Role of Cyclic Adenosine 3':5'-Monophosphate in the Action of 1,1,1-Trichloro-2,2-bis-(*p*-chlorophenyl)ethane (DDT) on Hepatic and Renal Metabolism

By SAM KACEW and RADHEY L. SINGHAL

Department of Pharmacology, Faculty of Medicine, University of Ottawa, Ottawa, Canada

(Received 28 January 1974)

The possibility whether alterations in the cyclic AMP–adenylate cyclase–phosphodiesterase system play a role in the action of 1,1,1-trichloro-2,2-bis-(*p*-chlorophenyl)ethane (DDT) on hepatic and renal carbohydrate metabolism was investigated. Administration of exogenous cyclic AMP (10mg/100g) was found to mimic the action of DDT which enhanced the activities of pyruvate carboxylase, phosphoenolpyruvate carboxylase, fructose 1,6-diphosphatase and glucose 6-phosphatase in both liver and kidney cortex, elevated the concentration of blood glucose and urea and decreased the amount of hepatic glycogen. Treatment with theophylline augmented the effects of a submaximal dose of this halogenated hydrocarbon on serum urea and glucose as well as the key gluconeogenic enzymes in liver and kidney cortex. Addition of DDT *in vitro* to liver and kidney homogenates resulted in a significant enhancement of adenylylase activity. Hepatic and renal slices from rats already treated with DDT displayed an increased ability to convert [³H]adenosine into cyclic [³H]AMP. Whereas kidney-cortex slices excised from rats given caffeine and DDT produced an even greater amount of cyclic [³H]AMP, imidazole, propranolol and hydrazine prevented the insecticide-stimulated rise in cyclic nucleotide production. In contrast, prostaglandin E₁ failed to exert any significant effect on DDT-induced increases in cyclic [³H]AMP synthesis from radioactive adenosine. The present study and our previous findings (Kacew & Singhal, 1973*e*) support the concept that the DDT-induced alterations in carbohydrate metabolism of liver and kidney cortex may be related to an initial stimulation of the cyclic AMP–adenylate cyclase system in these tissues.

Exposure to halogenated hydrocarbon insecticides is known to result in marked alterations in body metabolism. Cardiac damage (Hinshaw *et al.*, 1966), subnormal reproduction (Bitman *et al.*, 1968; Singhal *et al.*, 1970) and disturbances in central nervous system activity (Henderson & Woolley, 1970; Hrdina *et al.*, 1973) are some of the prominent pharmacological consequences attributed to pesticide usage. Hepatic and renal tissues are two of the major sites in which the organochlorine insecticides accumulate and induce morphological alterations (Buhler *et al.*, 1969; Deichmann *et al.*, 1969; Jefferies & French, 1969; Kimbrough *et al.*, 1971). Although the halogenated hydrocarbons such as DDT [1,1,1-trichloro-2,2-bis-(*p*-chlorophenyl)ethane] produce a variety of metabolic changes in kidney and liver (Hrdina *et al.*, 1974), the mechanism involved in triggering the action of these agents remains obscure.

We have shown that treatment of rats with either *op'*-DDT or *pp'*-DDT increased the urinary excretion of glucose and enhanced the glucose-synthesizing potential of liver and kidney cortex (Kacew *et al.*, 1972*a*). Since the cyclic nucleotide cyclic 3':5'-AMP has been shown to play a vital role in the actions of certain hormones and drugs that affect carbohydrate and lipid metabolism (Sutherland & Robison, 1969), it was decided to compare the effects of DDT with those exerted by exogenous cyclic AMP on liver and kidney. In addition, the question of whether DDT treatment (*in vivo*) could increase the ability of renal and hepatic slices to transform [³H]adenosine into cyclic [³H]AMP and augment the activity of adenylylase *in vitro* was examined. Preliminary accounts of portions of this work have appeared (Kacew & Singhal, 1973*e*; Singhal & Kacew, 1973).

Materials and Methods

Animals

Adult male Wistar rats (approx. 200g) maintained on Master Laboratory Chow and water *ad libitum* were used throughout this study. Both treated and control animals were starved overnight (16h) before treatment.

