

Unit 4– Cerebrospinal fluid (CSF)

**Session 12 – Collection,
composition, guidelines in handling,
processing and transport of CSF.**

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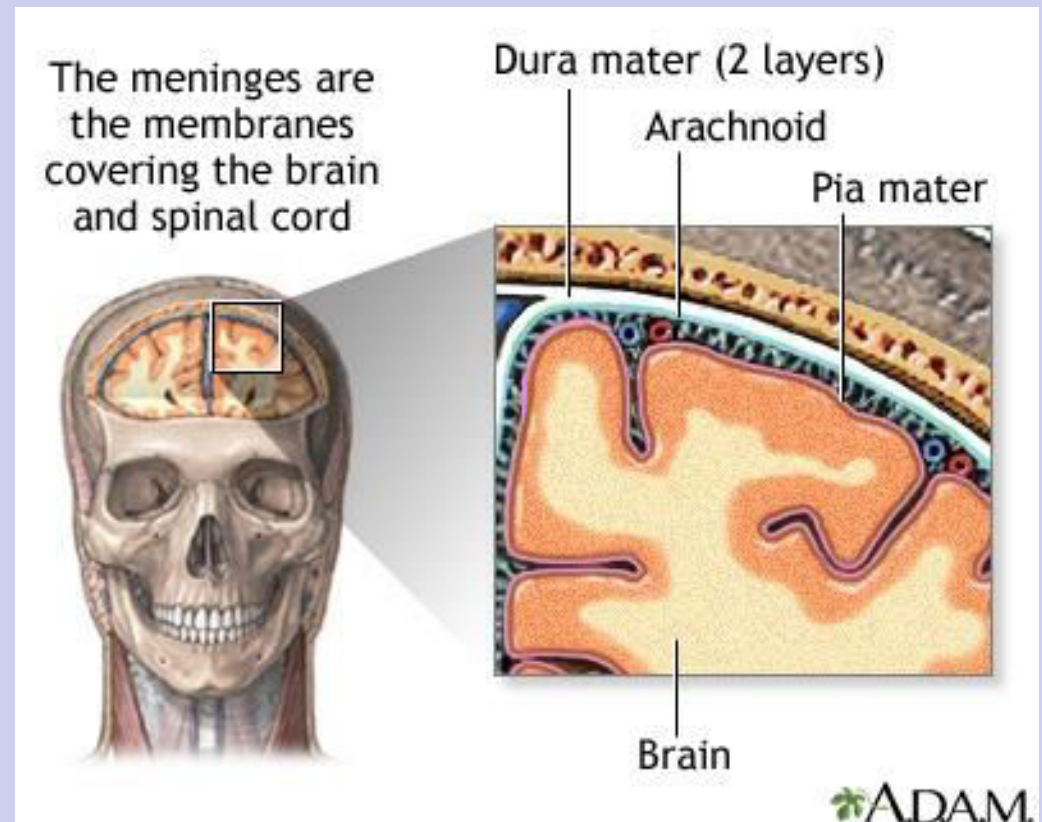
Introduction

- Diagnosis of diseases of the central nervous system
- Specimen collection procedure- Lumbar puncture
- Need sterile screw-capped specimen containers
- Should sent laboratory promptly

- Clues to underlying disease-
 - Variations of the CSF composition and / or
 - presence of abnormal constituents including organisms in the CSF

12.1 Formation of CSF – structural aspects

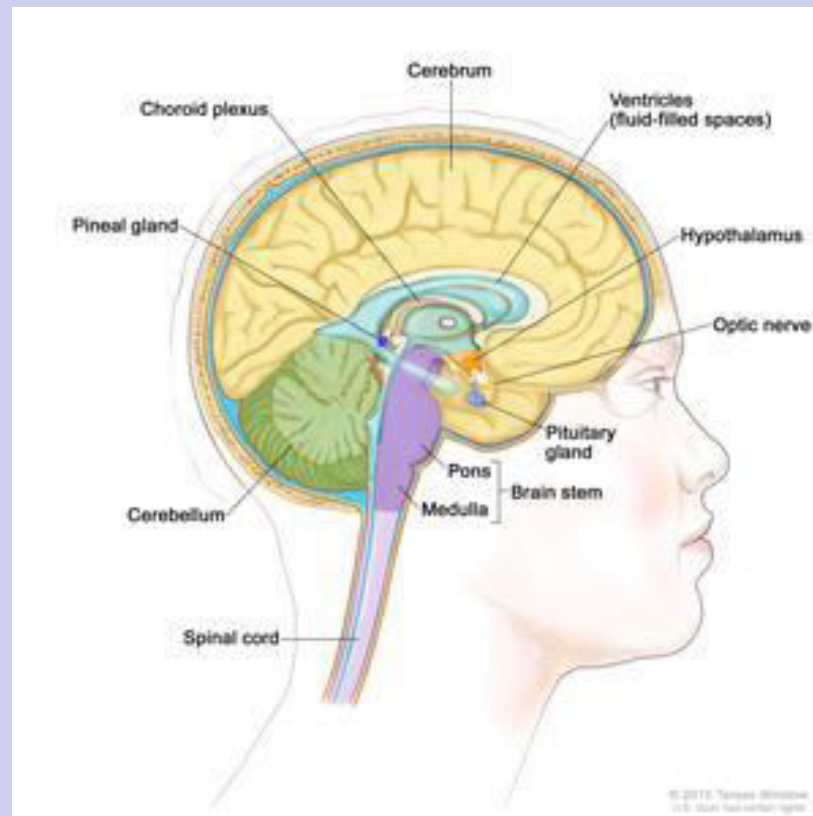
- Protective membranes that cover brain and the spinal cord: meninges
 - pia mater – *innermost covering*
 - arachnoid mater – *middle covering*
 - dura mater – *outermost covering.*



