Review of Literature
1. GASTRIC ACID SECRETION AND ITS REGULATION

The term fermentation was used in the 16th and 17th century for the changes that occur in the stomach after intake of food. One of the first studies was conducted by Reaumur (1683-1757) in birds. He demonstrated that the gastric juice was able to digest meat and turn blue litmus paper to red [10]. Lazaro Spallanzani (1729-1799) performed many experiments on himself and studied the digestive processes. The chemical analysis of gastric juice was done by William Prout in 1824 and HCl was isolated from gastric juice [10]. In the beginning of this century, Russian Physiologist, Ivan Pavlov and Heidenhain contributed to the knowledge of the mechanism of gastric secretion. They devised various pouches in the stomach of dogs and by Sham feeding described the neural and humoral control of gastric secretion [11].

The concept of gastric acid secretion as a regulated rather than a facultative process has its origin on a fateful day in June 1822, when a shot gun accidentally discharged, wounding a 18 year old boy Alexis St Martin. Under the care of William Beaumont, the boy survived but was left with a permanent gastric fistula. Over the subsequent decades Beaumont was able to observe and record the secretion of gastric acid through the fistula. From these observations, Beaumont deduced that the acid secretion is tightly regulated by a variety of mechanisms including components of central nervous system, the enteric nervous system, hormones, paracrine agents and intracellular messengers [12]. Golgi in 1893 postulated that the gastric parietal cell was the source of acid secretion based on
his observation of expansion of intracellular canaliculus of the parietal cell during active secretions [13].

**i) CENTRAL REGULATORY MECHANISMS**

The initial observation that emotional or mental factors could stimulate acid secretion, combined with the classical studies of Pavlov [14], established the concept that the central nervous system (CNS) participates in the initiation of acid secretion.

Central structures identified as key participants in the regulatory process include the dorsomotor nucleus of vagus (DMNV), the hypothalamus and the nucleus tractus solitarius (NTS). The final integration of central stimuli appears to occur in DMNV, which supplies stimulatory efferent fibres to the stomach via vagus nerve [15]. Destruction of DMNV eliminates central stimulation of acid secretion [16]. Several sites in the hypothalamus have been identified as having potential stimulatory and inhibitory influences on acid secretion. The abundance of visceral sensory input to the CNS is critical to the continuous central regulation of gastric function and integration of CNS and peripheral mechanisms. Sensory information from the stomach is relayed to the CNS by both vagal and sympathetic afferent fibres [17]. The vagal afferent fibres are found in the smooth muscle layers and the mucosa of the stomach. The receptors in the muscle layers are primarily tension or stretch receptors capable of detecting motility changes. Although the receptors detect and regulate motility of the muscle layers, they also are involved in the vagovagal reflexes (so-called long reflexes) associated with distension-dependent
secretory activity [18], an important element in the peripheral regulation of acid secretion.

**ii) PERIPHERAL REGULATORY MECHANISMS**

Mechanisms intrinsic to the stomach itself are capable of initiating and regulating acid secretion. These peripheral mechanisms include neural, hormonal, paracrine and autocrine elements. The common goal of peripheral regulatory mechanisms, is to modulate the levels of the stimuli acting directly on the parietal cell. The major stimuli acting on parietal cells are acetylcholine, histamine and gastrin [19].

**Acetylcholine:** The only source of acetylcholine (ACh) that can act directly on parietal cell is from the post-ganglionic fibres to the enteric nervous system. Accordingly, the regulation of ACh release depends on the synaptic connections and transmitters controlling enteric nerve activity. In addition to the direct stimulation of parietal cells, ACh released from enteric nerve fibres serves to regulate the activity of several endocrine cell types and thus exerts an indirect influence on the parietal cell. The efferent fibres of the vagus do not innervate the parietal cell directly but synapse with ganglion cells of the enteric nervous system [20].

Regional release of ACh activates the parietal cells directly by binding to M₃ subtype muscarinic receptor [21]. ACh stimulates the fundic enterochromaffin like (ECL) cells to release histamine which in turn stimulates the parietal cells by binding to H₂ histamine receptors. In the gastric antrum, ACh stimulates the G cells to secrete gastrin into the circulation which travels to the fundus to stimulate the ECL
cells to release histamine [22]. In the antrum, ACh also inhibits the release of somatostatin from D cells, releasing the tonic inhibition of gastrin release and thus augmenting the direct stimulation of G cell. Thus, while the direct action of ACh on the parietal cell is significant, its more important role lies in the direct and indirect stimulation of histamine release from the ECL cells [19].

**Histamine:** The role of histamine in gastric acid secretion has been controversial since the discovery by Papielski that histamine is a potent stimulus for acid secretion. In the 1970s, Black et al postulated that histamine stimulated acid secretion through a novel histamine receptor, the H$_2$ receptor and successfully developed antagonists that were selective for this receptor [23]. These antagonists proved to be potent inhibitors of acid secretion. The use of H$_2$ receptor antagonists revolutionised the clinical management of ulcer disease. Moreover, the ability of a selective anti-histamine to block physiologically activated acid secretion demonstrated that histamine plays a central role in the stimulation of acid secretion. The failure to observe histamine in the plasma during acid secretion continued to raise controversy, specifically as to the source of histamine and how this stimulus reached the parietal cells. The emerging concept of paracrine regulation led to the suggestion that histamine could be released within the gastric mucosa and act locally without reaching sufficient concentration to be detected in the blood [24].

Initially it was proposed that histamine is released from mucosal mast cells, but more recent studies indicate that gastric histamine is released from a specialised endocrine cell of the stomach, the ECL cells. The release of histamine from ECL cells is regulated by a complex set of mechanisms involving neurocine, endocrine, paracrine and autocrine pathways. Histamine is released from ECL cells
in vitro by gastrin at physiological concentrations [22] Histamine release from isolated ECL cells is also stimulated by ACh and by β-adrenergic agonists and other drugs which elevate cellular cAMP [22].

Gastrin: In the adult, gastrin is found primarily in the G-cells of gastric antrum and duodenum, with small amounts being located in the pituitary and some vagal fibres [25]. Physiologically gastrin is released from the antral G cells by the presence of food in the stomach. At least three stimulatory pathways associated with the ingestion of food, have been shown to be involved in the release of gastrin central and neural activation, distension of the antrum and specific chemical components in the food. At cellular level these pathways regulate release of gastrin through the action of ACh, gastrin releasing peptide, somatostatin and direct chemical effects of H⁺ ion and amino acids [19].

