

Module 3

The human nervous system. Neuron, action potential of brain, brain waves, types of electrodes, placement of electrodes, evoked potential, EEG recording, analysis of EEG(2 Hrs)

Electromyography: Nerve conduction velocity, instrumentation system for EMG. (1 Hr)

Physiology of respiratory system (brief discussion), Respiratory parameters, spirometer, body plethysmographs, gas exchange and distribution (2Hrs)

Physiology of respiratory system (brief discussion), Respiratory parameters, spirometer, body plethysmographs, gas exchange and distribution (2Hrs)

Instruments for clinical laboratory: Oxymeters, pH meter, blood cell counter, flame photometer, spectrophotometer (3 Hrs)

- To imagine how the nervous impulse happens, observe the figure below. The perception of acute pain when a sharp object penetrates your foot is caused by the generation of certain action potential in some nervous fiber in the skin. It is believed that the membranes of these cells have sodium channels that open when nerve terminals of the cell are stretched. The initial chain of events is:



1. Sharp object penetrates the skin;
2. The membranes of nerve fibers in the skin are stretched;
3. Channels permeable to sodium (Na^+) are opened.

- Due to the concentration gradient and negative charge in extracellular fluid, ions enter the fiber by means of these channels. Sodium entrance (yellow balls) depolarizes the membrane, that is, the side of membrane bathed by extracellular fluid becomes less negative in relation to the cell interior.
- If this depolarization, called generator potential, reaches a critical point, the membrane then generates an action potential. The critical point of depolarization that must be

overcome in order to fire an action potential is called threshold. The action potentials are caused by depolarization of the membrane above the threshold.

- In the repolarization phase, the sodium channel is deactivated and the conductivity of membrane for potassium increases. These positive ions follow its chemical and electrical gradient and exit from the cell, thus gradually inverting the membrane potential.
- In the recovery phase, the active sodium/potassium pump reestablishes the ionic levels which were altered during the action potential: for each sodium ion which is transported from the inside to the outside, a corresponding potassium is transported in the opposite direction.
- The skin perforation is transduced into signals that travel upwards via sensorial nerves. This information arrives at the spinal cord and is distributed to interneurons (neurons that provide intermediate connections with other neuron chains). Some of these neurons send axons to the sensorial region of the brain where the sensation of pain is registered. Other make synapses with motor neurons, which send signals downward to the muscles. The motor junctions command the muscle contraction and withdrawal of the foot. This is an example of a neuron chain called reflex arc.

Brain Waves

- Brainwaves are produced by synchronised electrical pulses from masses of neurons communicating with each other.
- Brainwaves are detected using sensors placed on the scalp. They are divided into bandwidths to describe their functions (below), but are best thought of as a continuous spectrum of consciousness; from slow, loud and functional - to fast, subtle, and complex.
- Our brainwaves change according to what we're doing and feeling. When slower brainwaves are dominant we can feel tired, slow, sluggish, or dreamy. The higher frequencies are dominant when we feel wired, or hyper-alert
- **Infra-Low brainwaves (<.5HZ)** (also known as Slow Cortical Potentials), are thought to be the basic cortical rhythms that underlie our higher brain functions. Very little is known about infra-low brainwaves. Their slow nature make them difficult to detect and accurately measure, so few studies have been done. They appear to take a major role in brain timing and network function.
- **Delta brainwaves(.5 TO 3.5 HZ)** are slow, loud brainwaves (low frequency and deeply penetrating, like a drum beat). They are generated in deepest meditation and dreamless sleep. Delta waves suspend external awareness and are the source of empathy. Healing and regeneration are stimulated in this state, and that is why deep restorative sleep is so essential to the healing process

- **Theta brainwaves (4 TO 7.5 HZ)** occur most often in sleep but are also dominant in deep meditation. Theta is our gateway to learning, memory, and intuition. In theta, our senses are withdrawn from the external world and focused on signals originating from within. It is that twilight state which we normally only experience fleetingly as we wake or drift off to sleep. In theta we are in a dream; vivid imagery, intuition and information beyond our normal conscious awareness. It's where we hold our 'stuff', our fears, troubled history, and nightmares.
- **Alpha brainwaves (8 TO 13 HZ)** are dominant during quietly flowing thoughts, and in some meditative states. Alpha is 'the power of now', being here, in the present. Alpha is the resting state for the brain. Alpha waves aid overall mental coordination, calmness, alertness, mind/body integration and learning.
- **Beta brainwaves (13 TO 38 HZ)** dominate our normal waking state of consciousness when attention is directed towards cognitive tasks and the outside world. Beta is a 'fast' activity, present when we are alert, attentive, engaged in problem solving, judgment, decision making, or focused mental activity. Beta brainwaves are further divided into three bands; Lo-Beta (Beta1, 12-15Hz) can be thought of as a 'fast idle', or musing. Beta (Beta2, 15-22Hz) is high engagement or actively figuring something out. Hi-Beta (Beta3, 22-38Hz) is highly complex thought, integrating new experiences, high anxiety, or excitement. Continual high frequency processing is not a very efficient way to run the brain, as it takes a tremendous amount of energy.
- **Gamma brainwaves (38 TO 42 HZ)** are the fastest of brain waves (high frequency, like a flute), and relate to simultaneous processing of information from different brain areas. Gamma brainwaves pass information rapidly and quietly. The most subtle of the brainwave frequencies, the mind has to be quiet to access gamma.
- Gamma was dismissed as 'spare brain noise' until researchers discovered it was highly active when in states of universal love, altruism, and the 'higher virtues'. Gamma is also above the frequency of neuronal firing, so how it is generated remains a mystery. It is speculated that gamma rhythms modulate perception and consciousness, and that a greater presence of gamma relates to expanded consciousness and spiritual emergence

WHAT BRAINWAVES MEAN TO YOU

- Our brainwave profile and our daily experience of the world are inseparable. When our brainwaves are out of balance, there will be corresponding problems in our emotional or neuro-physical health. Research has identified brainwave patterns associated with all sorts of emotional and neurological conditions.
- Over-arousal in certain brain areas is linked with anxiety disorders, sleep problems, nightmares, hyper-vigilance, impulsive behaviour, anger/aggression, agitated depression,

chronic nerve pain and spasticity. Under-arousal in certain brain areas leads to some types of depression, attention deficit, chronic pain and insomnia. A combination of under-arousal and over-arousal is seen in cases of anxiety, depression and ADHD(attention deficit hypertension disorder).

- Instabilities in brain rhythms correlate with tics, obsessive-compulsive disorder, aggressive behaviour, rage, bruxism, panic attacks, bipolar disorder, migraines, narcolepsy, epilepsy, sleep apnea, vertigo, tinnitus, anorexia/bulimia, PMT, diabetes, hypoglycaemia and explosive behaviour.

Types of Electrodes

- **Essentially, five types of electrodes are typically used:**

1. Scalp: silver pads, discs, or cups; stainless steel rods; and chlorided silver wires.
2. Sphenoidal: Alternating insulated silver and bare wire and chlorided tip inserted through muscle tissue by a needle.
3. Nasopharyngeal: silver rod with silver ball at the tip inserted through the nostrils.
4. Electrocorticographic: cotton wicks soaked in saline solution that rests on the brain surface (removes artifacts generated in the cerebrum by each heartbeat).
5. Intracerebral: sheaves of Teflon-coated gold or platinum wires cut at various distances from the sheaf tip and used to electrically stimulate the brain

Reusable disks

- These electrodes can be placed close to the scalp, even in a region with hair because they are small. A small amount of conducting gel needs to be used under each disk. The electrodes are held in place by a washable elastic head band. Disks made of tin, silver, and gold are available. They can be cleaned with soap and water
- The cost of each disk and lead is dependent on the type of metal used as a conductor, the gauge of wire used as a lead, and the type of insulation on the wire lead.

EEG Caps with disks

- Different styles of caps are available with different numbers and types of electrodes. Some
- caps are available for use with replaceable disks and leads. Gel is injected under each disk through a hole in the back of the disk.

- Since the disks on a region of the scalp covered with hair cannot be placed as close to the scalp as individual disc electrodes, a greater amount of conducting gel needs to be injected under each. After its use, more time is required to clean the cap and its electrodes, as well as the hair of the subject. Depending on the style and longevity of the cap and the electrodes, their expense can be moderate to high.

Adhesive Gel Electrodes

- These are the same disposable silver/silver chloride electrodes used to record ECGs and EMGs, and they can be used with the same snap leads used for recording those signals.
- These electrodes are an inexpensive solution for recording from regions of the scalp without hair. They cannot be placed close to the scalp in regions with hair, since the adhesive pad around the electrode would attach to hair and not the scalp.
- Small metal disks made of stainless steel, tin, gold or silver covered with AgCl coating are generally used for recording EEG
- They are placed on the scalp in special positions
- These positions are specified by international 10/20 system. Each electrode site is labelled by letter and number which indicates the area of the brain for eg F for frontal, T for temporal
- even numbers denote right side of head and odd numbers refer to left side of the head

Disk electrodes:



EEG cables with disc electrodes

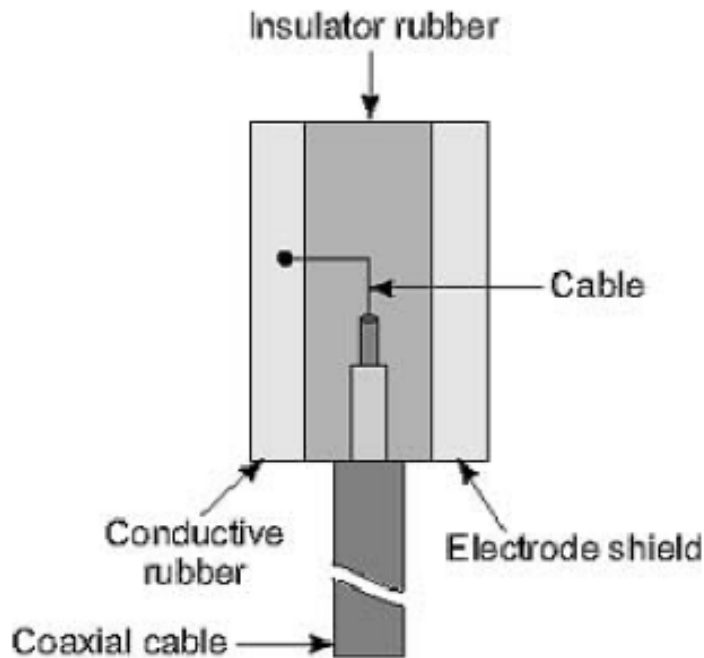
- This type of electrodes provide enough space to contain electrode gel
- In these electrodes the skin does not touch the electrode material directly
- The electrode tissue interface has impedance depending on various factors.

- Another type for recording EEG signals is the chloride silver disc having 6-8mm diameter.
- Contact with the scalp is made via an electrolytic paste through a washer.
- Small needle electrodes are used for carrying out EEG studies when they are inserted subcutaneously.
- Silver ball or pellet electrodes covered with a small cloth pad are used when electrical activity is recorded from the cortex.



