2 LITERATURE REVIEW

2.1 NITRIC OXIDE (NO)

The discovery that mammalian cells generate NO, a gas previously considered to be merely an atmospheric pollutant, is providing important information about many biological processes. Studies on the biological action of NO essentially began with the observation that NO gas, generated from an acidified nitrite solution, activated crude soluble preparations of guanylate cyclase [30]. In a series of pioneering experiments, Murad et al observed that NO could account for the ability of numerous chemically diverse, nitrogen containing compounds to activate cytosolic guanylate cyclase and elevate tissue levels of cGMP. The first observation that NO is a potent inhibitor of platelet aggregation came from Ignarro. In addition, Ignarro extended the requirement of tissue thiols for the vasodilator action of Nitroglycerin, forwarded by Needleman et al [31, 32].

With the findings that Nitroglycerin reacts with cysteine to yield s-nitroso cysteine, which is a labile but potent vascular smooth muscle relaxant that works through the action of liberated NO [32]. S-nitroso thiols were found to be labile precursors of NO that
activate cytoplasmic or cytosolic guanylate cyclase, elevate vascular and platelet levels of cGMP, and cause vascular smooth muscle relaxation, inhibition of platelet aggregation and profound hypotension in anaesthetized animals [33, 34].

NO can undergo numerous reactions, as it can act as both lewis acid and a lewis base. Since NO possesses an intermediate oxidation state, it can act both as an oxidizing and reducing agent. The electronic structure of NO has been represented as both of the following.

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\cdot &\text{N} \equiv & \cdot \text{O}
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I II

The resonating structure II is more favorable than structure I because structure I favors dimerization of NO. NO can be formed non-enzymatically in numerous laboratory reactions that would be most unlikely to occur in biological tissues. Enzymatic chemical reactions by which NO is generated are the likely source of NO in biological tissues. Enzymatic reactions known to generate NO involve azide anion (N\(^{-3}\)), hydroxyl amine (NH\(_2\)OH), hydrazine (NH\(_2\)NH\(_2\)), and
l-arginine. L-arginine can be converted to NO plus citrulline in a reaction catalyzed by an NADPH-dependant monooxygenase via enzyme NO synthase [35].

2.1.1 NITRIC OXIDE SYNTHASE (NOS)

In 1990 it has become apparent that there are two types of this enzyme one is constitutive, cytosolic, ca\(^{2+}\)/calmodulin dependant, and releases NO for short periods in response to receptor or physical stimulation. The NO released by this enzyme acts as a transduction mechanism underlying several physiological responses from vasculature, endothelial cells, platelets, epithelial cells, neuroblastoma cells and mast cells.

The other enzyme is induced after activation of macrophages, endothelial cells, and a number of other cells by cytokines and, once expressed synthesizes NO for longer periods. Furthermore, this enzyme is inducible (cytosolic), ca\(^{2+}\) independent and requires tetrahydrobiopterin as well as other cofactor, and its induction is inhibited by glucocorticoids [36].
**Similarities and Differences between the two NOS**

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<tr>
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<td>Cytosolic*</td>
<td>Cytosolic</td>
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<tr>
<td>NADPH dependant dioxygenase</td>
<td>NADPH dependant dioxygenase</td>
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<td>Inhibited by l-arginine analogues</td>
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<tr>
<td>Ca2+/calmodulin dependant</td>
<td>Ca2+/calmodulin independant</td>
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<tr>
<td>Picomoles NO released</td>
<td>Nanomoles NO released</td>
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<td>Short lasting release</td>
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<td>Unaffected by glucocorticoids</td>
<td>Induction inhibited by glucocorticoids</td>
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*-Evidence for particular form of this enzyme in the vascular endothelium has been reported.

**2.1.2 NOVEL INHIBITORS OF NOS**

L-arginine is a known synthesizer of NO via enzyme NOS. Other analogues of l-arginine have been described as inhibitors of NO generation. The various l-arginine analogues are as follows.

1. $\text{N}^G$- monomethyl – 1 - arginine (L-NMMA)
2. $\text{N}^G$- nitro – 1 - arginine (L-NNA)
3. N-iminoethyl – 1 - ornithine (L-NIO)
4. $\text{N}^G$- nitro – 1 - arginine methyl ester (L-NAME)
5. $\text{N}^G$- amino – 1 - arginine (L-NAA)
These enzymes induce enantiomerically specific effect in various tissues, and some enzyme is more potent than the others in various tissues. The different potency shown by these compounds in vitro and in vivo may also be due to differences in uptake, distribution, or metabolism of the compounds [37].

