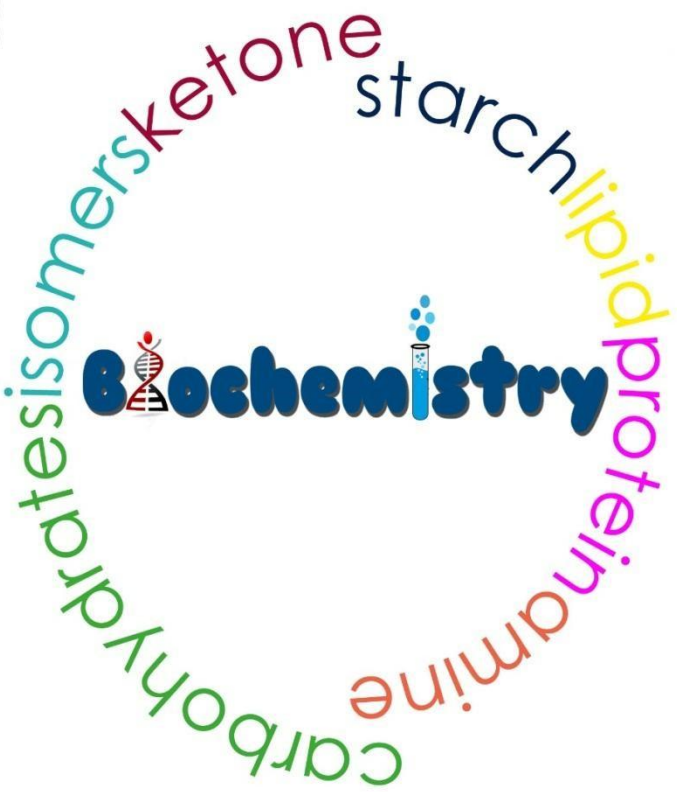


◉Sheet ○Slides

Subject :	Regulation of glycogen
Done by :	Ammar Ramadan
Corrected by :	Motasem Abu Mallouh & Yazeed Qudah
Number :	15

00:00 – 05:30 is discussed in the previous lecture. (For each glucose unit added to glycogen how much energy do we need?)

If we started with glucose 1-phosphate, how much energy do we need?

Glucose 1-phosphate + UTP → UDP-glucose + PPi

PPi + H2O → 2Pi

UDP-Glucose + Glycogen (n) → UDP + Glycogen (n+1)

Glucose 1-phosphate + UTP + Glycogen (n) → UDP + Glycogen (n+1)

So we need 1 ATP equivalent molecule ☺

▪When we degrade glycogen to glucose 1-phosphate by glycogen phosphorylase, we don't produce any ATP

So if the synthesis of glycogen from glucose 1-phosphate and the degradation of glycogen to glucose 1-phosphate happened at the same time, we would lose 1 ATP for each molecule. Therefore, regulation is needed to prevent the degradation and the synthesis from occurring together.

When glycogen synthesis is required, we have to inhibit the degradation and vice versa.

