CHAPTER - VI

EFFECT OF CARBOPLATIN ON LIVER AND KIDNEY OF RAT AND PIGEON

The mechanism of action of cisplatin (CDDP) has captured the interest of scientific community since the discovery of its antimetastatic properties. The second generation analogue, carboplatin (cis-diammine-1, 1-cyclobutanedicarboxylate Pt (II) (CBDCA), was found to be less toxic than cisplatin. CBDCA has been shown to have antimetastatic activity similar to that of cisplatin, although the chemotherapeutic dosage required is ten times greater than that of CDDP (Siddik et al., 1984). Carboplatin is well-tolerated and may have a role as a less toxic substitute for cisplatin in combination chemotherapy regiments for oesophageal cancer (Mannel et al., 1989). However, in rats a maximal tolerated dose of carboplatin has produced some toxic effects (Siddik et al., 1987).

Choie et al., (1980) reported that the kidney retains a greater concentration of cisplatin and its biological half-life is long in this organ. As compared with the above, no significant changes were noticed in renal function parameters after carboplatin treatment (Laznickava et al., 1989). Acid phosphatase which is present in lysosomes throughout the kidney increased substantially in quantity after cisplatin treatment; but only a slight increase was noted after CBDCA treatment (Batzer and Aggarwal, 1986). The increase in alkaline phosphatase activity in the urine, however, does

indicate that while both drugs have nephrotoxic effects, the CBDCA has much less degree of toxic effect.

One of the side effects of cisplatin is its effect on nerves. Neuropathy is predominantly on sensory fibres and the concentration of cisplatin in both spinal cord and brain tissue is 5-20 fold lower than elsewhere in the body. It is speculated that CDDP affects the sensory neurons or supportive cells in the dorsal root ganglia which are less well-protected by the blood brain barrier (Thompson, et al., 1984). Recently, Mollman (1990) reported that carboplatin appears to have antineoplastic activity equal to that of cisplatin at least in some tumours without neurotoxicity.

In the present study, attempt was made to study the effect of carboplatin treatment on glucose metabolism, AChE, protein and nucleic acid contents in liver and kidney of rats and pigeons.

MATERIALS AND METHODS

In each experiment, male albino rats and domestic pigeons were housed in appropriately sized cages and were allowed ad libitum access to food and water. After acclimatizing to the laboratory condition, rats and pigeons were divided into four groups.

Group I : Experimental rats and domestic pigeons received a single ip. dose of CBDCA (50 mg/kg b.w.)

Group II : Control rats and pigeons were given sucrose (vehicle) only.

Group III : Experimental rats and pigeons which received a single dose of CBDCA (50mg/kg b.w.) were given a glucose load of 2 gm/kg b.w. and 70 mg/100gm b.w. respectively. These glucose loaded experimental animals were subjected to GTT.

Group IV : Control rats and pigeons which received only sucrose were given a glucose load of 2gm / kg b.w and 70 mg/100gm b.w respectively. These control animals were subjected to GTT.

Carboplatin was not diluted with solutions containing chloride ions because of possible conversion of the compound to cisplatin (Cheung et al., 1987). After the administration of drug, all animals were returned to the respective cages. Animals were pair fed for 60 hrs, followed by an overnight (12 hr) fasting. Then, the rats were sacrificed by exsanguination and pigeons by decapitation (Groups I & II). Blood glucose tolerance was monitored in rats and pigeons (Groups III & IV) from samples taken through the orbital

sinus puncture and from the brachial vein respectively (details in Chapter I). The liver and kidney were rapidly extirpated from the sacrificed animals and processed for AChE and nucleic acids (chapter-I). Statistical analysis was carried out by Student's 't' test.

RESULTS

Acetylcholinesterase (AChE)

In rats carboplatin (CBDCA) treatment caused a nonsignificant increase in AChE activity in the liver while a notable decrease was observed in the kidney (fig-1). In pigeons, the AChE level in the kidney remained unaltered but in the liver the level showed a significant decrease (fig-1).

Glucose tolerance test (GTT)

The glucose tolerance curves of CBDCA treated rats and pigeons were similar to that of control animals but in both the animals a mild elevation in the basal glycaemic level was observed. Upon glucose loading, the glucose level markedly elevated within 30 minutes and this increase lasted till 60 minutes. The level gradually regained normalcy by 150 minutes (fig-2).