- **Subarachnoid space**

- space beneath the arachnoid membrane and above the pia mater
- contains CSF

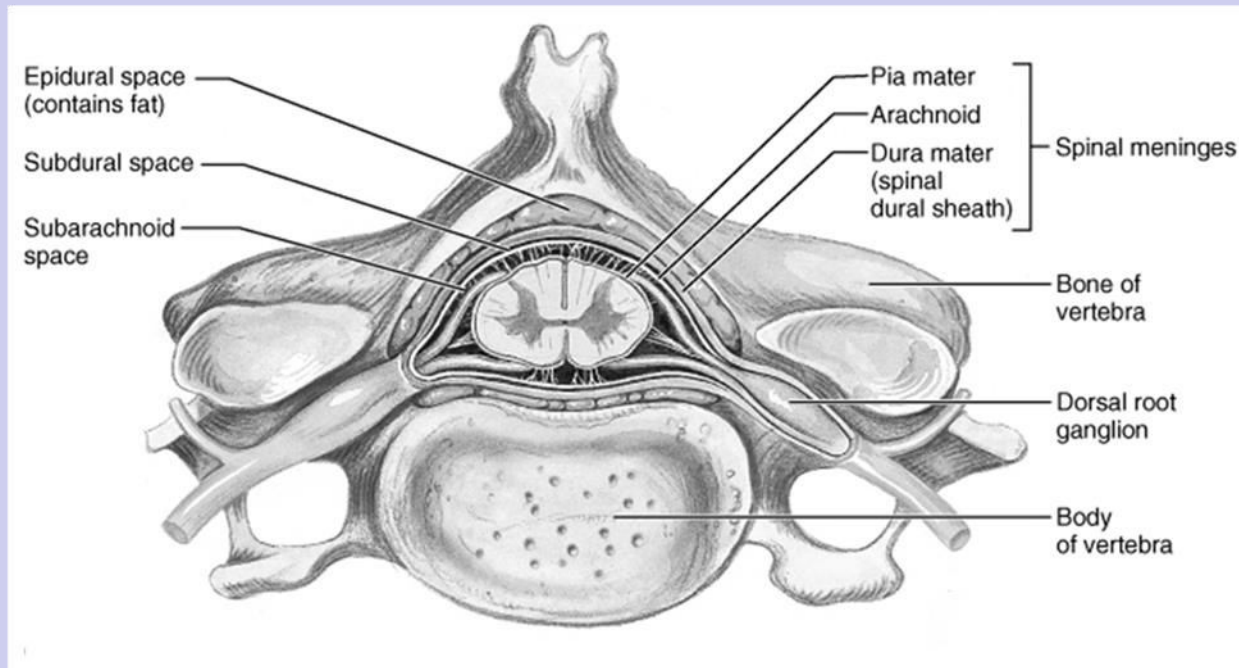
- Cerebrospinal fluid is formed in the brain, mainly in the **choroid plexus**

