CHAPTER - V

A STUDY OF PROTECTIVE EFFECT OF CALCIUM CHLORIDE ON CISPLATIN TOXICITY AND VAGOTOMY

Ringer's classical experiments have shown the fundamental role of calcium ions in the contraction of muscle fibres. Since then, a number of calcium regulated functions were discovered, such as secretion, motility and neuronal activity. The calcium ions play a significant role in plasma membrane permeability. This divalent cation also acts as the mediator of neuronal and hormonal control on the cells.

Calcium accumulation is critical for the activation of several enzymes; examples include : glyceraldehyde phosphate dehydrogenase, pyruvate dehydrogenase and α-ketoglutaric acid dehydrogenase. It has been suggested that in many cAMP mediated hormonal systems, Ca^{2+} and ICM (intracellular mediators) may play a key role in the suppression of hormonal activation (Rassmussen, 1981; Nishizuka, 1984). Many hormones evoke change in calcium fluxes and alters intracellular calcium metabolism (Berridge and Irvine, 1984). An example for this is the stimulatory effect of parathyroid hormone (PTH) and cAMP on gluconeogenesis in the renal proximal tubule.

Rassmussen et al. (1981) have shown that the stimulatory effect of PTH on gluconeogenesis in renal proximal tubules may be mediated through its effects to increase intracellular calcium. Calcium plays a major role in glycogenesis in liver (Hue and Vande, 1981). The involvement of calcium in the

alteration of membrane permeability and stimulus response coupling of many non-excitable tissues have been reviewed by Rasmussen and Wassman (1981).

The vagus nerve has a profound influence on the hepatic membrane permeability, as vagotomy produced an increased Ca^{2+} in liver (Pilo et al., 1982) while a decrease in serum calcium level was observed in the vagotomized pigeons (Verma et al., 1982). These evidences indicate cholinergic influence on calcium permeability of the hepatic membrane. Studies on various tissues have indicated that many abnormalities associated with calcium metabolism occur in diabetic animals. A significant decrease in active calcium uptake in slices of kidney cortex from streptozotocin induced chronic diabetic rats was seen prior to any evidence of nephropathy (Hoskin et al., 1979).

Recently, it has been found that verapamil and other calcium channel antagonists can substantially increase the cytotoxicity of different antitumour agents (Tsuruo et al., 1985, 1987; Onoda et al., 1986, 1988). This effect was correlated with a reduced drug efflux from the cells modulated by the calcium channel blockers. Although the precise mechanism of this effect remains unknown, it is speculated that a decrease in intracellular Ca^{2+} concentration is involved (Tsuruo et al., 1982). Cisplatin, the successful antitumour agent, induces suppression of Ca^{2+} channel functions (Vassileu et al., 1987). The other

intracellular effect of this drug includes suppression of mitochondrial respiration and mitochondrial Ca^{++} concentration (Gerald and Gathone, 1986). Calcium channel blockers potentiate the cytotoxic effects of these drugs on resistant cell lines (Tsuruo et al., 1985). CDDP treatment alters calcium homeostasis in gastric smooth muscles and depletes the plasma membrane-bound calcium in kidney cells (Aggarwal and Hammouda, 1980; James et al., 1984). Cisplatin taken up by the kidney cortical mitochondria inhibited the calcium uptake which may contribute to cisplatin nephrotoxicity (Gemba et al., 1987). Further, it has been shown that in isolated mitochondria, cisplatin causes calcium efflux and mitochondrial damage (Aggarwal et al., 1980). Cisplatin could be depleting the cellular Ca^{2+} stores indirectly by causing serum hypocalcemia, a drug effect reported in rats (Schaeppi et al., 1973, Rosen et al., 1980). These evidences suggest that there occurs an adverse influence on the calcium levels after cisplatin treatment and vagotomy. The present investigation deals with an exogenous supply of calcium to cisplatin treated and vagotomised animals to study the effect of calcium as a protective agent preventing biochemical changes, cholinergic actions and nucleic acid contents in liver and kidney of rats and pigeons subjected to CDDP treatment and vagotomy.

MATERIALS AND METHODS

Male albino rats and domestic pigeons were used for the experiments. For experimental purpose all animals were housed separately, divided into four groups and allowed ad libitum access to food and water (Chapter I).

Group I. Cisplatin (CDDP) was dissolved in normal saline by gently heating the solution. In rats and pigeons, 1.3% $CaCl_2$ was injected twice daily (morning and evening) immediately after CDDP administration.

Group II. Control rats and pigeons received injections of an equal volume of saline and $CaCl_2$.

Group III. Subdiaphragmatic vagotomy was performed in rats and injected 1ml of 1.3% of $CaCl_2$ twice daily until the day of sacrificing. Pigeons underwent cervical vagotomy and received similar dose of $CaCl_2$ solution.

Group IV. Rats and pigeons were sham operated and administered at a dosage 1ml of 1.3% $CaCl_2$ twice a day.

This group served as control animals.

All groups of animals were deprived of food while water was given ad libitum. Cisplatin treated rats and pigeons were sacrificed on the 4th day of injection. Vagotomized animals were sacrificed on the 3rd day of surgery. Pigeons were

sacrificed by decapitation and rats by exsanguination. Liver and kidney were quickly extirpated and processed for AChE and nucleic acid assay (details in Chapter I).