The interaction of the three endocrine cells, ECL cell, G cell and D cell serves to regulate the release of histamine. Histamine together with ACh and possibly gastrin in turn activates the parietal cell to secrete acid. Despite the obvious complexity of the mechanisms involved, overall goal of these processes is to regulate the acidity of the gastric contents. The implication of this goal is that the regulatory mechanisms must be able to detect the intragastric pH and respond appropriately. The only known mechanism corresponding to this requirement is the suppression of gastrin by pH <3.0. In fact low pH can be detected by the antral D cell and release of somatostatin results in suppression of gastric secretion [19].
Pariental Cell Receptors: The pariental cells appear to possess a variety of receptors for both stimulatory and inhibitory modulators.

Histamine receptor: The stimulating action of histamine is mediated by H₂ receptor as demonstrated by pharmacological studies. Histamine stimulation of acid secretion is completely inhibited by selective H₂ receptor types, e.g., cholinergic or adrenergic, indicating that histamine acts directly on the pariental cell. In contrast, H₂ receptor antagonists inhibit, at least partially, acid secretion stimulated by cholinergic agents and gastrin suggesting that these secretagogues act, in part, through histamine [23]. The role of histamine in mediating cholinergic and gastrin stimulation of acid secretion may be explained largely by the action of these agents to release histamine from ECL cells.

The histamine H₂ receptor has been cloned and expressed. The deduced amino acid sequence shows typical features of a G-protein-coupled receptor. This is consistent with the observation that the H₂ receptor is coupled to activation of adenyl cyclase and the formation of intracellular cyclic AMP. The quantitative correlation between stimulation of acid secretion, activation of adenyl cyclase and accumulation of cAMP shown by a variety of H₂ receptor antagonists and agonists provides the strong evidence that the H₂ receptor is coupled to activation of adenyl cyclase [26].

Acetylcholine receptor: The non-selective cholinergic antagonist, atropine, is well known for its ability to inhibit acid secretion in vivo [18]. Belladonna had been used to treat dyspepsia since the time of Roman Empire. Atropine which is its major...
component, was the primary medical treatment for peptic ulcer before the
development of H₂ receptor antagonists. In vitro, cholinergic stimulation of acid
secretion is weak and often transient, particularly in the presence of H₂ receptor
antagonists. Only that portion of cholinergic stimulation which is not inhibited by H₂
receptor antagonists is blocked by atropine. These results suggest that there is
some direct action of ACh on the panetal cells in addition to the observed
interaction with histamine. Pharmacological characterisation of cholinergic
stimulation of acid secretion in vitro indicates that panetal cell contains a M₃
muscarinic receptor subtype. Interestingly, cholinergic stimulation of acid secretion
in vivo appears to involve M₁ receptor as evidenced by high sensitivity to
pyrenzepine [27]

Gastrin receptor: There are reports indicating a direct action of gastrin on
panetal cell in the presence of gastrin binding sites on gastric mucosal membrane.
These binding sites are characterized as gastrin type or CCK-B, sites in that they
show equal affinity for gastrin and sulphated CCK [19]

Miscellaneous receptors: Panetal cells appear to contain receptors for
somatostatin, prostaglandins and epidermal growth factor (EGF). The possible
actions of prostaglandin are complex. At relatively high concentrations,
prostaglandin E₂ has been shown to increase production of cAMP, whereas at lower
concentrations prostaglandins inhibit cAMP formation. This effect is most likely
related to the inhibition of acid formation [19]. The conflicting responses of cAMP
formation to prostaglandins might be explained by the presence of multiple receptor
subtypes [28]. In the case of EGF and prostaglandins, it may be speculated that
the inhibitors which are known mediators of wound healing, act only during periods of mucosal injury to reduce acidity and allow more rapid healing.

**Intracellular Coupling**

**Intracellular messengers:** It has been firmly established that the gastric histamine H₂ receptor is coupled to the formation of cAMP [26]. In contrast, activation of panetal cell M₃ cholinergic receptor or CCK-B receptor leads to an elevation of Ca, [29] The elevation of cAMP through the H₂ receptor stimulation appears to result from direct coupling, via G protein to adenyly cyclase while the elevation of Ca, proceeds by a more complex mechanism. The release of Ca, appears to occur via a regulated calcium channel, two types of which have been identified. In non-excitable cells, presumably including the panetal cells and the prominent endoplasmic reticulum (ER), calcium channel appears to be regulated by inositol 1,4,5-triphosphate (IP₃) [30] The long standing observation that cholinergic and gastrin stimulation of acid secretion is potentiated by histamine or agents such as phosphodiesterase inhibitors which elevate the cAMP suggest that cAMP and Ca, interact at some level. Thus histamine, which acts primarily to elevate cAMP, has been shown to produce a small transient elevation of Ca, in panetal cells [26]. Furthermore, buffering Ca, below basal levels inhibit secretory responses to histamine. Accordingly, it may be suggested that both cAMP and elevated Ca, are necessary for an optimal secretory response in that a permissive level of one second messenger is required for a full response from the other [19].

**Intracellular coupling reactions:** The only known coupling action of cAMP is to activate cAMP dependent protein kinase (PKA) [31]. In the case of panetal cells,
histamine has been reported to selectively activate a soluble type I PKA as indicated by the presence of catalytic subunit.

Several protein kinases are known to be dependent on or activated by calcium, including the phospholipid dependent protein kinases (PKC) and several calcium/calmodulin-dependent protein kinases (Ca M kinases) [32]. Ca M kinase acts early in the coupling sequence, but the exact role remains unknown. It is noted, however, that calcium can act at a variety of intracellular sites independent of protein kinases, and this adds a level of complexity for defining the coupling reactions associated with this second messenger. Because many proteins appear to undergo phosphorylation, it is not clear which one of them are true intermediates in stimulating acid secretion as opposed to parallel events such as metabolic activation or transcriptional regulation. Although, these parallel events may be important aspects of the overall activation process, they are not directly involved in the activation of proton pump [19].

**Gastric Hydrogen – Potassium – Adenosine Triphosphatase**

The membrane bound enzyme responsible for gastric H⁺ transport has been identified as a K⁺ stimulated ATPase. In the presence of ATP, Mg⁺ and K⁺, isolated tubulovesicles are capable of transporting H⁺ into the vesicular interior [33] suggesting a K⁺/H⁺ exchanger pump. The K⁺ activated ATPase co-purified with membrane vesicles are capable of ATP-dependent proton transport and was soon identified as the enzyme responsible for H⁺ transport by gastric membranes [19]. Selective inhibitors of H⁺-K⁺-ATPase are now available and one of these,
OMEPRAZOLE [34] has proved to be highly valuable for investigating the mechanism of gastric acid regulation.

