CHAPTER - 6

TRANSAMINASES AND TRANSPORT ENZYMES IN THE KIDNEY OF VAGOTOMIZED AND CISPLATIN TREATED PIGEONS

Blood sugar regulatory mechanisms in birds are more sensitive or oriented towards glucagon. Birds quickly respond to hypoglycemia unlike mammals, which respond to an hyperglycaemic condition. Birds as such tolerate a very high blood sugar level whereas mammals are intolerant to hyperglycemia (Pilo and Verma, 1984). In comparison, avian pancreas has more A cells in the islets and the glucagon concentration too is higher than that of mammals.

Glucagon not only induces liver to release glucose from glycogen by activating glycogenolytic enzymes but also in due course of time activates gluconeogenesis. Glycogenolysis is also activated by catecholamines (released by adrenals or sympathetic nerve endings). Prolonged need of gluconeogenesis (during starvation) necessitates the recruitment of adrenocortical glucocorticoids. In the overall scheme of blood sugar regulation, these hormones play a vital role along with the autonomic nerve fibres belonging to sympathetic nervous system (SNS) and parasympathetic nervous system (PNS). These fibres also stimulate the release of hormones from endocrine glands and prime the tissue such as liver to become highly proficient in releasing into or absorbing more glucose from blood.

In birds, kidney is the dominant gluconeogenic tissue whose rate of glucose production from non-carbohydrate precursors surpasses even that of liver (Pearce, 1971).

Although the role of kidney in blood sugar regulation has been known since long, control of such activities in the kidney either by hormones or by neurons are not been much investigated. Histochemical changes in the pigeon kidney after vagotomy and chemical sympathectomy was investigated by Pilo et al. (1987). Mehta and Pilo (1987) found that chemical sympathectomy (6-OHDA treatment) elevated GOT and Na^+-K^+-ATPase activities in the pigeon kidney. Injections of catecholamines decreased GOT and Na^+-K^+-ATPase activities (Mehta and Pilo, 1989). Acetylcholine administration decreased GPT and Na^+-K^+-ATPase activities in kidney of pigeons (Pilo and Mehta, 1988). Glucagon administration decreased both GOT and GPT as well as Na^+-K^+-ATPase (Pilo and Mehta, 1989).

The various experiments referred above indicate that several hormones and the autonomic neurotransmitters exert influence on the kidney metabolic activities. Rate and response time or resistance of such biochemical activities depend on physiological state of the bird.

Vagotomy may produce an increase in the sympathetic tone or sympathectomy may produce an increased vagal tone. The resulting changes in the tissue may not only be due to absence of one of the division of ANS, but may be also and due to increased or uninhibited activity of the other autonomic division. Added to this, the changes in the rate of secretion of hormones through the activation of one or the other autonomic division will also alter the metabolic status of organs such as liver and kidney.

The autonomic dysfunction thus exerts tremendous effects on metabolic, homeostatic mechanisms. Autonomic neuropathy is a common feature in many diabetic patients. Similar neuropathy is also seen in cisplatin treated patients. As mentioned earlier, cisplatin is an antitumour drug that also cause several metabolic disorders such as clinical hyperglycemia and hypocalcemia along with other renal toxicities. Changes in the transaminases were also observed by Schaeppi et al. (1972). Treatment with calcium chloride was found to alluviate some of the side effects caused by cisplatin administration.

Studies in our laboratory have shown some fundamental similarities in the effects between vagotomy and cisplatin treatment (Parikh, 1992; Oommen, 1992). Hence it was of interest to undertake a comparative study of the effects of vagotomy and cisplatin treatment on kidney carbohydrate metabolism in pigeons. The protective function of calcium was also evaluated since these birds received calcium chloride along with vagotomy and cisplatin.

Materials and Methods

Adult pigeons weighing about 250-300 gms which were acclimated to the laboratory conditions were grouped into 8 sets, each set consisting of 6 birds.

Set 1. Birds were cervically vagotomized under mild anesthesia (see Chapter 1).

Set 2. Sham operated birds served as controls for Set 1.

Set 3. Along with vagotomy 1.3% $CaCl_2$ was administered (ip) for birds (1 ml) morning and evening for 48 hours.

