

Pharmacological Adrenalectomy with Mitotane

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The potential of mitotane (ortho, para'-DDD, commonly used to treat adrenal carcinomas in humans and dogs) was investigated as an alternative to surgical adrenalectomy in birds, salamanders, and lizards. House sparrows (*Passer domesticus*) were injected twice daily with vehicle or one of two doses of mitotane (225 or 450 mg/kg), and basal and stress-induced levels of corticosterone (CORT) were measured 3 and 5 days after injections. Mitotane reduced basal CORT levels to nondetectable and abolished stress-induced CORT increases by the 3rd day of treatment. In another study, a single injection of mitotane was effective in lowering endogenous CORT levels 36 h later, but levels had apparently recovered by 10 days after the injection. Mitotane did not effect testicular weights and had no detectable effect on testosterone levels. In contrast to its effects on house sparrows, mitotane did not lower endogenous CORT levels in either tiger salamanders (*Ambystoma tigrinum*) or tree lizards (*Urosaurus ornatus*), even at doses much higher than those used in house sparrows. © 2000 Academic Press

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In experimental endocrinology, it is often desirable to decrease the production of endogenous glucocorticoids. In many mammals this can be achieved by adrenalectomy. In most nonmammalian vertebrates, the adrenocortical tissue is partially embedded in the cardinal vein or the kidney and so is surgically inaccessible. As an alternative to surgical adrenalectomy, a number of chemical inhibitors of steroid production are currently used in clinical contexts, particularly in the treatment of steroid-producing cancers. Chemical

inhibitors frequently work by interfering with the enzymes involved in the production of steroid hormones. Most inhibitors (e.g., aminoglutethimide, ketoconazole) act on the early steps of steroid synthesis and result in decreased levels of a wide range of steroids, including glucocorticoids (Bossche, 1992). Metyrapone, which inhibits the transformation of 11-deoxy-corticosterone to corticosterone (CORT) and can block side-chain cleavage at higher doses (Roosendaal *et al.*, 1996), has been widely used to decrease glucocorticoid production. However, the drug has several drawbacks, including increased mortality or morbidity (C. Schreck and J. C. Wingfield, personal communication; M. C. Moore and R. Knapp, unpublished data) and a variable success rate in different species (Gray *et al.*, 1990; Savory and Mann, 1997; Scholnick *et al.*, 1997); also, it is no longer commercially available. In this paper, we explore the use of mitotane (ortho, para, dichlorodiphenyl dichloroethane; o, p'-DDD) for pharmacological adrenalectomy.

Mitotane is used to treat adrenal carcinomas in humans and dogs (i.e., Feldman *et al.*, 1992; Kintzer and Peterson, 1994; Wooten and King, 1993). Studies in mammals and birds (chickens) indicate that mitotane is metabolically activated by an adrenal-specific cytochrome P450 (Jonsson *et al.*, 1994; Martz and Straw, 1977); once in its activated form, it then blocks cytochrome P450-mediated reactions (Maher *et al.*, 1992; Martz and Straw, 1977), resulting in a reduction of glucocorticoid production and causing selective necrosis of adrenocortical tissue. Mitotane has been used in rats, guinea pigs, cows, and dogs in studies of

clinical treatment of adrenal cancer (Cai *et al.*, 1995; Glicksman *et al.*, 1982; Schteingart *et al.*, 1993; Watson, 1992). To our knowledge, mitotane has only been used once as an experimental tool in basic research: Akinci and Johnston (1993) tested the effects of acute swim stress on GABA(A) receptor binding in the forebrain of mitotane-treated mice.

We investigated the effect of mitotane on corticosterone levels in species representing three classes of nonmammalian vertebrates: house sparrows (*Passer domesticus*), tiger salamanders (*Ambystoma tigrinum*), and tree lizards (*Urosaurus ornatus*). In sparrows, we addressed whether a single injection or repeated injections of mitotane could significantly decrease CORT levels. Given that mitotane interrupts biosynthetic pathways in the adrenal, it may also effect other steroidogenic pathways. To address this, we measured testosterone (T) levels and testicular mass in male house sparrows after control or mitotane treatment. We expected that mitotane would reduce CORT production in all three species, producing a tool that would be useful in a wide variety of studies, including glucocorticoid receptor pharmacology, stress physiology, and behavioral experiments conducted with captive and free-living animals. However, we discovered that mitotane was effective only in house sparrows and was not effective in either tiger salamanders or tree lizards.

