Biochemical Pharmacology, Vol. 19, pp. 2145–2155. Pergamon Press. 1970. Printed in Great Britain

METABOLIC CONTROL MECHANISMS IN MAMMALIAN SYSTEMS—IX

ESTROGEN-LIKE STIMULATION OF UTERINE ENZYMES BY *o,p'*-1,1,1-TRICHLORO-2-2-BIS (*p*-CHLOROPHENYL) ETHANE*

RADHEY L. SINGHAL,† J. R. E. VALADARES‡ and WAYNE S. SCHWARK

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

(*Received* 17 *October* 1969; *accepted* 19 *December* 1969)

Abstract—The widespread occurrence of 1,1,1-trichloro-2-2-bis (*p*-chlorophenyl) ethane (DDT) in our environment and the possibility that this insecticide may have adverse effects on animal fertility led us to investigate the influence of this chlorinated hydrocarbon on carbohydrate metabolism in uteri of ovariectomized rats. Administration of *o,p'*-DDT (10 mg/100 g) significantly enhanced uterine weights, glycogen content and the activities of hexokinase, phosphofructokinase, aldolase and pyruvate kinase as well as of glucose 6-phosphate and 6-phosphogluconate dehydrogenases. *p,p'*-DDT treatment, on the other hand, resulted in only minor increases in uterine aldolase, pyruvate kinase and glucose 6-phosphate dehydrogenase without exerting any appreciable effect on the other enzymes or on glycogen. Time-course studies demonstrated that, while significant increases in uterine weight and in the activities of most enzymes occurred within 4 hr after a single injection of *o,p'*-DDT, maximal increases were attained at 16 hr. Chronic treatment with *o,p'*-DDT (5·0 mg/100 g) for 3 days produced increases in uterine enzyme activities greater than those observed after a single dose of the pesticide. Administration of either actinomycin or cycloheximide to *o,p'*-DDT-treated animals effectively blocked the insecticide-stimulated increases in uterine glycloytic and hexose monophosphate shunt enzymes, suggesting that acceleration of the synthesis of both new RNA and protein may be associated with the observed augmented enzymatic activities. Whereas concomitant treatment with estradiol-17β and *o,p'*-DDT produced effects on uterine enzymes which were somewhat additive, administration of progesterone resulted in an almost complete inhibition of the insecticide-stimulated enzyme increases. The present investigation indicates that DDT analogs possess uterotrophic activity and that, analogous to the action of estrogens on uterine tissue, they are capable of inducing new synthesis of several carbohydrate-metabolizing enzymes.

IT IS becoming increasingly apparent that several drugs as well as a variety of commonly used insecticides are capable of producing detrimental effects upon reproduction and fertility in several species. Several investigators have suggested that the chlorinated hydrocarbon, 1,1,1-trichloro-2-2-bis (*p*-chlorophenyl) ethane (DDT), may produce hormonal or antifertility effects in laboratory animals and in wildlife.[1–5] Treatment

* Supported in part by a grant from the Upjohn Company, the Medical Research Council of Canada and Eli Lilly & Co., Indianapolis, Ind. A preliminary account of portions of this work has appeared previously in *Proc. Fourth Int. Cong. Pharmac.*, Basel, Switzerland (1969).

† Medical Research Scholar of the Medical Research Council of Canada.

‡ Present address: Division of Medicine and Pharmacology, Drug Advisory Bureau, Food and Drug Directorate, Tunneys Pasture, Ottawa, Canada.

of immature female or ovariectomized rats with various isomers of DDT results in estrogenic effects that are more pronounced with *o,p'*-DDT than with *p,p'*-DDT. Administration of *o,p'*-DDT enhances uterine water imbibition, wet weight and incorporation of glucose-^{14}C into uterine protein, lipid and RNA.[4] In addition, Bitman *et al.*[5] demonstrated that this isomer of DDT is capable of augmenting glycogen accumulation in uteri of immature rats as well as in the oviduct of two avian species.