Nucleic acids (DNA and RNA)

CBDCA treatment caused a significant decrease in DNA content in the kidney of rats, while no significant change was

Table I **Effect of CBDCA on AChE activity in the liver and kidney of rat and pigeon, (Mean ± SEM).**

Treatment	Rat		Pigeon	
	Liver	Kidney	Liver	Kidney
Control	0.004 ± 0.0004	0.018 ± 0.0003	0.0159 ± 0.0001	0.0046 ± 0.00054
CBDCA	0.0042NS ± 0..00031	0.0013** ± 0.00007	0.0104**** ± 0.0006	0.0045 NS ± 0.0001

values are expressed as μg substrate hydrolysed/mg protein/min.

NS - not significant, ** $p < 0.02$ **** $p < 0.001$

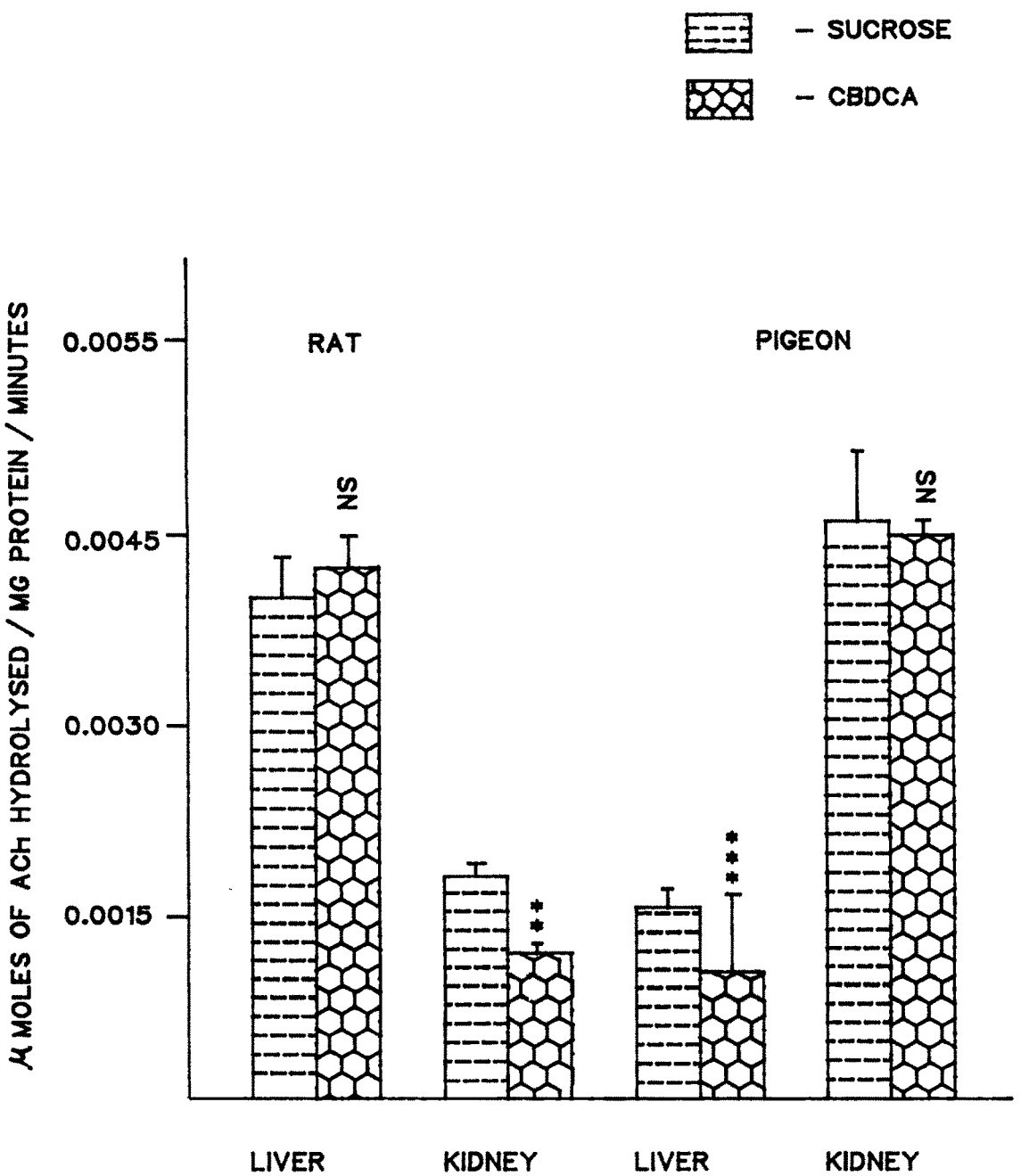

Fig. 1. EFFECT OF CBDCA ON AChE ACTIVITY IN LIVER AND KIDNEY OF RAT AND PIGEON RESULTS ARE EXPRESSED AS MEAN ± SEM. ** $P < 0.02$, **** $P < 0.001$; NS – NONSIGNIFICANT (N=6).

