GENERAL CONSIDERATIONS

It is well-known that two functional divisions of the autonomic nervous system, the parasympathetic (PNS) and sympathetic systems (SNS) with their respective transmitters, acetylcholine (ACh) and norepinephrine (NE), often have opposite actions on the effector organs. Due to their antagonistic actions, the two autonomic systems are able to regulate large number of physiological functions that contribute to the maintenance of a well-balanced internal environment or homeostasis.

Modulation of glucose homeostasis in blood is one of the important functions of the autonomic nervous system. Both liver and kidney have substantial role in controlling blood glucose level and these organs are mainly innervated by PNS and SNS. Stimulation of parasympathetic nerves to the liver produces a rapid and dramatic decrease in hepatic glucose output and activates glycogen synthetase which is accompanied by rapid glycogen deposition (Shimazu, 1971; Ottolenghi et al., 1971; Lautt, 1979). Stimulation of sympathetic nerves to the liver, on the other hand, leads to the activation of phosphorylase and G-6-Pase and release of glucose (Shimazu and Amakawa, 1968a, b). Both sympathetic and parasympathetic systems have their controlling centres in the hypothalamus. Hypothalamus receives information about glucose concentration in blood through its own glucose receptors as well as through (afferent) fibres coming from hepatic blood vessels (mainly portal vessels). According to the signal regarding the

glycaemic level, ventromedial hypothalamus - VMH (the sympathetic centre) gets activated. VMH through sympathetic nervous system can increase blood sugar level while lateral hypothalamic area (LHA) through PNS can decrease the blood sugar level. Lesions of VMH have been reported to produce hyperinsulinemia whereas electrical stimulation of this nucleus decreased the release of glucagon (Steffens et al., 1972). The stimulation of LHA-(parasympathetic centre), on the other hand, increased insulin release, glycogenesis in the liver and a fall in glycaemic level. LHA lesions naturally could lead to hypoinsulinemia as well as reduced glucose uptake by liver.

Hypoinsulinemia and the inability of the tissues to absorb glucose are the two main abnormalities in diabetes mellitus. These two conditions are found in vagotomized pigeon (Pilo et al., 1985).

Vagotomized pigeons also failed to bring about normoglycaemia within 60-90 minutes when challenged with a glucose load (Pilo et al., 1985). Thus vagal denervation can bring about hyperglycaemia. Recently it is recognised that many complications found in diabetes mellitus could be due to vagal cholinergic dysfunction (Lautt, 1980; Dobbs and Unger, 1982). In some cases, vagal neuropathy could also be considered as a reason for the manifestation of diabetes mellitus, especially insulin dependent diabetes mellitus (IDDM).

Although diabetic condition could be experimentally produced by alloxan and streptozotocin, which are selective B cell cytotoxic agents, it is difficult to produce experimentally a selective neuropathic condition. Lack of such experimental models where autonomic neuropathy could be induced has created a real vacuum in the knowledge of autonomic dysfunction as a causitive factor for the development of IDDM.

Reports that several antitumour drugs cause neural dysfunction and thereby disturb metabolic regulation, especially in the liver and kidney, attracted attention in our laboratory. Cisplatin (CDDP), a widely used chemotherapeutic agent, comes in this category (Rosenberg, 1985). Patients who have undergone cisplatin therapy have developed side effects very similar to that seen in diabetic autonomic neuropathy. Cisplatin can influence carbohydrate metabolism by their ability to alter insulin secretion (Goldstein et al., 1983). Nephrotoxicity has also been reported in many cases as one of the side effects of this drug (Dobyan et al., 1980). In animals, CDDP induces toxic side effects mainly in kidney, intestine and bone marrow (Lippman et al., 1973). Cisplatin has differential affinities for kidney and liver. It shows preferential localizations within the subcellular sites in the kidney. Kidney not only retains a great concentration of CDDP than liver, but the biological half-life of this drug is also

longer in the kidney (Choie et al., 1980). The fact that CDDP administration has resulted in hyperglucagonemia and impaired the insulin secretion (Goldstein et al., 1983) can be shown as an evidence of cisplatin's selective side effect on autonomic nervous system.

There are several clinical reports at hand that show peripheral neuropathy after high dose of CDDP. Richardson and Cantwell (1990) observed that some patients with metastatic germ-cell cancer have autonomic neuropathy based on CDDP.