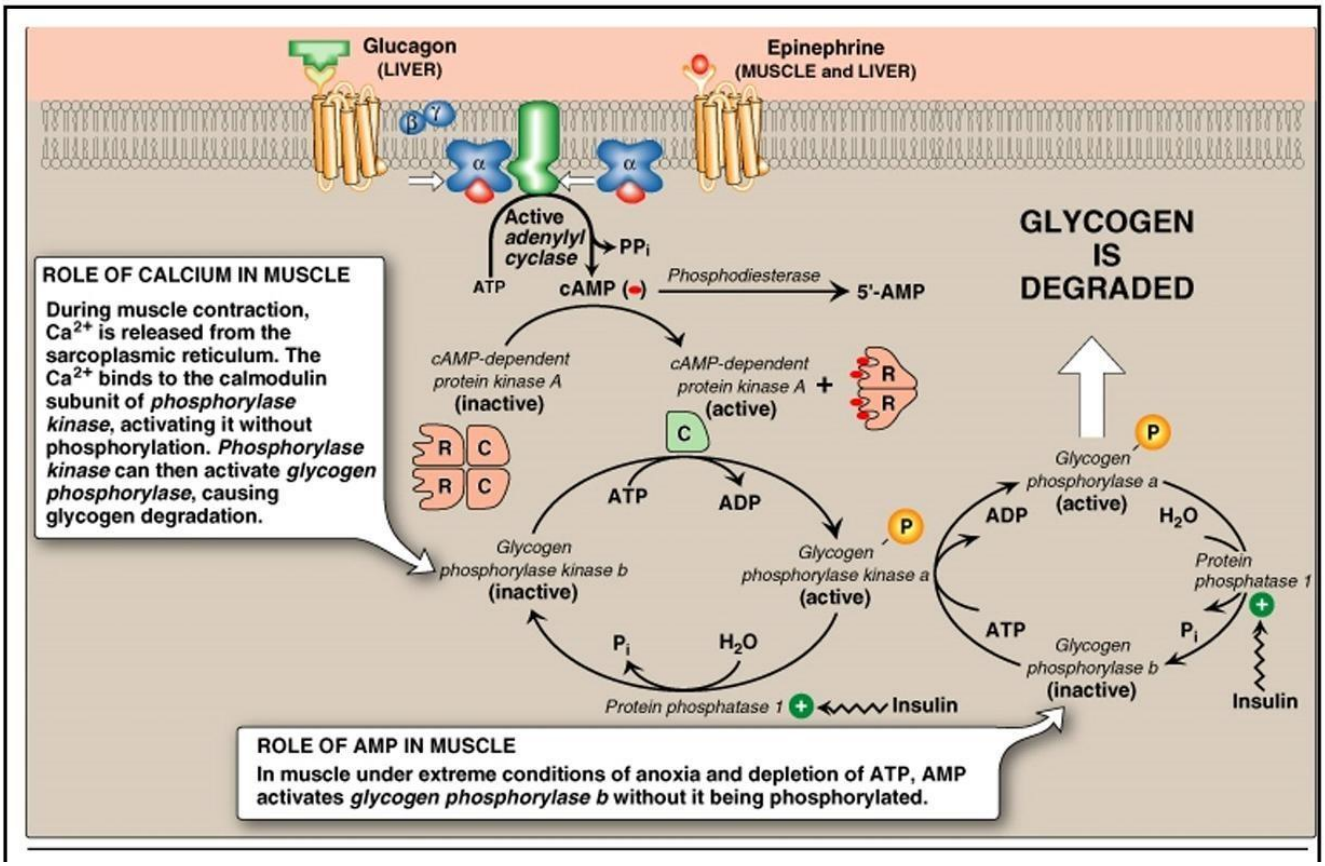
5:30 – 7:30

Regulation can be achieved by two mechanisms:

1- Interaction of cell with hormones (the regulation at the hormonal level regulates the function of the cell in response to bodily needs).
2- Allosterically

Regulation at the hormonal level:

▪If there is glucagon / epinephrine (during stress), it would indicate that blood glucose level is low. For that reason, cells respond by increasing glycogen degradation. (Here we are talking about glycogen



in the liver)

Mechanism:

▪ Glucagon binds to a specific receptor in the plasma membrane of liver cells to stimulate the G-protein (composed of alpha, beta and

gamma). Alpha subunit then binds to GTP and releases GDP leading to the activation and dissociation of the alpha subunit.

▪ Activated alpha subunit binds to Adenylyl cyclase which in turn converts ATP to cAMP (acts as a regulator)

▪ cAMP binds to the regulatory subunits of the cAMP-dependent protein kinase A (tetramer {2 regulatory subunits and 2 catalytic subunits}), this binding leads to the release of the catalytic subunits (protein kinase A).

▪ Protein kinase A (since it is kinase, it phosphorylates molecules by adding a phosphate group) phosphorylates some proteins to make them phosphorylated (either active or inactive). One of its substrates is the enzyme **glycogen phosphorylase kinase B**

▪ Once PKA phosphorylates **glycogen phosphorylase kinase B** using ATP, the enzyme is converted to an active form, **glycogen phosphorylase kinase A.** [phosphorylated form is active and the dephosphorylated form is inactive]

▪ **Glycogen phosphorylase kinase A** (active form) can now phosphorylate **glycogen phosphorylase B using ATP**, which is converted from the inactive form **glycogen phosphorylase B** to the active form **glycogen phosphorylase A** .

▪ **Glycogen phosphorylase A** then degrades glycogen.