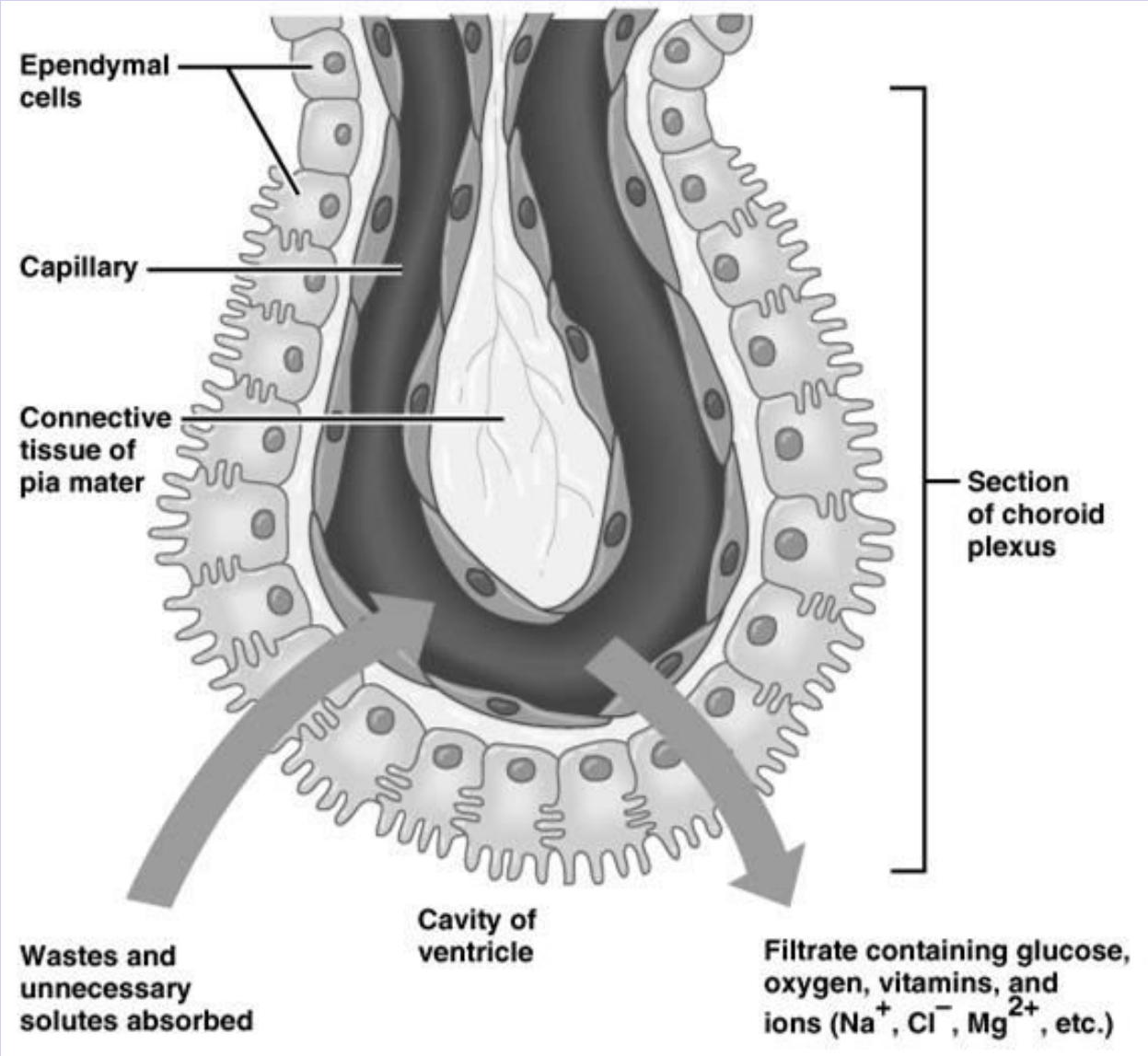


choroid plexus

- vascular network of capillaries project into each of the four ventricles of the brain

Figure 2. Transverse section across a vertebra revealing the spinal cord with the three meninges in cross-section.





Formation of CSF and moving out

https://www.youtube.com/watch?v=okD6_k9

[Xosk](#)

- in the adult -100mL to 150mL of CSF, in continuous turnover
- pathway

12.2 Functions of CSF

1. Protects the delicate nervous tissues from mechanical shock
 - a fluid cushion between brain substance and the rigid skull bone.
 - fluid cushion –
 - » a shock absorber -minimises the effects of mechanical shock
2. Provides buoyancy
 - brain floats in the CSF by supporting its weight

3. Nourish the cells of the CNS
 - second circulatory fluid -deliver oxygen and nutrients to the nervous tissue

12.3 Lumbar puncture to tap CSF

- Procedure done in the hospital by the doctor

12.3. a. Indications for lumbar puncture

1. Suspected CNS infection/ meningitis
2. Suspected subarachnoid hemorrhage
3. Therapeutic reduction of CSF pressure
4. Sampling of CSF for any other reason

Ex. Suspicion of central nervous system (CNS) diseases such as [Guillain-Barré syndrome](#) and carcinomatous meningitis

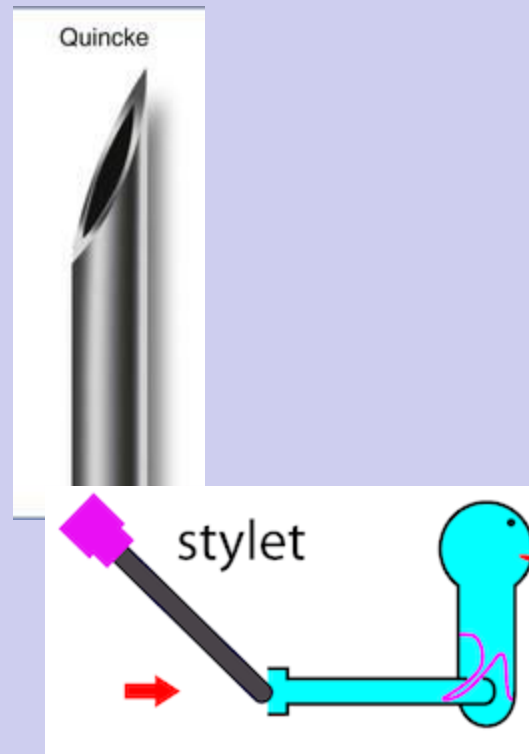
12.3. b. Contraindications for lumbar puncture

1. Local skin infections over the proposed puncture site
2. Raised intracranial pressure
3. Suspected spinal cord mass lesion or intracranial mass lesion
4. Uncontrolled bleeding tendency
5. Spinal column deformities
6. Lack of patient cooperation

12.3. c. Materials required for lumbar puncture

Lumbar puncture tray -
20-gauge or 22-
gauge spinal (Quincke)
needle with stylet

prep solution,
manometer, drapes,
and the local
anesthetic.



- The laboratory should provide
 - **sterile screw-cap CSF specimen containers**
appropriately numbered

Container Number	Purpose
1	Appearance and chemistry (e.g. glucose, protein, protein electrophoresis)
2	Microbiology (e.g. Gram's stain, bacterial and viral cultures)
3	Second CSF specimen for Microbiology
4	Microscopy (e.g. cell count and differential cell count).

