Biochemical Pharmacology, Vol. 22, pp. 47–57. Pergamon Press, 1973. Printed in Great Britain.

ADAPTIVE RESPONSE OF HEPATIC CARBOHYDRATE METABOLISM TO ORAL ADMINISTRATION OF *p,p'*-1,1,1-TRICHLORO-2,2-*BIS* (*p*-CHLOROPHENYL)ETHANE IN RATS*

SAM KACEW† and RADHEY L. SINGHAL

Department of Pharmacology, University of Ottawa, Faculty of Medicine, Ottawa, Canada K1N 6N5

(*Received* 10 *March* 1972; *accepted* 21 *July* 1972)

Abstract—The effects of an acute dose of *p,p'*-1,1,1-trichloro-2,2-*bis* (*p*-chlorophenyl)-ethane (*p,p'*-DDT) have been investigated on the activities of pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose 1,6-diphosphatase and glucose 6-phosphatase in rat liver. Administration of *p,p'*-DDT (600 mg/kg, p.o.) produced 2- to 2·5-fold stimulation of these key gluconeogenic enzymes in 5 hr, although statistically significant increases could be detected at 1 hr. Blood glucose increased as early as 0·5 hr, attained peak values at 1 hr, and then declined to reach control levels in 3–5 hr. In contrast, hepatic glycogen decreased gradually to 47 per cent of the control values in 1 hr, and was restored by 3 hr following treatment with this insecticide. Administration of *p,p'*-DDT to adrenalectomized rats produced similar changes in blood glucose and hepatic glycogen, suggesting that the insecticide-induced hyperglycemia and glycogenolysis may not be mediated by a release of catecholamines from the adrenals. Dose–response studies revealed that 100 mg/kg of *p,p'*-DDT was the minimal amount necessary to induce statistically significant increases in the activities of hepatic pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose 1,6-diphosphatase and glucose 6-phosphatase. Maximal stimulation of the four key gluconeogenic enzymes was seen generally at 5 hr when rats were treated with a 400–600 mg/kg dose of *p,p'*-DDT. Daily administration of small doses of *p,p'*-DDT (5 or 25 mg/kg) for 45 days also resulted in significant enhancement in the activities of various hepatic gluconeogenic enzymes. Actinomycin D, cycloheximide or ethionine failed to affect the basal levels of either of these hepatic enzymes, but effectively reduced the insecticide-induced increases in various enzyme activities. Treatment of adrenalectomized rats with *p,p'*-DDT enhanced various hepatic enzymes to a degree similar to that observed in intact animals. Furthermore, administration of triamcinolone to *p,p'*-DDT-treated adrenalectomized rats did not potentiate the action of the insecticide on any of the gluconeogenic enzymes examined. Our results suggest that treatment with *p,p'*-DDT produces marked increases in the quartet of key, rate-limiting gluconeogenic enzymes in hepatic tissue which are not mediated through a release of corticosteroids from adrenal glands.

THE USE OF chlorinated hydrocarbon insecticides has been shown to be associated with a variety of alterations in cellular metabolism. Administration of DDT enhances uterine water imbibition and wet weights as well as the incorporation of glucose-^{14}C into uterine protein, lipid and RNA.[1,2] In addition, Singhal *et al.*[3] demonstrated that administration of DDT produces a marked stimulation of uterine glycogen formation

* This study was supported by funds from the Public Health Research Grant (Project No. 605–7–711) of the National Health Grants Program of the Government of Canada and from the Medical Research Council of Canada.

† Predoctoral Trainee of the Medical Research Council of Canada.

as well as of several enzymatic responses in estrogen-deprived animals. Ratcliffe[4] related decreased eggshell thickness and weight as well as increased egg breakages to insecticide contamination and suggested that DDT may cause a disturbance in the estrogen–parathormone regulation of calcium metabolism in certain birds. In the male, pesticide administration was found to decrease testicular growth, inhibit the development of secondary male sexual characteristics, and interfere with the process of spermatogenesis.[5,6] In addition to the effects on animal reproduction, chlorinated hydrocarbon insecticides have been shown to exert prominent pharmacological actions on the cardiovascular and the central nervous system as well as on hepatic and renal tissues.[7–16] Deichmann *et al.*[11] reported that livers of rats exposed to DDT showed fatty infiltration, and Jefferies and French[12] found that the avian hepatic tissue was markedly enlarged after treatment with this hydrocarbon. Increases in hepatic smooth endoplasmic reticulum and atypical mitochondria also have been reported in rats given DDT.[13] In addition, administration of various chlorinated hydrocarbon insecticides was shown to enhance the activity of hepatic microsomal enzymes involved in the metabolism of barbiturates, corticosteroids and other compounds.[17–19]