Acid secretion is a discontinuous process, the rate of which is determined by its necessity following a meal. Consequently, the eventual result of the complex mechanisms for regulation of secretion is to activate H+-K+-ATPase. Substantial evidence has accumulated to indicate that activation of proton pump results from association of the H+-K+-ATPase with K+ permeability in the membrane of secretory canaliculus. It has been known for sometime that stimulation of the parietal cells leads to a change in the morphology of the cell involving the disappearance of cytoplasmic tubules and development of the secretory canaliculus [35]. The canaliculus forms as a result of incorporation of cytoplasmic tubules into the apical surface of the parietal cell. A clear consequence of this event is the translocation of H+-K+-ATPase molecules from the tubules into the canaliculus. When present in the cytoplasmic tubules, the H+-K+-ATPase is inactive as a proton pump due to lack of K+ access to the interior (extracystosolic) of the tubule. Translocation of the H+-K+-ATPase from the tubules to the canaliculus results in the activation of proton pump, since the canaliculus is permeable to K+ [19].

**Parietal Cell Homeostasis**

General consideration: The secretion of 160 mM HCl across the canalicular membrane of the parietal cell represents a significant challenge to the cell's ability to maintain pH and electrolyte balance. In the steady state, transporters located in the basolateral membrane must respond to this challenge by replacing the Cl− and removing the accumulated excess base. Secreting canalicular membrane contains
a KCl export pathway, a potassium reabsorptive pathway and a hydrogen secreting pathway (H⁺-K⁺-ATPase efflux pathway). Basolateral membrane contains a variety of potassium channels, perhaps a chloride conductance and Na⁺-H⁺ and Cl⁻-HCO₃⁻ exchangers in addition to H⁺-K⁺-ATPase (Hersy and Sachs [19]).

It is agreed generally that the activity of H⁺-K⁺-ATPase results in primary secretion of mM HCl into the secretory canaliculus. Because the H⁺-K⁺-ATPase is electroneutral [36], it is necessary that the KCl permeability pathway associated with the canaliculus, transfer a minimum of 160 mM KCl for each litre of fluid secreted. This is true whether the KCl pathway consists of conductive or electroneutral transporters. In fact, it is likely, that the KCl pathway allows transfer of a slight excess of KCl over the minimum required for the production of gastric HCl. In the absence of other mechanisms, the combined activity of the transporters at the apical pole of the parietal cell would rapidly lead to alkalinization of the cell and depletion of cellular Cl⁻ and H⁺. The potential disturbances in electrolyte balance are prevented by the activity of transporters at the basolateral membrane. These include an anion exchanger (AE), a Na⁺-H⁺ exchanger (NHE) and the H⁺-K⁺-ATPase [19].

Basolateral transporters: The existence of a basolateral AE in the parietal cell was postulated first by Rehm on the basis that conductance of the parietal cell for H⁺, OH⁻ and HCO₃⁻ was insufficient to account for the removal of intracellular base [37]. The simultaneous discovery of a mechanism in erythrocyte membranes that exchanges HCO₃⁻ for Cl⁻ led to the proposal that such a mechanism in the parietal cell could account both for the extrusion of base and for the replacement of secreted Cl⁻ [5]. The presence of a NHE in the parietal cells was demonstrated by
the finding that recovery of intracellular pH (pHᵢ) from an acid load is dependent on sodium in the medium and blocked by high concentrations of amiloride [38]. The two exchangers together with the H⁺-K⁺-ATPase appear to be the primary transporters responsible for maintaining the parietal cell homeostasis [19].

**Homeostatic response in the parietal cell:** Under non-secreting conditions, as with most cells, the parietal cells are most likely to maintain their electrolyte balance through activity of the sodium pump and nominal responses of the NHE and AE to incidental perturbations of intracellular pH [19]. Additionally, NHE activity is inhibited during steady state secretion [37] and this was attributed to the small rise of pHᵢ. Alternately, it has been proposed that the second messenger i.e. CAMP and Ca, that activate the proton pump may modulate the pHᵢ regulatory transporters directly by altering their pH set point [38]. Apart from a role in regulating pHᵢ in the resting parietal cell, NHE may also serve indirectly to supply K⁺ that is lost into the gastric secretion. Although the NHE activity is inhibited during secretion, it is not abolished. The exchange of cellular proton for Na⁺ by the NHE would lead to uptake of K⁺ by the H⁺-K⁺-ATPase and thus replace any net loss of K⁺ across the apical membrane. The responses of the AE, NHE and sodium pump are qualitatively sufficient to account for electrolyte balance in both the resting and the secreting parietal cell [19].
2. PROTECTIVE MECHANISMS OF GASTRIC MUCOSA

The gastric mucosa is under constant exposure to endogenous and exogenous injurious agents. The gastric mucosa possesses a number of defense mechanisms to protect itself from injury and eventual ulceration. The term "cytoprotection" was first introduced by Andre Robert in 1979 [6]. He used this term to refer to protection by prostaglandins against experimentally induced acute gastric lesions, in doses which do not affect gastric secretion in the rat. Now the term cytoprotection is used in a broader sense to mean protection against gastric mucosal injury by a mechanism other than inhibition or neutralisation of gastric acid. However it is found that surface cells are often not protected by cytoprotective agents though deep hemorrhagic necrosis is prevented. This made Szabo and Szelenyi suggest the term 'gastroprotection'. But the term "cytoprotection" continues to be more popular in literature [39].

i) MUCUS

The relative importance of mucus as a protective mechanism is still controversial. It has been shown that diffusion of hydrogen ions across mucus gel is four times slower than through a similar layer of water [5]. The mucus gel structure in patients with gastric ulcer has been found to be abnormal in that it contains less glycoprotein [40] and several cytoprotective drugs have been shown to increase mucus gel thickness like Carbenoxolone, prostaglandins, etc. Gastric mucus is a gel composed of 95% water and 5% mucopolysaccharides and glycoproteins. Mucus is produced from the cylindrical cells of the superficial
epithelium, by foveolar cells and by cells of glandular neck border, as well as mucous cells of gastric glands.

