CHAPTER - 7

EFFECT OF CARBOPLATIN ON PIGEON KIDNEY METABOLIC ACTIVITIES

Heavy metal platinum co-ordination complexes such as Cisplatin, Carboplatin, Iproplatin, Proplatin, etc. are attracting considerable attention as potential chemotherapeutic agents a number of tumors. Of these, one of the widely used compounds is Cisplatin (CDDP) which has chronic deleterious effects. Morphological damage to kidney is one of the several toxic effects met with after CDDP treatment. Proteinemia, Diarrhoea, Anorexia, Splenic atrophy, inhibition of cytokinesis, hypocellularity, hypocalcemia, hyperglycemia, severe nausea, etc. are some of the other ill effects caused by Cisplatin. Peripheral neuropathy and hypomagnesemia were also observed (Rosenberg, 1980; Aggarwal, 1974; Cowan, 1980; Schaeppi et al., 1973) with cisplatin-treatment. Nephotoxicity being severe, limits the chemotherapeutic usage of the drug (Goldstein et al., 1983; Mayor et al., 1983). Cytochemical studies by Aggarwal (1981) and fine structural studies using platinum co-ordination complexes suggests that these complexes might inhibit the mitochondrial enzymes and their production by complexing with RNA or DNA, thus resulting in kidney toxicity. A comparative study made by Batzer and Aggarwal (1986) on the toxic effects of various drugs, revealed that Carboplatin (CBDCA) became less toxic, compared to all other compounds. Analysis on various phosphatases revealed that compared to Cisplatin, Carboplatin showed very less side effects. Even the minimum dosage used in case of Cisplatin which was 5 mg/kg body wt. showed toxic signs than that of Carboplatin which was 50 mg/kg body wt. Alkaline phosphatase showed poor reaction with CDDP (10%) which in case of CBDCA was (100%). Ca^{+}-ATPase, Na^{+}-K^{+}-ATPase, and nucleases showed analogous response in their activity. Another

study by same authors in isolated kidney tubules showed no reaction with cisplatin while that with carboplatin averaged at 50%. Urine volumes monitored showed an increase in CDDP treatment while a decrease was observed after CBDCA treatment. Thus it was opined that CBDCA was much less toxic in comparison to CDDP.

Vagotomy and CDDP treatment showed very similar effects on the metabolic activities indicating that the effects of CDDP must be through parasympathetic inhibition (Chapters 5,6). Since CBDCA (carboplatin) is less toxic, it may not cause autonomic neural suppression as much as CDDP is capable of. To derive a comprehensive comparative data, it was thought worthwhile to repeat the experiments with carboplatin, and then to study pigeon kidney metabolic activities.

Materials and Methods

Adult blue rock pigeons weighing about 250-300 gms were acclimated to laboratory conditions and were divided into 2 groups of 6 each. One set received Carboplatin (CBDCA) injections (50 mg/kg body wt.) and a parallel set treated with Sucrose (0. 5%) served as controls. Food and water was provided ad libitum. All birds were sacrificed under mild anesthetia at 72 hours. Kidneys were excised from both sides and weighed. A piece of tissue was transferred to alcoholic KOH for estimating the glycogen content.

Enzymes like Glycogen synthetase, Glucose-6-phosphatase, Phosphorylase, Aldolase, Lactate dehydrogenase, Succinic dehydrogenase, Pyruvate

