CHAPTER - IV

IMPAIRMENT OF GLUCOSE HOMEOSTASIS DUE TO AUTONOMIC NERVOUS DYSFUNCTION IN RAT AND PIGEON

Modulation of glucose homeostasis during perturbations of the internal and external milieus is an important function of the autonomic nervous system (Cannon et al., 1929; Himms-Hagen,1967; Monnier, 1968; Frohman, 1971) and of organs such as liver and muscle (Bass and Hudicka, 1960). The neurally mediated changes in blood flow are also involved in this process (Jorfeldtt and Wahren, 1971) . Insulin release is initiated by stimulation of ventrolateral hypothalamic area which is regarded as the centre of parasympathetic nervous system (PNS) (Idahl and Martin, 1971). It is also well-established that ventromedial hypothalamic lesions enhance the secretion of insulin (Frohman et al., 1969; Hum and Frohman, 1970; Hustvedt, 1972). The stimulation of peripheral ends of vagus nerves produces prompt rise in arterial plasma insulin and pancreatic polypeptide (PP) in the conscious calf (Bloom and Edwards, 1981a; Andrian, et al., 1983) and the release of insulin which normally occurs in response to hyperglycaemia is mediated largely via the parasympathetic innervation to the pancreatic islets in these animals (Bloom and Edwards, 1981b).

Parasympathetic and sympathetic fibres are credited with the power of controlling glucose uptake and output by the liver. The antagonistic actions of these nerve fibres are also seen in their effect on glucose homeostasis and liver functions

(Lautt, 1983). While parasympathetic fibres induce glucose uptake, the sympathetic actions induce the liver to release glucose (Shimazu, 1983). It has been shown that bilateral vagotomy decreases glycogen content in the liver while ACh administration brings the deposition rate to normalcy. ACh mimics the action of insulin especially by the induction of liver cells to take up more glucose (Beyner and Geelen, 1982). Bilateral vagotomy also resulted in delayed accumulation of glycogen after glucose administration (Pilo and Verma, 1985). It is clear from the above observations that autonomic fibres are involved very much in the control of carbohydrate metabolism and their dysfunctions impair the animal's capacity to regulate blood sugar level. Similar impairment is also seen in the treatment with many drugs especially with the antitumor drug cisplatin (CDDP).

The divalent metals such as cadmium (Ghatghazi and Mennear, 1973), cobalt (Eaton, 1973) and Zinc (Horak and Sunderman, 1975a) are also known to induce hyperglycaemia. Golstein et al. (1982) reported that administration of cisplatin results in hyperglucagonemia in rats. Hyperglucagonemia may be a consequence of either increased glucagon biosynthesis and increased secretion by pancreatic A cells or decreased glucagon catabolism. Recent report by Richardson and Cantwell (1990) has indicated that one patient heavily treated with CDDP developed autonomic neuropathy. The mechanism of action of CDDP which involves changes in intracellular Ca^{2+} or its redistribution, has been championed

by a number of investigators (Gordan and Gattone, 1986; Vassilev et al., 1987).

The well-established role of glucose in maintaining insulin release is associated with an ability of the sugar to stimulate the retention of calcium in B cells (Bergstein, 1987; Hughs et al., 1987). Calcium has been proposed to act as a mediator for insulin action (Kissebah et al., 1975). More recently, an early effect of glucagon and epinephrine on calcium efflux was noted (Pilo and Mehan, 1987). The efflux of calcium is followed by the efflux of K^+, which is associated with hyperpolarization of the liver cell membrane. There are several enzymes in glycogenolytic and gluconeogenic pathways which are calcium sensitive. Cisplatin treatment alters calcium homeostasis in gastric smooth muscle. If such an effect is general to all smooth muscles and nerves, this could have a profound effect on neuromuscular function of the cisplatin treated animals (San-Antonio et al., 1989). The present study is therefore, designed to evaluate and compare the effects of CDDP and vagotomy on glucose metabolism through Glucose Tolerance Test (GTT), and also to understand whether glucose uptake failure by liver and kidney is manifested in cisplatin treated and vagotomised rat and pigeon. In addition, it was also of interest to determine the effect of exogenous calcium on glucose metablism in CDDP treated and VgX animals.