Thus, CDDP was found to cause (1) neuropathy, especially that of autonomic nervous system and (2) hyperglycaemia. Since autonomic nervous system is responsible for the regulation of glucose homeostasis, it is easy to believe that the hyperglycaemia seen in CDDP treated patients is due to autonomic neuropathy. A natural sequal to this contention is that CDDP can be considered as chemical agent that causes selective autonomic neuropathy. So far there is no concentrated research that relate autonomic neuropathy and glucose homeostatic mechanisms. Most of the work was based on vagal transection and chemical sympathectomy. The effect of vagotomy on glucose homeostasis has been extensively done in our laboratory. Vagotomy in pigeon have been shown to cause :

1. Hyperglycaemia (John et al., 1985)
2. Increased glucose uptake by liver (Pilo and Verma, 1985).
3. Decreased hepatic phosphorylase (Pilo et al., 1984).
4. Increased hepatic glycogen synthetase activity (Pilo et al., 1984).
5. Increased gluconeogenesis in liver (Pilo et al., 1984).
6. Decreased gluconeogenesis in kidney (Verma et al., 1984).

Bird's response to hyperglycaemia and hypoglycaemia are similar to that of mammals, at the molecular level but the temporal correlates of these responses and degree of tolerance to hypo-and hyperglycaemia are different in birds and mammals (Review : Pilo and Patel, 1985). The basic difference is that birds tolerate hyperglycaemia much more than hypoglycaemia. In fact, in birds the pancratic islets contain more A cells than B cells; reverse is true for mammals as they are less tolerant to hyperglycaemia and produce insulin more quickly and in larger amounts than glucagon. It was thus indeed deemed worth while to compare mammalian response to vagotomy and CDDP treatment with that of avian response. In this light, a series of experiments were designed to get comprehensive information about glycaemic response in vagotomised and CDDP treated rats and pigeons.

Vagotomy (VgX) caused hyperglycaemia in rats which is indicative of that fact that parasympathetic afferents and efferents are necessary for maintaining blood sugar level. CDDP treatment also elevated the blood glucose level in rat (Chapter II). A possibility of cholinergic dysfunction as the cause for the hyperglycaemia in CDDP treated rat could be envisaged as both vagotomy and CDDP treatment reduced the AChE activity in liver and kidney (Chapter II). Absence of ACh secretion from the vagal fibres in VgX and CDDP treated animals could be the reason for the reduced AChE activity in the liver and kidney. Experiments on smooth muscle strips from CDDP treated rats exhibited hypersensitivity to ACh. The muscle is hypercontractile to ACh because less of the neurotransmitter is hydrolysed due to the presence of less-active AChE (San Antonio and Aggarwal, 1984).

Response of pigeon to vagotomy and CDDP treatment is also similar to that of rat. Hyperglycaemia was accompanied by a decreased AChE activity in the liver and kidney of pigeon (Chapter II).

The hyperglycaemia response of VgX and CDDP treated rat and pigeon could be either due to increased glucose output by liver and kidney or due to decreased uptake of glucose by these tissues. It has been shown in our laboratory that the liver glycogen and glycogen synthetase activity levels decreased in VgX and CDDP treated rats. At the same time phosphorylase enzyme was very active (Parikh, 1992).

Gluconeogenesis and lactate utilization was also more in VgX and CDDP treated rat/pigeon liver (Parikh, 1992). Gluconeogenesis was not at the expense of labile proteins in the liver which was evident from the fact that protein content did not decrease in the liver of both rat and pigeon after VgX or CDDP treatment (Chapter III).

Protein and nucleic acid concentration also reflect on the overall state of multiplication of cells and maintenance of cell mass in any tissue. Hormones and nerves are equally involved in this trophic action. It has been suggested that insulin plays a major role during cholinergic differentiation (Tesoriere et al., 1992). Hence, the reduced level of DNA and RNA content in the liver of CDDP treated or VgX animals could be due to insufficiency of insulin as well as due to the decreased neural activity (Chapter III). The major effect of cisplatin in tumour cells is to inhibit DNA synthesis by cross linking the complimentary strands of nucleic acids (Roberts and Pascoe, 1972). Moreover, like CDDP vagal denervation also resulted in causing an impediment in DNA synthesis (Shimazu, 1983). Cellular toxicity of CDDP in pigeon was more than that of rat. Administration of CDDP reduced the DNA content in the kidney of both the rat and pigeon while DNA content did not alter in the kidney after vagotomy. Cisplatin treatment produced a drastic reduction in RNA content in the kidney of pigeons. Bilateral vagotomy inhibitited DNA synthesis during regeneration after partial hepatectomy (Kato and Shimazu, 1989).

