CHAPTER - 5

EFFECT OF VAGOTOMY AND CISPLATIN TREATMENT ON GENERAL CARBOHYDRATE METABOLISM IN PIGEON KIDNEY

Autonomic nervous system (ANS) has several goals to play in the regulation of blood sugar level. Since various organs such as liver, kidney, pancreas, adrenal, skin and adipose tissue have autonomic innervation, the functions of all these organs could be orchestrated by ANS for blood sugar homeostasis. While autonomic fibres reaching endocrine organs could regulate the secretion of hormones, those that innervate organs such as liver and kidney could induce metabolic changes directly. Since ANS has its origin in hypothalamus, there is an absolute coordination between various endocrine and neural stimulation of metabolic actions in tissues. Glucose release and glucose uptake are two opposing mechanisms controlled by separate neural and endocrine systems. Glucose release (from liver and kidney) are mediated by sympathetic system, which is also involved in the release of glycogenolytic hormones such as glucagon. The parasympathetic system induces the tissues to take up more glucose on one hand and stimulates the B cells to release insulin on the other hand. Thus both parasympathetic and sympathetic systems are involved in the maintenance of blood sugar level. Vagotomy resulted in hyperglycaemia in pigeons (Pilo et al., 1984). Several studies revealed that vagal denervation leads to impairment of glucose uptake by liver (see review by Pilo and Verma, 1985). Parasympathetic dysfunction or neuropathy could thus adversely affect glucose homeostasis.

Antitumor drug, Cisplatin (cis-diamminedichloroplatinum-II), not only caused peripheral neuropathy but also caused hyperglycaemia in patients (Goldstein et al., 1981) who were receiving treatment for cancer. The drug

further caused acute renal toxicity. It was observed by Schaeppi et al. (1972) that cisplatin treatment showed toxic signs including severe haemorrhagic enterocolitis, hypocellularity, renal lesions etc., leading to hyperchloremia, proteinuria and hypocalcemia. Nephrotoxicity of cisplatin (CDDP) is known to reduce the glucagon removal from the circulation by the kidney (Goldstein et al., 1983). High glucagon values are reported in cases of severe renal failure (LeFebvre and Luyakx , 1977). Thus cisplatin could effect the glucose homeostasis in several ways. Increased glucagon release and reduced glucagon removal combined with reduced uptake of glucose by the tissues together produced hyperglycaemia. In this case cisplatin acts both through neural and renal toxicity.

The side effects of cisplatin are due to its effects on physiology on one hand and toxicity on the other hand. Since cisplatin treatment results in hypocalcaemia (Schaeppi et al., 1973; Rosen et al., 1980) some of these side effects were eliminated or attenuated by calcium treatment (Aggarwal et al., 1980).

The present investigation is aimed at unearthing similarities and dissimilarities between vagal denervation and cisplatin treatment. At the same time it was also deemed worthwhile to investigate whether calcium could provide protection against the adverse effects of cisplatin treatment and vagal denervation in pigeons.

Materials and Methods

Adult domestic pigeons weighing around 250-300 gms were chosen for the study. They were acclimatised to the laboratory conditions and were divided into eight groups of six birds each. The first group of birds were subjected to cervical vagal denervation for which sham operated birds (second group) served as controls. The third set was vagally denervated and treated with 1.3% calcium chloride (1 ml) morning and evening for 48 hours. Sham operated controls (fourth group) were also administered with the same dosage of calcium chloride. The fifth set of birds were subjected to cisplatin treatment (5 mg/kg body wt. in 0.85% saline). Controls (sixth set) received plain injections (ip) of saline (0.85%). Seventh group of birds received calcium chloride injections (ip) 1.3% (1 ml) morning and evening along with the cisplatin treatment, whose controls (eighth group) were treated with calcium chloride after saline treatment.