TABLE 1 : EFFECT OF CARBOPLATIN ON PIGEON KIDNEY METABOLISM

GLYCOGEN	0.0016± 0.0001	0.0026±** 0.0003
GLYCOGEN-SYNTHETASE	0.0062± 0.0130	0.1011±** 0.0179
G-6-PASE	0.1376± 0.0117	0.1941±** 0.0282
PHOSPHORYLASE	18.1795± 0.3761	23.1582±**** 1.229
ALDOLASE	0.0008± 0.00003	0.0014±*** 0.0001
LDH	44.5024± 0.5665	34.675±*** 1.439
SDH	15.3869± 0.3806	13.0394±* 0.4956
PYRUVATE CARBOXYLASE	0.7836± 0.0241	0.2585±**** 0.0046
GPT	12.6595± 0.1792	12.3923±** 0.0719
GOT	8.2074± 0.4292	8.8417±** 0.8168
Na^+-K^+-ATPase	5.2432± 0.1687	5.4853±** 0.5171
ALKALINE PHOSPHATASE	2.0210± 0.0633	1.3725±**** 0.0651
ACID PHOSPHATASE	1.410± 0.0693	1.6953±* 0.0468
PROTEIN	10.7672± 0.1778	10.9376±** 0.3752

$P \leq 0.02^{*}$ $P \leq 0.05^{**}$ $P \leq 0.01^{***}$ $P \leq 0.001^{****}$

TABLE 2 : EFFECT OF CARBOPLATIN IN PIGEON KIDNEY METABOLISM

IN TERMS OF PERCENTAGE CHANGES.

GLYCOGEN	62.5	↑
GLYCOGEN-SYNTHETASE	1530	↑
G-6-PASE	41.06	↑
PHOSPHORYLASE	27.38	↑
ALDOLASE	75	↑
LDH	22.08	↓
SDH	1.56	↓
PYRUVATE CARBOXYLASE	67	↓
GPT	2.1	↓
GOT	7.7	↑
Na^+-K^+-ATPase	4.6	↑
ALKALINE PHOSPHATASE	32	↓
ACID PHOSPHATASE	20.2	↑
PROTEIN	1.58	↑

(% is corrected to nearest whole number, expressed as increase [↑], decrease [↓] in value of the group in parenthesis compared to its adjoining group)

$P \leq 0.02^{*}$, $P \leq 0.05^{**}$, $P \leq 0.01^{***}$, $P \leq 0.001^{****}$

EXPLANATION TO FIGURES

Effect of Carboplatin treatment with respect to:

Fig (1) : Glycogen content and activities of glycogen synthetase, glucose-6-phosphatase, phosphorylase in the kidney.

Fig (2) : Activities of aldolase, LDH, SDH, pyruvate carboxylase in the kidney.

Fig (3) : Protein content and activities of GPT, GOT, Na^+-K^+-ATPase, acid and alkaline phosphatases in the kidney.