Mucus is released by cells producing it via three mechanisms: (a) continuous exocytosis of a few granules at any given time; (b) explosive release from apical portion of older cells and (c) only minimally as a consequence of cellular exfoliation in the gastric area. The production of mucus is stimulated by both nervous and humoral mechanisms. Former includes stimulating action of the vagus and local action of the cholinergic mediators [41]. Way back in 1962, Hollander had observed that electrical stimulation of the coeliac ganglion resulted in viscous mucus secretion; a rapid stimulation of vagus produced a non-acid and viscous juice, whereas a prolonged stimulation resulted in production of hydrochloric acid and pepsin [42]. Some prostaglandins, (PGs) particularly PGE₂, has been shown to cause increase in the production of mucus in the rat. This has been confirmed by the evaluation of NANA (n-acetyl neuramic acid) and hexosamines in the gastric juice; the presence of which increases after the administration of synthetic PGs [43].

Gastric mucus possesses numerous properties that contribute to the defense of the mucosa. Because of its physicochemical characteristics, mucus exerts a lubricating action that facilitates the progress of alimentary bolus and protects the mucosa from the mechanical insult of ingested food. This function, defined as “indirect or passive protection” was attributed to the mucus gel adhering to the epithelial surface, as far back as the 1950s by Hollander [44].
The secondary role, the active protective property of mucus is by maintaining a pH gradient between the surface of epithelial cells and the gastric lumen. The presence of pH gradient for the gastric and duodenal mucosal surface was shown experimentally by using micro electrodes in the rat [45]. In fact, it has been observed that when the intra luminal pH is between 2 and 3, the pH level in the superficial epithelium is near neutral [46]. The quality of pH gradient depends on (a) secretion rate of bicarbonates; (b) the rate of diffusion of bicarbonate secretion through the mucus; (c) the rate of diffusion of acid-secretion through the mucus; (d) the thickness of mucus layer that depends directly on the balance between its synthesis and its mechanical and chemical degradation. The alteration in any of these elements can reduce the quality of pH gradient, thereby rendering the mucosal defense less effective. Another important property of mucus is its ability to direct hydrogen ions towards the lumen, give the presence of a flux to other cations (potassium, sodium) from the lumen towards the mucosa. This is because the mucus that originates from the mucous cells of the glandular neck border is very rich in sulphate groups. This allows the formation of a channel that ensures the conduction of hydrogen ions towards the gastric lumen, thus avoiding damage to adjacent cells.

Many studies have provided evidence that surface epithelial cells have intrinsic barrier properties and play an important role in the first line defense of the stomach. Although as far back as 1933 to 1939, Teorell observed the presence of an ionic flux through gastric wall [47], the expression of mucosal barrier was better defined later on, during the period between 1940 and 1953, by Glass, Boyd and Hollander. Hollander mentioned the concept of “mucus barrier”, identifying it as
components of the layer of secreted mucus deposited upon the superficial epithelium and the mucus deposited in the areas of superficial cells themselves. In 1964, Davenport et al describing the phenomenon of hydrogen ion back diffusion in an experimental model in the dog, coined the term “mucosal barrier” [48]. The apical membrane or tight junctions between epithelial cells are relatively impermeable to hydrogen ions and therefore form a physical barrier to back diffusion of acid [48].

More recent studies [49] have shown the existence of surface active phospholipids which form a hydrophobic lining on the luminal surface of the gastric epithelium and retard the passage of water soluble ions such as hydrogen ions. NSAIDS have been shown to eliminate surface hydrophobicity and disrupt the mucosal barrier to hydrogen ions. On the other hand, cytoprotective agents like prostaglandins increase the concentration of surface active phospholipids [50].

Flemstrom in 1977 [51] first demonstrated the existence of bicarbonate secretion from fundic and antral mucosa which occurs by a metabolically dependent process as well as by passive diffusion. Vagal stimulation increases both acid and alkali secretion. ‘This alkaline tide’ during hydrogen ion secretion increases bicarbonate delivery to the surface epithelium. However, the rate of bicarbonate secretion is only 5-10% of the maximal acid output [52]. Thus bicarbonate alone cannot lower sufficiently the hydrogen ion concentration but it can complement the action of mucus, forming what is known as the “mucus-bicarbonate barrier” [53]. This has been confirmed experimentally using pH sensitive micro-electrodes which have shown a marked pH gradient from lumen to cell surface [54].
ii) **GASTRIC MOTILITY**

Various studies have suggested that changes in gastric motility may play a role in the development and prevention of experimental gastric lesions. As the lesions occur at the site of greatest mechanical stress 'mucosal compression' by gastric hyper contraction probably accounts for necrosis and ulceration of epithelium [55]. Studies using prostaglandins and mast cell stabilisers, as well as sulphydryl compounds have confirmed that inhibition of gastric motility is associated with their cytoprotective action in the rat [55].

iii) **MUCOSAL BLOOD FLOW**

The capillary net work of mucosa arise from an arterial plexus at the glandular level. The capillaries run parallel in close proximity to the gastric glands, converge and drain into venules at the subepithelial luminal level. These venules pierce the mucosa without receiving further tributaries. The parietal cells produce equimolar amounts of H⁺, delivered to the gastric lumen and HCO₃⁻ which probably diffuses to nearby capillaries and enters the blood stream. Because of the microvascular architecture, HCO₃⁻ produced at the various levels of gastric gland will be directed towards luminal subepithelial stratum of the mucosa before reaching the venous return. According to this, HCO₃⁻ concentration is expected to be highest near the luminal surface where it is most needed to neutralise back diffusion of H⁺ ions [56].

Injurious agents usually attack the mucosa from the luminal side, and there are no blood vessels between the luminal fluid and the surface epithelium. Therefore, blood flow, however high, cannot prevent injurious substances in the
gastric lumen from reaching surface cells, although the flow may reduce the damaging effect by removing injurious agents penetrating into the mucosa. Injurious attack may take place under basal conditions where mucosal blood flow is low, and it is possible that vasodilatation is triggered by some cellular damage. Therefore, blood flow may not be an important factor in protection of the surface mucous cells. Mucosal injury leads to dilatation and increased flow in mucosal blood vessels left intact after damage. Increase in blood flow accompanying gastric mucosal damage is of vital importance for protection of gastric glands and for restitution of the surface epithelium after destruction of epithelial lining [57]