Note: epinephrine can also bind to its receptor (in the liver and the muscles) to activate the same cascade.

Note: epinephrine has 2 receptors in the liver (alpha and beta adrenergic receptors). Beta adrenergic receptor is the one we discussed above.

☆ In any regulatory mechanism there should be a way to reverse this regulation. For example, if blood glucose level is normal, we have to inhibit the glycogen phosphorylases.

1- If the receptor is empty the alpha subunit will hydrolyse GTP to make it GDP so the alpha subunit becomes inactive and binds to the beta-gamma complex.
2- **Phosphodiesterase** converts cAMP to 5'AMP (inactive). This way PKA will stay inactive, thus it will not activate glycogen phosphorylase kinase B.
3- **Protein phosphatase 1** removes the phosphate group - from the glycogen phosphorylase A and converts it to the inactive form
   - from the glycogen phosphorylase kinase A and converts it to the inactive form.

☆ Protein phosphatase 1 and phosphodiesterase are stimulated by insulin (high blood glucose level >> no need to degrade glycogen anymore)

☆ Protein phosphatase 1 can be inhibited by an **inhibitor protein** (if the inhibitor protein was activated (phosphorylated) by PKA.

4- Free glucose: binds to the enzyme **glycogen phosphorylase A**, once bound to it, glycogen phosphorylase A would be a good substrate for the enzyme **protein phosphatase 1**. Protein phosphatase 1 works better if the free glucose was bound to **glycogen phosphorylase A**. This only happens in the liver because there we have the enzyme glucose 6-phosphatase)

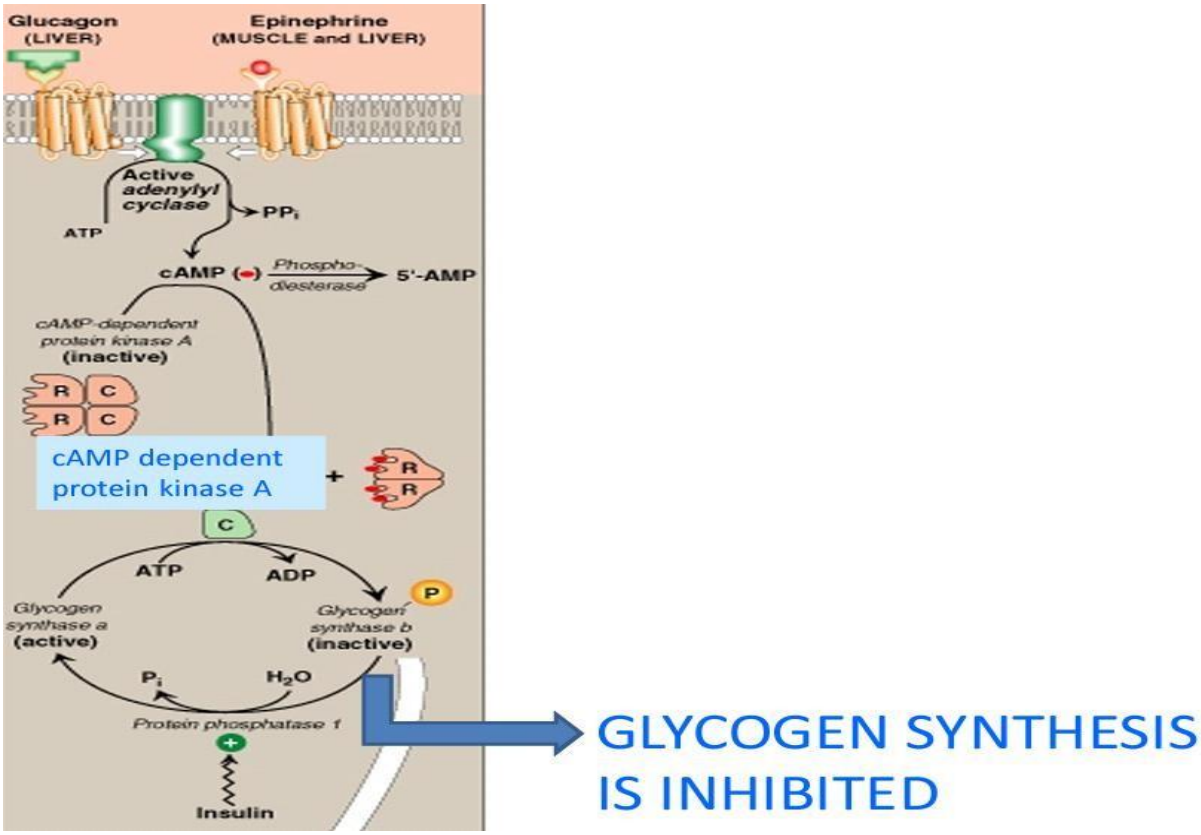
☆Free glucose indicates that we have a high blood glucose level, so we need to stop glycogen degradation.

