INTRODUCTION

A discovery in the middle of nineteenth century by Bernard (1954) that the puncture in the floor of fourth ventricle results in hyperglycaemia was a major breakthrough in neuroendocrinology. The finding stimulated many endocrinologists to do further experiments on glucose balance in blood after the stimulation of various areas of diencephalon. A fair appreciation of Bernard's observation was made by Cannon et al. (1924) who revealed that the sympathetic nervous system and adrenal glands are involved in glucose homeostasis. Compelling evidences were also derived from the parallel studies by Britton (1924) and other workers which implicated the involvement of parasympathetic nervous system, in particular the vagus nerve, in the control of insulin secretion.

Of the central neural structures the hypothalamus, the most vital part of brain, regulates the activities of sympathetic and parasymapthetic nerve fibres. These autonomic nerve fibres in turn, are credited with the power of controlling various physiological processes including emergency mechanism, repair and the preservation of constant internal milieu, and are thus likely to be important in modulating the metabolism in the visceral organs such as liver and kidney.

Maintenance of homeostasis is the main function of hypothalamus. In this brain region, a large number of physiological parameters are monitored on the basis of which

the regulation of body functions and metabolic reactions are carried out. The regulation of body functions by hypothalamus is done through both autonomic nerves as well as through hormones. The neurons that reach hypothalamus could regulate the release of releasing factors that are involved in the stimulation or inhibition of synthesis and release of hormones from adenohypophysis. The hypothalamus is also the seat of both divisions of autonomic nervous system (PNS and SNS).

On the basis of studies by Ban (1975) who evaluated measurements of blood pressure, gastric motility, respiratory movement, milk ejection, and urinary bladder function, the autonomic functions of the hypothalamus have been divided into 3 separate zones; (1) a parasympathetic zone involving the middle line periventricular stratum (2) a sympathetic zone located in the medial hypothalamus that included ventromedial nucleus, and (3) a parasympathetic zone corresponding to the lateral hypothalamic nucleus. According to the classical scheme of the autonomic innervation, the sympathetic influence is supplied through the splanchnic nerve and the parasympathetic through the vagus nerve. Preganglionic sympathetic fibres reach the coeliac ganglion through the bilateral greater and lesser splanchnic nerves. Postganglionic fibres arise in the coeliac ganglion forming an extensive plexus, the coeliac plexus. Parasympathetic system also has preganglionic and postganglionic fibres.

Preganglionic fibres run directly to the organs. Since the ganglia are situated on the wall or near the organ itself, postganglionic fibres are very short. Almost 75% of parasympathetic fibres are found in the 10th cranial (vagus) nerve which run down to the visceral region as right and left branches.

Parasympathetic system orginates from Lateral hypothalamic area (LHA) while sympathetic system from Ventromedial hypothalamus (VMH). Thus through parasympathetic system, the LHA and through sympathetic system, the VMH control the visceral functions including metabolic process (Shimazu, 1979, 1980, 1981, 1983). Gellhorn et al. (1941) further developed the concept of autonomic nervous control of blood glucose level and studied various influence on its two components, the sympatho-adrenal axis and the vago-insulin axis.

Autonomic dysfunction is observed in many diseases affecting human beings. In diabetes mellitus, the incidence of autonomic neuropathy is as high as 20% to 40% of all patients. In most cases, the autonomic neuropathy can be a consequence of diabetic conditions. Shimazu (1966) has investigated the relationship between changes in blood glucose level and the nuclei of the hypothalamus by selective stimulation of each nucleus in rabbits, and has shown that blood glucose increases after stimulation of the VMH (sympathetic area) while it decreases after stimulation of

the LHA (Parasympathetic area). In general, the roles of VMH and LHA in metabolic regulation as in other functions is reciprocal, particularly in the regulation of carbohydrate metabolism in the liver (Shimazu, 1977, Shimazu et al. 1977).

The autonomic centres of the hypothalamus probably receive a constant afferent influx of glucose related signals from the liver and hepatic vein, on the basis of which hypothalamic controlling mechanisms centrifugally influence the hepatic carbohydrate metabolism. The afferent fibres also provide feed-backs for the maintenance of glucose homeostasis. The liver is the central organ for metabolic homeostasis. In maintaining glucose concentration, for instance, the liver plays a dominant role since it can vary the amount of glucose that is being pumped into the general circulation, and hormonal messengers are involved in this mechanism. The liver receives its innervation from the hepatic plexus, which is one of the subsidiary of the coeliac plexus. The hepatic plexus (or nerve) contains among other fibres, the sympathetic and parasympathetic efferent nerve fibres (Lautt, 1980; Mc Cuskay, 1980 ; De Wulf and Carto, 1981). Lautt and Cote (1977) showed that hyperglycaemia following surgical trauma occurs in the adrenalectomized rat in response to reflex activation of hepatic sympathetic nerves.