All the birds were provided with water and was sacrificed by exsanguination at 48 hours. Kidneys from both sides were excised, weighed and processed for further investigations. A piece of tissue was weighed and treated in alcoholic potassium hydroxide (KOH) and processed for determining the glycogen content. Biochemical estimations were carried out for glycogen synthetase, glucose-6-phosphatase, phosphorylase, aldolase, lactate dehydrogenase (LDH), succinate dehydrogenase (SDH) and pyruvate carboxylase (PC) as per method described in Chapter 1.

Statistical Analysis :

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

Vagotomy (VgX) :

Vatogomy caused a decrease in the glycogen content of pigeon kidney. Correspondingly there was also a reduction in the glycogen synthetase activity of the kidney. Increased glycogenolysis and glucose release in VgX pigeons can be easily explained by the fact that glycogen phosphorylase and G-6-Pase enzymes became more active in the kidney. Together with increased glycogenolytic activities, VgX pigeon kidney also showed elevated glycolysis. This became evident from the increased LDH and aldolase levels. Elevated SDH and pyruvate carboxylase activities were also seen in the kidney of VgX birds.

Calcium chloride injection in VgX pigeons produced a slight reduction in glycogen content and a drastic reduction in the glycogen synthetase activity. However, phosphorylase, G-6-Pase, LDH, SDH and PC activities showed only a milder response compared to what observed in VgX pigeons. Aldolase was the only enzyme that did not show an opposite response in the $CaCl_2$ treated VgX pigeons, over and above the activity of this enzyme increased further.

TABLE 1 : EFFECT OF VAGOTOMY AND CISPLATIN ALONE AND IN COMBINATION WITH $CaCl_2$ IN PIGEON KIDNEY ON GLUCOSE METABOLISM

	GLYCOGEN	GLYSYNTH	G-6-PASE	PHOSPHORY-ASE	ALDOLASE	LDH	SDH	PC
VgS	0.0158± 0.0004	0.0326± 0.004	0.229± 0.011	90.198± 1.128	0.240± 0.052	83.455± 1.26	6.6606± 0.7048	0.400± 0.001
VgX	0.0118± 0.0004 ****	0.0136± 0.001 ****	0.367± 0.015 ***	104.884± 0.732 ***	0.430± 0.005 ***	102.62± 0.72 ****	21.4597± 1.370 ****	1.059± 0.001 ***
Con	0.022± 0.002	0.044± 0.001	0.216± 0.011	83.34± 1.44	0.170± 0.018	49.066± 2.43	7.5685± 1.041	0.514± 0.001
CDDP	0.0181± 0.0003 **	0.013± 0.0007 ****	0.358± 0.006 ****	106.177± 1.04 ****	0.230± *	53.89± 1.32 **	11.1358± 0.6191 **	0.590± 0.002 ****
VgS+Ca	0.0216± 0.008	0.023± 0.003	0.378± 0.014	32.454± 1.33	0.417± 0.024	75.861± 0.587	5.6060± 0.7705	0.342± 0.0009
VgX+Ca	0.0194± 0.0006 **	0.002± 0.0003 ****	0.045± 0.020 *	36.758± 1.55 #	0.865± 0.017 ****	77.111± 0.206 *	5.0475± 0.8703 **	0.755± 0.001 ****
Con-Ca	0.006± 0.0005	0.080± 0.001	0.118± 0.001	104.938± 1.88	0.010± 0.0002	17.156± 0.312	7.5731± 0.6977	0.593± 0.001
CDDP+Ca	0.007± 0.0003 **	0.015± 0.0007 ****	0.126± 0.0003 ****	119.33± 1.29 ****	0.70± 0.002 ****	37.90± 0.64 ****	3.8855± 0.2945 ****	0.548± 0.001 ****

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

TABLE 2 : EFFECT OF VAGOTOMY AND CISPLATIN ALONE AND IN COMBINATION WITH $CaCl_2$ IN PIGEON KIDNEY ON GLUCOSE METABOLISM IN TERMS OF PERCENTAGE CHANGES.

