CONTENTS

I. PHYSIOLOGY OF CIRCULATION

I.1. Cardiac physiology

I.1.1. *In situ* frog heart: cardiogram; temperature influence

Definition: cardiogram refers to the recording of mechanical activity of the heart
Principle: the mechanical manifestation of the *in situ* frog's beating heart is directly recorded using Marey cardiograph.
Materials required: Marey cardiograph, kymograph with smoked drum, dissecting set to obtain frog heart *in situ* preparation

- *Marey cardiograph* consists of two paddles, one fixed and one mobile connected to a writing pen (figure 1.1).

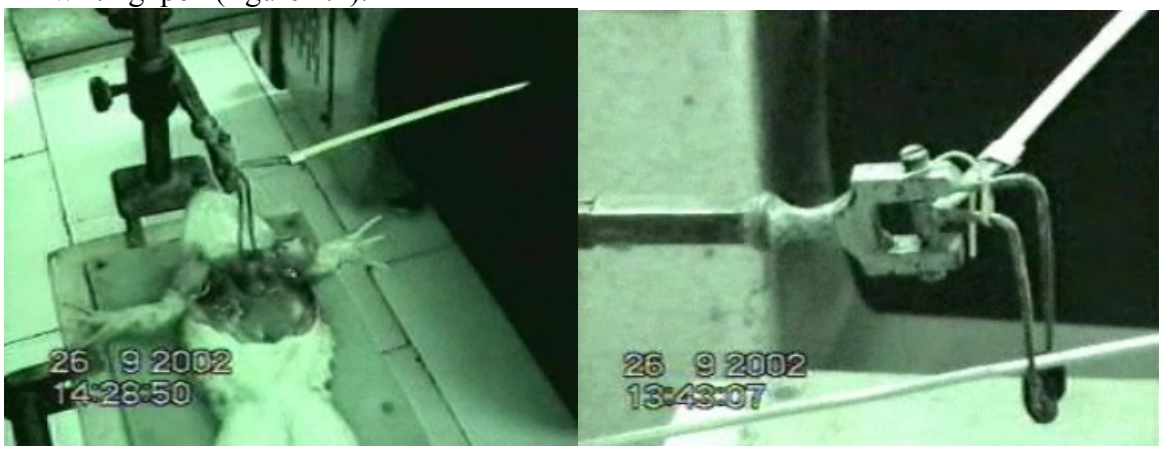


Figure 1.1. Marey cardiograph

- *Kymograph* (figure 1.2) entails a base with motor and a straight shaft over which the drum (rotating cylinder) is fixed. The drum can be moved up and down as desired by moving a screw on the top of the shaft. Below the base of the kymograph are present levelling screws. The drum is wrapped over by a glazed paper which is smoked uniformly and thin in order to obtain good tracings. The kymograph has provision for off and on, locking system, speed selector for rotating drum.

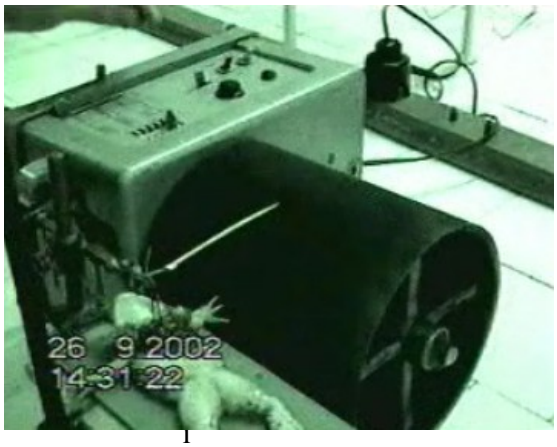


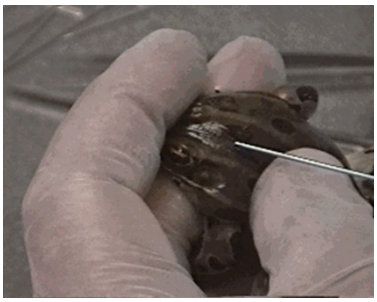
Figure 1.2. Kymograph

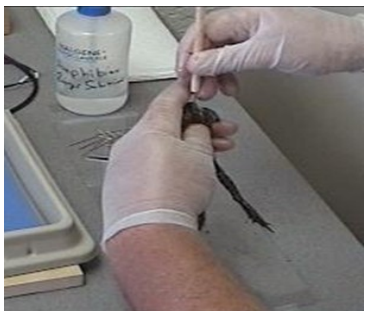
- *Frog heart in situ preparation*: fresh living frog, dissecting set (dissection board, scissors with blunt and sharp end, pins to fix the frog on the board), pithing needle, gloves, Ringer's solution (13g of NaCl, 0.28g of KCl, 0.3g of $CaCl_2 2H_2O$, 0.4g of $NaHCO_3$ for 2L of distilled water), Pasteur pipette, wipes.

Technique:

1. *pithing of the frog (destruction of spinal cord of the frog with a needle)*

- catch the frog gently, but firmly with a dry cloth or cotton
- hold the frog with left hand tightly and fix the head of the frog by keeping the index finger on the head, thumb on the back and other three fingers on the belly of the frog
- press between the head and trunk with index finger and feel the depression at the atlanto-occipital joint
- pass the needle through the depression and move it inside vertebral canal towards the lower side; rotate the needle in all directions to destroy the spinal cord properly (the needle is correctly positioned because immediately after the needle is directed into the spinal cord, the muscles of the trunk and lower limb become spastic; they become flaccid after destruction of spinal cord)
- after pithing the frog loses its voluntary and reflex movements, but is still alive and can be used for experiments.





2. dissection:

- place the frog on the dissecting board on its back (dorsal surface) with the pins in their extremities;
- lift the skin over the abdomen and cut abdominal wall with a pair of scissors towards the sternum;
- raise the xiphisternum with a blunt forceps and cut lateral the diaphragm and then cut laterally the chest cage towards pectoral girdle to remove the anterior wall of the thorax and expose the heart in the pericardial sac;
- carefully remove the pericardial sac and cut any connective tissue attachments so that the heart beats freely;
- keep the heart moist at all times with Ringer's solution applied with the Pasteur pipette;
- study the different parts of the heart: on anterior view it is observed the initial dilated portion of the aorta (bulbus arteriosus) which divides into 2 aortae; if the ventricle is lifted, on the posterior view it can be identify the sinus venosus separated from atria by a white crescentic line. Two superior vena cavae and the inferior vena cavae enter the sinus venous (figure 1.3).

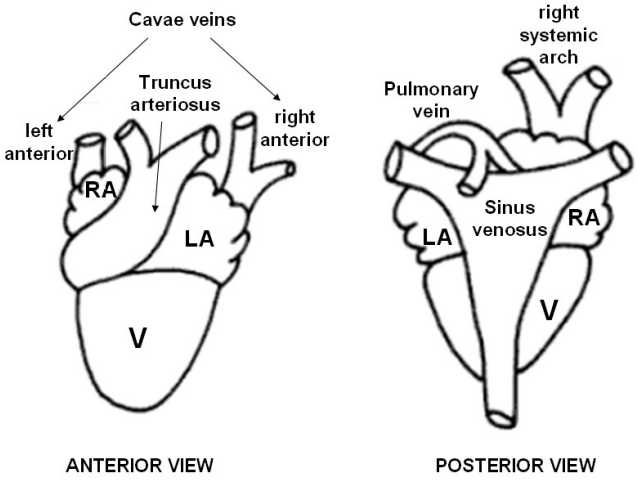


Figure 1.3. Frog heart: anterior and posterior view (V = ventricle, RA = right atrium, LA = left atrium)

- it can be observed the changes in volume and shape of the heart during cardiac cycle (systola and diatola)

3. the beating heart will be placed between the paddles of Marey cardiograph and the writing pen attached to mobile paddle will record on the rotating smoked drum of the kymograph a trace called cardiogram.

4. on cardiogram it can be observed the rate of the frog heart (frequency of the contractions on orizontal axis) and the amplitude of the contractions (on vertical axis, which is directly related with the force of the contraction or contractility of the frog myocardium)

5. record the heart contractions at room temperature;
- then drop warm (~30°C) frog Ringer's solution on the heart until significant changes are seen in rate and contractility; record contractions at this time;
- next, drop cold Ringer's (4-10°C) on the heart and record when changes are observed.

