

METABOLIC ALTERATIONS AFTER EXPOSURE OF RATS TO CERTAIN
CHLORINATED HYDROCARBON INSECTICIDES

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PC; Pyruvate carboxylase

FD-Pase; Fructose 1,6-diphosphatase

G6-Pase; Glucose 6-phosphatase

PK; Pyruvate kinase

PFK; Phosphofructokinase

HK; Hexokinase

DDD; 1,1-dichloro-2,2-bis(p-chlorophenyl)ethane

DDA; 1,1-dichloro-2,2-bis(p-chlorophenyl)acetic acid

DDE; 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene

NADH; Nicotinamide adenine dinucleotide (reduced)

Cyclic AMP; Adenosine 3',5'-monophosphate

AMP; Adenosine 5'-monophosphate

ATP; Adenosine 5'-triphosphate

ACTH; Adrenocorticotrophic hormone

DNA; Deoxyribonucleic acid

ADP; Adenosine 5'-diphosphate

GMP; Guanosine monophosphate

GDP; Guanosine diphosphate

GTP; Guanosine triphosphate

RNA; Ribonucleic acid

EEG; Electroencephalogram

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1. INTRODUCTION

Although man has been able to control some of the hostile forces that tend to envelop him, the technological achievements have led to vast proliferation of a variety of pollutants in the environment. One of the major accomplishments of modern day technology has been man's ability to increase food and fiber production through the use of chemical pesticides in the control of pest-vectored diseases. The widespread use of these toxic chemicals in crop spray programs has resulted in contamination of air, water and food ultimately leading to their presence in body tissues. According to Kraybill (1), contamination of environmental substrata by pesticides is in a sense comparable to radioactive fallout in that pesticides are ubiquitous, long-acting and involve populations throughout the world.

Evaluation of the safety of biologically active chemicals such as a pesticide in our environment, demands complete understanding of their metabolism and of the various physiological effects they produce on body functions. Since the discovery in 1939 of the insecticidal effectiveness of DDT, considerable research has been directed towards studying the effects of chlorinated hydrocarbon pesticides on biological systems. Some of the most prominent pharmacological actions of the chlorinated hydrocarbon insecticides include ventricular fibrillation (2) and central nervous system effects characterized by hyperexcitability, tremor, convulsions and hyperpyrexia (3). Pesticide treatment also affects reproductive function (4) and produces marked histopathological alterations in both kidney and liver. Pesticide administration has been shown to result in tubular

degeneration, parenchymous alterations and vascular congestion in the kidney (5-8) as well as fatty degeneration, atypical mitochondria and enlargement of the liver (8-10). Klaassen and Plaa (11) demonstrated that there was an increased excretion of glucose in the urine of mice that were treated with certain aliphatic chlorinated hydrocarbons. One of the major functions of liver and kidney is to convert noncarbohydrate precursors into glucose which serves as an energy fuel for the vital functioning of various cellular activities. In order to investigate whether acute administration of halogenated hydrocarbon insecticides is capable of altering the synthesis of glucose by liver and kidney cortex, the effects of these compounds were examined on the activities of pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose 1,6-diphosphatase and glucose 6-phosphatase, the four key, rate-limiting enzymes involved in the process of gluconeogenesis. In addition, the concentration of blood glucose and of serum urea, a product of protein catabolism, was determined following pesticide treatment. Since the amount of glucose in the blood can also be elevated through hepatic glycogenolysis, the acute effects of chlorinated hydrocarbon insecticides were also studied on glycogen content of this tissue.

The response of various body tissues following the chronic exposure to submaximal doses of chlorinated hydrocarbon insecticides is of vital importance in assessing the effects of these agents on cellular processes. Numerous investigations have shown that environmental stress induced by various chemicals, viruses and toxins produces marked alterations in cellular DNA and protein and over an extended period of time, can culminate into recognizable disease entities (1). In the present study, an attempt

was also made to investigate the effects of chronic exposure to daily small amounts of organochlorine pesticides on hepatic and renal carbohydrate metabolism. The effects of two doses of p,p'-DDT and α -chlordane given for 20 or 45 days were examined on the activities of pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose 1,6-diphosphatase and glucose 6-phosphatase in both kidney cortex and liver.

Recent studies indicate that hepatic and renal carbohydrate metabolism is regulated by a variety of hormones and nutritional factors. One of the mechanisms proposed for the control of glucose synthesis involves the hormonal stimulation of adenyl cyclase which increases the conversion of adenosine triphosphate into cyclic 3',5'-adenosine monophosphate. It is believed that the increase in cyclic AMP then elevates the synthesis of hepatic gluconeogenic enzymes, increases serum glucose and urea and depletes liver glycogen. Indeed, administration of exogenous cyclic AMP has been found to enhance renal and hepatic phosphoenolpyruvate carboxykinase and glucose 6-phosphatase, elevate the concentration of blood glucose and urea and to lower the amount of liver glycogen (12-15). The ability of cyclic AMP and organochlorine pesticides to produce similar changes in carbohydrate metabolism of kidney and liver prompted studies concerning the role of cyclic AMP in the mechanism of action of DDT on these tissues.

This dissertation is concerned primarily with an elucidation of the acute and chronic effects of chlorinated hydrocarbon insecticides on carbohydrate metabolism in kidney cortex and liver. In particular, the influence of p,p'-DDT, α -chlordane, heptachlor and endrin was investigated on hepatic and renal pyruvate carboxylase, phosphoenolpyruvate carboxy-

kinase, fructose 1,6-diphosphatase, glucose 6-phosphatase as well as on the concentrations of blood glucose, liver glycogen and serum urea. The effects of various insecticides given in vivo and in vitro were examined on the ability of liver and kidney to synthesize radioactive cyclic AMP from tritiated adenosine. The influence of propranolol and hydrazine, compounds which depress the synthesis of hepatic glucose and phosphoenolpyruvate carboxykinase (16,17), was also examined to determine whether they could interfere with the action of DDT on the synthesis of cyclic AMP- H^3 . The ability of phosphodiesterase inhibitors such as theophylline to potentiate the action of a submaximal dose of DDT was investigated on hepatic and renal carbohydrate metabolism. Finally, the effects of p,p'-DDT treatment were examined on the endogenous levels of cyclic AMP and the activities of renal and hepatic adenylyl cyclase and phosphodiesterase. Results of the present investigation suggest that the chlorinated hydrocarbon insecticide-induced alterations in carbohydrate metabolism of kidney cortex and liver may be associated with their ability to enhance the synthesis of cyclic AMP in these tissues.

2. LITERATURE REVIEW

I. SOME IMPORTANT FEATURES OF THE ACTION OF CHLORINATED HYDROCARBON PESTICIDES IN MAMMALS

The widespread use of chlorinated hydrocarbon insecticides has resulted in environmental pollution and has presented a variety of health hazards to both man and "wild-life." Although the organochlorine pesticides have been effective in accomplishing their objective of preventing crop loss, the continued use of these compounds without a complete understanding of their influence upon body function has created considerable public concern. Reports concerning the adverse effects of these compounds indicate that their withdrawal from the market has not deterred the irreversible damage which the environment has already suffered (18). Ecologists have attributed decline in the population of birds, fish and mammals to insecticide exposure in certain regions where extensive pesticide treatment programs were carried out (18). According to the estimates of Woodwell et al. (19), over two billion pounds of DDT alone are believed to currently exist in our biosphere. Fahim et al. (20) demonstrated that the average human breast-fed baby in the United States ingests twice the amount of DDT (0.1 mg/kg/day) deemed safe by the World Health Organization, while corresponding British and Swedish infants consume 0.017 mg of DDT/day/kg body weight. Neonates whose mothers were treated with organochlorine pesticides, presented evidence of tumorigenesis, depressed body growth and increased rate of mortality (20,21). The persistence in the body of such compounds as DDT, chlordane, heptachlor and endrin, as well as subsequent

chronic exposure of tissue cells to these substances and their metabolites, emphasize the need for obtaining additional information on the cellular responses during acute and chronic exposure to these insecticides.

A. General Toxicity

In mammals, administration of acute large doses of chlorinated hydrocarbon insecticides produces a variety of toxic effects that invariably lead to death. The LD_{50} following the oral administration of either DDT, chlordane, heptachlor or endrin to rats and other species is presented in Table 1. Among the various pesticides examined, endrin has been found to be the most toxic. It is of interest that the toxicity of various chlorinated hydrocarbon insecticides was dependent upon the age of the animal. Lu et al. (33) reported that the LD_{50} in one-day old rats was approximately 4 g/kg, whereas in 10-day old rats, it was 728 mg/kg (23). As the rats mature, it can be seen from Table 1 that the oral LD_{50} decreased to 200 mg/kg. Sex differences also play a role in the action of insecticides on mammalian organisms. Male animals were less resistant than females to the toxic effects of DDT and chlordane, whereas females were more sensitive to heptachlor and endrin. Finally, species is another factor which is involved in pesticide action. In general, large domestic animals showed greater resistance to acute toxic doses of organochlorine insecticides than the laboratory animals (24,34). In contrast to the large domestic animals, chickens have been found to be considerably more susceptible to acute doses of DDT than the usual laboratory species (24).

B. Tissue Distribution and Storage

Following absorption, the chlorinated hydrocarbons are widely distributed in the tissues; in fact, irrespective of the route of administration, it

TABLE 1
ACUTE TOXICITY OF CHLORINATED HYDROCARBON PESTICIDES

Insecticide	Species Examined	Oral LD ₅₀ (mg/kg)	Reference
DDT	Mice	400	22
1,1,1-Trichloro-2,2-bis (p-chlorophenyl)ethane	Rats (10 days old)	728	23
	Rats (adult)	200	22
	Guinea Pigs	400	22
	Rabbits	300	24
	Cats	300	25
	Monkeys	200	26
Chlordane	Rats	200	27
1,2,4,5,6,7,8,8-Octachloro- 3a,4,7,7a-tetrahydro-4,7- methanoindane	Rabbits	300	27
	Dogs	200-700	28
	Sheep	500-1000	29
Heptachlor	Rats	150	30
1,4,5,6,7,8,8-Heptachloro- 3a,4,7,7a-tetrahydro-4,7- methanoindene			
Endrin	Mice	3	31
1,2,3,4,10,10-Hexachloro- 6,7-epoxy-1,4,4a,5,6,7,8, 8a-octahydro-exo-1,4-exo- 5,8-dimethanonaphthalene	Rats	10	30
	Cats	3-6	32

Tissue distribution studies following daily administration of heptachlor to rats and dogs indicate that accumulation of this compound (as epoxide) occurred primarily in tissue fat, liver, kidney and muscle (45). The pattern of distribution of chlordane in various tissues was shown to be similar to that observed for DDT (46). As expected, the highest conc-

entration of endrin was present in body fat (47).

C. Biotransformation and Excretion

The biotransformation of DDT and related organochlorine insecticides has been extensively reviewed by Hayes (48) and O'Brien (49) and is only briefly summarized here. The major routes of DDT metabolism in mammals are oxidation to DDA; dehydrochlorination to DDE and finally, reductive dechlorination to DDD. In vertebrates, the major metabolite of DDT in feces (50) and urine (51) was found to be DDA. Of DDT-derived ether soluble material in the rat bile, DDT constituted about 3%, DDE 1% and free DDA, 25-35%; the remainder consisting of complexes of DDA or a closely related material (52). Hayes et al. (53) demonstrated that DDE was the principle storage form of ingested DDT in man. However, while man stored about 60% of DDT-derived material in the form of DDE, rats stored only about 22-29% of DDE (54), and monkeys converted little or no DDT to DDE (55). Reductive dechlorination of DDT to DDD in livers of rats was first described by Klein et al. (56). In addition, these authors made an important observation that an isomeric transformation of o,p'-DDT to p,p'-DDT occurred under in vivo conditions in fat stores and liver of the rat.

While our knowledge of the complete metabolic pathways of DDT biotransformation is incomplete, data on the metabolism of other chlorinated hydrocarbon insecticides are even more limited. Chlordane probably undergoes detoxification in the liver, since organically bound chlorine was extracted from the urine of rabbits fed this compound (27). The major metabolite of heptachlor found in dog and rat liver was heptachlor epoxide (57). Endrin accumulated in the body fat of cows and was excreted

unchanged in small amounts in their milk (47).

Excretion of chlorinated hydrocarbon insecticides occurs by way of expired air, urine, feces, milk and dermal secretion. Metabolic studies by Hayes (48) have shown that in the rat, fecal excretion of DDT-derived material exceeded urinary excretion irrespective of the route of administration. It appears that the principle source of DDT metabolites in the feces is bile, since it contained 65% of the intravenously injected radioactive DDT (52). The rate of excretion of organochlorine insecticides in the milk was proportional to their dosage (48).

D. Metabolic Effects of Organochlorine Insecticides

(a) Reproductive Tissues

Interference with reproductive performance and the consequent decline in the population of certain avian species have been recently attributed to environmental pesticide contamination (58-60). Wurster and Wingate (59) have suggested that the decline in the population of the Bermuda petrel may be related to the residues of DDT found in the eggs of this species. Ratcliffe (58) related decreased eggshell thickness and weight as well as increased egg breakages to insecticide contamination and suggested that the pesticide may cause a disturbance in the estrogen-parathormone regulation of calcium metabolism in certain birds. In addition, Burlington and Lindeman (61) demonstrated that DDT produces adverse effects in the cockerel inasmuch as it decreases testicular growth and inhibits the development of secondary sexual characteristics. It has been suggested that o,p'-DDT and the technical grade DDT may exert an estrogenic action on the uterus after undergoing metabolic conversion to an active metabolite by hepatic enzymes. Since most known estrogens possess a phenolic hydroxyl

group, it is conceivable that the various analogs of DDT are hydroxylated in the benzene ring to form estrogenic metabolites (62).

The similarity in configuration of DDT to the synthetic estrogen, diethylstilbestrol, as well as the possible physiological implications of chlorinated hydrocarbons on mammalian fertility, prompted several investigators to study the influence of pesticides on reproductive tissues. Bitman et al. (63) observed an increase in the weight, water, RNA and glycogen content in uteri of rats and oviducts of chickens treated with o,p'-DDT. In addition, Welch et al. (62) found that o,p'-DDT enhanced the incorporation of glucose-C¹⁴ into protein, lipid and RNA as well as augmented wet weight and water imbibition in uteri of ovariectomized and immature rats. It is of interest that the o,p'-isomer exerted more pronounced estrogenic effects than p,p'-DDT (62). Recently, Singhal et al. (64) demonstrated that administration of o,p'-DDT to ovariectomized rats produced a marked rise in uterine weight and glycogen content and stimulated the activities of hexokinase, phosphofructokinase, pyruvate kinase, glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase. As expected, the changes in uterine metabolism with o,p'-DDT were of a greater magnitude than those seen with the p,p'-isomer. It is interesting that administration of estradiol-17 β also produced similar responses in the uterus and that simultaneous treatment with this hormone and o,p'-DDT induced somewhat additive effects on uterine weight and enzyme activities (64). Singhal et al. (64) suggested that DDT produces de novo enzyme biosynthesis since prior administration of actinomycin D or cycloheximide effectively blocked the o,p'-DDT-stimulated rise in uterine glycolytic and hexose monophosphate shunt enzymes. Administration of

chlordanes, heptachlor or dieldrin stimulated the metabolism of estrone by hepatic microsomal enzymes and inhibited the estrone-induced increase in uterine weight of ovariectomized rats (66). In addition, Welch et al. (66) observed a decrease in reproductive success in chlordanes-treated female mice. Recently, Smith et al. (65) found a significant reduction in the ability of DDT-treated prostate glands to assimilate testosterone-1, 2- H^3 ; however, the inhibitory action of DDT upon male accessory glands did not appear to be related to its known inherent estrogenic properties.

(b) Central Nervous System

(i) Symptoms of pesticide-poisoning

Numerous studies in 1940's and 1950's showed that the principle symptoms of acute DDT poisoning result from its effects upon the central nervous system. After oral administration of a single large dose (300-600 mg/kg) of DDT to rats, there was a latent period of approximately 2 hours. Symptoms such as hyperexcitability to sound and tactile stimuli and fine tremors of the head were first observed between the 2nd and the 3rd hour. The tremors then progressed gradually and increased in intensity. Episodes of clonic convulsions occurred but the tremors were so intense that the convulsive episodes were often difficult to discern. The body temperature remained unaffected during the first 2 hours of DDT administration (600 mg/kg p.o.) but rose considerably within the next few hours to reach 40-41°C (3,67). It is of interest that the observed increase in body temperature coincided with the development of tremor. The occurrence and the severity of clinical symptoms depended on the dose of DDT used (68,69). With the 100 mg/kg dose, toxic symptoms occurred at about 4 hours after DDT administration; were maximal at about 8 hours, diminished

slightly at 12 hours and disappeared by 24 hours. After the 300 mg/kg dose, a great majority of rats died within 24 hours; the surviving animals continued to display marked tremors even after 24 hours which later subsided gradually (25). In rats given the 600 mg/kg dose of DDT, death occurred between the 5th and the 8th hour (3) which resulted from respiratory failure during extreme exhaustion. Hyperthermia also is an important factor contributing to death in acute DDT poisoning (3). It is of interest that basically, a similar symptomology has been seen during acute DDT poisoning in rats, rabbits, cats, dogs and monkeys (23,25,69-71).

(ii) Morphological alterations

In spite of the pronounced neurotoxicity of chlorinated hydrocarbon insecticides, studies concerning the pathological changes in the central nervous system of DDT-treated animals have yielded little of note. Administration of DDT was reported to produce degenerative changes in the anterior horn cells of the spinal cord whereas the cerebrum, cerebellum and spinal cord were unaffected in cats, rabbits and rats (72,73). In contrast, Haymaker et al. (71) found selective degeneration of the cerebellum with no associated alterations in the cerebrum, brain stem and peripheral nerves of DDT-treated dogs. Damage of the cerebellum was characterized by pycnosis of Purkinje cells and degeneration of the dentate and roof nuclei. These findings suggested that the cerebellum might be an important target portion of the brain for the neurotoxic action of DDT.

(iii) EEG changes

Administration of a single dose of DDT to cats and monkeys caused the normal pattern of irregular bursts of brain waves to be shifted to a persistent rhythm in the cerebral motor cortex, whereas in the cerebellum,

there was a progressive increase in amplitude of waves followed by a constant peak at the time the tremors appeared (70). Pollock and Wang (74) showed that cats ingesting DDT in the diet manifested EEG changes associated with ataxia and tremors. These were observed initially in the cerebellum and as the intensity of insecticide poisoning increased, convulsive activity was noted in the cerebral cortex. Similarly, an increase in the frequency and amplitude of brain waves was reported in DDT-treated rats (75,76). Woolley and Barron (68) found that DDT-induced changes in cerebellar electrical activity occurred sooner and were greater in magnitude than those observed in other brain areas such as the motor cortex and reticular formation. Further, the changes in cerebellar activity occurred before clinical symptoms (such as tremoring) became evident, suggesting that the cerebellum may be particularly sensitive to the effects of DDT. Haymaker et al. (71) indeed pointed out that the tremoring and ataxia seen during acute DDT poisoning were similar to the symptoms of cerebellar dysfunction. The EEG studies together with the pathological changes support the view that cerebellum and cerebral motor cortex might be two important areas for the action of DDT on the central nervous system.

(iv) Behavioural effects

Despite the apparent effects of chlorinated hydrocarbon insecticides on the central nervous system, little attention has been focussed on the influence of these compounds on mammalian behaviour. Scudder and Richardson (77) found a significant decrease in aggressive behaviour in males chronically exposed to DDT. Recently, Sobotka (78) reported alterations in several behavioural and neurophysiological parameters in mice after a single low dose of DDT. The open-field exploratory activity was signif-

icantly enhanced and the ability of animals to habituate to this situation was attenuated by DDT. In addition, changes in the maximum electrical shock seizure patterns reflected an increase in brain excitability suggesting that DDT may facilitate the central excitatory process, at least partially, by a disinhibitory mechanism (78).

(v) Changes in biogenic amines

Treatment with chlorinated hydrocarbon insecticides exerts pronounced effects on metabolism in the central nervous system. Administration of an acute oral dose of p,p'-DDT significantly lowered the concentration of striatal acetylcholine whereas dopamine levels in the striatum remained unaltered (3,69,79). Although the concentration of 5-hydroxytryptamine was not affected, DDT treatment enhanced the level of its metabolite, 5-hydroxyindoleacetic acid, in brain stem (79,80). It is interesting that the activities of brain stem monoamine oxidase, tryptophan hydroxylase and 5-hydroxytryptophan decarboxylase remained unaltered in DDT-fed rats. In contrast to the effects of p,p'-DDT on the central nervous system, administration of the o,p'-isomer of DDT failed to induce tremors, hyperthermia or any of the changes seen in biogenic amines (79).

Since it is the chronic exposure to small doses of chlorinated hydrocarbon insecticides which actually presents a potential health hazard, Hrdina et al. (81) examined the long-term effects of pesticides on the levels of acetylcholine and other biogenic amines in rat brain. Daily administration of small doses of p,p'-DDT produced a significant decrease in the concentration of striatal acetylcholine and an increase in brain stem 5-hydroxyindoleacetic acid. In contrast, α -chlordane failed to alter the concentration of brain acetylcholine and 5-hydroxyindoleacetic

acid.

In addition to the observed changes in biogenic amines, administration of various organochlorine pesticides produces changes in the concentration of brain ammonia, alanine, glutamine, pyruvate and lactate. St. Omer (82, 83) found that pesticide-induced seizures were accompanied by a marked rise in the concentration of brain ammonia and glutamine in rats and cockerels. Similar findings concerning insecticide-induced increases in brain ammonia have been reported by Hathway et al. (84). In contrast, Witter et al. (85) demonstrated elevated levels of alanine and γ -aminobutyric acid in brains of dieldrin-treated rats whereas DDT failed to produce any significant change in these amino acids. At present, the precise mechanism involved in pesticide-induced neurotoxicity is unknown but it appears that rather complex changes in brain biochemistry accompany the symptoms of acute and chronic poisoning.

(vi) Prevention of acute neurotoxic effects of DDT

Soon after the discovery that DDT and related chlorinated hydrocarbon insecticides produce severe neurotoxic effects, attempts were made to find antidote procedures which could be used in the treatment of acute poisoning. Physiological antagonists seemed to be the first logical choice. Indeed, central depressants such as urethane, barbiturates and magnesium sulphate as well as the anticonvulsant agent sodium dilantin all offered some degree of protection against neurotoxic symptoms and decreased the mortality of rats, cats, dogs and monkeys poisoned with DDT (25,36). Not all central depressants were equally effective. By far the most outstanding remedy tested was phenobarbital, which in non-anesthetic doses was capable of not only controlling tremors and convulsions but also preventing death

in many instances. Urethane barbitol and pentobarbital also reduced mortality but the control of tremors and convulsions required full anesthetic doses. Magnesium sulphate did not influence the mortality although it gave a brief protection against tremors and convulsions. In rats pre-treated with dilantin, the lethal effect of DDT was reduced but the neurotoxic signs of insecticide poisoning remained unaltered. In contrast, paraldehyde failed to provide any protection against DDT poisoning (25).

Administration of calcium either as gluconate or chloride has also been shown to exert an antidotal action against DDT in dogs, cats and rats (50,86,87). Calcium gluconate reduced the severity of neurotoxic effects of DDT in rats, however, the protection was only partial and best results were achieved when calcium was given before DDT. Treatment with glucose was found to decrease mortality and reduce the toxic symptoms observed after a large dose of DDT in dogs and cats (38,87). In contrast, Stohlman and Lillie (88) observed no protecting effect of glucose on DDT-induced neurotoxic symptoms and mortality in rabbits. Recently, Hrdina and Singhal (89) reported that administration of cycloheximide to rats given a lethal dose of DDT prevented the development of hyperthermia, tremors and convulsions as well as increased the survival time of DDT-poisoned rats by approximately 8 hours. Cycloheximide also prevented the drop in striatal acetylcholine as well as the rise in brain stem 5-hydroxyindoleacetic acid observed with the pesticide alone. In contrast, actinomycin D, another inhibitor of RNA and protein synthesis, failed to affect not only the DDT-induced tremors, convulsions and hyperthermia, but also the alterations in brain acetylcholine and 5-hydroxyindoleacetic acid. It was suggested that cycloheximide may exert its protective

action by interfering with the effects of DDT on enzymatic processes involved in the metabolism of 5-hydroxytryptamine and acetylcholine.

(c) Liver and Kidney

It has been well established that kidney and liver are two of the major tissues which retain organochlorine pesticides (90). Woolley and Talens (41) demonstrated that the high concentrations of DDT, DDE and DDD in hepatic and renal tissue were dependent on the amount of pesticide administered to pregnant female rats and that tissue retention in neonates was greater during lactation than during gestation. Brown (91) found that starvation produced an even greater retention of DDT in both liver and kidney. Since the chlorinated hydrocarbon insecticides are stored in kidney and liver, it is not surprising that pesticide treatment has been shown to produce marked morphological changes in these tissues. In various species of fish, pesticide administration resulted in tubular degeneration, necrosis and vascular congestion in the kidney (7,92,93). Similarly, parenchymous alterations, tubular degeneration, necrosis and occlusion of the renal tubules with debris were found in insecticide-treated animals (5,6,8,94-96). The histopathological changes produced by organochlorine pesticides in hepatic tissue include necrosis, hypertrophy of liver cells as well as fatty infiltration (8,9,82,93).

Recently, Kimbrough et al. (10) found that long-term feeding of dieldrin or DDT produced atypical mitochondria in rats. These pesticide-induced morphological changes in hepatic mitochondria were shown to be associated with abnormal respiration and a reduction in the activities of cytochrome oxidase, succinic dehydrogenase and NADH oxidase (97-99). Similarly, Kramp1 (100) examined the relationship between the histological

In higher organisms, the kidney cortex and liver appear to be unique in that they possess the full enzymatic potential for both glucose synthesis

from noncarbohydrate precursors, and glucose degradation via the glycolytic pathway of Embden, Meyerhof and Parnas. The end-product of glycolysis, glucose, is not only used to generate biological energy in the form of adenosine triphosphate, but also supplies precursors for fatty acid, protein and nucleic acid synthesis (Fig. 1). Glucokinase, phosphofructokinase and pyruvate kinase are believed to be the three key enzymes in the glycolytic sequence since they play a rate-limiting role in determining the overall activity of this metabolic pathway (109,110). It is of interest that in conditions of diabetes or starvation where insulin levels are low, the activities of hepatic glucokinase, phosphofructokinase and pyruvate kinase are markedly decreased (109). Administration of insulin to alloxan diabetic rats produced a rise in the triad of key glycolytic enzymes which was blocked by actinomycin D suggesting that insulin induced the de novo biosynthesis of these liver enzymes. Weber et al. (109) demonstrated that glucocorticoids failed to alter the insulin-induced biosynthesis of pyruvate kinase in diabetic rats and produced no changes in the activities of hepatic glucokinase, phosphofructokinase and pyruvate kinase in normal and hypophysectomized rats. These authors suggested that insulin and corticosteroid hormones regulate carbohydrate metabolism probably by different mechanisms.

In mammals, gluconeogenesis comprises the synthesis of glucose from lactate, pyruvate, glycerol and certain amino acids. The gluconeogenic pathway is essential for replenishing body glycogen stores and glucose during starvation and when carbohydrate intake from the alimentary tract is limited. During heightened sympathetic activity and exercise when the concentrations of lactate and glycerol rise, the formation of glucose

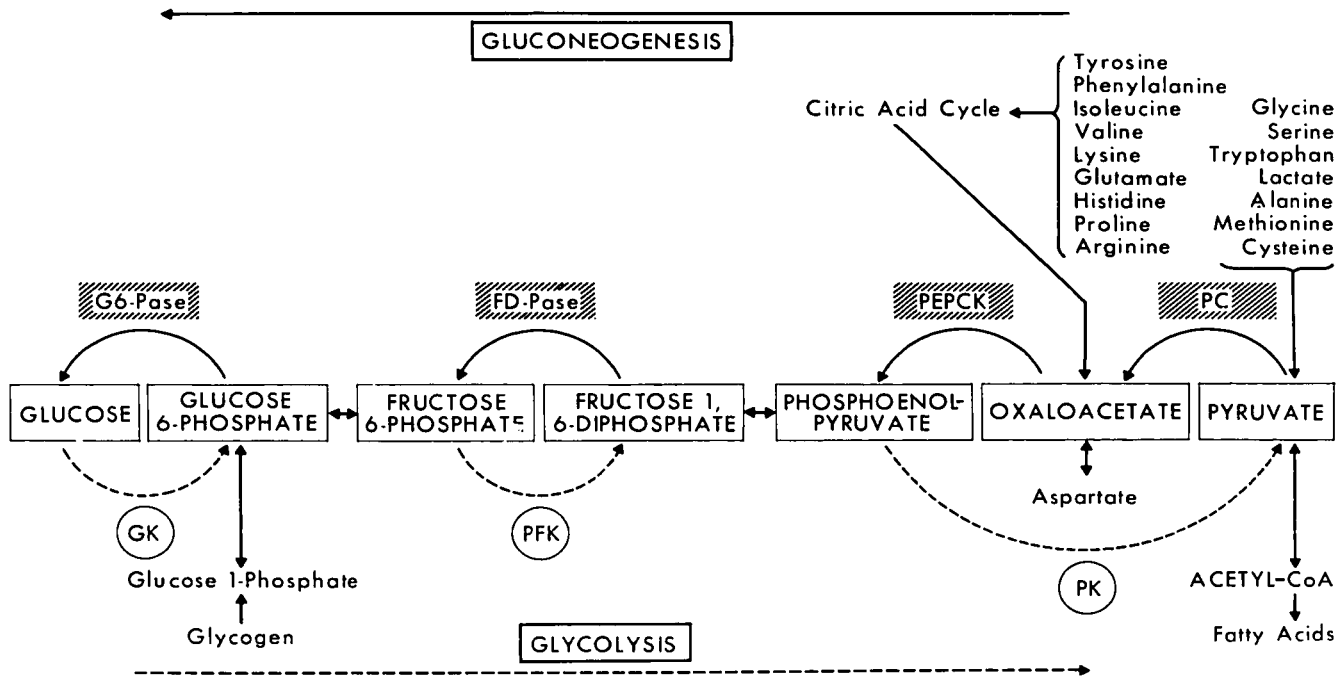


Fig. 1. Pathways of hepatic and renal carbohydrate metabolism showing the important enzymes involved in the process of glycolysis and gluconeogenesis. Abbreviations used: hexokinase (HK); phosphofructokinase (PFK); pyruvate kinase (PK); pyruvate carboxylase (PC); phosphoenolpyruvate carboxykinase (PEPCK); fructose 1,6-diphosphatase (FD-Pase); and glucose 6-phosphatase (G6-Pase).

serves to remove these substrates from the blood. In addition, gluconeogenesis provides increased amounts of ammonia for the kidney to counteract acidosis. Finally, amino acids, which are absorbed from the alimentary tract or released during protein catabolism, can be utilized by gluconeogenic enzymes to synthesize new glucose.

The location and the reactions catalyzed by the four key, rate-limiting gluconeogenic enzymes are illustrated in Fig. 2. Pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose 1,6-diphosphatase and glucose 6-phosphatase are the quartet of enzymes which play a rate-limiting role in glucose formation from noncarbohydrate sources, since they catalyze irreversible reactions, are involved in circumventing thermodynamic barriers and are located almost exclusively in organs (liver and kidney cortex) capable of gluconeogenesis (111). Recently, Crabtree et al. (112) also demonstrated the existence of pyruvate carboxylase, phosphoenolpyruvate carboxykinase and fructose 1,6-diphosphatase activities in muscles of vertebrates and invertebrates, however, the levels of gluconeogenic enzymes in muscle were comparatively lower than those obtained for hepatic and renal tissues. Pyruvate carboxylase is the enzyme involved in the synthesis of oxaloacetate from pyruvate (Fig. 2), whereas phosphoenolpyruvate carboxykinase is the enzyme involved in biotransforming oxaloacetate into phosphoenolpyruvate. In addition to pyruvate, malate and aspartate can also be converted into phosphoenolpyruvate. The other two important gluconeogenic enzymes are fructose 1,6-diphosphatase and glucose 6-phosphatase. Fructose 1,6-diphosphate is converted to fructose 6-phosphate by fructose 1,6-diphosphatase whereas glucose is the end-product of the reaction catalyzed by glucose 6-phosphatase.

