# CHAPTER - III

# EFFECT OF VAGOTOMY AND CISPLATIN TOXICITY ON NUCLEIC ACID AND PROTEIN CONTENT IN THE LIVER AND KIDNEY OF RAT AND PIGEON

Since both nerves and hormones can exert trophic influence on the tissues and organs, hypothalamus has several roles to play in regulation of growth and development. Release of growth hormone or somatotrophin is controlled by hypothalamus. Destruction of ventromedial nucleus in rats is followed by a decrease in liver growth which is not entirely corrected by treatment with growth hormone (Goldman et al., 1970) indicating a probable involvement of neural pathway in trophic stimulation of visceral organs. Apart from such observations, very little is known about the neural influence on growth of liver and kidney. Since nucleic acids are associated with increase in protein synthesis, (Riddiford, 1960; Hay and Fisherman, 1961; Ostein and Walkev, 1961), regulation of growth also has to be mediated through nucleic acids. Studies have pointed to the fact that autonomic innervation of the liver does influence DNA synthesis during liver regeneration after partial hepatectomy (Kato and Shimazu, 1982). The profound influence of nervous system on RNA synthesis was proved when electrical stimulation of the paraventricular hypothalamic nucleus was found to increase the synthesis of nuclear RNA in liver cells of intact and adrenalectomised rats and that this effect of hypothalamic stimulation is abolished by hepatic denervation (Tyulenev et al., 1980). Additional evidence for nervous influence on

nucleic acid metabolism in the liver was provided by Sumi and Umeda (1977., 1979).

Bilateral subdiaphragmatic vagotomy abolished DNA synthesis required during regeneration after partial hepatectomy (Kato and Shimazu, 1989). A similar reduction in DNA synthesis is reported in cisplatin therapy. Cisplatin has been reported to inhibit the synthesis of DNA, RNA and protein (Harder and Rosenberg, 1970; Scanlon et al., 1983). Cisplatin (cis-diaminedichloroplatinum II) (CDDP) is an antitumor drug with activity against several solid tumors. Its antitumor effect apparently derives from its ability to inhibit DNA synthesis. Several lines of evidence support the hypothesis that DNA is the most important cellular target for platinum (II) drugs (Harder and Rosenberg, 1970; Robert and Pera, 1983). Cisplatin in less than frankly toxic doses, has profound effects on cells grown in tissue culture. The nature of some of these biochemical change has been probed using the incorporation of radiolabelled precursors of such biopolymers as protein, RNA and DNA. Platinum drugs at concentrations higher than the therapeutic value were found to cause inhibition of RNA and then protein synthesis until finally, cell death occurs.

Platinum concentrations in kidney were significantly higher in nuclear and microsomal fractions than that in mitochondrial or plasma membrane fractions; but in liver, platinum concentrations were not significantly higher in

nuclei or microsomes than in mitochondria or plasma membrane (Choie et al., 1980). CDDP produces bifunctional reaction with DNA which appears critical to its toxic action (Eastman et al., 1988). Disappearance of platinum in DNA was faster in kidney than in liver or lung (Moustonen et al., 1988).

As autonomic neural denervation and CDDP treatment cause changes in nucleic acid and protein synthesis, it was thought worth while to compare these effects on vagotomized and CDDP treated animals.

## MATERIALS AND METHODS

Male albino rats and domestic pigeons (200-300 gm) were used in all experiments. The rats had free access to standard rat-chow and water while pigeons were maintained on a mixed grain diet. Rats and pigeons were divided separately into four groups of 6 animals each.

Group-I. CDDP dissolved in physiological saline and injected ip. at a dose of 7 mg/kg b.w. for rat and 5 mg/kg b.w. for pigeons.

Group- II. Animals were injected with the above dose of saline served as controls to the Group-I.

Group-III. Subdiahragmatic vagotomy was performed in rats while pigeons underwent bilateral cervical vagotomy.

Group-IV. Rats and pigeons were subjected to sham operation. These were used as control animals (chapter-I) to the Group-III.

The vagotomised animals were caged separately and were deprived of food. They were sacrificed at the end of the experiment (48 hrs). CDDP treated rats and pigeons were given food till 60 hrs and sacrificed at 72 hours after an overnight starvation of 12 hrs. The controls were pair fed till 60 hrs. All rats were killed by exsanguination and pigeons by decapitation. The liver and kidney were excised quickly from the animals. DNA and RNA were assayed by employing the method described by Schneider (1957). The protein contents of the homogenates were estimated by the method of Lowry et al. (1951) (chapter I). Statistical significance was evaluated using Student's 't' test.