12.3. d. Pre-procedural interaction with patient

- develop a rapport with the patient or the patient's caregiver
- obtain informed consent
- let the patient know of
 - possibilities of complications
 - bleeding, persistent headache, infection
 - how these complications would be taken care of
- informed of the
 - major steps of the procedure
 - positioning of patient
 - post-procedural care

12.3. e. Lumbar puncture to collect CSF

12.4 Composition of lumbar CSF in adults

- Protein-poor plasma filtrate

Appearance	Clear, colourless, sterile watery
Total solid dissolved	0.85 – 1.70 g/dL
Specific gravity	1.006 – 1.008
pH	7.35 – 7.40
Cellular elements	0 – 8 lymphocytes/mm ³ devoid of erythrocytes and neutrophils

Total protein concentration	80 – 320 mg/L
Predominant protein	Albumin 50 – 70% (100 – 300 mg/L)
	Does not contain fibrinogen, no clotting activity
Has all the globulin types	α 1 globulin = 3 – 9% α 2 globulin = 4 – 10% β globulin = 10 – 18% γ globulin = 3 – 9%

- CSF is a nutritive medium

Nutrient	Composition
Electrolytes	Na ⁺ = 136 – 150 mmol/L K ⁺ = 2.5 – 3.2 mmol/L Cl ⁻ = 118 – 132 mmol/L
Glucose	2.22 – 3.89 mmol/L
Amino acids	glutamine predominates (6 – 16 mg/dL)
Minerals	Fe, Mg

- Excretory compounds- small amounts
 - Urea
 - Uric acid
 - Metabolic waste (e.g. lactate) and
- Other compounds of importance
 - Immunoglobulins
 - hormones.

12.5 Guidelines in processing, handling and transport of CSF

- Considerations
 - critically ill patient
 - invasive procedure
 - ultimate measure towards arriving at a definitive diagnosis
 - hand delivery
 - prior notification
 - Immediate processing
 - potentially bio-hazardous
 - only a small volume is available
 - do not require pre-processing

- Special considerations
 - photo-labile chromogenic substances such as bilirubin then the CSF specimen container (container number 1) must be wrapped in a dark paper to keep the light out

12.6 Deterioration of CSF on standing

- Reasons to avoid delaying of testing
 1. Lowering of the CSF glucose concentration with time
 2. Disintegration of cells such as leukocytes
 3. Lysis of bacteria such as meningococci
 4. Death of *Neisseria meningitides* and *Neisseria gonorrhoea* when CSF is in contact with air.

12.7 CSF, pre-analytical short-term storage

- Immediate processing RECOMMENDED
- If an inadvertent delay
 - container 1 for chemistry (sugar, protein) can be kept under refrigeration
 - container 2 **must not** be refrigerated, must be kept in an incubator at a temperature 35°C to 37°C
 - specimens for virology
 - refrigerated up to 24-hours
 - frozen at -70°C for longer periods

Thank you!!!!!!

Unit 4– Cerebrospinal fluid (CSF)

Session 14 – Cytological Examination of CSF and Cytological Changes in CSF in Disease

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Introduction

- Usually devoid of cells.
- Only a few lymphocytes (about $5 - 8 \text{ ml}^{-3}$)
- Erythrocytes and leukocytes appear in the CSF due to haemorrhage
- Leukocytes present due to
 - infections : pathogenic bacteria, Fungi, Viruses
 - offensive agents: toxic heavy metals and chemicals.

- Leukocytes present in pathological CSF specimens
 - lymphocytes (elevation)
 - Monocytes
 - Neutrophils
 - Eosinophils
 - Basophils
- Polymorphonuclear leukocytes

- The basic diagnostic microscopic tests performed with pathological CSF - total leukocyte count, enumeration of the differential leukocyte count
- Similar to WBC/DC in blood

