PHYSIOLOGY TEAM 435

جامعة الملك سعود
King Saud University

Very important

Extra information

Physiology Practical

Objectives :

1. To measure the volumes and determine the compositions of urine excreted by volunteers who have:
    a. Taken no fluids for several hours (fasting or control subjects).
    b. Drunken 1 liter of water.
    c. Drunken 1 liter of isotonic saline.
    d. Taken 1 tablet of Lasix 40 mg (a diuretic drug).

2. To be able to discuss the mechanisms by which the body:
    a. Conserves fluids and sodium if neither food nor water is taken.
    b. Excretes more water when extracellular fluids are diluted by the ingestion of water.
    c. Slowly eliminates sodium and water when the extracellular fluid volume is increased without altering its osmolality.
    d. Excretes large quantities of both water and sodium after taking Lasix tablet.

Contact us : Physiology435@gmail.com

OVERVIEW

METHODS AND OBSERVATIONS:

Several students have volunteered to take an active part in this practical class. None of them have taken fluids or food after 8:00 am.

- They are divided into 4 groups :

1) **Group 1 :** Not given any water or solution or drugs
2) **Group 2 :** Drank 1 liter of water
3) **Group 3 :** Drank 1 liter of 0.9% saline (isotonic saline)
4) **Group 4 :** Swallowed a Lasix (Furosemide) tablet 40 mg with the help of 25 ml of water.

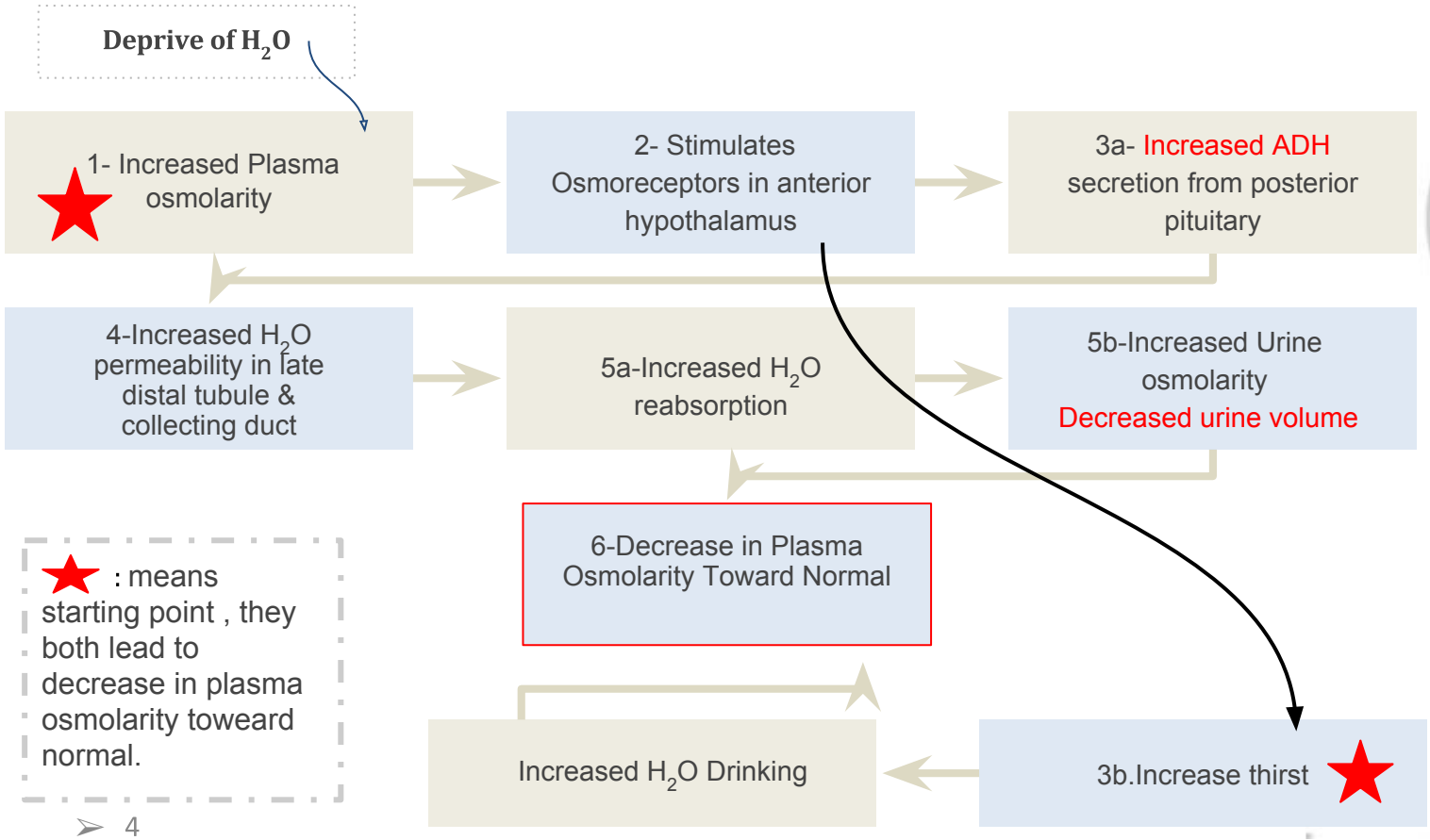
GROUP 1

1-Emptied their bladders at 8:00 am and discarded the urine.

2-From 8:00 they are restricted to take any fluids and they are asked to provide various urine samples for analysis at: 10:00 am, 12:00 noon, 2:00 pm and 3:00 pm.

What will happen?

Subsequent urine sample is lesser in volume and darker yellow in color that shows the kidneys try to conserve water in fasting state.



EXTRA

- **GROUP 1 (control group):**

Fasting all the night, he evacuate his bladder at the morning so he will give us the first sample that indicates the collection of urine overnight. We won't use this sample (discarded) . After one hour, we ask him to give another urine
Sample. Another 3 samples are taken for the next 3 hours.