Table II **Effect of carboplatin (CBCDA) on glucose tolerance in rat and pigeon**

Interval in Minutes	Rat		Pigeon	
	Control	CBDCA	Control	CBDCA
0	144.234 ± 6.84	175.962 ± 6.274	215.423 ± 8.758	246.322 ± 9.540
30	202.144 ± 5.83	215.459 ± 7.538	277.971 ± 6.459	310.398 ± 9.059
60	164.539 ± 7.865	180.682 ± 8.355	250.440 ± 8.207	273.924 ± 11.560
90	178.261 ± 5.65	191.009 ± 6.48	226.293 ± 7.178	250.228 ± 13.191
120	163.793 ± 7.55	190.274 ± 6.08	207.933 ± 5.037	289.404 ± 11.68
150	166.997 ± 6.85	195.608 ± 5.22	211.136 ± 3.95	248.418 ± 10.896

(Mean ± SEM) (Glucose mg/100 ml blood)

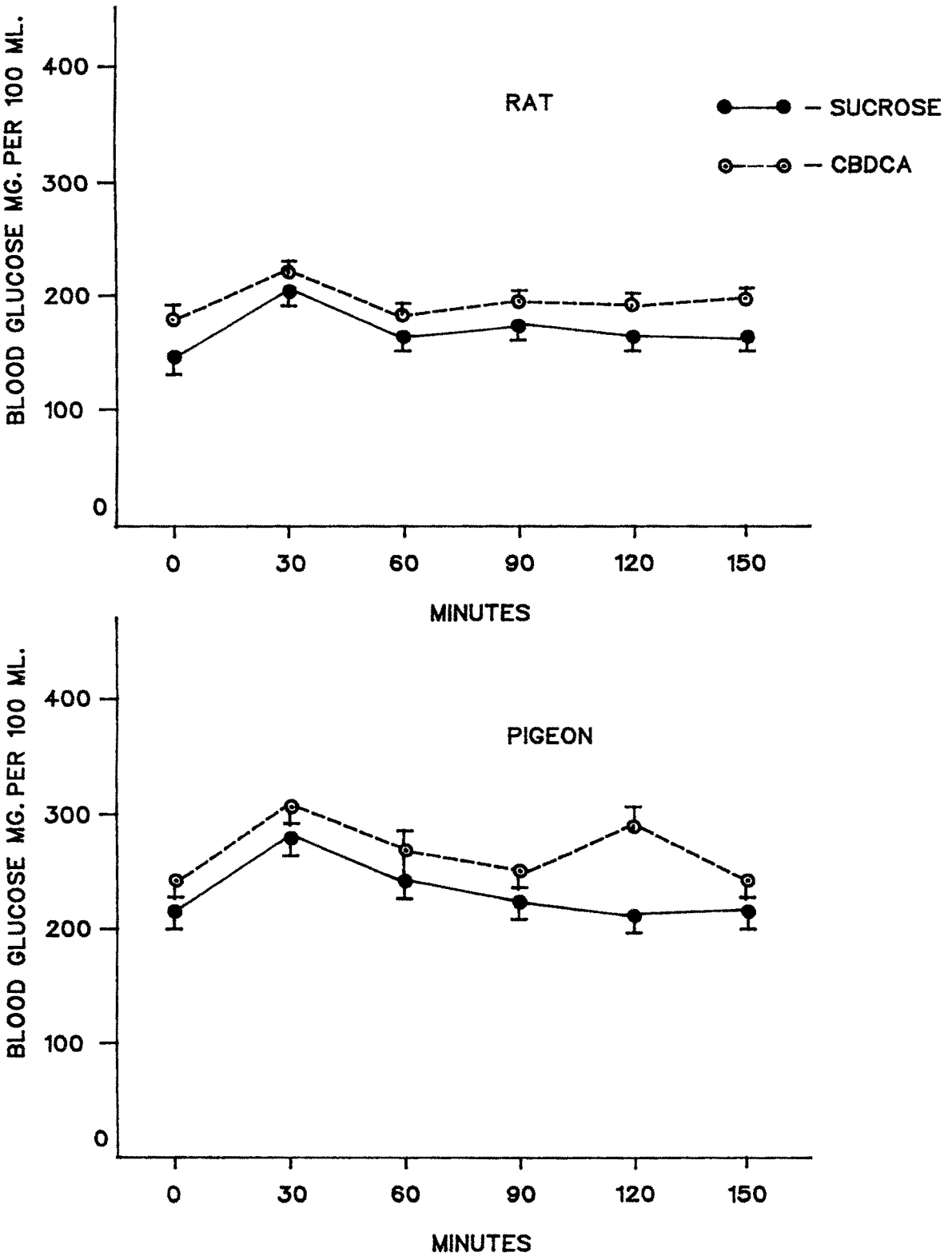

FIG. 2. GLYCAEMIC RESPONSE OF CBDCA IN RAT AND PIGEON. EACH POINT A VERTICAL LINE REPRESENTS THE MEAN ± SEM OF SIX DETERMINATIONS.

Table III **Effect of carboplatin (CBDCA) on the DNA and RNA content in the liver and kidney of rat and pigeon. (Mean ± SEM)**

Parameters		Rat		Pigeon	
		Liver	Kidney	Liver	Kidney
DNA	Control	0.1064 ± 0.005	0.1692 ± 0.0042	0.1246 ± 0.0038	0.1743 ± 0.0046
	CBDCA	0.1160NS ± 0.0043	0.1474** ± 0.0067	0.0767**** ± 0.0015	0.1467*** ±0.0032
RNA	Control	0.0682 ± 0.0016	0.0362 ± 0.0015	0.0611 ± 0.0018	0.0352 ±0.0005
	CBDCA	0.0616NS ± 0.0019	0.0314NS ± 0.0015	0.0562NS ± 0.0025	0.0314NS ±0.0004

Values expressed mg/100 mg of tissue.