**iv) ENDOGENOUS MEDIATORS OF GASTRIC CYTOPROTECTION**

Prostaglandins: Prostaglandins were the first endogenous compounds implicated in gastric cytoprotection [6]. Prostaglandins are derived from arachidonic acid, a fatty acid into endoperoxides, which are further metabolised into various prostaglandins (PGs). Several PGs have been shown to be synthesised by the gastric mucosa. Endogenous PGs E$_2$, F$_2$\textalpha, D$_2$ and I$_2$ in the gastrointestinal tract play an important role in preventing mucosal ulceration [58]. Gastroduodenal protection by PGs include both increase in mucosal resistance as well as decrease in aggressive factors mainly acid and pepsin. The term protection by PGs may apply both to the prevention of imminent damage and the enhancement of mucosal repair mechanisms after injury [59]. Enhancement of endogenous protective PGs may also account, in part, for the mechanism of action of other protective drugs such as sucralfate, bismuth and carbenoxolone [60].
The naturally occurring PGS are short acting, but their synthetic analogues have improved biological activity and long duration. Increased mucosal blood flow by prostaglandins has been suggested to be responsible for their gastroprotective effect [61]. However, various other mechanisms have also been postulated like dilution of noxious agents by prostaglandin-stimulated mucus secretion, stimulation of basic bicarbonate secretion, increase in concentration of surface active phospholipids, stimulation of cyclic AMP, stabilisation of lysosomes, decrease in gastric motility and dissolution of gastric mucosal folds and maintenance of mucosal sulphydryl groups. Prostaglandins probably also have a repair function by stimulating rapid resolution of disrupted surface epithelium (Fig.1) [8]. It has been shown that prior exposure of gastric mucosa to mild irritants protects it from damage by more noxious agents. This "adaptive" cytoprotection is mediated by prostaglandins [62].

Sulphydryls: Szabo et al observed that naturally occurring sulphydryl (SH) containing amino acids L-cysteine and methionine, as well as sulphydryl containing drugs protect rats from ethanol induced gastric lesions whereas sulphydryl blocking drugs counteract the cytoprotective effect of PGE\textsubscript{2}. They proposed that endogenous sulphydryls may be one of the mediators of cytoprotection [63].

Epidermal growth factor: This potent polypeptide inhibitor of gastric secretion is found in salivary glands as well as other sources such as duodenal mucosa and pancreas [64]. Studies have shown its efficacy in preventing stress ulcers in rats. At present, however, its exact role in human gastric cytoprotection is still being elucidated [65].
Fig. 1. Diagrammatic representation of gastric cytoprotective mechanisms [8]
v) **FREE RADICALS**

Disturbances of the balance between the production of reactive oxygen species such as superoxide, hydrogen peroxide, hypochlorous acid, hydroxyl, alkoxyl and peroxyl radicals and antioxidant defenses against them produce oxidative stress. Reactive oxygen species are constantly produced during normal aerobic metabolism and are safely removed by a variety of biological antioxidants [65].

Aerobic life uses oxygen to oxidise carbon and hydrogen rich substrates to obtain the chemical and heat energy essential for life. When oxygen is reduced by the stepwise addition of electrons, two free radicals ($\text{HO}_2^*$, $\cdot\text{OH}$) are formed, together with $\text{H}_2\text{O}_2$. At pH 7.4, the hydroperoxyl radical ($\text{HO}_2^*$) with a pKa of 4.8 (the pH value at equal concentration of acid ($\text{HO}_2$) and base ($\text{O}_2^-$) are present) dissociates to give the superoxide anion radical.

$$\text{O}_2 + e^- + \text{H}^+ \rightarrow \cdot\text{HO}_2^- (\text{hydroperoxyl radical})$$

$$\text{HO}_2^* \rightarrow \text{H}^+ + \text{O}_2^- (\text{superoxide radical})$$

$$\text{O}_2 + 2\text{H} + e^- \rightarrow \text{H}_2\text{O}_2 (\text{hydrogen peroxide})$$

$$\text{H}_2\text{O}_2 + e^- \rightarrow \text{OH}^- \cdot\text{OH} (\text{hydroxyl radical})$$

$$\cdot\text{OH} + e^- + \text{H}^+ \rightarrow \text{H}_2\text{O}$$

The hydroxyl radical is the most powerful oxidant formed in biological systems and can rapidly attack any biological molecule. Hydroxyl radicals can attack polyunsaturated fatty acids to initiate lipid peroxidation.

The detection and measurement of lipid peroxidation is the evidence most frequently cited to support the involvement of free radical reactions in toxicology.
and disease. When pro-oxidants increase or antioxidants fail, a situation of oxidative stress ensues that leads to excessive molecular damage and tissue injury. Antioxidant is a substance that, when present at low concentrations, compared with those of the oxidizable, considerably delays or inhibits oxidation of the substrate. Antioxidants can act by (a) removing oxygen or decreasing local oxygen concentrations; (b) removing catalytic metal ions; (c) removing key reactive oxygen species such as superoxide and hydrogen peroxide; (d) scavenging the initiating free radicals such as hydroxyl, alkoxyl and peroxyl species; (e) breaking the chain of an initiated sequence or (g) quenching or scavenging singlet oxygen [66].

Vitamin E (α-tocopherol), a fat soluble vitamin, is a poor antioxidant outside a membrane biolayer but is very effective when incorporated into the membrane. Antioxidants like vitamin E and selenium have been shown to have a protective effect on gastric mucosa against stress and chemically induced lesions [67]. β-carotene is a lipid soluble radical scavenger and ascorbic acid is an extracellular antioxidant scavenging free hydroxyl radical [66].

**vi) DECREASED RELEASE OF ENDOGENOUS MEDIATORS OF GASTRIC INJURY**

Mast cell stabilisers, vasoactive amines and leukotrienes have been proposed as endogenous mediators of acute gastric mucosal damage. Since the two products of arachidonic pathway, prostaglandins and leukotrienes have opposite effect on gastric mucosa it is possible that a balance between production of prostaglandins and leukotrienes may play an important role in mucosal integrity.
There is experimental evidence to indicate that decreased synthesis of leukotrienes may be more significant as compared to increased levels of prostaglandins [68].

**vii) CELLULAR TURNOVER**

Production of mucus and bicarbonates and cellular turnover are the fundamental mechanisms of cytoprotection. The mucous cells, particularly their number and constant turnover are anatomically the most relevant factor of cytoprotection [69]. These cells constantly produce mucus, composed of glycoproteins rich in carbohydrates, which, along with bicarbonates, form a continuous layer protecting the mucosa from corrosive agents.

