Isatins are a group of heterocyclic compounds with indole nucleus. The parent compound (2,3-dioxoindoline) was discovered independently in 1841 as an indigo derivative (Glover et al., 1991). Subsequent studies showed that isatin is a very reactive molecule that can be converted into many derivatives.

A. Physiochemical properties of Isatin:

Isatin (structure 1) itself has a distinctive bright orange/yellow colour. It is soluble in hot water and organic solvents. It dissolves in sodium or potassium hydroxide and on heating results in ring opening with the formation of salt of isatic acid. Acidification results in ring closure and isatin precipitates. At higher pH isatin has been shown to undergo ring cleavage (Sumpter et al., 1949).

Isatin possesses a highly reactive 3-oxo group on the planar structure of o-amino-phenylglyoxalic acid lactam that is responsible for its interactions with a host of diverse molecules, such as amines, amino acids, guanidines, glycols and thioglycols (Sumpter, 1959; Spande and Witkop, 1967). Isatin forms 1:1 addition products with mercaptans (Sumpter, et al., 1944) having a structure (2). With ammonia the product formed has the structure (3).

\[
\text{(1.)} \quad \text{(2.)} \quad \text{(3.)}
\]
Similarly, isatin reacts with alcoholic ethylamine to give isatin-β-ethylamine (4) and with phenol, it forms compound 5. Isatins have been reported to undergo condensation with acetone and related ketones (Popp et al., 1980) to give compounds of general structure (6).

\[
\begin{align*}
\text{(4)} & \quad \text{(5)} \\
\text{(6)} & \quad R = \text{C}_6\text{H}_5/\text{CH}_3, \\
& \quad 2\text{-Pyridyl}
\end{align*}
\]

The presence of such and related functional groups in amino acids side chains of proteins and enzymes and the reactivity of the 3-oxo-groups of isatin have been of special interest to enzymologists and protein chemists.

B. Biological properties of Isatin:

Isatin is a compound of immense biological importance. It has a distinct regional distribution in rat tissues and normal human urine as shown by mass spectrometry gas chromatography (Glover et al., 1988), where it forms a major proportion of the endogenous MAO inhibitor "tribulin".
Highest concentration have been found in vas deferens and seminal vesicles. In mammalian brain, highest levels are found in hippocampus, conceivably supporting links between isatin and anxiety. The distinct tissue distribution also reflects diverse physiological functions of isatin. The properties of isatin and its derivatives fall into two broad types: (a) actions in brain, and (b) to be protective against certain types of infections and effect on body physiology (Glover et al., 1991).

**Isatin effects on CNS**

Muller in 1962 reported that isatin could antagonise electrical or pentetrazol tonic seizures in mice. But indole was found to be highly toxic and did not manifest anticonvulsant activity. Sareen et al., (1962) reported that isatin had anticonvulsant action against maximal electroshock seizures in rats. They also showed that on N-alkylation or acylation isatin lost its anticonvulsant property. Ammonia is considered to be one possible endogenous neurotoxin associated with the neurochemical lesions in epileptogenic tissue. Activated keto-group in position 3 of isatin, readily reacts with ammonia to prevent its excessive accumulation in the brain.
Chocholova and Kolinova (1979) found that isatin significantly reduced the total incidence of audiogenic epileptic seizures in rats highly sensitive to an acoustic epileptogenic stimulus. Isatin reduces motor activity and muscular tone and inhibits certain autonomic functions (Klingberg and Muller, 1968).

One of the first effects of isatin to be described was its anticonvulsant effects (Muller, 1962) on convulsions induced either electrically or by metrazol and other chemicals. Isatin specifically modifies the tonic component of these convulsions and thus reduces mortality.

The effect of isatin on epileptic phenomena may be associated with its striking effect on electroencephalogram (EEG). Chochova and Kolinova (1979) reported that one hour after isatin administration, there was a marked increase in the rhythmic episodes against a background of desynchronised activity and a decrease in slow wave sleep.

Popp (1980) demonstrated that substituted 3-hydroxy oxindoles can be obtained by condensation of isatin with a series of ketones. These indoles act as potential anticonvulsants. 3-Hydroxy-3-acetonyl oxindole exhibited greater anticonvulsant activity in MES test than 3-hydroxy-3-phenacyl-oxindole (Popp & Donigon, 1979). 5-bromo, 3-hydroxy, 3-acetonyl oxindole is inactive in MES screen at 600 mg/kg, but exhibited activity at 100 mg/kg (Popp et al., 1980).
The antiepileptic potency of isatin was found to be less than dilantin, but due to its lower toxicity, it has an edge over other drugs (Issacson et al., 1956; Livingston, 1966). Since it can cross the blood brain barrier, it was thought to react with ammonia, that may have accumulated in excessive amounts in the brain of epileptic patients, and thus resulting in seizures.

Klingberg and Muller (1968) carried out electrophysiological studies of antiextensor action of isatin with respect to behavioral parameters. Isatin significantly reduced the heart rate and respiratory function of rats. It has been shown to decrease the ventricular contraction and cardiac output of frog heart (Singh, 1971), but has no effect on cardiovascular system in mammals.

Isatin has been shown to induce changes in serotonergic activity of rat brain, similar to those induced by cold immobilization stress (McIntyre & Norman, 1990). An increase in hypothalamic and cortical 5-hydroxy-tryptamine (5HT) levels, but no change in 5-hydroxy-indole acetic acid (5HIAA) levels was observed. Similarly synaptosomal 5HT uptake remained unchanged, but 5HT$_2$ receptor population was down-regulated (Sittner et al., 1985). These observations indicated that isatin significantly effected the 5HT activity, which may be responsible for some of the behavioral effects exhibited by it.
Possible role of isatin in anxiety or stress:

Clow et al., (1989) demonstrated that pentylene-tetrazole, an anxiogenic, proconvulsant agent, brought about a significant increase in isatin level in rabbit brain, but not in liver. Mean brain concentration rose from 49 to 71 ug/gm, whereas values in liver remained unchanged. These findings, raise the possibility that isatin or any of its metabolites play a role in generating the symptoms of anxiety and acting as an antiseizure agent. Recently, isatin exhibited significant anxiogenic and anxiolytic activity in rats and mice in doses between 15-20 mg/kg ip. However, these effects were not apparent on increasing the dose to more than 50 mg/kg ip (Bhattacharya, et al., 1991). These investigations indicate that isatin has significant anxiogenic effect and support the contention that it/or its biotransformation products may be responsible for at least part of the ability of tribulin demonstrated previously in animal models and in clinical situations of stress and anxiety.

Enzyme like activity:

(a) Dehydrogenase activity: Traube (1911) reported oxidation of amino acids by isatin and its derivatives like alloxan and quinone through a Strecker (1862), type reaction. It was observed that isatin readily converted both
amino-phenyl-acetic acid and benzyl-amine to aldehyde. Strecker reaction was shown to be fairly general for amino acids having the structure R-CH(NH₂)COOH and is brought about by substances possessing group -CO(CH-CH)CO- (Schonberg et al., 1948 and Abderheldane, 1938). The reactive amino acid is degraded to the corresponding aldehyde with one less carbon atom as reported by Abderheldane (1938). Langenbeck (1928, 1927) studied the catalytic dehydrogenation of amino acid, using isatin, N-methyl isatin and isatin 5-sulphonic acid and methylene blue as a hydrogen acceptor. He isolated isatide from the reaction of isatin with alanine in the absence of air. In presence of air or methylene blue, isatide was oxidized to isatin. One molecule of isatin was sufficient for the degradation of a number of amino acids. Giovannini et al., (1959), showed that apparent enzyme like activity of isatin was associated with its reactive 3-keto group and it could be modified by substitution of meta orienting groups in 4 and 6 positions of indole nucleus. The 4 and 6 carboxylic acids of isatin were found to be more effective as catalysts than the parent compound. Mix and Krause (1956) reported the dehydrogenase activity of various isatins by measuring the time required to decolorise a solution of alanine and methylene blue. The amino acid loses 2 hydrogen atoms, which are taken up by isatin or its derivatives and changes into imino acid which is immediately hydrolyzed to give an
aldehyde, CO₂ and NH₃ (Wieland and Bengel 1924). The following scheme shows the proposed mechanism for the dehydrogenase activity of isatin and its derivatives based on its reversible reduction to isatide (Sumpter and Muller, 1954), in which atmospheric O₂ or methylene blue could serve as H-acceptor.

\[
\begin{align*}
R-CH-COOH + 2 \text{NH}_2 &\rightarrow \text{AMINO ACID} \\
&\rightarrow \text{ISATIN} \\
&\rightarrow \text{ISATIDE} \\
&\rightarrow \text{LEUCOBASE} \\
&\rightarrow \text{KETO ACID} \\
\end{align*}
\]

The overall reaction is

\[
\begin{align*}
2 \text{Isatin} \\
R-CH-COOH (O_2) \text{ or } \text{M.B.} &\rightarrow RCHO+CO_2+NH_3+H_2O \\
\text{NH}_2 \quad &+ \text{Leucobase}
\end{align*}
\]

The dehydrogenation of amino acids by isatin is considered to proceed by series of reactions involving both
Cassebaun (1954, 1958) tried to correlate the dehydrogenase activity of isatins with their redox potentials. In the case of isatin much useful information was provided by polarographic and spectroscopic studies (O’Sullivan & Sadler, 1956). Langenbeck and his coworkers (1956), reported that dehydrogenase activity of isatin derivatives of similar molecular weight increased with increasing redox potential. An increase in molecular weight of substances of similar oxidation reduction potential decreases the dehydrogenase activity. They showed that dehydrogenation of amino acid was accelerated, if pyridine was used as a solvent.
Enzyme inhibiting activity: Isatin has the potential to alter the activity of variety of enzymes. Most of the actions of isatin are attributed to its 3-keto group, which is located in a favourable steric environment of planar structure of O-aminophenylglyoxalic acid lactam, which could reversibly bind to the -SH group or -NH₂ group in the active centre or allosteric site of the enzyme, thus altering the enzyme activity. Substitution at 3 position reduces its potential as a modifier of enzyme activity. Blocking of 2-keto group is not as effective as 3-keto group, due to its tautomerisation with the -NH group at position 1 to give enol form as shown below:

Muller and Schmiedal (1965) observed that isatin and N-methyl isatin inhibited MAO in liver homogenate, when injected intraperitonially to rats. The inhibitory effectiveness of unsaturated compound was significantly increased by the introduction of bromo group at 5 position.