NS - Not significant; **** $P < 0.001$; *** $P < 0.01$, ** $P < 0.02$

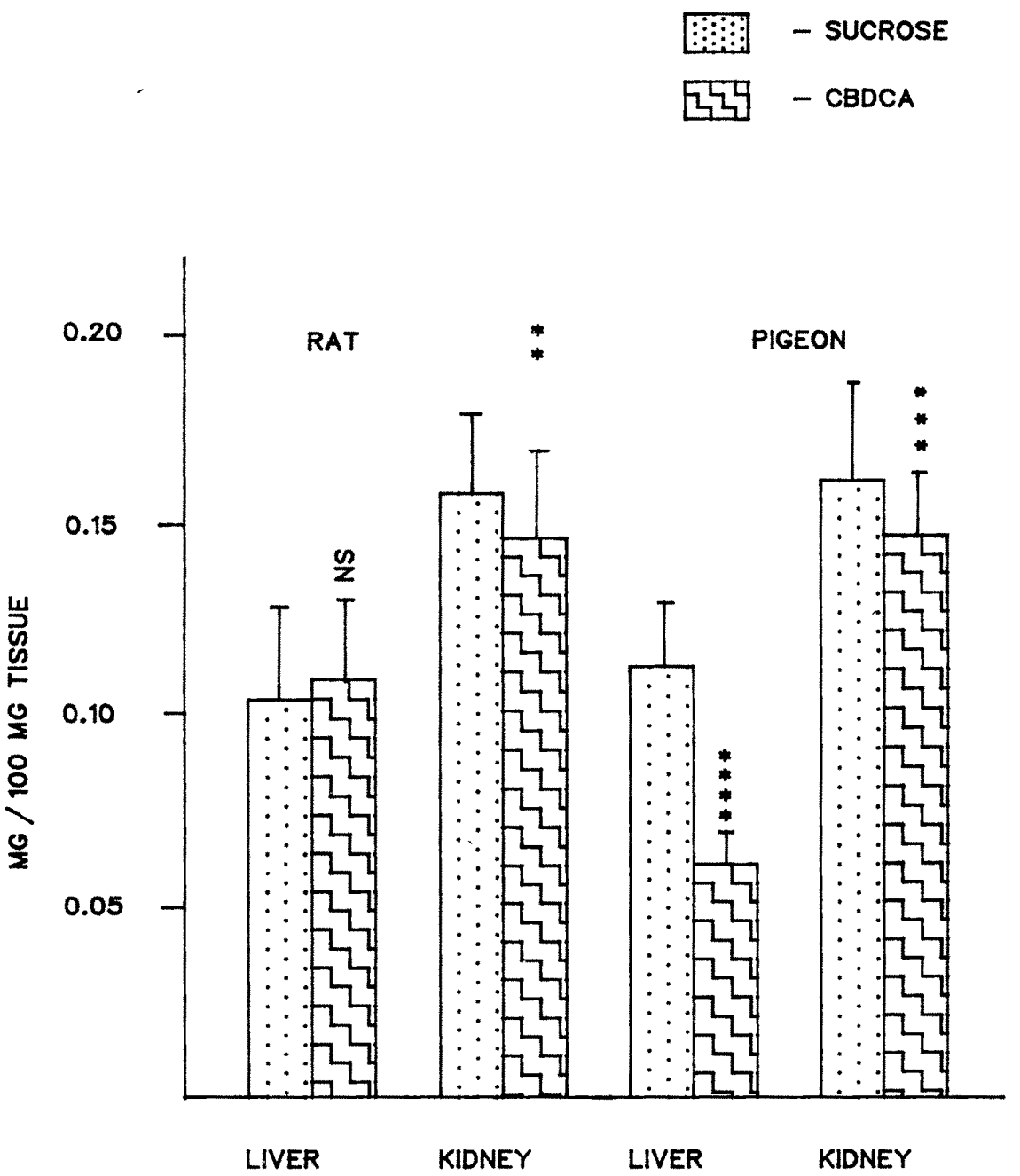

Fig. 3. EFFECT OF CBDCA ON DNA CONTENT IN LIVER AND KIDNEY OF RAT AND PIGEON. RESULTS ARE EXPRESSED AS MEAN ± SEM. *** $P < 0.01$, **** $P < 0.001$; NS – NONSIGNIFICANT (N=6).

