tected. No abnormal symptoms or effects on milk production were observed while conducting the feeding experiments.

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Mechanism of Dieldrin-Induced Fat Accumulation in Rat Liver

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Dieldrin was administered orally to male albino rats at a dose level of 30 mg/kg and the effects on hepatic lipid metabolism were determined. Liver total lipid content was increased ($p < 0.05$) and this change was confined only to the triglyceride fraction; phospholipid and cholesterol levels remained unaltered. This was paralleled by an increase in incorporation of glucose-^{14}C into glyceride-glycerol. The incorporation of the isotope into fatty acids and the activity of hepatic fatty acid synthetase were significantly reduced in insecticide-administered rats, indicating an inhibition of lipogenesis by dieldrin. The secretion of triglycerides into plasma is unaffected. Hence, the accumulation of fat in the liver during dieldrin toxicity is ascribed to enhanced hepatic synthesis of triglycerides, due to increased availability of free fatty acids and α-glycerophosphate.

Studies on the effect of chlorinated hydrocarbon insecticides on mammalian biochemical functions have, of late, generated considerable interest. In recent reports from this laboratory we demonstrated manifold disturbances in intermediary metabolism of rats receiving a single oral dose of dieldrin (Bhatia *et al.*, 1971, 1972a,b; Bhatia, 1972). The findings included hyperglycemia, lowered glucose tolerance, enhanced hepatic gluconeogenesis, deposition of glycogen in the liver, and an increase in plasma nonesterified fatty acid (NEFA) level. Stimulation of lipolysis resulting in elevated plasma NEFA has also been observed in DDT-intoxicated rats (Schwabe, 1964). The enhanced mobilization of fat, among other factors, is generally associated with conditions which lead to accumulation of fat in the liver (Isselbacher and Greenberger, 1964; Lombardi, 1966; Farber, 1967). In this context it is of great interest to examine the effect of dieldrin on hepatic lipid metabolism.

Our preliminary studies indicated a slight but significant increase in the liver total lipids of insecticide-treated rats. In this paper we have attempted to elucidate the mechanism by which dieldrin induces lipid accumulation in the liver.

EXPERIMENTAL

Wistar strain male albino rats weighing 100–120 g were used in the present investigation. The experimental rats were orally administered dieldrin dissolved in groundnut oil at a dose level of 30 mg/kg body weight. The corresponding group of control aimals received 0.2–0.3 ml of groundnut oil only. The rats were sacrificed 24 hr after treatment. During the posttreatment period the animals were kept fasted but had free access to water.

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For studies involving the incorporation of glucose-*U*-^{14}*C* into liver lipids, 20–22 hr-fasted animals were orally administered glucose-*U*-^{14}*C* (specific activity 30 μCi/200 mg) at a dose level of 30 μCi/100 g body weight and sacrificed 3 hr thereafter.

Rats were stunned by a blow on the head and blood was collected in heparinized tubes by cutting the jugular vein. Liver was removed and freed of adhering materials by dipping in chilled normal saline. Samples of the tissue (1–2 g) were dropped in 20 volumes of chloroform–methanol (2:1, v/v) mixture and finely ground with acid-washed sand. In the case of plasma, 1–2 ml of freshly separated plasma was pipetted out dropwise into a flask containing 20 ml of the chloroform–methanol mixture. Lipids were extracted and isolated according to the procedure of Folch *et al.* (1957). Final lipid solutions in chloroform were stored in sealed stoppered tubes at −20° until required for further estimations.

Total lipid content was determined gravimetrically. Phospholipid phosphorus was estimated according to the method of Bartlett (1959) as modified by Marinetti (1962). The phospholipid content was calculated by multiplying the phosphorus value by the factor of 25. Total cholesterol was determined by the procedure of Hanel and Dam (1955). Neutral glycerides, comprised predominantly of triglycerides, were calculated by substracting the sum of cholesterol and phospholipids from total lipid. The results are expressed as mg of glycerides/g of fresh liver.