RESULTS

Vagotomy + $CaCl_2$

Rat

Acetylcholinesterase (AChE) activity in the $CaCl_2$ treated VgX rat liver did not show variation from that of the controls (fig-1). The results obtained in AChE activity in the kidney of $CaCl_2$ treated VgX rat were also similar to that of liver (fig-2). So in both the liver and kidney of rat, calcium could nullify the effect of vagotomy as far as AChE is concerned.

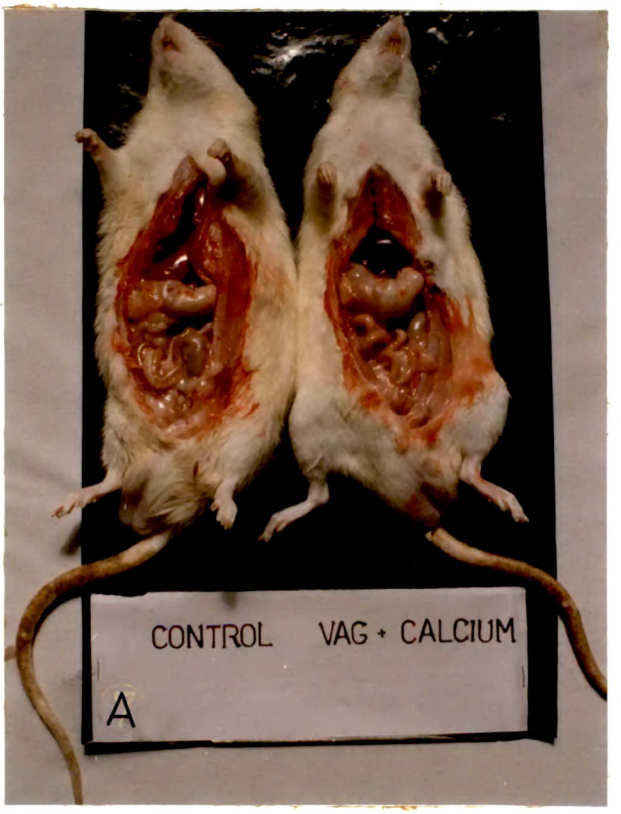
VgX rats treated with $CaCl_2$ showed a significant increase in the DNA contents in the liver and kidney (fig 3 & 4). $CaCl_2$ treatment thus had a beneficial effect on the DNA content in these organs. $CaCl_2$ administration to VgX rats also increased the RNA content of both the liver and kidney compared to sham operated rats administered with $CaCl_2$ (fig 5 & 6).

Pigeon

AChE in the liver of $CaCl_2$ treated VgX pigeons did not show any variation from that of sham operated pigeons (fig-1). In other words, $CaCl_2$ treatment prevented any increase in the AChE activity in the liver. However, in the kidney a similar

Explanation For Figures

Fig. A & B. Cisplatin treated and vagotomized rats were given exogenous supply of calcium chloride. Calcium supplementation reduces the stomach distention in cisplatin treated and vagotomized rats.


CONTROL VAG + CALCIUM
A


CONTROL CIS + CALCIUM
B

Table I **The effect of $CaCl_2$ administration on AChE activities in the liver and kidney of CDDP treated and VgX animals (Mean ± SEM)**

Treatments	Rat		Pigeon	
	Liver	Kidney	Liver	Kidney
Saline+$CaCl_2$	0.0028 ± 0.0002	0.0012 ± 0.00005	0.0069 ± 0.0004	0.0028 ± 0.0001
CDDP + $CaCl_2$	0.0035NS ± 0.0003	0.0012NS ± 0.00003	0.0047** ± 0.0001	0.0024NS ± 0.0002
VgS + $CaCl_2$	0.0032 ± 0.0009	0.0011 ± 0.00008	0.0094 ± 0.0007	0.0044 ± 0.00036
VgX + $CaCl_2$	0.0032NS ± 0.0001	0.0009NS ± 0.00007	0.0093NS ± 0.0009	0.0035** ± 0.00015