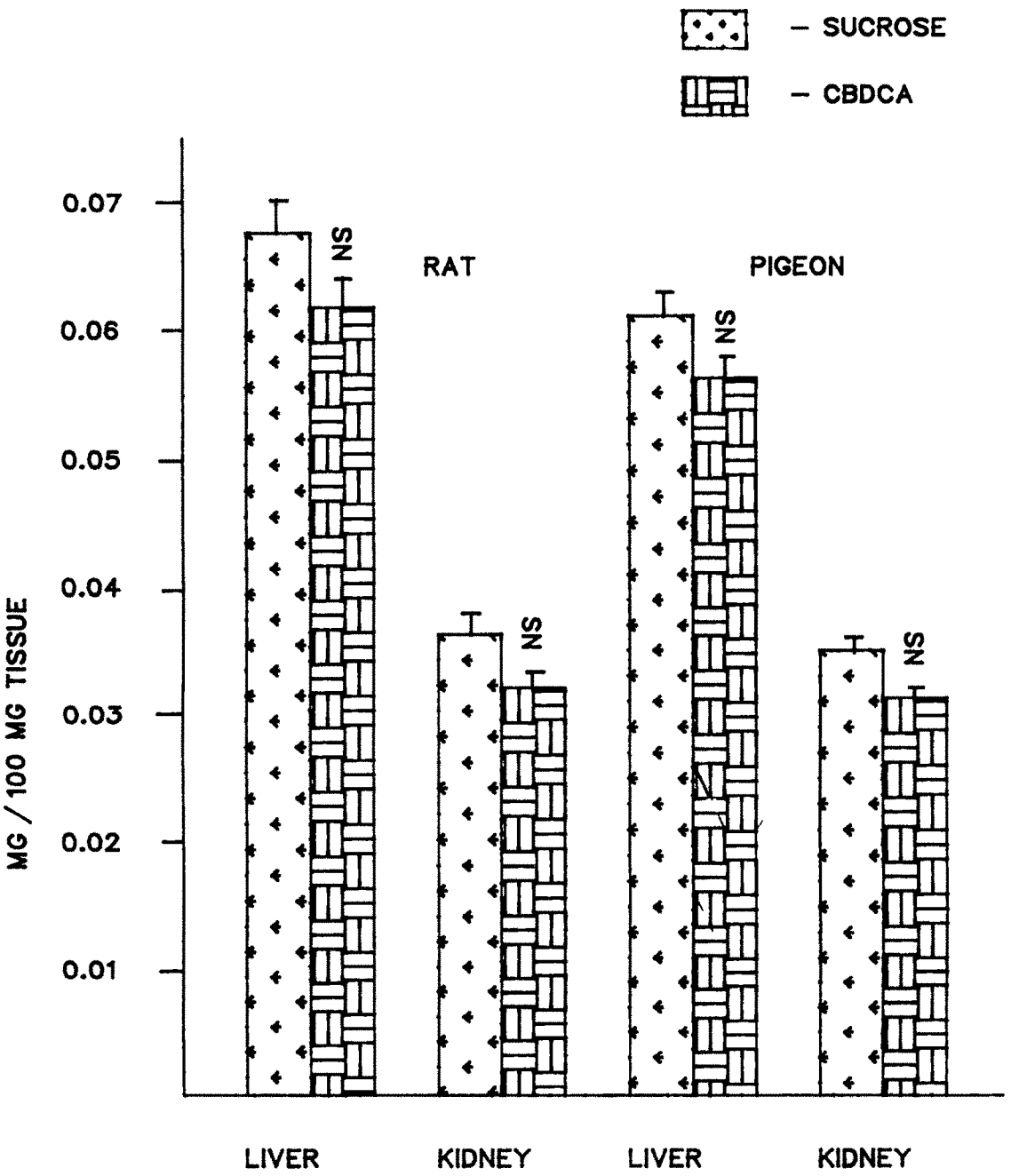

Fig. 4. EFFECT OF CBDCA ON RNA CONTENT IN THE LIVER AND KIDNEY OF RAT AND PIGEON. RESULTS ARE EXPRESSED AS MEAN ± SEM.
NS – NONSIGNIFICANT (N=6).

noticed in the liver (fig-3). In pigeons, DNA content in both the organs was found to be decreased significantly (fig-3). No significant differences were noticed in RNA levels in the liver and kidney of both the rats and pigeons due to carboplatin treatment (fig-4).

DISCUSSION

Diammine cyclobutanecarboxylate platinum (II), carboplatin (CBDCA) is known to have a subcellular mechanism of action very similar to that of cisplatin (CDDP), although its clinical spectrum of side effect differs somewhat from that of cisplatin. Clinical use of CDDP is limited by a number of drug-induced toxic reactions; the time course and frequency generally depending on the schedule of the drug delivery. These toxicities may include renal dysfunction, nausea, peripheral neuropathy, auditory impairment, visual impairment and rare case of siezures (Legha and Dimery, 1985; Tanaka et al., 1986; Coon et al., 1987). However, CBDCA treated rats were entirely free from toxic side effects like diarrhoea, nausea and stomach distention.

CBDCA treatment has a different effect on the levels of activities of various enzymes in the kidney and the liver. Equally, there were differences between rats and pigeons too. AChE activity in the liver of rats was unaffected due to CBDCA treatment but that of the kidney showed a significant decrease. In pigeons, while the liver AChE levels decreased

there was no significant change in the activity of the enzyme in the kidney. Since, Cholinergic parasympathetic innervation is found in both the liver and the kidney, the reduction in AChE could be considered as an index of reduced parasympathetic stimulation due to CBDCA treatment.