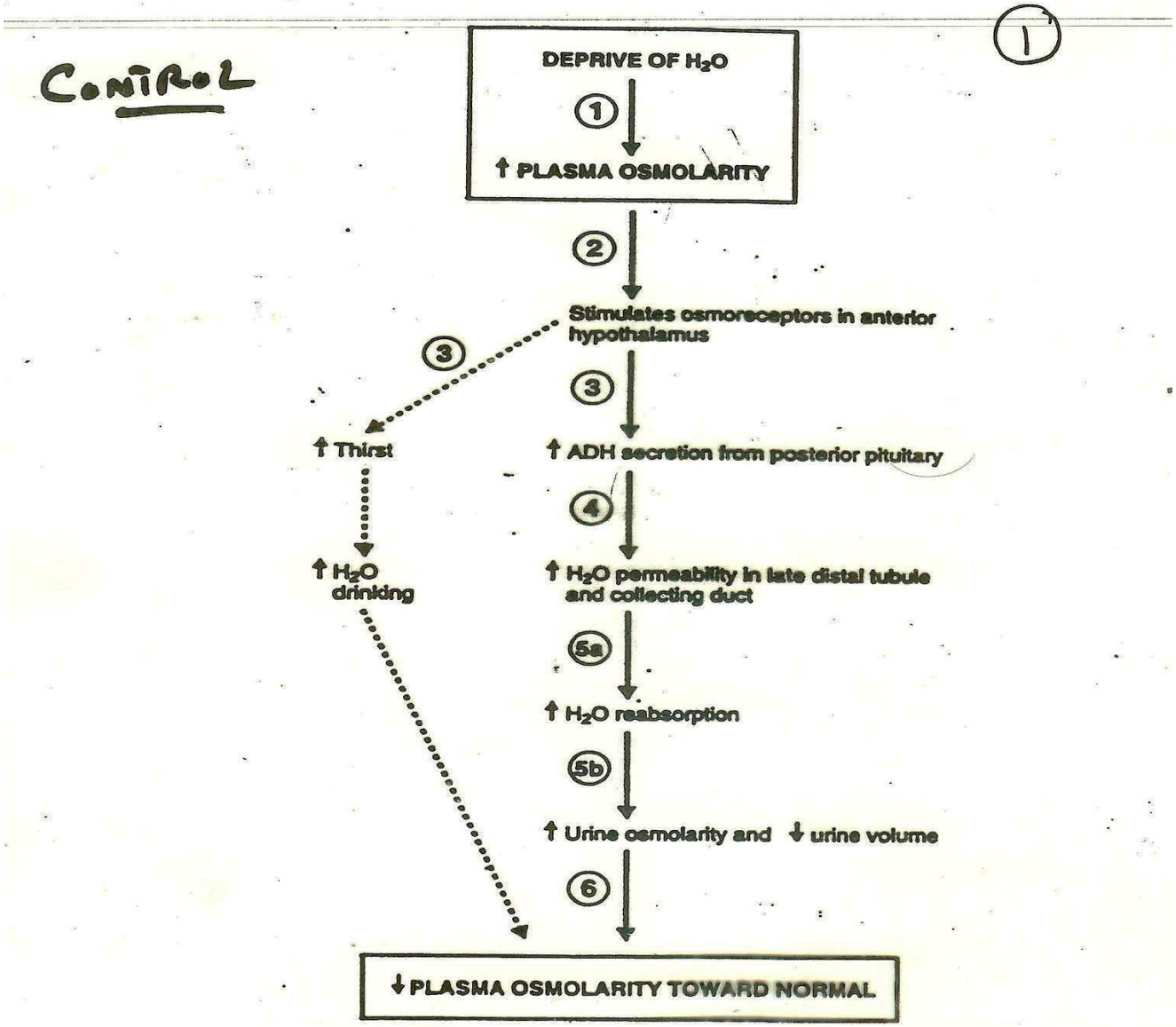
"not given any water or solution or drugs"

***Mechanism :**

First, the osmolarity of plasma will be increased ⟶ stimulation of osmoreceptors (located in hypothalamus) ⟶ stimulation of **ADH** (Secreted from posterior pituitary gland) increase water reabsorption ⟶ Decreased urine volume ⟶ Increased urine osmolarity Decreased plasma osmolarity.

In brief : Urine volume ↓ / Urine osmolarity ↑ / Plasma osmolarity ↓

summary of group 1



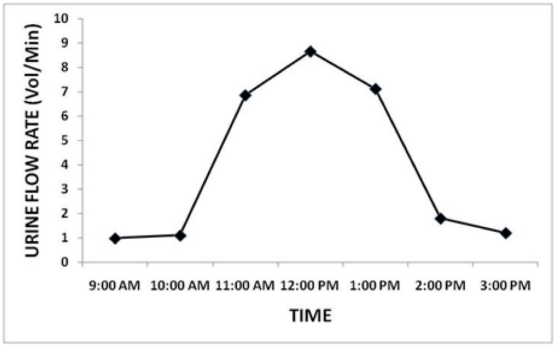
GROUP 2

1-Emptied their bladder at 8:00 am and discarded the urine

2-At 10:00 am emptied their bladder again, but this time they measured its volume and provided a sample for analysis. This sample will be pre-experimental sample

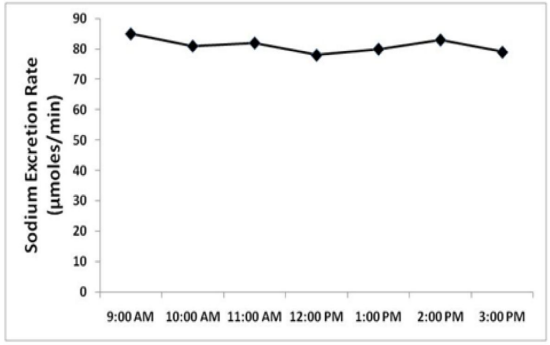
3- Drank 1 liter of water immediately after providing the pre-experimental sample.

4-Were then asked to empty their bladders and provide post-experimental samples every half an hour after drinking water until 3:00 pm. **you may wonder** why there is post-experimental point every hour instead of half an hour? it's because in the graph not all of the points appeared .