2.1.3 NO IN CARDIOVASCULAR SYSTEM

L-arginine was shown to be the precursor for the synthesis of NO by vascular endothelial cells, via the enzyme NOS. Data from experiments indicates that L-NMMA is a competitive inhibitor of the NOS. This compound has been a useful tool in the investigation of the biological significance of the l-arginine: NO pathway in the CVS.

NO released from non-adrenergic non-cholinergic (NANC) terminals may contribute to the regulation of blood flow and pressure [20]. NO also inhibits platelet aggregation by a mechanism dependant on cGMP and synergizes with prostacyclin, which inhibits the platelet aggregation by increasing their concentrations of cAMP [33]. Platelet themselves generate NO, which acts as a negative feedback mechanism to inhibit platelet activation.
NO may also be involved in the interaction of leukocytes with vessel walls, since it inhibits leukocyte activation [33]. Furthermore, it inhibits the proliferation of smooth muscle cells. NO participates in the general homeostatic control for the vasculature [34].

A decreased synthesis of NO contributes to the origin of conditions such as artherosclerosis and hypertension. Endogenous NO has been shown to inhibit the release of renin. NO releasing vasodilators inhibit proliferation of vascular smooth muscle cells by a cGMP-mediated process. Furthermore, NO has been reported to inhibit mitogen release from stimulated human platelets and sodium nitroprusside prolongs fibrinolysis, possibly by preventing the release from platelets of an inhibitor of tissue plasminogen activator.

2.1.4 NO IN CENTRAL NERVOUS SYSTEM

Accumulating evidence indicates that NO plays a part in the formation of memory [38]. In vitro, after specific receptor stimulation NO is released from a postsynaptic source to act presynaptically on one or more neurons in any direction. This leads to a further increase in the release of glutamate and, as a result, to a
stable increase in synaptic transmission, a phenomenon known as long-term potentiation, that is thought to be linked to memory formation [39]. NO may also have a role in vision [40], feeding behavior [41], nociception [42], and olfaction [43]. The L-arginine: NO pathway may also play a role in the pathology of the CNS. The Ca\(^{2+}\) influx that accompanies prolonged NMDA receptor activation is associated with degeneration of neurons. It is likely that excessive NMDA receptor activation, with the consequent increase in Ca\(^{2+}\) contributes to glutamate neurotoxicity by enhanced production of NO.

2.1.5 NO IN PERIPHERAL NERVOUS SYSTEM

In the gastrointestinal tract of rats, NO seems to mediate some forms of relaxation, including dilation of stomach to adapt to increase in intragastric pressure. In the taenia coli [44], the circular sigmoid muscle [45] in humans, inhibitors of NOS reduces electrically induced relaxation. The L-arginine: NO pathway is responsible for the relaxation of the corpus cavernosum and thus the development of penile erections in humans [46]. NO also contributes to the NANC vasodilatation and relaxation of guinea pig and human tracheal
Moreover, inhibiting the synthesis of NO in rats induces hyperactivity in the urinary bladder and decreases bladder capacity [37].

2.1.6 NO IN IMMUNITY AND INFLAMMATION

NO-dependent nonspecific immunity is a general phenomenon involving not only the reticuloendothelial system but also non-reticuloendothelial cells such as hepatocytes [47], vascular smooth muscle [48], and the vascular endothelium [49], in all of which the inducible NOS has been detected. The role of the lung and liver in NO-dependent non-specific immunity appears to be crucial, since both organs are strategically placed in the circulation to serve as immunologic filters [47, 49]. Increasing evidence indicates that NO may play a part in acute and chronic inflammation. Treatment with inhibitors of NOS reduces degree of inflammation in rats with acute inflammation or adjuvant arthritis, whereas L-arginine enhances the same [50, 51].

