CHAPTER - 3

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

The uptake of glucose in two diverse regions-peripheral and splanchnic-occurs by two different mechanisms : insulin mediated glucose uptake and non-insulin mediated glucose uptake. By defination insulin mediated glucose uptake occurs in insulin sensitive tissues, while non-insulin mediated glucose uptake occurs both in insulin sensitive and non-insulin sensitive tissues (Peters et al., 1991). DeFronzo et al. (1981) demonstrated that hyperglycemia enhances glucose uptake by both splanchnic and peripheral tissues. In Insulin Dependent Diabetes Mellitus (IDDM), glucose utilization is impaired during periods of poor control (Yki-Jarvinen, 1984). Stimulation of sympathetic nervous system or catecholamine administration inhibits ongoing insulin secretion (Roy et al., 1984; Ahren et al., 1986). Conversely, parasympathetic nervous stimulation via vagus (Ahren et al., 1987) as well as parasympathomimetic agents (Malaisse et al., 1987) as well as parasympathomimetic agents (Malaisse et al., 1966) potentiate glucose stimulated insulin release. Studies on hyperglycemia and glucose tolerance in obese rats with VMH lesions demonstrated that autonomic disorder may consist of defects in the tonic control by the hypothalamus or in preabsorptive autonomic reflexes essential for glucose homoeostasis (Balkan et al., 1991). Hyperglycemia, a common metabolic abnormality occurs as a general disorder following onset of diabetes mellitus.

Development of diabetes leads to variety of alterations not only in metabolic ways but also in many pathological ways. Clinical diabetic nephropathy and neuropathy are the most common effects seen. Hyperfilteration is observed in patients with clinical diabetic nephropathy

(Vance et al., 1984). Glomerular filteration rate tends to be greater than normal in some patients with diabetes mellitus. Renal hypertrophy is reported by Sochor et al. (1986) in experimental diabetes. Urinary volumes and higher levels of urinary glucose were observed in the kidney of alloxan treated diabetic rats (Andrew et al., 1983). Increase in urinary volume and glucosuria was observed in patients receiving Cisplatin against cancer (Goldstein et al., 1983; Schaeppi et al., 1972). Cisplatin administration is known to cause conditions akin to diabetes (Cacini and Singh, 1991).

Cisplatin, used as an anti-tumor agent, has its own toxic side effects. The maximum side effects observed in kidney lead to nephrotoxicity and chronic lesions. Proteinuria, increased blood urea nitrogen, glucosuria and hypocalcemia are also reported (Schaeppi, 1972). Cisplatin induces hyperglucagonemia (Thompson et al., 1988).

The mechanism of action in Cisplatin, streptozotocin, alloxan, etc. seems to be similar in nature. All these drugs break the DNA strands thereby modifying cellular effects. Renal abnormalities are reduced on administration of various chloride salts. The administration of NH_4Cl_2 reduced the cytotoxicity (Yates and McBrien, 1985) and $NaCl_2$ administration brought improved renal functions (Literest, 1981). Administration of $CaCl_2$ modified the functional status of kidney thereby reducing the deleterious effects (Aggarwal, 1980).

The similarity of the pathophysiology of vagotomized rats and CDDP treated rats are so great that (Chapter 2), it was but tempting to study the depth of such similarities in other metabolic activities such as activities of transaminases and phosphatases.

Materials and Methods

Male albino rats of the Charles-Foster strain were used for the study. Animals weighing about 250-300 gms were used, they were acclimatized in the laboratory conditions three weeks prior to the experiment. All these animals were caged into 8 groups of 6 animals each.

1) First set were vagotomized sub-diaphragmatically (see Chapter 1).

2) Second set were sham operated and served as control for set 1.

3) The rats in this set were given Cisplatin 7 mg/kg body wt. in 0.9% saline (single injection) as the vehicle.

4) The controls received injections of the vehicle alone.

5) Fifth set comprised of animals receiving $CaCl_2$ - 1.3% (1 ml morning and evening) for 48 hours after a vagal denervation and 72 hours after CDDP treatment.

6) Sham operated animals with $CaCl_2$ injections as described above remained the controls for fifth set.

7) Seventh set comprised rats treated with $CaCl_2$ along with the Cisplatin treatment.

8) Last set served as controls for the animals of seventh set which received plain saline injections along with $CaCl_2$.

