# INTRODUCTION

Regulation of glucose homeostasis is a complex process involving several components such as (1) a monitoring system-central (hypothalamus) and peripheral (in tissues) (2) regulating factors-nerves and hormones (3) tissues that can release or store glucose and (4) tissues that can effectively take up glucose and store or utilize it. The role of each component and the mechanism involved are well known. However, an integrated approach to the understanding of the complex process of blood sugar regulation is comparatively new. The role of insulin and glucagon in the maintenance of glucose level in blood has been under minutest scrutiny ever since insulin was identified as a hypoglycemic agent. The knowledge that insulin 'insufficiency' (either lack of its production or its failure to act) caused diabetic condition in human beings opened up a flood gate of research. Insulin and glucagon barring catecholamines are the few hormones that can be released and act in a very short span of time. The control of release of these hormones into the blood is the basic mechanism in the regulation of blood sugar regulation. These hormones influence large number of tissues and cells but the action on the liver assumes an over all importance as the liver is one of the few that can effectively store and release glucose at a rapid rate and with ease. Thus, as Shimazu (1983) puts it, "the liver is a central organ for metabolic homeostasis". He further states that, "in maintaining the homeostatis of the glucose concentration, the liver plays a dominant role, since it can vary the amount of glucose by

pumping it into circulation or by removing it from the circulation, in accordance with the requirements of the body, requirements that are transmitted by both neural and hormonal messengers".

The liver as a central organ of metabolic homeostasis:

The liver is an astonishing organ with a multitude of functions that are mostly associated with metabolism. It has all the biochemical machinery to deal with carbohydrate, lipid and protein metabolism along with many specifically predominant ones such as glycogen storage, glucose release mechanism, lactate recycling, ornithine cycle, gluconeogenic mechanism, lipogenesis, amino acid deamination and lipolysis. There are many other hepatic functions that are indirectly connected with metabolism such as release of plasma protein and enzymes, storage of vitamins, bile pigment and bile salt production, detoxification, deactivation of biologically active substances (hormones and neurotransmitters). Because of the strategic position, blood supply and close association with digestive system, most of the liver functions are invariably entwined with nutrition and metabolism. The most important metabolic homeostasis in the body is that concerned with glucose. The blood sugar is maintained at a constant level because the brain itself has no control over the uptake or entry of it. The more the glucose level in the blood, the more its entry into brain through the

barrier. When the blood sugar level drops the rate of entry of glucose will fall due to the absence of an inducible uptake against a concentration gradient such as one that is operating in muscle, adipose or liver, mediated by insulin. So the blood sugar level is controlled with the help of liver and other tissues. When the blood sugar level increases as is the case during post-prandial period, insulin is released which in turn induces tissues to take up more glucose. When the level dips, the liver is induced by glucagon, or catecholamines to release glucose into the blood. When the glycogen reserve is over, the liver resorts to produce glucose through gluceneogenesis. The activation of gluconeogenic pathway is done by hormones such as glucocorticoids (corticosterone in birds). Thus, the liver performs several metabolic adjustments ultimately resulting in the withdrawal of excess of glucose from blood or in the release of glucose into the blood to cover up the deficit. This is the reason why the liver is adjudged the 'central organ of metabolic homeostasis'.

The liver has an intrinsic capacity to function as a glucose homeostatic organ free of hormonal and neural influences (Glinsmann et al., 1969; Bucols et al., 1974). "Hormonal and neural influences", as Lautt and Wong (1978b) state, "appears to be superimposed upon this intrinsic glucostatic ability".