Results: The cardiogram presents 2 types of waves (figure 1.4):

- atrial activity (not always seen) with atrial systole (upward) and atrial diastole (downward)
- ventricular activity with ventricular systole (upward) and ventricular diastole (downward)

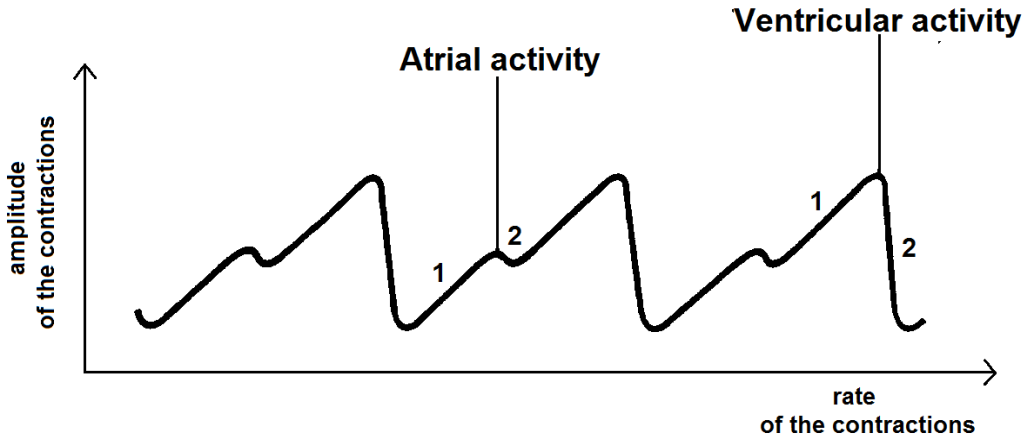


Figure 1.4. Frog cardiogram with mechanical activity of the atria
(1- atrial systole. 2- atrial dyastole) and mechanical ativity of the ventricles
(1- ventricular systole, 2 – ventricular dystole)

In the cold Ringer's solution, the frog heart beat slower than baseline and the amplitude of the contractions lower (figure 1.5).

In the warm Ringer's solution, the frog heart beat faster than baseline and the amplitude of the contractions higher (figure 1.5).

Discussions:
- Explain the effect that decreasing the temperature had on the frog heart. How do you think the human heart would respond?

Frog heart rate decreases with decrease in temperature because the frog has no internal homeostatic regulating mechanism, so the temperature will change depending on external environment. Human heart rate should not change because humans are homeothermic, i.e. are able to maintain a constant (~ 37°C) internal body temperature even though the external temperature is changing. A lower temperature for a human's heart would cause a decrease in heart rate and possibly hypothermia in extreme external temperature conditions.

- Explain the effect that increasing the temperature had on the frog heart rate. How do you think the human heart would respond?

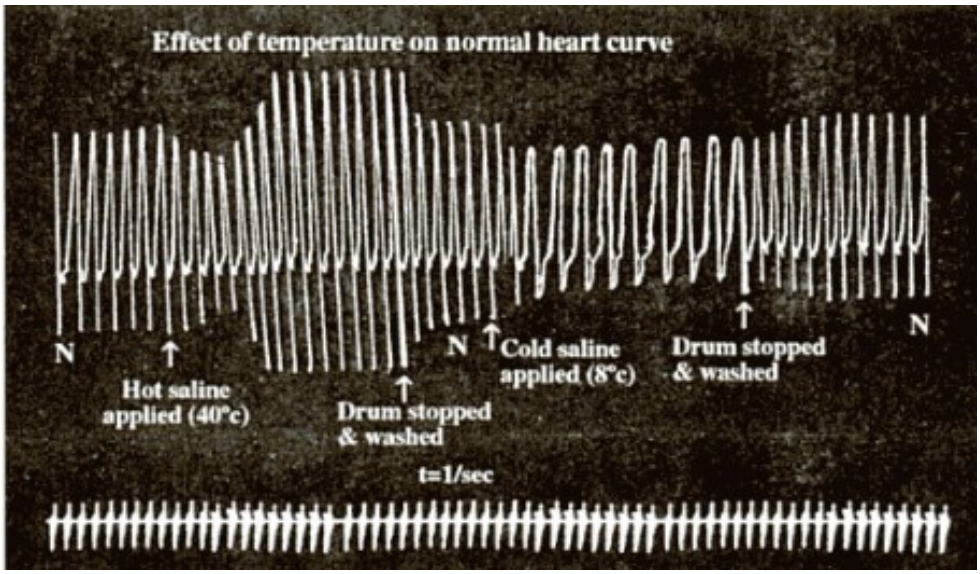


Figure 1.5. Effect of temperature on frog's heart.

Data interpretation/ Clinical significance:
Why do we choose the frog for this laboratory experiments?

The frog is an amphibian and it is a good choice for laboratory experiments because of its physiological characteristics:

- it has low metabolic needs
- it functions well at room temperature (poikilothermic vertebrate)
- it absorbs oxygen through its moist skin.

The frog heart has 3 chambers: two atria and a single ventricle.

The atria receive blood from venous sinus that collect deoxygenated blood from veins that drain the organs of the body and oxygenated blood from the lungs and skin (which also serves as a gas exchange organ in most amphibians). Both atria empty into the single ventricle. So when the ventricle contracts, the blood is send into the truncus arteriosus and then to all organs of the body to supply the needs

Differences between frog heart and human heart:

	Frog heart	**Human heart**
1. Heart chambers	3 chambers (2 atria + 1 ventricle)	4 chambers (2 atria + 2 ventricles)
2. Sinus venosus	Receive venous blod	Absent, venous blood is collected by the right atrium
3. Coronary arteries	Not well developed	Well developed
4. Blood in ventricle	Mixed (oxygenated and deoxygenated)	Not mixed
5. Myocardial perfusion	Directly from blood present into ventricle	Through coronary circulation
6. Pacemaker	Venous sinus	Sinoatrial node
7. White crescentic line	Function as a parasympathetic ganglion	Absent

The heart wall has 3 layers: pericardium (external layer), myocardium and endocardium (internal layer). The myocardium has some functional properties:

1. *Excitability (batmotropism)* - the ability of myocardium to respond to adequate stimuli by generating an action potential followed by a mechanical contraction.
2. *Automaticity (chronotropism)* - capacity of myocardium have the to generate spontaneous action potential without external influences (neuro - hormonal control);
3. *Rhythmicity* - the ability of myocardium to contract in a regular constant manner
4. *Conductivity (dromotropism)* - the ability of myocardium to conduct the action potential generated by the sino-atrial node (the pacemaker of the heart).
5. *Contractility (inotropism)* - the ability of the myocardium to develop tension (to convert chemical energy into mechanical work)
6. *Tonicity (tonotropism)* - the ability of myocardium to maintain a certain contraction state during diastole.

Automaticity and rhytmicity are *intrinsic properties*, specific for myocardium and the rest of the properties are *common with the other muscles*.

The properties of the heart that are studied in this experiment are:

- *automaticity* (by observing the automatic beating of the heart),
- *rhythmicity* (by observing the regular contractions of the heart),
- *conductivity* (conduction of impulse from the sinus venous to the ventricles; better demonstrated by Stannius ligatures; in this experiment - by observing the contraction of atria first followed by contraction of ventricle),
- *contractility* (by recording the systole and diastole in the tracings).

The low temperature decrease

- automaticity (negative chronotrop effect) because reduce the activity of the pace maker from venous sinus, so it decrease the heart rate
- rhythmicity
- conductivity (negative dromotrop effect)
- contractility (negative inotrop effect), so it decrease the amplitude of contractions.

All the effects of temperature on frog heart could be explained by directly influence of temperature on metabolism of cardiac cells. If the metabolism decrease, the exchanges between cells and environment decrease, including rapid transmembrane ionic exchanges (action potential).

The high temperature has opposite effect, it increases

- automaticity (positive chronotrop effect)
- rhythmicity
- conductivity (positive dromotrop effect)
- contractility (positive inotrop effect).