MATERIALS AND METHODS

Male albino rats and domestic pigeons (weighing 200-300g) were used for the studies. They were housed in animal cages in 12/12h light/dark cycle (for details see chapter-I). The animals were acclimatized in laboratory condition for 2 weeks and divided into eight separate groups. Each group comprised six animals.

Group-I : The platinum compound, CDDP was prepared in physiological saline. Each rat received a single ip. dose of cisplatin (7mg/kg b.w) and pigeons received 5mg/kg b.w.

Group-II : Controls received saline in a similar manner.

Group-III: Rats were subjected to subdiaphragmatic vagotomy (chapter-I) under ether anaesthesia, while pigeons were vagotomized bilaterally from the cervical region.

Group-IV: Control animals underwent sham operation.

Group-V : Cisplatin was injected (ip.) in rat and pigeon along with 1 ml of 1.3% $CaCl_2$ ip. $CaCl_2$ was injected twice daily until the day of sacrifice.

Group-VI : Controls received saline and calcium chloride.

Group-VII: Vagotomized animals received $CaCl_2$ twice daily until the day of scrifice.

Group-VIII: Sham operated rats and pigeons were received 1.3% calcium chloride.

Both CDDP treated and VgX animals were deprived of food. GTT was carried out following a single ip. glucose load. Prior to GTT, the animals were fasted for 4-5 hrs. Blood samples were collected via orbital sinus puncture from unanaesthetised rats. In pigeons, blood was collected from brachial vein. Blood samples were analysed for glucose by the method of Folin and Malmrose (1929)(Chapter-I).

Statistical Analysis

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

RESULTS

Cisplatin treated and vagotomized animals and their controls were subjected to a glucose load to obtain the glucose tolerance curve. The mean glucose level in the blood at different intervals after glucose load in various experimental groups are given in Tables I, II and III. The initial glucose level of CDDP treated rats and pigeons were higher than that of respective controls. Similarly, 48 hrs after vagotomy, the rat and pigeons also showed significant increase in the basal level of glucose value in comparison to sham operated controls.

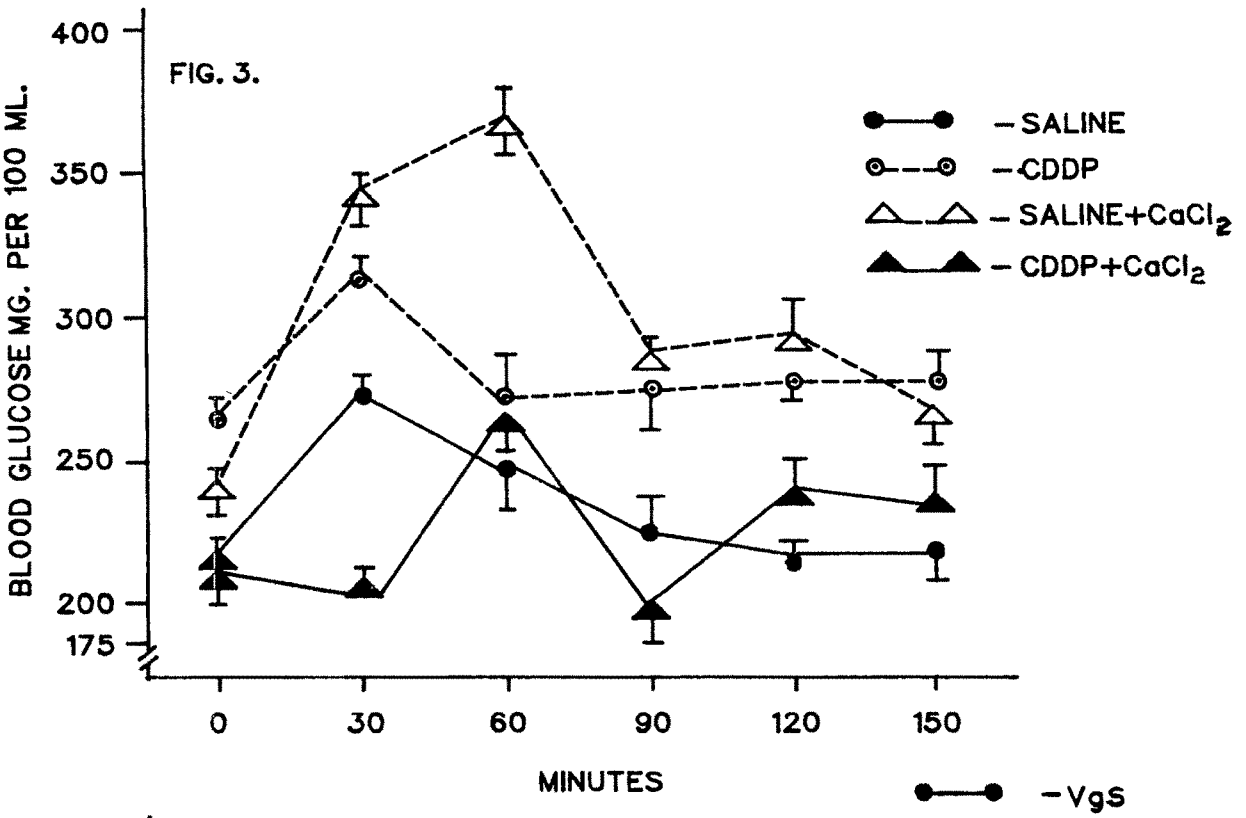
Table I Effect of CDDP and CDDP+$CaCl_2$ on glucose tolerance in rats (Mean ± SEM)