All the animals were provided with water adlibitum. They were sacrificed at 48 hours (VgX rats) and at 72 hours (CDDP treated rats), under mild ether anesthesia, by exsanguination. Kidneys from both sides were removed, weighed and processed for various assays.

Biological quantitative assays were carried out for GPT, GOT, Na^+-K^+-ATPase, Alkaline Pase and Acid Pase (Chapter 1).

Statistical Analysis :

All the data are expressed as mean ± SEM. Difference between the means were analysed statistically by students 't'-test. The 0.05 level of probability was used as the criterion of significance.

Results

Vagotomy :

In the kidney of vagotomized rats, GPT (alanine pyruvate transaminase) showed an increase compared to that of sham operated rat kidney. However, GOT (glutamate oxaloacetate transaminase) showed only a slight decrease.

Of the phosphatases, Na^+-K^+-ATPase showed a reduction in the activity in the VgX, rat kidney while alkaline phosphatase showed an appreciable

TABLE 1 : EFFECT OF VAGOTOMY AND CISPLATIN TREATMENT ALONE, AND IN COMBINATION WITH $CaCl_2$ IN RAT KIDNEY ON TRANSAMINASES, NON-SPECIFIC PHOSPHATASES, PROTEIN CONTENT

	GPT	GOT	Na^+-K^+-ATPase	ALKALINE PHOSPHATASE	ACID PHOSPHATASE	PROTEIN
VgS	16.492± 0.444	21.747± 2.916	6.605± 0.759	0.985± 0.079	2.119± 0.071	13.972± 0.370
VgX	25.594± 1.342	20.753± 2.446	4.594± 0.611	1.653± 0.152	2.210± 0.127	13.655± 0.307
Con	16.496± 1.458	35.995± 3.255	5.741± 0.485	1.893± 0.127	2.345± 0.085	12.797± 0.308
CDDP	41.295± 1.713	22.790± 1.184	5.431± 0.597	2.009± 0.043	2.319± 0.101	11.617± 0.287
VgS+Ca	16.578± 2.067	12.107± 0.488	6.506± 0.733	1.471± 0.033	1.337± 0.011	14.095± 0.345
VgX+Ca	26.063±± 0.932	23.853± 1.525	5.259± 0.366	1.641± 0.036	1.450± 0.021	14.079± 0.531
Con+Ca	14.083± 0.538	13.149± 0.988	4.802± 0.223	1.754± 0.044	1.353± 0.036	12.492± 0.504
CDDP+Ca	22.666± 3.035	7.175± 0.645	5.399± 0.151	1.353± 0.036	1.58± 0.046	11.789± 0.663

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

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

	GPT	GOT	Na^+-K^+-ATPase	ALKALINE PHOSPHATASE	ACID PHOSPHATASE	PROTEIN
VgS (VgX)	55.1 ↑	4.57 ↓	31.8 ↓	68.3 ↑	4.29 ↑	⟷
Con (CDDP)	150.39 ↑	36.6 ↓	5.3 ↓	5.8 ↑	1.10 ↓	↓
VgS+Ca(VgX+Ca)	57.2 ↑	97.1 ↑	20 ↓	14.2 ↑	7.6 ↑	⟷
Con+Ca(CDDP+Ca)	60.9 ↑	45.4 ↓	10.4 ↑	23.5 ↓	15.3 ↑	↓

% 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.

