KIDNEY PRACTICALS
1. DIURESIS – PART 1

OBJECTIVES:

1. To measure the volumes and determine the compositions of urine excreted by volunteers who have drunk:-
   a) no fluids for several hours
   b) a litre of water
   c) a litre of isotonic saline
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.

METHODS:

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

A. THOSE ACTING AS CONTROLS (taking no fluids)
   i. emptied their bladders at 8:00AM and discarded the urine they produced
   ii. at 10:00AM, 12:00 noon, 2:00PM and 3:00PM measured the volumes of urine they could produce and provided a sample for analysis

B. THOSE TAKING WATER
   i. emptied their bladders at 10:00AM and discarded the urine they produced
   ii. at 12:00 noon again emptied their bladders but this time they measured its volume and took a sample for analysis
   iii. drank one litre of water immediately after providing their urine samples
   iv. were asked to empty their bladders and provide samples every half hour after drinking water until 3:00PM

C. THOSE TAKING SALINE
   i. emptied their bladders at 8:00AM and discarded the urine they produced
   ii. collected all the urine volume that they could produce at 9:00AM and kept a sample for analysis
   iii. drank one litre of 0.9% saline (i.e. 154mM or isotonic) immediately after the 9:00AM sample
   iv. were asked to empty their bladders and provide samples every hour after drinking saline until 3:00PM

URINE WILL BE ANALYZED TO DETERMINE ITS:-

a) volume, simply by using a measuring cylinder
b) $[Na^+]$ and $[K^+]$ by flame photometry
c) $[H^+]$ using a pH meter
d) osmolality. The osmolality of urine (i.e. mosmoles per Kg water) can be determined by depression of freezing point measurements

A cautionary note

The instruments used to determine the composition of urine provide information about the concentrations of sodium etc. Such determinations can be misleading and lead an unwary student to the wrong conclusions. For example, a decrease in sodium concentration accompanied by an increase in urine volume may produce no change in sodium excretion. When studying sodium losses in this practical remember to calculate the amounts of sodium excreted.

Each experiment is supervised by at least one member of staff. Students will be divided into small groups and each group will have at least one volunteer associated with it. Make sure that you recognize what is happening in each of the experiments. A summary of the data collected and the conclusions reached will be given at approximately 3:15PM.

RESULTS:

PSL 131 – DIURESIS PRACTICAL 1

SUBJECT CONDITIONS

STARTED DATE

SAMPLE NO.	1	2	3	4	5	6	7	8
Time								
Collection Interval – min								
Volume (ml)								
Flow rate (ml/min)								
$[Na^+]$ mM								
Na + excretion Total – mmole								
Na + excretion µmole/min								
$[K^+]$ mM								
Osmolality Mosmol/kg								
pH								

NORMAL URINE

1. Urine production rate = approx. 1.5 litres/day (1ml/min).
2. pH of urine = slightly acidic (minimum value = 4.5)
3. Sodium excretion rate = 100-200 mmol/day
4. Potassium excretion rate = 25-100 mmol/day
5. Osmolality = 70-1200 mosmol/kg (average = 600).
   N.B. Remember plasma osmolality is 275-295 mosmoles/kg.

Specific gravity is the weight of a fluid as compared with an equal volume of water (i.e. g/ml).

Osmotic forces are of great importance for the movement of water between the different fluid compartments. If a solution and pure solvent are separated by a membrane that is permeable to the solvent but not to the solute then the solvent passes into the solution by osmosis. The osmotic pressure (force) is equivalent to the hydrostatic pressure that must be applied to prevent the movement of the solvent. Osmotic pressures depend on the number of particles per unit volume of solvent. One gram-molecular weight of glucose or any other non-dissociating compound consists of 6.023×10^{23} molecules and is called 1 osmole. If 1 osmole is dissolved in 1 kg of water an osmotic activity of 22.4 atmospheres is produced. 1 milliosmole is a thousandth of an osmole. If this is dissolved in 1 kg of water an osmotic force of 22.4 x 760 / 1000 = 17mmHg is produced.

Sodium chloride in solution, in contrast to glucose, dissociates into two ions. One gram-molecular weight, therefore, exerts an osmotic effect of almost 2 osmoles. (N.B. sodium sulphate in solution exerts an osmotic effect of about 3 osmoles).

The number of osmotic particles in solutions can be expressed in two ways i.e. osmolality and osmolarity. Osmolality is the number of osmoles per kilogram of solvent. Osmolarity is the number of osmoles per litre of solution. Osmolarity is affected by the volume of the various solutes in the solution. N.B. Osmolality and osmolarity are virtually the same when dilute solutions are considered and differences between these two measurements are small for body fluids.

The osmotic concentrations of solutions are usually measured in terms of depression of freezing point. Pure water freezes at 0°C. A solution containing 1 osmole of an undissociated solute in 1 kg of water freezes at –1.86°C. The freezing point of urine varies depending on its osmolality. A value of –0.25°C can be recorded in very dilute urine and about –2.6°C when urine is concentrated.

QUESTIONS AND PROBLEMS:

1. Why does the pH of urine not fall below 4.5? Is there any physiological significance to this finding?

2. Data collected in previous years is presented in the following tables. Note that the times at which urine was collected is not quite the same as in the experiments conducted this year. Assume that these volunteers would have continued to excrete water and sodium at the same rates they did before they drank water or saline.
   a) Calculate how much more (extra or additional) water this volunteer excreted in the 3 hours after drinking:-
      i. 1 litre of water

      ii. 1 litre of saline

   b) Calculate how much more (extra or additional) sodium this volunteer excreted in the 3 hours after drinking:
      i. 1 litre of water

      ii. 1 litre of saline

3. How quickly, and by what mechanisms can a man excrete 1 litre of water and 154 mmoles of sodium after he has ingested 1 litre of isotonic saline?

