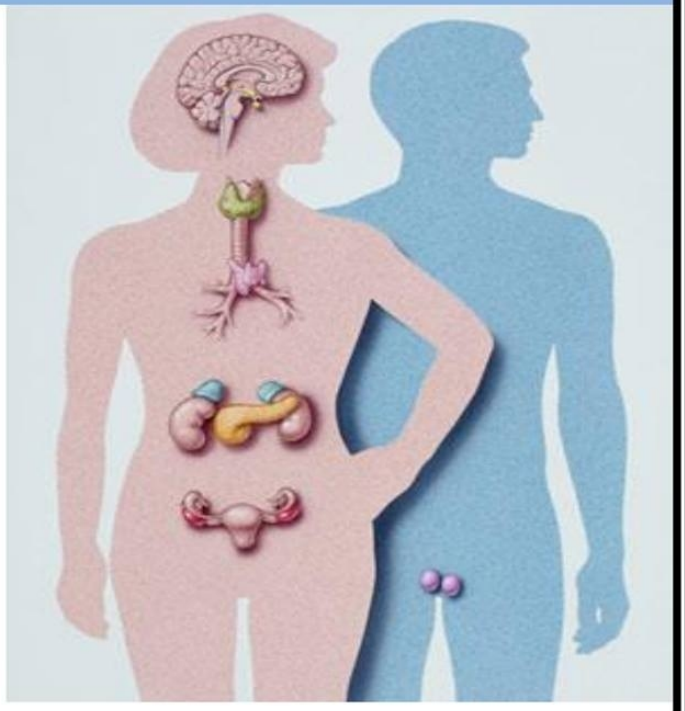
University of Jordan - Faculty of Medicine
(2013-19)

Medical Committee
The University of Jordan

Endocrine System



- ☐ Anatomy/Embryology/Histology
- ■ Biochemistry
- ☐ Physiology
- ☐ Pharmacology
- ☐ Pathology
- ☐ PBL

☐ Slide ■ Sheet ☐ Handout ☐ Other

Lecture #: **3**

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بسم الله الرحمن الرحيم

SIGNAL TRANSDUCTION CASCADES

- Recall:

- last lecture we talked about signal transduction and cyclic AMP cascade, starting with binding of the hormone to the cell surface receptor (7 transmembrane helix) leading to activation transmembrane G protiens , release of the alpha subunit , then binding of the alpha subunit to adenylate cyclase followed by release of cAMP molecule and activation of protien kinase A enzyme (its called protien kinase A because it is dependent on cAMP) .

And the example on a defect in this system was ***Cholera*** and **whooping cough السعال الديكي**.

That was the first cascade .

Today we will take in detail about another cascade.

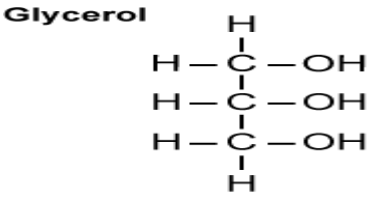
The pathway of the second cascade::

The hormone will bind to the receptor leading to the activation of the G protien , next is the separation of the alpha subunit and binding not to adenylate cyclase but to an enzyme called (**phospholipase C**).

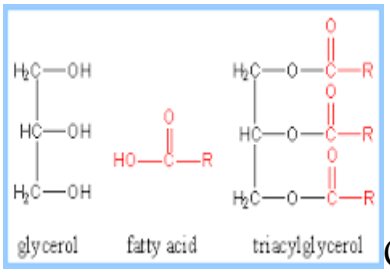
Function of phosphlipase C is to break phospholipids found in the membrane .

The substrate of the phospholipase C is phosophtidyl inositol bisphosphate.

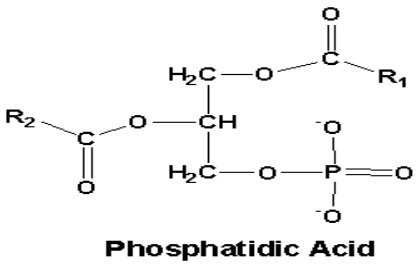
This substrate is composed of glycerol molecule binding two fatty acid molecules and inositol ring .



Glycerol is a three carbon molecule.