I.1.2. In situ/in vitro frog heart: demonstration of cardiac automatism (Stannius ligatures)

Definition: cardiac automatism refers to generation of spontaneous action potential without external influences.

A. Demonstration of cardiac automatism using *in situ* frog heart: Stannius ligatures

Principle: different parts of the heart has got different rates of impulse generation

Materials required: dissecting set to obtain frog heart *in situ* preparation, surgical thread

Technique and results (figure 1.6):
- obtain frog heart *in situ* preparation (see section I.1.1)
- *1st Stannius ligature*
    - site of ligature: at the junction of venous sinus and atria over the white crescentic line; the conduction of impulse from sinus to atria is mechanically blocked.
    - effect: the atria and ventricle stop beating while the venous sinus continue to beat as before.
    - conclusion: venous sinus contain a excitatory ganglion called Remak pacemaker (noted with +)

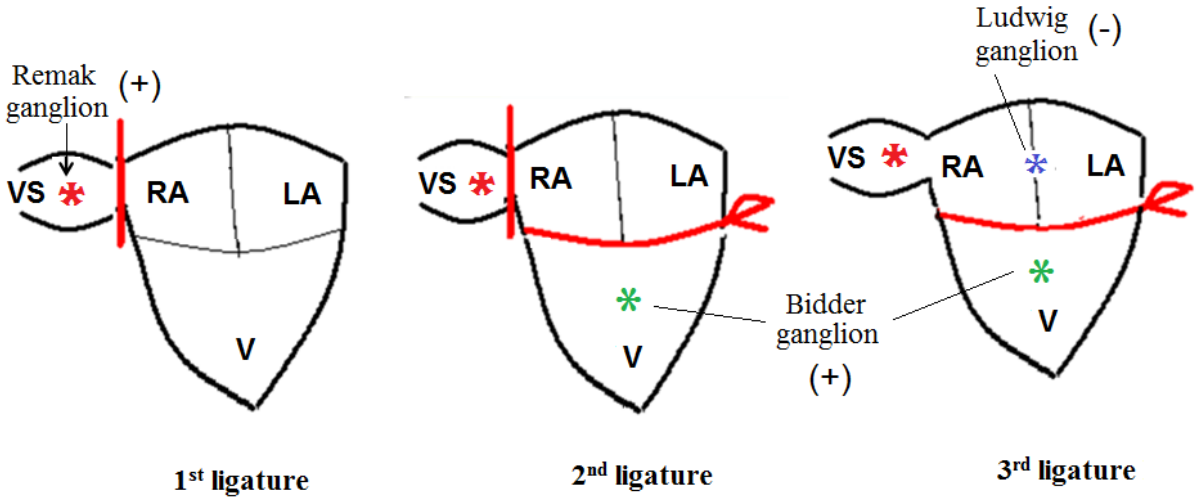


Figure 1.6. Stannius ligatures
(VS = venous sinus, RA = right atrium, LA = left atrium, V = ventricle)

- *2nd Stannius ligature*
    - site of ligature: the ligature is applied around atrioventricular groove (prevent the transmission of impulse from atria to ventricle) in the presence of the Ist ligature.
    - effect:
        - the venosus sinus continue to beat with its own rhythm generated by Remak's ganglion;
        - the atria stop beating (so here it is located an inhibitory ganglion called Ludwig);
        - the ventricle generate its own rhythm and beat independent of atria at much slower rate than venosus sinus rate (1/2 of rate of venosus sinus) because of another excitatory ganglion called Bidder;
        - the communications through conductive system between venous sinus, atria and ventricle has been blocked by the ligatures, so these three regions function independently.
    - conclusion:
        - Remak's ganglion has excitatory function (+)
        - Ludwig ganglion has inhibitory funtion (-)
        - Bidder ganglion has excitatory function (+) and its rate is 1/2 of rate of venosus sinus

- 3rd Stannius ligature

- site of ligature: is made on a new frog heart between atria and ventricle around the atrioventricular groove;
- is made to establish the relationship between Remak and Ludwig ganglions.
- effect:
  - the venosus sinus and atria continue to beat with the rate generated by Remak's ganglion (+)
  - the ventricle continue to beat with the rate generated by Bidder ganglion (+) (1/2 of rate of venosus sinus)
- conclusion: Remak's ganglion suppress Ludwig ganglion

Discussions:

So long as the atria are in connection both with the venous sinus and the ventricle, the excitatory ganglion Remak is sufficiently powerful to impose the cardiac rhythm because has the highest rate of generation action potentials. When the first ligature is applied, the inhibitory ganglion of the atria is supposed to be so powerful as to keep both it and the ventricle in a state of rest. When the ventricle is separated from the atria and their inhibitory influence removed, the ventricle begin to pulsate rhythmically.

In order to obtain a clearer idea of function of the conductive system of the heart, many variations of the above fundamental experiment have been made (many ligatures had been placed on the heart and their effects had been observed)

Data interpretation/ clinical significance:

The Stannius ligatures demonstrate the hierarchy of impulse generation as well as the ability of different parts of the heart to act as pacemakers, if required.

B. Demonstration of cardiac automatism using *in vitro* frog heart:

Principle: generation of spontaneous contraction without external influences.
Material required: dissecting set to obtain frog heart *in vitro* preparation, Petri dish, Ringer solution
Techique:

- obtain frog heart *in situ* preparation (see section I.1.1)
- remove the heart of the frog and put it into a Petri dish with Ringer solution

Results:

- observe the spontaneous contractions of frog heart without external influences

Interpretation:

- inside frog heart there is a structure that produce rhythmic heart beats.

Practical significance: Automatism (chronotropy) - pacemakers and impulse formation

Pacemaker represents the part of the heart that generates rhythmic impulses for regular heart contractions.

In the human heart the primary pacemaker is sino-atrial (SA) node because of the highest rate of impulse generation. So, in the human heart there is a hierarchy of pacemaker activity, where the SA node pacemaker normally suppresses the slower pacemaker activity in the atrio-ventricular (AV) node and Hiss bundle. The activity of the lower pacemakers become manifest only when they fire more rapidly than SA node, means that firing rate of SA node pacemaker is decreased or conduction of impulses generated in the SA node is blocked.

In normal resting human heart the firing rate of

- SA node is 60 - 80 / minute → give the normal rhythm of the heart → *sinusal rhythm*
- AV node is 40 - 60 / minute → impose *nodal rhythm*
- Hiss bundle is 20 -40 / minute → impose *idioventricular rhythm*

In amphibian heart, Remak ganglion from sinus venous acts as pacemaker, though other parts of the heart can also generates rhythmic impulses (Bidder ganglion from ventricle) proved by experiment above (Stannius ligatures)

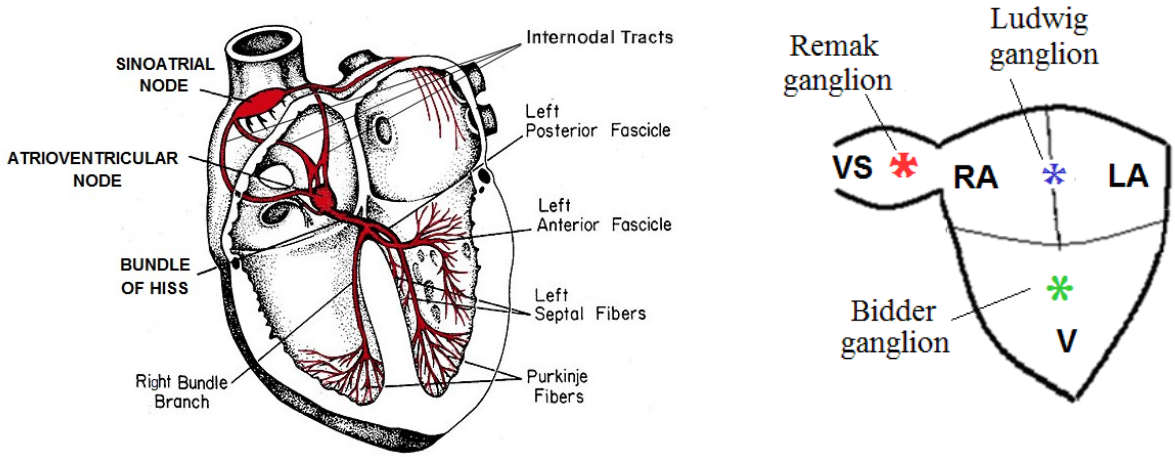


Figure 1.7. Conduction system of human heart (left) *versus* frog heart (right) (VS = venous sinus, RA = right atrium, LA = left atrium, V = ventricle)

I.1.3. In situ frog heart: influence of vagal stimulation and the "escape phenomenon"

Principle: recording the effect of vagal stimulation of heart on a rotating drum
Materials required: dissecting set to obtain frog heart *in situ* preparation, electrical circuit to stimulate the vagus nerve, signal marker
Technique and results:

- obtain frog heart *in situ* preparation (see section I.1.1)
- extend the incision to the lower jaw and cut the platysma. Expose petrohyoid muscle (has a shinning colour and insertion is from the base of the skull to posterior corner of hyoid bone). Superficial to the petrohyoid muscle are located the glossopharyngeal and hypoglossal nerves. Along the lower border of the petrohyoid muscle there is a neurovascular bundle formed by the laryngeal nerve, the carotid artery and the vagosympathetic trunk.