The possible physiological and ecological implications of this pesticide on animal fertility, as well as the similarity in configuration of DDT to the synthetic estrogen diethylstilbesterol, prompted us to examine the influence of DDT on several important carbohydrate-metabolizing enzymes in the uterus of the ovariectomized rat. Our earlier studies have shown that diethylstilbesterol, as well as the naturally occurring estrogens, regulates uterine glucose metabolism by exerting its effects on receptor sites at the source of enzyme formation to turn on the biosynthesis of certain enzymes, *viz.* hexokinase (HK), phosphofructokinase (PFK), aldolase and pyruvate kinase (PK), that are involved in the process of glycolysis.[6–12] In the present communication, we report that *o,p'*-DDT is capable of exerting an estrogen-like modulating influence on several glycolytic and hexosemonophosphate shunt enzymes in uteri of ovariectomized rats.

MATERIALS AND METHODS

Young female rats of the Wistar strain, weighing approximately 150 g at the time of surgery, were used in this study. The animals were ovariectomized bilaterally under light pentobarbital anesthesia and were used after a postoperative period of 2 weeks.[6, 7, 10] The following experimental procedures were used.

Comparison of the effects of two DDT analogs. The effects of *o,p'*-DDT and *p,p'*-DDT on uterine weight, glycogen content and the activities of several glycolytic and pentose shunt enzymes were studied in groups of ovariectomized rats. Single doses of 10 mg/100 g of each insecticide were administered i.m., and animals were sacrificed 16 hr later.

Time-course of o,p'*-DDT-induced changes.* Since the above study indicated that *o,p'*-DDT was the more potent isomer, the sequence of events after administration of this hydrocarbon was followed for a period of 24 hr. Groups of ovariectomized rats were given single i.m. injections of *o,p'*-DDT (10 mg/100 g) and killed after 4, 8, 16 and 24 hr.

Effects of actinomycin and cycloheximide. In order to investigate the nature of the *o,p'*-DDT-induced increases in uterine enzyme activities, the effects of two inhibitors of RNA and protein synthesis were examined. Four groups of ovariectomized animals were used: (1) control rats; (2) animals treated with *o,p'*-DDT; (3) and (4) *o,p'*-DDT-treated rats injected (i.p.) with either actinomycin or cycloheximide 30 min prior to administration of the insecticide. All animals were killed 16 hr after *o,p'*-DDT injection.

Influence of cycloheximide on chronic o,p'*-DDT treatment.* The effect of cycloheximide on uterine enzyme increases induced by treatment with *o,p'*-DDT for 3 days was studied in the following three groups of ovariectomized rats: (1) control rats; (2) animals injected with *o,p'*-DDT; (3) *o,p'*-DDT-treated rats given cycloheximide. *o,p'*-DDT (5 mg/100 g) was injected at 24-hr intervals for 3 days. Cycloheximide was administered daily 30 min prior to injection of the insecticide. Groups of rats were killed 24 hr after termination of *o,p'*-DDT treatment.

Effect of progesterone and estradiol-17β. In order to examine the effects of progester-

one and estradiol-17β on *o,p'*-DDT-induced changes in uterine weight and enzyme activities, the following six groups of ovariectomized rats were employed: (1) control animals; (2, 3 and 4) rats treated with *o,p'*-DDT (10 mg/100 g), progesterone (5 mg/100 g) and estradiol-17β (0·1 μg/100 g) respectively; (5 and 6) *o,p'*-DDT-treated animals injected 30 min earlier with either progesterone or estradiol-17β. All animals were killed 16 hr after administration of the insecticide.

Chemicals and dosages. Analytically pure *o,p'*-DDT and *p,p'*-DDT were obtained from the Aldrich Chemical Co., suspended in corn oil and administered i.m., in doses of 10 mg/100 g body weight. Actinomycin D (25 μg/100 g; Merck) and cycloheximide (70 μg/100 g; Upjohn) were dissolved in 0·9% saline and given by the i.p. route. Estradiol-17β (0·1 μg/100 g; Sigma) and progesterone (5 mg/100 g; Nutritional Biochemicals) were dissolved in ethanolic 0·9% saline and administered i.m.

Sample preparation and assay methods. All animals were stunned, decapitated and exsanguinated. Uteri were rapidly excised, trimmed of all extraneous tissue and weighed on a Roller Smith torsion balance. Pooled uteri were finely minced in a beaker immersed in crushed ice, and 5% homogenates and supernatant fluids were prepared as described in previous communications.[6, 7] The activities of hexokinase,[11] phosphofructokinase,[6] aldolase[12] and pyruvate kinase[10, 13] were assayed in the supernatant. Glucose 6-phosphate dehydrogenase (G6-PDH) and 6-phosphogluconate dehydrogenase (6-PGDH) were estimated according to the method of Glock and McLean,[14] as described previously.[15, 16] Enzyme activities were assayed under strictly linear kinetic conditions at 340 mμ in a constant recording Unicam spectrophotometer, model SP 800, set at 37° and calculated as micromoles of substrate metabolized per gram of tissue per hour times the weight of the uterus.[6–12] Uterine glycogen was assayed by the anthrone method of Seifter *et al.*,[17] and expressed as micrograms per total uterus. The results were subjected to statistical evaluation and significant differences between the means (calculated as P values) are shown. No statistical significance is indicated when the P value is $>0·05$.

