BODY FLUIDS AND ELECTROLYTES

(a) ***To explain the distribution of body fluids and their measurement.***

(I) Distribution of body fluids:

Distribution of total body water (TBW):

- TBW is 60% of body weight (42 L in 70 kg ♂ adult) → BUT this varies according to:
  - (i) Body fat content – ↑ fat content → ↓ %TBW of body weight
  - (ii) Age – Aging causes ↓ %TBW of body weight (due to ↑ fat content)
  - (iii) Gender – ♀ TBW is 55% body weight (due to ↑ fat content)
- TBW is broken into various "body fluid compartments" as per the table below:

Compartment	% TBW	Volume (L)	% body weight
ECF	45	19	27
- (i) Interstitial fluid	20	8.4	12
- (ii) Plasma volume	7.5	3.2	4.5
- (iii) Dense CT H_2O	7.5	3.2	4.5
- (iv) Bone H_2O	7.5	3.2	4.5
- (v) Transcellular fluid	2.5	1	1.5
ICF	55	23	33
TBW	100%	42 L	60%

Note – "Body fluid compartment" is defined as collections of "physiologically significant" fluid characterised as → (i) being easily defined, (ii) having a similar composition within the compartment, and (ii) behaving predictably to certain fluid interventions)

Intracellular fluid (ICF):

- ICF consists of all fluid within the cell membrane → 55% TBW (23 L); 33% BWgt
- ICF composition:
  - Mainly K^+ (150 mmol/L), PO_4^{3-} (100 mmol/L) and proteins (+++)
  - Contains smaller amounts of Na^+ (10 mmol/L), Cl^- (3 mmol/L), HCO_3^- (10 mmol/L), Mg^{2+} (0.5 mmol/L), Ca^{2+} (< 0.01 mmol/L)
  - Osmolality 290 mosm/kg H_2O
- It does NOT exist not a single united fluid compartment → exists as 10^{14} discrete separate packets (cells) of solution

Extracellular fluid (ECF):

- ECF consists of all fluids outside the cell membrane → 45% TBW (19 L) ; 27% BWgt
- ECF composition:
  - Mainly Na^+ (140 mmol/L) and Cl^- (100-105 mmol/L)
  - Contains smaller amounts of HCO_3^- (27 mmol/L), K^+ (3.5-5 mmol/L), Ca^{2+} (1.1 mmol/L), PO_4^{3-} (1.1 mmol/L), Mg^{2+} (0.5 mmol/L), protein (+)
  - Osmolality 290 mosm/kg H_2O
- Contains many sub-compartments:
  - (1) Interstitial fluid (20% TBW; 8.4 L; 12% BWgt)
    - Fluid that bathes all cells in body and links their ICF with PV → role in transfer of metabolic substrates (O_2/nutrients), waste products and chemical messengers
    - Similar composition to PV → BUT very ↓ protein composition cf. PV
    - Includes "lymph" (See below) → role in returning excess ISF and protein to circulation
  - (2) Plasma fluid (7.5% TBW; 3.2 L; 4.5% BWgt)
    - Role in transport function within body (Ie. of metabolic substrates, waste products, chemical messengers) → relies on high "bulk flow"
    - Similar composition to ISF → BUT ↑↑↑ protein content
  - (3) Dense CT H_2O (7.5% TBW; 3.2 L; 4.5% BWgt)

- (4) Bone H_2O (7.5% TBW; 3.2 L; 4.5% BWgt)
- (5) Transcellular fluid (2.5% TBW; 1 L; 1.5% BWgt)
  - Diverse group of small fluid collections (Eg. CSF, bile, joint fluid, aqueous humour, bowel fluid, bladder urine, body cavity fluids, Etc.)
  - This type of fluid is specially characterised by → (i) having special physiological roles in body, (ii) being in contact with ICF across an epithelial cell membrane (rather than ISF), and (iii) being formed by specific cellular transport activity in epithelial-lined spaces

Note → there are two groups of ECF:
- (1) "Functional ECF" (PV + ISFV → 27.5% TBW)
  - Kinetically active (fast) ECF → vital in determining compartment distribution of acutely infused fluids
  - Explains 2:1 ECF:ICF ratio with acute IVF intervention (as functional ECF:ICF ratio, rather than total ECF:ICF ratio which is 55:45)
- (2) "Non-functional ECF" (TCF + bone H_2O + dense CT H_2O → 17.5% TBW)
  - Kinetically inactive (slow) ECF → minor role in determining compartment distribution of acutely infused fluids

Blood volume:
- Blood volume (5 L) consists of BOTH ECF and ICF compartments → PV from ECF (3.2 L) and red cell volume from ICF (1.8 L)

Blood volume = PV (from ECF) + red cell volume (from ICF)

(II) <u>Factors controlling distribution of TBW</u>:

Important to note:
- All body fluid compartments are isotonic (or iso-osmotic in terms of "effective" osmoles) as H_2O easily and rapidly moves (via osmosis) across cell membranes
- Distribution of TBW in body fluid compartments is due to various factors (see below) that cause H_2O to shift across membranes into a specific compartment

- TBW distribution between ICF and ECF → determined by ECF content of Na^+ because:
  - (i) ECF Na^+ content is main determinant of ECF osmolality → this is because Na^+ and its associated anion (Cl^-) are the main osmotically active solutes in ECF (90% ECF osmolality) → but since Δ in ECF Cl^- content occur 2° to Δ in ECF Na^+ content, so ECF Na^+ content is the true determinant of ECF osmolality

    Remember → ECF Na^+ is controlled by arterial, venous and cardiac baroreceptors that measure ECFV (which is determined by ECF Na^+) → see "renal physiology"

  - (ii) ECF osmolality determines TBW distribution between ECF and ICF → this is because cell membranes are H_2O-permeable and thus Δ ECF osmolality cause H_2O movement across it via "osmosis" until both ICF and ECF osmolalities equalise (Ie. hypertonic ECF draws H_2O out from ICF until ICF/ECF osmolality are equal)

    Remember → Semi-permeable membrane (permeable to H_2O but impermeable to most solutes) allows osmotic gradients to develop on both sides of the membrane. "Osmosis" is a process where H_2O passively diffuses across the membrane from ↓ to ↑ osmolality until osmolality is same on both sides of the membrane

- TBW distribution between ISF and intravascular compartments → determined by "Starling forces" → balance of $P_{HYDROSTATIC}$ and $P_{ONCOTIC}$ across the capillary membrane determines net ultrafiltration of H_2O into ISF:

$$\text{Net } H_2O \text{ flux into interstitium} = K_F \bullet [(P_{CAP} - P_{ISF}) - \sigma(\pi_{CAP} - \pi_{ISF})]$$