Interval in minutes	Saline	CDDP	Saline+$CaCl_2$	CDDP+$CaCl_2$
0	140.64 ± 3.484	211.056**** ± 3.60	129.658 ± 3.804	229.062**** ± 7.935
30	207.93 ± 5.246	299.014**** ± 5.338	280.136 ± 5.606	238.478*** ± 6.758
60	164.418 ± 3.606	328.18**** ± 5.309	256.304 ± 7.525	294.632* ± 6.184
90	171.252 ± 6.385	307.588**** ± 5.274	220.616 ± 7.763	240.03NS ± 4.535
120	162.028 ± 3.32	273.154**** ± 6.485	234.076 ± 10.005	291.634* ± 6.2009
150	158.526 ± 5.137	265.586**** ± 5.63	232.456 ± 7.214	261.664NS ± 7.9432

(Glucose - mg/100 ml blood).

* $P < 0.05$; *** $P < 0.01$; **** $P < 0.001$;

NS - Not significant



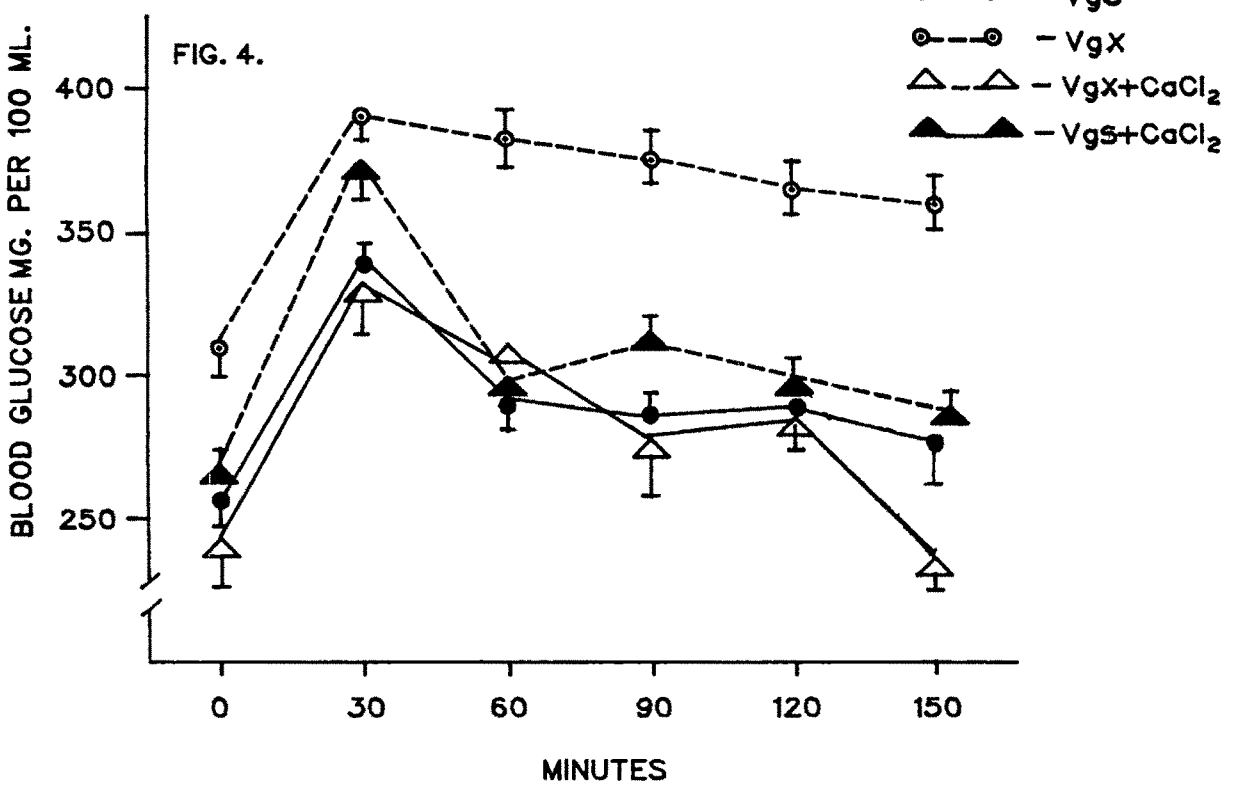


FIG. 3 & 4 GLYCAEMIC RESPONSE TO CISPLATIN AND VAGOTOMY IN PIGEON.