DIURESIS EXPERIMENT
SUBJECT DRANK A LITRE OF ISOTONIC SALINE

SAMPLE NO.	1	2	3	4	5	6	7
COLLECTION TIME – mins	120	30	30	30	30	30	30
VOLUME (ml)	125	39	50	42	47	32	45
VOLUME/MINUTE (ml/min)	1.04	1.30	1.67	1.40	1.57	1.07	1.50
[Na^+] (mM)	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 (µmoles/min)	105.2	127.4	186.7	152.6	188.0	146.1	190.5

2 HOURS BEFORE TAKING A LITRE OF SALINE (ISOTONIC)

3 HOURS AFTER TAKING SALINE

NOTE: THIS EXPERIMENT IS COMPARABLE WITH THE ONE IN WHICH WATER WAS DRUNK

DIURESIS EXPERIMENT
SUBJECT DRANK A LITRE OF WATER

SAMPLE NO.	1	2	3	4	5	6	7
COLLECTION TIME – mins	120	30	30	30	30	30	30
VOLUME (ml)	118	33	206	260	214	54	36
VOLUME/MINUTE (ml/min)	0.98	1.10	6.87	8.67	7.13	1.8	1.2
[Na^+] (mM)	87	56	12	9	10	25	53
TOTAL SODIUM EXCRETION – mmoles	10.3	1.8	2.5	2.3	2.1	1.4	1.9
SODIUM EXCRETION (µmoles/min)	85.6	61.6	82.4	78	71.3	45	63.6

2 HOURS BEFORE TAKING A LITRE OF WATER

3 HOURS AFTER TAKING A LITRE OF WATER

KIDNEY PRACTICALS
DIURESIS – PART 2

OBJECTIVES:

1. To measure the volume and determine the composition of urine excreted by volunteers who have:-
   a) drunk a litre of water and then continued with their normal activities (i.e. remained active)
   b) drunk a litre of water and then rested quietly (supine)
   c) swallowed a Lasix tablet (furosemide) with 25ml water
2. To be able to explain why a man who drinks a litre of water and remains active does not excrete the same:-
   a) volume of urine,
      or
   b) amount of sodium

   as a man who drinks the same volume of water but then rest quietly for the subsequent 3 hours.
3. To be able to discuss the mechanisms whereby a man taking Lasix excretes large quantities of both water and sodium.

METHODS:

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

A. THOSE TAKING WATER
   i. emptied their bladders at 10:00AM and discarded the urine they produced
   ii. at 12:00AM (noon) again emptied their bladders but this time they measured its volume and took a sample for analysis
   iii. drank one litre of water immediately after providing their urine samples
   iv. were asked to empty their bladders and provide samples every half hour after drinking water until 3:00PM

B. THOSE TAKING LASIX
   i. emptied their bladders at 8:00AM and discarded the urine they produced
   ii. collected all the urine that they could produce at 10:00AM and kept a sample for analysis
   iii. swallowed a Lasix tablet (furosemide), washing it down with 25ml water immediately after taking the 10:00AM sample
   iv. were asked to empty their bladders and provide samples every hour after taking Lasix until 12:00AM (noon) and then every half hour until 3:00PM

URINE WILL BE ANALYZED TO DETERMINE ITS:-

a) volume, simply by using a measuring cylinder
b) $[Na^+]$ and $[K^+]$ by flame photometry
c) $[H^+]$ using a pH meter
d) osmolality using an osmometer. The osmolality of urine (i.e. mosmoles per kg water) can be determined by depression of freezing point measurements

Each experiment will be supervised by at least one member of staff. Students will be divided into small groups and each group will have at least one volunteer associated with it. Make sure that you recognize what is happening in each of the experiments. A summary of the data collected and the conclusions reached will be given at approximately 3:15PM.

RESULTS:

PSL 131 – DIURESIS PRACTICAL 2

SUBJECT CONDITIONS

STARTED DATE

SAMPLE NO.	1	2	3	4	5	6	7	8
Time								
Collection Interval – min								
Volume (ml)								
Flow rate (ml/min)								
$[Na^+]$ mM								
Na + excretion Total – mmole								
Na + excretion µmole/min								
$[K^+]$ mM								
Osmolality Mosmol/kg								
pH								

(5)

QUESTIONS AND PROBLEMS:

Data collected in previous years is presented in the following tables. Assume that these volunteers would have continued to excrete water and sodium at the same rates they did before they drank water or took a Lasix tablet.

1. Calculate how much more (extra or additional) water this volunteer excreted in the 3 hours after taking Lasix.

Carefully explain how Lasix can increase the loss of body fluids.

2. What are the total volume of urine excreted in the 3 hours after drinking a litre of water and:-
   a) continuing normal activities

   b) resting

Is the total volume of urine excreted in the 3 hours after drinking water anywhere near a litre?

Have the rates of urine production returned to normal within this period of time?

If a litre of urine has not excreted what explanations can you offer?

3. How much extra sodium was excreted by the volunteer who drank water and then rested?

This volunteer had not ingested any sodium with the water. Why was he losing sodium?