Recently, Kacew *et al.*[20,21] demonstrated that treatment of rats with DDT resulted in an increased excretion of urinary glucose as well as in a marked stimulation of the gluconeogenic process in kidney cortex. Since in mammals, liver is the only other tissue, besides kidney, which possesses complete enzymatic potential for active gluconeogenesis, the present study was undertaken to investigate the influence of *p,p'*-1,1,1-trichloro-2,2-*bis* (*p*-chlorophenyl)ethane (*p,p'*-DDT) on the activities of the four hepatic enzymes which play a key, rate-limiting role in glucose formation from non-carbohydrate sources. Our results show that a single oral dose of *p,p'*-DDT in rats is capable of enhancing the activities of hepatic pyruvate carboxylase (PC),[1] phosphoenolpyruvate carboxykinase (PEPCK), fructose 1,6-diphosphatase (FD-Pase) and glucose 6-phosphatase (G6-Pase), and that the observed enzymatic responses are related to the dose of the insecticide and are not mediated through a release of adrenocortical steroids.

MATERIALS AND METHODS

Animals. Experiments were carried out in adult male rats of the Wistar strain weighing approximately 200 g and maintained on Master Laboratory chow and water *ad lib.* Some rats were bilaterally adrenalectomized under light pentobarbital anesthesia and used after a postoperative period of 8 days.[20,21] Before receiving any treatment, all animals were starved overnight (16 hr).

Chemicals and doses. All reagents were of the purest grade available. *p,p'*-DDT (Velsicol) was dissolved in corn oil and administered by the oral route. Control animals received an equal volume (1·0 ml) of corn oil. Actinomycin D (40 μg/100 g; Merck, Sharp & Dohme) was injected intraperitoneally in two equally divided doses, 0·5 hr before and 2·5 hr after the administration of the pesticide. Cycloheximide (70 μg/100 g; Upjohn) or ethionine (50 mg/100 g; Sigma) was also given intraperitoneally, 30 min prior to *p,p'*-DDT. Triamcinolone suspension (Lederle) was diluted in physiological saline and given by the intraperitoneal route in a dose of 10 mg/100 g body wt. For examining the chronic influence of *p,p'*-DDT on hepatic gluconeogenic enzymes,

rats were injected intramuscularly with this insecticide at a dose level of 5 or 25 mg/kg daily for 20 or 45 days and killed 24 hr after the last injection. All biochemicals and intermediates used for assaying various enzyme activities were purchased from the Sigma Chemical Co.

Sample preparation and assay methods. Rats were stunned, decapitated and bled. A small portion of liver was rapidly removed, weighed and immersed in 1·0 ml of 30% boiling KOH for assaying glycogen by the anthrone method of Seifter *et al.*[22] The concentration of liver glycogen was expressed as g per 100 g. The remainder of the tissue was also weighed, homogenized in 0·15 M KCl (pH 7·4) and 5% homogenates and supernatant fluids were obtained as described in earlier communications.[20,21] The activities of pyruvate carboxylase,[23] phosphoenolpyruvate carboxykinase[24] and fructose 1,6-diphosphatase[25] were assayed in the supernatant fluid, whereas glucose 6-phosphatase activity was estimated using the whole homogenate.[26] All enzyme assays were carried out under strictly linear kinetic conditions at 37°. Enzyme activities were calculated as micromoles of substrate metabolized per hour per gram of tissue and expressed as specific activity per milligram of protein. Protein was determined according to the method described by Lowry *et al.*[27] Blood glucose was measured according to the method of Somogyi[28] and expressed as milligrams per 100 ml. The results were subjected to statistical evaluation and significant differences between the means calculated as P values. No statistical significance is indicated when the P value is $> 0{\cdot}05$.

RESULTS

Effect of p,p′-*DDT on hepatic gluconeogenic enzymes, glycogen content and blood glucose.* In rats given a single, oral dose of 600 mg/kg of *p,p′*-DDT, consistent tremor, convulsions and hyperpyrexia were observed at 5 hr,[9,10,21] and death generally occurred between the fifth and the sixth hr. The sequential changes in the activities of hepatic pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose 1,6-diphosphatase and glucose 6-phosphatase after the administration of *p,p′*-DDT are shown in Table 1. When expressed per milligram of protein, enzyme activities increased significantly as early as 1 hr and rose progressively to peak values at 5 hr when pyruvate carboxylase was 244 per cent, phosphoenolpyruvate carboxykinase 197 per cent, fructose 1,6-diphosphatase 230 per cent, and glucose 6-phosphatase 217 per cent of the control values. A similar magnitude of response was noted when enzyme activities were calculated either per gram of tissue or per 100 g body wt.

The time-course of *p,p′*-DDT-induced changes in blood glucose and liver glycogen is illustrated in Fig. 1. After the oral intubation of this insecticide (600 mg/kg), blood glucose was increased significantly in 0·5 hr (143 per cent), attained peak values at 1 hr (160 per cent) and then declined to the control levels. In contrast, the amount of hepatic glycogen ($1{\cdot}6 \pm 0{\cdot}4$ g/100 g) decreased gradually to 47 per cent of the control values at 1 hr and was later restored by 3 hr. Our data show that treatment with *p,p′*-DDT results in stimulation of hepatic key gluconeogenic enzymes as well as in hyperglycemia and breakdown of liver glycogen.