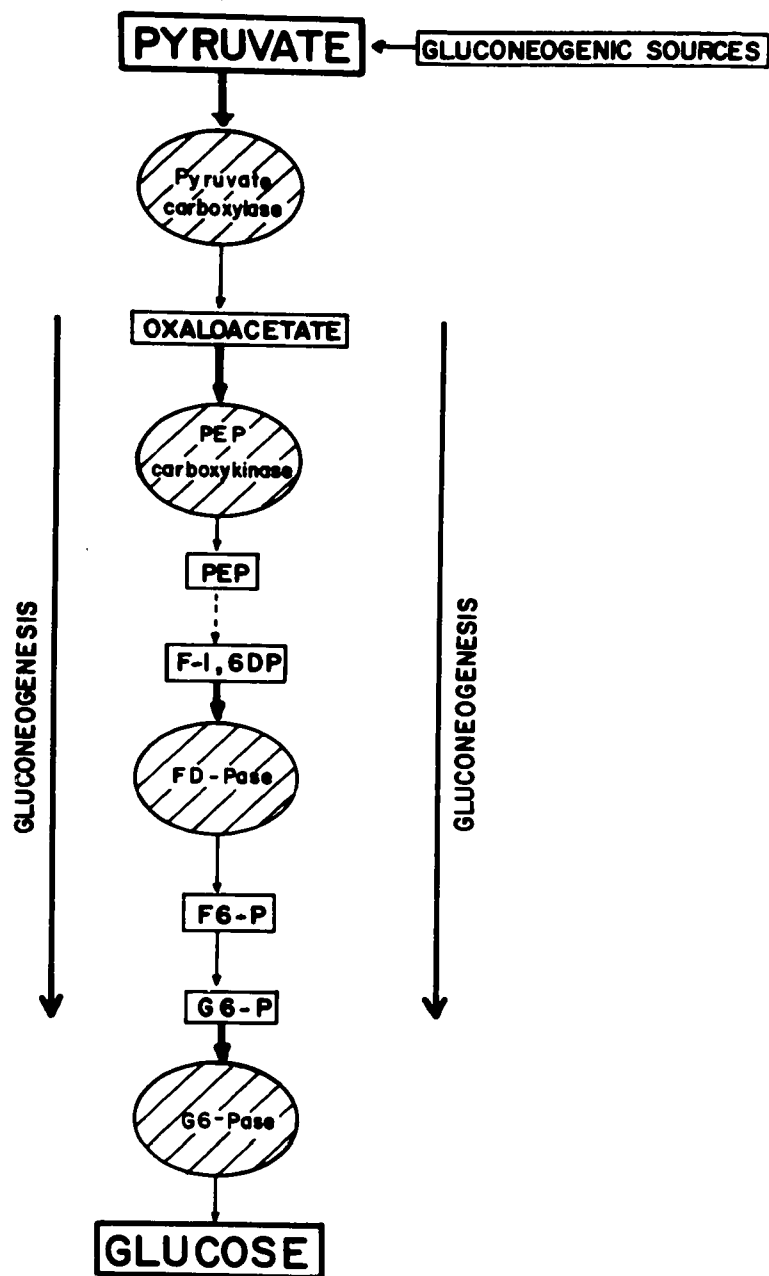


Fig. 2. The pathway of gluconeogenesis and the location of key rate-limiting enzymes. Abbreviations used: phosphoenolpyruvate (PEP); fructose 1,6-diphosphate (FDP); fructose 1,6-diphosphatase (FDPase); fructose 6-phosphate (F6-P); glucose 6-phosphate (G6-P); glucose 6-phosphatase (G6-Pase). Thick arrows indicate irreversible key rate-limiting steps whereas the interrupted arrow shows that a sequence of reactions is involved. Pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose 1,6-diphosphatase and glucose 6-phosphatase are the 4 enzymes which play a key rate-limiting role in the formation of glucose from non-gluconeogenic sources.

A. Factors Involved in the Regulation of Gluconeogenesis and Glycolysis

The synthesis of glucose in the body is known to be regulated by a variety of factors. Elucidation of various key steps involved in the gluconeogenic and glycolytic pathways provided basis for the formulation of current concepts of carbohydrate metabolism in mammalian tissues. During the last two decades, numerous studies on the regulation of carbohydrate metabolism have been carried out in order to understand various factors which affect the activity of key glycolytic and gluconeogenic enzymes in the body.

(a) Diet

It is well known that the levels of blood glucose vary greatly in different metabolic states. The diet plays an important role in determining whether glycolysis or gluconeogenesis will take place in the animal. A high carbohydrate diet stimulates the activities of hepatic glucokinase, phosphofructokinase and pyruvate kinase whereas the gluconeogenic enzymes, pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose 1,6-diphosphatase and glucose 6-phosphatase are inhibited (113). In contrast, feeding of a high-protein diet increased the activities of the four physiologically unidirectional gluconeogenic enzymes in liver tissue (114-116). In addition, high protein diet enhanced amino acid catabolism as evidenced by an increase in hepatic serine dehydrase, glutamic-pyruvic transaminase, glutamic-oxaloacetic transaminase, tyrosine- α -ketoglutarate transaminase and the activities of urea cycle enzymes (117-121). Stimulation of protein breakdown and elevation in serum urea indicate that amino acids were being utilized as noncarbohydrate precursors for gluconeogenesis under this dietary regime. Szepsi et al. (114) found

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that a high carbohydrate diet suppressed the activity of renal phosphoenolpyruvate carboxykinase whereas the activities of glucose 6-phosphatase, fructose 1,6-diphosphatase and phosphoenolpyruvate carboxykinase were enhanced by administration of a high protein diet. As in hepatic tissue, a high protein intake also stimulated the activities of arginase and arginine synthetase in rat kidney (114).

The frequency of food intake also has been shown to exert profound effects on metabolic processes. Teppermann and Teppermann (122) demonstrated a marked rise in the activities of hepatic pyruvate carboxylase and phosphoenolpyruvate carboxykinase in rats subjected to a 2-day fast followed by 1 day of feeding for a period of 3-10 weeks. Chakrabarty and Leveille (123) found that pyruvate carboxylase and phosphoenolpyruvate carboxykinase were also elevated in the livers of rats trained to eat only 2 hours a day. As in liver, Teppermann et al. (124) showed a similar enzymic pattern in the kidney cortex of meal-trained rats. A significant increase in the activities of pyruvate carboxylase and phosphoenolpyruvate carboxykinase was also observed in the kidneys of rats fed 1 out of every 3 days. It is of interest that in rats adapted to varying periods of feeding and starvation, the concentrations of blood glucose and hepatic glycogen were increased (125). In addition, Potter et al. (125) found an enhancement in the activities of hepatic serine dehydrase, tryptophan pyrrolase, tyrosine transaminase and ornithine transaminase, several enzymes which play a role in protein catabolism and thus supply precursors for gluconeogenesis.

Undoubtedly, the lack of food intake will also be expected to exert prominent effects on gluconeogenesis. Weber et al. (109) showed a marked

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decrease in the activity of hepatic glucokinase, phosphofructokinase and pyruvate kinase in 6-day starved rats. Refeeding of 6-day starved rats resulted in significant increase in the triad of glycolytic enzymes. It is interesting that pretreatment with actinomycin D or ethionine blocked the refeeding-induced rise in glucokinase, phosphofructokinase and pyruvate kinase suggesting that de novo enzyme biosynthesis was involved. In contrast, Schimmel and Knobil (126) found that starvation enhanced glucose-C¹⁴ production from labeled alanine, lactate, pyruvate and glycerol in hepatic tissue slices as well as serum glucose levels. Since measurements of tissue intermediates in livers perfused with lactate showed that fasting produced a marked rise in the rate of the reaction located between pyruvate and phosphoenolpyruvate, it was suggested that phosphoenolpyruvate carboxykinase played a key role in the adaptation of animals to fasting (127,128). Young and Scrimshaw (129) showed that starvation also stimulated the activities of pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose 1,6-diphosphatase and glucose 6-phosphatase. The rise in various gluconeogenic enzyme activities was accompanied by a fall in the concentration of valine and alanine (129). During starvation, there is a striking change in the role of the kidney cortex since it contributes almost as much as the liver to overall glucose production (130). This shift is believed to be attributed, at least in part, to changes in the acid-base balance in the blood caused by an increase in the body's production of ketone bodies. The consequence of alterations in acid-base balance upon renal gluconeogenesis will be discussed later.

(b) Hypoxia

The supply of oxygen to the liver by the portal venous system exerts profound effects on carbohydrate metabolism. Ballard (131) found that C^{14} -lactate, C^{14} -serine and C^{14} -aspartate was not incorporated into labeled glucose in 2-day old rats exposed to a 100% nitrogen atmosphere. Similarly, Philippidis and Ballard (132,133) found that gluconeogenesis was suppressed in fetuses subjected to hypoxia. Inhibition of gluconeogenesis and activation of glycolysis has also been demonstrated following hypoxia in perfused rat livers (134). Although the mechanism by which hypoxia suppressed gluconeogenesis is unknown, it is believed to be due to a fall in the concentrations of hepatic pyruvate and oxaloacetate (135,136).

(c) Pregnancy

As expected, marked metabolic alterations occur during pregnancy since the fetus has to depend upon the maternal substrates (137,138). Herrera et al. (139) found an enhanced gluconeogenesis in livers of pregnant rats administered C^{14} -pyruvate and suggested that the observed increase in gluconeogenesis was probably related to the large demand made by the fetus on the maternal glucose supply. However, Rixe (140) found no significant elevation in the rate of renal gluconeogenesis in pregnant dogs, as well as in the concentration of urinary glucose. It is of interest that lactation makes extra demands on glucose supply in mammals and the gluconeogenic pathway is the major pathway by which the organism is supplied with glucose in this condition (134).

(d) Exercise

The overall biochemical changes during physical exertion have also been investigated (113,134). Krebs et al. (141) demonstrated an enhanced

rate of gluconeogenesis from lactate, pyruvate and fumarate following muscular exercise. Sanchez-Medina et al. (142,143) not only found an increased production of glucose from lactate and pyruvate in exercised rats, but also stimulation of hepatic and renal phosphoenolpyruvate carboxykinase activity. An increase in the activity of hepatic glucose 6-phosphatase was also reported after exercise (144). Cristophe et al. (145) found that the activation of renal and hepatic gluconeogenesis in exercised rats was associated with changes in the concentration of amino acids in the plasma and kidney.

(e) Diabetes

Human as well as experimental diabetes is characterized by a disturbance in carbohydrate metabolism and is associated with secondary alterations in protein and fat metabolism (113). Weber et al. (109) demonstrated a marked decrease in the activities of hepatic glucokinase, phosphofructokinase and pyruvate kinase in alloxan-diabetic rats. Recently, Singhal et al. (146) found that administration of streptozotocin also induced diabetes in rats and significantly lowered the activities of key glycolytic enzymes in liver tissue. The enzymic changes in alloxan- and streptozotocin-induced diabetes were associated with a rise in blood glucose as well as a fall in hepatic glycogen (109,146). It is of interest that insulin treatment of diabetic rats restored changes in blood glucose and hepatic glycogen as well as in various enzyme activities (109, 146). Prior administration of either actinomycin, ethionine or cycloheximide effectively blocked the insulin-induced rise in the activities of hepatic glucokinase, phosphofructokinase and pyruvate kinase suggesting that the action of insulin on diabetic livers involved de novo enzyme

biosynthesis (109,146). In contrast to hepatic glycolytic enzymes, the activities of pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose 1,6-diphosphatase and glucose 6-phosphatase were markedly elevated in livers of alloxan- and streptozotocin-induced diabetic rats (110,128, 146-148). It is interesting that insulin treatment prevented the diabetic-induced rise in various gluconeogenic enzymes (110). Not only are the gluconeogenic enzymes increased in diabetic subjects but also the rate of incorporation of lactate, pyruvate and alanine into glucose (149,150). A similar increase in the rate of gluconeogenesis and stimulation in the activities of pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose 1,6-diphosphatase and glucose 6-phosphatase was observed in kidney cortex slices of alloxan diabetic rats (151,152). Unlike hepatic tissue, Joseph and Subrahmanyam (152) found that the renal glycolytic enzymes, hexokinase, phosphofructokinase and pyruvate kinase, were not affected by alloxan treatment.

(f) Acid-Base Balance

Disturbances in acid-base balance exert profound effects on carbohydrate metabolism in the kidney. Goodman et al. (153) demonstrated that in ammonium chloride-induced metabolic acidosis, there was an enhanced renal gluconeogenesis. They observed an increased incorporation of several noncarbohydrate precursors into glucose in rat renal cortical slices as well as elevated glucose levels. In addition, Alleyne and Scullard (154) noted a marked stimulation in the activity of renal phosphoenolpyruvate carboxykinase during metabolic acidosis induced by ammonium chloride. Recently, Cassin and Bunel (155) demonstrated an increased output of glucose by dog kidneys in metabolic acidosis although

the hepatic gluconeogenesis and the activity of phosphoenolpyruvate carboxykinase were not increased (156,157). In contrast, metabolic alkalosis induced by sodium bicarbonate loading was found to significantly depress the synthesis of glucose by renal cortical slices (153). As expected, Pilkington and O'Donovan (158) found that net glucose production in dog kidneys was decreased in metabolic alkalosis. However, Roxe et al. (159) were unable to detect any change in net glucose production in acidotic or alkalotic dog kidneys even though the injection of labeled precursors did enhance renal gluconeogenesis in metabolic acidosis.

(g) Glucocorticoids

In general, administration of glucocorticoids enhances gluconeogenesis in renal cortex and hepatic tissue slices (134,151). The activities of renal cortex and hepatic pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose 1,6-diphosphatase and glucose 6-phosphatase are all increased in corticosteroid-treated animals (110,152). In addition, Weber et al. (110) found that pretreatment with actinomycin or ethionine prevented the glucocorticoid-induced rise in the four gluconeogenic enzyme activities suggesting that the adrenal hormones stimulated de novo enzyme biosynthesis (110,111). However, the glucocorticoids differ in their action on carbohydrate metabolism from insulin in that glucokinase, phosphofructokinase and pyruvate kinase were not affected by triamcinolone (109).

(h) Glucagon

The action of glucagon on hepatic carbohydrate metabolism is believed to be antagonistic to that of insulin (160). Administration of glucagon was shown to augment hepatic gluconeogenesis from noncarbohydrate pre-

cursors in man (161) and in perfused livers (162). Glucagon treatment produced a rise in phosphoenolpyruvate carboxykinase and fructose 1,6-diphosphatase whereas the activities of hepatic phosphofructokinase and pyruvate kinase were decreased in the rat (128,160). A similar effect on these liver enzymes was found when glucagon was administered to humans (160). It is of interest that the concentration of glucose rises during glucagon perfusion and drops when the hormone is removed (162).

(i) Catecholamines

Exogenous epinephrine and norepinephrine have also been found to stimulate glucose production as well as the rate of gluconeogenesis in hepatic tissue (162). In addition, a marked rise in the concentration of blood glucose was noted following the administration of epinephrine (163). It is interesting that sympathetic stimulation via the splanchnic nerve also affected carbohydrate metabolism in liver (164). The release of norepinephrine from nerve terminals produced an increase in the concentration of blood glucose more rapidly than the intravenously injected catecholamine. Feldman et al. (165) showed that stimulation of the sympathetic center in the posterior hypothalamus of rats also elevated serum glucose levels and the rate of hepatic gluconeogenesis. From measurements of the levels of intermediates in the gluconeogenic pathway, Exton and Park (162) suggested that catecholamines activated the reaction between pyruvate and phosphoenolpyruvate. Indeed, Nagai et al. (166) found that epinephrine stimulated the activity of hepatic phosphoenolpyruvate carboxykinase.

(j) Thyroxine and Growth Hormone

The rate of gluconeogenesis in kidney cortex slices is enhanced

in both thyroxine- and growth hormone-treated rats (151). Recently, Joseph and Subrahmanyam (152) found that administration of growth hormone produced a rise in the activities of phosphoenolpyruvate carboxykinase, fructose 1,6-diphosphatase and glucose 6-phosphatase whereas the activities of phosphofructokinase and pyruvate kinase were depressed in renal tissue. Treatment with thyroxine also produced stimulation of hepatic phosphoenolpyruvate carboxykinase and glucose 6-phosphatase whereas pyruvate kinase remained unchanged (167-169). In addition, the thyroxine-induced increase in phosphoenolpyruvate carboxykinase was blocked by cycloheximide (168). It is of interest that thyroxine administration to normal rats significantly enhanced gluconeogenesis from lactate in the perfused liver (170).

(k) Miscellaneous Pharmacological Agents

There is evidence that the hypoglycemia induced by alcohol is due to reduced gluconeogenesis since ethanol inhibits the incorporation of noncarbohydrate precursors into glucose (134,171). In addition, ethanol was reported to inhibit glucose production by stimulating phosphofructokinase but depressed fructose 1,6-diphosphatase (172). Recently, Madappally et al. (173) found that the oral administration of acetylsalicylic acid also produced a fall in blood glucose levels associated with inhibition of hepatic and renal pyruvate carboxylase and phosphoenolpyruvate carboxykinase. In contrast, carbachol treatment decreased the activity of hepatic phosphoenolpyruvate carboxykinase and significantly elevated that of glucokinase (166).

Various hypoglycemic agents lower the concentration of blood glucose by acting at different enzymatic sites. Quinaldic acid and 5-methoxy-

indole-2-carboxylic acid inhibited hepatic gluconeogenesis via an action on pyruvate carboxylase, since mitochondrial oxidation and carboxylation of pyruvate was prevented (174). The hypoglycemia observed after hydrazine and quinolinic acid has been found to be associated with a fall in hepatic and renal phosphoenolpyruvate carboxykinase (17,175). Recently, Duncombe and Rising (176) found that the cyclopropanecarboxylate-induced inhibition of glucose production was due to depression of fructose 1,6-diphosphatase.

Several drugs that are used clinically also affect carbohydrate metabolism. Furosemide, a potent diuretic, stimulates the rate of glucose synthesis in kidney cortex by enhancing the activities of pyruvate carboxylase and phosphoenolpyruvate carboxykinase (177,178). Phenformin, used in the treatment of diabetes, was shown to inhibit hepatic gluconeogenesis in guinea-pigs (179). However, in normal and diabetic humans, evidence indicates that the major action of phenformin was stimulation of glycolysis in various tissues (134).

In short, mammalian carbohydrate metabolism can be altered by a wide variety of different mechanisms. Changes in gluconeogenesis are undoubtedly very important in metabolic derangements occurring in diabetes and disorders of glucocorticoid secretion; and indeed, the principle defect in alcohol hypoglycemia seems to be an impairment of glucose formation (134). Thus, there is a prime need for defining the hormonal and other factors involved in the regulation of gluconeogenesis and for elucidating the mechanisms by which the pharmacological agents modify this process.

III. THE IMPORTANCE OF CYCLIC AMP IN HORMONAL CONTROL OF METABOLIC PROCESSES

The discovery of cyclic adenosine 3',5'-monophosphate (cyclic AMP) 15 years ago and the gradual emergence of its biological significance has led to the belief that this cyclic nucleotide acts as an intracellular mediator in the action of many hormones and naturally occurring cell regulating substances. Cyclic AMP has been implicated in the mechanism of action of anterior and posterior pituitary hormones, biogenic amines, insulin, glucagon, angiotensin, prostaglandins, melatonin, hypothalamic releasing factors and parathyroid hormone. It appears that cyclic AMP is involved in the mediation of neural stimulatory actions as well as in the mediation of various hormonal effects in the body.

The biological importance of cyclic AMP has been extended from a relatively simple role in liver glycogenolysis to the point where it seems to be a key regulatory agent in most mammalian tissues. As a result of many extensive studies, the concept has evolved that many hormones act by a "two-messenger" system. According to this hypothesis, the "first messenger" is the hormone or the neurohormone itself which travels from the cells of its origin to the cells of target tissues to stimulate the formation therein of an intracellular "second-messenger." It is this "second-messenger" which then does the real work of the hormone and orders the affected cells to perform whatever function seems to be required at that particular time and place. This change in the level of "second-messenger" so alters cellular function (by changing enzyme activity, membrane permeability or other processes) that the cell responds in a characteristic manner to the hormone. To date, cyclic AMP is still

the only recognized second messenger, although it is safe to presume that other second messengers will be discovered eventually. Within this framework, the adenylyl cyclase system would seem to function both as a discriminator for environmental signals and as a signal generator. By this mechanism, the initial extracellular signal, represented by the first messenger, is changed to a signal (the second messenger) which is capable of acting within the cell (180). This signal may then undergo amplification in a variety of ways, the ultimate response depending upon the enzymatic profile of the type of cell involved. The concept of hormones as information transferring molecules was discussed extensively by Hechter and his colleagues (181,182).

A. Control of Cyclic AMP Formation

Cyclic AMP is known to be distributed very widely in nature. It is present in most mammalian tissues investigated including milk (183) and urine (184,185). The concentrations of cyclic AMP in blood and cerebrospinal fluid are ordinarily quite low but can be increased dramatically in response to pharmacological doses of hormones (186,187). This cyclic nucleotide seems to be absent in avian erythrocytes (188) and human platelets (189,190). Studies on the distribution of cyclic AMP also showed that its presence is not confined to mammalian tissues (191). It has been detected in several bacteria where it appears to play a role in the regulation of synthesis of inducible enzymes (192) and has been identified as the acrasin of slime molds (193). It has been reported to potentiate the anti-viral effects of interferon (194) and it is now believed that the level of cyclic AMP increases in some plant tissues in response to gibberellic acid (195). The occurrence of cyclic AMP in unicellular

organisms and in plants together with the evidence that it plays an important role in their metabolism suggests that the importance of cyclic AMP in regulatory mechanisms may indeed transcend its role in endocrine biology (180).

The formation of cyclic AMP in biological systems is catalyzed by the enzyme adenyl cyclase which is present in all animal cells examined except non-nucleated erythrocytes, and in most animal tissues it is located in the plasma membrane (188). Another feature which is common to adenyl cyclase from several animal sources is its sensitivity to stimulation by fluoride (196). Adenyl cyclase activity in liver preparations can be stimulated by epinephrine and other catecholamines (197,198), as well as by glucagon (199,200). Glucagon is much more potent than any of the catecholamines, not only in the sense that smaller concentrations of glucagon are required to stimulate the enzyme but also in that the maximal response in this case is much greater than the maximal response to catecholamines.

The other enzyme known to influence the level of cyclic AMP is a relatively specific phosphodiesterase which catalyzes the hydrolysis of cyclic AMP to 5'-AMP (201,202). This enzyme has been found in all animal tissues except non-nucleated erythrocytes and is present in both soluble and particulate fractions of most mammalian tissues. This enzyme is inhibited by several drugs and also by such metabolites as ATP and pyrophosphate (203,204). The drugs which have been most commonly used to inhibit phosphodiesterase are the methyl xanthines (e.g. theophylline) and it is generally true that hormones which act by stimulating adenyl cyclase are potentiated in the presence of one of these compounds. It is of interest that phosphodiesterase from unicellular organisms, unlike

the enzymes from higher species, is not susceptible to inhibition by methyl xanthines (205). Catecholamines and several other hormones which stimulate the accumulation of cyclic AMP in broken cell preparations and intact tissues appear to do so by stimulating adenyl cyclase and have not been shown to affect the activity of phosphodiesterase (206,207). However, certain hormones such as thyroid hormone and insulin can affect phosphodiesterase activity as has been shown by Mandel and Kuehl (208) and Schultz et al. (209).

B. Set of Criteria for Cyclic AMP Mediation

It is generally believed that several criteria must be fulfilled before the role for cyclic AMP as a mediator in the action of a given hormone or a drug can be established with any degree of certainty (206,207). Some of these have been satisfied for almost a score of hormones but all of them, for only a few. These criteria, most of which are self-evident, can be briefly summarized as follows: first, the effects of the hormone should be reproduced by exogenous cyclic AMP. This is generally considered to be the most difficult criterion to meet since cyclic AMP penetrates cells very poorly, and in some cases, probably not at all. Nevertheless, cyclic AMP has been shown to mimic hormonal actions in a variety of body tissues including the estrogen-dependent target organ, the uterus (210,211). The acylated derivative such as the N⁶,O^{2'}-dibutyryl analog of cyclic AMP has been found to be more potent than the parent compound and therefore, when tissues treated with dibutyryl cyclic AMP show the same response as seen with the hormone, this is taken as strong evidence that the hormone in question works through the cyclic AMP system. Secondly, the inhibitors of phosphodiesterase such as the methyl xanthines should be able to

potentiate the effects produced by a submaximal dose of the hormone. Indeed, the lipolysis in fat cells caused by catecholamines, glucagon and ACTH (212-215), the effects of vasopressin on the toad bladder (216), the cardiac inotropic responses to norepinephrine (217), uterine glycogenesis and glycolysis stimulated by estradiol (218) and the effects of isoproterenol on DNA synthesis in parotid glands (219) have all been shown to be potentiated by methyl xanthines. Thirdly, the first messenger (i.e. the hormone) should induce a measurable change in the concentration of tissue cyclic AMP. The endogenous levels of target tissue cyclic AMP should decrease upon hormonal deprivation such as should be the case following castration in male and female rats. Administration of the hormone should restore the drop in tissue cyclic AMP levels observed after castration. Ideally, the changes in cyclic AMP concentration should precede the metabolic responses that are presumed to be mediated by cyclic AMP. This change in the concentration of cyclic AMP should be tissue- and hormone-specific and correlate temporally and quantitatively with the physiological effects of the hormone. Finally, administration of the hormone should either enhance the activity of tissue adenylyl cyclase or inhibit phosphodiesterase or do both. The hormone should also stimulate the conversion of ATP (or adenosine) to cyclic AMP when added to broken cell preparations or slices of the target organ. It is not essential however, that the full metabolic response that occurs in intact cells be evoked in broken cells as this may be dependent upon total cellular integrity. Physiological concentrations of the hormone should be effective in such preparations and where a series of compounds have similar physiological effects on the intact tissue, their order of potency should correspond to the order of potency

on in vitro cyclic AMP synthesis.

C. Role of Cyclic AMP in Carbohydrate Metabolism

It has become increasingly apparent that there is a direct relationship between cyclic AMP and alterations in hepatic and renal carbohydrate metabolism. Some of the more prominent changes produced by cyclic AMP in liver include stimulation of phosphoenolpyruvate carboxykinase and glucose 6-phosphatase (12,220-223) and an enhanced incorporation of non-carbohydrate precursors into glucose (224-227). An increase in the incorporation of gluconeogenic precursors into glucose also was found in kidneys of rats treated with cyclic AMP (13,228-230). Recently, Weiss et al. (231) demonstrated that cyclic AMP augmented the entry and cumulative uptake of several amino acids into rat kidney cortex slices. From studies with gluconeogenic precursors, Guder et al. (232) suggested that the rate-limiting step in cyclic AMP-stimulated renal gluconeogenesis involved an enhanced formation of phosphoenolpyruvate by phosphoenolpyruvate carboxykinase. Since pretreatment with actinomycin D and cycloheximide prevented the cyclic AMP-induced rise in renal and hepatic phosphoenolpyruvate carboxykinase and glucose 6-phosphatase, evidence suggested that de novo enzyme biosynthesis was involved in the observed action of this cyclic nucleotide (12,220-223,233). It is of interest that cyclic AMP also produced a fall in hepatic glycogen content (15,225,234) and an elevation in the concentration of blood glucose (235-240).

Although considerable work has been carried out on the pathological changes produced by chlorinated hydrocarbon insecticides in renal and hepatic tissue, little attention has been focussed on the pesticide-induced metabolic alterations in these organs. In the present study, an attempt

has been made to examine the ability of various pesticides to increase hepatic and renal gluconeogenic enzymes, blood glucose and serum urea as well as to lower liver glycogen content. The changes in the biochemical parameters studied were found to be related to the tissues' ability to enhance cyclic AMP- H^3 production both in vivo and in vitro. The data obtained show that organochlorine insecticides also elevated endogenous hepatic and renal cyclic AMP levels and augmented both basal- and fluoride-stimulated adenylyl cyclase suggesting that the pesticide-induced changes in carbohydrate metabolism might be associated with an increased capacity of these tissues to synthesize cyclic AMP.

3. MATERIALS AND METHODS

I. ANIMALS

All animals were purchased locally from Bio-Breeding Laboratories of Canada Ltd. Male and female rats of the Wistar strain weighing approximately 200 g maintained on Master Laboratory Chow and water ad libitum were used in acute studies. In chronic experiments, rats of the Wistar strain weighing initially about 100 g were used. Animals in all groups were starved overnight (16 hours) before being sacrificed.

II. ADRENALECTOMY

Animals were adrenalectomized according to the method described by Zarrow et al. (241). A small cut was made through the muscle layer, taking care to avoid cutting the muscles around the vertebral column. The perirenal fat was grasped with forceps and carefully pulled through the opening. The fat below the gland was grasped with curved forceps and the adrenal was separated from the kidney with a second pair of forceps used to tear fat beneath the gland. The excised gland was placed on a moist filter paper and carefully examined to make sure that the entire gland had been removed. The incision was closed with silk sutures and the animals were given a single intramuscular injection of deoxycorticosterone acetate (100 µg) suspended in oil. A period of two weeks was allowed for recovery.

III. PREPARATION OF SAMPLES FOR ASSAYING GLUCOSE, GLYCOGEN, UREA AND GLUCONEOGENIC ENZYMES

Animals were killed by decapitation and blood was collected for esti-

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mation of glucose and urea. The kidneys were quickly excised and their cortices dissected and weighed on a torsion balance. A small portion of liver was rapidly removed, weighed and used for glycogen determination. The remainder of the liver was also removed, weighed and 5% homogenates of both kidney cortex and hepatic tissue were prepared in 0.15 M KCl, pH 7.4. Homogenization was effected with a chilled Potter-Elvehjem homogenizer (Fisher Scientific Co., Fairlawn, N.J.) fitted with a Teflon plastic pestle spinning at about 700 r.p.m. for exactly 90 seconds. After removing an aliquot of the homogenate for estimating glucose 6-phosphatase and protein, the homogenate was spun for 30 minutes at 100,000 X g at 0°C in a refrigerated International preparative model B-60 ultracentrifuge (International Equipment Co., Needham Heights, Mass.). The supernatant fluids were decanted and stored in glass vials immersed in crushed ice.

A. Blood Glucose, Serum Urea and Liver Glycogen Determinations

Blood glucose was measured according to the method of Somogyi (242) and expressed as milligrams per 100 ml. The concentration of urea was determined in serum according to the urease method of Brent and Bergmeyer (243), and given as milligrams per 100 ml. The anthrone method of Seifter et al. (244) was used to assay hepatic glycogen expressed as g per 100 g of liver. Protein was determined in both homogenate and supernatant fluids of kidney and liver according to the method of Lowry et al. (245).

B. Assay of Gluconeogenic Enzymes

The activities of pyruvate carboxylase, phosphoenolpyruvate carboxykinase and fructose 1,6-diphosphatase were determined in the 100,000 X g supernatant fluids. In contrast, the whole homogenate was used for the assay of glucose 6-phosphatase activity in liver and kidney cortex. Pyruvate

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carboxylase assays were conducted at 340 mμ in a constant recording Unicam spectrophotometer model SP 800 (Unicam Instruments Ltd., Cambridge, England) thermostated to run at 37°C. Preliminary experiments were performed to establish linearity with regard to time and amount of the tissue and in each case, enzyme activities were assayed under strictly linear kinetic conditions. Pyruvate carboxylase, phosphoenolpyruvate carboxykinase and fructose 1,6-diphosphatase activities were calculated as micromoles of substrate metabolized per hour per g of tissue and expressed as specific activity per milligram protein in the supernatant fluid. In contrast, glucose 6-phosphatase was calculated as micromoles of glucose 6-phosphate metabolized per hour per g of tissue and expressed as specific activity per milligram protein in the homogenate.