Glover et al., (1988) reported that it is a selective monoamine oxidase B inhibitor with an IC₅₀ of about 3 μM; its most potent in vitro action found to date.
Yuwiler (1990) reported that isatin produced a dose-dependent inhibition of MAO A and MAO B in broken cell preparations from rat brain and pineal. However, isatin administration (80-160 mg/kg) in vivo enhanced brain but not pineal serotonin levels. However, there was no change in 5HIAA or other indoles in either brain or pineal. In pineal organ culture, addition of isatin (< mM) had no influence on concentration of pineal indoles or the activities of MAO or serotonin N-acetyltransferase. Medvedev et al. (1992) studied in vitro effect of isatin and some of its derivatives like 5, 6 and 7 hydroxyisatin, N-methylisatin, isatic acid, dopamine-isatin, indole, oxindole on human MAO A and B. Most of the derivatives were less potent than isatin. Hydroxylation of the aromatic ring changed the inhibitory potency in favour of MAO A, with 5-hydroxy isatin being a potent and selective MAO A Inhibitor. Isatinic acid showed no inhibition. Isatin also inhibited rat liver MAO activity in a competitive and dose-related manner (Hamaue et al., 1992). Inhibition of liver MAO activity was significantly greater in rats receiving extracts of stroke-prone spontaneously hypertensive (SHRSP) rat brain and urine than in those receiving extracts of Wistar Kyoto rats (WKY) brain and urine. Purified extracts of both rat urine and human urine have been identified as istaint by gas liquid mass spectrometry. Acetylcarboxyanilide "an open drug of isatin" and 2-methacrylanilides were found to be more
efficient than isatin on their ability to inhibit alkaline phosphatase activity of Echinococcus multilocularis metaccetodes in vivo (Audin et al., 1992). Very recently, Kumar et al. (1993) reported the inhibition of rat brain acetylcholine sterase by isatin. The inhibition was irreversible and mixed in nature. The enzyme was equally sensitive to 5-bromoisatin, 5-methylisatin whereas 5-isatinsulphonic acid, 3-hydroxy-3-phenacyl oxindole (HPO) and 3-hydroxy-3-acetonyl oxindole (HAO) had no effect on rat brain acetylcholinesterase. Oral administration of isatin hydrazone, isatin-3-thiosemicarbazone, isatin-3-emicarbazone, isatin-3-phenylhydrozone, isatin oxime, and 3-hydroxy-3-acetonyl oxindole resulted in the metabolic excretion of isatin, anthranilic acid, tryptophan and nicotonic acid in rabbit urine. 3-hydroxy-3-acetonyl oxindole gave an additional metabolite, oxindole (Alam et al., 1990).

Bruns and Chaftan (1954), reported 73 percent inhibition of xanthine-oxidase in raw milk by isatin. This was similar to that caused by alloxan and ninhydrin. Inhibition was probably due to decarboxylation or deamination of terminal carboxyl or amino group.

Susheela et al., (1969), showed in vitro that inhibition of xanthine oxidase by isatin was of non-competitive type and highly dependent on concentration of inhibitor and pH. They also reported that isatin administration orally increased the specific activity of
liver and kidney xanthene-oxidase. The different behaviour of isatin in vitro and in vivo might be due to its decomposition under in vivo conditions.

Isatin is shown to inhibit rat testicular alkaline phosphatase activity (Kumar et al., 1978). Rat kidney alkaline phosphatase was also shown to be inhibited by isatin (Singh et al., 1979). Inhibition was concentration dependent and uncompetitive in nature.

Sluggo and Diagakh (1957), reported that isatin inhibited the breakdown of tryptophan to kynurenine by rabbit liver extract. Inhibition was completely overcome by the addition of glutathione.

Tonew and Schroeen (1980), showed that mengovirus RNA dependent RNA polymerase was inhibited by isatin B-thiosemicarbazone and a piperidine-thiocarbonylhydrazone derivative. The inhibitor reduced the synthesis of single stranded viral RNA. Bansal et al., (1983) studied developmental and structural changes in acid phosphatase of chick spleen. Inhibition of spleen acid phosphatase by istain in vitro increased with increasing age of chicks, while inhibition in case of jejunum and dodenum was independent of age. N-Methyl-isatin B-thiosemicarbazone has been reported as a new phosphodiesterase inhibitor in human lymphocytes by Webb and Hentry (1974).
Kumar et al., (1978), found that isatin activated (153%) rat testicular hyaluronidase after 2 hours and there was 59% decrease in enzyme activity after 24 hours of isatin in vivo (Kumar et al., 1976).

Singh et al., (1978), described that isatin (200 mg/kg body weight) decreased rat kidney alkaline phosphatase at 5 hrs, but increased the enzyme activity in jejunum and duodenum after 2 hrs and 5 hrs of administration of the drug. Nagpaul et al. (1985) described that isatin (6 mM) inhibited irreversibly both the sugar uptake and transmural (mucosal to serosal site) transport in intestine. Isatin inhibited the sugar uptake, similar to various -SH group reacting agents, presumably by binding to membrane sulphydryl groups. Inhibition of sugar uptake by isatin and harmaline was additive in nature, suggesting that these compounds interact at different sites on microvillus membrane surface.

**Physiology and Biochemistry of Intestine:**

The small intestine is the most indispensable site where the digestion of food is completed and its hydrolytic products are absorbed into the blood, through a specialized layer of absorbing cells, which cover the villi of the small intestine. The variety and complexity of transport processes in this cell is quite staggering. The plasma membrane of this versatile cell possesses the potential for virtually any type of transport mechanism known.
The structure of the small intestine is similar in most mammals. The intestinal wall is made up of an innermost mucosa, a middle muscular layer and an outer serosa (Trier 1968), but the absorptive function of the tissue is carried out by mucosa which is thrown into several folds called villi.

A fully developed epithelial cell (Fig.1a) is tall and narrow with nucleus situated deep into the cytoplasm, below a broad apical zone, richly supplied with mitochondria, endoplasmic reticulum and Golgi apparatus. The abundance of these organelles indicate intense oxidative and synthetic activity of the tissue. Just below the upper line or apex, each absorptive cell is bound to neighbouring cell by an exceptionally close juxtra position to cell membrane, known as tight junction. The most characteristic feature of the epithelial cells is the straited border, which is about 1.5 um wide on the top plane of the cells facing the lumen. The luminal surface of the epithelial cells is linked with finger like parallel projections called microvilli (Fig.1b) which are about 0.1 um in diameter and 0.5-1.5 um in length (Madara and Trier, 1987). Microvilli vary in different cell types with respect to their length and their frequency on the cell surface. The number of microvilli present per enterocyte vary from 600 to 1000 (Zetterquist, 1956) and they increase the surface area of the intestine by a factor of about 20.
STRUCTURE OF THE ABSORPTIVE CELL of the intestinal lining reflects its specialized function in digestion. The absorptive cell is one of the two main types of cell that constitute the epithelium of the villus. The other type is the goblet cell, which secretes mucus. Cells of both types are produced by cell division in the crypts and assume their mature structure during a two-day migration to the villus tip. Exposed in transit to the action of digestive fluids, some of the membrane covering the absorptive cell may be sloughed off. The cell is abundantly supplied with mitochondria, which provide energy for metabolic processes; with endoplasmic reticulum, where proteins (including digestive enzymes) are assembled, and with Golgi saccules, where carbohydrate side chains are attached to some proteins. Tight junctions and desmosomes bind the absorptive cells into a single sheet as they traverse the villus. The nucleus lies deep in the cell. The most striking feature of the cell is the brush border, a prominent structure on the luminal surface of the cell. The brush border is made up of rows of minute projections called microvilli. In the core of each microvillus is a bundle of fibers composed of protein. The fibers from all the microvilli on each cell extend down to the terminal web, a meshwork of fibers running parallel to the luminal surface of the cell.
Fig. 1b Anatomy of the Small Intestine (Moog, 1981).