(6)

DIURESIS EXPERIMENT
SUBJECT DRANK A LITRE OF WATER – NORMAL ACTIVITIES

SAMPLE NO.	1	2	3	4	5	6	7
COLLECTION TIME – mins	120	30	30	30	30	30	30
VOLUME (ml)	107	32	148	240	62	36	34
VOLUME/MINUTE (ml/min)	0.89						
[Na^+] (mM)	126	107	24	17	48	93	110
TOTAL SODIUM EXCRETION – mmoles	13.5						
SODIUM EXCRETION (µmoles/min)	112.4						

2 HOURS BEFORE TAKING A LITRE OF WATER

3 HOURS AFTER TAKING A LITRE OF WATER

DIURESIS EXPERIMENT
SUBJECT DRANK A LITRE OF WATER – THEN ASKED TO LIE QUIETLY

SAMPLE NO.	1	2	3	4	5	6	7
COLLECTION TIME – mins	120	30	30	30	30	30	30
VOLUME (ml)	124	41	200	375	327	216	88
VOLUME/MINUTE (ml/min)	1.03						
[Na^+] (mM)	118	117	34	21	17	25	55
TOTAL SODIUM EXCRETION – mmoles	14.6						
SODIUM EXCRETION (µmoles/min)	121.9						

2 HOURS BEFORE TAKING A LITRE OF WATER

3 HOURS AFTER TAKING A LITRE OF WATER

DIURESIS EXPERIMENT
SUBJECT TOOK A LASIX TABLET WITH 25ml OF WATER

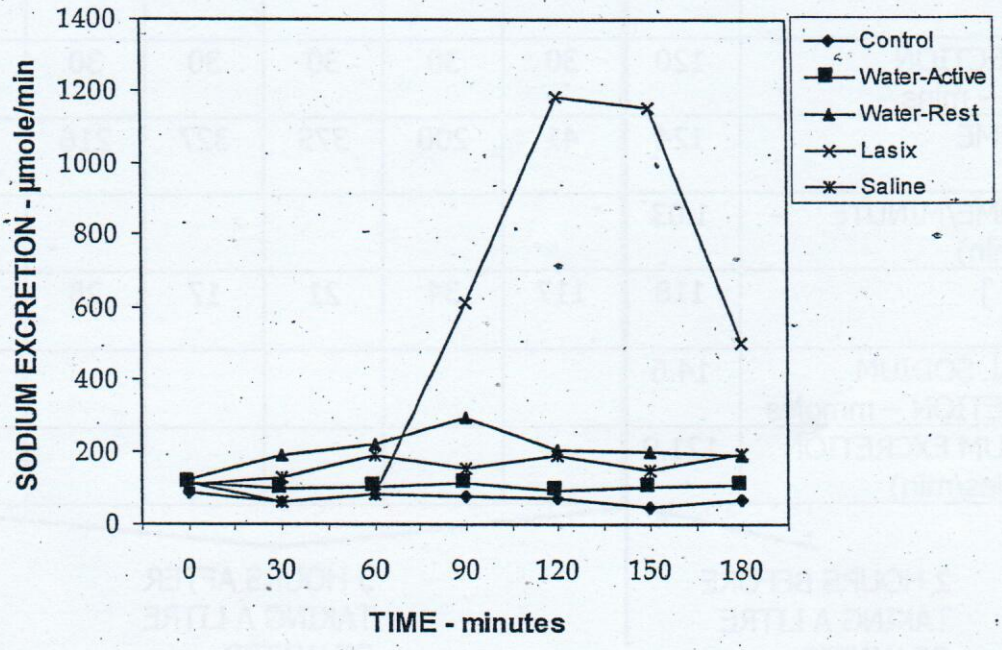
SAMPLE NO.	1	2	3	4	5	6	7
COLLECTION TIME – mins	120	60	42	18	30	30	
VOLUME (ml)	102	58	269	230	270	125	
VOLUME/MINUTE (ml/min)	0.85						
[Na^+] (mM)	132	107	121	115	121	117	
TOTAL SODIUM EXCRETION – mmoles	13.5						
SODIUM EXCRETION (µmoles/min)	112.2						

2 HOURS BEFORE TAKING LASIX

3 HOURS AFTER TAKING LASIX

DIURESIS EXPERIMENT

SODIUM AMOUNTS EXCRETED



Methods:

For all the five volunteers will ask them to empty their bladder two hours before the lab i.e at 11.00 am

And at 1.00 all will collect urine samples and these will be considered as the base line for each to which we will compare the other samples

1. Controls

Sample	1	2	3	4	5	6
Time	1.00	1.30	2.00	2.30	3.00	3.30
Collection interval (min)	120	30	30	30	30	30
Volume (ml)	84	15	11.4	13.8	16.5	
Flow rate (ml/min)						
[Na] mM	230	183	142	152	144	
Total Na excretion (mmole)						
Rate of Na excretion (µmole/min)						
[K] mM	39	63	83	81	79	
Osmolality (mosmol/kg)	720	666	728	812	800	
pH						

2. Water

a. Empty their bladder and discard rine at 11.00
b. Collect 1st sample at 1
c. Drink 1 liter of water at 1.00
d. Collect urine every half an hour

Sample	1	2	3	4	5	6	
Time	1.00	1.30	2.00	2.30	3.00	3.30	4.00
Collection interval (min)	120	30	30	30	30	30	30
Volume (ml)	107	32	148	240	62	36	34
Flow rate (ml/min)							
[Na] mM	126	107	24	17	48	93	110
Total Na excretion (mmole)							
Rate of Na excretion (µmole/min)							
[K] mM	7.4	12	3.9	3.6	12	45	
Osmolality (mosmol/kg)	220	239	69	56	135	435	
pH							

4. Saline

a. empty their bladder and discard urine at 11.00
b. collect 1st sample at 1
c. drink 1 liter of saline at 1.00
d. collect urine every half an hour

Sample	1	2	3	4	5	6	
Time	1.00	1.30	2.00	2.30	3.00	3.30	4.00
Collection interval (min)	120	30	30	30	30	30	30
Volume (ml)	125	39	50	42	47	32	45
Flow rate (ml/min)							
[Na] mM	101	98	112	109	120	137	127
Total Na excretion (mmole)							
Rate of Na excretion (µmole/min)							
[K] mM	43	7.7	33	30			
Osmolality (mosmol/kg)	837	169	639	700			
pH							

5. Lasix

a. empty their bladder and discard rine at 11.00
b. collect 1st sample at 1
c. swallow a lasix tablet with 25ml of water at 1.00
d. collect urine every half an hour

