Metallobiomolecules (Based on biological function)

- Transport & Storage proteins
- Enzymes
- Non protei[ns]

Transport & Storage proteins

- [E]lectron Carriers
  - → Cytochromes (Fe) — Respiration–e⁻ … $Fe^{II} \rightleftharpoons Fe^{III}$
  - → Iron-Sulfur (Fe) (Fe-S clusters, Oxi-red.)
  - → Blue copper (Cu) ($Cu^{I/II}$) $O_2 \rightarrow H_2O/H_2O_2$
- metal storage, Carrier & Structural
  - → Ferritin (Fe) → store iron & Release in controlled fashion.
  - → Transferrin (Fe) → Bind Fe & transport in body
  - → Ceruloplasmin (Cu) (Cu Carrying protein, Fe-metabolism)
- O_2 binding
  - → Hemoglobin (Fe)
  - → myoglobin (Fe)
  - → Hemerythrin (Fe) O_2
  - → Hemocyanin (Cu) (O_2 transport invertebrates, …

Enzymes

- Hydrolases
  - → Carboxypeptidases (Zn) — –C–NH– Pancreas, C-terminal –COOH, N-terminal –NH
  - → Aminopeptidases (Mg, Mn)
  - → Phosphatases (Mg, Zn, Cu)
    - Substrate –OP → (H_2O) → Substrate–OH + –O–P–OH
- Oxido-reductases O_2^-, H_2O/H_2O_2
  - → Oxidases (Fe, Cu, Mo)
  - → dehydrogenases (") $C_2H_5OH \rightarrow CH_3CHO$
  - → Hydroxylases (") (+ OH gp; Oxidation, detoxification, water soluble)
  - → oxygenases (Fe) — oxidizes substrate using molecular O_2/air, degrada[tion]
  - → Superoxide dismutase (Cu, Zn, Mn) $O_2^- \rightarrow O_2$ or H_2[O_2]
  - → Nitrogenases (Fe, Mo) $N_2 \rightarrow$ Fi[xation], Cyano[bacteria]
  - → Hydrogenases (Fe) $H_2 + A_{ox} \rightleftharpoons 2H^+ + A_{red}$
- Isomer[ases]
  - Synthe[tases]
  - → Vit.
  - Coenz[ymes] (C…

Non proteins

- metal transport & Structural
  - → Siderophores (Fe) — Fe-chelating compd, Fungi, bacteria → transport across cell mem.
  - → Skeletal (Ca, Si)
- photo-redox
  - → Chlorophyll (Mg)
  - → photosystem II (M[n])

⇒ Biochemistry of Na^+, K^+ & Cl^-

1) Source - $NaCl$, K^+
2) Absorption: ≈ 100%.
3) distribution: Na^+ extracellular, K^+ intracellular
4) functions:

a) → maintenance of normal hydration & osmotic pressure

The Sodium/ Potassium pump :-

Cells are enclosed by a membrane about 7000 P. (70 Å) thick

Composed of double layer of protein separated by lipids.

Cations cannot pass through the lipid layer without encapsulation & thus the enclosed cation must an organic, lipid-soluble surface to the membrane.

In most animal cells the conc. of K^+ is about 0.15 M & that of Na^+ is about ·01 M.

Outside — Na^+ = ·15 M
K^+ = less than ·004 M
(conc. rather close to those of seawater)