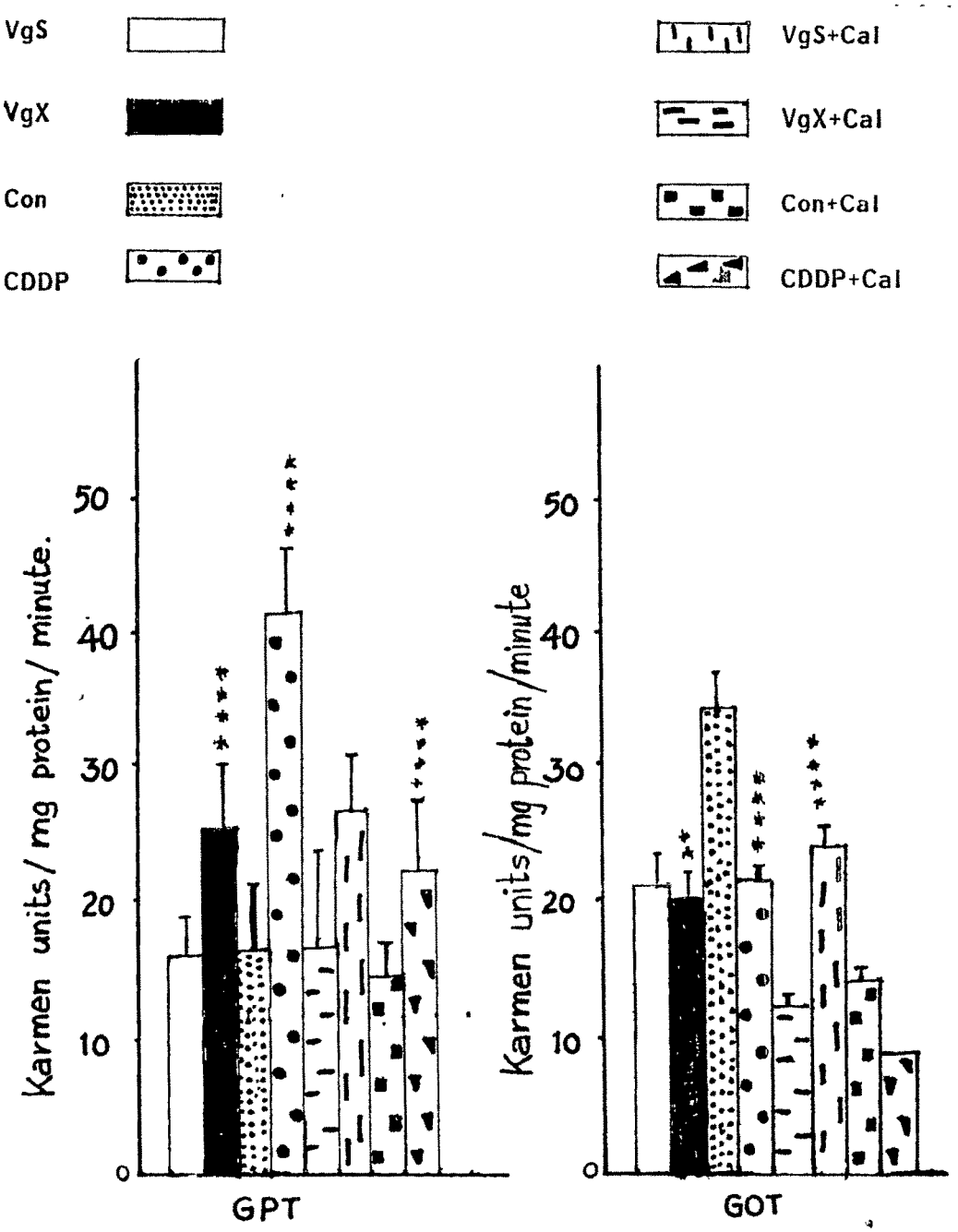

Fig: 1 Results given as mean ± SEM of six experiments.
$P<0.02^{*}$, $P<0.05^{**}$, $P<0.01^{***}$, $P<0.001^{****}$