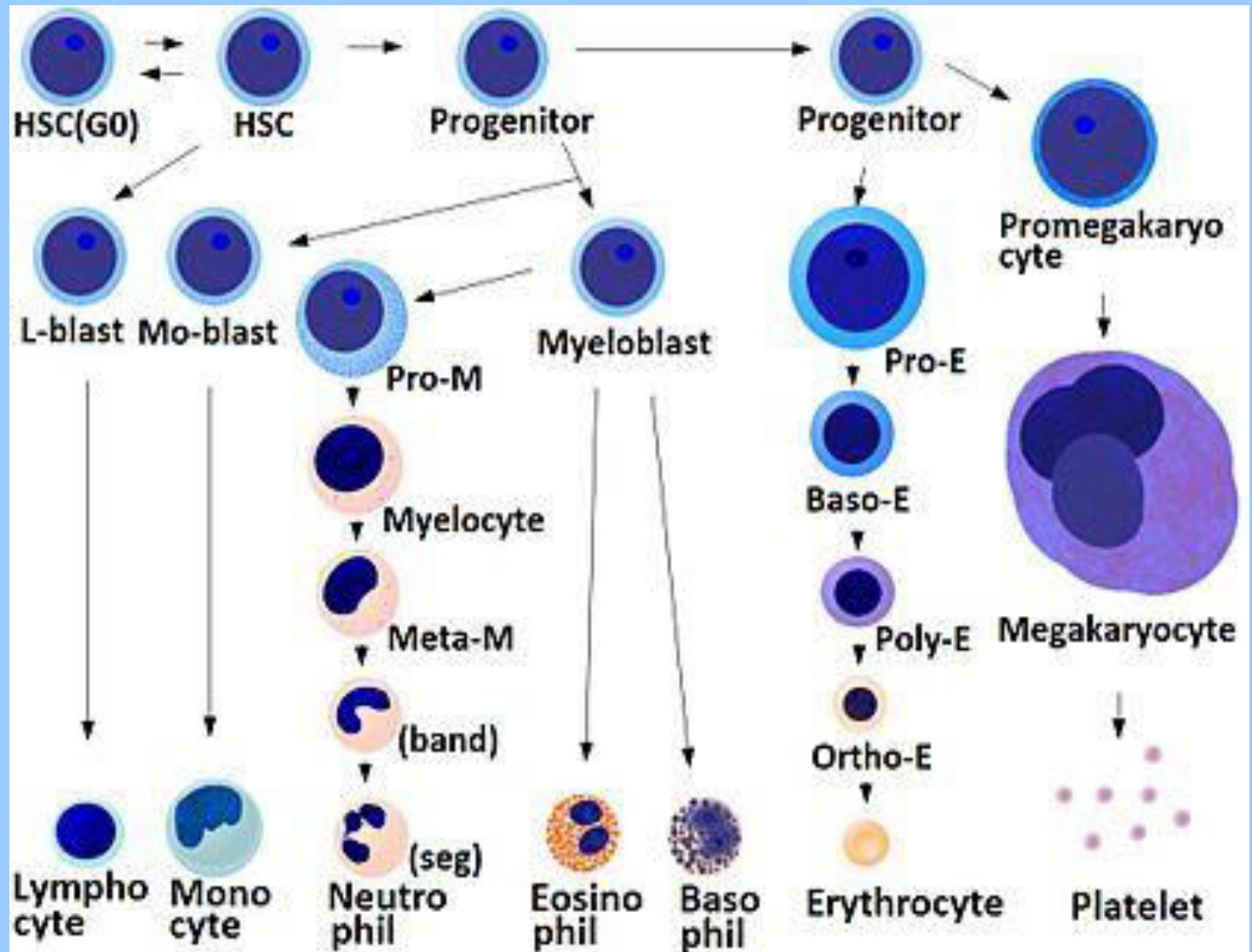
Cells in CSF : four categories

- Mature peripheral blood cells
- Immature hematopoietic cells
- Tissue cells
- Malignant cells

- Increased polymorphonuclear leukocytes : bacterial infection
- Lymphocyte elevations in different degrees: viral and fungal infections
- Precise laboratory diagnosis of infections of the CNS
 - leukocyte number concentration + differential leukocyte count + CSF appearance+ glucose concentration+ protein concentration

- Malignant cells are derived from: primary neural and extra-neural cancers
- Purposes of Cytological examination of CSF
 - diagnosis of neural and extraneural malignancies
 - establish the spread of cancer into the structures of the brain
- Malignant cell cytology- steps
 1. Concentration of malignant cells
 2. Spreading of a portion of the cell pellet on a slide
 3. Cell fixation
 4. Cytostaining
 5. Malignant cell identification and interpretation by a Cytopathologist.

14.1 Morphology of leukocytes and erythrocytes in CSF



Characteristic microscopic appearance of blood cells in the CSF

1. Lymphocytes

- small or large
- small lymphocyte
 - Diameter: 9 - 12 μ m
 - Nucleus: round with heavily clumped chromatin
 - Cytoplasm: scanty.
- large lymphocyte
 - Diameter: 12 - 16 μ m

2. Monocytes

- Diameter: 15 - 20 μ m
- kidney-shaped irregular nuclear shape
- cytoplasm : abundant, stains dull gray blue.

- Neutrophil
 - Diameter 15 - 20 μ m
 - Nucleus lobed, 2 – 5 lobes
 - Nuclear chromatin: dark purple clumps
 - Cytoplasm: numerous, fine, evenly distributed purplish granules



14.2 Determination of leukocyte number concentration

- Similar as WBC count in blood
- The counting must begin as the CSF sample arrives in the laboratory.
- **Delay in analysis** causes **disintegration of leukocytes.**

Correction of leukocyte count for contaminating blood in traumatic tap:

- CSF WBC count falsely elevate in a traumatic tap

$$\text{WBCs added in traumatic tap} = \text{WBC (blood)} \times \frac{\text{RBC (CSF)}}{\text{RBC (blood)}}$$

True CSF leukocyte count=

actual CSF WBC count - accidentally introduced WBCs

- When the patient's peripheral WBC and RBC counts are within normal limits true CSF leukocyte count calculation

Subtract one white cell from the CSF WBC count for every 750 RBC counted.

14.3 Determination of differential leukocyte count

- Concentrate the sample
- If the CSF is very cloudy or bloody,
 - run on an automated cell counter white cell count or
 - dilute, cytocentrifuge, and then stain
- **Flow-cytometry**
 - Detection, characterisation and enumeration of peripheral blood cells and cancer cells in CSF
 - Cell size and cellular granularity
 - Need intact and viable cells
 - Dead cells can falsify result interpretation by non-specific binding to monoclonal antibodies and emitting false fluorescent signals and falsely elevating cell counts.
 - Test without a delay

Safety Precautions

- Treated as potentially biohazardous
- Contaminated reusable pipettes, haemocytometer and coverslip must be soaked in 70% alcohol
- Hand washing
- Spinal fluids & disposable items should be placed in a biohazard container

14.4 Cells in pathological CSF

- Presence of erythrocytes in the CSF
- Elevation of lymphocytes
- Presence of polymorphs
- Presence of monocytes

14.5 Types of leukocytes differ in meningitis of different aetiologies

1. Polymorphs

- in pyogenic meningitis
- causative organisms are the bacteria,
 - *Neisseria meningitidis*
 - *Haemophilus influenza*
 - Pneumococci
 - Streptococci
 - Staphylococci
 - Coliforms

2. Presence of Neutrophils, lymphocytes and monocytes together - 'mixed reaction'

- Occur in
 - tuberculous meningitis
 - subacute bacterial meningitis
 - mycotic (fungal) meningitis
 - viral meningo-encephalitis

3. Monocytes and / or lymphocytes

- syphilitic meningitis
- tuberculous meningitis
- mycotic meningitis
- viral meningo-encephalitis.