Set 4. Sham operated pigeons with 1.3% $CaCl_2$ treatment remained the controls for Set 3.

Set 5. The set was subjected to cisplatin treatment with a dose of 5 mg/kg body weight in 0.85% saline.

Set 6. Controls received 0.85% saline alone.

Set 7. Cisplatin treated birds subjected to $CaCl_2$ treatment.

Set 8. Controls for Set 7 received $CaCl_2$ treatment after receiving 0.85% of saline.

All the birds were provided with water and were sacrificed by exsanguination under mild anesthesia at 48 hours (VgX) and 72 hours (CDDP). Kidneys from both sides were excised, weighed and processed for further investigations. Biochemical analysis was carried out for protein, transaminases, Na^+-K^+-ATPases, acid, alkaline phosphatases as per methods described in Chapter 1.

Results

Vagotomy (VgX) :

Vagotomized pigeon kidney showed increased GOT and GPT activities in comparision to that of sham operated kidneys. The increase of GOT was

more than 95% while that of GPT was 55%. All phosphatases studied (Na^+-K^+-ATPase, alkaline pase, acid pase) showed decrease in their level of activities in the kidney following vagotomy. Maximum reduction was that of Na^+-K^+-ATPase followed by acid pase and alkaline pase.

Vagotomy with calcium chloride (VgX + $CaCl_2$) :

$CaCl_2$ treatment to VgX pigeons reduced the level of increase of GPT activity in the kidney compared to that of $CaCl_2$ treated sham operated pigeons. GOT activity on the other hand remained high in the kidney of vagotomized pigeons inspite of $CaCl_2$ treatment. All phosphatases showed decreased activities in VgX pigeon kidney. However, in the $CaCl_2$ treated VgX pigeons this decrease was either small (Na^+-K^+-ATPase 21%) or practically nil as in (alkaline pase and acid pase).

Cisplatin (CDDP) :

Cisplatin treatment produced similar changes in the activities in GPT and GOT as seen in the kidney of vagotomized pigeons. Although both GPT and GOT increased in the CDDP treated pigeon kidney, the level of increase of GOT was much more than that of GPT which was also observed in vagotomized pigeon kidney. Just as in case of vagotomy, the three phosphatases showed decreased level of activities in the cisplatin treated pigeon kidney.

TABLE 2 : EFFECT OF VAGOTOMY AND CISPLATIN ALONE AND IN COMBINATION WITH $CaCl_2$ ON TRANSAMINASES, NON-SPECIFIC PHOSPHATASES, PROTEIN CONTENT - IN TERMS OF % CHANGES

	GPT	GOT	Na^+-K^+-ATPase	ALKALINE PHOSPHATASE	ACID PHOSPHATASE	PROTEIN
VgS (VgX)	55.4 ↑	97.8 ↑	90 ↓	37.3 ↓	65.2 ↓	↓
Con (CDDP)	56.6 ↑	161.3 ↑	54.4 ↓	48.1 ↓	35.1 ↓	↓
VgS+Ca (VgX-Ca)	21 ↓	127.5 ↑	21.2 ↓	1.13 ↓	5.5 ↑	↑
Con+Ca(CDDP+Ca)	33.1 ↑	44.72 ↑	48 ↓	4.7 ↓	8.3 ↑	↑

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

EXPLANATION TO FIGURES

Effect of Vagotomy and Cisplatin treatment alone, or in combination with calcium with respect to:

Fig (1) : Activities of GPT and GOT in the kidney.

Fig (2) : Activities of Na^{+}-K^{+}-ATPase and protein content in the kidney.

Fig (3) : Activities of acid and alkaline phosphatases in the kidney.