(a) Pyruvate Carboxylase

Pyruvate carboxylase activity was measured according to the method of Scrutton et al. (246). The assay was based on the rate of disappearance of NADH in an assay system coupled with malic dehydrogenase. The following components, at the designated final concentrations, were added in the reaction mixture (final volume, 1.0 ml) in the given sequence: Tris buffer (pH 7.8), 100 mM; $MgCl_2$, 5 mM; ATP, 1 mM; Na pyruvate, 20 mM; $KHCO_3$, 15 mM; cysteine, 20 mM; acetyl coenzyme A, 0.1 mM; NADH, 112.5 mM; malic dehydrogenase, 50 μg of protein. The reaction was initiated by addition of an appropriate dilution of the supernatant fluid and changes in optical density were recorded for at least 5 minutes against a blank which contained no pyruvate.

(b) Phosphoenolpyruvate Carboxykinase

This enzyme was determined according to the procedure of Phillips

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and Berry (247) based on the conversion of oxaloacetate to phosphoenolpyruvate which is subsequently allowed to react with alkaline hypiodide to produce the resultant inorganic phosphate. The reaction mixture (final volume, 2.0 ml) contained the following components added in the given order: Tris buffer (pH 7.5), 88 mM; $MnCl_2$, 40 mM; KF, 200 mM; inosine triphosphate, 45 mM; oxaloacetate (pH 7.0), 32 mM. The blanks were devoid of the substrate, oxaloacetate. The reaction was initiated by adding supernatant fluid corresponding to 5 mg wet weight of the tissue and stopped by 10% trichloroacetic acid. The supernatant was decanted, reacted with sodium hypiodide for 10 minutes, and then centrifuged at 1,000 X g using a clinical centrifuge (International Equipment Co., Needham Heights, Mass.). The supernatant was decanted to remove the precipitate (iodoform) and inorganic phosphate was estimated colorimetrically according to the method of Fiske and Subbarow (248) using a Klett-Summerson Photoelectric Colorimeter (Klett Manufacturing Co., N.Y.).

(c) Fructose 1,6-Diphosphatase

Fructose 1,6-diphosphatase activity was determined by measuring the release of inorganic phosphate from fructose 1,6-diphosphate and complexing the remaining substrate with borate to allow the reaction to proceed to completion according to the method of Weber and Singhal (249). The following components, at the designated final concentrations, were added to the reaction mixture (final volume, 1.0 ml) in the given sequence: glycylglycine buffer (pH 7.4), 10 mM; $MgSO_4$, 10 mM; sodium borate (pH 7.4), 30 mM; fructose 1,6-diphosphate, 50 mM. Blanks contained no fructose 1,6-diphosphate. The reaction was started by adding supernatant fluid corresponding to 5 mg wet weight of the tissue and terminated by 10% trichloro-

acetic acid. The supernatants were decanted and inorganic phosphate was measured according to the method of Fiske and Subbarow (248) using a Klett-Summerson Photoelectric Colorimeter (Klett Manufacturing Co., N.Y.).

(d) Glucose 6-Phosphatase

Glucose 6-phosphatase was measured according to the method of Singhal (250). The assay was based on the rate of formation of glucose and inorganic phosphate from glucose 6-phosphate. The reaction mixture (final volume, 1.0 ml) contained potassium citrate buffer (pH 6.7), 33 mM and glucose 6-phosphate, 10 mM. Blanks were devoid of glucose 6-phosphate. The reaction was initiated by adding the homogenate corresponding to 5 mg wet weight of the tissue and stopped by 10% trichloroacetic acid. The supernatants were decanted and inorganic phosphate was determined by the method of Fiske and Subbarow (248) using a Klett-Summerson Photoelectric Colorimeter (Klett Manufacturing Co., N.Y.).

IV. PREPARATION OF TISSUES FOR MEASURING CYCLIC AMP- H^3 SYNTHESIS FROM TRITIATED ADENOSINE

Animals were killed by decapitation, kidneys quickly excised and their cortices dissected. The livers were also removed and approximately 20 mg slices of renal cortex and hepatic tissue were preincubated for 30 minutes at 37°C in 2 ml of oxygenated (95% O₂ - 5% CO₂ gas phase) Krebs-Ringer bicarbonate buffer pH 7.4, and 10⁻⁹M adenosine- H^3 in a Dubnoff metabolic shaker (Eberbach Corp., Ann Arbor, Mich.) vibrating at 60 oscillations per minute at 37°C and then incubated in the presence of various drugs and insecticides for a period of another half hour (251). For in vivo studies, tissues were rapidly excised from insecticide-treated animals, and then

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incubated for 60 minutes in vials containing 2 ml of oxygenated Krebs-Ringer bicarbonate buffer pH 7.4 and 10^{-9} M adenosine- H^3 (specific activity, 17 Ci/mmole) in a shaker, as described above. After incubation, the tissues were removed, rinsed with 0.9% saline and immediately frozen in liquid nitrogen. The tissues were then homogenized in cold 10% perchloric acid and spun for 10 minutes at 1,000 X g in a clinical centrifuge (International Equipment Co., Needham Heights, Mass.). The supernatant fluids were obtained, decanted and neutralized with 5M K_2CO_3 . A summary of the experimental protocol used for studying the influence of various insecticides on cyclic AMP- H^3 synthesis from tritiated adenosine both in vitro and in vivo is presented in Figure 3.

A. Cyclic AMP- H^3 Analysis

A 10 μ l aliquot of the neutralized fraction was spotted on polyethyleneimine (PEI-cellulose) layers which contained fluorescent indicator (Brinkmann Polygram 300^R). Non-radioactive adenosine, cyclic 3',5'-AMP, 5'-AMP, ADP and ATP were added as carriers in order to obtain sufficient ultra-violet (UV) visualization. The thin-layer plates were developed for 30 minutes using a solvent system of 95% ethanol and 5M ammonium acetate in a ratio of 5:2. The spots were visualized and as illustrated in Figure 4, the R_f value of cyclic AMP was quite distinct from the motility rate of the other adenylates. The R_f value of cyclic AMP was 0.35, of adenosine was 0.65, whereas ATP, ADP and 5'-AMP remained at the origin. The radioactive cyclic AMP- H^3 spots were cut from the plate and placed in scintillation counting vials. The PEI-cellulose layer was removed from its plastic backing by sonicating with a sonic dismembrator

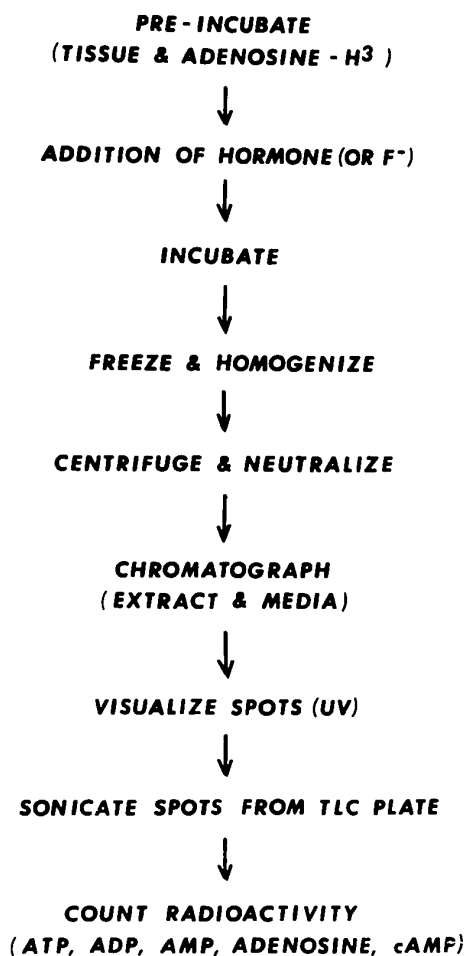
EXPERIMENTAL PROTOCOL

Fig. 3. Summary of the experimental protocol used for studying the formation of cyclic AMP-H³ from tritiated adenosine by renal and hepatic tissue. The thin layer plates were developed for 30 minutes using a solvent system of 95% ethanol and 5M ammonium acetate in a ratio of 5:2. Spots were visualized, cut from the plate and placed in scintillation counting vials. The cyclic AMP-H³ thus formed reflects the tissue's ability to biotransform tritiated adenosine into labeled adenosine triphosphate which is then converted subsequently into the cyclic nucleotide.

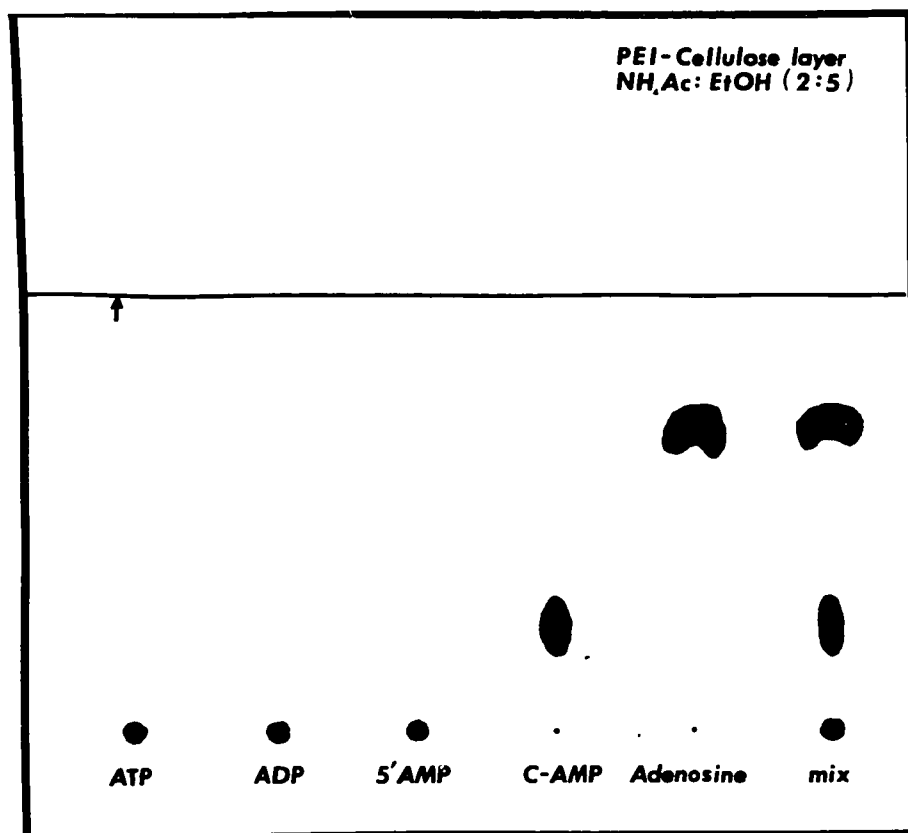


Fig. 4. Chromatographic separation of cyclic AMP from other adenylates. The R_f value of cyclic AMP = 0.35, of adenosine = 0.65, whereas ATP, ADP and 5'-AMP remained at the origin.

(Artek Systems Corp., Farmingdale, N.Y.) and the radioactivity was measured employing a suitable scintillation cocktail. In order to ascertain that the radioactivity measured was due to cyclic AMP, several other related substances were examined for their chromatographic characteristics. None of the compounds studied (including adenine, adenosine, 5'-AMP, ADP, ATP, guanine, guanosine, cyclic GMP, GMP, GDP, GTP, inosine, xanthine, hypoxanthine, theophylline and uric acid) possessed a R_f value similar to that obtained for cyclic AMP as had also been demonstrated previously by Smith (252). The results were expressed as disintegrations per minute per milligram wet weight of the tissue $\times 10^2$. The cyclic AMP- H^3 thus measured reflects the tissue's ability to biotransform tritiated adenosine into labeled ATP which is then converted subsequently into the cyclic nucleotide- H^3 .

V. PREPARATION OF TISSUE SAMPLES, PROTEIN INHIBITOR KINASE AND PROTEIN KINASE FOR ASSAYING ENDOGENOUS LEVELS OF CYCLIC AMP

Approximately 100 mg of renal cortex and hepatic tissue were quickly frozen in liquid nitrogen and homogenized in 3 ml of 5% trichloroacetic acid. Homogenization was effected with a chilled Potter-Elvehjem homogenizer (Fisher Scientific Co., Fairlawn, N.J.), fitted with a plastic Teflon pestle spinning at 700 r.p.m. for exactly 90 seconds. The homogenate was then spun for 15 minutes at 1,000 X g in a clinical centrifuge (International Equipment Co., Needham Heights, Mass.) and 0.2 ml of 1N HCl was added to the supernatant. The trichloroacetic acid was removed by extracting with anhydrous ether and the remaining supernatant was

lyophilized using a freeze-drying lyophilizer (Virtis Co., Gardiner, N.Y.). The freeze-dried residue was dissolved in 0.5 ml of distilled water and the concentration of cyclic AMP was determined according to the protein-binding method of Gilman (253).

A. Preparation of Protein Kinase Inhibitor

Protein kinase inhibitor was prepared according to the method of Appleman et al. (254). Fresh bovine muscle obtained from the slaughter house was minced and homogenized in 3 volumes of 10 mM Tris buffer (pH 7.5). Homogenization was carried out in a one-speed blender (Waring Blender, New York, N.Y.) for 2 minutes at 22°C. The homogenate was boiled for 10 minutes, filtered and precipitated with 50% trichloroacetic acid. The homogenate was spun for 30 minutes at 15,000 X g at 0° in a refrigerated International preparative model B-60 ultracentrifuge (International Equipment Co., Needham Heights, Mass.). The precipitate was collected, dissolved in distilled water, adjusted to pH 7.0, and dialyzed against distilled water at 22°C for 16 hours. The suspension was spun for 30 minutes at 15,000 X g at 0° in a refrigerated International preparative model B-60 ultracentrifuge and the remaining supernatant was lyophilized using a freeze-drying lyophilizer (Virtis Co., Gardiner, N.Y.). The freeze-dried residue was dissolved in distilled water such that the solution contained 300 µg protein kinase inhibitor per 0.1 ml.

B. Preparation of Cyclic AMP-Dependent Protein Kinase

This enzyme was prepared according to the method of Miyamoto et al. (255). Fresh bovine muscle was minced and homogenized in 3 volumes of 4 mM ethylenediamine tetraacetate (pH 7.0). Homogenization was carried out in a one-speed blender (Waring Blender, New York, N.Y.) for 2 minutes

at 22°C. The homogenate was spun for 30 minutes at 27,000 X g at 0°C in a refrigerated International preparative model B-60 ultracentrifuge (International Equipment Co., Needham Heights, Mass.). The supernatant was decanted, adjusted to pH 4.8 and centrifuged for 30 minutes at 27,000 X g at 0°C in an International preparative model B-60 ultracentrifuge. The supernatant was again decanted, adjusted to pH 6.5, precipitated with 2.5 M ammonium sulphate, stirred for 30 minutes, and then centrifuged for 20 minutes at 16,000 X g at 0°C in an International preparative model B-60 ultracentrifuge. The precipitate was dissolved in 5 mM potassium phosphate buffer (pH 7.0) to make a 6% solution which was dialyzed against potassium phosphate buffer (pH 7.0) for 14 hours. The dialyzed suspension was centrifuged for 30 minutes at 27,000 X g at 0°C in an International preparative model B-60 ultracentrifuge. The supernatant was decanted, applied to a column of DEAE cellulose (Whatman DE 11, 1 meq/g, 32 x 2.6 cm) previously washed with 5 mM potassium phosphate buffer (pH 7.0) and eluted with 100 mM potassium phosphate buffer (pH 7.0). The column was further eluted with 300 mM potassium phosphate buffer (pH 7.0). The eluted fraction contained 90 micrograms of binding protein per ml and 1 microgram of this protein was found to bind approximately 30 picomoles of cyclic AMP.

C. Cyclic AMP Determination

Cyclic AMP levels were measured according to the protein-binding method of Gilman (253). Cyclic AMP was allowed to compete with a known amount of tritiated cyclic AMP for binding sites on a cyclic AMP-dependent protein kinase in the presence of a protein kinase inhibitor. The cyclic

nucleotide-protein complex was adsorbed on a Millipore cellulose ester filter (Millipore Corp., Montreal, Que.) which was dissolved in 1.5 ml of methyl cellusolve (Fisher Scientific Co., Fairlawn, N.J.). The activity of this complex was then measured employing a suitable scintillation cocktail. The following components, at the designated final concentrations were added to the reaction mixture (final volume, 50 μ l) in the given sequence: Na acetate buffer (pH 4.0), 50 mM; protein kinase inhibitor, 15 μ g of protein; tritiated cyclic AMP, 3.6 picomoles; homogenate fluid corresponding to 10 mg wet weight of the tissue. The reaction was initiated by the addition of 1 μ g of binding protein and the concentration of cyclic AMP was calculated from a known set of standards counted under similar conditions and expressed as picomoles of cyclic AMP per milligram wet weight of the tissue.

VI. PREPARATION OF TISSUE SAMPLES FOR ASSAYING ADENYL CYCLASE AND PHOSPHODIESTERASE

5% homogenates of kidney cortex and liver were prepared in a homogenizing buffer containing NaCl, 20 mM; MgSO_4 , 1 mM; and glycyl-glycine buffer (pH 7.6), 2 mM. Homogenization was effected with a chilled Potter-Elvehjem homogenizer (Fisher Scientific Co., Fairlawn, N.J.) fitted with a plastic Teflon pestle spinning at about 700 r.p.m. for exactly 90 seconds. An appropriate aliquot of the homogenate was then used for the assay of adenylyl cyclase and phosphodiesterase activity.

A. Measurement of Adenylyl Cyclase Activity

Adenylyl cyclase activity was measured according to a modified procedure (256) of Sutherland et al. (257). The assay was based on the biotrans-

formation of adenosine triphosphate into cyclic AMP which was measured by the protein-binding method of Gilman (253) as described above. An inhibitor of phosphodiesterase, caffeine, was added to prevent the breakdown of the cyclic nucleotide. The reaction mixture (final volume, 1.0 ml) contained the following components added in the given order: Tris buffer (pH 7.5), 50 mM; MgCl_2 , 5 mM; ATP, 1 mM; caffeine, 7 mM; phosphoenolpyruvate, 10 mM; pyruvate kinase, 2 μg of protein; bovine serum albumin, 1 mg of protein. Sodium fluoride at a concentration of 10 mM was added to measure the fluoride-stimulated form of adenylyl cyclase. The reaction was initiated by the addition of homogenate fluid corresponding to 10 mg wet weight of the tissue, stopped by boiling for 3 minutes and centrifuged for 10 minutes at 1,000 X g using a clinical centrifuge. An appropriate aliquot of the supernatant was used for assaying adenylyl cyclase activity. Enzyme activity was calculated as picomoles of cyclic AMP formed per hour per mg wet weight of the tissue and expressed as specific activity per mg protein.

B. Assay of Phosphodiesterase Activity

This enzyme was assayed according to the procedure of Weiss (258) based on the rate of conversion of cyclic 3',5'-AMP to 5'-AMP which was reacted with alkaline phosphatase to yield inorganic phosphate. The assay medium contained the following in a total volume of 1.0 ml at the designated final concentration: Tris buffer (pH 8.0), 50 mM; cyclic AMP, 1 mM; MgSO_4 , 3 mM; alkaline phosphatase, 0.1 mg of protein. The reaction was started by the addition of homogenate fluid corresponding to 5 mg wet weight of the tissue and stopped by addition of 55% trichloroacetic acid. Inorganic phosphate was measured in the supernatant according to the method of Fiske and Subbarow (248) using a Klett-Summerson Photoelectric

Colorimeter (Klett Manufacturing Co., N.Y.). Phosphodiesterase activity was calculated as picomoles of cyclic AMP hydrolyzed per hour per mg wet weight of the tissue and expressed as specific activity per mg protein.

VII. CHEMICALS

All reagents were of the purest grade available and were dissolved in glass distilled water unless stated otherwise. Adenosine, ADP, ATP, AMP, cyclic 3',5'-AMP, pyruvate, acetyl CoA, NADH, malic dehydrogenase, inosine triphosphate, oxaloacetate, fructose 1,6-diphosphate, glucose 6-phosphate, phosphoenolpyruvate, pyruvate kinase, bovine serum albumin, and alkaline phosphatase were purchased from the Sigma Chemical Co. (St. Louis, Mo.). Caffeine (Matheson Co., Cincinnati, Ohio), imidazole (Sigma Chemical Co., St. Louis, Mo.), propranolol (Ayerst, McKenna and Harrison Ltd., Montreal, Que.), hydrazine sulphate (Fisher Scientific Co., Fairlawn, N.J.), prostaglandin E₁ and F₂ α (Upjohn Co., Kalamazoo, Mich.), actinomycin D (Merck, Sharp and Dohme, Rahway, N.J.), ethionine (Sigma Chemical Co., St. Louis, Mo.), cycloheximide (Upjohn Co., Kalamazoo, Mich.) and triamcinolone (Lederle Laboratories, Pearl River, N.Y.) were dissolved in physiological saline. Adenosine-H³ was purchased from the Amersham/Searle Corp. (Don Mills, Ont.). p,p'-DDT, α -chlordane, heptachlor and endrin (Velsicol Chemical Corp., Chicago, Ill.) were dissolved in corn oil and administered orally in a total volume of 1 ml in acute experiments. In chronic studies however, the hydrocarbon insecticides were injected by the intramuscular route in a final volume of 0.1 ml. Control animals always received an equal volume of corn oil. In in vitro experiments,

p,p'-DDT, α -chlordane, heptachlor and endrin were dissolved in 95% ethanol and added to the incubation medium in a total volume of 0.06 ml. Control tissue samples were also incubated with an equal amount of 95% ethanol.

VIII. STATISTICAL ANALYSIS

The results were subjected to statistical evaluation using a Student's "t-test" and significant differences between the means (calculated as p values) are shown. No statistical significance is indicated when the p value was >0.05 .

4. RESULTS

I. CHANGES IN KIDNEY CORTEX CARBOHYDRATE METABOLISM INDUCED BY ACUTE ADMINISTRATION OF DDT AND OTHER CHLORINATED HYDROCARBON INSECTICIDES

A. Effect of p,p'-DDT on Gluconeogenic Enzymes in Male Rats

In rats given a single, oral dose of p,p'-DDT (600 mg/kg), consistent tremor, convulsions and hyperpyrexia were observed at 5 hours (67,69,79) and death generally occurred between the sixth and the eighth hour. The results presented in Table 2 illustrate that p,p'-DDT treatment increases the activities of the four key gluconeogenic enzymes in kidney cortices of male rats. When expressed on a per milligram protein basis, renal cortex pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose 1,6-diphosphatase and glucose 6-phosphatase rose to 288%, 280%, 297% and 245% of the control values. Since a similar magnitude of response was noted when enzyme activities were calculated as per gram wet weight of the tissue, in all subsequent experiments, the activities are expressed only on a per mg protein basis.

B. Time-Course of the p,p'-DDT-Induced Stimulation of Gluconeogenic Enzymes

Results presented in Figure 5 illustrate the sequential changes of the p,p'-DDT-induced enhancement in the quartet of rate-limiting gluconeogenic enzymes in rat kidney cortex. Enzyme activities increased significantly as early as one hour when pyruvate carboxylase was 145%, phosphoenolpyruvate carboxykinase 172%, fructose 1,6-diphosphatase 147% and

TABLE 2

EFFECT OF p,p'-DDT TREATMENT ON KEY GLUCONEOGENIC ENZYME ACTIVITIES IN RAT KIDNEY CORTEX

Means $\pm$ S.E.M. represent four animals in each group. Rats were administered 600 mg/kg of p,p'-DDT orally and killed five hours later. Enzyme activities were calculated as micromoles of substrate metabolized per hour per gram of tissue and expressed as specific activity per gram and per milligram of protein. Data are also given in percentages (in parentheses) taking the values of control animals as 100%. Abbreviations used are: PC, pyruvate carboxylase; PEPCK, phosphoenolpyruvate carboxykinase; FD-Pase, fructose 1,6-diphosphatase; G6-Pase, glucose 6-phosphatase.

Treatment	PC		PEPCK		FD-Pase		G6-Pase	
	A/g	A/mg P	A/g	A/mg P	A/g	A/mg P	A/g	A/mg P
Control	7236 $\pm$ 0 (100)	120 $\pm$ 1 (100)	1110 $\pm$ 52 (100)	18 $\pm$ 1 (100)	197 $\pm$ 5 (100)	3.3 $\pm$ 0 (100)	1135 $\pm$ 19 (100)	12 $\pm$ 0.3 (100)
p,p'-DDT	19532 $\pm$ 1311 (269)*	344 $\pm$ 21 (288)*	2958 $\pm$ 42 (267)*	52 $\pm$ 1 (280)*	548 $\pm$ 14 (278)*	9.8 $\pm$ 0.3 (297)*	2634 $\pm$ 162 (233)*	29 $\pm$ 2 (245)*

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

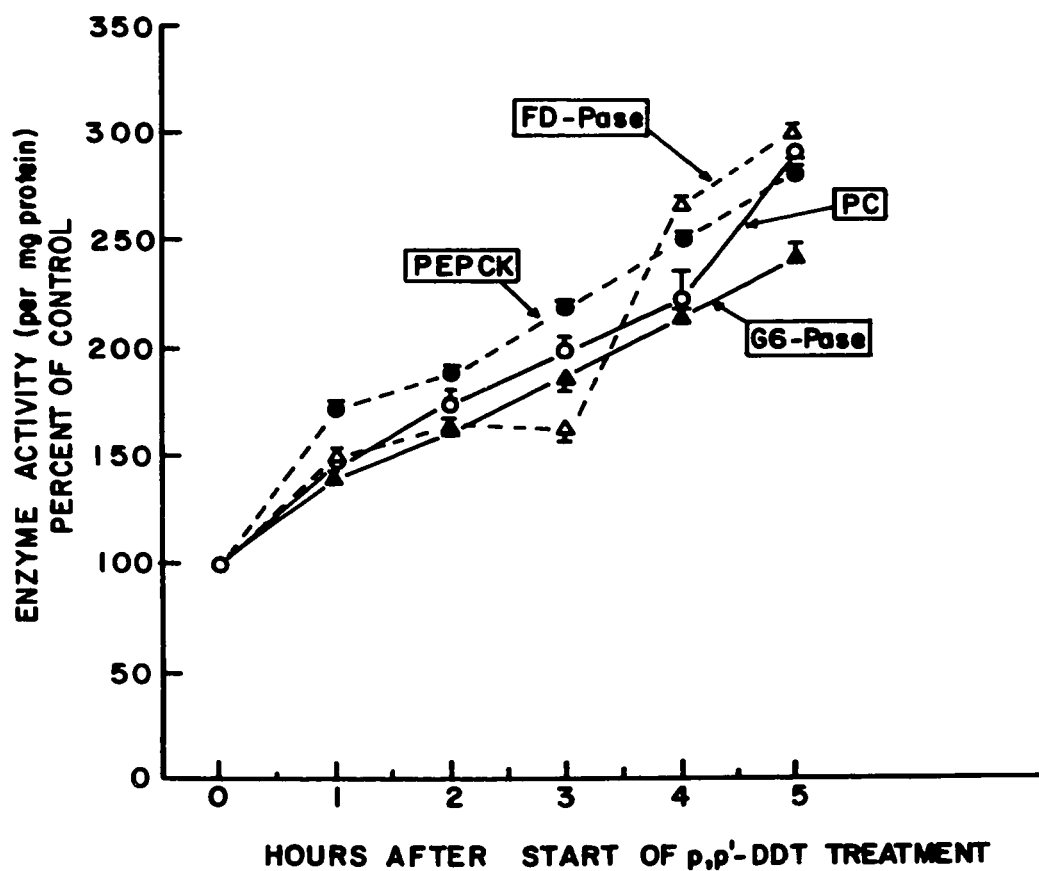


Fig. 5. Time-course of the p,p'-DDT-induced stimulation of kidney gluconeogenic enzymes. Each point represents mean $\pm$ S.E.M. of 4 rats in each group. Animals were administered p,p'-DDT (600 mg/kg) orally and killed 1, 2, 3, 4 and 5 hours after insecticide treatment. Enzyme activities were calculated as micromoles of substrate metabolized per hour per gram of tissue and expressed as specific activity per milligram protein. Data are given in percentages taking the values of control animals as 100%.

glucose 6-phosphatase 148% as compared to the control values. All renal gluconeogenic enzymes rose progressively at two, three and four hours and during the period of our experiment, reached highest values five hours after the administration of p,p'-DDT.

C. Influence of Varying Doses of p,p'-DDT

Since the administration of p,p'-DDT at a dose of 600 mg/kg resulted in marked increases in the activities of various renal gluconeogenic enzymes, a dose-response study was carried out to examine whether smaller doses of this insecticide also could produce significant increases in these enzymes. The results are reported in Table 3. A dose of 25 mg/kg p,p'-DDT, which exerted no effect on pyruvate carboxylase activity, increased phosphoenolpyruvate carboxykinase to 138%, fructose 1,6-diphosphatase to 125% and glucose 6-phosphatase to 126% of the control values. However, the increase was statistically significant only in the case of phosphoenolpyruvate carboxykinase. Table 3 also shows that increasing the dose of p,p'-DDT to 100 mg/kg resulted in significant increases in the activities of all four key gluconeogenic enzymes. Maximal stimulation of renal pyruvate carboxylase, fructose 1,6-diphosphatase and glucose 6-phosphatase was observed with the 400 mg/kg dose whereas phosphoenolpyruvate carboxykinase increased maximally when p,p'-DDT was administered at the dose level of 600 mg/kg body weight.

D. Comparison of the Effects of p,p'-DDT and o,p'-DDT in Male and Female

Rats

Since technical DDT contains approximately 80% p,p'-DDT and 20% o,p'-DDT, the influence of these two isomers on gluconeogenic enzymes was

TABLE 3

EFFECT OF VARYING DOSES OF p,p'-DDT ON KEY GLUCONEOGENIC ENZYMES
IN KIDNEY CORTEX

Means $\pm$ S.E.M. represent at least four animals in each group. Rats were administered with p,p'-DDT in doses ranging from 25 to 600 mg/kg orally and killed five hours after treatment. Enzyme activities were calculated as micromoles of substrate metabolized per hour per gram of tissue and expressed as specific activity per milligram of protein. Data are also given in percentages (in parentheses) taking the values of control animals as 100%. Abbreviations used are: PC, pyruvate carboxylase; PEPCK, phosphoenolpyruvate carboxykinase; FD-Pase, fructose 1,6-diphosphatase; G6-Pase, glucose 6-phosphatase.

Dose of p,p'-DDT (mg/kg)	PC	PEPCK	FD-Pase	G6-Pase
Control	137 $\pm$ 5 (100)	22 $\pm$ 1 (100)	4.8 $\pm$ 0.1 (100)	7.4 $\pm$ 0.1 (100)
25	138 $\pm$ 9 (100)	30 $\pm$ 1 (138)*	6.0 $\pm$ 0.6 (125)	9.3 $\pm$ 0.7 (126)
100	216 $\pm$ 9 (158)*	41 $\pm$ 0 (186)*	7.2 $\pm$ 0.4 (150)*	10.6 $\pm$ 0.2 (143)*
200	315 $\pm$ 5 (229)*	53 $\pm$ 1 (241)*	9.5 $\pm$ 0.3 (198)*	12.6 $\pm$ 0.3 (170)*
400	320 $\pm$ 10 (234)*	58 $\pm$ 3 (264)*	11.5 $\pm$ 1.0 (239)*	17.2 $\pm$ 0.5 (232)*
600	319 $\pm$ 17 (233)*	72 $\pm$ 4 (327)*	12.5 $\pm$ 0.4 (260)*	17.0 $\pm$ 0.1 (230)*

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

investigated in kidney cortices of both male and female rats. Data in Figure 6 compare the effects of p,p'-DDT and o,p'-DDT on kidney enzymes in male rats. In general, both isomers were found to be equally effective in increasing the activities of various gluconeogenic enzymes in the renal cortex. In addition, similar enzyme increases were observed when the two isomers were administered to female rats (Fig. 6), indicating that no sex difference existed for the action of either o,p'-DDT or p,p'-DDT in stimulating the process of kidney gluconeogenesis.