For determination of radioactivity of glucose-*U*-^{14}*C* incorporated into various lipid fractions, an aliquot of the lipid solution in chloroform was plated in stainless steel planchets and radioactivity was measured in a windowless gas flow counter. Another aliquot containing 5–7 mg of lipid was evaporated to dryness under nitrogen and lipids were saponified (Fain *et al.*, 1963). Nonsaponifiable materials and fatty acids were extracted into petroleum ether (boiling range 40–60°) from the alkaline alcoholic digest

Table I. Effect of Dieldrin on Body Weight, Liver Weight, and Major Chemical Constituents of Rat Liver

Item	Control	Dieldrin	p
Body weight, g			
Initial	118 ± 0.6 (36)	116 ± 0.5 (36)	>0.05
Fasted	106 ± 0.8 (36)	103 ± 0.6 (36)	>0.05
Liver weight, g	3.4 ± 0.05 (24)	4.0 ± 0.04 (24)	
Liver/100 g body			<0.01
weight, g	3.3 ± 0.05 (24)	4.0 ± 0.04 (24)	<0.01
Protein (mg/g			
fresh liver)	148 ± 3.2 (5)	153 ± 2.7 (5)	>0.05
Glycogen (mg/g			
fresh liver)	1.6 ± 0.11 (24)	5.1 ± 0.37 (12)	<0.01
Total lipid (mg/g			
fresh liver)	57.9 ± 1.2 (6)	63.0 ± 1.7 (6)	<0.05

Values given in the table are mean ± S.E. Figures in parentheses indicate number of animals. $p \leq 0.05$ is considered statistically significant.

(Entenman, 1957). Suitable aliquots of these fractions were plated in stainless steel planchets for radioactivity determination. Phospholipids were separated from neutral lipids on thin-layer chromatograms (Misra, 1968) and were eluted with chloroform–methanol–formic acid–water (97:97:4:2, v/v; Abramson and Blecher, 1964). An aliquot of the eluate was used for radioactivity determination and another for phosphorus estimation.

The activity of fatty acid synthesizing enzyme system (EC 6.4.1.2) was determined essentially as described by Abraham *et al.* (1960) in the 10,000 × *g* fraction of the liver homogenate prepared in 0.1 *M* phosphate buffer (pH 7.4). The enzyme assay system containing labeled acetate (2 μCi/6.67 μmol) was incubated aerobically at 37.5° for 60 min before alcoholic KOH was added to stop the reaction. The procedure for saponification, extraction, etc., was the same as described above. When boiled enzyme was added, no appreciable radioactivity was recorded in the fatty acid extracts. One unit of enzyme is that which incorporates one cpm/min into the fatty acids under the assay conditions.

Protein was estimated in the liver homogenates by the method of Lowry *et al.* (1951). Glycogen was isolated from alkali digests of liver (Good *et al.*, 1933), purified by reprecipitating twice with ethanol, and estimated by the phenol–sulfuric acid method of Montgomery (1957).

RESULTS AND DISCUSSION

Feeding of a single oral dose of dieldrin caused a significant ($p < 0.01$) increase in whole liver weight (Table I). This was true even when hepatic weight was expressed as a percent of body weight. The fasting liver glycogen content was increased by more than 200% on tissue weight basis. The concentration of hepatic protein was unaffected by dieldrin treatment. However, that of total lipid showed a slight but significant ($p < 0.05$) increase. This increase is all the more significant ($p < 0.01$) when results are expressed on body weight basis (Table II). On fractionating the lipids, it was observed that the dieldrin-induced changes in total lipid content were confined only to the triglyceride fraction; phospholipid and cholesterol levels remained unaltered. The increase in triglyceride concentration is very significant on both the parameters tested and is in keeping with the view that fatty liver caused by chemical toxicity (DDT, carbon tetrachloride, dimethylnitrosamine, aflatoxin, ethionine, puromycin, and ethanol) is manifested by an increased accumulation of triglycerides (Lombardi, 1966; Farber, 1967; Lieber, 1967; Judah *et al.*, 1970).

This view has led to the concept that the development of fatty liver is due to one or a combination of the following factors: increased fatty acid synthesis in the liver; enhanced mobilization of NEFA from the adipose tissue; decreased oxidation of fatty acids in liver; and impairment of the hepatic triglyceride secretory mechanism.

