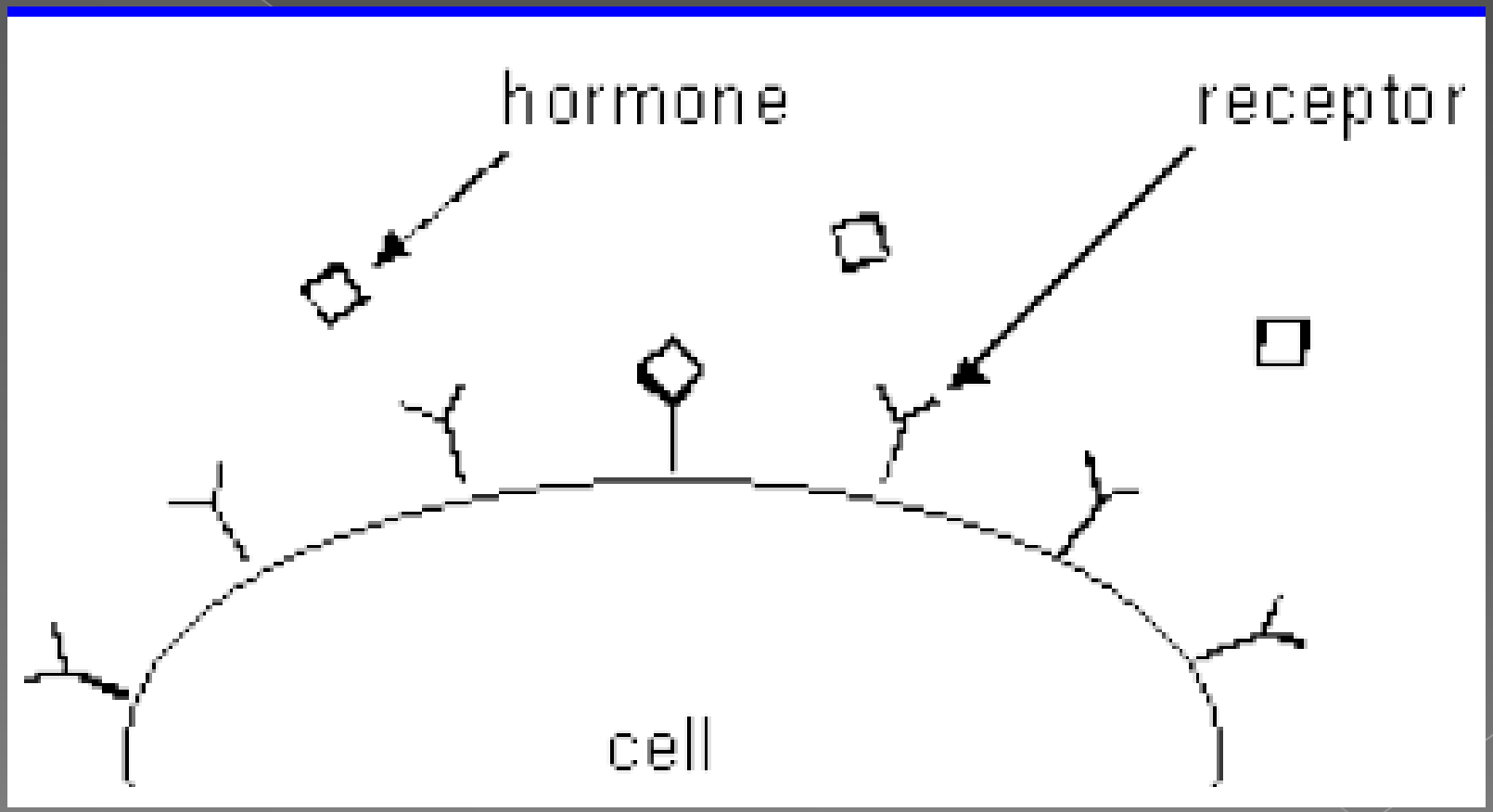
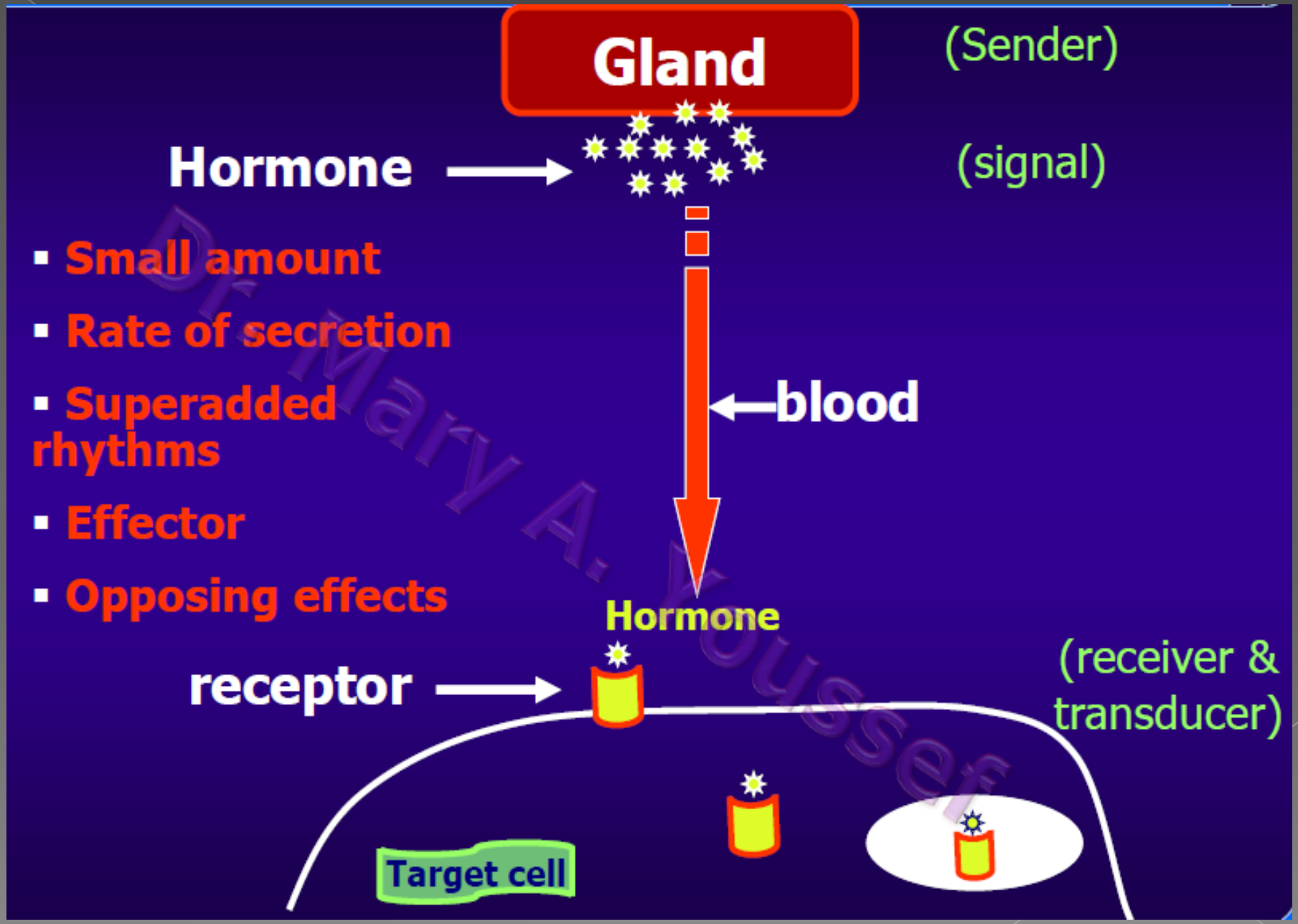
- Mechanism of action of hormones

Receptors for Chemical Messengers

- The recognition of chemical messengers by cells typically begins by interaction with a receptor at that cell.
- There have been over **20 families of receptors** for chemical messengers.
- These proteins are not static components of the cell, but their numbers increase and decrease in response to various stimuli, and their properties change with changes in physiological conditions.


hormone
receptor
cell


Gland
(Sender)
Hormone
(signal)
Small amount
Rate of secretion
Superadded rhythms
Effector
Opposing effects
blood
Hormone
receptor
(receiver & transducer)
Target cell
Dr Mary A. Youssef

When a hormone or neurotransmitter is present in excess, the number of active receptors generally decreases **(down-regulation),**

whereas in the presence of a deficiency of the chemical messenger, there is an increase in the number of active receptors **(up-regulation).**

angiotensin II is an exception; it increases rather than decreases the number of its receptors in the adrenal.