TABLE I: EFFECT OF VAGOTOMY AND CISPLATIN TREATMENT ON LIVER AND KIDNEY OF RAT AND PIGEON.

PARAMETERS.	RAT – LIVER		PIGEON – LIVER		RAT – KIDNEY		PIGEON – KIDNEY	
	VgX	CDDP	VgX	CDDP	VgX	CDDP	VgX	CDDP
BLOOD SUGAR	↓		↓					
AChE	■	■	■	↓	■	■	↓	■
DNA	↑	↑	■	↓	↑	■	↓	↓
RNA	↑	■	↑	↓	↑	■	↓	■

The decreased trophic action in the VgX and CDDP treated animals may be manifesting through two ways. Vagal denervation removes the direct trophic action of cholinergic fibres on one hand, and on the other hand, reduces the release of insulin and increases the release of glucocorticoids. Cisplatin has direct cytotoxic action as well as neurotoxicity, by which it reduces the neural stimulation of secretion of various hormones.

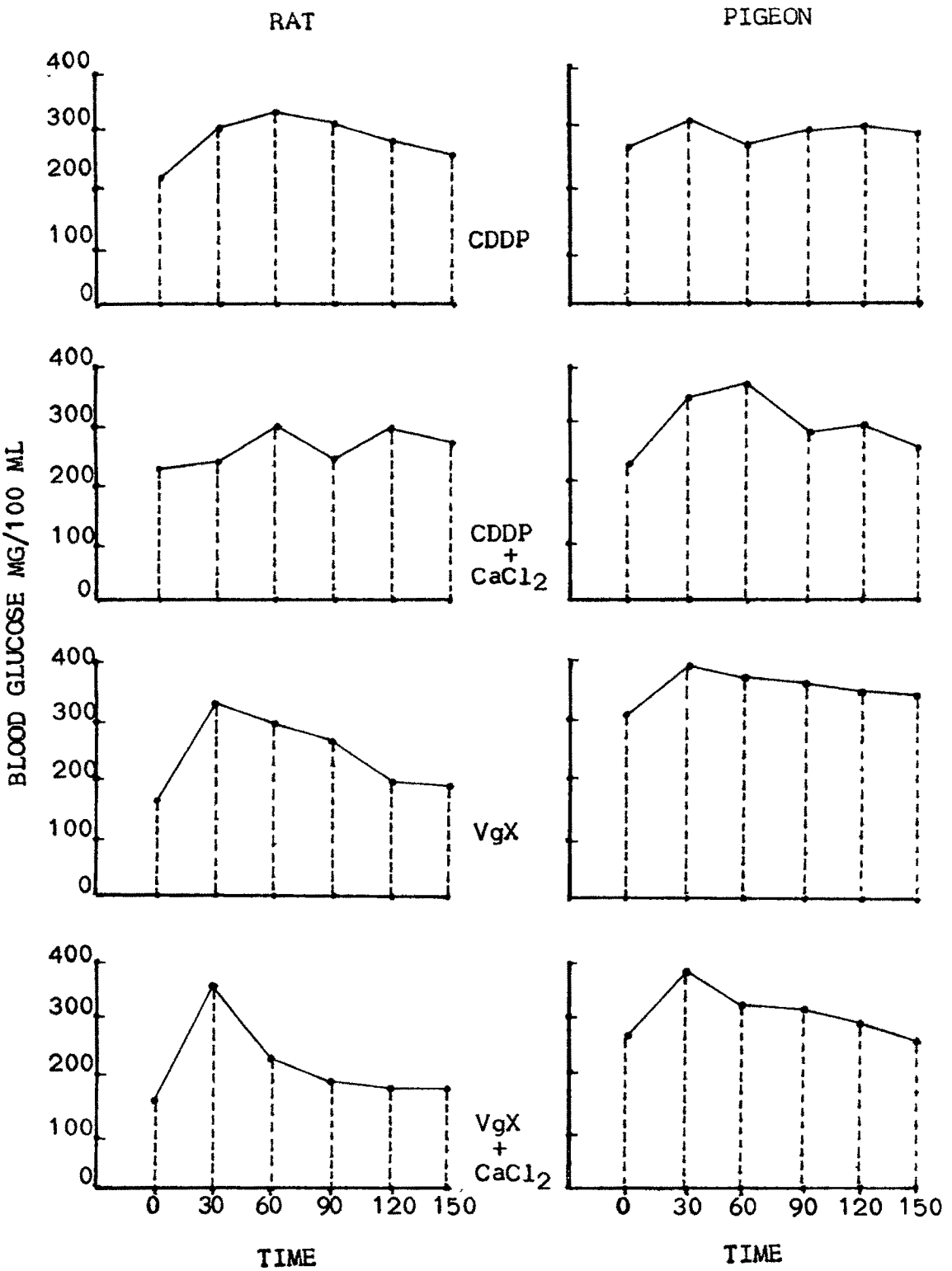
Vagotomy caused an increase in serum free fatty acid and blood sugar level in pigeon (John et al., 1985) which was attributed to an increased release of corticostreroids and NE (Viswamnathan et al., 1987). The glucocorticoids excert a very profound inhibitory effect on DNA synthesis (Tesoriere et al., 1992). Thus it is possible to conclude that in both rat and pigeon, nucleic acid metabolism was adversely affected under the influence of glucocorticoids that might have been released in greater amounts in VgX and CDDP treated animals.