The gastrointestinal tract and hemopoietic cells reproduce at a rapid rate. In the gastrointestinal epithelium, new cells develop in the lower portion of the glands and thereafter migrate upwards, thus causing the older cells to exfoliate. In this way, the superficial epithelium is completely renewed in matter of days [70]. The gastric surface is covered by a single layer of mucus-secreting cells, 20-25 μ thick, and generally deeper in the glandular crypts layered by parietal cells and all other mucous cells. Mucous cells of the glandular neck act as stem cells for all other types of cell. In the gastric mucosa of man, under normal conditions, 560,000 cells are exfoliated every minute in the gastric lumen. Entire turnover cycle lasts an average of 24 to 48 hours [69].

**viii) SURFACTANT**

In 1990, Hills demonstrated an oligolamellar lining which is probably phospholipid, on the gastric mucosal surface of the rat by transmission electron microscopy using fixation procedures specially developed to avoid the destruction
of hydrophobic surfaces [71]. Mammalian gastric mucosa is usually hydrophobic or non-wettable which may be an essential biophysical characteristic of the gastric mucosal barrier. This property may be attributable to an absorbed layer of surface active phospholipids (SAPL). Electron microscopic studies revealed that mucous cells and surface epithelial cells contain inclusion bodies associated with various subcellular organelles, e.g. nuclear envelope, endoplasmic reticulum, Golgi apparatus and its vesicles and mucus secreting granules [72].

Surface active phospholipids, chemically similar to pulmonary surfactants have been identified by thin layer chromatography in gastric juice and on the mucosal lining of five tissues in the canine gastrointestinal tract. These include oesophagus, oxyntic tissue, mid-duodenum, mid-jejunum and mid-colon. The most prominent mucosal phospholipid of GIT includes phosphatidylcholine (34-45%) and phosphatidylethanolamine (18-32%). The gastrointestinal mucosal surface also contains lesser amounts (5-10% each) of sphingomyelin, lysophosphatidylcholine, phosphatidyl inositol and phosphatidyleserine and small but detectable quantities of phosphatidyl glycerol and phosphatidic acid [73].

Several important properties have been attributed to the mucosal layer adjacent to the wall of the gastrointestinal tract, including the ability to lubricate the movement of intraluminal contents [74] and to act as a barrier to autodigestion and ulceration. The adsorbed surfactants at the pleural surface have also been identified in light scraping from gastric mucosal surface after removal of mucosal surface layer [75]. It is, therefore, conceivable that these phospholipids are acting as boundary lubricants along the gastrointestinal tract to facilitate motility at any point where the mucous lining has been penetrated or is pathologically absent. The same phospholipid has also been implicated in the cytoprotection of the gastric mucosal barrier.
3. PATHOGENESIS OF PEPTIC ULCER

Schwartz in 1910 stated that "peptic ulcer is a product of self digestion". It results from an excess autopeptic power in gastric juice over the defensive power of gastric and intestinal mucosa. Gastric and duodenal ulcer or peptic ulcer disease (PUD), Zollinger- Ellison Syndrome (ZES) and gastroesophageal reflux disease (GRD) are upper gastrointestinal disorders sharing a common abnormality; too much acid and pepsin activity for the degree of local tissue resistance. Gastric hypersecretion appears to be the primary causative event at one end of the disease spectrum as 93% of the patients afflicted with Zollinger Ellison Syndrome is characterised by single or multiple non-beta islet cell adenomas of the pancreas which release large amount of gastrin into the plasma resulting in 10-20 fold increase in the amount of acid secreted and a high incidence of ulcers. At the opposite end of the spectrum acid secretion plays a lesser role and mucosal resistance becomes more important. Some acid is, however, always required as peptic ulcer disease rarely develops in patients of achlorhydia. Many patients with gastric ulcer have normal or low acid secretion. These observations implicate altered mucosal defence in ulcer formation [76].

The other causes of peptic ulcer disease include Helicobacter pylori infection, non-steroidal anti-inflammatory drugs (NSAIDs) and malignancy. Helicobacter pylori is a gram negative spiral bacterium found in association with the gastric epithelium. It has been demonstrated in 90% of patients with duodenal ulcer and above 75% of gastric ulcer patients [77]. The NSAIDs when administered orally cause local irritation, allow back diffusion of acid into the gastric mucosa and
induce tissue damage, whereas parenterally administered NSAIDs can also cause gastric mucosal damage and bleeding correlated with the inhibition of the biosynthesis of gastric prostaglandins (PG) especially PGI and PGE [4].

i) CAUSES OF PEPTIC ULCER

Increased acid secretion: The formation of peptic ulcers depends critically on the presence of acid and peptic activity in gastric juice. About one-third of patients with duodenal ulcer, secrete excess gastric acid. Schwartz’s dictum “no acid, no ulcer” is more accurate if modified to “no acid and no peptic activity, no ulcer” as acid without pepsin has little digestive power. The dependence of peptic activity is supported by the therapeutic effects of the antacids and antisecretory drugs, anticholinergics, H₂ blockers and also the antisecretory drug omeprazole which completely inhibits the secretion of acid by blocking the hydrogen-potassium adenosine triphosphatase. However, these ulcers rapidly recur when therapy is stopped, reflecting the non-curative nature of antisecretory therapy alone [4].

Impaired mucosal defense: Peptic ulcer is a product of self-digestion and it results from an excess of auto-peptic power in gastric juice over the defensive power of gastric and intestinal mucosa. Two major factors appear to disrupt mucosal resistance to injury: NSAID and H. pylori infection. NSAIDs have been shown to eliminate the surface hydrophobicity and disrupt the mucosal barrier to hydrogen ions [4]. Although, occasionally ulcer results from large increase in the secretion of acid, peptic activity is also critical to the formation of ulcers. Generally, peptic ulcer develops only when mucosal defense is also compromised [76].
Lipid peroxidation: Reactive oxygen metabolites have been implicated in the pathogenesis of peptic ulcer [62]. Production of reactive oxygen species such as superoxide, hydrogen peroxide, hypochlorous acid, hydroxyl, alkoxyl and peroxyl radicals cause tissue damage [66]. The free radical oxidation of polysaturated fatty acids in biological systems is known as lipid peroxidation. Lipid peroxidation in biological membrane have been shown to cause fall in membrane potential, increased permeability to H\(^+\) ion and eventual rupture of the cell [66]. Increased lipid peroxidation has been suggested as one of the mechanisms by which NSAIDs cause peptic ulceration [4].