  - Hydrostatic pressure gradient ($P_{IV} - P_{ISF}$) → P_{IV} at arterial end and venous end of capillary is 35 mmHg and 15 mmHg, respectively, while P_{ISF} is 0 mmHg → thus, a $P_{HYDROSTATIC}$ gradient exists favouring net H_2O filtration into ISF (decreasing from 35 mmHg at start of capillary to 15 mmHg at the end)
  - Oncotic pressure gradient ($\pi_{IV} - \pi_{ISF}$) → plasma colloids (esp plasma proteins) cannot cross capillary membrane into ISF, so [protein] in capillaries is >> ISF (Ie. 80 vs 20 g/L) → π_{IV} >>. π_{ISF} (28 mmHg vs 3 mmHg) → constant but small net oncotic pressure gradient throughout capillary length favouring H_2O retention in intravascular space
  - K_F → = capillary surface area (↑↑↑) and its hydraulic permeability (membrane is semi-permeable → permeable to H_2O and most solutes EXCEPT large proteins)
  - σ → See "Reflection coefficient" below
  - Thus → NFP at arterial end of capillary is +10 mmHg (favouring filtration in interstitial space) while at venous end it is – 10 mmHg (favouring reabsorption in capillary) → there is net 2 mL/min fluid filtered into interstitial space
- TBW distribution between ECF sub-compartments → determined by:
  - (i) Starling forces → balance of $P_{HYDROSTATIC}$ and $P_{ONCOTIC}$ across the membrane determines ultrafiltration of H_2O across compartments (as above)
  - (ii) Active and passive transport of solute across the membrane (Eg. passive diffusion, facilitated transport, active transport) → influences osmotic gradient across the membrane and causes osmosis of H_2O between compartments
  - (iii) Gibbs-Donnan effect → non-diffusible ion in one compartment causes equal concentration ratios of diffusion ions across the membrane at equilibrium → results in ↑ osmotic pressure in compartment with non-diffusible ion → leads to osmosis of H_2O into it

(III) <u>Measurement of body fluid compartment volumes</u>:

Measuring body fluid compartment volume:

- "Dilution technique" is used to ascertain the volume of a given body fluid compartment → it involves (i) delivering a known amount of tracer, then (ii) measuring the [tracer] in the compartment being analysed (Nb. must allow for a sufficient time for uniform tracer distribution within compartment), and finally (iii) calculating the "volume of distribution" (V_D) of tracer within that compartment

$$\mathbf{V_D = \frac{\text{Amount of tracer given}}{\text{[tracer] in compartment}} = \text{Body fluid compartment volume}}$$

Requirements of the tracer substance:

- (1) Non-toxic
- (2) Rapidly and uniformly distributed within measured compartment, and confined only to compartment being measured
- (3) Not alter body fluid distribution
- (4) Not metabolised (Nb. if it is metabolised → assuming exponential decline (1st order kinetics), a series of measurements can be made and V_D can be determined by extrapolation back to zero time)
- (5) Not excreted (Nb. if it is excreted in urine → amount lost in urine can be measured and corrections made to V_D)
- (6) Easy to measure

Measuring TBW volume:

- Measure V_D of tracers that diffuse throughout and remain in TBW compartment
- Use H_2O isotopes (deuterium or tritium oxide) or aminopyrine → diffusion/equilibration in compartment takes 3-4 hrs (so V_D measured then)

Measuring ECF volume:

- Measure V_D of tracers that distribute throughout and remain in ECF compartment:
  - (i) Ionic tracers (Br, Cl, SO_4 isotopes) → small ions distribute readily throughout ECF BUT also distribute into ICF → overestimates "true" ECFV
  - (ii) Non-ionic tracers (inulin, mannitol, sucrose) → large crystalloids do not readily distribute throughout ECF (esp into "non-functional ECFV" a/w slow kinetics/long uptake times) → underestimates "true" ECFV
- ECFV is quoted as defined by tracer used and equilibration time prior to measurement (Eg. 20 hr Br space) → because tracers used do not actually measure "true" ECF (due to inaccuracies a/w tracers and different kinetics of functional vs non-functional ECF)

Measuring plasma volume:

- Measure V_D of tracers that distribute throughout and remain in intravascular compartment
- Use Evan's blue (binds plasma albumin) or radio-iodine labelled serum albumin (RISA)
- Issue → albumin rapidly distributes within intravascular space BUT some albumin is gradually lost into ISF (via 1st order kinetics → exponential ↓ plasma [tracer]) → corrected by collecting serial plasma samples and extrapolating plasma [tracer] back to zero time to estimate PV

Measuring ISF volume:

- There are no tracer distributed only within ISF compartment
- Thus, measured indirectly using ECFV and PV where → **ISFV = ECFV – PV**

Measuring TCF, dense CT and bone H_2O volumes:

- No tracers exist to measure these compartments

Measuring ICF volume:

- There are no tracers distributed only within ICF compartment
- Thus, measured indirectly using TBWV and ECFV where → **ICFV = TBWV – ECFV**

Measuring blood volume (BV):

- (i) Indirectly measured using known PV and Hcrt where → **BV = PV/(1 – Hcrt)**
- (ii) Directly measured using ^{51}Cr-labelled red cells
  - Patient's own RBC is labelled with ^{51}Cr and infused → distributes rapidly within BV (10 mins)
  - Issue – Calculating V_D of tracer to determine BV usin g measured blood [tracer] (amt of tracer in a volume of blood) is NOT accurate due to variations in Hcrt within the circulation → to avoid this issue, calculate "red cell volume" instead using "red cell mass" (amt of tracer in volume of red cells) → **BV = RCV/Hcrt**

Aside: Measuring Hcrt has issues too → as it varies within the circulation:

- Capillary blood has ↓ Hcrt than large vessels due to axial streaming of red cells (Eg. muscle capillary Hcrt only 0.2)
- Venous Hcrt ↑↑ cf. arterial because → (i) ↑ IC osmolality and H_2O content in venous RBC due to CO_2 transport in RBC, and (ii) 4-8% plasma trapped within red cells in tube → overestimate "whole body" Hcrt

"whole body" Hcrt = 0.91 x (venous Hcrt)

Aside: Determinants and control of intracellular volume, extracellular volume and body H_2O content.

(I) Determinants and control of ICF volume:

- Intracellular (cell) volume is maintained by a balance of IC and EC Gibbs-Donnan effect (see "Gibbs-Donnan Effect" in "Cellular Physiology"):
    - (1) IC Gibbs-Donnan effect → favours IC H_2O retention
        - Cells have a ↑↑↑ [colloids] (proteins and organic PO_4^{3-}) that cannot the cell membrane → they act as IC non-diffusible ions
        - At Gibbs-Donnan equilibrium → excess IC particles causes ↑ IC osmolality → favours $P_{OSMOTIC}$ gradient that causes IC osmosis of H_2O
    - (2) EC Gibbs-Donnan effect → favours EC H_2O extrusion
        - Na^+ is actively extruded extracellularly (by Na^+/K^+ ATPase) and has very low cell membrane permeability → it acts as an EC non-diffusion ion
        - At Gibbs-Donnan equilibrium → excess EC particles causes ↑ EC osmolality → favours $P_{OSMOTIC}$ gradient that causes EC osmosis of H_2O

> Aside: Inhibition of Na^+/K^+ ATP → favours IC H_2O retention due to IC Gibbs-Donnan effect → cell swelling and lysis!