Usually, hyperglycaemia is a result of several factors that operate differently depending on the physioloigcal state of the animal. During post-prandial conditions increased level of glucose in blood is effectively absorbed by tissues through the influence of insulin. If insulin defficiency or disruption of glucose uptake mechanism takes place, then hyperglycaemia lasts more than the usual period. Hyperglycaemia can also occur when excess release of glucose

manifests. This latter process found in stress, starvation or during exericise, is initiated by glucagon, NE and GH. Sustained glucose release under such conditions required gluconeogenesis which is stimulated by glucagon and glucocorticoids, in organs such as liver and kidney.

When glucose tolerance test (GTT) was undertaken after administration of glucose intraperitoneally, both VgX and CDDP treated pigeons and rats showed persisting hyperglycaemia during the period of tests indicating that glucose uptake in VgX and CDDP treated animals are very much reduced (Chapter IV). It has been reported that vagotomy produced a decrease in insulin level and impaired response to glucose load (Frohman et al., 1967). Even oral glucose loading in vagotomised rats did not elicite a proper insulin release response (Hamphrey et al., 1975). Impairment of glucose uptake mechanism was reported in vagotomized rats by Parikh (1992) in liver and Pillai (1992) in kidney. In both these organs a decreased glycogen synthetase and glycogen disposition was noticed following vagotomy and CDDP treatment. Moreover, there were increased activities of phosphorylase, G-6-Pase and LDH in these organs which explain the increased glucose release and the resultant hyperglycaemia. An overall decline in the activity of Na^+-K^+-ATPase and other phosphatases observed in the liver and kidney of VgX and CDDP treated rats (Parikh, 1992; Pillai, 1992) also indicated the decreased transport activities.

EFFECTS OF CISPLATIN AND VAGOTOMY ON GLUCOSE TOLERANCE IN RAT AND PIGEON



Effect of Vagotomy and Cisplatin Treatment on Liver and Kidney of rat.

Parameters	Liver *		Kidney **	
	VgX	CDDP	VgX	CDDP
Glycogen	↓	↓	↕	↕
Glycogen Synthetase	↓	↓	↕	↕
Phosphorylase	↑	↑	↑	↑
G-6-Pase	↕	↕	↕	↑
Aldolase	↕	↕	↑	↑
LDH	↑	↑	↑	↑
GOT	↕	↕	↕	↑
GPT	↕	↑	↑	↕
Na^{+}-K^{+} ATPase	↓	↓	↓	↕
Alkaline Pase	↓	↓	↑	↕
Acid Pase	↕	↕	↕	↕

* Parikh (1992); ** Pillai (1992)

↑ Increase

↓ Decrease

↕ No change

Activation and deactivation of enzymes as well as induction and repression of synthesis of enzymes are on the basis of regulation of glucose uptake or release at the cellular level.