Urine flow rate

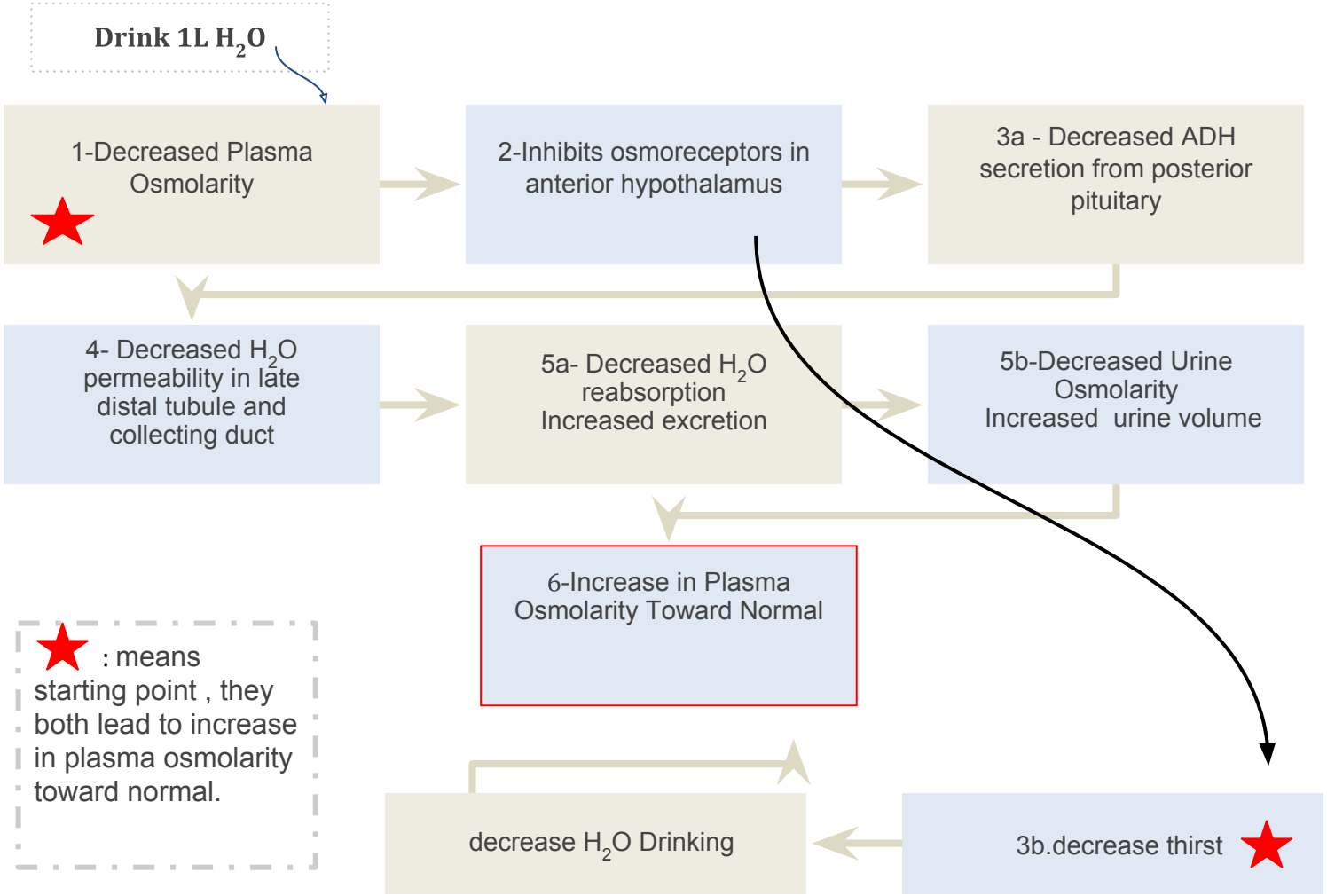
Urine volume will be about **the same** in the first post-experimental sample as of the pre- experimental sample, then will increase dramatically in the subsequent samples and will again decrease back to the level of pre- experimental sample in the last samples. It means that the healthy kidneys get rid of this 1 liter of water ingested by these volunteers in 3 hours and the mechanism starts after 30 minutes, as shown by the graph.



sodium excretion rate

Sodium concentration will remain constant

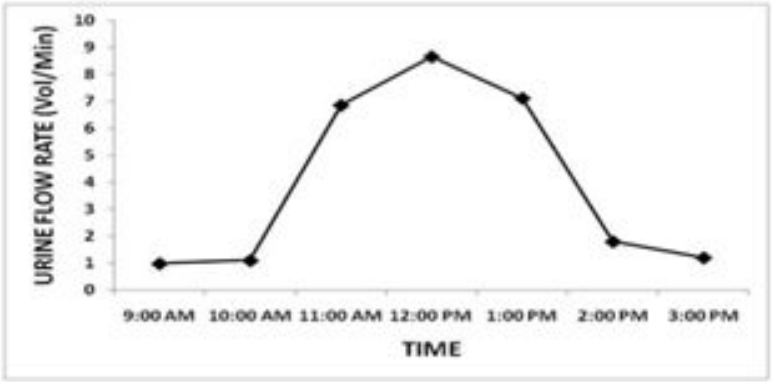
GROUP 2


Drink 1L H_2O
1-Decreased Plasma Osmolarity
2-Inhibits osmoreceptors in anterior hypothalamus
3a - Decreased ADH secretion from posterior pituitary
4- Decreased H_2O permeability in late distal tubule and collecting duct
5a- Decreased H_2O reabsorption Increased excretion
5b-Decreased Urine Osmolarity Increased urine volume
6-Increase in Plasma Osmolarity Toward Normal
: means starting point , they both lead to increase in plasma osmolarity toward normal.
decrease H_2O Drinking
3b.decrease thirst

EXTRA

- **GROUP 2 :**

Fasting overnight, he evacuate his bladder at 8 AM so he will give us the first sample that indicates the collection of urine overnight. We won't use this sample (discarded). At 10 AM, we ask him to give a *pre-experimental* sample (before the test) after that, we give him 1 liter of water then we take a new sample each hour.

