This is to certify that the

dissertation entitled

The Effect of Cisplatin on the Adrenal Gland and Corticosterone Concentration: An In Vivo and In Vitro Study

presented by

Casey Margaret Miller

has been accepted towards fulfillment
of the requirements for

Doctorate degree in Zoology

S. K. Agarwal
Major professor

Date 7/15/00

DATE DUE	DATE DUE	DATE DUE

THE EFFECT OF CISPLATIN ON THE ADRENAL GLAND AND CORTICOSTERONE CONCENTRATION: AN *IN VIVO* AND *IN VITRO* STUDY

By

Casey Margaret Miller

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Zoology

2000

ABSTRACT

THE EFFECT OF CISPLATIN ON THE ADRENAL GLAND AND CORTICOSTERONE CONTRATION: AN *IN VIVO* AND *IN VITRO* STUDY

By

Casey Margaret Miller

This study investigated the effect of the anticancer agent cisplatin on the adrenal gland and corticosterone in an attempt to find out if alterations in adrenal structure stimulated alterations in the level of corticosterone. Cisplatin (CDDP) has been shown to alter appetite, weight , water intake, urine output and adrenal structure *in vivo*. These effects can alter stress levels in the body and affect the concentration of corticosterone. After treatment with cisplatin (7mg/kg and 9mg/kg) the animals showed immediate decrease in appetite and extreme weight loss. Water intake was initially decreased with an increase as post-treatment time increased. Cisplatin acted as a diuretic initially and then as an antidiuretic in urine output. Plasma corticosterone concentration showed a trend of increase that coincided with an increase in adrenal secretion into media for the 9mg/kg group on day 3 and the 7mg/kg group on day 8. Further studies showed that there was cellular damage to the mitochondria in disruption of the cristae or total cavitation of the cristae from the mitochondria. There was enlargement and degradation of the endothelial lining of the capillaries in both the medulla and the cortex and an initial decrease in lipid droplets and then increase by day 8

(7mg/kg) post-treatment in the adrenal cortex. Organ culture studies verified cellular damage but did not confirm the changes in corticosterone levels. The longer-term cultures exhibited an overall decrease in corticosterone concentration whereas the shorter-term cultures exhibited no real change from the control. We feel that cisplatin appears to affect the adrenal gland directly by endothelial damage, which then causes further damage to the cell and also indirectly by degranulation of medullary and cortical secretory cells. We can not verify if this is the primary cause of the alterations in corticosterone concentration at this time.

Dedicated to the memories of my father, mother and brother who encouraged me, but could not see it to the end.

ACKNOWLEDGEMENTS

I want to express my sincere gratitude to my adviser, Dr. Surinder Aggarwal for taking me into his laboratory and giving me his support, guidance and patience. I would also like to thank Drs. R. Neal Band, Ved Gossain for serving on my committee. I would also like to thank Dr. Karen Klomparens for serving on my committee and for her mental and financial support. Their questions and ideas have been crucial in the completion of this thesis.

For their generosity in sharing laboratory resources I would like to thank Drs. Band, Kopachik and Freidman.

I also want to thank the Zoology staff Tracey, Judy, Lisa, Jan and Chris for all their help and support through the years and the Zoology faculty for their support. Past members of our laboratory have also been of immense help and understanding, Dr. James Fadool and Dr. JoAnn Palma who made class work and working in the lab and life in general more enjoyable. To all the friends I meant and shared science and turmoil with at Michigan State, thanks for your support and encouragement and to my family who helped me attain my goal.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Cisplatin (*cis*-dichlorodiammineplatinum II) is a heavy metal coordination complex that is now used as a major broad-spectrum anticancer drug in the treatment of ovarian, testicular, prostate, head and neck, and lung cancers [1]. Its proposed mechanism of action is to inhibit DNA synthesis by inducing crosslinking of the inter or intrastrands [2-6]. The therapeutic use of cisplatin is hampered by its severe side effects, which include nausea and vomiting [7-9], along with kidney damage [10-13]. It is known to cause stomach distention and ulceration [10, 14, 15], with treatments such as chemical, hormonal and vagotomy being used to alleviate these effects [16-22]. Steroids and catecholamines that are synthesized by the adrenal gland have been considered in the induction of ulcers [23] with stomach mucosa and gastric emptying being restored in rats after bilateral adrenalectomy [17]. Disease, infection [24], chemotherapeutic drugs [13, 25, 26], toxic chemicals [27, 28], drugs [29-32], hormones [33, 34] and peptides [35] can induce stress on the system at the physical, metabolic and cellular levels. The classic response to stress is for the system to greatly increase the production of glucocorticoids from the adrenal gland, which is the key endocrine gland to respond to these conditions [24, 36]. Stress also stimulates the immune system, which then can increase synthesis of glucocorticoids and the adrenal glands response to stress [24, 37-43]. This stimulation often overrides the normal feedback inhibition that glucocorticoids have on pituitary ACTH to allow prolonged production of glucocorticoids from the adrenal gland [37, 40, 44].

Stress can be expressed by altered body temperature [45], food intake,

weight loss [10, 46-54], water intake, urine output [10, 46, 54-58], or by stimulating or inhibiting steroid synthesis [13, 26, 29, 34, 59-61].

Cisplatin has been shown to act as a diuretic in Sprage-Dawley [47, 55] and Long-Evans Rats [46], while Wistar Rats respond to it as an antidiuretic [46]. Cisplatin and other chemotherapeutic drugs have been shown to alter water intake and urine output [10, 12, 46, 47, 62, 63]. Studies have shown that cisplatin and other heavy metals can alter the circulating concentration of corticosterone [27, 64] and testosterone [13, 26, 34, 60]. This can result by a direct interaction with the steroid or an indirect interaction in the stimulation or inhibition of other hormones that stimulate glucocorticoid synthesis or by affecting the structural integrity of the adrenal gland.

The adrenal gland is the major endocrine gland to show noxious compound induced changes in morphology [65]. Cisplatin [16, 66, 67] and other noxious chemicals have been shown to disrupt the adrenals ultra-structure by damaging the mitochondria in the zona fasciculata and their cristae and by enhancing or depleting lipid droplet formation which can affect both cellular structure and steroid synthesis [30, 68-77].

To narrow the effects of a compound that may be having on the adrenal gland and steroid concentration the adrenal gland should be removed from the *in vivo* system to stop all influence by uncontrolled hormonal, neurogenic or hemodynamic factors [78, 79]. Cell and organ culture of adrenal glands have shown that by treating the culture with toxic agents and/or other hormones that secretion of glucocorticoids continues, can be monitored and maintained [19, 24, 25, 41, 42, 80-87].

LIST OF REFERENCES

1. Calabresi, P. and R. Parks, *Antiproliferative agents and drugs used for immunosuppression*, in *The Pharmacological Basis of Therapeutics*, A.G. Gilman, *et al.*, Editors. 1985, MacMillan: New York. p. 1247-1307.
2. Harder, G. and B. Rosenberg, *Inhibitory effects of antitumor platinum compounds on DNA, RNA and protein syntheses in mammalian cells in vitro.* Int j Cancer, 1970. **6**: p. 207.
3. Heigre-Bernays, W., J. Essigmann, and S. Lippard, *Effect of the antitumor drug cis-diamminedichloroplatinum [II[and related platimun complexes on eudaryotic DNA replication.* Biochemistry, 1990. **29**: p. 8461.
4. Fichtinger-Schepman, A. and R. Baan, *Immunohistochemical quantitation of adducts induced in DNA by cis-diaminedichloroplatinum (II) and analysis of adduct-related DNA-unwinding.* Chem Biol Interact, 1985. **55**: p. 275.
5. Pinto, A.L., *et al.*, *Site-specifically platinated DNA, a new probe of the biological activity of platinum anticancer drugs.* J Am Chem Soc, 1986. **108**: p. 7405.
6. Prestayko, A.W., *et al.*, *Cisplatin (cis-diamminedichloroplatinum II).* Cancer Treat Rev, 1979. **6**: p. 17.
7. Smyth, J.F., *et al.*, *Does dexamethasone enhance control of acute cisplatin induced emesis by ondansetron?* Br Med J, 1991. **303**: p. 1423-26.
8. Loehrer, P.J. and L.H. Einhorn, *Cisplatin: diangosis and treatment. Drugs five years later.* Annals of Internal Medicine, 1984. **100**(4-6): p. 704-13.
9. Schaeppi, J., *et al.*, *Cis-dichlorodiammineplatinum (II) (NSD-119875): pre-clinical toxicologic evaluation of intravenous injection in dogs, monkeys and mice.* Toxicol Appl Pharmacol, 1973. **25**: p. 230-41.
10. Aggarwal, S.K. and J.M. Fadool, *Cisplatin and carboplatin induced changes in the neurohypophysis and parathyroid, and their role in nephrotoxicity.* Anti-Cancer Drugs, 1993. **4**: p. 149-62.
11. Fillastre, J.P. and G. Raguenez-Viotle, *Cisplatin nephrotoxicity.* Toxicol Lett, 1989. **46**: p. 163-75.
12. Aggarwal, S.K., *et al.*, *Platinum drugs: Combined anti-lymphoproliferative and nephrotoxicity assay in rats.* Cancer Chemother Pharmocol, 1980. **4**: p. 249-58.
13. Maines, M.D., *Effect of cis-platinum on heme, drug, and steroid metabolism pathways: possible involvement in nephrotoxicity and infertility.* Crit Rev Toxicol, 1991. **21**(1): p. 1-20.
14. Aggarwal, S.K. and G.K. Menon, *Ultrastructural localization of Ca^{2+} and its possible role in the amelioration of kidney toxicity due to cisplatin.* Journal of Clinical Hematology and Oncology, 1981. **11**(3): p. 73-84.

15. Contrans, R.S., V. Kumar, and S.L. Robbins, *Stomach*, in *Robbin's pathologic basis of disease*, B. Saunders, Editor. 1989, W.B. Saunders: Philadelphia. p. 837-907.
16. Aggarwal, S.K., *et al.*, *Cisplatin-induced peptic ulcers, vagotomy, adrenal and calcium modulation.* Anticancer Drugs, 1994. **5**(2): p. 177-93.
17. Broccardo, M. and G. Improta, *Pituitary-adrenal and vagus modulation of sauvagine- and CRF-induced inhibition of gastric emptying in rats.* European Journal of Pharmacology, 1990. **182**: p. 357-62.
18. Konturek, S.J., *et al.*, *Gastric cytoprotection by epidermal growth factor.* Gastroentrology, 1981. **81**: p. 438-43.
19. Nielsen, S.T. and T.S. Sulkowski, *Protetion by the calciium antagonist Wy-47,037 against stress ulceration in the rats.* Pharmacol Biochem Behav, 1988. **29**: p. 129-32.
20. Szabo, S., J.S. Trier, and P.W. Frankel, *Sulfhydryl compounds may mediate cytoprotection.* Science, 1981. **214**: p. 200-2.
21. Wilder-Smith, C.H., *et al.*, *Cinnariaine for prevention of nausea and vomiting during platin chemotherapy.* Acto Oncologica, 1991. **30**: p. 731-34.
22. Wilder-Smith, C.H., F. Halter, and H.S. Merki, *Tolerance and rebound to H2-receptor antagonists: intragastric acidity in patients with duodenal ulcer.* Dig Dis Sci, 1991. **36**(12): p. 1685-90.
23. Pfeiffer, C.J., *Drugs and peptic ulcer.* 1982, Boca Raton: CRC Press.
24. Harlin, C.A. and C.R. Parker, *Investigation of the effect of interleukin-1β on steroidogenesis in the human fetal adrenal gland.* 1991.
25. Dorfinger, K., *et al.*, *The effects of suramin on human adrenocortical cells in vitro: suramin inhibits cortisol secretion and adrenocortical cell growth.* Metabolism, 1991. **40**(10): p. 1020-24.
26. Sergejew, T.F. and R.W. Hartmann, *Effect of a diphenylethylenediamine platinum complex on steroidogenesis in rats.* J Steroid Biochem Mol Biol, 1996. **58**(2): p. 243-8.
27. Agrawal, R. and J.P.N. Chansouria, *Chronic Effects of mercuric chloride ingestion on rat adrenocortical function.* Bull Environ Contam Toxicol, 1989. **43**: p. 481-84.
28. Malendowicz, L.K., *et al.*, *Effect of bombesin on the structure and function of the rat adrenal cortex.* Res Exp Med, 1991. **191**: p. 121-28.
29. Aulakh, C.S., *et al.*, *Differential effects of antidepressant treatments on fenfluramine-induced increases in plasma prolactin and corticosterone in rats.* Pharmacology Biochemistry and Behavior, 1991. **39**: p. 91-95.
30. Robba, C., G. Mazzocchi, and G.G. Nussdorfer, *Effects of a prolonged treatment with aminoglutethimide on the zona fasciculata of rat adrenal cortex: a morphometric investigation.* Cell Tissue Res, 1987. **248**(3): p. 519-25.
31. Robba, C., *et al.*, *Effects of prolonged treatment with cyanoketone on the zona fasciculata of rat adrenal cortex. A combined morphometric and biochemical study.* Cell Tissue Res, 1987. **250**(3): p. 599-605.

32. Van Der Laan, J.W., *et al.*, *Differences in the duration of sedative and anxiolytic effects of desmethyldiazepam in two outbred wistar strains.* Pharmacology Biochemistry and Behavior, 1991. **39**: p. 149-53.
33. del Priore, G., *et al.*, *Adrenal function following high-dose steroids in ovarian cancer patients.* Gynecol Oncol, 1995. **59**(1): p. 102-4.
34. LeBlanc, G.A., *et al.*, *Platinum anticancer drugs modulate P-450 mRNA levels and differentially alter hepatic drug and steroid hormone metabolism in male and female rats.* Cancer Research, 1992. **52**: p. 540-47.
35. Itoh, S., *et al.*, *Effect of caerulein on plasma corticosterone concentration in the rat.* Life Sciences, 1980. **27**: p. 2205-10.
36. Long, J.A., *The adrenal gland*, in *Histology*, L. Weiss and R.O. Greep, Editors. 1977, McGraw-Hill: New York. p. 1099-118.
37. Besedovsky, H., *et al.*, *Changes in blood hormone levels during the immune response.* Proc Soc Exp Biol Med, 1975. **150**: p. 466.
38. Besedovsky, H. and A. del Rey, *Neuroendocrine and metabolic responses induced by interleukin-1.* J Neurosci Res, 1987. **18**: p. 172-78.
39. Krymskaia, L.G., N.Y. Gromykhina, and V.Q. Kozlov, *Interleukin-1 effect on adrenal gland function in mice.* Immunol Lett, 1987. **15**: p. 307-9.
40. Epstein, F.H., *Ubterkeyjub-1 and the pathogenesis of the acute-phase response.* N Engl J Med, 1984. **3111413-18**.
41. Roh, M.S., *et al.*, *Direct stimulation of the adrenal cortex by interleukin-1.* Surgery, 1987. **102**: p. 140-46.
42. Salas, M.A., *et al.*, *Interleukin-6 and ACTH act synergistically to stimulate the release of corticosterone from adrenal gland cells.* Clin Exp Immunol, 1990. **79**: p. 470-73.
43. Sapolsky, R., *et al.*, *Interleukin-1 stimulates the secretion of hypothalamic corticotropin releasing factor.* Science, 1987. **238**: p. 67-73.
44. Keller-Wood, M.E. and M.F. Dallman, *Corticosteroid inhibition of ACTH secretion.* Endocr Rev, 1984. **5**: p. 1-24.
45. Kent, S., *et al.*, *Sickness Behavior: A new target for drug development.* Trends Pharmacol Sci, 1992. **13**: p. 24-28.
46. Batzer, M.A. and S.K. Aggarwal, *An in vitro screening system for the nephrotoxicity of various platinum coordination complexes.* Cancer Chemotherapy and Pharmacology, 1986. **17**: p. 209-17.
47. Gordon, J.A., L.N. Peterson, and R.J. Anderson, *Water metabolism after cisplatin in the rat.* Amer J Physiol, 1982. **243**(1): p. E36-43.
48. Lang, I.M., *et al.*, *Effects of chronic administration of naltrexone on appetitive behaviors of rats.* Prog Clin Biol Res, 1981. **68**: p. 197-207.
49. Michajlovskij, N., *et al.*, *Effect of acute and repeated immobilization stress on food and water intake, urine output and vasopressin changes in rats.* Endocrinol Exp, 1988. **22**(3): p. 143-57.
50. Seefeld, M.D. and R.E. Peterson, *2,3,7,8-Tetrachlorodibenzo-p-dioxin-incuced weight loss: A proposed mechanism*, in *Human and Environmental Risks of Chloinated Dioxins and Related Compounds*, R.E. Tucker, A.L. Young, and A. Gray, Editors. 1983, Plenum: New York. p. 405-13.

51. Seefeld, M.D. and R.E. Peterson, *Digestible energy and efficiency of feed utilization in rats treated with 2,3,7,8-tetrachlorodibenzo-p-dioxin.* Toxicol Appl Pharmacol, 1984. **74**(2): p. 214-22.
52. Seefeld, M.D., R.E. Keesey, and R.E. Pererson, *Evidence of a reduced level of body weight regulation in rats treated with 2,3,7,8-tetrachlorodibenzo-p-dioxin.* Toxicol Appl Pharmacol, 1984.
53. Seefeld, M.D., *et al.*, *Characterization of the wasting syndrome in rats treated with 2,3,7,8,-tetrachlorodibenzo-p-dioxin.* Toxicol Appl Pharmacol, 1984. **73**: p. 311-22.
54. Carlberg, K.A., M.J. Fregly, and M. Fahey, *Effects of chronic estrogen treatment on water exchange in rats.* Am J Physiol, 1984. **247**(1 Pt 1): p. E101-10.
55. Fadool, J.M. and S.K. Aggarwal, *Immunocytochemical demonstration of vasopressin binding in rat kidney.* J Histochem Cytochem, 1990. **38**: p. 7-12.
56. Clifton, G.G., *et al.*, *Early polyuria in the rat following single-dose cis-dichlorodiammineplatinum (II).* Lab Clin Med, 1982. **100**(5): p. 659-70.
57. Fregly, M.J. and N.E. Rowland, *Bradykinin-induced dipsogenesis in captopril-treated rats.* Brain Res Bull, 1991. **26**(1): p. 169-72.
58. Ikonomov, O.C., A.G. Stoynev, and I.V. Kurtev, *Effect of morphine withdrawal on food and water intake, urine output and electrolyte excretion in the rat: participation of the renin- aldosterone-system in renal excretory changes.* Acta Physiol Hung, 1984. **63**(2): p. 137-46.
59. Berger, C.C., *et al.*, *Endocrinological late effects after chemotherapy for testicular cancer.* Br J Cancer, 1996. **73**(9): p. 1108-14.
60. Maines, M.D. and R.D. Mayer, *Inhibition of testicular cytochrome P-450-dependent steroid biosynthesis by cis-platinum.* J Biol Chem, 1985. **260**(10): p. 6063-68.
61. Malendowicz, L.K., *Sex differences in adrenocortical structure and function. XXIV. Comparative morphometric studies on adrenal cortex of intact mature male and remale rats of different strains.* Cell Tissue Res, 1987. **249**: p. 443-49.
62. Fadool, J.M., P.J. Boyer, and S.K. Aggarwal, *Effects of cisplatin treatment on the rat neurohypophysis.* Proc Elect Micro Soc Am, 1986. **44**: p. 122-3.
63. Sumpio, B.E., I.H. Chaudry, and A.E. Baue, *Reduction of drug-induced nephrotoxicity by ATP-MgClx. 1. Effects on the cis-diamminedichloroplatinum-treated isolated perfused kidneys.* J Surg Res, 1985. **38**: p. 429-37.
64. Burton, G.V. and A.W. Meikle, *Acute and chronic methyl mercury poisoning impairs rat adrenal and testicular function.* J Toxicol Environ Health, 1980. **6**(3): p. 597-606.
65. Ribelin, W.E., *The effects of drugs and chemicals upon the structure of the adrenal gland.* Fundamental and Applied Toxicology, 1984. **4**: p. 105-19.
66. Aggarwal, S.K., *Cisplatin-induced peptic ulcers and calcium modulation.* ICEM, 1994. **13-Paris**: p. 1079-80.

67. Miller, C.M., S.K. Aggarwal, and K.K. Klomparens, *Cisplatin-induced changes in the adrenal of the rat*, in *Proc Electron Microscope Society of America*, G.W. Bailey, J. Bentley, and J.A. Small, Editors. 1992, San Francisco Press: San Francisco. p. 1652-3.
68. de Castro, C.R., *et al.*, *Ultrastructural effects of nifurtimox on rat adrenal cortex related to reductive biotransformation.* Experimental and Molecular Pathology, 1990. **52**: p. 98-108.
69. de Castro, C.R., E.G.D. de Toranzo, and J.A. Castro, *Benznidazole-induced ultrastructural alterations in rat adrenal cortex. Mechanistic studies.* Toxicology, 1992. **74**: p. 223-32.
70. Magalhaes, M.M., *et al.*, *The effects of monensin on golgi complex of adrenal cortex and steroidogenesis.* Tissue and Cell, 1991. **23**(2): p. 209-15.
71. Malendowicz, L.K., *et al.*, *Analysis of the preventive action of ACTH on dexamethasone-induced adrenocortical atrophy in the rat.* Cytobios, 1992. **71**: p. 191-99.
72. Malendowicz, L.K., *Comparative studies on the effect of 4-APP(4-aminopyrazolopyrimidine) on hamster and rat adrenal cortex.* Exp Toxic Pathol, 1992. **44**: p. 134-37.
73. Mazzocchi, G., *et al.*, *Long term effects of the hypocholesterolaemic drug 4-aminopyrazolo- pyrimidine on the zona fasciculata of the rat adrenal cortex.* J Anat, 1986. **149**: p. 1-9.
74. Mazzocchi, G., *et al.*, *Effects of the hypolipidemic drug nafenopin on the zona fasciculata of the rat adrenal cortex: a correlated biochemical and stereological study.* The Anatomical Record, 1982. **204**: p. 245-54.
75. Lullmann-Rauch, R. and G.H. Reil, *Chlorphentermine-induced lipidosislike ultrastructural alterations in lungs and adrenal glands of several species.* Toxicology and Applied Pharmacology, 1974. **30**: p. 408-21.
76. Lullmann, G. and R. Lullmann-Rauch, *Tamoxifen-induced generalized lipidosis in rats subchronically treated with high doses.* Toxicology and Applied Pharmacology, 1981. **61**: p. 138-46.
77. Robba, C., *et al.*, *Opposed effects of chronic prolactin administration on the zona fasciculata and zona reticularis of the rat adrenal cortex: an ultrastructural stereological study.* J Submicrosc Cytol, 1985. **17**(2): p. 255-61.
78. Phelps, J.S., *et al.*, *Cisplatin nephrotoxicity: invitro studies with precision-cut rabbit renal cortical slices.* Toxicol Appl Pharmacol, 1987. **90**: p. 501-12.
79. Smith, J.H., *The use of renal cortical slices from the Fischer 344 rat as an in vitro model to evaluate nephrotoxicity.* Fundam Appl Toxicol, 1988. **11**: p. 132-42.
80. Calogero, A.E., *et al.*, *Cocaine stimulates rat hypothalamic corticotropin-releasing hormone secretion in vitro*.* Brain Research, 1989. **505**: p. 7-11.
81. Garrido, M.M., J. Manzanares, and J.A. Fuentes, *Hypothalamus, anterior pituitary and adrenal gland involvement in the activation of*

adrenocorticotropin and corticosterone secretion by gastrin-releasing peptide. Brain Research, 1999. **828**: p. 20-26.
82. Goodman, R., U. Otten, and H. Thoenen, *Organ culture of the rat adrenal medulla: a model system for the study of trans-synaptic enzyme induction.* J Neurochem, 1975. **25**(4): p. 423-7.
83. Hayashi, Y., *et al.*, *A nonisotopic method for determination of the in vivo activities of tyrosine hydroxylase in the rat adrenal gland.* Analytical Biochemistry, 1988. **168**: p. 176-83.
84. Mazzocchi, G., *et al.*, *Effects of galanin on the secretory activity of the rat adrenal cortex: in vivo and in vitro studies.* Res Exp Med, 1992. **192**: p. 373-81.
85. Rebuffat, P.m., L. K., *et al.*, *Morphology and functional responses of isolated inner adrenocortical cells of rats infused with interleukin-β.* Histol Histopath, 1992. **7**: p. 183-88.
86. Whitcomb, R.W., *et al.*, *Monocytes stimulate cortisol production by cultured human adrenocortical cells.* J Clin Endocrinol Metab, 1988. **66**: p. 22-28.
87. Williams, B.C., *et al.*, *Characterization of the steroidogenic responsiveness and ultrastructure of purified zona fasciculata/reticularis cells from bovine adrenal cortex before and after primary culture.* Journal of Endocrinology, 1989. **121**: p. 317-24.

CHAPTER ONE: CISPLATIN INDUCED PHYSIOLOGICAL RESPONSES AND ALTERATION OF CIRCULATING AND ADRENAL EXPLANT CORTICOSTERONE CONCENTRATIONS IN THE RAT

SUMMARY

Cisplatin has been shown to alter food consumption, body weight, and water intake and urine output. This study was designed to analyze these effects of the drug and the effect on corticosterone concentration in plasma and secreted from the adrenal gland into media. Wistar CRL:(WI)BR male rats were kept in metabolic cages with food consumption, weight, water intake and urine output being measured daily. Blood was collected on specific days for corticosterone analysis. Animals were injected with normal saline, 7mg/kg cisplatin or 9mg/kg of cisplatin via intraperitoneum. Animals were sacrificed on day three, five and eight for the 7mg/kg dose and on days three and five for the 9mg/kg dose. At sacrifice adrenal glands were collected , incubated in media and the medium collected for steroid analyses. The animal's appetite was suppressed in both cisplatin groups immediately after treatment with their weight decreasing 35% from their initial body weight. Water intake decreased to 0 by day 3 with an increase in intake by day 5 in the 7mg/kg group, 60% above that of the controls . Urine output was increased significantly on day 1 and then declined through day 4, with a return to that of the control by day 5. There was a trend of an increase in corticosterone concentration in plasma though it was not significant from the control. This increase did coincide with an increase in adrenal secretion of corticosterone into media for the 9mg/kg group on day 3 and the 7mg/kg group on day 8.