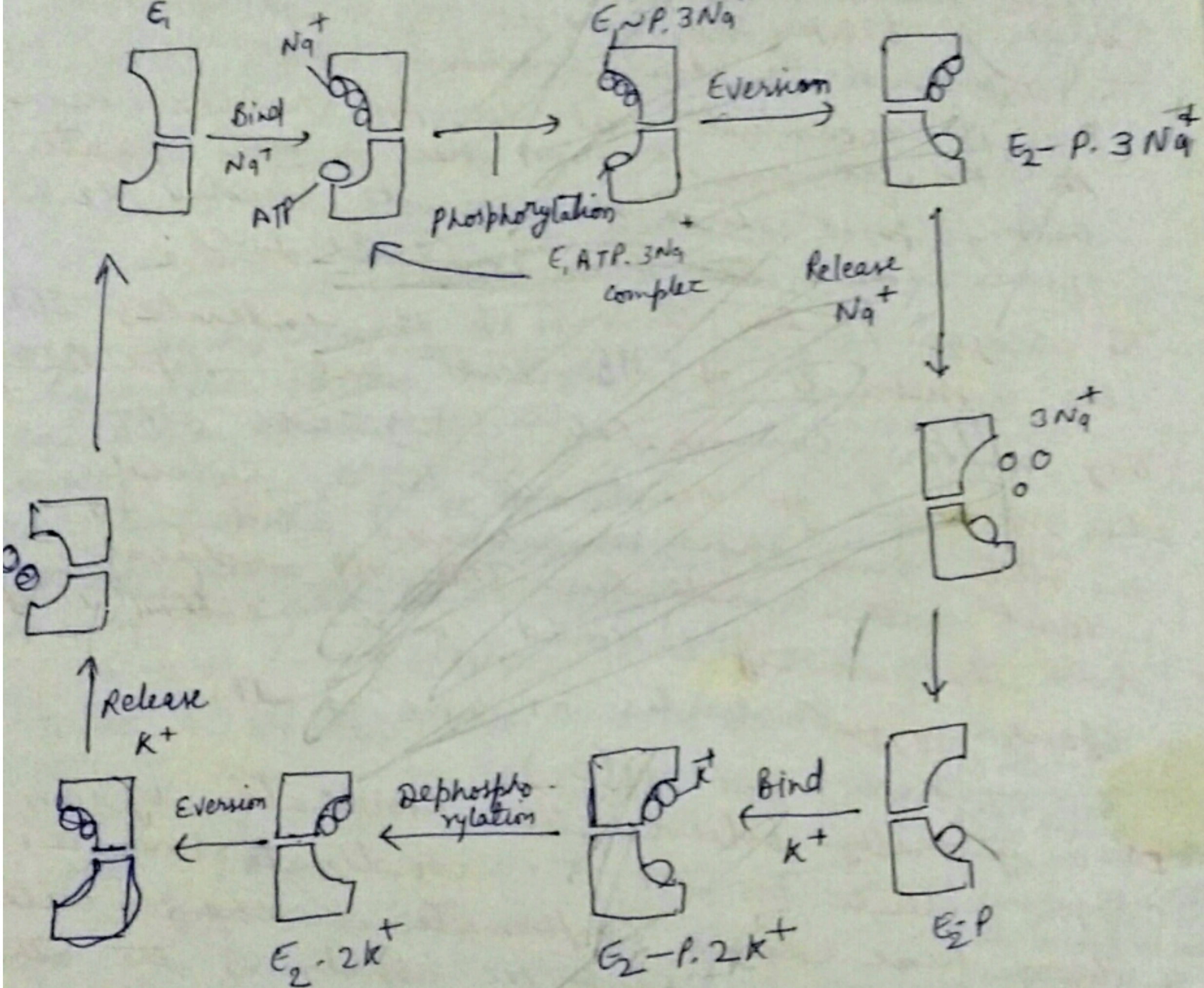
✓ Maintenance of these large-conc. gradients requires a (Na^+/K^+) pump.

Energy of transport of the ions is provided by the hydrolysis of ATP.

Each ATP molecule hydrolyzed transports $3Na^+$ out of the cell & $2K^+$ ($+H^+$) into the cell. The K^+ is required in the cell for glucose metabolism, protein synthesis and activation of some enzymes.

The transport of glucose & amino acid into the cell is coupled with Na^+ transports, c is favoured by great conc. gradient.

The Na^+ entering the cell in this way must be pump out.