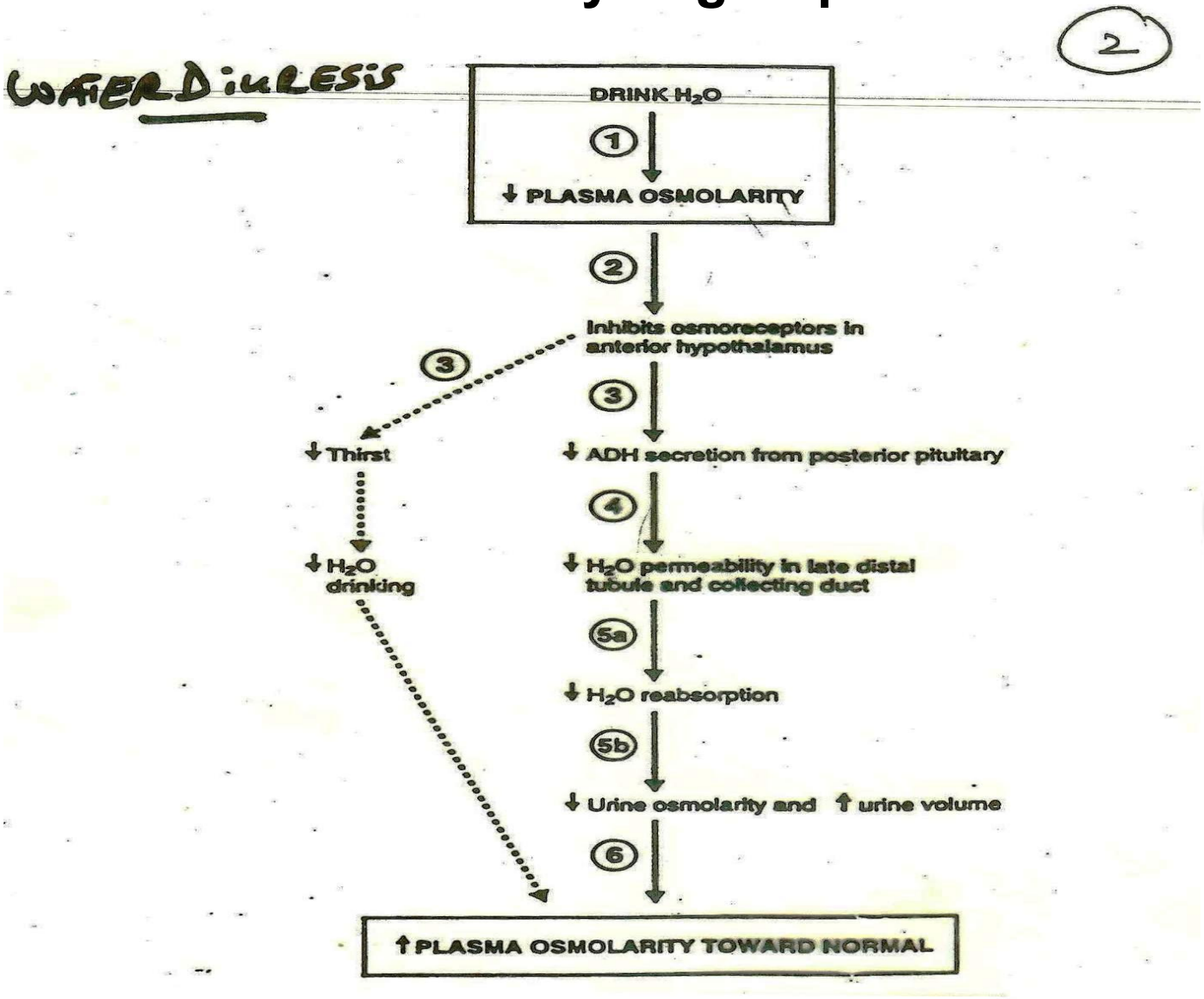


Urine volume increases gradually. Finally, it return back to its normal value which means that kidney can get rid of that liter of water within 4 hours.

***Mechanism :**

After drinking of water, plasma osmolarity will be decreased → inhibition of osmoreceptors
Decreased ADH → Decreased permeability of water → Increased Urine volume → Decreased Urine osmolarity → Increased plasma osmolarity.

summary of group 2



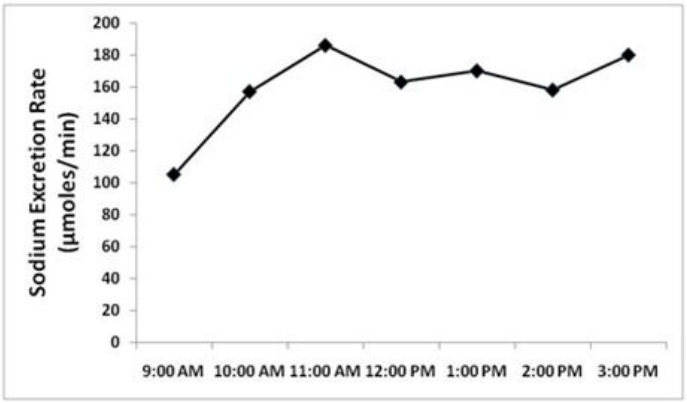
GROUP 3 [1L of Saline]

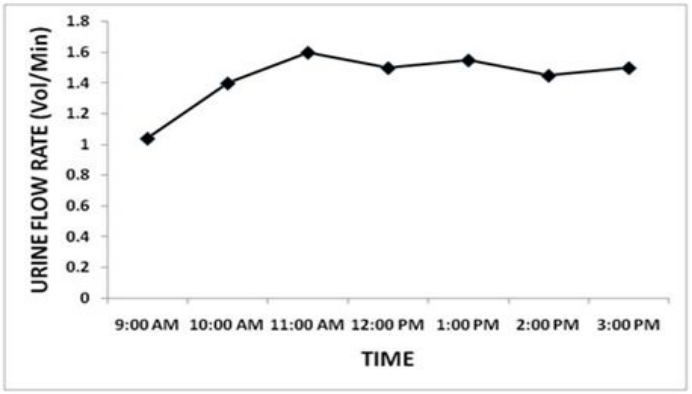
1-Emptied their bladders at 7:00 am and discarded the urine.

2-At 9:00 am emptied their bladder again, but this time they measured its volume and provided a sample for analysis. This sample will be pre-experimental sample.

3-Drank 1 liter of 0.9% saline (isotonic saline) immediately after providing the pre-experimental sample.

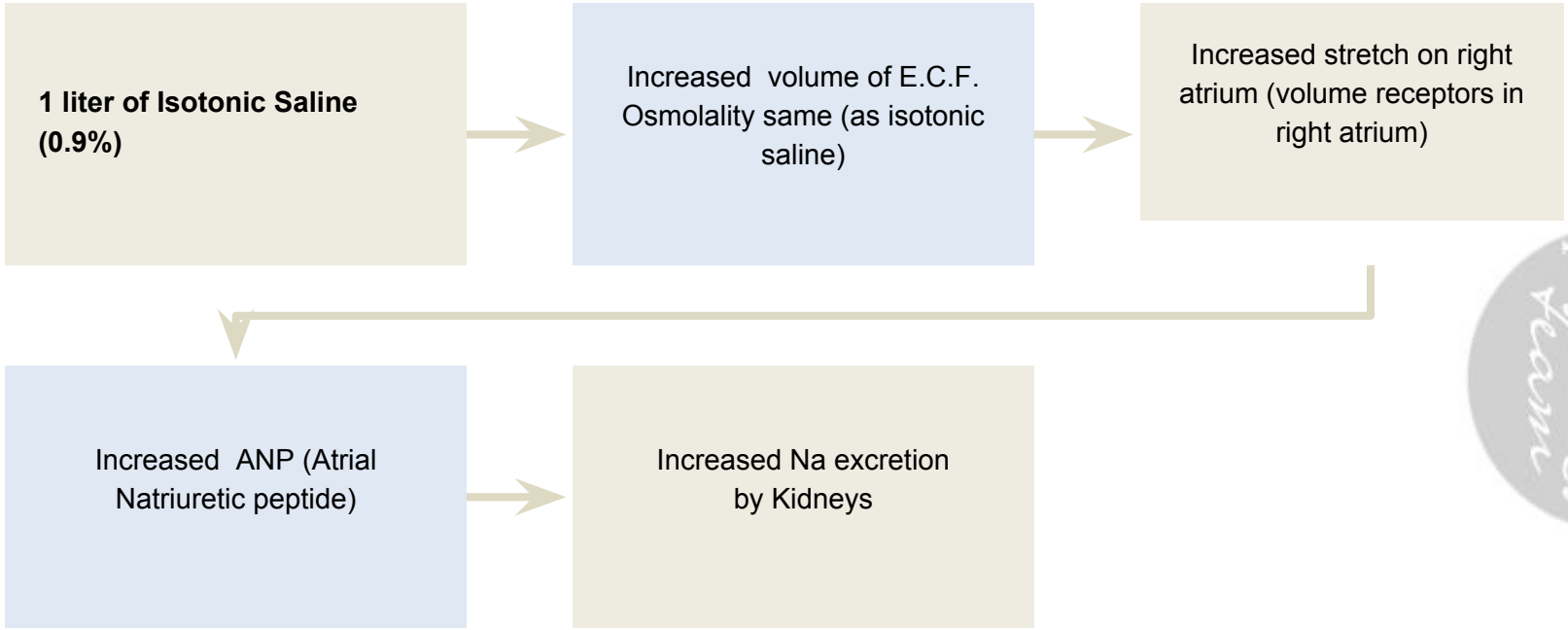
4-Were then asked to empty their bladders and provide post-experimental samples every hour after drinking saline until 3:00 pm.