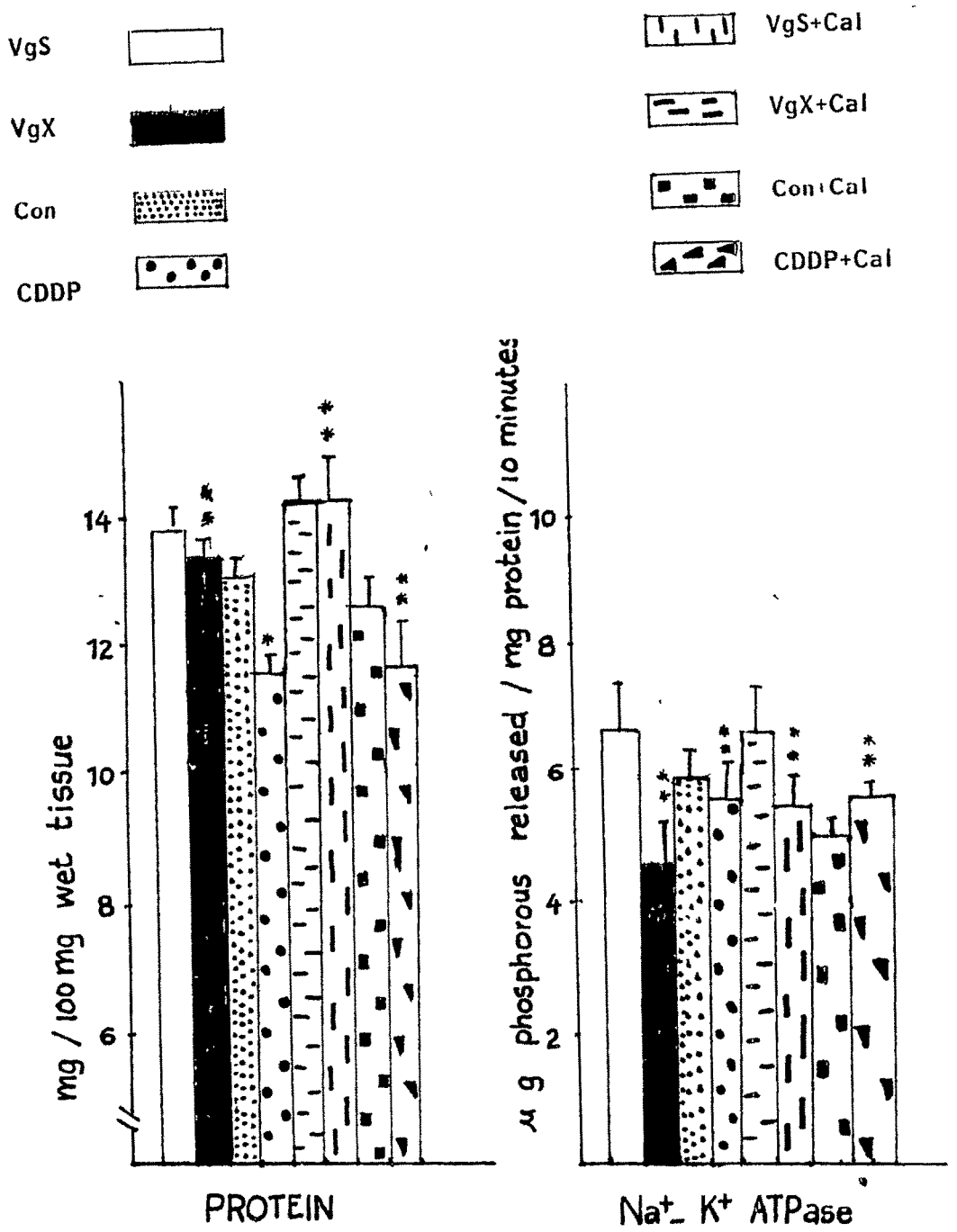

Fig:2 Results given as mean ± SEM of six experiments.
$P < 0.02^{*}$, $P < 0.05^{**}$, $P < 0.01^{***}$, $P < 0.001^{****}$