## RESULTS

A significant decrease in DNA content of the liver was observed in cisplatin treated and vagotomised rats and pigeons (fig.1). The DNA content in the kidney of rat was reduced by the administration of CDDP but it did not alter significantly by vagotomy (fig.3). After CDDP treatment, RNA content in the liver was significantly decreased in pigeon while it remained unchanged in rat (fig.2).However, vagotomy produced a drastic reduction in RNA content in the liver of rat and pigeon (fig.2). In contrast to that of the liver, vagotomy did not produce any change in the RNA content in

**Table I** **Effect of CDDP and Vagotomy on DNA content in liver and kidney of rat and pigeon. (Mean ± SEM).**

| Treatment | Rat | | Pigeon | |
|---|---|---|---|---|
| | Liver | Kidney | Liver | Kidney |
| Saline | 0.068 ± 0.0038 | 0.0872 ± 0.0033 | 0.0588 ± 0.002 | 0.0875 ± 0.0026 |
| Cisplatin | 0.0589 ± 0.0014**** | 0.0668 ±0.0021**** | 0.0453 ± 0.0031**** | 0.0723 ±0.0036**** |
| Sham | 0.0745 ± 0.0036 | 0.1008 ± 0.0032 | 0.0993 ± 0.0026 | 0.1249 ± 0.0078 |
| Vagotomy | 0.0592 ± 0.0016**** | 0.0981 ±0.0031NS | 0.07804 ± 0.004 **** | 0.1312 ±0.0037 NS |

Values expressed as mg/100mg tissue.

NS - Not significant **** - $P < 0.001$

**Table II** **Effect of CDDP and Vagotomy on RNA content in liver and kidney of rat and pigeon. (Mean ± SEM).**

| Treatment | Rat | | Pigeon | |
|---|---|---|---|---|
| | Liver | Kidney | Liver | Kidney |
| Saline | 0.0682 ±0.0016 | 0.0367 ±0.0009 | 0.0610 ±0.0018 | 0.0411 ±0.0009 |
| Cisplatin | 0.0687 ±0.014 NS | 0.024 ±0.0006**** | 0.0494 ±0.0061**** | 0.0326 ±0.0006**** |
| Sham | 0.0781 ±0.0012 | 0.0432 ±0.001 | 0.0529 ±0.0014 | 0.0478 ±0.0013 |
| Vagotomy | 0.0653 ±0.0021**** | 0.0427 ±0.001 NS | 0.0439 ±0.0012**** | 0.0442 ±0.0016 NS |

Values expressed as mg/100 mg tissue.