Values are expressed μ moles of ACh Hydrolysed / mg protein / min

** $P < 0.02$; **** $P < 0.001$; NS- Not Significant

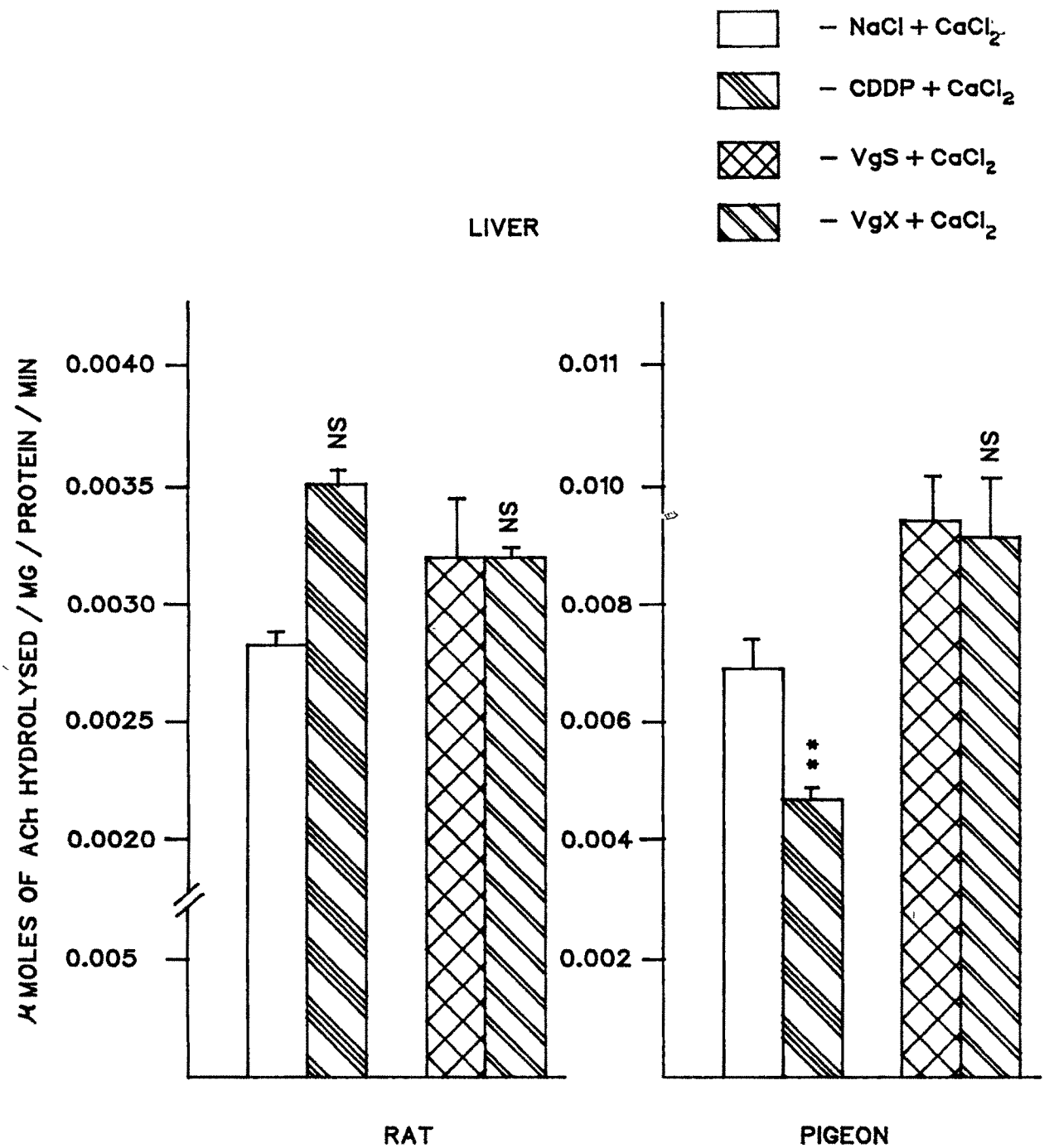


Fig. 1 EFFECT OF VAGOTOMY + $CaCl_2$ AND CDDP + $CaCl_2$ ON AChE ACTIVITY IN LIVER OF RAT AND PIGEON. EACH BAR REPRESENTS THE MEAN ± SEM OF AT LEAST SIX ANIMALS. NS – NON SIGNIFICANT; ** $P < 0.02$.