Since the early discovery of "puncture hyperglycaemia" by Claude Bernard, it has been assumed that the sympathetic nervous system participates in the genesis of hyperglycaemia

by promoting mobilization of hepatic glycogen, and that its effects depends almost exclusively on neuroendocrine mechanisms through the mediation of epinephrine (released from adrenal) and glucagon (from pancreas) (Frohman, 1971). Activation of principal sympathetic nerve innervating the liver, resulted in a marked increase in the activity of glycogenolytic enzymes, G-6-Pase and phosphorylase within 30 seconds after the onset of stimulation (Shimazu and Amakawa, 1968a, b). Sympathetic nervous system can activate hepatic glycogenolysis and rapidly supply the circulating blood with glucose, first directly through the hepatic innervation, second by the release of catecholamines from the adrenal medulla, and third by the release of glucagon from the pancreatic islets. The fact that hepatic glucose output is enhanced by the hepatic sympathetic stimulation has been confirmed by stimulating the splanchnic nerve in a perfused liver preparation isolated from the toad (Niijima and Fukuda, 1973a).

The administration of 6-hydroxydopamine (6-OHDA) selectively promotes acute degeneration of noradrenergic nerve terminals (Di Bona, 1978) and causes almost total depletion of noradrenalin in sympathetically innervated tissues (Soures-de-Silva and Davidson, 1985 ; Sourse-de-Silva and Azeuedo, 1988 ; Soures-de-Silva, 1986 ; 1987a, b). Cote et al. (1975) used intraportal injection of 6-OHDA to cause selective degeneration of the nerve endings within the liver of rat.

Direct stimulation of splanchnic nerves produced a marked increase in blood glucose which was greately reduced by cutting the hepatic nerves at the origin of the hepatic artery in adrenalectomized dogs (Edwards, 1972a) Lautt and Wong (1978) have shown that simultaneous action of sympathetic and parasympathetic fibres in the mixed hepatic nerve resulted in a complete domination by the sympathetic nerves. On the other hand, vagus plays an important role in the regulation of insulin secretion under physiological conditions (Idle, 1959; Frohman et al., 1967; Kaneto et al., 1967; Lee and Miller, 1985). Since acetylcholine (ACh) is released as a neurohumoral transmitter from the nerve ending in the effector cells upon physiological stimulation of the parasympathetic nervous system, the effects of vagus stimulation on insulin secretion would also be mimicked very faithfully by exogenous ACh or the closely related cholinomimetic agents (Kaneto et al., 1968; Ottolenghi et al., 1971). In the rat liver, stimulation of the parasympathetic nerves has direct effect on glucose metabolism synergic with insulin but antagonistic to glucagon (Glinsmann et al., 1969). Pilo and Patel (1978 b) suggested that in birds, the parasympathetic nerves may be the predominant inducer of glucose uptake by liver cells. Avian liver has rich cholinergic innervation and a high cholinergic activity (Pilo, 1969). Thus, parasympathetic system can influence the carbohydrate metabolism through its action on the release of insulin from pancreatic islets as well as through activation of hepatic enzymic machinery concerned

with glucose uptake and glycogen formation. Parasympathetic system can also inhibit the action of glucagon and sympathetic action in the post-prandial condition thereby decreasing glucose output into the circulation.

Apart from liver, vagal parsympathetic fibres also innervate kidney, pancreas, adrenal and thyroid. Kidney is an organ which controls blood volume and plasma components through its excretory and reabsortive capacity. Vagal cholinergic and adrenergic fibres are known to innervate kidney (Titurau, 1967; McKenna and Angelakar, 1968; Moffal, 1975) and control the activity of kidney by counter regulation (Pilo et al. 1987). Kidney is also known to play an important role in general metabolism particularly in glucose homeostasis (Krebs and Yoshida, 1963; Exton, 1971; Shen and Mistry, 1979). It is reported that kidney takes a compensatory role of producing more glucose when liver functions are altered as in diabetes, because it is an important site for gluconeogenesis. The reaction rate of gluconeogenesis in kidney acclerates during times of food deprivation. As kidney is a principal site of glucagon degradation as indicated by the relatively high renal extcretion rate and low urinary clearance of the hormone (Bastle et al., 1971), any renal pathologic condition could increase glucagon retention in blood and thereby producing hyperglycaemia.

The islets of Langerhans are richly vascularized and both the islet cells and blood vessels are intimately associated with the autonomic nerves. Lesions of the ventromedial or stimulation of the lateral hypothalamus within the diencephalon increases plasma insulin concentration; most probably by influencing neural outflow from the vagal nuclei to the pancreatic islets (Helman et al., 1982). Nerve to the pancreas contains preganglionic parasympathetic fibres from the dorsal trunk of the vagus and postganglionic sympathetic fibres from the greater and middle splanchnic nerves which originate in the coeliac and superior mesentric plexus (Bloom et al, 1974; Kaneto et al, 1974). The presence of both cholinergic and adnergic fibres within the islets has been established by fluoresence microscopy and enzymatic histochemistry (Coupland, 1958; Falck and Hellman, 1963; Cergrella, 1968; Terhuizen, 1968). VMH lesion brought about alterations in the central nervous system (CNS) controlled homeostasis that was responsible for the increase in the activity of the efferent vagus nerve that influence the endocrine pancreas (Bray et al, 1981; Rohner-Jeanrenaud et al., 1983). It is well-known that electrical stimulation of vagus nerve of normal rats favours not only insulin but also glucagon secretion; both process are inhibited by the cholinergic antagonist atropine (Miller, 1981; Helan et al., 1982. In view of all these observations, it seems clear that hypothalamus controls both liver glycogen metabolism and glucose homeostasis. The liver glycogen metabolism is