NS - Not significant **** - $P < 0.001$

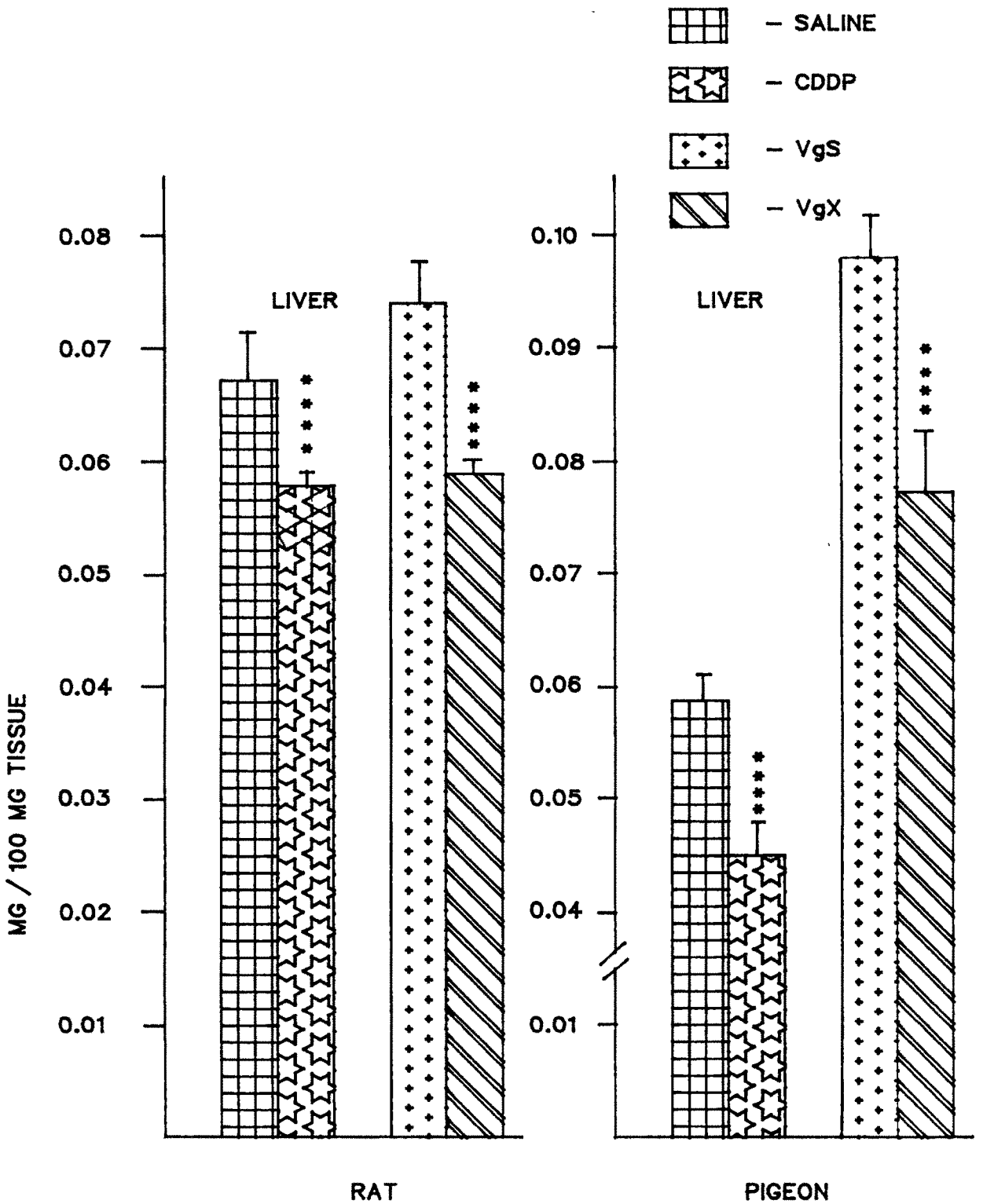


Fig.1 EFFECT OF CDDP AND VAGOTOMY ON DNA CONTENT IN LIVER OF RAT AND PIGEON. SIGNIFICANCE OF DIFFERENCE, DETERMINED BY STUDENT'S 't' TEST. **** $P < 0.001$. ($N = 6$).