Table II **Effect of Vagotomy and Vagotomy + $CaCl_2$ on glucose tolerance in rat. (Mean ± SEM)**

Interval in minutes	Sham	Vagotomy	Sham+$CaCl_2$	Vagotomy+$CaCl_2$
0	135.989 ± 3.740	173.63**** ± 5.340	115.874 ± 5.288	167.253**** ± 6.362
30	262.546 ± 6.475	340.828**** ±11.281	257.63 ± 7.247	363.14**** ± 6.741
60	293.089 ± 5.276	302.04NS ± 6.787	201.438 ± 5.625	235.76** ± 6.555
90	249.398 ± 5.262	276.10NS ± 5.262	223.854 ± 8.154	194.866NS ± 7.667
120	181.17 ± 7.40	198.58NS ± 4.582	175.572 ± 7.54	186.58NS ± 5.959
150	144.832 ± 5.95	197.014*** ± 8.508	177.562 ± 5.09	189.89 NS ± 5.850

(Glucose mg/100ml blood)

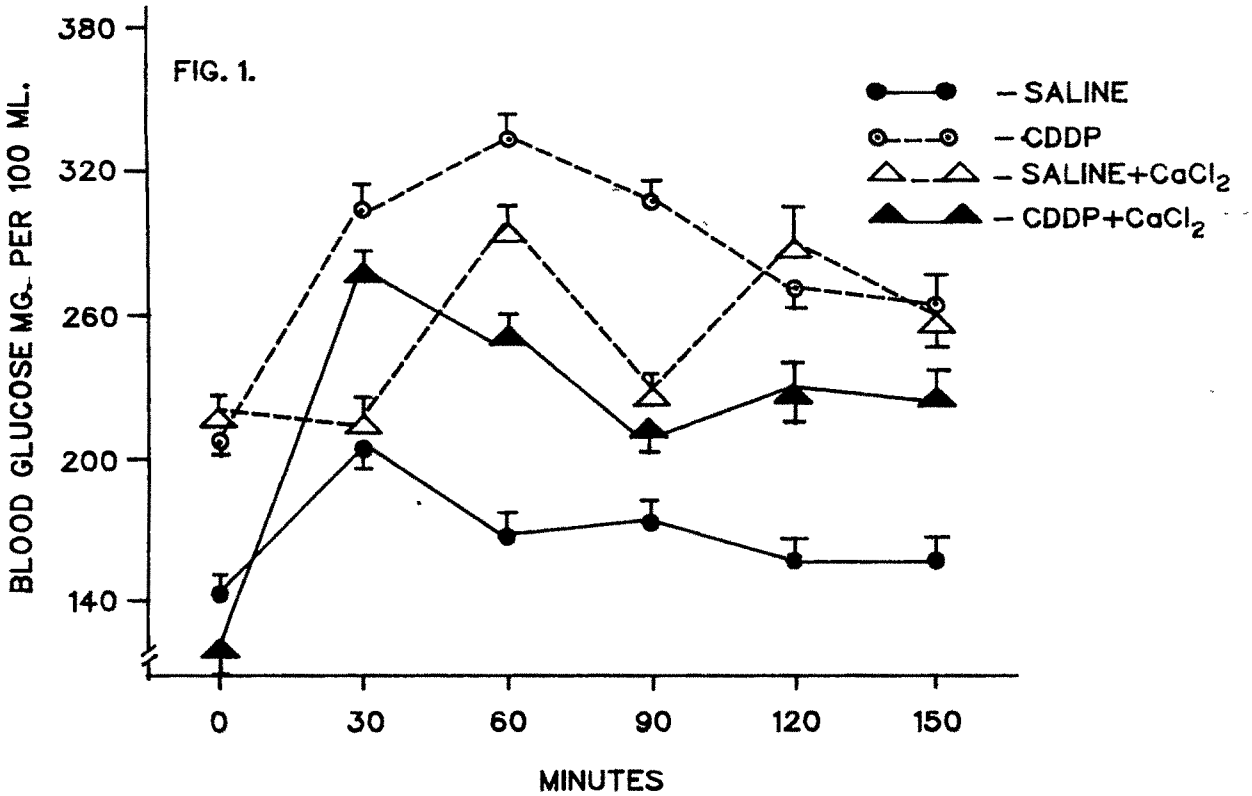
** $P < 0.02$; *** $P < 0.01$; **** $P < 0.001$;
NS - Not Significant