RESULTS AND DISCUSSION

Effects of DDT analogs on uterine weight, enzyme activities and glycogen content. Administration of *o,p'*-DDT (10 mg/100 g) to ovariectomized rats resulted in marked increases in uterine weight and in the activities of several uterine carboyhydrate-metabolizing enzymes (Table 1). Sixteen hr after injection of *o,p'*-DDT, uterine wet weight increased to 175 per cent, while hexokinase, phosphofructokinase, aldolase and pyruvate kinase activities were elevated to more than 200 per cent of the control values. The activity of G6-PDH was increased to 285 per cent and that of 6-PGDH to 197 per cent in uteri of rats treated with *o,p'*-DDT. Administration of *p,p'*-DDT also resulted in significant increases in the activities of hexokinase, phosphofructokinase, aldolase, pyruvate kinase and G6-PDH, although these were of a much smaller magnitude than those observed with *o,p'*-DDT. Uterine weight and the activity of 6-PGDH remained relatively unaffected by *p,p'*-DDT treatment. The effects of *o,p'*-DDT and *p,p'*-DDT on uterine glycogen content are also shown in Table 1. Whereas no change in uterine glycogen was evinced by the *p,p'*-isomer, *o,p'*-DDT increased glycogen content to 385 per cent of the control value.

Welch *et al.*[4] reported increases in the uterine weights of immature rats treated with purified *o,p'*-DDT as well as technical grade DDT, purified methoxychlor or *p,p'*-DDT.

TABLE 1. ESTROGEN-LIKE EFFECTS OF *p,p'*-DDT AND *o,p'*-DDT ON GLYCOGEN CONTENT AND SEVERAL CARBOHYDRATE-METABOLIZING ENZYMES IN THE UTERUS*

Treatment	Uterine wt. (mg)	Glycogen (μg)†	HK	PFK	Aldolase	PK	G6-PDH	6-PGDH
Control	77 ± 2 (100)	133 ± 27 (100)	3·2 ± 0·2 (100)	7·7 ± 0·3 (100)	9·2 ± 0·4 (100)	190·4 ± 0·3 (100)	6·6 ± 0·1 (100)	2·9 ± 0·1 (100)
p,p'-DDT	88 ± 3 (113)	129 ± 21 (93)	5·3 ± 0·1 (165)‡	9·2 ± 0·3 (119)‡	12·5 ± 0·1 (136)‡	278·6 ± 5·4 (150)‡	9·0 ± 0·1 (136)‡	3·2 ± 0·1 (110)
o,p'-DDT	135 ± 2 (175)‡	513 ± 46 (385)‡	8·6 ± 0·1 (269)‡	18·0 ± 1·5 (233)‡	25·1 ± 2·1 (273)‡	502·8 ± 60·0 (263)‡	18·8 ± 1·3 (285)‡	5·7 ± 0·6 (197)‡

* Each value represents the mean ± S.E. based on 3 determinations of enzyme activities in uteri pooled from 3 rats. Ovariectomized rats were injected with *p,p'*-DDT (10 mg/100 g) or *o,p'*-DDT (10 mg/100 g) i.m., and killed after 16 hr. Data are also given in percentages (in parentheses), taking the values of control rats as 100%. For abbreviations, see text.

† Mean ± S.E. represents four to six glycogen assays in each group.

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

Bitman *et al.*[5] also found that, whereas *p,p'*-DDT produced minimal effects, *o,p'*-DDT was capable of inducing significant increases in oviduct weight and glycogen content in two avian species as well as in uterine glycogen of immature rats. The results of the present study showing enhanced uterine weight and glycogen content in *o,p'*-DDT-treated ovariectomized rats confirm the findings of Bitman *et al.*[5] and support the conclusion that *o,p'*-DDT is the more potent isomer for eliciting uterotrophic effects.