Biochemical assays

For measuring the activities of the four key gluconeogenic enzymes, 5% (w/v) homogenates of kidney cortex and liver were prepared in 0.15M-KCl, pH 7.4. The activities of pyruvate carboxylase, phosphoenolpyruvate carboxylase, fructose 1,6-diphosphatase and glucose 6-phosphatase were assayed under strictly linear kinetic conditions at 37°C, calculated as μmol of substrate metabolized/h per g of tissue and expressed as specific activity/mg of protein as described earlier (Kacew & Singhal, 1973a,b,c; Kacew *et al.*, 1972b, 1973). To examine the effect of DDT *in vitro* on adenylate cyclase activity, various concentrations of the insecticide (10^{-8} , 10^{-6} and 10^{-5}M) were added to hepatic and renal-cortex homogenates obtained from normal untreated rats. Protein was determined by the Folin-phenol method of Lowry *et al.*, (1951). The anthrone method of Seifter *et al.* (1950) was used to measure the concentration of liver glycogen, expressed as g/100g. Blood glucose mg/100ml was measured by the method of Somogyi (1945). The concentration of serum urea (mg/100ml) was determined by using the urease method of Brent & Bergmeyer (1965). To examine

the influence of DDT treatment on cyclic [^3H]AMP synthesis from [^3H]adenosine, slices (approx. 20mg) of renal cortex and liver were incubated for 1h in vials containing 2ml of oxygenated [$\text{O}_2 + \text{CO}_2$ (95:5) gas phase] Krebs-Ringer bicarbonate buffer and 1nmol of [^3H]adenosine (sp. activity 17Ci/mmol) in a Dubnoff metabolic shaker (60 oscillations/min) at 37°C as described by Kacew & Singhal (1974). At the end of incubation, tissue homogenates were subjected to t.l.c., and cyclic AMP spots, detected ($R_F = 0.35$) with u.v. light, were cut from the plate and their radioactivity was measured with a suitable scintillant (Kacew, 1973). The results were expressed as d.p.m./mg wet wt. of tissue (Kacew & Singhal, 1974).

Drugs and Chemicals

All reagents were of the purest grade available and dissolved in double-glass-distilled water. Caffeine (Matheson Co., Cincinnati, Ohio, U.S.A.), imidazole and theophylline (Sigma Chemical Co., St. Louis, Mo., U.S.A.), propranolol (Ayerst, McKenna and Harrison Ltd., Montreal, Que., Canada) hydrazine sulphate (Fisher Scientific Co., Fairlawn, N.J., U.S.A.), prostaglandin E_1 (Upjohn Co., Kalamazoo, Mich., U.S.A.) and cyclic AMP (Calbiochem, San Diego, Calif., U.S.A.) were all dissolved in physiological saline and injected intraperitoneally. DDT (purity >99.8%; Velsicol Chemical Corp., Chicago, Ill., U.S.A.) was dissolved in corn oil and administered orally in a total volume of 1 ml. Control animals received an equal volume of the solvent. [^3H]Adenosine was purchased from the Amersham/

Table 1. Ability of exogenous cyclic AMP to mimic the action of DDT on hepatic and renal carbohydrate metabolism

Mean values $\pm$ S.E.M. represent at least five animals in each group. DDT (10mg/100g) was administered by the oral route whereas cyclic AMP (10mg/100g) along with theophylline (10mg/100g) was injected intraperitoneally in two equally divided doses at 0 and 2½ h. Control rats received the vehicle and all groups of animals were killed 5h later. Enzyme activities were calculated as μmol of substrate metabolized/h per g of tissue and expressed as specific activity/mg of protein. Blood glucose and urea are given in terms of mg/100ml whereas liver glycogen is expressed as g/100g. Results are also given in percentages (in parentheses) taking the values of control animals as 100%.

Tissue examined	Parameter examined	Treatment		
		Control	DDT	Cyclic AMP
Kidney cortex	Pyruvate carboxylase	110 $\pm$ 2 (100)	174 $\pm$ 14 (158)*	171 $\pm$ 8 (155)*
	Phosphoenolpyruvate carboxylase	22 $\pm$ 1 (100)	41 $\pm$ 0 (186)*	36 $\pm$ 1 (165)*
	Fructose 1,6-diphosphatase	4.8 $\pm$ 0.1 (100)	7.2 $\pm$ 0.4 (150)*	8.1 $\pm$ 0.4 (169)*
	Glucose 6-phosphatase	7.4 $\pm$ 0.1 (100)	10.6 $\pm$ 0.2 (143)*	12.5 $\pm$ 0.9 (169)*
Liver	Pyruvate carboxylase	245 $\pm$ 11 (100)	300 $\pm$ 5 (123)*	338 $\pm$ 15 (138)*
	Phosphoenolpyruvate carboxylase	14 $\pm$ 0.5 (100)	20 $\pm$ 0.6 (144)*	27 $\pm$ 1 (190)*
	Fructose 1,6-diphosphatase	4.2 $\pm$ 0 (100)	6.5 $\pm$ 0 (154)*	6.3 $\pm$ 0.3 (150)*
	Glucose 6-phosphatase	7.6 $\pm$ 0.2 (100)	9.2 $\pm$ 1.0 (121)	11.2 $\pm$ 0.4 (148)*
Blood	Glycogen	2.3 $\pm$ 0.2 (100)	1.3 $\pm$ 0.1 (57)*	1.5 $\pm$ 0.1 (65)*
	Glucose	80 $\pm$ 3 (100)	124 $\pm$ 2 (155)*	134 $\pm$ 4 (168)*
	Urea	24.5 $\pm$ 2 (100)	30.6 $\pm$ 0.3 (125)*	33.1 $\pm$ 2 (135)*