14.6 Leukocyte count in pathological CSF

- Differential diagnosis of disease

Category		Example
Normal		Subdural haematoma
Normal or elevated		Presence of brain tumour, spinal cord tumour and multiple sclerosis
Number concentration and Cell type	500 – 20,000mm ⁻³ polymorphs	Acute purulent meningitis

Number concentration and Cell type	10 – 500 mm ⁻³ Lymphocyte	Tuberculous meningitis
	25 – 2000mm ⁻³ Lymphocyte	Early acute syphilitic meningitis
	>2000 mm ⁻³	Viral meningoencephalitis

14.7 Variations of CSF constituents in bacterial, viral and fungal infections

Pathogen	CSF Appearance	CSF Glucose (mmol.L ⁻¹)	CSF Protein (mg.dL ⁻¹)	CSF Cells per mm ³
Bacterial	Opalescent, cloudy, purulent, clotting	Reduced or absent 0 – 2.5	Increased 50– 1000+	500– 2000 Mostly polymorphs
Tuberculous meningitis	As above + fibrin web	0 – 2.5	45 – 500+	50 – 500+ Mostly lymphocytes

Viral	Normal	2.5 – 5.5	Normal or slightly elevated	10 – 2000 mostly lymphocytes
Fungal	Slightly cloudy or clear	reduced	elevated	50–5000 lymphocytes

14.8 Cytology of malignant cells in CSF

- Immature hematopoietic cells
 - early nucleated RBC and WBC precursors,
- Tissue cells
 - ependymal cells lining the brain and ventricles
 - choroid plexus cells = found in the ventricles and are part of the blood brain barrier
- Malignant cells

- Malignant cells in the CSF are derived
 - from primary neural cancers
 - e.g. medulloblastoma = malignant brain tumor that originates in the cerebellum
 - teratoid/rhabdoid tumor = a highly malignant brain tumor)
 - from extraneural cancers
 - e.g. retinoblastoma = retinal tissues of the eye, metastatic melanoma

Flow-cytometry

A promising method for the detection of cancer cells in the CSF.

Proven acceptability in the detection of hematologic tumours (leukaemias and lymphomas) and has shown promise as a complementary method to cytology.

Thank You!!!!

Unit 4– Cerebrospinal fluid (CSF)

**Session 13 – Chemical Composition of
CSF and Chemical Analysis of
Cerebrospinal Fluid for the Diagnosis of
Central Nervous System Disorders**

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Introduction

- Routinely analysed major biochemical constituents - glucose and proteins
- Microscopic study- cellular elements and microbiology
- Alteration of the composition of glucose and protein
 - **no information of diagnostic relevance.**
 - But **direct the** selection of a **further testing** methods from among cytology, microbiology or virology for definitive diagnosis
- Newly arrived analytes of importance-
 - globulins
 - LDH
 - CPK
 - ICDH (Isocitrate dehydrogenase)
 - β -Human Chorionic Gonadotropin
 - β -hCG and other hormones

- CSF test panel
 - Gross examination
 - CSF blood cell identification
 - CSF blood cell counting
 - CSF differential blood cell counting
 - CSF microbiology
 - CSF virology
 - CSF cytology of malignant cells
- Appropriate diagnostic tests selection
 - relevant to patient's symptoms
 - relevant to tentative diagnosis of the patient's clinical condition

- CSF 'Full Report'
 - Gross physical examination
 - Total cell count
 - Differential cell count
 - Glucose concentration
 - Protein concentration

13.1 Normal Composition of selected constituents in the lumbar CSF.

Analyte (Unit of expression)	Concentration - range	Comment
Glucose (mg.L ⁻¹) [mmol.L ⁻¹]	400 – 700 [2.22 – 3.89] 600 – 800 [3.33 – 4.44]	Fasting adult* Child* *CSF [glucose] ∝ blood [glucose]
Protein total (mg.L ⁻¹)	80 – 320	
Albumin (mg.L ⁻¹)	100 - 300 Up to 450	Age > 4 years Up to 4 years
Sodium (mmol.L ⁻¹)	136 - 140	

Potassium (mmol.L ⁻¹)	2.5 – 3.2	~ 70% of plasma value
Chloride (mmol.L ⁻¹)	118 - 132	Fluctuates with plasma concentration

13.2 Glucose composition variations in disorders afflicting the CNS

- Decreased glucose- hypoglycaemia
- Increased glucose- hyperglycaemia

It is not necessary to do CSF glucose to diagnose diagnosis hypoglycaemia or hyperglycaemia

13.3 Protein composition variations in disorders afflicting the CNS

- Plasma proteins do not enter the CSF due to the presence of blood-CSF barrier.

Glucose composition in different conditions

Unaltered or normal (400 – 700 mg.L-1):	Late CNS syphilis, viral meningoencephalitis
Variable (150 -750 mg.L-1):	cerebral haemorrhage, subdural haematoma, early-acute syphilitic meningitis
Normal or elevated:	brain tumour, spinal cord tumour, subarachnoid block, multiple sclerosis, viral encephalitis
Elevated:	post-infectious encephalitis, (hyperglycaemic diabetic coma
Lowered (0 – 450mg.L-1):	acute purulent meningitis, tuberculous meningitis.

