SUMMARY

CHAPTER - 1

Various methods used for biochemical assays, drug dosages, experimental designs and methods for surgery are described in this chapter.

CHAPTER - 2

The effect of vagotomy on the carbohydrate metabolism in the kidney of rat was compared with that of Cisplatin treated rat. It is reported that CDDP, an antineoplastic drug produces side effects such as autonomic neuropathy and hyperglycemia. The effort of the study was to find similarity between vagotomy and CDDP treatment on the metabolic activities. Both VgX and CDDP treatment increased glycolysis. Inhibition of glycogen-synthetase activity was observed only in CDDP treated rat kidney.

It was reported earlier that $CaCl_2$ administration could protect the tissue from toxic effects of Cisplatin. In the present study $CaCl_2$ also prevented the kidney from such toxic conditions. This was more apparent with GS, G-6-Pase, SDH. In VgX rats also $CaCl_2$ administration reversed the effects on G-6-Pase & LDH. Calcium could thus somewhat prevent the side effects of not only CDDP but also could attenuate the effects of vagal denervation.

CHAPTER - 3

An intact parasympathetic functioning is essential for maintaining enzyme mediated transport activities across cell membranes. The functioning of

transport enzymes such as Na^+-K^+-ATPase, Alkaline Pase, Acid Pase were assayed in VgX as well as CDDP treated rats. The hyperglycemic response to these experimental procedures were also studied by observing the levels of transaminases such as GPT and GOT which are involved in interconversion of amino acids to glucose.

In both VgX and CDDP treated rats there were similarities in the pattern of response of the above mentioned enzymes. Transaminases showed a varied response in both cases. The data indicate that in the kidney certain amount of amino acids is being used up for gluconeogenesis while the uptake of glucose became reduced after the vagal denervation and Cisplatin treatment.

Na^+-K^+-ATPases showed a decrease in VgX as well as CDDP treated rat kidney indicating that permeability may be affected for the dysfunction of the parasympathetic system. The non-specific phosphatases also showed similarities in their response. Alkaline Pase showed an increase while Acid Pase activity showed no response.

Administration of $CaCl_2$ brought about reversal of response in enzymes of VgX and CDDP treatment. A slight improvement was observed in case of Na^+-K^+-ATPase and non-specific phosphatases, thereby suggesting an improved transport functioning. The transamination on the whole showed a slight change in response.

CHAPTER - 4

Hyperglycemia was observed as a common metabolic derrangement in various physiological circumstances. The administration of CDDP was causing neuropathies involving autonomic dysfunction. Chronic deleterious effects of the drug became a limiting factor in administering it against cancer. Hence, an equally potent but less toxic drug carboplatin is being used now a days. This drug has the same capacity in curing cancer but cause less neuropathological disorders. When dosage of CDDP was 5 mg/kg body wt., 50 mg/kg body wt. of carboplatin was safely administered.

All the enzyme parameters showed totally opposite response to that of CDDP treatment and vagal inhibition. The observed hyperglycaemia might have been due to increased glycogenolysis and glycolysis, although gluconeogenesis was inhibited. Though hyperglycemia prevented, it was not as much observed as in that of Cisplatin treated rats. The transamination and general transport enzyme activities remained unchanged.

It could be concluded that since the drug was causing less derangements in metabolism, the functional cholinergic pathway might not have been adversely effected.

CHAPTER - 5

Glucose homeostasis is brought mainly by Autonomic Nervous System. The involvement of the system in releasing their secretions from pancreas,

adrenal, liver, kidney etc. becomes utmost important in regulating the blood glucose release. Glucose release and glucose uptake are two opposing mechanisms controlled by separate neural and endocrine systems. In birds glucose release (from kidney and liver) is mediated by sympathetic system which involves the glycogenolytic hormone - glucagon. On the other hand parasympathetic system induces uptake of glucose via ACh or poorly by insulin too. The involvement of neural and endocrine regulation differs a lot in the avian and mammalian systems.

Glucagon helps in glucose release - which is higher in birds than that of mammals. The normoglycemic level in mammals are at 120 mg/100 ml where in birds the level is at 220-240 mg/100 ml. The blood glucose levels suggests that birds tolerate hyperglycamia.