We will find in these subjects that urine volume and osmolality will remain slightly increased in the post-experimental samples as compared to pre-experimental samples. It means that the kidneys are trying to get rid of this extra sodium chloride and water that has been ingested by these volunteers, but slowly. As a matter of fact, it will take 24 hours to excrete 1 liter of isotonic saline ingested by them. You can see the slightly increased urine volume and sodium excretion in the graphs.

GROUP 3 [1L of Saline]



what is Isotonic saline 0.9% ?

- Contains 154 mmol of NaCl, equivalent to 9 g of salt or 3.6 g of sodium.
- The sodium concentration of isotonic saline is equivalent to the normal sodium concentration of plasma water.

EXTRA

- **GROUP3 :**

They will drink 1 liter of saline (it contains the same conc. of plasma Na+)

Urine volume will be increased. After 4 hours, we will notice that the urine volume still increased! and it didn't return back to normal. In addition, sodium concentration will not return back to its normal value.

EXPLINATION :

the Na+ conc. in saline = Na+ conc. in plasma which will stimulates stretch receptors (in the right atrium) that will release ANP (Atrial natriuretic peptide) which will increase the blood flow to kidney thus increase the GFR (glomerular filtration rate)

→ increase the Na+ excretion by the kidney.

What is the difference between water and saline (group 2 & group 3) ?

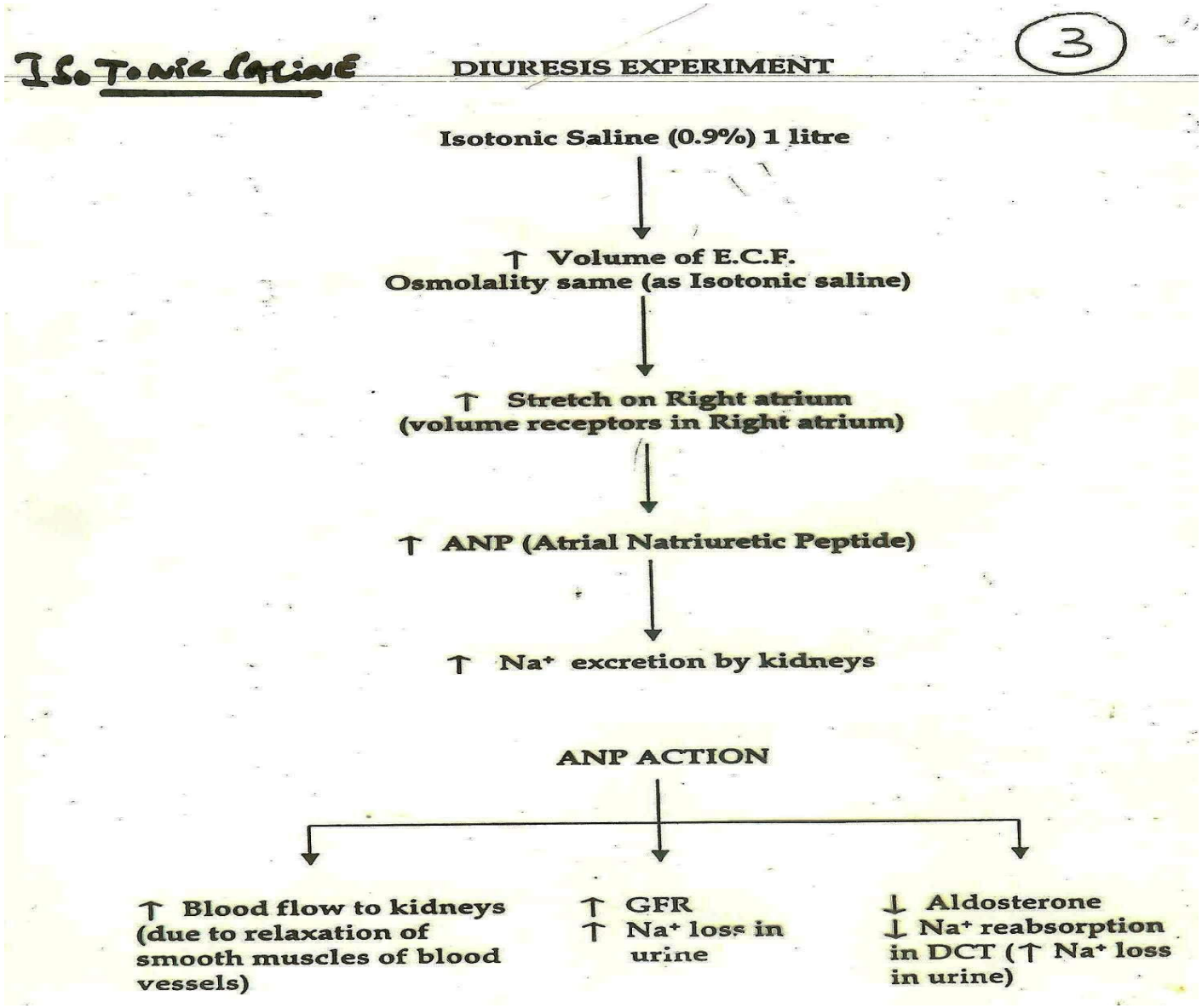
Both of them will increase the urine volume, the only difference is :

- Group 2 : urine volume will return back to its normal value.
- Group 3: urine volume will not return back to its normal value.

Sodium concentrations :

- Group 2 (water) : compensated.
- Group 3 (saline) : increased.

summary of group 3



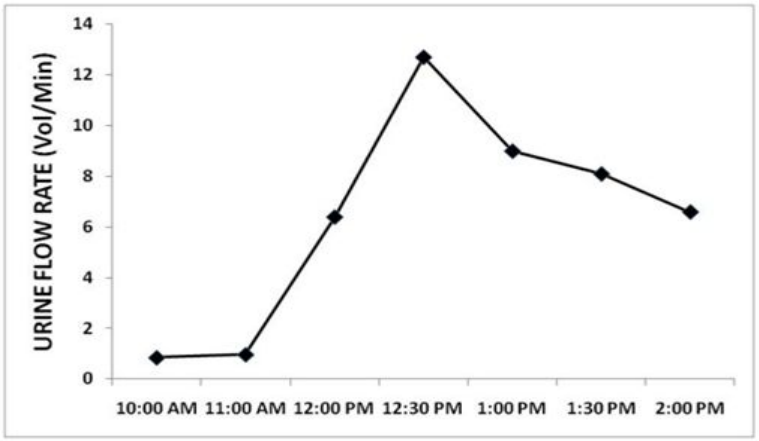
GROUP 4

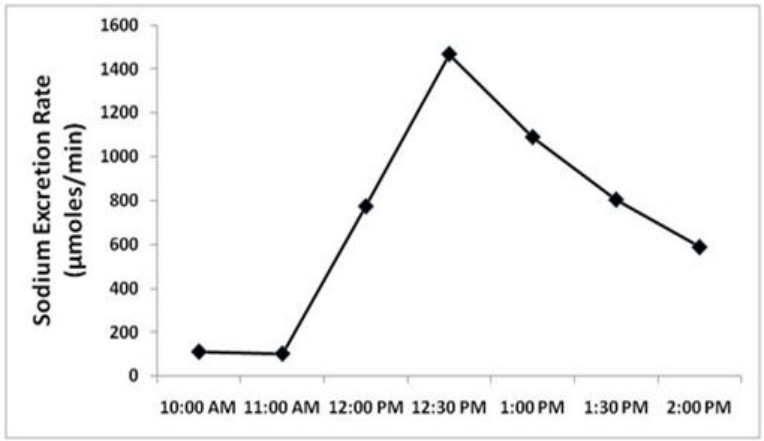
1–Emptied their bladder at 8:00 am and discarded the urine.