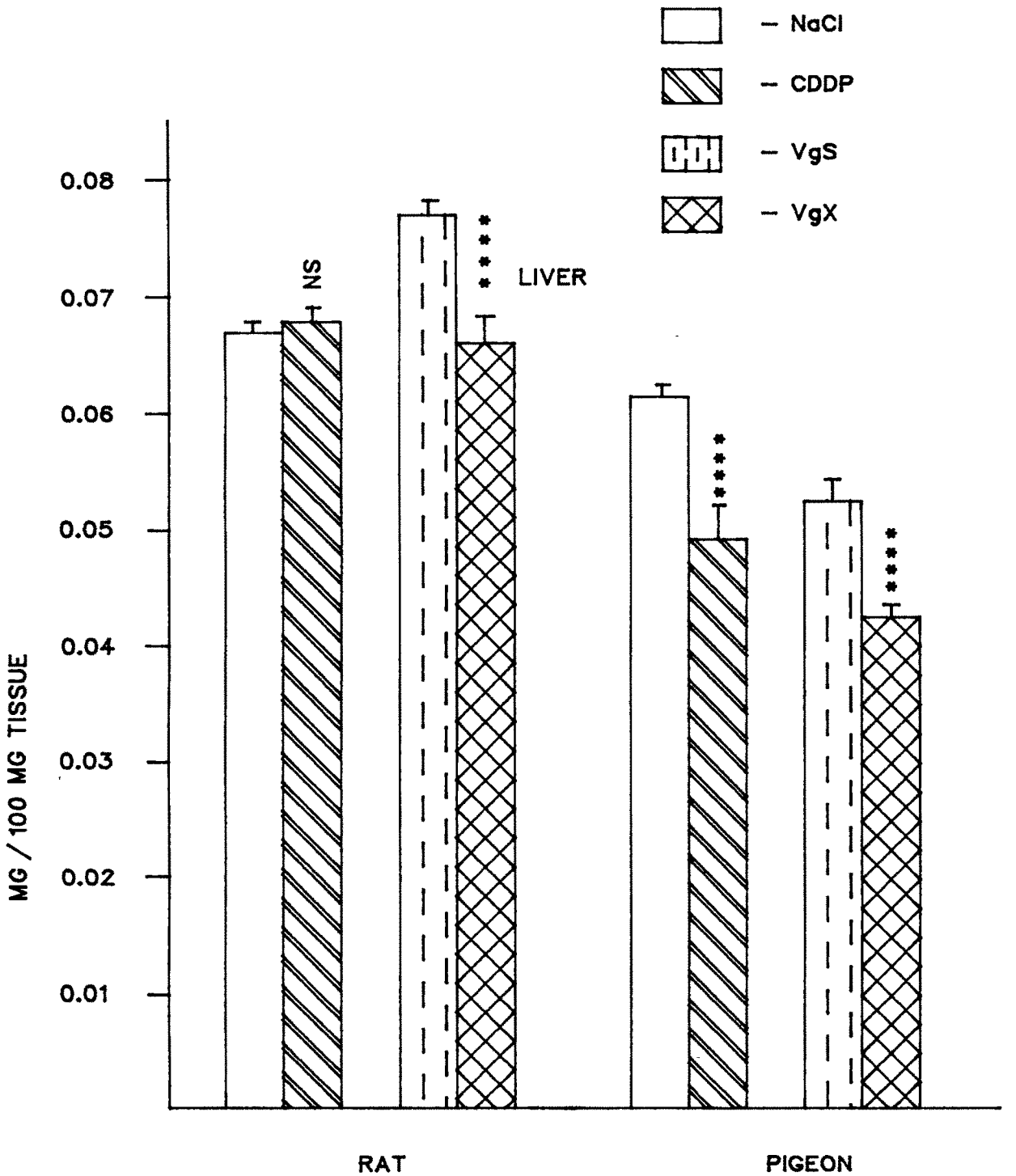


Fig.2 EFFECT OF CDDP AND VAGOTOMY ON RNA CONTENT IN LIVER OF RAT AND PIGEON. SIGNIFICANCE OF DIFFERENCE, DETERMINED BY STUDENT'S 't' TEST WAS: **** $P < 0.001$; NS – NONSIGNIFICANT. (N = 6).