INTRODUCTION

Cisplatin is an important chemotherapeutic agent which has dose limiting side effects of nausea and vomiting [1, 2] and kidney damage [3-11]. It also causes ulceration and distention of the stomach [12-16], with many measures being taken to modify this effect in patients undergoing cisplatin chemotherapy, protection of the mucosa by both chemical and hormonal means and control of acid secretion by means of vagotomy [13, 14, 17-24] are two such measures. Steroids and catecholamines produced by the adrenal gland have been considered to be a part of the induction of ulcers in rat's [25]. Ulceration and gastric emptying are restored when a rat has a bilateral adrenalectomy [17]. Chemotherapeutic drugs [10, 26-29], toxic chemicals [30-32]; drugs [33-38], hormones [39-46] and peptides [47] are known stressors. Stress can be expressed by altering food intake and/or weight loss [4, 5, 9, 48-54], water intake and/or urine output [4-6, 38, 50, 55-57] or by stimulating or inhibiting steroid production ([10, 26, 33, 36, 37, 43, 44, 47, 58-64]. This stress can arise from the animals response to the drug by either a direct or an indirect means. The adrenal gland is the key organ to respond to alarm, adaptation and exhilaration [65]. The initial response stimulates the release of cortocotropic releasing hormone (CRH) from the hypothalamus, enhancing the release adrenocortocotrophic hormone (ACTH) from the pituitary, enhancing the adrenal to synthesize corticosterone in the rat and cortisol in the human [66, 67]. As the

noxious compound induces physical, metabolic and/or cellular alterations to the system it may also have an affect on the concentration of circulating corticosterone. The present study was designed to analyze stressors that the chemotherapeutic agent, cisplatin may initiate physiologically and on steroid production. This was examined: (i) food consumption and weight loss, (ii) water intake and urine output and (iii) corticosterone concentration in circulating plasma (it is the primary glucocorticoid in rats [68]) and adrenal explants production into media.

MATERIALS AND METHODS

Animals and Treatment: Wistar Crl:(WI)BR male rats weighing 200 to 250 g each from Charles River were placed in metabolic cages, on a 12 hour light/dark cycle, with food and water adlib. Weight, food and water intake and urine output was measured daily at 6AM to follow the dinural rhythm of the rats. Animals were allowed to adapt to their new environment for three days prior to treatment, and were injected (Day 0) with a single dose via the intraperitoneum with normal saline (vehicle for cisplatin), 7mg/kg of cisplatin (CDDP) or 9mg/kg of CDDP. Animals were sacrificed by decapitation at three, five and eight days for the 7mg per kg treatment, and at days three and five for the 9mg per kg treatment.

Corticosterone Analysis Plasma: Blood was collected at 10AM from the tail artery on days –1, 1, 3, 4, 5, 7 and 8 for the 7mg per kg treatment group and on days –1, 1, and by cardiac puncture on days 3 and 5 for the 9mg per kg treatment group. Animals were anesthetized by inhalation of Metofane (Pitman/Moore), a tuberculin syringe coated with Heparin 1000 units/1ml (Elkins-Senn, Inc.) drew 3 to 4mls of blood. The plasma was collected and kept frozen at –70 ^{o}C until analysis could be conducted. The plasma was analyzed for corticosterone content using rat corticosterone-3H kit Radioimmunoassay (RIA) (ICN), read using a Beckman 7500 series scintillation counter.

Corticosterone Secretion from Adrenal Glands: Adrenal glands were removed from the animal, quartered and incubated for 30 minutes at 37 ^{o}C in 1

ml Krebs-Ringer bicarbonate (KRBG) solution with 0.3% glucose (Sigma) . The medium was discarded and new KRBG with 0.3% bovine serum albumin (fraction 5) (Sigma) was added. After a 30-minute incubation the medium was collected and stored at –70 °C until corticosterone analysis was conducted according to Malendowicz, L., *et al* (1992). The serum was analyzed for corticosterone content by a rat RIA and read using a Beckman 7500 series scintillation counter.

Statistical Analysis: Statistical analyses were performed using SAS Institutes Stat View for Windows. Food intake, weight, water intake, urine output, plasma corticosterone levels and adrenal serum corticosterone levels were analyzed using Student's *t* test, , ANOVA, or Student's *t* test, Mann-Whitney U rank-sum test and Kruskal-Wallis H-test as appropriate dependent on the sample size. The tests were two-tailed and significance was accepted at the $P \leq 0.05$ level.

RESULTS

Suppression or loss of appetite occurred in both cisplatin groups immediately after treatment. With food intake decreasing significantly from that of the controls 20-30mg/day to 0-5mg/day post-treatment by day 4 (Figure 1A). There also was a significant difference in food consumption between the two treatment groups on day 2. Both treated groups increased their food intake by day 5, continuing until the date of sacrifice when intake matched that of the controls. The initial weight loss correlates with the drop in food consumption (Figure 1A & B). Both CDDP treated groups had a 35% decrease in weight from their initial body weight, with little or no increase in weight prior to the date of sacrifice (Figure 1B). When sacrificed both groups had enlarged and distended stomachs that were full of food even on days 3 and 5 before food intake increased. This observation has been made previously and is caused by the lack of stomach emptying [12-14].

Water intake decreased to zero by day 3 for both treatment groups, and was significantly different from that of the controls on day 2-3 (Figure 2A). Water intake in the two treatment groups was significantly different on days 2 and 4, with water intake increasing for both groups' days 4-8. The 7mg/kg groups water intake increased by 60% from that of day 3 and 20% above that of the control prior to sacrifice. Urine output increased for both treatment groups and was significantly different from that of the control as of day 1 (Figure 2B). Urine output continued to decline for both treatment groups through day 4, with the

9mg/kg group being significant from the control. On day 5 the urine output of the treated groups begins approaching the level of the controls, with a significant 2-fold increase by day 6.

Corticosterone concentration was elevated significantly by day one in the 7mg/kg group (Figure 3). While the 9mg/kg group decreased (not significantly) from prior to treatment and that of the control on day one. The corticosterone concentration remained elevated for the remainder of the study in both treatment groups, though this elevation in concentration was not statistically significant. Corticosterone standards were unaffected by the addition of CDDP when analyzed. Secreted corticosterone into media from the adrenal glands of the 9mg/kg group had a significant increase from that of the 7mg/kg group and the control on day 3 (Figure 4). This coincided with an elevated concentration of corticosterone in the plasma on this day (Figure 3). There is a decrease in corticosterone secreted from the adrenal glands from both treatment groups on day 5, with the controls corticosterone increased though not significantly. The 7mg/kg group increased secretion from the adrenal glands significantly from the control on day 8, this coincides with an elevation in the concentration of corticosterone in the plasma.

Figure 1: Food Intake and % Weight Change of Control and CDDP Treated Rats.

A) Food intake (mg) of control and CDDP treated rats. The CDDP treated groups ceased eating immediately after treatment, with day-1 showing a significant difference from that of the control ($P \leq 0.05$) in the amount of food consumed. At days 2-4 both treatment groups are significantly different from the control group ($P \leq 0.001$) with their food consumption dropping to 0mg, with a significant difference between the two treatment groups at day-2 ($P \leq 0.005$) as well. The 7mg/kg group increased food consumption on days 6-7, with a significant difference from that of the control at day-6 ($P \leq 0.05$) and matching the controls food consumption by day 8. [Average ± S.D.]

B) The percent weight change of control and CDDP treated rats. The CDDP treated groups have a weight loss immediately after treatment with days 1-8 showing a significant difference from that of the control group ($P \leq 0.001$). [Average ± S.D.]

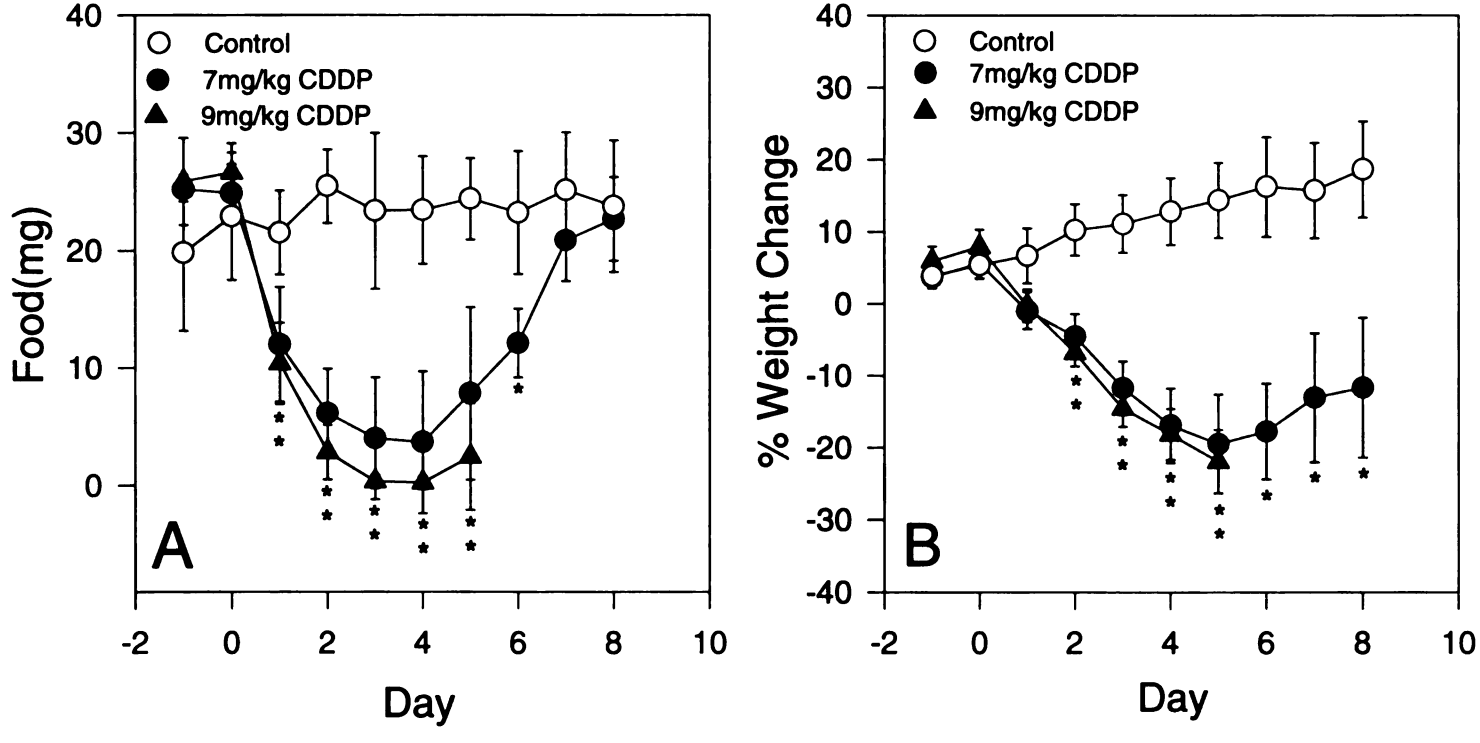
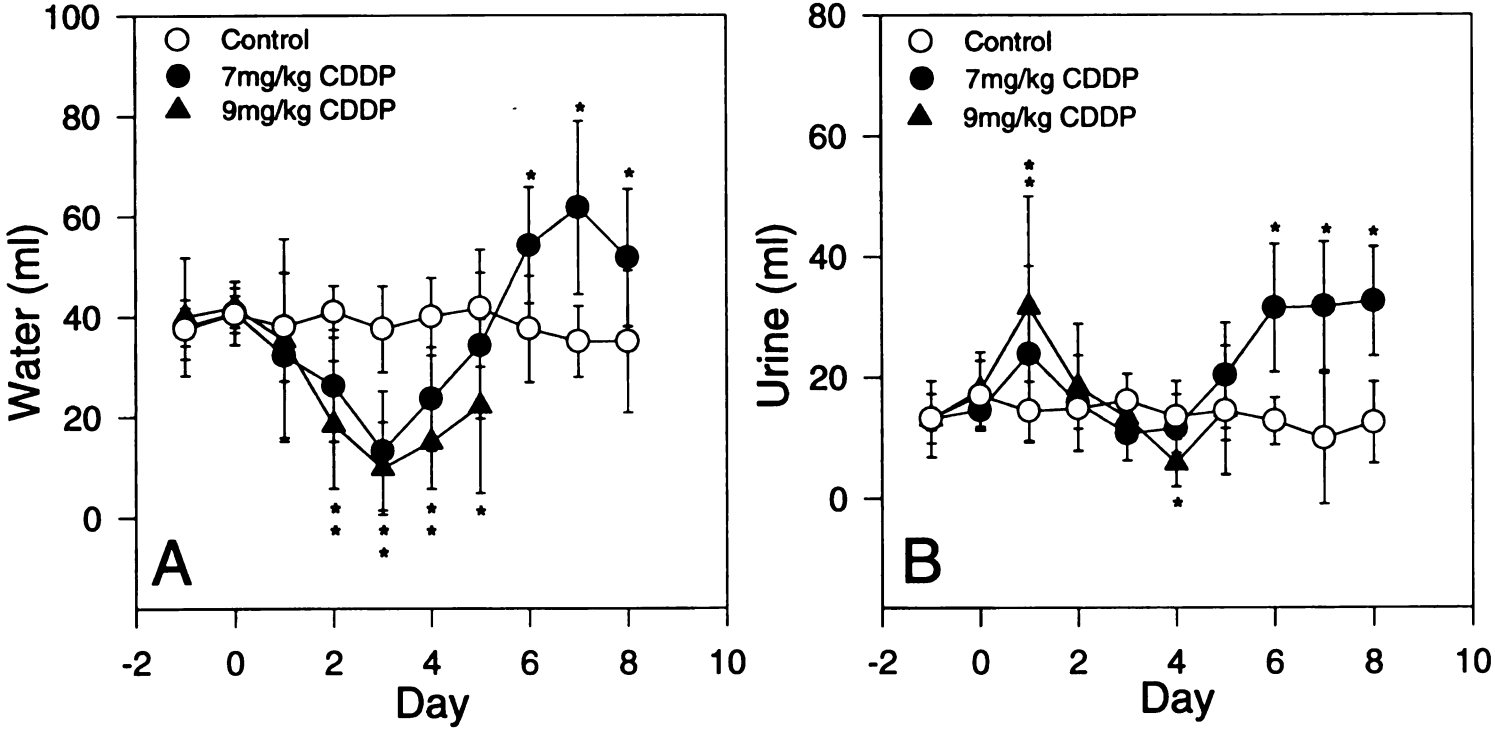

Control
7mg/kg CDDP
9mg/kg CDDP
Food(mg)
Day
A
% Weight Change
Day
B

Figure 2: Water Intake and Urine Output of Control and CDDP Treated Rats.

A) Water intake of control and CEEP treated rats. Water intake in both treatment groups decreased significantly (7mg/kg $P \leq 0.005$, 9mg/kg $P \leq 0.001$) by day 2 post-treatment and continues to decrease through day 3, with the two treatment groups also being significantly different ($P \leq 0.05$). Water intake starts to increase in both groups with the 9mg/kg group being significantly different from the control until sacrifice ($P \leq 0.0001$) and the 7mg/kg group ($P \leq 0.05$). The 7mg/kg group is significant only on day 4 ($P \leq 0.005$). By day 7 the 7mg/kg group has had a 4-fold increase in water intake from day 3. They are in taking a significantly ($P \leq 0.05$greater volume than the controls. [Average ± S.D.]

B) Urine output of control and CDDP treated rats. The treated groups had an increase in urine output by day-1 being significantly different from that of the control group (7mg/kg $P \leq 0.005$, 9mg/kg $P \leq 0.0001$). On day-2 the 9mg/kg group is significantly different ($P \leq 0.05$) from the control group in urine output but not the 7mg/kg group. The urine output on day-3 showed no difference between groups, with a difference appearing again on day-4 between the 9mg/kg treatment group and both the control ($P \leq 0.05$) and 7mg/kg group ($P \leq 0.05$). The 7mg/kg groups urine output increased greatly days 6-8 giving a significant difference from the control ($P \leq 0.05$). [Average ± S.D.]


Control
7mg/kg CDDP
9mg/kg CDDP
Water (ml)
Urine (ml)
Day
A
B

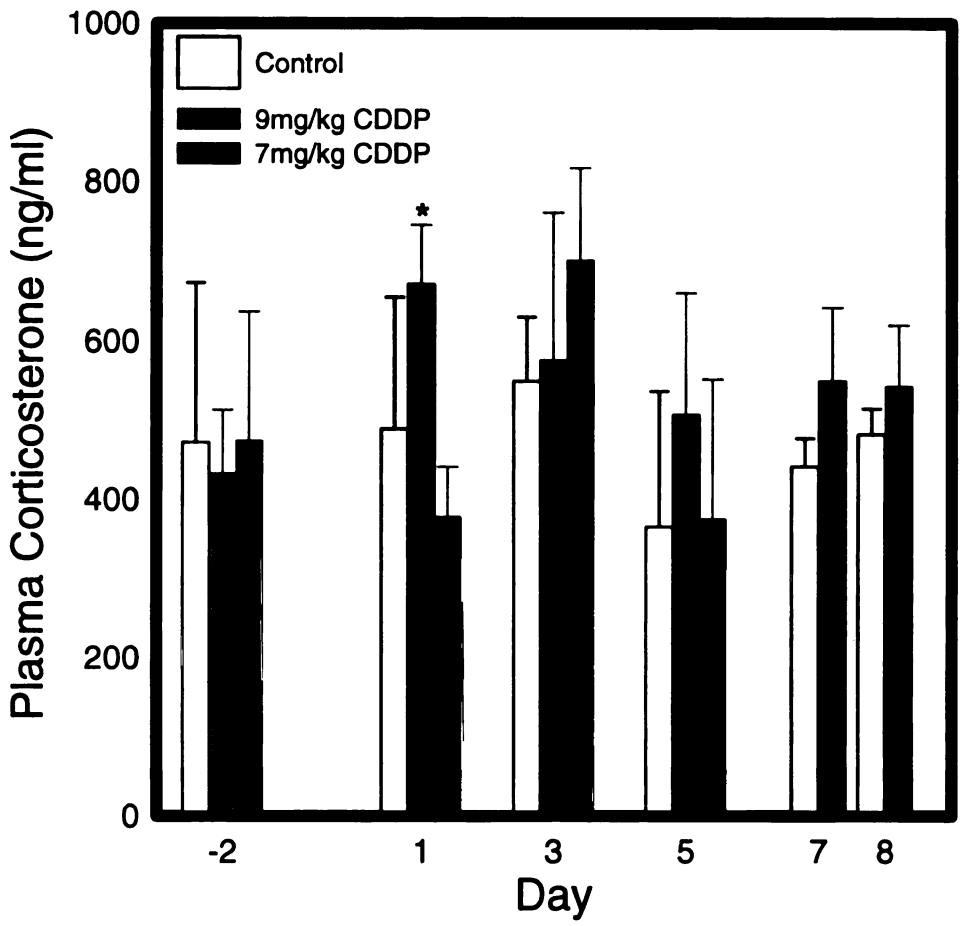


Figure 3: Plasma Corticosterone Concentration from Control and CDDP Treated Rats by Radioimmunoassay.

Plasma was collected on specified days and analyzed for the corticosterone concentration. On day 1 the 7mg/kg group showed a significant difference in the concentration of corticosterone from that of the control and the 9mg/kg group ($P \leq 0.005$). All other days showed no significant difference in the corticosterone concentration between groups. [Average ± S.D.]

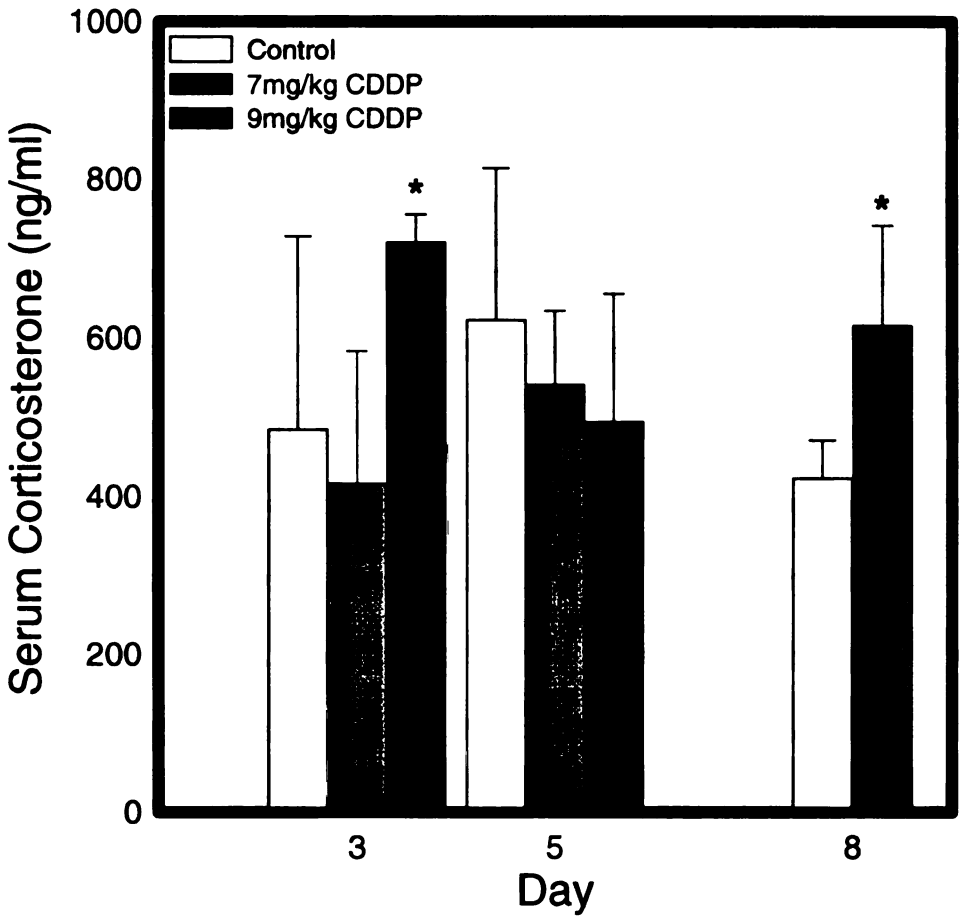


Figure 4: Corticosterone Concentration from Control and CDDP Treated Rats Adrenal Glands.

The serum was collected from processed from adrenal glands and analyzed for the level of corticosterone concentration. On day 3 the 9mg/kg group showed a significant difference in the amount of corticosterone present from the control and 7mg/kg group ($P \leq 0.05$). On day-8 the 7mg/kg group showed a significant difference in corticosterone concentration from that of the control group ($P \leq 0.05$). Day 5 shows no significant difference in the level of corticosterone between the groups. [Average ± S.D.]

DISCUSSION

Various chemotherapeutic agents [4, 6, 9, 13, 29, 69-72], toxic chemicals [30, 32, 51, 73] drugs [33, 36-38, 73] and hormones [43, 50, 74-76] or hormone-like substances [31, 47, 56, 64] are known to effect food consumption, weight loss or gain, water intake and urine output of experimental animals. In this present study, treatment with cisplatin resulted in a decrease in food consumption and subsequent weight loss in both treatments [4, 9]. It has been suggested that when noxious compounds are administered, weight loss may be due to a shift in the regulation level for body [9, 51, 52, 54] and that the weight reduction is a result of regulation by decreased food intake until such a level of regulation is reached that is induced by the dose of the drug [51, 54]. Although the animals have reduced their food consumption to 0mg a day on days 4-5, they show signs of stomach distention and ulceration at the time of sacrifice [13]. This is indicative of ulceration of the stomach, possibly related to the levels of circulating steroids [25].

Water intake and urine outputs were altered suggesting that there is an effect on hormones that cause dipsia and polyuria. Other groups have found that cisplatin or other chemotherapeutic drugs [4-7, 10, 11, 71, 72, 77-79], estrogen [50], pertussis toxin [55] and bradydinin [56] have effects on both water intake and urine output and cause nephrotoxicity. This pattern in water intake (decrease through day 3 and then increasing) has been seen previously after cisplatin administration [4, 6, 9].

Cisplatin has been shown to be diuretic in Sprage-Dawley [9, 78] and Long-Evans Rats [5], while it acts as an antidiuretic in Wistar Rats [5]. This study shows a diuretic effect on Wistar Rats as they have an increased urine output on day one, in spite of decreases in water intake. Urine output decreases through day 4, which parallels decreases in water intake. This suggests that it may not be the effect of cisplatin acting as a antidiuretic but due to the lower fluid intake. This is confirmed on days 5-8 when there is an increase in urine output that parallels an increase in water intake. This pattern has been seen in previous studies which showed an increase in serum creatinine which is indicative of a water concentration defect with later tubular changes in the kidney with two different doses of cisplatin [6, 9].

Concentrations of corticosterone in rats and cortisol in humans are changed by stress [66, 67, 80, 81] from internal and external sources. Cancer causes internal stress or a direct form, where treatment with chemotherapeutic drugs and the drugs to reduce the side-affects cause stress from external sources or an indirect form. Substances such as heavy metals [29, 30, 82], toxic compounds [31], drugs [33-38, 73, 83], hormones [10, 26, 29, 43, 44, 50, 59, 60, 76, 83] have an affect on the corticosterone levels in rats. Cisplatin is a heavy metal and chemotherapeutic agent known to effect the structure of the adrenal gland [13, 84], therefore there is good reason to believe that it could alter corticosterone concentration.

There was an increase in plasma corticosterone concentration with the 7mg/kg dose of CDDP on all days post-treatment compared with pre-treatment

levels. The secretion of corticosterone into media from the adrenal glands on day 8 confirmed these increases. On day 5 the secretion of corticosterone into media from the adrenal gland did not show an increase in corticosterone concentration compared to the control. The concentration in the culture medium does coincide with the plasma corticosterone levels. This pattern is repeated with the 9mg/kg group as well on day 5. The differences seen between the two concentrations could be explained by the stress to the animal when drawing blood, or stress they experience prior to sacrifice. The animals that received the 9mg/kg dose of cisplatin appeared to be irritable and perhaps more stressed that the group treated with the 7mg/kg dose. These animals were dehydrated making the blood draw difficult. Sergejew *et al* (1996), found that corticosterone concentration in plasma and that secreted into media from adrenal glands were increased, although they were not significant from the control. Basal levels in their studies differed in that they were of a lower concentration from those found in the present study but this could be attributed to strain and gender differences [85, 86]. Other studies using male Wistar Rats reported basal levels from 400 to 600 mg/ml that are similar to our findings [33, 35, 47].

The adrenal cortex has a large reserve of precursors for steroid production, which may cause problems with detecting changes in circulating corticosterone. This is seen by a study done by Xarli, et al (1978), in human autopsy material. They found that 1.1% percent of all cases examined had some type of adrenal hemorrhage with cellular degeneration (not the cause of death). None of these cases had clinical finding to suggest any adrenocortical

dysfunction. Based on this study an increase in corticosterone indicates either extreme stress, stimulation to steroid synthesis or damage to the adrenal gland on a cellular level.