# (I) NEURAL CONTROL OF HEPATIC METABOLISM

## (a) Hepatic innervation (Fig.1)

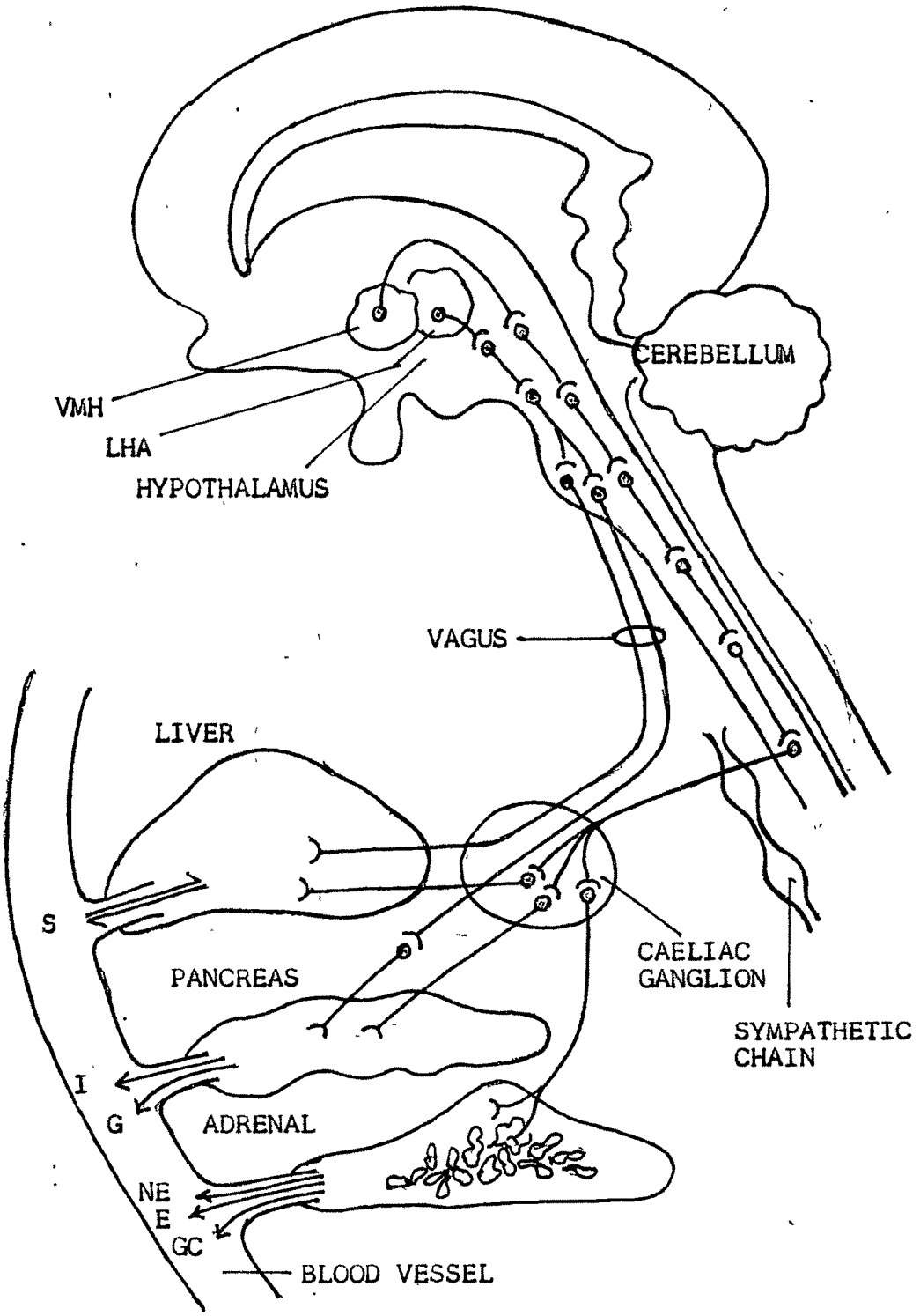
Several workers have given details of hepatic innervation (Alexander, 1940; Sutherland, 1964, 1965; Rappaport, 1975; Forssmann and Ito, 1977; Ito and Shibasaki, 1968; Nobin et al., 1977). Excellent recent reviews by Sawchenko and Friedman (1979), Lautt (1980) and Shimazu (1983) provide comprehensive picture and the present level of understanding of hepatic innervation. Nerves innervating the liver include parasympathetic, sympathetic and afferent fibres.

### (1) Parasympathetic and sympathetic fibres:

The parasympathetic fibres are from the right and left vagus nerves, and their pre-ganglionic fibres join the coeliac plexus. The sympathetic fibres to the liver are derived from the splanchnic nerves; pre-ganglionic sympathetic fibres reach the coeliac ganglia through the bilateral greater and lesser splanchnic nerves. The post-ganglionic fibres originate in the coeliac ganglia, forming a plexiform structure, the coeliac plexus. From the coeliac plexus, the hepatic plexus extends into the hepatic portal region. The hepatic plexus consists of post-ganglionic fibres of the splanchnic nerves via the coeliac ganglia and the pre-ganglionic fibers of the vagus nerves. The post-ganglionic parasympathetic fibers arise in the ganglion cells that are located close to the liver, i.e., in the hepatic

FIG. 1. SCHEMATIC PRESENTATION OF AUTONOMOUS NERVOUS SUPPLY TO LIVER, PANCREAS AND ADRENAL (Adapted from Shimazu, 1981).