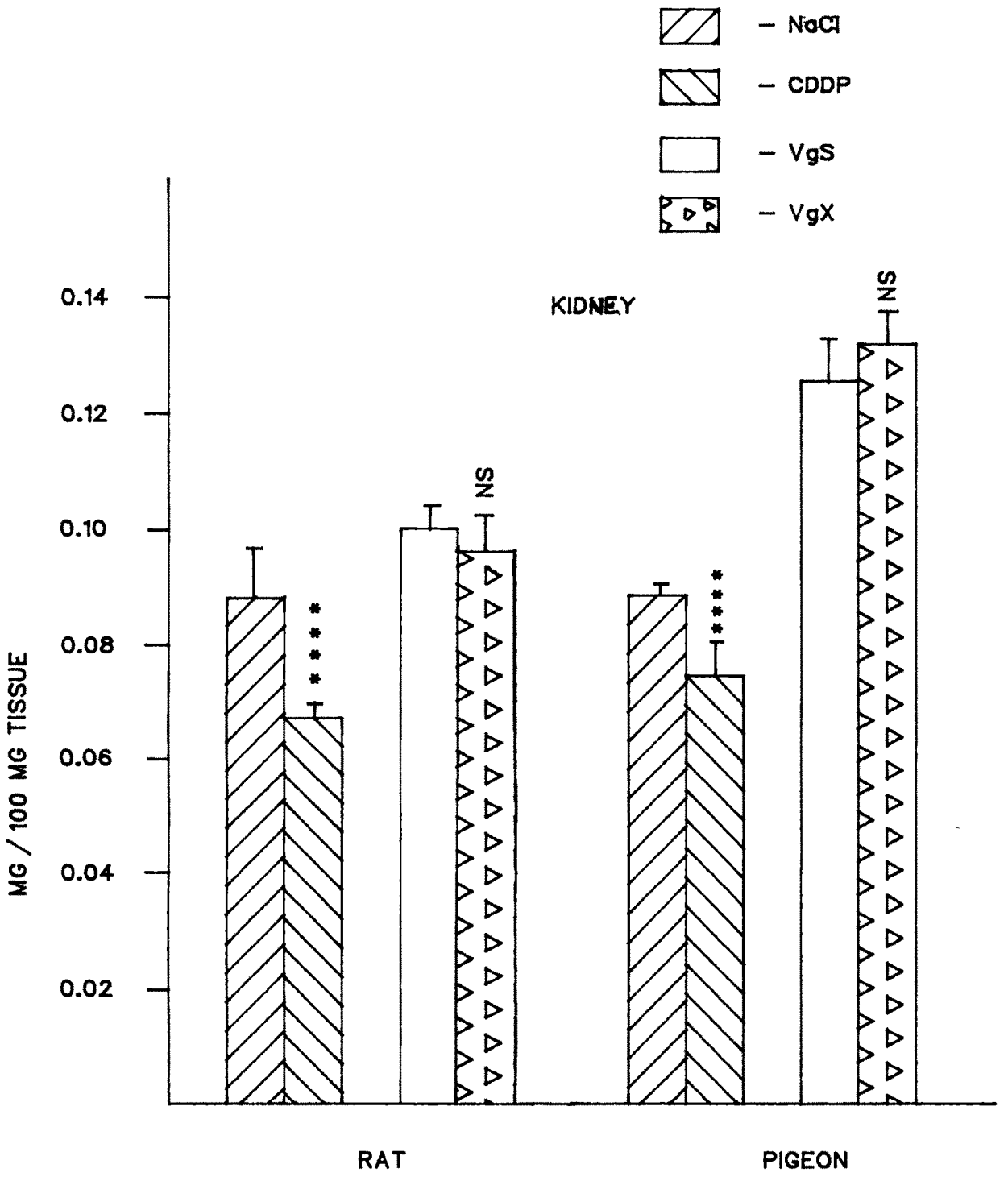


Fig. 3 EFFECT OF CDDP AND VAGOTOMY ON DNA CONTENT IN KIDNEY OF RAT AND PIGEON. SIGNIFICANCE OF DIFFERENCE, DETERMINED BY STUDENT'S 't' TEST WAS : **** $P < 0.001$; NS – NONSIGNIFICANT. ($N = 6$).