If glycogen degradation is active, glycogen synthesis should be inactive:

▪ Glucagon or epinephrine binds to its receptor (epinephrine binds to beta adrenergic receptors) in the plasma membrane of liver cells. This binding activates Adenylyl Cyclase, leading to the production of cAMP which activates PKA (same mechanism)

▪ PKA adds a phosphate group to **glycogen synthase A** converting it from an active to an inactive form. This way, glycogen synthesis is inhibited

Note: when insulin is available (inducer for the protein phosphatase), the protein phosphatase removes the phosphate group from the phosphorylated (inactive) glycogen synthase converting it to the active form to synthesize glycogen since we



have insulin (high blood glucose level)

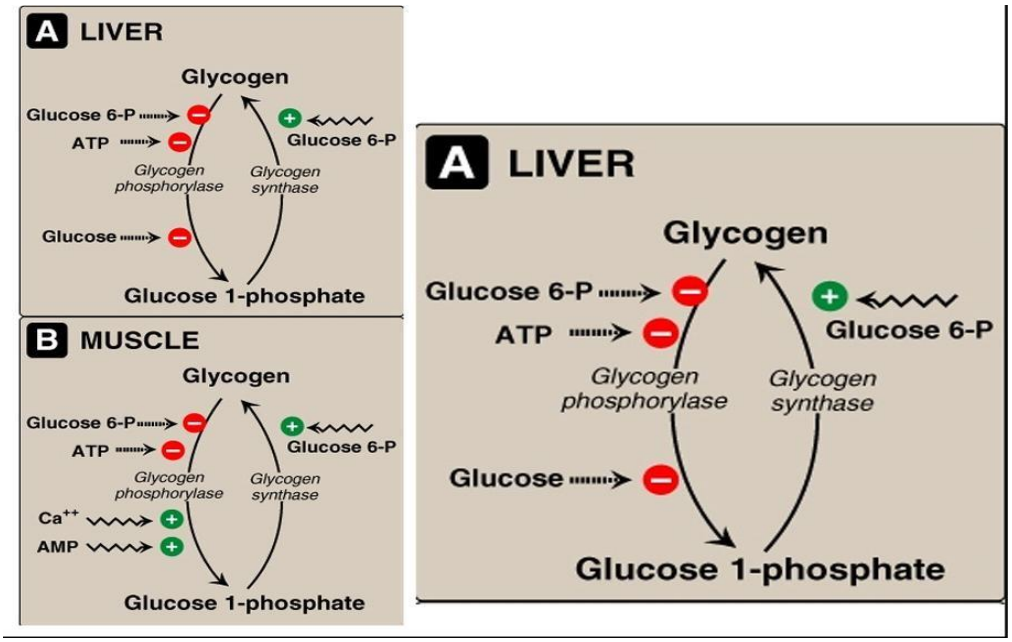
☆ Regulation at the hormonal level is done by covalent modification; a phosphate group is added to the protein by an ester bond (covalent bond) or removed in case insulin is available just like this example.

7:30 – 19:02

2-Allosteric regulations: binding of small molecules to the enzyme at allosteric sites by non-covalent bonding might increase or decrease the activity of the enzyme)

☆ Rapid response to cellular needs.

☆ Allosteric signals recognise the needs of the cell itself.