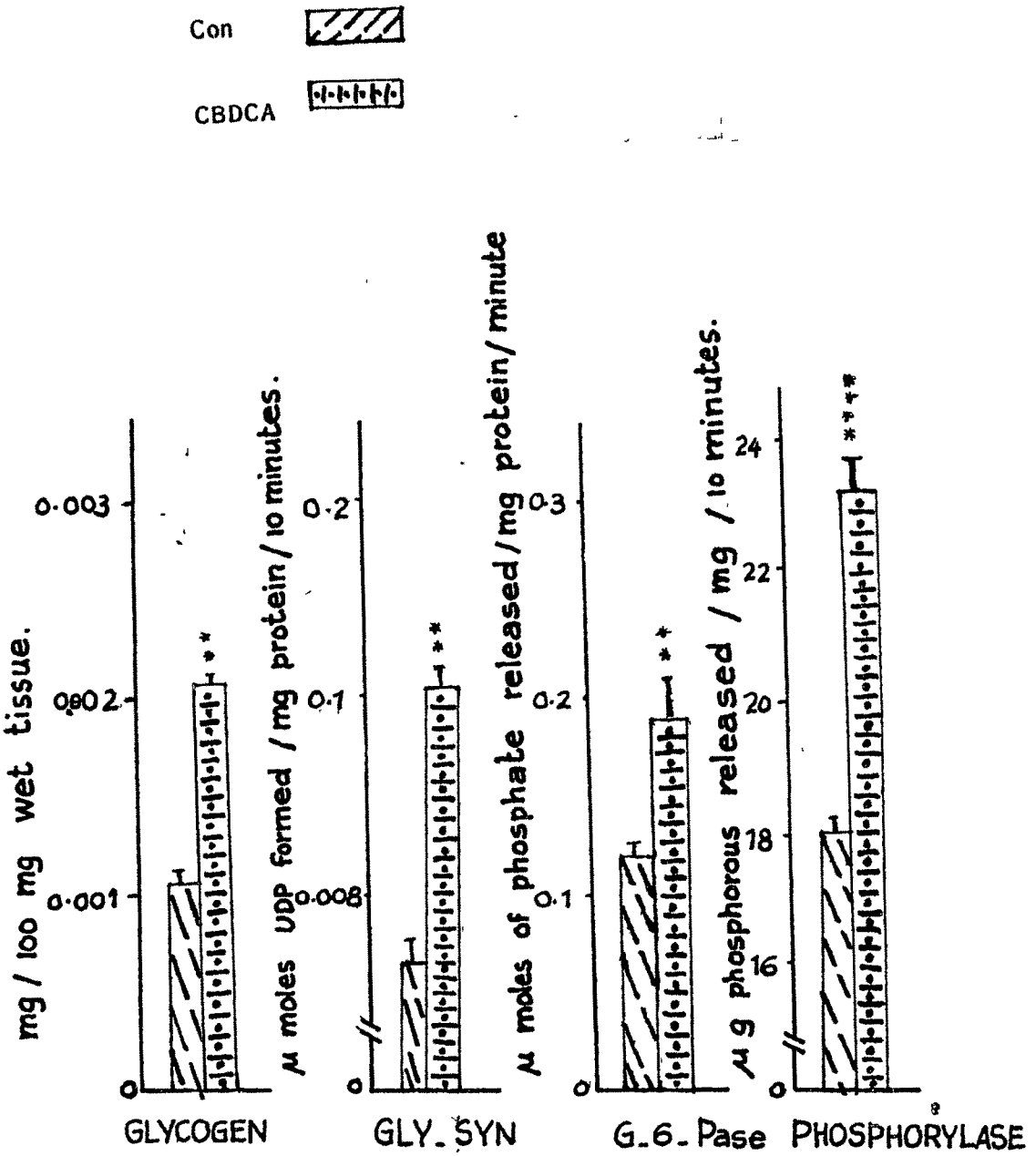

Fig : 1 Results given as mean ± SEM of six experiments. $P < 0.02^{*}$, $P < 0.05^{**}$, $P < 0.01^{***}$, $P < 0.001^{****}$