Other microscopic studies are required to examine the changes in structure, at light and EM levels, and explantation of the adrenal gland to organ culture to allow examination without uncontrolled factors such as changes in appetite, weight , water intake and urine output.

CONCLUSIONS

The physiological affects of alterations in weight and food, water and urine intake and/or output induce stress on the system and activate the corticosterone pathway. This gives rise to an increase in the concentration of corticosterone. To find if the corticosterone concentration increase is due to cisplatin directly or indirectly further studies will have to be conducted.

LIST OF REFERENCES

1. Smyth, J.F., *et al.*, *Does dexamethasone enhance control of acute cisplatin induced emesis by ondansetron?* Br Med J, 1991. **303**: p. 1423-26.
2. Schaeppi, J., *et al.*, *Cis-dichlorodiammineplatinum (II) (NSD-119875): pre-clinical toxicologic evaluation of intravenous injection in dogs, monkeys and mice.* Toxicol Appl Pharmacol, 1973. **25**: p. 230-41.
3. Aggarwal, S.K., *et al.*, *Platinum drugs: conbined anti-lymphoproliferative and nephrotoxicity assay in rats.* Cancer Chemother Pharmacol, 1980. **4**: p. 249-58.
4. Aggarwal, S.K. and J.M. Fadool, *Cisplatin and carboplatin induced changes in the neurohypophysis and parathyroid, and their role in nephrotoxicity.* Anti-Cancer Drugs, 1993. **4**: p. 149-62.
5. Batzer, M.A. and S.K. Aggarwal, *An in vitro screening system for the nephrotoxicity of various platinum coordination complexes.* Cancer Chemotherapy and Pharmacology, 1986. **17**: p. 209-17.
6. Clifton, G.G., *et al.*, *Early polyuria in the rat following single-dose cis-dichlorodiammineplatinum (II).* Lab Clin Med, 1982. **100**(5): p. 659-70.
7. Fadool, J.M., P.J. Boyer, and S.K. Aggarwal, *Effects of cisplatin treatment in the rat neurohypophysis.* Proc Elect Micro Soc Am, 1986. **44**: p. 122-3.
8. Fillastre, J.P. and G. Raguenez-Viotle, *Cisplatin nephrotoxicity.* Toxicol Lett, 1989. **46**: p. 163-75.
9. Gordon, J.A., L.N. Peterson, and R.J. Anderson, *Water metabolism after cisplatin in the rat.* Amer J Physiol, 1982. **243**(1): p. E36-43.
10. Maines, M.D., *Effect of cis-platinum on heme, drug, and steroid metabolism pathways: possible involvement in nephrotoxicity and infertility.* Crit Rev Toxicol, 1991. **21**(1): p. 1-20.
11. Hrushesky, W.J.M., *Selected aspects of cisplatin nephrotoxicity in the rat and man*, in *Platinum coordination complexes in cancer chemotherapy*, E.B. Double and I.H. Krakoff, Editors. 1984, Martinu Nimhoff: Boston. p. 165-86.
12. Aggarwal, S.K. and J. San Antonio, *Gastric ulcers produced by cisplatin.* Proc Electron Microscope Society of America, ed. G.W. Bailey. Vol. 39. 1981, Baton Rouge: Claitor Publishing Division. 582-3.
13. Aggarwal, S.K., *et al.*, *Cisplatin-induced peptic ulcers, vagotomy, adrenal and calcium modulation.* Anticancer Drugs, 1994. **5**(2): p. 177-93.
14. Aggarwal, S.K., *Cisplatin-induced peptic ulcers and calcium modulation.* ICEM, 1994. **13-Paris**: p. 1079-80.
15. Brzozowski, T., *et al.*, *Gastric adaptation to stress: role of sensory nerves, salivary glands, and adrenal glands.* Scand J Gastroenterol, 1995. **30**(1): p. 6-16.
16. Contrans, R.S., V. Kumar, and S.L. Robbins, *Stomach*, in *Robbin's pathologic basis of disease*, B. Saunders, Editor. 1989, W.B. Saunders: Philadelphia. p. 837-907.

17. Broccardo, M. and G. Improta, *Pituitary-adrenal and vagus modulation of sauvagine- and CRF-induced inhibition of gastric emptying in rats.* European Journal of Pharmacology, 1990. **182**: p. 357-62.
18. Chen, J.T., *et al.*, *Medical vagotomy: a new effective antiemetic therapy in cisplatin-induced nausea and vomiting.* Nippon sanka fujinka Gakkai Zasshi, 1987. **39**: p. 2231-2.
19. Konturek, S.J., *et al.*, *Gastric cytoprotection by epidermal growth factor.* Gastroentrology, 1981. **81**: p. 438-43.
20. Mohri, K., *Stress ulcer and unilateral truncal vagotomy*, in *Vagotomy*, F. holle and S. Anderson, Editors. 1974, Springer-Verlag: New York. p. 118-32.
21. Nielsen, S.T. and T.S. Sulkowski, *Protetion by the calciium antagonist Wy-47,037 against stress ulceration in the rats.* Pharmacol Biochem Behav, 1988. **29**: p. 129-32.
22. Szabo, S., J.S. Trier, and P.W. Frankel, *Sulfhydryl compounds may mediate cytoprotection.* Science, 1981. **214**: p. 200-2.
23. Wilder-Smith, C.H., *et al.*, *Cinnariaine for prevention of nausea and vomiting during platin chemotherapy.* Acto Oncologica, 1991. **30**: p. 731-34.
24. Wilder-Smith, C.H., F. Halter, and H.S. Merki, *Tolerance and rebound to H2-receptor antagonists: intragastric acidity in patients with duodenal ulcer.* Dig Dis Sci, 1991. **36**(12): p. 1685-90.
25. Pfeiffer, C.J., *Drugs and peptic ulcer.* 1982, Boca Raton: CRC Press.
26. Maines, M.D. and R.D. Mayer, *Inhibition of testicular cytochrome P-450-dependent steroid biosynthesis by cis-platinum.* J Biol Chem, 1985. **260**(10): p. 6063-68.
27. de Castro, C.R., E.G.D. de Toranzo, and J.A. Castro, *Benznidazole-induced ultrastructural alterations in rat adrenal cortex. Mechanistic studies.* Toxicology, 1992. **74**: p. 223-32.
28. de Castro, C.R., *et al.*, *Ultrastructural effects of nifurtimox on rat adrenal cortex related to reductive biotransformation.* Experimental and Molecular Pathology, 1990. **52**: p. 98-108.
29. Sergejew, T.F. and R.W. Hartmann, *Effect of a diphenylethylenediamine platinum complex on steroidogenesis in rats.* J Steroid Biochem Mol Biol, 1996. **58**(2): p. 243-8.
30. Agrawal, R. and J.P.N. Chansouria, *Chronic Effects of mercuric chloride ingestion on rat adrenocortical function.* Bull Environ Contam Toxicol, 1989. **43**: p. 481-84.
31. Malendowicz, L.K., *et al.*, *Effect of bombesin on the structure and function of the rat adrenal cortex.* Res Exp Med, 1991. **191**: p. 121-28.
32. Malendowicz, L.K., *Comparative studies on the effect of 4-APP(4-aminopyrazolopyrimidine) on hamster and rat adrenal cortex.* Exp Toxic Pathol, 1992. **44**: p. 134-37.
33. Aulakh, C.S., *et al.*, *Differential effects of antidepressant treatments on fenfluramine-induced increases in plasma prolactin and corticosterone in rats.* Pharmacology Biochemistry and Behavior, 1991. **39**: p. 91-95.

34. Feldman, D., *Imidazol antifungal drugs: New actions as steroidogenesis inhibitors and glucocorticoid antagonists*, in *Recent advances in adrenal regulation and function*, R. D'Agata and G.P. Chrousos, Editors. 1987, Raven Press: New York. p. 243-49.
35. Mazzocchi, G., *et al.*, *Effects of the hypolipidemic drug nafenopin on the zona fasciculata of the rat adrenal cortex: a correlated biochemical and stereological study.* The Anatomical Record, 1982. **204**: p. 245-54.
36. Robba, C., *et al.*, *Effects of prolonged treatment with cyanoketone on the zona fasciculata of rat adrenal cortex. A combined morphometric and biochemical study.* Cell Tissue Res, 1987. **250**(3): p. 599-605.
37. Robba, C., G. Mazzocchi, and G.G. Nussdorfer, *Effects of a prolonged treatment with aminoglutethimide on the zona fasciculata of rat adrenal cortex: a morphometric investigation.* Cell Tissue Res, 1987. **248**(3): p. 519-25.
38. Van Der Laan, J.W., *et al.*, *Differences in the duration of sedative and anxiolytic effects of desmethyldiazepam in two outbred wistar strains.* Pharmacology Biochemistry and Behavior, 1991. **39**: p. 149-53.
39. del Priore, G., *et al.*, *Adrenal function following high-dose steroids in ovarian cancer patients.* Gynecol Oncol, 1995. **59**(1): p. 102-4.
40. Goble, F.C., *Sex as a factor in metabolism, toxicity, and efficacy of pharmacodynamic and chemotherapeutic agents*, in *Advances in Pharmacology and Chemotherapy*, S. Garattini, *et al.*, Editors. 1975, Academic Press: New Your. p. 173-252.
41. Lesniewska, B., *et al.*, *Effects of arginine vasopressin on the pituitary-adrenocortical axix of intact and dexamethasone-suppressed rats.* Exp Pathol, 1991. **43**: p. 181-88.
42. Lis, M., C. Gilardeau, and M. Chretien, *Effect of prolactin on corticosterone production by rat adrenals.* Clin Res, 1973. **21**: p. 1027-31.
43. LeBlanc, G.A., *et al.*, *Platinum anticancer drugs modulate P-450 mRNA levels and differentially alter hepatic drug and steroid hormone metabolism in male and female rats.* Cancer Research, 1992. **52**: p. 540-47.
44. Mazzocchi, G., *et al.*, *Effects of testosterone on the zona fasciculata of the male rat adrenal cortex. A correlated stereological and biochemical study.* J Submicrosc Cytol, 1983. **15**(4): p. 991-1005.
45. Nussdorfer, G.G., *et al.*, *Effects of ACTH and dexamethasone on the zona glomerulosa of the rat adrenal cortex: an ultrastructural stereologic study.* Acta Endocrinol (Copenh), 1977. **85**(3): p. 608-14.
46. Szkudlinski, M., *et al.*, *Hydrocortisone inhibits the basal and angiotensin II-stimulated proliferation of rat adrenocortical cells in organ culture.* Exp Clin Endocrinol, 1990. **95**(2): p. 197-202.
47. Itoh, S., *et al.*, *Effect of caerulein on plasma corticosterone concentration in the rat.* Life Sciences, 1980. **27**: p. 2205-10.
48. Lang, I.M., *et al.*, *Effects of chronic administration of naltrexone on appetitive behaviors of rats.* Prog Clin Biol Res, 1981. **68**: p. 197-207.

49. Michajlovskij, N., *et al.*, *Effect of acute and repeated immobilization stress on food and water intake, urine output and vasopressin changes in rats.* Endocrinol Exp, 1988. **22**(3): p. 143-57.
50. Carlberg, K.A., M.J. Fregly, and M. Fahey, *Effects of chronic estrogen treatment on water exchange in rats.* Am J Physiol, 1984. **247**(1 Pt 1): p. E101-10.
51. Seefeld, M.D. and R.E. Peterson, *2,3,7,8-Tetrachlorodibenzo-p-dioxin-incuced weight loss: A proposed mechanism*, in *Human and Environmental Risks of Chloinated Dioxins and Related Compounds*, R.E. Tucker, A.L. Young, and A. Gray, Editors. 1983, Plenum: New York. p. 405-13.
52. Seefeld, M.D. and R.E. Peterson, *Digestible energy and efficiency of feed utilization in rats treated with 2,3,7,8-tetrachlorodibenzo-p-dioxin.* Toxicol Appl Pharmacol, 1984. **74**(2): p. 214-22.
53. Seefeld, M.D., *et al.*, *Characterization of the wasting syndrome in rats treated with 2,3,7,8,-tetrachlorodibenzo-p-dioxin.* Toxicol Appl Pharmacol, 1984. **73**: p. 311-22.
54. Seefeld, M.D., R.E. Keesey, and R.E. Pererson, *Evidence of a reduced level of body weight regulation in rats treated with 2,3,7,8-tetrachlorodibenzo-p-dioxin.* Toxicol Appl Pharmacol, 1984.
55. Anderson, R.J., *et al.*, *Effect of pertussis toxin on water metabolism in the rat.* Life Sciences, 1986. **38**: p. 15-19.
56. Fregly, M.J. and N.E. Rowland, *Bradykinin-induced dipsogenesis in captopril-treated rats.* Brain Res Bull, 1991. **26**(1): p. 169-72.
57. Ikonomov, O.C., A.G. Stoynev, and I.V. Kurtev, *Effect of morphine withdrawal on food and water intake, urine output and electrolyte excretion in the rat: participation of the renin- aldosterone-system in renal excretory changes.* Acta Physiol Hung, 1984. **63**(2): p. 137-46.
58. Berger, C.C., *et al.*, *Endocrinological late effects after chemotherapy for testicular cancer.* Br J Cancer, 1996. **73**(9): p. 1108-14.
59. Malendowicz, L.K., *et al.*, *Effects of neurotensin on the pituitary-adrenocortical axis of intact and dexamethasone-suppressed rats.* Exp Pathol, 1991. **43**: p. 205-11.
60. Malendowicz, L.K., *et al.*, *Neurotensin stimulates the growth and secretion of rat adrenal zona glomerulosa.* in vivo, 1992. **6**: p. 523-6.
61. Malendowicz, L.K., *et al.*, *Analysis of the preventive action of ACTH on dexamethasone-induced adrenocortical atrophy in the rat.* Cytobios, 1992. **71**: p. 191-99.
62. Malendowicz, L.K., *et al.*, *Neuromedin-N increases the levels of circulating ACTH and aldosterone without changing the blood concentration of corticosterone in rats.* Neuroendocrinol Lett, 1992. **14**(5): p. 343-48.
63. Mazzocchi, G., *et al.*, *Effects of prolonged infusion with endothelin-1 on the function and morphology of rat adrenal cortex.* Peptides, 1990. **11**: p. 767-72.

64. Mazzocchi, G., *et al.*, *Effects of galanin on the secretory activity of the rat adrenal cortex: in vivo and in vitro studies.* Res Exp Med, 1992. **192**: p. 373-81.
65. Selye, J., *The general adaptation syndrome.* J Clin Endocrinol, 1946. **6**: p. 117-230.
66. Greep, R.O., *The struture of the adrenal cortex*, in *The Adrenal Cortex*, H.D. Moon, Editor. 1961, Paul B. Hoeber, Inc.: New York. p. 23-45.
67. Long, J.A., *The adrenal gland*, in *Histology*, L. Weiss and R.O. Greep, Editors. 1977, McGraw-Hill: New York. p. 1099-118.
68. Dorfinger, K., *et al.*, *The effects of suramin on human adrenocortical cells in vitro: suramin inhibits cortisol secretion and adrenocortical cell growth.* Metabolism, 1991. **40**(10): p. 1020-24.
69. Prestayko, A.W., *et al.*, *Cisplatin (cis-diamminedichloroplatinum II).* Cancer Treat Rev, 1979. **6**: p. 17.
70. Ward, J.M., *et al.*, *Comparative nephrotoxicity of platinum cancer chemotherapeutic agents.* Cancer Treat Rep, 1976. **60**(1675).
71. Ward, J.M. and K.A. Fauvie, *The nephrotoxic effects of cis-diamminedichloroplatinum (II) (NCS-119875) in male F344 rats.* Toxicol Appl Pharmacol, 1976. **35**: p. 535.
72. Ribelin, W.E., *The effects of drugs and chemicals upon the structure of the adrenal gland.* Fundamental and Applied Toxicology, 1984. **4**: p. 105-19.
73. Lesniewska, B., *et al.*, *Comparison of the effects of neurotensin and vasopressin on the adrenal cortex of dexamethasone-suppressed rats.* Neuropeptides, 1992. **23**: p. 9-13.
74. Manuelidis, L. and P. Mulrow, *ACTH effects on aldosterone production and mitochondrial ultrastructure in adrenal gland cultures.* Endocrinology, 1973. **93**(5): p. 1104-8.
75. Robba, C., *et al.*, *Opposed effects of chronic prolactin administration on the zona fasciculata and zona reticularis of the rat adrenal cortex: an ultrastructural stereological study.* J Submicrosc Cytol, 1985. **17**(2): p. 255-61.
76. Aggarwal, S.K., *et al.*, *Platinum drugs: Combined anti-lymphoproliferative and nephrotoxicity assay in rats.* Cancer Chemother Pharmocol, 1980. **4**: p. 249-58.
77. Fadool, J.M. and S.K. Aggarwal, *Immunocytochemical demonstration of vasopressin binding in rat kidney.* J Histochem Cytochem, 1990. **38**: p. 7-12.
78. Sumpio, B.E., I.H. Chaudry, and A.E. Baue, *Reduction of drug-induced nephrotoxicity by ATP-MgClx. 1. Effects on the cis-diamminedichloroplatinum-treated isolated perfused kidneys.* J Surg Res, 1985. **38**: p. 429-37.
79. Vinson, G.P., B.J. Whitehouse, and J.P. Hinson, *The adrenal cortex.* 1992, Englewood Cliffs: Prentice Hall. 223.
80. Vivian, H.T.J., *The adrenal gland.* 1992, New York: Raven Press.

81. Burton, G.V. and A.W. Meikle, *Acute and chronic methyl mercury poisoning impairs rat adrenal and testicular function.* J Toxicol Environ Health, 1980. **6**(3): p. 597-606.
82. Mazzocchi, G., *et al.*, *Long term effects of the hypocholesterolaemic drug 4-aminopyrazolo- pyrimidine on the zona fasciculata of the rat adrenal cortex.* J Anat, 1986. **149**: p. 1-9.
83. Miller, C.M., S.K. Aggarwal, and K.K. Klomparens, *Cisplatin-induced changes in the adrenal of the rat*, in *Proc Electron Microscope Society of America*, G.W. Bailey, J. Bentley, and J.A. Small, Editors. 1992, San Francisco Press: San Francisco. p. 1652-3.
84. Kasprzak, A. and H. Nikicicz, *Sex differences in adrenocortical structure and function. IX. Stereologic studies on the effects of gonadectomy and testosterone or estradiol replacement on adrenal cortex of adult male and female hamsters.* Z mikr-anat Forsch, 1982. **96**: p. 91-102.
85. Malendowicz, L.K., *Sex differences in adrenocortical structure and function. XXIV. Comparative morphometric studies on adrenal cortex of intact mature male and remale rats of different strains.* Cell Tissue Res, 1987. **249**: p. 443-49.
86. Xarli, V.P., A.A. Stelle, and P.J. Davis, *Adrenal hemorrhage in the adult.*

Medicine, 1978. **57**: p. 211-22.

CHAPTER TWO: CISPLATIN-INDUCED STRUCTURAL CHANGES IN THE RAT *IN VIVO*

SUMMARY

Wistar CRL:(WI)BR male rats were treated with either normal saline (vehicle for cisplatin), 7mg/kg or 9mg/kg of cisplatin. Animals were sacrificed at 3, 7 and 8 days for the 7mg/kg group and at 3 and 5 days for the 9mg/kg group. Adrenals were processed for light and electron microscopy. Both doses showed disruption of the mitochondria and secretory cells, enlargement and endothelial lining degradation of the venous channels in both the medulla and cortex of the adrenal gland and by a decrease in lipid droplets and then increase by day 8 post-treatment. Cisplatin appears to affect the adrenal gland directly by endothelial damage, which then causes further damage to the cell, and indirectly by degranulation of medullary and cortical secretory cells.

INTRODUCTION

The structure of the adrenal gland plays a major role in steroidogenesis. The gland is divided into the medulla and the cortex. The medulla develops from ectodermal neurocrest cells, and synthesizes nitrogen containing hormones, norepinephrine and epinephrine [1]. The adrenal cortex develops from mesodermal coelemic epithelium and different hormones are synthesized in specific zonae [2]. Therefore the gland is divided into 3 zonae, the innermost being the zona reticularis which comprises about 7% of the cortex and synthesizes the sex hormones [3-6]. The middle zone, zona fasciculata comprises 70% of the cortex and synthesizes the glucocorticoids including cortisol and corticosterone [3-6]. The outermost zone the zona glomerulosa comprises 15% of the cortex and synthesizes the mineralocorticoid aldosterone [3-6]. The adrenal gland is very important maintaining the homeostasis of the body because its function is to respond to alarm, adaptation and exhilaration [7]. The different zonae and the medulla of the adrenal gland vary in their susceptibility to different noxious agents' [8]. The zona glomerulosa receives part of its circulatory supply from the capsule thus allowing it to be protected from infarctive effects that may damage the adrenal arterioles in the rest of the cortex or in the medulla [8]. The mitochondria of the medulla are less prominent and their function is related to the cellular energy [9]. The mitochondria of the different zonae vary in their design dependent on the hormone that they are producing [4]. In the zona fasciculata the mitochondrial membranes play a major role in steroid synthesis. Cytochrome

P450 resides on the inner mitochondrial membrane and is the major player in the first step of steroidogenesis, converting cholesterol to pregnenolone [4, 6, 9-11]. Steroidogenesis then moves to the mitochondrial outer membrane to start the second phase where it can receive a rapid supply of cholesterol.

Damage to the medulla can alter steroid production by releasing catecholamines into the medullary venous channel [8, 12]. Damage to the mitochondria within a specific zona of the cortex influences not only the hormone synthesis in that zona [11, 13-19], but can also have an effect on the medulla's synthesis of catecholamines [20]. Agents that increase lipid concentration, such as benznidazole, aminoglutethimide, ACTH, prolactin and testosterone in the adrenal cortex may be activating a degenerative process by destroying cellular structure and impair function as seen in the liver [17, 21-24]. On the other hand agents that reduce lipids, such as hypocholesteroloemic drugs, nifurtimox, monensin, 4-APP, tamoxifen can cause a shutdown in steroid synthesis [13, 25-28].

Present is an attempt to study any structural changes after cisplatin treatment and correlate these changes to alterations in corticosterone levels.

MATERIALS AND MEHTODS

Animals: Wistar Crl:(WI)BR male rats weighing 200 to 250 grams each from Charles River were placed 3 to a cage. Animals were maintained on a 12-hour day/night cycle, with food and water *ad lib*. Animals were allowed to adjust for three days to the new environment, after which time they were injected (Day 0) with a single dose via the intraperitoneal with normal saline (vehicle for cisplatin), 7mg per kg or 9mg per kg of cisplatin. Animals were then sacrificed by decapitation on days 3, 5 and 8 for the 7mg/kg treatment and days 3and 5 for the 9mg/kg treatment, the adrenal glands were processed for light and electron microscopy.

Light Microscopy: One adrenal was cut in half, and both halves fixed in 2% glutaraldehyde in 0.1M sodium phosphate buffer (PBS), pH 7.4, dehydrated in graded ethanol series and embedded in JB4 (glycol methacrylate, Polyscience, Inc.). Sections (2μm) were stained with hematoxylin and eosin for general histology. The other adrenal was cooled down and frozen in isopentane (Sigma), further frozen in liquid nitrogen and then stored in liquid nitrogen until cryosectioned. Sections (10μm) were fixed in 10% normal buffered formalin and stained with sudan black for lipid localization.

Electron Microscopy: A thin, sagittal slice was cut from each adrenal, fixed in 2% glutaraldehyde in 0.1M phophate buffered saline (PBS), and then cut into smaller sections. Samples were postfixed in 1% OSO_4, in PBS, dehydrated in graded acetone series and embedded in Araldite resin. Thick sections were

taken and stained with toluidine blue to localize the area for thin sectioning. Ultra-thin sections were made using an ultratome (LKB- Sweden) and stained with aqueous 2% uranyl acetate and lead citrate, and examined with a Philips CM 210 transmission microscope. Fifteen to twenty blocks were embedded for each of twelve animals in each group. Minimums of five blocks to a maximum of ten blocks were thick sectioned for each animal to pick areas for thin sectioning for each animal.

RESULTS

The adrenal gland consists of two major areas, the medulla and the cortex. The medulla synthesizes the hormone norepinephrine and epinephrine. The cortex is composed of three zones each having specific functions. The zona glomerulosa is the outer most area it compromises about 15% of the adrenal gland and synthesizes mineralocorticoids [3-6]. The zona fasciculata is the middle area and compromises about 70% of the adrenal gland and synthesis the glucocorticoids one of which is corticosterone [3-6]. The zona reticularis the innermost layer and borders the medulla, it comprises about 7% of the adrenal gland and synthesizes the sex hormones [3-6]. Mitochondria are prevalent in both the medulla and the cortex, although their function is somewhat different in each. The mitochondria of the medulla are less prominent and their function is related to the energy process of the cell [9]. The mitochondria of the cortex are very prevalent and play a major role in steroidogenesis because the hydroxylases required for steroid synthesis are located at the inner mitochondrial membranes [10].

The normal structure of the adrenal gland has been well defined and will not be repeated in this text [3-6, 29].

Medulla

The medullary secretory cell membranes in both of the CDDP treated groups were ill-defined compared to the controls with an apparent decrease in the number of nuclei on days 3 and 8 in the 7mg/kg dose (Figure 2-3) and days 3

and 5 in the 9mg/kg dose (Figure 4-5). There was an apparent increase in the number of nuclei on day 5 in the 7mg/kg dose (Figure 1-2). The medullary capillaries in the 7mg/kg dose were enlarged, have accumulated cellular debris within the lumens and the endothelial lining is degenerating (Figure 1-2). The venous channels of the 9mg/kg dose (Figure 4-5) did not have the aforementioned structural damage. At the EM level the mitochondria of both groups demonstrated an increase in damage as the days progressed post-treatment (Figure 6-9). This damage was apparent by the rarefaction and cavitation of the mitochondria. The granules within the norepinephrine and epinephrine cells were swollen and reduced in numbers on day 3 with the 7mg/kg dose (Figure 6 -9). By days 5 and 8 there was little distinction between the norepinephrine and epinephrine cells. Usually they vary in darkness with the norepinephrine cells appearing more white (the light cells) and the epinephrine cells appearing darker (the dark cells). Granules were swollen on all days and appeared to start to increase in number by day 8 with the 7mg/kg dose (Figure 7). The granules in the norepinephrine and epinephrine cells in the 9mg/kg dose were almost completely depleted.