- BUT ICF (or cell) volume is determined by Δ in ECF osmolality (which is determined by ECF Na^+ content) → such that:
    - (1) Acute ↑ ECF osmolality (Ie. mannitol infusion) → causes H_2O to shift from ICF to ECF → ↓ ICF (cell) volume (Ie. shrinks)
    - (2) Acute ↓ ECF osmolality (Ie. H_2O infusion) → causes H_2O to shift form ECF to ICF → ↑ ICF (cell) volume (Ie. swells)

> Mechanism → ICF and ECF osmolalities are EQUAL due to the H_2O-permeable nature of the cell membrane → so Δ ECF osmolality establishes a $P_{OSMOSTIC}$ gradient across the membrane → leads to osmosis of H_2O across it to dissipate the $P_{GRADIENT}$ (Ie. equalise ICF/ECF osmolalites)

- Compensatory mechanisms exist to minimise Δ ICF (or cell) volume due to long-term Δ ECF osmolality → achieves this by altering IC solute content (Ie. gain or lose IC solutes):
    - (1) Chronic ↑ ECF osmolality (Ie. chronic ↑ Na^+) → Cells gain a solute from either (i) ECF or (ii) ↑ production of an IC solute (Eg. brain cells can synthesis "Idiogenic osmoles") → Nb. this is why chronic ↑ Na^+ is better handled than acute ↑ Na^+
    - (2) Chronic ↓ ECF osmolality → Cells lose a solute (i) into ECF or (ii) due to ↓ production of an IC solute

(II) Determinants and control of ECF volume:

- ECFV is determined by the total amount of osmotically active solute present in this fluid compartment (ECF osmolality) → 1°ly Na^+ and Cl^- (90%) → BUT Δ Cl^- content occurs 2° to Δ Na^+ content → thus, ECF content of Na^+ is the main determinant of ECFV
- ECFV (as determined by ECF Na^+) influences PV → determines CVS $P_{HYDROSTATIC}$ (or MAP) → thus:
    - ↓ ECFV (a/w ↓ body Na^+ content) → ↓ PV → ↓ CVS $P_{HYDROSTATIC}$ (or MAP)
    - ↑ ECFV (a/w ↑ body Na^+ content) → ↑ PV → ↑ CVS $P_{HYDROSTATIC}$ (or MAP)
- ECFV is effectively controlled by a -ve feedback reflex system regulates MAP → involves sensing Δ in CVS $P_{HYDROSTATIC}$ via:
    - (i) Low pressure baroreceptors (atria and great vessels) → sense 5-10% Δ PV
    - (ii) High pressure baroreceptors (carotid sinus and aortic arch) → sense > 10% Δ PV
    - (iii) Intra-renal baroreceptors (JG cells in JGA) → sense Δ renal arterial BP

- Signals from these baroreceptors → sent to integration centres → effector responses modulate body Na^+ content (esp by kidneys) → restores ECFV (and PV) and Δ in MAP

> Note → Δ ECF Na^+ content will alter plasma osmolality → detected 1°ly by hypothalamic osmoreceptors (sensitive → detect 1% change) but also baroreceptors (mainly low-pressure BR) → lead to 2° changes in TBW content (hydration state) to normalise plasma osmolality

- Effector responses involve:
  - (1) Renal regulation of Na^+ (as most Na^+ is lost via renal excretion):

$$Na^+ \text{ excretion} = Na^+ \text{ filtered} - Na^+ \text{ reabsorbed}$$

    - (a) Control of GFR (minor) → influences amount of Na^+ filtered
      - (i) Intrinsic autoregulatory factors
        - Tubuloglomerular feedback → GFR maintained by regulating calibre of afferent arteriole via paracrine –ve feedback means according to tubular NaCl content at MD
        - Myogenic mechanism → afferent arteriolar blood flow maintained by reflex constriction in response to ↑ BP → maintains GFR
      - (ii) Extrinsic factors: Body Na^+ content (via ECFV)
        - Direct renal effects – ↓ [Na^+] (or ↓ ECFV) → results in ↓ GFR due to a ↓ glomerular capillary $P_{HYDROSTATIC}$ and ↑ glomerular capillary $P_{ONCOTIC}$ → ↓ GFR and Na^+ (and H_2O) filtered
        - Indirect renal effects – ↓ [Na^+] (or ↓ ECFV) → stimulates above BRR → neurohormonal response (↑ SNS, RAAS, ADH; ↓ ANP) → to ↓ GFR and Na^+ (and H_2O) filtered
    - (b) Control of Na^+ tubular reabsorption (major role)
      - (i) Glomerulotubular balance → intrinsic autoregulatory mechanism that minimises the effect of Δ in GFR on Na^+/H_2O excretion → PCT reabsorbs a constant proportion of glomerular filtrate (65% filtered Na^+/H_2O), rather than a constant amount

> So → ↑ GFR = ↑ glomerular filtration of Na^+/H_2O = ↑ tubular Na^+/H_2O reabsorption in PCT

      - (ii) Renal interstitial hydrostatic pressure → ↓ ECFV (and ↓ Na^+) results in ↓ MAP → leads to (i) ↓ $P_{HYDROSTATIC}$ and (ii) ↑ $\pi_{ONCOTIC}$ of peritubular capillaries → ↑ tubular Na^+ (and H_2O) reabsorption
      - (iii) Neurohormonal influences → RAAS, SNS activity, ADH, ANP (See below)
  - (2) Renin-angiotensin-aldosterone system:
    - Renin (glycoprotein) made in JG cells in afferent arteriole → secreted by:
      - (i) Renal SNS nerves and circulating catecholamines → via β1
      - (ii) ↓ renal arteriolar BP (as sensed by intrarenal baroreceptors)
      - (iii) ↓ tubular [NaCl] in TAL/EDCT (see TG feedback)
      - (iv) Prostacyclin
      - Nb. ADH and AII both inhibit renin secretion
    - Renin causes a cascade of events → it cleaves Angiotensinogen (liver) to AI → AI converted by ACE (lungs) to AII → AII
    - Effects of AII:
      - (i) ↑ renal tubular reabsorption of Na^+ and Cl^- (and ↑ ECVF) → (a) Direct effects on PCT, and (b) Indirect effects via ↑ aldosterone, ADH and SNS activity

- (ii) ↓ RBF and GFR (esp at ↑↑↑ levels) → ↓ glomerular Na^+ filtration via renal afferent and efferent arteriolar constriction and mesangial contraction
- (iii) ↑ body H_2O content (in relation to ↑ Na^+ content) via → ↑ thirst/H_2O intake (via hypothalamus), and ADH secretion

▪ Aldosterone → MOST important regulator of Na^+ content: → secreted from adrenal cortex (zona glomerulosa) due to → (i) ↑ AII (main), (ii) ↑ plasma [K^+] and (iii) ACTH → causes:

- (i) ↑ renal tubular Na^+ reabsorption (Principal cells of DCT and CCD → upregulates tubular basolateral Na^+/K^+ ATPase and luminal ENaC and K^+ channels)
- (ii) ↑ Na^+ reabsorption via GI tract, sweat and salivary glands

o (2) Renal SNS stimulation (NAd nerves and circulating NAd/Adr):

▪ Minor role at rest (tonic discharge) → but ↑ with (i) BRR (due to ↓ MAP), (ii) central SNS stimulation, and (iii) AII → causes:

- (i) Direct stimulation of Na^+ reabsorption at PCT (via α1 and β1), and indirect stimulation of Na^+ reabsorption via RAAS response (stimulates renin release)
- (ii) ↓ GFR (and RBF) → afferent and efferent arteriolar constriction and mesangial cell contraction → ↓ glomerular Na^+ filtration

o (3) ADH:

▪ Peptide hormone produced in hypothalamus → released from posterior pituitary mainly by ↑ plasma osmolality (hypothalamic osmoreceptors) but also BRR (triggered by ↓ MAP), AII, pain, N/V, Etc. → causes:

- (i) ↑ Na^+ reabsorption at the CCD (principal cells) via V2R → acts synergistically with aldosterone here
- (ii) ↓ GFR due to afferent arteriolar constriction and mesangial contraction via V1R → ↓ glomerular Na^+ filtration
- (iii) ↑ body H_2O content (in relation to ↑ Na^+ content) via → stimulates thirst response and renal H_2O reabsorption at CD (via insertion of luminal AQP2)

o (4) ANP:

▪ Peptide hormone produced by RA → released in response to ↑ RA stretching due to ↑ RA pressures (caused by ↑ MAP)

▪ Causes natriuresis via → (i) Inhibition of Na^+ reabsorption in the CDs, (ii) ↑ GFR (via afferent arteriolar dilation and mesangial cell relaxation → ↑ glomerular Na^+ filtration), and (iii) ↓ RAAS activity and ↓ ADH activity

(III) <u>Determinants and control of body H_2O content</u>:

- Body H_2O content (or TBW state) is determined by the body's H_2O balance (daily H_2O intake vs loss) → normally, it is balanced (as per table below):

Daily H_2O intake	
Drinking	1200 mL
Food	1000 mL
Metabolism (Eg. ETC)	350 mL
Total intake	*2550 mL/day (in 70 kg adult) → 25-35 mL/kg/day*
Daily H_2O loss	
Urine	1500 mL (includes obligatory loss ~ 430 mL)
Insensible losses (skin, lungs)	900 mL
Faecal	100 mL
Sweat	50 mL
Total loss	*2550 mL/day*

Note: Abnormal TBW states arise when an imbalance in body H_2O exists::
- (i) ↓ TBW ("H_2O deficit" → due to H_2O loss > intake) → results in ↑ plasma osmolality due to a relative ↑ plasma [Na^+] → associated with ↓ ECFV (and PV)
- (ii) ↑ TBW ("H_2O excess" → due to H_2O intake > loss) → results in ↓ plasma osmolality due to a relative ↓ plasma [Na^+] → associated with ↑ ECFV (and PV)

- TBW state is controlled via –ve feedback system as follows:
  - (1) Sensors:
    - (a) Osmoreceptors (anterior hypothalamus)
      - Responds to ↑ plasma osmolality. Very sensitive (detects 1% change) → threshold for stimulation is 280 mosm/kg (near lower normal limit) → steep linear rise in response > 290 mosm/kg
    - (b) Low-pressure baroreceptors (right atrium and great vessels)
      - Responds to ↓ plasma volume indirectly by ↓ CVS $P_{HYDROSTATIC}$ (↓ MAP) → ↓ sensitive cf. osmoreceptors (detects 5-10% Δ in PV)
    - (c) High-pressure baroreceptors (carotid sinus and aortic arch)
      - Responds to ↓ plasma volume indirectly by ↓ CVS $P_{HYDROSTATIC}$ (↓ MAP) → Even ↓ sensitive cf. osmoreceptors (detects > 10% Δ in PV → large H_2O deficits) → BUT its response overrides that of the osmoreceptors!
  - (2) Hypothalamus integrates afferent signals from these sensors and modulates an appropriate effector response that includes:
    - (a) Thirst response → triggered by (i) ↑ plasma osmolality, (ii) ↓ plasma volume (or ↓ MAP), (iii) AII (acting on circumventricular organs (SFO/OVLT)
    - (b) ADH
      - 9 a.a peptide hormone synthesised in hypothalamus (SON/PVN) → transported to posterior pituitary where it is secreted by:
        - (i) ↑ plasma osmolarity (main trigger)
        - (ii) ↓ plasma volume (or ↓ MAP) → note that LARGE Δ in PV (> 10%) can override response by osmoreceptors (Ie. ADH is secreted irrespective of plasma osmolality)
        - (iii) Other stimuli: AII, pain, nausea/vomiting, exercise
      - Effects:
        - (i) Via V1 receptor (GPCR via Gq → activates PLC to ↑ IP3 → ↑ IC [Ca^{2+}] → SM contraction) → causes ↓ GFR to ↓ glomerular filtration (and loss of) H_2O → via:
          - (a) Renal afferent arteriolar constriction
          - (b) Renal mesangial cell contraction
        - (ii) Via V2 receptor (GPCR via Gs → activates AC to ↑ cAMP → activates PKA) → this causes:
          - (a) Upregulates insertion of luminal AQP2 (stored in vesicles) in all parts of CD → ↑ H_2O permeability → ↑ H_2O reabsorption into hypertonic medullary interstitium
          - (b) Upregulates "urea transporters" in inner MCD → ↑ permeability to urea → ↑ urea absorption to maintain ↑ medullary osmolality (strengthens CCM) → promotes ↑ H_2O reabsorption
          - (c) ↑ Na^+ reabsorption and K^+ secretion by principal cells of CCD

(b) ***To describe the function, distribution, and physiological importance of sodium, potassium, magnesium, calcium and phosphate ions.***

(I) Sodium:

Distribution of Na^+ in body:

- Total body Na^+ → 4000 mmol (60 mmol/kg):
  - (i) Bone (45%)
  - (ii) ECF (50%) → 1°ly found in ECF (major EC cation) → [Na^+] 140 mmol/L
  - (iii) ICF (5%) → [Na^+] 10-15 mmol/L → kept low by (a) Na^+/K^+ ATPase (actively extrudes 3xNa^+) and (b) low membrane permeability to Na^+ (prevents influx of Na^+)

> Note: Two main pools of Na^+ → (i) Exchangeable Na^+ pool (70% from ECF, ICF, bone) and (ii) Non-exchangeable Na^+ pool (30% mainly as bone crystal)

Na^+ balance in body:

- Daily Na^+ intake → 1-1.4 mmol/kg/day (~ 100-300 mmol/day in 70 kg adult)
- Daily Na^+ loss via:
  - (i) Kidneys (main) → lose 150 mmol/day in urine
    - Glomerulus filters 25000 mmol Na^+/day → but tubular system reabsorbs 99.5% of it → only 0.5% excreted (150 mmol/day)
  - (ii) Sweat and GIT loss in faeces (minor) → lose 10 mmol/day

> Note → See "regulation of ECFV" above for body Na^+ regulation

Function and physiological importance of Na^+:

- (1) Main determinant of ECF osmolality (and ECF osmotic pressure and ECF tonicity) → because Na^+ (along with Cl^-) provides ECF with 90% osmotic solute load
- (2) Main determinant of ECFV → because Na^+ contributes much to ECF osmolality
- (3) Depolarisation in action potential 2° to ↑ Na^+ conductance (in excitable cells)
- (4) Co-transport of substances across membranes (Eg. glucose)
- (5) Involved in Na^+/K^+ ATPase in cell membranes

(II) Potassium:

Distribution of K^+ in body:

- Total body K^+ → 3200 mmol (40-45 mmol/kg):
  - (i) ICF (90%) → 1°ly found in ICF (major IC cation) → [K^+] 150 mmol/L
  - (ii) Bone (8%)
  - (iii) ECF (2%) → [K^+] 3.5-5 mmol/L