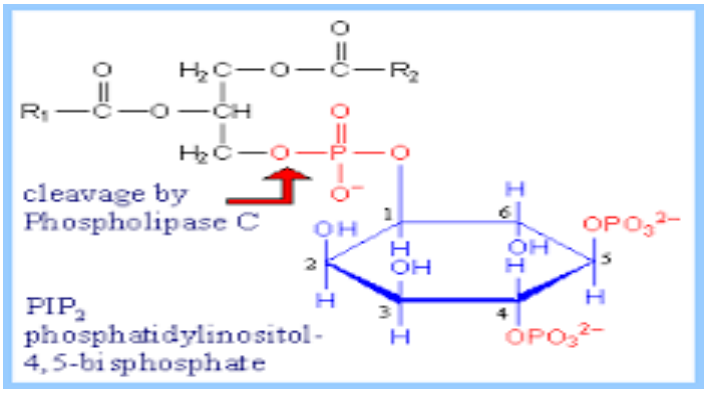


Glycerol binding three fatty acids called **triacylglycerid**.



Glycerol binding two fattyacids and phosphate

Called **phosphatidic acid** .



glycerol binding two fattyacids and an inositol ring with two phosphate called

<u>phosphatidylinositol4,5-bisphosphate (PIP$_2$)</u>

and this is the substrate **for phospholipase C**.

The phospholipase C has many isoforms, which are going to be discussed through the lecture .

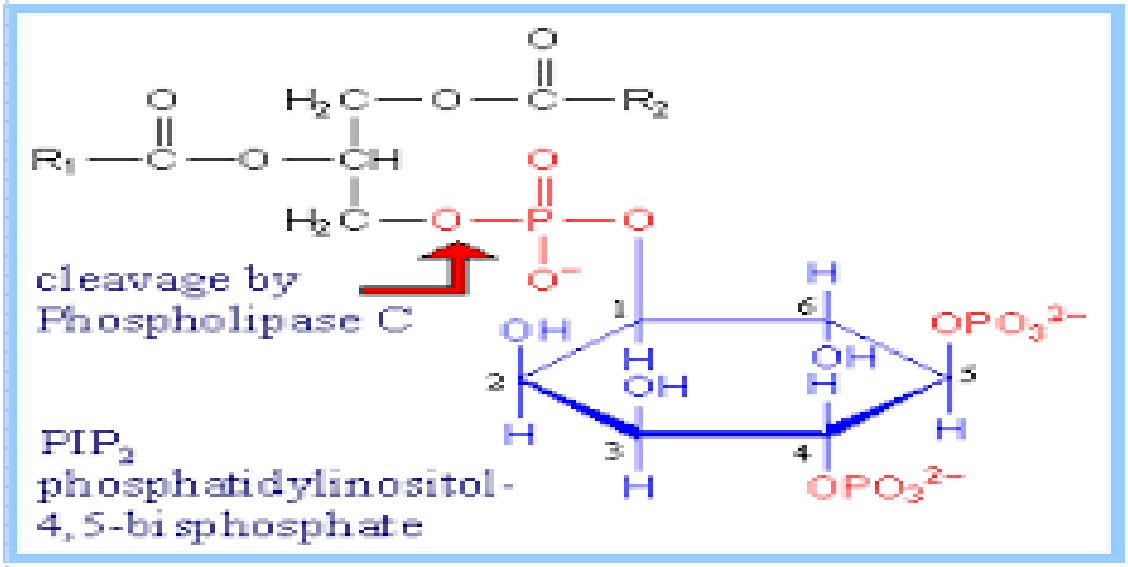
It breaks the phosphatidyl inositol 4,5-bisphosphate at the di-ester bond between the phosphate and the carbon which releases two products .

Leaving the glycerol back bone with the two fatty acids as the first product and the inositol ring with the three phosphates as the second product .

Products:

(1) Any structure with three or more carbon units is called acyl group . So the first product contain glycerol back bone and two acyl groups (diacyl glycerol **DAG**).

(2) The other molecule is an inositol ring with two phosphate and the third phosphate is from the **phosphatidic acid**.(inositol trisphosphate **IP3**).



The image demonstrates the cleavage and the products.