controlled by direct innervation of the liver, via the VMH-splanchnic and LH-vagus nerve pathways (hypothalamo hepatic axis) which directly controls the enzymes metabolizing glycogen in the liver and thus, is responsible for the initial and fine regulation of metabolic changes. Other mechanism is the hormonal regulation of glycogen breakdown and synthesis, which involves neural stimulation of the release of pancreatic hormones (hypothalamo pancreatic axis) and adrenomedullary catecholamine (hypothalamo-adrenal axis), which are responsible for the prolongation or consolidation of metabolic changes rather than their initiation.

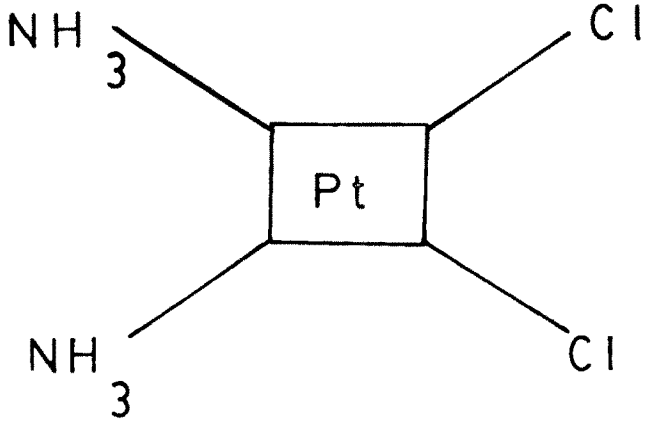
Glucose homeostasis could be severely affected if there are hypothalamic lesions especially in VMH and LHA or if the autonomic nervous system are not functioning properly (neuropathy). There are several useful drugs that cause autonomic neuropathy. Similarly, several divalent metal cations can also influence carbohydrate metabolism by their ability to inhibit insulin production. Of these, Cis-diamminedichloroplatinum-II (cisplatin, CDDP) is currently being used in the treatment of ovarian and testicular cancers (Rose, 1984). The drug is, however having certain toxic side effects in the kidney (proteinuria, morphological damage), intestine (diarrhoea and anorexia) and lymphatic system (splenic atrophy) (Aggarwal et al., 1980a,b; Schurig et al., 1980).

The drug cisplatin is most effective when administered either as intraperitoneal (ip.) or intravenous (iv.) injections. Subcutaneous and intramuscular injections were less effective and oral administration was not effective at all. The therapeutic dose is 7mg/kg which is less than LD_{10}. Studies by Woodman and Coworkers (1974) showed that cisplatin is able to act either additively or synergistically with a number of other anticancer drugs, yielding a substancial improvement in the treatment of animal tumors. Cisplatin was also effective in a variety of animal hosts such as mice, rats and chickens.

In the mouse, the first study on toxicities was done by Toth-Allen (1970) and in rat by Kociba and Sleight (1971). After administration of CDDP, Platinum is found to be distributed almost universally throughout the body with highest concentration found in kidney, liver, lung and ovary (Litterest et al.,1976; Pretorius et al., 1981). The dose - limiting side effect is due to the damage in kidney functions particularly in the proximal convoluted tubules. This causes a decrease in the filtering capacity of the kidney with a consequent elevation of the blood urea nitrogen and a decrease in the creatine clearance. Platinum drug produced mild damage to the blood forming cells too (Zak et al., 1972). One unusual toxicity of cisplatin that was first described in humans and then later detected in animals as well, is the destruction of haircells of the organ of Corti, leading to a loss in high frequency hearing and in some cases total deafness (Fleishman et al., 1975). In recent years,

CISPLATIN

STRUCTURE



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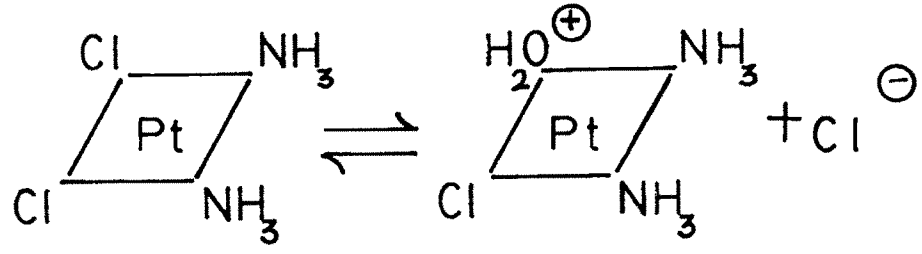
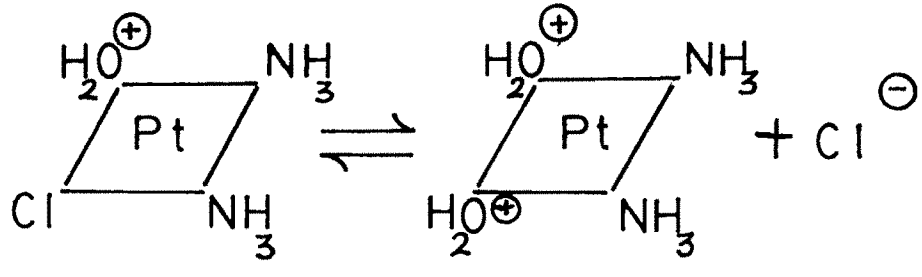
1. 