The present study showed importance of vagal involvement in blood sugar regulation in birds. Denervation of the vagi is known to cause hyperglycemia in pigeons. The denervation also caused a lot of neuropathological changes which adversely affected the glucose homeostasis. Peripheral neuropathy and hyperglycemia was observed in patients receiving Cisplatin against cancer. The drug caused acute renal toxicity leading to proteinuria, hypocalcaemia etc. The investigations unearthed the similarities and dissimilarities in the pathway involved in maintaining blood sugar level in vagotomized and CDDP treated pigeons. Hyperglycemia observed was due to glycogenolysis. Decreased glycogen content and glycogen synthetase, increased G-6-Pase and phosphorylase

activities were observed. Along with glycogenolysis active glycolysis was observed where LDH levels were enhanced. Glycolysis contributes substrates for gluconeogenesis, while pyruvate carboxylase caused an increase thereby helping in glucose release. The pathway by which glucose release effected remained similar in VgX birds and CDDP treated birds.

$CaCl_2$ administration brought a slight change in the hyperglycamic levels. Glycogenolysis was reduced but glycolysis was not much affected in CDDP treatment. In VgX pigeons though there was little change in glycogen deposition, the glycogenolytic enzymes were seen to have a reduced functioning, thereby reducing glucose release. Gluconeogenesis showed reduction but the glycolytic pathway remained unaltered.

CHAPTER - 6

Birds quickly respond to hypoglycemia but can tolerate very high glucose levels than mammals. In birds, kidney becomes a dominant gluconeogenic tissue. Gluconeogenesis generates glucose in most adverse conditions. Vagal and sympathetic actions have been known to cause changes in the rate of renal gluconeogenesis. Involvement of transport enzymes and transamination activities were observed in this study to evaluate the uptake characteristics after vagotomy and Cisplatin treatment.

The transaminase activities were enhanced in VgX kidney thereby suggesting the possibility of active gluconeogenesis. The oxalacetic transamination was more prominent than the pyruvate conversions. In CDDP

treatment both transaminases were enhanced. The analysis of non-specific phosphatases revealed a reduction in their activities. Na^+-K^+-ATPase being vital in permeability changes remained low, the Alkaline and Acid Pase too showed lower levels, suggesting that uptake metabolism was adversely effected in case of both VgX and CDDP treatment.

$CaCl_2$ administration in general reduced the effect of both VgX and CDDP toxicity on the renal machinery concerned with gluconeogenesis as well as transport of metabolites. In VgX pigeons $CaCl_2$ prevented enhancement of GPT levels, while GOT enhancement was curtailed after CDDP treatment. All the three phosphatases were found to remain unchanged over after $CaCl_2$ treatment.

It was concluded that, though transport enzyme activities and transamination showed similarities in their levels, their actions or pathway involved was slightly changed in VgX and CDDP treated pigeons.

CHAPTER - 7

This chapter deals with effects of Carboplatin treatment on pigeon kidney. Since Cisplatin treatment (Chapters 5,6) was found to cause deleterious effects, Carboplatin, a less toxic agent, was used. This anti-neoplastic agent is known to have little side effects.

The study dealt with effects of Carboplatin on CHO metabolism in pigeon kidney. There was an increase in glycogen, glycogen synthetase, phosphorylase, aldolase etc., while decrease in the activities were

observed in the case of LDH, SDH and PC. Transaminases did not show any alterations in their levels and transport enzymes showed varied response. Above all hyperglycemia was observed, but the glycemic index remained lower than that found in CDDP treatment. From the above inferences, one could summerize that partially glycogenolysis and glycolysis was increased whereas gluconeogenesis remained suppressed. The observed increase in blood glucose might have therefore been contributed by glycogenolysis.

ROLE OF AUTONOMIC NERVOUS SYSTEM ON RENAL CARBOHYDRATE METABOLISM AND GLUCOSE HOMEOSTASIS

A GENERAL CONSIDERATION

The autonomic nervous system (ANS) has both afferent and efferent nerve fibres innervating most of visceral organs. The major functions of ANS are to regulate heart beat, respiratory rate, smooth muscle contraction, glandular secretion, all of which ultimately help to maintain homeostasis. The two subdivisions of ANS, the parasympathetic and sympathetic systems are toxically active in all visceral organs and exert opposite effects in them. The coefficient ratio of these two opposing influences determines the dominating or controlling effects in the effectors. The two systems work autonomously by receiving impulses from the autonomous centre in the brain - the hypothalamus.