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

Fig. 1. Effect of theophylline on the DDT-stimulated increases in hepatic gluconeogenic enzymes

Each bar represents the mean value $\pm$ S.E.M. of four to five animals in each group. Rats were given a submaximal dose of DDT (10mg/100g) by the oral route and killed 5h later. Theophylline (10mg/100g) was injected intraperitoneally in two equally divided doses at 0 and 2½ h. Enzyme activities were calculated as μ mol of substrate metabolized/h per g of tissue and expressed as specific activity/mg of protein. Results in parentheses show percentages of control values taking the values of control rats as 100%. □, Control; ▤, DDT+theophylline; ▨, theophylline (10mg/100g); ▩, DDT (10mg/100g). A, pyruvate carboxylase; B, phosphoenolpyruvate carboxylase; C, fructose 1,6-diphosphatase; D, glucose 6-phosphatase. * Statistically significant difference when compared with the values of control animals ($P < 0.05$). † Statistically significant differences when compared with the values of DDT-treated rats ($P < 0.05$).

Fig. 2. Influence of theophylline on DDT-induced changes in renal cortical gluconeogenic enzymes

Each bar represents the mean value $\pm$ S.E.M. of five animals in each group. Animals were given DDT (10mg/100g) orally and killed after 5h. Theophylline (10mg/100g) was injected intraperitoneally in two equally divided doses at 0 and 2½ h. Enzyme activities were calculated as μ mol of substrate metabolized/h per g of tissue and expressed as specific activity/mg of protein. Results show percentages of control values in parentheses taking the values of control animals as 100%. □, Control; ▤, DDT+theophylline; ▨, theophylline (10mg/100g); ▩, DDT (10mg/100g). A, pyruvate carboxylase; B, phosphoenolpyruvate carboxylase; C, fructose 1,6-diphosphatase; D, glucose 6-phosphatase. * Statistically significant difference when compared with the values of control rats ($P < 0.05$). † Statistically significant difference when compared with the values of animals treated with DDT alone ($P < 0.05$).

Searle Corp. (Don Mills, Ont., Canada). All other biochemicals used for various assays were obtained commercially from Sigma Chemical Co.

Statistical analysis

The results were subjected to statistical evaluation and significant differences between the mean values calculated by Student's *t* test. No statistical significance is shown when the *P* value was >0.05.

Results

Effects of exogenous cyclic AMP and DDT

Table 1 shows a comparison between the effects of cyclic AMP and DDT treatment on the four renal and hepatic key gluconeogenic enzymes, liver glycogen, blood glucose and blood urea in male rats. Administration of cyclic AMP (10mg/100g) or DDT (10mg/100g) was equally effective in significantly increasing the activities of the quartet of kidney and hepatic gluconeogenic enzymes. Greater enzymic increases in both kidney cortex and liver have been noted in rats given a higher dose (60mg/100g) of DDT (Kacew *et al.*, 1972b; Kacew & Singhal, 1973c). Exogenous cyclic AMP also mimicked the effects of DDT by lowering hepatic glycogen and raising the concentration of blood glucose and urea.

Effect of theophylline and DDT

The question as to whether the pesticide-induced metabolic effects could be potentiated by using an inhibitor of phosphodiesterase was examined by studying the influence of concurrent treatment with theophylline and a submaximal dose of DDT (Kacew *et al.*, 1972b; Kacew & Singhal, 1973c). Fig. 1 shows that DDT significantly enhanced the activities of pyruvate carboxylase, phosphoenolpyruvate carboxylase and fructose 1,6-diphosphatase in rat liver. Likewise, when theophylline was injected, minor but statistically significant increases were noted in the activities of hepatic phosphoenolpyruvate carboxylase, fructose 1,6-diphosphatase and glucose 6-phosphatase. However, concurrent treatment with theophylline and DDT further augmented the activities of all four gluconeogenic enzymes in this tissue. Significant enhancement in glucose-synthesizing enzymes was also observed in kidney cortices of either DDT- or theophylline-treated rats (Fig. 2). Administration of theophylline to DDT-treated animals further increased the activity of renal pyruvate carboxylase to 193%, phosphoenolpyruvate carboxylase to 273%, fructose 1,6-diphosphatase to 240% and glucose 6-phosphatase to 200% of the control values. The increases observed in the presence of both theophylline and DDT were significantly higher than those seen with either agent alone. Table 2 shows that simultaneous treatment with DDT and theophylline also potentiated the insecticide-induced

