

# Oxidative Phosphorylation

- \* Mitochondria
- \* Electron transport chain  
its organization and function.
- \* Inhibitors of ETC.
- \* Uncouplers of ETC.
- \* Peter Mitchell's chemiosmotic hypothesis
- \* Proton Motive force
- \*  $F_0 F_1$  ATPase  
structure and mechanism of ATP synthesis
- \* Metabolite transporters in mitochondria.
- \* Regulation of oxidative phosphorylation.
- \* ROS production and antioxidant mechanism
- \* Thermogenesis.

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# Mitochondria

- \* They are central to neuronal and muscular function, and regulate whole body metabolism & body weight.
- \* ATP production, thermogenesis, steroid synthesis and apoptosis are the important functions of mitochondria.
- \* Human neurodegenerative diseases as well as cancer, diabetes and obesity are possible results of compromised mitochondrial function.
- \* Mitochondria is the site of oxidative phosphorylation in eukaryotes.
- \* Like gram negative bacteria, <sup>mitochondria</sup> have two membranes where the outer membrane is readily permeable to molecules having  $M_r < 5000$  and ions, which move through transmembrane channels called PORINS.



ATP synthase  
( $F_oF_1$ )

Cristae

## Outer membrane

Freely permeable to  
small molecules and ions

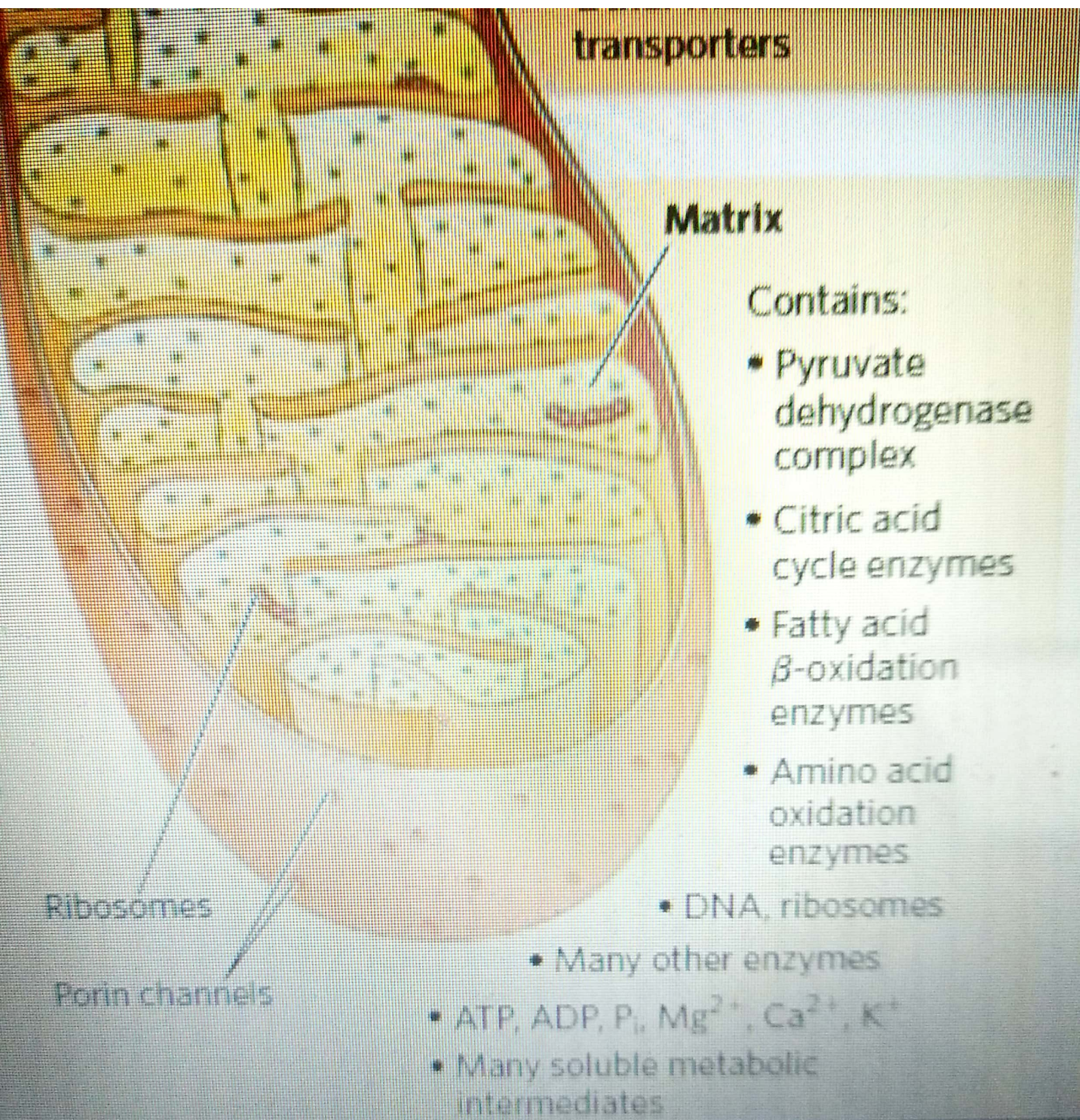
## Inner membrane

Impermeable to most  
small molecules and ions,  
including  $H^+$

Contains:

- Respiratory electron carriers (Complexes I-IV)
- ADP-ATP translocase
- ATP synthase ( $F_oF_1$ )
- Other membrane transporters







\* The inner membrane is impermeable to most small molecules and ions along with protons. Specific transporters are present on the membrane.

\* The inner membrane bears components of ETC and ATP synthase.

\* Mitochondrial matrix enclosed by inner membrane contains/houses:

✓ pyruvate dehydrogenase complex and enzymes of citric acid cycle.

✓ fatty acid  $\beta$ -oxidation pathway

✓ amino acid oxidation pathway.

\* Specific transporters carry pyruvate, fatty acids, and amino acids or their  $\alpha$ -keto derivatives into the matrix.

ADP &  $P_i$  are specifically transported into matrix as newly synthesized ATP is transported out.



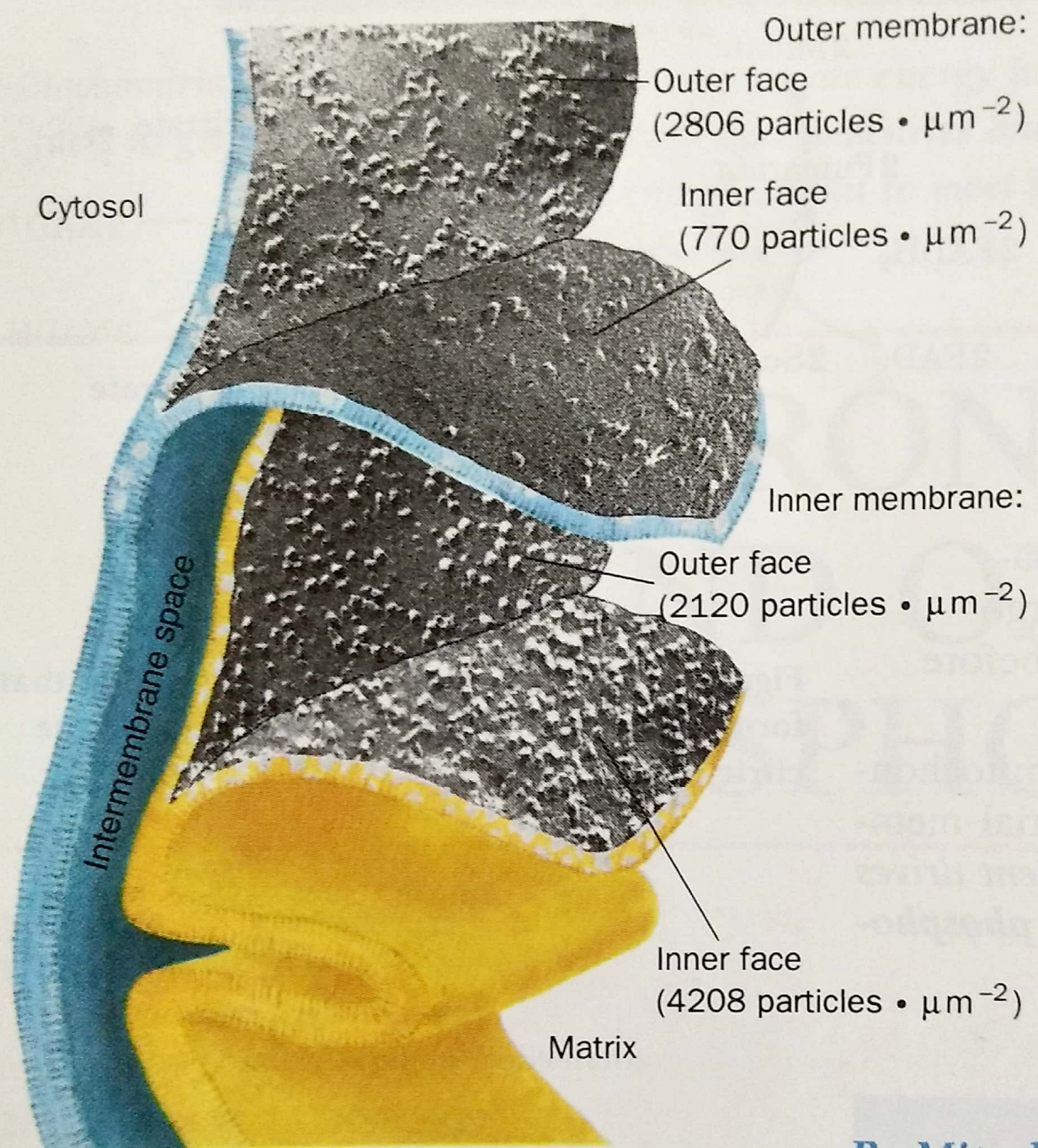
\* Tissues with a high demand of aerobic metabolism i.e. brain, skeletal, heart muscle, eye contain hundreds or thousands of mitochondria per cell and these cells have more densely packed cristae.