Down-regulation

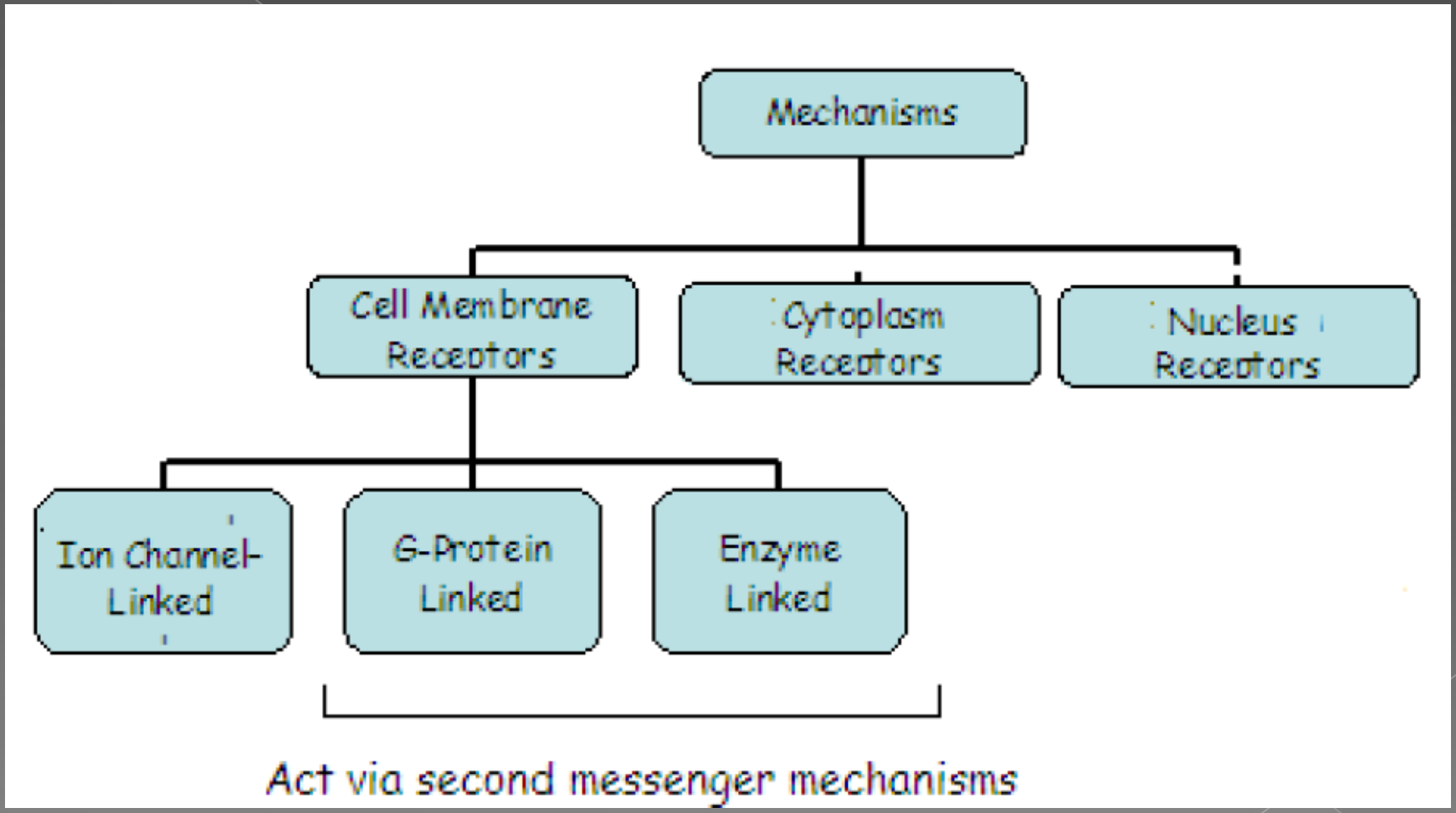
- (1) inactivation of some of the receptor molecules
- (2) inactivation of some of the intracellular protein signaling molecules
- (3) temporary sequestration of the receptor to the inside of the cell, away from the site of action of hormones that interact with cell membrane receptors
- (4) destruction of the receptors by lysosomes after they are internalized or
- (5) decreased production of the receptors.

- In each case, receptor down regulation decreases the target tissue's responsiveness to the hormone.

Up-regulation

- Stimulating hormone induces greater than normal formation of receptor
- or intracellular signaling molecules by the target cell
- or greater availability of the receptor for interaction with the hormone.
- When up-regulation occurs, the target tissue becomes progressively more sensitive to the stimulating effects of the hormone

Location of hormone receptors



Mechanisms by Which Chemical Messengers Act

- Receptor–ligand interaction is transduced into secondary responses within the cell that can be divided into four broad categories:
- (1) ion channel activation,
- (2) **G-protein** activation,
- (3) activation of enzyme activity within the cell,
- (4) direct activation of transcription.

I. Mechanism of action of protein & polypeptide hormones:

- 1. The hormone (1st messenger) binds to a cell membrane receptor of target cell

- 2. ion channel activation,
- **G-protein** activation,
- activation of enzyme activity within the cell

- 3. changing the activity of certain enzymes

Cell membrane hormone receptor system

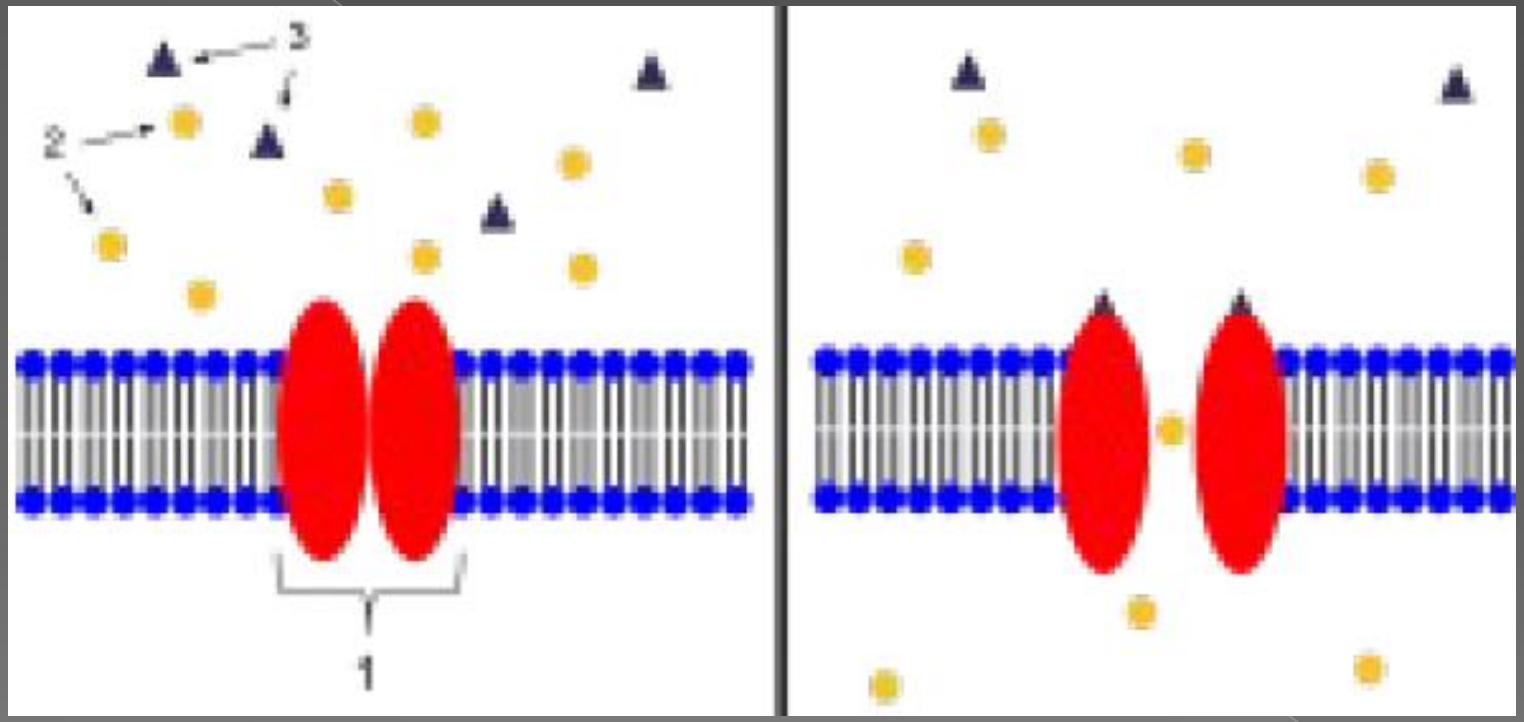
a. **Ion Channel–Linked Receptors:**

- Neurotransmitters like acetylcholine and norepinephrine

- combine with receptors in the post synaptic membrane → change in the structure of the receptor → opening and closing a channel for one or more ions → altered movement of these ions through the channels cause the subsequent effects on the post synaptic cells.

- open (or close) channels for sodium ions, others for potassium ions, others for calcium ions

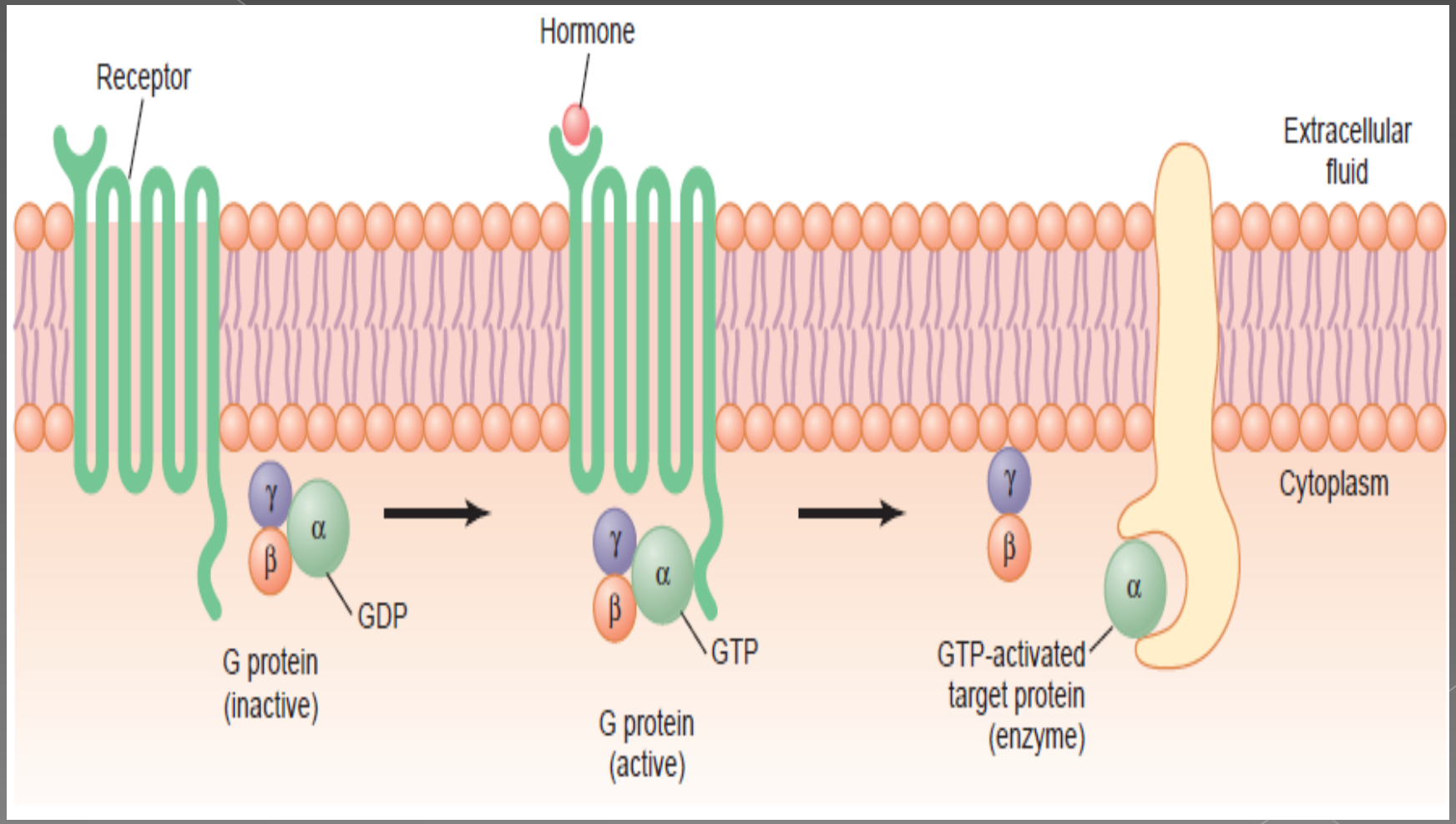
Ion Channel–Linked Receptors:

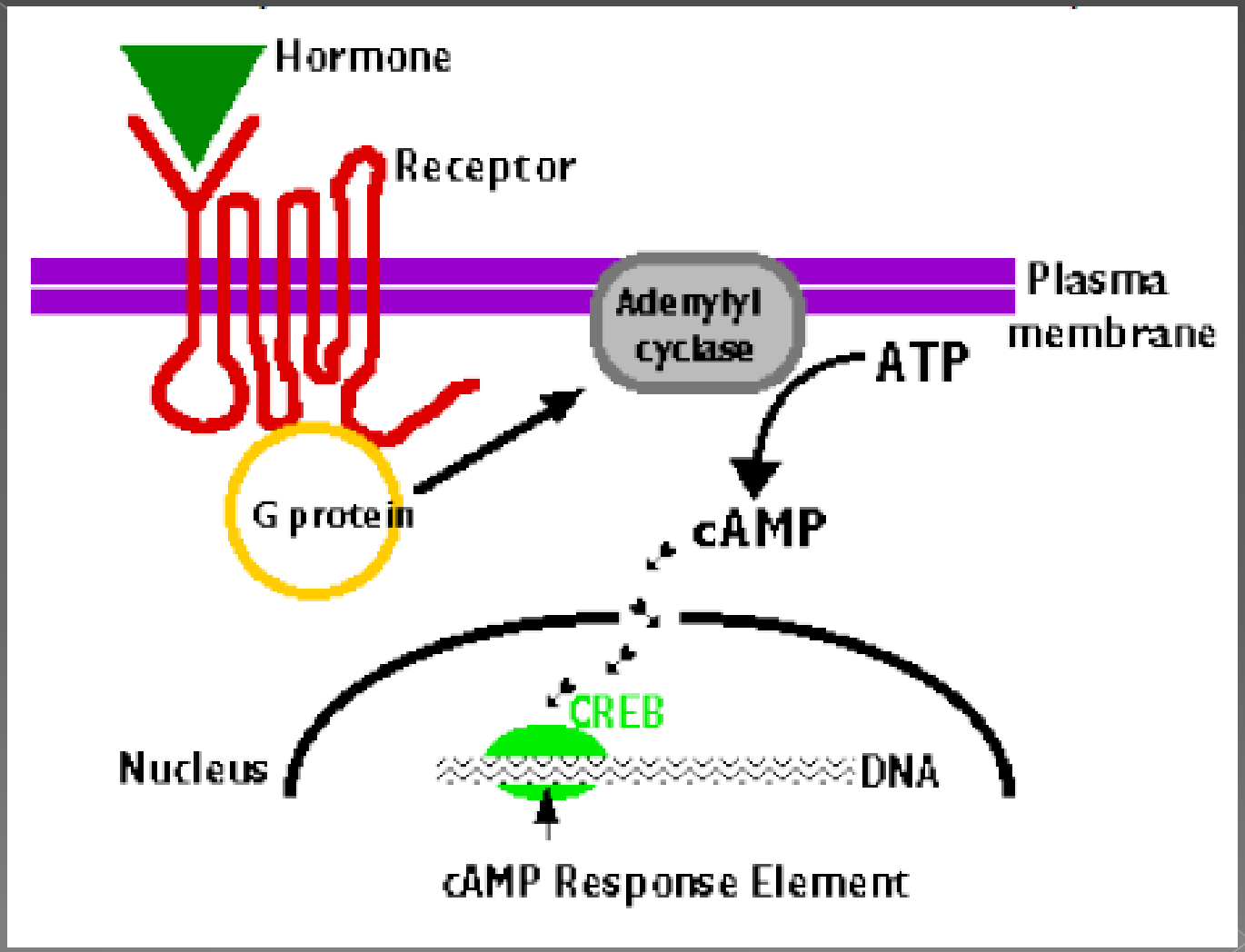


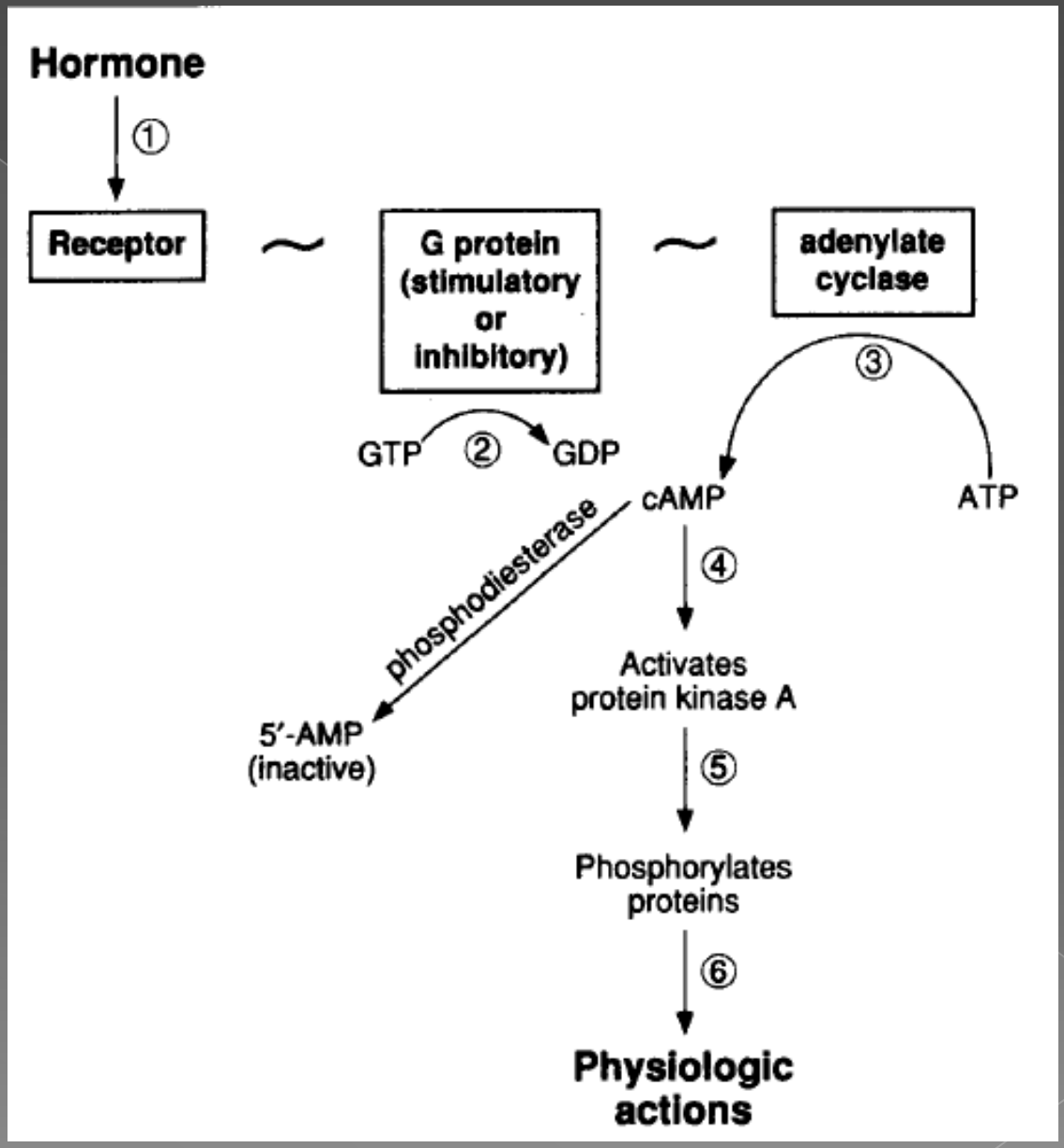
Cell membrane hormone receptor system
b. G-protein linked hormone receptor system

- Hormone + receptor → cell membrane G-proteins activated → alter the activity of ion channels or intracellular enzymes such as adenyl
- cyclase or phospholipase C → alters cell function.
- G-proteins link the receptor to membrane mechanisms that generate **second messenger.**
- **Examples**
- follicle stimulating hormone(FSH)
- Thyroid stimulating hormone (TSH)

G-protein linked hormone receptor system




Hormone
Receptor
Plasma membrane
Adenylyl cyclase
ATP
G protein
cAMP
CREB
Nucleus
DNA
cAMP Response Element


Hormone
①
Receptor
G protein (stimulatory or inhibitory)
adenylate cyclase
③
GTP
②
GDP
cAMP
ATP
phosphodiesterase
④
Activates protein kinase A
5′-AMP (inactive)
⑤
Phosphorylates proteins
⑥
Physiologic actions