Cortex

The medullary capillaries extend into and through the zona reticularis on days 3 and 5 of both treatments (Figure 10 and 11). The zona reticularis of the 9mg/kg dose (Figure 11) and the zona fasciculata (Figure 10) of the 7mg/kg dose on days 5 and 8 demonstrated clear areas that are indicative of an increase in the number of lipid compartments and/or an increase in the number of

mitochondrial lipid like inclusions. At the EM level (7mg/kg dose only) the medullary capillaries on days 5 and 8 were enlarged with accumulated cellular debris and endothelial lining degeneration (Figure 12 and 13). There was observed a disruption of both the medullary and zona reticularis mitochondria, increasing with time. The number of lipids in the zona reticularis increased by 2 fold by day 8 in the 7mg/kg dose compared to that of the control. This increase in lipids verifies what was seen at the light microscopic level.

The zona reticularis cells (Figure 14 and 15) in both treatment groups showed an apparent decrease in the number of nuclei, with day 5 and 8 (7mg/kg) showing some increase compared to the earlier treatments. The zona reticularis (9mg/kg) and the zona fasciculata with both treatment groups and after various days of post-treatment (Figure 14-18) showed disruption of the secretory cells with large clear areas which as mentioned previous indicate an increase in the number of lipid compartments and/or in the number of mitochcondrial lipid like inclusions. Lipids (7mg/kg dose only) have decreased by day 3 with an increase (Figure 14 and 15) by day 8. The 9mg/kg dose has decreased lipids on both days 3 and 5, shown previously [19]. At the EM level (Figure 19-22) there was observed damage to the mitochondrial cristae with some recovery after day 5 and 8 in the 7mg/kg dose with no recovery with the 9mg/kg dose. The capillaries were enlarged with cellular debris in the lumen. There was also an increase in the number lipids with time in the 7mg/kg dose only (Figure 19 and 20), where both doses of cisplatin showed some lipids cases with increased compartment size.

The zona glomerulosa on day 5 in the 9mg/kg dose appeared to have decreased cytoplasm and nuclei compared to that of the control. The adrenal capsules were intact and appear to be unaffected by the treatment of CDDP at either dose (Figure 23 and 24).

Figure 1: Light Micrographs of Sections (2μm) of the Adrenal Gland of Control (A) and After 7mg/kg CDDP Treatment at (B) 3 Days, (C) 5 Days, (D) 8 Days.

The capillaries (V) in the medulla (M) are enlarged and extending into the zona reticularis (zr). The zona reticularis has enlarged capillaries on day3, the zona fasciculata (ZF) by day 5 and the zona glomerulosa (ZG) by day 8. Methylene blue stain. Bar = 2mm

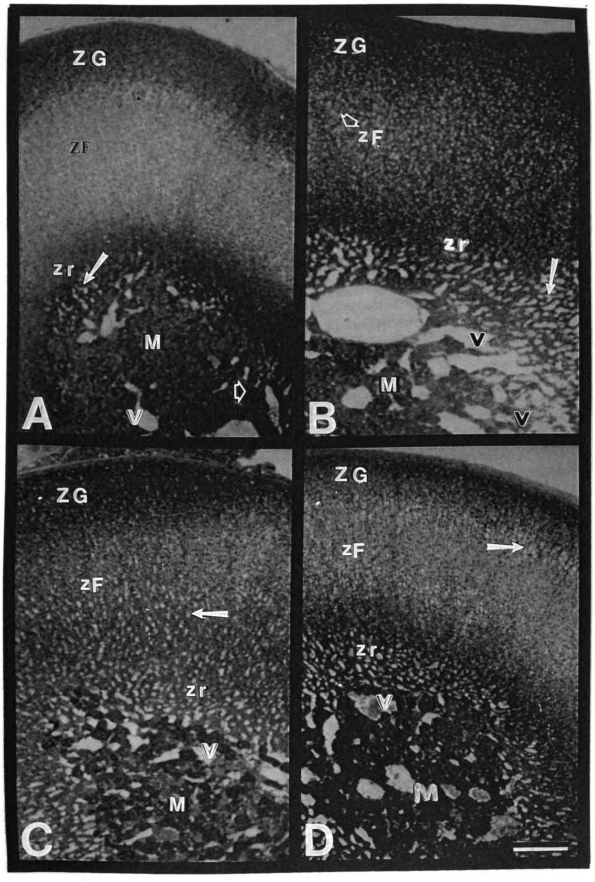

ZG
ZF
zr
M
V
A
ZG
zF
zr
V
M
V
B
ZG
zF
zr
V
M
C
ZG
zF
zr
V
M
D

Figure 2: Light Micrographs of Sections (2μm) of the Adrenal Medulla of Control (A) and After 7mg/kg CDDP Treatment at (B) 3 Days, (C) 5 Days, (D) 8 Days.

The secretory cell (arrows) membranes are not as defined, there appears to be fewer nuclei on days 3 and 8 with an increase on day 5. Note the enlarged capillaries (V) with red blood cells (R) and cellular debris within the lumen. The areas enclosed by square enclosures are enlarged in Figure 2. Hematoxylin and eosin stain. Bar = 0.02mm

A
B
C
D
V
V
V
V
V
V
R
R

Figure 3: Light Micrographs Showing the Magnified View of Various Cellular Changes Before (A) and After CDDP (7mg/kg) Treatment at (B) 3 Days, (C) 5 Days and (D) 8 Days.

The cell membranes are not as defined and the appearance of fewer nuclei (arrow) on day 3 and 8. The capillaries (V) are enlarged with red blood cells (R) and cellular debris with in the lumen. Hematoxylin and eosin stain. Bar = 0.0.006mm

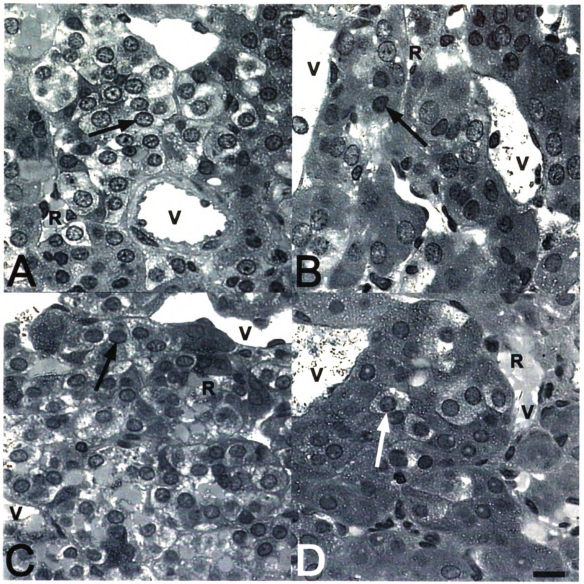

A
R
V
B
V
R
V
C
V
R
V
D
V
R
V

Figure 4: Light Micrographs of Sections (2μm) of the Adrenal Medulla of Control (A) and After 9mg/kg CDDP Treatment at (B) 3 Days and (C) 5 Days.

The medullary cell membranes are losing definition and there is a decrease in the number of nuclei (arrows) in the post-treatment. At this magnification there is no apparent damage to the capillaries (V). The areas enclosed by square enclosures are enlarged in Figure 5. Hematoxylin and eosin stain.
Bar = 0.02mm

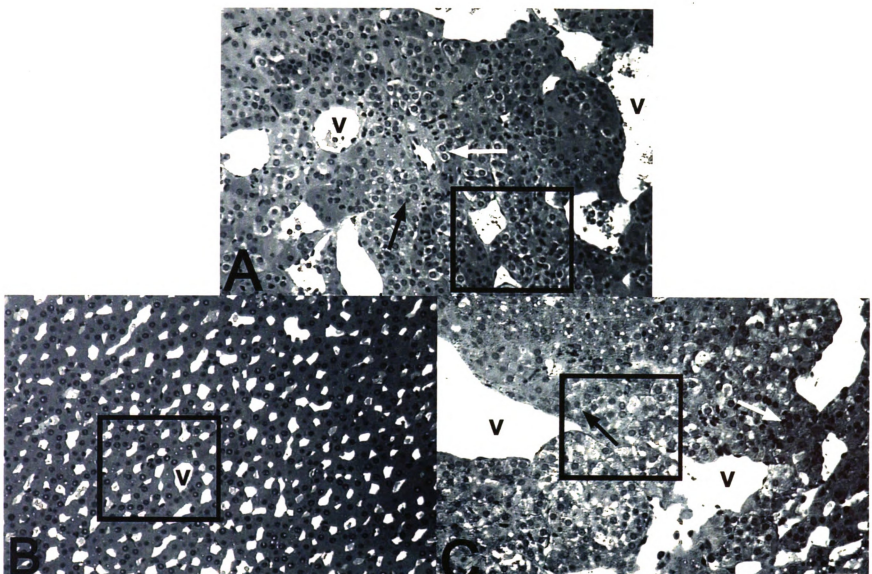

A
v
v
B
v
C
v
v

Figure 5: Light Micrographs Showing the Magnified View of Various Cellular Changes Before (A) and After CDDP (9mg/kg) Treatment at (B) 3 Days and (C) 5 Days.

Day 3 has no membrane distinction between the medullary cells compared to that of day 5. Both day 3 and 5 appear to have a decrease in the number of nuclei (arrows). The capillaries (V) show no structural damage. Hematoxylin and eosin stain. Bar = 0.006mm

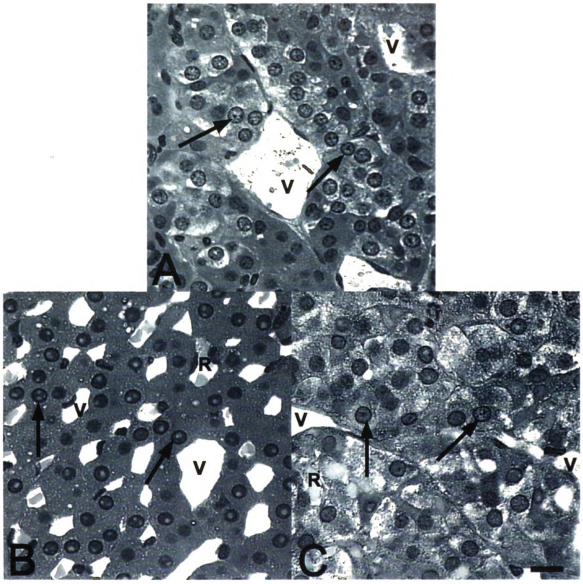

V
V
A
R
V
V
B
V
R
V
C

Figure 6: Electron Micrographs of Medullary Region of the Adrenal Gland of Control (A) and After 7mg/kg CDDP Treatment at (B) 3 Days, (C) 5 Days and (D) 8 Days.

The nuclei (N) and myelinated nerves (n) that are present show no change. The mitochondria (M) show increasing damage with time. This is evident in the cristae (arrowhead) or cavitation of the mitochondrial cells (no cristae). Chromaffin cells both norepinephrine (back arrow) and epinephrine (white arrow) have granules that appear swollen by day 3. By day 5 there is less differentiation between the norepinephrine (black arrow) and epinephrine (white arrow) cells, with fewer granules within the epinephrine cells. The medullary capillaries (V) are enlarged with red blood cells (R) in the lumen and the endothelial lining appears degenerated. By day 8 there are fewer granules in the norepinephrine cells (white arrow) while the epinephrine granules (black arrow) are swollen.
Bars = 400μm

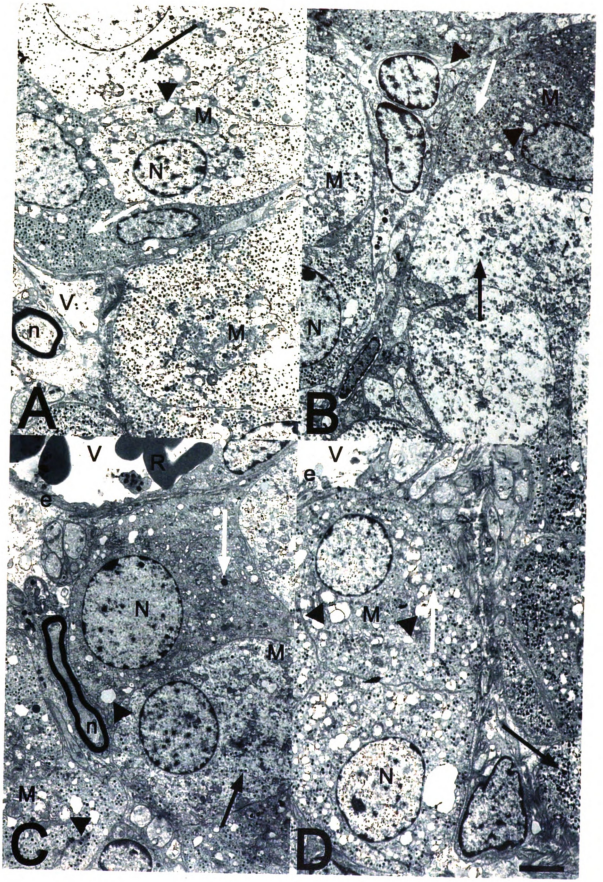

A
M
N
V
n
M
B
M
M
N
V
R
e
V
e
N
M
n
M
M
C
N
D

Figure 7: Selected Areas from Figure 6 Have Been Enlarged to Show Changes Before (A) and After CDDP (7mg/kg) Treatment at 3 Days (B), 5 Days (C) and 8 Days (D) in the Medulla.

Arrows (black) norepinephrine cell, (white) epinephrine cell, N = nucleus, n = myelinated nerve, V = capillary, M = mitochondria and arrowhead (black) cavitated mitochondria. Bars = 150μm

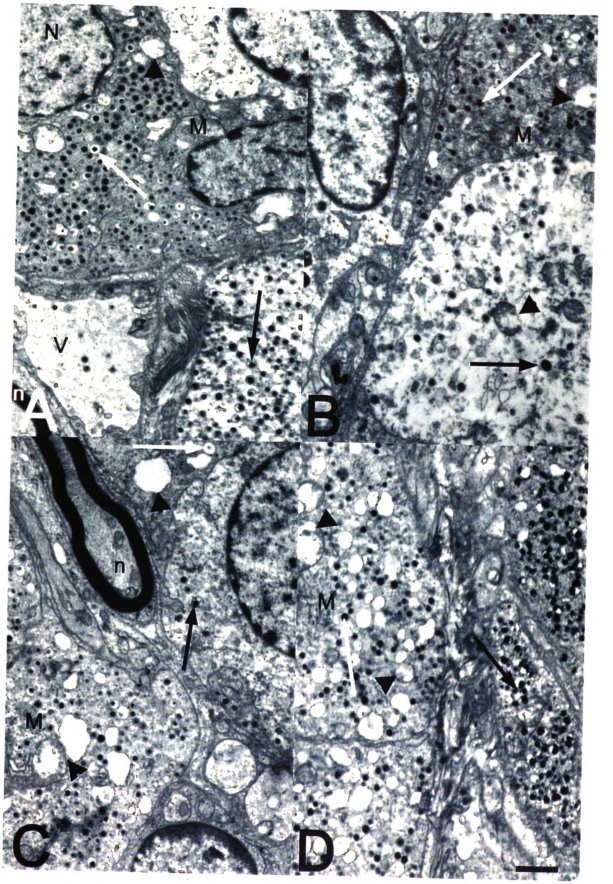

N
M
V
A
M
B
n
M
C
M
D

Figure 8: Electron Micrographs of Medullary Region of the Adrenal Gland of Control (A) and After 9mg/kg CDDP Treatment at (B) 3 Days, (C) 5 Days and (D) 8 Days.

The nuclei (N) show no change. There is progressing disruption of the mitochondria as shown by the cavitation (arrowhead) of the cell. This disruption appears to be more prevalent in the norepinephrine cells than in the epinephrine cells. The norepinephrine (black arrow) and epinephrine (white arrow) granules appear to be decreased in number as time progresses. Bar = 400μm

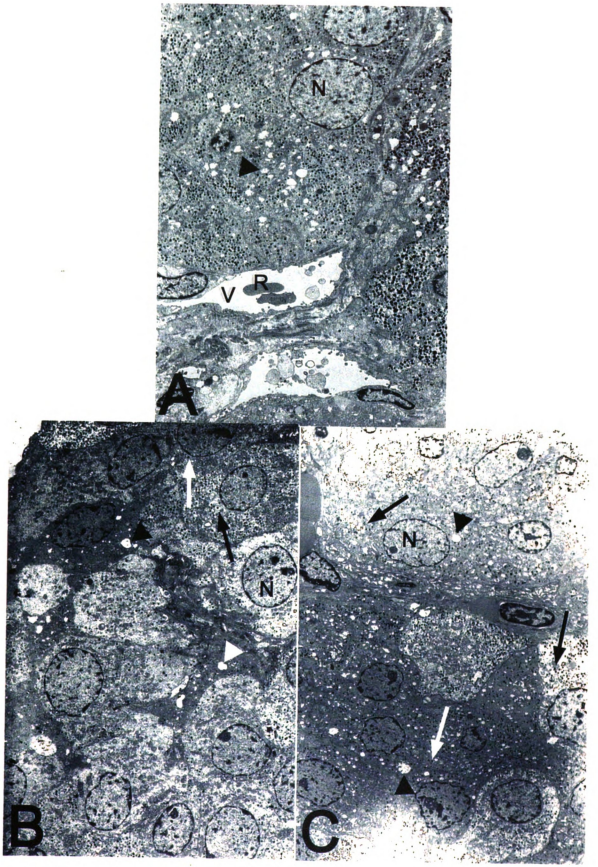

N
V
R
A
N
B
N
C

Figure 9: Selected Areas from Figure 8 Have Been Enlarged to Show Changes Before (A) and After CDDP Treatment at (B) 3 Days and (C) 5 Days, in the Medulla.

Arrows (black) norepinephrine cell, (white) epinephrine cell, N = nucleus, M = mitochondria and arrowhead cavitated mitochondria. Bar = 150μm

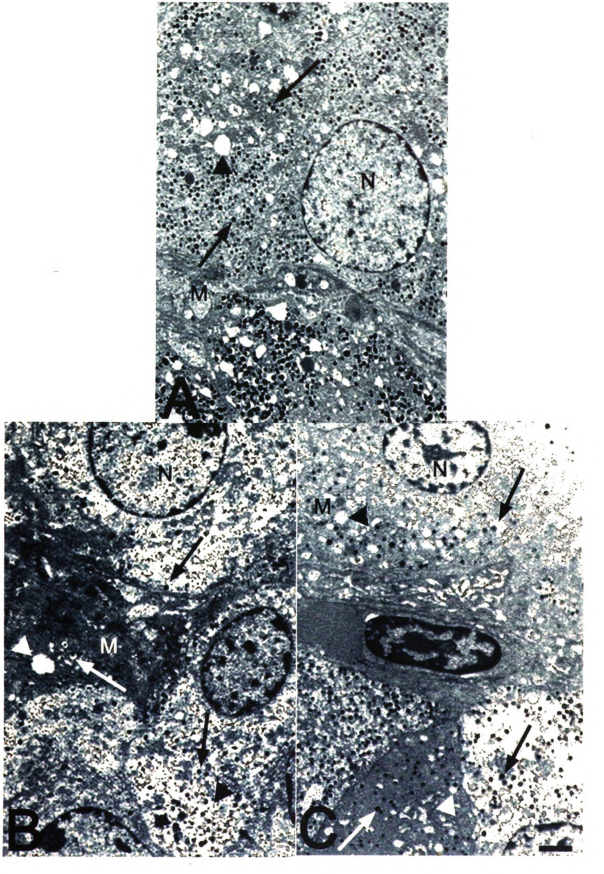

N
M
A
N
M
B
N
M
C

Figure 10: Light Micrographs of Sections (2μm) of the Medulla, Zona Reticularis and Zona Fasciculata of the Adrenal Gland of Control (A) and 7mg/kg CDDP Treatment at (B) 3 Days, (C) 5 Days and (D) 8 Days.

The medullary (M) cell membranes are less defined as in previous with an apparent decrease in the number of nuclei. Note the capillaries (V) that are extending through the zona reticularis (ZR). The zona fasciculata (ZF) has many clear areas (smaller areas) that are indicative of a higher number of lipid compartments and/or an increased number of mitochondrial lipid like inclusions on days 5 and 8 compared to that of day 3 and the control. Hematoxylin and eosin stain. Bar = 0.02mm

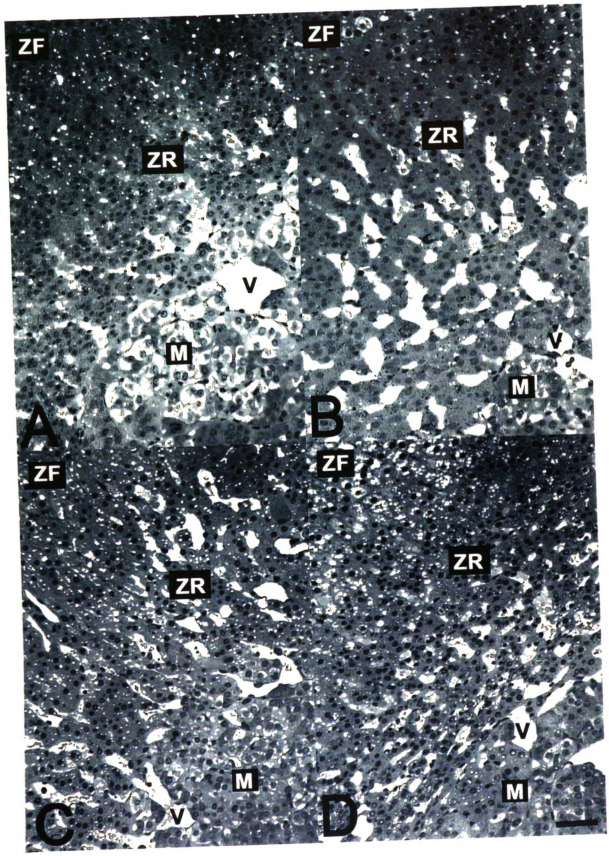

ZF
ZR
V
M
A
ZF
ZR
V
M
B
ZF
ZR
M
V
C
ZF
ZR
V
M
D

Figure 11: Light Micrographs of Sections (2μm) of the Medulla and the Zona Reticularis of the Adrenal Gland of Control (A) and After 9mg/kg CDDP Treatment at (B) 3 Days, (C) 5 Days and (D) 8 Days.

The medullary (M) cell membranes have very little to no distinction on day 3, with day 5 showing an improvement, there appears to be a decrease in the number of nuclei from the control. The medullary capillaries (V) have extended into the zona reticularis (ZR) by day 5. The zona reticularis has increased damage as post-treatment time progresses, with the appearance of small circular clear areas that are indicative of and increase in the number of lipid compartments and an increase in the number of mitochondrial lipid like inclusions. Hematoxylin and eosin stain. Bar = 0.02mm

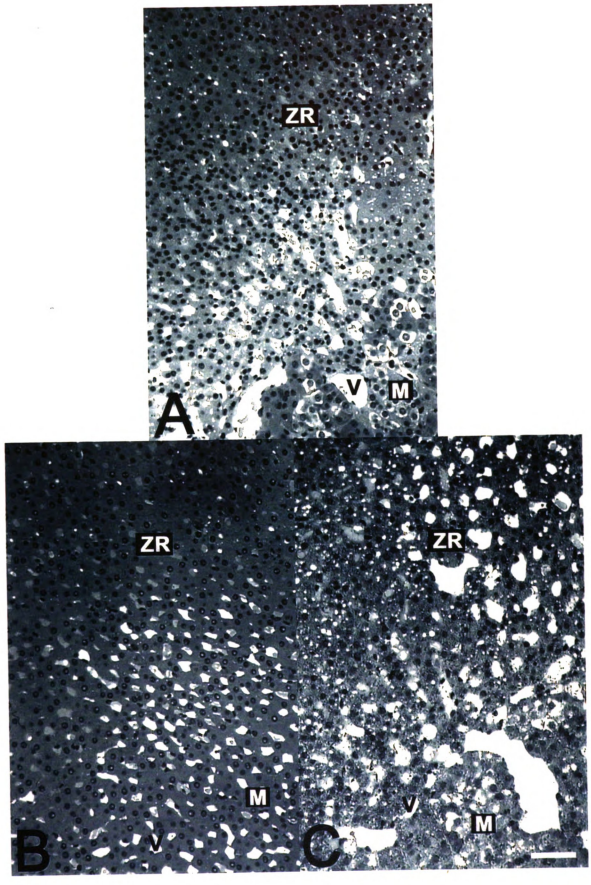

ZR
V
M
A
ZR
ZR
M
V
M
V
B
C

Figure 12: Electron Micrographs of the Medulla and Zona Reticularis of the Cortex of the Adrenal Gland of Control (A) and After 7mg/kg CDDP Treatment at (B) 3 Days, (C) 5 Days and (D) 8 Days.

The nuclei (N) in the medulla show no change in structure. The capillaries (V) are enlarged, with a red blood cell (R) present in the lumen of the control and an accumulation of cellular debris at days 5 and 8. The endothelial lining (e) has increased degeneration as time post-treatment progresses. The mitochondria (arrowheads) of the medulla have increased damage to their cristae time post-treatment progresses. The norepinephrine cells appear to have fewer granules (black arrow) at day 3 with an increase in the number of granules at day 5 and 8. All the granules within the norepinephrine cells from days 3-8 are swollen. The zona reticularis of the cortex, which consists of many mitochondria (arrowheads) shows increasing, damage as the time progresses. The number of lipids (L) has increased 2 fold by day 8 from the control. Bar = 400μm

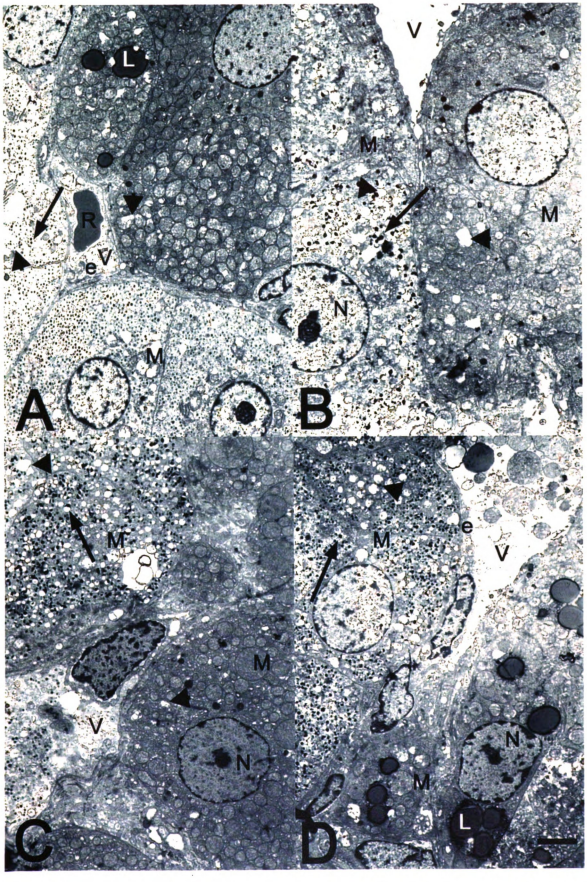

L
R
e
V
M
A
V
M
M
N
B
M
e
M
V
M
V
N
C
M
N
L
D

Figure 13: Selected Areas from Figure 12 Have Been Enlarged to Show Changes Before (A) and After CDDP (7mg/kg) Treatment at 3 Days (B), 5 Days (C) and 8 Days (D) in the Medulla and Zona Reticularis.