METHODS

House Sparrows

House Sparrows were mist-netted at a local feed lot in Chandler, Arizona (March, 1999). A baseline blood sample was collected from the wing vein within 3 min of capture. Sparrows were then held in cloth bags for 30 min, when a second "stressed" blood sample was collected. In the laboratory, birds were housed in an environmental chamber (2.2 × 2.4 × 2.0 m; 12L:12D h, to mimic natural photoperiod; 25°), with six birds per cage (50 × 50 × 88 cm). Animals were given commercial wild bird seed mix and water *ad libitum*.

Mitotane does not significantly affect aldosterone levels in dogs or humans (Kaminsky *et al.*, 1962; Knappe *et al.*, 1997; Plumb, 1991), but reduces aldoste-

rone production in porcine adrenal cell culture (Jager *et al.*, 1996). To be conservative, we put 0.75% NaCl into the drinking water of the mitotane-injected sparrows. For long-term and field studies, we recommend that the effect of mitotane on aldosterone secretion be determined.

Repeated injections. Sparrows were given one of three injections (into the pectoral muscle): control (100 μ l peanut oil), low mitotane (225 mg/kg sparrow = 100 μ l of 45 mg/ml mitotane in peanut oil), or high mitotane (450 mg/kg sparrow = 100 μ l 90 mg/ml mitotane in peanut oil). One hundred microliters is a frequently used injection volume for this size bird (Maney *et al.*, 1997). There were six birds in each group: four males and two females. Injections were given on the first evening of captivity and then twice a day (morning and evening) until the experiment was over. Basal and stressed blood samples were collected between 8:00 and 10:00 a.m. on days 3 and 5 of the experiment (day 1 = day of capture; see Fig. 1A). Blood samples were centrifuged at 1150g for 5 min and plasma was collected using a Hamilton syringe. Plasma samples were stored at -20° until assayed.

Single injection. To ascertain both how quickly one injection can lower blood levels of CORT and whether mitotane's effects are permanent, six house sparrows (three male, three female) were captured in the Tempe/Chandler area in January, 2000. These sparrows were subjected to the protocol described above, except that they were given only one injection of mitotane (100 μ l of 90 mg/ml in peanut oil) on the first evening of captivity. Basal and stress-induced blood samples were collected on days 1, 3, 5, and 10. Six control sparrows (three male, three female) were captured at the same time, with basal and stress-induced blood samples collected on days 1 and 10.

Testicular mass and testosterone level. Clinical studies suggest that chronic mitotane treatment can reduce testicular mass in human patients (Sparagana, 1987). In sparrows, we investigated the effect of mitotane treatment on both testicular mass and testosterone levels. We measured testicular mass in both control ($n = 8$) and mitotane-treated ($n = 8$) birds caught during the breeding season, 1999. We measured T levels in the same birds on day 1 (sample taken within 30 min of capture) and days 3 or 5 after capture. In control birds ($n = 4$), we compared day 1 and day 5 T levels within individuals. In the mitotane-treated

birds ($n = 4$, pooled from eight original samples to increase plasma volume for radioimmunoassay), we compared day 1 with plasma samples pooled from days 3 and 5. This analysis did not allow for direct comparisons between treatment groups, but determined whether captivity and/or mitotane treatment affected T levels within each group.

Tiger Salamanders

Juvenile tiger salamanders were purchased from a local bait shop. Animals were housed communally in circular tanks (132 cm diameter \times 60 cm deep) at ambient room temperature ($\sim 25^\circ$) until used in the experiment. Before the experiment began, animals were moved to small aquaria (26 \times 50 \times 31 cm; also at room temperature), with three or four animals per tank, and allowed to acclimate to the new housing for 3 or 4 days. Mitotane injections (four doses) were given i.p. once a day for 1–4 days (see Table 1). With each negative result in the salamanders, we increased the dose of mitotane and the duration of treatment in the next experiment. At the end of each experiment, animals were rapidly decapitated and trunk blood was collected. Blood samples were centrifuged at 1150g for 10 min and plasma was collected using a Hamilton syringe. Samples were stored at -20° until assayed. Juvenile salamanders were used in the first three experiments and adult salamanders in the last two experiments to ensure that we were not missing an effect of mitotane because of a change in physiology either before or after metamorphosis.

