Results obtained from the densitometry of the spots are remarkably close to those obtained from fatty acid analysis of the fractions. Figure 1 shows three selected patterns obtained by densitometry. The values in Table II, column C, are averages of 5 to 6 separate determinations made on each plate. The variations in each set ranged from 5% to 10%. Values obtained from densitometric curves represent groups of glycerides rather than one particular type. From the analytical results, it can be concluded the fatty acid distribution in liver fats follows the mathematical pattern of 2 random, 1-3 random, suggested by Vander Wal (15). Some differences were noted in the distribution of SSM and SMS and also between SMM and MSM, yet, the total amounts of glycerides in each fraction were not significantly different.

Jurriens and Kroesen (9) also found a similar agreement with the calculated values in lard, cocoa butter and palm oil. The results on rat liver triglyceride composition in rats presented here are in variance with these reported by Blank et al. (10). The only possible explanation appears to be the differences in the dietary fat and/or strains of experimental animals.

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Application of Thin-Layer Chromatography to the Quantitative Estimation of Tissue Triglycerides II. Influence of Methyl Parathion on the Composition of Liver Triglycerides in the Rat[1]

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Abstract

Methyl parathion fed at 10 ppm in a high protein low fat diet inhibited 46.9% of the total liver carboxylesterase activity. The total fatty acid composition of liver triglycerides was not significantly altered. However, the methyl parathion-fed rats showed a higher percentage of saturated acids in the 2-position of the glyceride molecule. Triglyceride analysis employing the multiple TLC-GLC technique (5) also showed a slightly higher percentage of saturated glycerides and those containing 1 and 2 double bonds than those in the control group. Triglyceride patterns in both groups were in general agreement with those calculated from the fatty acid distribution as suggested by Vander Wal (11).

IN A SERIES OF STARCH GEL electrophoretic studies McKinley and Read (1,2) demonstrated that organophosphorus pesticides inhibit liver carboxylesterases. Since some of the carboxylesterases may act as lipases the authors believe that the presence of an organophosphate might upset the enzyme balance influencing the triglyceride synthesis in the liver.

With the multiple technique employing thin-layer chromatography (TLC) and gas chromatography (GLC) reported by Jurriens and Kroesen (3), Blank et al. (4) and Sahasrabudhe (5) it has been possible to quantitatively determine the glyceride structure of tissue fats.

The experiments reported here were designed to investigate the influence of methyl parathion on the liver triglycerides in the rat.

Experimental

Weanling male rats of an inbred Wistar strain of the Food and Drug colony were divided into 2 groups and housed in individual cages. The two groups were fed ad libitum for 4 weeks a commercial diet containing 20% protein and 4% fat supplemented with 3% corn oil. One diet contained in addition 10 ppm methyl parathion. The pesticide was dissolved in corn oil and dispersed in the diet. At the end of the experimental period, the rats were anesthetised with chloroform and bled through the abdominal aorta. The livers were then excised, pooled in groups of 5 each, frozen at −70C and stored at −20C. The study was carried out in 2 separate series with 30 animals each.

Carboxylesterase Analysis

Representative samples were taken from each group. Liver tissues were homogenized and prepared for analysis as described by McKinley and Read (1). Quantitative estimation of the carboxylesterases was carried out according to the method of Main et al. (6) using a 1:25 dilution of the supernatant and o-nitrophenyl butyrate as the substrate. Liver carboxyl-

[1] Presented at the AOCS meeting in Houston, Texas, April, 1965.