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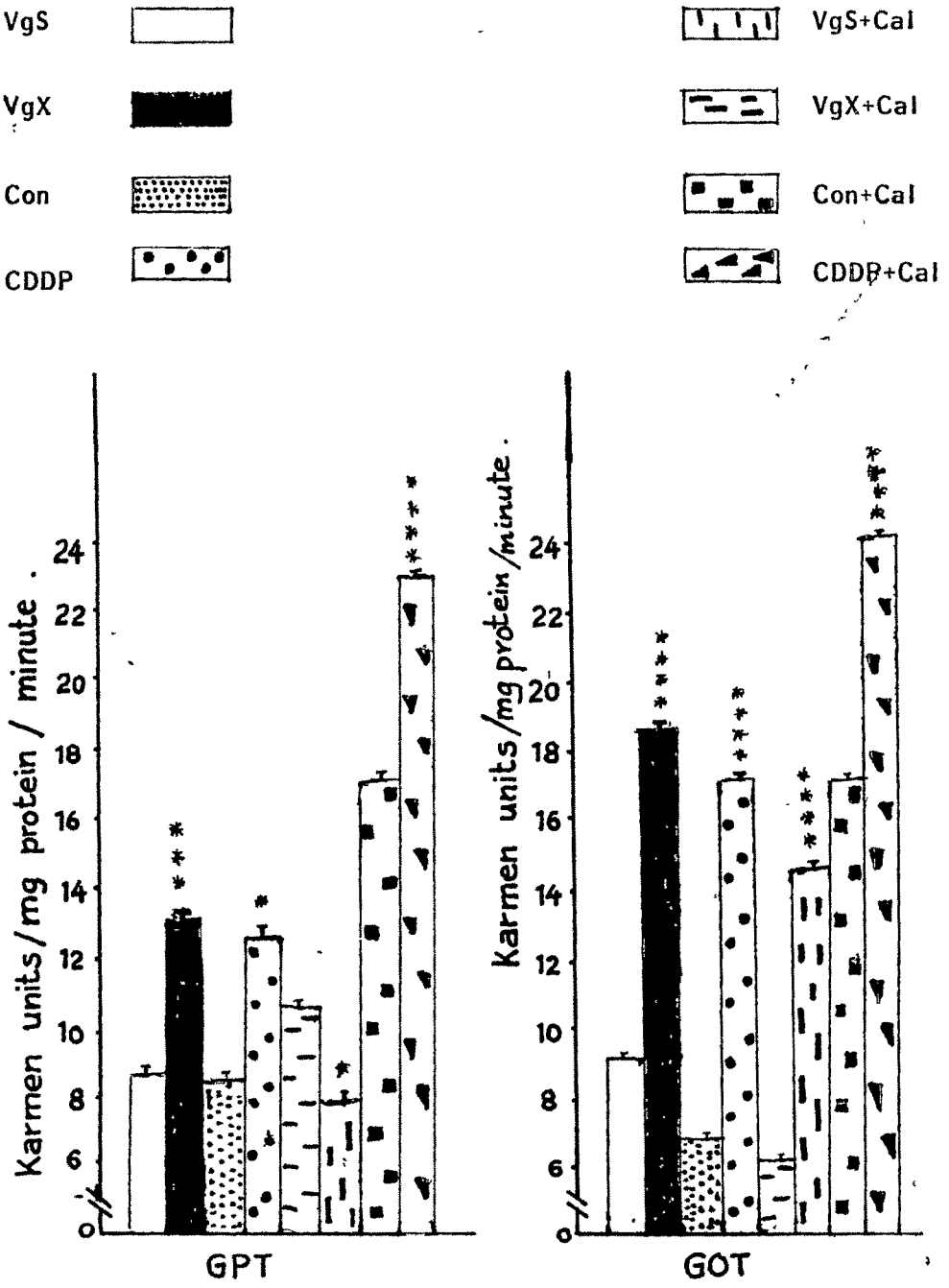

Fig: 1 Results given as mean ± SEM of six experiments.
$P < 0.02^{*}$, $P < 0.05^{**}$ $P < 0.01^{***}$ $P < 0.001^{****}$