25
Structure of the Microvillus Membrane (MVM)

MVM lining the mucosal surface, separates the luminal environment from the epithelial tissue. The surface of the epithelial cells, embedded in the muscular tissue is the basolateral membrane. These two components have different dimensions, enzyme composition and different mechanism by which substances enter and exit the cells. Further proteins of MVM are degraded fairly rapidly as compared to those of the basolateral membrane (Alpers, 1972).

Like any other plasma membrane the MVM is a typical trilaminar membrane having a thickness of 80-115 Å. The existence of a lipid bilayer has been confirmed by X-ray diffraction studies (Limbrick and Firean, 1970). Border surface frequently reveals a polysaccharide structure, which is about 60 Å in thickness extending from the membrane surface, this is referred to as "glycocalyx" or "fuzzy coat" (Ito, 1965). It is attached directly to the outer leaflet of the MVM (Madara and Trier, 1987), is rich in mucopolysaccharides and acts as a protective layer against proteolytic attack and performs some vital functions such as inter-cellular adhesion and recognition, as receptors for hormones, toxins, lectins and is involved in membrane transport phenomenon (Ugolev et al., 1979). A number of hydrolytic enzymes are adsorbed to the glycocalyx thus localizing the action of these enzymes close to the site of product absorption by the MVM.

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Using both histochemical and biochemical procedures, localization of various enzymes in the brush borders has been established (Ernst, 1975 and Nachlas et al., 1959). Functional proteins of MVM comprise various digestive enzymes and transport carriers (Crane, 1966, Kenny and Maroux, 1982). These enzymes are capable of digesting a wide variety of nutrients. Various enzymes localize in the MVM are listed in Table 2. Majority of the MVM enzymes are glycoproteins having molecular weight ranging upward from 80 KDa. The protein component of the glycoproteins penetrates into the lipid bilayer, while the carbohydrate component stretches into the lumen.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucoamylase</td>
<td>Kelly and Alpers (1973)</td>
</tr>
<tr>
<td>Trehalase</td>
<td>Sasajima et al. (1975)</td>
</tr>
<tr>
<td>Sucrase</td>
<td>Braun et al. (1975)</td>
</tr>
<tr>
<td>Isomaltase</td>
<td>Braun et al. (1975)</td>
</tr>
<tr>
<td>Lactase</td>
<td>Schlegel et al. (1972)</td>
</tr>
<tr>
<td>γ-glutamyltranspeptidase</td>
<td>Hyman et al. (1975)</td>
</tr>
<tr>
<td>Leucine aminopeptidase</td>
<td>Webster and Harrison (1969)</td>
</tr>
<tr>
<td>Leucine B-naphthyl amidase (LNase)</td>
<td>Takesue and Nishi (1978)</td>
</tr>
</tbody>
</table>

Table 2: Enzyme localized in Intestinal Microvillus Membrane
The molar ratio between cholesterol, phospholipids and glycolipids is of the order of 1:1:2 in rats (Forstner et al., 1968) and 1:1:1 in mice (Kawai et al., 1974). The glycolipids contribute some of the carbohydrates of the glycocalyx (Kim and Perdomo, 1974). The sugars identified in MVM include hexoamine, sialic acid and monosaccharides. The structure of the intestinal brush border membrane revealed regional differences in the lipid fluidity and composition of rat enterocyte microvillus membrane (Brasitus and Schachter 1980; Brasitus and Dudeja, 1985), and in basolateral membrane (Brasitus and Schachter, 1982).

**Mechanism of intestinal absorption of nutrients**

The primary function of the intestine is to absorb the digested products of carbohydrates, fats and proteins. In order to be transported from the lumen into the subepithelial spaces and then to the blood stream, these molecules must cross two in series plasma membranes i.e. the brush border and the basolateral membrane. Specific transport mechanisms are known to transport the digested products. There are at least three main pathways by which metabolites can pass through the intestine.

1. **Passive diffusion**: Simple diffusion is undoubtedly important pathway for absorption from small intestine for substances like water soluble non-electrolyte molecules, nucleic acid derivatives and many lipid soluble substances.
The rate of diffusion is quantitatively expressed by Fick's law (Fick, 1855):

\[ J = -D \frac{dc}{dx} \]

Where, \( J \) is the influx rate.

\( D \) is the diffusion coefficient

\( \frac{dc}{dx} \) is the change in the solute concentration at a particular distance \( x \).

The main characteristics of diffusion process are:

(a) Movement is not against the concentration gradient.
(b) The absorption rate is not inhibited by uncouplers of the energy process.
(c) There is a linear relationship between absorption rate and concentration gradient.

2. Facilitated Diffusion: Transport of polar solutes across the hydrophobic barrier of biological membranes is facilitated by a variety of specific translocation mechanisms. The term "facilitated diffusion" first was given by Danielli (1954). Facilitated transport systems have several general characteristics. Each transport system exhibits high structural specificity. The driving force for facilitated passive transport is the electrochemical gradient of the solutes that ultimately leads to equalization of concentrations. Such transport processes, which occur through a limited number of sites, occur in both directions (influx and efflux), and the rate of transport
exhibits saturation Kinetics, i.e. the rate of transport is limited by the number of transport sites. The characteristics of these transport systems are:

(1) Facilitated transport is driven only by the concentration gradient of the permeant. The initial steady-state rate of transport reaches a limiting rate at high permeant concentration (Saturation Kinetics). While the small size of the inner compartment would tend to promote a departure from the steady state condition. Metabolism of the translocated solute often tends to maintain the steady state concentration gradient for longer periods.

(2) A transporter exhibits a high substrate specificity i.e. $K_t$ which depends upon structure and conformation of the solute, tend, to be in the micro to millimolar range.

(3) The overall rate of exchange of solute $S$ between the 2 compartments is usually different from the rate of net flux. If the rate of translocation $CS$ complex is far greater than the rate of translocation of carrier $C$, the rate of net transport will be considerably slower than the rate of exchange.

(4) Transmembrane exchange of 2 different permeants species with different affinities can be mediated by
a carrier in opposite directions. If the rate of translocation of C is slow, counter transport of the two solutes is promoted under exchange conditions.

Temperature coefficients ($Q_{10}$) for most transporters are 2-3, that is, the rate of transport increases two to three fold for a $10^\circ$ increase in temperature. This corresponds to the activation energy greater than 10 Kcal/mole, compared with a value of less than 5 Kcal/mole for processes limited by diffusion in aqueous phase.

Binding of the permeant and translocation of the complex are two distinct steps in the overall transport cycle. This implies that the CS complex must assume proper conformation before it can translocate. It also raises the possibility of regulation at the binding as well as at the translocation level. Therefore, certain compounds may bind to the carrier, but the complex may not be translocated. In analogy with the behaviour of catalytic proteins, it is possible to inhibit the binding or the translocation step by competitive, noncompetitive, uncompetitive, or allosteric mechanisms. A high degree of structural specificity for a solute in the translocation step would therefore imply that the translocation step for the CS complex is not a simple diffusion from one interface to the other.
Specific facilitated transport in almost all known cases is mediated by proteins, therefore, such processes are inhibited by antibodies, protein reagents, and affinity labels.

Glucose transport across the human erythrocyte membrane is the best understood of all facilitated diffusion processes. The transport shows the classical signs of facilitative diffusion, i.e. saturation kinetics, competitive and non-competitive inhibition, trans stimulation, that is an increase of the net transport by the sugar when present (at lower concentration) on the side to which the sugar is transported. A temperature dependence of the transport process different from that of the simple diffusion further support the concept of the involvement of the transport system. The protein responsible for the transport has been purified and extensively characterized (Kasahara and Hinkel, 1977; Baldwin et al., 1982). The isolated protein retain ability to transport glucose, with kinetics similar to those seen in the intact erythrocytes (Wheeler and Hinkel, 1981; Connolly et al., 1985). The transporter is a glycoprotein and migrates as a broad band of apparent Mr 55000 on SDS-polyacrylamide gels (Boldwin et al., 1982; Sogin and Hinkel, 1978). Infrared spectroscopic and circular dichroism studies carried out by Alvarez et al (1987) and Chin et al (1986), have shown that
the protein is predominently x-helical in structure, with some random coil conformation. The transport protein has also been sequenced by Mueckler et al. (1985). The pattern of hydrophobicity in the amino acid sequence suggests that there may be 12 transmembrane x-helixes, a large extramembranous endofacial domain, and a smaller (glycosylated) exofacial domain.

The glucose transporter of human erythrocytes is specific for C1 conformation of D-glucose in which the hydroxyl group at position 2, 3, 4 and 5 are in equitorial conformation. A slightly strained 1C conformation assumed by L-glucose makes it a poor substrate. The transport system of human erythrocytes shows a high degree of specificity for D-glucose than L-glucose. The latter being within the range of simple diffusion permeability (Lidgard and Jones, 1975).