Hypothalamus, by regulating the sympathetic and parasympathetic nervous activities, preserves a constant internal environment as well as prepares the body to meet challenges during emergency. Hypothalamus performs this function by modulating hormonal secretion through hypothalamic releasing factors and by sending neural signals to the endocrine and other organs. Hormones and nerves can in their turn regulate the metabolic activities in the effector organs.

The hypothalamus contains several nuclei, but the two most active centres that are concerned with metabolism are ventromedial hypothalamic nucleus (VMH) and lateral hypothalamic area (LHA). These two centres act reciprocally in several regulatory functions (Table 1).

Table 1 : Reciprocal functions of the VMH and LHA :

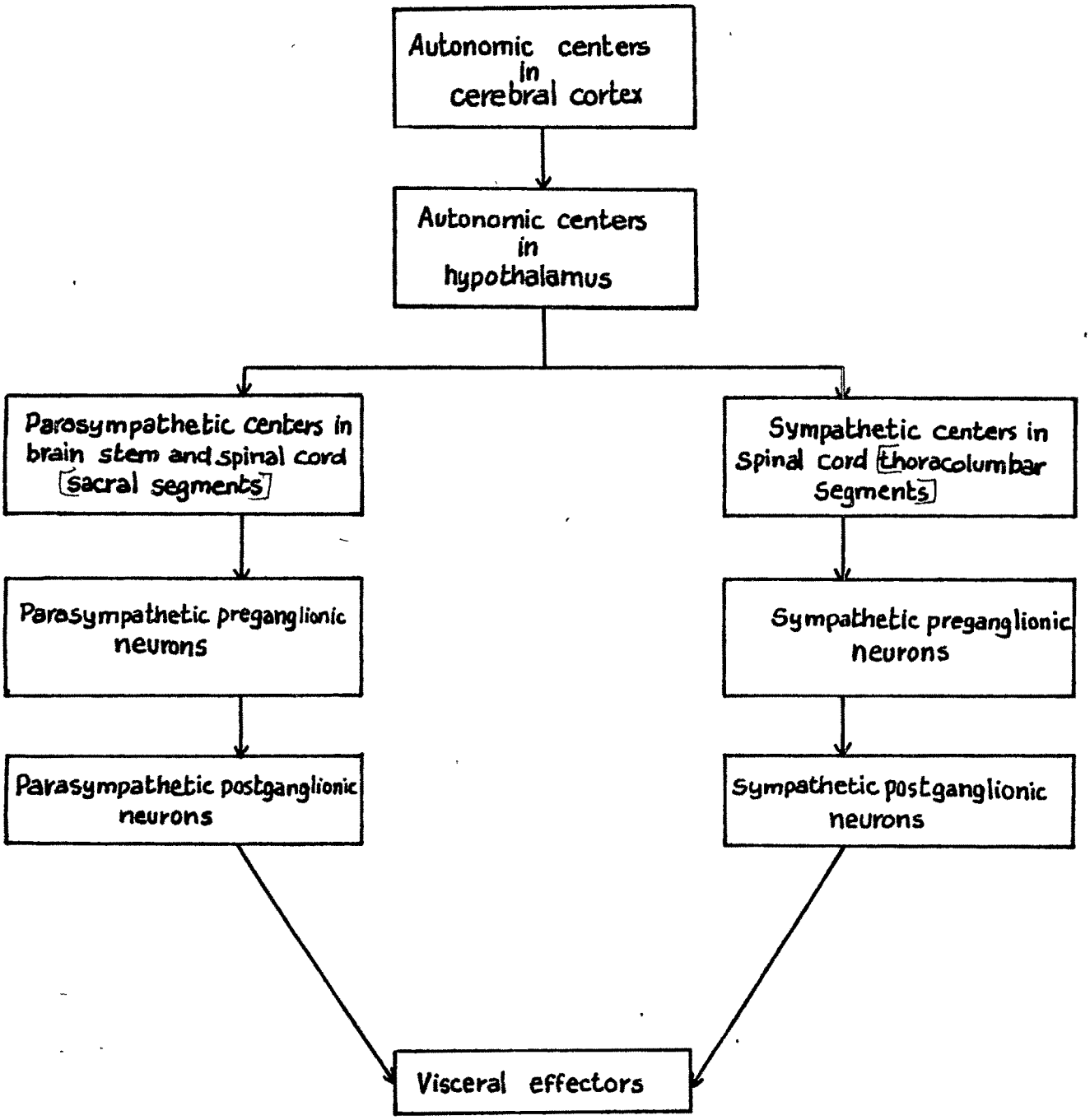
Function	VMH	LHA
Food intake control	Satiety	Feeding
Autonomic nervous system	Sympathetic	Parasympathetic
Metabolic regulation: liver	Glycogenolysis	Glycogenesis
	Gluconeogenesis	Inhibition of gluconeogenesis
	Hyperglycaemia	Hypoglycaemia
Pancreas	Glucagon release	Insulin release
Adipose tissue	Lipolysis	Lipogenesis

Adapted from Shimazu (1983).