It is well known that during lipogenesis glucose provides the carbon skeleton for fatty acids. In the present study dieldrin administration was found to cause a very significant decrease in the incorporation of ^{14}C of glucose-*U*-^{14}C into liver fatty acids (Table III). These findings are further substantiated by the observed decrease ($p < 0.01$) in the activity of the fatty acid synthesizing enzyme system on all the parameters tested (Table IV). This is indicative of an inhibition of fatty acid synthesis by dieldrin, thereby eliminating the possibility of enhanced lipogenesis being a causative factor in the hepatic triglyceride accumulation observed in the insecticide-fed rats.

From the present data the effect of dieldrin on fatty acid oxidation in the liver can not be ascertained. However, unlike the fatty liver caused by other chemicals, the lipid accumulation in the liver of dieldrin-fed rats can not possibly be ascribed to diminished lipoprotein formation and/or secretion into the blood for the following reasons. Liver is the major, if not the only, site for synthesis of plasma triglycerides. The triglycerides formed in the liver are released into the blood stream in the form of very low density lipoproteins (Farber, 1967; Fredrickson *et al.*, 1967). Thus, a diminution of lipoprotein synthesis and/or secretion would result in lowering of blood lipids, especially the plasma triglyceride content. In contrast, dieldrin-treated rats exhibit an increase ($p < 0.05$) in plasma triglyceride and phospholipid levels (Table V). These findings indicate that the mechanism of triglyceride secretion into blood is unaffected by dieldrin. Also, since the protein moiety of the very low density lipoproteins is synthesized mainly in the liver (Lombardi, 1966; Farber, 1967), a block in the synthesis and/or secretion of lipoproteins is ordinarily, though not always

Table II. Effect of Dieldrin Administration on Lipid Fractions of Rat Liver

Lipid fraction	mg/g of fresh liver		mg/100 g of body weight[a]	
	Control	Dieldrin	Control	Dieldrin
Total lipid	57.9 ± 1.2	63.0 ± 1.7	189 ± 8.53	249 ± 9.7
	($p < 0.05$)		($p < 0.01$)	
Phospholipids	36.6 ± 0.8	35.8 ± 0.4	119 ± 4.27	141 ± 2.87
	($p > 0.05$)		($p < 0.01$)	
Cholesterol	4.03 ± 0.06	3.96 ± 0.08	13.11 ± 0.50	15.62 ± 0.16
	($p > 0.05$)		($p < 0.01$)	
Neutral glycerides	17.3 ± 1.1	23.2 ± 1.6	56.56 ± 4.87	92.05 ± 7.6
	($p < 0.02$)		($p < 0.01$)	

[a] (mg/g of liver × whole liver weight/body weight) × 100. Values given in the table are mean ± S.E. of the results obtained from six animals. $p \leq 0.05$ is considered statistically significant.

Table III. Effect of a Single Oral Dose of Dieldrin on the Incorporation of Glucose-*U*-^{14}C into Lipid Fractions of Rat Liver

Lipid fraction	Radioactivity incorporated: cpm/g of fresh liver	cpm/100 g of body weight[b]
Total lipid		
Control	14,383 ± 1406	49,115 ± 3984
Dieldrin	16,658 ± 1355	66,941 ± 4603
	($p > 0.05$)	($p < 0.02$)
Cholesterol		
Control	1006 ± 74	3455 ± 260
Dieldrin	956 ± 120	3835 ± 451
	($p > 0.05$)	($p > 0.05$)
Total fatty acids		
Control	1257 ± 119	4297 ± 353
Dieldrin	610 ± 58	2452 ± 215
	($p < 0.01$)	($p < 0.01$)
Phospholipids		
Control	9145 ± 411	31,343 ± 1011
Dieldrin	7439 ± 333	29,359 ± 1976
	($p < 0.02$)	($p > 0.05$)
Neutral glycerides		
Control	4234 ± 1125	14,318 ± 3565
Dieldrin	8262 ± 1501	33,090 ± 5572
	($p > 0.05$)	($p < 0.05$)
Glyceride–glycerol[a]		
Control	12,121 ± 1327	43,736 ± 5960
Dieldrin	15,091 ± 1301	60,651 ± 4504
	($p > 0.05$)	($p > 0.05$)

[a] Obtained by subtracting cholesterol plus total fatty acids counts from total lipid radioactivity. [b] (cpm/g of liver × whole liver weight/body weight) × 100. Values given in the table are mean ± S.E. of the results obtained from five animals. $p \leq 0.05$ is considered statistically significant.