EEG electrode which can be applied to the surface of the skin by an adhesive tape

- Dry Type of electrodes does not require long set up time and can be used for long term recording.
- The electrode has 1.5mm thick conductive rubber shaped disc.
- The active side of electrode is capacitive and is coupled through insulating silicone rubber

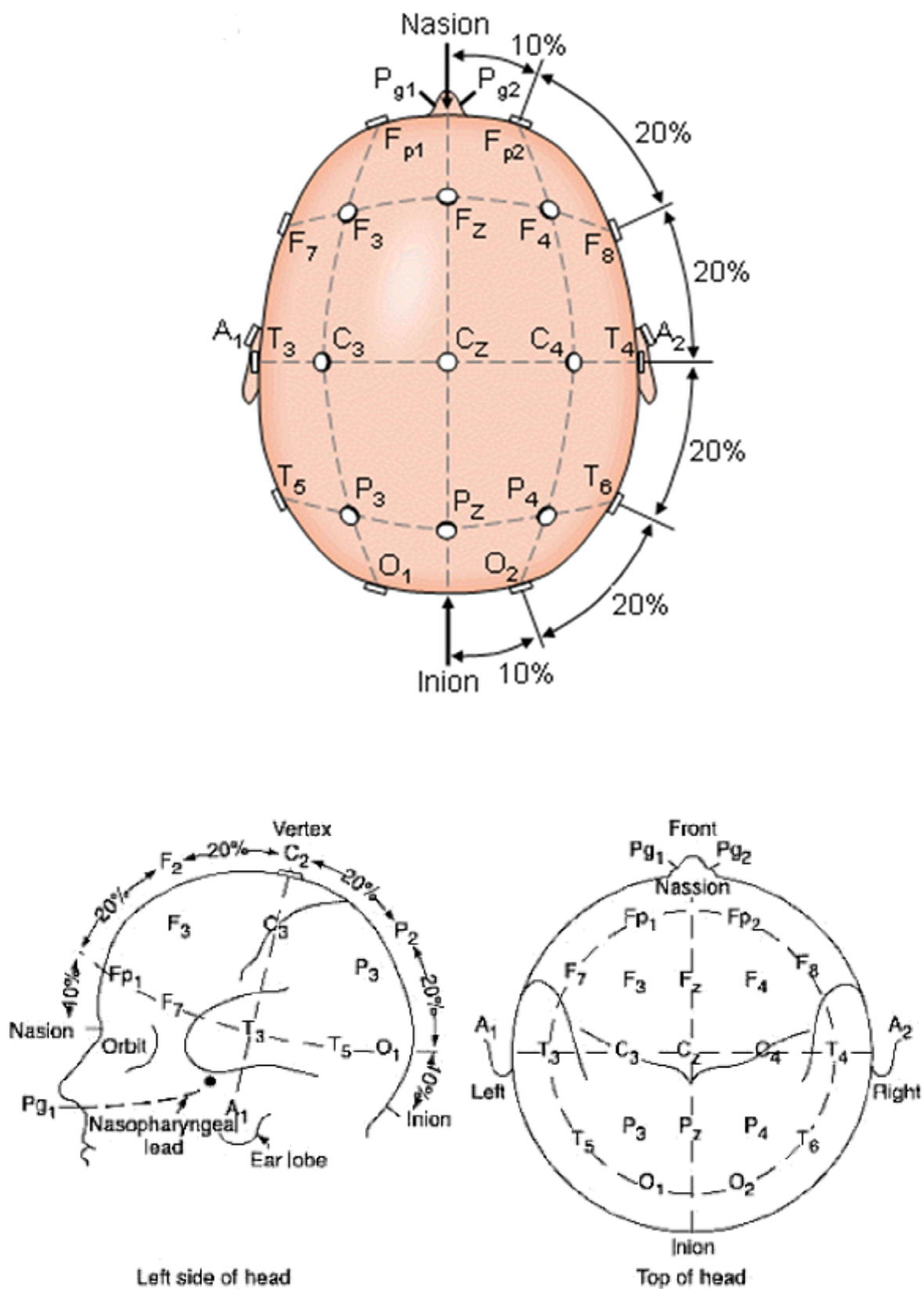


Construction of EEG dry electrode

- The EEG electrodes can be classified as disposable, reusable disc and cup shaped , subdermal needles (single used needle placed under the skin) and implanted electrodes.
- Needles are available with permanently attached wire leads , where the whole assembly is discarded.
- Some of the EEG electrodes are used for specific applications such as the implanted electrodes can be used to stimulate the brain and to relate the cortical and subcortical neurological functions.

Placement of electrodes

- Cerebrum has 4 lobes :
 - Frontal
 - Parietal
 - Temporal
 - occipital



10-20 System of placement of electrodes

- EEG electrodes are placed on the scalp according to a standard known as 10/20 system adopted by American EEG society.
- This system involves placement of electrodes at distances of 10% and 20% of the measured coronal, sagittal and circumferential arcs between landmarks on the cranium
- Traditionally there are 21 electrodes for the 10/20 system.
- Electrodes are identified according to their position on the head

Fp – frontal polar

F – Frontal

C – Central

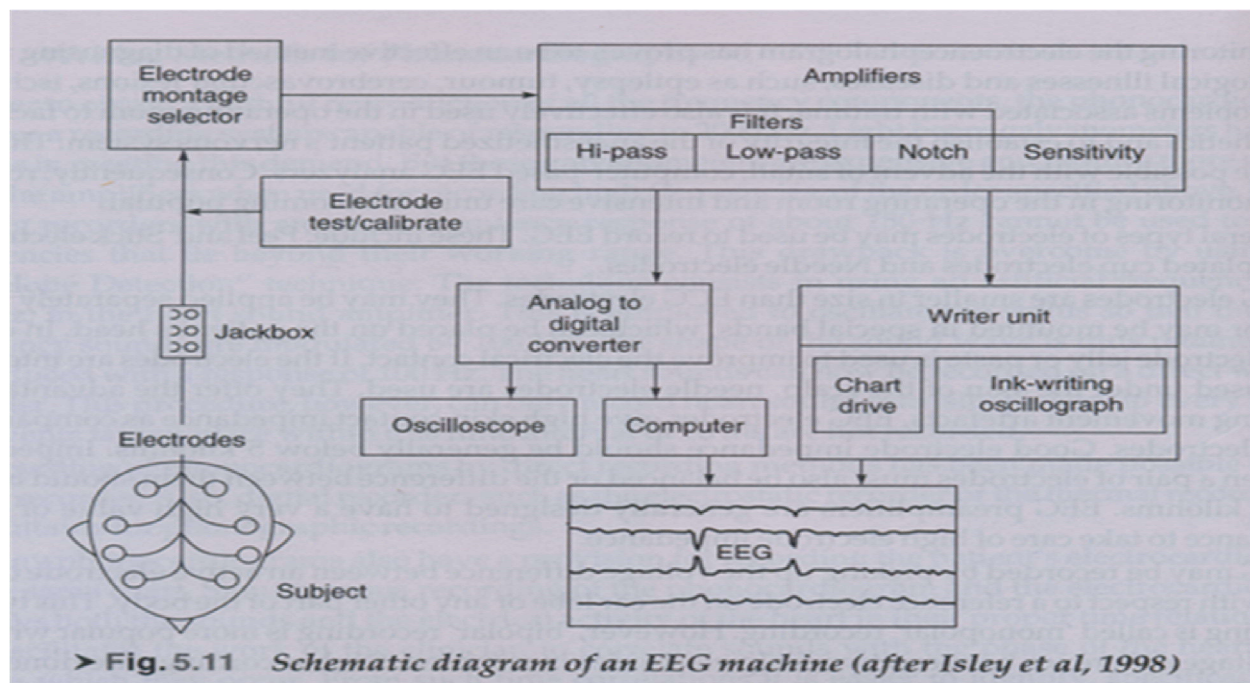
P – parietal

T – temporal

O – Occipital

- Odd numbers – electrodes on the left side of the head and even numbers- on the right side and Z – midline electrodes
-

EEG Recording



The above diagram shows both analog and digital components.

➤ Electrode montage selector:

- EEG signals are transmitted from the electrodes to the head box and then to the montage selector.

- Montage selector on analog EEG is a large panel having switches that allows user to select which electrode pair will have signals subtracted from each other to create array of channels of output called a montage.

- montage displays different spatial characteristics of the same data.

- Montages are either bipolar (made by subtraction of signals from adjacent electrode pairs) or referential (made by subtracting the potential of a common reference electrode from each electrode on the head)

➤ Preamplifiers:

- They must have high gain and low noise characteristics as EEG potentials are small in amplitude and high CMMR to minimize stray interference signals from power lines and other electrical equipments.

- The amplifier must be free from drift so as to prevent slow movement of the recording pen from its centre position as a result of changes in temperature.

- The individual EEG signals must be amplified at the bedside, for this purpose a specially designed connector box, has to be mounted near the patient, which is generally employed with EEG machines.

- The use of electrode amplifiers at the site also eliminates undesirable cross talk effects of the individual electrode potentials.

➤ Sensitivity control:

- the overall sensitivity of an EEG machine is the gain of the amplifier multiplied by the sensitivity of the writer.

- thus if writer sensitivity is 1cm/V , the amplifier must have an overall gain of 20,000 for a 50 microvolt signal.

- the various stages are capacitor coupled.

- EEG machine has 2 types of gain control, one is continuously variable and other operates in steps.

- one which is continuous is used to equalize the sensitivities of all channels and the next which operates in steps is used to increase or decrease the sensitivity of a channel by known amounts.

➤ Filters:

- EEG may contain muscle artifacts due to contraction of the scalp and neck muscles and this may be large and sharp.

- the muscle artifact may be removed by the low pass filters.

- Upper cut off frequency is controlled by high frequency filter.

-Some EEG machines have Notch filter tuned to 50hz to eliminate mains frequency interference

➤ Writing part:

- it is usually of the ink type direct writing recorder.

- The best types of pen motors used have a frequency response of about 90hz.

• Paper drive:

- This is provided by a synchronous motor.

-an accurate and stable paper drive mechanism is necessary and several paper speeds are available.

- Timing pulses are preferably generated independently of the paper drive mechanism in order to avoid difference in timing marks due to changes in paper speed.

• Channels:

-EEG machines have upto 32 channels although 8 or 16 channels are more common.

Recording of evoked Potential

➤ The electrical potentials after stimulation of specific neural tracts.

➤ If an external stimulus is applied to the sensory part of the brain it responds by producing an electrical potential known as the evoked potential.

➤ The evoked potentials classified as

➤ Sensory evoked

- Motor evoked
- Event related potentials
- The sensory evoked potentials include auditory evoked responses , visual evoked responses and somatosensory evoked potentials.
- The evoked potentials of EEG is in the order of 10 microvolts.
- The evoked potentials are generally superimposed with EEG.
- Signal averaging is done to resolve the low amplitude potentials.