Arrowheads = cavitated mitochondria, arrows = norepinephrine granules, M = mitochondria, L = lipids, G = golgi complex, N = nucleus, V = capillaries, R = red blood cell and e = endothelial lining. Bar = 150μm

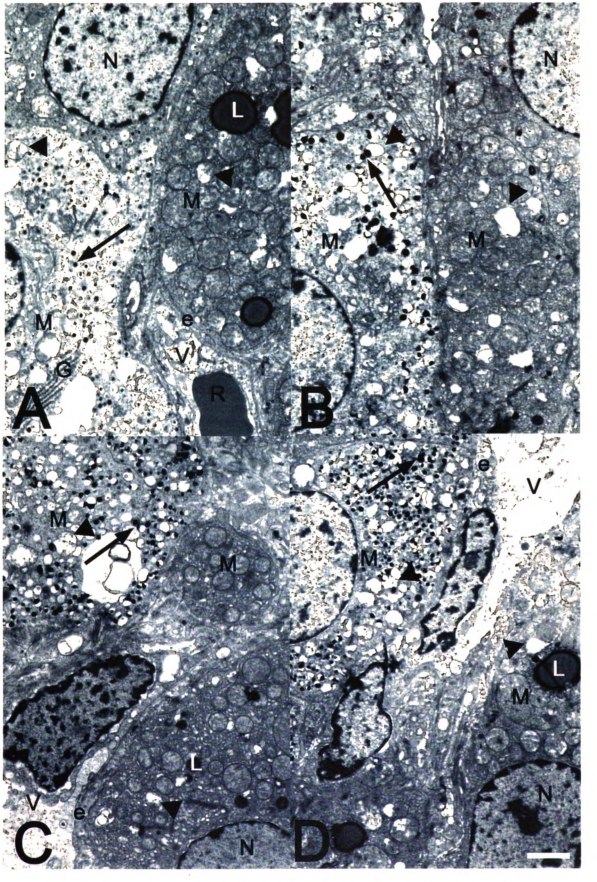

N
L
M
M
e
V
G
R
A
N
M
M
B
M
M
e
V
L
C
L
V
e
N
D
N

Figure 14: Light Micrographs of Sections (2μm) of the Zona Reticularis and Zona Fasciculata of the Adrenal Gland of Control (A) and After 7mg/kg CDDP Treatment at (B) 3 Days, (C) 5 Days and (D) 8 Days.

The network like clumps of secretory cells of the zona reticularis (ZR) are less defined and appear to have fewer nuclei on day 3 compared to days 5, 8 and the control. Days 5 and 8 appear to have an increase in the number of nuclei but no apparent improvement in the cellular organization. The narrow chords of secretory cells of the zona fasciculata (ZF) in all treatment groups show disruption with clear areas (small areas) (arrows) that indicate an increase in lipid compartments and an increase in the number of mitochondrial lipid like inclusions compared to that of the control. The areas enclosed by square enclosures are enlarged in Figure 15. Hematoxylin and eosin stain. Bar = 0.02mm

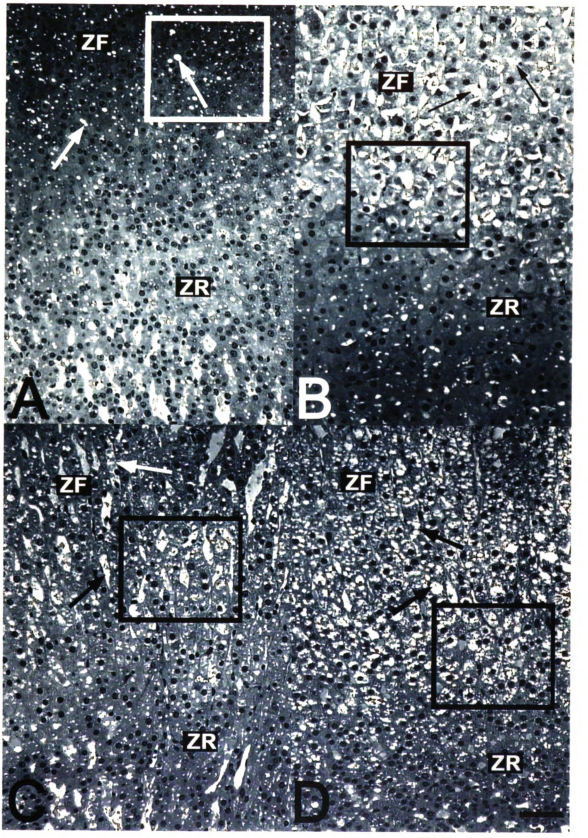

ZF
ZR
A
ZF
ZR
B
ZF
ZR
C
ZF
ZR
D

Figure 15: Light Micrographs Showing the Magnified view of Various Cellular Changes Before (A) and After CDDP (7mg/kg) Treatment at (B) 3 days, (C) 5 Days and (D) 8 Days..

There appears to be a decrease in the number of nuclei (arrows) from day 3-8, although day 5 and 8 have an increased number of nuclei compared to day 3. There is an increase in the number of small round clear areas as time post-treatment progresses. These areas are indicative of an increase in the number of lipid like compartments and an increase in the number of mitochondrial lipid like inclusions. Note the larger elongated clear areas (Va) which are capillaries some with red blood cells (R) in the lumens. Hematoxylin and eosin stain.
Bar = 0.007mm

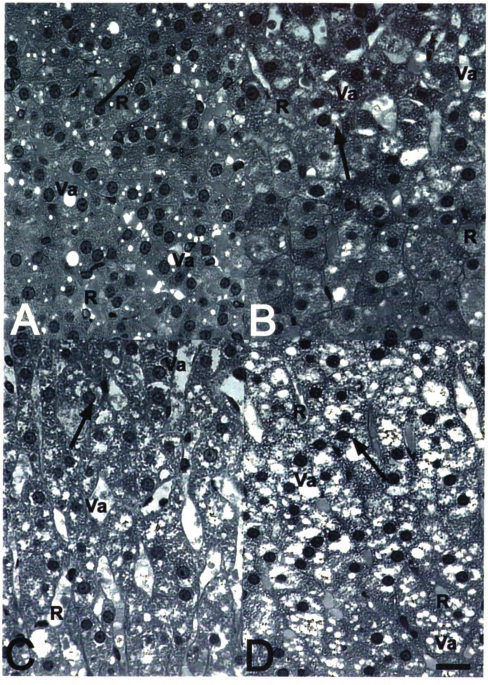

A
B
C
D
R
Va
Vz

Figure 16: Light Micrographs of Cryo-Sections (10μm) of the Zona Fasciculata and Zona Glomerulosa of the Adrenal Gland of Control (A) and After 7mg/kg CDDP Treatment at (B) 3 Days, (C) 5 Days and (D) 8 Days.

Clear areas within the zona fasciculata denote lipid deposition. There is a depletion of lipids on day 3, with an increasing number on day 5 and 8. Note the enlarged capillaries (arrow) on day 5 and 8. Sudan black stain. Bar = 0.2mm

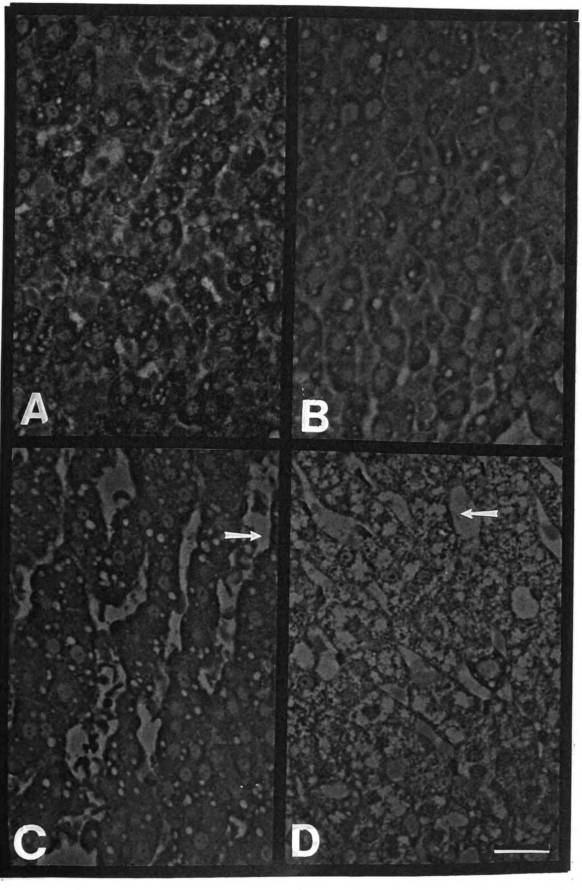

A
B
C
D

Figure 17: Light Micrographs of Sections (2μm) of the Zona Reticularis and Zona Fascicularis of the Adrenal Cortex of Control (A) and After 9mg/kg CDDP Treatment at (B) 3 Days and (C) 5 Days.

The zona reticularis (ZR) of the treated groups appear to have fewer nuclei. There is an increase in the number of small round clear areas (arrows) in both the zona reticularis and the zona fasciculata (ZF) of the treated groups with day 5 having a very high percentage of these areas. These areas indicate an increase in the number of lipid compartments and an increase in the number of mitochondrial lipid like inclusions. The areas enclosed by square enclosures are enlarged in Figure 18. Hematoxylin and eosin stain. Bar = 0.02mm

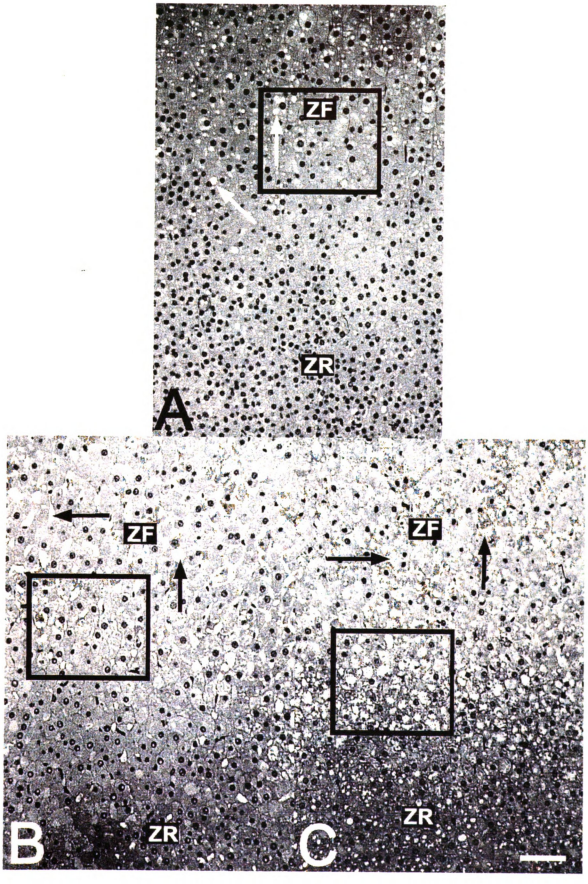

ZF
ZR
A
ZF
ZF
B
ZR
C
ZR

Figure 18: Light Micrographs Showing the Magnified View of Various Cellular Changes Before (A) and After CDDP (9mg/kg) Treatment at (B) 3 Days and (C) 5 Days.

The chords of secretory cells that make up the zona fasciculata are not as defined as the controls, and appear to have a decrease in the number of nuclei. There is a decrease in the number of small clear areas (arrow) as time progresses. These areas are indicative of lipid compartments. Note the larger elongated clear areas (Va) capillaries that are increasing in size, some with red blood cells within the lumen (R). Hematoxylin and eosin stain. Bar = 0.01mm

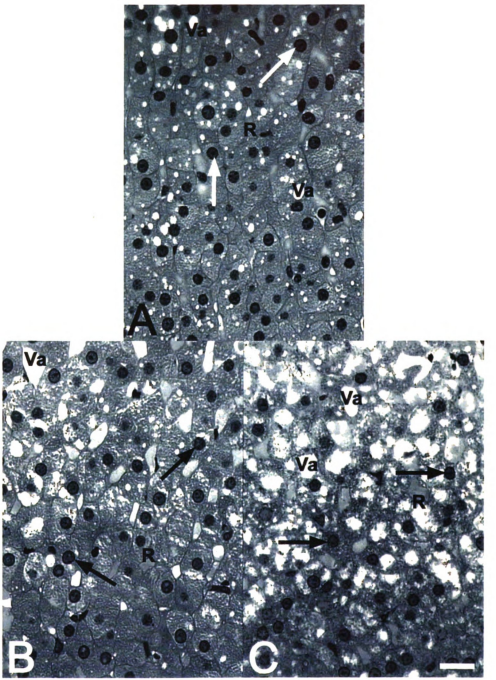

Va
R
Va
A
Va
Va
Va
R
R
B
C

Figure 19: Electron Micrographs of the Zona Fasciculata of the Adrenal Cortex of Control (A) and After 7mg/kg CDDP Treatment at (B) 3 Days, (C) 5 Days and (D) 8 Days.

The nuclei (N) show no alteration in structure. The mitochondria (M) have increased disruption to their cristae through day 5 with less apparent disruption on day 8. There is an increase in the number of lipids (L) as the post-treatment time progresses, with some showing an increase in the lipid compartment. The controls capillaries (S) has a red blood cell (R) within its lumen, but does not have the enlarged size or accumulation of cellular debris within the lumen, including platelets (p) that is seen in the treated. The endothelial lining (e) of the sinusoids are degenerate (arrow) in all treated groups. Bar = 400μm

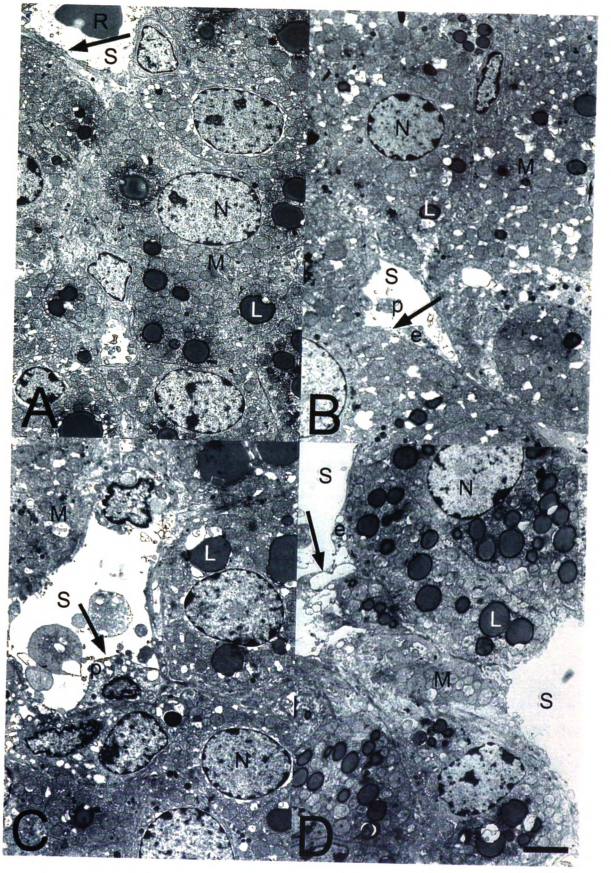

R
S
N
M
L
A
N
M
L
S
p
e
B
M
L
S
p
N
C
S
e
N
L
M
S
D

Figure 20: Selected Areas from Figure 19 Have Been Enlarged to Show Changes Before (A) and After CDDP (7mg/kg) Treatment at 3 Days (B), 5 Days (C) and 8 Days (D).

N = nucleus, M = mitochondria, arrowhead = cavitated mitochondria, arrow = mitochondria with damaged cristae, L = lipids, S = capillaries, e = endothelial lining and p = platelets. Bar = 150μm

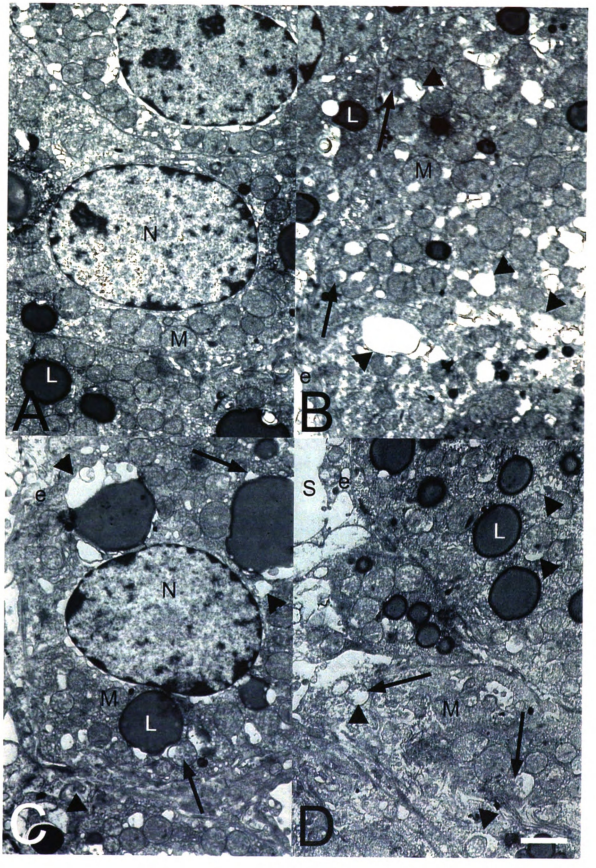

N
M
L
A
L
M
e
B
e
N
M
L
C
S
e
L
M
D

Figure 21: Electron Micrographs of the Zona Fasciculata of the Adrenal Cortex of Control (A) and After 9mg/kg CDDP Treatment at (B) 3 Days and (C) 5 Days.

The nuclei (N) show no apparent difference. The mitochondria (M) have irregular cristae or no cristae (arrowhead). The disruption of the mitochondria increases as time post-treatment progresses. There are less lipids (L) with the lipid size increasing by day 5. Bar = 400μm

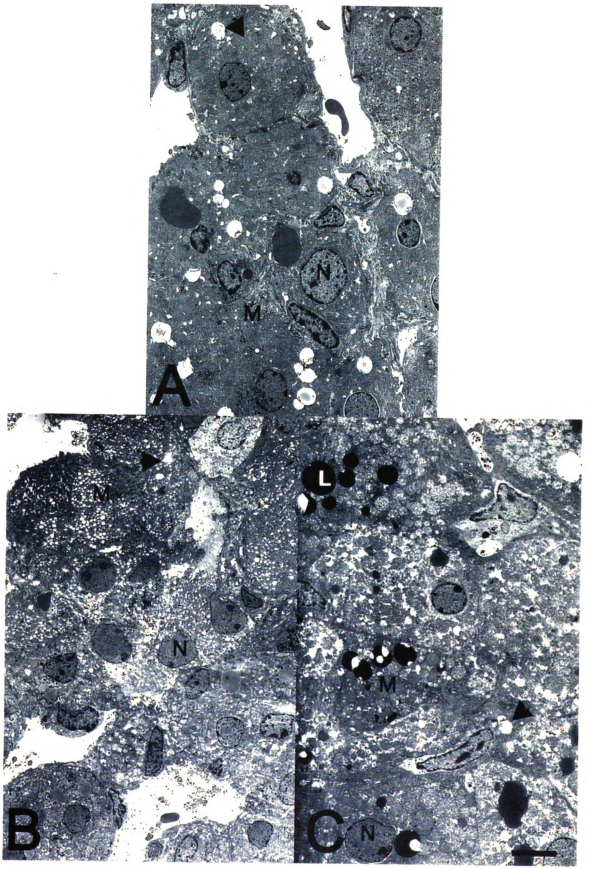

A
N
M
B
M
N
C
L
M
N

Figure 22: Selected Areas from Figure 21 Have Been Enlarged to Show Changes Before (A) and After CDDP Treatment at 3 Days (B) and 5 Days (C).

N = nucleus, M = mitochondria, arrowhead = cavitated mitochondri, L = lipids.
Bar = 150μm

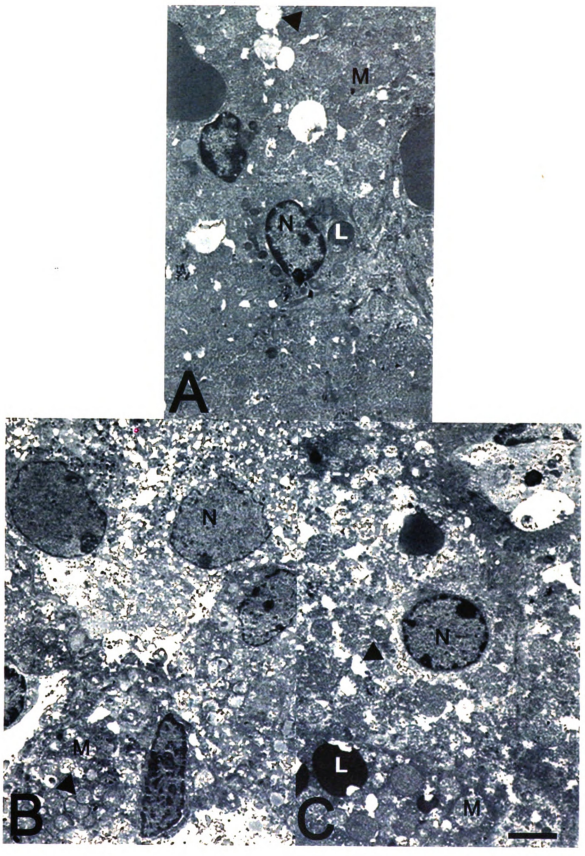

M
N
L
A
N
N
M
L
B
C
M

Figure 23: Light Micrographs of Sections (2μm) of the Zona Fasciculata and Zona Glomerulosa of the Cortex of Control (A) and After 7mg/kg CDDP Treatment at (B) 3 Days, (C) 5 Days and (D) 8 Days.

As described previously the zona fasciculata (ZF) appears to have a decreased number of nuclei (arrows) on days 3-8, although days 5 and 8 appear to have increased nuclei compared to day 3. There is an increased number of elongated clear areas (arrowheads) enlarged capillaries from the control. There is also an increased number of small round clear areas on days 5-8 which indicate an increase in the number of lipid compartments and an increase in the number of mitochondrial lipid like inclusions. There no apparent difference between groups in the zona glomerulosa (ZG) or the capsule (Cap). Hematoxylin and eosin stain. Bar = 0.02mm

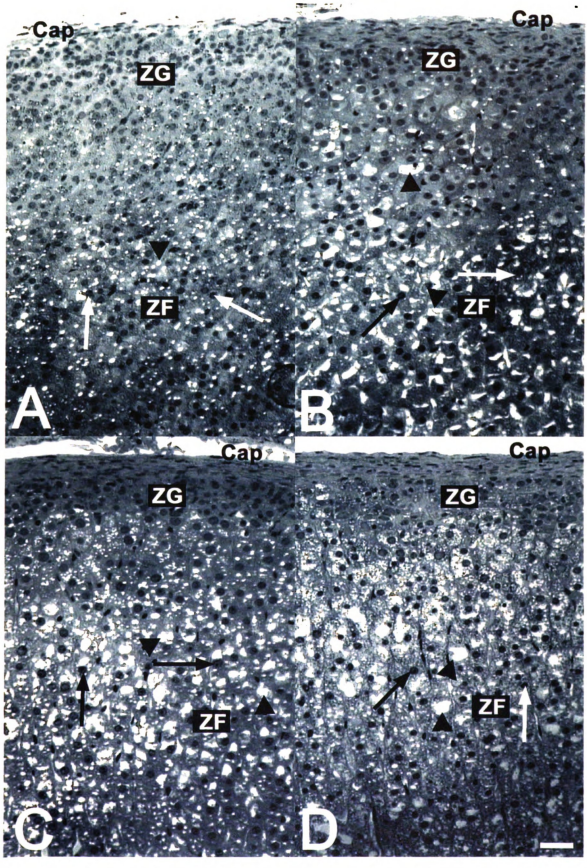
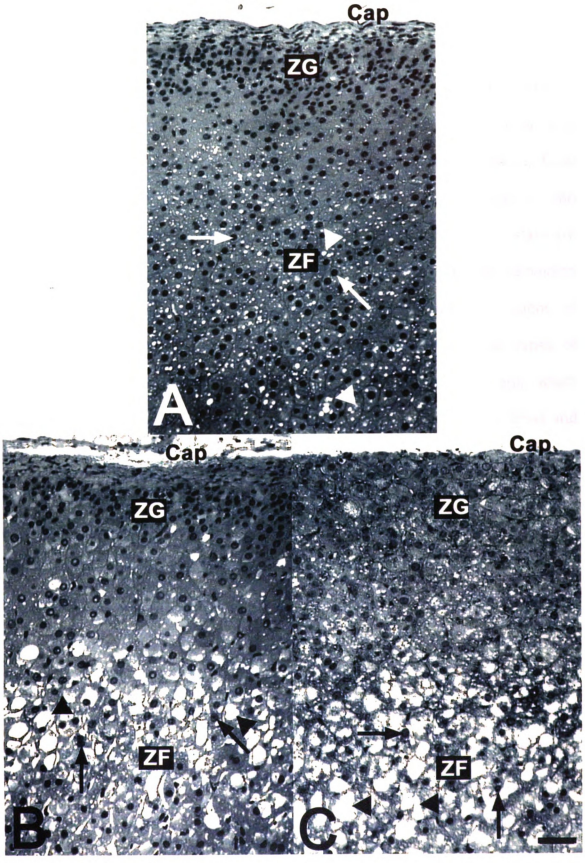

Cap
ZG
ZF
A
Cap
ZG
ZF
B
Cap
ZG
ZF
C
Cap
ZG
ZF
D

Figure 24: Light Micrographs of Sections (2μm) of the Zona Fasciculata and Zona Glomerulosa of the Adrenal Cortex of Control (A) and After 9mg/kg CDDP Treatment at (B) 3 Days and (C) 5 Days.