NO may play a part in tissue damage, for it may be cytostatic or cytotoxic not only for invading microorganisms but also for the cells that produce it and for neighboring cells. In some cases it may
interact with oxygen derived radicals to generate molecules that could enhance its cytotoxicity. There are also reports suggesting that both inhibitor of NOS and NO donors protects against some forms of injury. NO is therefore likely to have a multifaceted role in inflammatory reactions, ranging from the enhancement of vasodilatation and the formation of edema, through the modulation of sensory nerve ending and leukocyte activity to tissue cytotoxicity [52].

2.1.7 DEVELOPMENT OF NO AS A NANC TRANSMITTER

Two types of postsynaptic neurons, motor excitatory and motor inhibitory innervate the GI smooth muscle. The established physiologic neurotransmitter of the motor excitatory neurons is acetylcholine. The physiologic neurotransmitter/s of the inhibitory motor neurons that relaxes smooth muscle has not yet been identified. It is known however that these neurotransmitters are NANC [53].

The nature of the transmitter responsible for relaxation of contracted sphincters remained until, a controversial issue. ATP was first proposed as a possible transmitter, based on its ability to cause
relaxation and mimic inhibitory junction potentials that accompany the release of transmitter [10]. It proved difficult to demonstrate the presence of ATP or its release as a transmitter from nerve terminals. The role of ATP was progressively superseded by vasoactive intestinal peptide (VIP). VIP was shown to be a relaxant neuropeptide capable of eliciting low amplitude, slow inhibitory junction potentials. The projection of VIP neurons within the plexus and into the circular muscle layer was consistent with its role as an inhibitory transmitter [10, 54].

The discovery of a constitutive NOS, the enzyme responsible for the NO synthesis in neurons of the peripheral nervous system, raised the possibility that NO could also function as a transmitter. It was suggested soon that NO mediates relaxations elicited by NANC nerve stimulation [10, 11, 53].

Recent evidence suggest that NO serves as a NANC inhibitory transmitter in GIT, which is supported by the following –
1. Immunohistochemical studies have shown that the enzyme necessary for NO synthesis is expressed in enteric neurons. In vitro studies of muscles from all levels of GIT have also shown that arginine analogues, which inhibit NO synthesis, reduce inhibitory
effect of NANC neurotransmission. Effects of arginine analogues can be restored by addition of excess L-arginine, the substrate for NO synthesis. These data suggest that NO can be synthesized by enteric neurons [55].

2. Bioassays have demonstrated nerve-evoked release of a substance that has been identified as NO during NANC nerve stimulations. Oxyhemoglobin, known to bind to and sequester NO, also blocks NANC responses [56]. These data suggest that NO is released into extracellular fluid during nerve stimulation.

3. Addition of NO causes rapid hyperpolarisation of GI smooth muscle cells and relaxes muscle strips [57, 58]. These effects are similar to NANC nerve responses.

4. The pharmacology of NO and the NANC neurotransmitter in many preparations is similar [59].

In summary, it would appear that many of the criteria necessary for NO to be considered a neurotransmitter have been satisfied.
2.1.8 MECHANISM OF NO RELEASE IN GIT

NANC neurotransmission in the GIT is Ca^{2+} dependant and influx of Ca^{2+} into various sites during activation could increase synthesis of NO [58]. The NO produced would easily diffuse out of various sites and induce response in nearby effector cells as shown in figure.

**Schematic representation of NO release and action in GIT**
Schematics of various axon and smooth muscle are shown. Action potential involving various sites elicits inward Ca\(^{2+}\) current. Increase in Ca\(^{2+}\) in varicosity activates NOS. NO produced diffuses into extracellular space and then into nearby smooth muscle cells (for other cell type near varicosities). NO binds to hemoprotein and activates guanylate cyclase. Increase in cGMP can lead to activation of protein kinase G or have direct effect on ion channels. Although precise details of cGMP in dependant pathway are not yet known, an increase in cGMP is associated with decrease in Ca\(^{2+}\) sensitivity of contractile apparatus, and enhancement in potassium conductance i.e. production of inhibitory junction potentials. Either or both of these processes could contribute to NANC induced relaxations [59].

2.1.9  NANC TRANSMITTER IN GIT

2.1.9.1  LOWER ESOPHAGEAL SPHINCTER (LES):

Recent studies have quite convincingly shown that NO is probably that principle transmitter in LES in some species. Tottrup and colleagues [60] studied the opossum LES, a commonly used model of the LES, and found complete block of NANC nerve-induced relaxation with L-NNA. Similar experiment has been performed on
canine LES [61]. NO and field stimulation caused dramatic relaxation of muscle precontracted with 5-hydroxy-tryptamine. NO responses had a rapid onset and decayed rapidly.