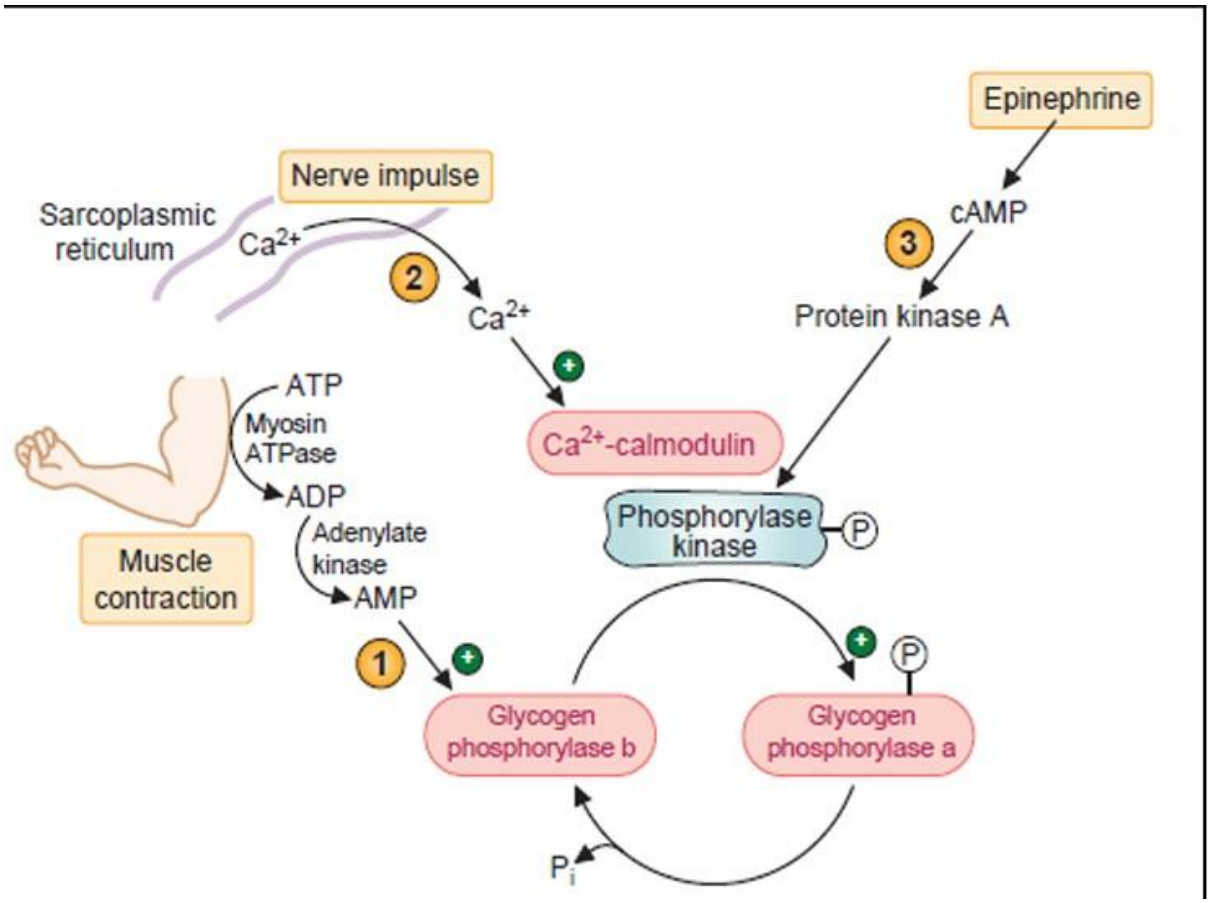
☆ High glucose-6-phosphate and ATP stimulate glycogen synthesis and inhibit glycogen degradation.

In **liver** and **muscle** cells, the enzyme that is regulated by allosteric signals is **glycogen synthase**. It is activated by glucose 6-phosphate even if it was phosphorylated. On the other hand, glucose 6phosphate and ATP inhibit the enzyme glycogen phosphorylase.

Glucose is also an inhibitor for the enzyme glycogen phosphorylase. (This happens in the liver only)

☆ These allosteric regulators of glycogen phosphorylase act without the need of phosphorylation.

In the **muscles** we have Ca+2 and AMP, these are allosteric activators for the enzyme **glycogen phosphorylase**. (High Ca+2 and AMP lead to an increase in the degradation of glycogen).