The intracellular actions of acetylcholine and many hormones are mediating through regulating free Ca^{2+} concentration in the cytosolic compartment (Pilo and Patel, 1978). Ca^{2+} is involved in the release of hormones such as insulin and activation of enzymes. Even several enzymes in turn have Ca^{2+} as second messengers. Even insulin acts through manipulating Ca^{2+} level in the cytoplasm (Kissebah et al., 1975; Laven 1974). It is conceivable that the adverse effects of vagotomy and CDDP treatment could be due to the failure of regulating Ca^{2+} efflux or release from a membrane bound state in the absence of acetylcholine.

In the wake of reports that CDDP treatment alters calcium homeostasis in smooth muscles and kidney cells (Aggarwal and Hammouda, 1980; Westfall, 1981) and decreases serum calcium level in rats (Rosen et al., 1980), it could be concluded that Ca^{2+} may play a decisive role in the manifestation of adverse effects of CDDP treatment. Administration of $CaCl_2$ to cisplatin treated rats did reduce the toxic side effects of this drug (Aggarwal and Fadood, 1981, Belay-Yates and Brien, 1985). Parathyroidectomy also was found beneficial to certain toxicity of cisplatin (Capasso et al., 1990). When $CaCl_2$ was administered to VgX and CDDP treated rats and

TABLE II: EFFECT OF CaCl2 ADMINISTRATION ON CISPLATIN TREATED/VAGOTOMIZED RAT AND PIGEON.

PARAMETERS.	RAT – LIVER		PIGEON – LIVER		RAT – KIDNEY		PIGEON – KIDNEY	
	VgX+ CaCl2	CDDP+ CaCl2	VgX+ CaCl2	CDDP+ CaCl2	VgX+ CaCl2	CDDP+ CaCl2	VgX+ CaCl2	CDDP+ CaCl2
BLOOD SUGAR								
AChE								
DNA								
RNA								

pigeons (Chapter V) the adverse effect of vagal ablation and cisplatin was found to be very much reduced. Both rat and pigeon showed no sign of hyperglycaemia following vagotomy and CDDP treatment when these experimental animals were also given $CaCl_2$. Even nucleic acid concentration was either maintained at the normal level or showed a slight increase. By and large acetylcholinesterase activity was also more or less maintained at the normal level when experimental animals were subjected to $CaCl_2$ administration. Thus many of the biochemical parameters were found to be reverted back to normal state or level in both $CaCl_2$ administered vagotomized and CDDP treated rats and pigeons. Parikh (1992) and Pillai (1992) also reported a similar protective action of $CaCl_2$ in the liver and kidney respectively, in rats subjected to vagotomy or CDDP treatment. Not surprisingly the VgX and CDDP treated rats and pigeons when given $CaCl_2$, responded almost normally to a glucose load (Chapter IV).

Calcium is also involved in the regulation of cell proliferation and hence, lowered calcium level in the serum caused by vagotomy (Verma *et al.*, 1982) and CDDP treatment (Schaeppi *et al.*, 1973; Rosen *et al.*, 1980) could also adversely affect the nucleic acid content of the liver and kidney. But $CaCl_2$ treatment reduced the adverse effect of both vagotomy and CDDP treatment (Chapter V). A significant increase in DNA content was observed in the liver of experimental rats and pigeons treated with $CaCl_2$. The effect of cisplatin on calcium ions is mediated through causing

serum hypocalcemia (Rose et al., 1980). The nephrotoxicity in CDDP treated animals could be the reason for increased calcium elimination. However, how vagotomy causes hypocalcemia needs further intensive studies. Probably the permeability of renal tubules are altered by ACh and in the absence of vagal innervation Ca^{2+} must be getting excerted more. Extraneous supply of calcium could elevate the serum level and thereby reverse, or protect the tissues from some of the effects of vagotomy and CDDP treatment that manifest through hypocalcemia.