The zona fasciculata (ZF) has increasing disruption to its secretory cells and appears to have fewer nuclei (arrows) as the time post-treatment progresses. There are a large number of elongated clear areas (arrowheads) enlarged capillaries compared to the control, which are increasing as time progresses. There is a decrease in the number of small round clear areas that are indicative of a decrease in the number of lipid compartments. The capillaries are starting to enlarge and also extend into the zona glomerulosa (ZG) by day 5 with an apparent decrease in the number of nuclei compared to day 3 and the control. The capsules (Cap) are intact with no apparent change in structure. Hematoxylin and eosin stain. Bar = 0.0


Cap
ZG
ZF
A
Cap
ZG
ZF
B
Cap
ZG
ZF
C

DISCUSSION

A number of drugs and noxious chemicals are know to alter the structural integrity of the adrenal gland by destruction (necrosis and hemorrhage), degeneration (senile nodular hypertrophy, telangiectasia, focal fatty changes), adaptive reactions (phospholipid lysomal inclusion) and neoplastic alterations [8, 12, 30]. The medullary mitochondria cristae are disrupted with the secretory cells having fewer granules, with the remaining granules being swollen. The capillaries are enlarged and have signs of endothelial lining degeneration. It has been suggested that some types of cortical endothelial injury may be secondary to the medullary damage, which subsequently releases catecholamine granules into the medullary capillaries and the cortical capillaries [8, 20, 31]. Cisplatin is known to cause severe telangiectasia and loss of the lipid vesicles from the zonae fasciculata and reticularis shortly after treatment and at the9mg/kg dose for the duration of the experiment, then increasing as time progresses post-treatment [18, 19]. This occurrence is also seen with other chemotherapeutic drugs [13], and toxic chemicals [21, 22, 25, 26, 32]. The loss of lipid vesicles could stimulate the system to retrieve new cholesterol for steroid production, which in rats is mainly from an exogenous source [33]. Lipids are necessary for the synthesis or steroid hormones because they carry the cholesterol that will be converted into pregnenolone [8, 11, 29, 34]. The mitochondria are the most susceptible and are altered to the point of being abnormal. The mitochondrial compartment is

enlarged with the matrix and inner membranes being completely disrupted. Other studies have reported these same results with chemotherapeutic agents [13, 21] other toxic chemicals ([16, 22] and hormones such corticotropin releasing hormone and adrenocorticotropic hormone [11, 15, 24, 35]. Cisplatin when hydroxylated is known to uncouple oxidative phophorylation in isolated mitochondria, a necessary part of the mitochondrial function [36]. The mitochondria of the zona fasciculata are the site of corticosterone synthesis thus any disruption can effect the concentration of corticosterone [6, 9-11, 20]. The disruption of the zona fasciculata at first may allow for release of corticosterone into the system, but then can interfere with steroid production by disrupting the contact between the smooth endoplasmic reticulum, mitochondria and the lipids. This contact is very important for the synthesis of steroids by allowing exchange of enzymes, cholesterol and intermediate products between these areas. We have previously shown that circulating corticosterone concentration is increased on day 1 and again at day 5 post-treatment in the lower dose of cisplatin (chapter 1) with corticosterone (ng/ml) output from adrenal explants being relatively the same. This is most likely due to the release of corticosterone due to an increase in stress and structural damage caused by CDDP at first and then to the increase in steroid synthesis seen by the increase of lipids in the cortex. Whereas corticosterone concentration decreased in the higher dose of CDDP on day 1 with an increase at day 3, this could be do to the difference in dose itself. The animals treated with the higher dose emulate a much worse overall condition.

Cisplatin appears to affect the adrenal gland directly by endothelial damage, which then causes further damage to the cell, and indirectly by degranulation of medullary and cortical secretory cells. With the release of epinephrine and corticosterone into the circulatory system and decrease in the lipid droplets that store cholesterol esters for steroidsynthesis [37] post-treatment with lipid recovery as days progress post-treatment. To determine the mechanism that alters adrenal gland function and corticosterone release, further studies are needed using adrenal organ culture or adrenal cell culture.

CONCLUSIONS

Cisplatin affects the adrenal gland structure by disruption of mitochondria and secretory cells, enlargement and endothelial lining degradation of the venous channels in both the medulla and cortex and by decreasing and then increasing lipid droplets in the cortex. These structural alterations then affect the hormonal release and synthesis in both the medulla and cortex changing the circulating concentration and secretion into media by adrenal glands of corticosterone. Further studies are required using adrenal organ culture and/or adrenal cell culture to verify these findings.

LIST OF REFERENCES

1. Levin, J.A., *Adrenal medulla*, in *The Adrenal Gland*, P.J. Mulrow, Editor. 1986, Elsevier: New York. p. 1-32.
2. Bourne, G.H., *Comparative anatomy and embryology of the adrenal glands*, in *The Adrenal Cortes*, H.D. Moon, Editor. 1961, Paul B. Hoeber, Inc.: New York. p. 1-22.
3. Greep, R.O., *The struture of the adrenal cortex*, in *The Adrenal Cortex*, H.D. Moon, Editor. 1961, Paul B. Hoeber, Inc.: New York. p. 23-45.
4. Long, J.A., *The adrenal gland*, in *Histology*, L. Weiss and R.O. Greep, Editors. 1977, McGraw-Hill: New York. p. 1099-118.
5. Vinson, G.P., B.J. Whitehouse, and J.P. Hinson, *The adrenal cortex.* 1992, Englewood Cliffs: Prentice Hall. 223.
6. Vivian, H.T.J., *The adrenal gland.* 1992, New York: Raven Press.
7. Selye, J., *The general adaptation syndrome.* J Clin Endocrinol, 1946. **6**: p. 117-230.
8. Ribelin, W.E., *The effects of drugs and chemicals upon the structure of the adrenal gland.* Fundamental and Applied Toxicology, 1984. **4**: p. 105-19.
9. Bornstein, S.R., *et al., Structure and dynamics of adrenal mitochondria following stimulation with corticotripin releasing hormone.* The Anotomical Record, 1992. **234**: p. 255-62.
10. Simpson, E.R. and M.R. Waterman, *Regulation of the syntheisi of steroidogenic enzymes in adrenal cortical cells by ACTH.* Ann Rev Physiol, 1988. **50**: p. 427-40.
11. Boshier, D.P., P. Rebuffat, and G.G. Nussdorfer, *Cellular responses of the rat adrenal zona fasciculata to acute acth stimulation: a morphometric study.* Endocrine Research, 1990. **16**(3): p. 377-89.
12. Szabo, S., *et al., Thioguanine-induced adrenocortical necrosis and its prevention by hypophysectomy in the rat.* Exp Mol Path, 1977. **26**: p. 155-68.
13. de Castro, C.R., *et al., Ultrastructural effects of nifurtimox on rat adrenal cortex related to reductive biotransformation.* Experimental and Molecular Pathology, 1990. **52**: p. 98-108.
14. Malendowicz, L.K., *Sex differences in adrenocortical structure and function. XXIV. Comparative morphometric studies on adrenal cortex of intact mature male and remale rats of different strains.* Cell Tissue Res, 1987. **249**: p. 443-49.
15. Mazzocchi, G., *et al., Investigations on the turnover of adrenocortical mitochondria. IV. A stereological study of the effect of chronic treatment with ACTH on the size and number of rat zona reticularis mitochondria.* Cell Tissue Res, 1976. **168**(1): p. 1-9.

16. Durham, S.K. and A. Brouwer, *3,4,3',4'-Tetrachlorobiphenyl distribution and induced effects in the rat adrenal gland.* Laboratory Investigation, 1990. **62**(2): p. 232-9.
17. Mazzocchi, G., *et al.*, *Effects of testosterone on the zona fasciculata of the male rat adrenal cortex. A correlated stereological and biochemical study.* J Submicrosc Cytol, 1983. **15**(4): p. 991-1005.
18. Miller, C.M., S.K. Aggarwal, and K.K. Klomparens, *Cisplatin-induced changes in the adrenal of the rat*, in *Proc Electron Microscope Society of America*, G.W. Bailey, J. Bentley, and J.A. Small, Editors. 1992, San Francisco Press: San Francisco. p. 1652-3.
19. Aggarwal, S.K., *et al.*, *Cisplatin-induced peptic ulcers, vagotomy, adrenal and calcium modulation.* Anticancer Drugs, 1994. **5**(2): p. 177-93.
20. Bornstein, S.R. and M. Ehrhart-Bornstein, *Ultrastructural evidence for a paracrine regulation of the rat adrenal cortex mediated by the local release of catecholamines from chromaffin cells.* Endocrinology, 1992. **131**(6): p. 3126-28.
21. de Castro, C.R., E.G.D. de Toranzo, and J.A. Castro, *Benznidazole-induced ultrastructural alterations in rat adrenal cortex. Mechanistic studies.* Toxicology, 1992. **74**: p. 223-32.
22. Robba, C., G. Mazzocchi, and G.G. Nussdorfer, *Effects of a prolonged treatment with aminoglutethimide on the zona fasciculata of rat adrenal cortex: a morphometric investigation.* Cell Tissue Res, 1987. **248**(3): p. 519-25.
23. Robba, C., *et al.*, *Opposed effects of chronic prolactin administration on the zona fasciculata and zona reticularis of the rat adrenal cortex: an ultrastructural stereological study.* J Submicrosc Cytol, 1985. **17**(2): p. 255-61.
24. Malendowicz, L.K., *et al.*, *Analysis of the preventive action of ACTH on dexamethasone-induced adrenocortical atrophy in the rat.* Cytobios, 1992. **71**: p. 191-99.
25. Mazzocchi, G., *et al.*, *Long term effects of the hypocholesterolaemic drug 4-aminopyrazolo- pyrimidine on the zona fasciculata of the rat adrenal cortex.* J Anat, 1986. **149**: p. 1-9.
26. Magalhaes, M.M., *et al.*, *The effects of monensin on golgi complex of adrenal cortex and steroidogenesis.* Tissue and Cell, 1991. **23**(2): p. 209-15.
27. Malendowicz, L.K., *Comparative studies on the effect of 4-APP(4-aminopyrazolopyrimidine) on hamster and rat adrenal cortex.* Exp Toxic Pathol, 1992. **44**: p. 134-37.
28. Lullmann, G. and R. Lullmann-Rauch, *Tamoxifen-induced generalized lipidosis in rats subchronically treated with high doses.* Toxicology and Applied Pharmacology, 1981. **61**: p. 138-46.
29. Rhodin, J.A.G., *The ultrastructure of the adrenal cortex of the rat under normal and experimental conditions.* J Ultrastructure Research, 1971. **34**: p. 23-71.

30. Szabo, S. and H. Selye, *Effects of phenobarbital and steroids on the adrenal apaplexy produced by acrylonitrile in rats.* Endocrinol Exp, 1972. **6**: p. 14L.
31. Szabo, S. and E.S. Reynolds, *Structure-activity relationships for ulecerogenic and adrenocorticolytic effects of alkyl nitriles, amines and thiols.* Environ Haskk Perspect, 1975. **11**: p. 135-40.
32. Robba, C., *et al.*, *Effects of prolonged treatment with cyanoketone on the zona fasciculata of rat adrenal cortex. A combined morphometric and biochemical study.* Cell Tissue Res, 1987. **250**(3): p. 599-605.
33. Boyd, G.S., *et al.*, *Cholesterol metabolism in the adrenal cortex.* J Steroid Biochem, 1983. **19**: p. 1017-27.
34. Rapp, j.P., *Adrenal steroid biosynthesis and metabolism*, in *The Adrenal Gland*, P.J. Mulrow, Editor. 1986, Elsevier: New York. p. 65-78.
35. Malendowicz, L.K., *et al.*, *Neuromedin-N increases the levels of circulating ACTH and aldosterone without changing the blood concentration of corticosterone in rats.* Neuroendocrinol Lett, 1992. **14**(5): p. 343-48.
36. Aggarwal, S.K., *Cisplatin-induced peptic ulcers and calcium modulation.* ICEM, 1994. **13-Paris**: p. 1079-80.
37. Black, V.H., R.I. Brody, and K.O. Martin, *3-Hydroxy-3-methylglutaryl coenzyme a reductase in outer versus inner cortices of the guinea pig adrenal: effects of adrenocorticotropin and dexamethasone.* Endocrinology, 1988. **122**(1): p. 296-305.

CHAPTER THREE: CISPLATIN – INDUCED ALTERATIONS IN CORTOCOSTERONE CONCENTRATION AND STRUCTURE OF ADRENAL GLAND CULTURES FROM THE RAT

SUMMARY

Adrenal glands and a pituitary were removed from Wistar CRL:(WI)BR rats for two groups, put into organ culture and treated with normal saline (vehicle for cisplatin) or 7• m/ml for specific times. Medium was collected for group 1 at 24, 0, 8, 20, 32, 56 and 80 hours to analyze for corticosterone concentration and adrenals were collected and processed for light microscopy at 32, 56 and 80 after treatment with cisplatin. Medium for group 2 was collected at 24, 0, 2, 4, 6 and 8 hours for corticosterone concentration and the adrenals collected and processed for light microscopy at 2, 4, 6 and 8 hours after treatment. The corticosterone concentration for group 1 showed a decrease from that of the control at all times except 32 hours. At this time the control and treated were at the same concentration. Both the control and treated showed a decrease in corticosterone concentration over the timeframe of the experiment, possibly due to a decrease in ACTH. Group 2 showed no real difference in corticosterone levels throughout the experiment. Microscopically the treated groups showed increased disruption of the secretory cells compared to that of the controls.

INTRODUCTION

Noxious compounds can create trauma and induce stress at the physical, metabolic and cellular levels in the *in vivo* system. The initial stress will cause the system to respond hormonally by the release of cortocotropic releasing hormone (CRH) from the hypothalamus, stimulating the pituitary to release adrenocortocotrophic hormone (ACTH) which stimulates the adrenal to synthesize corticosterone in rats and cortisol in humans [1-4]. Stress caused by disease, physical, metabolic and or cellular changes can also stimulate the immune system, which in turn increases the synthesis of glucocorticoids and the adrenal glands response to this stress [5-12]. This stimulation often overrides the normal feedback inhibition that glucocorticoids have on pituitary ACTH to allow prolonged production of glucocorticoids from the adrenal gland [5, 8, 13].

As a noxious compound induces physical, metabolic and/or cellular alterations to the system it may also have an affect on the concentration of circulating corticosterone. Is a change in the concentration of corticosterone due to the stress-related alterations in the body or due to the added noxious compound itself? To evaluate this problem the adrenal gland can be and examined at the cellular level or at the organ level. This allows for examination without the influence of uncontrolled hormonal, neurogenic or hemodynamic factors [14, 15].

Studies of adrenal organ, slice or cell culture have been used to look at chemotherapeutic agents [14, 16-20], noxious compounds [21, 22], hormones [20, 23-33], certain peptides [34-38] and interleukins [6-12, 39, 40] influence on catecholamine and steroid synthesis by secretion into the media and the structure of the adrenal gland. Other studies have examined specific points in steroid synthesis to find where stimulation and/or inhibitions take place [25, 26, 31, 32, 41-43]. In some studies (1) ACTH was supplemented to maintain a basal stimuli and secretion of corticosterone allowing any change in secretion to be the difference from the basal output [9, 11, 16, 26-28, 31, 35, 44, 45], (2) the intact pituitary was put into organ culture with the adrenal to achieve stimulus by a different means, this does not give a basal level that may be constant but allows more of the natural stimuli, though it can allow for more variability in the secretion of corticosterone [22, 29, 32], (3) or use no hormonal supplement so there is only the corticosterone and the effect of what compound you are supplementing the media with [9, 11, 16, 17, 25, 26, 30, 34, 35, 37, 40, 42, 45]. In cell or organ culture the concentration of the hormone secreted into the media varies. Sibley *et al.* [46], found that the variables in the relative amounts of steroids secreted into the incubation media may be a product not only of a change in synthesis of the steroid but a change in how it is secreted from the tissue.

It has been shown that treatment with cisplatin induces physical stress to the body by lowering body weight and food intake with alterations in water intake and urine output [47-51], metabolic alterations by the changes in corticosterone

concentrations and at the cellular level in the adrenal gland [52-54].To determine if these alterations are due to cisplatin induced stress on the system or on the adrenal gland itself, the organ was explanted and put into culture. Its activity was monitored both structurally and functionally at time intervals.

MATERIALS AND METHODS

Organ Culture: Wistar Crl:(WI)BR male rats weighing 200 to 250g each from Charles River were housed 3 to a cage with a light/dark cycle of 12 hours with food and water *ad lib.* Animals were allowed to adapt to their new environment for 3 days prior to adrenal culture. Animals were then decapitated; the pituitary and two adrenal glands were collected under sterile conditions and placed in HEPES buffer. Each adrenal gland was cut into 2, and these four pieces along with a pituitary were placed on a stainless steel mesh support within a culture dish, such that the medium under the support moistened the adrenal and pituitary explants for two separate Groups and timeframes were used (see Table 1 and Table 2). The timeframes for Group 2 were determined after the analyses of the corticosterone concentration in the culture medium from Group 1. The adrenal organ cultures were maintained at 37 °C in a humidified atmosphere of 5% CO_2 and 95% air. The culture medium was prepared following the protocol of Szkudlinski, M., *et.al.* (1989). Media was changed and collected after a specific time (see Table 1 and Table 2). The cultures were allowed to adapt to the environment for three days prior to treatment with CDDP, then treated with physiological saline or 7µm/ml media of CDDP for a specific time (see Table 1 and Table 2). The adrenals were collected and processed for light and microscopy.

Table 1: Group 1 Control and CDDP (7μm/ml) Adrenal Organ Culture Media Changes and Collection Times by Post-Treatment and Time from Last Media Change. Treatment time is 8 hours. (* = media contains saline or CDDP, **bold** = adrenal glands collected and processed for light microscopy)

Media	Time (Hours) ⇒								
Changed	-72	-48	-24	0	8*	12	12	24	24
Post-Treatment Hours					**8***	**20**	**32**	**56**	**80**
Collected (-80°C)			-24	0	8*	12	12	24	24

Table 2: Group 2 Control and CDDP (7μm/ml) Adrenal Organ Culture Media Changes and Collection Times. Treatment time is 2, 4, 6 and 8 hours. (* = media contains saline or CDDP, **bold** = adrenal glands collected and processed for light microscopy)

Media	Time (Hours) ⇒							
Changed	-72	-48	-24	0				
Collected (-80°C)			-24	0	**2***	**4***	**6***	**8***

Corticosterone Analysis: Corticosterone content in the serum was analyzed by a Rat Corticosterone-3H Kit (ICN) and read in a Beckman 7500 series scintillation counter as described earlier.

Light Microscopy: The adrenal halves were fixed in 2% glutaraldehyde in 0.1M sodium phosphate buffer (PBS), pH 7.4, dehydrated in graded ethanol series and embedded in JB4 (glycol methacrylate, Polyscience, Inc.). Sections (2μm) were stained with hematoxylin and eosin for general histology.

RESULTS

Corticosterone Concentration

The corticosterone concentrations in the media from Group 1 (Figure 1) decreased at 8, 20, 56 and 80 hours post-treatment. There was no significant difference between the control and CDDP treated cultures prior to treatment. At 32 hours post-treatment the control had a decrease in corticosterone concentration whereas the treated has an increase in concentration with no difference between the two. The CDDP treated cultures decrease in corticosterone revealed a trend towards significance, although the sample size was insufficient to determine this statistically. The control cultures also had a decrease in corticosterone concentration at day 0, 32 and 56 this could be due to a decrease in secreted ACTH from the pituitary gland.

The corticosterone concentration in the media from Group 2 (Figure 2) decreased 2, 6 and 8 hours into treatment with CDDP. The treated 4-hour culture exhibited a slight increase in corticosterone concentration, with no significant difference between the control and CDDP treated cultures prior to treatment. The sample number was insufficient to determine if the decreases or increase in corticosterone secretion at the given times is statistically significant.

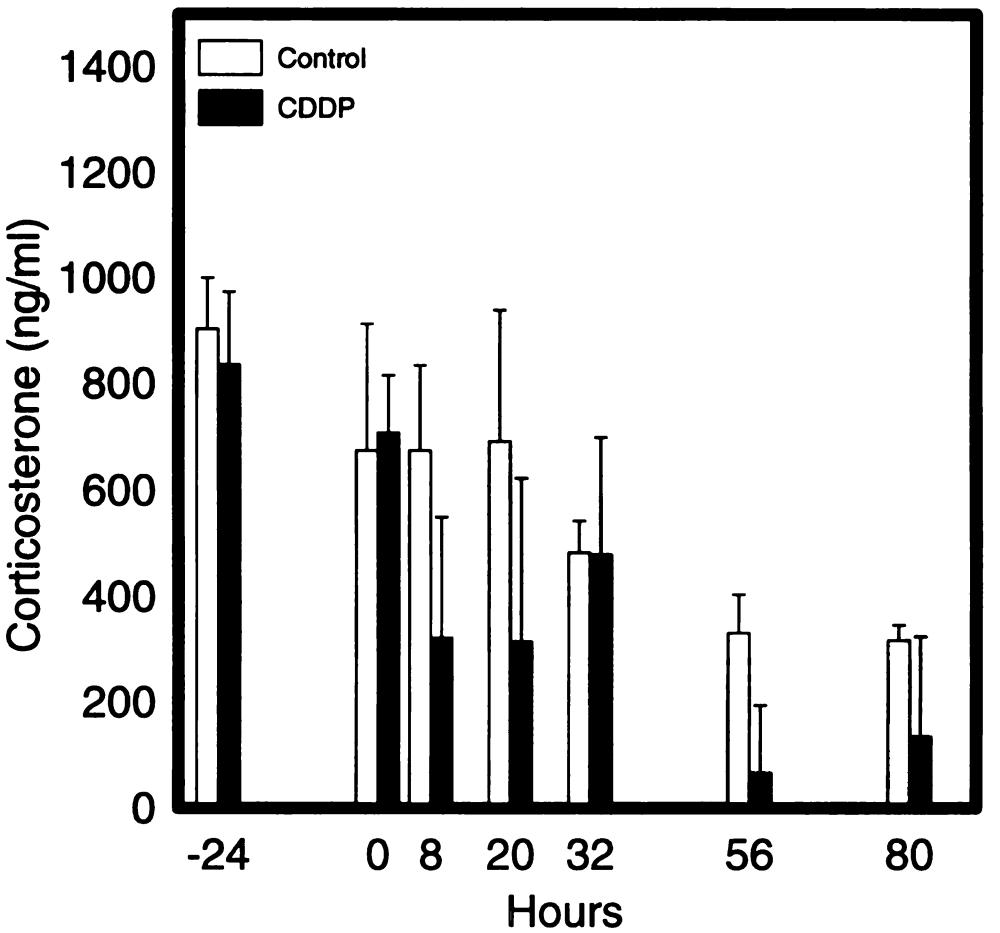


Figure 1: Corticosterone Concentration Present in the Media of Group 1 Adrenal Organ Cultures at Specific Times.

There is no significant difference in the concentration of corticosterone in the media between the treated and control groups prior to treatment or at 32 hours post-treatment. There does appear to be a difference in the corticosterone concentration present in the media at 8, 20, 56 and 80 hours post-treatment. The sample number was insufficient to determine whether this is statistically significant between the two groups. [Average ± S.D.]

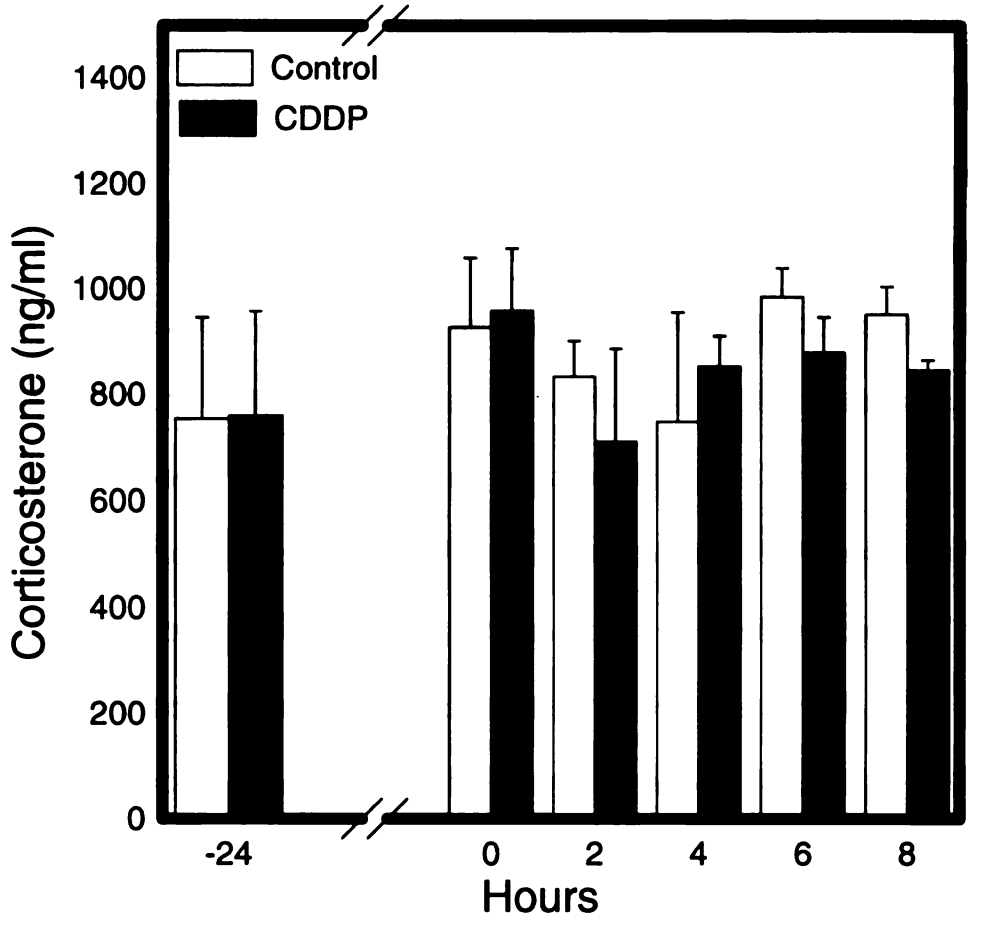


Figure 2: Corticosterone Concentration Present in the Media of Group 2 Adrenal Organ Cultures at Specific Times.

There appears to be no significant difference in corticosterone concentrations in the media at any treatment hour between CDDP treated and the control groups. The sample number was insufficient to determine whether there is a significant difference between the groups. [Average ± S.D.]

Group 1 Organ Culture

Medulla

The medullary secretory cells lacked cellular definition (Figure 3-6) in both the control and cisplatin (CDDP) cultures with membranes becoming more defined as the time of post-treatment progressed to 80 hours. There was an apparent decrease in cytoplasm in the CDDP culture at 32 hours post-treatment and the control and CDDP cultures at 56 hours post-treatment, whereas the 80-hour post-treatment culture appeared to have increased cytoplasm. There is apparent nuclear degeneration at 32 hours post-treatment in the CDDP cultures and the number of nuclei appeared increased as time progresses. The CDDP cultures capillaries had accumulated cellular debris within the lumens and were increased in number to that of the control at 56 hours at 80 hours post-treatment.