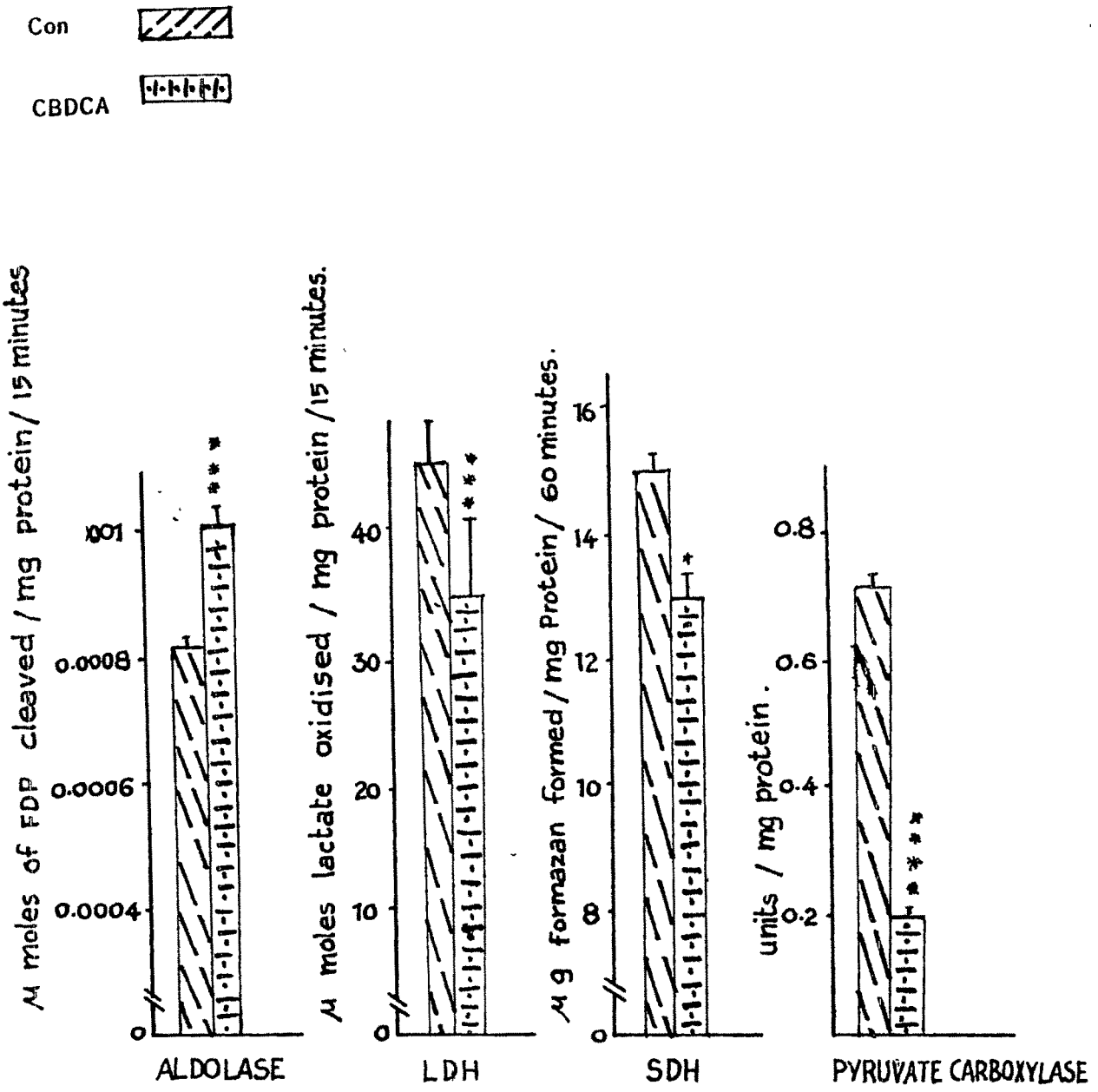

Fig :2 Results given as mean ± S E M of six experiments.
$P<0.02^{*}$, $P<0.05^{**}$, $P<0.01^{***}$, $P<0.001^{****}$