The feelings of satiety and hunger are are based on glucose level in the blood sensed by glucose sensitive neurons in the hypothalamus. Similarly the glucose level is also monitored to send appropriate signals to metabolically active tissues and endocrine glands in a bid to maintain constancy. Decrease in the blood sugar level will elicit sympathetic stimulation and glucagon release while an increase in glycaemic level will stimulate the parasympathetic activation and insulin release.

Although regulation of glucose level in the blood is done through several mechanisms, the neural elements and hormones impart a finer and time

CENTRAL NERVOUS SYSTEM HEIRARCHY THAT REGULATES AUTONOMIC FUNCTIONS



related control. Parasympathetic system and/or/through insulin activates the metabolic pathway that converts excess glucose into storage products (glycogen, fat, protein). In other words PNS activates anabolic or synthetic pathway. SNS on the other hand is involved in the 'emergency response'. Under stressed conditions (physical or emotional) sympathetic impulses are produced resulting in maximum release of glucose into the blood stream which could be used during fight or flight reaction (Cannon, 1968). Release of glucose is partially through the direct action of sympathetic nerves innervating the liver (adrenergic action) while the rest is through the actions of catecholamines (from adrenal medulla) and glucagon (from pancreas).

Thus, the actions of autonomic nerve fibres are through two ways : (1) a short term but quick direct action on the metabolic activities of effector organs such as liver and (2) by long term but slow action through the release of hormones. PNS, on one hand activates glucose uptake by activating glucokinase and glycogen synthetase thereby increasing glycogenesis. SNS on the other hand activates phosphorylase and thereby increasing glycogenolysis.

The glucose homeostasis thus require the concerted actions of :

1) hypothalamus
2) autonomic nervous system
3) A and B cells in the endocrine pancreas
4) liver and other metabolically active tissues.

The neural and hormonal factors exert the control over their target organs such as liver and other organs that figure in the maintenance of blood sugar level. Liver is the principal organ that is involved in the homeostasis of glucose. Involvement of kidney as a major gluconeogenic organ was discovered by Krebs (1964). He reported that though liver has gluconeogenic organ, kidney surpasses liver under most adverse situations. In fact kidney produces 20% more glucose then liver during stressful conditions. The ability of kidney to utilize lactate, pyruvate and alanine to produce glucose through gluconeogenic pathway, makes the organ equally important as liver in the glucose homeostasis. This gluconeogenic activity is under hormonal control, especially glucagon (Exton and Park, 1968).

Activation of pathways such as glucose uptake and glycogen deposition, glycogenolysis, glycolysis, gluconeogenesis etc. are generally done by hormones and neurons on a need basis. Since, sympathetic and parasympathetic autonomic fibres, as well as insulin and glucagon act reciprocally, the positive and negative controls of metabolism in tissues are based on the balance or tone created by the ratio between two opposing factors.

The complex mechanism in maintaining glucose homeostasis through hormonal and neural factors involve creating favourable ratio between, opposing factors. The absence of one factor not only eliminates its influence on the metabolism but also enhance the action of the opposing

factor. Thus in the absence of insulin, glucagon action is felt more. Deficiency of insulin (diabetes mellitus) not only reduces the capacity of tissues such as liver and kidney to absorb glucose but also increases the release of glucose into the blood stream, thereby compounding hyperglycaemic conditions. Insulin deficiency whether induced by antiinsulin serum, alloxan (Miller et al., 1971) streptozotocin (Meier et al., 1972) resulted in hyperglucagonemia irrespective of the degree of hyperglycaemia.