	GLYCOGEN	GLYSYNTH	G-6-PASE	PHOSPHORY-LASE	ALDOLASE	LDH	SDH	PC
VgS (VgX)	27 ↓	58.28 ↓	60.26 ↑	4.9 ↑	68 ↑	23 ↑	229 ↑	164.7 ↑
Con (CDDP)	17.7 ↓	70.45 ↓	65.7 ↑	27.3 ↑	35 ↑	10 ↑	47.2 ↑	14.78 ↑
VgS+Ca (VgX+Ca)	5.2 ↓	105 ↓	17.7 ↑	13.2 ↑	107 ↑	2 ↑	9.9 ↓	41.7 ↑
Con+Ca (CDDP+Ca)	16.6 ↑	700 ↓	8.4 ↑	13.7 ↑	640 ↑	120 ↑	95.1 ↓	7.5 ↓

% is corrected to nearest whole number, expressed as increase [↑], decrease [↓] in value of the group in parenthesis compared to its adjoining group.

EXPLANATION TO FIGURES

Effect of Vagotomy and Cisplatin treatment alone or in combination with calcium with respect to:

Fig (1) : Glycogen content and activity of glycogen synthetase in the kidney.

Fig (2) : Activities of glucose-6-phosphatase and phosphorylase in the kidney.

Fig (3) : Activities of LDH and aldolase in the kidney.

Fig (4) : Activities of SDH and pyruvate carboxylase in the kidney.