Cortex

Zona Reticularis

Both the control and CDDP cultures secretory cells (Figure 5-6) at 32 hours post-treatment demonstrated undefined cell membranes. At 56 hours post-treatment the control has better membrane definition with both the control and CDDP cultures having increased cytoplasm compared to the control and CDDP 32 hour cultures. The 32-hour CDDP culture had a number of intensely stained condensed nuclei that were not present in the other cultures. All cultures had enlarged cortical capillaries with the enlargement being more pronounced in the CDDP cultures as post-treatment time progresses.

Zona Fasciculata

The zona fasciculata (Figure 6-8) at 32 hours post-treatment lacked organization of the cells into the chord-like structure that is seen with intact adrenal glands in both the control and CDDP cultures. The CDDP culture at 32 hours has no cellular definition and has a number of intensely staining condensed nuclei. There was an increase in cellularity at 56 hours post-treatment in both the control and CDDP cultures from that of the 32-hour cultures, although there was still significant cellular disruption. The 56-hour post-treatment culture lacked membrane definition compared to its control culture. The zona fasciculata was more organized at 80 hours as shown by the chord-like seen in the intact adrenal gland. The control culture had better organization and cellular membrane definition than the 80 CDDP culture, although there was still significant cellular separation and disruption in both of the 80-hour post-treatment cultures.

Zona Glomerulosa and Capsule

The 32 hour controls zona glomerulosa (Figure 8) had better cellular definition with an apparent increased number of nuclei from the for the CDDP culture with both having a number of intensely stained condensed nuclei. The capsule was intact with an apparent increase in the number of nuclei in the CDDP culture. As post-treatment time progressed the zona glomerulosa in both the control and CDDP cultures had an apparent decrease in cytoplasm with increased cellular disruption at both 56 and 80 hours post-treatment with damage more pronounced in the CDDP cultures. The capsules were intact with an

apparent decrease in the number of nuclei as post-treatment time progressed with only a few visible by 80 hours in both the control and CDDP cultures.

Group 2 Organ Culture

Medulla

The cisplatin (CDDP) culture at 2 hours treatment (Figure 9-11) had apparent decreased cell membrane definition, cytoplasm and an accumulation of cellular debris with in the lumens of the capillaries. Both the control and CDDP cultures had condensed nuclei that were intensely stained and the control appeared to have and increased number of nuclei compared to the CDDP culture. Both the control and CDDP cultures at 4 hours treatment had very little membrane definition with intensely stained condensed nuclei and some nuclei that were degenerating. The 4-hour control had accumulated cellular debris within the lumen of the capillaries. As treatment time progressed the 6 and 8 hour treated cultures had increased membrane degradation. The 6-hour CDDP had an apparent increase in cytoplasm from the previous 2 and 4-hour cultures and to that of its control, although the control had increased cellular disruption and intensely stained condensed nuclei.

Zona Reticularis

The zona reticularis (Figure 11-12) in both the control and CDDP at 2-8 hours treatment had enlarged cortical capillaries going into the zona fascicularis, with areas of intensely stained nuclei at 2 hours treatment in the CDDP culture. The 4-hour CDDP treated culture had less membrane definition. At 6 hours

treatment the control culture had an apparent decrease in cytoplasm compared to the CDDP culture. The CDDP cultures at 6 or 8 hours treatment had areas of condensed nuclei that were intensely stained with the cells having no membrane definition.

Zona Fasciculata

After 2hrs of treatment (Figure 12-13) the CDDP culture had some condensed nuclei that were intensely stained. All cultures had an apparent decrease in cytoplasm, cellular separation and disruption, enlarged capillaries, a decrease in membrane definition and an increase in the number of nuclei as the treatment time progressed.

Zona Glomerulosa and Capsule

The zona glomerulosa in both the control and CDDP cultures at 2 and 4 hour treatment had apparent cytoplasmic loss with increased cytoplasm in the CDDP culture at 2 hours compared to the other cultures. Both the control and CDDP cultures at the above mentioned times had condensed nuclei that are intensely stained. There appeared to be an increase in cytoplasm at 6 hours in both the control and CDDP cultures with condensed intensely stained nuclei in both the zona glomerulosa and the capsule. At 8-hour treatment both the control and CDDP cultures appeared to have decreased cytoplasm with condensed intensely stained nuclei in both the zona glomerulosa and the capsule.

Figure 3: Light Micrographs of Sections (2μm) of the Medulla of Group 1 Adrenal Gland Organ Cultures.

A. Control, B. CDDP 32-Hours Post-Treatment:
The CDDP cultures medullary cell membranes are not well defined, with an apparent decrease in cytoplasm and fewer obvious nuclei (arrow) that are degenerating compared to the control. The treated cultures capillaries (V) have an accumulation of cellular debris within the lumens. The areas enclosed are magnified in figure 4.

C. Control, D. CDDP 56-Hours Post-Treatment:
Medullary cell number and nuclei (arrow) are relatively equal between the two cultures, although the CDDP culture appears to have a decrease in cytoplasm and an increase in the number of capillaries (V). The areas enclosed are magnified in figure 4.

E. Control, F. CDDP 80-Hours Post-Treatment:
Both the control and CDDP cultures show an increase in cellular membrane definition, and appear to have an increase in nuclei (arrow). The CDDP culture appears to have a decrease in cytoplasm, with an increase in and number and size of the capillaries (V) with an accumulation of cellular debris within their lumens. The areas enclosed are magnified in figure 4.

All are stained with hematoxylin and eosin. Bar = 0.01

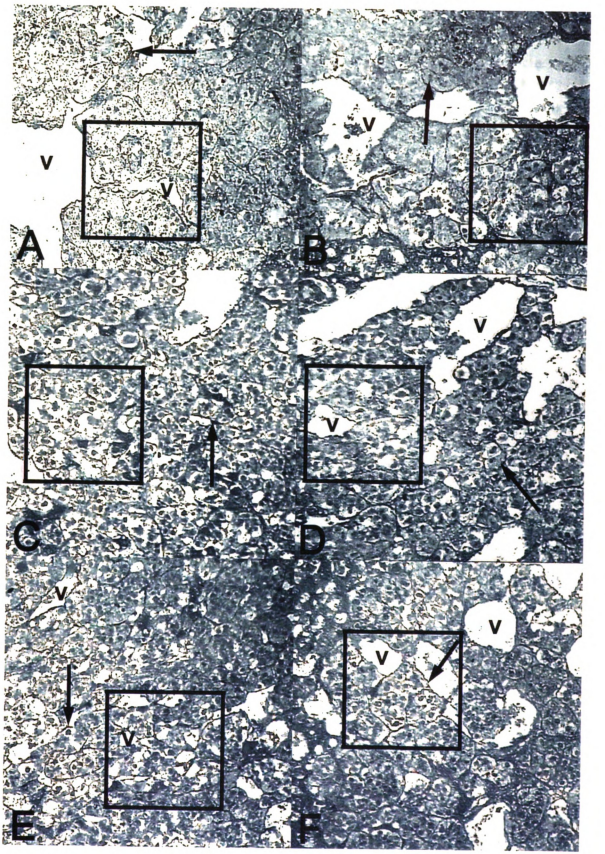

A
B
C
D
E
F
v

Figure 4: Light Micrographs of the Enclosed Areas Enlarged from Figure 3.

A. Control, B. CDDP 32-Hours Post-Treatment:
The cellular membranes have no definition with the cells merging together in the control and CDDP cultures. A number of nuclei (arrow) are condensing, with the CDDP culture appearing to have a decrease in the number of nuclei (arrow) and degeneration of those remaining. The CDDP culture also appears to have a decrease in cytoplasm. The controls capillaries are free of cellular debris unlike that of the CDDP culture (refer to Figure 3).

C. Control, D. CDDP 56-Hours Post-Treatment:
Both control and CDDP cultures secretory membranes are ill defined with degenerating nuclei (arrow) and loss of cytoplasm, although the CDDP culture is in worse condition. The two cultures capillary (V) and endothelial lining is intact with no apparent difference between the two.

E. Control, F. CDDP 80-Hours Post-Treatment:
The CDDP cultures medullary secretory cell membranes have better definition and appear to have an increase in the number of nuclei (arrow) compared to the control culture and previous cultures. The CDDP cultures capillaries (V) are have increased size with an accumulation of cellular debris within the lumens.

Bar = 0.005mm

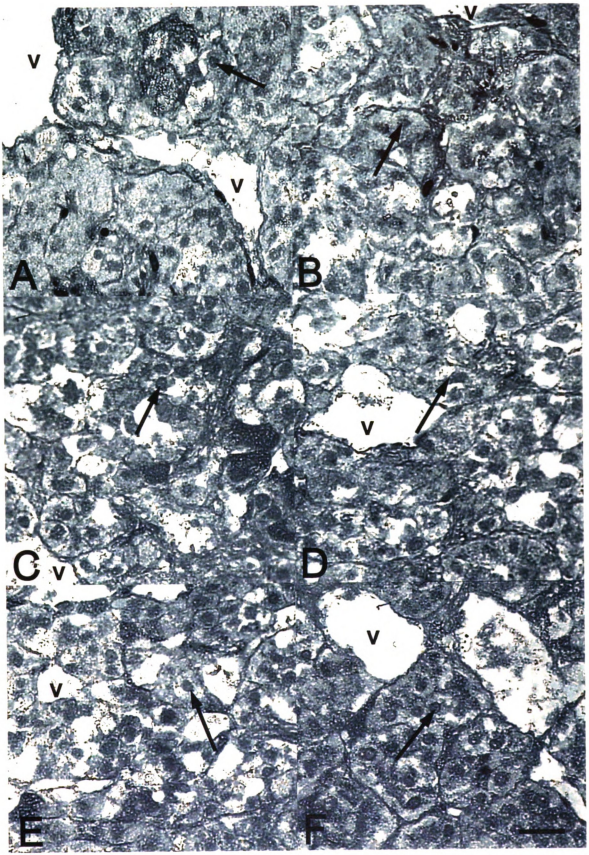

A
B
C
D
E
F
v

Figure 5: Light Micrographs of Sections (2μm) of the Medulla and Zona Reticularis (Junction of the Medulla and Cortex) of Group 1 Adrenal Gland Organ Cultures.

A. Control B. CDDP 32-Hours Post-Treatment:
The CDDP cultures medullary (M) cells are less defined; there appear to be fewer nuclei and a decrease in cytoplasm. The secretory cells of the zona reticularis (ZR) are undefined in both the control and CDDP cultures, although the CDDP culture has more enlarged capillaries (clear areas throughout the tissue).

C. Control D. CDDP 56-Hours Post-Treatment:
Medullary (M) cell number and nuclei are relatively equal between the control and CDDP cultures, although the CDDP culture appears to have a decrease in cytoplasm. The ZR secretory cell membranes have better definition in the control with the CDDP cultures capillaries being of increased size compared to the control (large clear areas throughout the tissue) and the previous cultures.

E. Control F. CDDP 80=Hours Post-Treatment:
There is improved medullary (M) membrane definition in both the control and CDDP cultures with the CDDP culture appearing to have a decrease in cytoplasm and an increase in the number and size of the capillaries (V). The ZR secretory cells are more defined with better membrane definition in the control with the CDDP culture having and increase in the size of the capillaries.

All are stained with hematoxylin and eosin. Bar = 0.015mm

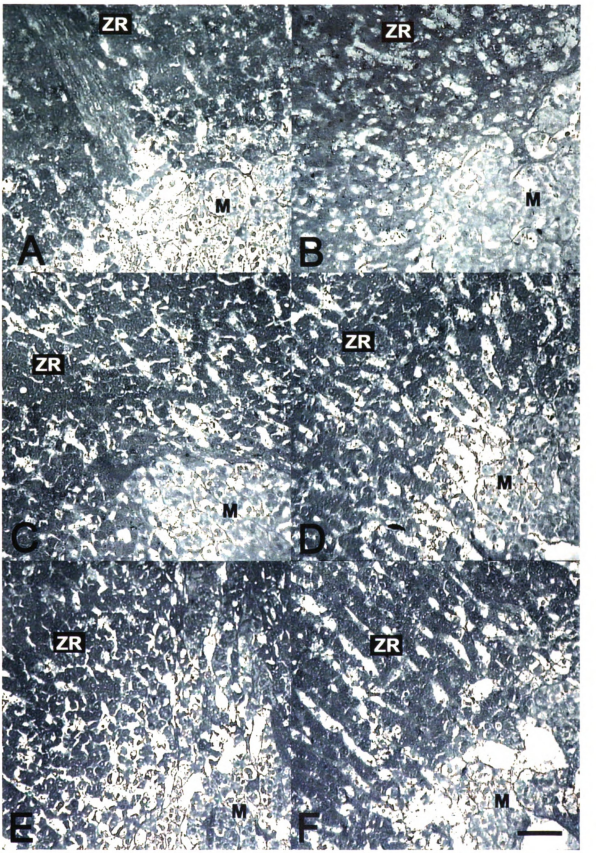

ZR
M
A
ZR
M
B
ZR
M
C
ZR
M
D
ZR
M
E
ZR
M
F

Figure 6: Light Micrographs of Sections (2μm) of the Zona Reticularis and Zona Fasciculata of Group 1 Adrenal Gland Organ Cultures.

A. Control B. CDDP 32-Hours Post-Treatment:
The controls zona reticularis (ZR) cells have better membrane definition with areas that have condensed nuclei in the CDDP culture. The CDDP culture has no cell membrane definition in either the ZR or the zona fasciculata (ZF), even though it appears to have more cytoplasm, although both cultures have large areas of cellular disruption (arrow). The areas enclosed are magnified in figure 7.

C. Control D. CDDP 56-Hours Post-Treatment:
There appears to be increased cellularity in both the ZR and the ZF from that of the 32-hour cultures, although the CDDP culture has very little cellular definition. Again the CDDP culture appears to have more cells with both control and CDDP cultures still having a significant amount of cellular disruption (arrow). The areas enclosed are magnified in figure 7.

E. Control F. CDDP 80-Hours Post-Treatment:
The secretory cells of the ZR and the ZF in the control culture have better organization and membrane definition than those of the CDDP culture and the previous cultures at 32 and 56 hours, although cellular disruption (arrow) is still seen in both cultures. The areas enclosed are magnified in figure 7.

All are stained with hematoxylin and eosin. Bar = 0.014mm

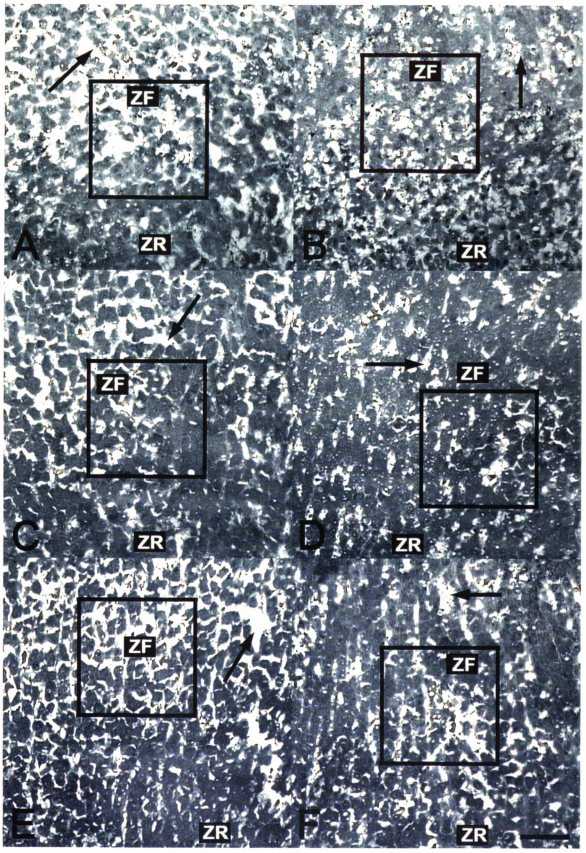

ZF
A
ZR
ZF
B
ZR
ZF
C
ZR
ZF
D
ZR
ZF
E
ZR
ZF
F
ZR

Figure 7: Light Micrographs of the Enclosed Areas Enlarged from Figure 6.

A. Control B. CDDP 32-Hours Post-Treatment:
There zona fasciculata has no cellular membrane definition and the nuclei are condensing in the CDDP culture, although the control appear to have less cytoplasmic area (arrow).

B. Control D. CDDP 56-Hours Post-Treatment:
There is an increase in cellularity of the ZF in both the control and CDDP cultures with the control having better cellular definition, although cellular damage (arrow) is still apparent in both.

E. Control F. CDDP 80-Hours Post-Treatment:
The secretory cells of the ZF are more organized in both the control and CDDP cultures compared to the previous cultures at 32 and 56 hours, this is apparent by the chord-like structure of the cells as is seen in the intact adrenal gland. The CDDP culture has little cellular membrane definition with both the control and CDDP cultures having separation of the cells and disruption (arrow).

Bar = 0.008mm

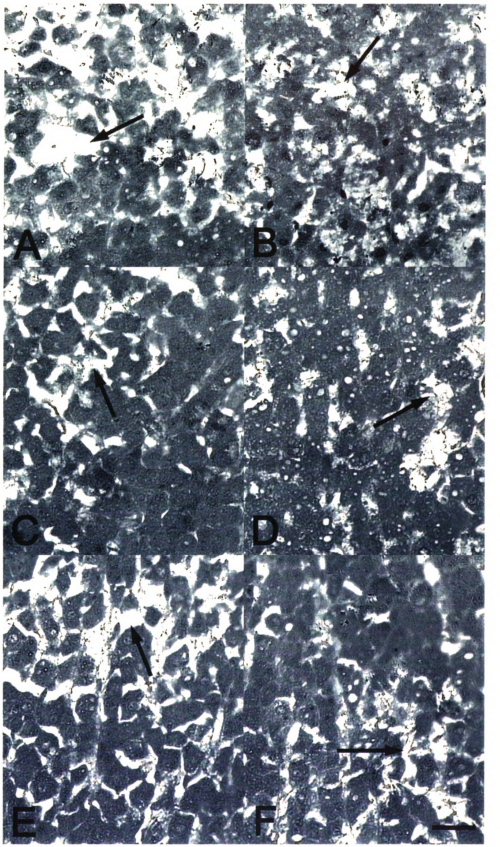

A
B
C
D
E
F

Figure 8: Light Micrographs of Sections (2μm) of the Zona Fasciculata, Zona Glomerulosa and Capsule Group 1 Adrenal Gland Organ Cultures.

A. Control B. CDDP 32-Hours Post-Treatment:
The secretory cells (arrow) of the upper regions of the zona fasciculata (ZF) closer to the zona glomerulosa (ZG) in both the control and CDDP cultures appear to have less cellular damage compared to lower regions of the ZF in previous figures. The cells are lacking the organization needed to form the chord-like structure that the ZF displays in the intact adrenal gland. There appears to be an increase in the number of nuclei in the ZG in the CDDP culture compared to the control with the nuclear condensation apparent in both the control and CDDP cultures. The adrenal capsule (Cap) appears to be intact with a number of intensely stained condensed nuclei in the CDDP culture.

C. Control D. CDDP 56-Hours Post-Treatment:
The control and CDDP culture appear to have a decrease in cytoplasm (arrow) with the CDDP culture having little cellular definition (arrow) in the ZF. There is increased disruption of the secretory cells of the ZG in both the control and CDDP cultures from that of the 32 hour cultures with some intensely stained condensed. The adrenal capsules (Cap) appear intact with a decrease in the number of nuclei from the 32-hour cultures.

E. Control F. CDDP 80-Hours Post-Treatment:
The secretory cells (arrow) of the ZF in both the control and CDDP cultures appear to be trying to organize into the chord-like structure apparent in an intact adrenal gland, although they are lacking cellular membrane definition. There appears to be a decrease in the number of secretory cells, organization and in the number of nuclei in ZG of both the control and CDDP cultures. The adrenal capsules (Cap) are intact but devoid of nuclei in both the control and CDDP cultures.

All are stained with hematoxylin and eosin. Bar = 0.01mm

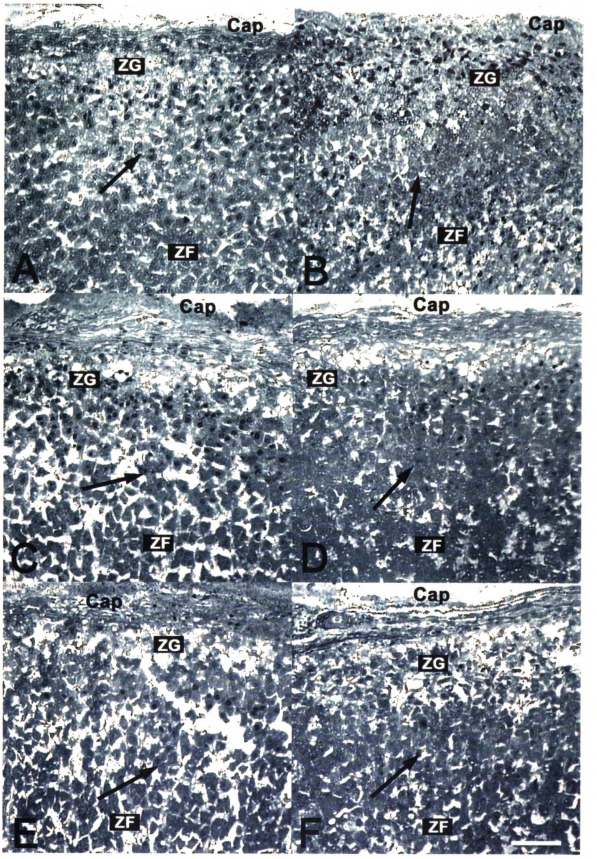

Cap
ZG
ZF
A
Cap
ZG
ZF
B
Cap
ZG
ZF
C
Cap
ZG
ZF
D
Cap
ZG
ZF
E
Cap
ZG
ZF
F

Figure 9: Light Micrographs of Sections(2μm) of the Medulla of Group 2 Adrenal Gland Organ Cultures.

A. Control B. CDDP 2-Hours Treatment:
The CDDP culture appears to have a loss of cytoplasm and membrane definition with both the control and CDDP cultures having condensed nuclei. The CDDP cultures capillaries (V) have accumulated cellular debris within the lumens. The areas enclosed are magnified in figure 10.

C. Control D. CDDP 4-Hours Treatment:
The CDDP culture has little membrane definition with the capillaries (V) having no apparent damage in either control or CDDP cultures. The areas enclosed are magnified in figure 10.

E. Control F. CDDP 6-Hours Treatment
The control appears to have an increased number of nuclei and cellular disruption with a decrease in the cytoplasm compared to the 2-hour cultures and its CDDP partner. The CDDP culture has an increase in cytoplasm. The capillaries (V) are intact with no apparent damage to either the control or CDDP cultures. The areas enclosed are magnified in figure 10.

G. Control H. CDDP 8-Hours Treatment:
Both the control and CDDP cultures have a loss of membrane definition, decreased nuclei with the CDDP culture having a decrease in cytoplasm. The venous channels (V) appear to be intact with no apparent damage in either control or CDDP cultures. The areas enclosed are magnified in figure 10.

All are stained with hematoxylin and eosin. Bar = 0.02mm

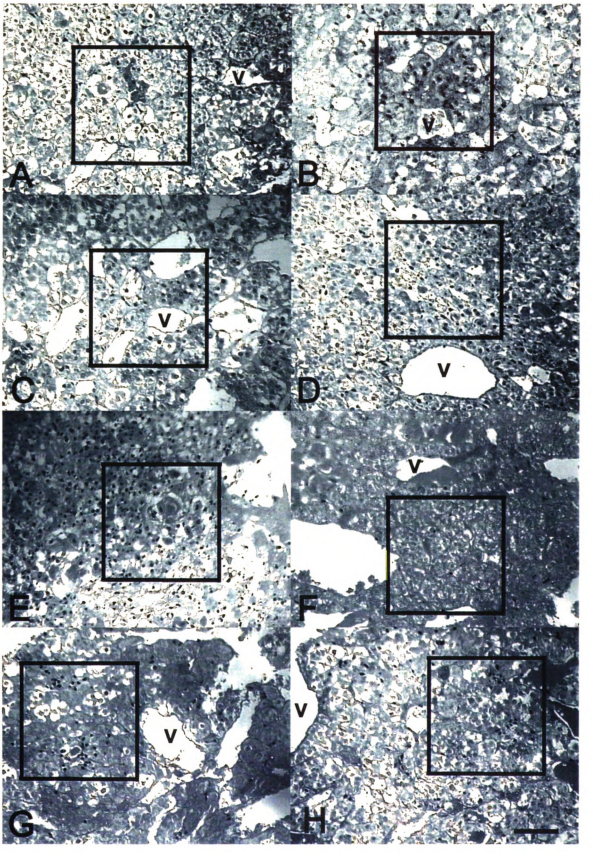

A
V
B
V
C
V
D
V
E
F
V
G
V
H
V

Figure 10: Light Micrographs of the Medulla Enclosed Areas Enlarged from Figure 9.

A. Control B. CDDP 2-Hours Treatment:

There is no membrane (arrow) definition, with condensed and degenerating nuclei, an apparent loss of cytoplasm and accumulated debris in the lumen of the capillaries (V) of the CDDP culture with the control having better membrane definition and increased pronounced nuclei.

C. Control D. CDDP 4-Hours Treatment:
There is no membrane (arrow) definition with condensed and degenerating nuclei in the CDDP culture, with both the control and CDDP cultures having an apparent loss of cytoplasm. The capillaries (V) of the control have accumulated some cellular debris.

E. Control F. CDDP 6-Hour Treatment:
Neither the control nor CDDP cultures have membrane (arrow) definition. The nuclei in the control are condensed, with an apparent loss of cytoplasm from the cells and the CDDP culture has nuclear degeneration.

G. Control H. CDDP 8-Hours Treatment:
Both the control and CDDP cultures lack membrane definition (arrow), have an apparent loss of cytoplasm and a decrease in the number of nuclei.