Since it is possible that the acute administration of *p,p′*-DDT might result in a release of catecholamines, which in turn could have produced the observed hyperglycemia and glycogenolysis, it was of interest to determine the effects of an acute

TABLE 1. TIME-COURSE OF *p,p'*-DDT-INDUCED STIMULATION OF HEPATIC GLUCONEOGENIC ENZYMES IN MALE RATS*

Enzymes	Time after administration of *p,p'*-DDT (hr)			
	0 (control)	1	3	5
PC	245 ± 11 (100)	365 ± 7 (149)†	438 ± 8 (179)†	598 ± 28 (244)†
PEPCK	13·9 ± 0·5 (100)	17·4 ± 0·2 (125)†	20·9 ± 0·8 (150)†	27·4 ± 2·9 (197)†
FD-Pase	2·8 ± 0 (100)	4·5 ± 0·1 (161)†	6·1 ± 0·1 (218)†	6·4 ± 0·4 (230)†
G6-Pase	3·8 ± 0·1 (100)	5·2 ± 0·2 (137)†	6·6 ± 0·5 (174)†	8·2 ± 0·5 (217)†

* Means ±S.E.M. represent four or more animals in each group. Rats were given 600 mg/kg of *p,p'*-DDT orally and killed either 1, 3 or 5 hr after treatment. Enzyme activities are expressed as micromoles of substrate metabolized per hour per milligram of protein. Data are also given in percentages (in parentheses), taking the values of control animals as 100 per cent. PC = pyruvate carboxylase; PEPCK = phosphoenolpyruvate carboxykinase; FD-Pase = fructose 1,6-diphosphatase; G6-Pase = glucose 6-phosphatase.

† Statistically significant difference when compared with the values of control rats ($P < 0·05$).

oral dose of this insecticide on the concentration of blood glucose and liver glycogen in adrenalectomized rats. Results presented in Table 2 demonstrate sequential changes in blood glucose and hepatic glycogen content after the administration of a 600 mg/kg dose of *p,p'*-DDT. In adrenalectomized animals, blood glucose increased to 219 per cent of the control values 0·5 hr after pesticide treatment and remained

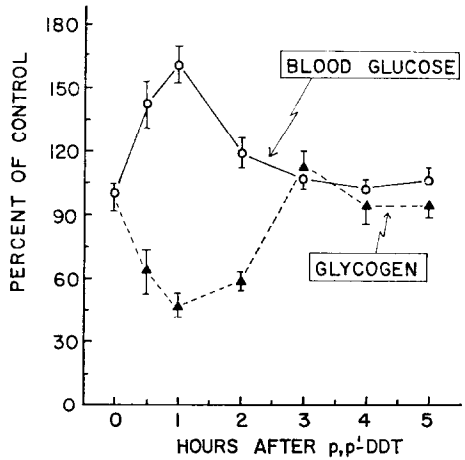

FIG. 1. Time-course of the *p,p'*-DDT-induced changes in blood glucose and liver glycogen. Each point represents the mean ±S.E.M. of four rats in each group. Animals were given *p,p'*-DDT (600 mg/kg) p.o. and killed 0·5, 1, 2, 3, 4 and 5 hr after insecticide treatment. Data are given in percentages, taking the values of control rats as 100 per cent.

TABLE 2. EFFECTS OF *p,p'*-DDT ON BLOOD GLUCOSE AND LIVER GLYCOGEN IN ADRENALECTOMIZED RATS*

Time (hr)	Blood glucose (mg/100 ml)	Liver glycogen (g/100 g)
0 (control)	46 ± 2 (100)	0·73 ± 0·03 (100)
0·5	101 ± 5 (219)†	0·54 ± 0·05 (74)†
1	100 ± 3 (218)†	0·48 ± 0·03 (66)†
2	86 ± 2 (187)†	0·56 ± 0·04 (77)†
4	79 ± 5 (172)†	0·50 ± 0·04 (68)†
5	79 ± 5 (172)†	0·56 ± 0·03 (77)†

* Means ±S.E.M. represent four animals in each group. Adrenalectomized rats were given *p,p'*-DDT (600 mg/kg) p.o. and killed 0·5, 1, 2, 4 and 5 hr after pesticide treatment. Data are also given in percentages (in parentheses), taking the values of control rats as 100 per cent. For abbreviations, see Table 1.