The DAG molecule is **lipophilic**, and the presence of the carbonyl groups and the oxygen made it an amphipathic molecule; in which most of the structure is lipophilic with hydrophilic head.

Where the inositol trisphosphate IP3 is **hydrophilic** molecule consists of a ring with hydroxyl groups and three phosphates .

These structure are within the membrane , the hormone binds to the 7 transmembrane helix ,then the G protein is activated releasing the alpha subunit , the alpha subunit enhance the function of **phospholipase C** enzyme and break the phosphotidyl inositol 4,5-bisphosphate giving **DAG** and **IP3 .**The hydrophilic molecules will be released to the cytoplasm.

When these molecules reach the cytoplasm it induces actions .

The actual second messenger is the IP3 which will reach the cytoplasm .

The DAG is amphipathic ;so it can stay within the membrane .

The pathway of each molecule will be discussed through the lecture .

Phospholipase C Isoforms :

This enzyme has many isoforms, however the **beta** isoform is the one which have the capability of binding with the G protein(**contain G protein interactive domain)** . So the beta isoform is the only one that will enter the cascade of the second messenger system .

The other isoforms can not bind to the G protein.

The phospholipase C contain multiple domains like the G protein binding protein **(why??)**

Because we need a **catalytic site** (a site for the action to occur) and we need domains for the attachment to the membrane like PH domain and the C2 domain, one of them attaches to phospholipid head .

groups and the other with lipids groups to anchor the membrane and activate the catalytic action .

When the G protein is active it will bind with the interaction G protein domain to induce the activity of the enzyme , once it is bound it induces conformational change within this enzyme to make the C2 domain and PH domain to bind to the membrane , once these two domains are bound to the membrane ,this induces a conformational changes within the catalytic domain to start the reaction .

Review

Hormones bind to (7 transmembrane helix) ⟶ activate G protein releasing alpha subunit ⟶ the alpha subunit activate phospholipase C enzyme ⟶ cleavage of phosphotidylinositol4,5-bisphospate to ⟶ DAG &IP3

What is the fate of DAG?

It will stay within the membrane .

What is the fate of the IP3?

It will be directed toward calcium channels and bind to them. We have calcium channels on the cell membrane and in the endoplasmic reticulum.

The endoplasmic reticulum contain high concentration of calcium as will as outside the cell; because of that we can use the calcium molecules in this system , we need to use a molecule with a large concentration difference between two compartments to be able to make a potent response .If the concentration is the same inside and outside the calcium will be with no use .

How many IP3 molecules should be bound to the Ca channels to be active?

The Ca channels are composed of 4 subunit , binding of 3 IP3 molecules will make them active . Binding of at least 3 will open them widely so as huge influx of Ca will come from the endoplasmic reticulum to the cytoplasm.

Why do I need Ca in the cytoplasm?

It will go and bind to protein kinase ,but the main thing they will bind to **calcium binding proteins** and from there those complexes will bind other proteins and enzymes to start the process of activation and deactivation, so as metabolism will go through.

Phospholipase C ----- IP3 ---------- Ca channels ---------- release of Ca to the cytoplasm (this is the IP3 pathway)

The other pathway is the DAG pathway ,which is the part remaining in the membrane . This DAG has the ability to enhance other kinase, which is **protein kinase C** .

The DAG can not enhance the protein kinase C without the presence of Ca which we will get it from the endoplasmic reticulum .(This is the way how we regulate this system)

Like the phospholipase C ,the protein kinase C has many isoforms and has domains within the protein, domains which are necessary for these proteins: Ca binding domain ,catalytic domain,C1 domain(responsible for binding of DAG), and membrane binding domain.

Along with these domains we have what we call it **pseudo substrate domain**.

Pseudo substrate domain: is amino acid sequence that mimics the sequence of the substrate for inhibition on the active site.

So this pseudo substrate works like a **competitive inhibitor.**

The sequence of the substrate contain serine and threonine to be phosphorylated, and the sequence of the pseudo substrate contain alanine(hydrophobic amino acid) which can not be phosphorylated and work as a cover on the active site.

Once the protein kinase C is activated by binding to Ca , then it go to the membrane and the C1 domain bind the DAG, it will pull the pseudo substrate domain and expose the active site and protein kinase C will function.