Analysis of EEG

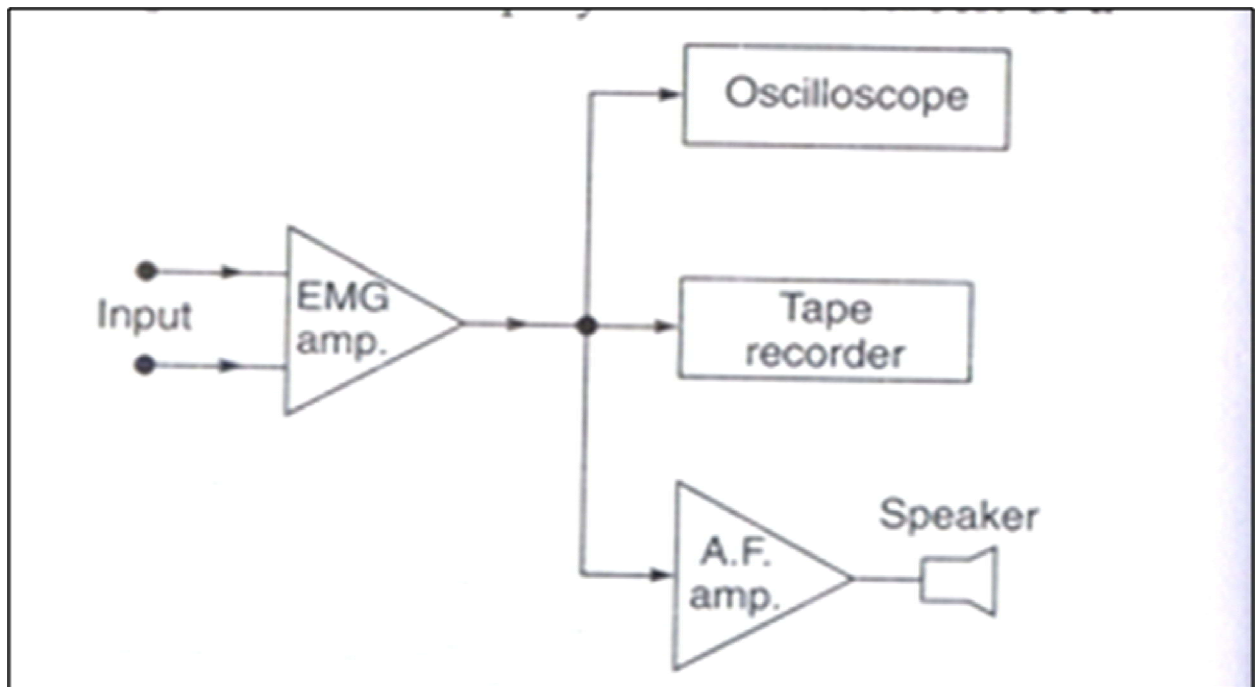
- For rapid and accurate interpretation , the frequency and amplitude of the EEG signals is essential.
- This requires constant analysis of the EEG signal by a skilled technician.
- The modern machines extract and present the frequency and amplitude information in simple and visually enhanced formats that can be interpreted directly by clinician.
- The analysis done are
 - Frequency analysis in which the raw EEG signals are broken into their component frequencies by method of FFT.
 - Amplitude Analysis : The amplitude changes result in change in power of the resulting frequency spectrum. The power spectrum is calculate by squaring the amplitudes of the individual frequency components.
 - **Compressed Spectral array:** In this format, a series of computer smoothed spectral arrays are stacked vertically with the recent EEG event at the bottom and the oldest at the top. Peaks will appear at the frequencies that contains more power or make larger contribution to the total spectrum.
 - **Dot-density modulated spectral array:** This is another method or for displaying the power spectra. This displays a power spectrum as a line of variable intensities .Areas of greatest density represent frequencies which make the greatest contribution to EEG power spectrum.

Electromyogram

- Used for recording the bioelectric potentials associated with the muscle activity whether muscle is contracting or not.

- Measured at the surface of the body near a muscle of interest or directly from the muscle by penetrating the skin with needle electrodes.(uses either surface electrodes or needle electrodes)
- Surface electrodes pick up many overlapping spikes and therefore produces an average voltage effect.
- Needle electrodes is in contact with a single muscle fiber and will pick up spike type voltages
- The action potential of a given muscle has a fixed magnitude , regardless of the intensity of stimulus that generates the response.
- The amplitude of the measured EMG waveform is the instantaneous sum of all the action potentials generated at any given time.
- These actions occur in both positive and negative polarities at given pair of electrodes, they sometimes add and sometimes cancel. Thus the EMG waveform appears very much like random noise.
- The instrument is useful for making a study of several aspects of neuromuscular function, neuromuscular condition, extent of nerve lesion , reflex responses etc.
- EMG measurements are also important of the myoelectric control of prosthetic devices(artificial limbs)

Block diagram of a EMG recorder



- EMG is usually recorded by using surface electrodes and more often using needle electrodes which are directly inserted into the muscle.
- A ground electrode is necessary for providing a common reference for measurement.
- These electrodes pick up the potential produced by the contracting muscle fibers.
- The signal is then amplified and displayed on the screen of a CRT.
- It is also applied to an audio amplifier connected to a loudspeaker.
- The oscilloscope displays EMG waveforms.
- Tape recorder is included to facilitate playback and study of EMG sound waveforms at a later time.

Nerve conduction studies (NCS)

- A nerve conduction study (NCS), also called a nerve conduction velocity (NCV) test--is a measurement of the speed of conduction of an electrical impulse through a nerve.
- NCS can determine nerve and muscle damage and destruction.
- During the test, the nerve is stimulated, usually with surface electrode patches attached to the skin.
- NCS tests can be done on peripheral nerves that are superficial to be stimulated through the skin.

- peripheral nerves are located outside of your brain and along your spinal cord. These nerves help you control your muscles and experience the senses.
- Healthy nerves send electrical signals more quickly and with greater strength than damaged nerves.
- This test is done commonly in the ulnar(wrist and elbow), median(wrist & elbow), peroneal (ankle &head of fibula), posterior tibial nerves (ankle).
- A direct stimulation is applied to a motor or sensory nerve and this initiates an impulse.
- The conduction velocity is calculated by recording evoked potential from an sensory nerve or the muscle innervated by the motor nerve.
- An EMG test records the electrical signals moving through your muscles. This helps to detect the presence, location, and extent of any disease that may damage the nerves and muscles.
- In general the normal conduction velocity is approximately 50 to 60 m/sec and may vary from individual to another.

Nerve conduction Velocity(NCV)

- The time taken for the impulse to travel from stimulation to recording site is called latency time measured in ms.
- The size of the response is called amplitude measured in mv.
- symptoms are as follows:
 - Slowing of NCS indicates there is damage to myelin.
 - Slowing of all nerve conduction in more than 1 limb indicates generalized peripheral neuropathy (eg. In diabetes)

Physiology of respiratory system

Functions of the Respiratory System

- Air Distributor
- Gas exchanger
- Filters, warms, and humidifies air
- Influences speech

- Allows for sense of smell

Divisions of the Respiratory System

- Upper respiratory tract (outside thorax)

Nose

Nasal Cavity

Sinuses

Pharynx

- Lower respiratory tract (within thorax)

Trachea

Bronchial Tree

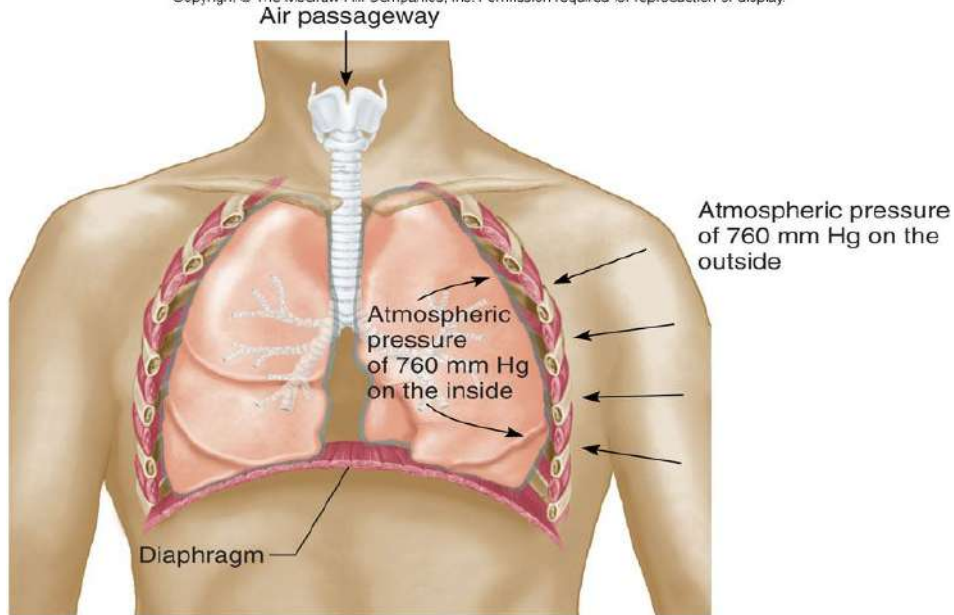
Lungs

Respiratory Physiology

- **Pulmonary Ventilation** = breathing Mechanism
 - Movement of gases through a pressure gradient - hi to low.
 - When atmospheric pressure (760 mmHg) is greater than lung pressure ---- air flows in = **inspiration**.

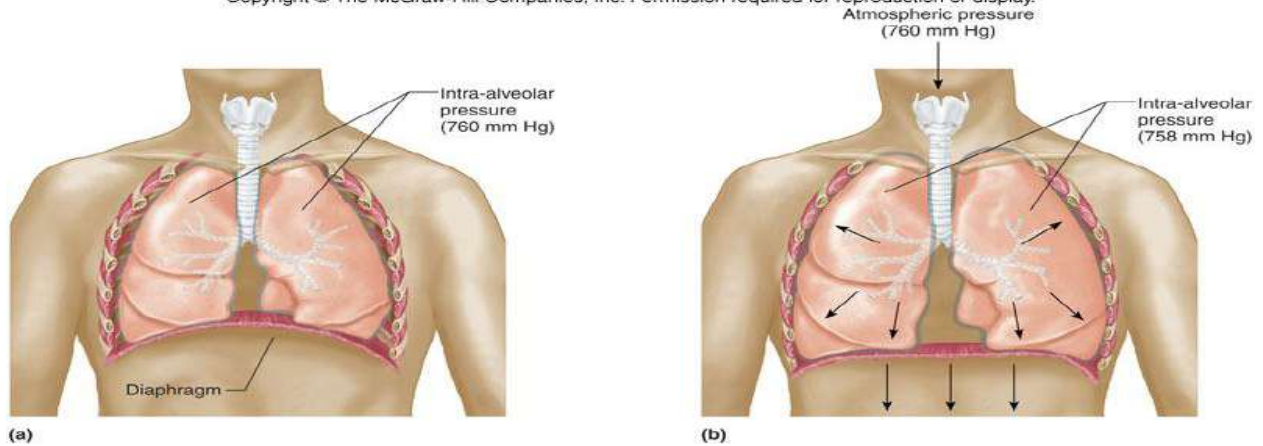
When lung pressure is greater than atmospheric pressure ---- air flows out = **expiration**