VMH- Ventromedial hypothalamus; LHA- Lateral hypothalamic area; S- Sugar; G- Glucagon; I- Insulin; GC- Glucocorticoids; NE- Norepinephrine; E- Epinephrine.



hilus and within the portal spaces. Fibers from the hepatic plexus enter the liver via hepatic artery and portal vein and supply the blood vessels and the bile ducts throughout the liver. Some parasympathetic fibers from left vagus may enter the liver without passing through hepatic plexus. The hepatic plexus are subdivisible into anterior and posterior plexus, both communicating with each other freely. The anterior hepatic plexus is composed of rami of the left vagus nerve. The posterior hepatic plexus is composed of right coeliac ganglion and the right vagus.

Histofluorescence studies have established that the liver has a rich supply of both adrenergic and cholinergic fibres. The degree of innervation of each varies according to species. The liver of man, monkey, tree shrew and guinea pig has a predominant adrenergic supply, while rat and several species have more cholinergic fibres.

(2) Afferent Nerve Fibres

Afferent nerve fibres from the liver are believed to carry information to hypothalamus regarding metabolic, osmotic or barometric changes taking place in the liver (Sawchenko and Friedman, 1979). Most of these afferent fibres belong to vagus (Adachi, 1981; Carobi and Magni, 1981; Niijima, 1981; Adachi and Niijima, 1982). Hence, via vagal sensory fibres, glucose related information is supplied to the hypothalamus. This feed

back information also figures in the over all glucose homeostatic control by hypothalamus.

(b) Neural Regulation of Glucose uptake and Glycogen Synthesis

The role of Parasympathetic nerves in glycogenesis was elucidated by Shimazu (1967, 1971) and Shimazu and Fujimoto (1971) through experiments in which vagus was stimulated electrically. The resulting increment in glycogen synthetase activity even in pancreactectomized animals explained the direct influence of vagus on glycogen synthesis. This effect of vagus on glycogen synthetase was counteracted by simultaneous stimulation of splanchnic nerve (Shimazu, 1967, 1971). Electrical stimulation of vagus also increased the rate of incorporation of radio active glucose into liver glycogen with a concomitant decrease in G-6-P and UDPG. Since acetyl choline is the most common neurotransmitter of parasympathetic system, investigation of the effect of acetyl choline on glycogenesis was a natural sequel. Acetyl choline was found to increase the activity of glycogen synthetase in isolated rat liver (Ottolenghi et al., 1971) and in isolated hepatocytes (Akpan et al., 1974). Vagotomy in rats reduced the hepatic glycogen deposition after glucose administration (Mondon and Burton, 1971). Acetyl choline release or vagus stimulation could also effectively inhibit glycogen breakdown and net glucose output (Lautt and Wong, 1978a).

(c) Neural Regulation of Glycogen break down and glucose output

The involvement of sympathetic nervous system in the genesis of hypo-glycemia through the mediation of epinephrine released from the adrenal gland and glucagon from the pancreas was recognized very early in the neuroendocrine investigative history (Cannon et al., 1924; Gellhorn et al., 1941; Celander, 1954; Frohman, 1971). The direct evidence of sympathetic control of hepatic glycogenolysis came from the study of Shimazu and Fakuda (1965) who showed the increase of phosphorylase and G-6-Pase and a decreased glycogen content in the liver following electrical stimulation of splanchnic nerve. This direct action was confirmed when similar results were obtained even in adrenalectomized and pancreatectomized rabbits. The increase in phosphorylase activity was due to conversion of inactive phosphorylase 'b' to active phosphorylase 'a'. Hepatic phosphorylase responded rapidly to sympathetic stimulation than to catecholamine infusion indicating that there may be two separate phosphorylase activating mechanisms, one through sympathetic fibres (norepinephrine- $\alpha$-adrenergic) and the other through circulating catecholamines (epinephrine-$\beta$-adrenergic) (Shimazu and Amakawa, 1968 a,b). Splanchnic nerve stimulation led to a prompt rise in blood glucose level and depletion of hepatic glycogen content (Edwards and Silver, 1970; Edwards, 1971, 1972a,b). As Shimazu (1983) puts it, "The physiological significance of the neurally mediated glucose output is that it provides a rapid supply of