Glucose transport activity of human erythrocytes is inhibited by several compounds. Cytochalasin B was shown to be a more potent inhibitor for glucose transfer in erythrocytes than phloretin (Tavera and Langdon, 1973; Bloch, 1973). Jenning and Solomon (1976) showed that phloretin rapidly penetrates red cell membrane. Basketter and Widdas (1977) suggested that phloretin must react at both inside and outside, since its inhibition is more effective than it would be expected from an inhibitor acting only outside. Steroids inhibits glucose transport in erythrocyte by binding to sites in the carrier which are
exposed on both outer and inner surface of the cell membrane (Krupka and Deves, 1980). Some steroids (androstendione and androstandione) bind almost exclusively at inner sites while others (corticostrone) bind on both sides of the membrane. Still other (deoxycorticostrone) exhibits a moderate preference for the internal site. 6-O-methyl-, 6-O-propyl-, 6-O-pentyl, 6-O-benzyl-D-glactose and 6-O-pentyl-D-glucose inhibit the glucose transport system of the human erythrocyte when added to the external medium (Barnett et al., 1973). Inhibition of sugar transport in human erythrocytes by sodium tetrathionate and iodoacetamide is due to modification of sulfhydryl group. 1-fluoro-2, 4-dinitro benzene and N-ethylmaleimide inhibit transport by reacting with an amino group (Bloch, 1973). 1-fluoro-2, 4-dinitrobenzene and N-ethylmaleimide also inhibited sugar transport suggesting that they act by modifying an essential sulfhydryl groups (Dawson and Widdas, 1963). A series of pyridine derivatives having structural similarity to glucose reversibly inhibit glucose transport in human erythrocytes (Hershfield and Richard, 1976). Lowe et al. (1991) demonstrated that ubiquinone Q\textsubscript{0} inhibits both glucose entry and glucose exit in human RBCs with kinetics consistent with the existence of ubiquinone binding sites at both the exofacial and endofacial sides of the transporter.
3. **Active Transport:** Active transport is an energy requiring process where by a substrate permeates across a membrane barrier from a lower to higher concentration (against electrochemical) gradient, yet the substance is neither bound on either side of the membrane nor produced or consumed during the transport. The energy for the active transport in intestine is usually derived from the equal distribution of Na\(^+\) ions across the microvillus surface and is independently linked to the hydrolysis of ATP. The carrier mediated active transport systems are undirectional (Ussing, 1949) and are inhibited by structurally similar compounds and metabolic poisons. This is a rapid and most efficient method for the absorption of many nutrients required for vital functions of body organs.

**Sodium Gradient Hypothesis:**

Sodium is a specific activator of the transport of a variety of substances across several cell membranes (Schultz and Curran, 1970). A hypothesis to explain the interactions between sodium and non-electrolyte transport in absorptive epithelia of small intestine was proposed by Crane in 1962. This model, now known as sodium-gradient hypothesis, has been intensely scrutinized in many tissues from a wide variety of experimental stand points. In small intestine, different classes of solute transport mechanisms require sodium. These include sugars, amino acids, bile
salts, uracil, sulphate, phosphate, calcium, ascorbic acid and biotin (Berger et al., 1972).

As early as 1902, Reid observed that presence of sodium in the intestinal lumen caused stimulation of glucose absorption. In 1958, Riklis and Questal observed that sugar absorption by guinea pig small intestine in vitro requires the presence of sodium in the mucosal medium. Bihler and Crane (1962) identified the entry of sugars across BBM of the epithelial cells as the primary site of sodium involvement in the overall process. A general model for sodium coupled transepithelial transport of solutes is depicted in the (Fig.2). Sodium and the substrate (S) enter the cell from mucosal solution by means of a specific transporter located in the BBM that couples the inward flow of S to the influx of sodium. The passive influx of sodium is driven by its electrochemical gradient across the apical membrane. The energy contained there in can drive the uphill cellular accumulation of S by virtue of the coupling mechanism. The sodium electrochemical gradient is maintained by the activity of Na\(^+\)-K\(^+\) ATPase pumping mechanism located in the basolateral membrane that extrudes Na\(^+\) into the serosal environment. Cell to serosa efflux of accumulated solute is by means of facilitated diffusion. Experimental evidence in support of Na\(^+\)gradient hypothesis has been provided by various workers (Alvarado, 1965; Crane, 1965; Eddy, 1968; Schultz and Curran, 1970).
FIG. 2. A MODEL FOR $\text{Na}^+$-GRADIENT HYPOTHESIS FOR THE $\text{Na}^+$-SUBSTRATE COTRANSPORT IN EPITHELIAL CELL.
Studies using membrane vesicles prepared from purified brush borders revealed that sugar and amino acid transport system are energized by the chemical and potential gradient due to Na\(^+\) ions across the BBM (Hopfer et al., 1973; Murer and Hopfer, 1974; Hopfer, 1978). Na\(^+\) dependent transport systems are rheogenic, which involves a net transport of positive charge with each molecule of the substrate entering the cells (Hopfer, 1977). To explain the transport characteristics of the Na\(^+\) and solute cotransport systems, various kinetic models have also been postulated (Schultz and Curran, 1970; Alvarado and Mahmood, 1974; Alvarado and Lherminier, 1982; Stevens and Wright, 1987).

Amino acid Transport across BBM:

(a) **Na\(^+\) dependent amino acid transport**: A prominent feature of amino acid absorption is dependence on sodium. Secondary active Na\(^+\) dependent transport was first shown for alanine in vesicles from rat intestine (Sigrist-Nelson et al., 1975). Similar to glucose transport, Na\(^+\)-dependent neutral amino acid transport is electrogenic (Gunter-Smith et al., 1982). The BBM contains at least four different Na\(^+\) dependent transport systems for neutral amino acids. They are specific for the BBM and consistent with genetic amino acid transport disorders that apparently affect only kidney and intestine (Hopfer, 1987).
(a) Most neutral amino acids are substrates for neutral brush border, NBB system. BCH, test substrate for L-lysine, also interacts with this system. MeAIB, a specific substrate for A system, is not transported by NBB. NBB system differs from ASC system by transporting glycine and phenylalanine.

(b) Phenylalanine and methionine are also transported by another Na\(^+\) dependent system termed PHE system.

(c) Transport capacity for imino acids is very high because of IMINO system. It also accepts MeAIB, but excludes alanine and other short chain amino acids.

(d) Munck (1985) has characterized a Na\(^+\) dependent B-alanine transport system in intact rabbit distal ileum.

According to Baker and George (1971), principally two transport systems are present for neutral amino acids, they are named as \(N_1\) and \(N_2\). The \(N_1\) system has high affinity for methionine and other neutral amino acids with non-polar side chains, while \(N_2\) system has more affinity for the imino acids, proline, hydroxyproline and N-substituted glycine derivatives such as betaine, and sarcosine (Wilson, 1962). Proline, hydroxyproline, alanine and leucine probably share both \(N_1\) and \(N_2\) systems (Munck, 1966).

Basic amino acids and cystine are handled by a separate transport system in the kidney and the intestine.
In initial experiments, Na⁺ dependence of lysine transport was not noted (Cassano et al., 1983; Schell et al., 1983). However, later on Wolfrans et al., (1984) have demonstrated the occurrence of Na⁺-dependent lysine transport in BBMs from rats fed high protein diet. It is possible that both, a Na⁺ dependent (Y⁺) and a Na⁺-dependent (y⁺) lysine transport system exist in BBM, either one or both are subject to regulation.

(b) Na⁺ independent amino acid transport: Na⁺-independent facilitated diffusion of amino acids is a minor pathway for amino acid absorption (Stevens et al., 1984). Its substrate specificity corresponds approximately to the L-system found ubiquitously in all animal cells. B-alanine was observed to be transported in an electrically silent manner in rabbit jejunal membranes (Schell et al., 1983).

Amino Acid Transport by γ-Glutamyl Cycle:

In 1970, Orlowski and Meister proposed the existence of γ-glutamyl cycle, advancing the hypothesis that, to some extent, cellular uptake of amino acids could be mediated by an energy-dependent series of reactions in which the tripeptide glutathathione was involved. In Meister’s formulation (Meister, 1973), γ-glutamyl transpeptidase functions as a membrane-localized transporter of amino acids from the extracellular space to the
intracellular one. The enzyme catalyze the transfer of \( \gamma \)-glutamyl groups of glutathione (or other \( \gamma \)-glutamyl containing compound) to a free amino acid or small peptide (acceptor), to form the dipeptide cysteiny1-glycine and \( \gamma \)-glutamyl-acceptor according to the reaction:

\[
\gamma\text{-Glu-Cys-Gly(glutathione)+acceptor} \rightleftharpoons \gamma\text{-Glu-acceptor+Cys-Gly}
\]

The cycle is represented in Fig.3. Considerably high, \( \gamma \)-glutamyl transpeptidase activity is present in plasma membranes of the tissues specialized in absorptive function such as kidney (Albert et al., 1961) and small intestine (Greenberg et al., 1967). The enzyme has been localized by histochemical techniques in the brush border region of the villus tips (Greenberg et al., 1967). The work of Garvey et al. (1976) firmly supports the fact that \( \gamma \)-glutamyl transpeptidase may be involved in intestinal amino acid and dipeptide uptake. Yet, there are certain major difficulties in accepting the involvement of this pathway in amino acid transport. One of the enzymes (5-oxoprolinase) has a very low activity relative to others and its substrate 5-oxoproline might be accepted to accumulate in cells if the system were overloaded with amino acids (Orlowski and Wilk, 1975). Some amino acids have this effect but others do not, and there is a poor correlation in these effects with the efficiency of different amino acids to be acceptor of the \( \gamma \)-glutamyl residues in
FIG. 3. GAMA-GLUTAMYL CYCLE FOR AMINO ACID TRANSPORT

1. γ-glutamyl transferase
2. γ-glutamyl cyclotransferase
3. 5-oxoprolinase
4. cysteinyl glycine synthetase
5. γ-glutamyl cysteine synthetase
6. glutathione synthetase
glutamyl transferase step e.g. \( \gamma \)-glutamyl-phenylalanine is not a substrate and hence phenylalanine could not be transported by this cycle. Characteristics of the amino acids transport system like \( \text{Na}^+ \) dependency, can not be accounted for \( \gamma \)-glutamyl cycle hypothesis \cite{Crane1977}. Non-involvement of \( \gamma \)-glutamyl-transferase in leucine and glucose transport in intestinal brush border vesicles has been demonstrated after papain treatment, which completely abolished the \( \gamma \)-glutamyl transferase activity without affecting the amino acid transport membrane vesicles \cite{Bertetool1980}.