A schematic diagram of the mechanism of sodium pumping highlighting the role of eversion of the enzyme

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INTRODUCTION

ATP DRIVEN ACTIVE AND PASSIVE TRANSPORT –

- The lipid bilayer of biological membrane is intrinsically impermeable to ions and polar molecules. Permeability is conferred by two classes of membrane proteins **pumps and channels.**

- Pumps use a source of free energy such as ATP to drive the thermodynamically uphill transport of ions and molecules.

- Pumps are energy transducers in that they convert one from of free energy into another.

- **Active transport is an endergonic process.** When a molecule moves from lower concentration to higher concentration as is often required in the cells, energy input is required and transport is described as **active transport.**

- Passive transport is along concentration gradient and does not require energy.

- Hydrolysis of ATP is coupled with the following transport system. Examples are Na^+- K^+ transport, H^+- K^+ transport, Ca^{++} transport, etc.

BASIC IDEA-

- All animals cells (especially eukaryotes) actively throw out Na^+ ions and take in K+ ions, the two processes being facilitated by integral protein called Na^+- K^+ ATPase or sodium pump. It is necessary because K^+ is needed for many functions for which Na^+ is inhibitory.

- Na^+, K^+, Cl^- and other ion gradients help neurons to communicate. It regulates cell volume and cell shape.

- In fact upto 20-40% of total metabolic energy of the cells is consumed in the maintenance of these ion gradients and this energy consumption requires 70% in the neural tissue.

- The ATPase hydrolyse ATP on cytoplasmic side of the membrane so that $3Na^+$ ion are transported out of the cells and two K^+ ions are transported into cell for each ATP molcule hydrolysed. Since this involves net movement of one positive charge outward per cycle, sodium pump is described as electrogenic in nature

The net process is as shown :

$$3\ Na^+ (in) + 2\ K^+ (out) + ATP + H_2O \rightleftharpoons 3\ Na^+ (Out) + 2\ K^+ (in) + ADP + Pi$$

Sodium- Potassium Pump : ($Na^+ - K^+$ ATPase System)

- This is an energy dependent ion pump. It lowers the conc. of ions inside the cell relative to outside favouring flow of water mol. from inside to outside.

- Membranes are structurally and functionally asymmetric. The outer and inner surfaces of all known biological membranes have different components and different enzymatic activities.

- An example is the pump that regulates concentration of Na^+ and K^+ in the cells.
  The ion gradients are generated by a specific transport system, an enzyme that is called the $Na^+ - K^+$ pump or the $Na^+ - K^+$ ATPase. The hydrolysis of ATP by the pump provides the energy needed.

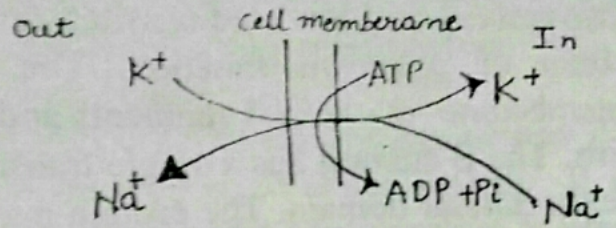
  The pump is called $Na^+ - K^+$ ATPase because the hydrolysis of ATP occurs only when Na^+ and K^+ are bound to pump.

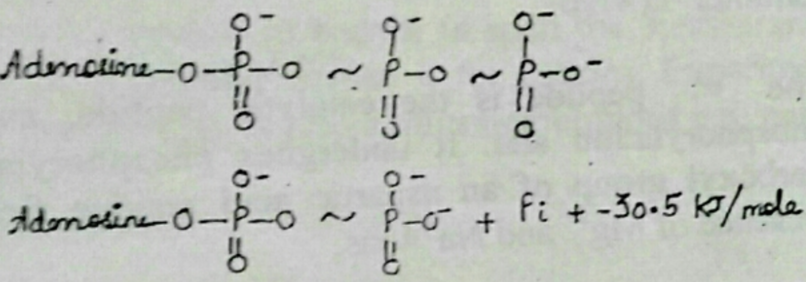
- Moreover it requires Mg^{2+}. Cells are enclosed by a membrane of about $70A^0$ thick and which is composed of double layers of protein separated by lipids, cation can not pass through the lipid (fatty) layer without encapsulation, lipid soluble surface to the membrane.