> Note:
> - Two main pools of K^+ → (i) Exchangeable K^+ pool (ICF/ECF) → 92%, and (ii) Non-exchangeable pool (bone) → 8%
> - Thus, effectively IC:EC ratio of K^+ = 90%:92% → thus %IC K^+ is 98% and %EC K^+ is 2%

K^+ balance in body:

- Daily K^+ intake → 0.7-0.9 mmol/kg/day (~ 60 mmol/day in 70 kg adult)
- Daily K^+ loss via:
  - (i) Kidneys → achieves body K^+ balance by excreting any excess K^+ not lost via sweat/GIT losses

        - Glomerular filters 900 mmol K^+/day
        - Tubular system reabsorbs nearly everything → PCT 55%, TAL of LoH 30%, CCD type A IC cell 10%, MCD 5%
        - Principal cell of CCD/LDCT secretes 0-15% depending on body K^+ balance (chiefly regulated by Aldosterone):
            - If excess, secretes up to 30% filtered K^+ (300 mmol/day)
            - If in loss, secretes only 5% of filtered K^+ (50 mmol/day)
            - Normally, secretes 10-20% filtered K^+ (100-200 mmol/day)
    - (ii) Sweat and GIT loss in faeces

> Note → See "renal physiology" for body K^+ regulation

Function and physiological importance of K^+:
- (1) Main determinant of ICF osmolality (and ICF osmotic pressure and ICF tonicity) → because K^+ is the main osmotic solute intracellularly
- (2) Regulates membrane potential (RMP) of excitable cells
- (3) Neuromuscular excitability → as determined by RMP
- (4) Role in action potential → repolarisation phase during AP
- (5) Secretion of insulin
- (6) Regulation of some IC processes (protein/glycogen synthesis)
- (7) Involved in Na^+/K^+ ATPase in cell membranes

(III) <u>Magnesium</u>:

Distribution of Mg^{2+} in body:
- Total body Mg^{2+} → 1000 mmol (12 mmol/kg):
    - (i) Bone (60%)
    - (ii) ICF (40% → esp organs/muscles)
        - Mg^{2+} is mainly an IC cation (cf. ECF)
        - Total IC [ ] 15 mmol/L → only 0.5 mmol/L free/ionised (rest is bound)
    - (iii) ECF (< 1%)
        - Plasma [ ] 0.5-1 mmol/L → 60% free/ionised, 33% protein-bound (esp albumin), 6% complexed (citrate/PO_4)

Mg^{2+} balance in body:
- Daily Mg^{2+} intake → 8-20 mmol/day (min 0.5 mmol/day)
- Daily Mg^{2+} loss → mainly by kidneys (normally 2.5-8 mmol/day)
    - Glomerular filtration of 100 mmol/day → 95% reabsorbed in tubules (PCT 15%, 70% LoH, 10% distal nephron) → 5% excreted
    - Minimal neurohormonal regulation of Mg^{2+} → b/c T_{MAX} of Mg^{2+} transporter near plasma Mg^{2+} levels → thus ↓ reabsorption with ↑ Mg^{2+} filtered (at ↑ plasma [ ])

Function and physiological importance of Mg^{2+}:
- (1) Co-factor in enzyme reactions (as a metallo-coenzyme), esp in PO_4 transfer reactions in formation/utilisation of ATP (Eg. Na^+/K^+ ATPase)
- (2) Energy storage, utilisation, transfer → due to role in formation/utilisation of ATP
- (3) Protein and nucleic acid synthesis → Mg^{2+} stabilises DNA and RNA structure
- (4) Mitochondrial reactions
- (5) Involved in Ca^{2+}/K^+ metabolism
- (6) ↓ membrane excitability (Eg. muscles/nerves)
- (7) ↓ NT release at cholinergic and adrenergic synapses
- (8) Antagonises Ca^{2+} activity (Eg. ECC)

(IV) Calcium:

Distribution of Ca^{2+} in body:

- Total body Ca^{2+} → 25,000 mmol (400 mmol/kg):
    - (i) Readily exchangeable pool (1%) → ECF (esp plasma) → immediate reserve for sudden Δ plasma $[Ca^{2+}]$:
        - Total plasma $[Ca^{2+}]$ ~ 2.12-2.65 mmol/L → ionised $[Ca^{2+}]$ ~ 1.2 mmol/L
        - There are two pools of Ca^{2+} in plasma:
            - (a) Diffusible Ca^{2+} (55%) → 45% free/ionised and 10% complexed (to citrate, carbonate, HPO_4)
            - (b) Non-diffusible Ca^{2+} (45%) → protein bound (esp to albumin) → pH-dependent (↑ binding with ↑ pH)

> Note: Corrected plasm Ca^{2+} → depends on plasma protein level (esp albumin):
> - 0.02 mmol/L correction factor added to measured plasma Ca^{2+} per 1 g/L ↑ plasma albumin (up to 40 g/L)
> - Eg. plasma Ca^{2+} of 1.83 and albumin 25 g/L → 1.83 + (40-25) x 0.02 = corrected Ca^{2+} of 2.13 mmol/L

    - (ii) Poorly exchangeable pool (99%) → in bone/teeth (as hydroxyapatite, phosphates, carbonates) → not available for rapid mobilisation

Ca^{2+} balance in body:

- Daily Ca^{2+} intake → 20 mmol/day (min 5 mmol/day)
- Bone liberates/reabsorbs 500 mmol/day from the "readily exchangeable pool"
- Daily Ca^{2+} loss via:
    - (i) Kidneys (40%) → 2.5-7.5 mmol/day
        - Glomerular filtration of 250 mmol/day → 95% reabsorbed by tubules (PCT 65% with Na^{2+}, TAL of LoH 20%, distal nephron 10%) → 5% excreted
        - Tubular reabsorption ↑ at LoH/distal nephron reabsorption due to (i) PTH and (ii) 1,25-dihydroxy-vitamin D
    - (ii) GIT in faeces (60%) → 6-14 mmol/day

> Note → See PTH, vitamin D and calcitonin regulation of Ca^{2+} in "Endocrine physiology"

Function and physiological importance of Ca^{2+}:

- (1) Mineralisation of bone (as $CaPO_4$ crystals)
- (2) Cell structure → Ca^{2+} is bound to cell surfaces to stabilise membranes and intercellular adhesions
- (3) Membrane excitation → Ca^{2+} influx across Ca^{2+} channels in cell membrane of excitable cells (esp nerve and muscle) causes cell membrane excitation
- (4) Muscle contraction (as part of ECC and Ca^{2+}-induced Ca^{2+} release (cardiac muscle))
- (5) Nerve conduction
- (6) IC 2nd messenger (Eg. part of Gq → PLC → DAG → ↑ IC Ca^{2+})
- (7) Enzyme phosphorylation (Eg. glycogen breakdown)
- (8) Co-factor in coagulation cascade
- (9) Excitation-secretion → Ca^{2+} influx induces secretion of endocrine/exocrine organs (also for NT release)

(V) Phosphate:

Distribution of PO_4^{3-} in body:

- Total body PO_4^{3-} → 25,000 mmol (400 mmol/kg):
  - (i) Bone (85%)
  - (ii) ICF (15% → esp soft tissue)
    - Mainly intracellular → major IC anion
    - Total IC [ ] 100 mmol/L → in organic form (Eg. ATP, ADP, Etc.)
  - (iii) ECF (<1%)
    - Plasma [ ] 0.8-1.45 mmol/L → in inorganic form → Protein-bound (15%), Complexed to Ca^{2+}/Mg^{2+} (45%), Free/ionised as HPO_4^{2-} (55%)