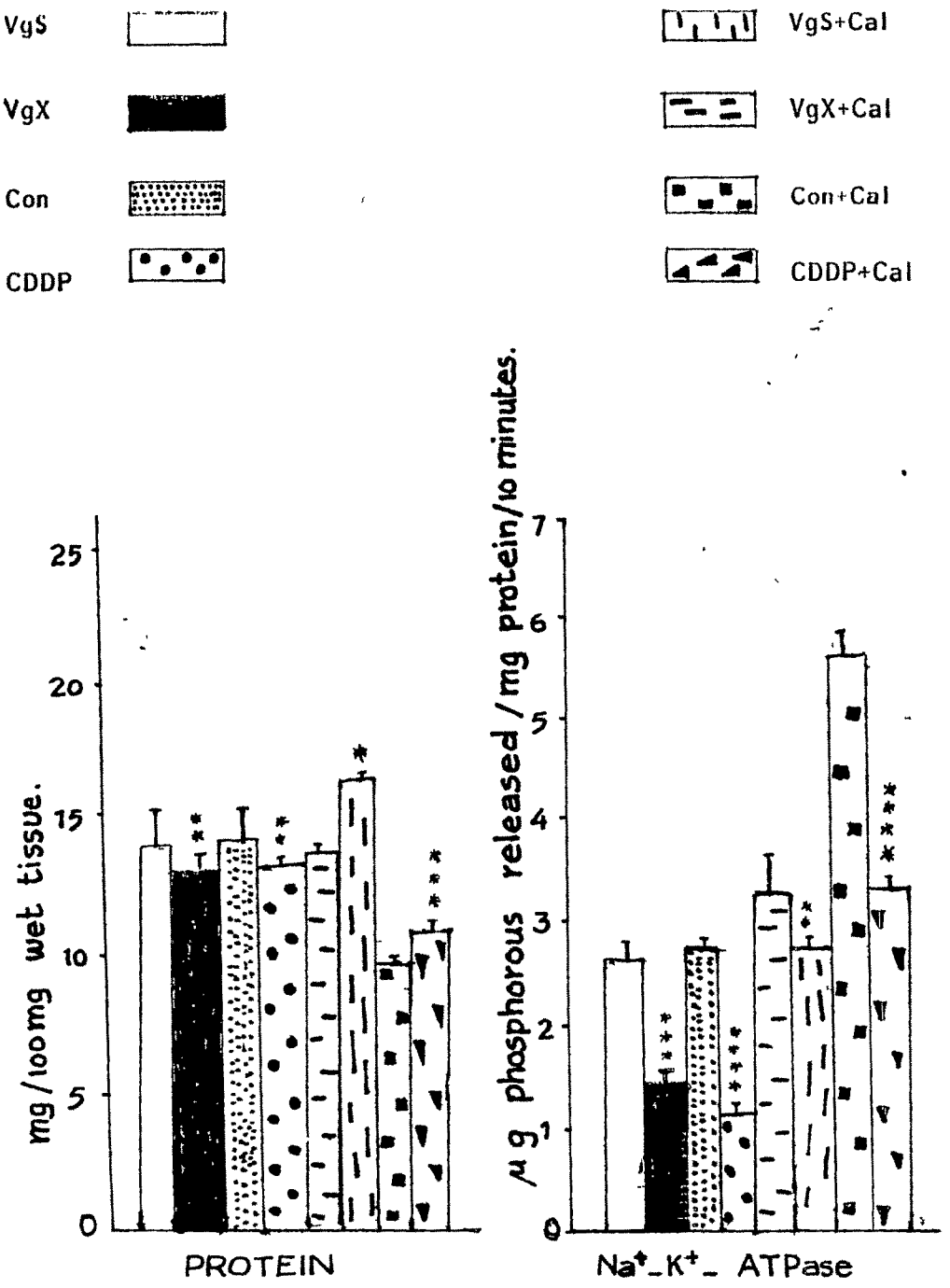

Fig : 2 Results given as mean ± SEM of six experiments.
$P < 0.02^{*}$, $P < 0.05^{**}$, $P < 0.01^{***}$, $P < 0.001^{****}$