It is well established that administration of estrogenic hormones produces marked increases in the activities of a number of enzymes in the uterus.[18–20] Earlier investigations from this laboratory have demonstrated that estrogens also are capable of stimulating the synthesis *de novo* of several uterine glycolytic and pentose phosphate shunt enzymes.[6–13, 16] Barker and Warren[21] have obtained similar evidence for the regulation of G6-PDH activity by estrogens in this tissue. The presently observed increases in the activities of these enzymes as a consequence of *o,p'*-DDT treatment are reminiscent of the action of estrogenic hormones on the target organ and may constitute a possible basis for further investigations on the estrogenicity of chlorinated hydrocarbon insecticides.

Time-course of o,p'-*DDT-induced changes.* The sequential changes in uterine weight and enzyme activities after a single dose of 10 mg/100 g of *o,p'*-DDT are shown in Fig. 1. Significant increases in uterine weight were observed as early as 4 hr (130%) while maximal increases were obtained 16 hr after administration of the insecticide (175%). Likewise, peak increases in the activities of the four glycolytic enzymes were obtained at 16 hr with hexokinase being elevated to 263 per cent, phosphofructokinase to 233 per cent, aldolase to 273 per cent and pyruvate kinase to 263 per cent of the control values, respectively. The activities of the two shunt enzymes also were increased significantly at 4 hr and rose progressively to 285 and 197 per cent in the case of G6-

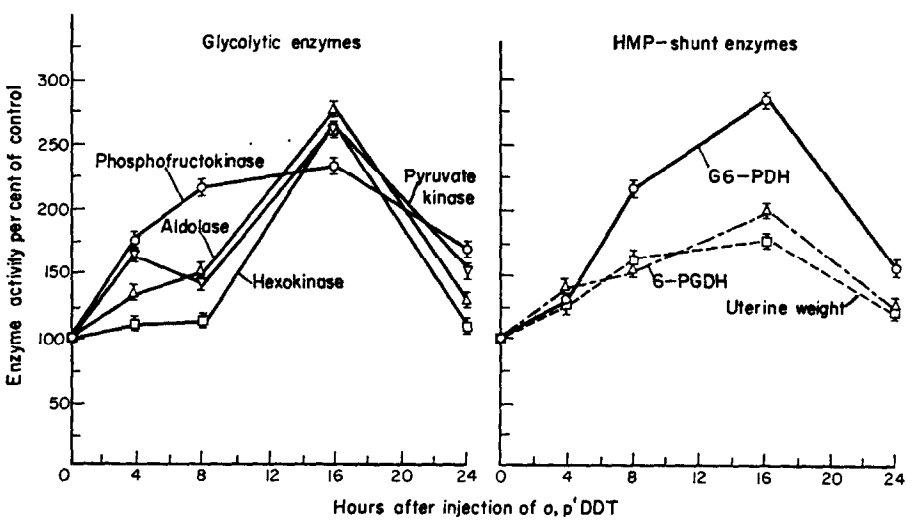

FIG. 1. Time-course of *o,p'*-DDT-induced increases in uterine weight and enzyme activities. Each point represents the mean and S.E. of three values, each obtained by pooling uteri from three rats. *o,p'*-DDT (10 mg/100 g) was administered (i.m.) to groups of ovariectomized rats which were killed 4, 8, 16 and 24 hr after injection of the insecticide. Data are given in percentages, taking the values of control rats as 100%.

PDH and 6-PGDH at 16 hr after *o,p'*-DDT injection. The present results on *o,p'*-DDT-induced stimulation of uterine enzymes resemble closely those obtained for the time-course of estradiol induction of uterine hexokinase, phosphofructokinase, aldolase and pyruvate kinase.[6–13] In all cases, statistically significant increases in these uterine enzymes were evident as early as 4 hr after hormone injection, with maximal increases being achieved at 16 hr.