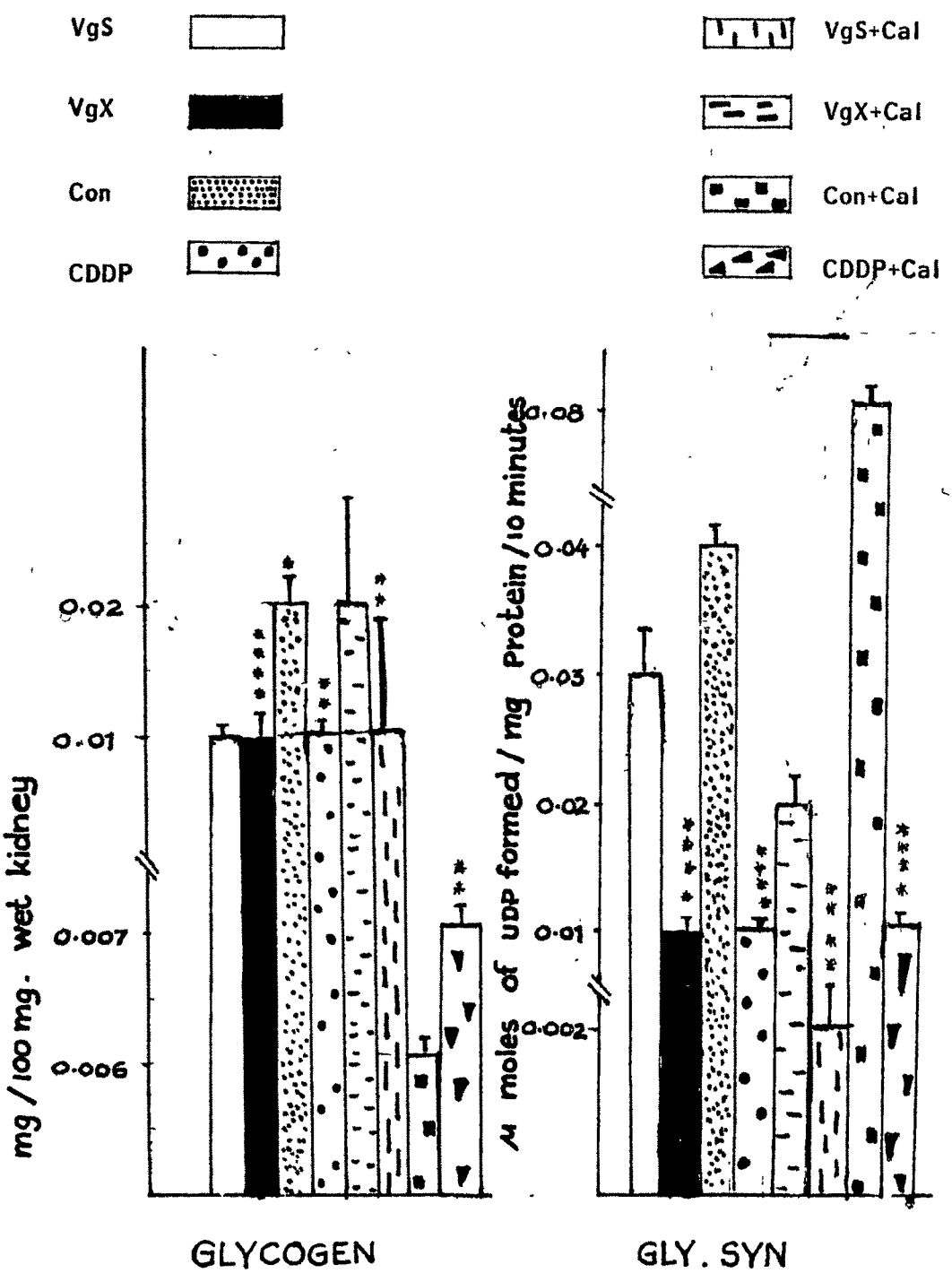

Fig:1 Results given as mean ± SEM of six experiments. $P<0.02^{*}$; $P<0.05^{**}$; $P<0.01^{***}$; $P<0.001^{****}$