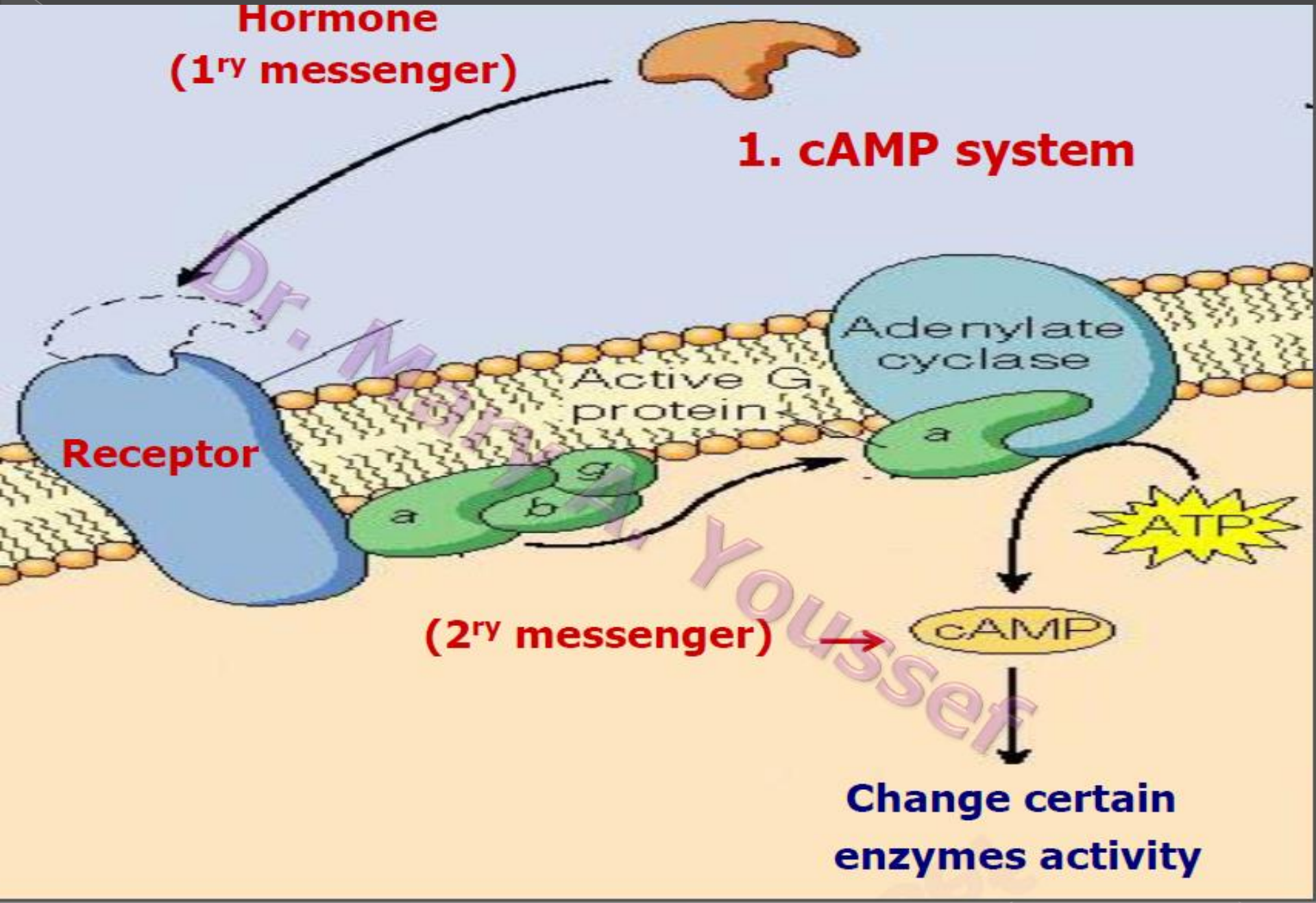
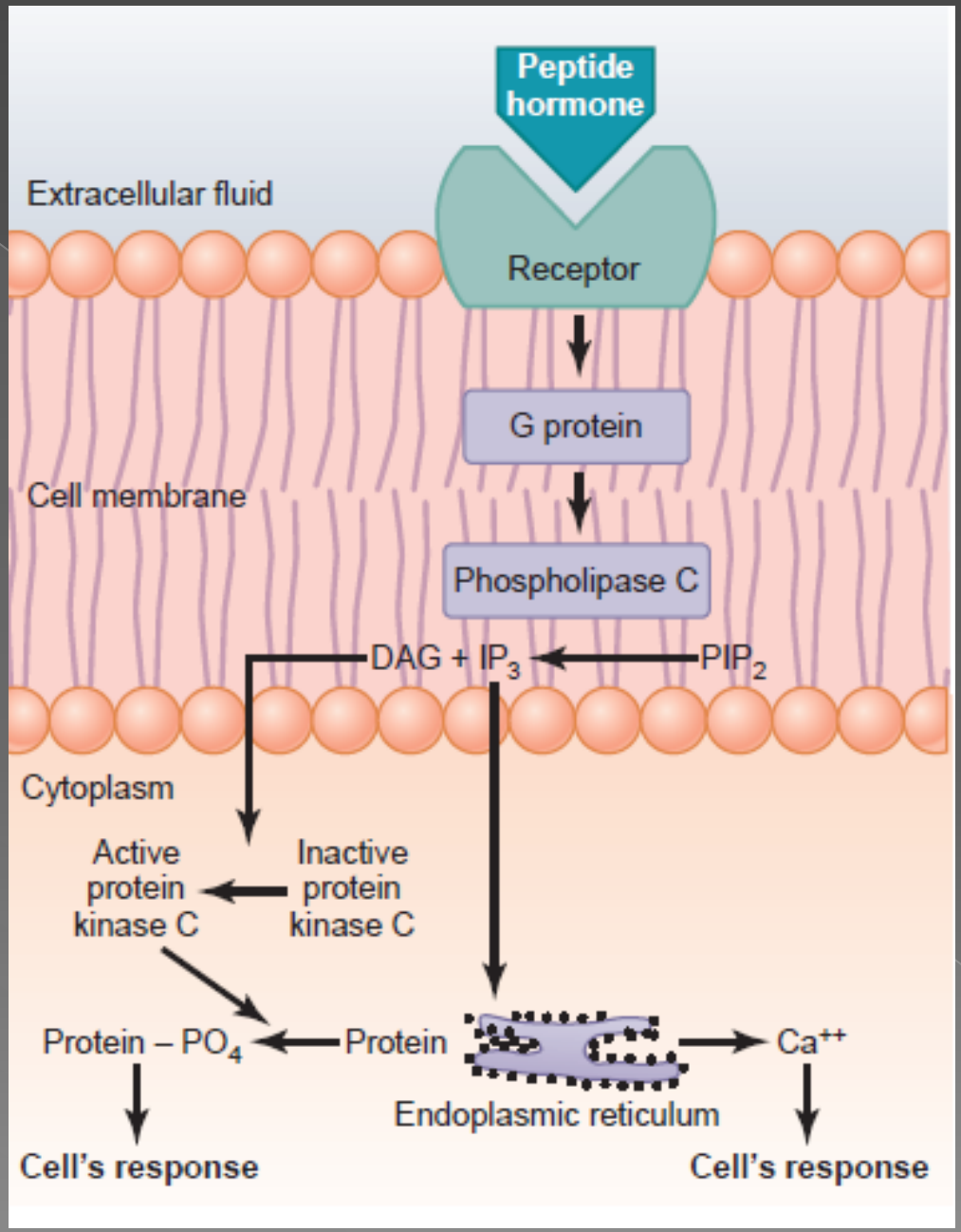
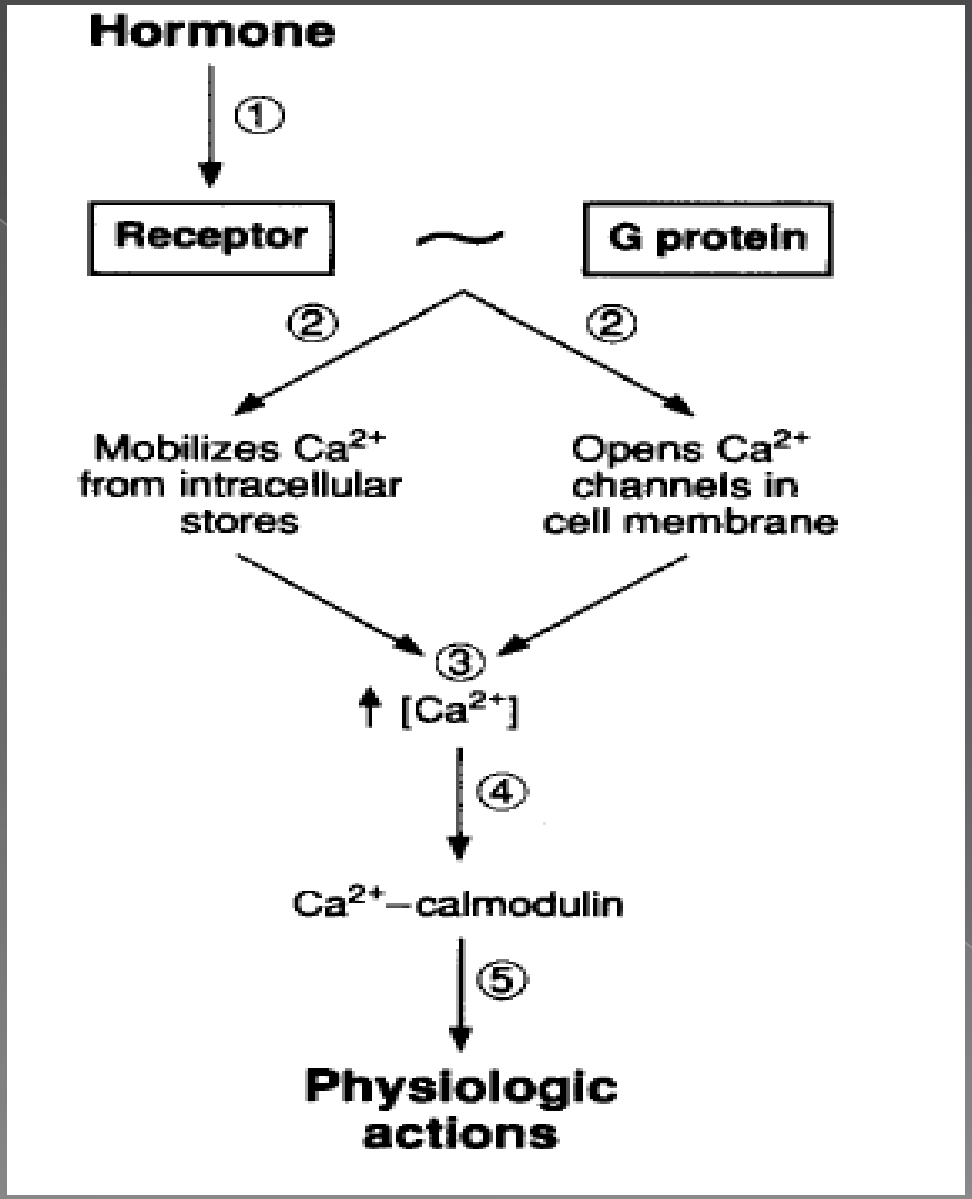
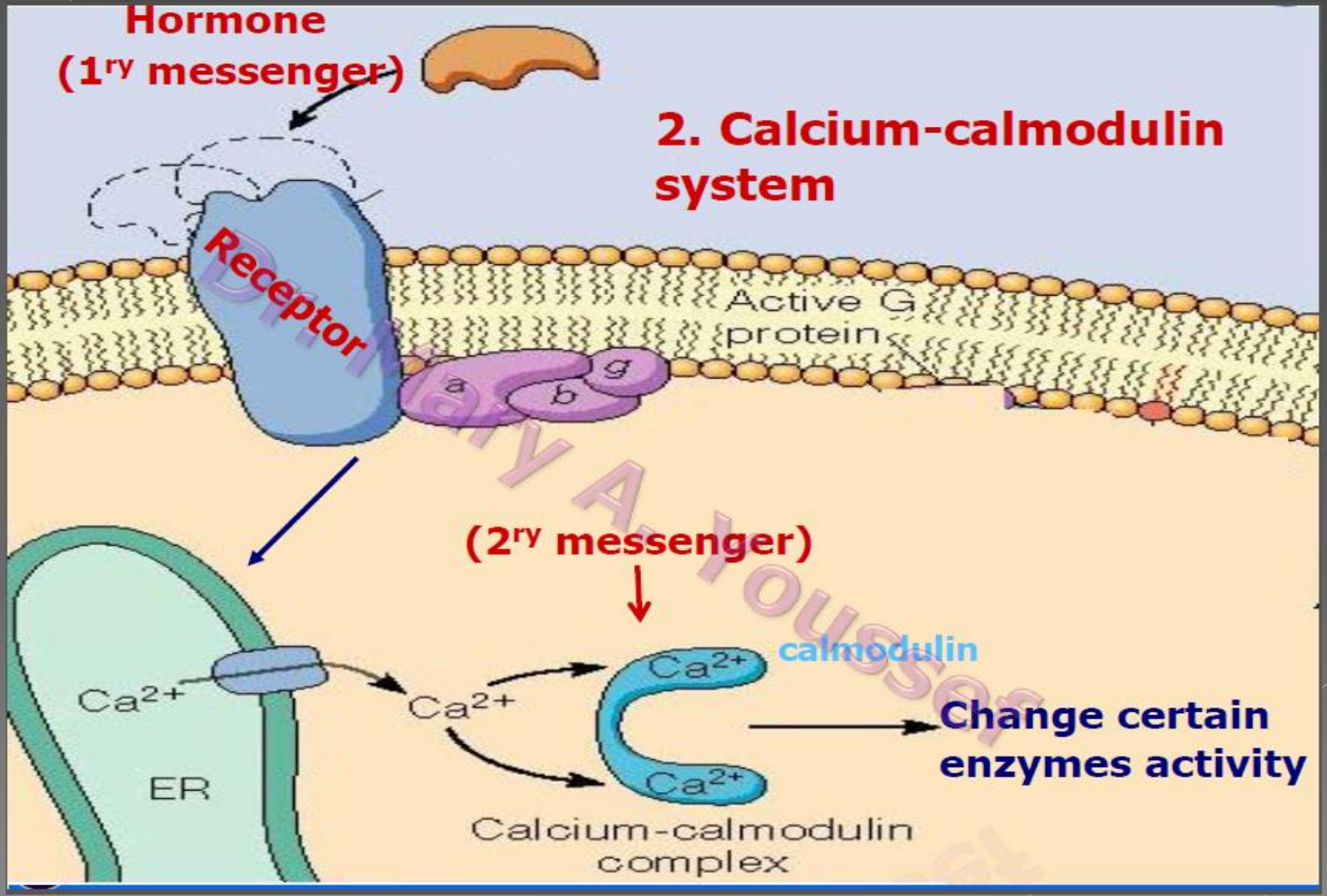