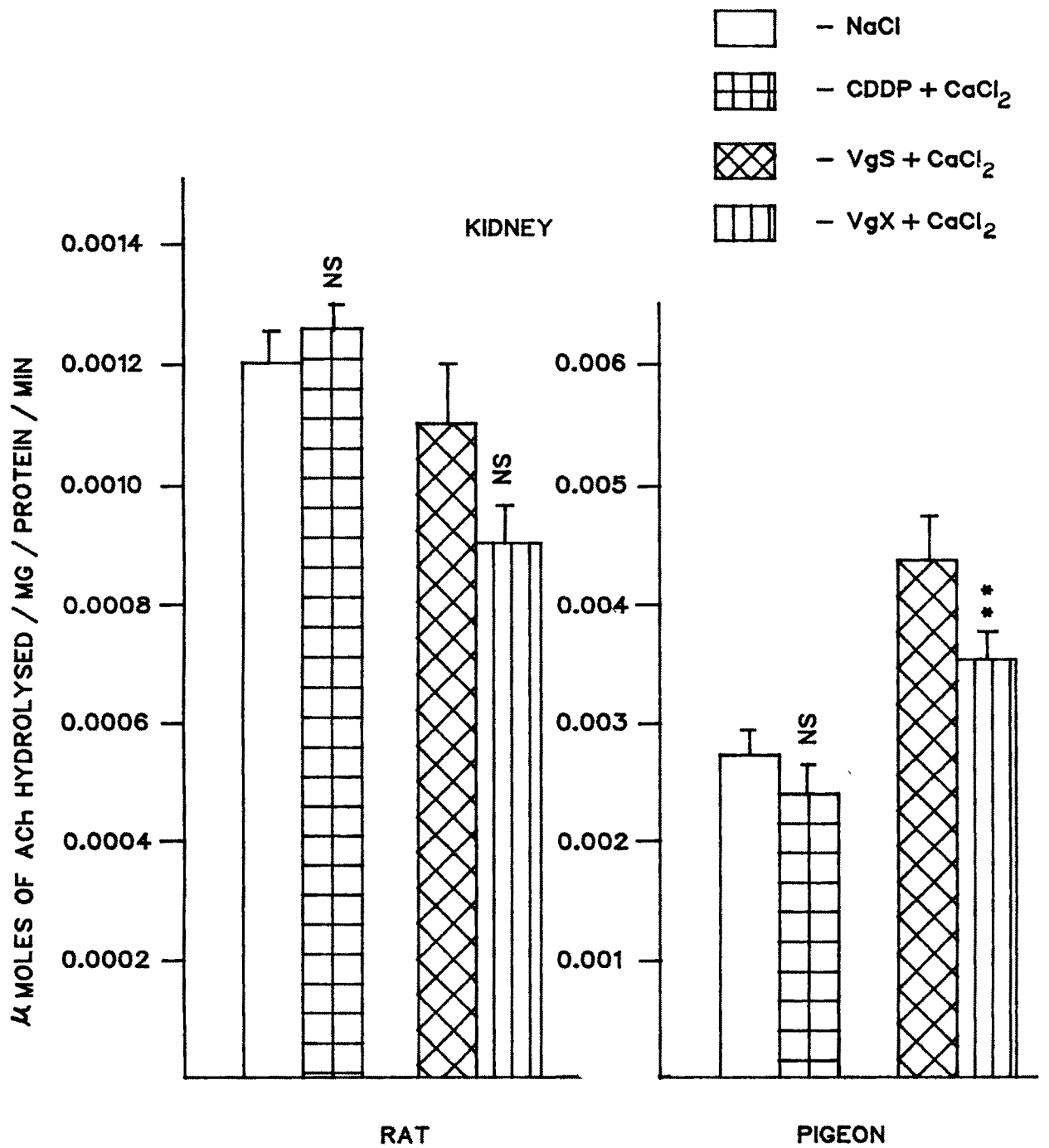


Fig. 2 EFFECT OF VAGOTOMY + $CaCl_2$ AND CDDP + $CaCl_2$ ON AChE ACTIVITY IN KIDNEY OF RAT AND PIGEON. EACH BAR REPRESENTS THE MEAN ± SEM OF AT LEAST SIX ANIMALS. NS – NON SIGNIFICANT; ** $P < 0.02$.

Table II Effect of $CaCl_2$ administration on DNA content in the liver and kidney of CDDP treated and VgX rats and pigeons (Mean ± SEM)

Treatments	Rat		Pigeon	
	Liver	Kidney	Liver	Kidney
Saline+$CaCl_2$	0.0576 ± 0.0012	0.0843 ± 0.0028	0.090 ± 0.0025	0.1038 ± 0.0037
CDDP+$CaCl_2$	0.0677* ± 0.0042	0.0885NS ± 0.0034	0.0703**** ± 0.0027	0.0891**** ± 0.0011
VgS+$CaCl_2$	0.0405 ± 0.0017	0.0820 ± 0.0028	0.0793 ± 0.0022	0.1058 ± 0.0043
VgX+$CaCl_2$	0.0518**** ± 0.0025	0.1028**** ± 0.0034	0.0777NS ± 0.0034	0.0856**** ± 0.0026

Values are expressed as / mg / 100 mg of tissue.

* $P < 0.05$; ** $P < 0.001$; NS - Not Significant

Table III Effect of $CaCl_2$ administration on RNA content in the liver and kidney of CDDP treated and VgX animals (Mean ± SEM)

Treatments	Rat		Pigeon	
	Liver	Kidney	Liver	Kidney
Saline+$CaCl_2$	0.0575 ± 0.0006	0.0344 ± 0.0009	0.0647 ± 0.0016	0.053 ± 0.0019
CDDP+$CaCl_2$	0.0603NS ± 0.001	0.0378NS ± 0.0013	0.0511**** ± 0.0012	0.0518NS ± 0.0006
VgS+$CaCl_2$	0.0499 ± 0.0007	0.0397 ± 0.0018	0.0679 ± 0.001	0.0511 ± 0.002
VgX+$CaCl_2$	0.0607** ± 0.0016	0.0471**** ± 0.0008	0.0837**** ± 0.0048	0.0469** ± 0.0009

Values expressed as mg/100mg of tissue.