Influence of actinomycin and cycloheximide. 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.[22–24] In order to investigate the nature of the *o,p'*-DDT-induced enzyme increases, the effects of actinomycin and cycloheximide were examined. The results illustrated in Fig. 2 demonstrate that

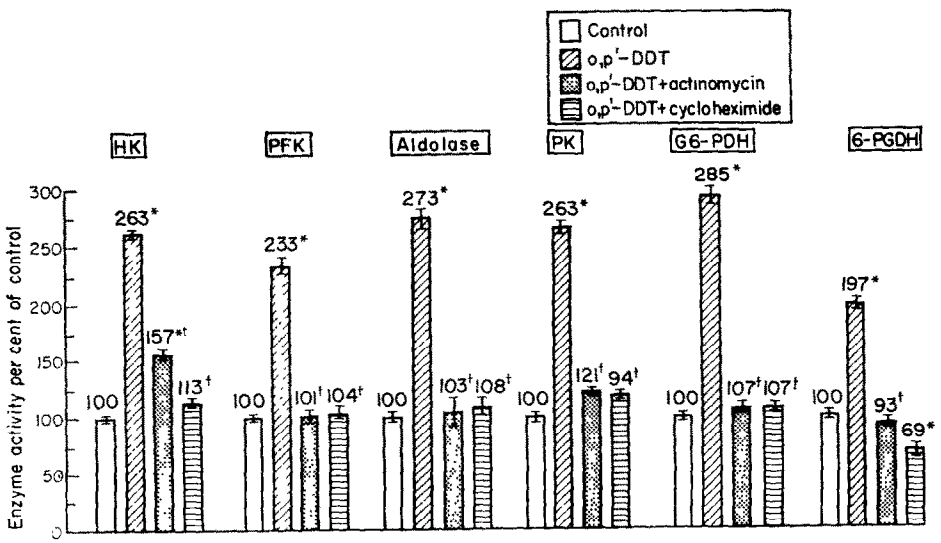

FIG. 2. Effect of actinomycin and cycloheximide on *o,p'*-DDT-induced increases in uterine enzymes. Bars represent the means and S.E. of three determinations of enzyme activity. Each assay was performed using uteri pooled from three rats. Ovariectomized rats were injected with *o,p'*-DDT (10 mg/100 g, i.m.) and killed after 16 hr. Actinomycin (25 μg/100 g) or cycloheximide (70 μg/100 g) was administered (i.p.) 30 min prior to *o,p'*-DDT injection. Data are expressed in percentages, taking the values of control rats as 100%.

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

† Statistically significant difference as compared with the values of *o,p'*-DDT-treated animals without actinomycin or cycloheximide administration ($P < 0.05$).

actinomycin, which is known to bind to DNA and block DNA-directed synthesis of nuclear RNA,[25, 26] significantly inhibited the insecticide-induced enzyme increases in the uterus. Cycloheximide, which blocks protein synthesis by inhibiting either the transfer of aminoacyl transfer-RNA to ribosomes or the formation of peptide bonds,[27, 28] prevented completely the *o,p'*-DDT-induced enzyme increases, suggesting that both new RNA and protein synthesis may be involved in the observed *o,p'*-DDT-stimulation of the uterine enzymes investigated.

Our earlier studies have shown that actinomycin is capable of inhibiting effectively

the estrogen-induced increases in hexokinase, phosphofructokinase, aldolase and pyruvate kinase in uteri of ovariectomized animals.[6–12] The ability of actinomycin to prevent the *o,p'*-DDT-induced increases in enzyme activities suggests that, in analogy to estrogenic hormones, the insecticide-stimulated alterations in uterine enzymes may also involve participation of messenger-RNA synthesis at the gene locus.[6, 7, 29]

Effect of cycloheximide during chronic o,p'*-DDT treatment.* The influence of cycloheximide on uterine enzymes in rats treated with *o,p'*-DDT (5 mg/100 g/day) for 3 days is shown in Fig. 3. Chronic treatment with the insecticide resulted in increases