2. 

EFFECT OF VAGOTOMY AND CISPLATIN TREATMENT IN ALBINO RATS

- IMPAIRED GLUCOSE TOLERANCE
- PERIPHERAL NEUROPATHY
- NAUSEA
- BLOATED STOMACH
- GASTROINTESTINAL MOBILITY (DECREASED)
- INHIBITION OF DNA SYNTHESIS
- PROTEINURIA
- DECREASED GLOMERULAR FILTRATION RATE
- INCREASED BLOOD UREA NITROGEN
- CHRONIC TISSUE LESIONS

peripheral neuropathy was discovered to be a significant toxicity when drug is given at higher dose levels (Kedar et al., 1978; Hoop et al., 1988; Mollman et al., 1988). Perpheral nerves from cisplatin treated patients showed axonal degeneration and secondary myelin breakdown (Thompson et al., 1984; Daugaarel et al., 1987; Koning et al., 1987). Richardoson and Cantwell (1990) recently showed some evidence of autonomic neuropathy in patients who received cisplatin.

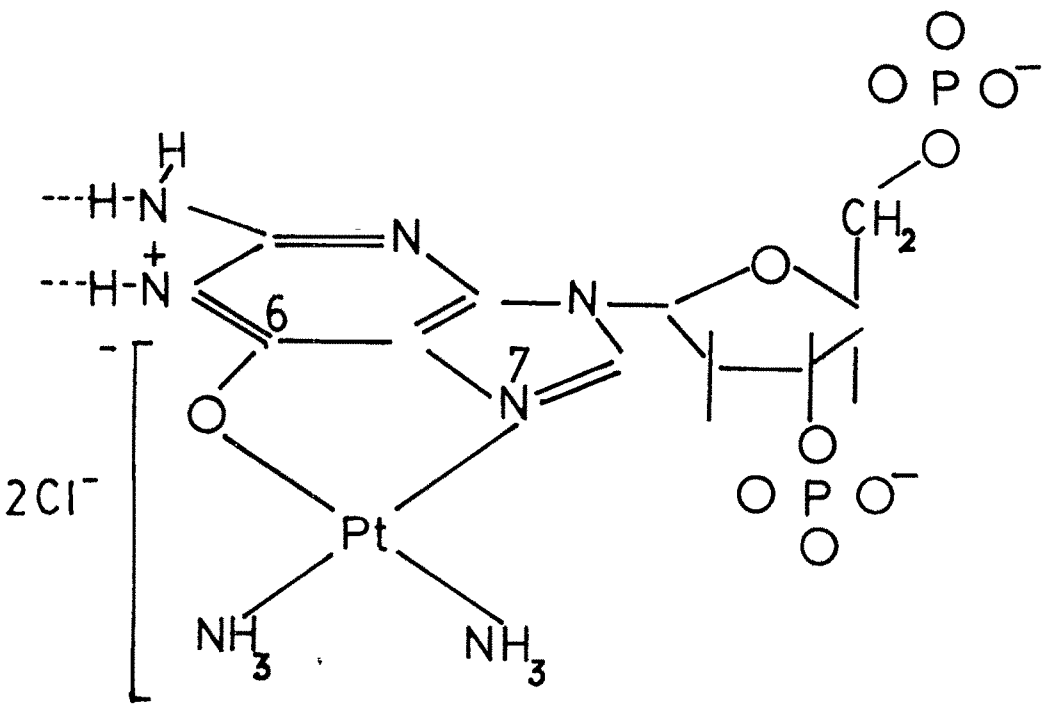
In the present study, a comparison was made between the effects of vagotomy and cisplatin treatment on the liver and kidney of rat and pigeon. The studies of the vagal ablation and cisplatin treatment have shown some similarities with respect to glucose homeostasis. Transection of vagal fibres can cause hyperglycaemia. Szabo and Szabo (1972, 1975) have claimed the existence of an insulin - sensitive central nervous system receptor which, when stimulated, suppressed the hepatic glucose output through a cholinergic and presumably vagal mediated mechanism. Diabetic autonomic neuropathy is a pathological state of variable severity and extent which can vary from asymptomatic anomalies of a parasympathetic test to a severe symptoms with abnormal sympathetic and parasympathetic tests (Clarke et al, 1979). Manifestation of diabetic neuropathy can occur in almost every system of the body and this complication of diabetes can mimic a large catogory of neurological diseases. In the present study the parasympathetic dysfunction was perceived from the decreased activity of acetylcholinesterase in liver

and kidney of vagotomized and cisplatin treated animals (Chapter II).