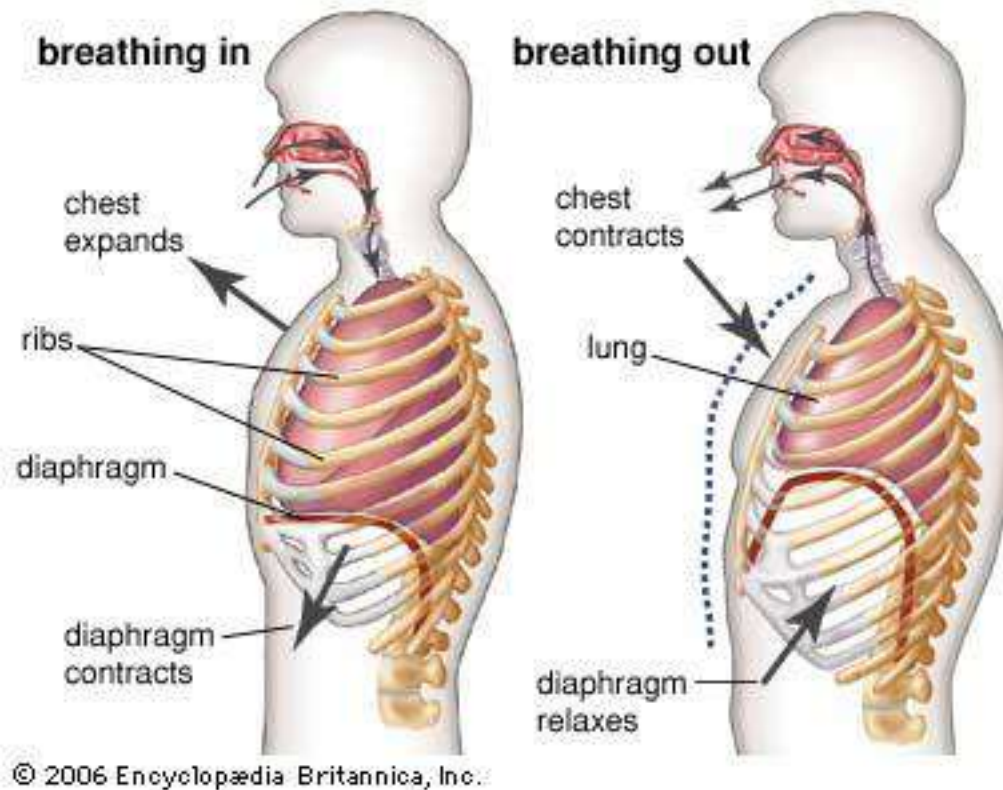
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- **Pressure gradients are established by changes in thoracic cavity.**
 - increase size in thorax = a decrease in pressure --- air moves in.
 - Decrease size in thorax = increase in pressure --- air moves out.

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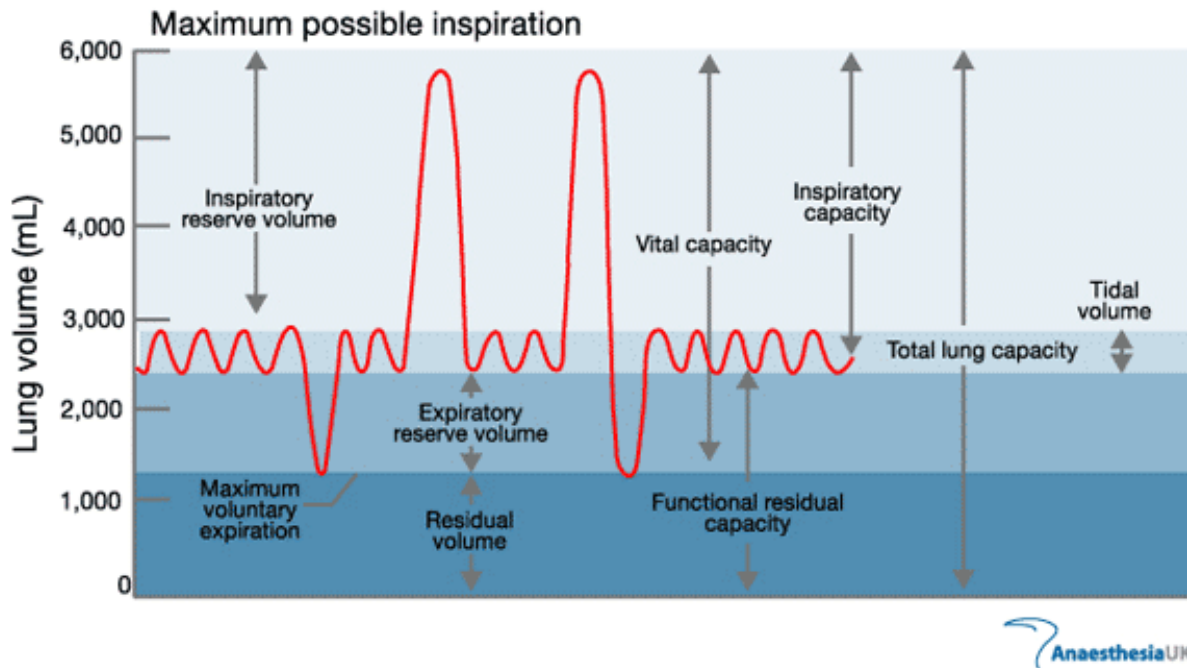


- **Inspiration** -contraction of diaphragm and intercostal muscles
- **Expiration** - relaxation of diaphragm and intercostal muscles

Respiratory parameters

- The respiratory activity involves supply of oxygen to and removal of CO_2 from the tissues.
- These gases are carried in the blood, O_2 from lungs to tissues and CO_2 from tissues to the lungs.
- During quiet breathing , ordinary intake of air or tidal volume is 0.5l.
- 3 basic measurements made in pulmonary clinic.
 - Ventilation- deals with measurement of the body as an air pump, determining the ability to move volumes of air and the speed with which it moves air
 - Distribution –quantify degrees of lung obstructions and also determines the residual volume which is the amount of air that cannot be removed from the lungs
 - Diffusion – identifies the rate at which gas is exchanged with the blood stream
- The ability of the pulmonary system to move air and exchange oxygen and carbon dioxide is affected by the air passages, diaphragm, rib cage and its associated muscles.
- The basic tests performed are those which help in determining the volumes and capacities of the respiratory system.

Lung Volumes and Capacities



➤ **Respiratory volumes:**

- **Tidal volume** : The volume of gas inspired or expired during normal quiet breathing
- **Minute Volume**: The volume of gas exchanged per minute during quiet breathing. It is equal to the tidal volume * the breathing rate.
- **Alveolar Ventilation** : The volume of fresh air entering the alveoli with each breath.

$$AV = \text{breathing rate} * (\text{tidal volume} - \text{dead space})$$

dead space is the volume of air which is inhaled that does not take part in the gas exchange, either because it (1) remains in the conducting airways, or (2) reaches alveoli that are not perfused or poorly perfused.

- **Inspiratory reserve volume (IRV)** The volume of gas which can be inspired between a normal end tidal volume (the additional amount of air that can be inhaled after a normal inhalation)

$$IRV = VC - (TV + FRC)$$

- **Residual Volume** : The volume of gas remaining in the lungs after a forced expiration
- **Expiratory Reserve volume**: The volume of gas remaining after normal expiration less the volume remaining after forced expiration. (the additional amount of air that can be expired from the lungs by determined effort after normal expiration)

$$ERV = FRC - RV$$

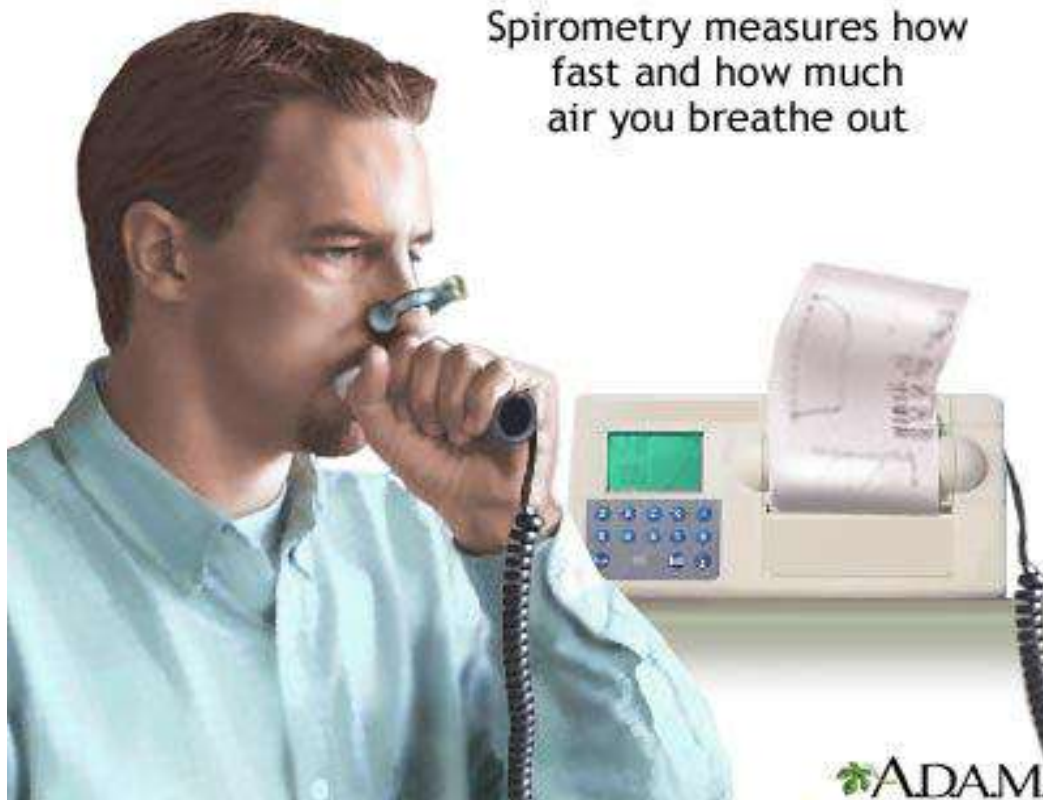
➤ **Respiratory Capacities:**

- **Functional Residual Capacity (FRC):** The volume of air in the body at the end of passive exhalation, including expiratory reserve & residual volumes ($FRC = ERV + RV$)
- **Total Lung Capacity (TLV)=** The sum of inspiratory reserve volume, tidal volume, expiratory & inspiratory reserve volume and residual volume ($TLC = IC + FRC$).
- **Vital Capacity (VC)=** The maximum volume of air that can be exchanged during respiration is the difference between TLC and RV.
- **Inspiratory Capacity (IC):** The maximum volume that can be inspired from the resting end expiratory position
- **Dead space :** It is the functional volume of the lung that does not participate in gas exchange.