Note: the binding of the IP3 to the Ca channels is cooperative binding which means it's very hard the first molecule but the second molecule will bind easier and the third is much easier to bind.(after the first molecule

binding within a short period of time you will have a full opening of the channel and release of the Ca) . ((sigmoidal shape plot))

<u>After we delivered the massage how can we terminate the action ??</u>

Previously we took the termination of the cyclic AMP by phosphodiesterase (enzyme which break cyclic AMP) .

IP3 action can be terminated by two ways :

1) Remove the phosphate groups, which are found on the inositol ring one by one by phosphatase .Removal of the first phosphate will make the structure inactive.
2) Addition of one phosphate . It will become tetraphosphate and this form is inactive . Then metabolism will occur by removing phosphates under one condition , which is the first phosphate to be removed is one from the original ones and NOT the last one added (the original phosphates are on carbon 1+4+5 and the new one is on carbon 3, so we start to remove one phosphate from carbons number 1 or 4 or 5 , so this new molecule is not the same as IP3 ,so it can't induce action in the cell) .

Note: some patients who suffer from depression and psychological disorders can use **lithium ion** which inhibits phosphatases, which remove phosphates from the inositol , so inositol will stay active and the second messenger system will stay in action and cell metabolism is good lifting up of the patient status will occur.

Why calcium is the input in this process ??

1) Because of the concentration difference between the endoplasmic reticulum and cytoplasm (this difference is about 10000 folds).

Within the cell we have Micro molar range but outside the cell its Milli molar range . (much larger concentration outside the cell and in the endoplasmic reticulum even through influx of calcium to the cell ,the outside and in ER will always stay larger in concentration)

To maintain this activation ,Ca channels pumps should open immediately to get the Ca outside the cell to maintain this concentration difference .

2)Ca is bulky molecule , when it bind to a protein it will induce conformational change .

3) It has a positive charge , so it can bind negatively charged amino acids within proteins and ligate itself with water ,with negative polar amino acids ,so it can be stable with in proteins.

4)Tight binding with negatively charged amino acids and polar amino acids (stability).

We said that Ca mainly will bind to certain proteins called **Ca binding proteins** .

One of these proteins called **calmodulin , tropnin c , Parvalbumin**

All of these protein structures composed of alpha helices.

Helices are stable and rigid due to the extensive number of hydrogen bonds.

Also these proteins should have negatively charged regions or crystallized water or good number of polar amino acids for Ca binding and ligation.

The proparties of these molecules:

Within there structure they have what we call it **EF hand** (two alpha helices one of them is short and the other is long and the area between is where the Ca binds, this area between is either **turn** super secondary structure or **loop** ,both of them can fit).But most of the time the two alpha helices are connected through **loops** which are coordinated to fit the Ca molecule by containing the negatively charged amino acids like Aspartic and glutamic acids ,water and other polar amino acids(usually the two alpha helices are not connected through **turns,** because turns are short and can't fit to the Ca) .

Parvalbumin structure is the first protein to be discovered of the Ca binding proteins , it contains 6 domains , these 6 domains were named depending on the alphabtic order A,B,C,D,E,F .

When this structure was studied ,the noted thing was that the EF hand(like the thumb and index and the area between for binding Ca) domain was repeated.

The **helix loop helix** is very similar to the **helix turn helix** structure**.**

The difference is that the turn contains only 4 amino acids while the loop contain more(longer).

Helix turn helix ,we find it usually in the DNA binding proteins while most of the Ca binding proteins contain the helix loop helix structure**(why??).**

Due to the need of large numbers of amino acids which has the ability to bind to the bulky Ca molecule and permit movement to induce conformational changes in the protein.

Calmodulin:

It 17 KD protein.

The name came from (calcium modulated protein), it's a protein which changes it shape and get modulated by binding Ca .

It is composed of two large globular domains on both sides and connected with a loop in between.

Each globular domain contain two EF hands, each EF hand can bind 1 Ca molecule , so in total number of 4 Ca molecules for each calmodulin protein.

*Binding of Ca will induce conformational change in the protein and the shape will change completely, the two globular domains are connected to each other through a loop.