Table 2. Effect of theophylline on DDT-induced changes in blood glucose, liver glycogen and serum urea

Mean values $\pm$ S.E.M. represent at least five animals in each group. Rats were administered DDT (10mg/100g) orally and killed 5h later. Theophylline (10mg/100g) was injected intraperitoneally in two equally divided doses at 0 and 2½h either alone or concurrently with DDT. Results are also given in percentages (in parentheses) taking the values of control animals as 100%.

Treatment	Blood glucose (mg/100ml)	Liver glycogen (g/100g)	Serum urea (mg/100ml)
Control	81 $\pm$ 2 (100)	2.2 $\pm$ 0 (100)	26.1 $\pm$ 0.7 (100)
DDT	124 $\pm$ 2 (153)*	1.3 $\pm$ 0.1 (59)*	30.6 $\pm$ 0.3 (117)*
Theophylline	92 $\pm$ 3 (114)*	2.7 $\pm$ 0.3 (123)	29.9 $\pm$ 0.9 (114)*
DDT + theophylline	143 $\pm$ 5 (177)*†	1.8 $\pm$ 0.2 (82)	39.6 $\pm$ 2.0 (152)*†

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

† Statistically significant difference when compared with the values of DDT-treated animals (*P* < 0.05).

Table 3. Activation of hepatic and renal adenylate cyclase by DDT *in vitro*

Each mean value $\pm$ S.E.M. represents five separate determinations of adenylate cyclase activity. Various concentrations (10^{-8} , 10^{-6} and 10^{-5} M) of DDT dissolved in 95% ethanol were added to the incubation medium in a volume of 0.06ml, just before the enzyme assay. An equivalent amount of 95% ethanol was added to control samples. The activity of adenylate cyclase was calculated as pmol of cyclic AMP formed/h per mg of tissue and expressed as specific activity/mg of protein. Results are also given in percentages (in parentheses) taking the values of control animals as 100%.

Concn. of DDT (M)	Adenylate cyclase activity	
	Liver	Kidney
Control	1.48 $\pm$ 0.14 (100)	2.83 $\pm$ 0.38 (100)
10^{-8}	1.43 $\pm$ 0.31 (97)	3.65 $\pm$ 0.90 (129)
10^{-6}	3.33 $\pm$ 0.53 (225)*	8.27 $\pm$ 0.53 (292)*
10^{-5}	1.70 $\pm$ 0.1 (119)	5.31 $\pm$ 0.82 (188)*

* Statistically significant difference when compared with the control values (*P* < 0.05).

rise in blood glucose and urea. In contrast, the decrease in liver glycogen content observed with DDT alone was not markedly altered by theophylline.

Activation of hepatic and renal adenylate cyclase by DDT *in vitro*

To examine the ability of DDT to enhance cyclic

Fig. 3. Time-course of DDT-stimulated increases in the synthesis of cyclic [³H]AMP by rat kidney cortices

Each point represents the mean value $\pm$ S.E.M. of four rats in each group. Animals were fed on DDT (10mg/100g) and killed 0.5, 1, 2, 3 and 5 h after insecticide treatment. Renal-cortex slices (approx. 20mg) were incubated with Krebs-Ringer bicarbonate buffer containing [³H]adenosine for 1 h at 37°C.

Fig. 4. Sequential changes in hepatic cyclic [³H]AMP production after DDT treatment

Each point represents the mean value $\pm$ S.E.M. of four rats in each group. Animals were given DDT (10mg/100g) and killed 0.5, 1, 3 and 5 h after insecticide treatment. Hepatic tissue (approx. 20mg) was incubated with Krebs-Ringer bicarbonate buffer containing [³H]adenosine for 1 h at 37°C.

AMP formation, the influence *in vitro* of this organochlorine pesticide was investigated on adenylate cyclase activity in liver and kidney-cortex homogenates obtained from normal untreated rats.