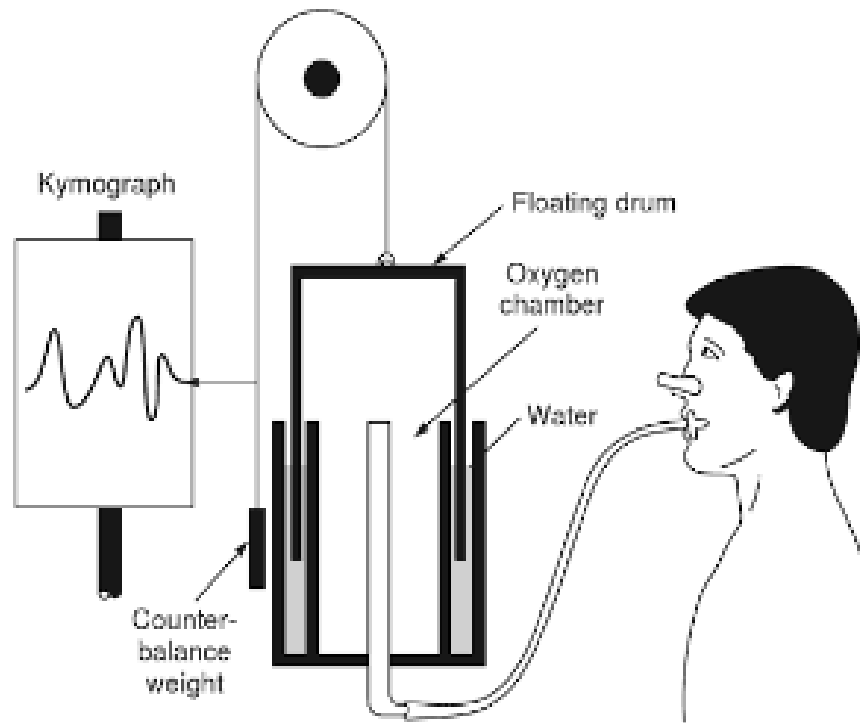
Spirometry

- It is the most common of the pulmonary function tests (PFTs), measuring lung function, specifically the amount (volume) and/or speed (flow) of air that can be inhaled and exhaled.
- Spirometry is an important tool used for generating pneumotachographs, which are helpful in assessing conditions such as asthma, pulmonary fibrosis, cystic fibrosis, and COPD(chronic obstructive pulmonary disease).
- The mouthpiece of the spirometer is placed in the mouth of the subject, whose nose is blocked.
- As gas moves into and out of the spirometer, the pressure of the gas in the spirometer changes.
- During resting breathing, the pressure changes in the gas within the spirometer can be considered negligible
- Therefore, only the temperature, average ambient pressure, and change in volume are needed to estimate the amount of gas exchanged with the spirometer.
- Spirometry is a painless study of air volume and flow rate within the lungs.
- Spirometry is frequently used to evaluate lung function in people with obstructive or restrictive lung diseases such as asthma or cystic fibrosis.
- Spirometry is the most common of the **lung function tests**. These tests look at how well your lungs work
- Spirometry shows how well you breathe in and out.
- Breathing in and out can be affected by lung diseases such as chronic obstructive pulmonary disease (COPD), asthma, pulmonary fibrosis and cystic fibrosis.
- **How is it done?**
- If it has not already been done, you will have your weight and height measured.
- For the spirometry itself, you need to breathe into the spirometer machine.
- First you breathe in fully and then seal your lips around the mouthpiece of the spirometer.

- You then blow out as fast and as far as you can until your lungs are completely empty.
- This can take several seconds.
- You may also be asked to breathe in fully and then breathe out slowly as far as you can.
- A clip may be put on to your nose to make sure that no air escapes from your nose.
- The measurements may be repeated two or three times to check that the readings are much the same each time you blow into the machine.



Basic Spirometer



Basic water sealed spirometer

-
- This has an upright , water filled cylinder containing an inverted counter weighted bell
 - Breathing into the bell changes the volume of gases trapped inside and the change in volume is translated into vertical motion , which is recorded on the moving drum of a kymograph.
 - The exertion of the bell will be proportional to the tidal volume.
 - An electrical signal proportional to volume displacement can be got by linear potentiometer connected to the pulley portion of the spirometer.
 - Two types of spirometers available:
 - 1. Wedge spirometer
 - 2. Ultrasonic spirometer
 - Spirometry helps in
 - Early **detection** of airflow obstruction (often late signs and symptoms)
 - Better **diagnosis** (helps to distinguish between asthma and COPD)

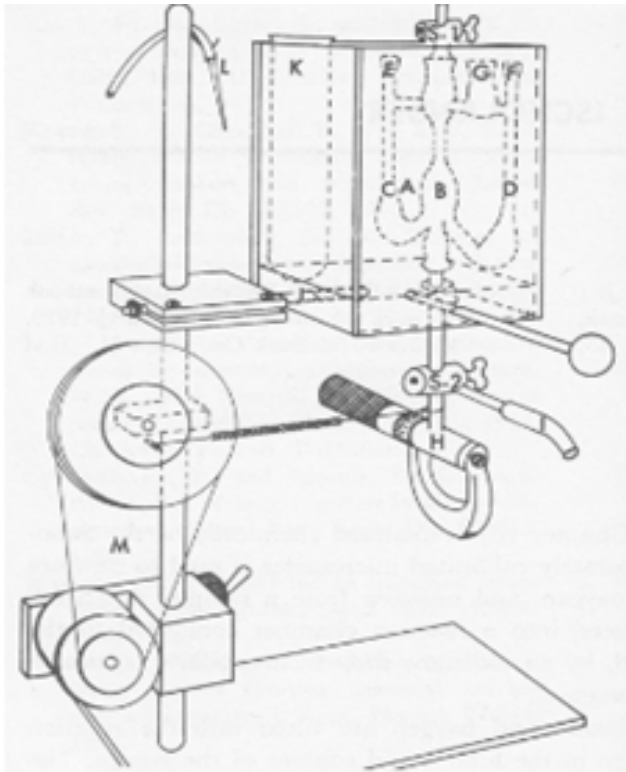
- Correct *follow-up* (compare with blood pressure meter in hypertension patients)

Gas exchange and Distribution

- A knowledge on the inspired gas and expired gas is important in finding out the investigations connected with respiratory physiology.
- The gases of interest for measurement and analysis are carbon dioxide, carbon monoxide, nitrous oxide and halothane .
- The mixing of gases within the lungs , the ventilation of the alveoli and the exchange of oxygen and carbon dioxide between the air and blood in the lungs takes place through diffusion process.
- Diffusion is the movement of gases from a point of higher pressure to a point of lower pressure to equalize the pressure difference.
- Measurements required for determining the amount of diffusion involve the partial pressures of oxygen and carbon dioxide.

Chemical analysis methods:

- In this device , a gas sample of approx 0.5ml is introduced into a reaction chamber by use of a transfer pipette at the upper end of the reaction chamber capillary.
- An indicator droplet in this capillary allows the sample to be balanced against a trapped volume of air in the thermobarometer.
- Absorbing fluids for CO₂ and O₂ can be transferred in from side arms without causing any change in the total volume of the system.
- The micrometer is adjusted so as to put mercury into the system in place of the gases being absorbed .
- The volume of the absorbed gases is read from micrometer barrel calibration.



A- reaction chamber

B- 2 side chambers ,**C** with CO_2 and with O_2

E and **F** two ends closed with stoppers.

H- burette

M consists of motor that drives counter wheel at a speed of 600rpm

Suction line **L** used for sucking off waste fluids

K suspended in the water bath is used for rinsing acid solutions.

Diffusing capacity using CO infrared analyzer

- To determine the efficiency of perfusion of the lungs by blood and the diffusion of gases, the most important test are those that measure O_2 , CO_2 , pH and bicarbonate in arterial blood.
- Co resembles oxygen in its solubility and molecular weight and also combines with hemoglobin .

- Its affinity for hemoglobin's 200 to 300 times that of oxygen.
- CO can thus be used as a tracer gas in measuring the diffusing capacity of the lung.
- It passes from alveolar gas into the alveolar walls, then into plasma from which it enters the RBC, where it combines with hemoglobin.
- The diffusing capacity of the alveolar membrane and the rate at which CO combines with hemoglobin in alveolar capillaries is expressed by relationship

$$\frac{1}{TF} = \frac{1}{Dm} + \frac{1}{\theta Vc} \text{ mmHg} / \text{ml} / \text{min}$$

TF = Diffusing capacity for the lung for CO

Dm=Diffusing capacity for the alveolar membrane

Vc= volume of blood in the capillaries

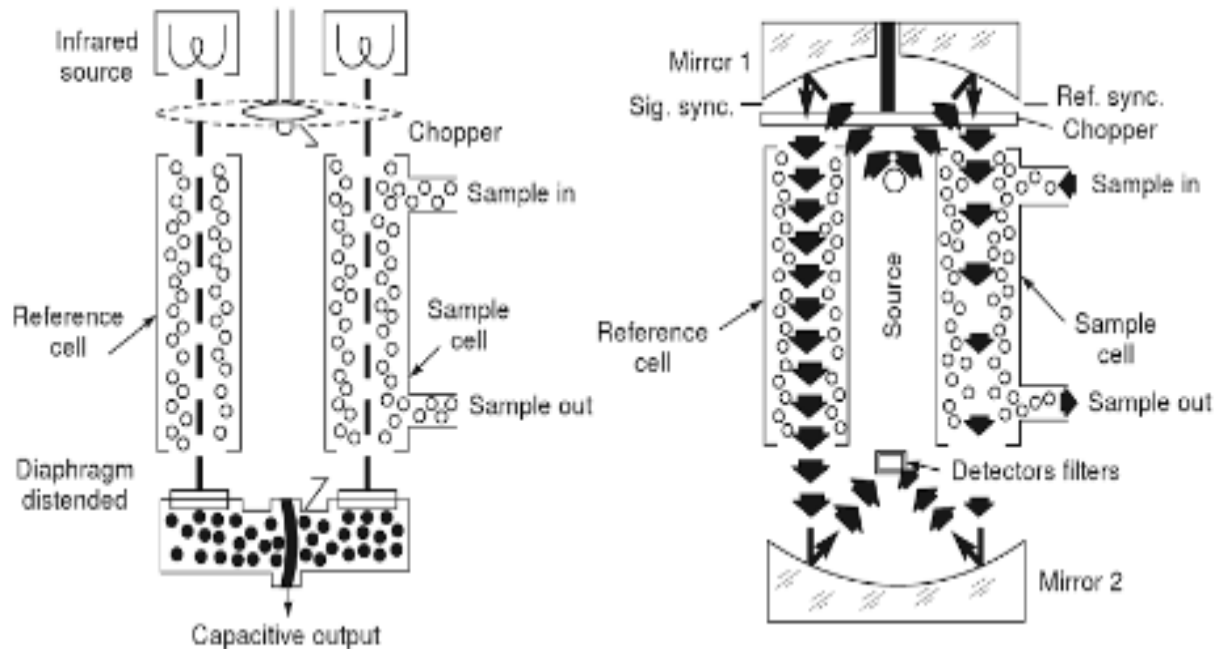
Theeta= reaction rate of CO with ox hemoglobin

TF=(ml CO taken up/min) /Pco in alveoli (mm/Hg)

Infrared Gas analyzer:

This works on the fact that some gases and vapors absorb specific wavelengths of infrared radiation.