### 2.1.9.2 STOMACH

Boeckxtaens and co-workers [62] found that L-NMMA and L-NNA increased basal tension in muscles of the canine gastric fundus, and these effects were partially reversed by l-arginine but not D-arginine. The fundus released a labile factor on electric stimulation that was bioassayed with rings of rabbit aorta from which endothelia had been removed. The factor was identified as NO or a NO donor.

### 2.1.9.3 SMALL BOWEL AND ILEOCOLONIC SPHINCTER

An early suggestion that NO serves as a NANC transmitter in GI muscles came from studies of longitudinal muscles of canine duodenum [57]. When prestimulated with bradykinin, these muscles relaxed in response to field stimulation of NANC nerves. The relaxation was blocked by L-NNA and by oxyhemoglobin and was reversed by l-arginine. Similar relaxations were produced by NO and nitroglycerin [56, 57].
The canine ileocolonic sphincter was one of the first and has been the most thoroughly characteristic preparations in terms of the role of NO as the NANC neurotransmitter. Exogenous NO caused concentration dependant relaxation of muscles from the ileocolonic junction [56]. L-NNA and L-NMMA also increase basal tension, and this effect was partially reversed by L-arginine but not D-arginine. The identity of the substance that mediates NANC relaxations in ileocolonic muscles was investigated with bioassays [56, 57].

2.1.9.4 COLON AND INTERNAL ANAL SPHINCTER

NANC responses were studied in intertaenia longitudinal muscles from the guinea pig colon [63]. L-NNA and hemoglobin reduced the amplitude of NANC mediated relaxations. L-NAME reduced NANC nerve induced relaxations in the distal colon and small bowel by 30-40%. NANC relaxations in the internal anal sphincter of the opossum also appear to be mediated by NO. L-NNA reduced relaxation responses to field stimulation and this effect were reversed by l-arginine in a concentration dependant manner.
In summary, it appears that many cells have the ability to synthesize NO and the summed output of this substance may have total inhibitory effects on GI muscles.

2.2 PHYSIOLOGY OF GASTROINTESTINAL MOTILITY

Control of GI function is accomplished by a diverse extrinsic autonomic innervation, sophisticated intrinsic neural system, extramural humoral influences, and a plethora of hormones produced by gastrointestinal organs themselves. Because drugs generally do not create any new physiological or biochemical functions they are useful only in so far as they modify the ongoing processes. In the GIT drugs modify mainly the various mechanisms that control secretion and motility [64]. The primary myogenic control of GI motility results from the electrical slow waves generated in the longitudinal layer of smooth muscle. Neurogenic control is exerted primarily via the neurons of the myenteric plexus and determines whether spike potentials will be initiated by the depolarization phase of the slow wave spike potentials all associated with muscle contractions [65].
Excitatory stimuli, such as release of acetylcholine from myenteric nerves, raise the level of excitability of the muscle and promote spike bursts and contraction. The distribution in time and space of contraction is determined by the slow waves or control electrical activity. Propulsion of intestinal contents requires a net pressure gradient and results from the sum of many separate events. These events require neural, muscular, and temporal integration for proper regulation of flow. The integration occurs over large periods of time and across large distances.

2.2.1 EXTRINSIC CONTROL
Neural control of GI motility occurs primarily from the activity of the intrinsic nerves, which are subject to modulation by the extrinsic innervation. Electrical stimulation of vagal or sympathetic nerves or local field stimulation can provoke both excitatory and inhibitory effects in the stomach and intestine. Signals over the extrinsic nerves originate in the CNS after appropriate afferent input [16].

2.1.2 INTRINSIC CONTROL
The most familiar intrinsic neural reflex of the intestine is the peristaltic reflex. The reflex requires simultaneous ascending
contraction and descending inhibition of the circular muscle [66]. The neural basis for descending inhibition has been established by intra cellular recording from the myenteric plexus [55]. In the cat colon, atropine can impair propulsion by selective blockade of the descending inhibition, which thus seems to contain muscarinic cholinergic receptor links. The descending inhibition associated with propulsion in the large intestine appears to involve a non-adrenergic inhibitory system [67].