Hormone
(1ry messenger)
1. cAMP system
Adenylate cyclase
Active G protein
Receptor
a
g
a
b
ATP
(2ry messenger)
cAMP
Change certain enzymes activity

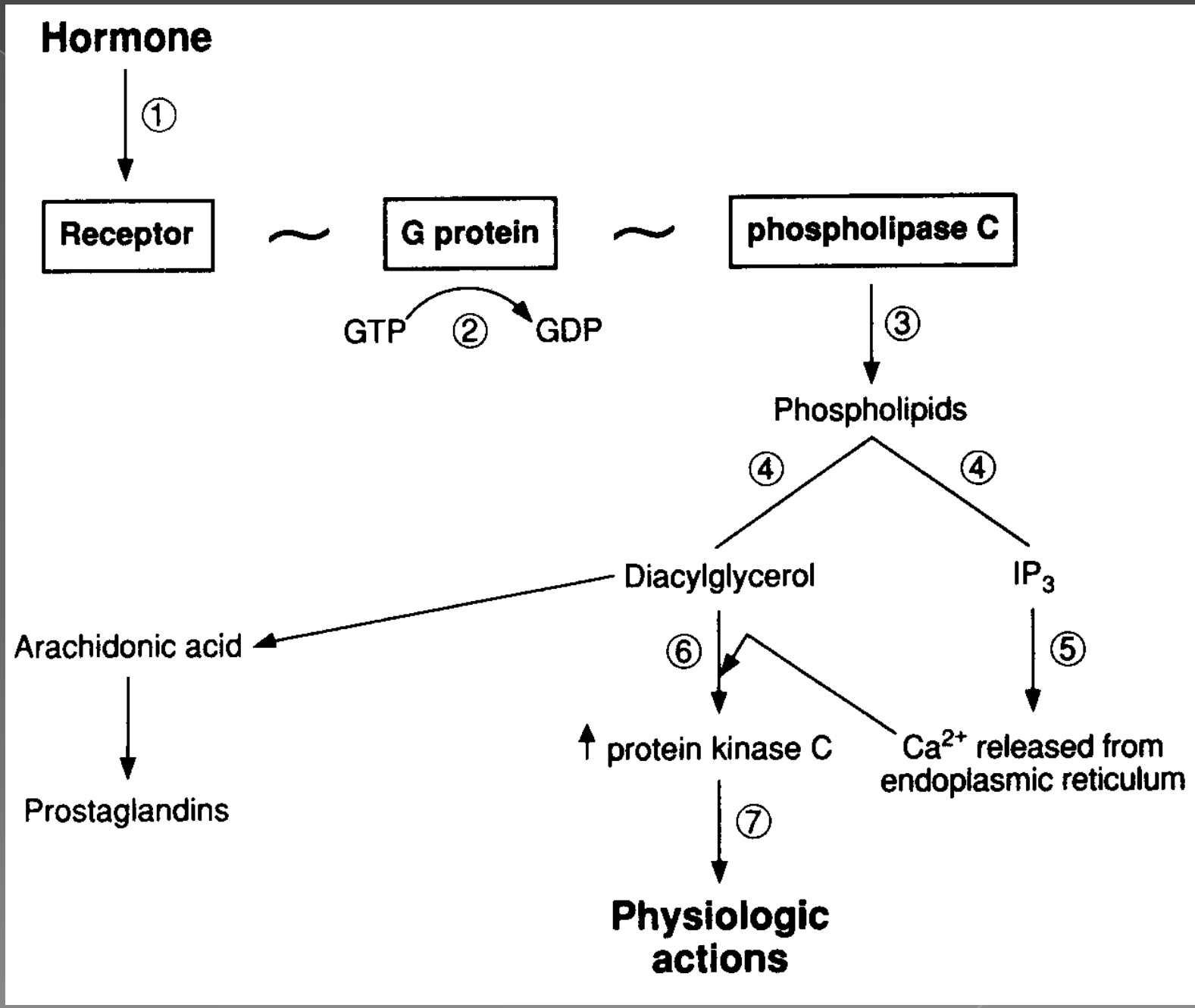
Table 75-3 Hormones That Use the Adenylyl Cyclase–cAMP Second Messenger System

Adrenocorticotropic hormone (ACTH)
Angiotensin II (epithelial cells)
Calcitonin
Catecholamines (β receptors)
Corticotropin-releasing hormone (CRH)
Follicle-stimulating hormone (FSH)
Glucagon
Growth hormone–releasing hormone (GHRH)
Human chorionic gonadotropin (hCG)
Luteinizing hormone (LH)
Parathyroid hormone (PTH)
Secretin
Somatostatin
Thyroid-stimulating hormone (TSH)
Vasopressin (V_2 receptor, epithelial cells)


Peptide hormone
Extracellular fluid
Receptor
G protein
Cell membrane
Phospholipase C
DAG + IP3
PIP2
Cytoplasm
Active protein kinase C
Inactive protein kinase C
Protein – PO4
Protein
Ca++
Endoplasmic reticulum
Cell's response
Cell's response


Hormone
①
Receptor
G protein
②
②
Mobilizes Ca^{2+} from intracellular stores
Opens Ca^{2+} channels in cell membrane
③
↑ $[Ca^{2+}]$
④
Ca^{2+}–calmodulin
⑤
Physiologic actions


Hormone
(1ry messenger)
2. Calcium-calmodulin system
Receptor
Active G protein
a
b
g
(2ry messenger)
Ca2+
ER
Ca2+
Ca2+
Ca2+
calmodulin
Change certain enzymes activity
Calcium-calmodulin complex