TABLE I

Fatty Acid Composition of Rat Liver Triglycerides

Fatty acids		Control				Methyl parathion			
		Total		2-monoglyceride		Total		2-monoglyceride	
S	C10:0	0.28	(0.1–0.4)	t	(t–0.1)	0.16	(0.1–0.3)	t	(t–0.1)
	C12:0	0.37	(0.2–0.6)	0.30	(0.2–0.4)	0.33	(0.2–0.4)	0.31	
	C14:0	0.47	(0.2–0.8)	1.0	(0.8–1.2)	1.25	(1.0–1.6)	3.52	(3.0–4.2)
	C15:0	—		—		—			
	C16:0	20.82	(19.5–23.8)	9.7	(8.5–10.2)	23.6	(20.5–26.7)	16.41	(14.0–18.1)
	C17:0	0.16	(0.1–0.2)	t	(t–0.1)	3.2	(2.9–3.9)	—	
	C18:0	2.81	(2.5–3.9)	0.8	(0.6–1.0)	0.8	(0.4–2.0)	1.80	(1.6–2.2)
	C20:0	0.19	(0.1–0.3)					—	(t–0.1)
M	C14:1	0.49	(0.1–0.6)	0.4	(0.1–0.8)	—			
	C16:1	0.94	(0.6–1.3)	0.4	(0.2–0.6)	1.3	(1.2–1.4)	0.64	(0.4–0.8)
	C17:1	0.35	(0.3–0.4)	—		—		—	
	C18:1	25.45	(23.5–26.6)	26.01	(25.2–26.8)	23.6	(21.9–24.2)	24.61	(24.0–25.1)
	C20:1	0.47	(0.2–1.0)	0.2	(0.1–0.3)	1.0	(0.8–1.4)	—	
D	C18:2	41.75	(39.9–43.0)	58.10	(56.3–59.2)	36.23	(35.2–37.1)	48.00	(47.2–48.8)
T	C18:3	1.10	(0.8–1.6)	0.60	(0.3–0.8)	1.62	(1.0–3.0)	2.00	(1.0–2.8)
X	C20:4	4.37	(3.9–4.6)	1.5	(1.0–2.1)	5.00	(3.6–6.0)	4.00	(3.1–5.1)
	SSS[a]	1.16				2.25			
	SSU	4.94				9.98			
	SUS	9.04				9.06			
	UUS	39.52				35.00			
	USU	5.27				9.55			
	UUU	41.02				33.80			

Fatty acids are mole %; Figures in parenthesis are ranges; S—Saturated; M—Monoenoic; D—Dienoic; T—Trienoic; X—others; U—Total Unsaturated; t—trace; [a]—Triglycerides as calculated according to Vander Wal.

esterase activity was calculated in kilo units from the formula:

$$KU = \frac{\text{optical density per gram liver X liver wt. (g)}}{\text{optical density per 1 } \mu\text{mole o-nitrophenol X reaction time (min)}}$$

Starch gel electrophoresis was carried out on undiluted supernatant and the carboxylesterases detected as red brown bands by coupling the hydrolysis product (α-naphthol) with Azoene Fast Blue RR Salt (1).

Triglyceride Analysis

Livers in groups of 5 were extracted with methanol-chloroform (7) and the extracted lipids were fractionated by TLC. Details of the procedure are described in the previous paper (5). The triglyceride fraction obtained from TLC was analysed for total fatty acids and those in the 2-position of the glyceride molecule (8). The fatty acids were estimated as methyl esters by GLC (5). The triglycerides were then fractionated by TLC on Silica-gel-G impregnated with 12% $AgNO_3$ into different types based on the degree of unsaturation. The various fractions were then analysed for the total fatty acids and those in the 2-position as above.

Results and Discussion

Methyl parathion was chosen on the basis of the findings of McKinley and Read (1). When several organophosphorus pesticides were studied in vitro for their influence on carboxylesterases of rabbit livers, methyl parathion showed the maximum inhibition. Observations on rat livers have also been reported by the same authors on parathion (9,10).

The growth rates of rats in the control and the methyl parathion fed groups did not show any significant difference. Increase in body weights for the two groups over the 4-week experimental period was 71.8 ± 3 and 73.5 ± 2 g, respectively. Liver weights were also similar (6.38 ± 0.2 and 6.47 ± 0.2). Livers showed no gross pathological defects in either group.

Figure 1 shows the carboxylesterase patterns of livers. A general inhibition of all carboxylesterases is observed. KU values for the control and the parathion fed group were found to be 9.45 ± 0.7 and5.02 ± 0.5. respectively, indicating a 46.9% inhibition of carboxylesterase activity by methyl parathion.

Table I gives the fatty acid distribution in the overall triglycerides. Values reported are the average of 3 in each group. Differences in the total fatty acid make-up are not significant. The group fed methyl parathion shows a higher proportion of $C_{16:0}$ in the 2-position as compared to the control.