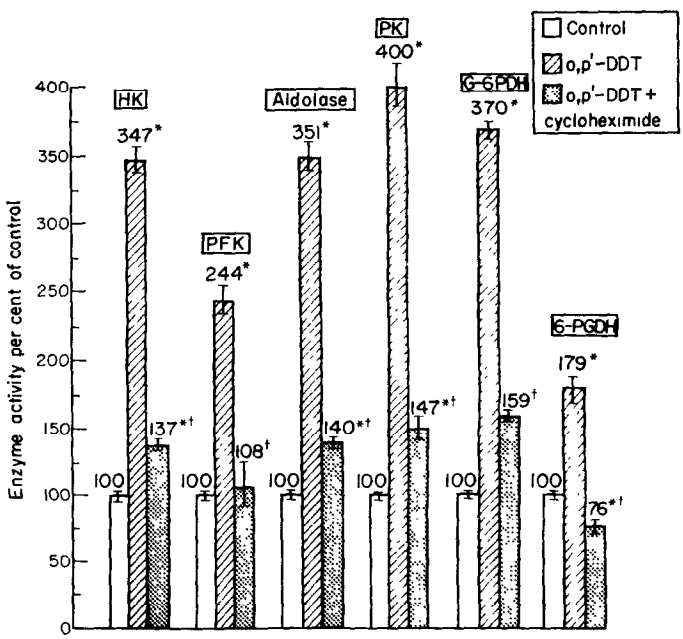

FIG. 3. Influence of cycloheximide administration on uterine enzyme activities in rats treated with *o,p'*-DDT for 3 days. Bars represent the means and S.E. of three determinations of enzyme activity. Each assay was performed using uteri pooled from three rats. Ovariectomized rats were injected with *o,p'*-DDT (5 mg/100 g/day, i.m.) for 3 days, and killed 24 hr after the last injection. Cycloheximide (70 μg/100 g) was administered (i.p.) 30 min prior to the injection of *o,p'*-DDT each day. Data are expressed in percentages, taking the values of control rats 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 *o,p'*-DDT-treated animals without cycloheximide administration ($P < 0.05$).

in the activities of hexokinase, aldolase, pyruvate kinase and G6-PDH that were more pronounced than those observed after a single dose of *o,p'*-DDT (Table 1). Phosphofructokinase and 6-PGDH were increased to virtually the same extent as that seen with a single dose of the pesticide. However, when cycloheximide and the insecticide were injected concurrently for 3 days, the *o,p'*-DDT-stimulated increases in uterine hexokinase, phosphofructokinase, aldolase, pyruvate kinase, G6-PDH and 6-PGDH were significantly inhibited and the values remained close to the normal range. The

present results showing greater increases in uterine enzyme activities after chronic *o,p'*-DDT treatment are in accord with our earlier findings demonstrating the greater effectiveness of estradiol in inducing uterine phosphofructokinase and phosphohexose isomerase when administered for 3 days.[6, 7] The inhibition by cycloheximide of the enzyme increases induced by chronic *o,p'*-DDT treatment resembles the inhibitory action of actinomycin on the increases observed in certain uterine enzymes induced by chronic administration of estradiol.[6, 7] Since cycloheximide is a potent inhibitor of protein synthesis, the observed inhibition by this antibiotic of the increases in uterine enzymes elicited by chronic *o,p'*-DDT treatment lends additional support to the view that the augmented uterine enzyme activities involve enzyme synthesis *de novo*.

Influence of progesterone and estradiol. Since *o,p'*-DDT appeared to mimic the effects of estrogens on uterine enzymes, it was of interest to examine the influence of estradiol-17β on the *o,p'*-DDT-stimulated changes in glycolytic and hexose monophosphate shunt enzymes. Administration of estradiol (0·10 μg/100 g) enhanced uterine weights as well as enzyme activities to about the same extent as did the 10·0 mg/100 g dose of *o,p'*-DDT. However, when estradiol and the insecticide were administered concomitantly, the magnitude of uterine enzyme induction, as well as the increases in tissue wet weight, was greater than with either compound injected alone (Table 2). Since progesterone has been shown previously to inhibit the estradiol induction of several uterine enzymes,[6, 7, 10, 12] we considered it pertinent to examine the effects of progesterone on the changes in uterine enzymes induced by *o,p'*-DDT. Administration of progesterone alone resulted in minor but statistically significant increases in uterine wet weight as well as in phosphofructokinase, aldolase, G6-PDH and 6-PGDH. However, when progesterone was administered 30 min prior to the injection of *o,p'*-DDT, the insecticide-induced stimulation of all uterine enzymes was blocked effectively and the values remained close to those observed in control rats. The ability of progesterone to block the estrogen-like effects of *o,p'*-DDT on uterine weight and enzyme activities suggests that this hormone may compete with the insecticide in a manner similar to that reported previously for estradiol-17β.[6, 7, 10, 12]

In contrast to the antagonistic action of progesterone, it is of interest that concomitant administration of estradiol-17β and *o,p'*-DDT produces somewhat additive effects on uterine weights and enzyme activities. Ui and Mueller[30] demonstrated that estradiol stimulates the incorporation of various precursors into uterine RNA and protein. Similarly, Welch *et al.*[4] have shown that treatment of immature female rats with purified *o,p'*-DDT causes a several-fold stimulation of the incorporation *in vitro* of glucose-^{14}C into uterine lipids, protein and RNA. The results of the present study demonstrate that estradiol-17β and *o,p'*-DDT produce additive effects on uterine weights and enzyme activities and indicate further that the hormone and the insecticide educe similar metabolic responses in uterine tissue.