Hormone
①
Receptor
G protein
phospholipase C
GTP
②
GDP
③
Phospholipids
④
④
Diacylglycerol
IP3
Arachidonic acid
⑥
⑤
Prostaglandins
protein kinase C
Ca2+ released from endoplasmic reticulum
⑦
Physiologic actions

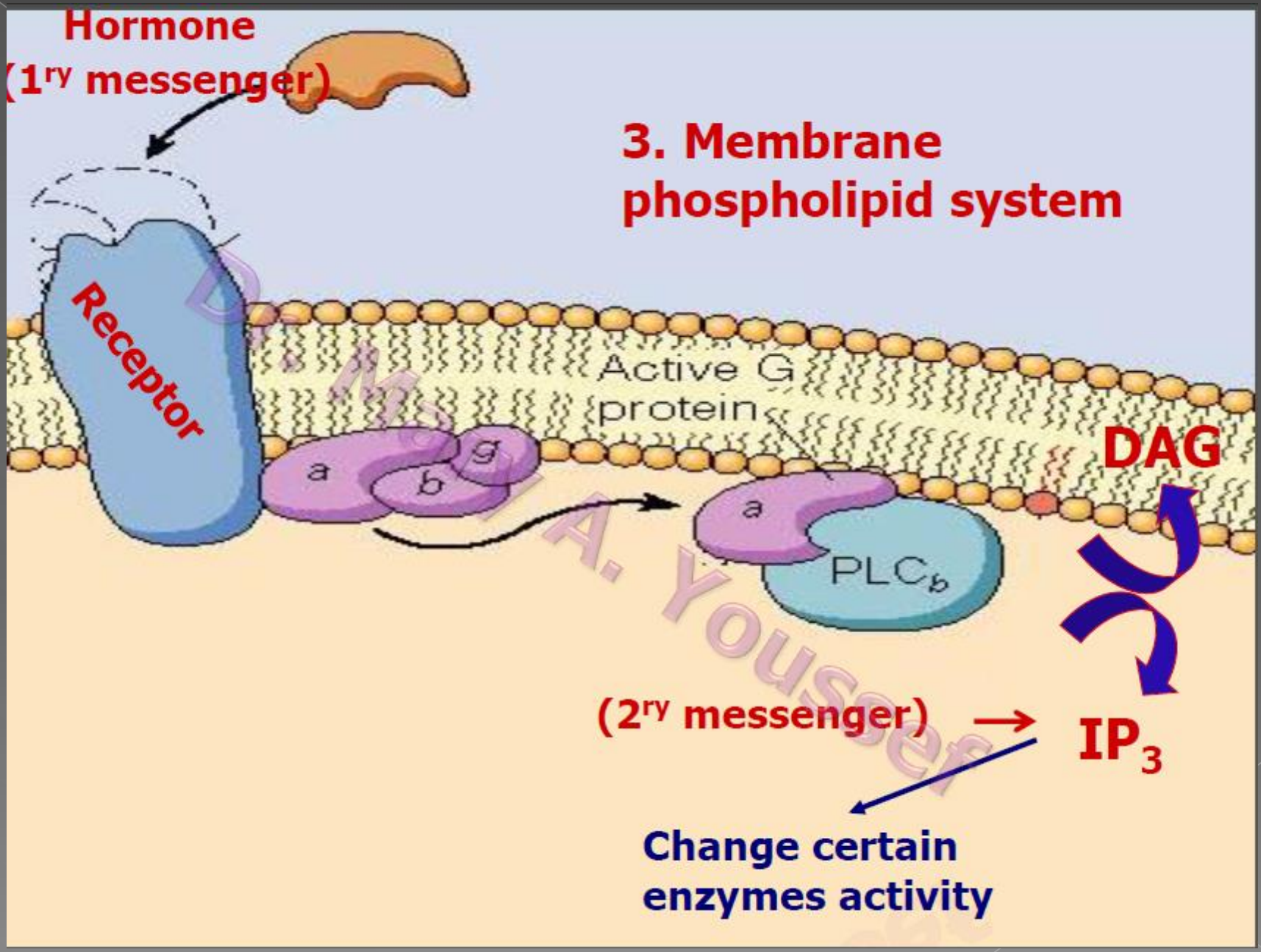

Hormone
(1ry messenger)
3. Membrane phospholipid system
Receptor
Active G protein
a
b
g
a
PLCb
DAG
(2ry messenger)
IP3
Change certain enzymes activity

Table 75-4 Hormones That Use the Phospholipase C Second Messenger System

Angiotensin II (vascular smooth muscle)
Catecholamines (α receptors)
Gonadotropin-releasing hormone (GnRH)
Growth hormone–releasing hormone (GHRH)
Parathyroid hormone (PTH)
Oxytocin
Thyrotropin-releasing hormone (TRH)
Vasopressin (V_1 receptor, vascular smooth muscle)

c. Enzyme linked hormone receptor

- Hormone + receptor → receptors act as enzymes or enzyme inside the cell membrane is activated → produce changes in cell function
- Cyclic AMP or cycle guanosine monophosphate (cGMP) is involved as a second messenger in
- enzyme linked hormone receptor system.
- **Examples**
- insulin
- leptin

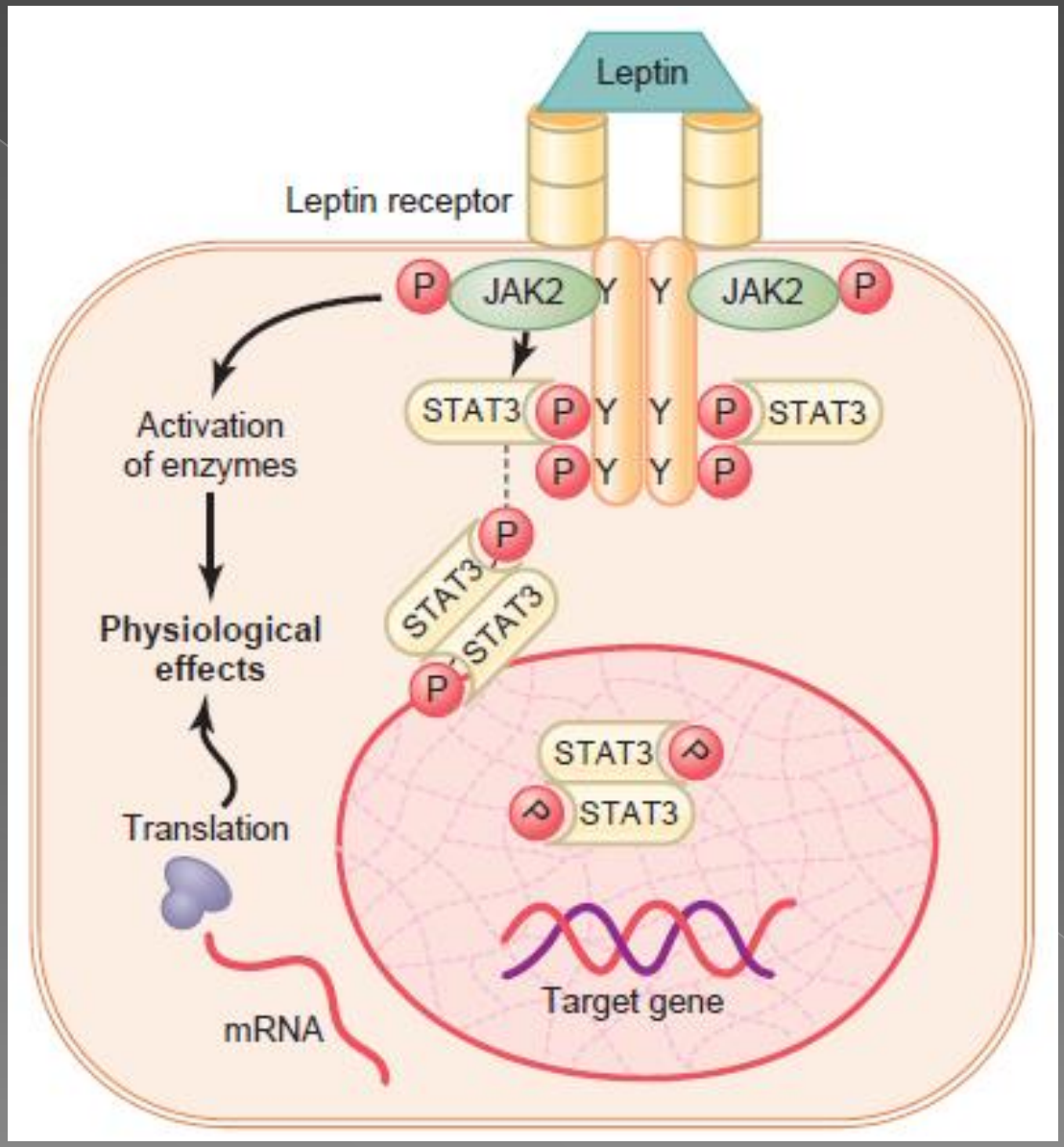
- ***leptin receptor***
- Leptin is a hormone secreted by fat cells and has many physiological effects, but it is especially important in regulating appetite and energy balance
- The leptin receptor is a member of a large family of cytokine receptors that do not themselves contain enzymatic activity but signal through associated enzymes.
- In the case of the leptin receptor, one of the signaling pathways occurs through a tyrosine kinase of the janus kinase (JAK) family, JAK2.

- The leptin receptor exists as a dimer (i.e., in two parts),
- binding of leptin to the extracellular part of the receptor alters its conformation,
- phosphorylation and activation of the intracellular associated JAK2 molecules.
- The activated JAK2 molecules then phosphorylate other tyrosine residues within the leptin receptor-JAK2 complex to mediate intracellular signaling.