Table 3 shows that 1 μ M-DDT significantly increased the activity of adenylate cyclase in both tissues, although no significant change could be noted when 10nM pesticide was added to the incubation mixture. Although 10 μ M-DDT produced a significant rise (88%) in renal adenylate cyclase, maximum stimulation of the enzyme activity in liver (125%) and kidney cortex (192%) was seen in the presence of 1 μ M pesticide.

Effect of DDT on cyclic [³H]AMP synthesis from [³H]adenosine

Since DDT treatment increased the amount of endogenous cyclic AMP (Kacew & Singhal, 1973e) probably by stimulation of adenylate cyclase, it was decided to examine whether this halogenated hydrocarbon could augment the capacity of liver and kidney-cortex slices to convert [³H]adenosine into labelled cyclic AMP. Fig. 3 shows the sequential changes of the DDT-stimulated rise in kidney cyclic [³H]AMP synthesis. The production of labelled cyclic nucleotide increased significantly in tissue slices

Table 4. Effects of certain pharmacological agents on renal cyclic [³H]AMP synthesis

Mean values $\pm$ S.E.M. represent at least five animals in each group. Animals were administered DDT (10mg/100g) orally and killed 1 h later. Caffeine (5.0mg/100g), imidazole (40mg/100g), propranolol (0.3mg/100g), hydrazine (26mg/100g) or prostaglandin E₁ (10 μ g/100g) was injected intraperitoneally either alone or concurrently with DDT. Renal-cortex slices were incubated in Krebs-Ringer bicarbonate buffer containing [³H]adenosine for 60min at 37°C. Cyclic [³H]AMP formation is expressed as d.p.m./mg of kidney cortex. Results are also given in percentages taking the values of control rats as 100%.

Treatment	10 ² × Cyclic [³ H]AMP formation	
	(d.p.m./mg)	(% of control value)
Control	337 $\pm$ 24	100
DDT	530 $\pm$ 13	157*
Caffeine	440 $\pm$ 22	130*
DDT + caffeine	610 $\pm$ 10	181*†
Imidazole	365 $\pm$ 11	108
DDT + imidazole	311 $\pm$ 11	93†
Propranolol	400 $\pm$ 17	119
DDT + propranolol	395 $\pm$ 11	117†
Hydrazine	387 $\pm$ 12	115
DDT + hydrazine	421 $\pm$ 30	125*
Prostaglandin E ₁	360 $\pm$ 47	107
DDT + prostaglandin E ₁	500 $\pm$ 40	148*

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

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

obtained from rats treated with DDT for as little as 30min. It may be noteworthy that the observed pesticide-induced increase in cyclic [^3H]AMP synthesis was maintained for up to 5h in kidney slices obtained from rats treated with DDT. Fig. 4 shows that statistically significant increases in cyclic nucleotide formation were also noted in liver slices excised from animals at $\frac{1}{2}$, 1 and 3h after administration of the pesticide, although maximum stimulation was seen in 30min samples.

Effect of various agents on kidney cyclic [^3H]AMP formation

Table 4 shows the influence of several agents, known to affect carbohydrate and cyclic AMP metabolism, on the ability of liver and kidney to synthesize labelled cyclic AMP from [^3H]adenosine. As expected DDT significantly enhanced the capacity of kidney cortex to form cyclic [^3H]AMP from labelled adenosine. Whereas administration of caffeine (5.0mg/100g) produced a significant rise in the synthesis of renal cyclic [^3H]AMP, imidazole (40mg/100g), propranolol (0.3mg/100g), hydrazine (26mg/100g) and prostaglandin E_1 (10 μg /100g) treatment failed to affect its basal production by this tissue. However, treatment of DDT-fed rats with caffeine further augmented the action of this hydrocarbon on cyclic [^3H]AMP formation. In contrast, whereas administration of either imidazole, hydrazine or propranolol to rats given DDT resulted in a significant lowering of the pesticide-stimulated enhancement in renal cyclic [^3H]AMP synthesis, prostaglandin E_1 failed to exert any significant effect.

Discussion

Pyruvate carboxylase, phosphoenolpyruvate carboxylase, fructose 1,6-diphosphatase and glucose 6-phosphatase are four key rate-limiting enzymes involved in the synthesis of glucose from non-carbohydrate precursors. Our results demonstrate that cyclic AMP produced significant enhancement in the activities of these liver and kidney gluconeogenic enzymes, increased the concentration of blood glucose and urea and decreased hepatic glycogen content. It is noteworthy that cyclic AMP administration has also been shown to stimulate the activities of renal and hepatic phosphoenolpyruvate carboxylase and glucose 6-phosphatase (Guder & Wieland, 1971; Greengard, 1969a; Pagliara & Goodman, 1969; Wicks, 1969). In addition, this cyclic nucleotide was found to elevate the concentration of serum urea and glucose and significantly increase the conversion of non-carbohydrate precursors into glucose (Menahan & Wieland, 1967; Exton & Park, 1968; Weiss *et al.*, 1972). In contrast, a marked fall in glycogen content has been observed in livers of rats injected with cyclic AMP (Exton *et al.*, 1966, 1971). Results of the present