Table IV. Activity of Fatty Acid Synthesizing Enzyme System in the 10,000 × *g* Fraction of Livers of Control and Dieldrin-Treated Rats

Parameter	Enzyme activity (units): Control	Dieldrin	*p*
Per g of fresh liver	546 ± 45	207 ± 18	<0.01
Per 100 g of body weight[a]	1760 ± 138	821 ± 72	<0.01
Per 100 mg of protein	713 ± 58	265 ± 25	<0.01

[a] (units/g of liver × whole liver weight/body weight) × 100. Values given in the table are mean ± S.E. of the results obtained from five animals. $p \leq 0.05$ is considered statistically significant.

Table V. Effect of Dieldrin on Plasma Lipid Fractions of Rat

Lipid fraction	Control	Dieldrin	*p*
	mg/100 ml plasma		
Total lipid	318 ± 11.9	354 ± 11.9	>0.05
Phospholipids	84.8 ± 3.0	97.5 ± 4.7	<0.05
Cholesterol	64.1 ± 1.4	61.7 ± 2.1	>0.05
Neutral glycerides	169 ± 7.7	195 ± 7.4	<0.05

Values given in the table are mean ± S.E. of the results obtained from six animals. $p \leq 0.05$ is considered statistically significant.

(Deamer *et al.*, 1965), accompanied by a diminished synthesis of protein in the liver. It is now well documented that organochlorine insecticides induce hepatic drug metabolizing enzymes, resulting in an increase in microsomal protein synthesis (Street, 1969). Our experiments on the effect of a single oral dose of dieldrin on protein biosynthesis reveal increased *in vivo* incorporation of leucine-*1*-^{14}C into microsomal protein (Bhatia, 1972). Thus, these findings provide strong evidence that the synthesis and secretion of lipoproteins is unaffected by the insecticide and that a factor other than these is responsible for the accumulation of triglycerides in the livers of dieldrin-fed rats.

The development of fatty liver can be ascribed to yet another factor, *viz.*, an enhanced rate of lipolysis. This seems plausible in the present investigation because rats administered a single oral dose of dieldrin exhibit a 45% increase in plasma NEFA level (Bhatia *et al.*, 1972a).

The synthesis of hepatic triglycerides is brought about by the esterification of fatty acids and α-glycerophosphate (Kennedy, 1961) and the rate of this synthesis is directly proportional to the concentration of both substrates (Steinberg, 1963; Nikkila and Ojala, 1965). Further, the uptake of fatty acids by cells is proportional to the concentration of fatty acids in plasma outside the cells (Fritz, 1967). Thus, the increased plasma NEFA level encountered during dieldrin toxicity would be expected to provide fatty acids for the enhanced synthesis of triglycerides.

Another prerequisite for fatty acid synthesis is the generation of α-glycerophosphate. This metabolite can be formed in the liver by the action of glycerophosphate dehydrogenase on the dihydroxyacetone phosphate formed during glycolysis or gluconeogenesis (Stein and Shapiro, 1957) or by the isomerization of glyceraldehyde phosphate formed through the hexose monophosphate (HMP) pathway. Since dieldrin stimulates gluconeogenesis and HMP pathway (Bhatia *et al.*, 1972b), the incorporation of glucose-^{14}C into glyceride–glycerol was therefore determined to assess the availability of α-glycerophosphate for triglyceride synthesis. The findings presented in Table III reveal a considerable increase in the incorporation of radioactivity into glycerol moiety of hepatic glycerides in dieldrin-fed animals. This increase in incorporation, though statistically not significant, was consistently observed in the insecticide group. Further, the counts in the triglyceride fraction of the dieldrin-treated rats are almost twice those observed in the corresponding control animals. It is of interest to note that the incorporation of radioactivity into phospholipids is inhibited by dieldrin, thereby indicating that the glycerol moiety is preferentially utilized for the synthesis of triglycerides. Thus, the increased availability of free fatty acids and glycerophosphate in the liver of insecticide-treated rats leads to an enhanced rate of esterification, and this is the major, if not the only, factor contributing to the accumulation of triglycerides.