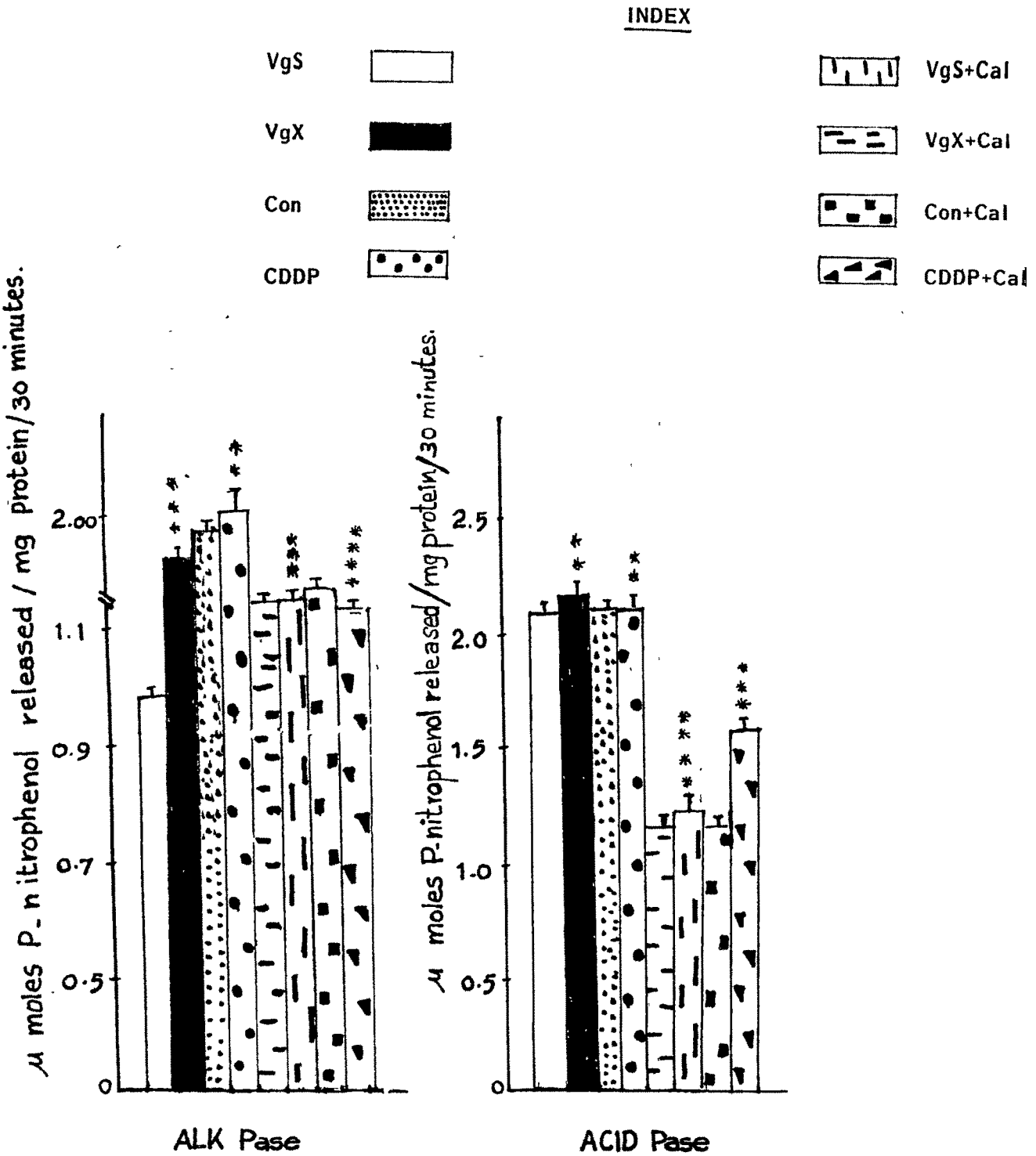

Fig: 3 Results given as mean ± SEM of six experiments.
$P<0.02^{*}$, $P<0.05^{**}$, $P<0.01^{***}$, $P<0.001^{****}$

increase. Acid pase did not respond to vagotomy.

Calcium chloride administration to VgX rat produced no effect on GPT, but GOT showed a reverse response to what was shown by VgX rat kidney. The increase in the GOT activity was almost two fold (97.1%).

Calcium chloride administration to VgX rats also reversed the response of acid pase. However, $CaCl_2$ could nullify the responses of Na^+-K^+-ATPase and alkaline pase to vagotomy to a great extent.

Cisplatin Treatment :

The response of GPT is cisplatin treatment was three fold (150%) increase while GOT showed a decreased activity in the kidney. Responses of Na^+-K^+-ATPase, alkaline pase and acid pase were very mild.

$CaCl_2$ administration to CDDP treated rats reduced the response of GPT to a certain extent, but GOT response remained unchanged. All the phosphatases showed reversal in their response when $CaCl_2$ was administered.

Discussion

The release of insulin and glucagon from the pancreatic A and B cells depend upon the appropriate integration of metabolic, neurotransmitter, hormonal and enteric signals. The counter regulatory responses to hypo- and hyperglycaemia consists of a cascade of neurohormonal responses and metabolic events, involving activation of the sympathetic and

parasympathetic nervous system, pancreatic hormones, catecholamine secretions and release of corticosteroides and growth hormone. Plasma glucose level has been established as the major signal for pancreatic A-cell function. However, in several physiological and pathological states, this feedback mechanism appears to breakdown with manifestation of hyper glucagonemia or even hyperglycaemia (Unger et al., 1975). Hyperglycaemia is known to occur due to a dysfunction of pancreas or dysfunction of the hypothalamic centers which regulate carbohydrate metabolism. Vagal denervation or parasympathetic blockade is known to cause increase in glucose level in the blood. VMH lesions brought about alterations in the central nervous system homoeostasis that were responsible for the increase in activity of the efferent vagus nerve that influences the endocrine pancreas (Bray et al., 1981; Jeanrenaud et al., 1983).