study show that the cyclic AMP-induced metabolic alterations in kidney and liver are qualitatively similar to those seen in organochlorine pesticide-treated animals. It is noteworthy that actinomycin D and cycloheximide can effectively prevent both the cyclic AMP- and the DDT-stimulated increases in the activities of hepatic and renal phosphoenolpyruvate carboxylase and glucose 6-phosphatase (Kacew *et al.*, 1972b; Kacew & Singhal, 1973c; Yeung & Oliver, 1968a; Greengard, 1969b).

If cyclic AMP were to mediate the observed effects of organochlorine insecticides on hepatic and renal carbohydrate metabolism, treatment with a phosphodiesterase inhibitor such as theophylline would be expected to enhance the effects of a small dose of DDT (Kacew *et al.*, 1972b; Kacew & Singhal, 1973c). The present investigation shows that theophylline can further augment the action of a submaximal dose of DDT on renal and hepatic gluconeogenic enzymes, blood urea and glucose. It is noteworthy that incubation of renal slices with caffeine and DDT potentiated the insecticide-stimulated increase in cyclic [^3H]AMP formation from [^3H]adenosine *in vitro* (Kacew & Singhal, 1974). Yeung & Oliver (1968b) demonstrated that the presence of caffeine potentiated the cyclic AMP-stimulated rise in hepatic phosphoenolpyruvate carboxylase. In addition, the lipolysis in fat-cells caused by catecholamines, glucagon and adrenocorticotrophic hormone has been shown to be potentiated by methyl xanthines (Butcher *et al.*, 1968; Blecher *et al.*, 1968; Halkerston *et al.*, 1966). Similarly, theophylline is known to augment the diuretic effect of vasopressin on the toad bladder, the cardiac inotropic response to noradrenaline and the stimulatory effect of isoproterenol on DNA synthesis in parotid glands (Orloff & Handler, 1962; Rall & West, 1963; Malamud, 1969). Singhal *et al.* (1971) demonstrated that treatment with theophylline potentiated the effects of a submaximal dose of testosterone on prostatic and vesicular weights and on the activities of hexokinase, phosphofructokinase, pyruvate kinase, glucose 6-phosphate dehydrogenase and glyceraldehyde 3-phosphate dehydrogenase in orchidectomized rats. Administration of this inhibitor of phosphodiesterase to ovariectomized rats was also found to enhance the oestradiol-stimulated increases in uterine weights and in the activities of several key rate-limiting glycolytic enzymes (Singhal & Lafreniere, 1972).

In view of the various criteria emphasized by Sutherland *et al.* (1969) which must be met to establish the role of cyclic AMP in the action of a given compound, it is important to show that DDT can increase the amounts of endogenous cyclic nucleotide through stimulation of renal and hepatic adenylate cyclase. Administration of DDT was found to enhance the activity of basal and fluoride-stimulated forms of adenylate cyclase and elevate the endogenous amounts

Fig. 5. Possible role of cyclic AMP in the mechanism of action of DDT on hepatic and renal carbohydrate metabolism

of cyclic AMP in both kidney cortex and liver (Kacew & Singhal, 1973e). The present study shows that a marked rise in the activity of adenylate cyclase can be seen when renal and hepatic homogenates are incubated with DDT *in vitro*. In contrast with the observed changes in adenylate cyclase, the activity of phosphodiesterase remained mostly unaffected when rats were treated with DDT (Kacew & Singhal, 1973e). The present results also show that this halogenated hydrocarbon markedly increased the capacity of both renal and hepatic tissue slices to convert [^3H]adenosine into labelled cyclic AMP. Whereas inhibition of phosphodiesterase by caffeine further augmented the DDT-induced rise in kidney cyclic [^3H]AMP synthesis, imidazole prevented the insecticide-stimulated increase in cyclic nucleotide production. It is noteworthy that activation of phosphodiesterase by imidazole has been shown to block the cyclic AMP-induced changes in adipose tissue lipolysis as well as the increase in serum calcium (Goodman, 1969; Avery *et al.*, 1971). Concurrent treatment with DDT and compounds such as hydrazine or propranolol, which depress the synthesis of glucose and decrease phosphoenolpyruvate carboxylase activity (Berk *et al.*, 1970; Fortney *et al.*, 1967; Nakamura *et al.*, 1972; Ray *et al.*, 1970), inhibited the insecticide-stimulated rise in cyclic [^3H]AMP formation. Yeung & Oliver (1968b) found that administration of prostaglandins markedly decreased the activity of phosphoenolpyruvate carboxylase in rat liver. However, Exton *et al.* (1971) reported that prostaglandins failed to alter the gluconeogenic process in hepatic tissue. Our results demonstrate that the DDT-stimulated increase in cyclic [^3H]AMP synthesis from [^3H] adenosine remains unaffected by prostaglandin E_1 .