† Statistically significant difference when compared with the values of control animals ($P < 0·05$).

significantly elevated throughout the experimental period. In contrast, the concentration of hepatic glycogen declined to 74 per cent at 0·5 hr, reached the minimal level (66 per cent) at 1 hr, and remained below the control values during the 5-hr period. Since *p,p'*-DDT elevated blood glucose and decreased hepatic glycogen, even in the absence of the adrenal glands, the results seem consistent with the suggestion that the insecticide-induced hyperglycemia and hepatic glycogenolysis are not mediated by the release of catecholamines in response to the stress induced by this chlorinated hydrocarbon.

Effect of varying doses of p,p'-*DDT on hepatic gluconeogenic enzymes.* Since various enzyme activities increased markedly in livers of rats given 600 mg/kg of *p,p'*-DDT, dose–response studies were carried out to ascertain whether smaller amounts of the pesticide also could produce significant responses. Results presented in Fig. 2 show that a 25 mg/kg dose of *p,p'*-DDT failed to exert any measurable effect on any of the enzymes studied. Significant enhancement of pyruvate carboxylase (123 per cent), phosphoenolpyruvate carboxykinase (144 per cent) and fructose 1,6-diphosphatase (154 per cent) was observed with the 100 mg dose of *p,p'*-DDT. In contrast, a 200 mg/kg dose of the pesticide was required to produce statistically significant stimulation (160 per cent) in the activity of hepatic glucose 6-phosphatase. Figure 2 also shows that maximal increases in various gluconeogenic enzymes were observed generally with a 400–600 mg/kg dose of *p,p'*-DDT. These results indicate that the observed enhancement of hepatic enzymes is related to the dose of the insecticide and that statistically significant increases in four gluconeogenic enzymes can be seen with a 100–200 mg/kg dose of *p,p'*-DDT.

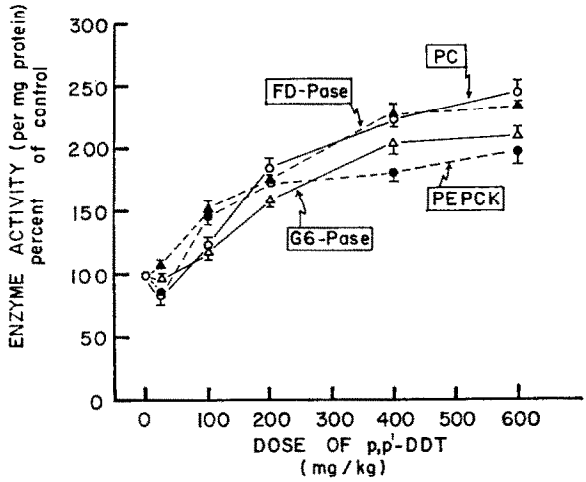

FIG. 2. Effects of varying doses of *p,p'*-DDT on the activities of four key gluconeogenic enzymes in rat liver. Each point represents the mean ±S.E.M. of four or more animals in each group. Rats were given *p,p'*-DDT by the oral route in doses ranging from 25 to 600 mg/kg and killed after 5 hr. Enzyme activities were expressed as micromoles of substrate metabolized per hour per milligram of protein. Data are expressed in percentages, taking the values of control animals as 100 per cent.

Effects of inhibitors of RNA and protein synthesis. In order to examine the nature of the *p,p'*-DDT-stimulated increases in the activities of hepatic pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose 1,6-diphosphatase and glucose 6-phosphatase, the effects of three compounds known to interfere with RNA and protein synthesis were investigated. Results are presented in Table 3. Treatment with either actinomycin D, cycloheximide or ethionine failed to produce any significant change in the basal levels of any of the enzymes studied. However, prior administration of anyone of these compounds to *p,p'*-DDT-treated animals effectively prevented the insecticide-induced enhancement of various gluconeogenic enzymes. Table 3 also shows that among the three inhibitors used, cycloheximide and ethionine seemed to be slightly more effective than actinomycin D in blocking the *p,p'*-DDT-induced increases in hepatic pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose 1,6-diphosphatase and glucose 6-phosphatase.

Effect of p,p'-*DDT in adrenalectomized rats.* Since various glucocorticoid hormones have been shown to act as inducers of hepatic gluconeogenic enzymes,[25,29,30] it was of interest to investigate whether a release of these hormones from adrenal glands was responsible for the enzymatic stimulation observed with *p,p'*-DDT. The influence of *p,p'*-DDT was therefore investigated in adrenalectomized rats and the results are shown in Fig. 3. When *p,p'*-DDT was administered to these rats, activities of the four hepatic gluconeogenic enzymes were elevated to approximately the same extent as that observed in intact animals. Thus, treatment of adrenalectomized rats with *p,p'*-DDT enhanced pyruvate carboxylase to 197 per cent, phosphoenolpyruvate carboxykinase to 227 per cent, fructose 1,6-diphosphatase to 203 per cent, and glucose 6-phosphatase to 196 per cent of the respective control values, indicating that the observed enzyme increases are not affected by adrenalectomy. Further evidence for a lack of adrenal