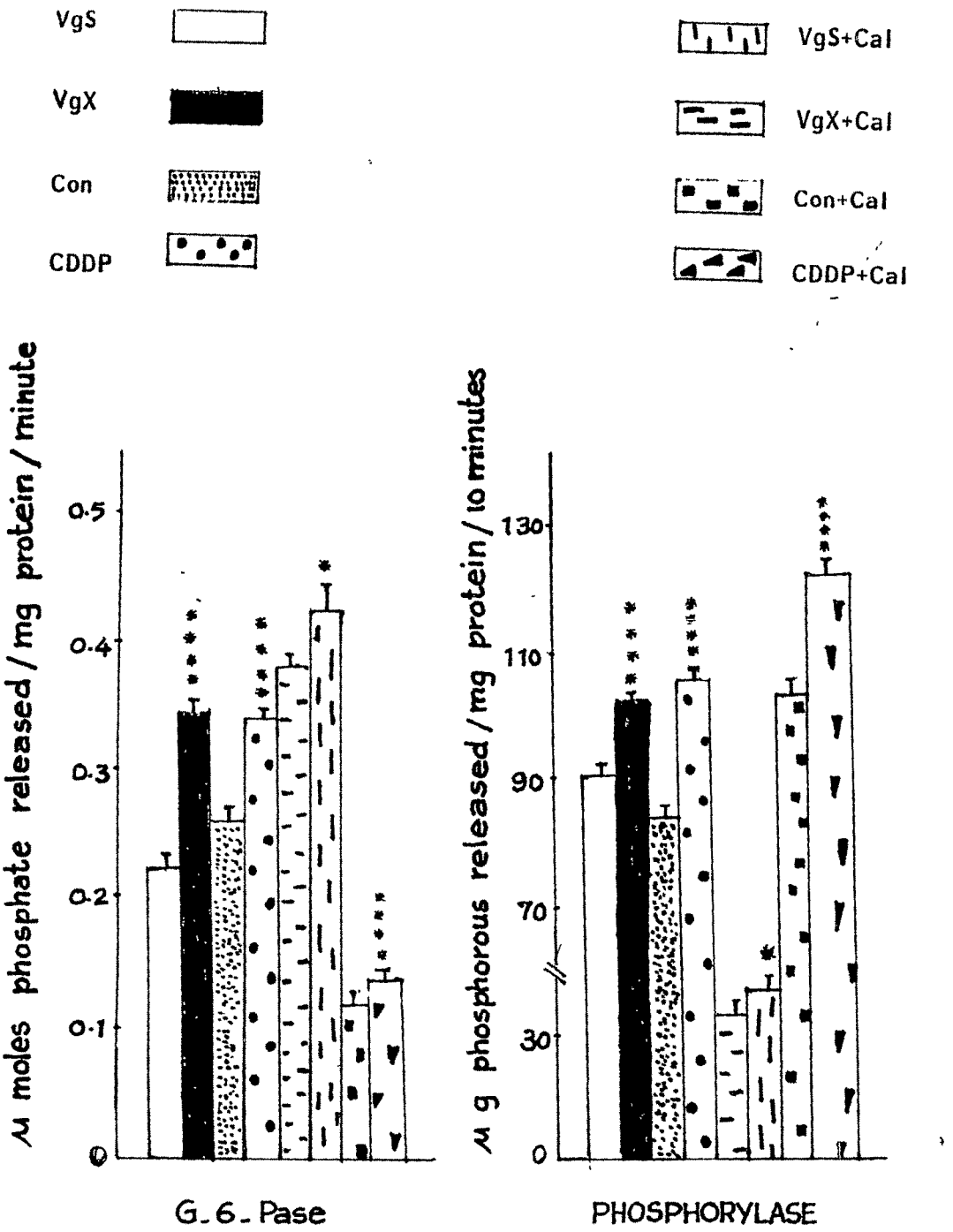

Fig : 2 Results given as mean ± SEM of six experiments. $P<0.02^{*}$, $P<0.05^{**}$, $P<0.01^{***}$, $P<0.001^{****}$