The neuropathy of one autonomic system would lead to increased tone of the other system. Vagotomy has produced in pigeon not only hyperglycaemia but also increased catecholamine, growth hormone and corticosterone release into the blood stream (Viswanathan et al., 1987; John et al., 1985). These lead to increased glucose release from liver and kidney. Role of autonomic nerve fibres and hormones in regulating kidney gluconeogenesis was investigated in our laboratory. Verma et al. (1984) and Pilo et al. (1987) studied the effect of vagotomy on the kidney metabolism. The studies on the influence of glucagon (Pilo and Mehta, 1989), catecholamines (Mehta and Pilo, 1989), insulin (Mehta and Pilo, 1988), thyroid hormones (Pilo and Mehta, 1988) as well as acetylcholine (Pilo and Mehta, 1988) established that the kidney metabolism, especially that of carbohydrate are regulated by both nerves and hormones.

Dysfunction of autonomic nervous system could thus also severely affect glucose homeostasis in the following manner :

1. No efferent input into the brain about glycaemic level

2. No direct stimulation of metabolic reactions in tissues

3. No stimulation of hormones from endocrine glands

It is easy to believe than that autonomic neuropathy could also bring about metabolic disorders such as diabetes mellitus. Most of the information about diabetic condition is derived from studies conducted by administering B cell cytotoxic agents such as alloxan and streptozotocin. These drugs, through their action on DNA could be also used as antitumourdrugs (Okomato) but their side effects are more problematic. Many antineoplastic drugs such as cisplatin also cause hyperglycaemia (Rosenberg, 1965; Goldstein et al., 1982). The other side effect of cisplatin is autonomic neuropathy. A drug that cause selective autonomic neuropathy could provide immense information about the role of autonomic neuropathy in the development diabetes.

The effects of vagotomy and cisplatin treatment in rats and pigeons were compared with a view to understand the mechanism of manifestation of autonomic neuropathy. Mammals tolerate hypoglycaemia while birds tolerate hyperglycaemia (Pilo and Patel, 1978; Pilo and Verma, 1984). Mammals maintain low blood sugar level (100 mg/dl) while birds' normoglycaemic level is above 200 mg/dl. The kidney of both these groups also show variation in its gluconeogenic capacity. Avian kidney has higher capacity for gluconeogenesis than that of mammals, due to the presence of inducible cytosolic form of PEPCK (Watford et al., 1981).

Both vagotomy and cisplatin caused hyperglycaemia (Oommen, 1992) and the liver responded with elevated release of glucose (Parikh, 1992). The present study on the effect of vagotomy and cisplatin treatment on the kidney metabolism in rat and pigeon revealed that both vagotomy and cisplatin caused similar metabolic changes in the kidney. This was evident in glycogen level and activities of glycogen synthetase, G-6-Pase, phosphorylase, aldolase, LDH, SDH (Chapters 2,5) transaminases and phosphatases (Chapters 3,6).

In general vagal denervation and cisplatin treatment caused reduced glucose uptake by kidney, increased glycogenolysis and gluconeogenesis (Table 2).

Since the effects of cisplatin treatment was very similar to that of vagotomy (Fig 2,3), it could be reasoned that most of the effects of cisplatin is through its action of inhibition of cholinergic fibres. The few differences are due to the fact that vagotomy removes both efferent and afferent fibres while cisplatin may be only adversely affecting afferent neural branches.

Though cisplatin showed several metabolic side effects, the effects of carboplatin and later generation of platin compounds were much less. Carboplatin, although produced mild hyperglycaemia, did not drastically influence the enzyme activities, thereby affecting metabolic pathways, in the kidney.

Many of the enzymes are activated or inhibited through Ca^{2+} dependent stimulation mediated through phosphorylase kinase (Shimazu, 1983). Since, cisplatin is known to all· the Ca^{2+} level in the blood due to nephropathy, some of the side effects of cisplatin could be due to the effect of Ca^{2+} deficiency on the neuronal activity. Both neural activity and cellular activity need proper Ca^{2+} concentration. $CaCl_2$ administration has reduced the adverse influence of both vagotomy and cisplatin (Fig 4, 5.).

The present study very well indicate that the kidney in both mammals and birds are involved in glucose homeostasis and a healthy kidney is necessary for maintaining glucose level in the blood. The kidney carbohydrate metabolism, especially the gluconeogenesis is under both neural and hormonal control. Both the autonomic denervation (vagotomy) autonomic neuropathy (caused by cisplatin treatment) result in hyperglycaemia. The kidney effectively contributes to this hyperglycaemia by increasing glycogenolysis as well as gluconeogenesis. The cisplatin side effects are manifested through parasympathetic neuropathy. The effect on metabolic pathway by vagotomy on autonomic neuropathy is through interference with the maintenance of intracellular Ca^{2+} concentration.