Sample	1	2	3	4	5	6
Time	1.00	1.30	2.00	2.30	3.00	3.30
Collection interval (min)	120	30	30	30	30	30
Volume (ml)	102	58	269	230	270	125
Flow rate (ml/min)						
[Na] mM	132	107	121	115	121	117
Total Na excretion (mmole)						
Rate of Na excretion (µmole/min)						
[K] mM	25	23	13	38	39	
Osmolality (mosmol/kg)	360	257	190	309	361	
pH						

Measurement:

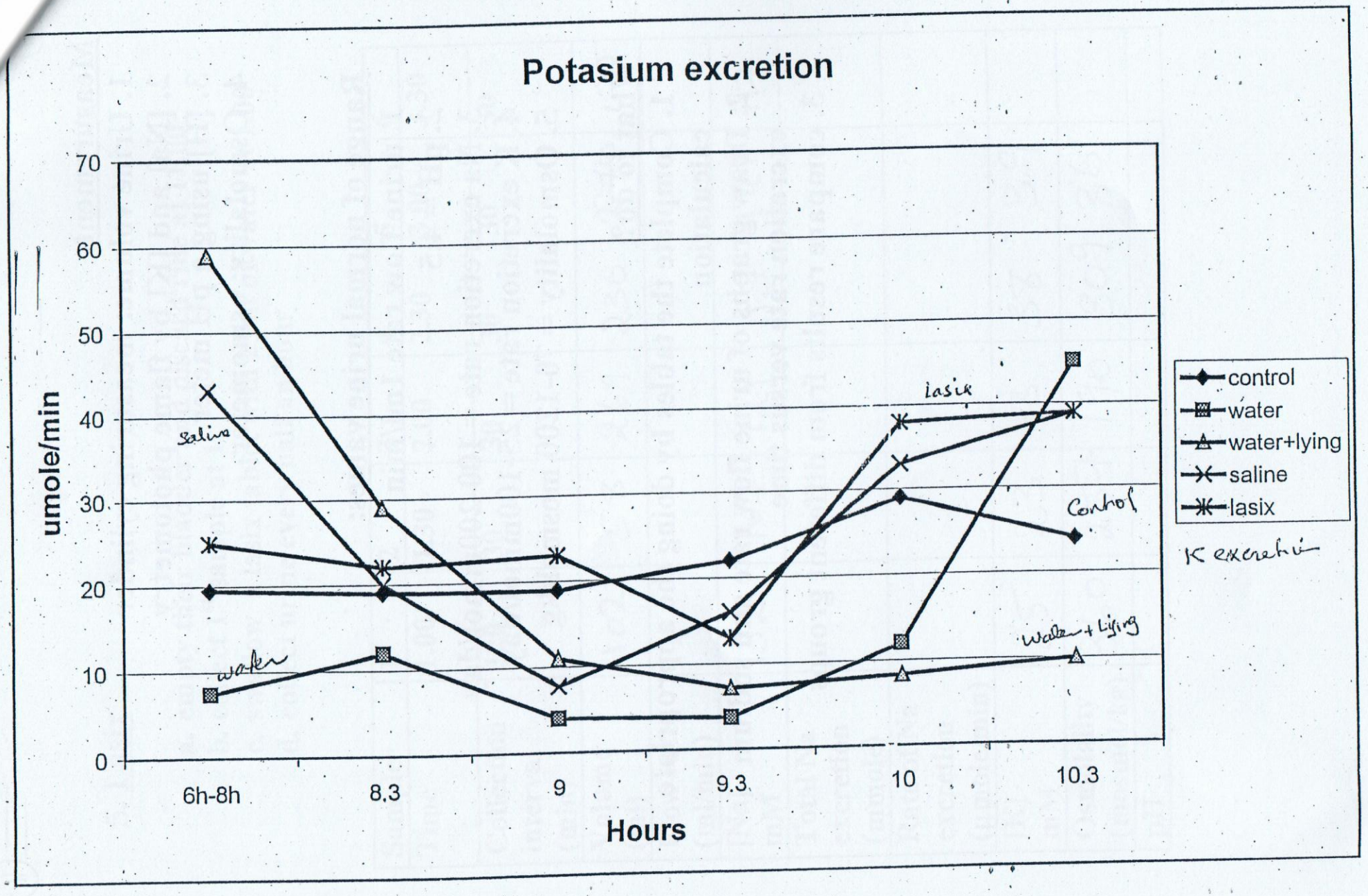
1. Urine volume: measuring cylinder
2. [Na] and [K] by flame photometry
3. [H] using a pH meter
4. Osmolality: osmometer

Range of normal urine values:

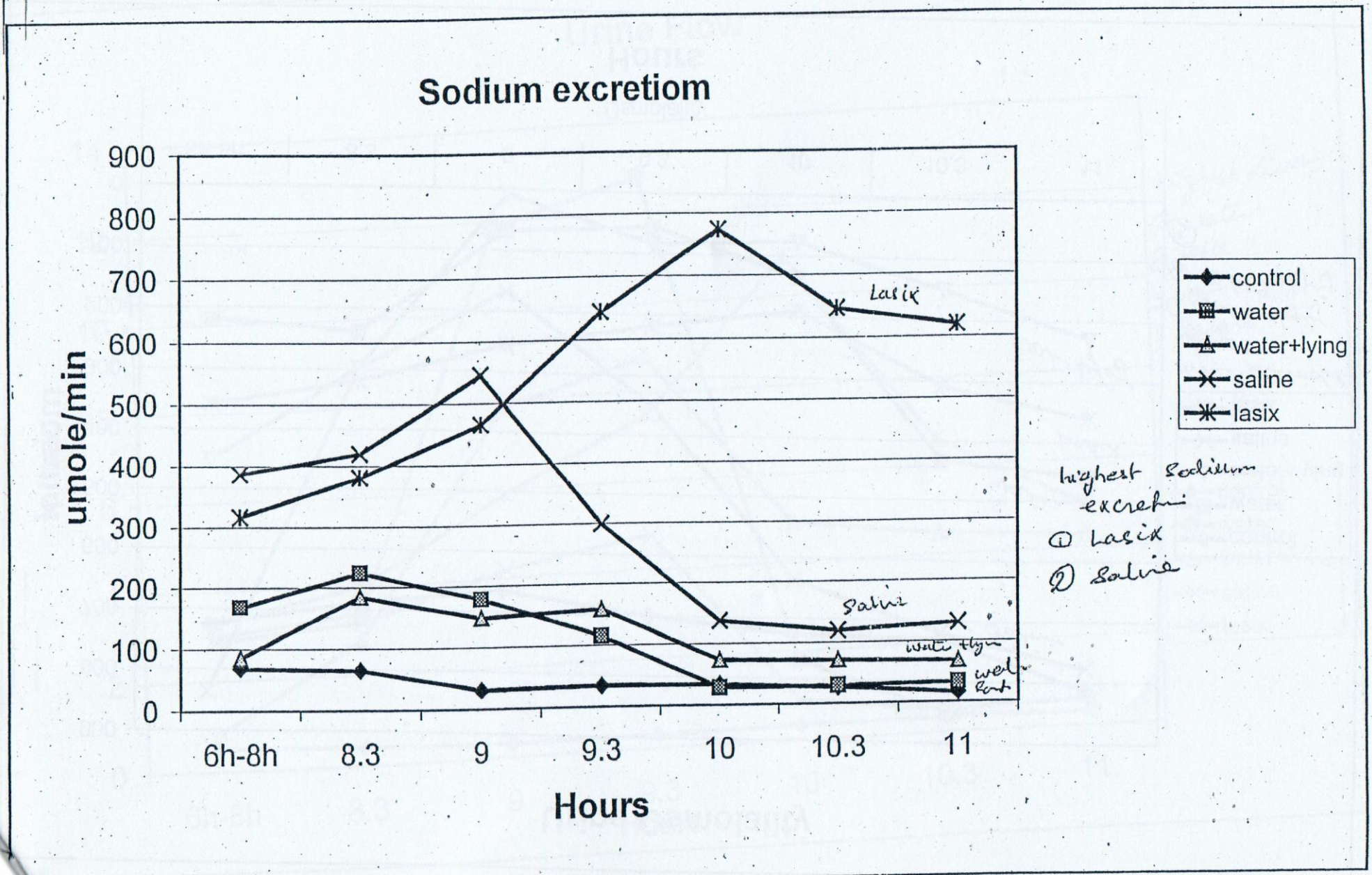
1. urine flow rate 1ml/min
2. pH = 4.5
3. Na excretion rate = 100-200mmol/day
4. K excretion rate = 25-100mmol/day
5. Osmolality = 70-1200 mosmol/kg

What to do?

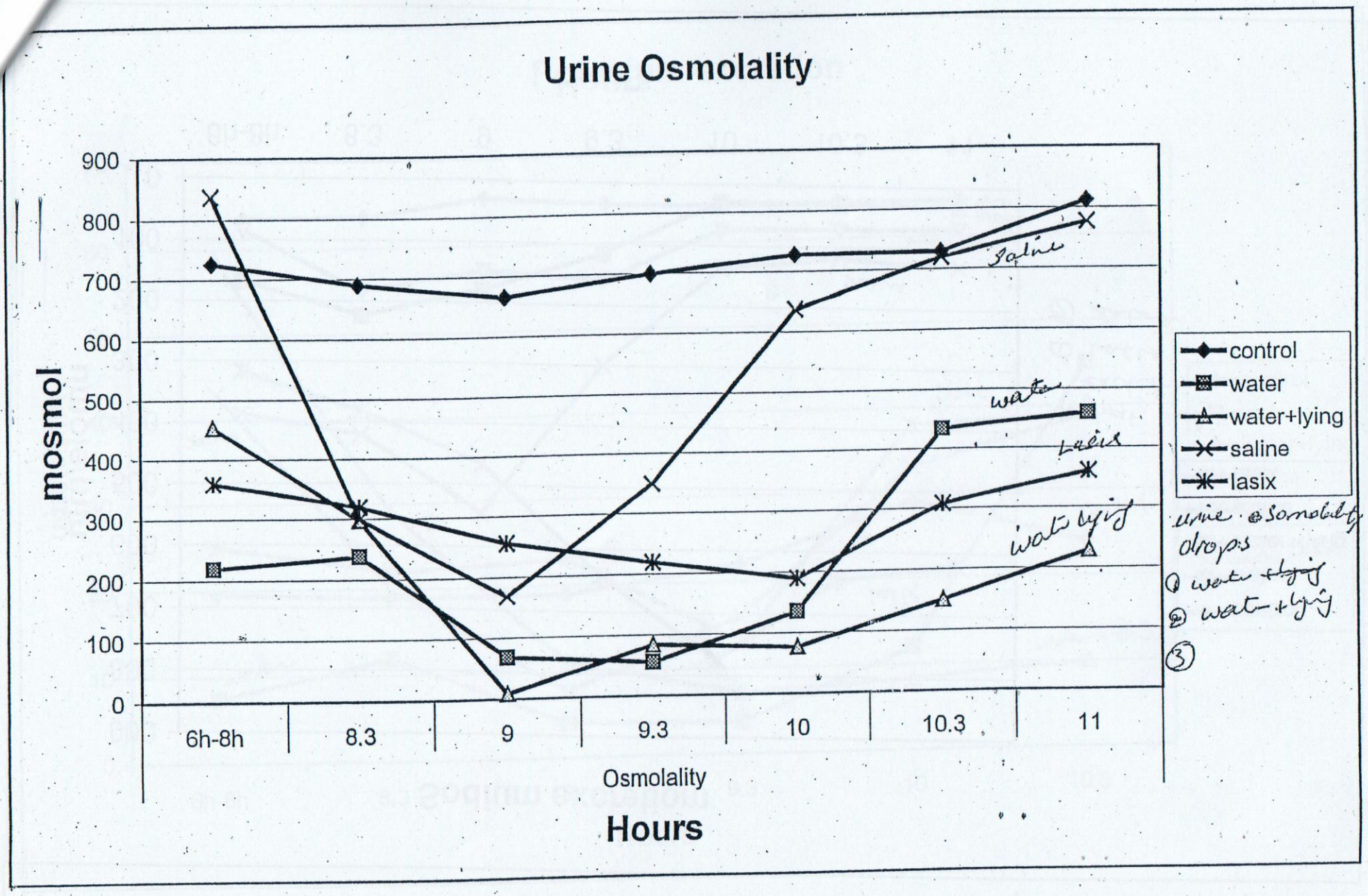
1. Complete the tables by doing the appropriate calculation
2. Draw graphs of urine flow rate and sodium excretion rate versus time
3. compare results from different groups