2–At 10:00 am emptied their bladder again, but this time they measured its volume and provided a sample for analysis. This sample will be pre-experimental sample.

3–Swallowed a Lasix (Furosemide) tablet 40 mg with the help of 25 ml of water immediately after providing the pre-experimental sample.

4–Were then asked to empty their bladders and provide post-experimental samples every hour after taking Lasix until 12:00 noon and then every half an hour until 3:00 pm.





We will find in these subjects that urine volume and osmolality dramatically increased after 1 hour of taking Lasix tablet and remained increased for further duration of experiment. What we know about the effect of Lasix is that it usually starts 1-2 hours after ingesting it and lasts for 4-6 hours. Because in this experiment both urine volume and osmolality will increase, this type of dieresis is called OSMOTIC DIURESIS, while the diuresis that occurred in those subjects who drank water is called WATER DIURESIS, as the increase in the urine volume was not accompanied by simultaneous increase in urine osmolality in those subjects. You can see the changes in urine volume and sodium excretion in these subjects taking Lasix tablet in the graphs.

GROUP 4

1 tablet of Lasix (furosemide) (40mg) with 25ml of water

→

Action starts 1-2 hours and lasts for 4-6 hours **(1/2 life of furosemide is 6hr)**

→

Acts **on thick ascending limb of loop of Henle** and blocks the Na-K-2Cl co-transport (called loop diuretic)

↓

Increased Na excretion in urine and water excretion (osmotic drug)

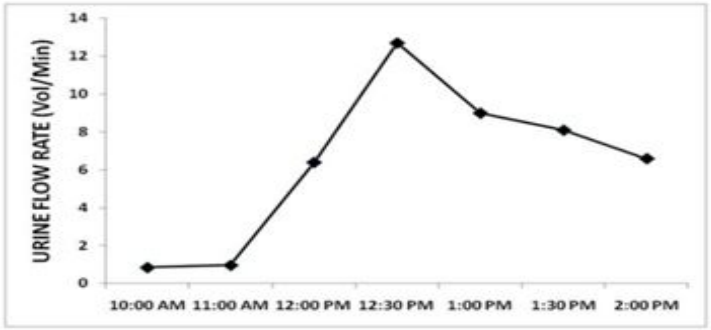
What is Lasix?

Furosemide (lasix) is a loop diureticused in the treatment of hypertension, congestive heart failure and edema it inhibits the sodium-potassium-chloride co-transport system located within the ascending limb of the Loop of Henle.

EXTRA

- **GROUP 4 :**

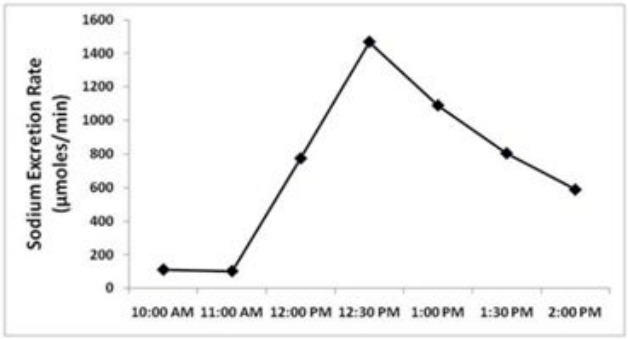
They will take Lasix . *Lasix (furosemide) is a loop diuretic (water pill) that prevents your body from absorbing too much salt, allowing the salt to instead be passed in your urine.*



Sharp or dramatic increase in urine volume

. Furosemide action start after 2 hours of administration

(the urine volume reach its maximum value) and its duration of action is 4-6 hours, so during this period the urine volume will not return back to normal.



Sharp or dramatic increase in sodium excretion.

It will not return back to its normal value until the end of duration of Furosemide.

summary of group 4

4

Lasix tab

DIURESIS EXPERIMENT

1 tab of Lasix (Furosemide)
(40mg) with 25ml of water

↓

Action starts after 1 – 2 hours and lasts for 4 – 6 hours

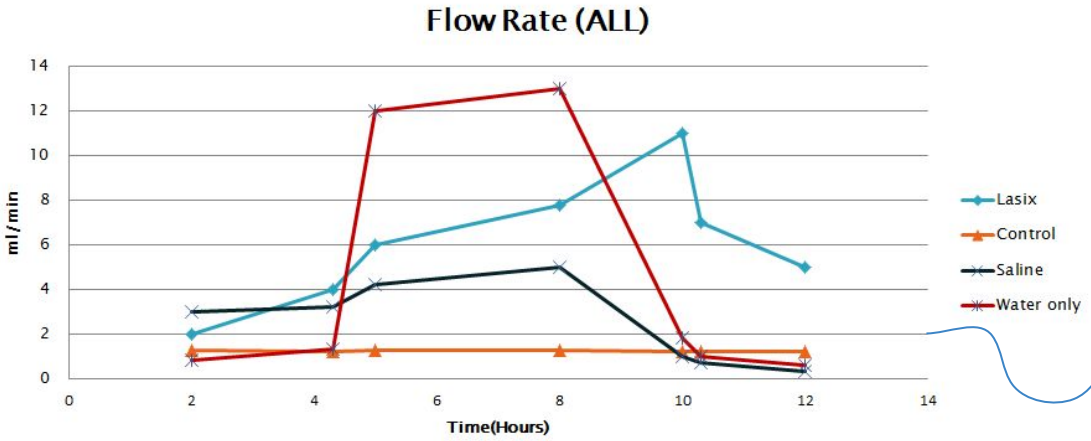
↓

Acts on thick ascending limb of loop of Henle and blocks the
$Na^+ - K^+ - 2Cl^-$ Co-transport (called loop diuretic)

↓

↑ Na^+ excretion in urine
↑ water excretion

GRAPHS



After 4 hours is the flow will be back to normal?
No, why? Because the action of diuretic works for 4-6 hours.

- The action start from 1-2 hours, and that's why after 2 hours we find the maximum increase in the urine volume.
- The volume might be back after the test if the patient doesn't take any fluid.

The Na+ excretion is the same the diuretic increase like the volume and it'll never back to the normal during the test.

- This situation called osmotic diuresis.