- In most animal cells the concentration gradient of K^+ is 0.15M and that of Na^+ is about .0 1 M. in the fluids outside the cells the conc. of Na^+ is 0.15 M and that of K^+ is less than .004M. Maintenance of these large conc. gradient requires a $Na^+ - K^+$ pump.

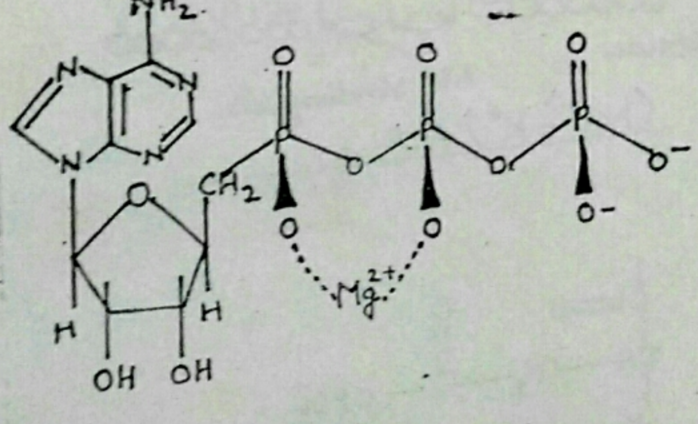
- It is important to know that ATP stores energy for the cells in the form of free energy that can be released on hydrolysis of P-O-P phosphate bond of the phosphate polymer. The P-O-P bond is formed by condensation of two P-OH units with loss of H_2O, and reaction is controlled by pH and electrostatic charge.

➤ The high concentration gradient of Na^+ and K^+ that exist across the cell membrane are maintained by activity of an energy requiring pump that transports Na^+ out of the cell in exchange for K^+ as most animal cells have high conc of K^+ inside cell membrane than outside and high conc of Na^+ outside the cell than inside **ATP is hydrolysed to ADP and inorganic phosphate.**





Sodium and potassium ion-activated adenosine triphosphatase is a complex, membrane bound eneyme that catalyzes the transport of three sodium ions out of cell and two potassium ions into cell with associated hydrolysis of MgATP.

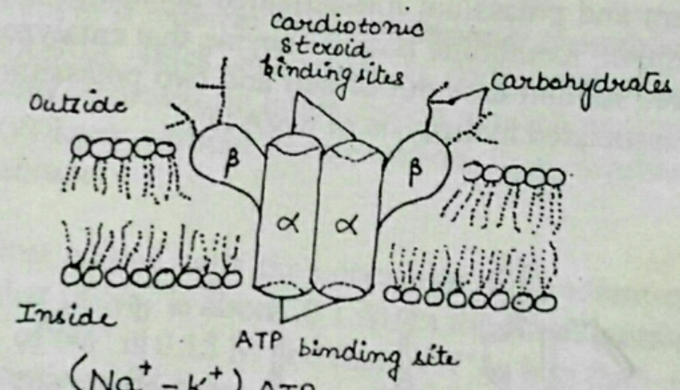


- Enzyme consist two different polypeptides with Molecular weights about 100,000 & 50,000, designated as the α- & β- peptides respectively.

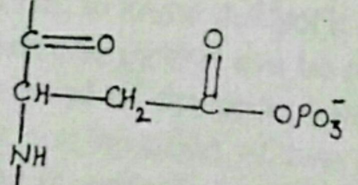
Structure of $Na^+ - K^+$ ATPase :

- **One of the most thoroughly studied active transport systems is the ($Na^+ - K^+$) ATPase in the plasma memberane of higher eukaryotes which was first characterized by Jens Skou.** This transmemberane protein consists of two types of subunits : a 110-kD nonglycosylated α subunit that contains the enzymes catalytic activity and ion-binding sites, and a 55 kD glycoprotein β submit of unknown function. The α subunit has eight transmemberane α- helical segments and two large cytoplasmic domains. The β subunit has a single transmemberane helix and a large extracellular domain. The protein may function as an $(\alpha\beta)_2$ tetramer in vivo.