Bar = 0.007mm

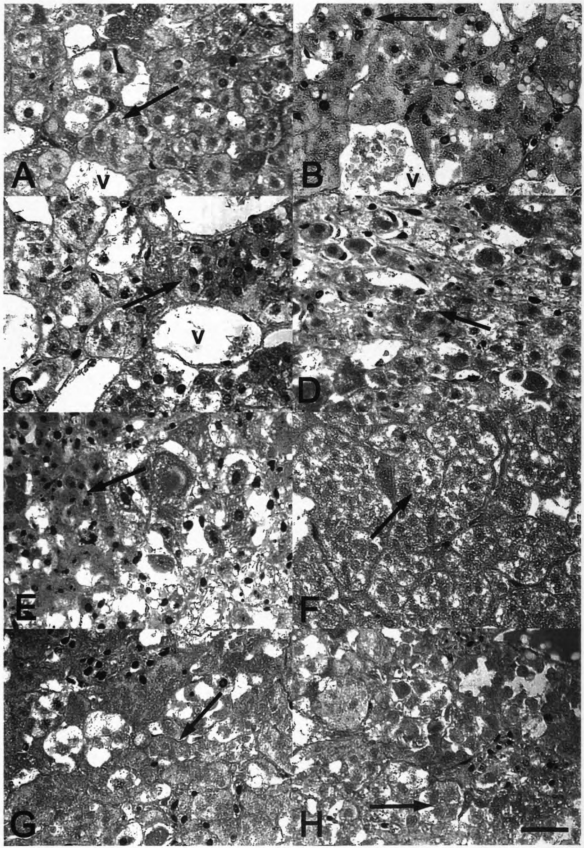

A
V
B
V
C
V
D
E
F
G
H

Figure 11: Light Micrographs of Sections (2μm) of the Medulla and Zona Reticularis of Group 2 Adrenal Gland Organ Cultures.

A. Control B. CDDP 2- Hours Treatment:
The CDDP cultures medullary (M) cells appear to have decreased cytoplasm and membrane definition. The zona reticularis (ZR) in both the control and CDDP cultures have enlarged capillaries (clear areas) with the treated cultures being more pronounced and having a number of nuclei that are condensed.

C. Control D. CDDP 4-Hours Treatment:
Both the control and CDDP cultures have an apparent loss of cytoplasm in the medulla (M) with the ZR having enlarged capillaries (clear areas) with the CDDP culture having no cellular membrane definition. The medullary capillaries (V) are intact with no apparent damage in either the control or CDDP cultures.

E. Control F. CDDP 6-Hours Treatment:
The controls medulla (M) and areas of the ZR appear to have decreased cytoplasm and a number of condensed nuclei with both the control and CDDP cultures having enlarged capillaries (clear areas). The medullary capillaries (V) in the treated culture have no apparent damage to the endothelial lining.

G. Control H. CDDP 8-Hours Treatment:
The control has an apparent decrease in cytoplasm in the medulla (M) and the ZR has areas of intensely stained nuclei that are condensed. The CDDP culture appear to have an increase in cytoplasm with no cellular membrane definition and the medullary capillaries (V) have no apparent damage to the endothelial lining.

All are stained with hematoxylin and eosin. Bar = 0.02mm

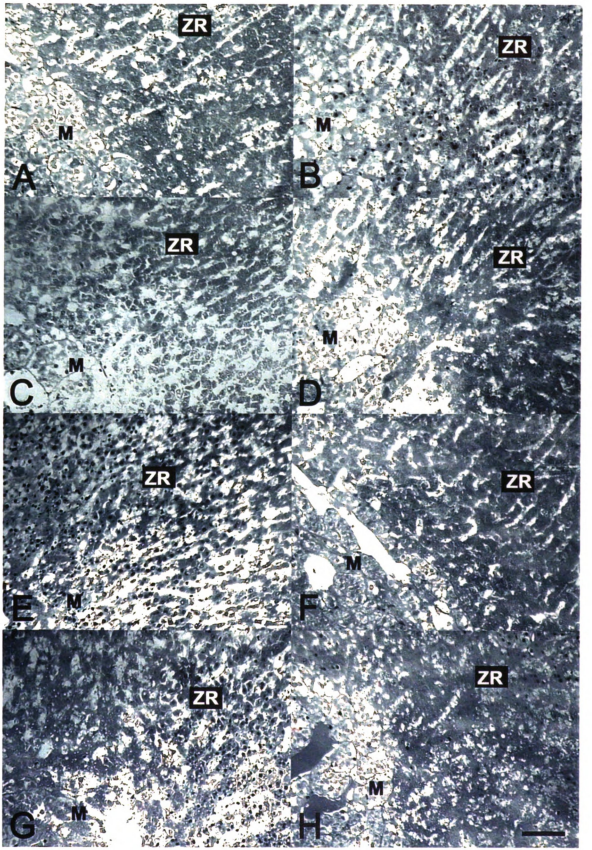

ZR
M
A
ZR
M
B
ZR
M
C
ZR
M
D
ZR
M
E
ZR
M
F
ZR
M
G
ZR
M
H

Figure 12: Light Micrographs of Sections (2μm) of the Zona Reticularis and the Zona Fasciculata of Group 2 Adrenal Gland Organ Cultures.

A. Control B. CDDP 2-Hours Treatment:
The zona reticularis (ZR) in both the control and CDDP cultures have enlarged capillaries and cellular separation (clear areas) that go into the zona fasciculata (ZF). The CDDP culture has no cellular definition and areas with condensed nuclei. The areas enclosed are magnified in figure 13.

C. Control D. CDDP 4-Hours Treatment:
The ZR in both the control and CDDP cultures has enlarged capillaries (clear areas) that go into the ZF, being more pronounced in the control. The CDDP culture to have an increase in cytoplasm but the cells have no membrane definition. The areas enclosed are magnified in figure 13.

E. Control F. CDDP 6-Hours Treatment:
Both the control and CDDP cultures have enlarged capillaries and cellular separation (clear areas), that are more pronounced in the ZR of the control and the ZF of the treated. The cell membranes have no apparent definition in either the control or CDDP cultures. The areas enclosed are magnified in figure 13.

G. Control H. CDDP 8-Hours Treatment:
The cells of the ZR and the ZF in both the control and CDDP cultures have no membrane definition, with the enlarged capillaries being more pronounced in the control culture. The areas enclosed are magnified in figure 13.

All are stained with hematoxylin and eosin. Bar = 0.02mm

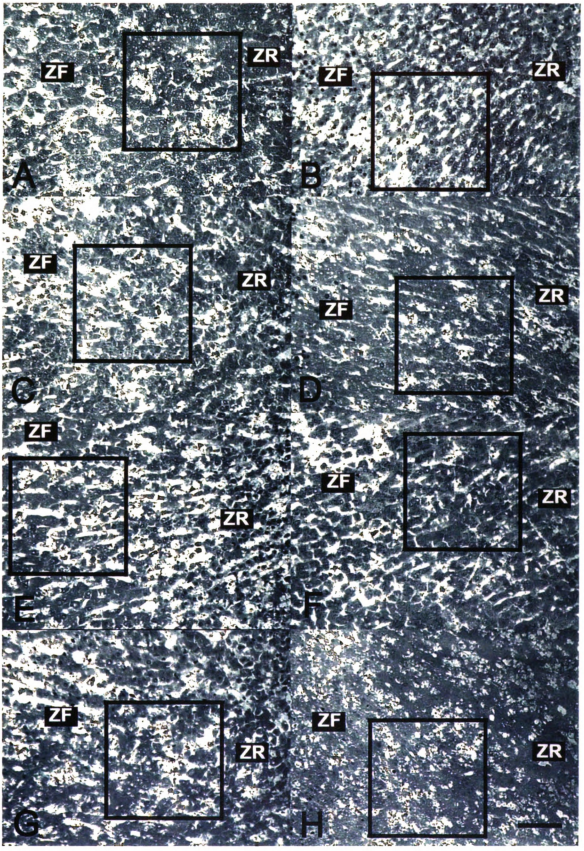

ZF
ZR
A
ZF
ZR
B
ZF
ZR
C
ZF
ZR
D
ZF
ZR
E
ZF
ZR
F
ZF
ZR
G
ZF
ZR
H

Figure 13: Light Micrographs of the Enclosed Areas Enlarged from Figure 12.

A. Control B. CDDP 2-Hours Treatment:

C. Control D. CDDP 4-Hours Treatment:

E. Control F. CDDP 6-Hours Treatment:

G. Control H. CDDP 8-Hours Treatment:

The ZF appears to have a decrease in cytoplasm, enlarged capillaries (arrow) with separation of cells and a decrease in membrane definition and nuclei as time in culture progresses in both the control and CDDP cultures.

Bar = 0.007mm

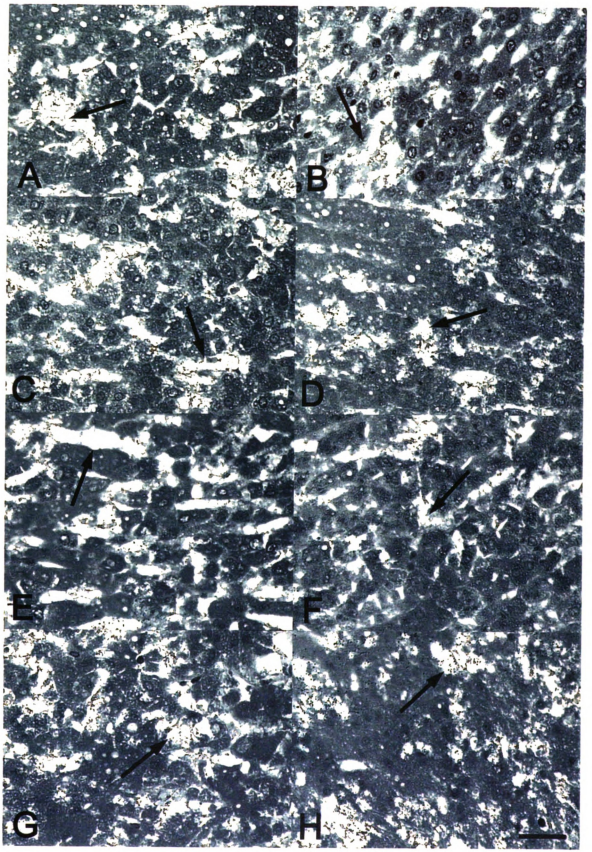

A
B
C
D
E
F
G
H

Figure 14: Light Micrographs of Sections (2μm) of the Zona Fasciculata, Zona Glomerulosa and Capsule Group 2 Adrenal Gland Organ Cultures.

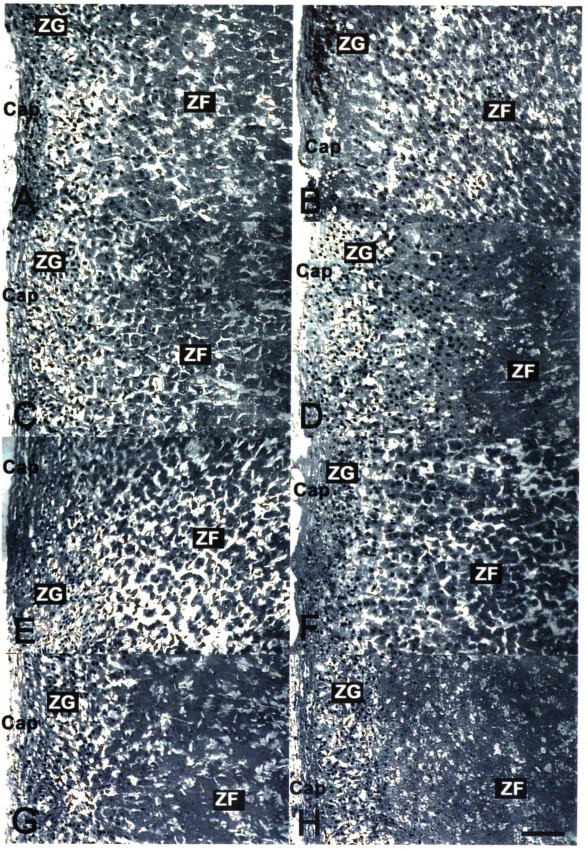
A. Control, B. CDDP 2-Hours Treatment:
Both the control and CDDP cultures have enlarged capillaries and an apparent decrease in cytoplasm through the zona fasciculata (ZF). The control and CDDP cultures have areas of condensed nuclei in the ZF, zona glomerulosa (ZG) and the capsule (Cap) with the control appearing to have a greater loss of cytoplasm in the ZG, but allover better membrane definition than the CDDP culture.

C. Control, D. CDDP 4-Hours Treatment:
There are enlarged capillaries that go from the ZF into the ZG, with cytoplasmic loss and condensed nuclei in the upper ZF, ZG and the Capsule (Cap), in both the control and CDDP cultures with the CDDDP culture having no membrane definition.

E. Control, F. CDDP 6-Hours Treatment:
There appears to be a decrease in cytoplasm, enlarged capillaries, cellular separation and condensed nuclei in the ZF, with increased cytoplasm and a number of condensed nuclei in the ZG in both the control and CDDP cultures. The Capsule (Cap) is intact with a number of intensely stained condensed nuclei in both the control and CDDP cultures.

G. Control, H. CDDP 8-Hours Treatment:
There is an apparent increase in cytoplasm with no cellular membrane definition; the cells are merged together in the ZF in both the control and CDDP cultures. The ZG and capsule (Cap) have a loss of cytoplasm with an increase in the number of intensely stained condensed nuclei in both the control and CDDP culture.

All are stained with hematoxylin and eosin. Bar = 0.02


ZG
ZF
Cap
A
B
C
D
E
F
G
H

DISCUSSION

Corticosterone concentration for the Group 1 cisplatin treated decreased throughout the experiment except for at 32 hours where it was equal to the control. In the controls, corticosterone concentrations also declined throughout the experiment. This may be indicative of the drop in ACTH synthesis from the pituitary in culture with the adrenal gland and/or the length of time in culture. Some studies have shown that a constant concentration of ACTH is necessary to maintain the adrenal glands cellular structure and that without it apoptosis will become activated [25, 26]. Group 2, which was in culture for a shorter time did not show this decline in corticosterone concentration in either the control or the cisplatin treated. There were differences in corticosterone concentration between the treated and the control, but they do not appear to be significant.

Cellular damage was apparent in all the organ cultures, with a trend of increasing damage as time in culture progressed. The zona glomerulosa is usually spared the infarctive effects of damage that the arterioles have in the other zonae [55]. This difference is due to the zona glomerulosa receiving part of its vascular supply from the capsule [55] thus sparing it from catecholamines and cellular debris being dumped into the arterioles. There is apparent cytoplasmic loss and an increased number of condensed nuclei in the zona glomerulosa of the organ cultures. Thus it has lost its protection in culture and has induced apoptosis. The morphology of the organ cultures does not show the fine structure that is in the intact gland.

Our *in vivo* studies showed an overall increase in corticosterone concentration which conflict with the results from the *in vitro* studies. Conflicting results in corticosterone concentrations between in vivo and in vitro studies have been seen before [36, 37]. The main thought for this occurrence is the alteration in the hypothalamic-pituitary-adrenal axis, which is non-existent in organ culture. Interleukin 1β and interleukin 6 have been found to stimulate corticosterone concentration *in vivo* [6, 7, 10-12, 39], with variable results *in vitro* [9].

Further studies are needed to verify the results found in this study. Organ cultures should be repeated with addition of ACTH to stimulate corticosterone synthesis and the cellular integrity of the culture. Additional in vivo studies should be done with rats that are on a constant level of dexamethasone, a synthetic glucocorticoid agonist to analyze if cisplatin effects it. Studies of the immune system's interaction with the Hypothalamus-Pituitary-Adrenal axis and corticosterone concentration would be beneficial to how the system relates to stress and the toxic effects of the cisplatin.

CONCLUSIONS

This study has left more unanswered questions than answers. The immune systems interaction with the hypothalamus-pituitary-adrenal axis (HPA) and corticosterone concentrations in the induction of prolonged stress would be of great importance in contributing to the knowledge of how the system deals with disease and the toxic effects of treating that disease.

LIST OF REFERENCES

1. Greep, R.O., *The struture of the adrenal cortex*, in *The Adrenal Cortex*, H.D. Moon, Editor. 1961, Paul B. Hoeber, Inc.: New York. p. 23-45.
2. Long, J.A., *The adrenal gland*, in *Histology*, L. Weiss and R.O. Greep, Editors. 1977, McGraw-Hill: New York. p. 1099-118.
3. Vinson, G.P., B.J. Whitehouse, and J.P. Hinson, *The adrenal cortex.* 1992, Englewood Cliffs: Prentice Hall. 223.
4. Vivian, H.T.J., *The adrenal gland.* 1992, New York: Raven Press.
5. Besedovsky, H., *et al.*, *Changes in blood hormone levels during the immune response.* Proc Soc Exp Biol Med, 1975. **150**: p. 466.
6. Besedovsky, H. and A. del Rey, *Neuroendocrine and metabolic responses induced by interleukin-1.* J Neurosci Res, 1987. **18**: p. 172-78.
7. Krymskaia, L.G., N.Y. Gromykhina, and V.Q. Kozlov, *Interleukin-1 effect on adrenal gland function in mice.* Immunol Lett, 1987. **15**: p. 307-9.
8. Epstein, F.H., *Ubterkeyjub-1 and the pathogenesis of the acute-phase response.* N Engl J Med, 1984. **3111413-18**.
9. Harlin, C.A. and C.R. Parker, *Investigation of the effect of interleukin-1β on steroidogenesis in the human fetal adrenal gland.* 1991.
10. Roh, M.S., *et al.*, *Direct stimulation of the adrenal cortex by interleukin-1.* Surgery, 1987. **102**: p. 140-46.
11. Salas, M.A., *et al.*, *Interleukin-6 and ACTH act synergistically to stimulate the release of corticosterone from adrenal gland cells.* Clin Exp Immunol, 1990. **79**: p. 470-73.
12. Sapolsky, R., *et al.*, *Interleukin-1 stimulates the secretion of hypothalamic corticotropin releasing factor.* Science, 1987. **238**: p. 67-73.
13. Keller-Wood, M.E. and M.F. Dallman, *Corticosteroid inhibition of ACTH secretion.* Endocr Rev, 1984. **5**: p. 1-24.
14. Phelps, J.S., *et al.*, *Cisplatin nephrotoxicity: invitro studies with precision-cut rabbit renal cortical slices.* Toxicol Appl Pharmacol, 1987. **90**: p. 501-12.
15. Smith, J.H., *The use of renal cortical slices from the Fischer 344 rat as an in vitro model to evaluate nephrotoxicity.* Fundam Appl Toxicol, 1988. **11**: p. 132-42.
16. Dorfinger, K., *et al.*, *The effects of suramin on human adrenocortical cells in vitro: suramin inhibits cortisol secretion and adrenocortical cell growth.* Metabolism, 1991. **40**(10): p. 1020-24.
17. Magalhaes, M.M., *et al.*, *The effects of monensin on golgi complex of adrenal cortex and steroidogenesis.* Tissue and Cell, 1991. **23**(2): p. 209-15.
18. Sergejew, T.F. and R.W. Hartmann, *Effect of a diphenylethylenediamine platinum complex on steroidogenesis in rats.* J Steroid Biochem Mol Biol, 1996. **58**(2): p. 243-8.
19. Zelazowski, A.J., J.S. Garvey, and J.D. Hoeschele, *In vivo and in vitro binding of platinum to metallothionein.* arch Biochem Biophys, 1984. **229**(1): p. 246-52.

20. Zhang, J.G., *et al.*, *Protection effects of procaine on oxidative stress and toxicities of renal cortical slices from rats caused by cisplatin in vitro.* Archives of Toxicology, 1992. **66**(5): p. 354-58.
21. Azhar, S., *et al.*, *Okadaic acid interferes with lipoprotein-supported corticosterone production in adrenal cells.* Biochemical and Biophysical Research Communications, 1991. **179**(2): p. 726-33.
22. Szkudlinski, M. and A. Lewinski, *Indomethacin inhibits the basal and angiotensin II-stimulated mitotic activity of adrenocortical cells in vivo and in organ culture.* Res Exp Med, 1989. **189**: p. 173-80.
23. Black, V.H., R.I. Brody, and K.O. Martin, *3-Hydroxy-3-methylglutaryl coenzyme a reductase in outer versus inner cortices of the guinea pig adrenal: effects of adrenocorticotropin and dexamethasone.* Endocrinology, 1988. **122**(1): p. 296-305.
24. Branchaud, C.L., *et al.*, *Contribution of exogenous progesterone to human adrenal cortisol synthesis in vitro: a comparison of early gestation fetal and adult tissues.* Steroids, 1986. **47**(4-5): p. 269-85.
25. Carsia, R.V., *et al.*, *Models to elucidate the regulation of adrenal cell death.* Endocr Res, 1998. **24**(3-4): p. 899-908.
26. Carsia, R.V., K.I. Tilly, and J.L. Tilly, *Hormonal modulation of apoptosis in the rat adrenal gland in vitro is dependent on structural integrity.* Endocrine, 1997. **7**(3): p. 377-81.
27. Manuelidis, L. and P. Mulrow, *ACTH effects on aldosterone production and mitochondrial ultrastructure in adrenal gland cultures.* Endocrinology, 1973. **93**(5): p. 1104-8.
28. Ali, S. and J.R. Bassett, *The release of corticosterone and a corticosterone-binding protein by incubated rat adrenal slices.* J Steroid Biochem Molec Biol, 1991. **39**(1): p. 119-29.
29. Szkudlinski, M., *et al.*, *Hydrocortisone inhibits the basal and angiotensin II-stimulated proliferation of rat adrenocortical cells in organ culture.* Exp Clin Endocrinol, 1990. **95**(2): p. 197-202.
30. Vinson, G.P., *et al.*, *The role of the tissue renin-angiotensin system in the response of the rat adrenal to exogenous angiotensin II.* J Endocrin, 1998. **158**: p. 153-59.
31. Yamamoto, M., K. Arishima, and Y. Eguchi, *The sensitivity of the fetal rat adrenal gland to adrenocorticotropic hormone in vivo and in vitro.* Biol Neonate, 1986. **50**(1): p. 48-54.
32. Yamamoto, M., *et al.*, *Sensitivity of the fetal rat pituitary-adrenal system to corticotripin-releasing factor in organ culture.* Acta Anat, 1992. **145**: p. 88-92.
33. Zhu, Y.S., *et al.*, *Time course of enkephalin mRNA and peptides in cultured rat adrenal medulla.* Molecular Brain Research, 1992. **12**: p. 173-80.
34. Cameron, L.A. and J.P. Hinson, *The role of nitric oxide derived from L-arginine in the control of steroidogenesis, and perfusion medium flow rate in the isolated perfused rat adrenal gland.* J Endocrinol, 1993. **139**: p. 415-423.

35. Garrido, M.M., J. Manzanares, and J.A. Fuentes, *Hypothalamus, anterior pituitary and adrenal gland involvement in the activation of adrenocorticotropin and corticosterone secretion by gastrin-releasing peptide.* Brain Research, 1999. **828**: p. 20-26.
36. Malendowicz, L.K., *et al.*, *Effect of bombesin on the structure and function of the rat adrenal cortex.* Res Exp Med, 1991. **191**: p. 121-28.
37. Mazzocchi, G., *et al.*, *Effects of galanin on the secretory activity of the rat adrenal cortex: in vivo and in vitro studies.* Res Exp Med, 1992. **192**: p. 373-81.
38. Porter, I.D., *Effects of dopamine, high potassium concentration and field stimulation on the secretion of aldosterone by the perfused rat adrenal gland.* J Edocrinology, 1992. **133**: p. 275-82.
39. Nakamura, H., S. Motoyoshi, and T. Kadokwa, *Anti-inflammatory action of interleukin 1 through the pituitary-adrenal axis in rats.* Eur J Pharmacol, 1988. **151**: p. 67-73.
40. Rebuffat, P.m., L. K., *et al.*, *Morphology and functional responses of isolated inner adrenocortical cells of rats infused with interleukin-β.* Histol Histopath, 1992. **7**: p. 183-88.
41. Goodman, R., U. Otten, and H. Thoenen, *Organ culture of the rat adrenal medulla: a model system for the study of trans-synaptic enzyme induction.* J Neurochem, 1975. **25**(4): p. 423-7.
42. Raven, P.W., M.E. McAuley, and G.P. Vinson, *Serine proteases selectively control the output of 18- hydroxycorticosterone and aldosterone in stimulated zona glomerulosa tissue of the rat adrenal.* J Endocrinol, 1983. **99**(1): p. 13-22.
43. Unsicker, K., *et al.*, *Embryonic rat adrenal glands in organ culture: effects of dexamethasone, nerve growth factor and its antibodies on pheochromoblast differentiation.* Cell Tissue Res, 1985. **241**: p. 207-17.
44. Carsia, R.V., *et al.*, *Apoptotic cell death in the rat adrenal gland: an in vivo and in vitro investigation.* Cell Tissue Res, 1996. **283**(2): p. 247-54.
45. Williams, B.C., *et al.*, *Characterization of the steroidogenic responsiveness and ultrastructure of purified zona fasciculata/reticularis cells from bovine adrenal cortex before and after primary culture.* Journal of Endocrinology, 1989. **121**: p. 317-24.
46. Sibley, C.P., *et al.*, *Studies on the distribution of steroid between medium and tissue after incubation of rat adrenal glands in vitro [proceedings].* J Endocrinol, 1978. **79**(2): p. 63P-64P.
47. Aggarwal, S.K. and J.M. Fadool, *Cisplatin and carboplatin induced changes in the neurohypophysis and parathyroid, and their role in nephrotoxicity.* Anti-Cancer Drugs, 1993. **4**: p. 149-62.
48. Batzer, M.A. and S.K. Aggarwal, *An in vitro screening system for the nephrotoxicity of various platinum coordination complexes.* Cancer Chemotherapy and Pharmacology, 1986. **17**: p. 209-17.
49. Clifton, G.G., *et al.*, *Early polyuria in the rat following single-dose cis-dichlorodiammineplatinum (II).* Lab Clin Med, 1982. **100**(5): p. 659-70.

50. Fadool, J.M., P.J. Boyer, and S.K. Aggarwal, *Effects of cisplatin treatment on the rat neurohypophysis.* Proc Elect Micro Soc Am, 1986. **44**: p. 122-3.
51. Gordon, J.A., L.N. Peterson, and R.J. Anderson, *Water metabolism after cisplatin in the rat.* Amer J Physiol, 1982. **243**(1): p. E36-43.
52. Aggarwal, S.K., *et al.*, *Cisplatin-induced peptic ulcers, vagotomy, adrenal and calcium modulation.* Anticancer Drugs, 1994. **5**(2): p. 177-93.
53. Aggarwal, S.K., *Cisplatin-induced peptic ulcers and calcium modulation.* ICEM, 1994. **13-Paris**: p. 1079-80.
54. Miller, C.M., S.K. Aggarwal, and K.K. Klomparens, *Cisplatin-induced changes in the adrenal of the rat*, in *Proc Electron Microscope Society of America*, G.W. Bailey, J. Bentley, and J.A. Small, Editors. 1992, San Francisco Press: San Francisco. p. 1652-3.
55. Ribelin, W.E., *The effects of drugs and chemicals upon the structure of the adrenal gland.* Fundamental and Applied Toxicology, 1984. **4**: p. 105-19.