Whereas tissues with less active metabolism like skin have fewer mitochondria with fewer cristae.

\* During cell growth & division, just like bacteria, mitochondria divide by fission.

Likewise, they can also fuse to form longer, more-extended structure.





**Figure 17-3. Freeze-fracture and freeze-etch electron micrographs of the inner and outer mitochondrial membranes.** The inner membrane contains about twice the density of embedded particles as does the outer membrane. [Courtesy of L. Packer, University of California at Berkeley.]

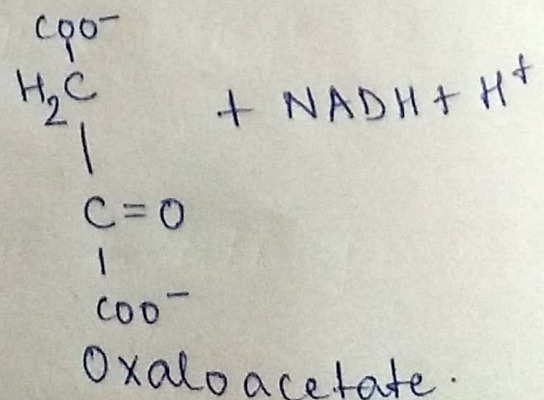
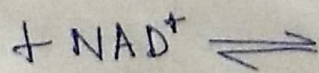
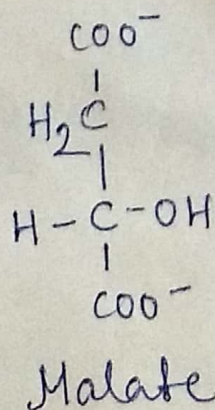
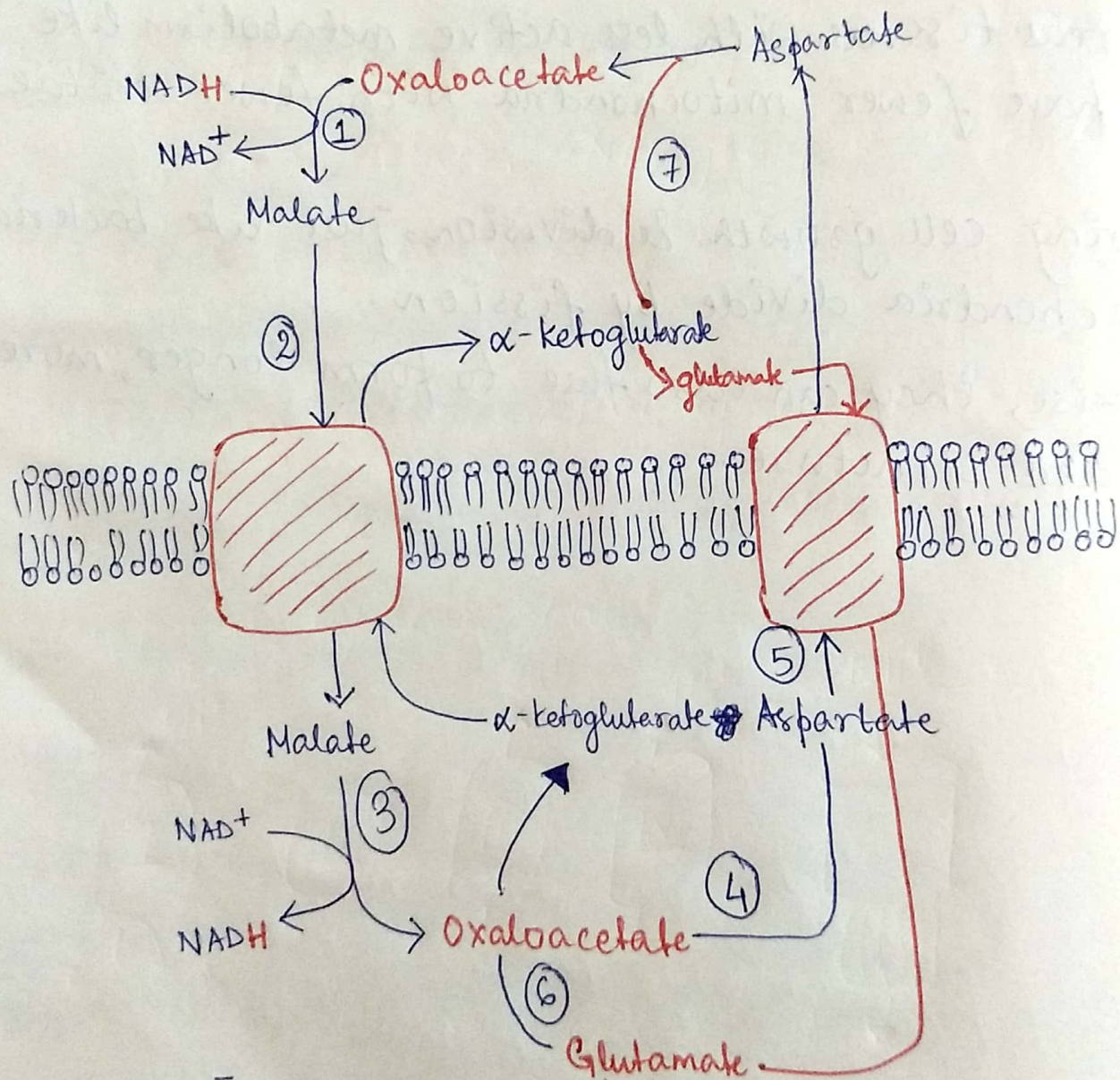
## B. Mitochondria

Like bacteria, mitochondria contain porins in their outer membrane, which is the only membrane that is permeable to small molecules.



# Metabolite transporters in mitochondria

## MALATE ASPARTATE SHUTTLE





The Malate - Aspartate shuttle is required to transport NADH into the matrix of mitochondria, in **cardiac muscle cells and liver cells**.

### Steps involved in the shuttle

Step 1: NADH produced in glycolysis reduces oxaloacetate into malate by transferring the electron pair and itself oxidizing into  $\text{NAD}^+$ .

Step 2: The malate moves into the matrix via an antiporter transport protein in exchange of  $\alpha$ -ketoglutarate.

Step 3: Malate is then oxidized back into oxaloacetate whereas  $\text{NAD}^+$  is again converted back into NADH catalyzed by the enzyme mitochondrial malate dehydrogenase.

Step 4: Since ~~a~~ oxaloacetate cannot move across the inner membrane it is converted to aspartate by a **transamination reaction**.

Step 5: Aspartate can flow out of inner membrane through antiporter in exchange of glutamate.