A possible scheme to help understand the role of cyclic AMP in the mechanism of action of DDT on

liver and kidney carbohydrate metabolism is proposed in Fig. 5. The sequence of events which leads to ultimate changes in hepatic and renal metabolism is initiated when DDT stimulates the synthesis of cyclic AMP from ATP by activating the enzyme, adenylate cyclase. The insecticide-induced rise in endogenous amounts of cyclic AMP subsequently results in marked increases in the activities of hepatic and renal pyruvate carboxylase, phosphoenolpyruvate carboxylase, fructose 1,6-diphosphatase and glucose 6-phosphatase, which can be blocked by inhibitors of RNA and protein synthesis (Kacew *et al.*, 1972b; Kacew & Singhal, 1973c). This scheme shows also that propranolol and hydrazine can interfere with the DDT-stimulated enhancement of cyclic [^3H]AMP synthesis in rat kidney cortex. Whereas imidazole effectively prevented the pesticide-induced rise in cyclic AMP, methyl xanthines potentiated the effects of a submaximal dose of DDT on cyclic AMP synthesis as well as on renal and hepatic gluconeogenic enzymes. All these results taken together would tend to support the concept that the DDT-induced changes in carbohydrate metabolism might involve alterations in the cyclic AMP–adenylate cyclase system of kidney cortex and liver.

This investigation was supported by grants from the Public Health Research Grant (Project no. 605–7–711) of the National Health Grants Program of the Government of Canada and is taken, in part, from a dissertation presented by S. K. (M.R.C. Fellow) to the Graduate School of the University of Ottawa in partial fulfilment of the requirements for the degree of Ph.D. We are grateful to Dr. Percy B. Polen, Principal Regulatory Scientist, Velsicol Chemical Corporation, Chicago, Ill. for generous supplies of DDT and to Dr. John E. Pike, Upjohn Company, Kalamazoo, Mich., for donating prostaglandin E_1 .