A number of studies have shown that the cholinesterase activity varies during cell growth and maturation. AChE is also elevated in various primary tumours and in the sera of patients with certain carcinomas (Small, 1990). Acetyecholine may also be important for the development of neurons and in neurite outgrowth in the central and peripheral nervous system. Cisplatin, in less than frankly toxic doses, has profound effects on cells grown in tissue culture. Harder and Rosenberg (1970) and simultaneously, Howle and Gule (1970) reported a selective and persistent inhibition of new DNA synthesis without concomitant inhibition of transcription and translation.

However, recent studies have suggested that autonomic innervation of the liver does influence DNA synthesis during liver regeneration after partial hepatectomy (Kato and Shimazu, 1982). Similarly electrical stimulation of paraventricular hypothalamic nuleus leads to an increase in the synthesis of nuclear RNA in liver cells of intact rats and this effect is abolished by hepatic denervation (Shimazu, 1983). The biologic action of Pt(II) complexes are due to displacement reaction, which causes the platinum to become stably bound to DNA, RNA, protein or other critical biomolecules (Pinto and Lippard, 1985). Many studies have attempted to relate cisplatin induced DNA lesions to cytotoxicity. As cisplatin can cause parasympathetic

PROPOSED METHOD OF CDDP
BINDING TO PURINE



dysfunction, it was thought worthwhile to investigate vagal parasympathetic influence on nucleic acid contents in liver and kidney of rat and pigeon (Chapter III).

Both vagotomy and CDDP treatment reduced the influence of parasympathetic system on liver and other tisues such as kidney (Chapter II). Parasympathetic system becomes active when a glucose challenge is made. In the absence of PNS-influence glucose uptake will be affected maximum. To evaluate the extend of adverse effect of vagotomy and CDDP on glucose uptake mechanism, the experimental animals (rats and pigeons) were subjected to glucose tolerance (GT) Test (Chapter IV). A persistant hyperglycaemia was noticed in both Vagotomized and CDDP treated rats and pigeons after glucose administration. This indicates that either uptake mechanism is failed to get activated or is very effectively inhibited. Hyperglucagonemia found in rats after CDDP treatment (Goldstein et al., 1983) may explain the failure of liver to absorb glucose to produce normoglycaemia.

Vagotomy produced several digestive, metabolic and physiologic problems in the experimental animals. The digestive problem included the lack of food movement from stomach, stomach distension and fluid retention. Many of this problems that observed in vagotomized animals, were also manifested in cisplatin treated animals. Hence, a comparison of vagotomy and cisplatin toxicity was found necessary to establish whether cisplatin toxicity was due to ANS

neuropathy or dysfunction. Both experimental groups of rats and pigeons showed distended stomach indicating the manifestation of effects of vagal ablation and cisplatin toxicity. This dysfunction can be suppressed by loading the animals with calcium chloride intraperitoneally during CDDP treatment and after vagotomy (Chapter V). Cisplatin treatment alters calcium homestasis in gastric smooth muscles (San Antonio et al.,1984). Onoda et al. (1986) reported that calcium channel blocker nifedipine can enhance the cytotoxicity of cisplatin. Vagotomy also produced many abnormalities associated with calcium metabolism (Pilo et al., 1982; Verma et al., 1982). A detailed study was carried out (Chapter V) after an exogenous supply of calcium chloride for reducing the cellular toxicity of CDDP and dysfunction caused by vagotomy in the rat and pigeon. The changes in intracellular Ca^{2+} can affect glycogenolysis in hepatocytes and such changes can contribute to the glycogenolytic action of the - adrenergic agonist phenylephrine, but not that of glucagon (Jeannet et al., 1977). Glucagon promotes cAMP generation and some of its action are mediated by that nucleotide, whereas direct glucose stimulation of the B cells requires mediation of extracellular Ca^{2+} which cAMP is effective only in its absence. Martin (1985) reported that high concentration of Ca^{2+} can directly promote insulin secretion.

The second generation analogue caboplatin (cis-diammine-1-cyclobutanedicarboxylateplatinum II, CBDCA) is reported to be

45 times less effective in cell-killing compared to cisplatin (Hussain et al., 1988). Ozols et al. (1985) reported that there seems to be a substantial clinical cross resistance between cisplatin and carboplatin and that there was no clinically apparent nephrotoxicity or neurotoxicity from carboplatin. Since the degree of toxicity is low in carboplatin treated patients, it was thought worth while to study whether this drug has any toxic effect on parasympathetic nerve fibres. The action of CBDCA in rats and pigeons was studied in order to understand how far vagal fibres are affected by the toxic action of carboplatin and also to appreciate whether carboplatin can be used as an alternative to cisplatin (Chapter VI).