- CSF glucose determination alone is **not** diagnostic of the above conditions

13.3 Protein composition variations in disorders afflicting the CNS

- 80 – 320 mg.L-1
- Albumin and Globulins
- Plasma proteins **do not** enter the CSF - presence of blood-CSF barrier

Elevation of CSF protein-

1. influx syndrome

- *i.e. breakdown of blood-CNS barrier*
 - meningitis
 - injury to brain tissue
 - bleeding into the subarachnoid space
 - disruption of the blood-brain barrier due to offensive agents such as bacteria, fungi, viruses, toxic heavy metals, chemicals and drugs

2. Presence of blood in the CSF

The **level of total protein** increase is categorised according to the **amount of protein**

<u>Level of increase</u>	<u>Total protein concentration (mg.L⁻¹)</u>	Possible causes
Slight	600 – 750	viral meningitis, neurosyphilis, subdural haematoma, cerebral thrombosis, brain tumour and multiple sclerosis
Moderate	750 – 1500	bacterial meningitis, tuberculous meningitis, cerebral haemorrhage, subarachnoid block, spinal cord tumour

Marked	>1500	Acute purulent meningitis, tuberculous meningitis and early acute syphilitic meningitis
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CSF protein determination alone is **not** diagnostic of the above conditions

- Of total protein, predominant protein- albumin 56 – 76% (100 – 300 mg/L).
- Immunoglobulin-G (IgG) elevation –
 - acute bacterial meningitis
 - neurosyphilis
 - multiple sclerosis
 - CNS demyelinating disease

13.4 Enzyme (activity) variations in disorders afflicting the CNS

- Level of enzymes elevates due to **brain and spinal cord malignancies**
 - Lactate Dehydrogenase (LDH)
 - Creatine Phosphokinase (CPK)
 - Glutamate Oxaloacetate/ Aminotransferase (GOT or AST)
 - Isocitrate Dehydrogenase (ICDH)
- Provide further evidence for the presence of bacterial meningitis.

13.5 Multiple-marker testing with CSF to detect CNS Disorders

- Purpose- to arrive at a more accurate diagnosis of a CNS disease
 - Ex. Multiple sclerosis: elevate glucose, total protein, lactate, IgG, and CPK
- CSF multi-marker assay
 - comprise glucose, total protein, lactate, IgG, and CPK
 - improved analytical methods
 - requiring micro-litre volumes of CSF and reagents
 - Ability to assay batch wise
 - completed within a short time in the same multi-channel analyser.

13.6 Visual examination of CSF

- The **first mandatory test** that must be performed
- Information leading to the
 - selection of the type of testing
 - omission of un-required testing
- Physical appearance indicates
 - **presence of** a number of **abnormal constituents** such as blood, bilirubin and cellular elements
 - **increased presence of** a constituent **normally present** in the CSF, e.g. protein.

CSF appearance observation

Feature	Observation	
	Yes	No
Clear		
Opalescent		
Turbid		
Purulent (containing pus)		
Pale pink / reddish colour ¹		
Pale yellow colour ²		
Fibrin threads or clot		

1 Erthrochromasia

2 Xanthochromia

- Clear as water in tube- has a negligible number of cells and trace protein
- CSF is colourless- not contain pigment (chromogen) such as blood (haemoglobin) or bile pigment (bilirubin)
- Turbidity- CSF protein is elevated+ cell count increased
- Chromogenic substances that can be present in pathological conditions-
 - blood (haemoglobin)
 - bacterial chromogens
 - bilirubin (in neonatal jaundice= kernicterus and adult hyperbilirubinaemia)
 - carotene (excessive consumption of yellow fruits, carrot juice)
 - melanin (melanin producing tumours = melanomas in brain and meninges)

- Erythrochromasia
 - Blood stained CSF
 - orangish or reddish
 - become xanthochromia after a few days due to the conversion of haemoglobin to bilirubin
- Xanthochromia
 - the **yellow discoloration** indicating the **presence of bilirubin** in the **CSF**
 - could also be due to the entry of bilirubin from the blood stream into CSF
- Presence of **high amounts of protein - pinkish** appearance.
- Presence of **bacterial chromogen- greenish** discoloration

- The presence of fibrin threads and clots
 - due to the presence of blood in CSF introduced inadvertently at CSF withdrawal (traumatic tap)
 - bleeding into the subarachnoid space (subarachnoid haemorrhage)
 - fibrin meshwork takes the appearance of a **cobweb** it is suggestive of **tuberculosis meningitis**.

- If CSF is turbid, colour differentiation can be done using supernatant of CSF centrifuged at 3000 rpm for 5 minutes.

Extension of preliminary visual examination findings

Turbid CSF-

- May be due to
 - Increased CSF protein
 - bacterial infection: bacterial cells
- Thus guide to
 - (a) determination of the leukocyte number concentration
 - (b) microbiology for identification of pathogenic bacterium or bacteria

- Presence of blood
 - check (visually) whether all the CSF containers 1, 2, 3 and 4 are similarly blood stained : subarachnoid haemorrhage or cerebral haemorrhage
 - if the orangish or reddish coloration is present in specimen container 1 only: a traumatic tap

13.7 CSF glucose concentration determination

Specimen container:

- Glucose concentration can decrease on standing of CSF after collection due to cellular consumption of glucose (blood cells, tumour cells, bacterial cells and fungal cells).
- Can be overcome by collecting in to a container having the correct amount of sodium fluoride (NaF).
- The F⁻ ions of NaF inhibit cellular consumption of glucose by inhibiting the glycolysis pathway operating in the cells.
- NaF concentration has to be exact
- If excess- unintended inhibition of enzymes used in the enzyme-based glucose assay
- The exact amount of NaF to use is 0.5mg per 0.5mL of CSF

- Dissolve the NaF into the CSF by gentle inversion of the specimen container 6 – 8 times immediately after CSF collection.

Your duties!!

- Issue the glucose container (together with the other numbered containers 1, 2, 3 and 4) **with the level marks** to which the CSF is allowed to drip.
- Affix a label on the CSF specimen containers
 - patient's name
 - Bed-Head Ticket Number (BHT) or the Reference Number
 - Date and Time of collection of the specimen

- Acceptance/ rejection
 - ensure the labelling information is available
 - ensure at least the minimum volume (100 μ L) of CSF is there in the specimen container for glucose determination.
- Whenever CSF glucose concentration is expected, request to provide a blood sample drawn concurrently for glucose determination, as CSF glucose concentration is better judged in relation to blood glucose.