2.2.3 HUMORAL CONTROL

The GI hormones deserve special attention as potential physiological modulators that are subject to pharmacological intervention. They may also be important as mediators of some indirect actions of existing GI drugs. The actions of catecholamine on GI motility have been reviewed previously [62].

Gastrin increases tone of LES, increase antral slow wave activity, increase force of antral contraction, yet delay gastric emptying and in relatively high doses increase spike bursts and contractile activity of intestine [68].
Secretin reduces gastric motility, delays gastric emptying decreaseintestinal motility and causes relaxation of the LES. As in the case ofgastric acid secretion, secretin appears to be a competitive inhibitor of the stimulant effect of gastrin on the LES. Cholecystokinin,caerulein, and the c-terminal octapeptide of cholecystokinin exhibitessentially identical diverse actions of GI motility. The intestinaleffect of cholecystokinin and related peptides seems similar in some respects to those of 5-HT, which may act upon intramuralcholinergic ganglia by activation of a receptor distinct from the nicotinic cholinergic terminals [69]. Gastric inhibitory peptide,which is secreted by the mucosa of the upper small intestine mainly in response to fat but to a lesser extent in response to carbohydrate,had a mild effect in decreasing motor activity of the stomach and therefore slowing the emptying of gastric contents into the duodenum when the upper small intestine is already oversupplied with food products [70]. Motilin a peptide containing amino acid residue has been extracted from duodenal mucosa. It stimulates gastric acid secretion [71].
2.3 DIABETES AND GASTROINTESTINAL MOTILITY

Abnormalities of gastrointestinal motility are common in diabetes mellitus, in particular diarrhea and disturbances of gastric emptying occur in patients with this disease [72, 73]. Although diabetes is associated with degenerative changes in the peripheral nervous system, there is conflicting information on the involvement of diabetic neuropathy in the pathogenesis of GI disorders [73]. Since knowledge of changes in gut motility during the progression of the disease might be important in the understanding of diabetic gastroenteropathy, stomach to caecum transit time (SCTT) was measured one to eight weeks after induction of diabetes in the rat. These times were chosen as raised levels of pancreatic glucagon after one week have been reported [74], whereas morphological and functional evidence for autonomic neuropathy is also a feature at eight weeks [75].

The pathophysiology of impaired gastrointestinal functions in both chronic diabetes and acute ketoacidosis [74], remains undefined, although, several mechanisms have been implicated. These include autonomic neuropathy [75], microangiopathy [73], changes in
insulin and glucagons release, and acute metabolic disturbance. In recent years various new GI hormones have been described, although their physiological importance remains to be determined [68]. It is likely that both the chronic and acute metabolic changes found in diabetes alter their effects on the gut. The myenteric plexus plays a crucial role in the motility of the GIT. Because morphological abnormalities of the small intestinal and colonic myenteric plexuses have been described in a variety of motor disorder such as chronic intestinal pseudo obstruction and severe idiopathic constipation [55].

The entire GIT may be involved in diabetic autonomic neuropathy features of dysfunction are manifested as reduced peristalsis and dilation of the esophagus, delayed gastric emptying, disordered small bowel movement, and atony of the large bowel, which occasionally results in gross dilation of the colon [76]. Clinical studies have provided conflicting information concerning the nature of changes that occur in the GIT during diabetes mellitus. While increased peristaltic activity rapid transit and decreased intestinal tone have been reported in diabetic diarrhea, another study has
shown delayed transit through the ileum and no change in intestinal tone in this condition [77]. The relationship of an abnormal postprandial colonic response and bowel dysfunction has been observed in the colonic disorder. In chronic idiopathic intestinal pseudo obstruction the intestinal transit also was delayed, and a similar diminution in the colonic response was noted [78, 79].

Previous studies suggested that GI motility disturbances in diabetes mellitus are due to a neural dysfunction [80], small intestine motility studies in diabetics with diarrhea have shown a decreased response to intestinal distention but a normal response to the administration of catecholamine or cholinergic drugs. Therefore, neural dysfunction may play an important part in GI motility disorder in diabetes mellitus, but the smooth muscle appears to be normally responsive. It has been suggested that diabetes may develop a significant abnormality in colonic motility presumably related to autonomic neuropathy of the GIT. Because the colonic smooth muscle can contract after maximal pharmacological stimulation, severely symptomatic patients may have a therapeutic benefit from drugs,
which act at the myoneural junction or directly on the smooth muscle [81].