**Inhibitors of the Transport Systems:**

The investigation of complex processes, such as transport of substances across epithelia, is often greatly facilitated, if there are specific inhibitors available which block individual steps in the overall event. Unfortunately the number of such inhibitors are limited and every additional compound by which the number could be extended would be of great value.

Harmaline, a hallucinogenic alkaloid, inhibits sugar and amino acid transport by the guinea pig intestinal mucosa in vitro during short and long incubations. It effects sodium-dependent transport systems in other tissues such as dog colonic mucosa and renal cortex slices, but does not affect sodium-independent transport. It inhibits sodium
entry into intestinal rings, both in the presence and absence of non-electrolytes. It has been proposed that harmaline interacts with the sodium binding site of the transport carrier (Sepulveda and Robinson, 1974). The same workers (Sepulveda and Robinson, 1976) proposed that the harmaline effects the first step of the transport process, namely the translocation of the substrate across the brush border membrane of the enterocytes. Sepulveda et al. (1976) further reported that both the Na\(^+\),K\(^+\)-ATPase activity and the uptake of L-phenylalanine in guinea-pig intestinal mucosa are inhibited by harmaline. The latter effect is not a direct consequence of the former, since higher concentrations are needed to inhibit the enzyme than the influx into the mucosa. Furthermore, the uptake is still sensitive to harmaline when the Na\(^+\),K\(^+\)-ATPase has been fully inhibited by ouabain. The inhibitory action of harmaline on L-phenylalanine uptake by guinea-pig intestinal rings is fully reversible provided only low concentrations of inhibitor are used (Buclon et al., 1977). However, Samarzija et al. (1977) suggested that harmaline does not specifically compete with Na\(^+\) for transport sites. They indicated that harmaline may block the Na\(^+\)-glucose entry step and simultaneously alter one or more other parameters of the system which happen to cancel in their effect on the cell potential. The involvement of sulfhydryl groups in sugars
and amino acids transport processes is reasonably well substantiated and a number of compounds are known to effect the transport by reacting with -SH groups present in the carrier molecule (Stribing, 1975; Schaeffer et al., 1973 and Lerner et al., 1977). Klip et al. (1979) found p-chloromercuribenzoate and p-chloromercuriphenylsulfonate to be powerful inhibitors for D-glucose uptake and phlorizin (a competitive inhibitor of D-glucose transport) binding in brush borders. Klip et al. (1980) have suggested the involvement of sulfhydryl group in the inhibitory process by HgCl₂. Phlorizin is generally believed to bind at the outer surface of the brush border membrane (Toggenburger, 1982), but the evidence supporting this contention is not conclusive. Because the -SH group that is affected by the mercurials is not located at this surface, it appears that the reaction takes place at a site other than the glycoside-binding site. The inhibitory effect of blood-glucose lowering biguanides (phenethyl- and butylbiguanide) on active transport of methionine, leucine, glycine and x-aminoisobutyric acid of hamster small intestine has been reported by Caspary and Creutzfeldt (1973).

Intestinal Brush Border Sucrase:

Among the constituents of the small-intestinal brush border membrane, the dimeric enzyme complex sucrase-
isomaltase (Sucrose x-D-glucohydrolase, EC 3.2.1.48 and oligo-1,6-glucosidase, EC 3.2.1.10, respectively) is the most abundant protein accounting for approximately 10% of the intrinsic proteins. The enzyme complex accounts for all of the sucrase activity, approximately 90% of isomaltase and 70-80% of the maltase activity of the small intestine (Semenza, 1991).

The sucrase-isomaltase (SI) activities have been found in the Brush Border Membrane (BBM) of small intestine of several mammalian species, (Doel et al., 1965). However, isomaltase free of sucrase activity was identified in a single human intestinal preparation (ASP & Dahlqvist 1973). The two enzymes have been studied as a complex in rabbit (Kolinska & Semenza 1967), rat (Kolinska et al., 1972), man (Conklin et al., 1975) and pig intestine (Sjostrom et al., 1980).

It was possible to separate the subunits by the action of denaturating agents. The denaturation being irreversible (Mosimann et al., 1973).

Takesue (1969), purified rabbit intestinal sucrase and its molecular weight was estimated to be 23500. It hydrolyzed maltose, x-methyl glucoside and sucrose but not x'x-trehalose, B-glucoside and x and B galactosides. Maltase activity in the homogenate paralleled to the sucrase activity during purification implying that the enzyme is glucoside-sucrase or an x-glucosidase.
The sucrase-isomaltase complex (SI) is an intrinsic glycoprotein of brush border membrane which is synthesized as a single chain precursor of approximately 260,000 daltons with both enzymatically active sites on the mature protein (Hunziker et al., 1986). This glycoprotein is composed of two similar but not identical subunits of molecular weight approximately 112,000, one splitting sucrose and the other isomaltose x-glucosides. The enzyme complex is anchored to the intestinal BBM by a hydrophobic segments accounting to about 10% of the total protein mass which is located near the NH₂ terminal end of the isomaltase subunit (Brunner et al., 1977).

Further investigation on rabbit SI complex by Cogoli et al., (1972) showed that this glycoprotein is rich in acidic amino acids, it has no free thiols in the native state but denaturation makes six thiols. The tyrosine/tryptophan ratio is 2. The sugar moities (15%) is composed of D-glucose, D-galactose, D-mannose, fucose, glucosamine and galactosamine. The SI complex was shown to contain neuraminic, uronic acid, lipids, phosphate, sulphate or zink. The molecular weight of rat SI complex was found to be 215,000 by Sasaki et al., (1979). The Km values of the enzyme was 18 mM (sucrase) and 4 mM (isomaltase).
Catalytic properties of sucrase:

The SI complex is a membrane bound protein which translocates some sugars across natural and artificial membrane (Braun et al., 1975). Sucrase is an \( \alpha \)-glucosidase as indicated by its substrate specificity. In addition to sucrose, it splits maltose, maltitol, but no fructoside other than sucrose. The transglucosidase activity of sucrase and the fructose-disaccharide exchange indicate the intermediary formation of a glucose enzyme complex. It is possible to inactivate the sucrase activity in the complex without affecting isomaltase activity (Cogoli et al., 1973). Steady state Cleland Kinetic analysis of rabbit small intestine sucrase and isomaltase has shown that the most likely kinetic mechanism is ping-pong bi-bi (for transglucosidation) reducing to ordered uni-bi for hydrolysis (Semenza 1991). Tris competes with the substrate for the glucose substrate site as reported by Semenza and Balthazar (1974). The given scheme is:

\[
\begin{align*}
A & \quad (\text{Sucrose } S) \\
P & \quad (\text{Fructose } F) \\
B & \quad (\text{\textquoteleft Water\textquoteright or acceptor, } X) \\
Q & \quad (\text{Glucose, G transglucosidation product, } G_X) \\
\text{E} & \quad \text{ES} \\
\text{EG}'F & \quad \text{EG}'X \\
\text{E} & 
\end{align*}
\]

Scheme 1 Ping-Pong bi-bi
The above mechanism reduced in the presence of an excess of water and in the absence of any other acceptor, to an ordered uni-bi mechanism (Cleland, 1963).

\[
\text{--- A ---} \text{ (Sucrose, S)} \quad \text{--- P ---} \text{ (Fructose, F)} \quad \text{--- Q ---} \text{ (Glucose, G)}
\]

\[
E \quad \text{ES} \quad \text{EG'} \quad E
\]

Scheme II Uni-bi

The glucose arising from the hydrolysis of sucrose by BBM sucrase was demonstrated to have the kinetic advantages for transport in comparison with glucose added in the free form to the media (Miller and Grane 1961). In addition the glucose generated by the hydrolytic action of other BBM enzymes were not found to have similar advantage of being transported.

