by Morell[3] in which DDT was seen to stimulate the synthesis of microsomal DDT metabolizing enzymes over a 96-hr period. This efficiency is absent in control samples.

Acknowledgements—The authors thank J. M. Witt and E. J. Apple for their advice during the course of these experiments.

This work was supported in part by Grant No. EF-00627-02 from USPHS.

Department of Dairy Science,
The University of Arizona,
Tuscon, Arizona, U.S.A.

D. A. Vessey
L. S. Maynard*
W. H. Brown
J. W. Stull

* Department of Entomology, The University of Arizona, Tuscon, Arizona.

REFERENCES

1. V. H. Parker, *Biochem. J.* **77**, 74 (1960).
2. I. J. Tinsley, *Biochem. Pharmac.* **14**, 874 (1965).
3. A. Morello, *Can. J. Biochem. Physiol.* **43**, 1289 (1965).
4. L. G. Hart and J. R. Fouts, *Proc. Soc. exp. Biol. Med.* **114**, 388 (1963).
5. W. C. Schneider and G. H. Hogeboom, *J. biol. Chem.* **183**, 123 (1950).
6. J. M. Witt, W. H. Brown, G. I. Shaw, L. S. Maynard, L. M. Sullivan, F. M. Whiting and J. W. Stull, *Bull. envir. Contam. Toxic.* **1**, 887 (1966).
7. J. M. Witt, F. M. Whiting, W. H. Brown and J. W. Stull, *J. Dairy Sci.* **49**, 370 (1966).
8. C. M. Menzie, *Special Scientific Report—Wildlife No. 96.* U.S. Dept. of the Interior, Washington, D.C. (1966).
9. M. C. Bowman, F. Acree and M. K. Corbett, *J. agric. Fd Chem.* **8**, 406 (1960).
10. J. E. Peterson and W. H. Robison, *Toxic. appl. Pharmac.* **6**, 321 (1964).
11. R. S. Barker, F. O. Morrison and R. S. Whitaker, *Nature, Lond.* **205**, 621 (1965).
12. W. J. Hayes, *A. Rev. Pharmac.* **5**, 27 (1965).