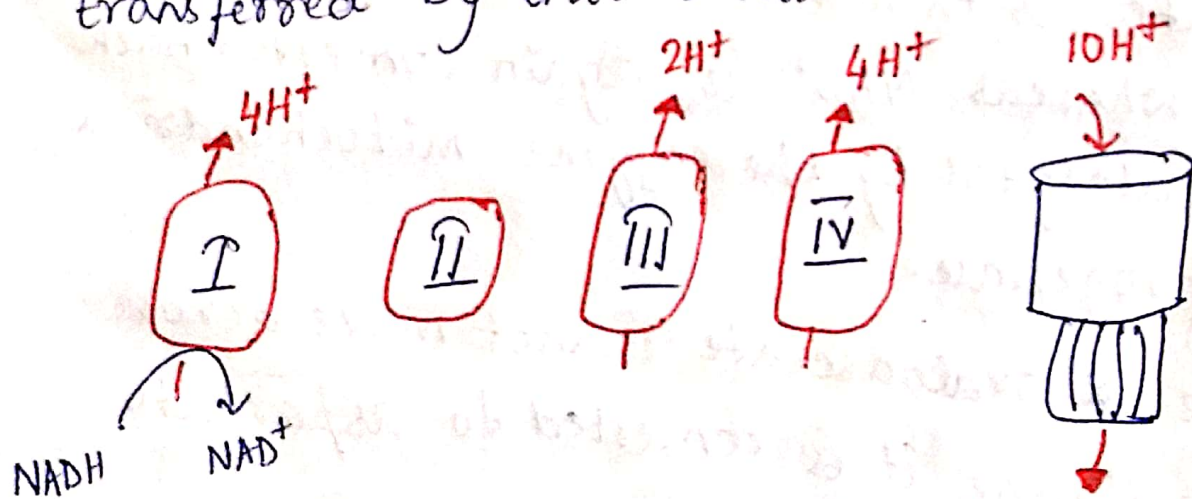


Step 6: Glutamate transfers  $-NH_2$  group onto oxaloacetate and forms aspartate and  $\alpha$ -ketoglutarate.

Step 7: Aspartate in the cytoplasm is deaminated to form oxaloacetate.  $-NH_2$  group is used by glutamate to form  $\alpha$ -ketoglutarate.

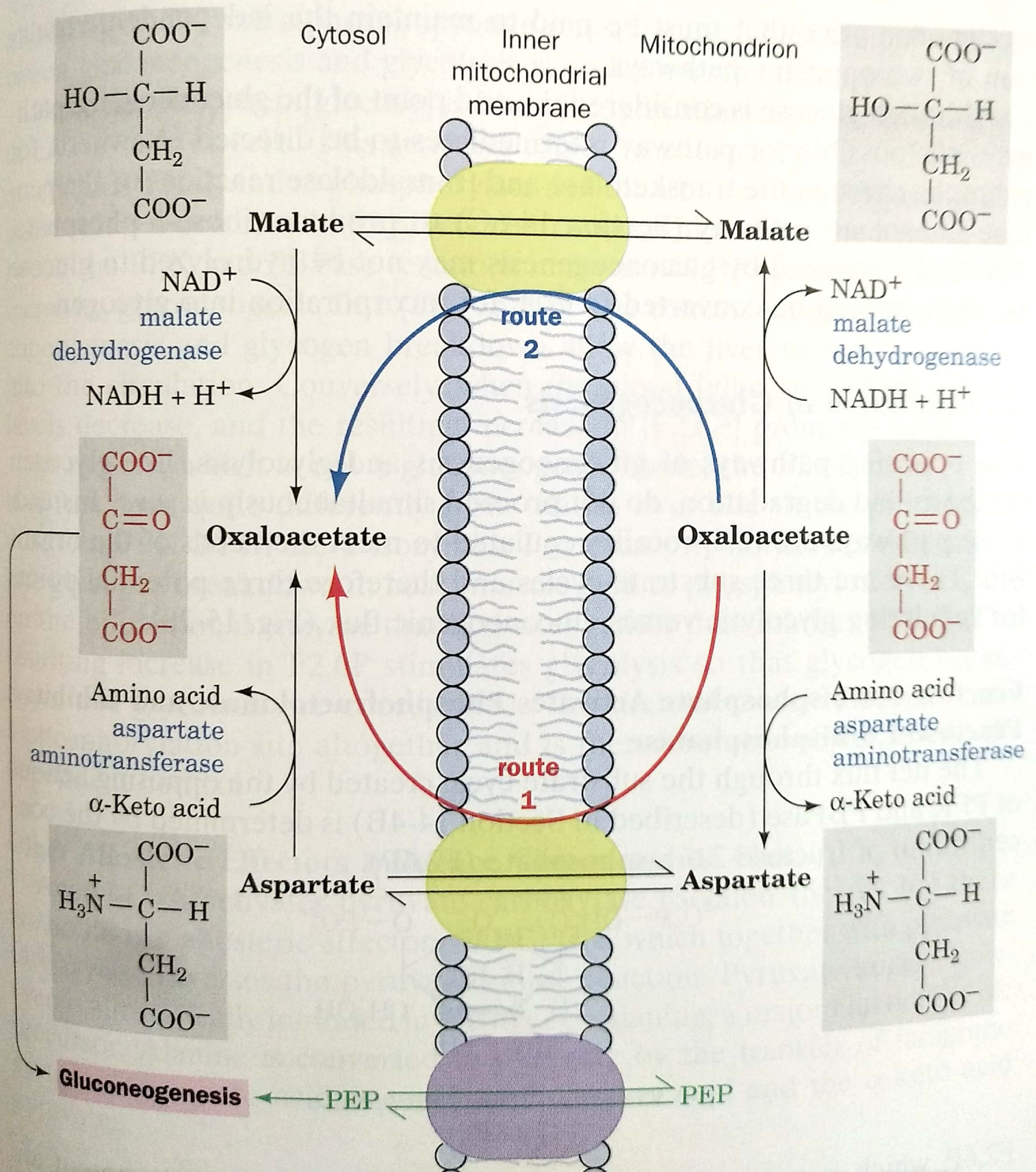
### Calculation for generation of ATP molecules

How many ATP formed from the NADH transferred by this shuttle.



$10 H^+$  pass through  $ATP$  synthase to form  $2.5$   $ATP$  molecules,  
Since  $4H^+$  is required for  $1$   $ATP$  production

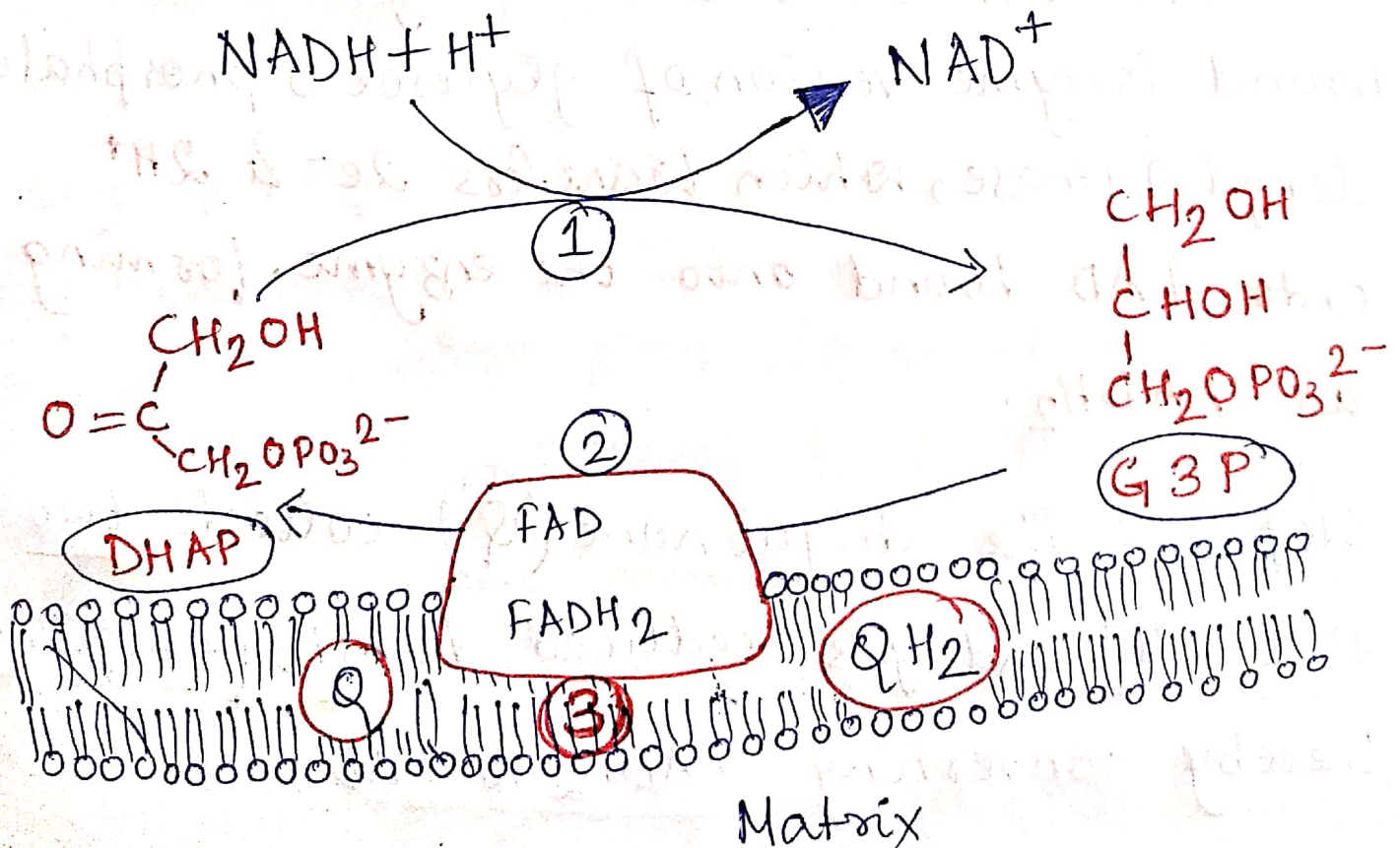






## Glycerol-3-Phosphate Shuttle

- \* During glycolysis, the NADH molecules produced has to be transported into the mitochondria and this allows to synthesize ATP and also regenerate  $\text{NAD}^+$  required to continue glycolysis.
- \* However, inner mitochondrial membrane is impermeable to  $\text{NAD}^+/\text{NADH}$ , so a special transport system is needed.