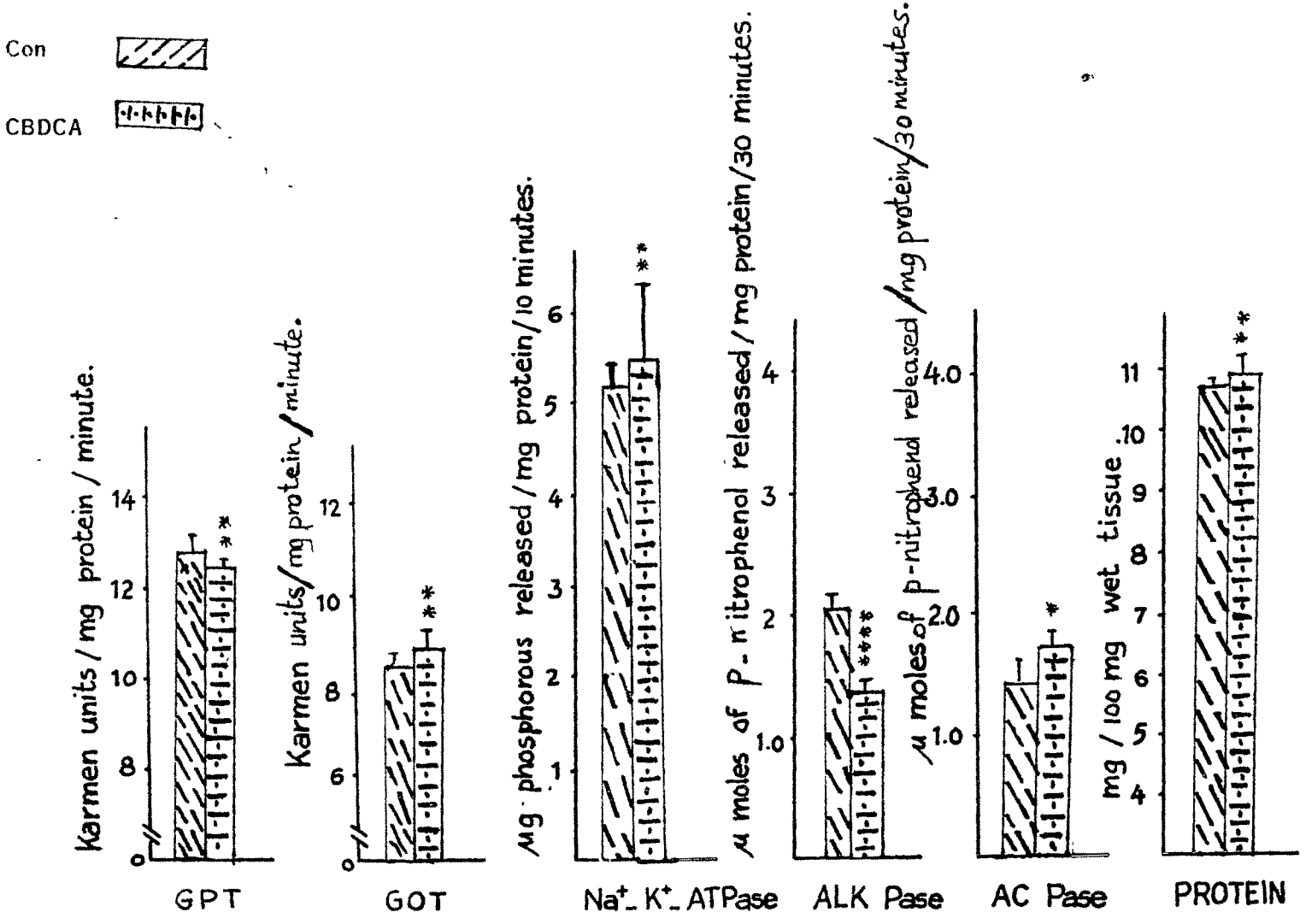

Fig :3 Results given as mean ± SEM of six experimements.
$P<0.02^{*}$, $P<0.05^{**}$, $P<0.01^{***}$, $P<0.001^{****}$

carboxylase, Transaminases and various phosphatases (Na^+-K^+-ATPase, Alkaline and Acid Pases) along with protein content were also assayed.

Results

Glycogen content in the pigeon kidney along with glycogen synthetase activity showed an increase. G-6-Pase though showed slight but non-significant increase compared to controls. Phosphorylase and Aldolase compared to controls showed higher values while, Lactate dehydrogenase, Succinate dehydrogenase and Pyruvate carboxylase showed lower levels. Transaminases (GOT and GPT) and protein remained mostly unchanged compared to controls. Transport enzymes showed varied responses to CBDCA treatment as activities of Na^+-K^+-ATPase remained unaltered, while Alkaline Pase diminished and Acid Pase increased. On the whole the pattern of response of CBDCA was in many respects opposite to what was observed with CDDP treatment (Chapters 5,6).