** $P < 0.02$; **** $P < 0.001$; NS - Not Significant

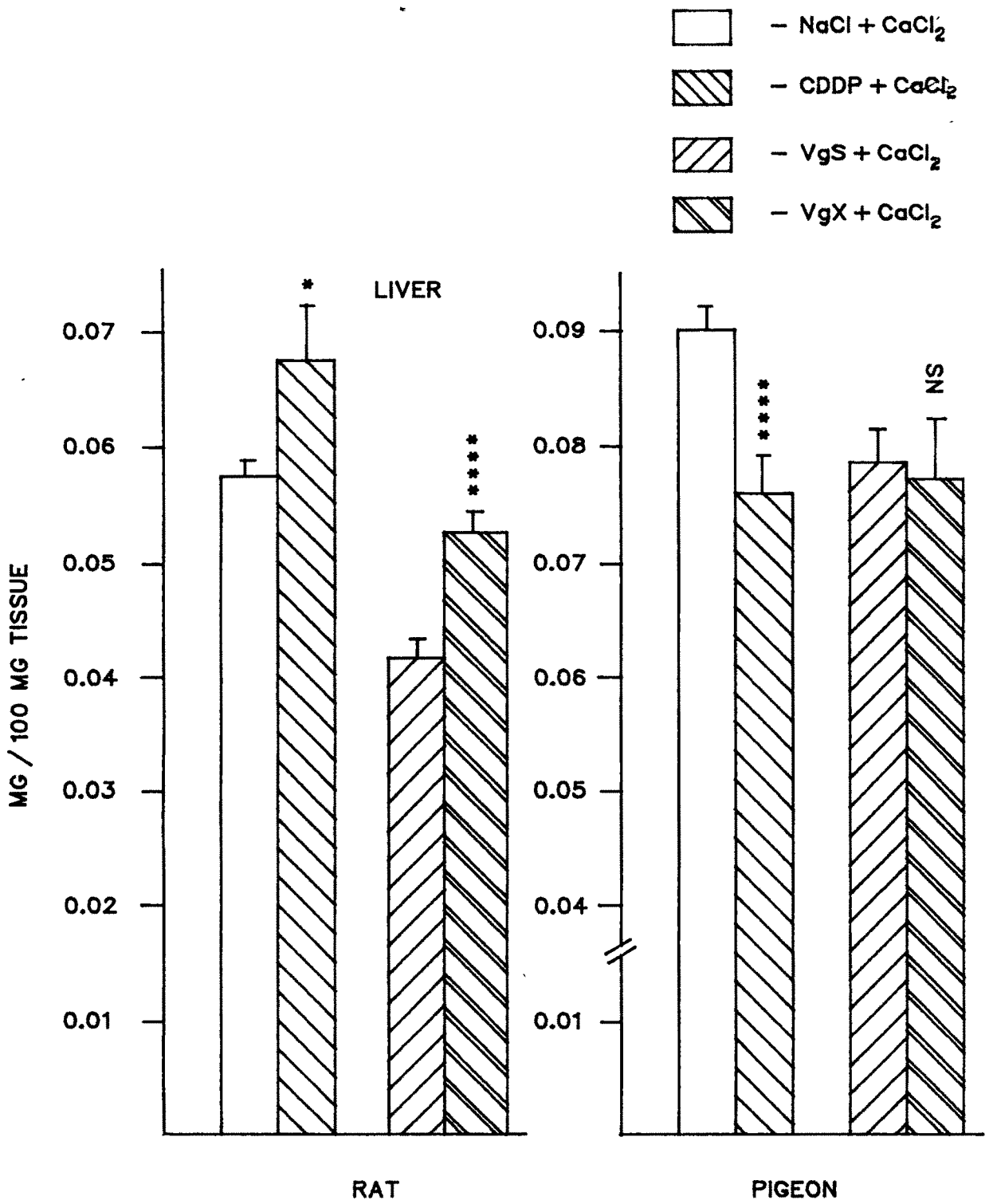


Fig. 3 EFFECT OF $CaCl_2$ ADMINISTRATION ON DNA CONTENT IN LIVER OF VgX AND CDDP TREATED RAT AND PIGEON. EACH BAR REPRESENTS THE MEAN ± SEM OF AT LEAST SIX ANIMALS. NS – NON SIGNIFICANT; **** $P < 0.001$; * $P < 0.05$.