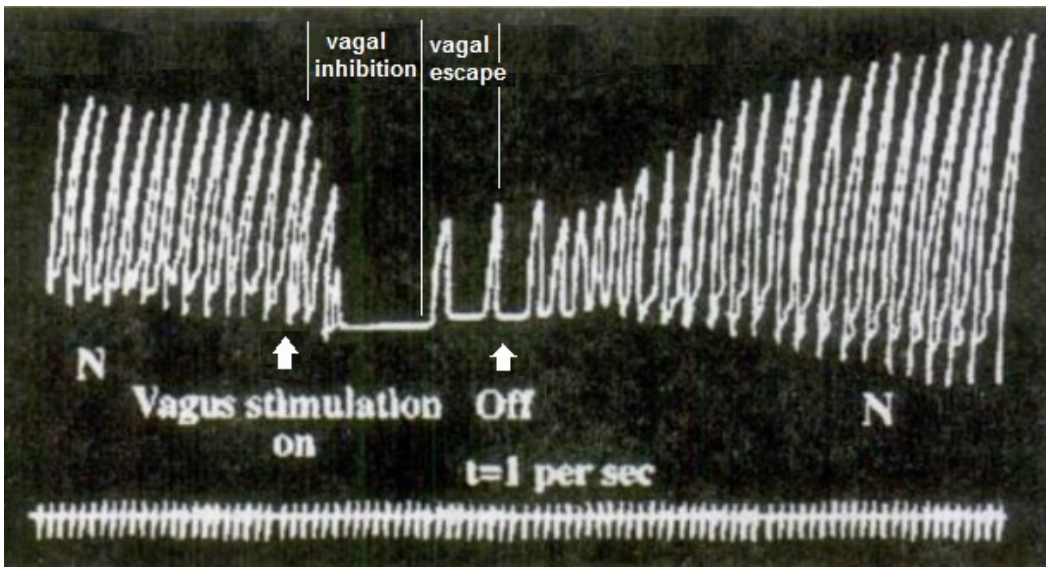


Figure 1.8. Effect of vagal stimulation on frog heart.

- isolate the vagus nerve carefully with a thin glass rod and pass a thread below the nerve and place the stimulation electrodes under the vagus nerve;
- record a normal cardiogram;
- stimulate the vagus nerve with a lower strength of stimulus and record the effect; increase gradually the intensity of stimuli till the heart stops in diastole; the decreasing heart rate, force of contraction and finally stopping of the heart in diastole due to vagal stimulation is called *vagal inhibition* (figure 1.8);
- after the heart stops, continue vagus nerve stimulation and observe that heart starts to beat again with a lower rate than normal (because the escape rhythm is a ventricular rhythm) – phenomenon called *vagal escape* (escape of heart from the inhibitory influence of the vagus nerve) (figure 1.8)

Discussions and clinical significance:

- the vagal inhibition occurs due to release of acetylcholine at the nerve endings. Acetylcholine will increase the potassium conductance in the nodal tissue by opening the K^+ACh channels (↑ K^+ efflux → hyperpolarisation) → decrease the firing rate. Also, acetylcholine binds muscarinic receptors and through Gi proteins inhibits adenilat cyclase, so the concentration of cAMP in the cell decreases and slows the opening of the calcium channels → decrease the firing rate in the conduction system. A decrease in calcium concentration in the myocardial cells decreases the force of the contraction (amplitude of systole and diastole on cardiogram)
- *vagal escape*: a strong vagal stimulation may lead to stoppage of the heart. But, the heart recovers automatically, even after the continuation of vagal stimulation. Different explanations have been given to clarify the abolition of the inhibitory effect of vagal stimulation:
    - *idioventricular rhythm* generated by the excitatory ventricular pacemaker (half rate than normal rate of venous sinus). Strong vagal stimulation stops venous sinus and atria. The ventricle also stop due to the lack of pacemaking activity from venous sinus. But, the ventricle is not supplied by the vagus nerve, so the ventricular pacemaker starts generate the impulses that give it own intrisec rhythm called idioventricular rhythm.
    - *depletion of acetylcholine* released at the nerve endings of the vagus nerve due to prolonged stimulation. Acetylcholine is degraded rapidly by the acetylcholinesterase in the synaptic cleft, the nerve-endings are depleted of acetylcholine, the acetylcholine receptors are no longer stimulated and the vgal stimulation loses its inhibiting effect.
    - *sympathetic stimulation*. The vagus nerve is accompanied by sympathetic nerve fibers in a common bundle called vagosympathetic trunk. After vagal inhibition, when the heart stopped, continuing stimulation of vagosympathetic trunk activates the sympathetic nerve fibers and heart starts to beat again.

Vagal tone: normal heart rate is lower than intrinsic heart rate in a denervated heart. This decrease from intrinsic heart rate suggests a tonic influence of the vagus nerve on heart – called vagal tone.

In clinics, parasympathetic stimulation is obtained through different *vagal maneuvers*: Valsalva maneuver, carotid sinus massage, viscero-vagal reflex *(see lecture and minimum knowledges.*

I.1.4. In situ frog heart: extrasystole

Definition: extrasystole (premature beat, premature contraction or ectopic beat) represents a contraction of the heart outside the normal rate. Usually, extrasystole occurs prior to the time that normal contraction would have been expected.
Principle: record the frog heart cardiogram, apply electrical stimuli at different phases of cardiac cycle and observe in which phase the response appears.

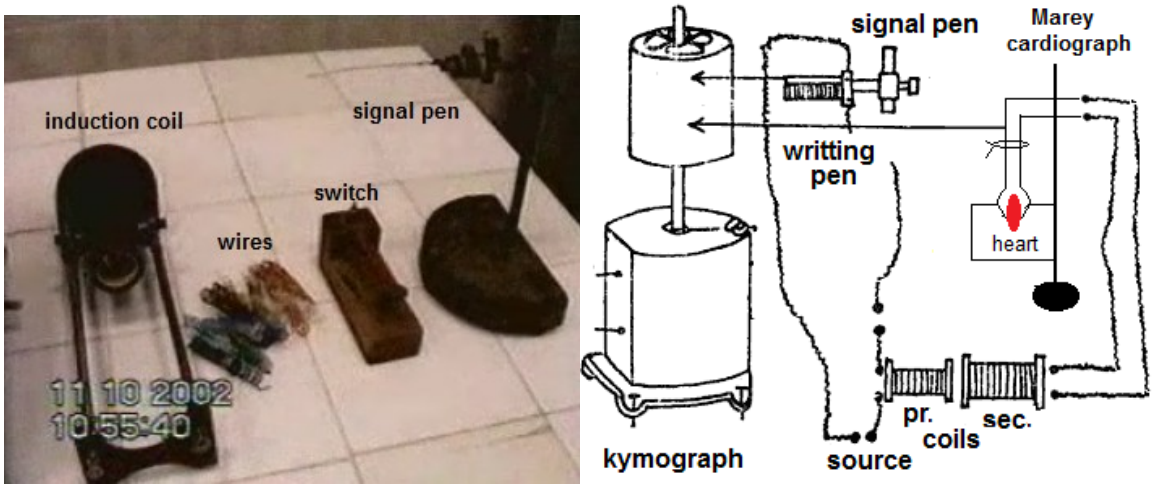


Figure 1.9. The set up for recording extrasystole

Materials required:
- dissecting set to obtain frog heart *in situ* preparation (see section I.1.1);
- Marey cardiograph;
- kymograph with smoked drum;
- electrical circuit for heart stimulation: electrical source, induction coil (to establish stimulus intensity) with primary coil connected to signal pen (that mark the moment of stimulation) and with secondary coil connected to cardiograph (the paddels constitutes the stimulationg electrodes used to apply ectopic stimuli).