The study, interestingly points to the role of autonomic neuropathy in the development of diabetic condition especially the type I diabetes or IDDM. A drug that selectively inhibit parasympathetic afferent fibres could provide more information. Cisplatin, although found to cause autonomic

neuropathy has many other side effects especially nephropathy. The study mainly indicates that intensive investigation is needed to understand the intriguing intricate relationship between autonomic fibres and pancreatic hormones in the development of diabetes especially the IDDM.

Table 2 :

Effect of VgX, CDDP, CBDCA on renal metabolism in rats and pigeons.

	Pigeons			Rats		
	VgX	CDDP	CBDCA	VgX	CDDP	CBDCA
Glycogen	↓	↓	↑	↑	↔	↓
GS	↓	↓	↑	↑	↓	↑
G-6-Pase	↑	↑	↑	↓	↑	↓
Phosphorylase	↑	↑	↑	↑	↑	↓
Aldolase	↑	↑	↑	↑	↑	↑
LDH	↑	↑	↓	↑	↑	↑
SDH	↑	↑	↓	↑	↓	↓
PC	↑	↑	↓	↑	↓	↓
GPT	↑	↑	↓	↑	↑	↓
GOT	↑	↑	↑	↓	↓	↑
Na^+-K^+-ATPase	↓	↓	↑	↓	↓	↑
Alk Pase	↓	↓	↓	↑	↑	↑
Ac Pase	↓	↓	↑	↑	↓	↑
Protein	↓	↓	↑	↔	↓	↑

Increase ↑
Decrease ↓
No change ↔

Table 3 :

$CaCl_2$ administration

	Pigeons		Rats	
	VgX	CDDP	VgX	CDDP
Glycogen	↓	↑	↓	↓
GS	↓	↓	↑	↑
G-6-Pase	↑	↑	↓	↑
Phosphorylase	↑	↑	↑	↑
Aldolase	↑	↑	↑	↑
LDH	↑	↑	↓	↑
SDH	↓	↓	↑	↑
PC	↑	↓	↑	↑
GPT	↓	↑	↑	↑
GOT	↑	↑	↑	↓
Na^+-K^+-ATPase	↓	↓	↓	↑
Alk Pase	↓	↓	↑	↓
Ac Pase	↑	↑	↑	↑
Protein	↑	↑	↔	↓

Increase ↑
Decrease ↓
No change ↔

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

EFFECT OF VAGOTOMY AND CISPLATIN TREATMENT ON BLOOD PARAMETERS

PARAMETERS	VAGOTOMY	CISPLATIN
BLOOD SUGAR	↑	↑
IMMUNOREACTIVE GLUCAGON	æ↑	ø↑
IMMUNOREACTIVE INSULIN	æ↓	ø↓
SERUM THYROXINE T_4	↓	↓
SERUM T_3	↑	↑
T_3 / T_4	↑	↑
HAEMATOCRIT (PACKED CELL VOLUME)	↓	↓

æ FROM SHIMAZU [1983]

ø FROM GOLDSTEIN et al [1983]

EFFECT OF VAGOTOMY AND CISPLATIN TREATMENT ON LIVER AND KIDNEY

PARAMETERS	LIVER		KIDNEY	
	VGX	CIS	VGX	CIS
GLYCOGEN	↓	↓	↕	↕
GLYCOGEN SYNTHETASE	↓	↓	↕	↕
PHOSPHORYLASE	↑	↑	↑	↑
GLUCOSE-6- PHOSPHATASE	↑	↕	↕	↑
ALDOLASE	↕	↕	↑	↑
LACTATE DEHYDROGENASE	↑	↑	↑	↑
GOT	↑	↕	↕	↑
GPT	↕	↑	↑	↑
Æ DNA	↓	↓	↕	↓
Æ RNA	↓	↕	↕	↓
PROTEIN	↓	↕	↕	↕
Na^+ - K^+ - ATPASE	↓	↓	↓	↕
ALKALINE PHOSPHATASE	↓	↓	↑	↕
ACID PHOSPHATASE	↕	↕	↕	↕
Æ ACETYLCHOLINESTERASE	↓	↕	↓	↓