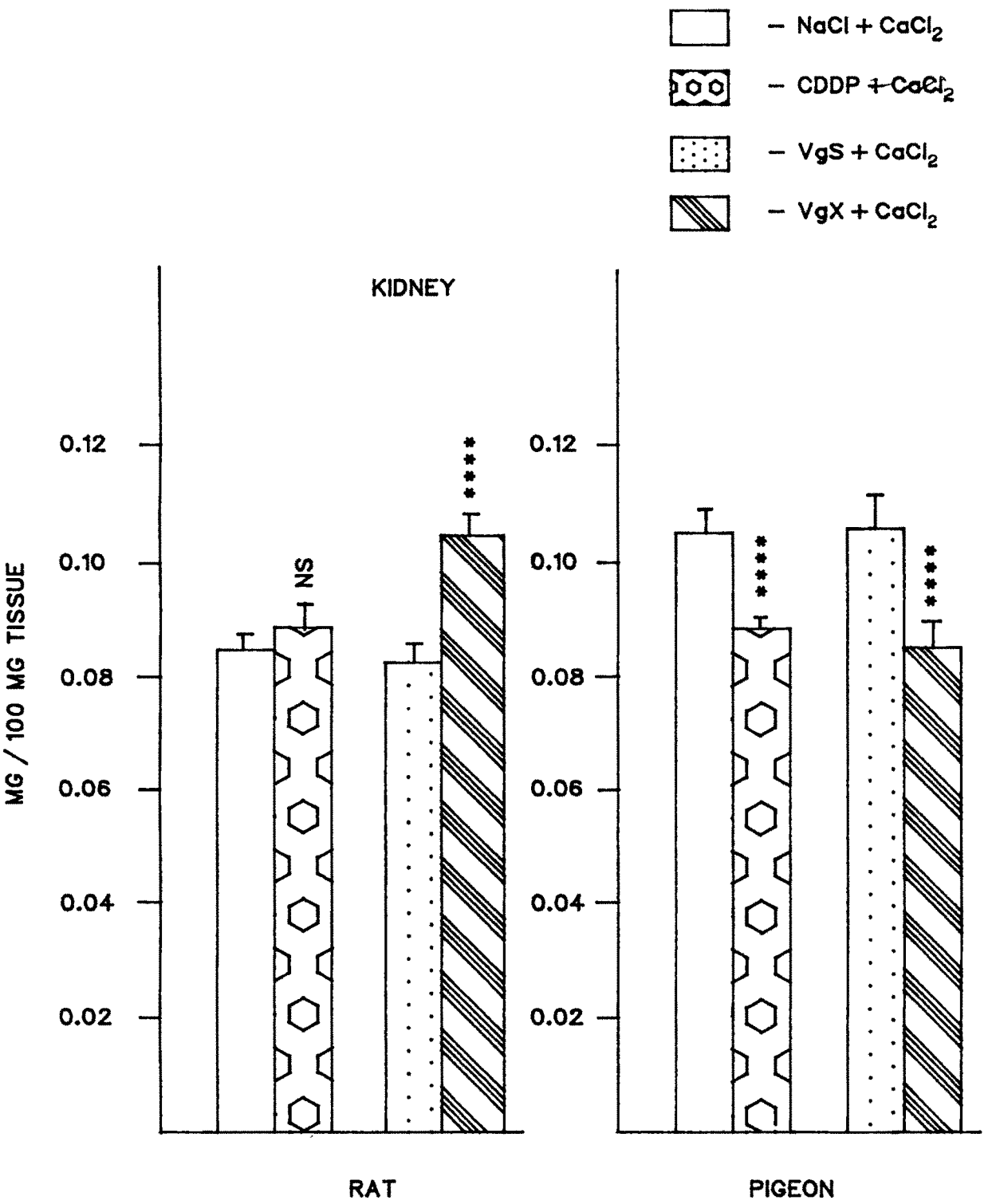


Fig. 4 EFFECT OF $CaCl_2$ ADMINISTRATION ON DNA CONTENT IN THE KIDNEY OF VgX AND CDDP TREATED RAT AND PIGEON. EACH BAR REPRESENTS THE MEAN ± SEM OF AT LEAST SIX ANIMALS. NS – NON SIGNIFICANT; **** $P < 0.001$.