In rats the nucleic acid levels showed that, DNA content in the liver was not affected by carboplatin treatment but the same in kidney was decreased. The decrease in kidney DNA levels were much less than that of cisplatin treated rats (chapter- III). In pigeons, DNA levels both in the liver and kidney were found to be decreased. DNA is the principal biologic target of CDDP (Roberts and Pera, 1983). Micetich et al. (1985) studied the comparative cytotoxicity and DNA damaging effect of cisplatin and carboplatin. They reported that CBDCA is 45 times less toxic than CDDP when compared on a molar basis. From the studies of nucleic acid, it is clear that both DNA and RNA content in the liver of rats did not fluctuate significantly after CBDCA treatments. The decrease in DNA content in the kidney of both rat and pigeon, however, does indicate that the drug is inducing some cellular toxicity in this organs but at a lower rate compared to cisplatin. On the contrary, in pigeons, DNA levels decreased both in the liver and kidney after CBDCA administration. This reveals that an altogether different pattern of toxicity might be resulting from administration of platinum compounds in birds and mammals. In rats, carboplatin is having less

toxic side effects than cisplatin. In the light of above findings, it could be stated that CBDCA, by and large, is a promising second generation platinum analogue with respect to the decreased toxicity in rats. Carboplatin may therefore be an alternative to cisplatin in circumstances where renal and neural toxicity are of dose limiting consideration.