**Activators and Inhibitors of sucrase activity:** All small-intestinal sucrase so far investigated are activated by sodium, although the kinetic overall mode of activation and with in a given species, it is dependent on pH (Semenza 1991). At the optimum pH, rabbit sucrase is activated by about 30% with some effect on the apparent km for glucose (Semenza, 1991). Potassium has a higher, Li$^+$ a lower affinity than sodium, the sequence of affinities of the
cations of the first group corresponds to one of Eisenman’s series (Semenza, 1991), and indicates the participation of infinitely spaced -COO\(^-\) groups as ligands (Semenza, 1991). Such ligands can bind H\(^+\) also (a number of less biologically relevant cations). An accurate kinetic analysis of alkali metals and H\(^+\) activation has been carried out by Alvarado’s group (1988). They have demonstrated that the alkali metal ions Na\(^+\) and Li\(^+\) have a concentration dependent biphasic effect on the reaction catalysed by this enzyme. At low concentrations, they facilitate the release of H\(^+\) (a key proton), resulting in K type inhibition. On the basic side of the pH spectrum, the existence of separate non-competitive effects of alkali metal ions, particularly Li\(^+\) was confirmed (Alvardo and Mohmood, 1979). It was reported by Semenza and Balthazar (1974), that the site of activation by Na\(^+\) was located at a level prior to the further transformation of the glucose enzyme complex and also that Tris competes with the substrate for the glucose sub site. Heavy metal ions inhibited the enzyme activity either completely (Ag\(^+\) & Hg\(^+\)) or partially (Cu\(^{2+}\) & Zn\(^{2+}\)) (Sasako et al., 1979). The inhibition by some metals, lack of inhibition by other metals or by P-chloromercuribenzoate indicate activity, where an imidazol group may be required (Kolinska & Semenza 1967). The inhibitory effect of harmaline on intestinal brush border sucrase activity was reported by Mahmood and Alvarado.
(1977), the inhibition was due to interaction of harmaline with alkali metal ions for their binding site because of its positive charge.

It has been reported by Hanozet et al., (1981), that of the fully competitive inhibitors of small intestinal sucrase, acarbose, nojirimycin and deoxynojirimycin have the highest affinity for the enzyme. Their interaction with the enzyme is found to be slow. Their overall "on" and "off" contrast were small which indicated that a conformational change accompanied the interaction of these substances with the active site of intestinal sucrase. Sucrase inhibition by Tris, PD (2-amino-2-methyl-1-3-propanediol), PM (2-amino-2-methyl-1-prapanol) and Li⁺ has also been described by Vasseur et al. (1990). At pH 5.2 PM caused activation of the enzyme whereas Tris had a concentration dependent biphasic effect, first causing activation, then fully competitive inhibition. Above pH 6.8 both Tris and PM acted as fully competitive inhibitors. Li⁺ (300 mM) activated sucrase between pH 5.4 and 7.4 but behaved as an inhibitor at either side of these pH values.

An allosteric, non-compulsory mechanism involving two distinct sites, one for sucrase and other for the metal activator has been described by Mahmood and Alvarado (1975), K⁺ was shown to have 10 times more affinity for the metal site than Na⁺. Later on an extensive investigation on the
quantitative analysis of the mixed activating effects of the alkali metals ions on the rabbit BBM sucrase at pH 5.2 was reported (Alvarado et al., 1980).

**Physiological function of intestinal sucrase:**

In general, disaccharidases are the enzymes that split 12-carbon sugar molecule into 6-carbon subunits. Most enzymes are specific against a specific glycosidic bond. Sucrase is an exception, as it can hydrolyse both maltose and sucrose (Gray et al., 1979). Gray and Coworkers concluded from study of rat SI that, this enzyme accounts for the total hydrolysis of anylase products. Whelan and Coworker (1984), studying pig enzyme demonstrated that SI activity accounts for the hydrolysis of the smallest x-limit dextrin 6'-x-glycosyl-maltotriose.

The turnover of disaccharidases is more rapid than that of the total brush border proteins. Sucrase-isomaltase turnover has been recorded to be as short as 4.5 hours (Das and Gray, 1970). Intestinal sucrase-isomaltase from hamster jejunum did not hydrolyse L-sucrose and the enzyme was reported to be stereospecific (Dinda et al., 1982). Sucrose mediated changes in sucrase activity of jejunal mucosa in the rat were shown to initiate at the level of crypt epithelial cell and was expressed after the latent period of 18-24 hrs. during which these cells matured and migrated to the villus tip (Martin and Grand 1979).
Development of Intestinal Brush Border Sucrase:

The enzymatic development of the rat small intestine is characterized by a group of enzymes like lactase and lysosomal hydrolases that show a high activity at birth and then decline during the third week of life (Henning 1981). These postnatal changes in the enzyme activity have been established by the localization of the enzyme along the crypt-villus axis of the rat on sequentially isolated cells (Webster and Harrison, 1969; Weiser, 1973) or on horizontal sections (Nordstrom et al., 1967). In the suckling rats, sucrase-isomaltase activity first appeared in intestine crypt cells (Herbst and Koldovsky, 1972; Henning et al., 1975; Raul et al., 1977). At 17 days of age, the decline of lactase activity in the crypt zone with the simultaneous appearance of sucrase in crypt cells was observed by Simon et al., (1979). In the adult rat, negligible amounts of active sucrase are present in the crypt cell fraction. Sucrase in villus tip has been found to be 31-fold than that in the crypt base (Webster and Harrison, 1969; Nordstrom et al., 1967). Also Weiser (1973) found sucrase activity to be highest in the upper villus zone and almost absent in the crypts. Using cryostat transversal sections of Fresh-Frozen intestine, either a weak sucrase activity (Fortin-Magana et al., 1970) or a complete absence of this enzyme activity was found in crypt cells (De Both and Plaisier, 1974).
In addition, glucocorticoids and thyroxine were reported to modulate the postnatal rat intestinal sucrase and lactase activity. Cortisone was found to induce coordinated increases in sucrase and lactase activities and in corresponding mRNA levels. Thyroxine, on the other hand, only enhanced cortisone induced sucrase expression and antagonized cortisone by depressing lactase activity post-translationally (Yeh et al., 1991). Insulin administration to mice has also been shown to procociously induce sucrase activity in intestine (Menard and Malo, 1979).

**Molecular genetics of sucrase Isomaltase complex (SI)**

Sucrase isomaltase is an intestine specific gene product that catalyzes the hydrolysis of the disaccharides sucrose and maltose into their component monosaccharides before absorption by enterocytes (Semenza and Auricchio, 1989). The enzyme is synthesized as a single polypeptide, glycosylated in the endoplasmic reticulum and Golgi, and transported to the apical membrane of enterocytes (Brunner et al., 1983; Hunziker et al., 1986 and Semenza and Auricchio 1989). Both subunits are initially synthesized as a single large polypeptide (Pro-SI), which is split into the final forms by pancreatic proteinases when inserted at the luminal cell surface membrane (Hauri et al., 1982 and Grand et al., 1985). Hunziker and his co-workers (1986) have
cloned and sequenced cDNA encoding the rabbit prosucrase-
isomaltase. The nucleotide sequence and derived amino acid sequence of a cDNA clone encoding most of the N-terminal, isomaltase region of human sucrase-isomaltase was reported by Green et al., (1987). Molecular cloning and characterization of rat intestinal sucrase isomaltase cDNA has been done by Broyart et al., (1990). They demonstrated that the induction of sucrase-isomaltase activities is directly associated with an increase in sucrase-isomaltase mRNA levels. They also suggested that circadian modulation of SI transcription may occur in basic SI gene expression. Expression and synthesis of SI were studied in human jejunum and in the colon tumor cell lines CaCO-2 and HT-29 by Beaulieu et al., (1989). It has been shown that sucrase-isomaltase is synthesized by both crypt and villus cells, but processing of the co-translationally glycosylated high mannose precursor is dependent on the state of differentiation of the enterocytes. It represents a general mechanism for the regulation of expression of differentiated cell products at the post translational level. Expression of SI in human small intestine was confirmed by Gorvel et al. (1991), using monoclonal antibodies. They produced monoclonal antibody (8A9) against the human sucrase-isomaltase complex and one (4H3) against the human dipeptidylpeptidase IV in the rat. Both enzymes were found to be expressed in the
poorly differentiated crypt cells of the small intestine as well as in the mature villus cells, and very low levels were found to be expressed in the colon. Homogeneous immunolabeling of the whole clonic epithelium with the monoclonal antibody 4H3 was often observed whereas labeling with the monoclonal antibody 8A9, if any, was either restricted to a few crypts and plateaus. These co-workers further used the two antibodies to perform specific immunoprecipitation of the corresponding antigen, the N-terminal sequence of which was determined after sodium dodecyl sulfate-poly-acryl-amide gel electrophoresis purification and electroblotting, and were compared with those of other species. In secretor blood group A humans both sucrase-isomaltase and the dipeptidyl peptidase IV have type 3 blood groups A determinants. In addition a panel of monoclonal antibodies specific for sucrase-isomaltase, but differing in their ability to stain the proliferative crypt cells in human jejunum, was used by Beaulieu et al., (1990), to investigate expression of this enzyme in adult human colon and colonic tumors. Their results demonstrated that colonic crypt cells and some benign tumor cells synthesize and express at their cell surface sucrase-isomaltase immunologically distinct from that present in the brush borders of small intestine villus cells.

Traber et al., (1992) have examined the distribution of SI mRNA along the crypt villus axis of human
small intestine using isolated epithelial cells and *in situ* hybridization. cDNA to 5' portion of the human SI mRNA was amplified using polymerase chain reaction. Hybridization analysis of the RNA extracted from human intestinal epithelial cells showed that the cloned cDNA recognized a single 6.5-kb mRNA. *In situ* hybridization of duodenal biopsy specimens was performed using a single standard RNA probe derived from this cDNA. Their analysis showed that there was little SI mRNA in crypt cells and appearance of mRNA in enterocytes located at the crypt-villus junction. The mRNA levels were maximal in lower and mid villus cells with decreased levels noted in villus tip cells.