Tree Lizards

Adult male *Urosaurus ornatus* were collected in the Tonto National Forest within a 5-km radius of the intersection of Sugarloaf Mountain–Sycamore Creek Road with Arizona State Highway 87. Animals were collected in May and August, 1999. Animals were housed in wire cages (37 \times 42 \times 45 cm) on a 14L:10D h photoperiod at 26° . A 25-W heat lamp suspended above each cage allowed animals to behaviorally thermoregulate. Animals were housed two per cage and given food (crickets) and water *ad libitum*. Mitotane injections were given i.p. once a day for either 3 or 5 days (Table 1). All animals were of average body condition, based on visual inspection during dissec-

tion. Trunk blood was collected following decapitation; all samples were collected within 5 min of capture and blood was kept on ice until processing. Blood samples were centrifuged at 1150g for 5 min and plasma was removed using a Hamilton syringe. Samples were stored at -20° until assayed.

Mitotane

Mitotane, purchased in crystalline form from Sigma (St. Louis, MO) (catalog No. C3010; purity = 99.6%, as provided by manufacturer), was dissolved in peanut oil (by 10–20 min of sonication) on the day of the first injection. We stored the mitotane solution at $0-4^\circ$ between injections and made up fresh solution every 3–4 days (as recommended by Sigma).

To estimate the timing and concentration of the mitotane dose for nonmammalian vertebrates, we used an equation developed for use in veterinary medicine which incorporates allometric scaling into dose rate and dose frequency calculations (Sedgwick, 1993). This equation uses a control species for which the correct dosage is known and estimates dosage for a different species, taking into consideration average weight of both control and new species and differences in metabolic rates in animals in discrete groups (placental mammals, marsupial mammals, passerine birds, nonpasserine birds, and reptiles).

Steroid Hormone Assays

Radiolabeled steroids [$1,2,6,7,16,17-^3\text{H}$] testosterone ($[^3\text{H}]\text{T}$; specific activity = 116–121 Ci/mmol) and [$1,2,6,7-^3\text{H}$] corticosterone ($[^3\text{H}]\text{CORT}$; specific activity = 88 Ci/mmol) were purchased from New England Nuclear (Boston, MA).

Plasma corticosterone levels were determined following the methods of Wingfield *et al.* (1992). Briefly, samples were allowed to equilibrate overnight with 2000 cpm of corticosterone for determination of individual recoveries. Each sample was extracted with 4.0 ml of dichloromethane, dried under nitrogen, and re-suspended in phosphate-buffered saline with 1% gelatin. Samples were assayed in duplicate, and assay values were corrected for plasma volume and individual recoveries following extraction (standard curve range (2000–1.25 pg), detectability (1.2 pg/tube), accuracy (87%)). Intraassay coefficients of variation were

1.0 and 2.3% (for two assays on sparrow plasma) and 7.9 and 14.5% (for two assays including lizard and salamander plasma). Interassay coefficient of variation was 9.2% (sparrow) and 10.6% (lizard and salamander).

Plasma testosterone levels were determined by radioimmunoassay following chromatographic separation (Moore *et al.*, 1991). Briefly, plasma samples were allowed to equilibrate overnight with 2000 cpm of radioactive testosterone for determination of individual recoveries. Each sample was then extracted twice with 2.0 ml diethyl ether, dried under nitrogen, and resuspended in 10% ethyl acetate in isooctane. Testosterone was separated from dihydrotestosterone and from neutral lipids using celite minicolumns (3 g celite:1 ml ethylene glycol:propanediol mixture (1:1) over 3 g celite:1 ml water). Testosterone was collected after stepwise elution using increasing concentrations of ethyl acetate in isooctane. The testosterone fraction was dried under nitrogen, resuspended in assay buffer (phosphate-buffered saline containing 1% gelatin), and assayed in duplicate. Assay values were corrected for plasma volume and individual recoveries following chromatography (standard curve range (500–0.3 pg), detectability (0.3 pg/tube), accuracy (96%)). Intraassay coefficient of variation was 17.3%.