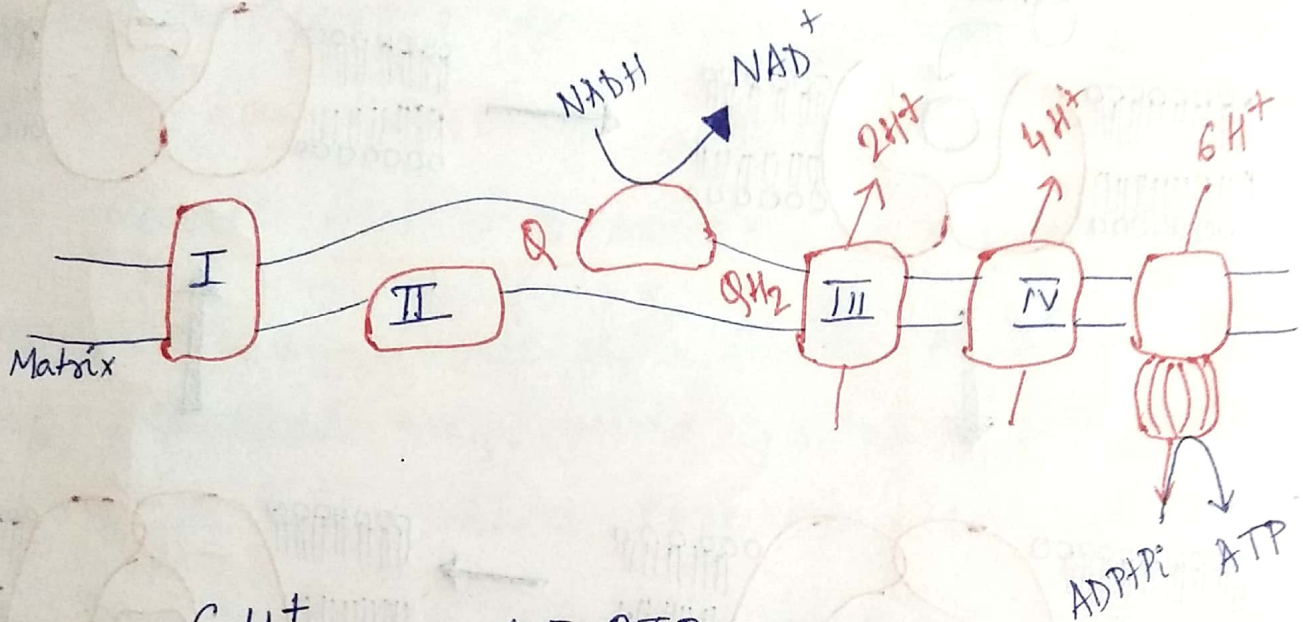
Step 1: NADH produced during glycolysis is oxidized back into  $\text{NAD}^+$  by reducing DHAP to G-3-P. This is catalyzed by enzyme found in cytoplasm called cytoplasmic glycerol-3-phosphate dehydrogenase.

Step 2: The G3P then moves into the intermembrane space of mitochondria where it is oxidized back to DHAP by a membrane bound isozyme version of glycerol 3 phosphate dehydrogenase, which transfers  $2e^-$  &  $2H^+$  onto FAD bound onto the enzyme, forming an  $\text{FADH}_2$ .

Step 3: The ubiquinone (Q) collects two  $e^-$  &  $2H^+$  and gets reduced to  $\text{QH}_2$  (ubiquinol) thereby converting  $\text{FADH}_2$  to FAD.



## calculation for generation of ATP molecule:



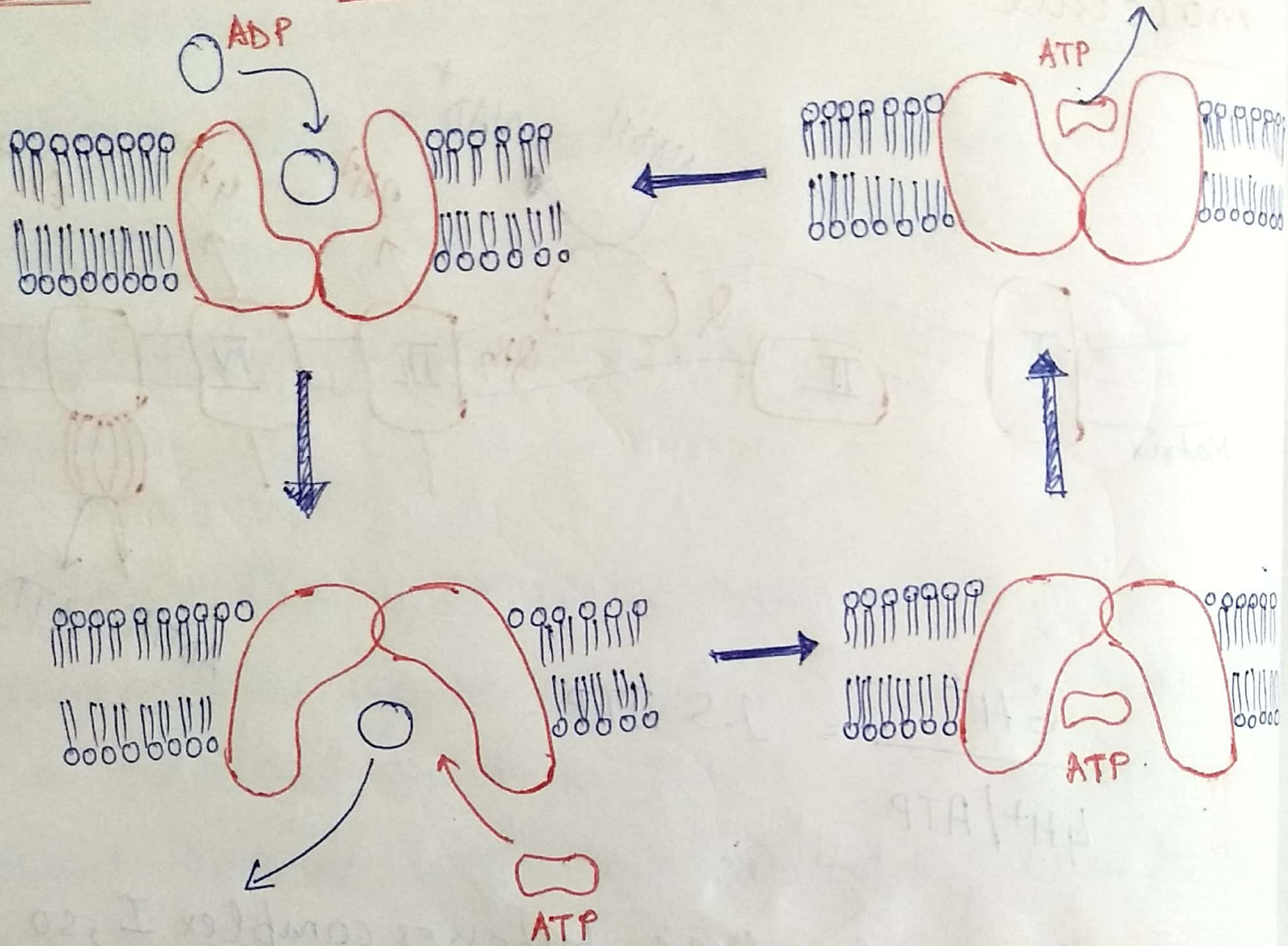
$$\frac{6 H^+}{4 H^+ / \text{ATP}} = 1.5 \text{ ATP}$$

\* NADH from glycolysis bypasses complex I, so it produces only 1.5 ATP molecules per NADH. This is in contrast to a net result of 2.5 ATP formed from 1 NADH as in TCA cycle.