PO_4^{3-} balance in body:

- controlled by PTH => urinary phosphate excretion
- Daily PO_4^{3-} intake → 20-40 mmol/day
- Daily PO_4^{3-} loss via:
  - (i) Kidneys (66%) → 30 mmol/day
    - Glomerular filtration of 180 mmol/day → 90-95% reabsorbed by tubules (PCT 70-85%, distal nephron 20%) → 5-10% excreted
    - Tubular reabsorption controlled by (i) PTH → inhibits PCT Na^+/PO_4^{3-} co-transport (↓ reabsorption), and (ii) Vitamin D → ↑ distal reabsorption
  - (ii) GIT in faeces (33%) → 15 mmol/day

> Note → See PTH, vitamin D and calcitonin regulation of PO_4^{3-} in "Endocrine physiology"

Function and physiological importance of PO_4^{3-}:

- (1) Mineralisation of bone (as $CaPO_4$ crystals)
- (2) Cell structure → phospholipids in cell membrane
- (3) Energy storage as ATP
- (4) Buffer system (Eg. IC and urinary buffers)
- (5) O_2 transport (Eg. in RBC as 2,3-DPG)
- (6) Enzyme regulation

(c) ***To outline the composition and functions of lymph.***

Lymph:

- Lymph refers to ISF within lymphatic vessels

Lymphatic system:

- Lymphatic capillaries are present in nearly all tissues (EXCEPT in cartilage, BM and CNS) → characterised by:
  - (i) being blind-ending
  - (ii) possessing "flap valves" → permit one-way entry of ISF only
  - (ii) low-pressure system (1 mmHg at rest)
- These capillaries drain into → lymph venules and veins → drain into LNs → return to venous circulation via (i) thoracic duct (at junction of left SCV and IJV) and (ii) right lymphatic duct

Lymph production and flow:

- Lymph is formed from ISF due to an excess net efflux of fluid across the capillary wall:
  - At rest – Net capillary filtration at arterial end of capillary is 20 mL/min → BUT only 18 mL/min returns to circulation at venous end of capillary → thus, 2 mL/min of lymph is produced (= 120 mL/hr or 3 L/day of lymph production)

> Note – 10% of fluid filtered at capillaries returns to circulation as lymph!

  - With exercise – ↑ lymph production due to ↑ $P_{HYDROSTATIC}$ in capillary systems and excess fluid filtration
- Lymph flow is dependent on two factors:
  - (1) Generation of a +ve pressure gradient within lymphatic vessels via:
    - (i) Lymph vessel wall SM contraction
    - (ii) External +ve pressure → caused by muscle and intestinal contraction, and neighbouring arterial vessel pulsations
    - (iii) External –ve intrathoracic pressure in thorax
  - (2) One-way valves to prevent retrograde flow into interstitium → via valves within lymphatic vessels and "flap valves" at entry points into lymphatic system
- Total lymph flow is 120 mL/min (= lymph production rate) → 80% of it re-enters circulation via thoracic duct (100 mL/hr) and 20% enters via right lymphatic duct

Contents of lymph:

- Its contents are the SAME as ISF (as it is derived from it) → noteworthy points are:
  - (i) ↓ protein content (cf. plasma)
    - Generally 20 g/L of protein (similar to ISF)
    - EXCEPT in thoracic duct lymph (↑ protein content ~ 50 g/L) → due to protein-rich hepatic lymph that contributes 50% of total body lymph flow
    - Hepatic lymph is protein-rich (60 g/L) because → hepatic sinusoids are very permeable (↓ σ)
  - (ii) Contains all CFs except has ↓↓↓ fibrinogen content (cf. plasma)
    - Fibrinogen is large (340kDa) → difficult to cross capillary membrane
    - BUT lymph can still clot due to presence of CFs
  - (iii) Contains ↑↑↑ fat content
    - Due to TG/cholesterol within chylomicrons → from lymph draining bowel after a fatty meal
    - Causes lymph to appear "milky"
  - (iv) Contains bacterial and cell fragments

Functions of lymph and the lymphatic system:

- (1) Return of excess fluid/protein to circulation from ISF → prevents interstitial oedema

    - Keeps low ISF protein [ ] at 20 g/L → maintains oncotic pressure gradient across capillary membrane → prevents interstitial pressure from ↑↑↑
    - This effectively prevents net fluid accumulation in ISF
- (2) Role in fat transport form small intestine
    - 90% of fat extruded from bowel epithelial cells enters into ISF via central lacteal vessels in the intestinal villi
    - Fat is incorporated in chylomicrons for transport in lymph ("milky" appearance)
- (3) Immunological role
    - Filter/remove bacteria by macrophages in LN (reticulo-endothelial system)
    - Promotes lymphocyte circulation throughout blood and lymph
    - Activation/proliferation of lymphocytes in LN in response to Ag in lymph

(d) ***To define osmotic pressure and to explain the factors that determine it.***

Definition of osmotic pressure:

- The $P_{HYDROSTATIC}$ required to oppose movement of solvent (such as H_2O) across a semi-permeable membrane along its thermodynamic activity gradient
- This thermodynamic activity gradient is established by a $P_{OSMOTIC}$ gradient across the membrane → determined by the difference in osmolality (specifically "effective" osmolality or tonicity) of the two solutions across the membrane
- It is a type of "colligative property" → meaning that it is a property of solution that is dependent only on particle [ ] (# particles per unit volume) in solution (Ie. osmolality)

> Aside – "Colligative properties" of a solution:
> - Properties of a solution that depend only on the particle concentration (Ie. osmolality) → meaning # particles per unit volume → and NOT the type or nature of the particles!
> - Types of colligative properties – (i) Osmotic pressure, (ii) Vapour pressure depression, (iii) Freezing point depression, (iv) Boiling point elevation

Explanation of osmotic pressure:

- Imagine two solutions separated by a semipermeable membrane where → (i) "Reference solution" containing only H_2O, (ii) "Test solution" containing dissolved particles, and (iii) membrane is only permeable to H_2O (and not the dissolved particles)
- Dissolved particles in the "test solution" are "effective" osmoles → contribute to ↑ "effective" osmolality (which in this case = "total" osmolality) → establishes "osmotic gradient" across membrane (as the H_2O-only "reference solution" has zero osmolality)
- H_2O diffuses passively across membrane via osmosis from "test solution" → "reference solution" until the osmotic gradient dissipates (Ie. both solutions have = osmolality)
- Applying $P_{HYDROSTATIC}$ to the "test solution" will prevent osmosis of H_2O from the "reference solution" → the $P_{HYDROSTATIC}$ at which there is no net H_2O movement across the membrane is EQUAL to the $P_{OSMOTIC}$ of the "reference solution" (which is a measure of the solution's osmolality)

> Aside: "Osmosis" is defined as:
> - A passive process by which a solvent (such as H_2O) moves across a semi-permeable membrane due to a thermodynamic activity gradient for that solvent → this gradient is established by a $P_{OSMOTIC}$ gradient across the membrane
> - Causes solvent to flow from a solution of ↓ $P_{OMSOTIC}$ (hypotonic solution) to one of ↑ $P_{OSMOTIC}$ (hypertonic solution) until the $P_{OSMOTIC}$ gradient dissipates