Parasympathetic system is known to bring about reduction in glucose level in blood, through its action on glucose uptake by the liver as well as on the release of insulin from pancreas. Sympathetic system has opposing or counter regulating actions. Liver, pancreas and kidney are subjected to direct sympathetic actions just as parasympathetic nerves. Hence, an attempt was made to investigate the influence of sympathetic nerve on liver and kidney of rat and pigeon (Chapter VII) through chemical sympathectomy using 6-Hydroxydopamine (6-OHDA) which causes destruction of terminal endings of sympathetic neurons (Porter et al., 1963). The neurotoxic effects of 6-OHDA depend on its uptake by adrenergic nerve endings (Sachs and Johnson, 1975). Reserpine, a drug that causes striking depletion of

catecholamine from the nerve endings was also administered along with 6-OHDA to eliminate residual adrenergic actions. The lack of catecholamine response to insulin hypoglycaemia found in the patients with sympathetic neuropathy is well-known and has been reported in patients with idiopathic or diabetic orthostatic hypotension (Polinsky et al., 1980; Hoeldtke et al., 1982). Previous studies indicated that ACh and catecholamines have reciprocal effects on the kidney metabolism and thereby affects the homeostasis of blood sugar (Mehta and Pilo, 1987). In vivo studies in the duck by Gross and Mialhe, (1982a, b) showed that pancreatic A and B cells are sensitive to a moderate rise in plasma glucose concentration and that insulin secretion could be inhibited by adrenergic actions. Other studies have shown that there is a sympathetic denervation in diabetes (Ewing et al., 1973; Duchen et al., 1980; Hilsted, 1980) and it leads to autonomic neuropathy (Nawar et al., 1975). Some of the actions exhibited by 6-OHDA treated rats and pigeons are perhaps due to increased vagal tone. The increased RNA content of liver and kidney of both rat and pigeons can be correlated with increased trophic actions by cholinergic nerves as well as by insulin.

A separate section is given, at the end, as general consideration in which the major inferences of the present investigation have been highlighted and compared with various lines of information from the relevant studies.

CHAPTER - I

MATERIALS AND METHODS

SECTION - I

ANIMALS

The rats (Rattus norvegicus albinus) used in this study were male Charles - Foster strain, aged 6 months with average body weight of 200-300 gms. They were housed in cages with a 12L:12D light cycle. The animals had access to food and water ad libitum. The domestic pigeons (Columba livia) weighing 200-300 gms were procured from a local animal dealer and acclimatized to the laboratory condition with 12L:12D light cycle and food and water ad libitum. The birds were acclimatized for a minimum period of 15 days prior to experiment.

SECTION II

I Surgical Procedures for Vagotomy

(a) Subdiaphragmatic Vagotomy in Rat :

In rats, subdiaphragmatic vagotomy and sham operation were performed under ether anaesthesia. The fur over the abdomen was shaved off and an incision (6 cm) was made starting just below the xiphisternum. The liver and intestine were gently displaced to provide access to the lower oesophagus and the dorsal and ventral abdominal vagal trunks were mobilised over a distance of approximately 1 cm and ligated and a segment of nerve at least 5 mm long was removed between the ligatures. If a hepatic branch was observed leaving the ventral vagal

trunk, the trunk was ligated and sectioned above this division. In all the above animals, the gastroesophageal junction region was stripped of any strands of tissue that might have contained vagal fibres.

In sham operated rats, the liver and gastrointestinal tract were manipulated but the vagi were not sectioned. In both groups, the incision was closed by suturing and all animals recovered consciousness within 20 minutes after surgery. No deaths were resulted from vagotomy and all rats were seen to be eating very small amount of food within 2-3 hrs after recovery from anaesthesia. The controls were subjected to pair feeding till 60 hrs. Both experimentals and controls were sacrificed at 72 hrs after a 12 hr overnight starvation.

(b) Bilateral Cervical Vagotomy in Pigeons :

The pigeons were anaesthetized with ether and a 15 mm incision was made on the dorsal side of the cervical region. The vagal trunk of either side were separated from the surrounding tissues and jugular vein. Thereafter, approximately 5 mm of vagal trunk was removed. The incision was closed by suturing and the birds were allowed to recover from anaesthesia. Sham vagotomy involved similar procedure except that the vagal trunks were lifted and left back intact. Necessary post operative care was taken for all the pigeons. The vagotomized and sham operated pigeons were maintained in cages without food for 48 hours.

II Cisplatin and Carboplatin Treatments

Cisplatin Cis-dichlorodiammine platinum (II) (CDDP), was obtained from Sigma Chemical Co., St. Louis, USA. Carboplatin (Cis-diamine-1, 1-cyclobutanedicarboxylate platinum (II) (CBDCA), was kindly gifted by Prof. S.K. Aggarwal, Michigan State University, USA.

(i) Cisplatin (CDDP) Administration in Rats and Pigeons :

Cisplatin was administered in rats at a clinical dosage of 7 mg/kg body weight in 0.9% saline. In pigeons the dosage was 5 mg/kg body weight in 0.85% physiological saline. The drug was injected at a single dose intraperitoneally in both rats and pigeons. Control animals received an equivalent volume of saline. The pigeons were deprived of food until they were sacrificed. The drug treated rats and controls were subjected to pair feeding till 60 hrs and kept without food overnight (12 hrs) till 72 hrs. Cisplatin treated rats and pigeons were sacrificed by decapitation under mild anaesthesia after 72 hrs of drug treatment. Liver and kidney were quickly extirpated from the sacrificed animals and used for various assays.