\* The glycerol 3-phosphate shuttle is used predominantly by skeletal muscle cells. It allows them to quickly generate NAD<sup>+</sup> and synthesize ATP.



# ATP-ADP Translocase



\*

For the Electron transport chain ~~needs~~ to be efficient:  
the ADP levels in the mitochondrial matrix  
should be high to ensure ATP synthase can  
use the PMF to generate ATP.

and,

as the ATP are generated, it needs to be  
transported into the cytoplasm.



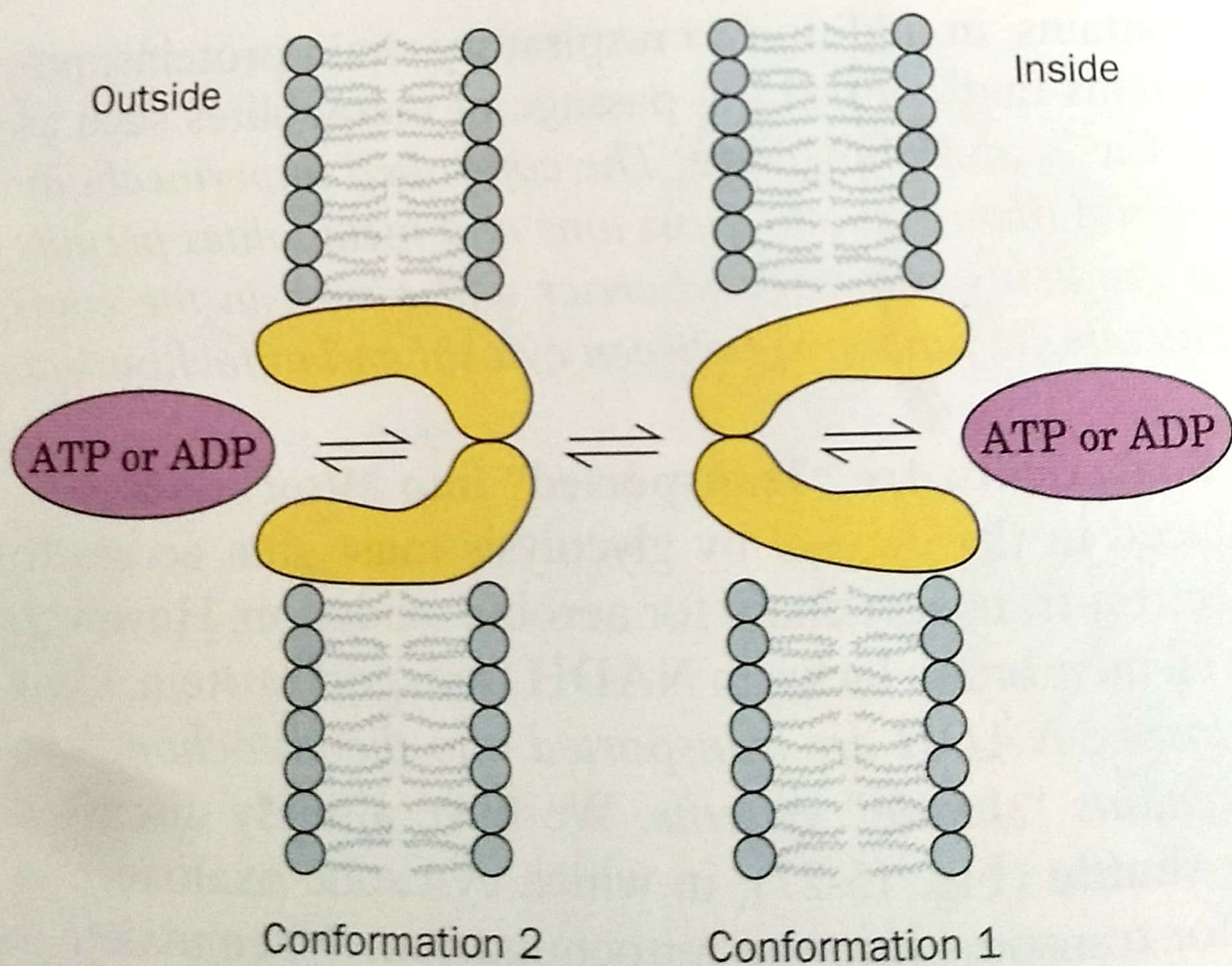
\* But both ADP & ATP are charged molecules unable to cross the mitochondrial membrane. So the antiporter transport protein called ATP-ADP translocase is required.

It imports ADP into matrix and exports ATP out.

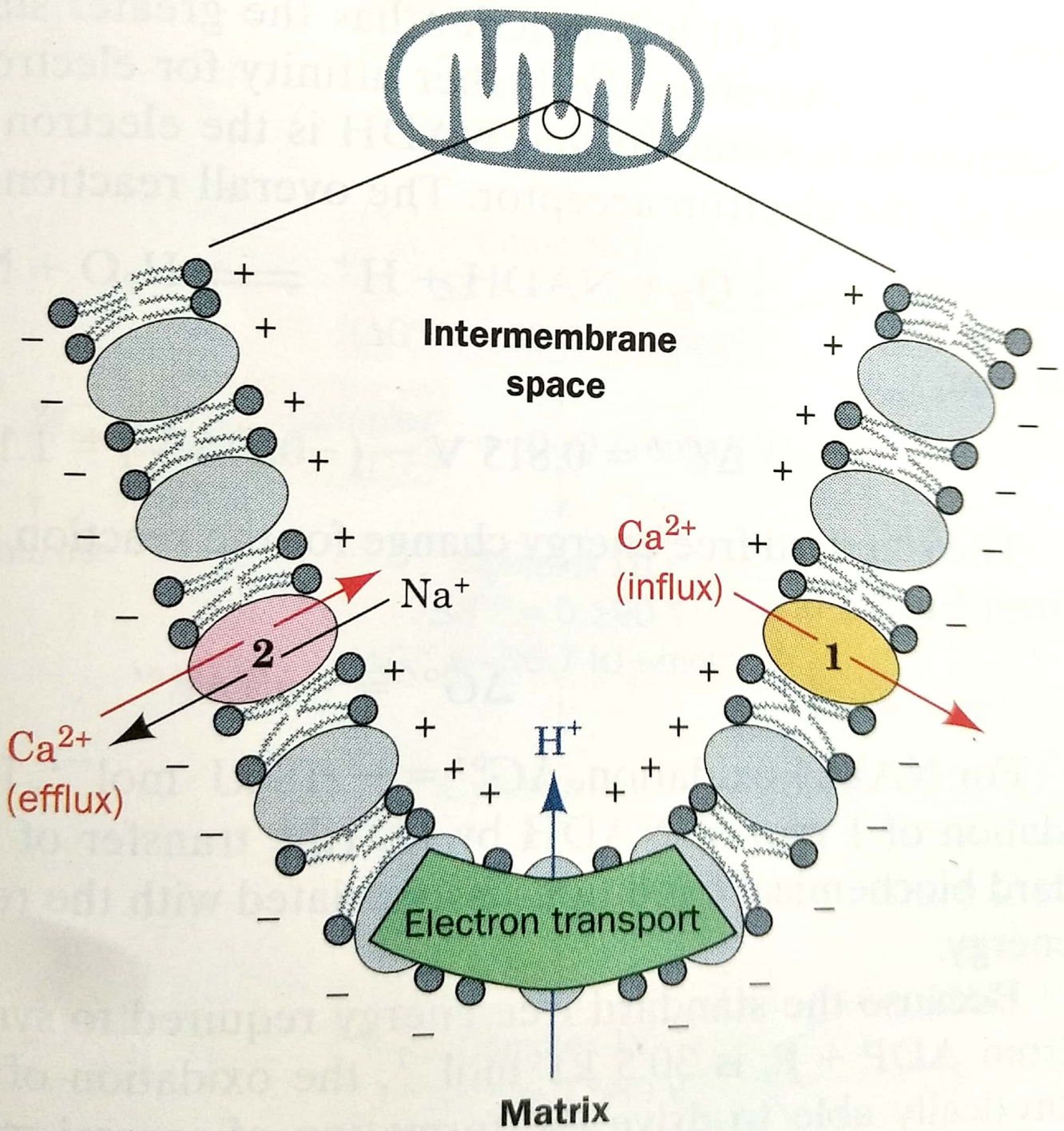
\* ATP-ADP translocase is a dimer that consists of 2 identical polypeptide chains. Each chain consists of 6  $\alpha$ -helices that span the membrane.

\* The two subunits create a binding pocket that alternates between matrix & cytoplasmic side of inner membrane.