The commonly measured gas using infrared absorption is carbon dioxide.



Principle of infrared gas analyzer: conventional (two sources) and improved design (with single source) (Courtesy: Infrared Industries, USA)

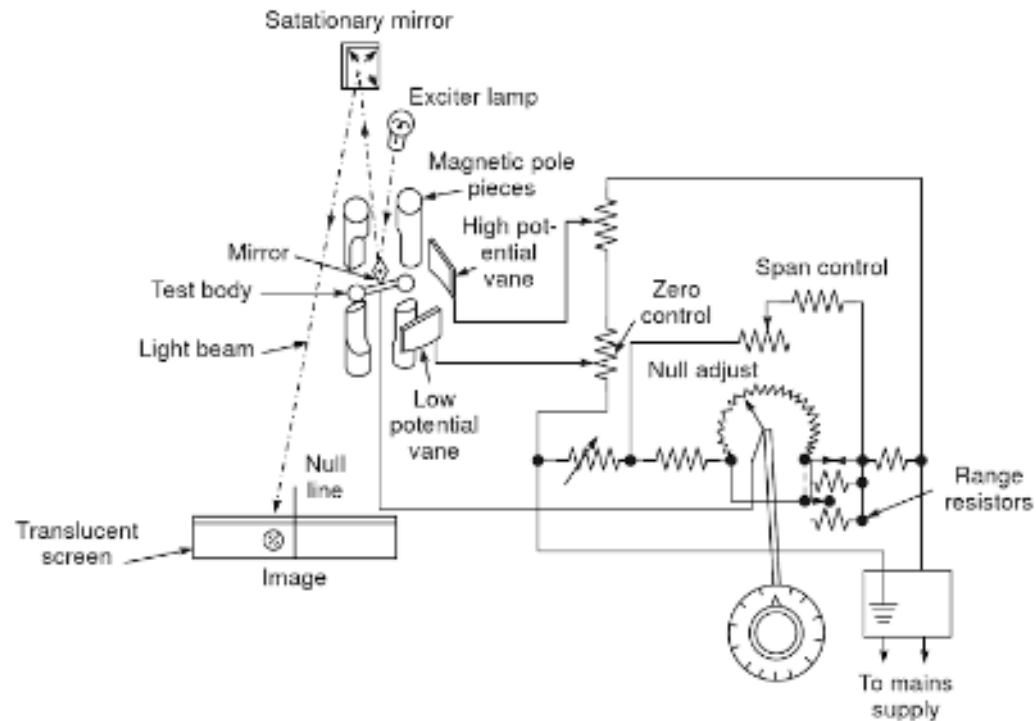
- This method uses double beam infrared spectrometer having a pair of matched gas cells in the two beams.
- One cell is filled with reference gas which is non absorbing gas like nitrogen , and measuring cell contains the sample.
- The difference in optical absorption of the sample detected between the two cells is a measure of the absorption of the sample a particular wavelength.
- Infrared analyzers are used for measuring carbon dioxide in the respired air.

Gas Chromatograph:

- The quantities of various gases in the expired air can also be determined by means of gas chromatograph in which the gases are separated as the air passes through a column containing various substances that interact with the gases.
- The reactions cause different gases to pass through the column at different rates so that they leave the column at different times.
- The quantity of each gas is measured as it emerges.

- To identify the gases in the expired air other than oxygen , nitrogen or co2, a mass spectrometer is used in conjunction with the gas chromatograph.
- The mass spectrometer identifies the ions based on their mass/charge ratio.

Paramagnetic oxygen analyzer



Functional diagram of Oxygen analyzer based on magnetic susceptibility (Courtesy: Beckman Instruments Inc., USA)

-
- The paramagnetic oxygen analyzer is based on the scientific principle that oxygen is a paramagnetic material
 - When sample containing oxygen is introduced into the test cell, the oxygen in the sample is attracted to the point of maximum field strength.
 - The magnitude of the dumbbell placement is proportional to the amount of oxygen in the sample.
 - The movement of the dumbbell is detected by a light beam from a light source exterior to the test cell.
 - The light beam is reflected from a mirror on the dumb bell body to an exterior photocell.

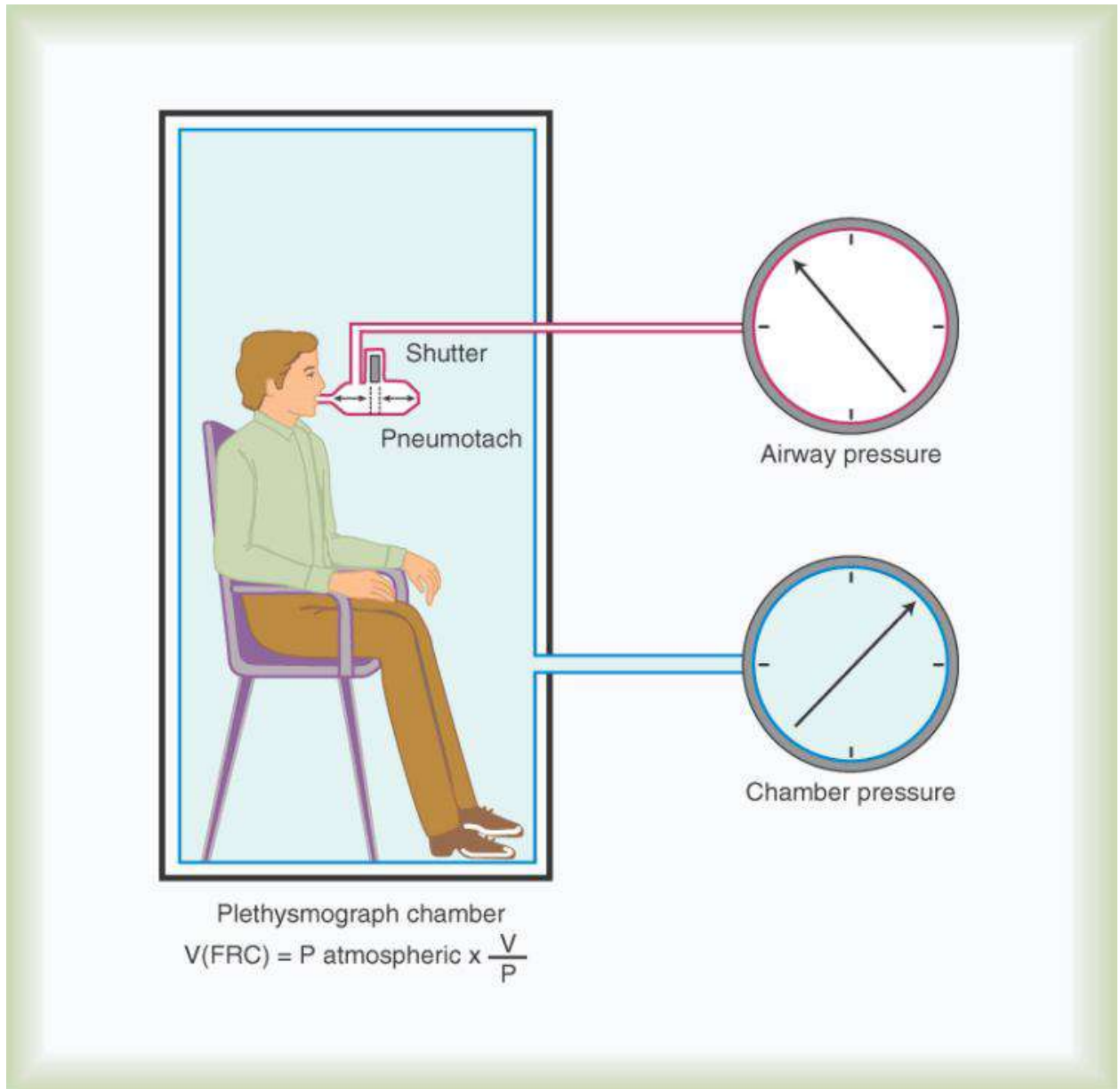
- The output of the photocell is amplified and transmitted to an indicating unit that is calibrated to read out the oxygen content in the test sample in percentage.

Measurement of gas distribution

- The distribution of oxygen from lungs to tissues and CO_2 from tissues to the lungs takes place in the blood.
- Oxygen is carried by the hemoglobin of the red blood cells, CO_2 and water combine to form carbonic acid which is dissolved in the blood.
- The amount of carbonic acid in the blood affects the pH of the blood

BODY PLETHYSMOGRAPHY

- The primary function of wholebody plethysmographs is the measurement of intrathoracic gas volume (TGV) and volume change.
- Different models of whole-body plethysmograph (or “body box”) are used to measure changes in lung volume, from mL to L.
- In body plethysmography, the patient sits inside an airtight box, inhales or exhales to a particular volume (usually FRC, functional residual capacity), and then a shutter drops across their breathing valve.
- The subject makes respiratory efforts against the closed shutter causing their chest volume to expand and decompressing the air in their lungs.
- The increase in their chest volume slightly reduces the box volume and thus increases the pressure in the box.
- This method of measuring FRC actually measures all the conducting pathways including abdominal gas; the actual measurement made is VTG (Volume of Thoracic gas).



(From Wilkins RL, Stoller JK, Scanlan CL: *Egan's fundamentals of respiratory care*, ed 8, St Louis, 2003, Mosby.)

- This utilizes Boyle's law (a constant temperature, the volume of gas varies inversely with the pressure), the ratio of the change in lung volume to change in mouth pressure is used to determine the thoracic gas volume.
- The patient breathes air from within the box through a tube containing an airflow transducer and a shutter to close off the tube for certain portions of the test.
- Pressure transducer measures the air pressure in the breathing tube on the patient's side of the shutter and inside the boxes.

- The amount of air in the box , including that in the patients lungs , remains constant, since there is no way for air to escape or enter.
- When a patient compresses that air in his lungs during expiration , his total body volume is decreased.hus educing pressure in the box.
- When patient inhales by educing the pressure in his thoracic region , his body volume increases and increases the box pressure
- The FRC is measured with the shutter in the breathing tube closed.
- The patient is instructed to pant at a lower rate against closed shutter . As he does so , he alternately expands and compresses the air in the lungs.

By measuring the changes in mouth pressure and corresponding changes in intrathoracic volume, it is possible to calculate the intrathoracic volume.