(ii) Carboplatin (CBDCA) Administration in Rats and Pigeons:

Carboplatin was injected intraperitoneally in both rats and pigeons at a single dosage of 50 mg/kg body weight. The drug was dissolved in 5% sucrose as vehicle. Both the control groups received equivalent amount of vehicle only.

III Chemical Sympathectomy

6-Hydroxydopamine hydrobromide (6-OHDA.HBr) used for chemical sympathectomy, and reserpine were purchased from Sigma Chemical Co., St. Louis, USA.

(i) 6-OHDA Administration in Rats :

In rats, 6-OHDA was injected intraperitoneally at a total dosage of 30 mg/kg body weight in two equal doses of 24 hrs apart. The drug was prepared immediately before injection by dissolving in 0.9% saline with 1% ascorbic acid as stabilizer. Control rats received equal volume of saline containing ascorbic acid. The animals were deprived of food but had access to water.

Resperine was administered at 36th hour at a dose of 5 mg/kg body weight. Resperine was prepared by dissolving the drug in 2-3 drops of Tween-80 and then made upto the volume with 0.9% saline. Controls received vehicle only. The control and sympathectomized rats were sacrificed by exsanguination at 48 hrs under ether anaesthesia. Liver and kidney were quickly excised for various assays.

(ii) 6-OHDA Administration in Pigeons :

Pigeons were chemically sympathectomized with 6-OHDA at a dosage of 40 mg/kg body weight. The drug was injected intraperitoneally in two equal doses 24 hrs apart. Saline (0.85%) containing 1% ascorbic acid served as vehicle. Control groups received only the vehicle. At 36th hour post

sympathectomy the pigeons were given resperine treatment. Resperine was injected intraperitoneally at a dosage of 1.2 mg/kg body weight. The drug was dissolved in 2-3 drops of Tween -80 and made up to the volume with 0.85% saline. Control groups received an equal volume of vehicle. Both control and experimental groups were deprived of food but given access to water only. At 48 hrs, control and sympathectomized groups were sacrificed by decapitation under mild ether anaesthesia.

SECTION III

(i) Histofluorescence Studies of Catecholamines

The extent of sympathectomy in rats and pigeons were assessed through the histofluorescence localization of catecholamines in cornea.

The corneas from rats and pigeons were dissected out and immersed in ice-cold 0.1 M phosphate buffer (pH-7.3) containing 2% glyoxalic acid. After 5 min of immersion the cornea was taken, blotted dry, stretched on a glass slide and dried under warm air. The completely dried tissues were exposed to glyoxalic acid vapours at 100^{o}C for 5 min and mounted in Entellen (Terro, 1977). The slides were observed under Carl-Zeiss fluorescence microscope with epi-illumination. The filter settings were B 224 (440 nm) excitation filter and G 247 (510 nm) barrier filter.

(ii) Estimation of Blood Glucose

Blood was obtained from different experimental and control groups of rats and pigeons by cardiac puncture. The blood was collected in test tubes containing EDTA and analysed for glucose by the micromethod of Folin and Malmaros (1929) and the concentration is expressed in mg glucose/100 ml of blood.

(iii) Glucose Tolerance Test (GTT)

(a) Rats :

After different drug treatment and surgery, the glucose tolerance was evaluated by GTT. Following 4-5 hrs of fast, blood was drawn from orbital sinus puncture by using a heparinized capillary tube. Immediately after the first sampling the animals were subjected to an intraperitoneal glucose load (2 gm/kg body weight). Blood samples were then collected at intervals of 30, 60, 90, 120 and 150 min. The blood glucose was estimated by the method of Folin and Malmaros (1929).

(b) Pigeons :

A similar procedure, as in the case of rats, was followed. Pigeons were subjected to an intraperitoneal glucose load of 70 mg/100 gm body weight. The blood was drawn from brachial veins.

IV Biochemical Assay of Acetylcholinesterase (AChE)

AChE activity in tissues was assayed by the method of Ellman et al. (1961).

The tissue was homogenized in 0.02 M potassium phosphate buffer pH - 7.0 and to this is added Triton X 100 at the level of 1%. Tissue homogenates were centrifuged at 14000 rpm for 30 min at 4°C. The resultant supernatent was collected and assayed for AChE.

The assay system consist of :

0.1 M Potassium phosphate buffer (pH - 8.0)	2.7 ml
0.1 M DTNB (5' 5' Dithiobis 2, Nitrobenzoic acid)	0.1 ml
Enzyme I - 10 dilution	0.2 ml
Acetylthiocholine Iodide (14.4 mg/ml)	0.02 ml
Total volume	3.02 ml

The intensity of the colour developed was measured at 412 nm in a spectrophotometer at intervals of 1 min.