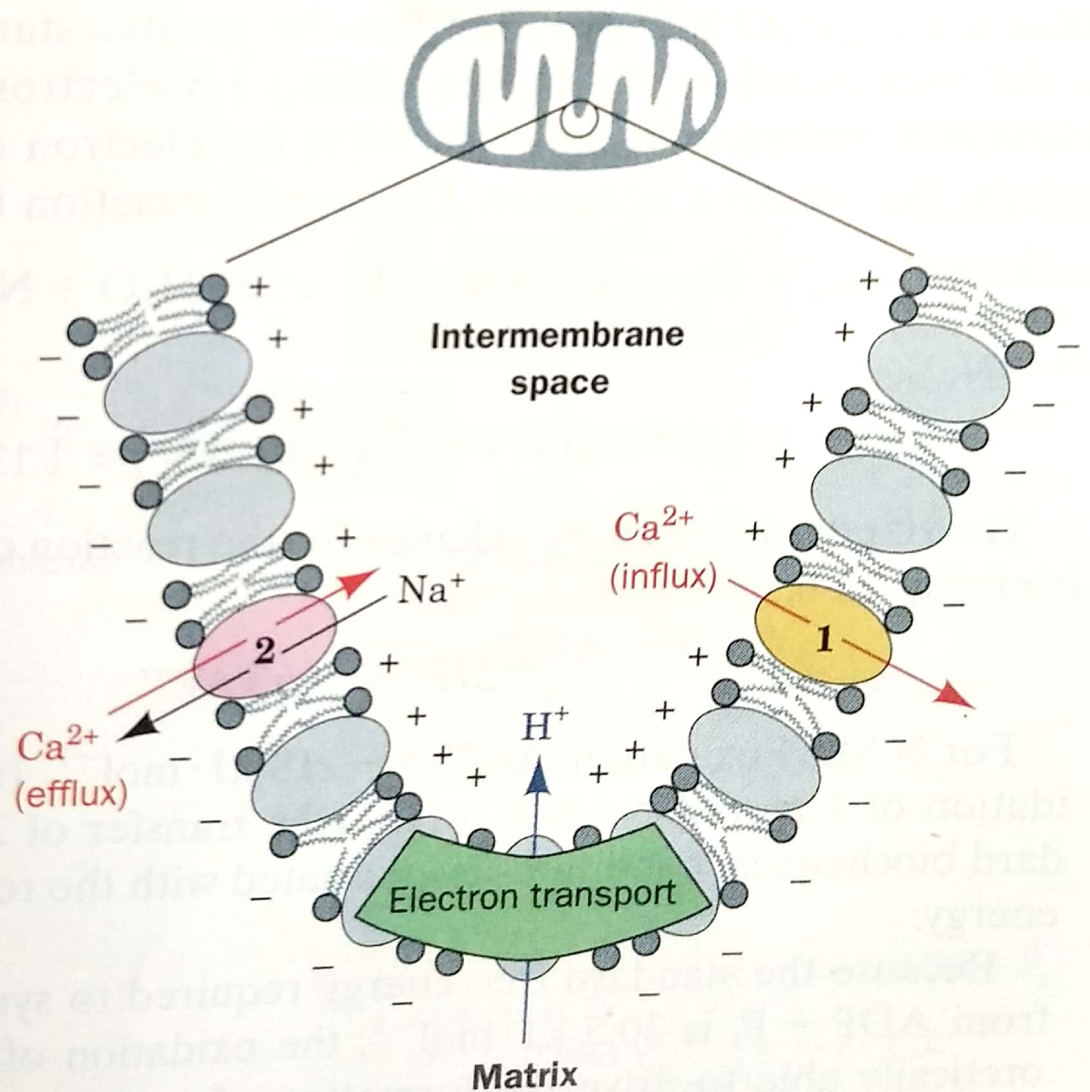


## **Ca<sup>2+</sup> Transport**

Separate systems in the inner mitochondrial membrane mediate the influx and efflux of Ca<sup>2+</sup> (Fig. 17-6). The Ca<sup>2+</sup> influx is driven by the membrane potential ( $\Delta\Psi$ , negative inside), which attracts positively charged ions. The rate of influx varies with the external [Ca<sup>2+</sup>] because the  $K_M$  for Ca<sup>2+</sup> transport by this system is greater than the cytosolic Ca<sup>2+</sup> concentration.

Ca<sup>2+</sup> exits the matrix only in exchange for Na<sup>+</sup> (antiport). This exchange process normally operates at its maximum velocity. *Mitochondria (as well as endoplasmic reticulum and sarcoplasmic reticulum) therefore can act as a "buffer" for cytosolic Ca<sup>2+</sup>*: If cytosolic [Ca<sup>2+</sup>] rises, the mitochondrial Ca<sup>2+</sup> influx increases while Ca<sup>2+</sup> efflux remains constant, resulting in a net influx of Ca<sup>2+</sup>. The mitochondrial [Ca<sup>2+</sup>] therefore increases while the cytosolic [Ca<sup>2+</sup>] decreases to its original level (its set-point). Conversely, a decrease in cytosolic [Ca<sup>2+</sup>] reduces the influx, causing net efflux of Ca<sup>2+</sup> from the mitochondrion and an increase of cytosolic [Ca<sup>2+</sup>] back to the set-point. When cytoplasmic [Ca<sup>2+</sup>] rises, for example, during increased muscle activity, the matrix [Ca<sup>2+</sup>] also rises, thereby activating the enzymes of

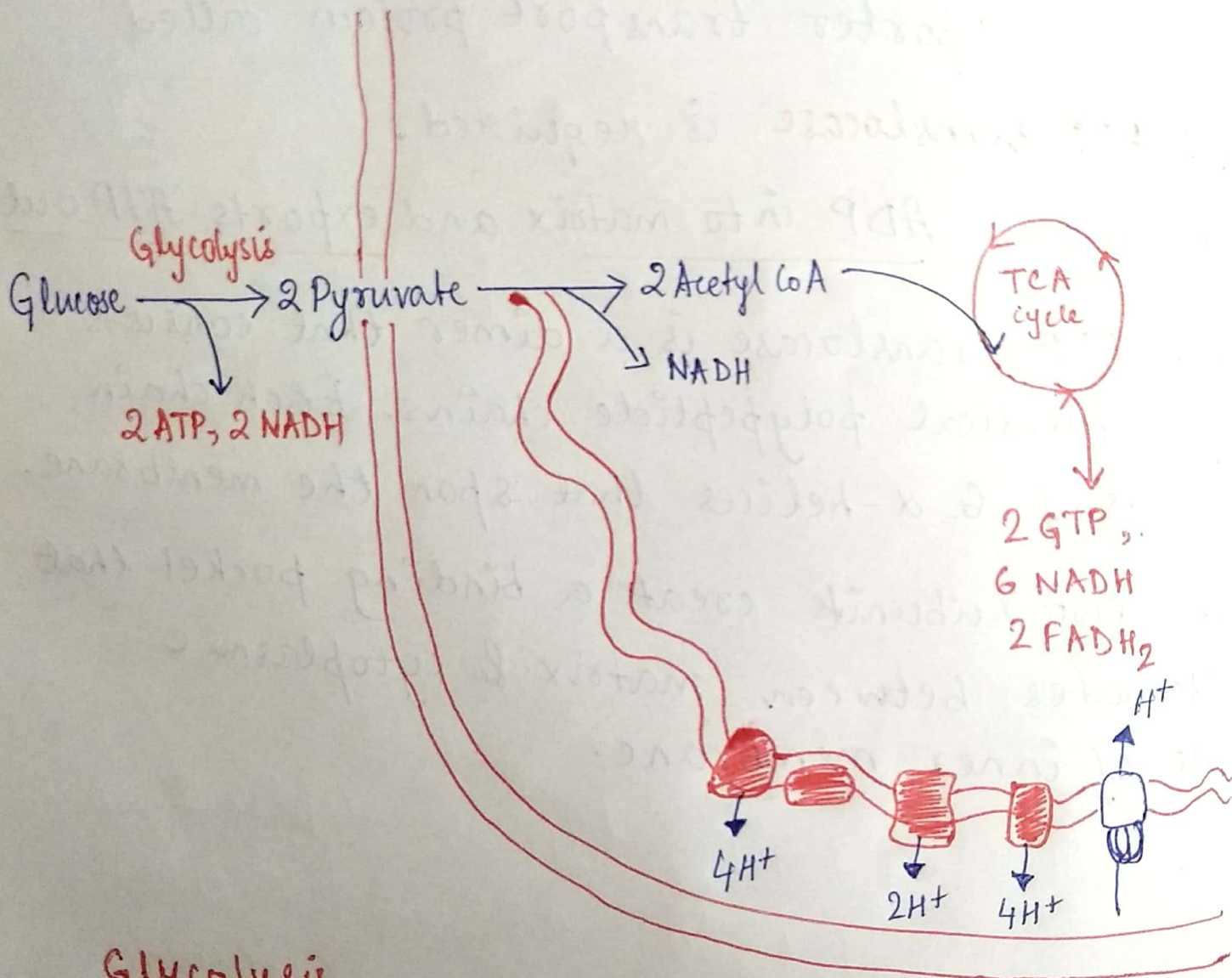




the citric acid cycle (Section 16-4B). This leads to an increase in the level of NADH, whose reoxidation by the mitochondrial electron-transport system generates the ATP needed for muscle contraction.