glucose to the circulation under emergency situation". Since sympathetic stimulation can also cause glucagon release from pancreatic A cells, there could be at least three separate mechanisms by which sympathetic nerve could induce hyperglycemic response : (1) directly through hepatic innervation on glycogenolysis and release of glucose (2) through release of catecholamines from the adrenal medullary cells and (3) by the release of glucagon from the pancreatic islets. The sympathetic nerve mediated phosphorylase activation in the liver was found to be cyclic AMP independent (Shimazu and Amakawa, 1975). They also suggested that $Ca^{++}$ ions may be involved in the activation of phosphorylase rather than cAMP in the sympathetic nerve stimulated mechanism (Shimazu 1981; Shimazu and Usami, 1982; Proost et al., 1979; Lautt, 1979; Seydoux et al., 1979; Hartman et al., 1982). The mobilization of intracellular $Ca^{++}$, instead of an influx of extracellular $Ca^{++}$, which also leads to cytosolic free $Ca^{++}$ increase, is reported to be the cause of phosphorylase activation by $\alpha$-adrenergic mediated sympathetic nerve stimulation.

Thus, it is clear that hepatic nerves could directly control glucose metabolism in the liver. Glycogenesis is activated by parasympathetic system (through the activation of glycogen synthetase) and glycogenolysis and glucose output are stimulated by sympathetic system (through the activation of phosphorylase and glucose-6-phosphatase).

## (3) Hypothalamic control of hepatic carbohydrate metabolism

### (a) Hypothalamo-hepatic neural mechanism

The knowledge that the liver glycogen metabolism is under hypothalamic control came from the study of Shimazu et al. (1966) who observed that the ventromedial hypothalamus (VMH) and lateral hypothalamic area (LHA) act reciprocally in regulating the blood glucose level and liver glycogen content in rabbits. Electrical stimulation of the VMH caused glycogenolysis in the liver while stimulation of LHA resulted in glycogenesis in the liver. The hyperglycaemic response following VMH stimulation has been confirmed by several workers (see Frohman, 1980; Oomura, 1980).

Further experiments established that electrical stimulation of the VMH causes glycogenolysis in the liver by rapid activation of phosphorylase, leading to a prompt rise in blood glucose, while stimulation of the LHA promotes glycogenesis in the liver by activation of glycogen synthetase which in turn leads to a slight decrease in blood sugar level. VMH stimulation in essence resembles sympathetic stimulation and LHA stimulation, that of parasympathetic (Shimazu, 1977; Shimazu et al., 1978; Ishikawa and Shimazu, 1976, 1980).

Direct application of acetylcholine or Carbocol on LHA produced a fairly specific activation of glycogen synthetase in the liver indicating that acetylcholine sensitive

(muscarinic) neurons in the LHA are involved in the regulation of glycogen synthesis in the liver. This effect was blocked by cholinoceptive blockade (atropine) (Matsushita et al., 1979; Shimazu et al., 1976; 1977a). The LHA must be receiving afferent inputs from the glucoceptive fibres in the liver. Szabo and Szabo (1972, 1975) and Szabo and Szabo (1975) reported that an insulin-sensitive glucoregulator centre in the hypothalamus is under cholinergic influence. Microinjections of small quantities of insulin into LHA and VMH result in rapid lowering of hepatic venous glucose concentration, the insulin effect being greater and more sensitive on the VMH than on the LHA (Iguchi et al., 1981; Storlien et al., 1975). The activation of liver phosphorylase to noradrenergic stimulation of the VMH was suppressed by the treatment of VMH neurons with Propranolol ($\beta$-adrenergic blocker) and not phentolamine ( $\alpha$-adrenergic blocker) (Matsushita and Shimazu, 1980) indicating that norepinephrine-sensitive neurons with $\beta$-adrenergic receptors in the VMH are involved in the regulation of liver phosphorylase.

(b) Hypothalamic control of endocrine pancreas

Recent studies have shown that stimulation or ablation of the hypothalamus in the VMH and LHA modify the secretion of both glucagon and insulin from the pancreas. This modulatory influence of hypothalamus is mainly exerted through autonomic nerves that regulate the release (either accelerating or inhibiting) of hormones from the islets. Secretion of glucagon

from A cells is enhanced by activation of the splanchnic sympathetic fibres, while secretion of insulin from B cells is inhibited by sympathetic stimulation (See Woods and Porte, 1974, 1978; Gerich et al., 1976; Miller, 1981). Electrical stimulation of VMH elicits a rapid rise in plasma glucagon with relative suppression of insulin release (Frohman and Bernardis, 1971; Frohman, 1980). Conversely, lesions of the VMH lead to an acute as well as chronic increase in the circulating level of insulin, and this effect of VMH lesion is due to increased activity of parasympathetic nervous system. VMH is the hypothalamic component of sympathetic system, and the destruction of VMH thus, leads to relative predominance of the parasympathetic nervous system. Indeed, hyperinsulinemia produced by VMH destruction has been shown to be reversed by sub-diaphragmatic vagotomy (Inoue and Bray, 1977; Berthoud and Jeanrenaud, 1979).