In the present investigation, the bilateral sub-diaphragmatic vagotomy in rats resulted in inactivation of the parasympathetic system leading to several changes. Distention of the stomach, partial digestion of the food, less food intake, nausea, diarrhoea, softness in the tissues etc. were observed. Similar inference were made on rats treated with Alloxan, STZ, etc. Damage to the nephron, tubular atrophy, dilation, glomerular sclerosis, etc. were also observed after Alloxan treatment (Evan et al. 1983). Renal hypertrophy in long term and experimental diabetes was observed by Steer et al. (1982). A correlation between kidney growth and blood glucose values over a wide range of blood glucose concentrations were observed by Seyer-Hansen (1977). Differential response of the adult and

immature rats on the renal effects of experimental diabetes was observed by Sochor et al. (1986). Renal lesions, glomerular infilterations, hypertrophy, etc. were noticed in rats treated with Cisplatin (Goldstein et al 1981). In all the above mentioned experiments hyperglycemia remained as a common disorder. Hyperglycaemia was also observed in vagotomized (Ommen, 1992; Parikh, 1992) and cisplatin treated rats (Ommen, 1992; Parikh, 1992).

Of the activities of transaminases assayed in rat kidney after vagal denervation and Cisplatin treatment, pyruvate transaminase (GPT) showed an increase in the level. Elevated transaminases denote utilization of amino acids to form glucose. The vagal inhibition can cause activation of adenylate cyclase and can inhibit cAMP-PDE thereby enhancing the gluconeogenic activities (Verma et al., 1984). The level of oxaloacetic transaminase (GOT) was found to decrease in both VgX and CDDP treated rats. The activity of this enzyme was measured by Szepesi et al. (1970) in the rat kidney and opined that GOT activity appears to be divided between cortex and medulla. The activity was confined more in cortex than in medulla due to the presence of cytoplasmic and mitochondrial isoenzymes in the former and only the cytoplasmic forms in the latter. The transaminases are generally known to have a role in the amino acid catabolism. The observations here suggest that the kidney is able to respond to increase the gluconeogenic enzymes when in need.

The pattern of response to vagotomy and CDDP treatment slightly changed after the administration of $CaCl_2$. As mentioned earlier there might have

occured an increase in the glucagon secretion due to vagal inhibition and Cisplatin treatment. Hyperglucagonemia is known to cause efflux of calcium (Pipeleers et al., 1985). Influence on the fluctuation of intracellular A-cell Ca^{2+} by glucagon produces a stimulus for amino acid coupling. It would be suggested that hyperglucagonemia might have evoked the release of Ca^{2+} and hence when a dose of extracellular $CaCl_2$ was given, the enzyme showed a reverse response in some cases.

The selective transport of various molecules and ions acros the cell membrane is a ubiquitous property of living cells. Three types of transport systems predominate for this function. Translocation of a particular species may occur down its own concentration gradient (facilitated diffusion); some may be coupled to the concentration gradient of a concomittantly transported ion (co-transport); and some may be driven by the hydrolysis of ATP (active transport) (Walmsley, 1988). Active transport involves the ATPases, of which Na^+-K^+-ATPase being a vital one. In hyperglycemia, osmotic diuresis, renal hypertrophy, renal hyperfunction, increase in the glomerular filtration rate, increased renal Na^+ pump activity, etc. are reported by many authors (Spiro et al., 1971; Rasch and Norgaord, 1982; Brown et al., 1982). Diarrhoea, proteinuria, anorexia, morphological damage, etc. were observed after CDDP treatment (Goldstein et al., 1983).

In the present study the Na^+-K^+-ATPase activity in the kidney recorded a decrease in case of VgX and CDDP treated rats. Alterations in the Na^+-K^+-ATPases in experimental diabetes raised the interesting possibilities that

opposite changes in the sodium pump in different regions of the nephron could contribute to distinct pathological alterations in renal functions of this disease (David Ku et al., 1987). The hormonal influence of Na^+-K^+-ATPase was studied by Pippard and Baylis (1984) using cytochemical bioassays in the rat medullary thick ascending limb. Charlton and Baylis (1990) suggested that Argenine-Vasopressin can stimulate rat renal medullary Na^+-K^+-ATPase. Insulin increases the ATPase activity in cell membranes of normal rats but fails to do so in membranes of non-independent diabetic rats (NIDDM) (Levy et al., 1990). Inactivation of membrane ATPase would tend to lead an ionic imbalance, which is probably responsible for cellular mortality in CDDP treated rats (Stekhovens and Bonting, 1981). CDDP administration is known to inhibit ATPases (Anderson et al., 1990).