E. Effect of p,p'-DDT in Adrenalectomized Rats

In order to examine whether adrenal glands are involved in the observed enhancement of kidney gluconeogenic enzymes, the effects of p,p'-DDT were investigated in adrenalectomized rats. Data in Figure 7 show that when p,p'-DDT was administered to these rats, the activities of pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose 1,6-diphosphatase and glucose 6-phosphatase were elevated to about the same extent as that observed in the cortices of intact rats. When a potent glucocorticoid, triamcinolone (10 mg/100 g) was injected, minor but statistically significant increases were seen in the activities of renal phosphoenolpyruvate carboxykinase and fructose 1,6-diphosphatase without any concomitant change in the other two enzymes investigated. It is of interest that treatment of p,p'-DDT-injected animals with triamcinolone did not augment further the effects of this insecticide on any of these key gluconeogenic enzymes except in the case of phosphoenolpyruvate carboxykinase. These results suggest that the increases in the activities of various gluconeogenic enzymes produced by p,p'-DDT in the kidney cortex are not mediated through glucocorticoids.

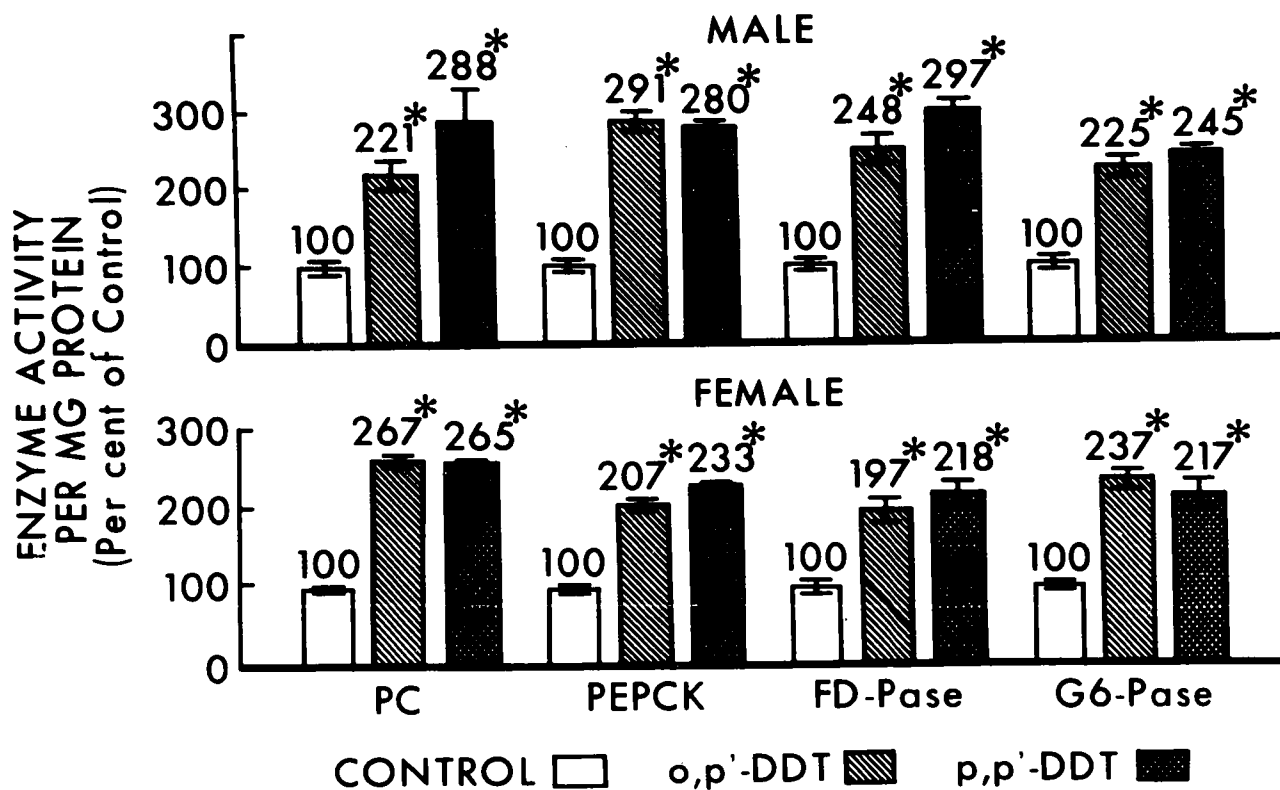


Fig. 6. Comparative effects of o,p'-DDT and p,p'-DDT on key gluconeogenic enzymes in kidney cortex of male and female rats. Bars represent the mean $\pm$ S.E.M. of 4 values in each group. Rats were given either o,p'-DDT or p,p'-DDT (600 mg/kg) orally and killed after 5 hours. Enzyme activities were calculated as micromoles of substrate metabolized per hour per gram of tissue and expressed as specific activity per milligram protein. Data are given in percentages taking the values of control animals as 100%.

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

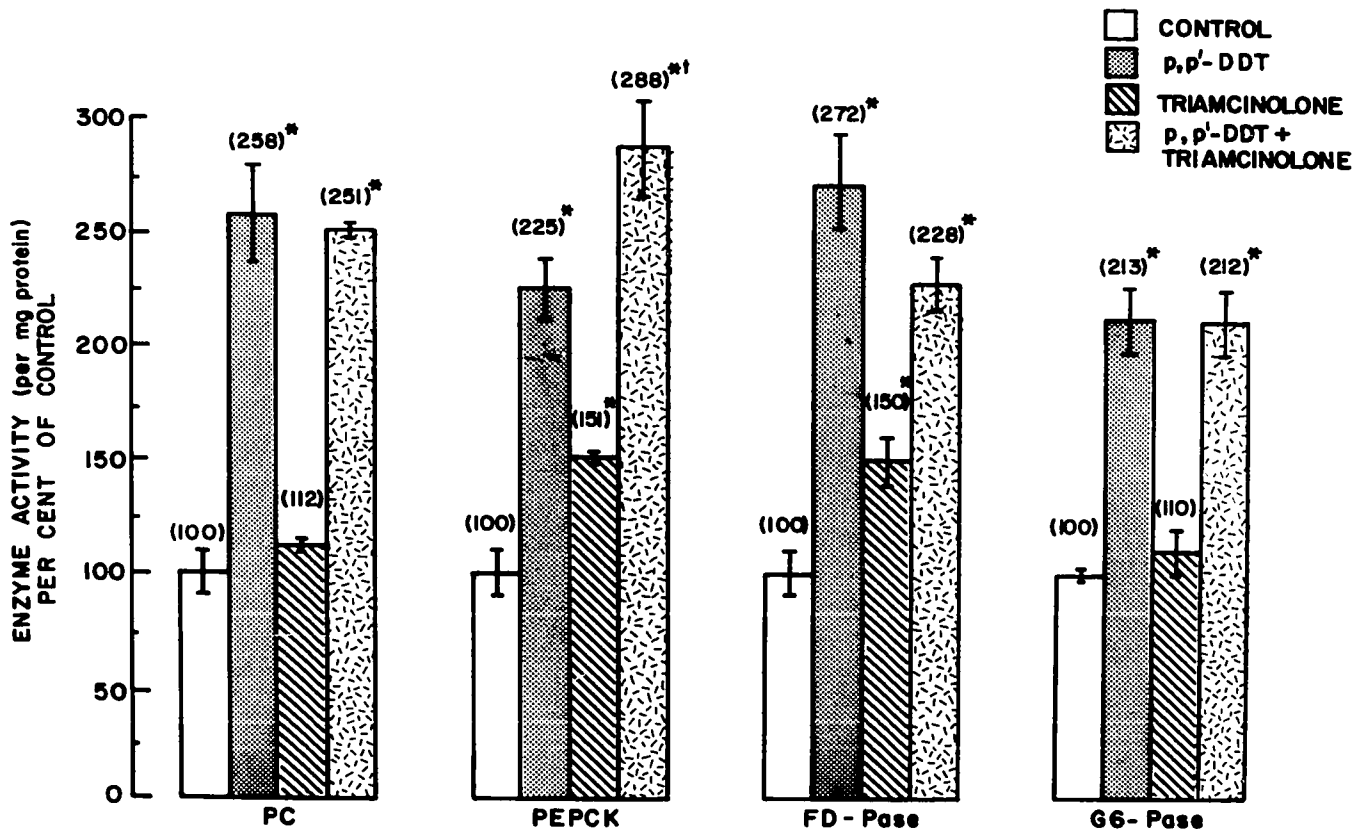


Fig. 7. Independence of p,p'-DDT-induced changes in kidney gluconeogenic enzymes from adrenal function. Each bar represents a mean value $\pm$ S.E.M. of at least 4 adrenalectomized animals in each group. Adrenalectomized rats were administered p,p'-DDT (600 mg/kg) orally and killed 5 hours later. Triamcinolone (10 mg/100 g) was injected either alone or concurrently with p,p'-DDT. Enzyme activities were calculated as micromoles of substrate metabolized per hour per gram of tissue and expressed as specific activity per milligram protein. Data are expressed in percentages, taking the values of control animals as 100%.

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

†Statistically significant difference when compared with the values of rats given p,p'-DDT alone ($p = <0.05$).

F. Influence of Inhibitors of RNA and Protein Synthesis

The nature of the p,p'-DDT-induced increases in the activities of various kidney gluconeogenic enzymes was studied with the use of compounds that are known to inhibit RNA and protein synthesis. The data presented in Figure 8 show that administration of either actinomycin D, cycloheximide or ethionine produced no significant changes in the basal levels of any of the enzymes studied. However, prior administration of either of these compounds to p,p'-DDT-treated animals significantly reduced the insecticide-stimulated increases in the activities of pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose 1,6-diphosphatase and glucose 6-phosphatase. Among the three inhibitors of RNA and protein synthesis used, cycloheximide seemed most effective in abolishing the p,p'-DDT-induced enzymic increases and the values in this group of rats remained close to those noted for controls.

G. Comparative Effects of Various Insecticides on Kidney Gluconeogenic Enzymes

In order to compare the effects of p,p'-DDT on the quartet of gluconeogenic enzymes with other organochlorine insecticides, rats were administered p,p'-DDT (600 mg/kg), α -chlordane (200 mg/kg), heptachlor (200 mg/kg) or endrin (50 mg/kg) by the oral route and killed 3 hours later. Data presented in Table 4 demonstrate that treatment with either of these insecticides significantly increased the activities of renal cortex pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose 1,6-diphosphatase and glucose 6-phosphatase. The most marked increase was seen in the case of phosphoenolpyruvate carboxykinase in rats treated with p,p'-DDT. The

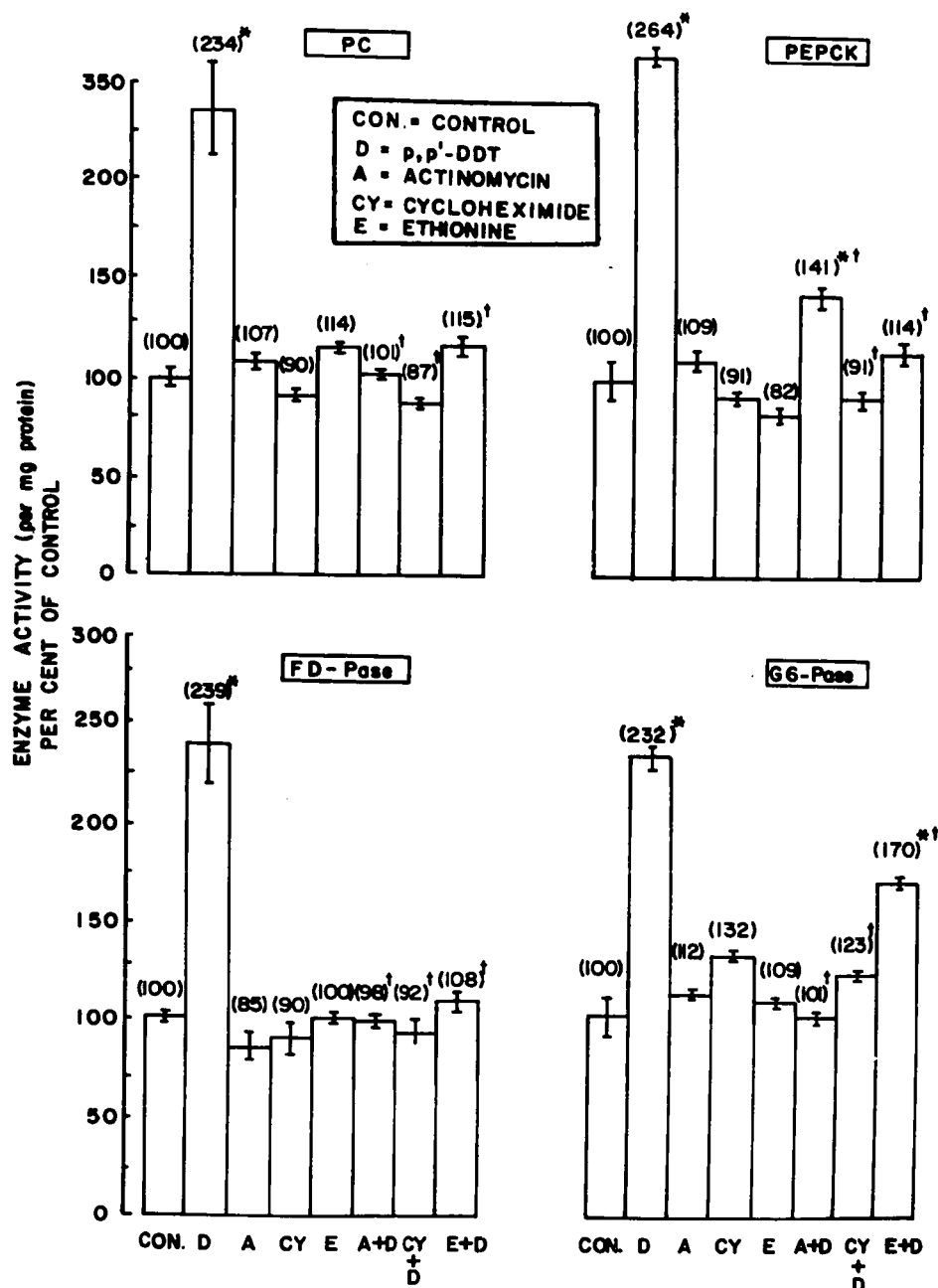


Fig. 8. Effect of inhibitors of RNA and protein synthesis on p,p'-DDT-induced increases in renal gluconeogenic enzymes of male rats. Each bar represents the mean $\pm$ S.E.M. of at least 4 values in each group. p,p'-DDT was administered orally in a dose of 400 mg/kg and the animals were killed after 5 hours. Actinomycin D (40 μ g/100 g) was given in two divided doses 30 minutes before and 2½ hours after DDT treatment. Cycloheximide (70 μ g/100 g) and DL-ethionine (50 mg/100 g) were injected intraperitoneally 30 minutes before DDT administration. Enzyme activities were calculated as micromoles of substrate metabolized per hour per gram of tissue and expressed as specific activity per milligram protein. Data are given in percentages (in parentheses) taking the values of control animals as 100%. *Statistically significant difference when compared with the values of control rats (p = <0.05). †Statistically significant difference when compared with the values seen with p,p'-DDT alone (p = <0.05).

TABLE 4

EFFECT OF VARIOUS INSECTICIDES ON KEY GLUCONEOGENIC ENZYMES
IN KIDNEY CORTEX OF RATS

Means $\pm$ S.E.M. represent four animals in each group. Rats were administered either p,p'-DDT (600 mg/kg), α -chlordane (200 mg/kg), heptachlor (200 mg/kg) or endrin (50 mg/kg) orally and killed 3 hours later. Enzyme activities were calculated as micromoles of substrate metabolized per hour per gram of tissue and expressed as specific activity per milligram of protein. Data are also given in percentages (in parentheses) taking the values of control animals as 100%.

Treatment	PC	PEPCK	FD-Pase	G6-Pase
Control	120 $\pm$ 1 (100)	18 $\pm$ 1 (100)	6.6 $\pm$ 0 (100)	8 $\pm$ 0.3 (100)
p,p'-DDT	238 $\pm$ 5 (199)*	40 $\pm$ 1 (219)*	10.4 $\pm$ 0.3 (158)*	15 $\pm$ 0.8 (189)*
α -Chlordane	187 $\pm$ 0 (156)*	32 $\pm$ 1 (178)*	12.5 $\pm$ 0.2 (189)*	14 $\pm$ 0.4 (175)*
Heptachlor	200 $\pm$ 5 (167)*	32 $\pm$ 1 (178)*	12.4 $\pm$ 0.4 (188)*	11 $\pm$ 0.1 (138)*
Endrin	206 $\pm$ 15 (172)*	32 $\pm$ 1 (178)*	9.8 $\pm$ 0.3 (148)*	13 $\pm$ 0.3 (163)*

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

results show that the various structurally-unrelated pesticides examined are equally effective in augmenting the activities of the four key renal gluconeogenic enzymes.

II. ALTERATIONS IN HEPATIC CARBOHYDRATE METABOLISM INDUCED BY ACUTE ADMINISTRATION OF DDT AND OTHER ORGANOCHLORINE INSECTICIDES

A. Time-Course of the p,p'-DDT-Induced Changes in Blood Glucose and Liver Glycogen in Normal Rats

The time-course of p,p'-DDT-induced changes in blood glucose and liver glycogen is illustrated in Figure 9. Following the oral intubation of this insecticide (600 mg/kg), blood glucose was increased significantly in $\frac{1}{2}$ hour (143%), attained peak values at 1 hour (160%) and then declined to the control levels. In contrast, the amount of hepatic glycogen (1.6 ± 0.4 g%) decreased gradually to 47% of the control values at 1 hour which was also later restored by 3 hours. These 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.

B. p,p'-DDT-Induced Changes in Blood Glucose and Liver Glycogen in Adrenalectomized Rats

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 oral dose of this insecticide on the concentration of blood glucose and liver glycogen in adrenalectomized rats. Results presented in Table 5 demonstrate sequential changes in blood glucose and hepatic glycogen content following the administration of 600 mg/kg

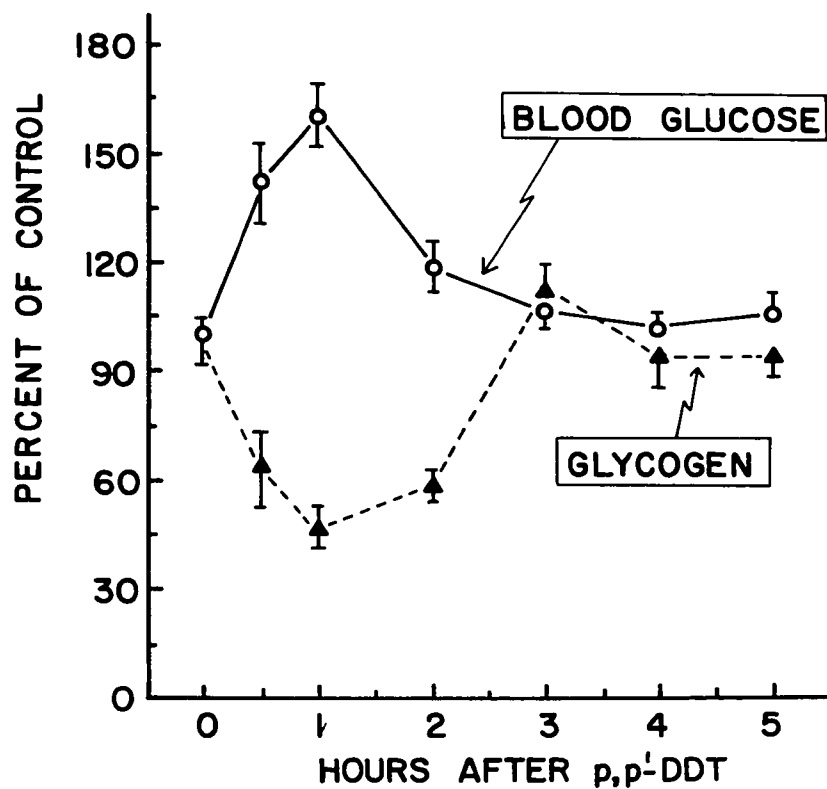


Fig. 9. Time-course of the p,p'-DDT-induced changes in blood glucose and liver glycogen. Each point represents the mean $\pm$ S.E.M. of 4 rats in each group. Animals were administered p,p'-DDT (600 mg/kg) orally and killed $\frac{1}{2}$, 1, 2, 3, 4 and 5 hours after insecticide treatment. Data are given in percentages taking the values of control rats as 100%.

TABLE 5

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

Means $\pm$ S.E.M. represent four animals in each group. Adrenalectomized rats were administered p,p'-DDT (600 mg/kg) orally and killed $\frac{1}{2}$, 1, 2, 4 and 5 hours after pesticide treatment. Glucose concentration is expressed as milligrams per 100 milliliters of blood whereas glycogen as grams per 100 grams of liver. Data are also given in percentages (in parentheses) taking the values of control rats as 100%.

Time (hr)	Blood Glucose (mg/100 ml)	Liver Glycogen (g/100 g)
0 (Control)	46 $\pm$ 2 (100)	.73 $\pm$.03 (100)
$\frac{1}{2}$	101 $\pm$ 5 (219)*	.54 $\pm$.05 (74)*
1	100 $\pm$ 3 (218)*	.48 $\pm$.03 (66)*
2	86 $\pm$ 2 (187)*	.56 $\pm$.04 (77)*
4	79 $\pm$ 5 (172)*	.50 $\pm$.04 (68)*
5	79 $\pm$ 5 (172)*	.56 $\pm$.03 (77)*

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

dose of p,p'-DDT. In adrenalectomized animals, blood glucose increased to 219% of the control values $\frac{1}{2}$ hour after pesticide treatment and remained significantly elevated throughout the experimental period. In contrast, the concentration of hepatic glycogen declined to 74% at $\frac{1}{2}$ hour, reached the minimal level (66%) at 1 hour and remained below the control values during the 5 hour period. Since p,p'-DDT elevated blood glucose and decreased hepatic glycogen even in the absence of 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.

C. Time-Course of the p,p'-DDT-Induced Stimulation of Hepatic Gluconeogenic Enzymes

The sequential changes in the activities of hepatic pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose 1,6-diphosphatase and glucose 6-phosphatase following the administration of 600 mg/kg of p,p'-DDT orally are shown in Table 6. When expressed as per mg protein, enzyme activities increased significantly as early as 1 hour and rose progressively to peak values at 5 hours when pyruvate carboxylase was 244%, phosphoenolpyruvate carboxykinase 197%, fructose 1,6-diphosphatase 230% and glucose 6-phosphatase 217% of the control values.

D. 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 Figure 10 show that 25 mg/kg dose of p,p'-DDT failed to exert any measurable effect on any of the

TABLE 6

TIME-COURSE OF p,p'-DDT-INDUCED STIMULATION OF HEPATIC
GLUCONEOGENIC ENZYMES

Means $\pm$ S.E.M. represent four or more animals in each group. Rats were administered 600 mg/kg of p,p'-DDT orally and killed either 1, 3 or 5 hours after the treatment. Enzyme activities were calculated as micromoles of substrate metabolized per hour per gram of tissue and expressed as specific activity per milligram of protein. Data are also given in percentages (in parentheses) taking the values of control animals as 100%.

Enzymes	Time in hours after the administration of p,p'-DDT			
	0 (Control)	1	3	5
PC	245 $\pm$ 11 (100)	365 $\pm$ 7 (149)*	438 $\pm$ 8 (179)*	598 $\pm$ 28 (244)*
PEPCK	13.9 $\pm$ 0.5 (100)	17.4 $\pm$ 0.2 (125)*	20.9 $\pm$ 0.8 (150)*	27.4 $\pm$ 2.9 (197)*
FD-Pase	2.8 $\pm$ 0 (100)	4.5 $\pm$ 0.1 (161)*	6.1 $\pm$ 0.1 (218)*	6.4 $\pm$ 0.4 (230)*
G6-Pase	3.8 $\pm$ 0.1 (100)	5.2 $\pm$ 0.2 (137)*	6.6 $\pm$ 0.5 (174)*	8.2 $\pm$ 0.5 (217)*

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

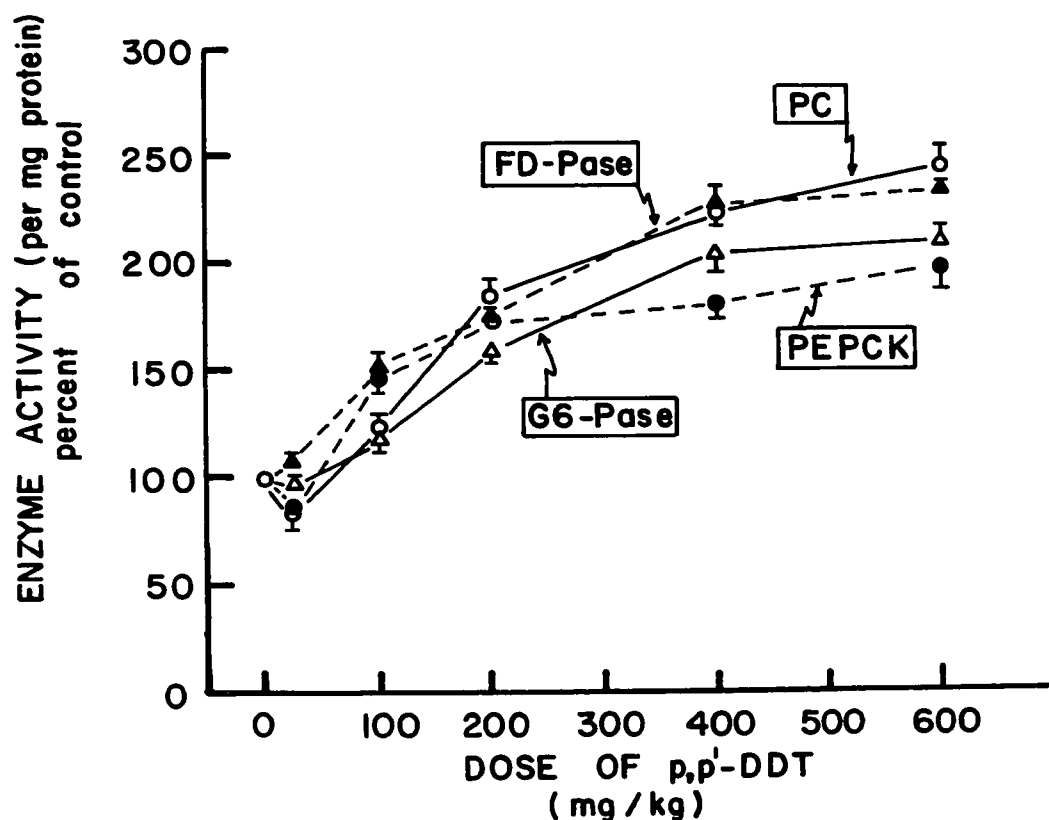


Fig. 10. Effects of varying doses of p,p'-DDT on the activities of four key gluconeogenic enzymes in rat liver. Each point represents the mean $\pm$ S.E.M. of 4 or more animals in each group. Rats were given p,p'-DDT by the oral route in doses ranging from 25 to 600 mg/kg and killed after 5 hours. Enzyme activities were calculated as micromoles of substrate metabolized per hour per gram of tissue and expressed as specific activity per milligram protein. Data are expressed in percentages taking the values of control animals as 100%.

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enzymes studied. Significant enhancement of pyruvate carboxylase (123%), phosphoenolpyruvate carboxykinase (144%) and fructose 1,6-diphosphatase (154%) 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%) in the activity of hepatic glucose 6-phosphatase. Figure 10 also shows that maximal increases in various gluconeogenic enzymes were observed generally with 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 100-200 mg/kg dose of p,p'-DDT.

E. Effect of p,p'-DDT in Adrenalectomized Rats

Since various glucocorticoid hormones have been shown to act as inducers of hepatic gluconeogenic enzymes (128,249,259), it was of interest to investigate whether a release of these hormones from adrenal glands was responsible for 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 Table 7. 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%, phosphoenolpyruvate carboxykinase to 227%, fructose 1,6-diphosphatase to 203% and glucose 6-phosphatase to 196% of the respective control values, indicating that the observed enzyme increases are not affected by adrenalectomy. Further evidence for a lack of adrenal mediation in DDT action on hepatic gluconeogenesis was obtained

TABLE 7

INDEPENDENCE OF p,p'-DDT-INDUCED STIMULATION OF HEPATIC GLUCONEOGENIC ENZYMES FROM ADRENAL FUNCTION

Means $\pm$ S.E.M. represent at least four animals in each group. Adrenalectomized rats received p,p'-DDT (600 mg/kg) orally and killed 5 hours later. Triamcinolone (10 mg/100 g) was injected intraperitoneally either alone or concurrently with p,p'-DDT. Enzyme activities were calculated as micromoles of substrate metabolized per hour per gram of tissue and expressed as specific activity per milligram of protein. Data are also given in percentages (in parentheses) taking the value of control animals as 100%.

Treatment	PC	PEPCK	FD-Pase	G6-Pase
Control	177 $\pm$ 8 (100)	8.8 $\pm$ 0.2 (100)	3.0 $\pm$ 0.2 (100)	2.6 $\pm$ 0.2 (100)
p,p'-DDT	348 $\pm$ 18 (197)*	20.0 $\pm$ 1.1 (227)*	6.1 $\pm$ 0.4 (203)*	5.1 $\pm$ 0.3 (196)*
Triamcinolone	224 $\pm$ 0 (127)*	14.7 $\pm$ 0.7 (167)*	4.7 $\pm$ 0.2 (157)*	4.3 $\pm$ 0.4 (165)*
p,p'-DDT + Triamcinolone	354 $\pm$ 18 (200)*	22.5 $\pm$ 0.9 (256)*	6.7 $\pm$ 0.2 (223)*	5.1 $\pm$ 0.1 (196)*

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

from experiments in which the ability of triamcinolone to potentiate the action of this insecticide was investigated. Table 7 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 similar magnitude as 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.

F. 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 8. 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 either of these compounds to p,p'-DDT-treated animals effectively prevented the insecticide-induced enhancement of various gluconeogenic enzymes. Table 8 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

TABLE 8

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

Means $\pm$ 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 hours. Actinomycin D (40 μ g/100 g) was given in 2 divided doses, 30 minutes prior and 2½ hours following DDT treatment. Cycloheximide (70 μ g/100 g) and DL-ethionine (50 mg/100 g) were injected intraperitoneally 30 minutes prior to DDT administration. Enzyme activities were calculated as micro-moles of substrate metabolized per hour per gram of tissue and expressed as specific activity per milligram of protein. Data are also given in percentages (in parentheses) taking the values of control animals as 100%.

Treatment	PC	PEPCK	FD-Pase	G6-Pase
Control	245 $\pm$ 11 (100)	13.9 $\pm$ 0.5 (100)	2.8 $\pm$ 0 (100)	3.8 $\pm$ 0.1 (100)
p,p'-DDT	544 $\pm$ 19 (222)*	25.8 $\pm$ 1 (185)*	6.3 $\pm$ 0.5 (225)*	7.8 $\pm$ 0.3 (205)*
Actinomycin D	238 $\pm$ 17 (97)	15 $\pm$ 1 (107)	2.1 $\pm$ 0.2 (75)	4.8 $\pm$ 0.6 (126)
Cycloheximide	258 $\pm$ 20 (105)	16 $\pm$ 1 (114)	2.6 $\pm$ 0.1 (93)	4.2 $\pm$ 0.5 (111)
Ethionine	260 $\pm$ 15 (106)	15 $\pm$ 1 (107)	3.0 $\pm$ 0.1 (107)	4.9 $\pm$ 0.4 (129)
Actinomycin D + p,p'-DDT	294 $\pm$ 14 (120)†	16 $\pm$ 1.3 (115)†	3.0 $\pm$ 0.1 (107)†	4.7 $\pm$ 0.2 (124)†
Cycloheximide + p,p'-DDT	265 $\pm$ 11 (108)†	15 $\pm$ 1 (107)†	2.7 $\pm$ 0.2 (96)†	4.0 $\pm$ 0.1 (100)†
Ethionine + p,p'-DDT	220 $\pm$ 11 (90)†	15 $\pm$ 1 (107)†	3.3 $\pm$ 0.3 (118)†	3.9 $\pm$ 0.5 (103)†

*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 ($p = <0.05$).

increases in hepatic pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose 1,6-diphosphatase and glucose 6-phosphatase.