The effect of stimulation of LHA is not very consistent. LHA stimulation produces increased insulin and in some cases even glucagon release from pancreas. Since parasympathetic fibres also secrete several neuropeptides (such as C-terminal tetrapeptide amide of cholecystokinin and gastrin, Try-Met-Asp-Phe-$NH_2$), some of which are potent islet hormone releasers, the non-cholinergic, non-adrenergic stimulation of hormones is also possible (Rehfeld et al., 1980).

Chemical stimulation of VMH with norepinephrine (DeJong et al., 1977) or epinephrine (Shimazu and Ishikawa, 1981)

induced secretion of both glucagon and insulin. Microinjection of acetyl choline into the VMH resulted in a selective release of glucagon with parallel suppression of insulin release (Shimazu and Ishikawa, 1981). The release of glucagon following acetyl choline application on VMH was antagonized by hexamethonium and not atropine, indicating that the principal action of acetyl choline in this response was nicotinic and not muscarinic. Chemical stimulation of LHA with NE (DeJong et al., 1977) or epinephrine (Shimazu and Ishikawa, 1981) was found to induce a selective rise in the plasma insulin level without a significant change in the glucagon level. Vagotomy completely abolished the insulin response to norepinephrine activation of LHA (Steffens, 1981).

Apart from the release of insulin and glucagon, the adrenal action is also modulated by VMH and LHA through autonomic nervous system. Adrenalectomy did not inhibit the initial rise in glucose level after VMH stimulation but impaired the response after 10 and 15 minutes (Frohman and Bernardis, 1971).

"In view of these observations", states Shimazu (1983), "it seems clear that the hypothalamus has a dual mechanism of control of liver glycogen metabolism and glucose homeostasis: one mechanism is by direct neurogenic innervation of the liver via the VMH-splanchnic and the LHA-vagus nerve pathways ("hypothalamohepatic axis"), which directly controls the enzymes metabolizing glycogen in the liver and thus is responsible

FIG. 2. ROLE OF HYPOTHALAMIC CENTRES, AUTONOMIC NERVOUS SYSTEM AND HORMONES IN THE REGULATION OF BLOOD SUGAR LEVEL ( ADAPTED FROM SHIMAZU, 1983)

| HYPOTHALAMIC CENTRE | AUTONOMOUS NERVOUS SYSTEM/ NEUROTRANS-MITTER/ HORMONES | BLOOD SUGAR LEVEL | LIVER GLY-COGEN | PHOSPHO-RYLASE | GLYCOGEN SYNTHE-TASE | PLASMA GLUCAGON | PLASMA INSULIN |
|---|---|---|---|---|---|---|---|
| VMH | SYMPATHETIC NE & E | ↑ | ↓ | ↑ | | ↑ | ↓ |
| LHA | PARA-SYMPATHETIC ACh | ↓ | ↑ | ± | ↑ | ± | ↑ |
| | INSULIN | ↓ | ↑ | ↓ | ↑ | | |
| | GLUCAGON | ↑ | ↓ | ↑ | ↓ | | |

VMH- Ventromedial hypothalamus; LHA- Lateral hypothalamic area; NE- Norepinephrine; E- Epinephrine; ACh- Acetylcholine.

for the initial and fine regulation of metabolic changes. The other mechanism is the neural-hormonal regulation of glycogen breakdown and synthesis which involves neural stimulation of the release of pancreatic hormones ("hypothalamo-pancreatic axis"), and of adrenomedullary catecholamines ("hypothalamo-adrenal axis"), and which is responsible for prolongation or consolidation of metabolic changes rather than for their initiation".

## (4) Hormonal control of hepatic carbohydrate metabolism

### (a) Hormonal control of Glycogenolysis

Hepatic glycogen degradation may be rapidly stimulated by a wide variety of hormones which are released in response to different stimuli. Glucagon is the most effective hormone that could decrease glycogen in the hepatocytes, increase the phosphorylase 'a' activity and release glucose. Another well known catabolic hormone is adrenalin which stimulates glycogen degradation in the liver. Noradrenalin too could induce the same effect. Recently the peptide hormones such as vasoperessin, angiotensin II and oxytocin were found to be effective in bringing about activation of phosphorylase and hepatic glycogenolysis (Whitton, 1981). Parathyrin is another hormone which can stimulate release of glucose from the liver, but activation of phosphorylase has not been reported. A number of other hormones have been reported to affect hepatic glycogenolysis. Most of the gastointestinal hormones such as secretin, gastrin

and vasoactive intestinal polypeptide (VIP) promote hepatic glucose release. Some of these hormones mentioned above can also inhibit glycogen synthesis on a reciprocal basis. Net synthesis of glycogen in the liver of the starved rat is inhibited by vasopressin, adrenalin and oxytocin and parathyrin. Vassopressin, adrenalin and glucagon increase hepatic glycogen synthetase activity.