Urine sample examination

- Volume → measuring cylinder "1"
- Sodium and potassium concentration → flame photometry "2"
- PH → PH meter "3"
- Osmolality → Osmometer "4"

1

2

3

4

physiology team

Sodium excretion

Δ Total sodium excretion is obtained by applying following equation:

$$\text{Sodium excretion} = \frac{\text{Sodium concentration x Volume of urine}}{1000}$$

Δ Sodium excretion rate (that means divided on time) is obtained by applying the following equation:

$$\text{Sodium excretion rate} = \frac{\text{Sodium concentration x Volume of urine}}{\text{Time}}$$

EXTRA

- **When we get the sample we should look at :**

    1) Urine volume (By Measuring cylinder).
    2) Na+ and k+ concentration in the blood.
    3) PH.
    4) Osmolality.

- **PH meter :**

{ This part will be immersed in the urine sample, the result will be shown on the screen }.

- **Flame photometry :**

Closed digitalized system that measures sodium and potassium concentrations. (same as the blood test)

- **Osmometer :**

To measure urine osmolality.

{ This part will pull the urine sample }

*Note : we have to do the 4 measurements for *each sample* .

GROUP 2

SAMPLE NO.	1	2	3	4	5	6	7
COLLECTION TIME (minutes)	120	30	30	30	30	30	30
VOLUME OF URINE (ml)	118	33	200	280	240	60	50
URINE FLOW RATE (ml / min)	0.98	1.1	6.66	9.33	8	2	1.66
SODIUM CONCENTRATION (mmoles/liter)	87	65	12	10	8	30	40
TOTAL SODIUM EXCRETION (mmoles)	10.3	2.2	2.4	2.8	1.9	1.8	2.00
SODIUM EXCRETION RATE (µmoles/min)	85.6	71.5	80	93.3	64	60	66.7

Measured by Flame Phtometery

$$\text{Sodium excretion} = \frac{\text{Sodium concentration} \times \text{Volume of urine}}{1000}$$

$$\text{Sodium excretion rate} = \frac{\text{Sodium concentration} \times \text{Volume of urine}}{\text{Time}}$$

GROUP 3

$$\text{Sodium excretion rate} = \frac{\text{Sodium concentration x Volume of urine}}{\text{Time}}$$

$$\text{Sodium excretion} = \frac{\text{Sodium concentration x Volume of urine}}{1000}$$

SAMPLE NO.	1	2	3	4	5	6	7
COLLECTION TIME (minutes)	120	30	30	30	30	30	30
VOLUME OF URINE (ml)	125	39	50	42	47	32	45
URINE FLOW RATE (ml / min)	1.04	1.30	1.67	1.40	1.57	1.07	1.50
SODIUM CONCENTRATION (mmoles/liter)	101	98	112	109	120	137	127
TOTAL SODIUM EXCRETION (mmoles)	12.6	3.8	5.6	4.6	5.6	4.4	5.7
SODIUM EXCRETION RATE (µmoles/min)	105.2	127.4	186.7	152.6	188.0	146.1	190.5

GROUP 4

Example

Let's take **Sample No.2 in group 4** as an example:

Sodium Excretion Rate = 107 x 0.97 = 103 µmoles/min

Sodium Excretion = 107 x 58 / 1000 = 6.2 mmoles

SAMPLE NO.	1	2	3	4	5	6
COLLECTION TIME (minutes)	120	60	42	18	30	30
VOLUME OF URINE (ml)	102	58	269	230	270	125
URINE FLOW RATE (ml / min)	0.85	0.97	6.4	12.7	9.0	4.2
SODIUM CONCENTRATION (mmoles/liter)	132	107	121	115	121	117
TOTAL SODIUM EXCRETION (mmoles)	13.5	6.2	32.5	26.4	32.6	14.6
SODIUM EXCRETION RATE (µmoles/min)	112.2	103	774	1467	1089	487.5

VERY IMPORTANT!!

Glomerular Filtration Rate (GFR)

Measure something according to a time unit.

Definition:

It is the volume of fluid filtered **from** the renal glomerular capillaries **into** the Bowman's capsule per unit time.

$$GFR = \frac{\text{Urine Concentration X Urine Flow}}{\text{Plasma Concentration}}$$

Normal result:

GFR ranges from **90** to **120** ml/min/1.73 m^2

Abnormal result:

a) Chronic kidney disease

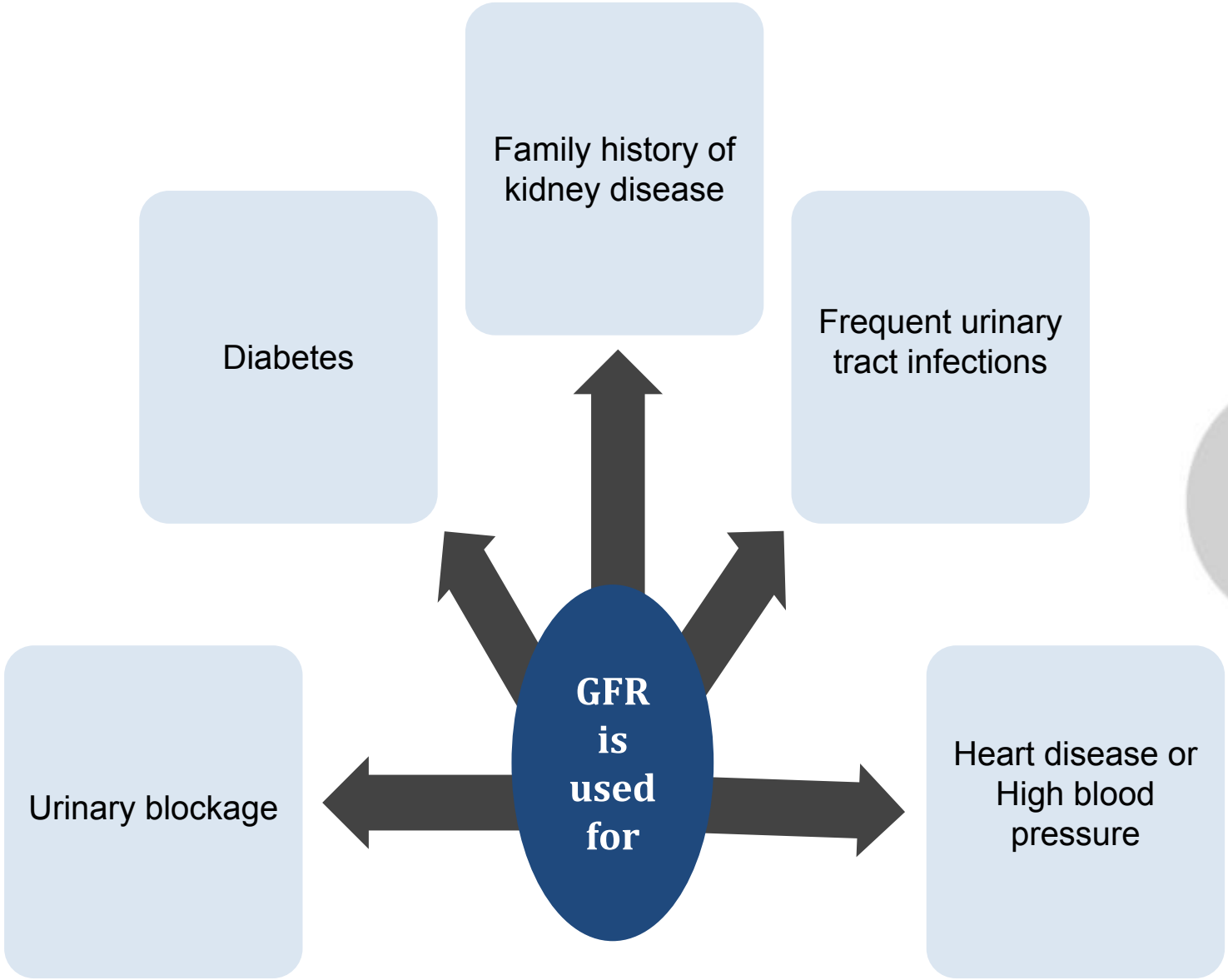
GFR **<** **60** mL/min/1.73 m^2 for 3 or more months