# Yield of ATP in Aerobic Cell Respiration



## Glycolysis

\* 2 ATP

\* 2 NADH { 5 ATP via Malate Aspartate Shuttle  
3 ATP via G3P shuttle }

## Pyruvate Decarboxylation

\* 2 NADH (5 ATP).



## Citric Acid Cycle

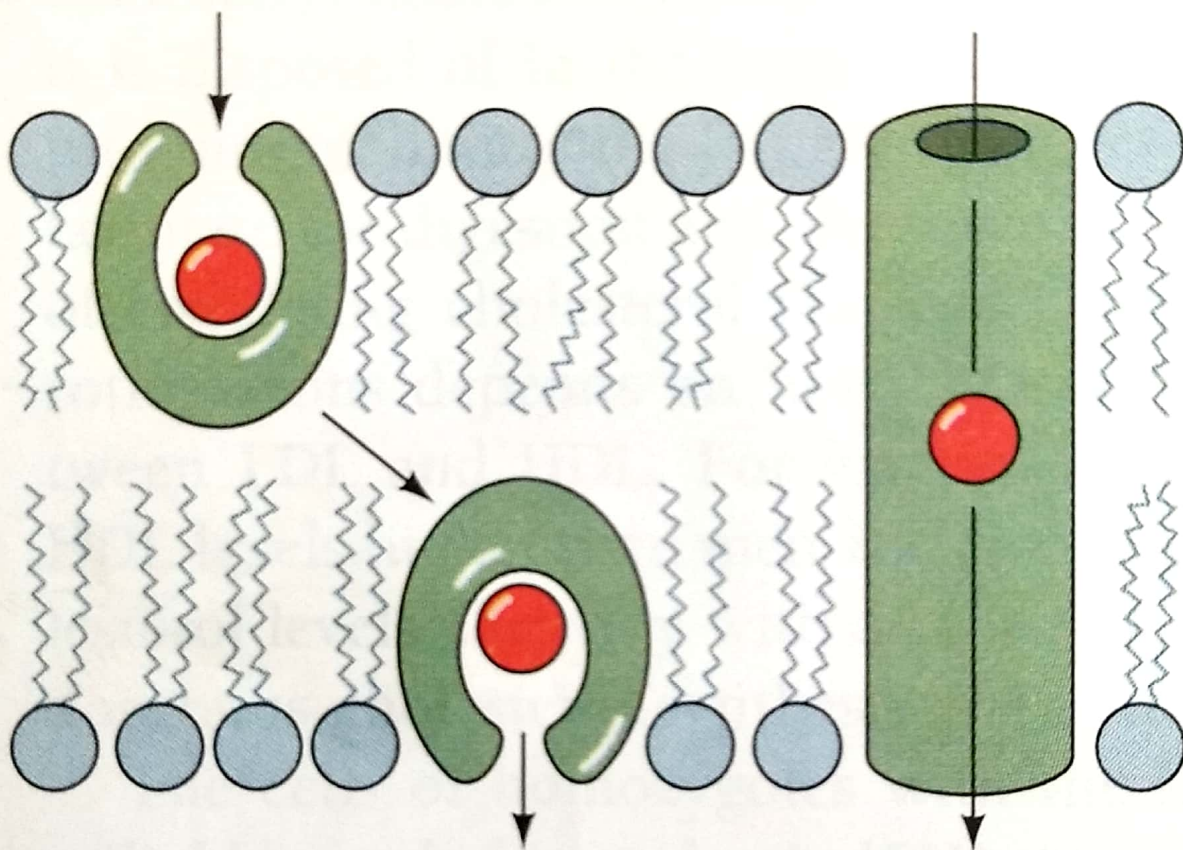
- \* 2 GTP (2 ATP)
- \* 6 NADH (15 ATP)
- \* 2 FADH<sub>2</sub> (3 ATP)

Net 30-32 ATP are formed for one glucose in aerobic cell respiration.



(a) Carrier ionophore

(b) Channel-forming ionophore



**Figure 10-29. Ionophore action.** (a) Carrier ionophores transport ions by diffusing through the lipid bilayer. (b) Channel-forming ionophores span the membrane with a channel through which ions can diffuse.



## B. Mechanisms of Mediated Transport

Substances that are too large or too polar to diffuse across lipid bilayers on their own may be conveyed across membranes in complex with carrier molecules that are variously called **carriers**, **permeases**, **porters**, **translocases**, and **transporters**.

### Ionophores Facilitate Ion Diffusion

One type of carrier molecule is an **ionophore**, an organic molecule—in many cases an antibiotic of bacterial origin. **Carrier ionophores** increase the permeabilities of membranes to a particular ion by binding the ion, diffusing through the membrane, and releasing it on the other side (Fig. 10-29a). For net transport to occur, the uncomplexed ionophore must then return to the original side of the membrane ready to repeat the process. The ionic complexes of all carriers must therefore be soluble in nonpolar solvents. A second type of ionophore, **channel-forming ionophores**, form solvent-filled, transmembrane channels or pores through which their selected ions can diffuse (Fig. 10-29b).

Even small amounts of an ionophore greatly increase the permeability of a membrane toward a specific ion. For example, a single molecule of the carrier antibiotic **valinomycin** transports up to  $10^4$   $K^+$  ions per second across a membrane. Channel formers, such as the antibiotic **gramicidin A**, have an even greater ion throughput, over  $10^7$   $K^+$  ions per second. The antibiotic properties of these ionophores arise from their ability to discharge the ion concentration gradients that healthy cells actively maintain. Note that since ionophores passively permit ions to diffuse across a membrane in either direction, they can only dissipate—not generate—an ion concentration gradient.



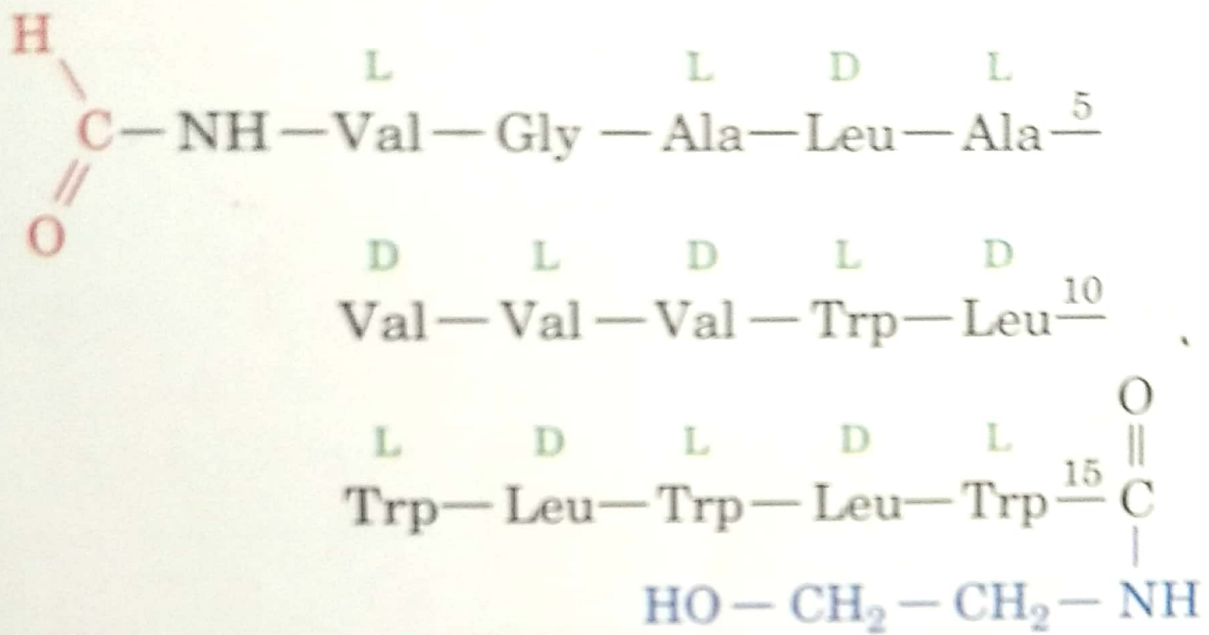
Valinomycin, one of the best characterized ionophores, specifically binds  $K^+$ . It is a cyclic molecule containing D- and L-amino acid residues that participate in ester linkages as well as peptide bonds (Fig. 10-30). The X-ray structure of valinomycin's  $K^+$  complex (Fig. 10-31) indicates that the  $K^+$  ion is octahedrally coordinated by the carbonyl groups of its six Val residues, and the cyclic valinomycin backbone surrounds the  $K^+$  coordination shell. The methyl and isopropyl side chains project outward to provide the complex with a nonpolar exterior that makes it soluble in the hydrophobic cores of lipid bilayers.