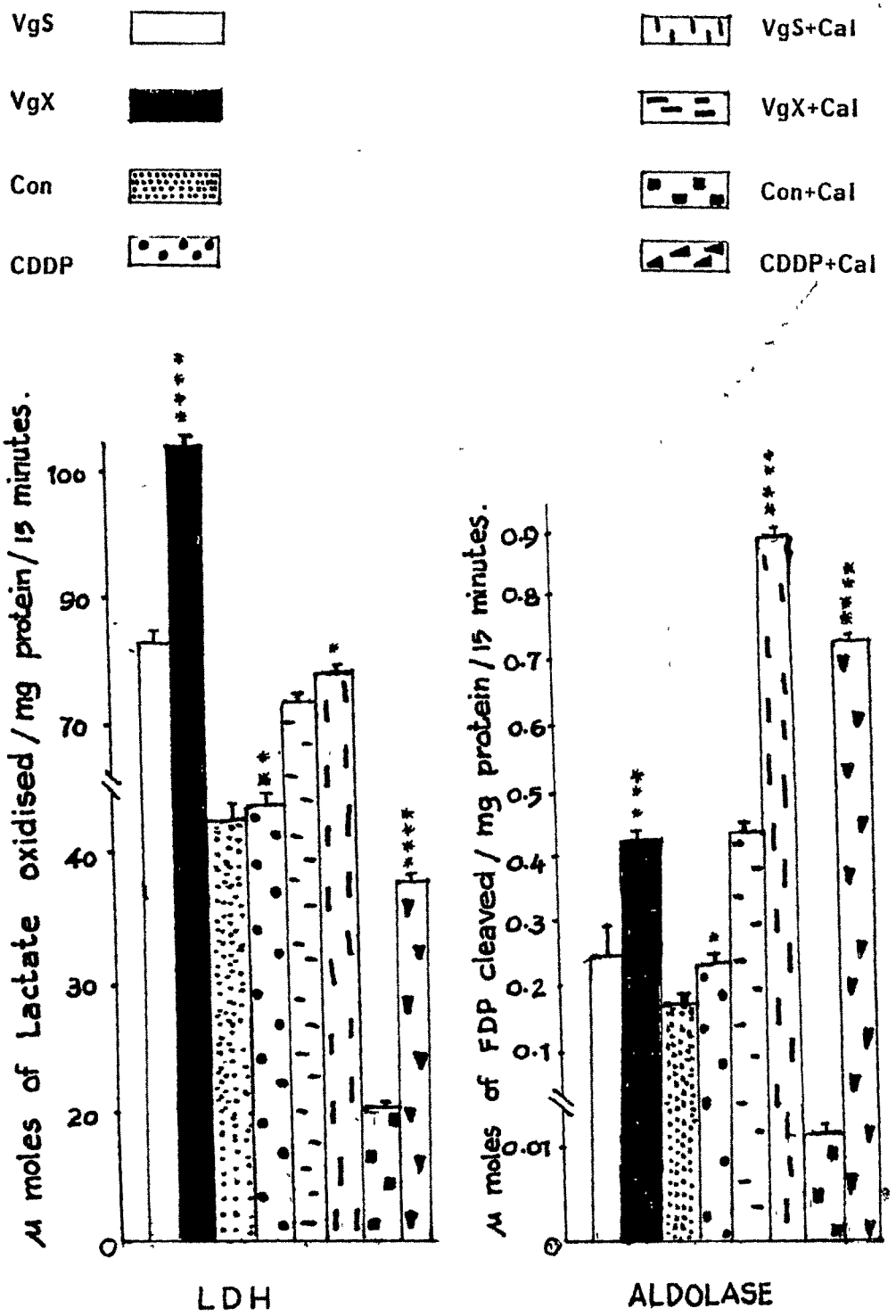

Fig:3 Results given as mean ± SEM of six experiments. $P<0.02^{*}$, $P<0.05^{**}$, $P<0.01^{***}$, $P<0.001^{****}$