Review of glucose assay methods

- Similar to/ suitable to use plasma glucose analysis techniques- enzymatic colorimetric method: glucose oxidase method

Basis of glucose concentration determination assay methods:

(a) The ortho-tolidine method

CSF glucose (protein free) & O-tolidine in glacial acetic acid was brought to boil green colour formed due to the formation of glycosylamine, directly proportional glucose concentration determined photometrically.

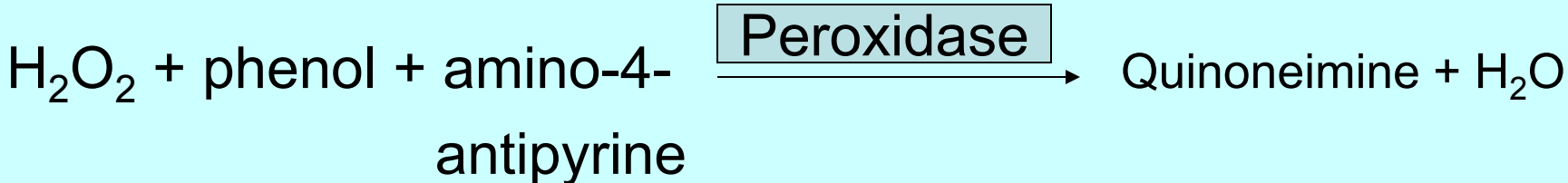
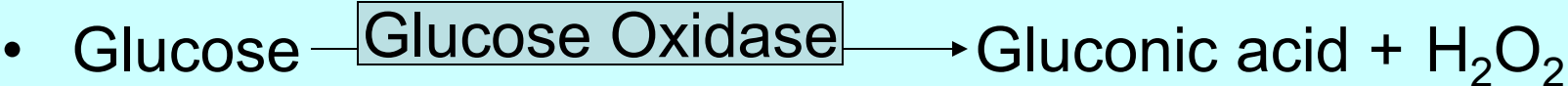
(b) The copper reduction method

glucose in protein-free CSF filtrate was brought to a highly reactive species (enediol form) by alkalinising the reaction medium, which on boiling with Cu^{2+} (salt = copper tartrate) reduced Cu^{2+} to Cu_2O (cuprous oxide). The Cu_2O formed was reacted with phosphomolybdic acid ($\text{H}_4[\text{P}(\text{Mo}_3\text{O}_{10})_4]$) to generate a blue colour complex, whose colour intensity was directly proportional to glucose concentration.

- Glucose + cupric \longrightarrow cuprous + gluconic acid
(Alkaline medium)
- Cuprous + phosphomolybdic acid \longrightarrow cupric + molybdenum blue

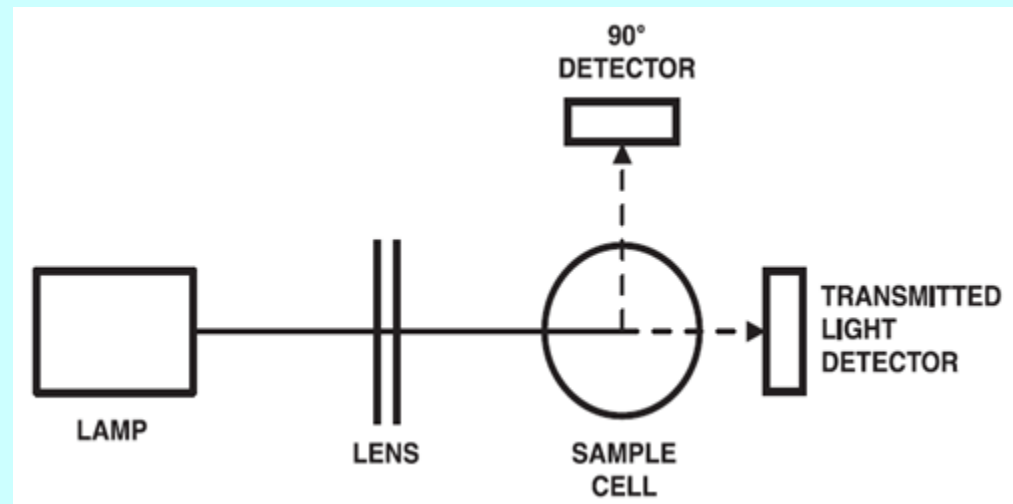
(b) The glucose oxidase method

CSF is incubated at a fixed pH (buffered) with an enzyme combination comprising of glucose oxidase and peroxidase. The nascent oxygen generated enzymatically is made to oxidise a chromogenic dye, whose colour concentration (absorbance) determined photometrically is directly proportional to CSF glucose concentration.



13.8 CSF protein concentration determination

- Similar to/ suitable to use plasma glucose analysis techniques
- A variety of assay methods (turbidimetry, nephelometry, photometry) are available for CSF protein concentration determination
- Turbidimetric method with sulphosalicylic acid (SSA) – commonly used method



The sulphosalicylic acid – turbidimetric method

- of when mixed with form a, yielding.
CSF Proteins+ sulphosalicylic acid \longrightarrow fine precipitate
with
turbidity
Turbidimetry at 640 nm
- Kingsbury's method -A series of protein concentrations (protein standards) treated with SSA is kept (in sealed glass containers) for visual comparison
- As visual comparison is subjective: turbidimetric method erases out observer variability.
- Also available as an automated version.

Thank You!!!!