Results (figure 1.10)*:*
- when the heart is stimulated with an external stimulus (electrical current E1) during systole (ascending limb of cardiogram), the stimulus fails to induce contraction, the cardiogram remain unchanged because the heart is unexcitable during systole;
- when the heart is stimulated with an electrical stimulus E2 during or at the end of the diastole (on descending limb or at the end of cardiogram), a premature contraction is recorded. This contraction, called *extrasystole* is followed by a pause named *postextrasystolic pause*.

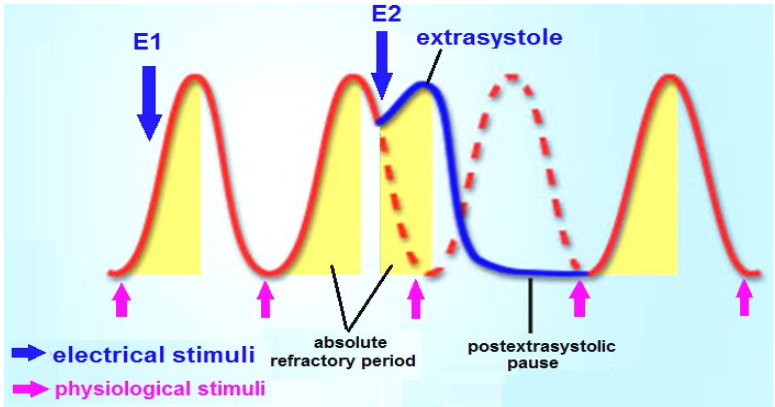


Figure 1.10. Extrasystole diagram.

Discussions and clinical significance (figure 1.11):

When the heart contractions are caused by other stimuli than those generated by physiological pacemaker of the heart (Remak ganglion of venous sinus in frog heart; SA node in human heart), these stimuli are called *ectopic stimuli* and the heart contractions determined by them – *extrasystoles*.

If the ectopic stimulus E1 is applied during systole, it remains without response, because the heart is unexcitable being in *absolute refractory period* (law of periodic inexcitability of the heart: while depolarized the heart cannot respond to other stimulus, no matter how intense it will be – *see lecture*).

If the ectopic stimulus E2 falls during diastole it catches the heart during excitable period, in *the relative refractory period* and produce an extrasystole followed by a *postextrasystolic pause*. The postextrasystolic pause appears because the normal stimulus fired by physiological pacemaker catches the myocardium in the absolute refractory period of the extrasystole and it fails to induce a response (heart contraction). Like any contraction (or systole), the extrasystole has an absolute refractory period when the myocardium is unexcitable. The pause begins from the end of the extrasystole and last till the next normal heart contraction induced by next stimulus fired by physiological pacemaker. Next stimulus fired by normal cardiac pacemaker finds the heart in the postextrasystolic pause, specifically in the relative refractory period of the extrasystole.

If the ectopic stimulus is applied at the beginning of diastole, it will determine a weak premature contraction (because the ventricle does not have time to fill with blood ➔ low end diastolic volume ➔ low stroke volume) with low amplitude on cardiogram followed by a long postextrasystolic pause.

If the ectopic stimulus is applied at the end of the diastole, it will determine a strong premature contraction followed by a short postextrasystolic pause.

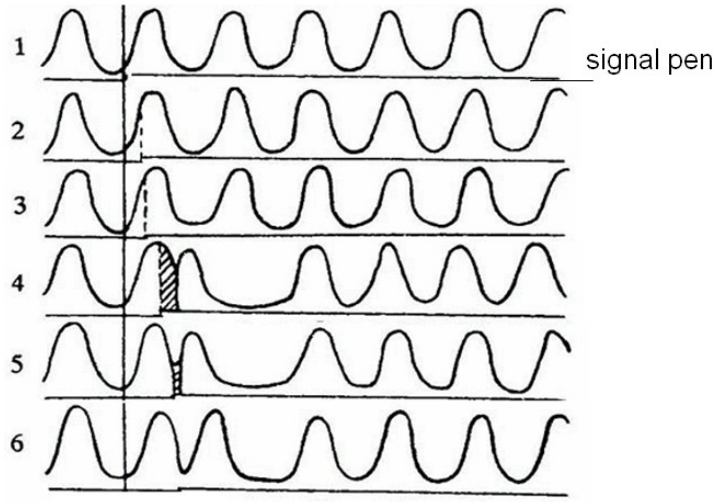


Figure 1.11. Extrasystole traces.

Signal pen mark the moment of stimulation with electrical current.
1: ectopic stimulus coincides with normal stimulus;
2, 3: ectopic stimuli are applied during absolute refractory period;
4, 5, 6: ectopic stimuli are applied during relative refractory period

In human cardiac pathology and even on normal individuals, the extrasystoles generated by ectopic areas are a common clinical manifestation of a disturbance of myocardial excitability.

I.1.5. In situ/in vitro frog heart: Frank-Starling law

Definition: Frank-Starling law of the heart states

- that the force of contraction of the myocardium is proportional with the degree of cardiac filling, so with the initial length of the myocardial fibers
- states that within physiological limits the force of cardiac contraction is proportional to its end diastolic volume

Principle: heart has the ability to pump all the blood received. The myocardium has the ability to adjust its force of contraction according to (i) degree of diastolic filling and (ii) peripheral resistance

Materials required:

- dissecting set to obtain frog heart *in situ* preparation (see section I.1.1)
- perfusion circuit (cannula, tube connection, perfusion bottle) and drainage circuit (canula, tube connection, glass tubes with lateral branches) (figure 1.12)

Technique:

- obtain frog heart *in situ* preparation (see section I.1.1)
- the venous sinus and one of the aorta will be cannulated (the other aorta will be ligated)
- the venous sinus will be connected to *a perfusion circuit* that mimics venous return and the cannulated aorta will be connected to *a drainage circuit* that mimics peripheral resistance;
- to cannulate venous sinus and aorta pass a thread around the venous sinus and with a sharp scissors make a small slit and introduce the cannula; tie the thread around the neck of the cannulae;
- connect the cannula of venous sinus with the perfusion bottle containing frog's Ringer solution; the connection tube has a clip and by pressing it can be controlled the *input flow in the perfusion* circuit which correspond to venous return changes in the body;

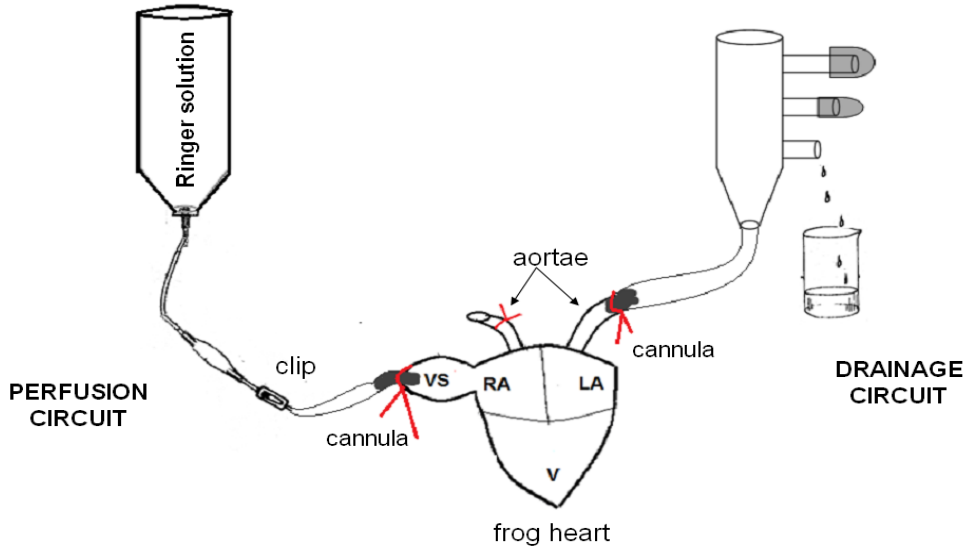


Figure 1.12. Diagram for experimental set up to demonstrate Frank-Starling law on frog heart (VS = venous sinus, A = atrium, V = ventricle)