Statistical Analysis

For statistical purposes, nondetectable hormone samples were set to the limit of detectability of the assay (converted to ng/ml). Comparison of plasma hormone levels in sparrows were made using repeated-measures ANOVA. Significant differences between groups were identified with Tukey's HSD posthoc analysis. Unpaired *t* tests were used to detect a treatment effect of mitotane in sparrow testicular mass and hormone data from lizards and salamanders.

RESULTS

Repeated Injections

Mitotane injections decreased CORT levels in sparrows within 36 h (Fig. 1). Both basal and stress-induced CORT levels were below detectable limits by

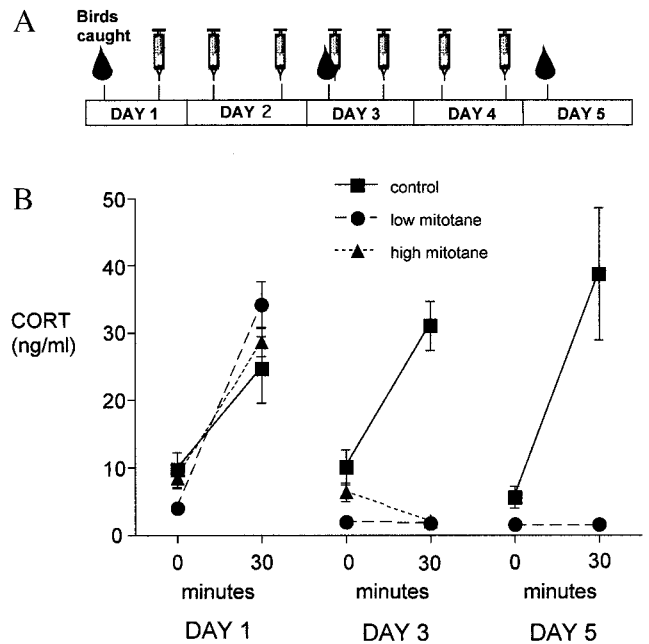


FIG. 1. Mitotane decreases endogenous CORT levels in house sparrows. (A) Schedule of injections (shown by needles) and bleeds (droplets) for this experiment. (B) Level of circulating CORT at first capture (day 1—collected in the field) and on days 3 and 5 (collected in the lab) of the injection protocol. The 0 sample represents baseline levels of CORT. CORT levels are significantly reduced by day 3 in both low- and high-mitotane treatments (repeated-measures ANOVA: significant interaction of treatment, day, and time (min); $P < 0.0001$, $F = 29.97$, Tukey's HSD post hoc analysis).

day 3 in the low-mitotane treatment group. In the high-mitotane treatment group, basal levels of CORT were above detectable limits and not significantly different from those of controls, but stress-induced levels were undetectable. Blood samples were not taken from the high-mitotane group on day 5 because the animals showed symptoms of distress on day 3 (feathers ruffled, immobile). Low-mitotane-treated sparrows showed similar behavior starting on day 5. These responses are consistent with those displayed by adrenalectomized rats, which often become sick or die when stressed, unless they can increase blood glucose levels through food intake (Bruce McEwen, personal communication). In four subsequent experiments (60 sparrows total) we supplemented their diet with fresh apple. In these experiments, mitotane injections reduced CORT to undetectable levels, and morbidity rate was reduced from 50% (without apple) to 5% (with apple).