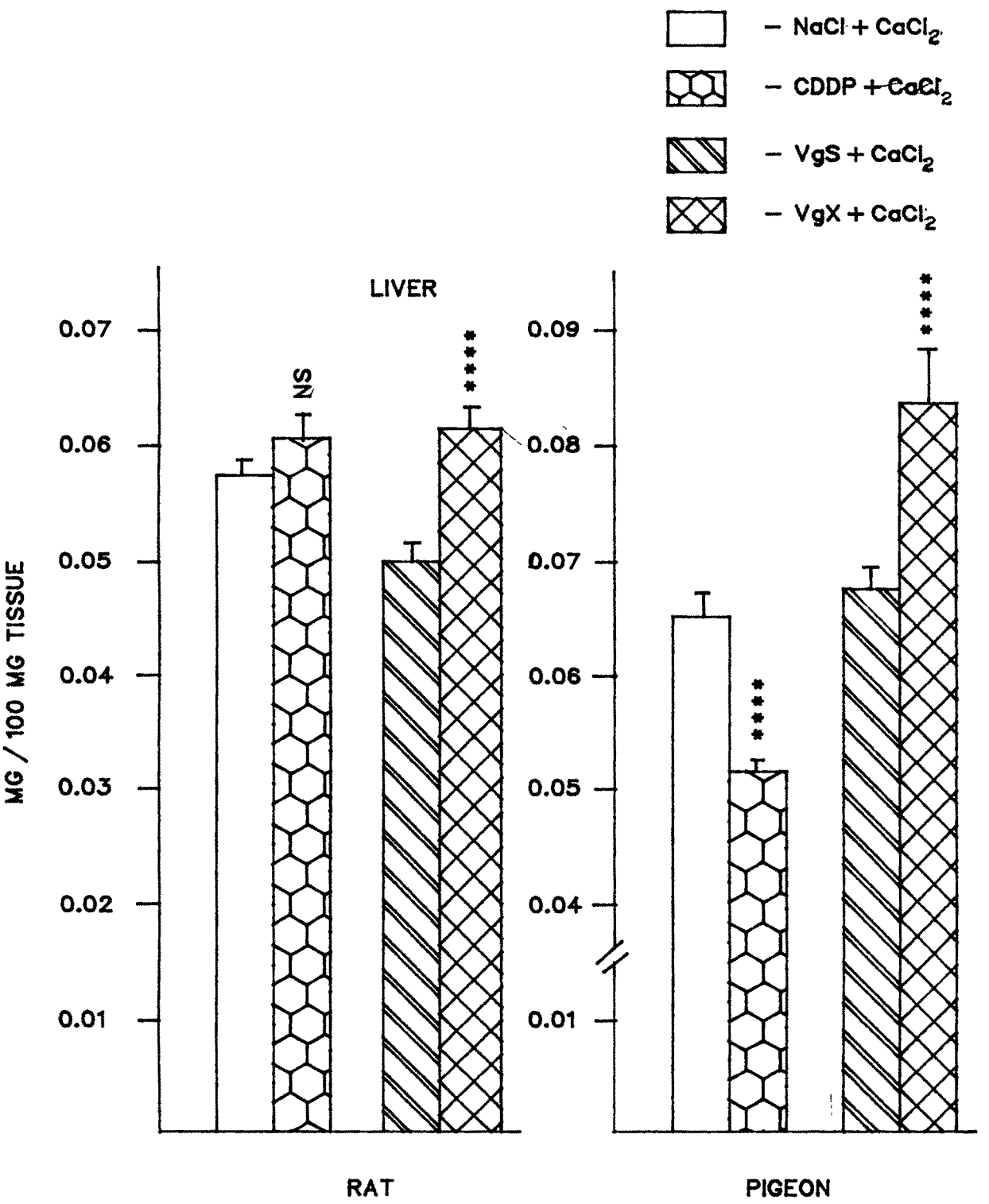


Fig. 5 EFFECT OF $CaCl_2$ ADMINISTRATION ON RNA CONTENT IN THE LIVER OF VgX AND CDDP TREATED RAT AND PIGEON. EACH BAR REPRESENTS THE MEAN ± SEM OF AT LEAST SIX ANIMALS. NS – NON SIGNIFICANT; **** $P < 0.001$.