- connect the cannula of aorta with the glass tube with lateral branches to create the drainage circuit. This circuit (i) permits *flow output* assessment by the number of drops per unit time through glass tube branches and (ii) it allows *changes in flow resistance* through the hydrostatic effect that correspond to variations of peripheral resistance;
- lateral branches of the glass tubes are similar to arterial branches and the variations of peripheral resistance correspond to the ability to force the liquid column to rise at different levels in the lateral branches of the glass tube.
- changing either venous return, either peripheral resistance it will be observed how the heart adapts to new conditions.

Results:

1. *increasing of venous return* (leaving unchanged peripheral resistance) by increasing the input flow in the perfusion circuit (by rising the number of Ringer's solutions drops) will determine identical increasing of the output flow on the drainage circuit (evaluated by increasing the number of drops pumped by the heart through the first lateral branch of the glass tube). Also, it is observed that the heart will increase in volume due to higher ventricular filling (an increased end diastolic volume). So, the heart pumps the entire quantity of liquid received. This phenomenon is based on distension of ventricular myocardium by the increased *end diastolic volume* (i.e. preload) which will lengthen the cardiac fiber and therefore increases systolic myocardial force of contraction, producing an increased stroke volume (proportional with increase of venous return). This ability of the heart is limited. So, a large increase of venous return will reveal limited ability of the heart to adjust the force of contraction to myocardial fibers length.

2. *increasing of peripheral resistance* (leaving unchanged venous return) by blocking the first lateral branch of the glass tube we require the heart to pump fluid received to a higher level. In this situation, it is observed on the following systoles that the output flow decrease and the heart increase in volume. Initial heart fails to pump systolic volume against this new resistance. So in the heart remains, after the first systole an increased residual volume called end systolic volume. To this volume will add a similar volume on the second systole. It will therefore cause an increase in end diastolic volume witch will lengthen the myocardial fiber. As a result, the force of contraction will increase (Frank Starling mechanism) and the heart will pump through the second lateral branch of the glass tube the same amount of blood, but against a higher outflow resistance. So the output flow remains constant similar to the one before the change in peripheral resistance.

Discussions and clinical significance (see the lecture):

Starling's law of the heart establishes that the force of heart contraction is proportional to the initial length of the cardiac muscle fiber. On *in situ* frog heart, the length of the cardiac muscle fiber is increased by an increase in diastolic filling of the heart.

Frank-Starling law states that the greater the volume of blood entering the heart during diastole (end-diastolic volume), the greater the volume of blood ejected during systolic contraction (stroke volume) and vice-versa.

This allows the cardiac output to be synchronized with the venous return, arterial blood supply and humeral length without depending upon external regulation to make alterations.

I.1.6. In situ frog heart: action of the main extracellular ions; effects of some chemical mediators

Principle: the effect of various extracellular ions and chemical mediators on in situ frog heart preparation is studied and recorded on a moving drum.

Materials required:

- dissecting set to obtain frog heart *in situ* preparation (see section I.1.1);
- perfusion circuit (cannula, tube connection, perfusion bottle) and drainage circuit (canula, tube connection, glass tubes with lateral branches) (see section I.1.5);
- heart lever, hook, kymograph with smoked drum;
- reagents: extracellular ions (CaCl2 1% , KCl 1%o, NaOH 1%o), chemical mediators (adrenaline, 1/2000; acetilcolină 1%o, atropine 1 mg/ml)

Technique:

- obtain frog heart *in situ* preparation connected to perfusion and drainage circuit like in section I.1.5;
- take a wire that end with a hook: attach the hook to the apex of the heart and the other end of the wire attach it to the heart lever. The lever has a writing arm at one end and counter-weight at the other end;
- the writing arm of the lever will record the cardiogram on the rotating smoked drum;
- in the perfusion fluid of the heart will add different solutions and their effect will be observed on cardiogram.

Results and discussions:

- *Potassium chloride* – an increase in extracellular [K+] determines an increase in heart volume with a decrease of heart rate and amplitude of the contractions (the force of contractions decrease) till heart stops in diastole (this phenomenon is called "potassium inhibition"); K+ decrease resting membrane potential.
- *Calcium chloride* – an increase in extracellular calcium concentration increases the amplitude of contraction (increases force of contraction), moderate increases of heart rate, increases of basal tonus of the heart and finally the heart stops in systole ((this phenomenon is called "calcium rigor").
- *Sodium chloride* – Na+ ions decrease the amplitude of contractions (decrease the force of contraction) and when there is an excess of sodium the heart stops in diastole. Sodium ions decrease excitability (membrane hyperpolarization) and contractility of the myocardium by competing with calcium ions.
- *Adrenaline* - increase both the rate and the force of contraction of myocardium. Adrenaline is a sympathomimetic drug. It (i) stimulates the venous sinus pacemaker ➔ increase the heart rate ➔ positive chronotropic effect (mechanism: increase intracellular cAMP which causes an increase in calcium ions influx through L-type calcium channels ➔ the slope of pacemaker potential becomes more abrupt leading to an increase of heart rate); (ii) acts on myocardium and increase the force of contraction ➔ positive inotropic effect (mechanism: binds beta 1 receptors ➔ increases the influx of calcium ions ➔ increases force of contraction).
- *Acetylcholine* is a parasympathomimetic drug which decreases the heart rate and force of contraction (see the effect of vagal stimulation)
- *Atropine* is a parasymptholitic drug which blocks acetylcholine muscarinic receptors. It increases the rate and force of contraction of heart. Thus, following atropine administration, vagal stimulation loses the cardioinhitory effect and have no influence on heart rate.

I.1.7. Electrocardiographical method

***Electrocardiography*:** is the graphic record on the lead axes of the projection of the electrical activity vectors of the heart.

An electrical activity (depolarization and repolarization) may be revealed in the muscle fibers of the heart. This activity is the consequence of heart muscle activation by stimuli originating in the conduction system of the heart, and the cause of heart contractions.
There are several theories grounded on the electric phenomena occurring in the cardiac fiber membranes:

A. Single-Phase Wave Theory
B. Dipole Theory
C. Vectorial Theory

A. Single-Phase Wave Theory

- at rest, the deactivation or repolarization phenomenon triggered by an electrode placed on both cell ends causes a curve called single-phase wave (curve in a particular direction).
- the simultaneous recording of the electrical events arising on both ends causes 2 identical single-phase curves, which occur at different moments in time as the activation and repolarization process reaches the distal cell end with difficulty.

Both depolarization and repolarization processes occur in the heart and are recorded as diphasic waves. A diphasic wave is achieved by placing the electrode on the surface so as to simultaneously record the events that take place on both ends.

A stimulus applied on one end, triggers the onset of area depolarization, whereas the distal end is still undepolarized. Meanwhile, repolarization begins on the first end (the wave has not yet reached the distal end), and the surface electrodes detect potential difference.

The end where the activation started enters into the reversion process, which has not yet reached the distal end, and hence a potential difference is no longer detected. The repolarization process has begun and the device records a reversion tendency, which materializes into a negative deflection read by the electrodes. The reversion continues on the distal end, and both surface electrodes record a diphasic curve, whereas the two (distal and proximal) end electrodes detect a single-phase wave.

B. Dipole Theory

In biological systems, the current flows through conductors that are made up of electrolytic solutions with different conductibility depending on the tissue. When a dipole is placed in a homogeneous conductive environment, an electric field is generated around it, which includes a series of lines of force.