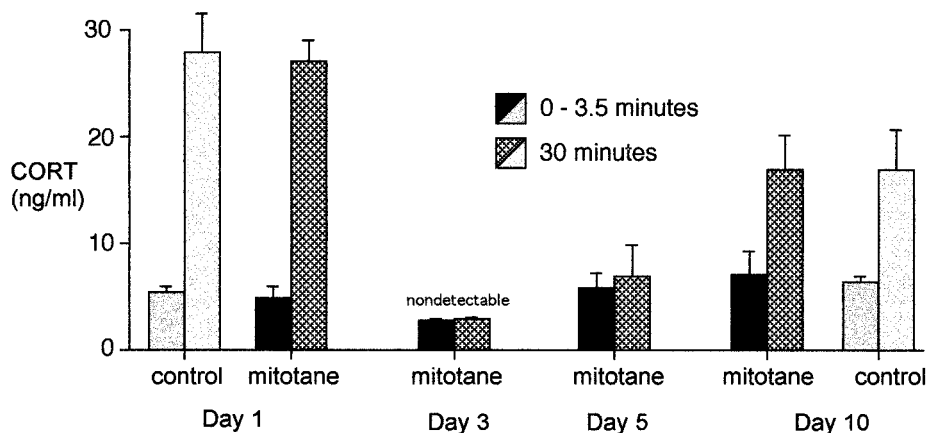


FIG. 2. One injection of mitotane (100 μ l of 90 mg/ml) decreases basal and stress-induced CORT levels to below detectable levels within 36 h of injection. Day 1 samples were collected in the field at first capture; days 3, 5, and 10 were collected from the same birds in captivity. By day 5, baseline levels returned to control levels, but there was no stress response. By day 10, adrenal function apparently recovered.

One Injection

One injection of mitotane decreased CORT to nondetectable levels by the 3rd day of the experiment (36 h after the injection). By day 5 CORT levels were once again detectable, although there was no adrenocortical response to stress. By the 10th day following the single injection, basal and stress-induced levels of CORT were near control levels (Fig. 2). On day 10, only two of the six mitotane-treated sparrows are included in the basal CORT data; it took longer to get basal CORT levels on those days, and only two of the six birds had samples taken within 3.5 min. Basal samples from all six control birds were obtained within 3.5 min of entering the chamber.

Testicular Mass and Testosterone Level

Three days of mitotane treatment had no detectable effect on either testicular mass or testosterone level. Testicular mass in control animals (0.391 ± 0.08 g; $n = 8$) was not significantly different from testicular mass in mitotane-treated animals (0.427 ± 0.076 g; $n = 8$; t test: $P = 0.75$, $t = -0.322$). T levels decreased from 1.13 ± 0.27 ng/ml on the 1st day of capture to below detectable limits by the end of the experiment in both control and mitotane groups (data not shown).

Tiger Salamanders and Tree Lizards

Mitotane did not reduce CORT levels in tiger salamanders or tree lizards (Table 1). Trials were run

using 100-fold differences in mitotane concentration. In one case, mitotane injection (2500 μ g/g body weight) increased CORT levels significantly (t test: $P < 0.001$, $t = 6.415$, $df = 19$).

DISCUSSION

The general goal of this study was to develop a method of pharmacological adrenalectomy for animals in which surgical adrenalectomy is difficult or impossible. In our experiments, mitotane injection reduced CORT production in house sparrows. Repeated injections of mitotane reduced CORT to below detectable levels within 36 h of the first injection; CORT levels did not increase while injections continued. A single injection of mitotane also lowered CORT levels below detectability within 36 h, but CORT secretion slowly recovered over the subsequent 8 days. While mitotane treatment eliminated CORT production in sparrows, it was ineffective in tiger salamanders or tree lizards.

We examined testosterone levels and testicular weight in sparrows to determine whether mitotane affected components of the hypothalamic-pituitary-gonadal axis. There is evidence that chronic use of mitotane decreased testicular size in a human patient (Sparagana, 1987). This may be because mitotane is a metabolite of DDT, which shows estrogenic activity. Over the past 5 years, environmental toxicologists

TABLE 1

Mitotane Treatment Does Not Lower Endogenous CORT Levels in Tiger Salamanders (*Ambystoma tigrinum*) or Tree Lizards (*Urosaurus ornatus*)

Animal	μg Mitotane/g body weight ^a / injection ($\mu\text{g}/\text{g}$)	Days of injection	N ^b	Basal CORT after treatment (ng/ml) ^c		Stress-induced CORT (ng/ml)	
				Control	Mitotane	Control	Mitotane
<i>A. tigrinum</i>	6.9	1	4	0.61 \pm 0.2	0.45 \pm 0.2		
<i>A. tigrinum</i>	6.9	2	4	0.79 \pm 0.3	0.74 \pm 0.3		
<i>A. tigrinum</i>	20.6	3	6	1.27 \pm 0.3	0.99 \pm 0.1		
<i>A. tigrinum</i> (adults)	41.2	4	5	1.54 \pm 0.5	1.52 \pm 0.4		
<i>A. tigrinum</i> (adults)	150.0	4	3, 4	1.11 \pm 0.1	3.12 \pm 1.2	20.67 \pm 7.7	18.13 \pm 3.3
<i>U. ornatus</i>	25	3	14, 18	18.49 \pm 3.9	18.12 \pm 3.3		
<i>U. ornatus</i> *	2500	5	9, 12	22.19 \pm 5.6	73.50 \pm 5.5		

^a Body weight was averaged within each group to obtain this measure (variation in body weight between individuals was minimal).