TABLE 3. INFLUENCE OF INHIBITORS OF RNA AND PROTEIN SYNTHESIS ON, *p,p'*-DDT-INDUCED INCREASES IN HEPATIC GLUCONEOGENIC ENZYMES*

Treatment	PC	PEPCK	FD-Pase	G6-Pase
Control	245 ± 11 (100)	13·9 ± 0·5 (100)	2·8 ± 0 (100)	3·8 ± 0·1 (100)
p,p'-DDT	544 ± 19 (222)†	25·8 ± 1 (185)†	6·3 ± 0·5 (225)†	7·8 ± 0·3 (205)†
Actinomycin D	238 ± 17 (97)	15 ± 1 (107)	2·1 ± 0·2 (75)	4·8 ± 0·6 (126)
Cycloheximide	258 ± 20 (105)	16 ± 1 (114)	2·6 ± 0·1 (93)	4·2 ± 0·5 (111)
Ethionine	260 ± 15 (106)	15 ± 1 (107)	3·0 ± 0·1 (107)	4·9 ± 0·4 (129)
Actinomycin D + *p,p'*-DDT	294 ± 14 (120)‡	16 ± 1·3 (115)‡	3·0 ± 0·1 (107)‡	4·7 ± 0·2 (124)‡
Cycloheximide + *p,p'*-DDT	265 ± 11 (108)‡	15 ± 1 (107)‡	2·7 ± 0·2 (96)‡	4·0 ± 0·1 (100)‡
Ethionine + *p,p'*-DDT	220 ± 11 (90)‡	15 ± 1 (107)‡	3·3 ± 0·3 (118)‡	3·9 ± 0·5 (103)‡

* Means ±S.E.M. represent at least four animals in each group. *p,p'*-DDT was administered orally in a dose of 400 mg/kg and the animals were killed after 5 hr. Actinomycin D (40 μg/100 g) was given in two divided doses, 30 min before and 2·5 hr after DDT treatment. Cycloheximide (70 μg/100 g) and DL-ethionine (50 mg/100 g) were injected intraperitoneally 30 min prior to DDT administration. Enzyme activities are expressed as micromoles of substrate metabolized per hour per milligram of protein. Data are also given in percentages (in parentheses), taking the values of control animals as 100 per cent. For abbreviations, see Table 1.

† Statistically significant difference when compared with the values of control rats ($P < 0·05$).

‡ Statistically significant difference when compared with the values of rats treated with *p,p'*-DDT alone ($P < 0·05$).

mediation in DDT action on hepatic gluconeogenesis was obtained from experiments in which the ability of triamcinolone to potentiate the action of this insecticide was investigated. Figure 3 shows that a single intraperitoneal injection of triamcinolone (10 mg/100 g) to adrenalectomized rats produced small, yet statistically significant, increases in the activities of all four hepatic gluconeogenic enzymes. However, when triamcinolone was administered to *p,p'*-DDT-treated animals, it failed to augment the effects of the pesticide on any of the enzymes investigated. Alterations in the activity of hepatic pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose 1,6-diphosphatase and glucose 6-phosphatase in rats given both *p,p'*-DDT and triamcinolone were of a magnitude similar to that seen in animals receiving *p,p'*-DDT alone. Data suggest that the *p,p'*-DDT-induced stimulation of various key gluconeogenic enzymes is independent of the presence of adrenal glands.

Influence of chronic treatment. In order to examine whether the stimulation of hepatic gluconeogenic enzymes could be produced also by long-term exposure to this pesticide, rats were injected intramuscularly with either a 5 or 25 mg/kg dose of *p,p'*-DDT daily for 20 or 45 days and killed 24 hr after the last injection. Results presented in Table 4 demonstrate that *p,p'*-DDT, when administered in relatively small amounts over a prolonged period was capable of enhancing the activities of all four hepatic gluconeogenic enzymes. Thus, in rats treated with the 5 mg/kg dose of *p,p'*-DDT for