Discussion

Responsiveness to platinum complexes may depend on the presence of certain biochemical characteristics that may render certain types of cells unusually sensitive to this class of agents. Different tumors may differ greatly in drug sensitivity.

The antitumor activity of platinum complexes were discovered as a result of fortuitious observations by Rosenberg during the study of effects of

electric current on growing bacteria. He suggested that an active substance may have entered the medium, possibly through the release of soluble platinum compounds from the electrodes. Usage of various platinum compounds as antitumor agents have been reported by various authors, of which cisplatin was found to be the most effective but had deleterious effects (Chapters 2,3). Since the toxicity was a limiting factor, carboplatin (CBDCA), a less toxic substitute was suggested for the treatment.

Diamminecyclobutane-dicarboxylatoplatinum-II (CBDCA), Carboplatin seems to have subcellular mechanism of action very similar to that of Cisplatin, although its chemical spectrum of side effects differ somewhat from that of Cisplatin. Alkaline elution studies by Micetich et al. (1985) on the comparative effects of cytotoxicity between CDDP and CBDCA suggests that CBDCA was 45 times less toxic than Cisplatin. Infusion studies on CBDCA by Curt et al. (1983) and Reece et al. (1987) suggested that the mean renal clearance of the drug was only 61% of the total body clearance. Ozols and colleagues (1987) reported that patients with ovarian cancer can safely receive 800 mg of Carboplatin per m^2 per cycle in 2-12 hours. Thus CBDCA treatment shows a very 'insignificant' effect on the distribution of various enzymes. Batzer and Aggarwal (1986) who studied the comparative effects between CDDP and CBDCA treatment on certain enzymes in rats found that the effects are same in both in vivo and in vitro conditions.

In the present study, CBDCA administration caused an increase in the level of Glycogen, Glycogen Synthetase, Phosphorylase and Aldolase indicating that active glycogenolysis along with a glycolysis must be occuring, though G-6-Pase remained unaltered. Further the diminished activities of LDH, SDH and PC suggests that gluconeogenic machinery did not operate as effective as in the case of CDDP treated birds and VgX birds. The concomitant increase of glycogenolysis and glycolysis must have contributed to the observed increase in glucose release, although glucose level was not very high compared to that of CDDP treated birds (Oommen, 1992). The fact that both transaminases remain unaltered validates the view that the gluconeogenic mechanisms were not very active in CBDCA treated birds. However, the response of transport enzymes indicate that even the uptake mechanisms were affected adversely.

In case of Acid Pase lysosomal build-up occurs until the cells lyse, thereby releasing the enzyme into urine. The decrease in Alkaline Pase correspondingly explains that membrane enzyme levels were high. An opposite effect was observed by Batzer and Aggarwal (1986), in case of rats. The difference here could be possible due to the changes which are met within the avian system. ATPase being a membrane bound enzyme plays a vital role in maintaining ionic stability. Depending on the strength by which it is bound it can be activated or inactivated. Stekhoven and Bonting (1981) have opined that inactivation of ATPase would lead to ionic imbalance thereby causing a cell kill. These altered responses were quite conspicuous in case of CDDP treatment and VgX birds (Chapters 5,6).

An evaluation made by Prajda et al. (1989) on side effects of platinum complexes suggested that the side effects were recovered completely by 96 hours of treatment. They used Carboplatin and Iproplatin in comparison to Cisplatin; where toxicity of CBDCA was of the least when observed. Foster et al. (1985) and Ozol et al. (1987) have opined that there seems to be substantial clinical differences between Cisplatin and Carboplatin. Ozol et al. (1985) and Reed et al. (1988) reported that there was no clinically apparent nephrotoxicity or neurotoxicity observed in their investigations with CBDCA. The present results too reveal that usage of Carboplatin was causing less metabolic derangements in comparision to that of Cisplatin and VgX birds.

In conclusion, it could be stated that when neurotoxic side effects are minimum as in CBDCA treatment the metabolic derangements would also be minimum.