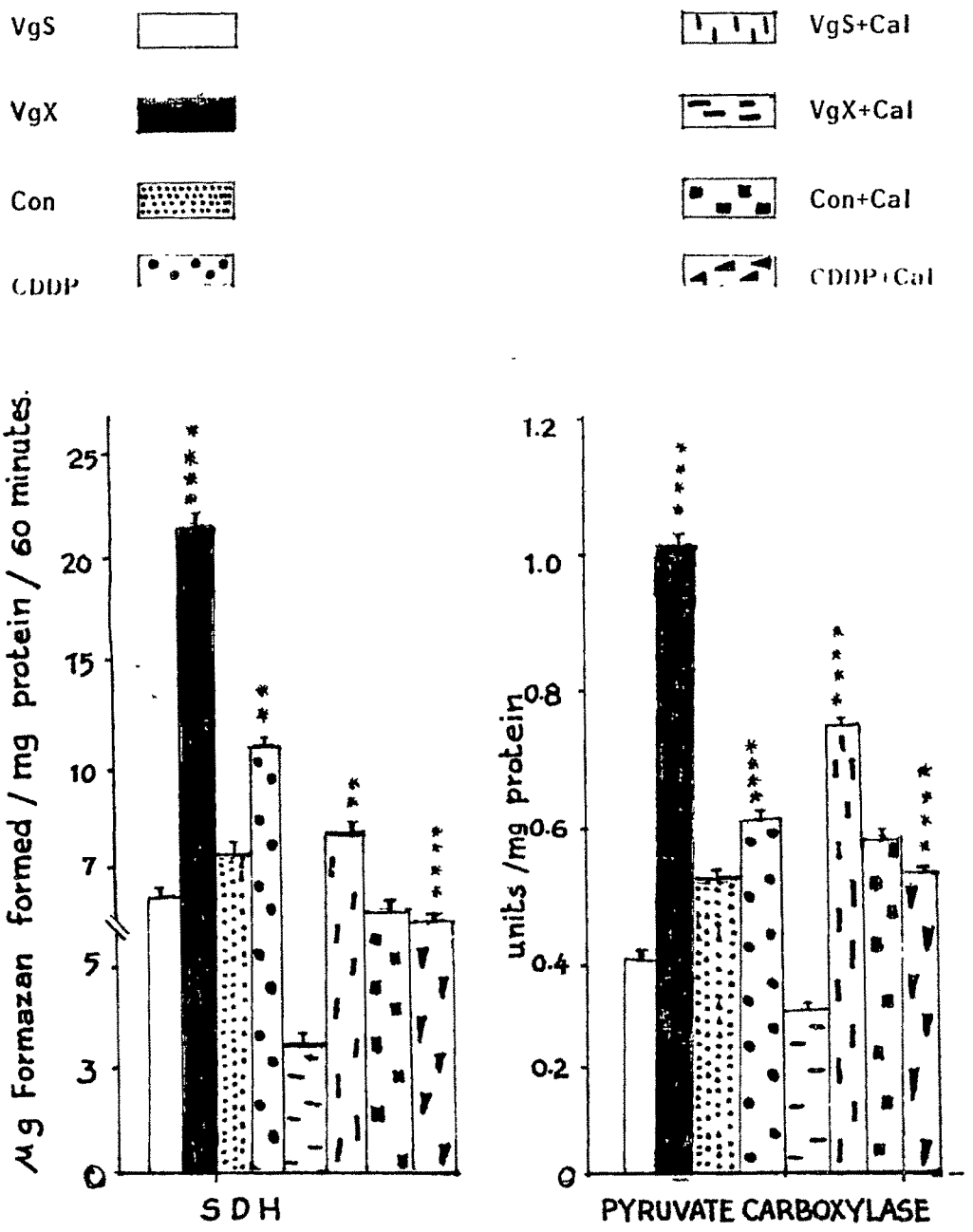

Fig : 4 Results given as mean ± SEM of six experiments. $P<0.02^{*}$, $P<0.05^{**}$, $P<0.01^{***}$ $P<0.001^{****}$

Cisplatin Treatment :

Just as in the case of VgX pigeons, CDDP treated pigeons showed a reduction in the glycogen content and glycogen synthetase activity in the kidney. All other enzymes studied (phosphorylase, G-6-Pase, LDH, Aldolase, SDH and pyruvate carboxylase) showed varying degree of activation. As in the case VgX pigeons, in CDDP pigeons also calcium chloride provided some protection as the increase in the enzyme activities were not as much noticed in CDDP treated pigeon kidney, except in the case of LDH and aldolase where the percentage increase was much more than what was observed in CDDP treated pigeon kidney.

Discussion

Major or striking effect of vagotomy in pigeon is the hyperglycaemia (Pilo et al., 1977) together with increased hepatic glycogenolysis and glucose release (Vostikov, 1978). Obviously the parasympathetic denervation has caused a destruction in the mechanism that regulates glucose level in blood. Vagal cholinergic fibers could initiate an uptake of glucose by tissues either by directly stimulating the uptake mechanism or by releasing insulin. These cholinergic fibers also may inhibit the glucose release mechanism either directly or through suppressing the release of glucagon. The hyperglycaemia following vagotomy indicates that the vagal suppression of glucose release from tissues, elevated sympathetic actions release of glucagon by A cells, catecholamines from adrenal medulla and glucocorticoids from adrenal cortex, gets removed, resulting in ultimately an increased level of glucose in blood. A prolonged release of

catecholamines, glucagon and glucocorticoids singly or collectively will activate gluconeogenesis (Hers and Hue, 1983; Kneer et al., 1974).

Gluconeogenesis is a process by which glucose is formed from substrate other than carbohydrates. Liver and Kidney are the major gluconeogenic organs. Gluconeogenesis is resorted to when food intake is curtailed or the food has very little carbohydrates. Kidney plays a vital role in glucose homeostasis through intense gluconeogenic activity (Krebs, 1963; Exton, 1971; Shah and Mistry, 1979; Pilo and Mehta, 1986). Generally, kidney takes up a compensatory role of producing more glucose when liver functions are altered as in diabetes (Kida et al., 1978). During normal conditions, kidney provides only 20% of glucose, while glucose production almost equal as in liver during prolonged starvation inspite of low levels of glycogen content in the kidney (Exton, 1980). In birds the kidney is the predominant tissue for gluconeogenesis (Pearce, 1971).

The immediate response to hypoglycemia is mediated by sympathetic system, while the hyperglycemia activates parasympathetic system. Both have the origin in hypothalamus. The hypothalamic nucleus, Ventromedial Hypothalamus (VMH), regulates the sympathetic system and Lateral Hypothalamic Area (LHA) regulates parasympathetic system. When VMH was lesioned, a rise in insulin level was observed even in the absence of a glucose challenge, and this hyperinsulinemic response was abolished by vagal denervation (Tokunaga et al., 1986).