The enzyme activity is expressed as μ moles of substrate hydrolysed /mg protein /min.

V Estimation of Nucleic Acids

Nucleic acid contents in the tissues were estimated according to the method of Schneider (1957). The method is based on

the extraction of nucleic acid by cold acid and further hydrolysis in hot acid. In acid solution, the straight chain form of a deoxypentose is converted to the highly reactive B-hydroxylevulinaldehyde which reacts with diphenylamine to give a blue complex.

10 % of tissue was homogenized in chilled distilled water using cold mortar and pestle. From this homogenate, 1 ml of the sample was pipetted out and to this added 2.5 ml of cold 10% trichloroacetic acid (TCA) and centrifuged for 15 min at 3000 rpm. The supernatent containing acid soluble fractions were discarded, and pellet was suspended again in 2.5 ml of 10% TCA. After centrifugation, the supernatent was discarded. The resultant pellet was suspended in 95% ethyl alcohol and centrifuged at 3000 rpm for 15 min (2 times) to remove the phospholipid fraction. The pellet was then suspended in 5% TCA and hydrolysed at 90^{o}C for 30 min. in a water bath. After hydrolysis, the tubes were cooled and centrifuged at 3000 rpm for 15 min and the supernatent was collected. This final superatent was used for the estimation of nucleic acid.

Estimation of DNA :

1% of diphenylamine, prepared in glacial acetic acid and to this was added 2.5 ml of conc. H_2SO_4. To 1 ml of each sample, 2 ml of DPA solution was added and boiled in a waterbath for 10 min. The intensity of the colour developed was read at 600 nm in a spectrophotometer against a DPA blank.

Preparation of a Standard Graph of DNA Solution :

A standard graph is prepared by using Calf-thymus DNA (Sigma Chemical Co. USA.). Calf-thymus DNA is dissolved in 0.03 N KOH at a concentration of 1 mg/ml and dissolved by warming. From this stock solution, assays were performed on 0, 10, 20, 40, 60, 80, 100 pg of DNA. The DNA content was estimated using DPA reaction. The optical density was plotted against DNA concentration.

Estimation of RNA :

The orcinol reaction of Ceriotti (1939) was employed for the determination of RNA content. 1.5 gm of orcinol was dissolved in 100 ml of conc. HCl and to this was added 0.75 gm of anhydrous $FeCl_3$. This solution was prepared immediately before use. To 1 ml of aliquot a 1.5 ml reagent was added and heated in a boiling water bath for 20 min. The extinction was read at 660 nm in a spectrophotometer.

Preparation of Standard Graph :

A standard graph was prepared by using Calf-liver RNA (Sigma Chemical Co. USA). RNA was dissolved in water at a conc. of 1 mg/ml. From this, aliquote were taken as 0, 10, 20, 40, 60, 80 and 100 µg RNA and assayed in presence of orcinol reagent. The RNA concentration was determined by plotting optical density against concentration.

VI Estimation of Protein

Protein content in tissues was estimated by the method of Lowry *et al*. (1925) using Bovin Serum Albumin (BSA) as standard, and expressed as gm percentage of wet tissue.

Statistical Analysis

Student's 't' test was used to calculate the statistical significance of difference between control and experimental values (Fisher, 1950). The standard error of mean ($\pm$ SEM) was used for the calculation of significance with 95% confidence level and expressed as P values.

ABBREVIATIONS USED IN TEXT

ACh	-	Acetylcholine
AChE	-	Acetylcholinesterase
cAMP	-	Adenosine 3',5' cyclic monophosphate
CBDCA	-	Cis - diammine -1-1- cyclobutane di carboxylate Pt-II
CDDP	-	Cis diammine dichloroplatinum (II)
Ca^{2+}	-	Calcium ions
$CaCl_2$	-	Calcium chloride
CNS	-	Central nervous system
DNA	-	Deoxy ribonucleic acid.
DTNB	-	5'5' dithiobis 2' nitrobenzoic acid
DA	-	Dopamine
E	-	Epinephrine
GH	-	Growth hormone
GTT	-	Glucose tolerance test
ip.	-	Intraperitoneal
ICM	-	Intracellular mediators
LH	-	Lateral hypothalamus
NE	-	Norepinephrine
NH_4Cl_2	-	Ammonium chloride
6-OHDA	-	6-Hydroxy dopamine
PNS	-	Parasympathetic nervous system
PTH	-	Parathyroid hormone
RNA	-	Ribnucleic acid.
SNS	-	Sympathetic nervous system
TCA	-	Trichloroacetic acid
VMH	-	Ventromedial hypothalamus
VgX	-	Vagotomy
VgS	-	Sham vagotomy

UNIT OF ACTIVITIES OF ENZYMES

AND VALUES OF METABOLITES.

PARAMETERS	UNITS
Protein	gm % of wet tissue
Acetyl Cholinesterase	μ moles of ACh hydrolysed/mg protein/minute
Glucose	mg/100 ml blood.
Deoxyribonucleic acid	mg/ 100 mg tissue
Ribonucleic acid	mg/100 mg tissue.