Acute toxicity of cisplatin is one of the reason why this drug is not widely used as a chemotherapeutic agent to control selected neoplastic growth. Carboplatin (CBDCA) a second generation analogue was found to be less toxic than cisplatin. Since carboplatin was found to have less toxic side effects, it was thought worth while to study the effect of carboplatin on glycaemic control. In both rat and pigeon a mild elevation of glycaemic level was noticed after CBDCA administration. Acetylcholinesterase activity in the liver of rats was unaltered after CBDCA treatment while in pigeons a decrease in AChE level was noted. However, CBDCA reduced AChE level in the kidney of the rat which could be related to the higher retention of CBDCA there. A comparision of CDDP treatment and CBDCA has shown that reduction in AChE enzyme activity is very much less in CBDCA treatment. DNA and RNA contents in the liver of rats did not fluctuate after CBDCA treatment as much as that was found with CDDP treatment.

TABLE III: EFFECT OF CARBOPLATIN TREATMENT ON LIVER AND KIDNEY OF RAT AND PIGEON.

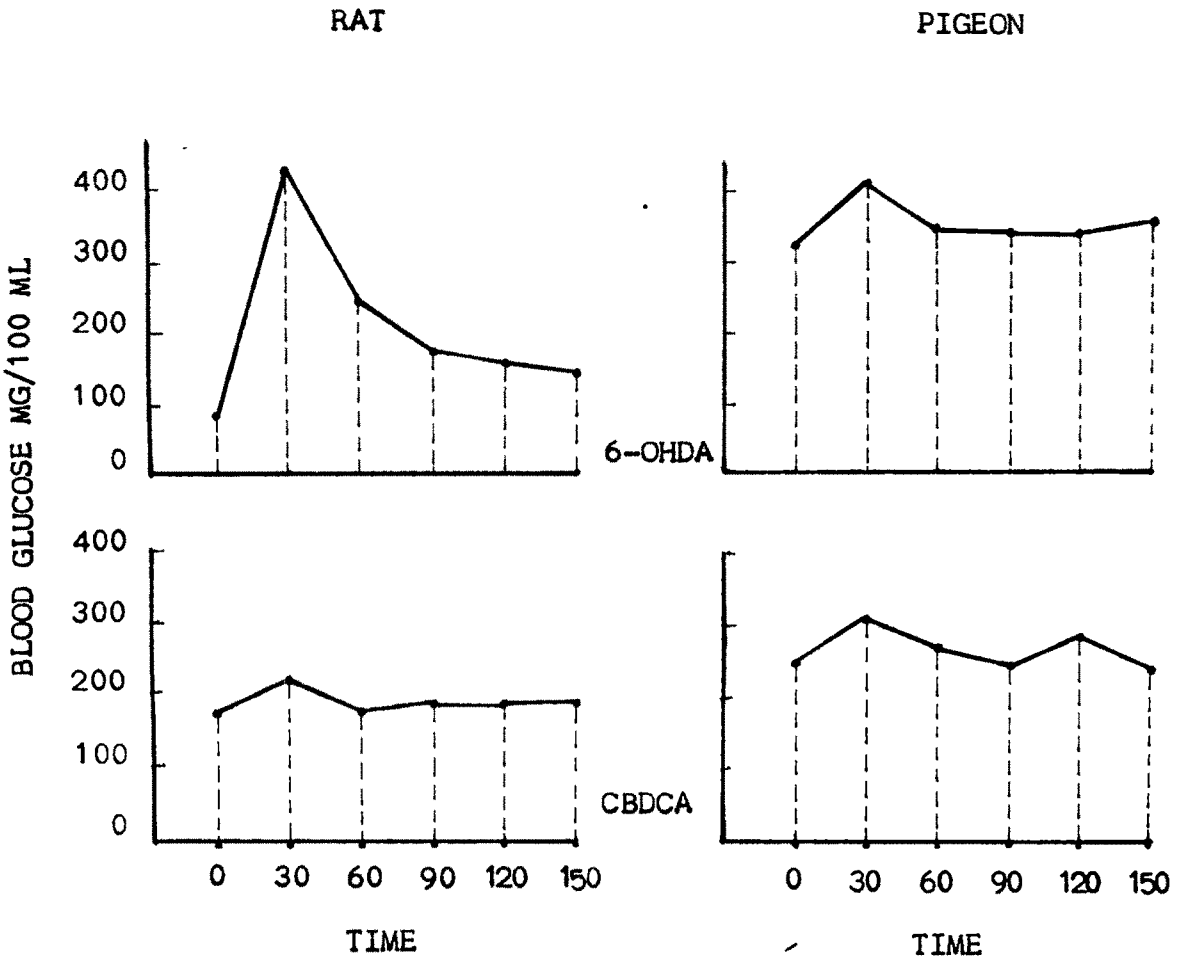
PARAMETERS.	RAT		PIGEON	
	LIVER	KIDNEY	LIVER	KIDNEY
BLOOD SUGAR				
AChE				
DNA				
RNA				