Table III Effect of Cisplatin and Cisplatin + $CaCl_2$ on glucose tolerance in pigeons. (Mean ± SEM)

Interval in minutes	Saline	CDDP	Saline+$CaCl_2$	CDDP+$CaCl_2$
0	215.423 ± 8.758	263.253** ± 6.44	215.996 ± 5.616	239.052NS ± 9.381
30	277.972 ± 6.459	316.817**** ± 6.932	225.364 ± 4.092	343.506**** ± 9.69
60	250.442 ± 8.266	272.99NS ± 9.989	265.656 ± 7.034	365.654**** ±11.021
90	226.293 ± 7.178	277.55** ± 9.367	235.436 ± 6.666	284.994* ± 6.876
120	217.658 ± 3.410	280.213**** ± 6.688	243.318 ± 8.045	295.72* ±10.886
150	220.575 ± 7.205	276.031** ± 8.29	238.96 ± 8.995	264.89 NS ± 6.79

(Glucose - mg/100ml blood)

* $P < 0.05$; $P < 0.02$; **** $P < 0.001$



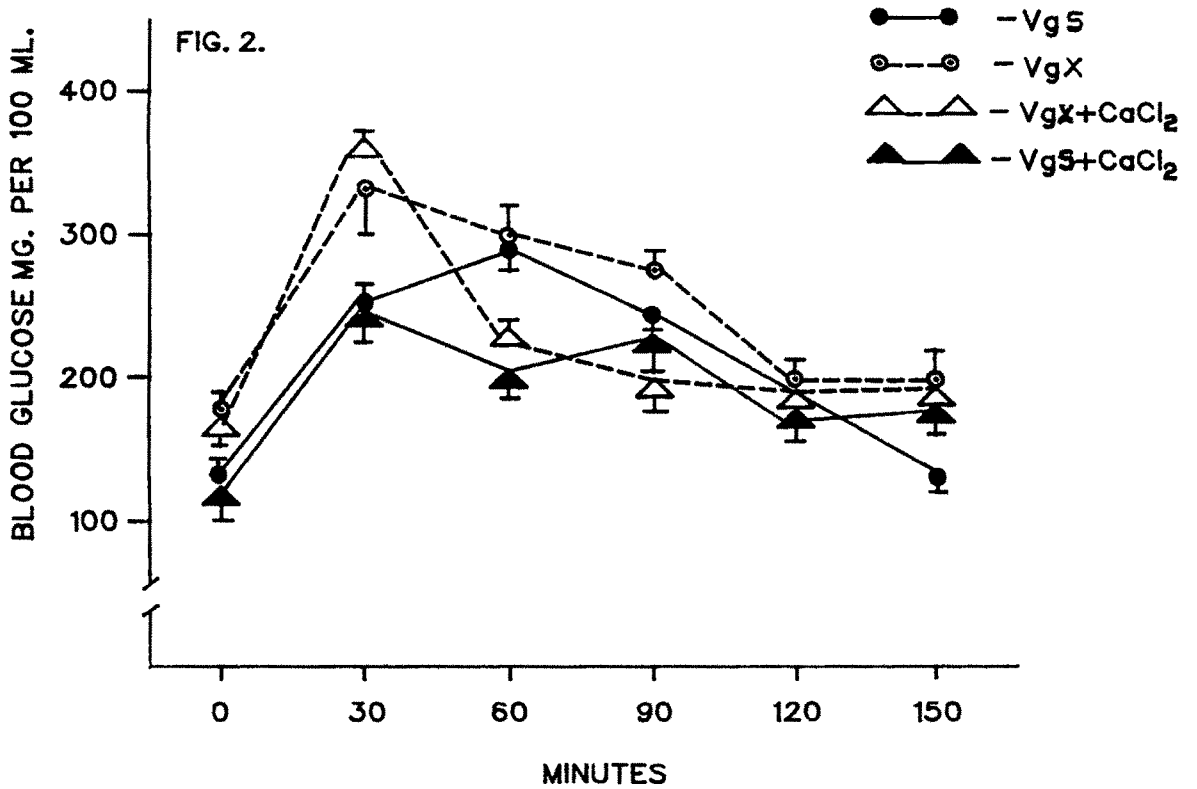


FIG. 1 & 2 GLYCAEMIC RESPONSE TO CISPLATIN AND VAGOTOMY IN RAT.

Table IV Effect of Vagotomy and Vagotomy + $CaCl_2$ on glucose tolerance in pigeons (Mean ± SEM)

Interval in minutes	Sham	Vagotomy	Sham+$CaCl_2$	Vagotomy+$CaCl_2$
0	261.46 ± 8.304	312.185** ± 7.470	239.048 ± 8.42	264.38 NS ± 9.395
30	338.977 ± 6.913	395.964*** ± 7.345	333.196 ± 10.563	374.186 NS ± 7.5
60	295.411 ± 5.63	384.01**** ±13.50	307.528 ± 10.119	315.474NS ± 1.639
90	285.26 ± 5.755	377.252**** ±14.258	274.806 ± 8.771	312.608NS ±10.411
120	296.943 ± 5.123	368.35*** ±13.135	284.958 ± 7.587	295.556NS ± 7.138
150	278.976 ±11.66	363.185** ±12.401	236.016 ± 9.744	285.902* ± 7.261

(Glucose mg/100ml blood)

* $P < 0.05$; ** $P < 0.02$; *** $P < 0.01$; **** $P < 0.001$.

GTT in CDDP Treated Rats and Pigeons