The direct action of many of these hormones mentioned above are experimentally seen in in vitro conditions. However, in in vivo conditions the action of each one of them depends on the presence or absence of many blood borne or putative factors, for example, the glycogenolytic effect of glucagon can be largely prevented by insulin, and in all possibility the concerted and antagonistic action of the two hormones plays a major role in hepatic glycogenolysis and glucose homeostasis. The glucagon : insulin ratio, rather than the absolute concentration of both hormones, is probably the controlling influence that determines the metabolic response of the liver. Insulin can also suppress adrenalin-induced glucose release as well as decrease the effects of submaximal concentrations of vasopressin on hepatic glucose release. However, it is not clear whether, insulin as a sole hormone can exert an anti-glycogenolytic action. In in vivo conditions insulin can suppress hepatic glycogenolysis, glucose release and phosphorylase activity, and in diabetic condition the glycogen synthetase

activity is depressed while phosphorylase activity is enhanced. Some of these *in vivo* effects of insulin are not reproducible in *in vitro* conditions and some of the *in vitro* effects are not seen in *in vivo* conditions. The presence or absence of antagonist or agonist and presence or absence of co-factors are believed to be responsible for these anamolies (Whitton, 1981). Interestingly the glucocorticoid could be a likely co-factor in the glycogen anabolic reactions, but then one could not reconcile with 'co-factor' effects of this adrenal steroid in the actions of glucagon, adrenalin and vasopressin.

(b) Hormonal control of Glycogenesis

Insulin is the only hormone that could facilitate glucose transport, increase phosphorylation and utilization of glucose for glycogen synthesis. The significant effect of insulin is thus on the glucokinase, glycogen synthetase, and glycogen phosphorylase. The two former enzymes are activated while the latter is inhibited by insulin. The activation or inactivation of glycogen synthetase and phosphorylase depend upon the concentration of cAMP. Increased cAMP concentration activates protein kinase which could phosphorylate glycogen synthetase and phosphorylase enzymes. When the 'active' glycogen synthetase 'a' is phosphorylated it becomes an inactive glycogen synthetase 'b'. On the other hand when 'inactive' phosphorylase 'b' is phosphorylated, the enzyme becomes an active phosphorylase 'a'. Insulin is known to decrease the concentration of cAMP through its action on

phosphodiestrase. The counteraction of glucagon effect by insulin is also mediated through the latter's cAMP suppression action. The possible effects of insulin in the regulation of metabolism, however, not only include changes in cyclic nucleotides, but also many other factors such as ionic movements, redox state of membrane thiol groups and the turn over of membrane phospholipid (Stalmans and Van de Werve, 1981). The most striking effects of insulin which are involved in glycogen synthetase are the changes in the concentrations of cyclic AMP and $Ca^{++}$. Others may be involved in glucose uptake, utilization of glucose for lipid synthesis, amino acid uptake and suppression of glucose release.

(c) Hormonal control of Gluconeogenesis

When blood glucose concentration becomes low, the liver produces glucose for the benefit of extra hepatic tissues that have an absolute requirement for the sugar. This glucose comes from glycogen and from precursors such as lactate, pyruvate, glycerol, some amino acids and fatty acids. The metabolic process by which glucose is synthesized from non-carbohydrate moieties is called gluconeogenesis. The role of gluconeogenesis in maintaining blood sugar level during starvation is obviously critical to animals, particularly when the liver glycogen stores are depleted. Gluconeogenic pathway is also useful in the utilization of amino acids. Gluconeogenesis is controlled by hormones such as glucagon, insulin, adrenalin, noradrenalin,

vasopressin, growth hormone and glucocorticoids.

Stimulation of gluconeogenesis by glucagon results from a concerted mechanism involving (1) stimulation of pyruvate transport and of pyruvate carboxylation in the mitochondria (2) cyclic AMP-dependent phosphorylation and inactivation of pyruvate kinase resulting in a rerouting of phosphoenol pyruvate towards glucose (3) decreased activity of phosphofructokinase resulting from the disappearance of its potent stimulator, fructose-6-diphosphate (4) probably an increased activity of fructose diphosphatase, and (5) stimulation of glucose-6-phosphatase activity resulting from the accumulation of glucose-6-phosphate (Hue et al., 1981).