However, a slight decline in DNA content in both rat and pigeon denotes that CBDCA has slight cellular toxicity in liver and kidney though with a less intensity compared to CDDP.

Some of the metabolic effects caused by ablation of vagus nerve and CDDP treatment seem to be correlated with activation of sympathetic nervous system (SNS) and release of glucagon, catecholamines and thyroid hormones. Reduction on parasympathetic tone (due to vagotomy or cisplatin neuropathy) as such can cause adrenergic sympathetic tone to assume a dominant role. Glucose homeostasis is modulated by a coefficient correlation of these two opposing autonomic divisions. Activation of PNS or inhibition of SNS could bring the same set of responses in many tissues. As mentioned earlier, hyperglycaemia in VgX and CDDP treated rats and pigeons could be due to increased sympathetic activity (tone).

The role of sympathetic nervous system can not be studied early through surgical ablation unlike with PNS. Chemical sympathectomy is the choicest method. Administration of 6-OHDA selectively disturbs the hepatic sympathetic nerve and also restrains the neuronal stimulation of glucose release (Lautt and wong, 1978). A comparative study was undertaken to understand the role of SNS in blood sugar regulation in birds and mammals (Chapter VII). Chemical sympathectomy resulted in hypoglycaemia in rats while pigeons showed

EFFECTS OF 6-OHDA AND CARBOPLATIN (CBDCA) ON GLUCOSE TOLERANCE IN RAT AND PIGEON



hyperglycaemia. Both parasympathetic and sympathetic nervous systems may be intimately involved in the control of hormone secretion. In mammals, insulin, glucagon and catecholamines play major role in the minute to minute regulation of hepatic glucose output under in vivo condition (Gerich et al., 1981). Increased vagal cholinergic action could deduced by the increased AChE activity in the liver of sympathectomized rat. The decreased blood sugar level in rat could be thus due to the increased vagal tone as well as due to increased insulin secretion after sympathectomy. These lead to increase operation of glucose uptake mechanism in the rat liver resulting in a lower glycaemic level. In contrast, chemical sympathectomy produced hyperglycaemia in pigeon. Unlike in rat, there was no increased AChE activity in the liver of pigeon after sympathectomy. Lack of activation of PNS could be taken as a reason why there was no increased glucose uptake and a resultant hypoglycaemia one would rather expect. GTT in 6-OHDA treated pigeons showed that glucose uptake mechanism is not at all affected by sympathectomy as a normal glucose curve was noted in the experimental birds. The increased glucose level in the pigeon treated with 6-OHDA could be due to increased glucocorticoids releases under stress.

Similar to vagotomy, sympathectomy also did affect the DNA and RNA contents in the liver and kidney of rat; while in pigeon it was not much affected. Enhanced content of nucleic

TABLE IV: EFFECT OF 6-OHDA ADMINISTRATION ON LIVER AND KIDNEY OF RAT AND PIGEON.

PARAMETERS.	RAT	PIGEON
	LIVER – KIDNEY	LIVER – KIDNEY
BLOOD SUGAR		
AChE		
DNA		
DNA		
PROTEIN		

acids in the liver and kidney of rat could be due to the increased insulin release after 6-OHDA treatment. The major influence of insulin in the liver on protein synthesis is attributed to the augmentation of RNA synthesis (Innu and Ishioka, 1983a, b; Rannels et al., 1977). The insignificant role of insulin in maintaining glucose level in pigeons was also reflected in nucleic acid metabolism. The decrease in DNA content in the liver and kidney of pigeon after sympathectomy could be primarily due to the elevated release of glucocorticoids. The high dose of corticosroids injection in rats produced an inhibitory effect on protein synthesis. Manchester, 1959; Rannels et al., 1978).