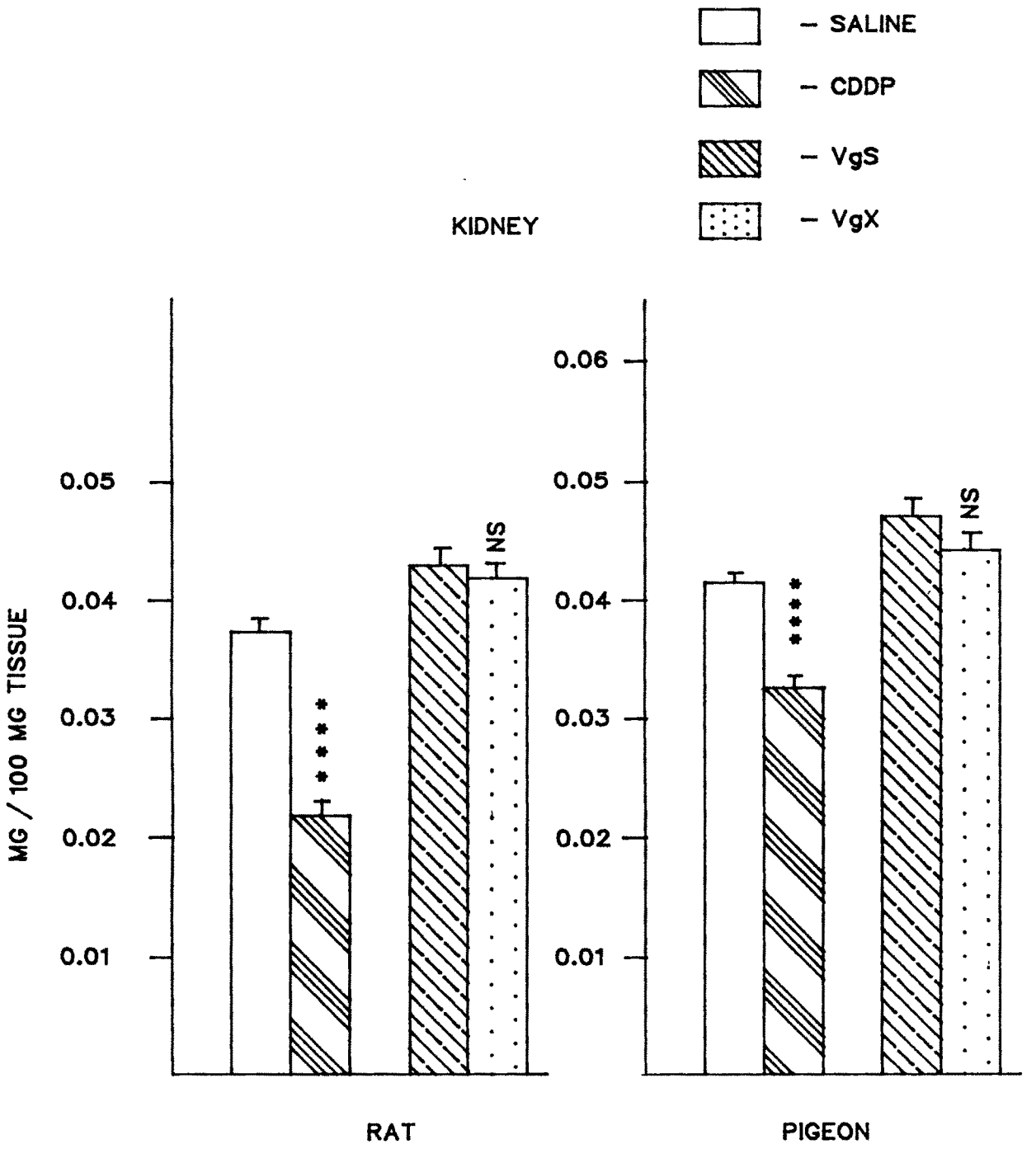


Fig.4 EFFECT OF CDDP AND VAGOTOMY ON RNA CONTENT IN KIDNEY OF RAT AND PIGEON. SIGNIFICANCE OF DIFFERENCE, DETERMINED BY STUDENT'S 't' TEST WAS; **** $P < 0.001$. NS — NON SIGNIFICANT ($N = 6$).

**Table III** **Effect of CDDP and Vagotomy on protein content in liver and kidney of rat and pigeon (Mean ± SEM).**

| Treatment | Rat | | Pigeon | |
|---|---|---|---|---|
| | Liver | Kidney | Liver | Kidney |
| Saline | 8.8332 ±0.7645 | 7.902 ±0.357 | 14.440 ± 0.3223 | 13.894 ± 0.3612 |
| Cisplatin | 9.3918 ±0.3632 NS | 5.971 ±0.224**** | 13.516 ± 0.5808NS | 10.4482 ±0.3878**** |
| Sham | 11.293 ± 0.555 | 11.2784 ± 0.7829 | 15.333 ± 0.8364 | 14.566 ± 0.7829 |
| Vagotomy | 11.720 ± 0.825 NS | 11.2716 ± 0.7858 NS | 14.592 ± 0.405 NS | 17.289 ± 0.913 NS |

Values expressed as mg/100 mg tissue.