Shimazu et al. (1966) reported that prolonged stimulation of vagus nerve in rabbits increased glycogen deposition. In pigeons, the observations that cervical vagotomy produced a reduction in glycogen content as well as glycogen synthetase activity indicate that along with vagal denervation, there was also a sympathetic activation. Increased phosphorylase activity in kidney also indicate the participation of glucagon as well as catecholamines. Glycogenolysis initiated by glucagon is accompanied by glycogen depletion (Glinsmann and Mortimore, 1968; Exton and Park, 1972; Hutson et al., 1976; Hems et al., 1978). Epinephrine and Norepinephrine also stimulate glycogen degradation (Sherline et al., 1972; Exton and Harper, 1975; Saitoh and Ui, 1975; Hems et al., 1976; Hutson et al., 1976).

In vagotomized pigeon kidney, along with depletion of glycogen, G-6-Pase activity also increased indicating that glucose release is also concurrently augmented. The VgX pigeon kidney not only contributed glucose to the blood stream but also stepped up glycolysis. This was indicated by the fact that aldolase activity increased almost two fold. There was also increased LDH activity in the VgX pigeon kidney. Similar increase in aldolase and LDH was noticed in the liver of VgX pigeon (Verma and Pilo, 1984). Glycolysis also provides substrate for gluconeogenesis (gng). Satisfactory oxygenation of the tissue is a pre-requisite for gluconeogenesis (Krebs, 1963). In gluconeogenesis, pyruvate is transformed into oxaloacetate by pyruvate carboxylase. This enzyme was found to become active more than two fold in the VgX kidney. Energy for converting

pyruvate to glucose comes from fatty acid oxidation. An increased oxidative metabolism was evident in pigeon kidney by the increased SDH activity observed in the experimental birds. During vagal inhibition, catecholamines remain undisturbed in their release or are having compensatory role to maintain the glucose regulation. Norepinephrine stimulates SDH activity in kidney, heart, liver etc. (Shivramakrishnan and Ramasarma, 1982).

In conclusion it could be stated that vagotomy in pigeons induced hyperglycaemia as reported earlier by (Pilo et al., 1977) and increased glycogenolysis, lipolysis and gluconeogenesis. In other words vagal denervation has removed the inhibition over these reactions or activated sympathetic system through which such reactions were enhanced. Almost similar metabolic response was shown by CDDP treated pigeons. Hyperglycaemia and hepatic glycolysis were reported in CDDP treated rats (Parikh, 1992). In the kidney of CDDP treated pigeons increased glycogenolysis, glucose output, glycolysis and gluconeogenesis were observed just as in the case of VgX pigeon kidney. CDDP treatment probably inhibits the cholinergic activity or stimulates the sympathetic system.

Many side effects of cisplatin were found to be attenuated by $CaCl_2$ treatment (Agarwal, 1983). Calcium deficiency or hypocalcemia must be taking place in CDDP treated animals (Schaeppi et al., 1982). In the present experiment, $CaCl_2$ treatment to CDDP treated pigeons protected the

metabolic reactions, especially glycogenolysis and glucose release as both phosphorylase and G-6-Pase activities declined. Glycogenolysis was also reduced in the $CaCl_2$ treated pigeon kidney while glycolysis was not much affected by $CaCl_2$ treatment to CDDP pigeons.

In the VgX pigeon kidney too, $CaCl_2$ has provided some kind of protection over the side effects. There was no improvement in glycogen value or glycogen-synthetase which were usually activated by vagal stimulation. However, activities of glycogenolytic enzyme phosphorylase and glucose releasing enzyme G-6-Pase were very much reduced by $CaCl_2$ treatment. Glycolysis as in the case of CDDP treated pigeon did not show any change while oxidative metabolism and gluconeogenesis showed decrease by $CaCl_2$ treatment.