G. Comparative Effects of Various Chlorinated Hydrocarbon Insecticides on Hepatic Gluconeogenic Enzymes

The effects of p,p'-DDT on the activities of the four key, rate-limiting gluconeogenic enzymes in liver were compared with those seen with α -chlordane, heptachlor and endrin. Data presented in Table 9 demonstrate that 3 hours after oral intubation of either of these insecticides, the activities of hepatic pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose 1,6-diphosphatase and glucose 6-phosphatase were significantly elevated. The most marked increase was seen in the case of fructose 1,6-diphosphatase in rats treated with heptachlor. The results show that various structurally-unrelated pesticides examined are equally effective in augmenting the activities of the quartet of hepatic key gluconeogenic enzymes.

H. Changes in Blood Glucose, Serum Urea and Liver Glycogen Produced by Various Pesticides

Since p,p'-DDT treatment elevated serum glucose and the activities of renal and hepatic gluconeogenic enzymes, it was of interest to examine the influence of α -chlordane, heptachlor and endrin on the concentration of blood glucose, serum urea and liver glycogen. The results are presented in Table 10. One hour after treatment, fasting blood glucose levels were increased from 81 ± 2 mg% in controls to 124 ± 2 , 119 ± 1 , 120 ± 1 and 128 ± 2 mg% in rats fed p,p'-DDT, α -chlordane, heptachlor and endrin, respectively. In contrast, all four chlorinated hydrocarbon insecticides produced a significant drop in the concentration of liver glycogen. Table 10 also

TABLE 9

COMPARATIVE EFFECT OF VARIOUS CHLORINATED HYDROCARBON INSECTICIDES
ON HEPATIC GLUCONEOGENIC ENZYME ACTIVITIES

Means $\pm$ S.E.M. represent four animals in each group. Rats were given either p,p'-DDT (600 mg/kg), α -chlordane (200 mg/kg), heptachlor (200 mg/kg) or endrin (50 mg/kg) by the oral route and killed 3 hours after treatment. Enzyme activities were calculated as micromoles of substrate metabolized per hour per gram of tissue and expressed as specific activity per milligram of protein. Data are also given in percentages (in parentheses) taking the values of control rats as 100%.

Treatment	PC	PEPCK	FD-Pase	G6-Pase
Control	245 $\pm$ 11 (100)	14 $\pm$ 1 (100)	2.8 $\pm$ 1 (100)	3.8 $\pm$ 0.1 (100)
p,p'-DDT	438 $\pm$ 8 (179)*	21 $\pm$ 1 (150)*	6.1 $\pm$ 0.1 (218)*	6.6 $\pm$ 0.5 (174)*
α -Chlordane	449 $\pm$ 5 (183)*	27 $\pm$ 1 (193)*	6.6 $\pm$ 0.1 (236)*	6.9 $\pm$ 0.3 (182)*
Heptachlor	488 $\pm$ 21 (199)*	28 $\pm$ 1 (200)*	6.7 $\pm$ 0.1 (239)*	6.0 $\pm$ 0.2 (158)*
Endrin	414 $\pm$ 2 (169)*	22 $\pm$ 1 (157)*	5.7 $\pm$ 0.1 (204)*	7.3 $\pm$ 0.3 (192)*

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

TABLE 10

EFFECTS OF VARIOUS INSECTICIDES ON BLOOD GLUCOSE,
HEPATIC GLYCOGEN AND SERUM UREA

Means $\pm$ S.E.M. represent four or more animals in each group. Rats were administered either p,p'-DDT, α -chlordane, heptachlor or endrin orally and killed 1 hour later. Blood glucose and serum urea are expressed as milligrams per 100 milliliters of blood whereas liver glycogen as grams per 100 grams of tissue. Data are also given in percentages (in parentheses) taking the values of control rats as 100%.

Treatment	Blood Glucose (mg/100 ml)	Liver Glycogen (g/100 g)	Serum Urea (mg/100 ml)
Control	81 $\pm$ 2 (100)	2.2 $\pm$ 0 (100)	26.1 $\pm$ 0.7 (100)
p,p'-DDT (600 mg/kg)	124 $\pm$ 2 (153)*	1.3 $\pm$ 0.1 (59)*	30.6 $\pm$ 0.3 (117)*
α -Chlordane (200 mg/kg)	119 $\pm$ 1 (147)*	1.4 $\pm$ 0.2 (64)*	32.6 $\pm$ 0.6 (125)*
Heptachlor (200 mg/kg)	120 $\pm$ 1 (148)*	1.3 $\pm$ 0.1 (59)*	31.0 $\pm$ 0.7 (119)*
Endrin (50 mg/kg)	128 $\pm$ 2 (158)*	1.4 $\pm$ 0.1 (64)*	32.9 $\pm$ 1.0 (126)*

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

shows that p,p'-DDT, α -chlordane, heptachlor or endrin significantly increased the levels of serum urea by approximately 20% at 1 hour, when compared with the values seen in control rats.

III. METABOLIC ALTERATIONS INDUCED BY CHRONIC PESTICIDE TREATMENT

A. Influence of Chronic p,p'-DDT Treatment on Renal Gluconeogenic Enzymes

Since it is the long-term exposure to small amounts of chlorinated hydrocarbon insecticides which actually presents a potential health hazard, it was of interest to examine whether administration of doses as low as 5 and 25 mg/kg p,p'-DDT could also produce stimulation of the four key gluconeogenic enzymes in rats. Results presented in Figure 11 demonstrate that p,p'-DDT, when administered in a small dose over a prolonged period, was capable of producing stimulation of renal gluconeogenic enzymes. Whereas treatment for 20 days did not affect various enzyme activities, the 5 mg/kg dose of p,p'-DDT given for 45 days produced a significant rise in renal pyruvate carboxylase to 150%, phosphoenolpyruvate carboxykinase to 173%, fructose 1,6-diphosphatase to 134% and glucose 6-phosphatase to 133% of the control values. In rats treated with 25 mg/kg dose of p,p'-DDT for 20 days, there was already significant enhancement in all four gluconeogenic enzymes in the kidney cortex although, greater enzymic increases were produced with the same dose when the period of treatment was extended for 45 days.

B. Effect of Chronic p,p'-DDT Treatment on Hepatic Gluconeogenic Enzymes

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 either 5 or 25 mg/kg dose of p,p'-DDT

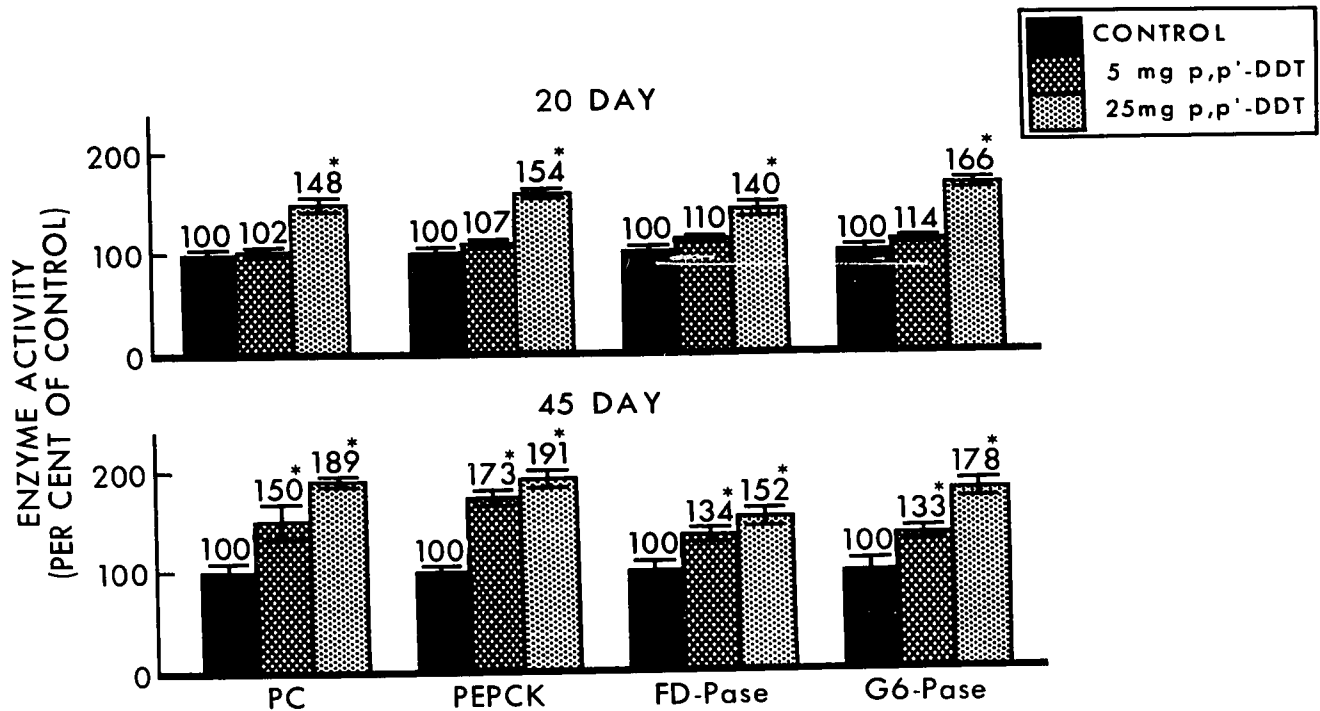


Fig. 11. Effects of chronically administered p,p'-DDT on gluconeogenic enzymes of kidney cortex. Each bar represents the mean $\pm$ S.E.M. of 4 or more animals in each group. Rats were injected intramuscularly, daily with p,p'-DDT (5 or 25 mg/kg) for either 20 or 45 days and killed 24 hours after the last injection. Data are given in percentages taking the values of control animals as 100%.

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

daily for 20 or 45 days and killed 24 hours after the last injection. Results presented in Table 11 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 5 mg/kg dose of p,p'-DDT for 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 5 mg/kg dose of p,p'-DDT raised pyruvate carboxylase to 138%, phosphoenolpyruvate carboxykinase to 142%, fructose 1,6-diphosphatase to 120% and glucose 6-phosphatase to 167% 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.

C. Influence of Chronic α -Chlordane Treatment on Renal Gluconeogenic Enzymes

In order to examine whether prolonged administration of small doses of a cyclodiene insecticide also enhanced the four rate-limiting gluconeogenic enzymes in kidney cortex, rats were injected daily with 5 and 25 mg/kg α -chlordane for 20 and 45 days. The chronic influence of two different doses of α -chlordane on the activities of various key gluconeogenic enzymes of kidney cortex is shown in Table 12. A 5 mg/kg dose of α -chlordane for 45 days produced statistically significant increases in all gluconeogenic enzymes examined, whereas only fructose 1,6-diphosphatase activity was elevated in rats given this amount of insecticide for 20 days. When the daily dose of α -chlordane was raised to 25 mg/kg, the activities of the

TABLE 11

EFFECT OF CHRONIC ADMINISTRATION OF p,p'-DDT ON HEPATIC GLUCONEOGENIC ENZYMES

Means $\pm$ S.E.M. represent at least four animals in each group. Groups of rats were injected daily by the intramuscular route with either 5 or 25 mg/kg of p,p'-DDT for either 20 or 45 days and killed 24 hours after the last injection. Enzyme activities were calculated as micromoles of substrate metabolized per hour per gram of tissue and expressed as specific activity per milligram of protein. Data are also given in percentages (in parentheses) taking the values of control animals as 100%.

Treatment	Dose (mg/kg/day)	Enzymes			
		PC	PEPCK	FD-Pase	G6-Pase
	0 (Control)	210 $\pm$ 7 (100)	9.3 $\pm$ 0.2 (100)	3.3 $\pm$ 0.2 (100)	2.8 $\pm$ 0.1 (100)
p,p'-DDT 20 days	5	241 $\pm$ 7 (115)*	11.5 $\pm$ 0.5 (124)*	4.4 $\pm$ 0.1 (133)*	3.1 $\pm$ 0.1 (111)
	25	286 $\pm$ 9 (136)*	13.1 $\pm$ 0.4 (141)*	5.1 $\pm$ 0.1 (155)*	4.2 $\pm$ 0.2 (150)*
	0 (Control)	284 $\pm$ 19 (100)	9.3 $\pm$ 0.2 (100)	4.1 $\pm$ 0.2 (100)	2.8 $\pm$ 0.1 (100)
p,p'-DDT 45 days	5	392 $\pm$ 11 (138)*	13.2 $\pm$ 0.1 (142)*	4.9 $\pm$ 0.1 (120)*	4.7 $\pm$ 0.2 (167)*
	25	489 $\pm$ 11 (172)*	16.6 $\pm$ 0.9 (178)*	6.8 $\pm$ 0.4 (166)*	5.2 $\pm$ 0.3 (187)*

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

TABLE 12

EFFECTS OF CHRONIC α -CHLORDANE TREATMENT ON RENAL CORTEX
GLUCONEOGENIC ENZYMES

Means $\pm$ S.E.M. represent at least four rats in each group. Groups of rats were injected daily by the intramuscular route with either 5 or 25 mg/kg of α -chlordane for either 20 or 45 days and killed 24 hours after the last injection. Enzyme activities were calculated as micromoles of substrate metabolized per hour per gram of tissue and expressed as specific activity per milligram of protein. Data are also given in percentages (in parentheses) taking the values of control animals as 100%.

Treatment	Dose (mg/kg/day)	Enzymes			
		PC	PEPCK	FD-Pase	G6-Pase
	0 (Control)	113 $\pm$ 4 (100)	13 $\pm$ 0.5 (100)	7.0 $\pm$ 0.2 (100)	6.1 $\pm$ 0.4 (100)
α -Chlordane 20 days	5	107 $\pm$ 2 (95)	14 $\pm$ 0.5 (107)	8.9 $\pm$ 0.4 (127)*	7.0 $\pm$ 0.4 (114)
	25	133 $\pm$ 4 (118)*	16 $\pm$ 0.6 (123)*	9.9 $\pm$ 0.4 (141)*	9.0 $\pm$ 0.3 (147)*
	0 (Control)	133 $\pm$ 5 (100)	11 $\pm$ 0 (100)	5.6 $\pm$ 0.2 (100)	3.6 $\pm$ 0.3 (100)
α -Chlordane 45 days	5	206 $\pm$ 17 (155)*	18 $\pm$ 1.0 (164)*	7.1 $\pm$ 0.4 (127)*	5.5 $\pm$ 0.3 (153)*
	25	237 $\pm$ 12 (178)*	19 $\pm$ 1.0 (173)*	8.3 $\pm$ 0.5 (148)*	5.7 $\pm$ 0.2 (158)*

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

four renal enzymes were enhanced after 20 days although as expected, the degree of stimulation was much greater in rats receiving 25 mg of α -chlordane for a period of 45 days.

D. Effect of Chronic α -Chlordane on Hepatic Gluconeogenic Enzymes

Since the contribution of liver to overall gluconeogenesis is considerably greater than that of renal cortex, the effects of chronic α -chlordane treatment on gluconeogenic enzymes also were examined in hepatic tissue. Figure 12 shows that treatment with 25 mg dose of α -chlordane for 20 days produced significant stimulation of all four gluconeogenic enzymes examined. In contrast, the smaller dose of the insecticide (5 mg/kg) increased hepatic glucose 6-phosphatase and fructose 1,6-diphosphatase but failed to exert any significant effect on the activities of pyruvate carboxylase and phosphoenolpyruvate carboxykinase. When the time of exposure to α -chlordane was extended to 45 days, both doses of the insecticide significantly enhanced the levels of all four enzymes when compared with the control values.

IV. EFFECT OF p,p'-DDT ON CYCLIC AMP- H^3 SYNTHESIS FROM TRITIATED ADENOSINE

"IN VITRO"

A. Influence of p,p'-DDT on Kidney Cortex Cyclic AMP- H^3 Synthesis

The organochlorine pesticide-induced alterations in renal and hepatic carbohydrate metabolism closely resemble those seen after the administration of cyclic AMP. Some of the most prominent changes produced by cyclic AMP treatment include stimulation of hepatic and renal gluconeogenic enzymes, an elevation in the level of blood glucose and depletion of liver glycogen (12-15). The data presented in preceeding sections show that halogenated

EFFECTS OF CHRONIC α -CHLORDANE TREATMENT ON HEPATIC GLUCONEOGENIC ENZYMES

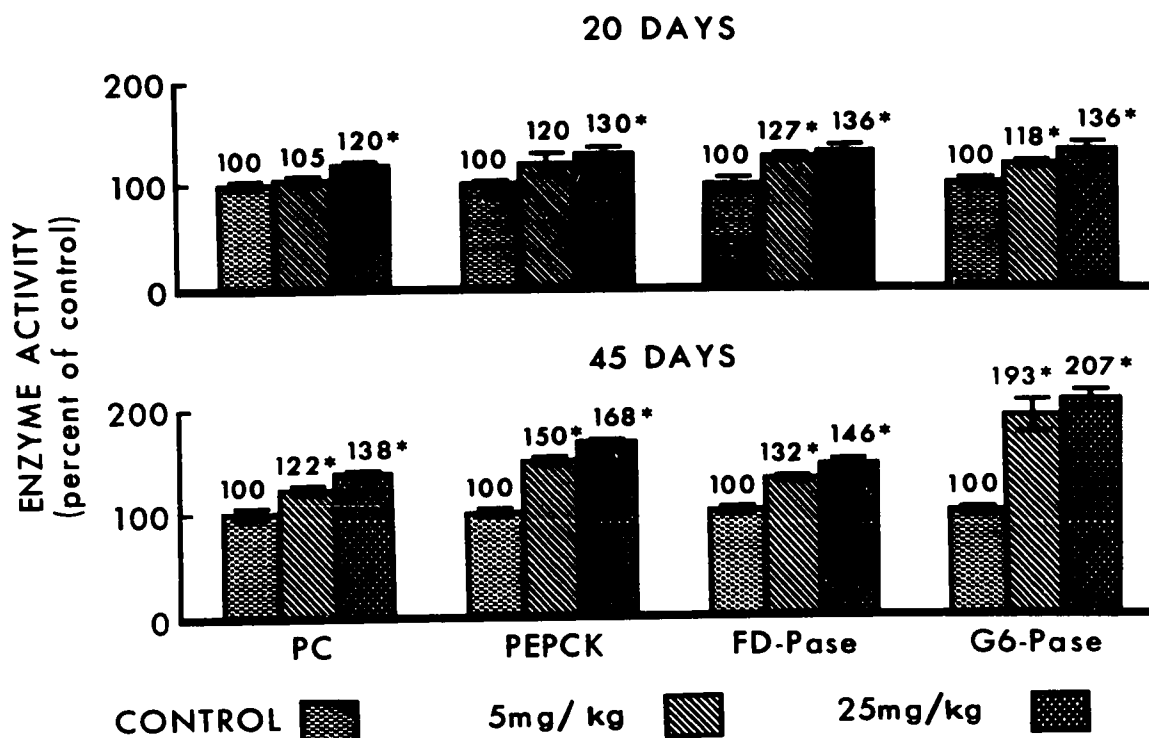


Fig. 12. Effects of chronically administered α -chlordane on key gluconeogenic enzymes in rat liver. Each bar represents the mean $\pm$ S.E.M. of at least 4 rats in each group. Animals were given α -chlordane daily by the intramuscular route in a dose of 5 or 25 mg/kg for either 20 or 45 days and killed 24 hours after the last injection. Data are expressed in percentages taking the values of control animals as 100%.

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

hydrocarbons also stimulated the gluconeogenic enzymes in kidney cortex and liver, and produced hepatic glycogenolysis and a significant rise in blood glucose. In order to examine whether the pesticide-induced changes in kidney cortex carbohydrate metabolism were associated with the capacity of this tissue to synthesize cyclic AMP- H^3 from tritiated adenosine, renal cortices were incubated with varying concentrations of p,p'-DDT ($10^{-9}M$ to $10^{-5}M$ for 30 minutes). Figure 13 shows that addition of p,p'-DDT in vitro resulted in a significant increase in cyclic AMP- H^3 formation from radioactive adenosine in kidney cortex. A statistically significant rise in cyclic AMP- H^3 synthesis to 466 ± 12 DPM/mg $\times 10^2$ from the control value of 324 ± 30 DPM/mg $\times 10^2$ was noted with $10^{-7}M$ concentration of p,p'-DDT. A maximal stimulation to 543 ± 25 DPM/mg $\times 10^2$ cyclic AMP- H^3 resulted when renal cortices were incubated with $10^{-6}M$ p,p'-DDT. It is of interest that increasing the concentration of p,p'-DDT to $10^{-5}M$ did not further augment the formation of kidney cyclic AMP- H^3 .

B. Effect of Caffeine on p,p'-DDT-Induced Changes in Renal Cyclic AMP- H^3 Production

The possibility whether an inhibitor of phosphodiesterase such as caffeine would potentiate the action of a submaximal dose of p,p'-DDT on kidney cyclic AMP- H^3 formation was examined. The results are presented in Table 13. Incubation of kidney cortex slices with p,p'-DDT (10^{-9} or $10^{-8}M$) did not significantly alter cyclic AMP- H^3 formation. However, in the presence of 0.5 mM caffeine, the amount of cyclic AMP- H^3 formed increased to 130% of the control values. This table also shows that simultaneous exposure of renal cortices to both p,p'-DDT and caffeine produced a definite augmentation in cyclic AMP- H^3 formation from tritiated adenosine.

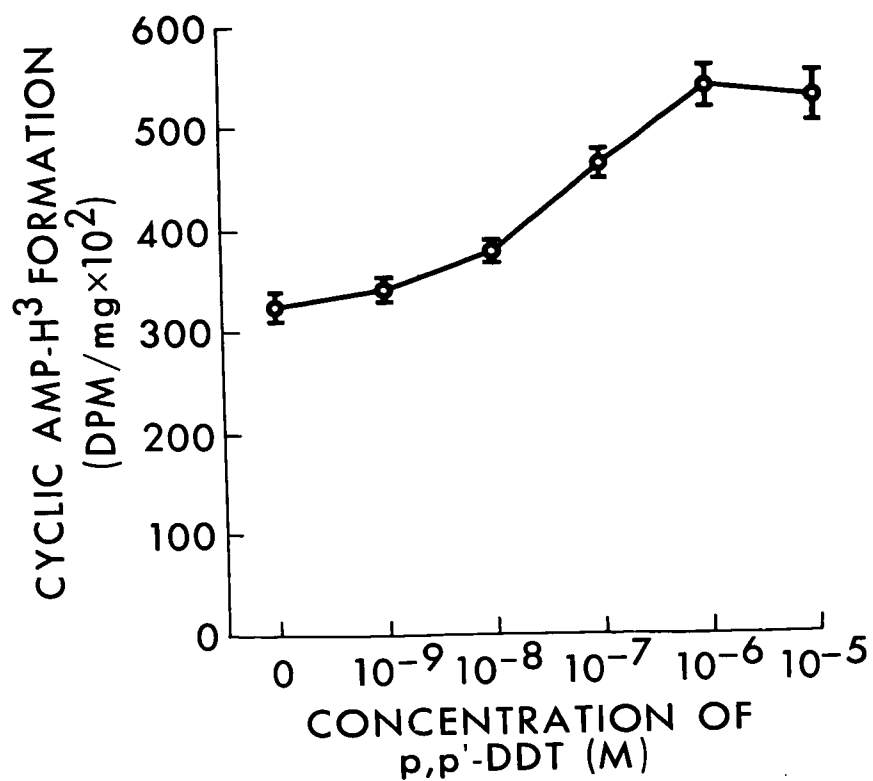


Fig. 13. Effect of varying concentrations of p,p'-DDT on the cyclic AMP-H³ synthesis by kidney cortex. Each point represents the mean $\pm$ S.E.M. of 4 determinations of cyclic AMP-H³. Renal cortices were incubated in presence of varying concentrations of p,p'-DDT (10⁻⁹ to 10⁻⁵M). Cyclic AMP-H³ formation is expressed as disintegrations per minute per milligram of tissue $\times 10^2$ (DPM/mg $\times 10^2$).

TABLE 13

EFFECT OF CAFFEINE AND p,p'-DDT ON RENAL CYCLIC AMP-H³ FORMATION

Means $\pm$ S.E.M. represent at least five determinations of cyclic AMP-H³ formation in rat kidney cortex. Renal cortical slices were incubated in Krebs-Ringer bicarbonate buffer containing H³-adenosine and either 0.5 mM caffeine, 10⁻⁸M p,p'-DDT, 10⁻⁹M p,p'-DDT or simultaneously with caffeine and p,p'-DDT for 30 minutes at 37°C. Data are also given in percentages taking the values of control animals as 100%.

Treatment	Cyclic AMP-H ³ Formation (DPM/mg X 10 ²)	Percent of Control
Control	319 $\pm$ 9	100
0.5 mM Caffeine	415 $\pm$ 19	130*
10 ⁻⁸ M p,p'-DDT	360 $\pm$ 5	116
10 ⁻⁸ M p,p'-DDT + Caffeine	603 $\pm$ 21	189*†
10 ⁻⁹ M p,p'-DDT	335 $\pm$ 10	105
10 ⁻⁹ M p,p'-DDT + Caffeine	553 $\pm$ 39	173*†

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

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

C. Effect of Imidazole on p,p'-DDT-Induced Changes in Cyclic AMP-H³

In order to examine whether the observed enhancement in cyclic AMP-H³ formation could be prevented by stimulating phosphodiesterase activity, renal cortices were incubated in presence of both imidazole and p,p'-DDT. The results presented in Table 14 show that imidazole (10^{-2} M) decreased the amount of cyclic AMP-H³ from $319 \pm 8 \times 10^2$ to 284 ± 8 DPM/mg $\times 10^2$. Incubation with p,p'-DDT (10^{-6} M) produced a marked rise in renal cyclic AMP-H³; however, concurrent treatment with imidazole and the insecticide significantly lowered the p,p'-DDT-induced increase in the amount of cyclic AMP-H³ formed from radioactive adenosine.

D. Influence of Propranolol on the p,p'-DDT-Stimulated Rise in Kidney Cyclic AMP-H³ Formation

Since propranolol is known to lower blood glucose levels and inhibit the hyperglycemic response of cyclic AMP (16,260), the possibility whether this β -adrenergic blocking agent would interfere with the ability of p,p'-DDT to enhance kidney cortex cyclic AMP-H³ formation was investigated. Figure 14 shows that propranolol, by itself, at a concentration of 10^{-6} M or 10^{-4} M did not significantly alter the basal levels of renal cyclic AMP-H³. When kidney cortices were incubated with equimolar quantities of propranolol (10^{-6} M) and p,p'-DDT (10^{-6} M), the β -adrenergic blocking agent failed to prevent the insecticide-induced increase in cyclic AMP-H³ formation. However, raising the concentration of propranolol to 10^{-4} M significantly lowered the amount of cyclic AMP-H³ formed to 357 ± 25 DPM/mg $\times 10^2$ when compared with the value of 536 ± 25 DPM/mg $\times 10^2$ seen with p,p'-DDT alone.

TABLE 14

EFFECT OF IMIDAZOLE ON THE p,p'-DDT-INDUCED INCREASE IN
CYCLIC AMP-H³ SYNTHESIS

Means $\pm$ S.E.M. represent at least five determinations of cyclic AMP-H³ formation in rat kidney cortex. Renal cortical slices were incubated in Krebs-Ringer bicarbonate buffer containing H³-adenosine and either 10⁻⁶M p,p'-DDT, 10 mM imidazole or a combination of both for 30 minutes at 37°C. Data are also given in percentages taking the values of control animals as 100%.

Treatment	Cyclic AMP-H ³ Formation (DPM/mg X 10 ²)	Percent of Control
Control	319 $\pm$ 8	100
Imidazole	284 $\pm$ 8	89
p,p'-DDT	536 $\pm$ 25	168*
Imidazole and p,p'-DDT	319 $\pm$ 25	100†

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

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

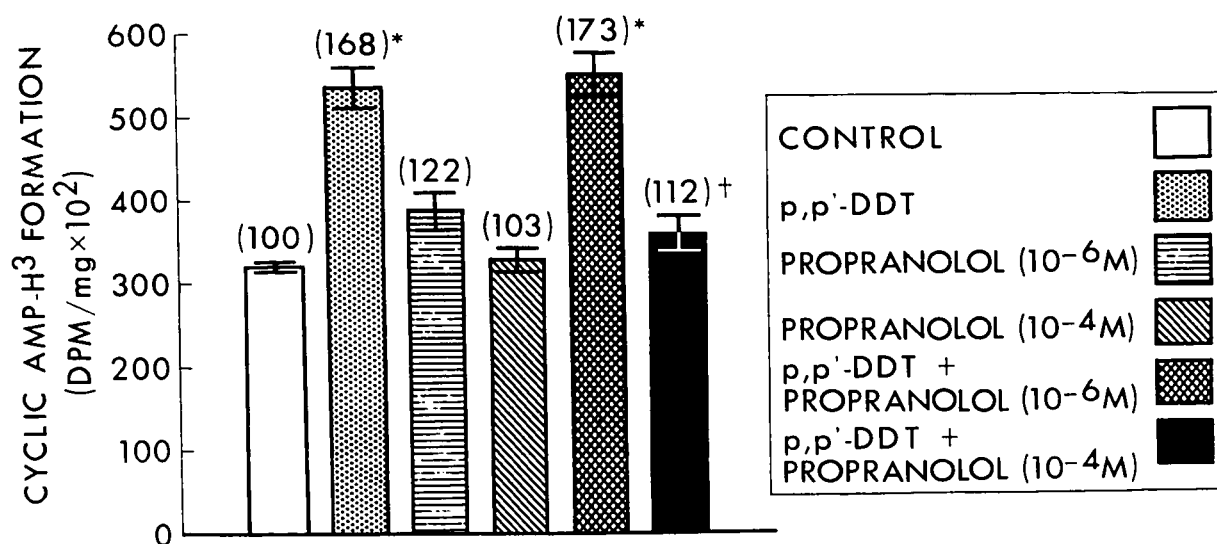


Fig. 14. Effect of propranolol on p,p'-DDT-induced stimulation of cyclic AMP-H³ formation by kidney cortex. Each bar represents the mean $\pm$ S.E.M. of 4 determinations of renal cyclic AMP-H³. 10⁻⁶M p,p'-DDT was added to the medium either alone or simultaneously with 10⁻⁶M or 10⁻⁴M propranolol. The formation of cyclic AMP-H³ is expressed as disintegrations per minute per milligram of tissue $\times 10^2$ (DPM/mg $\times 10^2$). Data are also given in percentages taking the control values as 100%.

*Statistically significant difference when compared with the control values ($p = <0.05$).

†Statistically significant difference when compared with the values seen with p,p'-DDT alone ($p = <0.05$).

E. Inhibition by Hydrazine of the DDT-Induced Increase in Renal Cyclic AMP-H³ Synthesis

Because hydrazine decreases the blood sugar level and noncompetitively inhibits the activity of phosphoenolpyruvate carboxykinase (17), it was of interest to determine whether this hypoglycemic agent also would prevent the p,p'-DDT-induced increase in renal cyclic AMP-H³ formation. As seen in Figure 15, hydrazine at a concentration of 10^{-5} M produced no significant change in the cyclic AMP-H³ levels of kidney cortex. As expected, incubation with p,p'-DDT (10^{-6} M) elevated cyclic AMP-H³ formation from a control value of $324 \pm 30 \times 10^2$ to 543 ± 25 DPM/mg $\times 10^2$. However, simultaneous incubation with p,p'-DDT and hydrazine resulted in a significant decrease in the amount of cyclic AMP-H³ formed when compared to the values seen with the pesticide alone.

F. Effect of Prostaglandins on the p,p'-DDT-Stimulated Rise in Cyclic AMP-H³ Production by Kidney Cortex

Results presented in Table 15 demonstrate that incubation of renal cortices with either prostaglandin E₁ or F₂ α (10^{-4} M) produced no significant change in cyclic AMP-H³ production by kidney cortex. Furthermore, when kidneys were incubated concurrently with p,p'-DDT and either of the two prostaglandins, the pesticide-induced rise in cyclic AMP-H³ formation remained completely unaffected.