In the early stages of diabetes variation in the gut hormones has also been reported namely glucagons, GIP and motilin. In man as well as in animal’s glucagons inhibits gastric secretion and gastric motility. The inhibitory effects of glucagon on the stomach seem to be a direct one, and not secondary to the hyperglycemia. The effect of glucagon on intestinal motility has previously been studied only in animals. Sporn and Necheles, studied gastric and colonic motility in non-anaesthetized dogs with the aid of balloon kymograph and found that glucagon inhibited motility for 35-40 min. after the injection. There is evidence that glucagon may have a wider role in the intestinal response to diabetes, pronounced changes in intestinal nutrient absorption have been reported in rats treated with pancreatic glucagons [82], and it has been suggested that this hormone may affect the increased nutrient transport in diabetes [83]. Glucagon, by its effect on motility, may also play a part in the hypertrophy, which is a characteristic feature of the diabetic small intestine [84].
2.3.1 HYPERGLYCEMIA AND GASTROINTESTINAL MOTILITY

Recently, several studies have shown that the gastrointestinal motor responses to various stimuli are impaired during acute hyperglycemia in both healthy subjects and diabetic patients. It has been demonstrated that acute hyperglycemia impairs esophageal peristalsis, reduces the LES pressure, delays gastric emptying, slows intestinal transit and reduces gall bladder contraction in response to various stimuli in healthy subjects. In diabetic patients gastric emptying and gall bladder contraction have been shown to be impaired during hyperglycemia with regard to the mechanism of action. It has been suggested that hyperglycemia may affect gastrointestinal function through vagal cholinergic inhibition of alteration in serum osmolality or perhaps by alterations in gastrointestinal hormone secretion [83]. The inhibitory effect of hyperglycemia on gastric motility has been recognized for many years. Buleto and Carson first reported that hyperglycemia inhibited hunger contractions in the fasted dog and insulin induced hypoglycemia produced gastric hypermotility that could be inhibited by intravenous glucose administration [85].
More recently, Macgregor et al [86] induced acute hyperglycemia intravenously in healthy humans during the ingestion of various liquid test meals. A significant decrease in the rate of gastric emptying of meals containing fat and protein was observed. Many of these studies, however, measured gastric emptying instead of motility, and the earlier studies measured motility using imprecise techniques. The infusion of concentrated glucose in normal volunteers caused a definite elevation in serum insulin levels. It is possible that hyperinsulinaemia secondary to hyperglycemia was responsible for inhibition of antral motility. This is unlikely because previous work indicates a stimulatory role for insulin on GIT motility [87].

Macgregor et al also found that injection of insulin into fasted dogs resulted in premature phase iii activity. Other investigators have noted disruption of the MMC (migrating motor complex) and development of intestinal hyperactivity characteristics of a feed like pattern during insulin infusion [86]. The mechanism responsible for the inhibitory action of hyperglycemia on gastric motility and plasma motilin concentrations in not known [88]. Cholinergic
blockade with atropine prevents the occurrence of spontaneous and motilin induced MMCs. Hence impairment of cholinergic transmission provides a common mechanism to inhibit gastric motility and plasma motilin. Recently it has been shown that glucose infusion suppresses efferent activity in the vagus nerve [89].

In experimental diabetes, nerve conduction velocity is impaired by hyperglycemia. The postulated mechanism involves alterations in tissue myoinositol and sodium potassium stimulated adenosine triphosphatase concentrations [90]. Hyperglycemia may cause reduced nerve myoinositol by competitively inhibiting sodium dependant myoinositol uptake, and increasing activation of the polyol pathway low tissue myoinositol leads to a decrease in sodium potassium stimulated adenosine triphosphatase activity, which results in slowed nerve conduction velocity. This may explain the impaired gastric motility observed during the hyperglycemic period.

Abnormal nerve conduction and clinical neuropathy in human diabetes are related to the severity of hyperglycemia [91]. Also measurements of abnormal nerve function in these patients have been shown to improve coincident with tighter blood glucose control.
The mechanism by which hyperglycemia induces changes in gastric emptying remains speculative. It has been reported that plasma motilin levels are suppressed when I.V. glucose is infused. Motilin has been shown to stimulate contraction of the stomach and also to affect intestinal motility [94]. It is the gastrointestinal hormone most likely to play a physiological role in altering gut motility, and hence the rate of absorption of nutrients and it is possible that changes observed with I.V. glucose are mediated via changes in motilin.