Studies by Amirov et al in 1987 have shown that all types of ulcerogenesis in the gastric mucosa led to the decrease in lysosomal membrane stability to mechanical stress in the course of lysosomal fractionation. In addition, there was a substantial release of lysosomal enzymes into the gastric cavity in different types of ulcerogenesis. The decrease in lysosomal membrane stability combined with a subsequent development of ulcers in the gastric mucosa seems indicative of the fact that lysosomal enzymes take part in the initial formation of ulcers in the gastric mucosa [78]. In 1995, Kedziora-Komatowska showed that patients with peptic ulcers have high malonyl dialdehyde in blood platelet and decreased superoxide dismutase activity when compared to control group. They suggested that superoxide anion is more destructive and harmful to cells, because the enzymatic antioxidative defense is decreased. The increase of lipid peroxidation in patients with peptic ulcer disease also supports this conclusion [79].
**ii) PREDISPOSING FACTORS FOR PEPTIC ULCERATION**

Non-steroidal anti-inflammatory drugs: Non-steroidal anti-inflammatory drugs like aspirin and salicylates produce injury to the gastroduodenal mucosa. Aspirin and most other NSAIDs are weak organic acids. A solution of two aspirin tablets in 100 ml of drinking water results in a pH of about 2.5 [80]. Aspirin and other NSAIDs also increase basal and maximally stimulated acid secretion which may contribute to NSAID induced damage [81].

Aspirin and other NSAIDs damage the gastric mucosal barrier by altering cell membrane permeability, allowing “back diffusion” of hydrogen ions. Davenport concluded that aspirin damages the mucosal barrier as it is absorbed by an unknown mechanism that renders the mucosa abnormally permeable to water soluble hydrogen ions. Back diffusion of strongly acidic gastric juice then leads to mucosal damage, including erosion and bleeding [82] (Fig.2). These experiments may explain why achlorhydric patients are less susceptible to aspirin-induced gastric injury than normal persons and why damage from aspirin is markedly reduced when gastric secretions are buffered to pH 6.7. In addition, the aspirin-induced increase in hydrogen ion permeability observed experimentally has been confirmed in normal volunteers. It has been demonstrated for other NSAIDs including indomethacin and fenoprofen [83]. Fenoprofen causes less hydrogen ion flux than indomethacin and aspirin but more than control preparation. Mayor et al showed that aspirin disrupts the tight junctions between cells [84]. Hingson and Ito experimented with other weak organic acids to demonstrate that the mechanism of damage is not unique to aspirin. Aspirin inhibits gastric mucus secretion. Aspirin
Fig.2. Aspirin induced back diffusion. 1. Absorption, 2. Ionisation and entrapment of aspirin and 3. Abnormal ion flux across the gastric mucosa. Back diffusion of hydrogen ion from the lumen leads to gastric erosion and bleeding [82]
increases pepsin mediated proteolysis of mucus, decreases mucus viscosity and increases the permeability of mucosa to hydrogen ion. Aspirin inhibits active bicarbonate secretion by the gastric mucosa [85].

In 1971, Vane proposed that the biological actions of NSAIDs resulted from inhibition of prostaglandin synthesis, a theory that has given wide spread acceptance [86]. Prostaglandins are known to be cytoprotective [6]. Further, they protect against NSAID-induced gastric injury [87] and accelerate the healing of gastric and duodenal ulcers when administered exogenously. Prostaglandins have numerous mucosal protective properties, impairment of which contribute to NSAID injury. It is not clear which is the most important injurious factor affecting patients taking NSAIDs. Intrinsic irritancy/toxicity probably accounts for the greater mucosal toxicity of aspirin, although it is not clear whether this is due to a direct (non-prostaglandin-dependent) effect on mucus or bicarbonate secretion, membrane integrity, interference with intermediary metabolism or the production of toxic products [88].

The first enzyme in the synthetic pathway of prostaglandin is prostaglandin endoperoxidase synthetase, or fatty acid cyclo-oxygenase. This enzyme converts arachidonic acid to the unstable intermediates PGG2 and PGH2. There are two forms of cyclo-oxygenase, termed cyclo-oxygenase-1 (COX-1) and cyclo-oxygenase-2 (COX-2). COX-1 constitutes isoform found in the blood vessels, stomach and kidney. While COX-2 is induced on settings of inflammation by cytokines and inflammatory mediators. The fate of PGG2/PGH2 cyclo-oxygenase products differ in different tissues. NSAIDs which inhibit cyclo-oxygenase, block the production of PGE2 and PGI2 in the gastric mucosa. NSAID-induced gastric
damage occurs as a result of a dual insult. 1. The direct effects of the acidic drug on membrane permeability (which in turn is influenced by the rate of absorption of the drug) and 2. drug related effects on prostaglandin production, which may be of particular significance in the early stages of NSAID drug induced injury [89] (Fig.3). It appears that the development of gastric mucosal damage by aspirin and possibly other ulcerogenic NSAIDs, involve hyperproduction of tissue-destructive free radicals which may come from (a) enhanced conversion of hydroperoxy to hydroxy fatty acids in lipo-oxygenase accelerated xanthine-oxidase activity in the mucosa and (c) possibly by drugs themselves (like salicylate) [4].

Helicobacter pylori: Gastric colonisation with H. pylori has been reported in 90-95% of patients with duodenal ulcer and 60-70% of patients with gastric ulcer. Healthy persons less than 30 years of age have prevalence rates of gastric colonisation with H.pylori of approximately 10%. Gastric colonisation increases with age. Most patients having gastric colonisation with H.pylori never develop ulceration and remain asymptomatic. The organism resides in the mucus gel coating, the epithelial cells, with a minor portion of H.pylori directly adherent to the epithelial cells [3].