NS - Not significant **** - $P < 0.001$

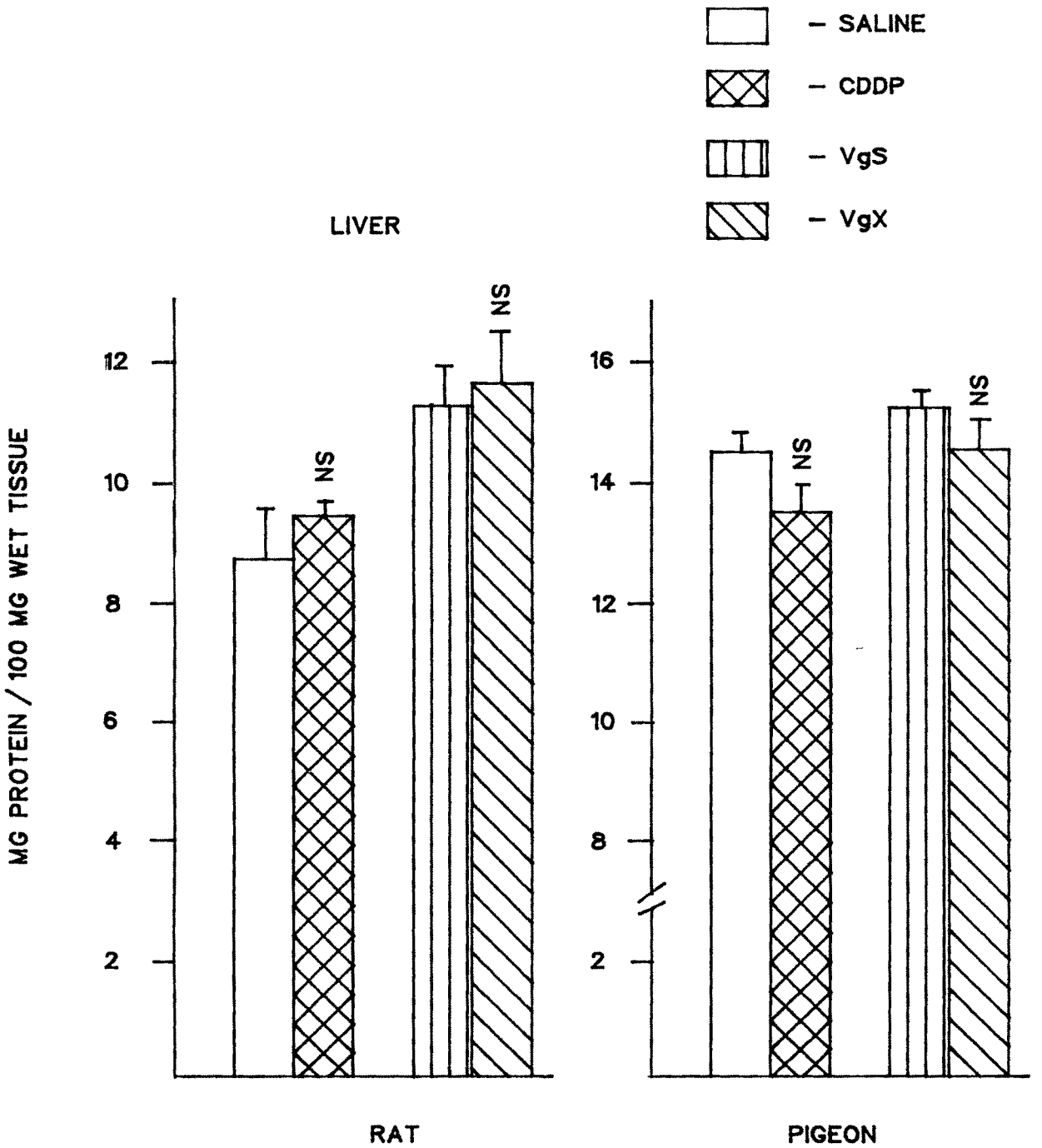


Fig.5 EFFECT OF CDDP AND VAGOTOMY ON PROTEIN CONTENT IN THE LIVER OF RAT AND PIGEON. SIGNIFICANCE OF DIFFERENCE, DETERMINED BY STUDENT'S 't' TEST. NS – NONSIGNIFICANT. (N = 6).