The calmodulin calcium complex can go and bind kinases, enzymes or other proteins, it either **activate** or **deactivate** them.

The most important targets in which this complex go to is **ca++ calmodulin dependent protien kinase**, this kinase has the ability of phosphorylating a lot of proteins.

Note: Even if the calcium influx is stopped ,this kinase will still be active for a period of time inside the cell. **Why?** Because it works as a sort of memory;

the activity of the kinase will indicate that there was a calcium signal before a while.

After the release of calcium from the reservoir (ER) to the cytoplasm, the concentration of the calcium in the cytoplasm will increase and the cell will recognize this manipulation in concentrations and bring back the calcium to the ER to maintain high concentration within it and the activity of the calcium.

Note: high concentration of calcium in the cytoplasm will inhibit its activity and to maintain the potency of calcium, meaning we need any influx of calcium to be effective

Calcium calmodulin complex will bind to calcium ATPase pump to return calcium to its origin, accordingly decrease calcium concentration within the cells.

The calcium transporters and calcium ATPase pumps consume a lot of energy, because we are transporting calcium against concentration gradient. (One ATP molecule is needed per two calcium molecules getting in).

Transporters are found in huge amount, 80% of the proteins which are present in the membrane and ER are considered as calcium transporters. So the function of ER is to release calcium and return it back.

If there was a depletion in ATP ,the Ca ions cannot be transported outside the cell and that will cause muscles contraction (tetany) and after death it will cause rigor mortis (Temporary stiffness of joints and muscular rigidity occurring after death).

What are The conformational changes?

1)A long alpha helix between the two globular domains is now found (not found before Ca+ binding)

2)Exposure of some hydrophobic amino acid to the surface (hydrophobic pouches). This exposure will help calmodulin to bind other proteins through hydrophobic interactions.

Review : signal transduction starts when hormone bind to a receptor called 7 transmembrane helix → the massage will go to G protein → then the massage will be sent to → Adenylate cyclase (this pathway deal with c AMP)

→ Phospholipase C (this pathway break PIP2 and deal With Ca ,IP3.DAG)

*There is another option where the hormones do not bind to 7 tranmembrane helix receptor but they bind **Receptor Tyrosine Kinase**

Receptor Tyrosine Kinase :this receptor is responsible for phosphorylation and it has Tyrosine involved in the process.

The hormone will bind the receptor which has thyrosine, the thyrosine will be activated and then phosphorylated , and then this structure will phosphorylate other enzymes and proteins .

*These receptors consist of :

Either one polypeptide chain ,one monomer with cytoplasmic domain, extracellular domain and helix within the membrane.

Or dimers has the same shape connected together by disulfide bridges like (Insulin receptor).

Or monomeric with disulfide bridges making super secondary structures .

*The extracellular portion of the receptor is responsible for binding of the hormone (recognition domain), and the portion found inside is the portion containing tyrosine which will be phosphorylated (tyrosine kinase domain-intracellular)

The monomeric receptor mechanism :

We have several classes : class1 ,class 3.
like epidermal growth factor receptor, endothelial growth factor receptor, fibroblast growth factor receptor.

*The hormone binds one monomer and induces conformational change and induces dimer formation.

*When the dimer is formed the receptor will become active.

*When it is active it can do autophosphorylation process of the receptor itself on the tyrosine residues.

*When it is autophosphorelated it is now fully active ,and can act as a kinase enzyme which can phosphorylate other proteins and enzymes.

Proteins which can be phosphorylated by this mechanism :

1)Phospholipase C (Can be activated by receptor tyrosine kinase).

2)Protein phosphatase1 (which is linked it Insulin metabolism ,once insulin is bound to protein phosphatase1 it enhance glycogen synthesis)

**Hormones which bind to this type of receptors are usually growth hormones , platelet derived growth factors ,epidermal growth factors.

Review: hormone bind to receptor ⟶ induce dimerization ⟶

Autophosphorelation process occur ⟶ phosphorelation of other proteins and enzymes.

Example: Growth Hormone

*It is a small subunit hormone , size is about 200 amino acid.

*Its receptor is almost 3 times larger (600 amino acid) than the hormone itself .

*Growth hormone receptor contains 3 domains : extracellular domain, intracellular domain contain tyrosine residues and domain within the membrane.