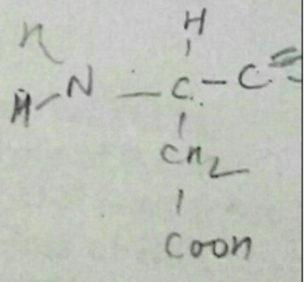
- The α peptide is the catalytic peptide, and contains the phosphorylation site. It undergoes phosphorylation at the β-Carboxyl group of an aspartic acid residue from ATP in the presence of Mg^{2+} and Na^+ ions.



($Na^+ - K^+$) ATPase



ASPARTYL PHOSPHATE RESIDUE



Aspartic a

- The phosphatase reaction requires K^+ . The α - peptide also contains the binding site for cardiac glycoside inhibitors such as **ouabain.** The β - peptide is a glycoprotein whose function is uncertain.

- $Na^+ - K^+$ ATPase protein consists of two components, a 100 – kilodalton catalytic subunit and 45-kilodalton associated glycoprotein, organized into and $\alpha_2\beta_2$ tetramer.

- Controlled hydrolysis with trypsin in presence of K^+ or Na^+ gives different results for loss of ATPase activity. It is so because enzyme exists in two different conformation in presence of these cations. The α peptide in known to span the memberane, and so can provide a pathway across it for cations. Experiments have shown β polypeptide is also a transmembranous polypeptide.

Working and Mechanism

➢ **The Na^+ - K^+ ATPase is an antiport** that generates a charge seperation across the membrane. The key to the $Na^+ - K^+$ ATPase is the phosphorylation of a specific Asp residue of the transport protein. ATP phosphorylates the transporter only in the presence of Na^+ whereas the aspartyl phosphate residue is subject to hydrolysis only in the presence of K^+. **This suggests that Na^+ - K^+ ATPase has two conformational states (called E_1 and E_2) with different structures, different catalytic activities and different ligand specificities.** The protein is thought to operate in the following manner :

Operation of pump :

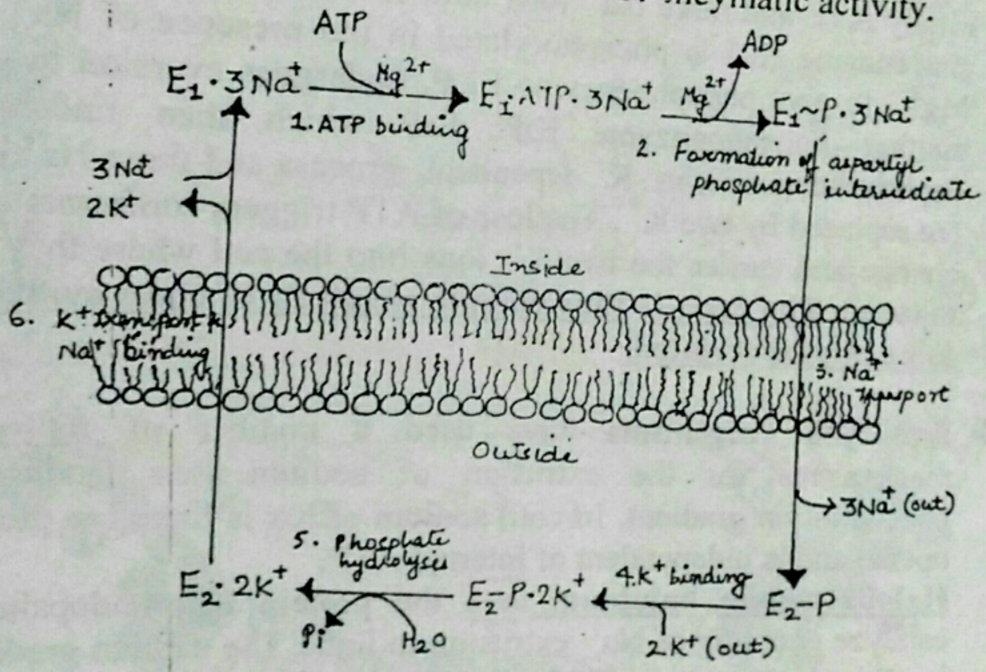
1) The transporter in E_1 state binds three Na^+ ions inside the cell and then binds ATP to yield an E_1. ATP. $3Na^+$ complex.
2) ATP hydrolysis produces ADP and a 'high energy' aspartyli phosphate intermediate $E_1 \sim$ p. 3 Na^+ ('~' indicates high energy bond).
3) This high energy intermediates relaxes to its low energy conformation, E_2 – P. 3 Na^+ and relaxes its bound Na^+ outside the cell.
4) E_2 – P binds two K^+ ions from outside the cell to form an E_2- P. 2 K^+ complex.
5) The phosphate group is hydrolysed, yielding E_2 . 2 K^+.
6) E_2. 2 K^+ changes conformation, releases its two K^+ ions inside the cell and replaces them with three Na^+ ions, thereby completing the transport cycle.