2.4 METHODS TO MEASURE GI MOTILITY

The propulsive performance of the intestine in vivo is usually assessed by measuring the transit of non-absorbable markers along the alimentary canal of animals [95]. Most available data produced by different laboratories use the long established charcoal meal test [96] or related methods with rats or mice.

In these methods, transit along the small intestine of non-absorbable marker fed by stomach tube is generally measured form the percentage of the total length reached by the marker in a given time. The marker may be introduced through a duodenal cannula in
chronically implanted animals, the presence or absence of the marker in the caecum can be taken as an all or none response, the slope produced by linear regression analysis of the cumulative percentage of radioactive marker passing through the each of several intestinal segments or the geometric center of the distribution of radioactivity throughout the intestine are the scored end points [97].

An animal model has been developed to investigate the mechanism of the ileal brake, using the breath hydrogen technique to monitor stomach to caecum transit and thus demonstrate the influence of ileal lipid infusion. In another experiment, rats were simultaneously housed in separate perspex chambers linked to the exhaled hydrogen monitor. Effluent gases from each chamber were analyzed serially at present intervals. Each chamber was perfused with room air at constant flow rate of 100 ml/min. The gases within the chamber were continuously mixed by means of small fans and sampled through perforated Perspex rods running the length of the chamber. Animals were anaesthetized with sodium pentobarbitone (60mg/kg) and prepared with chronic indwelling small intestinal cannulae exteriorized between the scapulae permitting the infusion of solution
into ileum or duodenum while monitoring breath hydrogen from within the chamber [98].

In Porereca and Burks (1983) method each mouse received a single injection of sodium chromate (0.5cr, 0.2ml / mouse). Animals were killed by cervical dislocation and stomach and intestine removed, divided into equal 10 portions and placed into culture tubes and radioactivity in each tube is determined by gamma counting for 1min. Transit or marker along the intestine was calculated using G.C.method [98].

Small intestinal transit was assessed in diabetic patients and healthy controls by measuring the breath hydrogen appearance time after the ingestion of lactulose. After an overnight fast, all subjects were given a solution of lactulose to drink (13 g lactulose as Duphaloc syrup / 20ml diluted with water to 130 ml.). Breath samples were obtained every 10 minutes by end expiratory sampling and analyzed for hydrogen content using the apparatus described by Bergman et al. sampling was continued with the subject inactive until a definite and sustained rise in breath hydrogen concentration was observed. The point was defined as the hydrogen appearance time [99].
2.5 EXPERIMENTAL MODEL OF DIABETES

In diabetes mellitus there is deficiency of insulin, the similar pathology is produced in rendering the animals diabetic. As insulin is secreted by beta cells of pancreas, insulin deficiency, therefore, can be produced by surgical removal of pancreas or by partial/total destruction of pancreas by means of drugs.

2.5.1 SURGICAL METHODS

2.5.1.1 TOTAL EXCISION OF THE PANCREAS:

Total excision of the pancreas can be successfully carried out in bigger animals like dogs and the cat. The smaller animals like mice and rat are not suitable for this procedure although rats are used occasionally. Diabetes after total removal of the pancreas is severe from the first day and persists at maximum intensity until the death of animals [99].

2.5.1.2 PARTIAL EXCISION OF THE PANCREAS:

Diabetes is produced by removing nine tenth of the pancreas. The remaining one tenth retained is that around the junction of wirsung’s canal with the duodenum. An important operational technique consists in retaining the unicinate process of the pancreas and
extirpating the rest. This part retains its normal blood supply through its own vascular pedicle and is then placed under the skin of the abdomen. The time period between subtotal pancreatectomy and the appearance of diabetes is shorter in animals left with a fragment of pancreas around wirsing’s canal because of the progressive sclerosis that involves that fragment of pancreas placed in the thickness of the abdominal wall. Onset of diabetes is variable. It depends on the various factors in particular on the number of beta insulin secreting cells that persist in the stump of pancreas, on the degree at their resistance to diabetogenic influence and on the diet of animals [99].