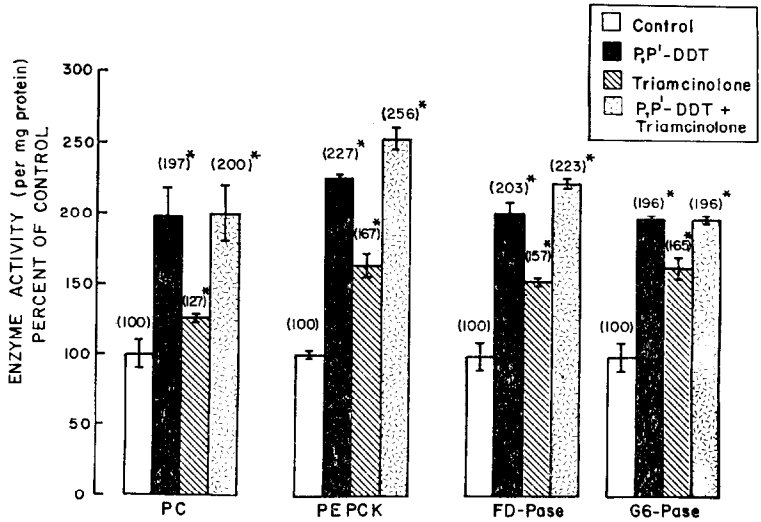

FIG. 3. Independence of *p,p'*-DDT-induced stimulation of hepatic pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose 1,6-diphosphatase and glucose 6-phosphatase from adrenal function. Each bar represents the mean ±S.E.M. of four or more adrenalectomized rats in each group. Adrenalectomized animals were treated with a 600 mg/kg dose of *p,p'*-DDT and killed after 5 hr. Triamcinolone (10 mg/100 g) was administered intraperitoneally, either alone or concurrently with the insecticide. Enzyme activities were expressed as micromoles of substrate metabolized per hour per milligram of protein. Enzyme activities in rats given triamcinolone and *p,p'*-DDT were not different significantly when compared with the values of rats receiving *p,p'*-DDT alone ($P < 0.05$). Data are expressed in percentages, taking the values of control rats as 100 per cent. The asterisks indicate a statistically significant difference when compared with the values of control animals ($P < 0.05$).

TABLE 4. EFFECT OF CHRONIC ADMINISTRATION OF *p,p'*-DDT ON HEPATIC GLUCONEOGENIC ENZYMES*

Enzymes	Treatment for 20 days			Treatment for 45 days		
	Control	*p,p'*-DDT (5 mg/kg)	*p,p'*-DDT (25 mg/kg)	Control	*p,p'*-DDT (5 mg/kg)	*p,p'*-DDT (25 mg/kg)
PC	210 ± 7 (100)	241 ± 7 (115)†	286 ± 9 (136)†	284 ± 19 (100)	392 ± 11 (138)†	489 ± 11 (172)†
PEPCK	9·3 ± 0·2 (100)	11·5 ± 0·5 (124)†	13·1 ± 0·4 (141)†	9·3 ± 0·2 (100)	13·2 ± 0·1 (142)†	16·6 ± 0·9 (178)†
FD-Pase	3·3 ± 0·2 (100)	4·4 ± 0·1 (133)†	5·1 ± 0·1 (155)†	4·1 ± 0·2 (100)	4·9 ± 0·1 (120)†	6·8 ± 0·4 (166)†
G6-Pase	2·8 ± 0·1 (100)	3·1 ± 0·1 (111)	4·2 ± 0·2 (150)†	2·8 ± 0·1 (100)	4·7 ± 0·2 (167)†	5·2 ± 0·3 (187)†

* Means ±S.E.M. represent at least four animals in each group. Rats were injected daily with a 5 or 25 mg/kg dose of *p,p'*-DDT intramuscularly, for either 20 or 45 days, and killed 24 hr after the last injection. Enzyme activities are expressed as micromoles of substrate metabolized per hour per milligram of protein. Data are also given in percentages (in parentheses), taking the values of control animals as 100 per cent. For abbreviations, see Table 1.

† Statistically significant difference when compared with the values of control rats ($P < 0.05$).

20 days, there was already a significant rise in hepatic pyruvate carboxylase, phosphoenolpyruvate carboxykinase and fructose 1,6-diphosphatase, although greater increases in all four gluconeogenic enzymes were produced with the higher dose of the insecticide (25 mg/kg). When the period of treatment was extended for 45 days, even the 5 mg/kg dose of *p,p'*-DDT raised pyruvate carboxylase to 138 per cent, phosphoenolpyruvate carboxykinase to 142 per cent, fructose 1,6-diphosphatase to 120 per cent, and glucose 6-phosphatase to 167 per cent of the control values. As expected, the 25 mg/kg dose of *p,p'*-DDT given for 45 days resulted in more marked enzymatic increases in hepatic tissue. Both doses of *p,p'*-DDT elevated the concentration of urinary glucose (more than 2 per cent, as indicated on the Lilly Tes-Tape) 45 days after the start of insecticide treatment. However, an increase in urinary glucose was detectable as early as 20 days in rats receiving the 25 mg/kg dose of *p,p'*-DDT.[20]