b) kidney failure

GFR **<** **15** mL/min/1.73 m^2

- **NOTE :**

According to the National Kidney Foundation, normal results range from 90 - 120 ml/min/1.73 m^2 . Less than 60 indicates chronic kidney disease, less than 15 indicates renal failure


Family history of kidney disease
Diabetes
Frequent urinary tract infections
GFR is used for
Urinary blockage
Heart disease or High blood pressure

Creatinine Clearance (C_{Cr})

Definition:

The volume of blood plasma that is cleared of creatinine per unit time.

$$C_{Cr} = \frac{U_{cr} \times V}{P_{Cr}}$$

U_{Cr} → Creatinine concentration in the collected urine

V → Urine flow rate

P_{Cr} → Plasma concentration

Normal values:

Male: 97 to 137 ml/min.

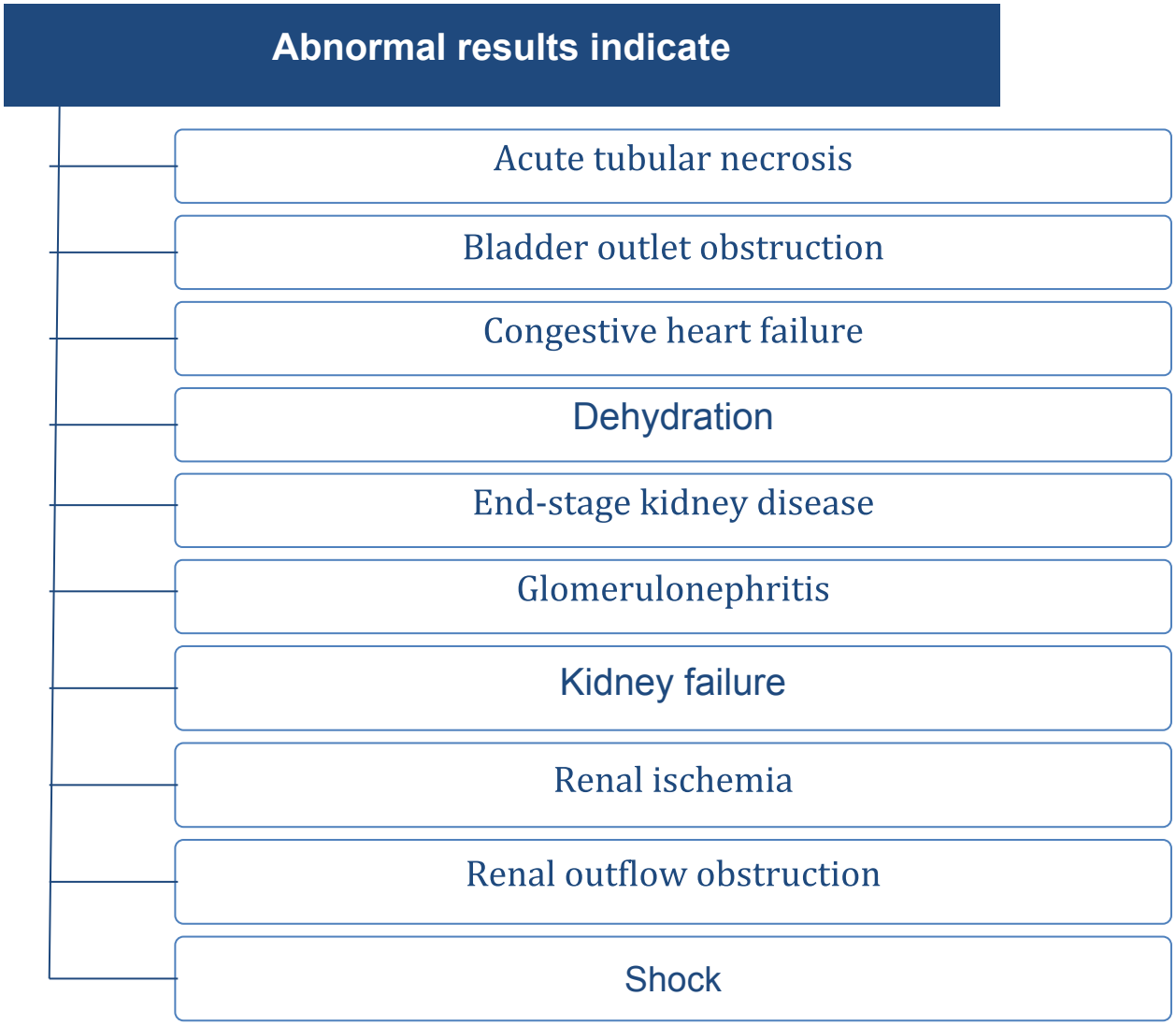
Female: 88 to 128 ml/min.

Example:

A person has a plasma creatinine concentration of 0.01 mg/ml and in 1 hour produces 60 ml of urine with a creatinine concentration of 1.25 mg/mL.

$$C_{Cr} = \frac{1.25mg/mL \times \frac{60mL}{60min}}{0.01mg/mL} = \frac{1.25mg/mL \times 1mL/min}{0.01mg/mL} = \frac{1.25mg/min}{0.01mg/mL} = 125mL/min$$

Creatinine Clearance (C_{Cr})



important

نقاط مهمة تأكدوا من إلمامكم بها :

- في كل قروب: لازم تعرفون أشكال graphs و أبرز الصفات فيها , كما هو موضح سابقا.
- ركزوا جيدًا على mechanisms (الشرح المفصل).
- التركيز على القوانين و طريقة حسابها.
- Normal Vs. Abnormal ranges, and the related diseases.

Physiology Leader:

لُجين السواط

Members :

- خولة العماري
- منيرة السلولي
- نورة القحطاني
- منيرة العُمري
- ديما الراجحي
- سارة العنزي
- العنودالسلمان

اللّهم انفعنا بما علمتنا و زدنا علمًا