2.5.2 DRUG INDUCED DIABETES

2.5.2.1 ALLOXAN INDUCED DIABETES:

Diabetes can be produced in various species of animals by the intravenous injection of alloxan. The dose varies with species, age and route of administration. In dog the dose is about 50 mg/kg of body weight given intravenously. In rabbits it is 150 mg/kg body weight intravenously, in rat’s 60 mg/kg body weight intravenously [99]. The diabetes that appears after the injection of alloxan varies in intensity according to whether it has or not destroyed a significant
number of beta cells in the islets of langerhans. When alloxan is injected parenterally (I.V. or I.P.) a series of responses observed include

I. Hyperglycemia believed to be due to epinephremia and possibly to a direct effect on the liver.

II. Hypoglycemia which probably represent a response to a sudden release of insulin into the blood stream from damaged beta cells and finally,

III. Chronic hyperglycemia presumably due to irreversible beta cell damage [99].

It is proposed that alloxan produces diabetes mellitus in animals by virtue of

I. Its molecular shape resembling glucose molecules leading to their uptake by the glucose receptor in the plasma membranes of beta cells to damage them.

II. Its inhibitory effects on adenylyl cyclase and

III. Its effects on increasing the concentration of hydrogen peroxide, superoxide anions leading to the destruction of the pancreatic beta cells [99].
2.5.2.2 STEPTOZOTOCIN INDUCED DIABETES:
The dose varies with species, age and route of administration. The mode of action is by destruction of beta cells. In rats, the dose is about 50 mg/kg body weight intraperitoneally. Generally the streptozotocin is used in citrate buffer, pH 4.2. The onset of hyperglycemia is indicated within 24 hours of drug administration. In mice the I.V.dose of 200-mg/kg-body weight is reported to induce the highest percentage (80%) of diabetes and the lowest level of mortality (10%) [95].

2.5.2.3 GROWTH HORMONE INDUCED DIABETES:
Administration of growth hormone to experimental animals produces so-called idio-hypophyseal diabetes. This is not a permanent form of diabetes. The doses to be injected vary according to the animals. The repeated and prolonged administration of growth hormones produces so-called meta-hypophyseal diabetes, which is a permanent form of diabetes [99]. The characteristics resemble closely to alloxan diabetes. Pituitary growth hormones containing so-called diabetogenic factor causes variable degree of carbohydrate intolerance. The permanently diabetic animals have shown a
permanent reduction in the number and size of islets and reduction of the number of beta cells in each islet. Thus pituitary diabetes is also a form of pancreatic diabetes.

2.5.2.4  STEROID DIABETES:
Diabetes appears in certain species during administration of glucocorticoids especially if the animal has been sensitized to diabetes by a subtotal pancreatectomy. This is not a true diabetes mellitus because, this hormone produces only temporal shift in metabolism but no permanent damage sufficient to cause permanent diabetes after hormone is discontinued.

2.5.2.5  SPONTANEOUS DIABETES:
Certain animals, notably the dog and the Chinese hamster born to diabetic parents develop spontaneous diabetes [99] however this method is not routinely used to study the hypoglycemic agents.

2.5.2.6  IMMUNOGENIC DIABETES:
This type of diabetes can be produced by the I.V. administration of anti-insulin serum. This model of transient diabetes has limited to short-term experiments, so does not help to study or explain the
chronic degenerative diabetes mellitus. The disease is produced by immunologic inactivation of circulating insulin. The secondary changes in the pancreatic islets. Subsequent anti-insulin serum injection leads to compensatory attempt to increase insulin secretion [99]. While, chronic administration induces an inflammatory reaction containing eosinophils around the islets resembling the reaction found in the pancreas of an infant of a diabetic mother. Another approach is the administration of exogenous insulin with adjuvant in the hope, that the antibodies, which are produced, will be cross reactive and cause diabetes. It is well established that exogenous insulin, either homologous or heterologous, will produce antibodies to insulin in the most species [100]. However, attempts have been failed to produce permanent diabetes with this technique.

Looking into the above description we can say that it is evident that surgical procedures are laborious requiring special skill and larger animals like cat and dog while the drug induced diabetes can be easily induced even in smaller animals like mice and rats. In the present study alloxan was used in the dose 60 mg/kg body weight intravenously to produce diabetes in rats.