Determinants of osmotic pressure:

- $P_{OSMOTIC}$ is calculated by the "van't Hoff equation" → states that $P_{OSMOTIC}$ is directly proportional to – (i) # of particles the substance dissociates into, (ii) [ ] (or molarity) of solution, and (iii) temperature – and inversely proportion to the MWT of the molecules

$$\text{Osmotic pressure } (\pi) = n \times (c/M) \times RT$$

n = # particles into which substance dissociates
c = [ ] (in g/L)
M = MWT of molecules
R = Universal Gas Constant (= 0.082)
T = Temp. (in K)

> Note: 1 mol of any solute dissolved in 22.4 L of solvent at 0°C → generated $P_{OSMOTIC}$ = 1 atm

- In a mixed solution → Total $P_{OSMOTIC}$ = ∑ of individual $P_{OSMOTIC}$ of each solute in solution

Osmotic pressure of plasma:

- Total osmotic pressure of plasma is **5545 mmHg (or 7.3 atm)** at 37°C (given a plasma osmolality = 287 mosm/kg) → as determined by van't Hoff equation
- Note – 99% of total osmotic pressure is due to electrolytes dissolved in plasma (while only 0.5% is due to plasma proteins (see colloid osmotic pressure below))

(e) ***To outline the significance of oncotic pressure, colloid osmotic pressure and reflection coefficients.***

(I) Oncotic (or colloid osmotic) pressure:

Definition of oncotic pressure (or colloid osmotic pressure):

- Defined as the component of total osmotic pressure (or total osmolality) of a solution that is due to colloids dissolved as a solute in solution

> Aside → "Colloids" are defined as large MWT (> 30kDa) particles

Plasma oncotic (or colloid osmotic) pressure:

- Colloids in plasma (mainly plasma proteins) contribute an osmotic pressure in plasma of ~ 25-28 mmHg → Note that this is a very SMALL fraction (0.5%) of total osmotic pressure of plasma (5545 mmHg)!
- Factors determining plasma oncotic (or colloid osmotic) pressure:
    - (1) Plasma proteins (15-19 mmHg → main determinant):
        - (a) Albumin → main contributor to plasma oncotic pressure (65-75%) due to → (i) ↑ plasma [ ], (ii) ↓ MWT, and (iii) –ve charge ("Donnan excess pressure")
        - (b) Globulins (intermediate contributor → ↓ plasma [ ] and ↑ MWT)
        - (c) Fibrinogen (minor contributor → ↓↓ plasma [ ] and ↑↑ MWT)
    - (2) Gibbs-Donnan effect (7-8 mmHg)
        - -ve charged plasma proteins are a non-diffusible ion that causes net retention of Na^+ in plasma at Gibbs-Donnan equilibrium
        - This leads to ↑ # of particles in plasma → ↑ plasma osmolality (by 0.4 mosm/L) → ↑ $P_{ONCOTIC}$ of plasma ("Donna excess pressure")
    - (3) Excluded volume effect (minor)
        - Refers to the effect of large size of protein which occupies a large volume in plasma → responsible for discrepancy in colloid osmotic pressure as calculated by van't Hoff equation

> Aside → Calculating plasma colloid osmotic pressure using van't Hoff equation (using plasma protein [ ] alone) produces a $P_{OSMOTIC}$ of only 15-19 mmHg → rest of $P_{OSMOTIC}$ due to (i) Gibbs-Donnan effect and (ii) Excluded volume effect

- Low plasma oncotic pressure is VITAL in transcapillary fluid dynamics:
    - Since capillary membranes are largely impermeable to proteins (but permeable to most other solutes) → proteins are the only solutes in plasma that can effectively retain H_2O within intravascular space of capillaries
    - Thus, they have a significant role in maintaining circulating volume!

> Aside: ↓ plasma oncotic pressure (Ie. due to loss of albumin)
> - Consequences of ↓ plasma oncotic pressure:
>   - ↑ H_2O loss from capillaries due to ↑ net filtration into ISF (↑ NFP) → results in ↓ IVV and ↓ ECFV → ↓ CVS $P_{HYDROSTATIC}$ (and MAP)
>   - This is detected by low- and high-pressure baroreceptors (arterial, venous, cardiac) → response is net Na^+/H_2O retention to offset change in IVV
>   - BUT this leads to ↑ net filtration into ISF and ↑ ISFV → interstitial oedema occurs ONLY when plasma oncotic pressure < 11 mmHg (occurs at plasma albumin < 20 g/L) → this means a LARGE safety margin against oedema (cf normal pressures of 25-28 mmHg!)
> - Factors that protect again interstitial oedema:
>   - (i) ↑ lymph flow → return excess ISF back to systemic circulation
>   - (ii) ↑ tissue $P_{HYDROSTATIC}$ 2° to ↑ ISFV → retards net filtration (↓ NFP)
>   - (iii) ↓ ISF [protein] 2° to ↓ albumin leaking out of capillaries → ↓ $\pi_{INTERSTITIUM}$ → retards net filtration (↓ NFP)

Measuring oncotic pressure (or colloid osmotic pressure):

- "Oncometer" is used to measure oncotic pressure:
    - Two chambers separated by semi-permeable membrane (permeable to everything BUT colloids → so colloids are only solutes present that can exert an osmotic force across membrane) → (i) "reference chamber" contains isotonic saline, (ii) "test chamber" contains test solution
    - IF the test solution has colloids → H_2O diffuses across membrane into test solution via osmosis → Δ pressure test chamber is measured by a pressure transducer, which effective measures the oncotic pressure!

(II) <u>Reflection coefficient</u>:

- "Reflection coefficient" is a correction factor applied to measured oncotic pressure gradient ($\pi_{CAP} - \pi_{ISF}$) across capillary wall
- It accounts for the "ineffectiveness" of some oncotic pressure gradient due to protein leakage across the capillary membrane (Ie. 2° to ↑ capillary permeability or "leakiness") → causes protein to no longer act as an "effective" osmole (Ie. cannot generate osmotic force or pressure)

$$\text{"Reflection coefficient"}\ (\sigma) = \frac{\#\ \text{particles unable to cross membrane}}{\text{Total \# of particles}}$$

If all particles reflected by membrane → $\sigma = 1$ (Eg. brain, glomerulus)
If none reflected by membrane → $\sigma = 0$ (Eg. liver)

Note:

- ↑ σ occurs due to ↑ $P_{OSMOTIC}$
- ↓ σ occurs due to capillary damage (Eg. sepsis, inflammation)

(f) ***To describe the measurement of osmolality and the control mechanisms involving the regulation of osmolality.***

(I) Mole vs Osmole:

- Mole:
  - A mole is a SI unit for the amount of substance that contains # of molecules equal to Avogadro's number (6.02×10^{23})
  - Note → mass (in grams) of a mole of a substance = atomic mass unit (or MWT in grams) of the substance
- Osmole:
  - An osmole is 1 mole of a substance containing Avogadro's # of osmotically active particles → it is independent of the nature of particle present (Eg. type, size, weight, Etc.), such that many different particles types may be present
  - For example – 1 mmol glucose contributes 1 mosm in solution, while 1 mmol NaCl contributes 2 mosm in solution (due to dissociation into Na^+ and Cl^-)
  - It is measured by "freezing point depression" → 1 mol of an "ideal" solution depresses freezing point by 1.86 deg K → thus, "x" mosm/L = freezing point depression/0.00186