^b Numbers of control and experimental animals are the same unless two numbers are noted, in which case the first represents control animals and the second represents experimental animals.

^c *t* Tests run on each experiment show no significant difference due to treatment except in the last case, wherein mitotane treatment significantly increased CORT level ($*P < 0.001$, $t = 6.415$, $df = 19$).

have investigated the steroidal effects of mitotane (and other derivatives of DDT) in a number of systems. Although the reports are contradictory, mitotane appears not to have estrogenic properties (Chen *et al.*, 1997; Johnson *et al.*, 1992; Kramer and Giesy, 1999; for exception, see Vonier *et al.*, 1996) but, in some systems, may act as an androgen and/or progesterone receptor antagonist, although only at very high (μM) concentrations (Gaido *et al.*, 1997; Klotz *et al.*, 1997; Maness *et al.*, 1998). In our experiments, mitotane did not effect testicular weight in sparrows. Testosterone levels decreased to undetectable levels in both control and mitotane-treated birds during the 5 days of captivity, making it difficult to determine whether mitotane also decreased T levels. The decrease in T is possibly due to the lack of environmental cues present in the laboratory (discussed in more detail in Breuner *et al.*, 1999). However, if mitotane acted as an androgen receptor antagonist (as suggested by Maness, 1998), T levels would be expected to increase in response to treatment; this did not occur.

The ability to eliminate CORT production in sparrows opens up a variety of experimental possibilities in both the lab and the field. For example, in the laboratory mitotane is currently used in conjunction with glucocorticoid receptor pharmacology/radioligand binding experiments. Endogenous CORT can significantly decrease estimations of total intracellular receptor number; baseline CORT levels can result in

almost nondetectable levels of the high-affinity receptors. By reducing CORT to nondetectable levels, mitotane permits the characterization of the high-affinity glucocorticoid receptors in the avian brain. Mitotane could also be used to control CORT levels more precisely in behavioral experiments. For example, differences in basal and stress-induced CORT levels in individuals can introduce unwanted variation; a combination of mitotane injections with silastic implants of CORT could minimize individual variation in CORT levels. Mitotane is also a potential tool for short-term field studies of behavioral endocrinology. A single injection of mitotane would reduce CORT levels over the subsequent 5 days, allowing observations of behavioral responses in the absence of stress-induced CORT. As it appears that both basal and stress-induced adrenal activity recover within 9 days of injection, recovery of the behavior of interest could also be tested. In field studies, glucose and saline cannot be easily supplemented, as they were in the lab studies. Although it would need to be empirically determined, it is possible that free-living birds can adjust their diet to meet the greater need for glucose and/or salt if the need exists. Before extensive use in the field, mitotane's effects on other steroids, including testosterone and aldosterone, should be tested in free-living animals. Whether it is used in lab or field studies, the effective dose would need to be determined for each new species.

Mitotane did not reduce CORT levels in either tiger salamanders or tree lizards, which may reflect differences in adrenal pharmacology. Mitotane is thought to be activated by an adrenal-specific cytochrome P450-dependent mechanism (Jonsson *et al.*, 1994); once activated, it then blocks cytochrome P450-mediated reactions (Maher *et al.*, 1992). We suspect, therefore, that salamanders and lizards have alternate forms of cytochrome P450 that either cannot metabolically activate mitotane or are not sensitive to mitotane once it is activated. It would be interesting to know whether mitotane is ineffective across ectothermic vertebrates or retains action in specific groups.