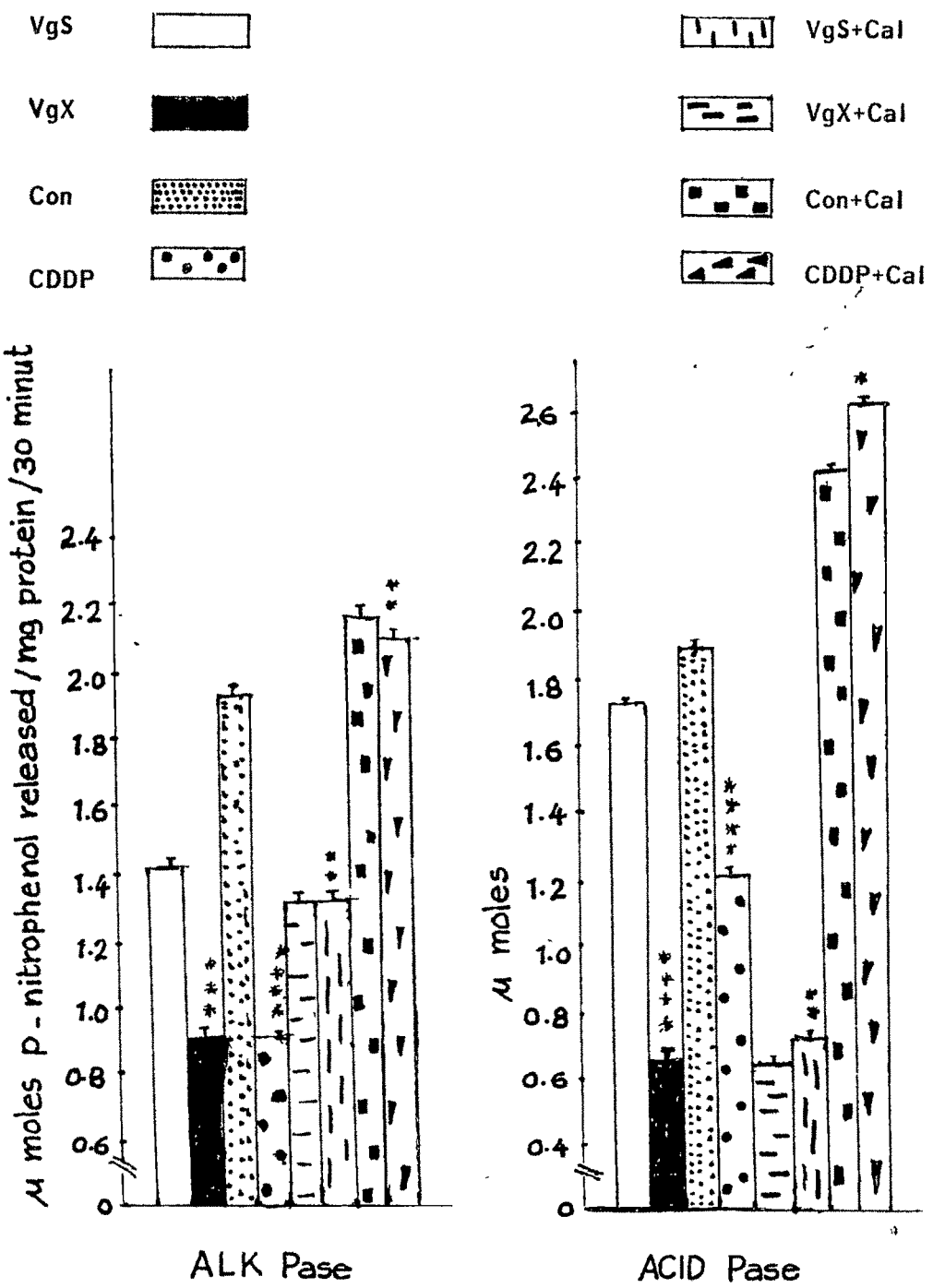

Fig : 3 Results given as mean ± SEM of six experiments.
$P < 0.02^{*}$, $P < 0.05^{**}$, $P < 0.01^{***}$, $P < 0.001^{****}$

Cisplatin and $CaCl_2$:

$CaCl_2$ treatment minimized the level of increase in GOT in the CDDP treated pigeon kidney. The degree of reduction was found to be more in GOT activity than in GPT activity. $CaCl_2$ administration to cisplatin treated pigeons also prevented the decrease in the activities of Alkaline Pase and Acid Pase as seen in CDDP treated pigeon kidney.