Administration of glucose to CDDP treated rat produced a progressive increase in the glycaemic level from 0 hr till 90 minutes. Although value decreased by 120 minutes, the value did not reach the initial (0 hr) level even by 150 minutes (fig.1).

CDDP treated pigeons showed a peak glucose level at 30 minutes after the glucose load but the level began decreasing by 60 minutes (fig.3).

GTT in Vagotomized Rats and Pigeons

Glucose loading in VgX rats resulted in a peak glucose level by 30 minutes and thereafter the level began declining, reaching the 0 hr value by about 120 minutes (fig.2).

Vagotomized pigeons responded to the glucose load with a peak glycaemia at 30 minutes and the hyperglycaemia persisted even at 150 minutes. Sham operated pigeons, although showed the peak at 30 minutes, glucose concentration in them began to decrease to the normal level by 60 minutes (fig.4).

GTT in CDDP + $CaCl_2$ Treated Rats and Pigeons

CDDP + $CaCl_2$ treated rats when confronted with the glucose load showed a peak glycaemic level at 60 minutes. But thereafter (by 90 minutes) the level began to decrease (fig.1).

In CDDP + $CaCl_2$ treated pigeons, the administration of glucose further pushed up the glucose level, reaching a maximum level at 60 minutes. However, the lowering of the glucose level took place very fast.

GTT in VgX + $CaCl_2$ Treated Rats and Pigeons

Although VgX +$CaCl_2$ rat showed a peak glycaemic response by 30 minutes, the decline was equally fast. Somewhat similar response was also shown by VgX +$CaCl_2$ pigeons subjected to glucose load (fig.4).