The pathway:

1)Once this receptor is monomeric it is inactive ,when the growth hormone bind to the receptor it activates it (cooperative binding).

2)Next, induction of dimerization .

3)Then autophosphorylation occur and the receptor will become fully active.

4)How it can phosphorylate other proteins??

On the receptor we have kinase which phosphorylates proteins and enzymes .

The main enzyme that is phosphorylated is called **JANUS kinase** (connected to the phosphorylated tyrosine residues and only bind them when they are phosphorylated) , and this kinase contain two parts , one interact with the membrane and the other interact with receptor tyrosine kinase.

Why it is called JANUS kinase?

JANUS is the goddess of the beginning and the end(اله البداية والنهاية) .This goddess has two similar heads similar to the kinase ,which can bind to tyrosine in receptor tyrosine kinase and get activated

*After autophosphorylation and phosphorylation of JAKs , autophosphorylation and cross phosphorylation each subunit will phosphorylate the other one , now JANUS kinase is active.

5)Now JANUS kinase will enhance STAT (signaling molecule activators of transcription). STAT will increase the transcription of mRNA from the gene **.(how??)**

*Two monomers dimerized to be active by different dimerization process.

*If the receptor stayed active in a pathological way or the JAK stayed active the transcription process will proceed further causing cancer formation.

*The phosphorylation of the STAT monomer : each STAT monomer contain phosphorylation domain and tyrosine residues , phosphorylation of tyrosine and binding of this tyrosine to the other monomer to complete the dimerization .

Note: dimerization is induced by phosphorylated tyrosine monomer ,while each tyrosine will bind the other monomer to induce the cross binding between the two monomers forming STAT dimer .

*Most of these proteins are active through dimerization.

Hormones using receptor tyrosine kinases other than growth hormone is the epidermal growth factor:

Pathway:

1-Hormone binds monomer receptor. 2-induce dimerization.

3-Autophosphorylation. 4-Cross phosphorylation.

5-Activation of other proteins and enzymes.

Is dimerization enough to induce the activity??

No, it is not sufficient to induce activity. (why????)

Because after dimerization we should have phosphorylation to have full activity.

Insulin:

-It is a hormone which uses receptor tyrosine kinase ,the only difference is the insulin receptor is not a monomer ,it is dimeric in origin .

-Each monomer consist of alpha and beta subunits bound through disulfide bridges ,so it is more like a monomers and after activation they will get closer together and the affinity increases.

-They are already bound to each other, binding of the hormone to the alpha subunit from outside , induces conformational change within the beta subunit , this change in shape will induce autophosphorylation process and we will have phosphorylated tyrosine within the receptors which induce other proteins and enzymes to come and get phosphorylated and activated or inactivated, playing in the metabolism .

*Receptor tyrosine kinase enhance proteins and enzymes ,one of the downstream proteins is RAS protein (after activation of the receptor we have second and other proteins called RAS)

*RAS is a form of G protein, but it differs from G protein that it consists from only one subunit molecule and not three subunits.

*We talked about RAS here ;because it is one of the downstream proteins which are activated and phosphorylated by receptor tyrosine kinase .

*RAS proteins when it bind GTP it becomes active and when it bind GDP it become inactive. It has GTPase activity , when it is activated it induce a lot of effects inside the cell and through the intrinsic GTPase activity it can come back to its original inactive structure.

Eicosanoids:

*They are lipidic structures.

*The name came from (Ecosa=twenty) meaning these structures are composed of twenty carbon units molecules.

*They work in similar manner as hormones ,the difference is hormones work in distance, but eicosanoids work in autocrine and paracrine fashion on adjacent cells.

*They have several classes, which are derived from Archodinic acid (twenty carbon units molecule).