From the values in Table I the glyceride distribution was calculated as suggested by Vander Wal (11). Glyceride types in the individual TLC fractions containing 0–3 double bonds are shown in Table II along with those calculated from the densitometric curves. Values obtained from densitometric curves represent

TABLE II

Glyceride Distribution in Rat Liver Triglycerides (%)

		Control			Methyl parathion		
		A	B	C	A	B	C
0	SSS	1.1	0.9	1.5	2.5	3.5	4.0
1	SSM	2.0	2.5		4.0	3.2	
	SMS	2.8	2.0		2.8	3.6	
		4.8	4.5	4.8	6.8	7.0	7.0
2	SMM	4.8	5.0	4.5	4.4	4.0	6.2
	MSM	0.9	1.1		1.6	3.0	
	SSD	2.4	2.5	9.0	4.6	6.0	11.0
	SDS	5.9	5.8		5.6	4.8	
		14.0	14.4	13.5	16.2	17.8	17.2
3	SMD	5.7	6.2	15.4	5.1	3.0	16.2
	SDM	10.4	8.6		8.7	10.4	
	MSD	2.1	1.6		3.6	3.8	
	MMM	2.1	2.0		1.7	3.2	4.2
	SST	0.1	0.1	5.6	0.2	0.2	
	STS	++	++				
		20.4	18.5	20.0	19.5	20.6	20.4
4	MMD	5.1		10.6	4.0		8.0
	MDM	4.5			3.4		
	SDD	12.4			10.0		
	DSD	1.3		12.4	2.0		
	MST	0.1			0.2		
	STM	0.1			0.4		9.0
	SMT	0.3			0.2		
	SSX	0.1			1.0		
	SXS	0.1			0.9		
		24.3	25.0	24.0	22.1	16.2	17.0
5	MDD	10.8			7.8		
	DMD	3.0		16.1	2.3		
	SMX	1.0			0.8		14.6
	SXM	0.1			1.2		
	MSX	0.4			0.7		
	STD				0.4		
	SDT	0.4			0.5		
	DST	0.1			+		
	TMM	0.2			0.2		
	MTM	0.1			0.1		
	DDD	6.5		20.1	4.5		21.8
	Others	12.7			13.8		
		35.4	37.2	36.2	32.9	35.1	34.4

A, Calculated according to Vander Wal.
B, Calculated from fatty acid analysis of fractions.
C, Densitometry.
S = Saturated, M = Monoenoic, D = Dienoic, T = Trienoic, X = Others.
+ = less than 0.05, ++ = 0.05 to 0.10.

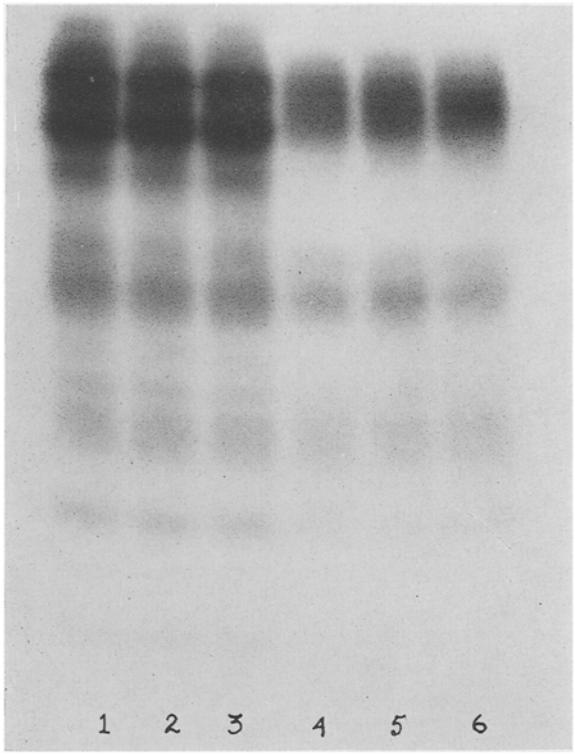

FIG. 1. Vertical starch gel electrophoretic patterns of rat liver carboxylesterases. Samples 1, 2 and 3 from control animals; 4, 5 and 6 from animals fed 10 ppm methyl parathion. Analysis conditions: Borate buffer pH 8.4; temperature during run 8C; voltage 200 v; current 18 ma; anode end at the bottom of photograph.

groups of glycerides rather than one particular type. The methyl parathion group showed differences in the proportions of different types in each class, particularly ratios of SMM: MSM and SMD: SDM; the total amount of each type in a particular fraction, however, is similar to that calculated according to Vander Wal.

The experimental group shows a higher proportion of saturated glycerides and those with 1 and 2 double bonds as compared to the control. This, in part, can be explained on the basis of the higher proportion of saturated fatty acid in the 2-position. In general, the glyceride distribution follows the pattern calculated according to Vander Wal. It can also be concluded that the presence of an organophosphate influences the mechanism by which the fatty acids are distributed in the three positions of the glyceride molecule.

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