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REFERENCES

- Akinci, M. K., and Johnston, G. (1993). Sex-differences in the effects of acute swim stress on GABA(A) receptor-binding in mitotane treated and or gonadectomized mouse forebrain. *J. Neurochem.* **61**, S133-S133.
- Bossche, H. V. (1992). Inhibitors of P450-dependent steroid biosynthesis: From research to medical treatment. *J. Steroid Biochem. Mol. Biol.* **43**, 1003-1021.
- Breuner, C. W., Wingfield, J. C., and Romero, L. M. (1999). Diel rhythms of basal and stress-induced corticosterone in a wild, seasonal vertebrate, Gambel's white-crowned sparrow. *J. Exp. Zool.* **284**, 334-342.
- Cai, W., Counsell, R. E., Djanegara, T., Schteingart, D. E., Sinsheimer, J. E., and Wotring, L. L. (1995). Metabolic-activation and binding of mitotane in adrenal-cortex homogenates. *J. Pharm. Sci.* **84**, 134-138.
- Chen, C. W., Hurd, C., Vorojeikina, D. P., Arnold, S. F., and Notides, A. C. (1997). Transcriptional activation of the human estrogen receptor by DDT isomers and metabolites in yeast and MCF-7 cells. *Biochem. Pharmacol.* **53**, 1161-1172.
- Feldman, E. C., Nelson, R. W., Feldman, M. S., and Farver, T. B. (1992). Comparison of mitotane treatment for adrenal tumor versus pituitary-dependent hyperadrenocorticism in dogs. *J. Am. Vet. Med. Assoc.* **200**, 1642-1647.
- Gaido, K. W., Leonard, L. S., Lovell, S., Gould, J., Baba, Y. D., Portier, C. J., and McDonnell, D. P. (1997). Evaluation of chemicals with endocrine modulating activity in a yeast-based steroid hormone receptor gene transcription assay. *Toxicol. Appl. Pharmacol.* **143**, 205-212.
- Glicksman, A. S., Bliven, S. F., and Leith, J. T. (1982). Modification of radiation damage in rat spinal cord by mitotane. *Cancer Treat. Rep.* **66**, 1545-1547.
- Gray, J. M., Yarian, D., and Ramenofsky, M. (1990). Corticosterone, foraging behavior, and metabolism in dark-eyed juncos, *Junco hyemalis*. *Gen. Comp. Endocrinol.* **79**, 375-384.
- Jager, L. P., De Graaf, G. J., and Widjaja-Greefkes, H. C. A. (1996). Screening for drug-induced alterations in the production and release of steroid hormones by porcine adrenocortical cells *In Vitro*. *Toxicol. In Vitro* **10**, 595-608.
- Johnson, D. C., Sen, M., and Dey, S. K. (1992). Differential effects of dichlorodiphenyltrichloroethane analogs, chlordecone, and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on establishment of pregnancy in the hypophysectomized rat. *Proc. Soc. Exp. Biol. Med.* **199**, 42-48.
- Jonsson, C. J., Lund, B. O., Brunstrom, B., and Brandt, I. (1994). Toxicity and irreversible binding of 2 DDT metabolites 2-methylsulfonyl-DDE and *O,P'*-DDD in adrenal interrenal cells in birds. *Environ. Toxicol. Chem.* **13**, 1303-1310.
- Kaminsky, N., Luse, S., and Hartroft, P. (1962). Ultrastructure of adrenal cortex of the dog during treatment with *o,p'*-DDD. *J. Natl. Cancer Inst.* **29**, 127-159.
- Kintzer, P. P., and Peterson, M. E. (1994). Mitotane treatment of 32 dogs with cortisol-secreting adrenocortical neoplasms. *J. Am. Vet. Med. Assoc.* **205**, 54-61.
- Klotz, D. M., Ladlie, B. L., Vonier, P. M., McLacklan, J. A., and Arnold, S. F. (1997). *o,p'*-DDT and its metabolites inhibit progesterone-dependent responses in yeast and human cells. *Mol. Cell. Endocrinol.* **129**, 63-71.
- Knappe, G., Gerl, H., Ventz, M., and Rohde, W. (1997). Long-term treatment of hypothalamic-pituitary Cushing's syndrome with mitotane (*o,p'*-DDD). *Deutsche Med. Wochenschrift* **122**, 882-886.
- Kramer, V. J., and Giesy, J. P. (1999). Specific binding of hydroxylated polychlorinated biphenyl metabolites and other substances to bovine calf uterine estrogen receptor: Structure-binding relationships. *Sci. Total Environ.* **233**, 141-161.
- Maher, V. M. G., Trainer, P. J., Scoppola, A., Anderson, J. V., Thompson, G. R., and Besser, G. M. (1992). Possible mechanism and treatment of *O,P'*-DDD-induced hypercholesterolemia. *Q. J. Med.* **84**, 671-679.
- Maness, S. C., McDonnell, D. P., and Gaido, K. W. (1998). Inhibition of androgen receptor-dependent transcriptional activity by DDT isomers and methoxychlor in HepG2 human hepatoma cells. *Toxicol. Appl. Pharmacol.* **151**, 135-142.
- Maney, D. L., Richardson, R. D., and Wingfield, J. C. (1997). Central administration of chicken gonadotropin-releasing hormone-II enhances courtship behavior in a female sparrow. *Horm. Behav.* **32**, 11-18.
- Martz, F., and Straw, J. A. (1977). The *In Vitro* metabolism of 1-(*o*-chlorophenyl)-1-(*p*-chlorophenyl)-2,2-dichloroethane (*o,p'*-DDD) by dog adrenal mitochondria and metabolite covalent