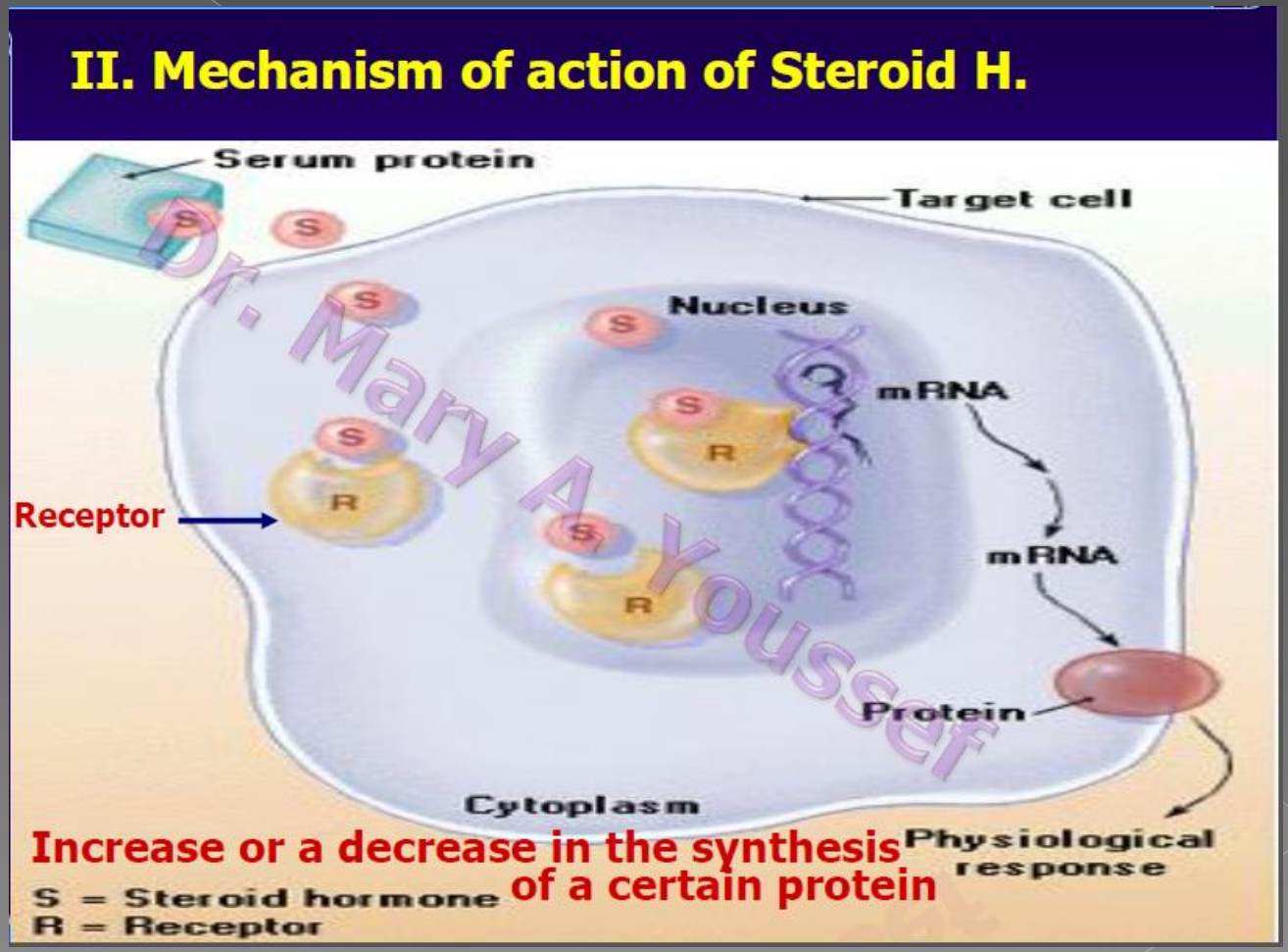
- The intracellular signals include phosphorylation of signal transducer and activator of transcription (STAT) proteins, which activates transcription by leptin target genes to initiate protein synthesis.

- Phosphorylation of JAK2 also leads to activation of other intracellular enzyme pathways such as mitogen-activated protein kinases (MAPK) and phosphatidylinositol 3-kinase (PI3K).

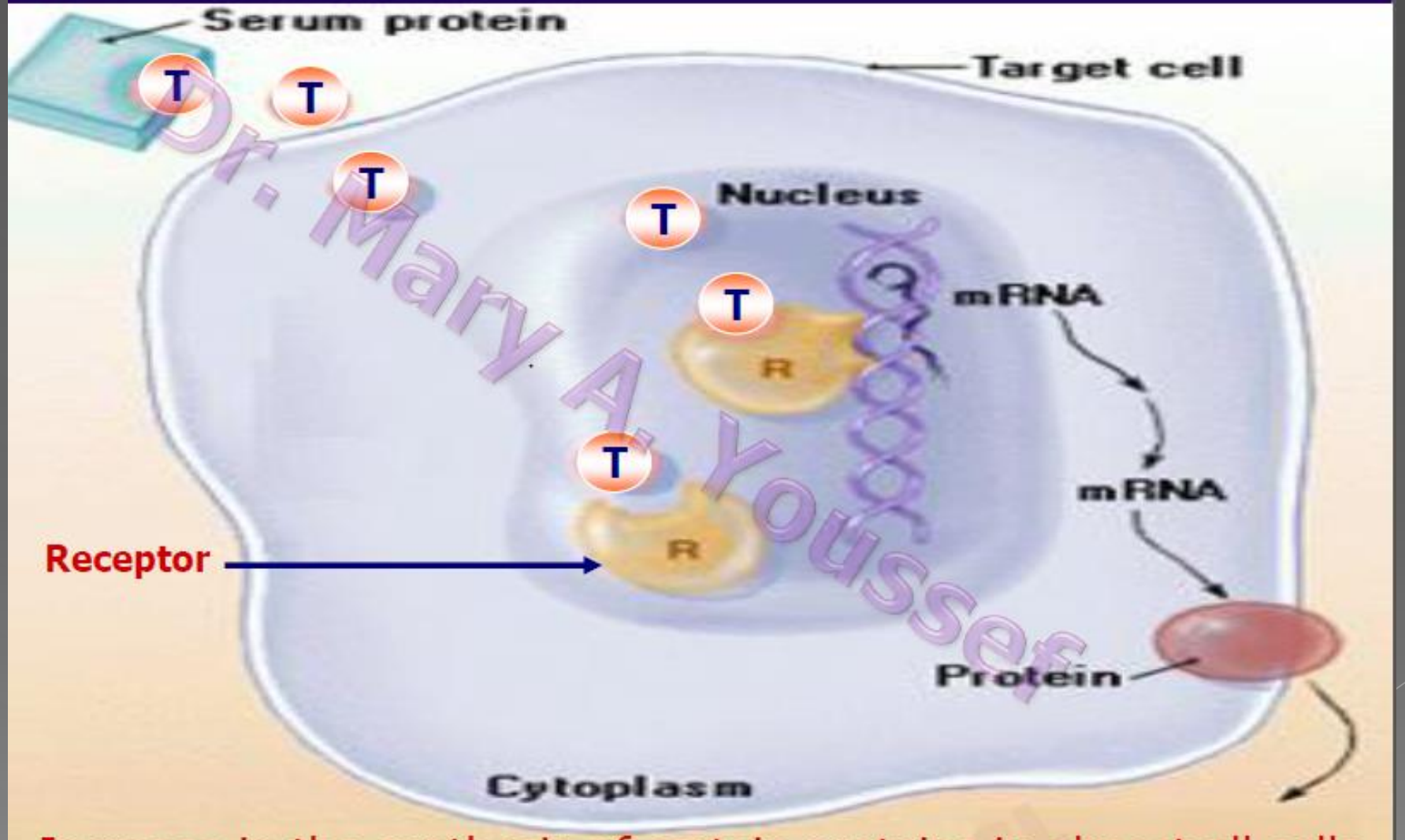
- Some of the effects of leptin occur rapidly as a result of activation of these intracellular enzymes, whereas other actions occur more slowly and require synthesis of new proteins.


Leptin
Leptin receptor
P
JAK2
Y
Y
JAK2
P
Activation of enzymes
STAT3
P
Y
Y
P
STAT3
P
Y
Y
P
Physiological effects
P
STAT3
STAT3
P
STAT3
P
P
STAT3
Translation
mRNA
Target gene

Intracellular Hormone Receptors and Activation of Genes



III. Mechanism of action of Thyroid H.



Increase in the synthesis of certain proteins in almost all cells

“Clearance” of Hormones From the Blood.

Two factors can increase or decrease the concentration of a hormone in the blood.

- One of these is the rate of hormone secretion into the blood.
- The second is the rate of removal of the hormone from the blood, which is called the metabolic clearance rate. This is usually expressed in terms of the number of milliliters of plasma cleared of the hormone per minute.

- To calculate this clearance rate, one measures the
- 1. rate of disappearance of the hormone from the plasma (e.g., nanograms per minute) and
- 2. the plasma concentration of the hormone (e.g., nanograms per milliliter of plasma).

- the metabolic clearance rate is calculated with use of the following formula:

- Metabolic clearance rate=
- Rate of disappearance of hormone from the plasma/Concentration of hormone

- A purified solution of the hormone to be measured is tagged with a radioactive substance.

- Then the radioactive hormone is infused at a constant rate into the blood stream until the radioactive concentration in the plasma becomes steady. At this time, the rate of disappearance of the radioactive hormone from the plasma equals the rate at which it is infused, which gives one the rate of disappearance.

- At the same time, the plasma concentration of the radioactive hormone is measured using a standard radioactive counting procedure. Then, using the formula just cited, the metabolic clearance rate is calculated.

Hormones are "cleared" from the plasma in several ways, including

- (1) metabolic destruction by the tissues,
- (2) binding with the tissues,
- (3) excretion by the liver into the bile, and
- (4) excretion by the kidneys into the urine.

a decreased metabolic clearance rate may cause an excessively high concentration of the hormone in the circulating body fluids.

this occurs for several of the steroid hormones when the liver is diseased because these hormones are conjugated mainly in the liver and then "cleared" into the bile.

- Hormones are sometimes degraded at their target cells by enzymatic processes that cause **endocytosis** of the cell membrane hormone-receptor complex*

- Most of the peptide hormones and catecholamines are water soluble and circulate freely in the blood. They are usually degraded by **enzymes in the blood and tissues** and rapidly excreted by the **kidneys and liver**. For example, the half-life of angiotensin II circulating in the blood is less than a minute.