> Aside – There are two types of osmoles:
> - "Effective" osmoles:
>   - Substances capable of exerting an osmotic force across a cell membrane b/c they cannot cross it easily (most solutes such as Na^+, Cl^-, Ca^{2+})
>   - Thus → ↑ osmolality (and tonicity) of solution containing them → creates $P_{OSMOTIC}$ gradient → results in osmosis of H_2O from ↓ to ↑ $P_{OSMOTIC}$ until the gradient dissipates
> - "Ineffective" osmoles
>   - Substances incapable of exerting an osmotic force across a cell membrane b/c the can cross it easily (Eg. urea)
>   - Thus → substance diffuses across membrane until its [ ] gradient is equal in both solutions → so the osmolality of both solution ↑ proportionately and no $P_{OSMOTIC}$ gradient is generated (Ie. both solutions isotonic) → no osmosis of H_2O

(II) Molality vs Molarity:

- Molality:
  - Defined as the number of moles of solute per kg solvent (mol/kg) → independent of temperature and Δ solvent volume (due to Δ temperature)
  - Represents the # particles of a particular substance present in a given weight of solvent
- Molarity:
  - Defined as the number of moles of solute per L of solvent (mol/L) → dependent on temperature (due to Δ solvent volume with Δ temperature)
  - Represents the # particles of a particular substance present in a given volume of solvent

(III) Osmolality vs Osmolarity:

- Osmolality:
  - Defined as the number of osmoles of solute per kg solvent (Osm/kg) → independent of temperature and Δ solvent volume (due to Δ temperature)
  - Represents the # osmotically active particles present in a given weight of solvent → it is (i) independent of the nature of the particle (Ie. there can be several different types of particles present in an osmole), and (ii) a measure of ALL solute particles in solution (Ie. both "effective" and "ineffective" osmoles → cf. tonicity which only measures former)
  - Forms basis of "Colligative property" of a solution (See above)

ECF and ICF osmolality:
- ECF osmolality = 285-290 mOsmoles/kg → determined 1°ly by ECF Na^+ content (See above) → urea and glucose contributes a small amount (3%) only
- ICF osmolality is SAME as ECF osmolality → because H_2O easily diffuses across the membrane to dissipate any $P_{OSMOTIC}$ gradient between ICF and ECF!

Regulation of ECF/plasma osmolality:
- ECF/plasma osmolality is determined by <u>ECF Na^+ contents</u> (see reasons above) → it is thus determined by mechanisms that regulate ECF Na^+ → relies on <u>sensing Δ ECFV and regulating it</u> (See "Regulation of ECFV" above)
- It is also regulated by ADH/thirst response that <u>modulates TBW content according to plasma osmolality</u> (See "Regulation of TBW content" above)

Measuring serum osmolality:
- (i) "Osmometer" in lab
- (ii) Calculating sum of plasma solute [ ] = 2x [Na^+] + [glucose] + [urea] = 2x140 + 5 + 5 = 290 mosm/kg
- Nb. "Osmolar gap" = difference between calculated and measure osmolality

- Osmolarity:
  - Defined as the number of osmoles of solute per L of solvent (Osm/L) → dependent on temperature (due to Δ solvent volume with Δ temperature)
  - Represents the # osmotically active particles present in a given volume of solvent → like osmolality, it is (i) independent of the particle's nature (Ie. can be several types of different particles in an osmole) and (ii) measures all solutes in solution (Ie. both effective and ineffective osmoles)

Important to note – Since a L of H_2O = 1 kg → osmolality and osmolarity for dilute solutions ALMOST the same!

(IV) <u>Tonicity</u>:
- Definition → A measure of "effective" osmolality of a solution (Ie. part of total osmolality due to "effective" osmoles) → in other words, a measure of only the particles in solution that can exert an osmotic force (and produce an osmotic pressure gradient) across a semi-permeable membrane
- It is often defined in relation to another fluid across a membrane:
  - (i) Isotonic (both fluids have same tonicity or "effective" osmolality)
  - (ii) Hypertonic (↑ tonicity or "effective" osmolality cf. other fluid)
  - (iii) Hypotonic (↓ tonicity or "effective" osmolality cf. other fluid)
- Implication → determines H_2O distribution across a cell membrane (Ie. whether EC solution will cause cell to Δ volume) as → H_2O flows across a membrane via osmosis from a hypotonic solution (↓ $P_{OSMOTIC}$) across a semi-permeable membrane to a hypertonic one (↑ $P_{OSMOTIC}$) until both solutions are isotonic (no $P_{OSMOTIC}$ gradient)
- Measuring serum tonicity:

Plasma tonicity = (Plasma osmolality) – [urea] – [glucose]

Nb. Urea and glucose are "ineffective" osmoles present in any significant [ ] in plasma)

Aside: Notable clinical relevance of tonicity
- Eg 1. Urea is isotonic to most cells in body as it is permeable to their membranes (Ie. an ineffective osmole) and does not cause any H_2O transfer → EXCEPT for brain cells for which it is hypertonic to as urea is impermeable to BBB (Ie. now an effective osmole) → generates an osmotic pressure gradient across it and causes H_2O transfer out of brain → thus hypertonic urea used to treat ↑ ICP!
- Eg 2. In DM patients, glucose is hypertonic (Ie. an effective osmole) to insulin-sensitive cells (fat/muscle) that cannot take up glucose → generates osmotic pressure gradient that draws H_2O out of these cells → cause dehydration as fat/muscle is large % of body!

Aside: Sweat

Overview of sweat:

- Sweat is the secretion from eccrine sweat glands in the skin → involves the loss of water, electrolytes (esp Na^+) and heat
- Main role → heat loss in situations of heat stress

Insensible water loss from skin vs sweating:

- Sweat differs from "insensible water loss from skin" as follows:
    - (1) IWL is solute-free and only water is lost (cf. sweat contains electrolytes)
    - (2) IWL from skin involves water that has diffused through skin (transepidermal diffusion) and is lost by evaporation from skin surface (cf. sweat is produced from eccrine sweat glands)
    - (3) IWL cannot be prevented (minimal IWL from skin is 450 mL)

> Remember → Total IWL = 450 mL from skin + 450 mL evaporative water loss from respiratory tract during ventilation = 900 mL total

Control of sweating:

- Thermosensitive neurons (sense core body temperature) → send afferent signals to hypothalamus → innervate eccrine sweat glands (distributed over 99% skin surface) via sympathetic cholinergic (muscarinic) nerves

Contents of sweat:

- H_2O loss from sweating is ~ 50 mL/day → varies (depending on environment) with maximal rate of 1.5-2 L/hr (or 12 L/day)!
- $[Na^+]$ ~ 30-65 mmol/L → varies from 5-350 mmol lost/day → Na^+ loss is ↓ with aldosterone
- H_2O and Na^+ lost in sweat depends on "acclimatisation" of the subject (adaptive changes that occur over period of time in sweat mechanisms when moving from cold to hot climate) such that:
    - (i) Maximal rate of sweating increases markedly
    - (ii) $[Na^+]$ decreases markedly
    - Note → this allows maximal heat loss without extreme loss of solutes