G. Comparative Effects of Various Organochlorine Insecticides on Renal Cyclic AMP-H³ Synthesis

In addition to p,p'-DDT, other insecticides such as α -chlordane and dieldrin were found to stimulate renal and hepatic gluconeogenesis as well as increase the levels of blood glucose (261-263). A comparison

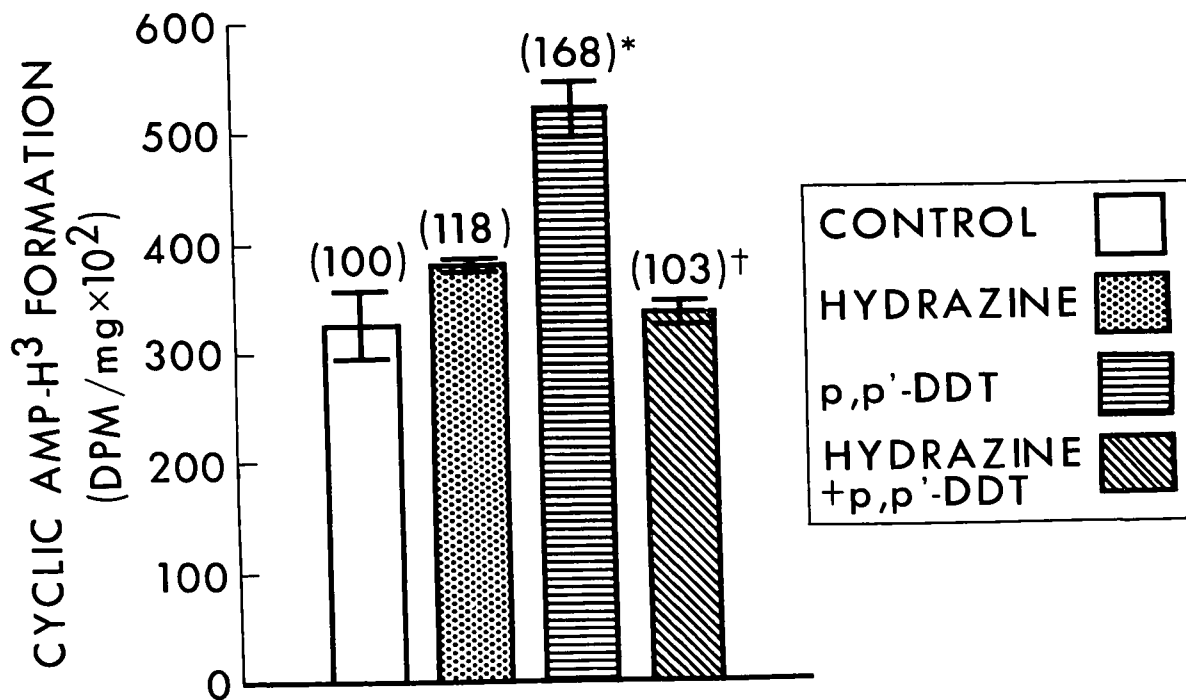


Fig. 15. Inhibition by hydrazine of the p,p'-DDT-induced stimulation of cyclic AMP-H³ formation by renal cortex. Each bar represents the mean $\pm$ S.E.M. of 4 determinations of cyclic AMP-H³. Hydrazine (10^{-5} M) was added to the incubation medium either alone or concurrently with p,p'-DDT (10^{-6} M) for 30 minutes. Cyclic AMP-H³ formation is expressed as disintegrations per minute per milligram of tissue $\times 10^2$ (DPM/mg $\times 10^2$). Data are also given in percentages taking the control values as 100%.

*Statistically significant difference when compared with the control values ($p = <0.05$).

†Statistically significant difference when compared with the values seen with p,p'-DDT alone ($p = <0.05$).

TABLE 15

EFFECT OF PROSTAGLANDINS ON THE p,p'-DDT-INDUCED INCREASE IN CYCLIC AMP-H³ FORMATION BY KIDNEY CORTEX

Means $\pm$ S.E.M. represent at least five determinations of cyclic AMP-H³ formation by rat kidney cortex. Renal cortices were incubated in Krebs-Ringer bicarbonate buffer containing adenosine-H³ and either 10⁻⁶M p,p'-DDT, 10⁻⁴M prostaglandin E₁, 10⁻⁴M prostaglandin F₂ α or simultaneously with p,p'-DDT and prostaglandin for 30 minutes at 37°C. Data are also given in percentages taking the values of control animals as 100%.

Treatment	Cyclic AMP-H ³ Formation (DPM/mg $\times$ 10 ²)	Percent of Control
Control	324 $\pm$ 30	100
10 ⁻⁶ M p,p'-DDT	543 $\pm$ 25	168*
10 ⁻⁴ M Prostaglandin E ₁	379 $\pm$ 12	117
10 ⁻⁴ M Prostaglandin E ₁ + 10 ⁻⁶ M p,p'-DDT	468 $\pm$ 18	144*
10 ⁻⁴ M Prostaglandin F ₂ α	373 $\pm$ 25	115
10 ⁻⁴ M Prostaglandin F ₂ α + 10 ⁻⁶ M p,p'-DDT	545 $\pm$ 22	168*

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

of the effects of p,p'-DDT with α -chlordane, endrin and heptachlor on cyclic AMP-H³ formation by kidney cortex was therefore undertaken and the results are presented in Table 16. In general, the in vitro addition of either of these chlorinated hydrocarbons stimulated to about the same extent, the synthesis of renal cyclic AMP-H³ from radioactive adenosine.

H. "In Vitro" Effect of p,p'-DDT on Liver Cyclic AMP-H³ Formation

Since p,p'-DDT enhanced the activities of both renal and hepatic gluconeogenic enzymes as well as increased kidney cyclic AMP-H³ formation, it was of interest to investigate whether this pesticide would also affect cyclic AMP-H³ synthesis in hepatic tissue. The results are presented in Figure 16. A statistically significant rise in cyclic AMP-H³ formation by liver to 281 ± 9 DPM/mg $\times 10^2$ from the control value of 190 ± 14 DPM/mg $\times 10^2$ was noted with 10^{-7} M concentration of p,p'-DDT. Maximal stimulation to 318 ± 31 DPM/mg $\times 10^2$ cyclic AMP-H³ was obtained when liver slices were incubated with 10^{-6} M p,p'-DDT.

V. STIMULATION OF CYCLIC AMP-H³ FORMATION BY "IN VIVO" ADMINISTRATION OF ORGANOCHLORINE PESTICIDES

A. Time-Course of the p,p'-DDT-Stimulated Rise in Renal Cyclic AMP-H³ Formation

Since the in vitro addition of pesticides enhanced kidney cortex and liver cyclic AMP-H³ synthesis, it was of interest to examine whether renal cortices excised from rats which have already been treated with a single, oral dose of p,p'-DDT also could biotransform a greater amount of adenosine-H³ into the labeled cyclic nucleotide. The sequential changes of the p,p'-DDT-stimulated rise in renal cyclic AMP-H³ formation in vivo are illustrated in Figure 17. Labeled cyclic nucleotide production

TABLE 16

EFFECT OF VARIOUS INSECTICIDES ON CYCLIC AMP-H³ FORMATION
IN RAT KIDNEY CORTEX

Means $\pm$ S.E.M. represent at least five determinations of cyclic AMP-H³ formation in rat kidney cortex. Renal cortices were incubated in Krebs-Ringer bicarbonate buffer containing H³-adenosine and 10⁻⁶M of either p,p'-DDT, α -chlordane, endrin or heptachlor for 30 minutes at 37°C. Data are also given in percentages taking the values of control animals as 100%.

Treatment	Cyclic AMP-H ³ Formation (DPM/mg X 10 ²)	Percent of Control
Control	324 $\pm$ 30	100
p,p'-DDT	543 $\pm$ 25	168*
α -Chlordane	526 $\pm$ 41	162*
Endrin	539 $\pm$ 22	166*
Heptachlor	479 $\pm$ 34	148*

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

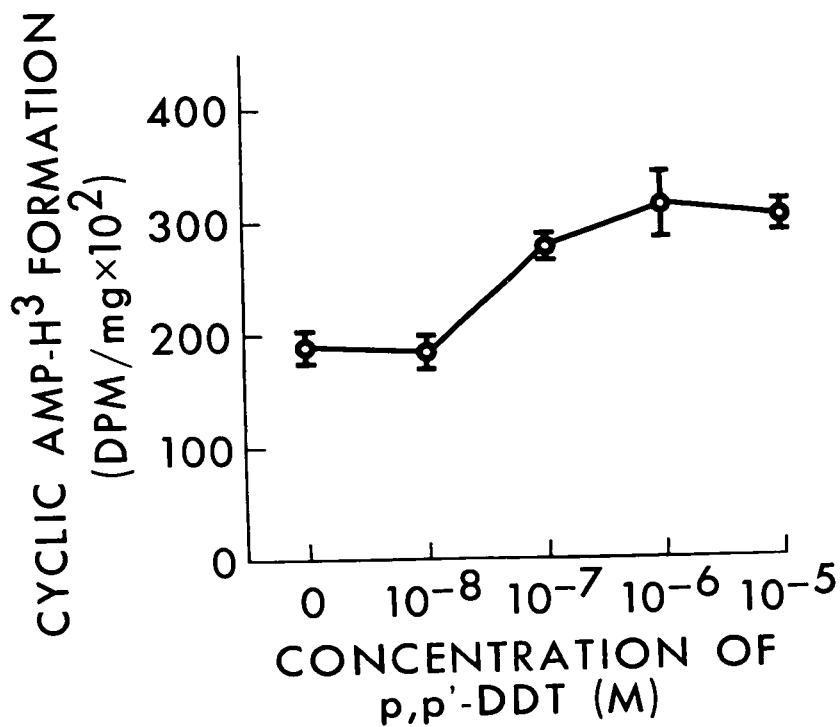


Fig. 16. Effect of varying concentrations of p,p'-DDT on liver cyclic AMP-H³ formation. Each point represents the mean $\pm$ S.E.M. of 4 determinations of cyclic AMP-H³. The tissue was incubated with varying concentrations of p,p'-DDT (10⁻⁸ to 10⁻⁵M) for 30 minutes. Cyclic AMP formation is expressed as disintegrations per minute per milligram of tissue $\times 10^2$ (DPM/mg $\times 10^2$).

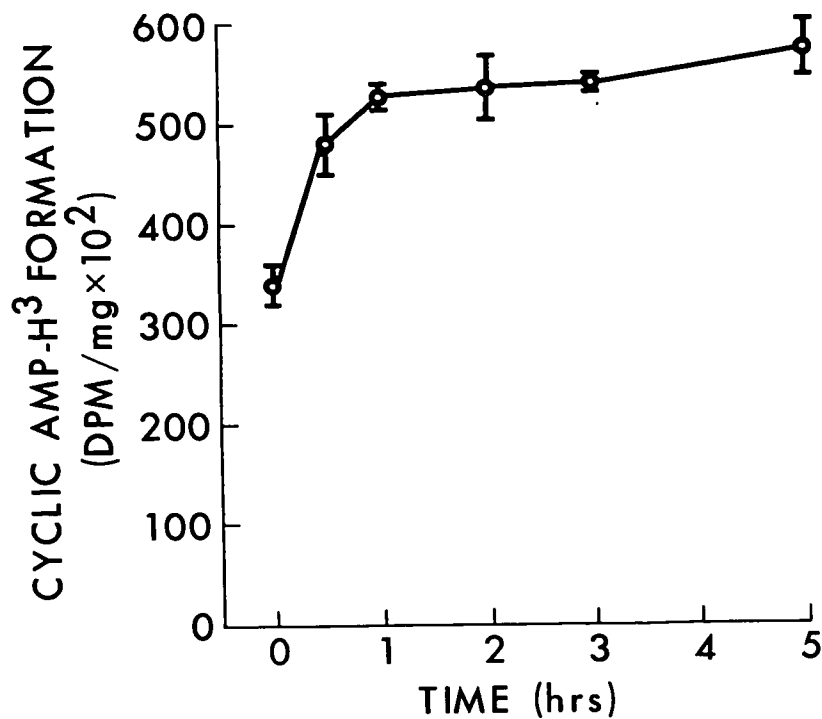


Fig. 17. Time-course of cyclic AMP-H³ formation in kidney cortex of p,p'-DDT-treated rats. Each point represents the mean $\pm$ S.E.M. of 4 rats in each group. Animals were fed p,p'-DDT (600 mg/kg) orally and killed 0.5, 1, 2, 3 and 5 hours following insecticide treatment. Approximately 20 mg renal cortex slices were incubated with Krebs-Ringer bicarbonate buffer containing adenosine-H³ for 1 hour at 37°. Data are expressed as disintegrations per minute per milligram of tissue $\times 10^2$ (DPM/mg $\times 10^2$).

increased significantly as early as 30 minutes after p,p'-DDT to 479 ± 33 DPM/mg $\times 10^2$ from a control value of 337 ± 24 DPM/mg $\times 10^2$. There was a progressive rise in cyclic AMP- H^3 synthesis at one, two and three hours which reached highest values five hours after the administration of p,p'-DDT.

B. Influence of Varying Doses of p,p'-DDT on Renal Cortex Cyclic AMP- H^3 Synthesis

Since the administration of p,p'-DDT at a dose of 600 mg/kg produced a marked rise in renal cyclic nucleotide synthesis, a dose-response study was undertaken to determine whether smaller doses of this pesticide also could elicit a significant response. The results are presented in Table 17. A statistically significant increase in renal cyclic AMP- H^3 synthesis was observed in rats receiving p,p'-DDT in a dose of 100 mg/kg, whereas the smaller (25 mg/kg) dose of the insecticide produced no effect. Table 17 also shows that maximal stimulation of cyclic AMP- H^3 formation was observed in renal cortices of rats treated with 600 mg/kg dose of p,p'-DDT.

C. Effect of Caffeine on the p,p'-DDT-Induced Rise in Cyclic AMP- H^3 Formation

In order to examine whether inhibition of phosphodiesterase by methyl xanthines could potentiate the pesticide-induced increase in renal cyclic AMP- H^3 formation, the effects of p,p'-DDT were investigated in rats pretreated with caffeine. The results presented in Table 18 illustrate that p,p'-DDT (600 mg/kg) significantly enhanced kidney cyclic AMP- H^3 formation in one hour. When caffeine (50 mg/kg) was injected, a significant increase from 337 ± 24 to 440 ± 22 DPM/mg $\times 10^2$ cyclic AMP- H^3 was observed. It is of interest that pretreatment of p,p'-DDT-fed rats with caffeine further

TABLE 17

EFFECT OF VARYING DOSES OF p,p'-DDT ON CYCLIC AMP-H³ FORMATION
IN RAT KIDNEY CORTEX

Means $\pm$ S.E.M. represent five or more determinations of cyclic AMP formation in rat kidney cortex. Groups of animals received varying amounts of p,p'-DDT (25-600 mg/kg) orally and were killed five hours later. The kidneys were excised and renal cortex slices incubated with Krebs-Ringer bicarbonate buffer containing H³-adenosine for 60 minutes at 37°C. Data are also given in percentages taking the values of control rats as 100%.

Dose of p,p'-DDT (mg/kg)	Cyclic AMP Formation (DPM/mg $\times 10^2$)	Percent of Control
Control	337 $\pm$ 24	100
25	369 $\pm$ 6	110
100	437 $\pm$ 25	130*
200	518 $\pm$ 27	154*
400	534 $\pm$ 40	159*
600	578 $\pm$ 29	172*

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

TABLE 18

THE INFLUENCE OF CAFFEINE ON THE p,p'-DDT-INDUCED INCREASE IN
CYCLIC AMP-H³ FORMATION BY KIDNEY CORTEX

Means $\pm$ S.E.M. represent at least four determinations of cyclic AMP-H³ formation by kidney cortex. p,p'-DDT was administered orally in a dose of 600 mg/kg and the animals were killed one hour after treatment. Caffeine (50 mg/kg) was given intraperitoneally 30 minutes prior to pesticide administration. Renal cortical slices were incubated in Krebs-Ringer bicarbonate buffer containing adenosine-H³ for 60 minutes at 37°C. Data are also given in percentages taking the values of control rats as 100%.

Treatment	Cyclic AMP-H ³ Formation (DPM/mg X 10 ²)	Percent of Control
Control	337 $\pm$ 24	100
Caffeine	440 $\pm$ 22	130*
p,p'-DDT	530 $\pm$ 13	157*
p,p'-DDT + Caffeine	610 $\pm$ 10	181*†

*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 rats ($p = <0.05$).

augmented the action of this hydrocarbon, showing that the p,p'-DDT-induced rise in renal cyclic AMP- H^3 formation is potentiated by methyl xanthines.

D. Inhibition by Imidazole of the DDT-Stimulated Rise in Cyclic AMP- H^3 Production

Since phosphodiesterase inhibition by a methyl xanthine was found to potentiate the p,p'-DDT-induced rise in kidney cyclic AMP- H^3 formation, the influence of phosphodiesterase stimulation by imidazole on cyclic nucleotide synthesis was also investigated. Data in Figure 18 show that imidazole by itself exerts no significant effect on kidney cyclic AMP- H^3 formation. However, prior administration of imidazole in p,p'-DDT-treated rats significantly prevented the pesticide-stimulated enhancement in renal cyclic AMP- H^3 synthesis.

E. Blockade by Propranolol of the p,p'-DDT-Induced Increase in the Synthesis of Renal Cyclic AMP- H^3

The possibility whether propranolol, a compound known to lower blood glucose levels and inhibit cyclic AMP-induced hyperglycemia (16,260) would interfere with the ability of p,p'-DDT to enhance renal cyclic AMP- H^3 synthesis was investigated. Figure 19 shows that propranolol, by itself, did not significantly alter the basal levels of kidney cyclic AMP- H^3 . However, prior administration of propranolol to p,p'-DDT-fed rats markedly lowered the amount of cyclic AMP- H^3 formed to 395 ± 11 DPM/mg $\times 10^2$ when compared with the value of 530 ± 13 DPM/mg $\times 10^2$ seen with p,p'-DDT alone.

F. Effects of Hydrazine and Prostaglandin E_1 on the DDT-Stimulated Increase in Kidney Cyclic AMP- H^3 Production

Since hydrazine has been shown to lower serum glucose and inhibit phosphoenolpyruvate carboxykinase activity (17), it was of interest to

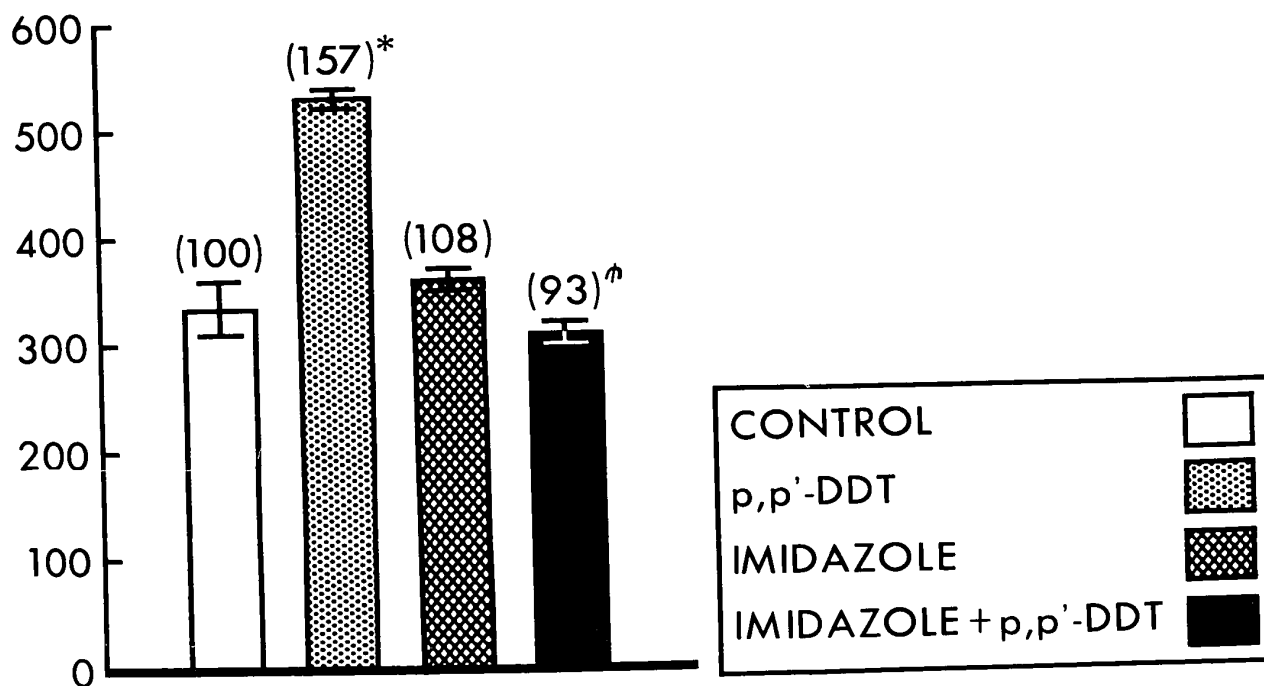


Fig. 18. Inhibition by imidazole of the p,p'-DDT-induced stimulation of cyclic AMP-H³ formation by renal cortex. Each bar represents the mean $\pm$ S.E.M. of 4 rats in each group. Animals were given p,p'-DDT (600 mg/kg) orally and killed one hour later. Imidazole (400 mg/kg) was injected intraperitoneally either alone or concurrently with p,p'-DDT. Approximately 20 mg slices of kidney cortex were incubated with Krebs-Ringer bicarbonate buffer containing adenosine-H³ for 1 hour at 37°. Cyclic AMP formation is expressed as disintegrations per minute per milligram of tissue $\times 10^2$ (DPM/mg $\times 10^2$). Data are also given in percentages (in parentheses) taking the values of control animals as 100%.

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

†Statistically significant difference when compared with the values seen with p,p'-DDT alone ($p = <0.05$).

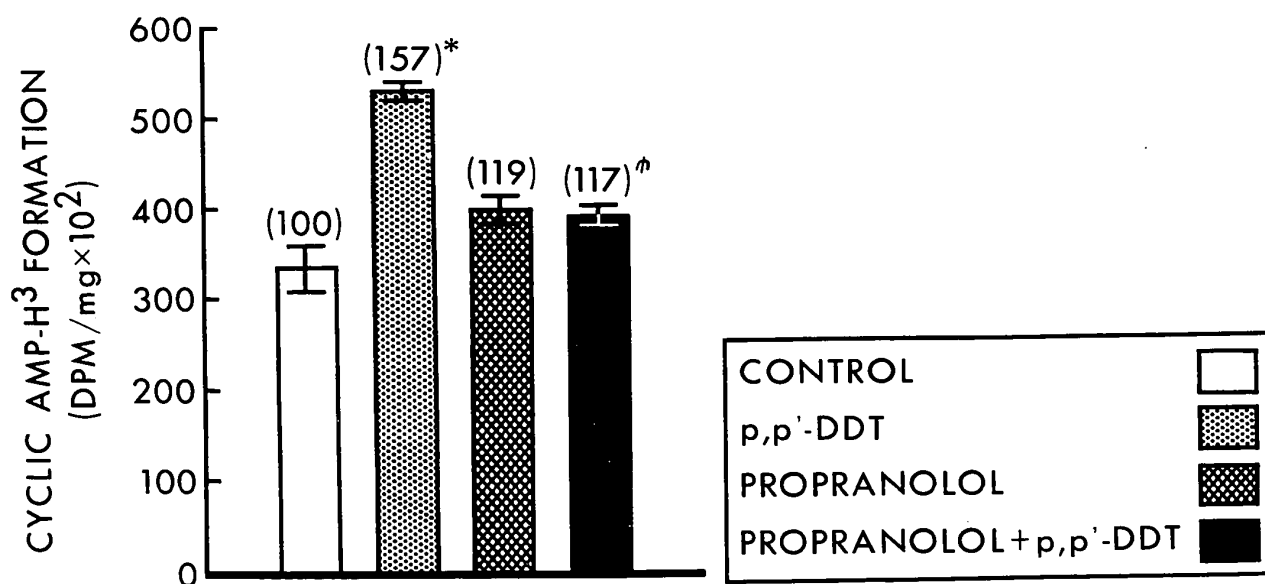


Fig. 19. Blockade by propranolol of the DDT-stimulated rise in renal cyclic AMP-H³ synthesis. Each bar represents the mean $\pm$ S.E.M. of 4 rats in each group. p,p'-DDT was administered by the oral route in a dose of 600 mg/kg and animals were killed after 1 hour. Propranolol (3 mg/kg) was injected intraperitoneally either alone or concurrently with p,p'-DDT. Approximately 20 mg kidney cortex slices were incubated with Krebs-Ringer bicarbonate buffer containing adenosine-H³ for 1 hour at 37°. Cyclic AMP formation is expressed as disintegrations per minute per milligram of tissue $\times 10^2$ (DPM/mg $\times 10^2$). Data are also given in percentages (in parentheses) taking the values of control rats as 100%.

*Statistically significant difference when compared with the control values ($p = <0.05$).

†Statistically significant difference when compared with the values seen with p,p'-DDT alone ($p = <0.05$).

examine whether this hypoglycemic agent would also block the p,p'-DDT-induced rise in kidney cyclic AMP-H³ formation. The results are presented in Table 19. Whereas hydrazine, by itself, did not significantly alter the cyclic nucleotide synthesis, this compound effectively lowered the amount of cyclic AMP-H³ synthesized in renal cortices of p,p'-DDT-fed animals. In contrast to hydrazine, prior administration of prostaglandin E₁ to DDT-treated rats did not affect the insecticide-induced rise in cyclic AMP-H³ production (Table 19).

G. Influence of p,p'-DDT Treatment on Cyclic AMP-H³ Synthesis by Rat Liver

Since administration of p,p'-DDT to rats augmented kidney cortex cyclic AMP-H³ formation, it was of interest to examine whether this hydrocarbon could also enhance hepatic cyclic nucleotide synthesis. The results presented in Figure 20 illustrate that p,p'-DDT significantly elevated liver cyclic AMP-H³ formation from control values of 180 ± 26 DPM/mg $\times 10^2$ to 440 ± 10 DPM/mg $\times 10^2$ in 30 minutes. Significant increases in hepatic cyclic AMP-H³ formation were also observed after one and three hours although, maximal stimulation was seen 30 minutes after treatment with p,p'-DDT.

H. Effect of Various Organochlorine Pesticides on Hepatic and Renal Cyclic AMP-H³ Formation

In order to examine whether the prior in vivo treatment with several other chlorinated hydrocarbon insecticides would promote the ability of renal cortex and hepatic tissue to synthesize radioactive cyclic nucleotide from adenosine-H³, groups of rats were given orally either p,p'-DDT, α -chlordane, heptachlor or endrin and killed an hour later. Data presented

TABLE 19

EFFECTS OF HYDRAZINE AND PROSTAGLANDIN E₁ ON THE p,p'-DDT-INDUCED STIMULATION OF RENAL CYCLIC AMP-H³ SYNTHESIS

Means $\pm$ S.E.M. represent at least four determinations of cyclic AMP-H³ formation by kidney cortex. Rats were administered 600 mg/kg of p,p'-DDT orally and killed one hour later. Hydrazine sulphate (260 mg/kg) or prostaglandin E₁ (100 γ /kg) was injected intraperitoneally 30 minutes prior to pesticide treatment. Renal cortical slices were incubated in Krebs-Ringer bicarbonate buffer containing adenosine-H³ for 60 minutes at 37°C. Data are also given in percentages taking the values of control rats as 100%.

Treatment	Cyclic AMP-H ³ Formation (DPM/mg X 10 ²)	Percent of Control
Control	337 $\pm$ 24	100
p,p'-DDT	530 $\pm$ 13	157*
Hydrazine	387 $\pm$ 12	115
Prostaglandin E ₁	360 $\pm$ 47	107
Hydrazine + p,p'-DDT	421 $\pm$ 30	125†
Prostaglandin E ₁ + p,p'-DDT	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 ($p = <0.05$).

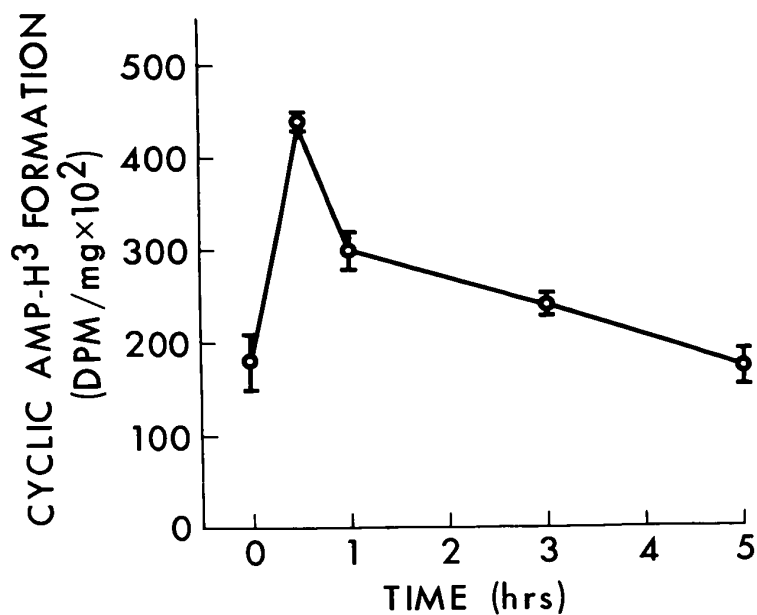


Fig. 20. Sequential changes in hepatic cyclic AMP-H³ production following p,p'-DDT treatment. Each point represents the mean $\pm$ S.E.M. of 4 rats in each group. Animals were fed p,p'-DDT (600 mg/kg) orally and killed 0.5, 1, 3 and 5 hours after insecticide treatment. Approximately 20 mg tissue was incubated with Krebs-Ringer bicarbonate buffer containing adenosine-H³ for 1 hour at 37°. Cyclic AMP formation is expressed as disintegrations per minute per milligram of tissue $\times 10^2$ (DPM/mg $\times 10^2$).

in Table 20 show that administration of p,p'-DDT produced a significant rise in tritiated cyclic AMP formation from the control values of 337 ± 24 to 530 ± 13 DPM/mg $\times 10^2$. Treatment with α -chlordane, heptachlor or endrin also elevated renal cyclic AMP levels by 49%, 59% and 90% respectively, when compared with the controls. In addition, a marked stimulation of 66%, 46%, 48% and 64% was observed in cyclic AMP- H^3 synthesis by livers of rats treated with either p,p'-DDT, α -chlordane, heptachlor or endrin, respectively. In general, the in vivo treatment with various chlorinated hydrocarbons seemed to produce a similar degree of enhancement in the synthesis of radioactive cyclic AMP from tritiated adenosine by both kidney cortex and liver.

VI. ALTERATIONS IN CARBOHYDRATE METABOLISM PRODUCED BY EXOGENOUS CYCLIC AMP AND THEOPHYLLINE

A. Stimulation of Renal Gluconeogenic Enzymes

In order to establish whether cyclic AMP is involved in the action of p,p'-DDT on renal carbohydrate metabolism, it was considered essential to determine whether treatment with exogenous cyclic AMP by itself, could also increase the activities of the four key gluconeogenic enzymes in rat kidney cortex. It is of interest that the activity of renal phosphoenolpyruvate carboxykinase has already been shown to be enhanced by cyclic AMP treatment (13,232,233). The results presented in Figure 21 illustrate that the administration of cyclic AMP in conjunction with theophylline produced increases in the activities of all four renal gluconeogenic enzymes studied. A significant rise in kidney pyruvate carboxylase to 155%, phosphoenolpyruvate carboxykinase to 165%, fructose 1,6-diphosphatase to

TABLE 20

EFFECT OF SEVERAL ORGANOCHLORINE PESTICIDES ON CYCLIC AMP-H³
FORMATION BY KIDNEY CORTEX AND LIVER

Means $\pm$ S.E.M. represent at least four animals in each group. Rats were administered either p,p'-DDT, α -chlordane, heptachlor or endrin orally and killed one hour later. Renal cortex or hepatic tissue slices were incubated in Krebs-Ringer bicarbonate buffer containing adenosine-H³ for 60 minutes at 37°C. Data are also given in percentages taking the values of control rats as 100%.