DISCUSSION

Cisplatin treatment for 72 hours caused the elevation of blood sugar level in both rat and pigeon. Several divalent metal cations are found to influence the carbohydrate metabolism by their ability to alter insulin/or glucagon synthesis and release (Eaton, 1973; Bloom et al., 1974; Ghafghazi and Menonear, 1975; Herack and Sunderman, 1975 a & b). Therefore it can be postulated that, a divalent platinum compound such as CDDP may also affect glucose homeostasis through affecting insulin and/or glucagon synthesis or release. Vagotomy also induced elevation in glucose level in rat and pigeon. Thus CDDP treatment and vagotomy, both could impair the glucose homeostasis mechanism.

In normal rat, a glucose load elevates the blood sugar to a peak level by 30 minutes and the level returns to the normoglycaemic range by 60 to 90 min. In pigeon also, similar glucose tolerance curve was observed. A glucose load to CDDP treated rats produced a prolonged hyperglycaemic level, indicating an increased tolerance of glucose. Pigeons treated with CDDP too showed similar impaired glucose tolerance response. The CDDP treatment has thus reduced the glucose uptake by liver and other tissues. Probably insulin release itself might be getting affected. Both could be as the result of dysfunction of vagal cholinergic fibres.

Vagal fibres play a direct role in the uptake of glucose by liver by activating glucokinase and glycogen synthetase enzymes (Pilo and Verma, 1985). There are glucose sensitive neuronal elements in the hepatic portal vagal afferent fibres which convey the signals to the brain about the glucose level, on the basis of which the brain initiates regulatory mechanisms to maintain glucose homeostasis.

Vagotomy in rats and pigeons also eliminates the participation of vagal afferents in glucose related information transfer to brain as well as participation of vagal efferents in inducing the liver cells to take up more glucose.

CDDP and vagotomy can also cause disturbances in the insulin release from B cells of pancreatic islets. These disturbances are also caused through the absence of vagal

cholinergic stimulation of cells. In VgX animals (rats and pigeons) the vagal innervation to both liver and pancreas is totally removed. CDDP, on the other hand, must be causing selective neuropathy of cholinergic fibres. Autonomic neuropathy has been reported in cisplatin treated animals (Thompson et al. 1984; Richardson, 1990; Hanson, 1990). In diabetic condition also, peripheral and autonomic neuropathy has been commonly reported (Watkens, 1990).

Cholinergic dysfunction can directly produce hyperglycaemia. Administration of cholinergic antagonist atropine, considerably weakened the insulin secretion and glucose tolerance in animals with intact vagus nerve (Zelinic, 1986). In dogs, acute vagotomy produced a transient decrease in insulin levels with impaired response to intravenous glucose load (Frohman et al., 1967). It is also reported that in rats vagotomy decreases insulin response to oral glucose load (Hamphrey et al., 1975).

Vagotomy in pigeon can produce an elevation of sympathetic tone and a more than five fold increase of norepinephrine in the blood (Viswanathan et al., 1987). Pilo et al. (1986) have shown an increase in corticosteroid concentration in vagotomized pigeons. Increased sympathetic tone and corticosterone concentration could thus be the cause of hyperglycaemia in VgX pigeons. A similar explanation could also be extended to VgX rats. Similarly CDDP treatment may be causing selective cholinergic neuropathy because of which

glucose uptake by liver and insulin release by pancreas were severely affected.

Reported findings indicate that toxic side effects of CDDP can be suppressed by loading the animals with intravenous infusion of calcium gluconate either before or during cisplatin treatment (Aggarwal et al., 1980). It is also reported that cisplatin treatment causes excess elimination of calcium by kidney. Disturbances in Ca^{2+} conductance in neurons could be one of the reasons for the autonomic dysfunction in cisplatin treated animals. The synchronous release of acetylcholine in response to a nerve impulse depends upon Ca^{2+} concentration in the extracelluar fluid. Calcium is virtually the only ion required in the bathing fluid to evoke ACh release (Katz and Miledi, 1969). In the present study also, calcium chloride infusion has protected the tissues from CDDP toxicity. Even in VgX animals calcium infusion evoked partial recovery from the adverse effect of vagotomy on glucose tolerance.

The results of these studies clearly indicate that both CDDP treatment and VgX could affect the glucose tolerance adversely and these effects could be reversed to some extent if the animals are given exogenous calcium.