References

- Avery, S. H., Bell, N. H. & Stern, P. H. (1971) *Endocrinology* **89**, 951-957
- Berk, J. L., Hagen, J. F. & Beyer, W. H. (1970) *Horm. Metab. Res.* **2**, 277-281
- Bitman, J., Cecil, H. C., Harris, S. J. & Fries, G. F. (1968) *Science* **162**, 371-372
- Blecher, M., Merlino, N. S. & Ro'Ane, J. T. (1968) *J. Biol. Chem.* **243**, 3973-3977
- Brent, E. & Bergmeyer, H. U. (1965) in *Methods of Enzymatic Analysis* (Bergmeyer, H. U., ed.), pp. 401-406, Academic Press, New York
- Buhler, D. R., Ramusson, M. E. & Shanks, W. E. (1969) *Toxicol. Appl. Pharmacol.* **14**, 535-555
- Butcher, R. W., Baird, C. E. & Sutherland, E. W. (1968) *J. Biol. Chem.* **243**, 1705-1712
- Deichmann, W. B., Keplinger, M., Dressler, I. & Sala, F. (1969) *Toxicol. Appl. Pharmacol.* **14**, 205-213
- Exton, J. H. & Park, C. R. (1968) *J. Biol. Chem.* **243**, 4189-4196
- Exton, J. H., Jefferson, L. S., Butcher, R. W. & Park, C. R. (1966) *Amer. J. Med.* **40**, 709-715
- Exton, J. H., Robison, G. A., Sutherland, E. W. & Park, C. R. (1971) *J. Biol. Chem.* **246**, 6166-6177
- Fortney, S. R., Clark, D. A. & Stein, E. (1967) *J. Pharmacol. Exp. Ther.* **156**, 277-284
- Goodman, H. M. (1969) *Proc. Soc. Exp. Biol. Med.* **130**, 97-100
- Greengard, O. (1969a) *Biochem. J.* **115**, 19-24
- Greengard, O. (1969b) *Science* **163**, 891-895
- Guder, W. & Wieland, O. (1971) *Diabetologia* **7**, 482-483
- Halkerston, I. D. K., Feinstein, M. & Hechter, O. (1966) *Proc. Soc. Exp. Biol. Med.* **122**, 896-900
- Henderson, G. L. & Woolley, D. E. (1970) *J. Pharmacol. Exp. Ther.* **175**, 60-68
- Hinshaw, L. B., Solomon, L. A., Reins, D. A., Fiorica, V. & Emerson, T. E. (1966) *J. Pharmacol. Exp. Ther.* **153**, 225-236
- Hrdina, P. D., Singhal, R. L., Peters, D. A. V. & Ling, G. M. (1973) *Toxicol. Appl. Pharmacol.* **25**, 276-288
- Hrdina, P. D., Singhal, R. L. & Ling, G. M. (1974) *Advan. Pharmacol. Chemother.* in the press
- Jefferies, D. J. & French, M. C. (1969) *Science* **166**, 1278-1280
- Kacew, S. (1973) Ph.D. Thesis, University of Ottawa
- Kacew, S. & Singhal, R. L. (1973a) *Toxicol. Appl. Pharmacol.* **24**, 539-544
- Kacew, S. & Singhal, R. L. (1973b) *Environ. Physiol. Biochem.* **3**, 148-153
- Kacew, S. & Singhal, R. L. (1973c) *Biochem. Pharmacol.* **22**, 47-57
- Kacew, S. & Singhal, R. L. (1973d) *Fed. Proc. Fed. Amer. Soc. Exp. Biol.* **32**, 802
- Kacew, S. & Singhal, R. L. (1973e) *Life Sci.* **13**, 1367-1371
- Kacew, S. & Singhal, R. L. (1974) *J. Pharmacol. Exp. Ther.* **188**, 265-276
- Kacew, S., Singhal, R. L. & Ling, G. M. (1972a) *Can. J. Biochem.* **50**, 225-229
- Kacew, S., Singhal, R. L., Hrdina, P. D. & Ling, G. M. (1972b) *J. Pharmacol. Exp. Ther.* **181**, 234-243
- Kimbrough, R. D., Gaines, T. B. & Linder, R. E. (1971) *Arch. Environ. Health* **22**, 460-467
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265-275
- Malamud, D. (1969) *Biochem. Biophys. Res. Commun.* **35**, 754-758
- Menahan, L. A. & Wieland, O. (1967) *Biochem. Biophys. Res. Commun.* **29**, 880-885
- Nakamura, Y., Takemura, Y. & Yamada, T. (1972) *Proc. Soc. Exp. Biol. Med.* **139**, 579-581
- Orloff, J. & Handler, J. S. (1962) *J. Clin. Invest.* **41**, 702-709
- Pagliara, A. S. & Goodman, A. D. (1969) *J. Clin. Invest.* **48**, 1408-1412
- Rall, T. W. & West, T. C. (1963) *J. Pharmacol. Exp. Ther.* **139**, 269-274
- Ray, P. D., Hanson, R. L. & Lardy, H. A. (1970) *J. Biol. Chem.* **245**, 690-696
- Seifter, S., Dayton, S., Novic, B. & Muntwyler, E. (1950) *Arch. Biochem.* **25**, 191-200
- Singhal, R. L. & Kacew, S. (1973) *J. Cell Biol.* **59**, 321a
- Singhal, R. L. & Lafreniere, R. T. (1972) *J. Pharmacol. Exp. Ther.* **180**, 86-97
- Singhal, R. L., Valadares, J. R. E. & Schwark, W. S. (1970) *Biochem. Pharmacol.* **19**, 2145-2155
- Singhal, R. L., Parulekar, M. R., Vijayvargiya, R. & Robison, G. A. (1971) *Biochem. J.* **125**, 329-342
- Somogyi, M. (1945) *J. Biol. Chem.* **160**, 69-73
- Sutherland, E. W. & Robison, G. A. (1969) *Diabetes* **18**, 797-819
- Sutherland, E. W., Hardman, J. G., Butcher, R. W. & Broadus, A. E. (1969) in *Progress in Endocrinology* (Gual, C., ed.), pp. 26-29, Excerpta Medica Foundation
- Weiss, I. W., Morgan, K. & Phang, J. M. (1972) *J. Biol. Chem.* **247**, 760-764
- Wicks, W. D. (1969) *J. Biol. Chem.* **244**, 3941-3950
- Yeung, D. & Oliver, I. T. (1968a) *Biochem. J.* **108**, 325-331
- Yeung, D. & Oliver, I. T. (1968b) *Biochemistry* **7**, 3231-3239