There are simultaneous activated and non-activated areas in the heart (negative and positive load torques), hence the resulted dipoles. The cardiac dipole varies considerably in time and experiences a perpetual motion $\Rightarrow$ hence creating a field of electric stresses; the potential difference between the 2 dipoles is 0.

The potential intensity in the electric field is high around the pole and decreases by the square of the distance from the pole as we move away from it. The axis linking the 2 poles is the electric axis, whereas the electric potential 0 axis is perpendicular to the former. The activation area is delimitated by the non-activated area.

A. Vectorial Theory

It relies on the principle of dipoles graphically represented as vectors (which have: datum, sense, direction, size). Vectors may be both instantaneous (instantaneous dipole) and resultant.

The size of the projection depends on the angle defined by the vector and the reference axis (vector's position in relation to the axis). If the vector is parallel will have maximum projection (figure 1.13).

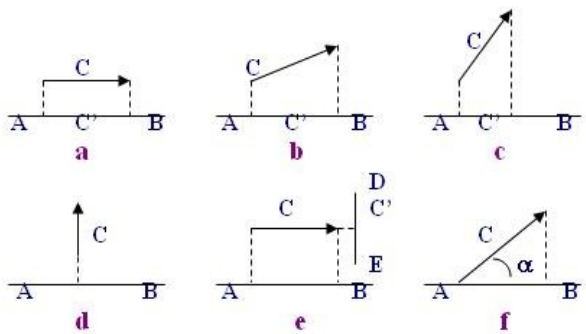


Figure 1.13. The size of vector's projection on axis.

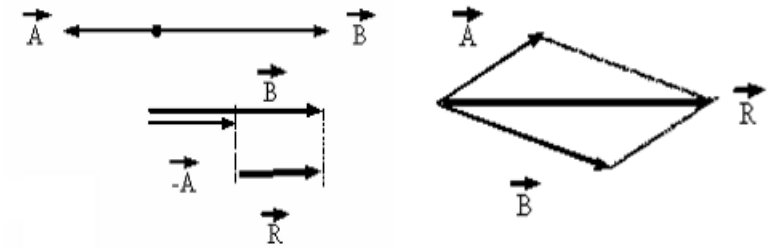


Figure 1.14. Vectors summation

Lead = space-based relation between 2 points where the recording electrodes are placed; this relation determines the specificity of the electric phenomena recorded in that lead.
Lead components:

- 2 points where the electrodes are placed:
    - located on the human body
    - the potential difference is read between them
- lead axis (imaginary line linking the 2 electrodes)
- lead direction:
    - positive – upwards from the isoelectric line (any vector projected in this area allows the detection of a positive deflection)
    - negative – downwards from the isoelectric line

The electric phenomena occurring in the myocardium cause an electric field that may be captured by electrodes. These electrodes turn the electric potential difference generated by the field into electric current, the intensity of which may be measured and may be graphically represented at the same time. Surface ECGs are achieved when these electrodes are placed on the patient's body. The recording electrodes are stainless steel plates placed on the limbs or on the thorax (precordial). Precordial electrodes are smaller as they are designed for a more precise location on the explored area. As for esophageal readings, a small metal part attached to the proximal end of the exploration probe plays the role of electrode. Cardiac potential may be recorded by placing electrodes on the limbs, since the human body is a conductive environment.

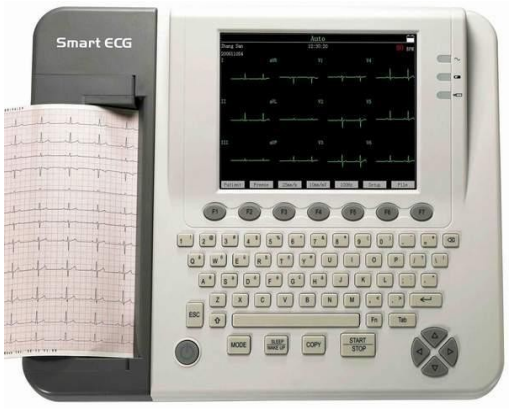


Figure 1.15. Electrocardiograph (ECG machine)

Technical ECG recording conditions:

- patient in the supine position, in a state of physical and mental relaxation;
- the recording occurs at comfortable room temperature (20^{o} C)
    - the teguments are wiped using alcohol before placing the electrodes;
    - the electrodes are wrapped in cloth soaked in sodium chloride meant to enhance conductibility.
- the electrodes are placed observing a set of criteria:
    - the teguments are wiped using alcohol before the electrodes are placed;
    - the electrodes are placed on muscular areas, avoiding hairy or bony areas;
- standard checks (1 mV current):

- a reference curve is recorded, which will allow assessing subsequent deflection amplitudes;
- the recording pen excursion will be 10 mm for a potential difference of 1 mV;
- the standard checks are conducted before each recording session;

- time recording:
  - time is recorded with the electrocardiogram path (the recording paper has specific both vertical and horizontal lines).
- The space between 2 horizontal lines (1 mm) is the wave amplitude and it corresponds to 1 mV, whereas the space between 2 thin lines (1 mm) is time and its values depend on the paper unwinding rate during the recording session.

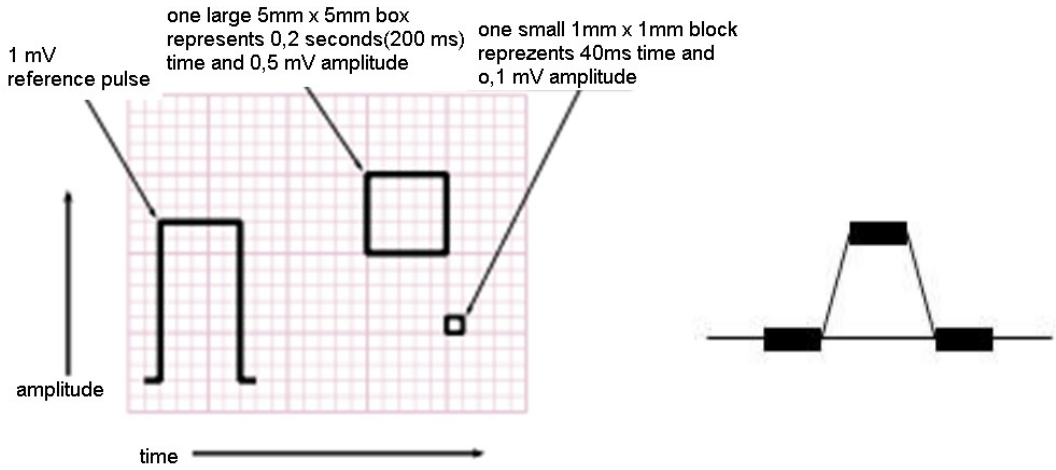


Figure 1.16. Standard checks: 1mV current

Artifacts = ECG tracing deformations due to the "parasites" caused by the alternating current:

Electrocardiographic leads:
There are several types of such leads:

- frontal (6):
  - standard or bipolar limb leads (3)
  - monopolar limb leads (3)
- horizontal (6): precordial or chest leads

Bipolar limb leads (standard lead, SD) (figure 1.17)

These leads occur by placing the electrodes on the left and right forearm, and on the left leg.

The electrocardiograph records the difference between the potential detected on the positive end and the one read on the negative end of that lead.

The electrodes that form these signals are located on the limbs one on each arm and one on the left leg. The limb leads form the points of what is known as Einthoven's triangle.

- Lead I is the voltage between the (positive) left arm (LA) electrode and right arm (RA) electrode: **Lead I** = LA-RA

- Lead II is the voltage between the (positive) left leg (LL) electrode and the right arm (RA) electrode: **Lead II= LL-RA**

- Lead III is the voltage between the (positive) left leg (LL) electrode and the left arm (LA) electrode: **Lead III = LL-LA**

Simplified electrocardiograph sensors designed for teaching purposes at e.g. high school level are generally limited to three arm electrodes serving similar purposes.
The general rule of Einthoven's triangle: the projection on the lead D II is the algebraic sum of the projections of the same vector in D I and D III.