Oxymeters

The need for real-time oxygen saturation monitoring

- Respiratory failure & pulmonary disease
- Intensive care (especially neonatal)
- Anaesthesia

1. Oximeters

- Oximetry refers to the determination of the percentage of oxygen situation of the circulating arterial blood .
- Oxygen saturation = $\frac{Hbo2}{[Hbo2+Hb]}$
 Where Hbo2 is oxygenated haemoglobin
 Hb is deoxygenated haemoglobin
- When blood is withdrawn from the subject under anaerobic conditions and measurement of oxygen saturation is done at a later time in lab , the procedure is called In Vitro Oximetry.
- When oxygen saturation measurement is done while blood is flowing through the vascular system, the procedure is called In Vivo oximetry
- There are different types of oximeters

Ear Oximeter

Pulse Oximeter

Skin reflectance Oximeter

Intravascular Oximeter

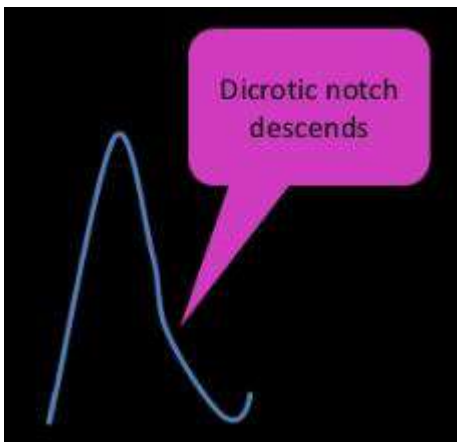
Pulse Oximeter

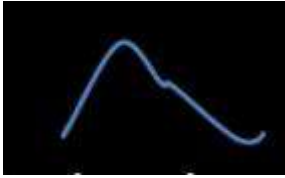


- It is one of the essential monitors for routine use in anesthesia and intensive care.
- A non invasive technology to monitor oxygen saturation of the haemoglobin.
- It measures the oxygen saturation of haemoglobin in arterial blood.
- It also measures heart rate.
- It gives an idea about the tissue perfusion by pulse waveform



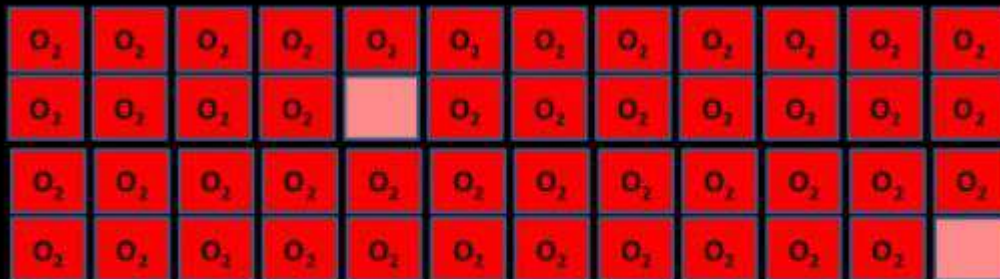
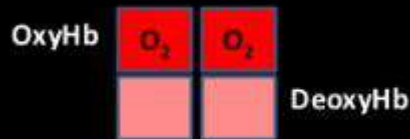
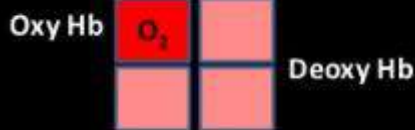
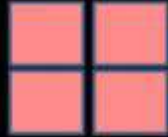
- Increased amplitude indicates vasodilatation (dilation of blood vessels, decrease in bp)
- Decreased amplitude indicates vasoconstriction
- Area under the curve indicates stroke volume





- Can be used for assessment of blood flow in revascularised limb, reanastomosed limb, in a digit after or during surgery

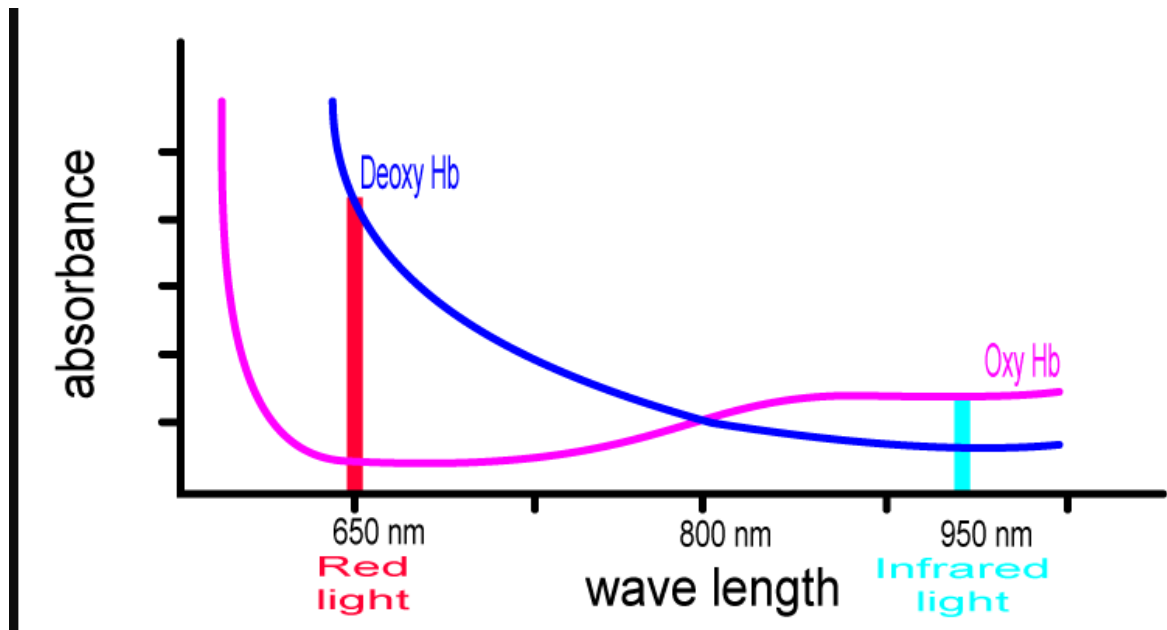
• What is oxygenation of a Hb ?



$$\text{Oxygen saturation} = \frac{\text{Oxygen content}}{\text{OxyHb} + \text{DeoxyHb}} \times 100$$

- oxygen saturation tells you the percentage of the total hemoglobin that is carrying oxygen.
- Pulse oximetry uses light to work out oxygen saturation. Light is emitted from light sources which goes across the pulse oximeter probe and reaches the light detector.

- If a finger is placed in between the light source and the light detector, the light will now have to pass through the finger to reach the detector. Part of the light will be absorbed by the finger and the part not absorbed reaches the light detector.
- The amount of light that is absorbed by the finger depends on many physical properties and these properties are used by the pulse oximeter to calculate the oxygen saturation.
- Hemoglobin (Hb) absorbs light. The amount of light absorbed is proportional to the concentration of Hb in the blood vessel. In the diagram below, the blood vessels in both fingers have the same diameter. However, one blood vessel has a low Hb concentration (i.e. low number of Hb in each unit volume of blood) and the other blood vessel has a high Hb concentration (i.e. high number of Hb in each unit volume of blood). Each single Hb absorbs some of the light, so more the Hb per unit area, more is the light is absorbed. This property is described in a law in physics called “Beer’s Law”.
- Beer’s Law: Amount of light absorbed is proportional to the concentration of the light absorbing substance
- By measuring how much light reaches the light detector, the pulse oximeter knows how much light has been absorbed. More the Hb in the finger , more is the light absorbed.
- Lambert’s Law: Amount of light absorbed is proportional to the length of the path that the light has to travel in the absorbing substance.
- The pulse oximeter uses two lights to analyze hemoglobin.
- One is a red light, which has a wavelength of approximately 650 nm. The other is an infrared light, which has a wavelength of 950 nm. (Throughout our description, we will show the infrared light in light blue. In reality, infrared light is invisible to the human eye.)
- Oxy Hb absorbs more infrared light than red light
- Deoxy Hb absorbs more red light than infrared light

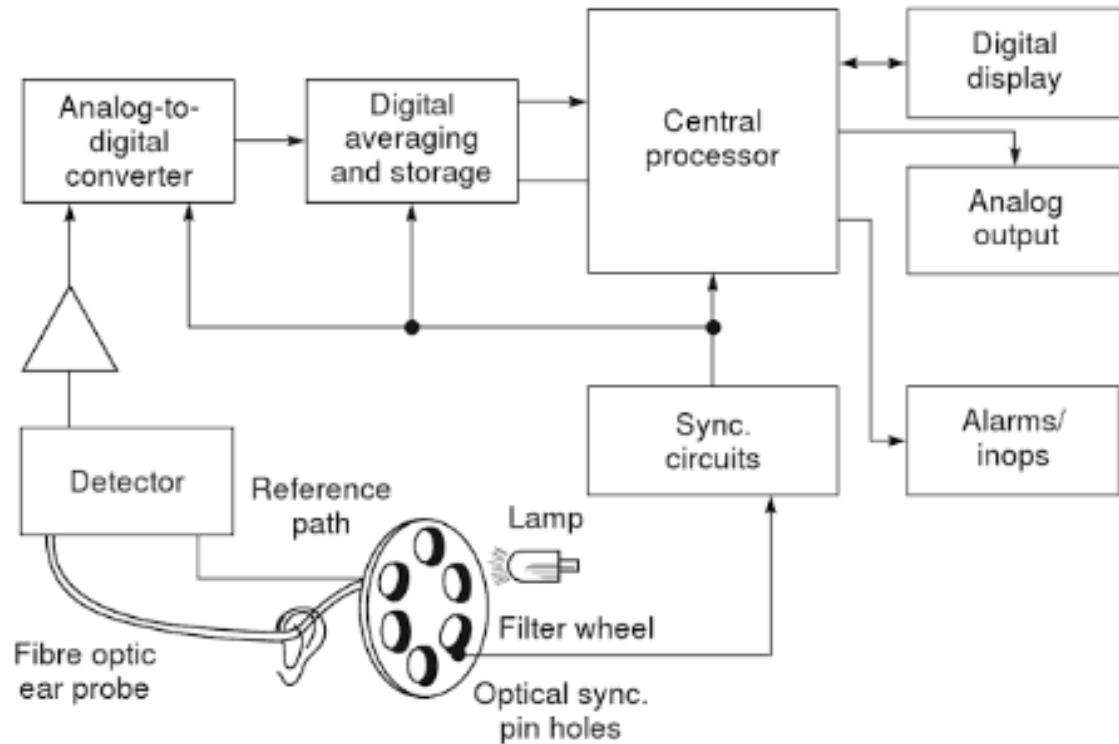


Limitations:

- Ambient lights have been shown to interfere with the measurement, therefore cover the cuff with an opaque material to prevent such interference.
- Motion artifact is also a potential problem.

EAR OXIMETER

- In this case, the pinna of the ear acts as a cuvette.
- Blood in the ear must be made similar to arterial blood in composition.
- This is done by increasing the flow through the ear without appreciably increasing the metabolism.
- Maximum vasodilatation is achieved by keeping the ear warm. It takes about 5 or 10 min for the ear to become fully dilated after the ear unit has been put up in place and the lamp turned on.



Block diagram of ear oximeter Model 47201A H.P.