In 1981, H.pylori was first cultured by Marshall, a research registrar. H.pylori is a spiral or curved micro-aerophilic gram negative rod, equipped with 4-6 flagellae at one end. Even though, gastric acid does kill most of the bacteria, gastric helicobacters have evolved some special features which allow them to live in this acidic media. Urea diffuses freely from plasma into gastric juice. H.pylori produces large amounts of nickel containing enzyme urease, which digests urea to produce
Fig. 3. NSAID induced damage by dual insult mechanism [89]
one carbon dioxide and two ammonia molecules, leading to net production of alkali [90].

\[ \text{NH}_2 \cdot \text{CO} \cdot \text{NH}_2 + \text{H}_2\text{O} \rightarrow \text{CO}_2 + 2\text{NH}_3 \]

Then, spontaneously, at neutral pH

\[ \text{NH}_3 + \text{H}_2\text{O} \rightarrow \text{NH}_4\text{OH} \rightarrow \text{NH}_4^+ + \text{OH}^- \]

Without urea, H.pylori are intolerant to acid, but in its presence, the bacterium prefers mildly acid conditions even though it can withstand a pH as low as 1.5 [91]. The gastric epithelium secretes bicarbonates into the mucus layer at about 10% of the rate of acid secretion so that the pH on the surface of epithelial cells is close to neutral. H.pylori colonizes in this zone, although, by doing so they may increase the permeability of the mucus layer to back diffusion of acid. H.pylori releases several factors which attract and activate leucocytes. It also possesses the lipolytic enzyme phospholipase. This digests phospholipids, which are important components of plasma membranes. One product of digestion of phospholipids is lysolecithin, which is cytotoxic [92].

Ammonium from the actions of urease from H.pylori may damage gastric mucosa. Ammonia is also an important ingredient for the production of monochloramine which is more toxic [93]. Monochloramine is produced when ammonia reacts with chloride ions in the presence of oxygen free radicals formed by neutrophils. H.pylori also produces a phospholipase which can digest the protective mucus layer as well as cell membrane itself [94]. H.pylori either releases platelet activating factor (PAF) itself or causes mast cells to release it [95]. Activation of platelet by PAF is likely to thrombose mucosal capillaries leading to
local hypoxia. *H. pylori* endotoxin might also cause microthrombosis by causing endothelial damage.

Gastric ulcers are more prevalent in patients affected by both NSAIDs and *H. pylori* than in patients who have only one of these precipitating factors. *H. pylori* infection increases the risk of developing an ulcer when NSAIDs are administered concurrently. However, *H. pylori* eradication does not influence healing or ulcer recurrence in NSAID induced ulcers [96]. Studies by Hills have shown that Helicobacter may act as an aggressive agent by ingesting a gastric mucosal barrier of gastric surfactant, exposing the surface to attack by acid, while simultaneously rendering it less hydrophobic. There is also evidence to show that *H. pylori* avoid their own digestion by coating themselves with essentially the same barrier of gastric surfactant, probably derived from the host [97].

**Genetic factors:** Peptic ulcer is common in individuals of blood group 'O' and in non-secretors. There is no evidence that the blood or secretor status per se predispose to the disease. It is possible that the genes controlling blood group are linked to those predisposing to peptic ulcer. Autosomal dominant inheritance of hyperpepsinogenemia I is common in duodenal ulcer. Several rare genetic syndromes may also be associated with peptic ulcer.

**Cigarette smoking:** Many studies have proved the association of cigarette smoking with peptic ulcer disease. Numerous mechanisms have been proposed to explain the effects of smoking on peptic ulcer. These include stimulation of acid secretion, alteration of blood flow or motility, induction of bile reflux and reduction in generation of prostaglandins. The peptic ulcer formation has been shown to be due to decreased prostaglandin synthesis and mucosal defense [98].
**Dietary factors:** Alcohol, coffee and tea increase gastric secretion. Studies have demonstrated that in peptic ulcer patients, there was a relief of symptoms by supplementation with fresh rice bran [99]. Studies have shown that alcohol causes plasma membrane changes leading to mucosal erosion. Gastric lesion caused by ethanol have been attributed to free radical damage which results in lipid peroxidation [4].

**Psychosomatic factors:** It was seen that anxiety and conflicting feelings associated with it were invariably accompanied by hyperaemia, hypersecretion and hypermotility of the stomach. Recurrence of peptic ulcer is often seen during periods of long standing emotional stress suggesting that psychological factors may be involved in its causation. Long term emotional stress may lead to persistent enhanced activity of the vagus nerve. The stomach, therefore, responds to vagal impulses by increased secretion. When secretion occurs in response to food, it acts on the food, sparing the gastric mucosa. But secretion taking place in the absence of food may break through the mucoprotective mechanisms causing ulceration.

Many studies suggest an association between psychological stress and peptic ulcer disease. Stress ulceration of the stomach is associated with clinical conditions like trauma, head injury, burns, shock and neurological disorders. It has been suggested to result from interactions between mucosal, vascular, neurohumoral factors and autonomic nervous system. Stimulation of gastric mucosa due to stress is transmitted by cerebral marginal system and hypothalamus to the medulla oblongata and spinal cord. Medulla oblongata stimulates the vagus which increases the gastric secretion and augments gastric motility. The spinal
cord causes the stimulation of the splanchnic nerve to produce a disturbance in circulation due to functional constriction of the gastric vessels which leads to a diminution of gastric blood flow. The function of anterior pituitary also gets disturbed due to stress releasing adrenocorticotropic hormone (ACTH) which ultimately leads to increased gastric secretion and reduced gastric mucosal resistance. Circulatory disturbances and nutritional deficiency are thus induced in the local tissue, which are then followed by a rapid appearance of a deep ulcer [100].

Diseases: Certain diseases like chronic pancreatitis, Crohn's disease and pulmonary diseases have been associated with peptic ulcer [4].
Fig. 4. A model of the pathogenesis of peptic ulcer
4. REVIEW OF GENERAL FUNCTIONS OF VITAMINS A, C AND E

i) VITAMIN A

Frederick Gowland Hopkins in England found that a growth stimulating principle in milk was present in the alcoholic extract, rather than in the ash, and soon thereafter, Stepp in Germany identified one of these so called "minimal qualitative factors" as a lipid. In 1913, E.V.McCollum and Marguerite Davis in Wisconsin showed that butter or egg yolk, but not lard, contained an essential growth factor for rats. They termed this factor "fat soluble A". Concomittantly, Osborn and Mendel in New Haven found a similar fat soluble growth factor in cod liver oil and butter. Thus, 1913 marks the beginning of the modern nutritional history of vitamin A [101].

Moore in England showed that β carotene was converted biologically to a colourless form of vitamin A which was then stored in liver tissue. In 1930, Karrer and his colleagues in Switzerland determined the structure of both vitamin A and β carotene. Five years later, Wald identified the chromophore of visual pigments as retinene, now termed retinal, thereby defining one of the primary functions of the vitamin.

Vitamin A is considered chemically as a subgroup of retinoids. The term vitamin A is used as a generic descriptor for retinoids exhibiting qualitatively the biologic activity of retinol. Most, if not all, of the vitamin A and β-carotene are available commercially. These synthetic compounds are identical in every way,