Mainly, gluconeogenesis is stimulated by activating the specific gluconeogenic enzyme such as phosphoenol pyruvate carboxykinase (PEPCK). Glucagon, cAMP, adrenalin, and glucocorticoids are known to activate PEPCK. Activation of some of the transaminases and inactivation of pyruvate kinase are also key steps in the regulation of gluconeogenic pathway. Insulin is known to antagonize the effects of glucagon by preventing the accumulation of cyclic AMP and the cyclic AMP-dependent inactivation of pyruvate kinase.

(5) Neural and hormonal control of glucose transport across hepatic cell membrane

The metabolic processes in the liver such as glycogen deposition and breakdown, glucose production by glycogenolysis or gluconeogenesis and glucose release or uptake, are activated with respect to glucose level in the blood. The activation of these reactions and pathways are carried out by interplay of several neural and hormonal factors. However, some of these reactions and pathways are activated or inhibited by allosteric influence of glucose itself or other intermediates or even ions. The rate and total quantity of glucose entering the cell itself could activate or inactivate the reactions. In some cases, all that neural or hormonal factors do is to induce the movement of glucose across the membrane.

The uptake of glucose by the liver cells is the difference between the phosphorylation of glucose by glucokinase and the hydrolysis of glucose-6-phosphate by glucose-6-phosphatase. The activity of glucokinase is controlled both by the level of glucose in the blood and by yet poorly understood factors which influence the affinity of the enzyme for glucose (Hers, 1981). In isolated hepatocytes, the cooperative effect and the affinity for glucose can be modified by the ionic environment of the cell (Bontemps et al., 1978). A change in the concentration of glucose from 5 mM to 20 mM can increase the rate of glucose phosphorylation 3-6 fold, according to the proportion

of $K^+$ and $Na^+$ in the external medium. The entry of glucose into liver cells could be greatly influenced by ionic levels in extra and intracellular compartments. The electrolytic gradient differences could be altered by changing the permeability of the membrane and the resulting ionic movements could trigger the sodium pump activation. The extrusion of $Na^+$ and the influx of $K^+$ are active processes mediated by $Na^+$ $K^+$ ATPase. Glucose and amino acids are known to be moved into the cell interior together with $K^+$, a process commonly known as flow coupled transport. An alteration of the permeability of the hepatic membrane is all that is required for the flow coupled transport of glucose provided glucose is above the normal level.

In mammals, glucokinase is sensitive to insulin. Insulin is also known to alter $Ca^{++}$ levels. Acetylcholine can also release $Ca^{++}$ or increase $Ca^{++}$ movement into the cell. $Ca^{++}$ is believed to be involved in the mediation of several actions of neurotransmitters and hormones. In mammalian liver insulin is more effective in inducing glucose uptake while in avian liver, acetylcholine could be equally effective (Pilo and Patel, 1979). There are, thus several points of differences in the manner in which glucose transport into liver cells is induced in mammals and birds.

## The role of insulin and glucagon in the blood sugar regulation in birds

Birds tolerate a very high glucose level in the blood compared to mammals. One explanation to this fact is that birds have less insulin secreting B cells in the pancreas and the secretion of insulin in response to glucose is very slow (Hazelwood, 1973). The fact that insulin sensitive glucokinase is absent or negligibly present in the avian liver (Ureta,1972) could be a consequence of sluggish and restricted insulin release. Because of predominance of A cells in the pancreas (Sitbon and Mialhe, 1980), and the ease with which glucagon is released, birds respond quickly to hypoglycaemia. As such glucagon/insulin ratio is high in birds, and total pancreatectomy induces a fatal hypoglycemia due to decrease in glucagon level in the blood (Sitbon and Mialhe, 1980). A subordinate function is all that could be credited to insulin in the blood sugar regulation in birds. However, birds do respond to hyperglycemia as effectively as mammals. Direct action of parasympathetic, cholinergic nerve fibres on the liver (vide supra) probably could account for the efficient control of hyperglycemia in birds.