**Na⁺-K⁺-ATPase:**

The Na⁺, K⁺ transporting adenosine triphosphatase (Na⁺,K⁺-ATPase; EC 3.6.1.3) also known as sodium potassium pump is an enzyme, found in nearly all animal cell membranes. It utilizes energy from the hydrolysis of intracellular ATP to transport Na⁺ ions outwards and K⁺ ions inwards. The enzyme may be thought of having three substrates namely ATP, intracellular Na⁺ ions and extracellular K⁺ ions and four products (ADP, orthophosphate, extracellular Na⁺ ions and intracellular K⁺ ions). The physiological significance of the sodium pump is directly or indirectly connected to the maintenance of transmembrane gradients of Na⁺ and K⁺. This is basis for
vital cell functions such as regulation of cell volume
maintenance of transmembrane potential and excitability,
transepithelial salt and water transport, regulation of
transport via co- and counter-transport coupled to the
\( \text{Na}^+ \) - and \( \text{K}^+ \) -gradients and possibly more (Cornelius, 1991). In intestine, enzyme is located at the basolateral site of
the enterocytes and is indirectly linked to \( \text{Na}^+ \) dependent
organis solute uptake transport system (Stirling, 1972).

**Characterization of \( \text{Na}^+,\text{K}^+\)-ATPase**

Among the mammalian tissues, kidney is the only
source of pure \( \text{Na}^+,\text{K}^+\)-ATPase; Jorgensen, (1980). But
immunological studies show that antibodies to pure renal
\( \text{Na}^+,\text{K}^+\)-ATPase) crossreact with \( \text{Na}^+,\text{K}^+\)-pumps in other
mammalian tissues including human erythrocytes
(Jorgensen et al., 1973). This identity of \( \text{Na}^+,\text{K}^+\)-ATPase
from different mammalian tissues is of general biochemical
significant since the information about the structure and
function of the pure \( \text{Na}^+,\text{K}^+\)-ATPase becomes relevant for the
\( \text{Na}^+,\text{K}^+\)-pumps in cells from which the enzyme may never be
purified (Jorgensen, 1980).

The purification and characterization of
\( \text{Na}^+,\text{K}^+\)-ATPase from canine kidney by zonal centrifugation
density gradient showed that almost all proteins accounted
for by two polypeptides with molecular weights of 105,000
and 58,000 and that the mass ratio of the large to small
peptide was 82:18 (Hayashi et al, 1977). The enzyme purified from human cadaver renal tissue exhibited a linear reaction rate with time (Braughler and Corder, 1977). There was no detectable Mg$^{2+}$-ATPase in the final purification SDS-disc gel electrophoresis yielded three protein peaks of 117,000, 92,000 and 56,000 daltons. The peptide band corresponding to 92,500 daltons undergoes Na$^+$-dependent phosphorylation with [γ-$^{32}$P]-ATP. The band for 56,000 daltons is stained for glycoprotein. The Km for Na$^+$ in the presence of 20 mM K$^+$ is 16 mM and the K$_m$ for K$^+$ in presence of 100 mM Na$^+$ is 1.5mM. The temperature optimum is 51°C and the pH optimum 7.00 (Braughler and Corder 1977).

In addition, a particulate enzyme complex from rabbit intestinal mucosa exhibiting Na$^+$,K$^+$-ATPase activity has been isolated and partially purified by Richardson (1968). The ATPase activity exhibits an assymetric stimulatory response to Na$^+$ and K$^+$ and is inhibited by ouabain and Ca$^{2+}$. It was shown that ATPase activity was the moiety of the membrane associated ion translocase system of the rabbit intestinal mucosa and that mucase activity in the culture filtrate from vibrio cholerae could selectively destroy or inactivate this enzyme in vitro. A high specific activity membrane bound Na$^+$,K$^+$-ATPase isolated from rat intestinal mucosal cells was examined for its enzymatic properties by Quigley and Gotterer (1969). The stimulated
enzyme activity required the combined presence of Na⁺ and K⁺ in physiological concentrations and exhibited high sensitivity to K⁺ and an absolute requirement for Na⁺. A Mg²⁺/ATP ratio of 1.0 was necessary for optimal activity and the Km for ATP was found to be 0.1 mM. ATP was the only nucleo triphosphate hydrolyzed. These results indicated that the Na⁺,K⁺-ATPase properties are very similar to those found for similar enzymes isolated from the other sources (Quigley and Gotterer, 1969). The major portion of the Na⁺,K⁺-ATPase in the rat intestinal mucosal cells has been isolated in fraction (M-I) which contains less than 2% of the total cellular protein. The enzyme is tightly bound to a membrane which is not mitochondrial, microsomal or brush border in nature.

Cation Activation of the Enzyme:

The activation by cytoplasmic Na⁺, K⁺ exchange has been studied by Karlish and coworkers in proteolipids (Karlish and Pick, 1981; Karlish and Stein, 1985) and this effect was found to be transmembrane allosteric effect. Cytoplasmic cation activation was also studied by Apell and Marcus (1986) on reconstituted Na⁺, K⁺ ATPase from outer medulla of kidney. At 16°C they found hyperbolic activation of turn-over by cytoplasmic Na⁺ with a Km=15 mM. They also measured activation curves where cytoplasmic Na⁺ and K⁺ were varied inversely, their sum held constant at 150 mM. This kind of "mixed activation curves" have been studied
previously in detail by Skou (1975), on membrane bound enzyme, where it reveals the sidedness of the effects of cations in an unsided preparation of Na\(^+\), K\(^+\)-ATPase due to the spread of affinities for Na\(^+\) and K\(^+\) on the two aspects of the enzyme. The reconstituted kidney enzyme shows typically 'Napoleon's hat' - properties of an unsided preparation (Apell and Marcus, 1986), it is activated steeply at low K\(^+\), a plateau is established in the middle region and at K\(^+\) concentration higher than 20 mM deactivation begins and activity gradually declined to zero at 120 mM K\(^+\), 30 mM Na\(^+\). The activation by cytoplasmic K\(^+\) obtained with saturating extracellular K\(^+\) is rather surprising. Yoda and Yoda (1987) also found activation of Na\(^+\)/K\(^+\) exchange by cytoplasmic K\(^+\) at higher ATP due to an acceleration of E\(_1\)P-E\(_2\)P conversion. Also the mentioned observation by Apell and Marcus (1986) of hyperbolic cytoplasmic activation by Na\(^+\) is typical. This can, however, be explained by the spread in the affinities for Na\(^+\) on cytoplasmic binding sites, where only the relatively low-affinity site is revealed. A clear dependence of the turn-over rate on pH in the range between 5-7.5 of cytoplasmic Na\(^+\) ion was also observed. This competition between H\(^+\) and Na\(^+\) was of the mixed type. These finding can most readily be explained as a reflection of the H\(^+\)/K\(^+\) exchange mode first described in proteoliposomes by Hara and Nakao (1986).
The mechanism by which Li+ stimulates the Na+,K+-ATPase activity of rat brain has been reported by Robinson (1975). It was suggested that Li+ might stimulate the enzyme activity by relieving inhibition due to K+ and Na+: (i) by competing with K+ for the site on the enzyme through which K+ decreases the apparent affinity for Mg2+ATP and (ii) by competing with Na+ at low affinity inhibitory sites, which, represent the external sites at which Na+ is discharged by the membrane Na+/K+ pump.

Cation Inhibition of the Pump:

During the steady state kinetic analysis of Na+,K+-ATPase by Rossi and Garrahan (1989), it was shown that at lower Na+, Mg2+ becomes a low-affinity inhibitor of Na+,K+-ATPase. Inhibition followed a pattern that was different from inhibition of Na+,K+-ATPase activity and was consistent with a mechanism in which Mg2+ acts both as dead end and as product inhibitor. Further the kinetic analysis in term of a product inhibitor (Robinson and Pratap, 1991) by Mg2+, were shown to require Mg2+ release at multiple steps. It was suggested that three alternative points for release, before Mg2+-ATP binding, K+ release and Na+ binding. The later alternative necessitates two Mg2+ ions bound simultaneously to the enzyme, presumably to divalent cation-sites associated with the phosphate and the nucleotide domains of the active site. Experiments with the
reconstituted Na\(^+\), K\(^+\)-ATPase show that besides the ATP dependent cytoplasmic Na\(^+\), K\(^+\)-competition for Na\(^+\) activation, there is a high affinity inhibitory effect of K\(^+\) (Cornelins and Skou, 1991). The high affinity inhibition was also observed with Cs\(^+\), Li\(^+\) or K\(^+\) as the extracellular cation, but the fractional inhibition was much less pronounced than with Na\(^+\) as the extracellular cation. It was suggested that either there are two populations of enzyme, one with normal ATP dependent Na\(^+\)/K\(^+\) competition and other which due to the preparative procedure has lost this ATP sensitivity or that the normal enzyme has two pathways for the transition from E\(_2\)P to E\(_1\)ATP. One on which, the enzyme with the translocated ion binds cytoplasmic K\(^+\) with a high affinity but not ATP and another on which ATP is bound but not K\(^+\). The suggested kinetic model is as follows (Fig.4).