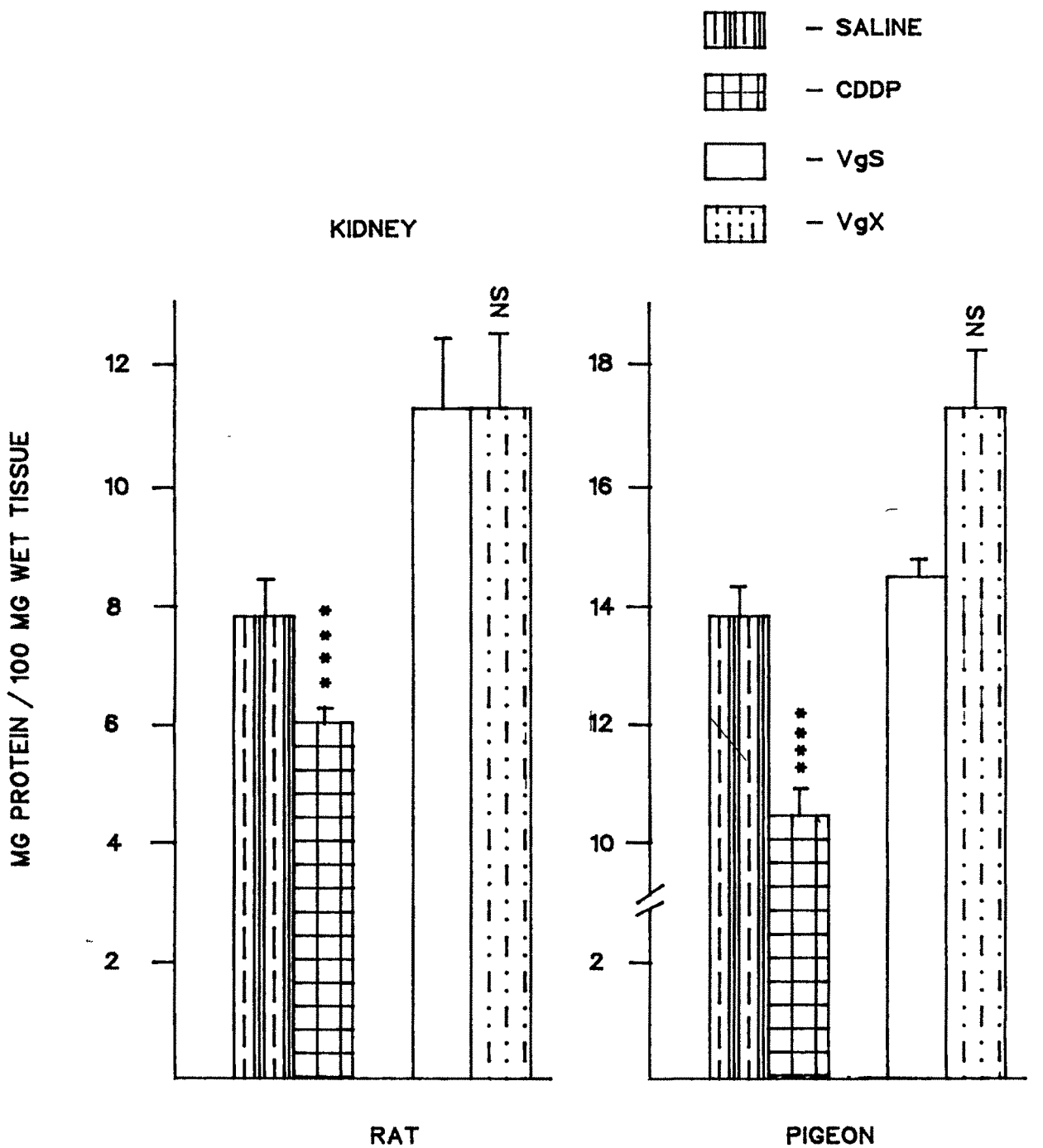


Fig.6 EFFECT OF CDDP AND VAGOTOMY ON PROTEIN CONTENT IN KIDNEY OF RAT AND PIGEON. SIGNIFICANCE OF DIFFERENCE, DETERMINED BY STUDENT'S 't' TEST. **** P< 0·001. NS-NON SIGNIFICANT (N=6).

the kidney of rat or pigeon while cisplatin treatment produced significant reduction in RNA content in the kidney of pigeons (fig.4).

Table III shows the effect of vagotomy and CDDP treatment on protein metabolism. When rats and pigeons were treated with cisplatin, a significant decrease in protein content was observed in kidneys of both animals. Vagotomy or CDDP treatment had no effect on protein metabolism in the liver of rat or pigeon. No significant difference in protein content was found between the kidney of VgX and VgS groups.

## DISCUSSION

A single dose of cisplatin injection as well as vagotomy in rats and pigeons resulted in a drastic reduction in DNA content in the liver. Shimazu (1983) showed that increase in DNA synthesis following partial hepatectomy was suppressed and delayed by subdiaphragmatic vagotomy. As shown in fig. I, DNA content in the liver, after CDDP treatment and vagotomy, was significantly reduced in rats and pigeons when compared with that of controls. The vagal denervation in rats and pigeons resulted in a decline in RNA content in the liver. Tanaka (1987) observed that subdiaphragmatic vagotomy in rats delayed and suppressed hepatic DNA synthesis. Recent studies by Nakamura and Schinichirou (1988) have demonstrated that nuclear DNA and cellular RNA contents of the individual cell after CDDP administration were arrested in S and $G_2$ phases.


The vagal denervation also results in an increased sympathetic tone in liver and kidney, and reduced insulin secretion (Sakaguchi, 1981; Miller, 1981). It has been suggested by many authors that insulin plays a role during cholinergic differentiation. Insulin may serve as a neuromodulator in the adult CNS and as a signal to promote cholinergic differentiation and neuronal maturation in the developing brain and retina (Orierc et al., 1992). Vost and Hollenberg (1970) reported that insulin is necessary for DNA synthesis and hyperinsulinism enhances stromal DNA synthesis in the rat adipose tissue. It has been previously demonstrated that a direct pancreatic effect of CDDP causes an impaired insulin secretion. Hence the reduced level of DNA and RNA content in the liver of CDDP treated and VgX animals could be due to the insufficiency of insulin as well as due to reduced neural stimulation.

Recently, studies have shown an involvement of glucocorticoid in DNA synthesis. The steroids like glucocorticoids exert a very pronounced inhibitory effect on DNA synthesis (Tesoriere et al., 1992). It has been observed in pigeon that vagotomy produces significant increase in corticosterone level (Viswanathan et al., 1987). It appears possible to conclude that in pigeons, DNA synthesis could be under the control of glucocorticoids, which seems to be functionally correlated to insulin. On the other hand, results showed that vagotomy did not influence DNA and RNA content in the kidney. It can be concluded that reduction in RNA and DNA content in the kidney

could be due to direct cytotoxic action of cisplatin on kidney. Inhibition of replication due to inactivation of DNA as a template has been proposed to be responsible for the cytotoxicity of CDDP (Roberts and Pera, 1983).

Cisplatin reduced the protein content in the kidney of rat and pigeon while no significant increase or change in protein content was observed in vagotomised animals. Withdrawal of insulin and impaired use of glucose can cause a decrease in protein synthesis. Insulin deficiency also increased the rate of protein excretion (Kern and Engerman, 1988).

The conclusion drawn from this study is that parasympathetic nerves have a profound influence on the nucleic acid metabolism while cisplatin can inhibit nucleic acid metabolism through its cytotoxic action.