DISCUSSION

The pathway of gluconeogenesis, the series of steps converting pyruvate into glucose, involves several reversible and four irreversible reactions.[31] From the point of view of metabolic control, considerable attention has been focused on the one-way reactions catalyzed by pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose 1,6-diphosphatase and glucose 6-phosphatase. These enzymes are considered to play a rate-limiting role in gluconeogenesis because they all are involved in circumventing thermodynamic barriers, catalyze one-way reactions, and are organ specific, since they occur only in liver and kidney where this process can take place actively.[31,32] Results of the present study demonstrate that treatment with *p,p'*-DDT results in a marked enhancement of various key gluconeogenic enzymes in rat liver. The increases in hepatic enzyme activities were time-dependent, and significant alterations could be detected as early as 1 hr after the insecticide. In addition, elevation in hepatic gluconeogenic enzymes was related to the dose of *p,p'*-DDT, and statistically significant enhancement in all enzyme activities was produced with the 200 mg/kg dose. When animals were treated over a prolonged period with relatively small doses of this insecticide (5 or 25 mg/kg), a significant rise was observed in the activities of the various hepatic enzymes studied. Recently, Bhatia *et al.*[33] demonstrated that hepatic gluconeogenesis was significantly enhanced in rats fed dieldrin. It is of interest that the hepatic key gluconeogenic enzymes also increased in rats exposed to *o,p'*-DDT,[20] another DDT isomer known to be present to the extent of 15–20 per cent in technical grade DDT.[1] Recently, Kacew *et al.*[21] found that both *p,p'*-DDT and *o,p'*-DDT were equally effective in enhancing gluconeogenic enzymes in kidney cortices of male and female rats. The responsiveness of liver and kidney cortex to *o,p'*-DDT and *p,p'*-DDT seems to differ from the action of these two DDT isomers on other tissues. Several investigators demonstrated that in mammalian and avian species, *o,p'*-DDT was more potent than the *p,p'*-isomer in eliciting an estrogen-like response on uterine and oviduct carbohydrate metabolism.[1–3] In addition, Henderson and Woolley[8] and Hrdina *et al.*[9] found that in the central nervous system, *p,p'*-DDT was responsible for producing various neurotoxic symptoms such as hyperpyrexia, tremor and convulsions, whereas the *o,p'*-isomer was completely devoid of any such effects.

The present study also demonstrates that administration of either actinomycin D,

cycloheximide or ethionine can prevent the *p,p'*-DDT-stimulated rise in the activities of hepatic pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose 1,6-diphosphatase and glucose 6-phosphatase seen at 5 hr. The results lend support to the view that new RNA and protein synthesis may be involved in the observed increases in hepatic gluconeogenic enzyme activities induced by *p,p'*-DDT. Recently, Kacew *et al.*[21] found that administration of inhibitors of RNA and protein synthesis significantly blocked the insecticide-induced increases in various gluconeogenic enzymes of rat kidney cortex. Similarly, Singhal *et al.*[3] demonstrated that the elevation in several uterine glycolytic and hexose monophosphate shunt enzymes induced by *o,p'*-DDT was prevented when ovariectomized rats were treated with actinomycin D or cycloheximide. Our results seem consistent with the suggestion that the *p,p'*-DDT-induced increases in the activities of hepatic and renal cortex pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose 1,6-diphosphatase and glucose 6-phosphatase are due to enzyme synthesis *de novo*.

Since *p,p'*-DDT enhanced the four key gluconeogenic enzymes in livers of adrenalectomized rats to the same extent as in normal animals, data indicate that the stimulation of hepatic gluconeogenesis observed after DDT is not dependent upon adrenal function. The observation that a potent glucocorticoid, triamcinolone, failed to potentiate the action of DDT on various hepatic enzymes lends additional support to the view that the observed pesticide-induced effects are not mediated through a release of corticosteroid hormones from the adrenal glands. A similar independence from adrenal function was shown recently by Kacew *et al.*[20,21] for the action of *o,p'*-DDT and *p,p'*-DDT on key gluconeogenic enzymes in kidney cortex.

p,p'-DDT is known to produce marked neurotoxic symptoms characterized by intense tremor and convulsions as well as a marked rise in body temperature.[8–10] Since glucose is not only the major source of energy in the central nervous system but also serves as a fuel for skeletal muscle under anaerobic conditions, it has been suggested that hyperglycemia might be a physiological response of the animal to meet the critical demand for increased energy due to enhanced muscular activity.[34] The increased glucose might then be consumed rapidly by muscles during involuntary contractions after acute DDT poisoning. In the present study, *p,p'*-DDT was found to produce a mild transient hyperglycemia as well as a rapid breakdown of liver glycogen. The observation that *p,p'*-DDT produced both hyperglycemia and hepatic glycogenolysis in adrenalectomized rats suggests that these effects induced by this chlorinated hydrocarbon insecticide are independent of any catecholamine release from the adrenal glands. It is of interest that an intravenous injection of DDT also produced elevation of blood sugar levels in rabbits.[34] The increase was detectable 10–30 min after DDT injection and was maximal after 1–2 hr. Our present observation that chronic administration of *p,p'*-DDT produced an increased excretion of glucose in the urine is in agreement with those reported previously.[20] Pesticide administration results in tubular degeneration and parenchymous alterations as well as vascular congestion in the kidney.[14–16] Why DDT produces only a transient hyperglycemia, despite sustained stimulation of hepatic and kidney cortex gluconeogenesis, in normal intact rats is not entirely clear, but it is conceivable that the renal tubular damage produced by DDT might actually prevent the reabsorption of glucose into the blood stream. Our results are in accord with the suggestion that elevation in urinary glucose levels observed after pesticide treatment may be related to renal tubular changes

as well as to a synchronous induction of all four key gluconeogenic enzymes in liver and kidney cortex.