- Hormones that are bound to plasma proteins are cleared from the blood at much slower rates and may remain in the circulation for several hours or even days.

- The half-life of **adrenal steroids** in the circulation, for example, ranges between 20 and 100 minutes, whereas the half-life of the protein-bound **thyroid hormones** may be as long as 1 to 6 days.

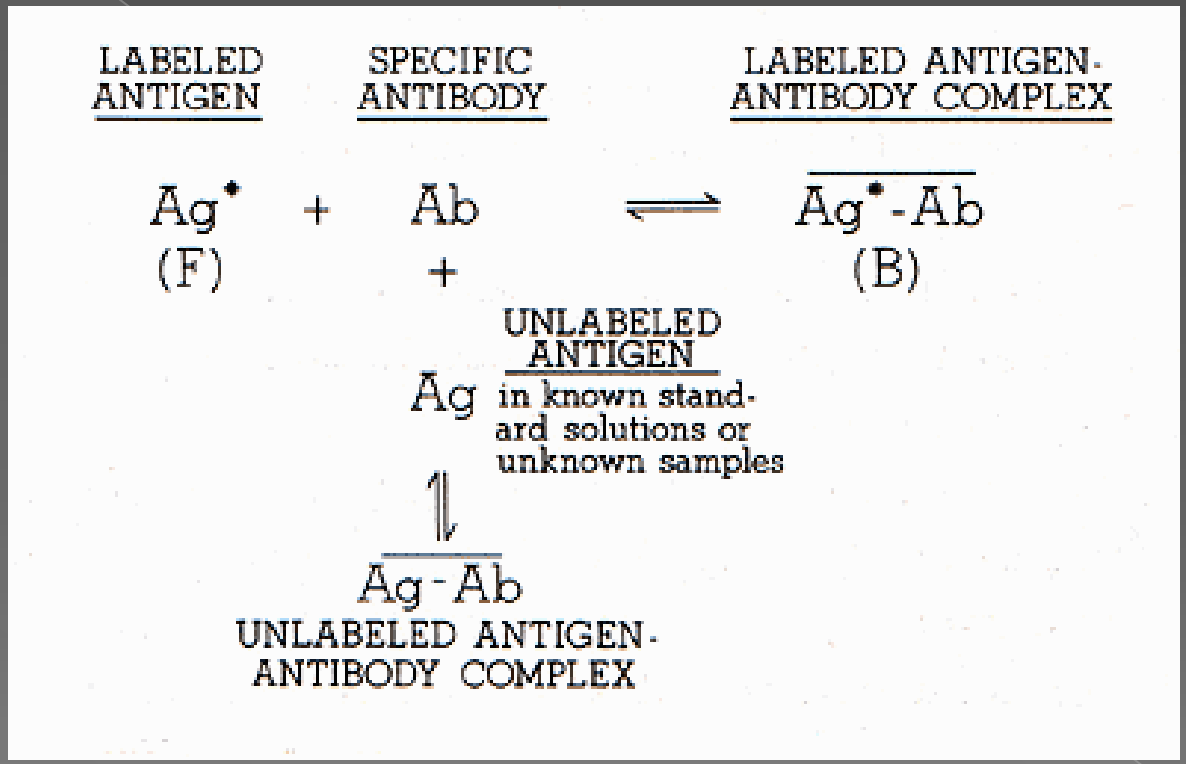
Measurement of Hormone Concentrations in the Blood

- Most hormones are present in the blood in extremely minute quantities; some concentrations are as low as one billionth of a milligram (1 picogram) per milliliter.

Radioimmunoassay

1) an antibody that is highly specific for the hormone to be measured is produced
2) Second, a small quantity of this antibody is mixed with a quantity of fluid from the animal containing the hormone to be measured
3) mixed simultaneously with an appropriate amount of purified standard hormone that has been tagged with a radioactive isotope. However, one specific condition must be met:

- There must be too little antibody to bind completely both the radioactively tagged hormone and the hormone in the fluid to be assayed.*


LABELED ANTIGEN
SPECIFIC ANTIBODY
LABELED ANTIGEN-ANTIBODY COMPLEX
Ag*
+
Ab
Ag*-Ab
(F)
+
(B)
UNLABELED ANTIGEN
Ag
in known standard solutions or unknown samples
Ag-Ab
UNLABELED ANTIGEN-ANTIBODY COMPLEX

- In the process of competing, the quantity of each of the two hormones, the natural and the radioactive, that binds is proportional to its concentration in the assay fluid.

- Third, after binding has reached equilibrium, the antibody-hormone complex is separated from the remainder of the solution, and the quantity of radioactive hormone bound in this complex is measured by radioactive counting techniques.

- If a large amount of radioactive hormone has bound with the antibody, it is clear that there was only a small amount of natural hormone to compete with the radioactive hormone, and therefore the concentration of the natural hormone in the assayed fluid was small.

- Conversely, if only a small amount of radioactive hormone has bound, it is clear that there was a large amount of natural hormone to compete for the binding sites.

- Fourth, to make the assay highly quantitative, the radioimmunoassay procedure is also performed for "standard" solutions of untagged hormone at several concentration levels. Then a "standard curve" is plotted,

- By comparing the radioactive counts recorded from the "unknown" assay procedures with the standard curve, one can determine within an error of 10 to 15 percent the concentration of the hormone in the "unknown" assayed fluid. As little as billionths or even trillionths of a gram of hormone can often be assayed in this way.

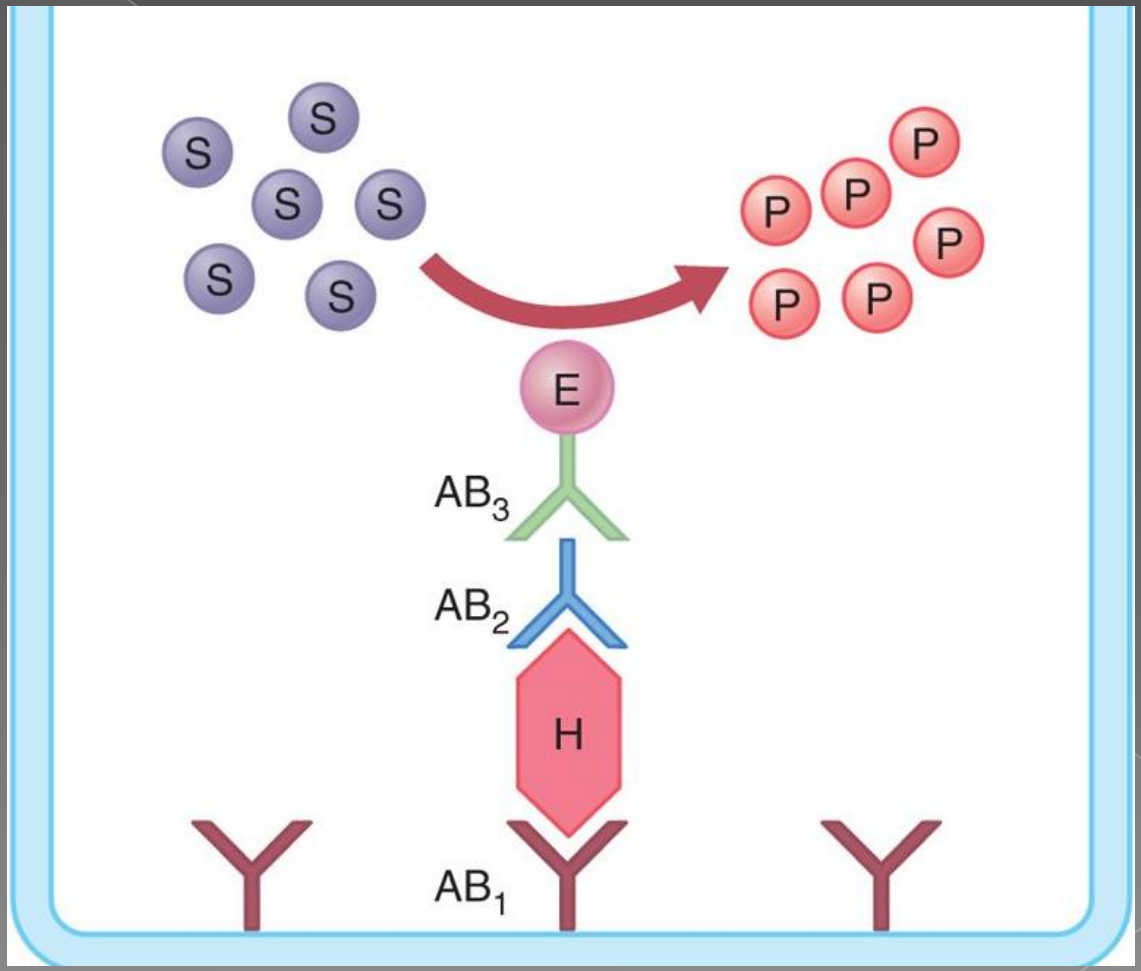
ELISA

- Enzyme-linked immunosorbent assays (ELISAs) can be used to measure almost any protein, including hormones. This test combines the specificity of antibodies with the sensitivity of simple enzyme assays.
- Each well is coated with an antibody (AB_1) that is specific for the hormone being assayed. Samples or standards are added to each of the wells, followed by a second antibody (AB_2) that is also specific for the hormone but binds to a different site of the hormone molecule.
- A third antibody (AB_3) that is added recognizes AB_2 and is coupled to an enzyme that converts a suitable substrate to a product that can be easily detected by colorimetric or fluorescent optical methods.

- Because each molecule of enzyme catalyzes the formation of many thousands of product molecules, even small amounts of hormone molecules can be detected.

- In contrast to competitive radioimmunoassay methods, ELISA methods use excess antibodies so that all hormone molecules are captured in antibody-hormone complexes.

- Therefore, the amount of hormone present in the sample or in the standard is proportional to the amount of product formed.


S
S
S
S
S
S
P
P
P
P
P
P
E
AB3
AB2
H
AB1

The ELISA method has become widely used in clinical laboratories because

(1) it does not employ radioactive isotopes,

(2) much of the assay can be automated using 96-well plates, and

(3) it has proved to be a cost-effective and accurate method for assessing hormone levels.