- binding to mitochondrial macromolecules. *Drug. Metab. Dispos.* **5**, 482–486.
- Moore, M. C., Thompson, C. W., and Marler, C. A. (1991). Reciprocal changes in corticosterone and testosterone levels following acute and chronic handling stress in tree lizards, *Urosaurus ornatus*. *Gen. Comp. Endocrinol.* **81**, 217–226.
- Plumb, D. C. (1991). "Veterinary Drug Handbook." Pharmavet Publishing, White Bear Lake, MN.
- Rooszendaal, B., Bohus, B., and McGaugh, J. L. (1996). Dose-dependent suppression of adrenocortical activity with metyrapone: Effects on emotion and memory. *Psychoneuroendocrinology* **21**, 681–693.
- Savory, C. J., and Mann, J. S. (1997). Is there a role for corticosterone in expression of abnormal behaviour in restricted-fed fowls? *Physiol. Behav.* **62**, 7–13.
- Scholnick, D. A., Weinstein, R. B., and Gleeson, T. T. (1997). The influence of corticosterone and glucagon on metabolic recovery from exhaustive exercise in the desert iguana *Dipsosaurus dorsalis*. *Gen. Comp. Endocrinol.* **106**, 147–154.
- Schteingart, D. E., Sinsheimer, J. E., Counsell, R. E., Abrams, G. D., McClellan, N., Djanegara, T., Hines, J., Ruangwises, N., Benitez, R., and Wotring, L. L. (1993). Comparison of the adrenalytic activity of mitotane and a methylated homolog on normal adrenal cortex and adrenal cortical carcinoma. *Cancer Chemoth. Pharmacol.* **31**, 459–466.
- Sedgwick, C. J. (1993). Allometric scaling and emergency care: The importance of body size. In "Zoo and Wild Animal Medicine: Current Therapy" (M. Fowler, Ed.), Vol. 3, pp. 34–37. Saunders, Philadelphia.
- Sparagana, M. (1987). Primary hypogonadism associated with *o,p'*-DDD (mitotane) therapy. *Clin. Toxicol.* **25**, 463–472.
- Vonier, P. M., Crain, D. A., McLachlan, J. A., Guillette, L. J., and Arnold, S. F. (1996). Interaction of environmental chemicals with the estrogen and progesterone receptors from the oviduct of the American alligator. *Environ. Health Perspect.* **104**, 1318–1322.
- Watson, A. D. J. (1992). Bioavailability and bioequivalence of drug formulations in small animals. *J. Vet. Pharmacol. Therap.* **15**, 151–159.
- Wingfield, J. C., Vleck, C. M., and Moore, M. C. (1992). Seasonal changes of the adrenocortical response to stress in birds of the sonoran desert. *J. Exp. Zool.* **264**, 419–428.
- Wooten, M. D., and King, D. K. (1993). Epidemiology and treatment with mitotane and a review of the literature. *Cancer* **72**, 3145–3155.