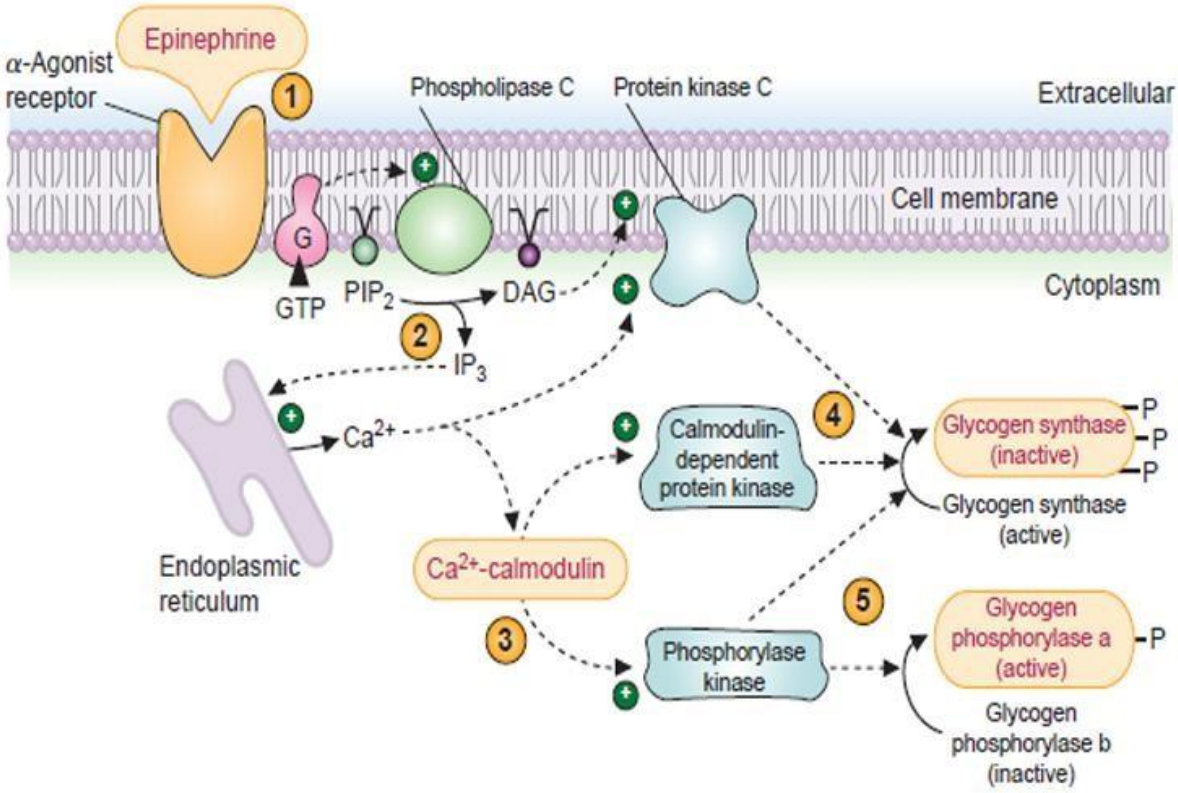


Muscles contract by nerve impulses , when the nerve impulse reaches the cell it stimulates the release of Ca+2 from the sarcoplasmic reticulum leading to an increase in the concentration of Ca+2 in the cytoplasm leading to the contraction of the muscle. With this, ATP consumption increases causing an increase in ADP concentration. ADP is then converted to AMP by adenylate kinase (ADP + ADP → ATP + AMP)

▪ The Ca+2 released from the SR binds to a protein call **calmodulin (CaM)**, a protein found in all cells and its role is to bind with Ca+2. Four ions of Ca+2 bind to calmodulin. After binding, the activated

Ca+2-calmodulin complex binds to **glycogen phosphorylase kinase** which is activated even without phosphorylation.

So **glycogen phosphorylase kinase** can be activated either by adding phosphate or by binding to Ca+2-calmodulin.



So this regulation happens indirectly through calcium binding proteins like calmodulin.

▪ High AMP in muscles reflects a low energy state (low ATP). This leads to the activation of **glycogen phosphorylase** without phosphorylation.

Ca+2 activation of liver **phosphorylase kinase**:

▪ Epinephrine binds to alpha 1 adrenergic receptor leading to the activation of g-protein which in turn activates phospholipase C. The activated phospholipase C then degrades PIP2 to DAG and IP3.