Treatment	Cyclic AMP-H ³ Formation (DPM/mg X 10 ²)			
	Kidney Cortex	Percent of Control	Liver	Percent of Control
Control	337 $\pm$ 24	100	180 $\pm$ 26	100
p,p'-DDT (600 mg/kg)	530 $\pm$ 13	157*	299 $\pm$ 20	166*
α -Chlordane (200 mg/kg)	503 $\pm$ 45	149*	263 $\pm$ 8	146*
Heptachlor (200 mg/kg)	537 $\pm$ 27	159*	267 $\pm$ 8	148*
Endrin (50 mg/kg)	640 $\pm$ 21	190*	296 $\pm$ 11	164*

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

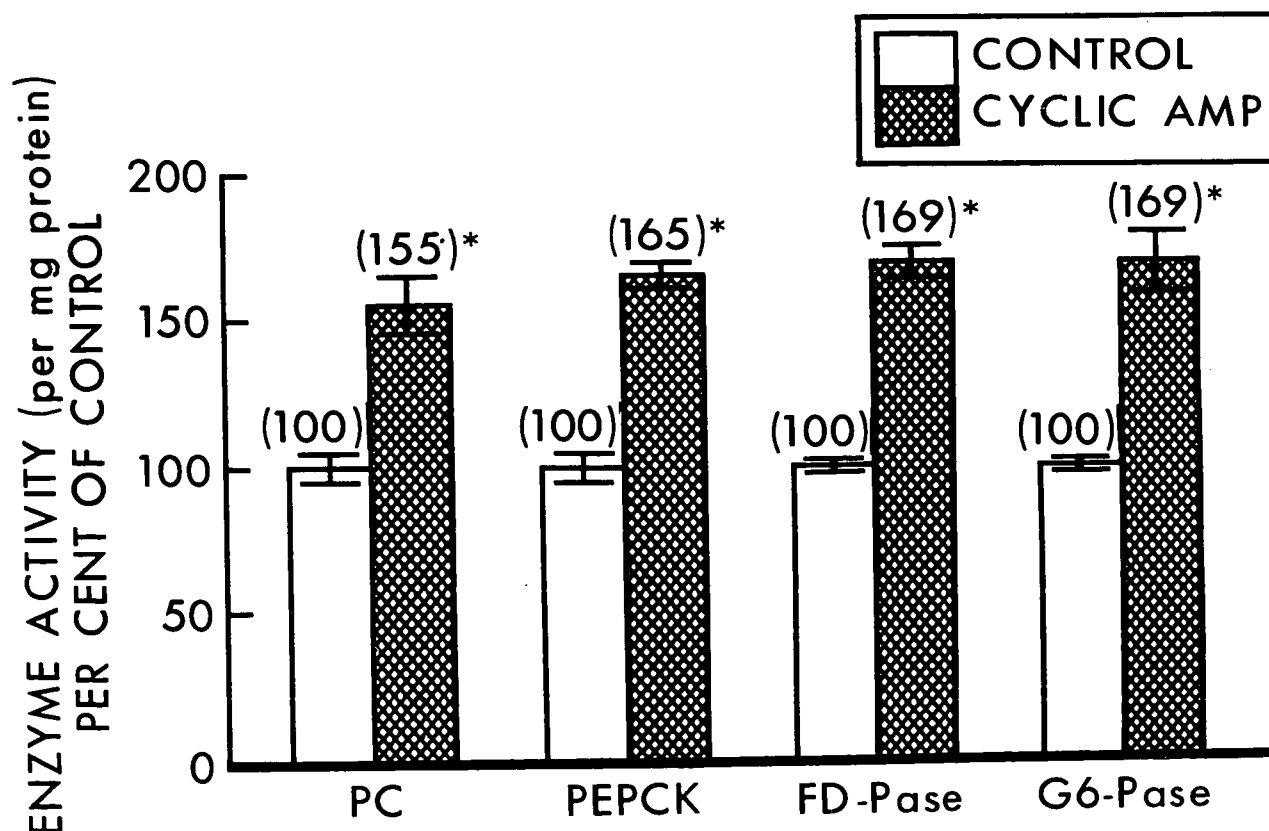


Fig. 21. Effect of exogenous cyclic AMP on key gluconeogenic enzymes of rat kidney cortex. Each bar represents the mean $\pm$ S.E.M. of 5 rats in each group. Cyclic AMP (10 mg/100 g) along with theophylline (10 mg/100 g) was injected intraperitoneally in two divided doses at 0 and 2½ hours and animals were killed 5 hours after the start of treatment. Enzyme activities were calculated as micromoles of substrate metabolized per hour per gram of tissue and expressed as specific activity per milligram protein. Data are given in percentages (in parentheses) taking the values of control rats as 100%.

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

169% and glucose 6-phosphatase to 169% of the control values was noted in rats treated with cyclic AMP and theophylline.

B. Effect of Theophylline on the p,p'-DDT-Induced Elevation in Renal Gluconeogenic Enzymes

If cyclic AMP indeed were to act as an intermediary in the action of p,p'-DDT on renal carbohydrate metabolism, inhibition of endogenous phosphodiesterase by theophylline would be expected to potentiate the effects of a submaximal dose of p,p'-DDT on renal gluconeogenic enzymes. Data in Figure 22 show that treatment with 100 mg/kg dose of p,p'-DDT significantly elevated the activities of renal cortical pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose 1,6-diphosphatase and glucose 6-phosphatase. Likewise, when theophylline (10 mg/100 g) was injected, minor but statistically significant increases were noted in all four gluconeogenic enzymes. It is of interest that treatment of p,p'-DDT-fed animals with theophylline augmented the effects of the low dose of the insecticide on the quartet of gluconeogenic enzymes in rat kidney cortex.

C. Cyclic AMP-Induced Stimulation of Hepatic Gluconeogenic Enzymes

Since p,p'-DDT produced stimulation of renal cortex and hepatic gluconeogenic enzymes and elevated cyclic AMP- H^3 formation, it was of interest to examine whether cyclic AMP, by itself, could also enhance the levels of these key enzymes in liver tissue. The results are presented in Figure 23. Administration of cyclic AMP and theophylline (10 mg/100 g) each significantly increased the activities of hepatic pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose 1,6-diphosphatase and glucose 6-phosphatase to 138%, 190%, 150% and 148% of the control values, respectively.

POTENTIATION BY THEOPHYLLINE OF p,p'-DDT-INDUCED INCREASE IN RENAL GLUCONEOGENIC ENZYMES

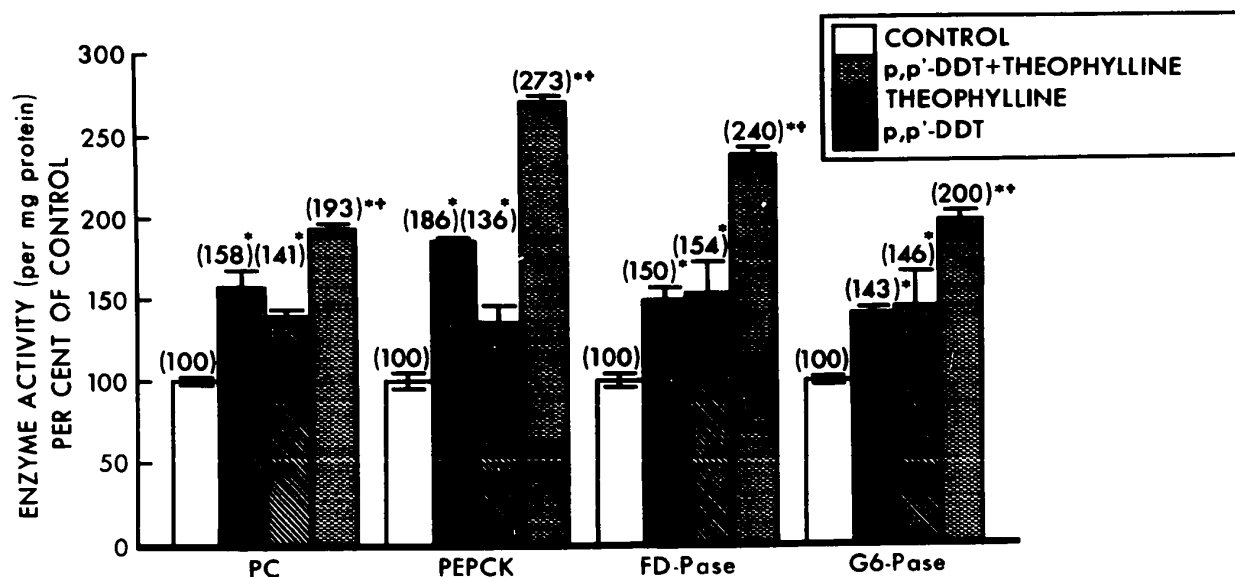


Fig. 22. Influence of theophylline and a submaximal dose of p,p'-DDT on renal gluconeogenic enzymes. Each bar represents the mean $\pm$ S.E.M. of 5 animals in each group. Animals were given a small dose (100 mg/kg) of p,p'-DDT orally and killed after 5 hours. Theophylline (10 mg/100 g) was injected intraperitoneally in two equally divided doses at 0 and 2½ hours. Enzyme activities were calculated as micromoles of substrate metabolized per hour per gram of tissue and expressed as specific activity per milligram protein. Data are given in percentages (in parentheses) taking the values of control animals as 100%.

*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 p,p'-DDT alone ($p = <0.05$).

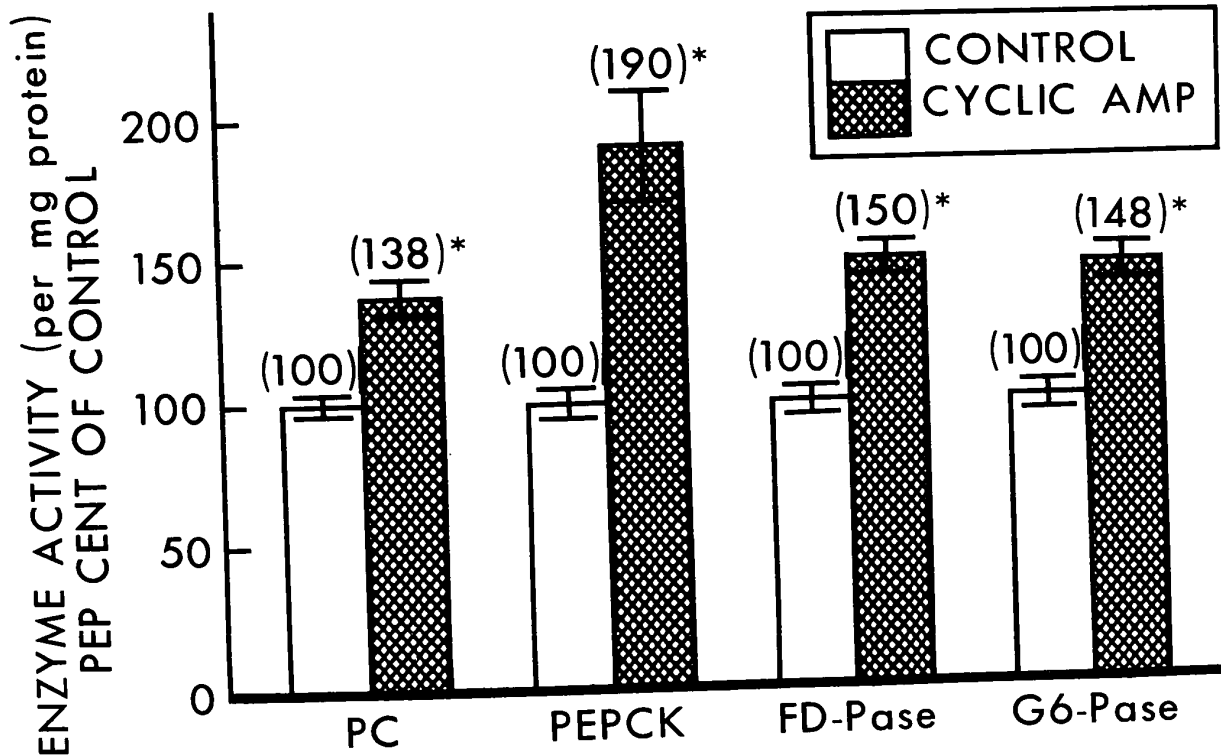


Fig. 23. Cyclic AMP-induced changes in key gluconeogenic enzymes of rat liver. Each bar represents the mean $\pm$ S.E.M. of 5 animals in each group. Cyclic AMP (10 mg/100 g) and theophylline (10 mg/100 g) were injected by the intraperitoneal route in two equally divided doses at 0 and 2½ hours and rats were killed 5 hours after the start of treatment. Enzyme activities were calculated as micromoles of substrate metabolized per hour per gram of tissue and expressed as specific activity per milligram protein. Data are given in percentages (in parentheses) taking the values of control rats as 100%.

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

D. Effect of Theophylline on the Stimulation of Hepatic Gluconeogenic

Enzymes by p,p'-DDT

Data presented in Figure 24 show that administration of p,p'-DDT (100 mg/kg) significantly increased pyruvate carboxylase, phosphoenolpyruvate carboxykinase and fructose 1,6-diphosphatase activities in hepatic tissues. Whereas theophylline failed to exert any appreciable effect on hepatic pyruvate carboxylase, a statistically significant rise was noted in the activities of phosphoenolpyruvate carboxykinase, fructose 1,6-diphosphatase and glucose 6-phosphatase in this tissue. A further augmentation in the activities of various key gluconeogenic enzymes was seen in rats treated simultaneously with p,p'-DDT and theophylline.

E. Cyclic AMP-Induced Changes in Serum Glucose and Urea and Hepatic Glycogen

Results in Table 21 show the effects of exogenously administered cyclic AMP and theophylline (10 mg/100 g each) on blood glucose, serum urea and liver glycogen. Just as in the case of DDT, cyclic AMP produced a rise in blood glucose and serum urea to 168% and 135% of the control values, respectively. In contrast, the concentration of liver glycogen decreased to 65% when compared to the values seen in control rats.

F. Effect of Theophylline and p,p'-DDT on Blood Glucose, Liver Glycogen and Serum Urea

The results presented in Table 22 demonstrate that the administration of p,p'-DDT (100 mg/kg) elevated blood glucose and urea as well as significantly lowered the concentration of liver glycogen. Treatment with theophylline (10 mg/100 g) produced minor, yet statistically significant increases in the amount of both glucose and urea in the serum. The administration of theophylline to p,p'-DDT-fed rats further augmented the action

EFFECTS OF THEOPHYLLINE ON THE STIMULATION OF HEPATIC GLUCONEOGENIC ENZYMES PRODUCED BY A SUBMAXIMAL DOSE OF p,p'-DDT

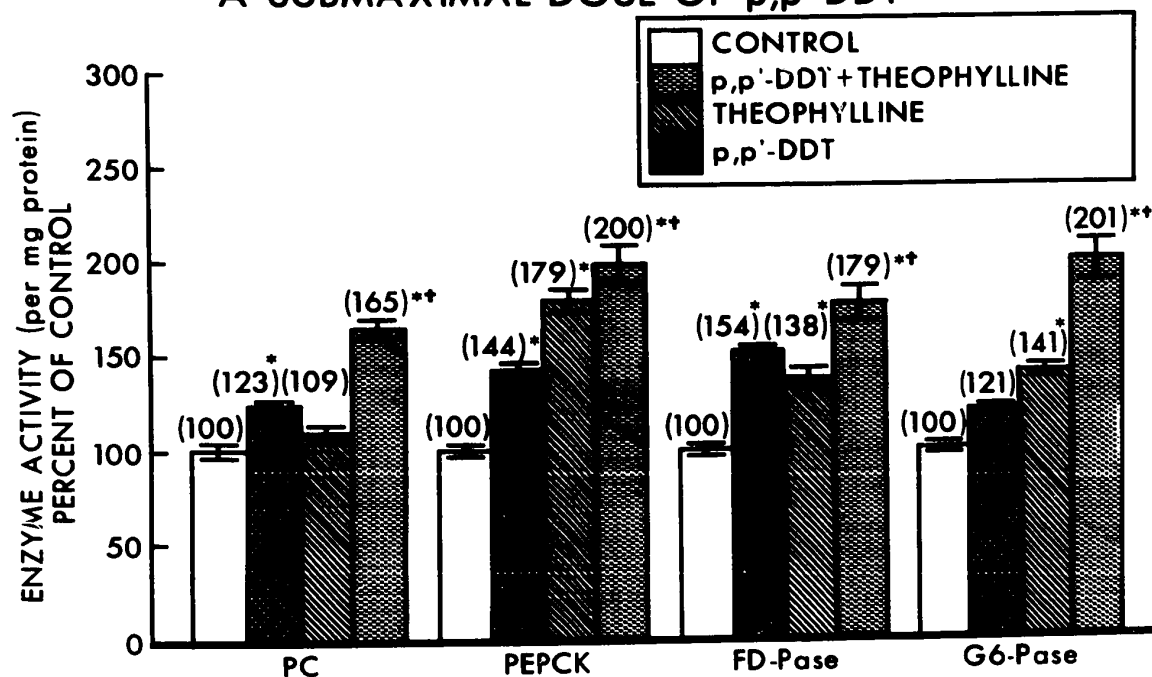


Fig. 24. Effect of theophylline on the p,p'-DDT-stimulated increases in hepatic gluconeogenic enzymes. Each bar represents the mean $\pm$ S.E.M. of 5 or more animals in each group. Rats were given a submaximal dose of p,p'-DDT (100 mg/kg) by the oral route and killed 5 hours later. Theophylline (10 mg/100 g) was injected intraperitoneally in two divided doses at 0 and 2½ hours. Enzyme activities were calculated as micromoles of substrate metabolized per hour per gram of tissue and expressed as specific activity per milligram protein. Data are given in percentages (in parentheses) taking the values of control rats as 100%.

*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 ($p = <0.05$).

TABLE 21

EFFECT OF CYCLIC AMP ON BLOOD GLUCOSE, SERUM UREA
AND LIVER GLYCOGEN

Means $\pm$ S.E.M. represent five animals in each group. Rats were administered cyclic AMP (10 mg/100 g) intraperitoneally and killed five hours later. Cyclic AMP was injected in 2 equally divided doses concurrently with theophylline (10 mg/100 g) at 0 and 2½ hours. Glucose and urea levels are expressed as milligrams per 100 milliliters of blood whereas glycogen as grams per 100 grams of liver. Data are also given in percentages (in parentheses) taking the values of control rats as 100%.

Parameters Examined	Control Rats	Cyclic AMP- Treated Rats
Blood Glucose (mg/100 ml)	80 $\pm$ 3 (100)	134 $\pm$ 4 (168)*
Serum Urea (mg/100 ml)	24.5 $\pm$ 2 (100)	33.1 $\pm$ 2 (135)*
Liver Glycogen (g/100 g)	2.3 $\pm$ 0.2 (100)	1.5 $\pm$ 0.1 (65)*

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

TABLE 22

EFFECT OF p,p'-DDT AND THEOPHYLLINE ON BLOOD GLUCOSE,
LIVER GLYCOGEN AND SERUM UREA

Means $\pm$ S.E.M. represent at least five animals in each group. Rats were administered p,p'-DDT (100 mg/kg) orally and killed 5 hours later. Theophylline (10 mg/100 g) was injected intraperitoneally in 2 equally divided doses at 0 and 2½ hours either alone or concurrently with p,p'-DDT. Blood glucose and serum urea levels are expressed as milligrams per 100 milliliters of blood whereas liver glycogen as grams per 100 grams of tissue. Data are also given in percentages (in parentheses) taking the values of control animals as 100%.

Treatment	Blood Glucose (mg/100 ml)	Liver Glycogen (g/100 g)	Serum Urea (mg/100 ml)
Control	81 $\pm$ 2 (100)	2.2 $\pm$ 0 (100)	26.1 $\pm$ 0.7 (100)
p,p'-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)*
p,p'-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 p,p'-DDT-treated animals ($p = <0.05$).

of this hydrocarbon on blood glucose and serum urea; however no potentiating effect of theophylline could be seen with respect to depressed liver glycogen after p,p'-DDT.

VII. INFLUENCE OF DDT ON ENDOGENOUS CYCLIC AMP, ADENYL CYCLASE AND PHOSPHODIESTERASE ACTIVITY OF LIVER AND KIDNEY

A. p,p'-DDT-Stimulated Increases in Renal Adenyl Cyclase and Cyclic AMP

Among the various criteria which must be fulfilled before accepting the role of cyclic AMP in the action of a given substance, it is essential to show that the compound increases the endogenous levels of the tissue cyclic nucleotide either by enhancing adenyl cyclase or inhibiting phosphodiesterase. In order to further demonstrate the involvement of cyclic AMP in DDT action on carbohydrate metabolism, the influence of this halogenated hydrocarbon was investigated on the endogenous levels of cyclic AMP as well as on the activity of adenyl cyclase in rat kidney cortex. Data presented in Figure 25 illustrate that p,p'-DDT administration in vivo significantly elevated (165%) the levels of kidney cortex cyclic AMP. In addition, pesticide treatment produced a marked enhancement in the activity of both basal- and fluoride-stimulated forms of adenyl cyclase. These results suggest that DDT is capable of increasing endogenous cyclic AMP synthesis in renal cortex which probably is achieved through stimulation of adenyl cyclase activity.

B. Effects of p,p'-DDT on Hepatic Adenyl Cyclase and Cyclic AMP

Since p,p'-DDT in vivo and in vitro enhanced the biotransformation of adenosine-H³ into labeled cyclic AMP by hepatic tissue. It was of interest to examine the influence of this hydrocarbon insecticide on adenyl

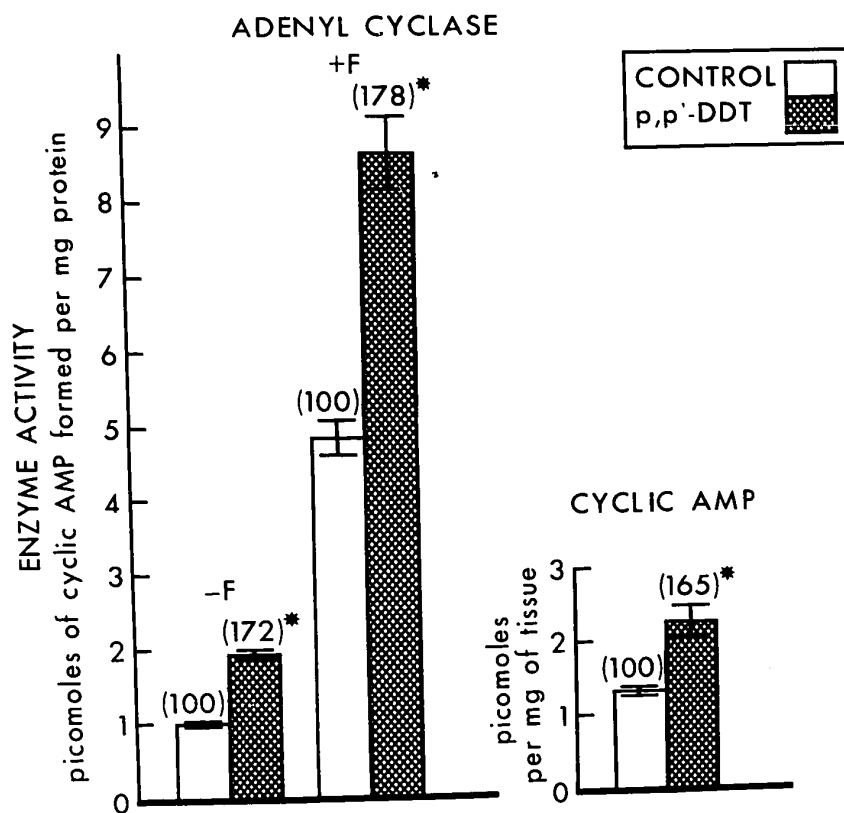


Fig. 25. Effect of p,p'-DDT treatment on the endogenous levels of cyclic AMP and adenyl cyclase activity of rat kidney cortex. Each bar represents the mean $\pm$ S.E.M. of 4 or more animals in each group. Rats were fed p,p'-DDT (600 mg/kg) orally and killed after 1 hour. Adenyl cyclase activity was calculated as picomoles of cyclic AMP formed per hour per milligram of tissue and expressed as specific activity per milligram protein. The concentration of cyclic AMP is given as picomoles per milligram of renal cortex. Data are shown in percentages (in parentheses) taking the values of control rats as 100%.

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

cyclase activity and endogenous cyclic AMP levels of rat liver. The results presented in Table 23 show that there was a significant rise in hepatic cyclic AMP levels (131%) in DDT-fed animals. Enhancement in the activity of both basal- and fluoride-stimulated forms of adenylyl cyclase was also observed after pesticide treatment.

C. Effect of p,p'-DDT on Renal and Hepatic Phosphodiesterase

In order to examine whether the observed DDT-induced elevation in endogenous cyclic AMP was also due to an inhibition of phosphodiesterase activity, the effect of this insecticide was investigated on the cyclic AMP-degrading enzyme in liver and kidney cortex. Data presented in Figure 26 demonstrate that p,p'-DDT treatment failed to affect the activity of hepatic phosphodiesterase. Kidney cortex phosphodiesterase, on the other hand, was slightly decreased (14%) in DDT-treated animals when compared with the control values.

TABLE 23

EFFECTS OF p,p'-DDT TREATMENT ON ADENYL CYCLASE ACTIVITY AND CYCLIC AMP LEVELS IN RAT LIVER

Means $\pm$ S.E.M. represent four animals in each group. Rats were administered 600 mg/kg of p,p'-DDT orally and killed after one hour. The activity of adenylyl cyclase was calculated as picomoles of cyclic AMP formed per hour per milligram of tissue and expressed as specific activity per milligram of protein. The concentration of cyclic AMP is given as picomoles per milligram of hepatic tissue. Data are also presented in percentages (in parentheses) taking the values of control rats as 100%.

Treatment	Adenylyl Cyclase		Cyclic AMP
	-F	+F (10 mM)	
Control	0.56 $\pm$ 0.01 (100)	3.24 $\pm$ 0.3 (100)	1.22 $\pm$ 0.08 (100)
p,p'-DDT	0.78 $\pm$ 0.01 (140)*	6.78 $\pm$ 0.6 (209)*	1.60 $\pm$ 0.06 (131)*

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

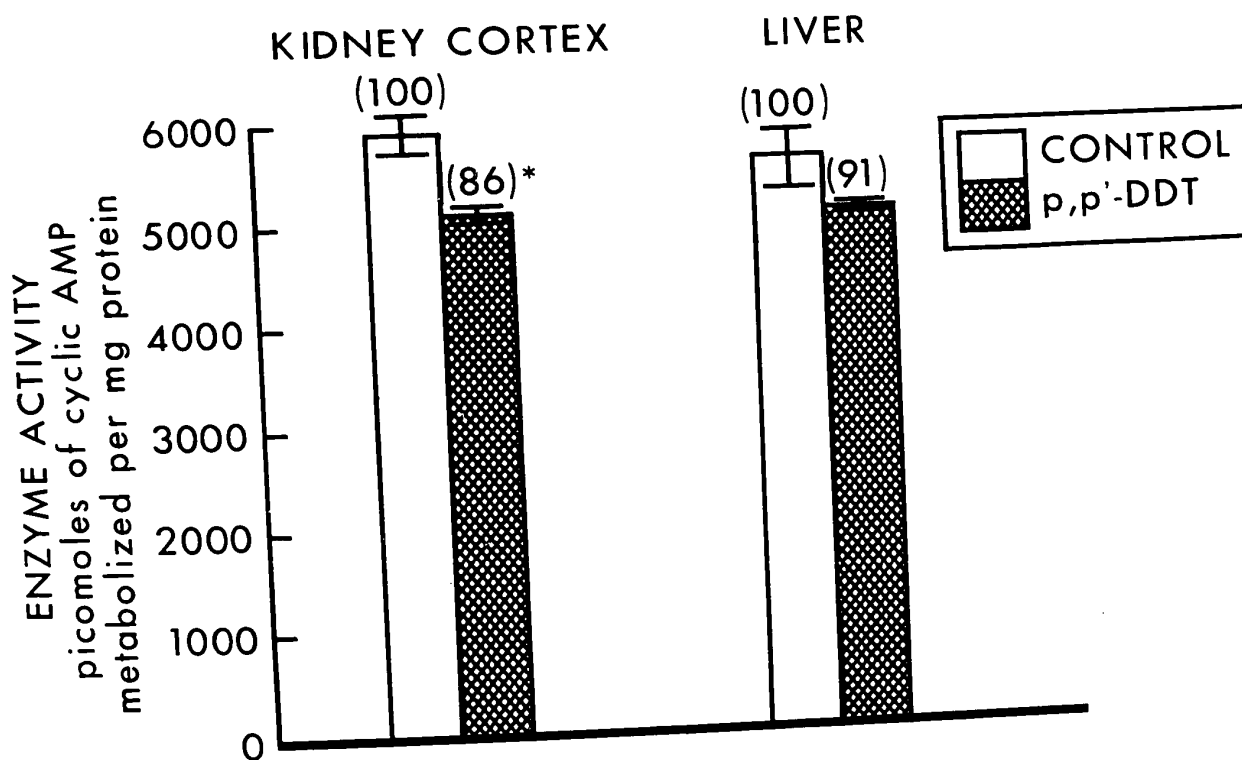


Fig. 26. Influence of p,p'-DDT on phosphodiesterase activity of liver and kidney cortex. Each bar represents the mean $\pm$ S.E.M. of 4 animals in each group. p,p'-DDT (600 mg/kg) was administered by the oral route and the rats were killed an hour later. Enzyme activity was calculated as picomoles of substrate metabolized per hour per milligram of tissue and expressed as specific activity per milligram protein. Data are given in percentages (in parentheses) taking the values of control animals as 100%.

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

5. DISCUSSION

I. EFFECT OF VARIOUS INSECTICIDES ON CARBOHYDRATE METABOLISM IN KIDNEY AND LIVER

The pathway of gluconeogenesis, the series of steps converting pyruvate into glucose, involves several reversible and four irreversible reactions (264). From the point of view of metabolic control, considerable attention has been focussed 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 mostly in liver and kidney where this process can take place actively (111,264). Results of the present study demonstrate that treatment with p,p'-DDT results in a marked enhancement of various key gluconeogenic enzymes in rat kidney cortex and liver. The increases observed in renal and hepatic enzyme activities were time-dependent and significant alterations could be detected as early as 1 hour after the insecticide. In addition, elevation in kidney and liver gluconeogenic enzymes was related to the dose of p,p'-DDT. Statistically significant enhancement of all four enzyme activities was produced with 100-200 mg/kg dose of this halogenated hydrocarbon (265-267). The present study also shows that both p,p'-DDT and o,p'-DDT were equally effective in enhancing renal gluconeogenic enzymes in male and female rats. The observed effects on kidney and liver gluconeogenesis appeared to be specific since the activities of several key glycolytic and hexose monophosphate shunt enzymes as well as of α -glycero-

phosphate dehydrogenase and glyceraldehyde 3-phosphate dehydrogenase remained completely unaltered following treatment with p,p'-DDT (265,268). It is of interest that the hepatic key gluconeogenic enzymes also increased in animals given o,p'-DDT (268), the DDT isomer known to be present to the extent of 15-20% in technical grade DDT (63). 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. o,p'-DDT was found to be more effective than the p,p'-isomer in stimulating the activities of certain important uterine enzymes involved in glucose metabolism. Singhal et al. (64) reported that o,p'-DDT produced a marked estrogen-like stimulation of uterine glycogen and of key glycolytic and hexose monophosphate shunt enzymes in ovariectomized rats whereas p,p'-DDT was only weakly estrogenic. In addition, Hrdina et al. (3) demonstrated that it was the p,p'-isomer which was responsible for the neurotoxic effects of technical DDT. In contrast, o,p'-DDT was shown to be void of any apparent neurotoxic effects. It is difficult to account, at present, for the observed differences in the responsiveness of various tissues to the two isomers contained in technical DDT (265,266).