The administration of $CaCl_2$ prevented the levels of ATPase activity from decreasing in VgX and Cisplatin treated rats. There was an increased ATPase activity in CDDP rats when compared with its own control. In the kidney, the proximal convoluted tubules have the higher concentrations of Acid and Alkaline phosphatases. During abnormal conditions Acid Pase, being a lysosomal enzyme, would show higher levels. In diabetes too, such a situation can be expected. In diabetes, wound healing takes a longer time. Shah et al. (1974, 1976) have shown in alloxan treated diabetic rats that the wound healing was delayed due to defective cell proliferation. Shevuck (1973) has shown sharp reduction in Alkaline Pase while an increased Acid Pase was reported in Alloxan diabetic rats (Sneer et al., 1970).

Acid Pase is involved in phagocytosis (Klockars and Wegelius, 1969), dissolution of tissue components (Weber and Nichus, 1961) synthetic activities (Sauter, 1967; Mishra and Mohanty, 1967) protein synthesis (Vorbdrot, 1958) and differentiation (Ghiretti, 1950). The involvement of the enzyme in repair was demonstrated by Carranza and Cabrini (1962) in diabetic rats.

The alkaline pase is reported to be associated with carbohydrate metabolism (Cori and Cori, 1952; Cusworth, 1958; Duncan, 1959; Rosenthal et al., 1960). The alkaline pase is also involved in DNA metabolism (Rogers, 1960) and passage of metabolites across cell membrane (Bradfield, 1950; Danielli, 1954). The enzyme activity generally is low in mammalian liver (Wachestein, 1963) but an increase was noticed in experimental condition such as bileduct obstruction (Hawkins and Hard, 1950; Kaplan and Righeth, 1970) and partial hepatectomy (Pekarthy et al., 1972).

Since both the enzymes were involved in wound healing and transport activities, it was expected that during metabolic derangements there would be alterations at tissue level activity. The present observation however, showed that in vagotomized rats and CDDP treated rat kidney alkaline Pase activity increased. Whereas, in both the situations acid Pase remained unaltered. The administration of $CaCl_2$ brought about a decrease in levels of Alkaline Pase in CDDP treated rat kidney when compared to its respective control. The $CaCl_2$ administration brought about an increase in levels of Acid Pase in both vagotomized and CDDP treated rat kidney

compared to sham operated and saline treated $CaCl_2$ groups. In other words the $CaCl_2$ administration could reverse the pattern of response or diminish the adverse affects.

Diabetic rats showed reduced levels of Acid Pase activity (Sneer et al., 1970). It seems that diabetic condition in some way inhibits or retards enzyme synthesis (Shah et al., 1977). They have further opined that this reduction could be due to poor protein synthesis in these cells. Vagal denervation has caused similar changes in levels of these enzymes (Verma et al., 1984; Pilo and Mehta, 1985). Cisplatin treatment brought about a similar effect. The delay in onset of the peak response of the enzyme was observed by Batzer and Aggarwal (1986) after cisplatin treatment.

The increased levels of Alkaline Pase suggest that in kidney, at the membrane level the enzyme remained active. Such active enzyme level could facilitate increased glucose uptake by brush border of proximal convoluted tubules (PCT) (Danielli, 1954; Chitnis et al., 1978). Cisplatin is known to cause efflux of Calcium (Aggarwal, 1980). The vagotomized and cisplatin treated conditions also provided such evidence in birds (Chapter 6). Decrease in the amount of calcium bound to kidney plasma membrane cells also have been observed (Aggarwal and Hammouda, 1980). Hypercalciuria in rats has been reported in tumor conditions by Adler et al. (1991).

The hormonal influence on calcium homoeostasis was shown by Reinhart et al. (1983) and Blackmore et al. (1982). They opined that rate of

hormone induced and basal cycling of calcium is dependent on extracellular calcium concentrations and calcium movements are part of a more general mechanism whereby hormone-receptor interactions are translated into cellular responses. One could finally conclude that in rats after vagal inhibition and cisplatin treatment, there was a change in gluconeogenesis; though both transaminases showed different responses. The transport enzymes were functioning at a lower level but, a $CaCl_2$ administration could mask the overall effect and improved the renal functioning especially in cisplatin treated rat kidney.