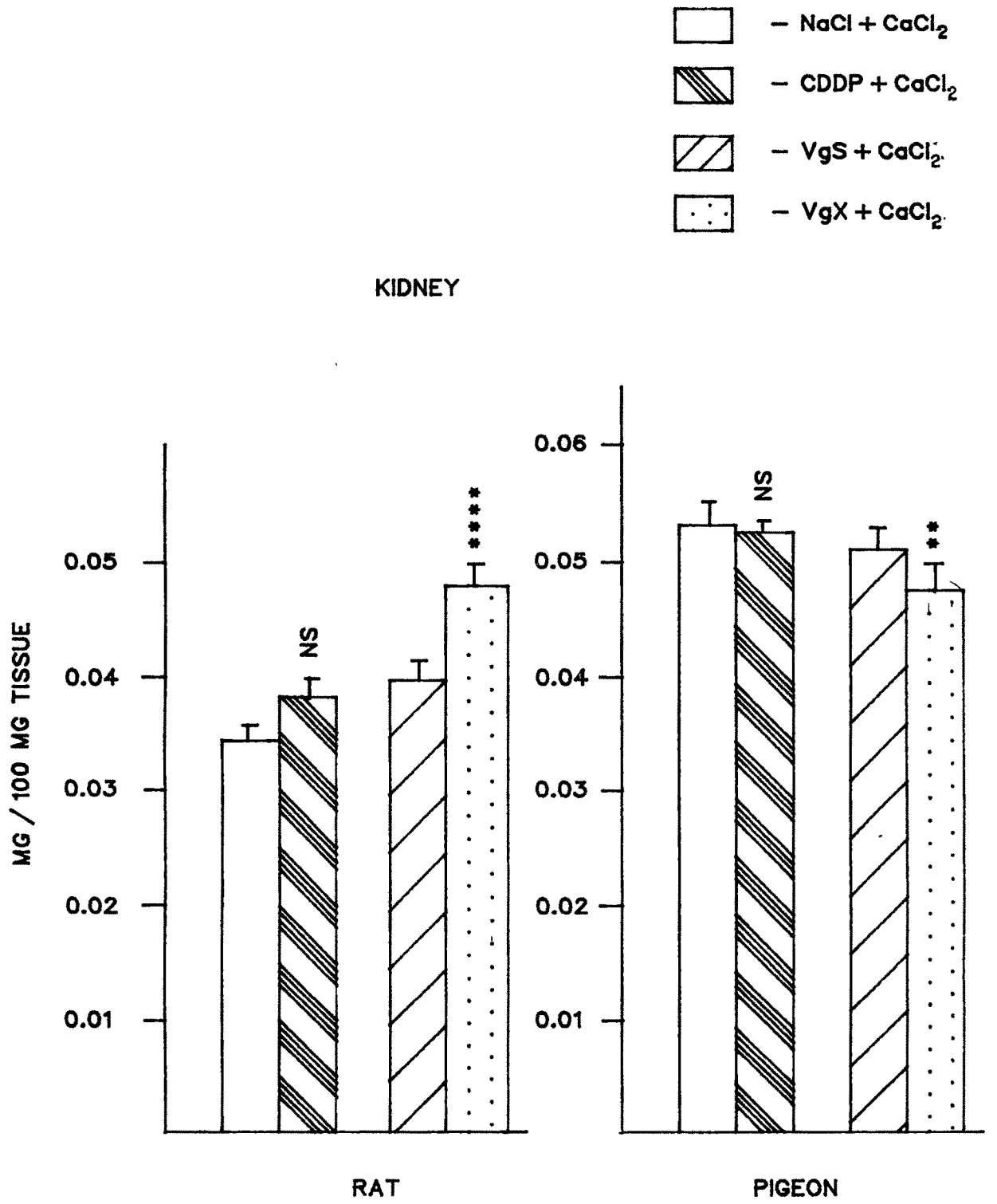


Fig. 6 EFFECT OF $CaCl_2$ ADMINISTRATION ON RNA CONTENT IN THE KIDNEY OF VgX AND CDDP TREATED RAT AND PIGEON. EACH BAR REPRESENTS THE MEAN ± SEM OF AT LEAST SIX ANIMALS. NS — NON SIGNIFICANT; ** $P < 0.02$; **** $P < 0.001$.

action of $CaCl_2$ was not observed as the kidney AChE activity showed a significant decrease (fig-2).

DNA content of the liver of $CaCl_2$ treated VgX pigeon did not show any variation from that of $CaCl_2$ treated sham operated pigeon (fig-3), indicating that in the liver, $CaCl_2$ is preventing or delaying the changes that were bound to take place due to vagotomy. DNA content in the kidney, however, showed a decrease (fig-4). RNA content, on the other hand, showed an increase in the liver of $CaCl_2$ treated VgX pigeons while it showed a decrease in the kidney (fig 5 & 6).

By and large, the results indicate that VgX effect is prevented or delayed from manifesting in the liver but in the kidney, $CaCl_2$ did not provide such protection.

Ciplatin (CDDP) treatment + $CaCl_2$

Rat

$CaCl_2$ treatment to CDDP rats produced non-significant increase in AChE activity in the liver and kidney (fig 1 & 2). DNA content in the liver of $CaCl_2$ + CDDP rats showed an increase but in the kidney it did not show any change (fig 3 & 4). RNA content, however, did not vary in both the liver and kidney from that of the control animals (fig 5 & 6).

Pigeon

$CaCl_2$ + CDDP treated pigeon exhibited a decreased AChE activity in the liver while in the kidney this enzyme did not show any variation from that of the respective $CaCl_2$ treated control birds (fig 1 & 2). DNA contents of both liver and kidney of $CaCl_2$ + CDDP pigeons decreased compared to that of control birds (fig 3 & 4). RNA content of liver decreased just as in the case of DNA but in the kidney RNA level remained unchaged (fig 5 & 6).

DISCUSSION

Previous study (chapter-II) has shown that therapeutic dosage of cisplatin administration induced in the liver and kidney a dysfunction in cholinergic systems as indicated by decreased AChE levels. In the present study, it has been noticed that injection of calcium chloride during cisplatin treatment retains the normal level of AChE in the liver and kidney of rats. Present findings support the earlier observations that $CaCl_2$ or NH_4Cl_2 play protective roles in cisplatin toxicity (Aggarwal and Fadood, 1981; Delay-Yates and me Brien, 1985). Capasso *et al*. (1990) reported that parathyroidectomy has a beneficial effect on experimental cisplatin induced nephrotoxicity. Vagotomy induced a decrease in AChE levels in the liver and kidney of rats (chapter-II), whereas $CaCl_2$ administration to vagotomised rats could reverse these effects. The acetylcholine released from the vagal fibres has been found to influence the permeability of the

hepatomembranes. These permeability changes of ions across the plasma membrane of hepatocytes probably occur through the release of membrane-bound calcium (Pilo and Patel 1978). Acetylcholine can also release Ca^{2+} or increase calcium movement into cells. Vagal ablation must be decreasing most of these cholinergic actions and adversely affecting calcium ion movements across the plasma membrane.

Vagotomy has been found to increase the sympathetic tone and plasma NE concentration in pigeon (Viswanathan et al., 1987) An increase in NE can cause influx of Ca^{2+} ions across plasma membrane. It appears that the administration of exogenous calcium resulted in a net increase in serum calcium while vagotomy increased the sympathetic tone and released more epinephrine into circulation which modulated the influx of Ca^{2+} into the cells. Present findings prove that $CaCl_2$ injection gives some protection against parasympathetic neuropathy in rats.

Cisplatin has been reported to inhibit the synthesis of DNA, RNA, protein and uptake of amino acids. It is to be seen whether this inhibition is through the drugs effect on Ca^{2+} level. A wealth of information is available on the role of Ca^{2+} in regulation of cell proliferation. Calcium administration to CDDP treated rats showed that the nucleic acid synthesis could be accelerated by exogenous calcium. A signficant increase in DNA content was observed in the liver of rats, while a prominent increase in DNA levels in both organs were noted in VgX + $CaCl_2$ treated rats. From these

evidences it is quite apparent that $CaCl_2$ administration to CDDP treated and vagotomised rats gives protection to a limited extent.

The present findings reveal that the exogenous supply of calcium chloride could reduce the cellular toxicity of CDDP and dysfunction due to vagotomy in rats. This might be occurring either through elevating the serum calcium levels and/or through the increased cellular uptake of calcium. However, such protective roles of $CaCl_2$ were not observed in CDDP treated and vagotomised pigeons.