The administration of a single oral dose of dieldrin has brought to light a noteworthy feature, *viz.*, inhibition of lipogenesis. This is evidenced by the decreased incorporation of glucose-^{14}C into fatty acids (Table III) and the reduced activity of fatty acid synthesizing enzyme system (Table IV). Since the incorporation of glucose-^{14}C into hepatic cholesterol is not affected by dieldrin on any of the parameters tested, the block in fatty acid synthesis can be visualized to be located at some step or steps subsequent to acetyl CoA formation, either at the acetyl CoA carboxylase reaction or the subsequent condensation of acetyl CoA and malonyl CoA. However, since the acetyl CoA carboxylase has been reported to be the rate-limiting step in fatty acid biosynthesis (Vagelos, 1964), a block at this step is conceivable. In view of the fact that fatty acids and fatty acyl CoA inhibit fatty acid biosynthesis (Vagelos, 1964), the dieldrin-mediated inhibition of lipogenesis can be attributed to increased availability of fatty acids as a result of their enhanced mobilization from adipose tissue.

From the foregoing observations it is evident that despite alterations in lipid metabolism induced by dieldrin, the accumulation of lipids in liver is not of a high magnitude.

The increase in triglycerides, though small, is significant at a level of $p < 0.05$. This is paralleled by an increase in the incorporation of glucose-^{14}C into glyceride–glycerol. Hence, it can be concluded that animals exposed to a single oral dose of dieldrin for 24 hr exhibit only a tendency to accumulate fat. It is likely that with continual exposure to the insecticide for long periods of time fatty changes in the liver would tend to become more severe. This facet of metabolism merits further investigation.

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Influence of Herbicides as Single Applications or Mixtures on Fatty Acid Composition of Cottonseed Oil

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Cottonseed (*Gossypium hirsutum* L. cv Atlas 67) oil composition from crops treated with 13 herbicides representing eight families of compounds was analyzed by gas–liquid chromatography. Minor changes in cottonseed oil fatty acid composition were caused by herbicide applications. However, none of the herbicides caused changes in the composition of cottonseed oil as large as were produced by season, edaphic characteristics, or location.

Current cotton (*Gossypium hirsutum* L.) production practices utilize several herbicides during the growing season. Due to the selectivity patterns of individual herbicides, use of several herbicides is often required to control a broad weed spectrum. Therefore, concomitant application of herbicides as mixtures often offers means of reducing machinery field time and the utilization of pesticides, giving a broad spectrum of weed control. Occasionally, when herbicides are applied as single-tank mixtures, reduced rates of the individual herbicides in a mixture result in weed control equivalent to higher rates of each herbicide independently. A continuous search goes on for new herbicides or improved application methods for established herbicides. Consequently, efficacy of herbicide mixtures or new compounds continues to be a major question on cotton production.

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However, influence of herbicide mixtures or new compounds on crop quality and quantity are separate questions from herbicide efficacy. Increased crop yield resulting from herbicide applications would be of little consequence if the quality of the crop were adversely affected. Wilkinson and Hardcastle (1971) reported that individual applications of nine herbicides did not alter the fatty acid composition of cottonseed oil. Wilkinson and Hardcastle (1972) reported that the influence of eight sequential herbicide application patterns utilizing six commercial herbicides did not alter cottonseed oil composition. However, applications of herbicide mixtures could possibly cause a modification of cottonseed oil fatty acid composition not observed in single or sequential herbicide applications.

Therefore, combinations of established commercial herbicides and several promising new herbicides were applied to field-grown cotton. Values for cottonseed oil fatty acid composition are reported herein.