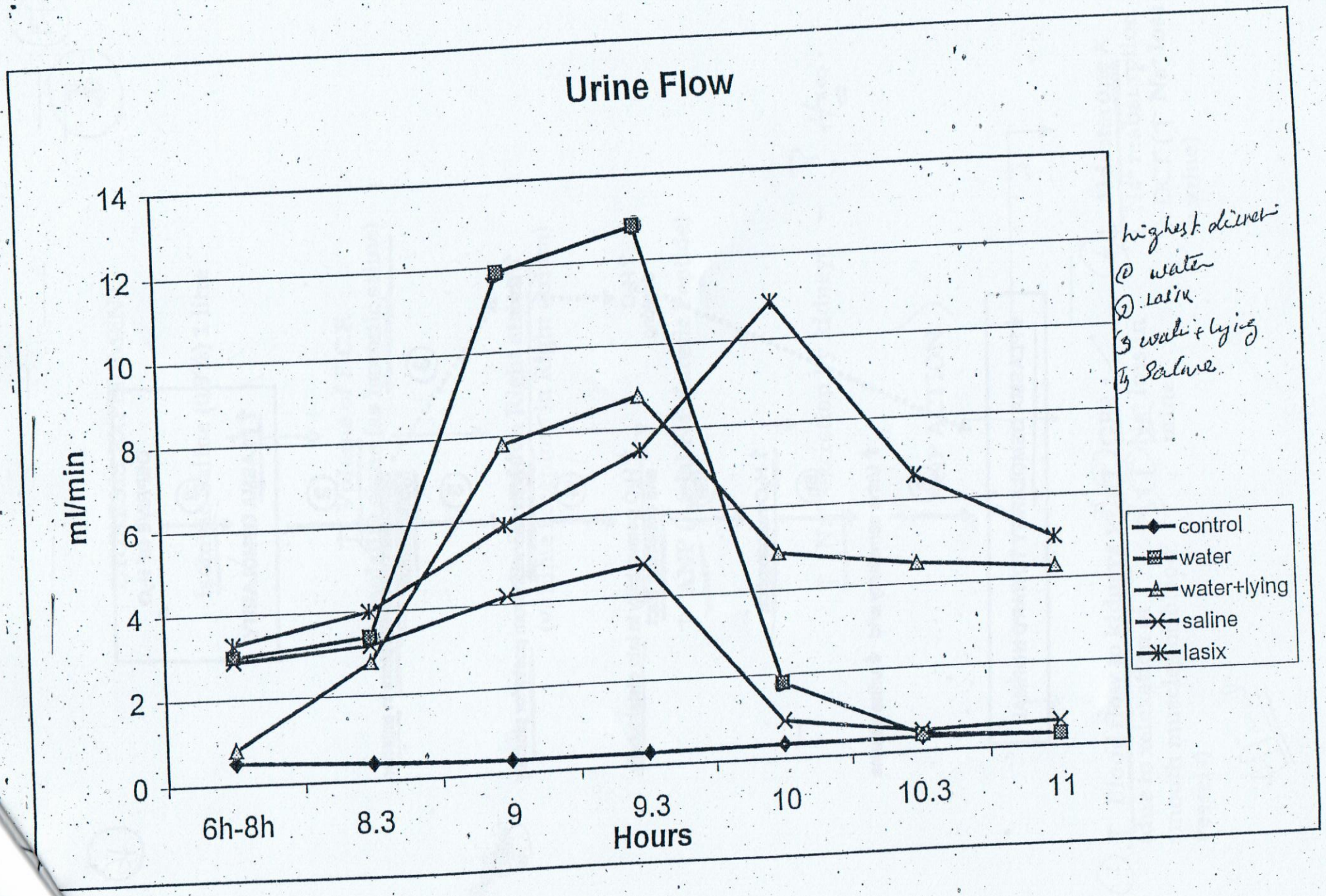

Potasium excretion
umole/min
70
60
50
40
30
20
10
0
6h-8h
8.3
9
9.3
10
10.3
Hours
control
water
water+lying
saline
lasix
saline
lasix
control
water
water+lying
K excretion