Since p,p'-DDT enhanced the four key gluconeogenic enzymes in livers and kidney cortices of adrenalectomized rats to the same extent as in normal animals (265,266), 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. It is interesting that p,p'-DDT elevated blood glucose and decreased hepatic glycogen even in the absence of the adrenal glands suggesting that the insecticide-induced hyperglycemia and liver glycogenolysis are not mediated by a release of catecholamines from adrenals in response to the stress induced by this chlorinated hydrocarbon. A similar independence from adrenal function was shown for the effects of o,p'-DDT in stimulating renal gluconeogenesis (268).

The use of inhibitors of RNA and protein synthesis has provided considerable insight into the mechanism of action of a variety of hormones and drugs at the molecular level (269,270). Treatment with actinomycin D, cycloheximide or ethionine prevented significantly the insecticide-stimulated rise in gluconeogenic enzyme activities observed 5 hours after p,p'-DDT. The present study indicates that new RNA and protein synthesis may be involved in the observed p,p'-DDT-induced increases in the activities of pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose 1,6-diphosphatase and glucose 6-phosphatase. Since actinomycin D inhibits DNA-dependent synthesis of RNA by binding to the deoxyguanosine bases of DNA (271), the inhibition of the insecticide-induced increases in various gluconeogenic enzymes produced by this antibiotic indicates the participation of messenger RNA synthesis at the gene locus (272). Cycloheximide, an antibiotic that suppresses protein synthesis by inhibiting either the transfer of aminoacyl-transfer RNA to ribosomes or the formation of peptide bonds (273,274), also prevented the DDT-stimulated rise in various gluconeogenic enzymes of kidney and liver. Additional evidence for the involvement of both RNA and protein synthesis in the observed increases in the four renal and hepatic gluconeogenic enzymes induced by

p,p'-DDT was obtained by the use of ethionine. This compound is thought to inhibit the synthesis of protein through an inhibition of RNA synthesis as well as lowering ATP concentrations (275,276). Ethionine, when administered to pesticide-treated animals, effectively prevented the DDT-induced rise in kidney and liver gluconeogenic enzyme activities. It is of interest that the o,p'-DDT-induced enhancement in the activities of several glycolytic and hexose monophosphate shunt enzymes was also blocked by actinomycin D and cycloheximide in uteri of ovariectomized rats (64). Recently, Hrdina et al. (89) found that pretreatment with cycloheximide effectively prevented the p,p'-DDT-induced hyperpyrexia, tremor, convulsions and decrease in striatal acetylcholine. In contrast, actinomycin D failed to affect the pesticide-induced alterations in striatal acetylcholine as well as the development of tremor and convulsions. It is interesting that Peters et al. (80) observed that the marked increase in the concentration of 5-hydroxyindoleacetic acid in the brain stem of DDT-treated rats also remained unaffected by actinomycin D but was completely blocked by prior treatment with cycloheximide. Our results seem consistent with the hypothesis 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 (265,266).

Results of the present study also show that administration of α -chlordane, heptachlor or endrin stimulated the process of gluconeogenesis in both liver and kidney to about the same extent as that observed with p,p'-DDT. Treatment with either of these insecticides was found to be equally effective in increasing the activities of renal and hepatic pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose 1,6-diphosphatase

and glucose 6-phosphatase. In addition, these insecticides produced a similar magnitude of response as far as the depression of liver glycogen and the elevation in the concentration of serum glucose and urea were concerned (263). It is of interest that Bhatia et al. (261,262) also demonstrated an increase in blood glucose levels and an enhancement in the activities of several important hepatic gluconeogenic enzymes in dieldrin-fed rats. Recently, enhanced protein breakdown as indicated by a rise in the concentration of several amino acids in the serum was noted after treatment with DDT and dieldrin (277,278). Hrdina et al. (69) found that whereas the administration of an acute dose of α -chlordane, heptachlor or endrin produced hypothermia, p,p'-DDT resulted in a marked increase in body temperature. In addition, chronic treatment with p,p'-DDT produced a small but statistically significant increase in brain stem 5-hydroxyindoleacetic acid as well as reduced the levels of acetylcholine in the striatum (79,81,279). In contrast, the concentration of striatal acetylcholine and brain stem 5-hydroxyindoleacetic acid remained completely unaltered in rats given daily intramuscular injection of α -chlordane for 45 days (81,279). Recently, Bercken (280) found that p,p'-DDT exposure of myelinated nerve fibers of the clawed toad, Xenopus Laevis, produced repetitive firing by inducing a large negative after-potential. However, dieldrin, even at much higher concentrations than DDT, failed to alter myelinated nerve fiber activity. It is of interest that α -chlordane, heptachlor and endrin all were found to be equally effective in preventing the estrone-induced increases in uterine weights (66). In contrast, Singhal et al. (64) reported that the estradiol-stimulated increases in uterine weights of ovariectomized rats were further potentiated by an insecticide

of the DDT family. In addition, DDT increased the incorporation of glucose- C^{14} into protein, lipid and RNA as well as the activities of several rate-limiting glycolytic and hexose monophosphate shunt enzymes in uteri of estrogen-deprived rats suggesting that this halogenated hydrocarbon by itself, possesses marked estrogenic properties (62,64).

The present investigation demonstrates that when rats were treated over a prolonged period with relatively small doses of p,p'-DDT (5 or 25 mg/kg), a significant rise was observed in the activities of renal and hepatic pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose 1,6-diphosphatase and glucose 6-phosphatase. Long-term exposure to small amounts of α -chlordane also resulted in statistically significant enhancement in the quartet of key gluconeogenic enzymes in both liver and kidney cortex (281). It is interesting that chronic exposure produced changes in various hepatic and renal gluconeogenic enzymes which were qualitatively similar to those seen after an acute oral dose of either insecticide. Chronic treatment with 5 or 25 mg dose of either DDT or α -chlordane also resulted in a significant rise in body temperature (80), although the degree of hyperpyrexia was much greater in rats administered a single large dose of this insecticide.

Some of the more prominent pharmacological actions associated with the use of halogenated hydrocarbon insecticides include hyperexcitability, convulsions, an increase in involuntary muscular activity as well as hyperpyrexia (79). 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 (88). 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 (266). It is of interest that an intravenous injection of DDT also produced elevation of blood sugar levels in rabbits (88). The increase was detectable 10-30 minutes following DDT injection and was maximal after 1-2 hours. Since amino acids serve as a source of noncarbohydrate precursors utilized in gluconeogenesis, pesticide treatment may be expected to result in enhanced protein breakdown. Indeed, increased protein catabolism as indicated by a rise in the concentration of several amino acids in the serum has been noted in rainbow trout after treatment with DDT and dieldrin (277). Recently, Wassermann et al. (278) also showed that the administration of dieldrin markedly increased the level of serum protein in rabbits.

Evidence indicates that a close relationship exists between altered renal function and the changes in gluconeogenic capacity of kidney cortex and liver (153,282). Pesticide treatment results in tubular degeneration, parenchymous alterations and vascular congestion in the kidney (5-8), as well as fatty degeneration, atypical mitochondria and enlargement of the liver (8-10). Klaassen and Plaa (11) demonstrated that there was an increased excretion of glucose in the urine of mice that were treated with certain aliphatic chlorinated hydrocarbons. Administration of p,p'-

DDT also was found to result in an increased concentration of urinary glucose (265,268). The possibility exists that the elevation in urinary glucose levels after DDT treatment is related to the observed stimulation of gluconeogenic enzymes in kidney cortex and liver and to the decreased glucose reabsorption associated with renal tubular pathology (265,266).

II. INFLUENCE OF ORGANOCHLORINE PESTICIDES ON THE SYNTHESIS OF CYCLIC AMP-H³ FROM TRITIATED ADENOSINE

The present study also suggests that the action of chlorinated hydrocarbon pesticides on hepatic and renal metabolism may be related to changes in the capacity of these tissues to synthesize cyclic AMP from radioactive adenosine. The increase in the in vitro synthesis of cyclic AMP by both liver and kidney was dependent upon the concentration of the pesticide used and a statistically significant elevation was seen with 10^{-7} M p,p'-DDT (283,284). Stimulation of cyclic AMP-H³ synthesis was also related to the in vivo dose of p,p'-DDT and significant increases were noted in livers and kidney cortices of rats given 100 mg/kg dose of the chlorinated hydrocarbon insecticide. In addition, the enhancement of labeled cyclic nucleotide formation from tritiated adenosine was time-dependent and significant changes in cyclic AMP-H³ levels could be detected in animals treated with p,p'-DDT for 30 minutes.

The intracellular concentration of tissue cyclic AMP is dependent, at least partially, on the rate of hydrolysis by the enzyme phosphodiesterase. As expected, inhibition of phosphodiesterase by caffeine elevated the concentration of kidney cyclic AMP-H³ and potentiated the p,p'-DDT-induced rise in nucleotide synthesis in vitro. A similar augmentation of the

action of p,p'-DDT on tritiated cyclic nucleotide formation was noted in renal cortices of insecticide-fed rats pretreated with caffeine. Yeung and Oliver (221) also found that caffeine potentiated the cyclic AMP-stimulated rise in phosphoenolpyruvate carboxykinase. In contrast to caffeine, stimulation of phosphodiesterase by imidazole was found to prevent both the pesticide-induced rise in kidney cyclic AMP-H³ production. It is of interest that activation of phosphodiesterase by imidazole has also been shown to block the cyclic AMP-induced changes in adipose tissue lipolysis as well as the increase in serum calcium (285,286).

The present investigation also shows that chlorinated hydrocarbon insecticides whether belonging to the cyclodiene family such as α -chlordane, heptachlor or endrin or to the DDT group (p,p'-DDT) are equally effective in elevating the formation of cyclic AMP-H³ from radioactive adenosine by renal and hepatic tissues. It is of interest that qualitatively similar increases in the quartet of renal and hepatic key gluconeogenic enzymes, rise in blood glucose and urea as well as fall in hepatic glycogen were also observed after exposure to various pesticides (263). Additionally, these chlorinated hydrocarbons were found to be equally effective in producing proliferation of the smooth endoplasmic reticulum and stimulating the hepatic microsomal enzymes involved in steroid and drug metabolism (287). In contrast, whereas chronic treatment with p,p'-DDT produced elevation in the concentration of brain stem 5-hydroxyindoleacetic acid and reduction in striatal acetylcholine, no such changes were noted in animals injected with α -chlordane (81). Similarly, Bercken (280) found that DDT differed from dieldrin in its action on the nervous system in that only p,p'-DDT produced repetitive firing of myelinated nerve fibers

of the clawed toad. Recently, Singhal et al. (64) demonstrated that DDT produced estradiol-like stimulation of uterine weight and carbohydrate metabolism in ovariectomized rats. However, α -chlordane, heptachlor or endrin were found to be equally effective in blocking the estrone-induced increases in uterine weights (66).

In view of the evidence that cyclic AMP plays an important role in the regulation of kidney and liver carbohydrate metabolism (13,225,232), it appears possible that alterations in cyclic AMP concentration or its biochemical function might be associated with changes produced by p,p'-DDT (283,284). Indeed, compounds such as propranolol and hydrazine which depressed the synthesis of glucose and phosphoenolpyruvate carboxykinase (16,17,260,288), also blocked the p,p'-DDT-induced increase in cyclic AMP- H^3 formation. Treatment with propranolol depressed the concentration of blood glucose and prevented the hyperglycemic response seen with the dibutyryl analog of cyclic AMP (16,260). The present study demonstrates that only the higher in vitro concentration of propranolol ($10^{-4}M$) was capable of preventing the insecticide-induced rise in renal cyclic AMP- H^3 synthesis. Since propranolol in this high concentration is known to exhibit many effects unrelated to β -adrenergic blockade, the possibility exists that the observed inhibition of DDT-induced kidney cyclic nucleotide formation by this compound might not involve adrenergic receptors. The observation that p,p'-DDT administration to adrenalectomized or normal rats produced similar changes in blood glucose, liver glycogen and enhancement of various renal and hepatic gluconeogenic enzymes also suggests that the insecticide-induced alterations in carbohydrate metabolism may not be mediated by a release of catecholamines from the adrenals (265,266).

It is of interest that high concentrations of propranolol inhibited the vasopressin-induced increase in toad bladder permeability (289,290). However, since catecholamines in general did not affect the transport of water in the bladder, these authors suggested that the observed effects of propranolol may not involve an interaction with adrenergic receptors. Recently, Yeung and Oliver (221) found that administration of prostaglandins markedly decreased the activity of phosphoenolpyruvate carboxykinase in rat liver. However, Exton et al. (291) found that prostaglandins failed to alter the gluconeogenic process in hepatic tissue. Our results demonstrate that the pesticide-stimulated increase in cyclic AMP- H^3 synthesis remains unaffected by prostaglandin E_1 and $F_2\alpha$. The possibility exists that the changes in carbohydrate metabolism produced by the organochlorine insecticides may be related to the capacity of liver and kidney cortex to synthesize cyclic AMP- H^3 (263,284).

III. ROLE OF CYCLIC AMP IN ORGANOCHLORINE PESTICIDE ACTION ON CARBOHYDRATE METABOLISM

Although definitive evidence for any single triggering event involved with the action of chlorinated hydrocarbon insecticides on carbohydrate metabolism is lacking, our results indicate that these compounds might exert their effects by stimulating the cyclic AMP-adenyl cyclase system of liver and kidney cortex. If cyclic AMP indeed were to mediate the observed effects of organochlorine insecticides, treatment with a methyl xanthine such as caffeine, would be expected to enhance the influence of small doses of pesticides on renal and hepatic carbohydrate metabolism. The present investigation shows that caffeine is capable of potentiating

the action of a submaximal dose of p,p'-DDT on the four key gluconeogenic enzymes of kidney and liver. In addition, the insecticide-induced rise in the concentration of blood glucose and urea was further augmented by caffeine. It is of interest that caffeine also potentiated the p,p'-DDT-stimulated increase in cyclic AMP- H^3 formation from tritiated adenosine (283,284). The lipolysis in fat cells caused by catecholamines, glucagon and ACTH has been shown to be potentiated by methyl xanthines (212-215). In addition, theophylline further augmented the diuretic effect of vasopressin on the toad bladder (216), the cardiac inotropic response to norepinephrine (217) and the stimulatory effect of isoproterenol on DNA synthesis in parotid glands (219). Recently, Singhal et al. (292) demonstrated that treatment with theophylline potentiated the effects of a submaximal dose of testosterone on prostatic and vesicular weights and the activities of hexokinase, phosphofructokinase, pyruvate kinase, glucose 6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, α -glycerophosphate dehydrogenase and glyceraldehyde 3-phosphate dehydrogenase in orchidectomized rats. Administration of this inhibitor of phosphodiesterase to ovariectomized rats also was found to potentiate the estradiol-stimulated increases in uterine weights and in the activities of hexokinase, phosphofructokinase and pyruvate kinase (210,211).

Results of the present study also demonstrate that exogenous cyclic AMP can mimic the effects of chlorinated hydrocarbon insecticides on renal and hepatic gluconeogenic enzymes, blood glucose and urea as well as on liver glycogen. Injection of cyclic AMP and theophylline was found to significantly enhance the activities of pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose 1,6-diphosphatase and glucose 6-phosphatase

in both liver and kidney cortex. In addition, the cyclic AMP-induced elevation in the concentration of serum glucose and urea and the fall in hepatic glycogen was similar to that seen after the treatment with organo-chlorine pesticides. It is interesting that administration of cyclic AMP has been reported to stimulate the incorporation of non-carbohydrate precursors into glucose in both kidney and liver (224,225,228,232). In addition, several investigators have reported that administration of cyclic AMP produced a significant rise in renal and hepatic phosphoenolpyruvate carboxykinase and glucose 6-phosphatase which was inhibited by actinomycin D and cycloheximide (220-223). Since cyclic AMP also increased the concentration of glucose and urea in the blood, enhanced the synthesis of glucose from non-carbohydrate precursors and lowered the levels of hepatic glycogen (13,235-240), it would appear that the insecticide-induced changes in carbohydrate metabolism are similar to those produced by exogenously administered cyclic AMP (265,266,284).

Sutherland et al. (206) emphasized the variety of criteria which must be met in order to establish the role of cyclic AMP in the action of a given drug or a hormone. Results of this investigation show that the essential criteria for the involvement of cyclic AMP in insecticide action on hepatic and renal carbohydrate metabolism have been fulfilled, at least partially. Administration of DDT enhanced the endogenous levels of cyclic AMP in kidney cortex and liver and produced significant increases in the activities of both basal- and fluoride-stimulated forms of adenyl cyclase. In contrast, the activity of hepatic phosphodiesterase was not altered in DDT-fed animals although, this enzyme was decreased slightly in renal cortex. Administration of various insecticides increased the

capacity of kidney and liver to biotransform tritiated adenosine into labeled cyclic AMP. In addition, exogenously administered cyclic AMP was able to mimic the effects of chlorinated hydrocarbon insecticides on renal and hepatic gluconeogenic enzymes, blood glucose and urea as well as on liver glycogen. Furthermore, treatment with an inhibitor of phosphodiesterase potentiated the action of a submaximal dose of DDT on carbohydrate metabolism in both kidney cortex and liver.

A possible scheme to help visualize the extent of cyclic AMP involvement in the action of DDT and other chlorinated hydrocarbon pesticides on carbohydrate metabolism is proposed in Figure 27. The sequence of events which leads to ultimate changes in hepatic and renal carbohydrate metabolism is initiated when DDT stimulates the synthesis of cyclic AMP from ATP by activating the enzyme, adenyl cyclase. The insecticide-induced rise in endogenous levels of cyclic AMP subsequently results in marked increases in the activities of hepatic and renal pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose 1,6-diphosphatase and glucose 6-phosphatase which can be blocked by a variety of compounds known to inhibit the synthesis of RNA and protein. Figure 27 also shows that propranolol and hydrazine can interfere with the DDT-stimulated enhancement of cyclic 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 and renal and hepatic carbohydrate metabolism. All these results would tend to support the view that the insecticide-induced changes in carbohydrate metabolism might involve alterations in the cyclic AMP-adenyl cyclase system of liver and kidney cortex.

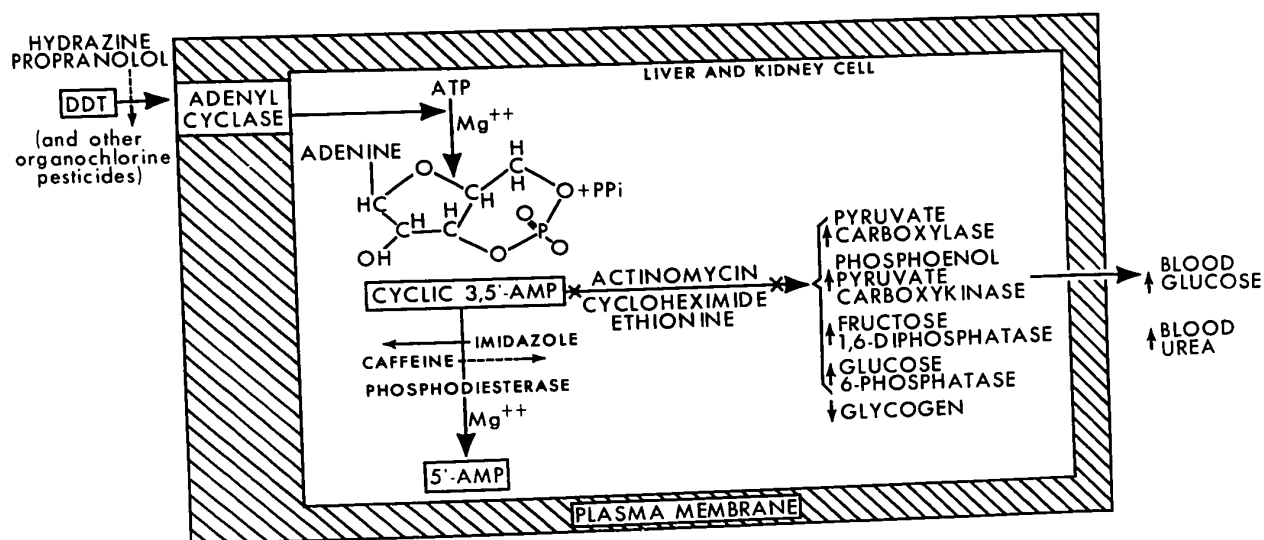


Fig. 27. Possible role of cyclic AMP in the action of DDT and other organochlorine pesticides on carbohydrate metabolism. The pesticide-induced rise in blood glucose and urea may be initiated when DDT stimulates adenylyl cyclase increasing the cyclic AMP levels which then enhances the activities of pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose 1,6-diphosphatase and glucose 6-phosphatase. This figure also shows that propranolol and hydrazine can interfere with the DDT-stimulated enhancement of cyclic AMP synthesis in rat kidney cortex. Whereas imidazole effectively prevents the pesticide-induced rise in cyclic AMP, methyl xanthines potentiate the effects of a submaximal dose of DDT on cyclic AMP formation and renal and hepatic gluconeogenic enzymes.

6. SUMMARY

The complex interaction between biological systems and environmental pollutants such as DDT and related organochlorine insecticides remains a problem of considerable interest. Over the past three decades, the pharmacological effects of various halogenated hydrocarbons on the functioning of the central nervous system and reproductive organs seem to have attracted major attention. The purpose of this dissertation was to examine the acute and chronic influence of certain chlorinated hydrocarbon pesticides on carbohydrate metabolism and to study the mechanism by which they enhance the synthesis of glucose from non-carbohydrate precursors in kidney cortex and liver. Administration of a single, oral dose of p,p'-DDT (600 mg/kg) produced marked increases in the activities of pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose 1,6-diphosphatase and glucose 6-phosphatase in rat kidney cortex and liver. Statistically significant increases in various renal and hepatic key, rate-limiting gluconeogenic enzymes were produced regardless of whether the activities were calculated as per g wet weight of tissue or per milligram protein. The observed effects on the quartet of gluconeogenic enzymes were time-dependent and were related to the dose of the insecticide. Both o,p'-DDT and p,p'-DDT were equally effective in enhancing the activities of various key gluconeogenic enzymes in renal cortices of male and female rats indicating the lack of any sex difference in the action of the two isomers contained in technical DDT.

Treatment of p,p'-DDT-fed rats with actinomycin D, cycloheximide or ethionine, compounds that are known to inhibit the synthesis of RNA and

protein, generally blocked the ability of this halogenated hydrocarbon to stimulate the activities of hepatic and renal pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose 1,6-diphosphatase and glucose 6-phosphatase. Cycloheximide appeared to be slightly more effective than actinomycin D or ethionine in inhibiting the p,p'-DDT-induced increases in gluconeogenic enzymes. The results suggest that stimulation of the synthesis of new RNA and protein might be involved in observed increases in the activities of hepatic and renal gluconeogenic enzymes after DDT treatment.

Administration of p,p'-DDT to adrenalectomized rats enhanced the four key gluconeogenic enzymes to about the same extent as in normal intact animals indicating that the stimulation of hepatic and renal gluconeogenesis by DDT may be independent of adrenal function. The finding that a potent glucocorticoid, triamcinolone, failed to potentiate the action of p,p'-DDT on various kidney and liver gluconeogenic enzymes lends further support to the view that the observed pesticide-induced alterations are not mediated through a release of corticosteroids from the adrenal glands. That the action of chlorinated hydrocarbon pesticides on carbohydrate metabolism is independent of adrenal function was also suggested from the experiment in which p,p'-DDT was shown to produce an equal hyperglycemic response in normal and adrenalectomized rats.

Treatment with an acute oral dose of p,p'-DDT (600 mg/kg), α -chlordane (200 mg/kg), heptachlor (200 mg/kg) or endrin (50 mg/kg) resulted in qualitatively similar increases in renal and hepatic pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose 1,6-diphosphatase and glucose 6-phosphatase. In addition, the various organochlorine pesticides elevated blood glucose and urea and lowered the concentration of hepatic glycogen

to about the same extent. Data indicate that p,p'-DDT, α -chlordane, heptachlor and endrin are equally effective in augmenting the gluconeogenic potential of both kidney cortex and liver. Exposure to small daily doses of p,p'-DDT (5 or 25 mg/kg) for a period of 20 or 45 days also resulted in significant increases in the activities of various hepatic and renal gluconeogenic enzymes. A similar enhancement in the activities of pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose 1,6-diphosphatase and glucose 6-phosphatase was noted in livers and kidney cortices of rats chronically injected with small doses of α -chlordane. Evidence suggests that prolonged treatment with relatively small amounts of p,p'-DDT or α -chlordane is also capable of enhancing the process of gluconeogenesis in both renal and hepatic tissue.

Treatment with p,p'-DDT, in vivo and in vitro, increased the formation of labeled cyclic AMP from tritiated adenosine by liver and kidney cortex. The p,p'-DDT-induced rise in renal and hepatic cyclic AMP-H³ production was time-dependent and was related to the amount of pesticide present. A similar increase in cyclic AMP-H³ synthesis was seen when rats were given either p,p'-DDT, α -chlordane, heptachlor or endrin suggesting that various pesticides are equally effective in enhancing the formation of hepatic and renal tritiated cyclic nucleotide. Administration of caffeine to p,p'-DDT-treated animals potentiated the insecticide-stimulated rise in the production of cyclic AMP-H³ whereas concurrent treatment with the hydrocarbon and imidazole, propranolol or hydrazine effectively blocked the observed response produced by p,p'-DDT. In contrast, prostaglandin E₁ and F₂ α failed to alter the ability of the insecticide to enhance cyclic AMP-H³ synthesis by kidney cortex. Data suggest that the organochlorine

insecticide-induced changes in hepatic and renal carbohydrate metabolism might be associated with their ability to increase cyclic AMP- H^3 synthesis from tritiated adenosine by rat kidney and liver.

Additional evidence for the involvement of cyclic AMP in insecticide action was obtained from experiments in which exogenous cyclic AMP was shown to mimic the effects of DDT on hepatic and renal metabolism. Treatment with cyclic AMP resulted in statistically significant changes in the activities of pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose 1,6-diphosphatase and glucose 6-phosphatase which were qualitatively similar to those seen with various insecticides. A rise in blood glucose and urea as well as a reduction in hepatic glycogen content was also observed in rats injected with cyclic AMP. Treatment with theophylline, an inhibitor of the enzyme phosphodiesterase, enhanced the effects of a submaximal dose of p,p'-DDT on various gluconeogenic enzymes, blood glucose and urea and hepatic glycogen. Administration of p,p'-DDT produced a significant rise in the endogenous levels of renal and hepatic cyclic AMP and increased the activities of both basal- and fluoride-stimulated forms of adenyl cyclase. Although p,p'-DDT treatment did not alter phosphodiesterase activity in liver, this enzyme was decreased slightly in kidney cortex. Results presented in this dissertation support the concept that alterations in the cyclic AMP-adenyl cyclase system of liver and kidney might play an important role in the mechanism of action of organochlorine pesticides on carbohydrate metabolism of these tissues.

In conclusion, the effects of DDT and other organochlorine insecticides have been investigated on certain aspects of carbohydrate and cyclic AMP metabolism in rat liver and kidney cortex. Statistically significant

alterations in hepatic and renal key gluconeogenic enzymes, blood glucose and urea and in liver glycogen were noted following treatment with chlorinated hydrocarbon pesticides. The observed changes in carbohydrate metabolism may be related to an enhanced capacity of liver and kidney to synthesize labeled cyclic AMP from tritiated adenosine. Evidence has also been presented which suggest that changes in the endogenous levels of cyclic AMP and the activity of adenyl cyclase might be involved in DDT-induced stimulation of hepatic and renal gluconeogenesis. The results provide further knowledge of the acute and chronic effects of chlorinated hydrocarbon insecticides on kidney and liver and suggest directions along which future work may be carried out concerning pesticide-induced metabolic alterations in mammalian systems.

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ABSTRACT

Administration of a single, oral dose of p,p'-DDT (600 mg/kg) produced marked increases in the activities of pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose 1,6-diphosphatase and glucose 6-phosphatase in the rat kidney cortex and liver. The observed effects on the quartet of gluconeogenic enzymes were time-dependent and were related to the dose of the insecticide. Treatment of p,p'-DDT-fed rats with actinomycin D, cycloheximide or ethionine, compounds that are known to inhibit the synthesis of RNA and protein, generally blocked the ability of this halogenated hydrocarbon to stimulate the activities of hepatic and renal pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose 1,6-diphosphatase and glucose 6-phosphatase. Administration of p,p'-DDT to adrenalectomized rats enhanced the four key gluconeogenic enzymes to about the same extent as in normal intact animals indicating that the stimulation of hepatic and renal gluconeogenesis by DDT may be independent of adrenal function. The finding that a potent glucocorticoid, triamcinolone, failed to potentiate the action of p,p'-DDT on various kidney and liver gluconeogenic enzymes lends further support to the view that the observed pesticide-induced alterations are not mediated through a release of corticosteroids from the adrenal glands. Treatment with an acute oral dose of p,p'-DDT (600 mg/kg), α -chlordane (200 mg/kg), heptachlor (200 mg/kg) or endrin (50 mg/kg) resulted in qualitatively similar increases in renal and hepatic pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose 1,6-diphosphatase and glucose 6-phosphatase. In addition, the various organochlorine pesticides elevated blood glucose and urea and lowered the concentration of hepatic glycogen to about the same extent. Exposure to small daily doses of p,p'-DDT (5 or 25 mg/kg) for a period of 20 or 45 days also resulted in significant increases in the activities of various hepatic

and renal gluconeogenic enzymes. A similar enhancement in the activities of pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose 1,6-diphosphatase and glucose 6-phosphatase was noted in livers and kidney cortices of rats chronically injected with small doses of α -chlordane. Evidence suggests that prolonged treatment with relatively small amounts of p,p'-DDT or α -chlordane is also capable of enhancing the process of gluconeogenesis in both renal and hepatic tissue.

Treatment with p,p'-DDT, in vivo and in vitro, increased the formation of labeled cyclic AMP from tritiated adenosine by liver and kidney cortex. The p,p'-DDT-induced rise in renal and hepatic cyclic AMP- H^3 production was time-dependent and was related to the amount of pesticide present. A similar increase in cyclic AMP- H^3 synthesis was seen when rats were given either p,p'-DDT, α -chlordane, heptachlor or endrin suggesting that various pesticides are equally effective in enhancing the formation of hepatic and renal tritiated cyclic nucleotide. Administration of caffeine to p,p'-DDT-treated animals potentiated the insecticide-stimulated rise in the production of cyclic AMP- H^3 whereas concurrent treatment with the hydrocarbon and imidazole, propranolol or hydrazine effectively blocked the observed response produced by p,p'-DDT. In contrast, prostaglandin E_1 and $F_{2\alpha}$ failed to alter the ability of the insecticide to enhance cyclic AMP- H^3 synthesis by kidney cortex. Data suggest that the organochlorine insecticide-induced changes in hepatic and renal carbohydrate metabolism might be associated with their ability to increase cyclic AMP- H^3 synthesis from tritiated adenosine by rat kidney and liver. Additional evidence for the involvement of cyclic AMP in insecticide action was obtained from experiments in which exogenous cyclic AMP was shown to mimic the effects of DDT on hepatic and renal metabolism. Treatment with cyclic AMP resulted

in statistically significant changes in the activities of pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose 1,6-diphosphatase and glucose 6-phosphatase which were qualitatively similar to those seen with various insecticides. A rise in blood glucose and urea as well as a reduction in hepatic glycogen content was also observed in rats injected with cyclic AMP. Treatment with theophylline, an inhibitor of the enzyme phosphodiesterase, enhanced the effects of a submaximal dose of p,p'-DDT on various gluconeogenic enzymes, blood glucose and urea and hepatic glycogen. Administration of p,p'-DDT produced a significant rise in the endogenous levels of renal and hepatic cyclic AMP and increased the activities of both basal- and fluoride-stimulated forms of adenyl cyclase. Although p,p'-DDT treatment did not alter phosphodiesterase activity in liver, this enzyme was decreased slightly in kidney cortex. Results presented in this dissertation support the concept that alterations in the cyclic AMP-adenyl cyclase system of liver and kidney might play an important role in the mechanism of action of organochlorine pesticides on carbohydrate metabolism of these tissues.

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