## The role of vagus or acetylcholine on the blood sugar regulation in birds

Avian liver has a rich cholinergic innervation and a high cholinesterase activity (Pilo, 1969). Vagal activity was found

to increase when glucose was injected, as judged from the fact that stimulation of LHA, the hypothalamic centre from where the vagal nerves originate, increased AChE activity (Woods and Porte, 1974; Pilo and Patel, 1977). Acetylcholine was also found to induce glucose uptake by pigeon liver (Pilo and Patel, 1978). Involvement of cholinergic vagal nerves in the uptake of glucose and thus in the regulation of blood sugar level in birds was suggested by Pilo and Patel (1979). Further confirmation came from the studies by Verma (1982) who reported that bilateral vagal transection delayed the onset of normoglycaemia or prolonged hyperglycaemia in pigeons after a glucose load. He also reported decreased glycogen content in the liver, decreased activities of AChE, glycogen synthetase, lipogenic enzymes, LDH and acid phosphatase, in vagotomized pigeons. Verma (1982) also showed that vagotomy in pigeons increased hepatic gluconeogenesis, lipolysis and glucose release. His studies also conclusively proved that vagus has a profound influence on the hepatic membrane permeability, as vagotomy produced an increased $Ca^{++}$ and $Na^{+}$, and decreased $K^{+}$ levels in the liver. The membrane permeability changes were evident from the decreased $Na^{+}$ $K^{+}$ ATPase activity, decreased $K^{+}$ content and increased $Ca^{++}$ and $Na^{+}$ contents in the liver of vagotomized pigeons (Verma, 1982).

Thus, the present state of understanding of direct neural involvement in the blood sugar regulation or hepatic

metabolism in birds is still sketchy. However, it is beyond doubt that vagal cholinergic nerves have a direct influence on the hepatocytes to induce an uptake of glucose, glycogen deposition and to inhibit glucose release. But the neural activity is a short term measure and cannot provide an all prevailing control over cellular functions in the presence of hormones or when physico-chemical microenvironment of cells are altered. In an *in vivo* condition, several factors such as hormones or the metabolic state of the liver itself could modify (suppression or acceleration) the action of nerves. In the present study, a somewhat detailed attempt has been made to fathom the agonism or antagonism of ions, several hormones and few other pharmacologically active substances towards acetylcholine and insulin under *in vitro* and *in vivo* conditions. Attempt is also made to derive tangible information regarding efficacy of cholinergic agonists such as choline chloride in vagotomized pigeons under *in vivo* conditions.

Since active transport of glucose involves movement of ions across hepatic cell membrane, the extracellular and intracellular concentrations of ions may exert specific influence on the glucose movement. Chapter 2 deals with the experiments with isolated liver slices incubated in the KRB medium with $Na^{+}$, $K^{+}$, or $Ca^{++}$ ions at hypertonic concentrations. The effect of ions on the glucose uptake or release was measured by estimating glucose in the medium before and after incubation.

To understand whether the effects of these ions singly or collectively on the glucose uptake or release are modified by acetylcholine or insulin, the slices were incubated with ions along with insulin or acetylcholine (Chapter 3). The movement of ions was found to be involved in the glucose uptake mediated by acetylcholine or insulin. Further evidence was obtained by inactivating pigeon liver slices with ACh or insulin in the presence of specific inhibitors such as ouabain and phlorizin (Chapter 4). To understand how far the actions of insulin or ACh are modified (inhibited or accelerated) by other factors, liver slices were incubated in KRB medium containing ACh or insulin along with thyroxine (Chapter 5), glucagon (Chapter 6), ACTH (Chapter 7) and dexamethasone (Chapter 8). The action of acetylcholinesterase inhibitors such as monocrotophos, acothion, and prostigmine, on the glucose movement in and out of liver cells was investigated, and presented in Chapter 9. The action of acetylcholine, choline chloride, insulin, glucagon, and dexamethasone on glucose tolerance in normal, sham operated and vagotomized pigeons was studied in order to understand how far vagal fibres are involved in modulating the actions of other hormones in the control of glycaemia in birds (Chapter 10). Recent reports show that the action of acetylcholine in tissues such as liver may be mediated through choline chloride, which is the degradation product of acetylcholine. To understand the action of choline chloride on the blood sugar regulation, glycaemic level as well as activities of several enzymes in the liver

were estimated in normal, sham operated and vagotomized pigeons (Chapter 11). The action of choline chloride on the membrane was investigated with the help of measurement of phospholipids and $Na^+$ $K^+$ ATPase in normal and vagotomized pigeons (Chapter 12).

Each chapter is prepared as seperate entity for clarity of expression and explanation. Under these circumstances, certain amount of repetitions become unavoidable in each chapter. The data obtained in each set of experiments are discussed within the scope of each chapter. However, interrelationships, generalized discussions and summing up of all data are presented in "General Considerations".