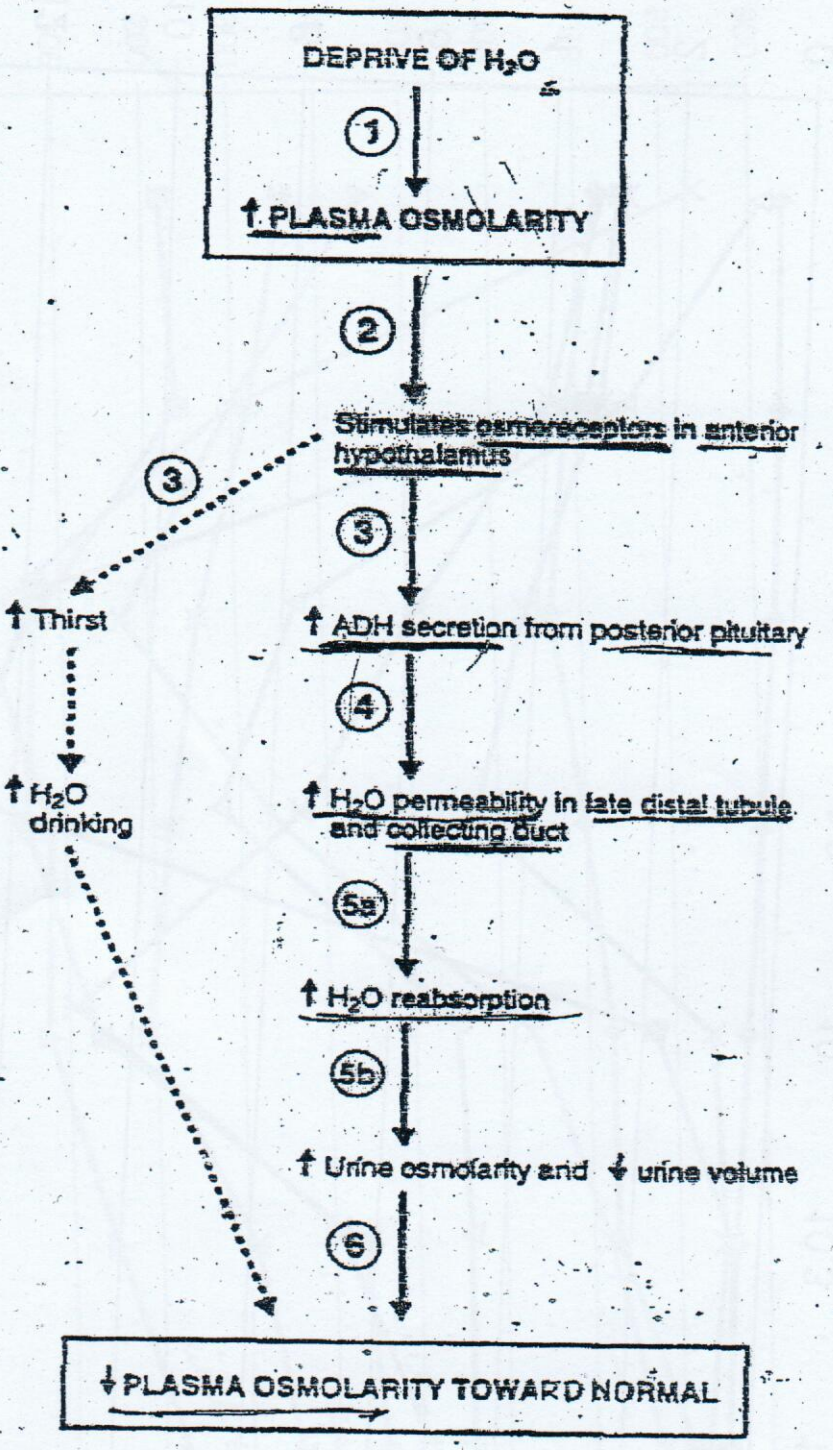
(13)



highest Sodium excretion
① Lasix
② Saline


Urine Osmolality
900
800
700
600
500
400
300
200
100
0
mosmol
6h-8h
8.3
9
9.3
10
10.3
11
Osmolality
Hours
control
water
water+lying
saline
lasix
saline
water
Lasix
water lying
urine osmolality
drops
1 water +lying
2 water +lying
3


Urine Flow
14
12
10
8
6
4
2
0
ml/min
6h-8h
8.3
9
9.3
10
10.3
11
Hours
control
water
water+lying
saline
lasix
highest diuresis
① water
② lasix
③ water + lying
④ Saline


DEPRIVE OF H_2O
1
↑ PLASMA OSMOLARITY
2
Stimulates osmoreceptors in anterior hypothalamus
3
3
↑ Thirst
↑ ADH secretion from posterior pituitary
4
↑ H_2O drinking
↑ H_2O permeability in late distal tubule and collecting duct
5a
↑ H_2O reabsorption
5b
↑ Urine osmolarity and ↓ urine volume
6
↓ PLASMA OSMOLARITY TOWARD NORMAL

17

B

DIURESIS EXPERIMENT

Isotonic Saline (0.9%) 1 litre

↓

↑ Volume of E.C.F.
Osmolality same (as Isotonic saline)

↓

↑ Stretch on Right atrium
(volume receptors in Right atrium)

↓

↑ ANP (Atrial Natriuretic Peptide)

↓

↑ Na^+ excretion by kidneys – ? H_2O

(ANP ACTION)

↓

(1) ↑ Blood flow to kidneys (due to relaxation of smooth muscles of blood vessels)

(2) ↑ GFR

(3) ↑ Na^+ loss in urine

(4) ↓ Aldosterone
↓ Na^+ reabsorption in DCT (↑ Na^+ loss in urine)

(1B)

DIURESIS EXPERIMENT

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

½ use of Frusemide in 6'