**The Role of Lipids in Na\(^+\),K\(^+\)-ATPase Activity:**

In the cell membrane the lipids apart from functional protein-lipid interactions serve as solvent for the intrinsic proteins which can diffuse laterally. The lipids themselves also show lateral diffusion but undergo relational and transverse motions (flip-flop) too.

Pure membrane-bound preparations of Na\(^+\),K\(^+\)-ATPase contains many molecules of cholestrol and both neutral and acidic phospholipids, for each molecule of Na\(^+\),K\(^+\)-ATPase, but it is clear that much of this lipid is unnecessary for
Cation-loading sites are accessible from the exterior and prefer K⁺ to Na⁺

Cation-loading sites are occluded

Cation-loading sites are accessible from the interior and prefer Na⁺ to K⁺

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the enzymes function. Several methods have been used to investigate the lipid requirements of the enzyme and the role of this lipids in enzyme activity. The oldest and the most straightforward approach is to delipidate the enzyme with detergents or organic solvents and then to see which lipids are able to restore activity. Application of these methods has led to the following general conclusions:

1. The lipid requirements are most stringent for Na\(^+\),K\(^+\)-ATPase activity and less stringent for the various partial reactions, indeed, ATP can be bound with a high affinity even by completely delipidated enzyme.

2. Activation of delipidated enzyme by lipid shows positive cooperativity.

3. There seems to be no absolute requirement for any particular phospholipid. For maximal activity, however, negatively changed phospholipids seem to be necessary, and the use of spin-labeled phospholipid shows that the protein possesses sites which bind negatively charged species preferentially. Gonzalez and Zambrano (1983) showed that the breakdown of sulphatide of membrane rich in ATPase is accompanied by inactivation of the ouabain-sensitive phosphatase activity. However, the sole addition of sulphatide in the medium was able to protect the phosphatase enzyme. Hansson et al (1978), and Zambrano et al. (1981) also suggested the specific role for sulphatides.
The fluidity and thickness of the lipid bilayer are also important in determining enzyme activity. Johannsson et al. (1981) reported that changes in membrane protein activities caused by extraneous agents are frequently attributed to changes they induce in bilayer fluidity. Agents which increase bilayer fluidity will often be perferentially distributed towards the centre of the bilayer where the structure is most liquid-like and the entropy is relatively high. They are, therefore, likely to increase the thickness of the bilayer and as well as its fluidity parameters in modulating membrane protein functions.

**Inhibitors of the Na\(^+\),K\(^+\)-ATPase:**

Several blocking agents have been used to study the functioning of the sodium pump, Ouabain, oligomycin and N-ethylmaleimide seem to be the most useful inhibitors of the Na\(^+\),K\(^+\)-ATPase.

Considerable evidence indicated that ouabain inhibits Na\(^+\),K\(^+\)-ATPase by binding to the phosphorylated form of the enzyme, but does not interfere with its formation (Charnock and Potter, 1963; Sen et al., 1969). Mg\(^{2+}\) ions appears to be a prerequisite for the ouabain-enzyme interaction (Skou et al., 1971). Phosphorylation of ATP or Pi drastically increase the binding rate,
yielding complexes with the same dissociation constant (Schonfeld et al., 1972). The number of ouabain binding sites equals the number of ATP binding and phosphorylation sites (Erdmann and Schoner, 1974). The rate constants for association and dissociation are determined by the structure of the drug. The dissociation rate constant also depends on whether the enzyme has been phosphorylated by ATP or Pi. The association rate constant merely depends on the steroid portion of the ouabain regardless of whether phosphorylation takes place by ATP or Pi (Yoda, 1974).

In erythrocytes, the penetrating sulfhydryl reagent NEM also inhibits ouabain binding, but not the non-penetrating sulfhydryl reagents ethacrynic acid and p-chloromercuriphenyl sulfonate (Hoffman, 1973). Thus ouabain binding to the outside is influenced by substrates and sulfhydryl reagents acting on the inside. The activating cations also affect ouabain binding. Internal Na⁺ and K⁺ as well as external K⁺ inhibit ouabain binding whereas external Na⁺ promotes binding by competition with external K⁺.

Reaction of Na⁺,K⁺-ATPase with agents modifying the sulfhydryl groups inhibit the enzyme activity. Noticeable differences in the inhibitory effects on overall and partial reactions have various essential sulfhydryl groups. Hart and Titus (1973) reported the presence of 2-6 sulfhydryl groups per active centre, and this -SH group in ATP binding site may interact with 6-amino group of ATP
Titration of the sulfhydryl groups with 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB) in the presence of sodium dodecyl sulfate (SDS) reveals that 36 free -SH groups are present per mole of Na⁺,K⁺-ATPase (250,000 mol.wt), 34 on the 100,000 mol.wt. subunit and only 2 on the 50,000 mol.wt. glycoprotein subunit (Schoot et al., 1979). Modification of essential group inside the ATP binding centre by means of 6-mercapto derivative of ATP results in nonparallel inhibition of Na⁺,K⁺-ATPase activity (Palzelt-Wenczler et al., 1975). Reaction with DTNB results in a similar nonparallel inhibition of Na⁺,K⁺-ATPase activity (Schoot et al., 1978). Various effects of NEM on the Na⁺,K⁺-ATPase activity have been described. Banerjee et al. (1972) reported the enhancement of the ATP-ADP phosphate exchange activity, due to an inhibition of the E₁ P-E₂P transition of NEM for a crud enzyme preparation. On the other hand, parallel inhibition of the Na⁺,K⁺-ATPase activity and the partial reactions is found in a purified enzyme preparation (Schoot et al., 1977). In the latter case, a second essential -SH group, outside the high-affinity ATP binding site is also involved. The effect of other sulfhydryl reagents, such as chlorpromazine, ethacrynic acid, and organomercurithiosalicylate and dansyleysleinylmucuric chloride have also been studied. A common effect of sulfhydryl reagents is the reduction in the number of ATP and ADP binding sites (Norby and Jensen, 1974; Erdmann and
Schoner, 1973). The exposure of the various vital -SH groups to the reagents apparently depends not only on reaction temperature and purity of the preparation but also on change in enzyme conformation, induced by various ligands active in overall ATPase reaction such as ATP, ADP, Pi, Na⁺,K⁺ and Mg²⁺ (Hart and Titus, 1973). Na⁺ and Mg²⁺, give rise to E₁.Na and E₂.Mg conformations respectively, which increase the inhibition by NEM, DTNB (Schoot, 1978). Phosphorylation by ATP or Pi, giving the E₂-P.Mg conformation also enhances ethacrynic acid inhibition (Banerjee et al., 1971) but has little or no effect on the inhibition of NEM (Banerjee et al., 1972).

Canessa et al. (1973) reported that harmaline is an inhibitor of Na⁺,K⁺-ATPase specially the Na⁺-dependent phosphorylation step. Harmaline at 0.3 to 3 mM inhibited several membrane ATPase preparations such as those from human erythrocytes, rat brain and squid retinal axons. It is thought that this drug is a competitive inhibitor at sodium site. However, Robinson (1975) have shown that harmaline inhibits the Na⁺,K⁺-ATPase activity by affecting both Na⁺ and K⁺ activation.

Oligomycin is another well known potent inhibitor of Na⁺,K⁺-ATPase activity. Sachs (1980) suggested that oligomycin must bind preferentially to the enzyme in E₁P form. Phosphorylation is not essential for oligomycin binding or restricted to the blocking of the conversion of
\[E_1^P \rightarrow E_2^P\] by this compound. Using tryptic digestion, Robinson (1982) showed that oligomycin held the dephosphoenzyme in the \(E_1\) form even in presence of \(K^+\). Esman (1983) found that when \(K^+\) was added to the enzyme suspended in a medium containing \(Mg^{2+}\) and \(Na^+\), oligomycin reduced the rate of conversion of enzyme to \(K^+\) form by three orders of magnitude. However, Askari and Koyal (1971) reported that low (but not high) concentration of oligomycin stimulate \(P\)-nitrophenylphosphatase activity in presence of \(Na^+\), and low concentration of \(K^+\) ions in rat brain \(Na^+\),\(K^+\)-ATPase. Whittington (1973) also suggested that oligomycin could stimulate \(Na^+\),\(K^+\)-ATPase activity if either ATP or \(Na^+\) concentration were very low. Tobin and Akera (1975) showed that showdomysin (a nucleoside antibiotic containing a maleimide ring) inhibits \(Na^+\),\(K^+\)-ATPase activity by reacting with specific chemical group or groups at the nucleotide-binding site on this enzyme. Inhibition by showdomycin appeared to be more selective to this site than due to tetrathionate or NEM. SPAI-1, a peptide isolated from procine duodenum, has also been shown to inhibit \(Na^+\),\(K^+\)-ATPase activity in vitro (Araki et al., 1989). SPAI-1 inhibited \(Na^+\),\(K^+\)-ATPase preparations from various organs of dog and rat or from sheep kidney with similar potency. Three isoforms of rat \(Na^+\),\(K^+\)-ATPase had similar sensitivity to inhibition by SPAI-1 although this isoforms had remarkable differences in their sensitivity to the inhibitory effect of ouabain (Ishizuka et al., 1991).