Acknowledgement—We are grateful to Dr. Percy B. Polen, Principal Regulatory Scientist, Velsicol Chemical Corp., Chicago, Ill., for the generous supply of *p,p'*-DDT.

REFERENCES

1. J. Bitman, H. C. Cecil, S. J. Harris and G. F. Fries, *Science, N.Y.* **162,** 371 (1968).
2. R. M. Welch, W. Levin and A. H. Conney, *Toxic. appl. Pharmac.* **14,** 358 (1969).
3. R. L. Singhal, J. R. E. Valadares and W. S. Schwark, *Biochem. Pharmac.* **19,** 2145 (1970).
4. D. A. Ratcliffe, *Nature, Lond.* **215,** 208 (1967).
5. H. Burlington and V. F. Lindeman, *Proc. Soc. exp. Biol. Med.* **74,** 48 (1950).
6. L. Z. McFarland and P. B. Lacy, *Toxic. appl. Pharmac.* **15,** 441 (1969).
7. L. B. Hinshaw, L. A. Solomon, D. A. Reins, V. Fiorica and T. E. Emerson, *J. Pharmac. exp. Ther.* **153,** 225 (1966).
8. G. L. Henderson and D. E. Woolley, *J. Pharmac. exp. Ther.* **175,** 60 (1970).
9. P. D. Hrdina, R. L. Singhal, D. A. V. Peters and G. M. Ling, *Eur. J. Pharmac.* **15,** 379 (1971).
10. D. A. V. Peters, P. D. Hrdina, R. L. Singhal and G. M. Ling, *J. Neurochem.* **19,** 1131 (1972).
11. W. B. Deichmann, M. Keplinger, I. Dressler and F. Sala, *Toxic. appl. Pharmac.* **14,** 205 (1969).
12. D. J. Jefferies and M. C. French, *Science, N.Y.* **166,** 1278 (1969).
13. R. D. Kimbrough, T. B. Gaines and R. E. Linder, *Archs envir. Hlth* **22,** 460 (1971).
14. E. Danopoulos, K. Milissinos and G. Katsas, *Archs ind. Hyg.* **8,** 582 (1953).
15. N. J. Smith, *J. Am. med. Ass.* **136,** 469 (1948).
16. D. R. Buhler, M. E. Ramusson and W. E. Shanks, *Toxic. appl. Pharmac.* **14,** 535 (1969).
17. L. G. Hart and J. R. Fouts, *Proc. Soc. exp. Biol. Med.* **114,** 388 (1963).
18. A. H. Conney, R. M. Welch, R. Kuntzman and J. J. Burns, *Clin. Pharmac. Ther.* **8,** 2 (1967).
19. C. O. Abernathy, E. Hodgson and F. E. Guthrie, *Biochem. Pharmac.* **20,** 2385 (1971).
20. S. Kacew, R. L. Singhal and G. M. Ling, *Can. J. Biochem.* **50,** 225 (1972).
21. S. Kacew, R. L. Singhal, P. D. Hrdina and G. M. Ling, *J. Pharmac. exp. Ther.* **181,** 234 (1972).
22. S. Seifter, S. C. Dayton, B. Novic and E. Muntwyler, *Archs Biochem.* **25,** 191 (1950).
23. M. C. Scrutton, M. R. Olmsted and M. F. Utter, in *Methods in Enzymology* (Eds. S. P. Colowick and N. O. Kaplan), p. 235. Academic Press, New York (1969).
24. L. J. Phillips and L. J. Berry, *Am. J. Physiol.* **218,** 1440 (1970).
25. G. Weber and R. L. Singhal, *J. biol. Chem.* **239,** 521 (1964).
26. R. L. Singhal, *J. Geront.* **22,** 77 (1967).
27. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193,** 265 (1951).
28. M. Somogyi, *J. biol. Chem.* **160,** 69 (1945).
29. E. Shrago, H. A. Lardy, R. C. Nordlie and D. O. Foster, *J. biol. Chem.* **238,** 3188 (1963).
30. H. V. Henning, I. Seiffert and W. Seubert, *Biochim. biophys. Acta* **77,** 345 (1963).
31. G. Weber, R. L. Singhal and S. K. Srivastava, *Proc. natn. Acad. Sci. U.S.A.* **53,** 96 (1965).
32. G. Weber, R. L. Singhal, E. Fisher and M. A. Mentendick, *Adv. Enzyme Regulat.* **2,** 1 (1964).
33. S. C. Bhatia, S. C. Sharma and T. A. Venkitasubramanian, *Proc. biochem. Soc., Lond.* 77 (1971).
34. E. F. Stohlman and R. D. Lillie, *J. Pharmac. exp. Ther.* **93,** 351 (1948).