↓

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

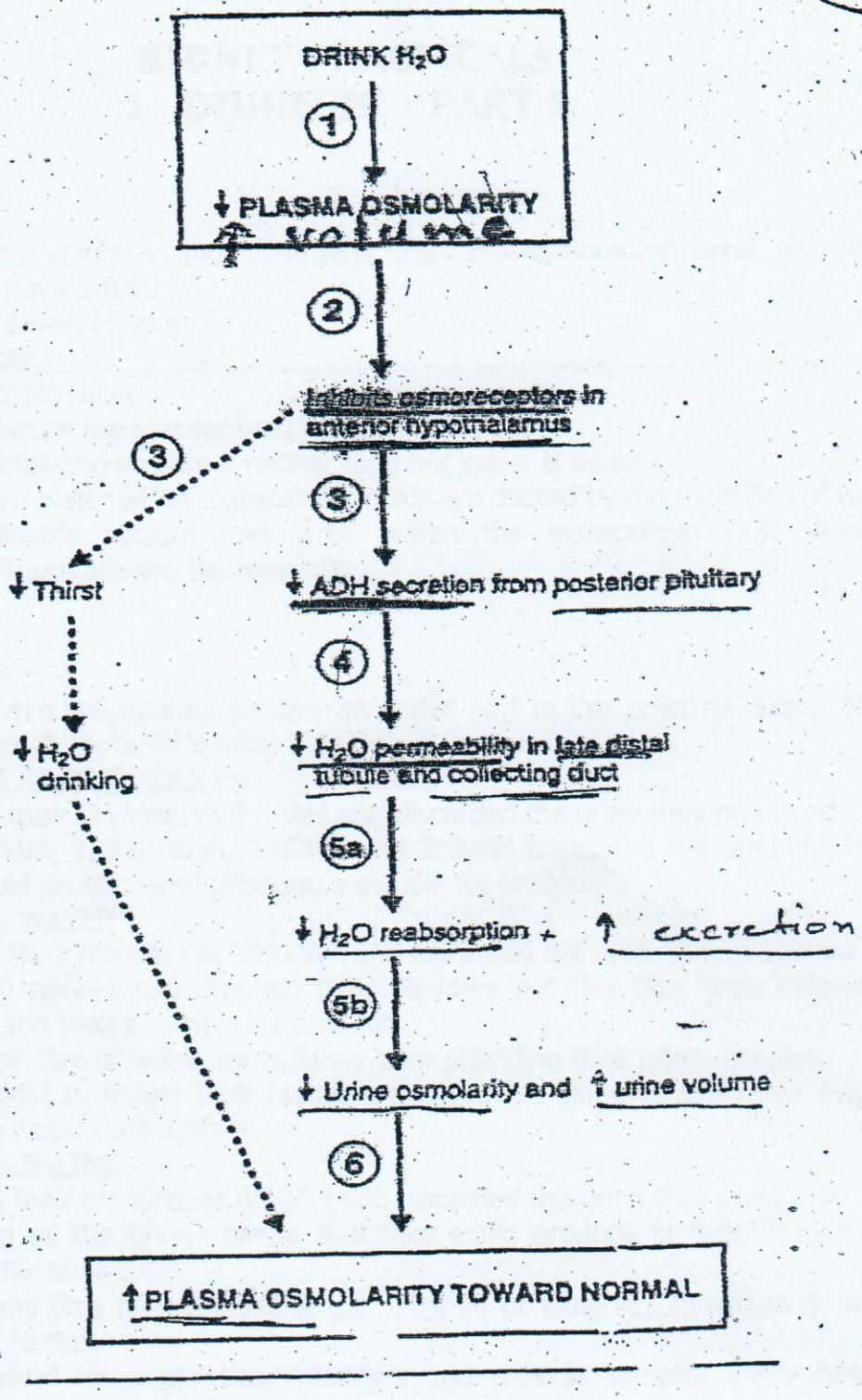
↓

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 → (osmotic drag).


DRINK H_2O
1
↓ PLASMA OSMOLARITY
↑ volume
2
Inhibits osmoreceptors in anterior hypothalamus
3
3
↓ Thirst
↓ ADH secretion from posterior pituitary
4
↓ H_2O drinking
↓ H_2O permeability in late distal tubule and collecting duct
5a
↓ H_2O reabsorption + ↑ excretion
5b
↓ Urine osmolarity and ↑ urine volume
6
↑ PLASMA OSMOLARITY TOWARD NORMAL

Control

Sampel nom	1	2	3	4
time	12	12.5	1	1.5
Collection interval min	120	30	30	30
Volume ml	14.8	5	5.5	5
Flow rate ml/min	.12	.16	.18	.16
[Na]mM	230	183	142	152
Na excretion total mmole	3.4	.9	.8	.8
Na excretion	28.4	30.5	26	25.3
[K]mM	39	63	83	81
OsmolalityMosoml/kg	861	920	948	135

Water

Sampel nom	1	2	3	4
time	12	12.5	1	1.5
Collection interval min	120	30	30	30
Volume ml	35	8	80	170
Flow rate ml/min	.29	.27	2.67	5.67
[Na]mM	126	107	24	17
Na excretion total mmole	4.4	.9	1.9	2.9
Na excretion	36.8	28.5	64	96.3
[K]mM	74	12	3.9	3.6
OsmolalityMosoml/kg	1042	1021	288	170

6.C

Lasix

Sampel nom	1	2	3	4
time	12	12.5	1	1.5
Collection interval min	120	30	30	30
volume	170	6	70	91
Flow rate=v/t	1.42	.2	2.3	3.03
[Na]mM	132	107	121	115
Na excretion total=vc/1000	22.4	.6	8.5	10.5
Na excretion Mmol=vc/t	187	21.4	282.3	348.8
[k] mM	25	23	13	39
Osmolality Mosmol/Kg	631	1021	407	372

Saline

Sampel nom	1	2	3	4
time	12	12.5	1	1.5
Collection interval min	120	30	30	30
volume	45	35	150	205
Flow rate=v/t	.4	1.2	5	6.8
[Na]mM	101	98	112	109
Na excretion total=vc/1000	4.5	3.4	16.8	22.3
Na excretion Mmol=vc/t	37.9	114.3	560	744.8
[k] mM	43	7.7	33	30
smolality Mosmol/Kg	67	105	218	560