The  $K^+$  ion (ionic radius,  $r = 1.33 \text{ \AA}$ ) fits snugly into valinomycin's coordination site, but the site is too large for  $Na^+$  ( $r = 0.95 \text{ \AA}$ ) or  $Li^+$  ( $r = 0.60 \text{ \AA}$ ) to coordinate with all six carbonyl oxygens. Valinomycin therefore has 10,000-fold greater binding affinity for  $K^+$  than for  $Na^+$ . No other known substance discriminates so acutely between  $Na^+$  and  $K^+$ .

Gramicidin A is a 15-residue linear polypeptide consisting of alternating L and D residues, all of which are hydrophobic (Fig. 10-32a). NMR and X-ray crystallographic evidence indicates that gramicidin A dimerizes in a head-to-head fashion to form a transmembrane channel (Fig. 10-32b). The alternating L and D residues of gramicidin A allow it to form a 4- $\text{\AA}$ -diameter helix with a nonpolar exterior and a polar central channel that facilitates the passage of  $Na^+$  and  $K^+$  ions.

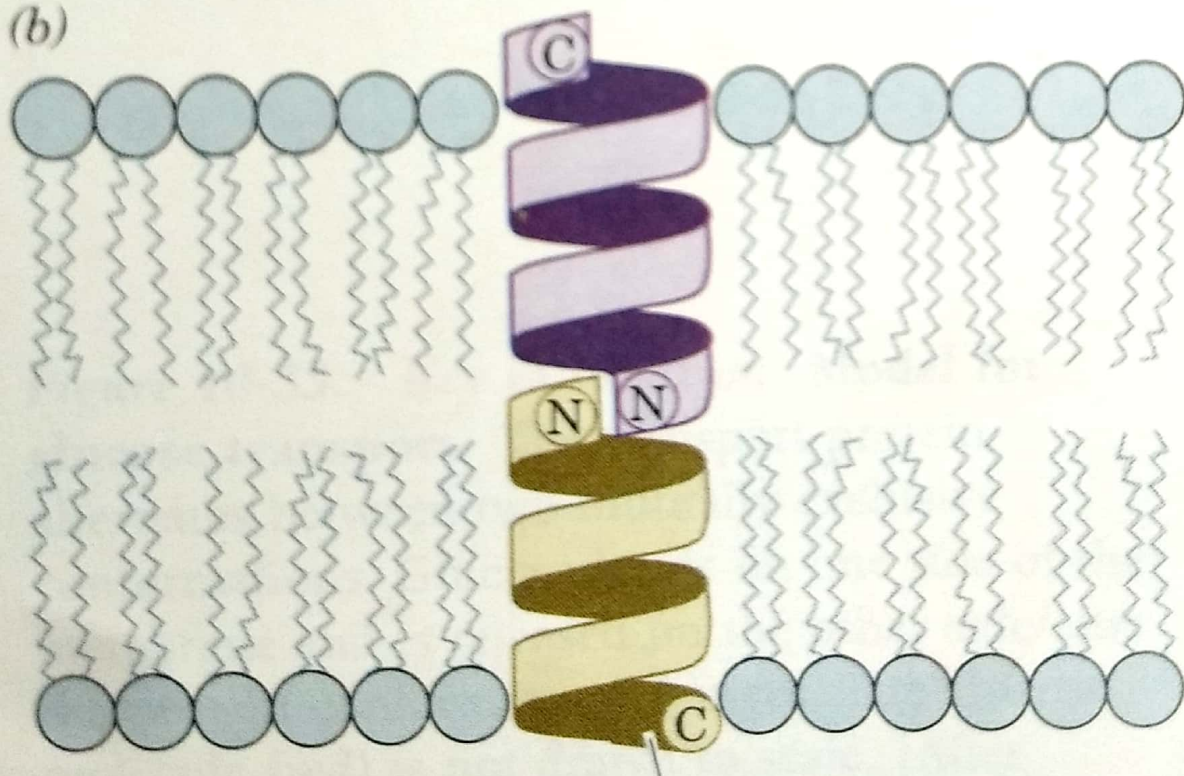


(a)



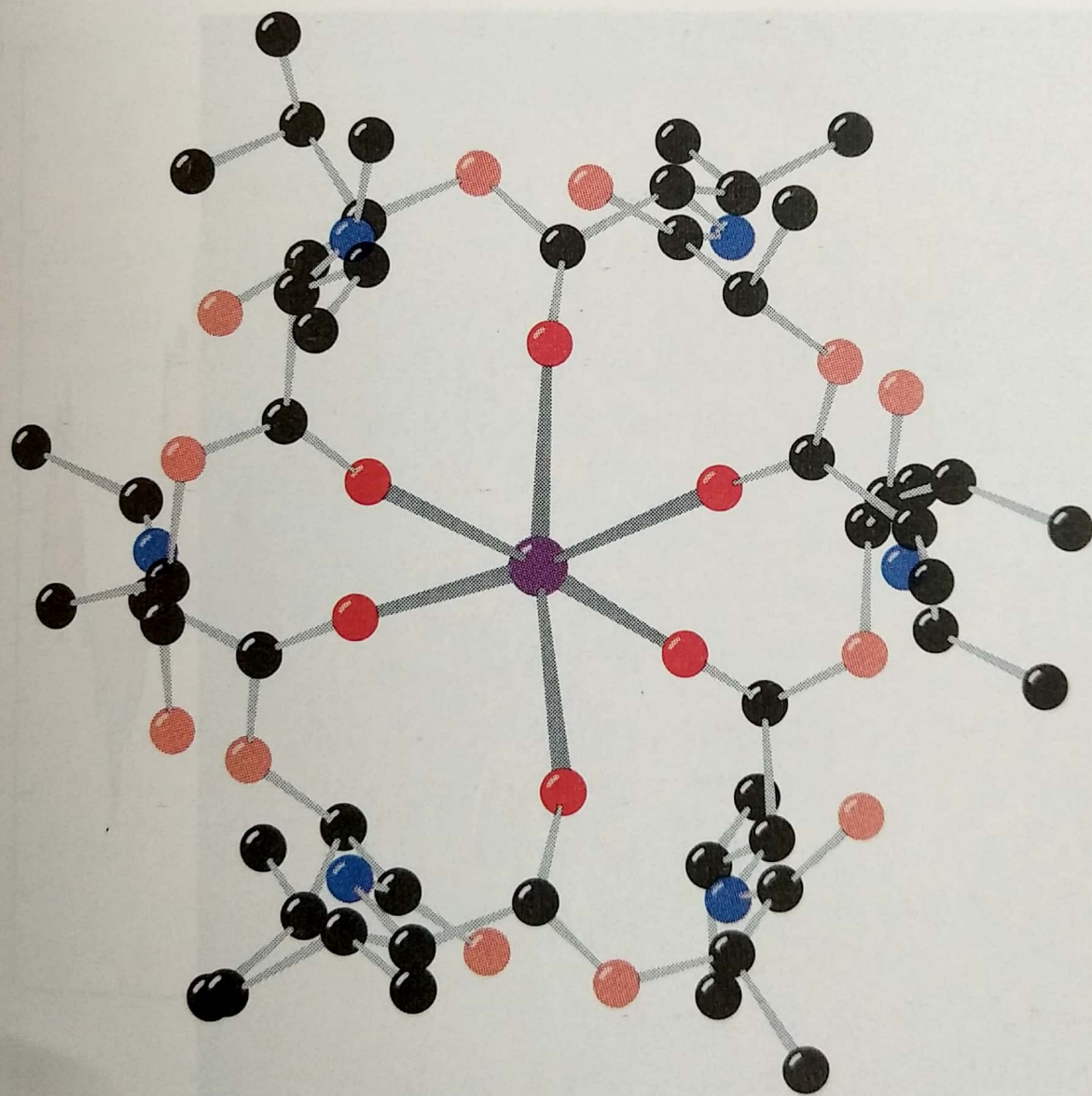
Gramicidin A

(b)



Gramicidin A  
dimer





**Figure 10-31.** X-Ray structure of valinomycin in complex with a  $K^+$  ion. Six oxygen atoms (dark red) octahedrally coordinate the  $K^+$  ion (purple). [After Neupert-Laves, K. and Dobler, M., *Helv. Chim. Acta* 58, 439 (1975).]