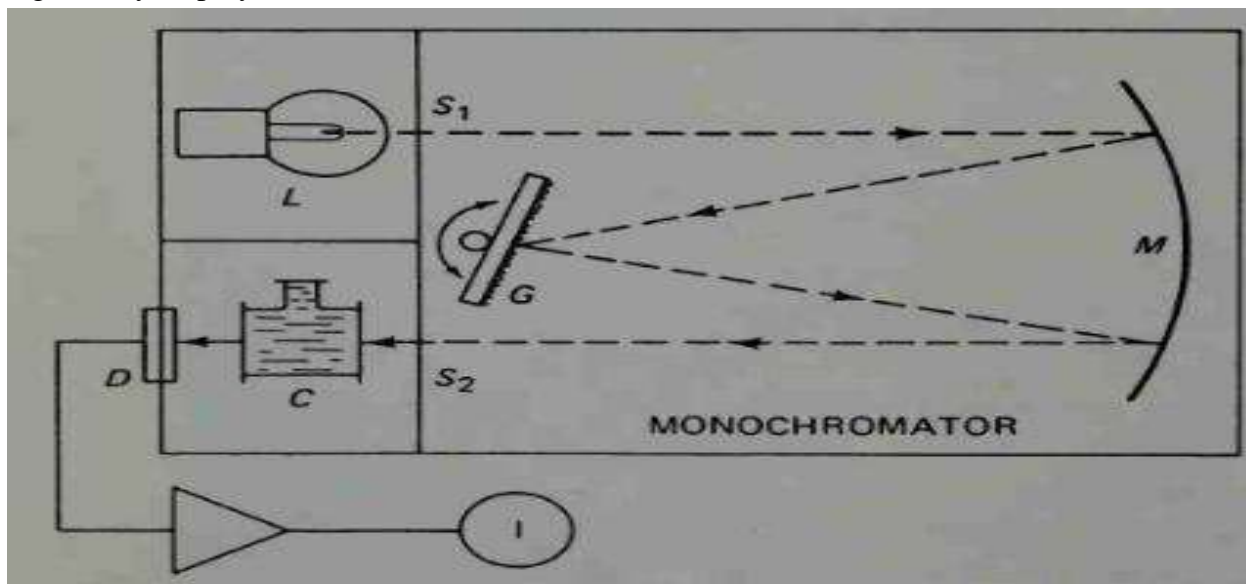
- Figure above explains the basic operation of the instrument. The light source is a tungsteniodinelamp that has a high output in the spectrum of interest.
- A lens system collimates the light beam and directs it through thin-film interference filters that provide wavelength selection.
- These filters are mounted in the periphery of a wheel rotating at 1300 rpm and thus cut the light beam sequentially. The filtered light beam then enters a fibre optic bundle that carries it to the ear.
- Another fibre optic bundle carries the light passing through the ear back to a detector in the instrument.
- A second light path is developed with a beam splitter in the path of the collimated light beam near the source. This path also passes through the filter wheel and then through a fibre optic bundle directly to the photodetector. So, the detector receives two light pulses for each wavelength
- The processor takes the ratio of two pulses as the measured value; so readings are compensated for any changes in the spectral characteristics of the light source and optical system.

- The current developed at the photodetector is only 0.5 nA or less during a light pulse. This is amplified in a high gain amplifier and then converted to a 16-bit digital form by an A-D converter synchronized with the wheel rotation.
- The 16-bit words are given to a digital signal averager that performs two functions. First, it averages out the noise content of the signal with a time constant of 1.6s and secondly it serves as a buffer to hold information till it is required for computation.
- Computation of percent oxygen saturation is accomplished by a 24-bit algorithmic-state machine.
- It uses serial processing with the program stored in ROM and the necessary coefficients of the equations stored on a field programmable ROM. The computation circuits also derive the quantity of total haemoglobin seen within the field of view of the earpiece. If this quantity is low, the instrument displays an 'Off Ear' indication.
- From the computational section data is transferred in pulse-decimal form to the output circuit board where it is converted to BCD for the front panel digital display
- The patient related part consists of arterializing blood flow in the pinna by a brisk 15 s rub.
- Application of the probe to the ear results in a suitable display in about 30s. A built-in heater regulated 41° maintains arterialization. Restandardization is not required when the instrument is to be used on other patients
- Measurements at eight wavelengths provide a great deal of information, which makes it possible to account for eight unknowns. This is sufficient to take into consideration the patient to patient variables and account for the various forms of haemoglobin.
- The procedure is simple, requiring only the storage of initial light intensities at each of the eight wavelengths. However, it is still necessary to arterialize blood flow by warming the ear, and a large ear probe incorporating fibre optics is necessary to make the system work.

2. SPECTROPHOTOMETERS:

- A spectrophotometer is an instrument which isolates monochromatic radiation in a more efficient and versatile manner than colour filters used in filter photometers
- The substances that can be quantitatively analyzed by spectrophotometers are numerous. They include: hemoglobin, erythrocytes, hematocrit, amylase, bilirubin, cholesterol, glucose, urea, creatinine, lipase, triglyceride, albumin, alcohol, ammonia, copper, magnesium, lactate, calcium, iron, magnesium, aluminium, sodium carbonate, carbon monoxide and even certain enzymes.
- In these instruments, light from the source is made into a parallel beam and passed to a prism or diffraction grating, where light of different wavelengths is dispersed at different angles.
- The amount of light reaching the detector of a spectrophotometer is generally much smaller than that available for a colorimeter, because of the small spectral band width.

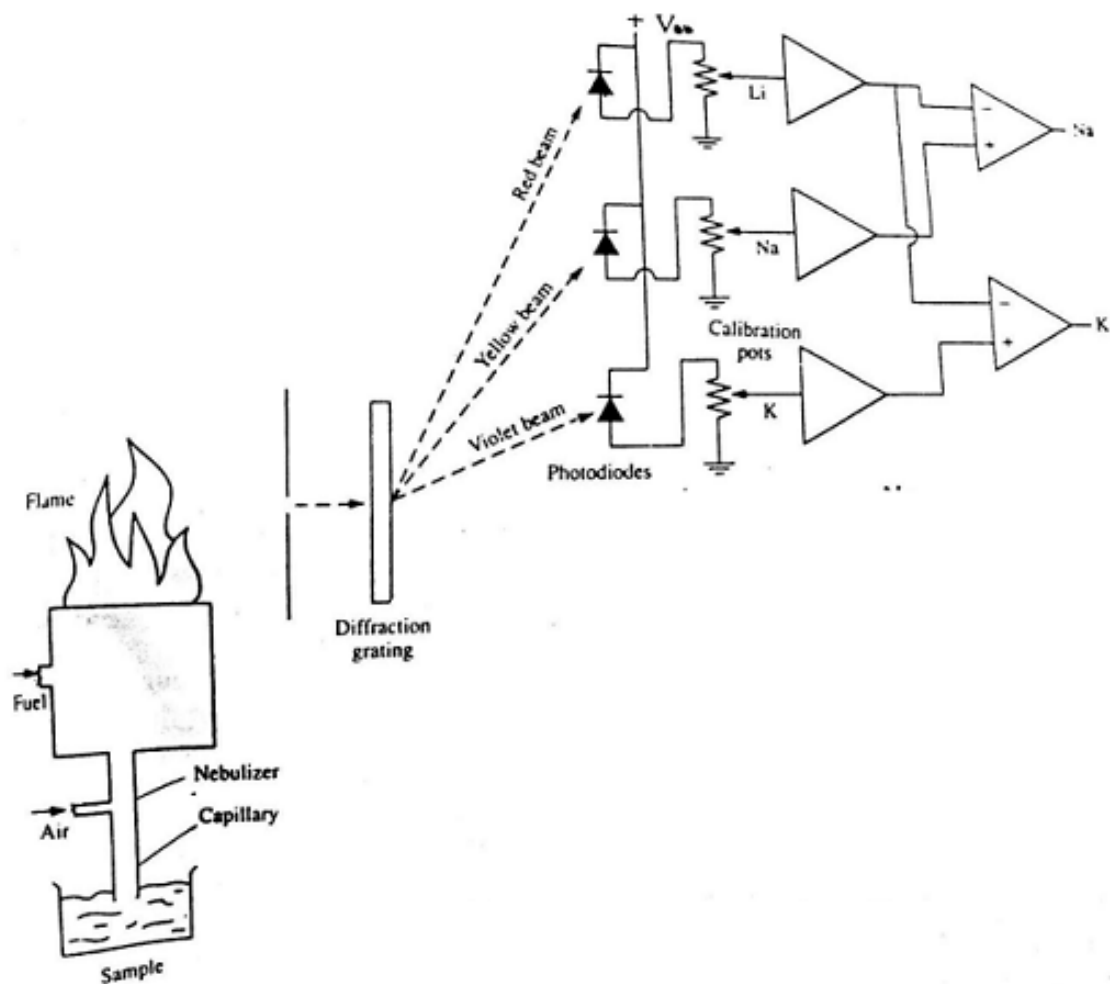
- Therefore, a more sensitive detector is required. A photomultiplier or vacuum photocell is generally employed



- In this device the simple selection filter of the colorimeter is replaced by a monochromator.
- A monochromator uses a diffraction grating G (or a prism) to disperse light from a lamp that falls through an entrance slit S, into its spectral components
- An exit slit S₂ selects a narrow band of the spectrum, which is used to measure the absorption of a sample in cuvette C.
- The narrower the exit slit, the narrower the bandwidth of the light, but also the smaller its intensity.
- A sensitive photodetector D (often a photomultiplier) is therefore required, together with an amplifier and a meter, which is calibrated in units of transmittance or absorbance. The wavelength of the light can be changed by rotating the grating.
- The spectrophotometer allows the determination of the absorption of samples at various wavelengths. The light output of the lamp, however, as well as the sensitivity of the photodetector and the light absorption of the cuvette and solvent, varies when the wavelength is changed
- This situation requires that, for each wavelength setting, the density reading be set to zero, with the sample being replaced by a blank cuvette, usually filled with the same solvent as used for the sample.
- In double-beam spectrophotometers this procedure is done automatically by switching the beam between a sample light path and a reference light path, generally with a mechanical shutter or rotating mirror. By using a computing circuit, the readings from both paths are compared and only the ratio of the absorbances (or the difference of the densities) is indicated.

3. FLAME PHOTOMETER

- A flame photometer is used to analyse urine or blood in order to determine the concentration of potassium (K), sodium (Na) and lithium (Li).
- Sometimes lithium is used as a calibration substance in the analysis of the other three substances.
- A known amount of lithium is added to the sample and the emitted light intensity of the sample under analysis is measured relative to that of the lithium.
- By this way any error due to the varying flame temperature is eliminated.
- Using an atomizer, the liquid sample is sprayed into fine droplets by passing oxygen or air past the opening in it.
- A combustible gas like acetylene is also added with air.



- The sample-air mixture is burnt out and the light emitted in the flame is passed through a narrow slit and then to diffraction grating.
- The diffracted colours are incident on various photodiodes.
- The concentration of potassium ions is detected by observing the peak height of the spectral line corresponding to it.
- For potassium, the wavelength that we are interested is 4047 \AA (violet).
- For sodium, the interested wavelength is 5890 \AA (yellow).
- For lithium, it is 6708 \AA (red).
- Separate photodetector is used for each channel.
- The photodetector circuit consists of a reverse biased diode in which current flow increases as the intensity of light incident upon it increases