Studies concerning the effects of organochlorine insecticides on reproductive processes in avian and mammalian species have recently been gaining considerable attention. Interference with reproductive performance and a consequent decline in the population of certain avian species have been attributed to environmental pesticide contamination.[1, 3, 31] According to the estimates of Woodwell *et al.*,[32, 33] over one billion pounds (453×10^6 kg) of DDT exists at present in the biosphere. Since technical grade DDT contains *o,p'*-DDT to the extent of 15–20 per cent, almost 200 million pounds of an active estrogen may be present in the environment.[5] Wurster and

TABLE 2. INFLUENCE OF ESTRADIOL-17β AND PROGESTERONE ON *o,p'*-DDT STIMULATION OF UTERINE ENZYMES*

Treatment	Uterine wt. (mg)	HK	PFK	Aldolase	PK	G6-PDH	6-PGDH
Control	79 ± 2 (100)	3·2 ± 0·2 (100)	7·3 ± 0·2 (100)	11·9 ± 0·2 (100)	165 ± 12 (100)	7·0 ± 0·8 (100)	2·1 ± 0·2 (100)
Estradiol-17β	134 ± 2 (170)†	7·9 ± 0·6 (247)†	21·5 ± 0·6 (294)†	24·2 ± 1·7 (212)†	255 ± 15 (155)†	18·0 ± 0·9 (256)†	4·5 ± 0·3 (214)†
o,p'-DDT	135 ± 2 (171)†	8·6 ± 0·1 (263)†	18·0 ± 1·5 (246)†	25·1 ± 2·1 (212)†	330 ± 15 (200)†	18·8 ± 1·3 (269)†	5·7 ± 0·6 (271)†
Estradiol-17β + *o,p'*-DDT	165 ± 9 (207)‡	9·7 ± 0·8 (303)‡	29·7 ± 1·2 (407)‡	34·9 ± 1·4 (393)‡	347 ± 15 (210)‡	24·2 ± 2·0 (344)‡	6·0 ± 0·5 (286)‡
Progesterone	98 ± 1 (124)†	3·0 ± 0·1 (94)	10·8 ± 1·4 (148)†	17·7 ± 1·8 (149)†	207 ± 16 (125)	10·7 ± 0·5 (152)†	3·5 ± 0·3 (167)†
Progesterone + *o,p'*-DDT	80 ± 2 (101)§	2·2 ± 0·3 (70)§	7·9 ± 0·5 (108)§	15·6 ± 1·5 (131)§	192 ± 3 (116)§	9·3 ± 0·1 (132)§	2·8 ± 0·2 (133)§

* Each value represents the mean ± S.E. based on three to four determinations of enzyme activity in uteri pooled from 3 rats. Ovariectomized rats were injected with *o,p'*-DDT (10 mg/100 g) and sacrificed after 16 hr. Estradiol-17β (0·10 μg/100 g) or progesterone (5 mg/100 g) was also given by the i.m. route 30 min prior to the administration of *o,p'*-DDT. Data are also given in percentages (in parentheses), taking the values of control rats as 100%. For abbreviations, see text.

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

‡ Statistically significant difference when compared with the values of rats injected with estradiol alone ($P < 0·05$).

§ Statistically significant difference when compared with the values of rats receiving *o,p'*-DDT without the administration of progesterone ($P < 0·05$).

Wingate[3] have recently 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[1] 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[2] have 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.[4] Bitman *et al.*[5] and Welch *et al.*[4] have described some estrogen-like properties of *o,p'*-DDT in the rat, chicken and quail. The results of the present investigation confirm their findings and demonstrate that, like estrogens, *o,p'*-DDT is capable of augmenting several glycolytic and hexose monophosphate shunt enzymes in the uterus of the ovariectomized rat. These *o,p'*-DDT-stimulated increases in uterine enzymes can be blocked effectively by actinomycin and cycloheximide, two inhibitors of RNA and protein biosynthesis, as well as by progesterone. The presently observed estradiol-like action of *o,p'*-DDT on several uterine enzymes involved in carbohydrate metabolism suggests the need for closer observation of similar effects that may be exerted by other insecticides.