Discussion

It is clear from several studies that normal functions of autonomic nervous system is equally important as that of endocrine system in the regulation of blood sugar (Woods and Porte, 1974). Hypothalamic centres, LH and VMH, through parasympathetic and sympathetic fibres control the release of glucostatic hormones such as insulin and glucagon and exert direct influence on the liver and kidney metabolic reactions. Stimulation of vagal fibres increased the glycogen synthesis in liver (Shimazu, 1971; Shimazu and Fujimoto, 1971) while stimulation of VMH or sympathetic fibres increased glycogenolysis (Shimazu and Amakawa, 1968a; Frohman and Bernardis, 1971). Alterations in the renal gluconeogenesis was shown by Verma et al. (1984) following vagotomy in pigeons. Pilo et al. (1987) showed that vagotomy has reduced the activity of AChE in the kidney while chemical sympathectomy increased AChE level. Decreased AChE activity is indicative of reduced cholinergic stimulation.

Bilateral cervical vagotomy in pigeons produced total parasympathetic blockade or inactivation leading to several changes such as fluid accumulation in the crop, aphagia and hypoglycaemia. Similar side effects were noted in patients receiving cisplatin treatment. It is a known fact that, a wide spectrum of drugs used in treatment of diabetes and cancer have their own ill effects leading to various pathological situations. Streptozotocin (STZ) is one such compound causing hyperglycaemia when used in treatment against cancer (Prestakyo, 1971). Since the mode of response remains analogous in case of VgX, CDDP and STZ treatment, it could be assumed that the possible mechanism by which these drugs become operative might be through influencing parasympathetic pathway.

Hyperglycaemia observed following vagotomy (Viswanathan et al., 1987) and cisplatin treatment (Goldstein et al., 1983) could be due to several reasons such as, decreased glucose uptake by tissues or increased release of glucose from the tissues. Sustained hyperglycaemia without a digestive supply can only be met by a stepped up gluconeogenesis in liver and kidney.

In vagotomized pigeon kidney, the transaminase activity was enhanced suggesting the possibility of gluconeogenesis. Elevated transaminase activity indicates utilization of amino acids to form glucose. Sustained higher levels of GOT over GPT indicates that oxaloacetate transformation was enhanced than the pyruvate conversions. Transamination is facilitated by production of pyruvate from alanine, oxaloacetate from aspartate and alpha-

ketoglutarate from glutamate. Amongst various steps during gluconeogenesis the most significant being the formation of oxaloacetate by the carboxylation of pyruvate, catalysed by pyruvate carboxylase, which showed higher levels (Chapter 5). Increased levels of GOT and GPT in the kidney of purely insectivorous birds (swifts) were reported by Pilo and Mehta (1985). The presence of fairly high activities of these enzymes in pigeon kidney is probably due to its involvement in converting amino acids to glucose.

CDDP treated birds also showed higher levels of GOT and GPT as seen in VgX birds. Investigation by Matsunotetsuya (1989) in the chicken hepatoma showed that glutamine oxidation proceeded in tumor mitochondria exclusively via a pathway involving GOT, which could be accounted for the increase observed in CDDP treated condition also.

Measurement of the overall rate of gluconeogenesis in kidney slices under a variety of conditions show that the rate depends on the physiological circumstances. Rats kept on a low carbohydrate diet increase the capacity of kidney gluconeogenesis (Krebs, 1963). In VgX pigeons elevated rate of gluconeogenesis is indicative of increased glucose formation which may be due to less carbohydrate intake or increased catecholamines secreted either from sympathetic nerve endings or liberated from adrenal medullary cells. The absence of ACh secretion (VgX) which inhibits adenylate cyclase and activates cAMP-PDE resulting in a decreased cAMP level could ultimately enhance the rate of gluconeogenesis (Verma et al., 1984).

Pagiliara and Goodman (1969) showed an increased gluconeogenesis in renal cortical slices after addition of cAMP thus accounting for elevated levels of transaminases.

Bilateral vagotomy also resulted in an alteration of the enzyme activities concerned with movement of metabolites. Non-specific acid and alkaline phosphatases indicated a decrease in their activities compared to respective controls. Na^+-K^+-ATPase too showed lower levels. Alkaline Pase activity which occurs in the brush border of proximal convoluted tubules (PCT) (Chitnis et al., 1978) mostly helps in reabsorption of glucose from the lumen by transphosphorylation reactions (Danielli, 1954). On the other hand, Acid Pase activity is localized in glomeruli, PCT and luminal border of collecting ducts. Significant reduction of Alkaline Pase activity in the VgX kidney is indicative of decreased reabsorption of glucose from the lumen. Cyclic AMP and its derivatives dibutaryl cAMP stimulates active cellular entry and cumulative uptake of several amino acids in rat kidney cortical slices (Weiss et al., 1972). In VgX pigeon kidney the enzymic level suggests reduction in active entry or uptake of metabolites. Na^+-K^+-ATPase being a membrane bound enzyme is mainly concerned with active uptake of ions and metabolites. This enzyme is localized in the thick ascending limb lying in the outer medulla where the filtrate gets concentrated. The reduction of enzyme activity caused due to VgX can be correlated as a reduction in active transport of Na^+ ions and other metabolites.