▪ IP3 binds to the endoplasmic reticulum causing the release of Ca+2. After that, Ca+2 binds to calmodulin to produce Ca+2-calmodulin.

▪ Ca+2-calmodulin binds to **calmodulin dependent protein kinase** to activate it. Once activated, calmodulin dependent protein kinase adds a phosphate group to glycogen synthase to inactivate it.

DAG and Ca+2 bind to protein kinase C and activate it. Activated kinase C phosphorylates glycogen synthase so that it is further inactivated.

In this cascade, three enzymes have been activated (protein kinase C, calmodulin dependent protein kinase and glycogen phosphorylase kinase) and these enzymes will phosphorylate glycogen synthase causing its inactivation.

19:02-29:00

Metabolism of monosaccharides: -

- Major dietary source of energy for human beings is glucose followed by fructose.
- Fructose is more abundant than galactose in our diet.
- We get fructose from sucrose, honey, fructose corn syrup and fruits.
- Entry of fructose to cells isn't insulin dependent. Diabetic patients may take fructose as substituent of glucose ;because it is rapidly metabolised and doesn't promote secretion of insulin.

Mechanism of the metabolism of fructose:

▪ Fructose is phosphorylated by **fructokinase** to fructose 1-phosphate. (In our bodies, there isn't an enzyme that phosphorylates fructose 1phosphate to fructose 1,6-bisphosphate)

[Recall: in glycolysis, fructose 6-phosphate can be converted to fructose 1,6-bisphosphate]

▪ Fructose 1-phosphate is cleaved by **aldolase B** into dihydroxyacetone phosphate (DHAP) and glyceraldehyde.

▪ Glyceraldehyde is phosphorylated at C-3 to give glyceraldehyde 3phosphate which can be metabolized through two pathways, glycolysis and gluconeogenesis.

These 3 reactions are unique for fructose metabolism.

Note: fructose can be converted to fructose 6-phosphate by **hexokinase** but with low affinity (high km) (not significant).

Note: if the substrate is fructose 1,6-bisphosphate, aldolase a, b and c cleave it to dihydroxyacetone phosphate and glyceraldehyde 3phosphate. However, Aldolase b is specific for the cleavage of fructose 1-phosphate.

Aldolase B	Aldolase A
Cleavage of fructose 1,6-bisphosphate Cleavage of fructose 1phosphate	Cleavage of fructose 1,6-bisphosphate
Liver, kidney and small intestine (where fructose is metabolized)	In most tissues

Note: aldolase C is found in the brain. It can cleave fructose 1,6-bisphosphate but it cannot do so for fructose 1-phostphate.

Disorders of fructose metabolism:

In any enzyme deficiency, an accumulation of the substrate occurs.

If fructokinase is deficient, accumulation of fructose occurs. It would appear in the urine (fructosuria). [Benign condition]

If aldolase B is deficient, accumulation of fructose 1-phosphate takes place. This leads to a decrease in the amount of Pi in the cell (because they are bound to the fructose at C-1) causing a decrease in the amount of ATP molecules. This leads to impaired gluconeogenesis giving rise to hypoglycemia and lacticacidemia.

(No gluconeogenesis results in the accumulation of lactic acid).

Here are some others symptoms written in the book: in the absence of Pi, AMP is degraded, causing hyperuricemia (and lactic acidosis).

The decreased availability of hepatic ATP affects gluconeogenesis (causing hypoglycemia with vomiting), and protein synthesis (causing a decrease in blood clotting factors and other essential proteins). Kidney function may also be affected.

This deficiency of aldolase B is called **hereditary fructose intolerance** (HFI) (fructose poisoning) with an incidence of 1/20.000. So, we have to screen for the deficiency of this enzyme. [Severe disturbance in liver and kidney metabolism]

Aldolase B is produced by two genes, one from the father and another from the mother. Since the disease is recessive, if one of the genes was defective, the disease would not appear. It can only happen when the two defective genes are present together)

As a rule, enzyme deficiency is always recessively inherited.

The first symptoms of HFI appear when a baby is weaned from milk and begins to be fed food that contains sucrose or fructose.

With HFI, sucrose and fructose must be removed from the diet to prevent liver failure and possible death.