REFERENCES

1. D. A. RATCLIFFE, *Nature, Lond.* **215**, 208 (1967).
2. H. BURLINGTON and V. F. LINDEMAN, *Proc. Soc. exp. Biol. Med.* **74**, 48 (1950).
3. C. F. WURSTER JR. and D. B. WINGATE, *Science, N.Y.* **150**, 979 (1968).
4. R. M. WELCH, W. LEVIN and A. H. CONNEY, *Toxic. appl. Pharmac.* **14**, 358 (1969).
5. J. BITMAN, H. C. CECIL, S. J. HARRIS and G. F. FRIES, *Science, N.Y.* **162**, 371 (1968).
6. R. L. SINGHAL, J. R. E. VALADARES and G. M. LING, *J. biol. Chem.* **242**, 2593 (1967).
7. R. L. SINGHAL and J. R. E. VALADARES, *Biochem. Pharmac.* **17**, 1251 (1968).
8. R. L. SINGHAL and J. R. E. VALADARES, *Proc. Fourth Int. Congr. Pharmac.* **4**, 196 (1969).
9. R. L. SINGHAL, J. R. E. VALADARES and G. M. LING, *Am. J. Physiol.* **217**, 793 (1969).
10. R. L. SINGHAL and J. R. E. VALADARES, *Am. J. Physiol.* **218**, 321, (1970)
11. J. R. E. VALADARES, R. L. SINGHAL and M. R. PARULEKAR, *Science, N.Y.* **159**, 990 (1968).
12. W. S. SCHWARK, R. L. SINGHAL and G. M. LING, *Biochim. biophys. Acta* **192**, 106 (1969).
13. R. VIJAYVARGIYA, W. S. SCHWARK and R. L. SINGHAL, *Can. J. Biochem.* **47**, 895 (1969).
14. G. E. GLOCK and P. MCLEAN, *Biochem. J.* **55**, 400 (1953).
15. R. L. SINGHAL and G. M. LING, *Can. J. Physiol. Pharmac.* **47**, 233 (1969).
16. R. L. SINGHAL, J. R. E. VALADARES and W. S. SCHWARK, *J. Pharm. Pharmac.* **21**, 194 (1969).
17. S. SEIFTER, S. C. DAYTON, B. NOVIC and E. MUNTWYLER, *Archs. Biochem.* **25**, 191 (1950).
18. A. HERRANEN and G. C. MUELLER, *Biochim. biophys. Acta* **24**, 223 (1957).
19. D. J. MCCORQUODALE and G. C. MUELLER, *J. biol. Chem.* **232**, 31 (1958).
20. B. ECKSTEIN and C. A. VILLEE, *Endocrinology* **78**, 409 (1966).
21. K. L. BARKER and J. C. WARREN, *Endocrinology* **78**, 1205 (1966).
22. H. G. WILLIAMS-ASHMAN, *Cancer Res.* **25**, 1096 (1965).
23. D. W. NEBERT and H. V. GELBOIN, in *Microsomes and Drug Oxidations*, p. 389. Academic Press, New York (1969).
24. R. L. SINGHAL and J. R. E. VALADARES, *Biochem. J.* **110**, 703 (1968).
25. E. REICH, R. M. FRANKLIN, A. J. SHATKIN and E. L. TATUM, *Science, N.Y.* **134**, 556 (1961).
26. T. TAMAOKI and G. C. MUELLER, *Biochem. biophys. Res. Commun.* **9**, 451 (1962).

27. H. L. Ennis and M. Lubin, *Fedn Proc.* **23**, 269 (1964).
28. M. R. Siegel and H. D. Sisler, *Nature, Lond.* **200**, 675 (1964).
29. M. A. Lea, R. L. Singhal and J. R. E. Valadares, *Biochem. Pharmac.*, **19**, 113 (1970)
30. H. Ui and G. C. Mueller, *Proc. natn. Acad. Sci. U.S.A.* **50**, 256 (1963).
31. R. D. Porter and S. N. Wiemeyer, *Science, N.Y.* **165**, 199 (1969).
32. G. M. Woodwell, *Scient. Am.* **216**, 24 (1967).
33. G. M. Woodwell, C. F. Wurster, Jr. and P. A. Isaacson, *Science, N.Y.* **156**, 821 (1967).