*Archodinic acid gives eicosanoids subclasses :

-prostaglandins -thromboxines -leukotrines

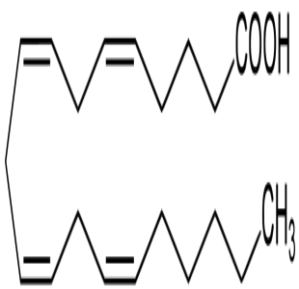
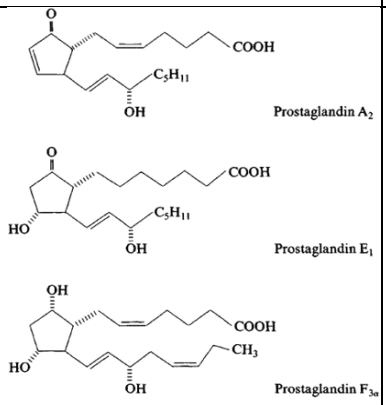
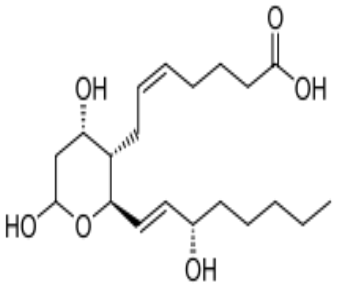
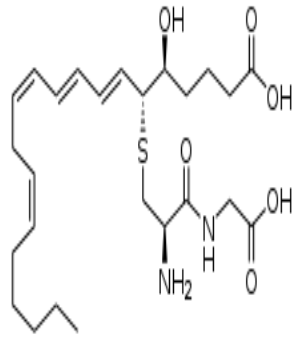
*These subclasses are found in all tissues ,they are potent molecules secreted in very small amounts and have huge effects.

*There function:Some of the prostaglandins increase vasodilatation and others decrease vasodilatation ,some increase the platelet aggregation and others decrease platelet aggregation ,meaning they work as antagonists to each other.

*The #number following the name indicate how many double bonds are within this structure .

***Note: you should be able to identify the structures when you look at them and these are classical questions in the exam.

All of them contain twenty carbon units

1)Archodinic acid	_2)prostaglandins	3)thromboxines	4)leukotrines
20 carbons	20 carbons	20 carbons	20 carbons
4 double bonds	2 double bonds	2 double bonds	At least 3 conjugated double bonds
no ring	Five membered ring	6 membered ring with oxygen Included	No ring
			

All are derivatives of archadonic acid .

From where we get the Archadonic acid ?? it is not essential molecule we can get it from lieonlic acid through elongation and saturation .

*The archadonic acid is within the structure of the membrane and it can get out of the membrane by phospholipase A , this enzyme work on carbon #2 and the archadonic acid becomes free.

*Now we can use it in producing prostaglandins and other derivatives.

*Note :we can get eicosanoids from molecules(fatty acids with 20 carbons) containing three or five double bonds , this is important in health ,the metabolism of these molecules within the cell. With every step you are removing two double bonds from the structure and make saturation to two double bonds , so the molecules which contain three double bonds they will be converted to molecules with one double bond, and the ones contain 4 double bonds will be converted to molecules with two double bonds , and the ones containing 5 double bonds will be converted to molecules containing 3 double bonds .

**e.g. on three double bonds is thromboxine B3 which decreases platelet aggregation –omega 3 from sea food- (good for health and prevent clotting and myocardial infarction)

**e.g. on two double bonds is thromboxine B2 –omega 6- increase the platelet aggregation (not good for health).

(اللهم اني اعوذ بك من علم لا ينفع)

اولا:اسفة على الشيت الطويل الي تعبتني كتيير قبل ما تتعبكم .

ثانيا: فقرة إهداء الشيت

اولا بهدي هاي الشيت لنفسي لانو بعتبرها من انجازاتي

ثانيا بهديها لكل من:

رفاء شكرا لانك خلتيني استعير مقعدك في المحاضرة

جارتي غيداء نجداوي

ميرنا بشناق happy birthday

الثنائي أسيل أحمد و مرح أبو رمان

المجموعة التحشيشية :أمل عرابي ،سندس هلسي ،مروى الشيخ و نور أبو غنيم

لين ياسين <3

سجى عاشور <3

و تحية خاصة لطلاب شعبة 1 البواسل

بعرف انكم بتيجو مواصلين فيعطيكم العافية

و تحية لاصدقائي العزيزين في شعبة 2

و still تحية ل إيمان العموش و رنيم بدر و دمتم ساااالمين

و كل عام و انتم بخير

و عيد فطر مبارك