➢ Although each of the above reaction steps is individually reversible, the cycle circulates only in the clockwise direction under normal physiological conditions. This is because ATP hydrolysis and ion transport are coupled unidirectional processes.

Role of Mg^{2+} :

➢ **For the ATPase activity of the enzyme, Mg^{2+} is required.** The activating effect of Mg^{2+} upon the cleavage of phosphoryl group from the ATP could reflect the enhancement of an SN^2 reaction at the phosphorus by electron withdrawal and charge neutralization via coordination to the metal.

Mg^{2+} also has a role as a regulator of encymatic activity.



SCHEME FOR ACTIVE TRANSPORT OF Na^+ AND K^+

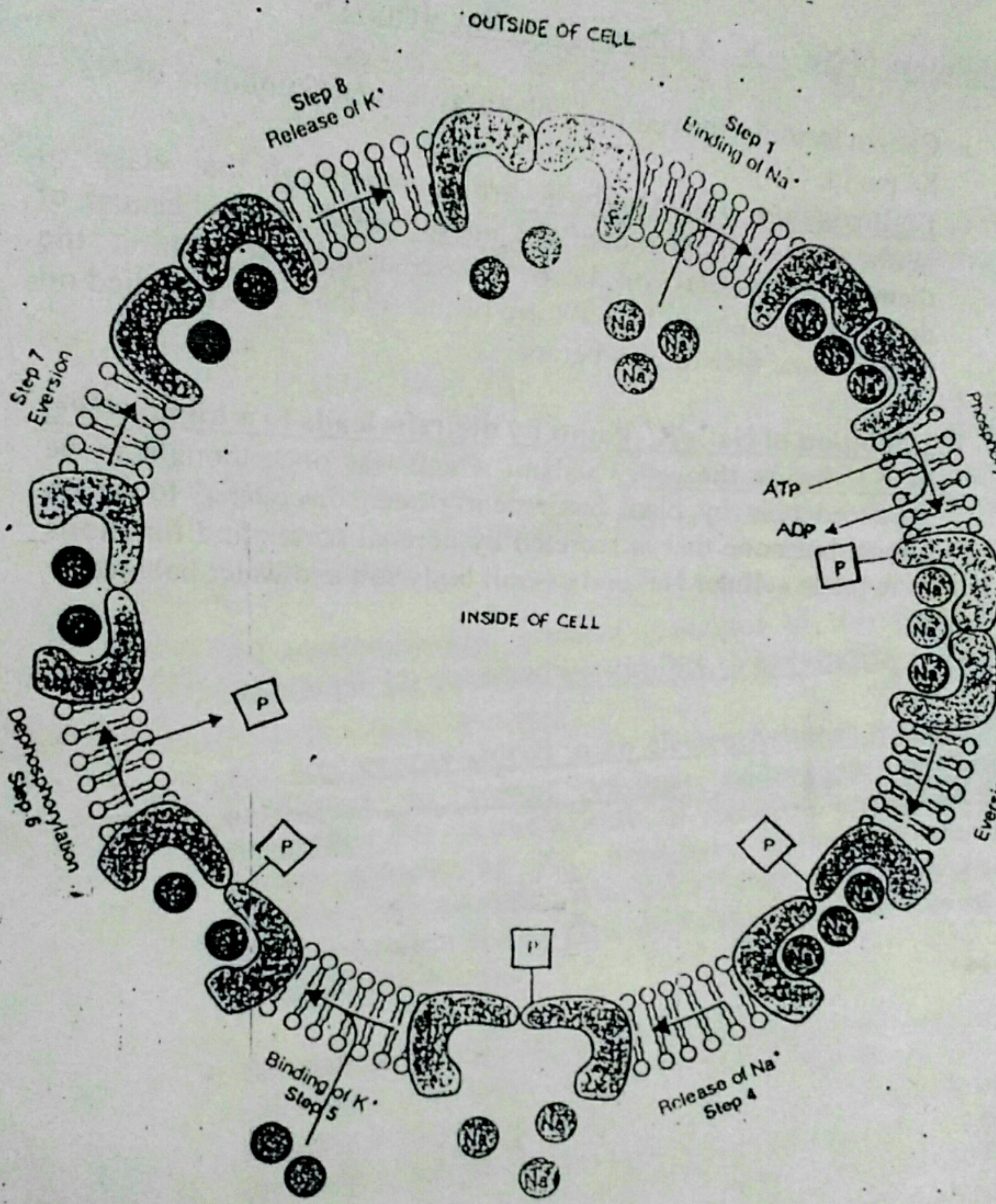
➢ Sodium binding to the inner side of the memberane facilitates phosphorylation of a specific aspartic-acid residue on the enzyme. This process then includes a conformational change that leads to transport of the three sodium ions across the memberane. Next potassium binding on the outer memberane catayzes dephosphoryltion of the enzyme, followed by inverse conformational change to transport the two potassium ions into the cell.

- The conductance of both Na^+ and K^+ increases greatly within 0.1 ms, leading to a large inward current of Na^+ and a smaller outward current of K^+ Acetylcholine which is a neurotransmitter, opens a singles kind of cation channel, which is almost equally permeable to Na^+ and K^+. This change in ion permeability is mediated by acetylcholine receptor.

- Based on reaction sequence, the mechanism can be outlined as. Firstly ATP and three Na^+ ions bind to inside of the memberane and enzyme (E_1) is phosphorylated in the presence of Na^+ and Mg^{2+} to give phosphoenzyme E_1 P. undergoes eversion to give another phosphoenzyme E_2P. E_2P which then undergoes dephosphorylation in K^+ dependent process and three Na^+ ions are replaced by two K^+. The loss of ATP triggers conformational change and carries the two K^+ ions into the cell where they are released. **The cycle is thus complete and E_1 is again available to be phosphorylated.**

- Prokaryotic organisms have used a number of transport mechanisms for the extrusion of sodium ions against a concentration gradient. In E.coli, sodium efflux is linked to proton uptake and is independent of internal ATP.

Halobacterium halobium uses the protein halorhodopsin to catalyze coupling of Na^+ extrusion to light. The sodium gradient in the methanogen **Methanococcus voltae** is exploited in transport of isoleucine as a positively charged complex.



SCHEME DEPICTING THE STEPS IN ATP-DEPENDENT EXPORT OF SODIUM AND POTASSIUM IONS BY Na^+-K^+ ATPase