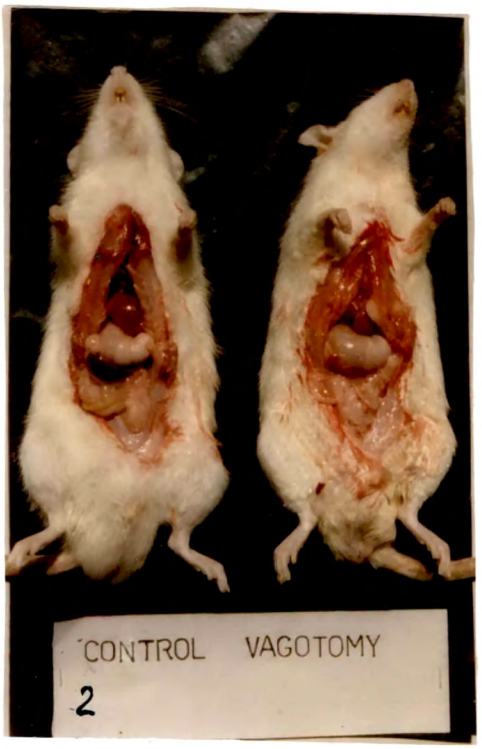
Æ. From Oommen [1992]

From Parikh [1992]

Fig.2: Sham operated (control) and vagotomized rats.

Fig.3: Saline (control) and cisplatin treated rats.

Distented stomach in vagotomized and cisplatin treated rats is clear.


CONTROL VAGOTOMY
2

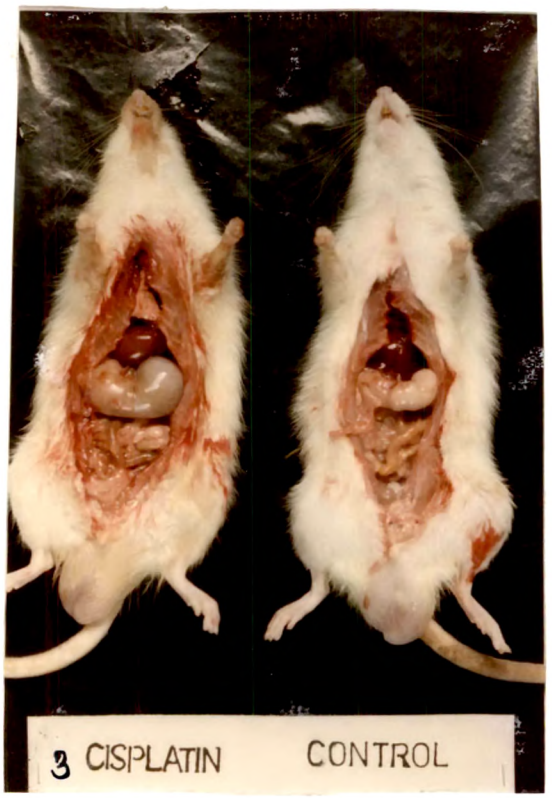
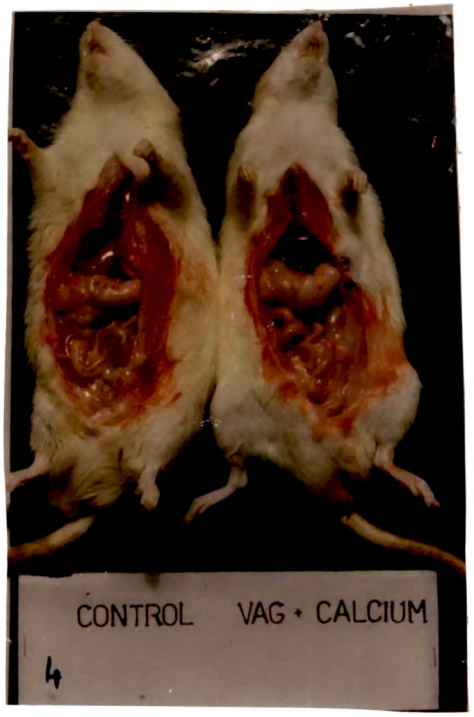

3 CISPLATIN CONTROL

Fig 4,5: Vagotomized and cisplatin treated rats were given an exogenous supply of calcium chloride. Calcium supplementation helps in reducing the stomach distention.


CONTROL VAG + CALCIUM
4


CONTROL CIS + CALCIUM
5