Transport enzyme activities are of major biological importance within the kidney and elsewhere throughout the organism (Goldin et al., 1979). They are responsible for metabolite exchange and have been implicated in the determination of normal and metastatic phenotypic states (Kramer et al., 1978). The transport enzymes have also been thought to interfere in the mitotic apparatus assembly and disassembly, thus interfering the cytokinesis (Aggarwal, 1974; 1979). Inactivation of these enzymes would tend to alter both the function and viability of kidney tubules.

The mechanism of interaction / inactivation of membrane bound enzymes may be manifold. Depending on the strength of membrane attachment, the enzymes can be removed, yet remain functional, inactivated but not removed (ATPases) or be removed and inactivated or stimulated to increase in quantity (Acid Pase). Inactivation of membrane ATPase would tend to lead to an ionic imbalance which is probably responsible for cellular mortality (Stekhoven and Bonting, 1981). CDDP tends to show significant decrease of transport enzyme activity within the cell membranes as well as corresponding increase in similar urinary enzyme activity. Studies in rats by Batzer and Aggarwal (1986), showed that delayed peaks in acid and alkaline pase was observed in CDDP treatment suggesting that renal alkaline pase corresponds to a decrease in the membrane enzyme levels in the kidney, possibly pointing the discharge into the urine. This increase in discharge of enzymes was also found in cultured kidney tubules showing that a similar situation occurs in the in vitro model too. Administration of calcium chloride improved the impaired functional status of kidney

(Aggarwal, 1980).

Distribution of calcium plays a vital role in the mechanism whereby alpha adrenergic agonists induce many hepatic responses (Review, Exton, 1981; Williamson et al., 1981; Taylor et al., 1983). Calcium ion movements are part of a general mechanism whereby hormone receptor interaction are translated into cellular responses (Reinhart et al., 1984). Aspects regarding the redistribution of calcium is through intracellular pools of calcium (Reinhart et al., 1982) and a rise in cytoplasmic free calcium concentration (Murphy et al., 1980; Barritt et al., 1981).

Various pathological situations develop in patients receiving treatment for diabetes and cancer. The most reported amongst being hypocalcaemia. Prolonged hyperglycemia has lead to deleterious side effects where hypocalcemia was prominent (Hoskin et al., 1981). Diabetes caused by streptozotocin, alloxan etc. also caused hypocalcemia (Onskov et al., 1965). Hypocalcemia in CDDP treatment was first reported by Schaeppi et al. (1973) and Rosen et al. (1980). CDDP causes an efflux of calcium from isolated mitochondria (Aggarwal, 1980). Investigations by James and Aggarwal (1984) on the gastric smooth muscle sensitivity after CDDP treatment enabled them to put forth a statement that $CaCl_2$ administration improves the hypocalcemia prevalent after CDDP treatment.

In the present study also, $CaCl_2$ administration in general, reduced the effect of both vagotomy and cisplatin toxicity on the kidney metabolic

machinery concerned with gluconeogenesis as well as transport of metabolites. There are of course minor differences in $CaCl_2$ protection between vagotomized and cisplatin treated pigeons. In VgX pigeons $CaCl_2$ prevented GPT activity from increasing while in CDDP treated pigeon kidney, it was GOT that was more restrained.

Thus in the kidney of both VgX and CDDP treated pigeons, there occured several changes in the rate of activity of metabolic reactions indicating that a functional parasympathetic system is absolutely necessary for glucose homeostasis. Parasympathetic dysfunction must be affecting the activity of several enzymes concerned with glucose uptake and release in organs such as liver and kidney, probably through influencing intracellular calcium ion concentrations.