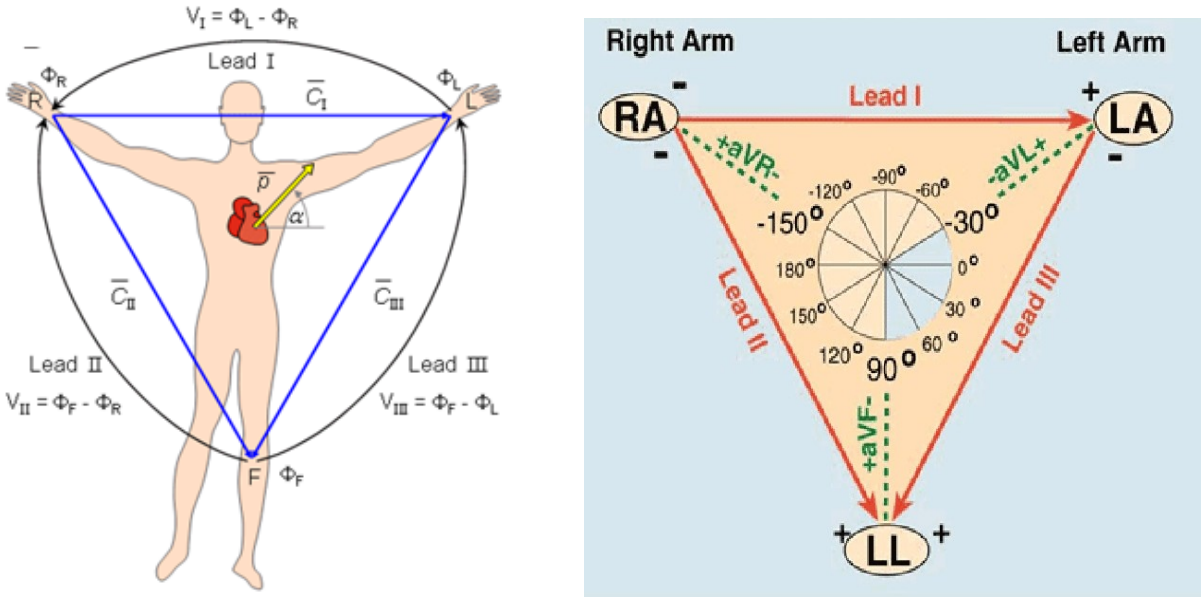


Figure 1.17. Bipolar limb leads (standard leads)

According to a set of mathematical rules and their corresponding graphic representation, the axes of the three standard leads may be represented as lines of a triangle (Einthoven's triangle) and the triangle angles are the 3 electrode locations in standard leads.

Monopolar limb leads

When forming these leads, one of the electrodes records a zero potential all the time.

This is called indifferent electrode and corresponds to the negative end of the lead axis.

Thus, the potential actually recorded by the device fully coincides with the potential read on the other (positive) end of the lead axis by the exploration electrodes.

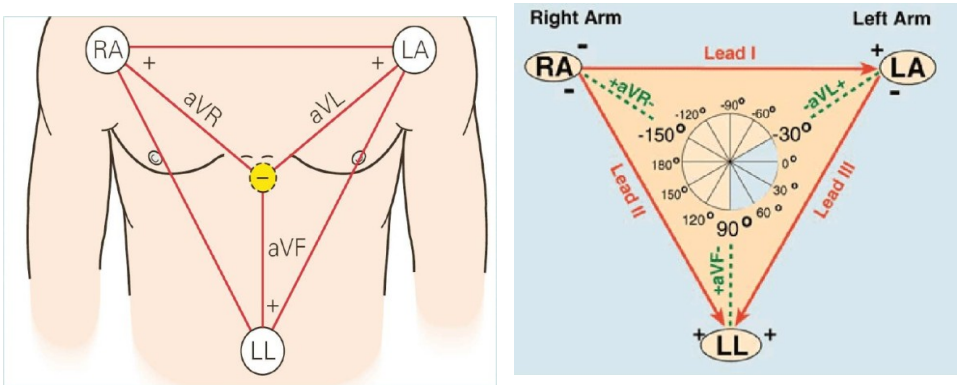


Figure 1.18. Monopolar limb leads

The 0 potential of the indifferent electrode may be achieved by short-circuiting the 3 electrodes used for SD, by placing 5000 ohm (Wilson) resistances on each cable (figure 1.19).

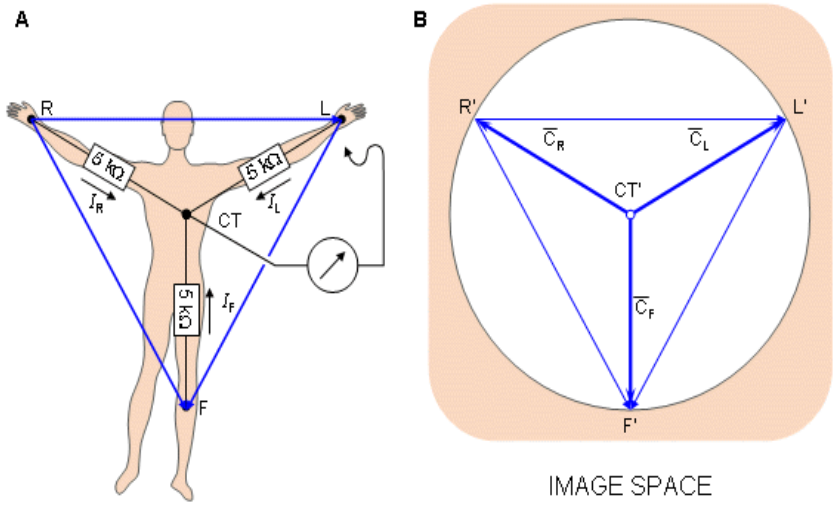


Figure 1.19 (A) The circuit of the Wilson central terminal (CT).
(B) The location of the Wilson central terminal in the image space (CT'). It is located in the center of the Einthoven triangle.

- *Augmented lead right (aVR)* has the positive electrode (white) on the right arm. The negative electrode is a combination of the left arm (black) electrode and the left leg (red) electrode, which "augments" the signal strength of the positive electrode on the right arm:

  aVR = RA - ½(LA+LF)

- *Augmented lead left (aVL)* has the positive (black) electrode on the left arm. The negative electrode is a combination of the right arm (white) electrode and the left leg (red) electrode, which "augments" the signal strength of the positive electrode on the left arm:

  aVL = LA - ½ (RA+LF)

- *Augmented lead foot (aVF)* has the positive (red) electrode on the left leg. The negative electrode is a combination of the right arm (white) electrode and the left arm (black) electrode, which "augments" the signal of the positive electrode on the left leg:

  AVF = LF - ½ (RA+LA)

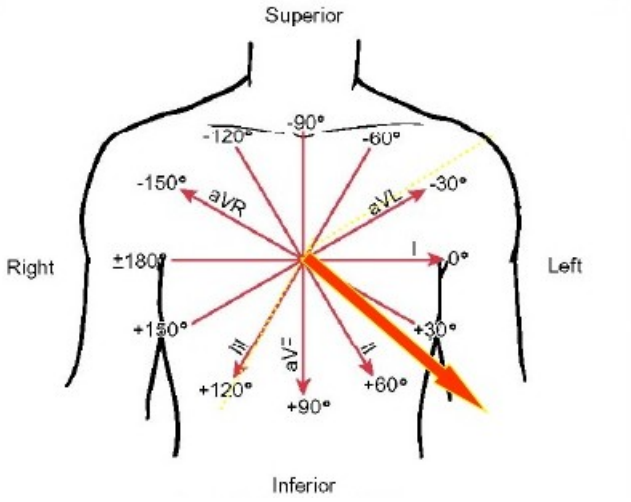


Figure 1.20. Hexaxial system

The augmented limb leads aVR, aVL, and aVF are amplified in this way because the signal is too small to be useful when the negative electrode is Wilson's central terminal.

Together with leads I, II, and III, augmented limb leads aVR, aVL, and aVF form the basis of the hexaxial reference system, which is used to calculate the heart's electrical axis in the *frontal plane*. The aVR, aVL, and aVF leads can also be represented using I and II limb leads:

- aVR = - I+II/2
- aVL = I – II/2
- aVF = II – I/2

General Rule monopolar limb leads: aVR + aVL + aVF = 0 (the sum of the projections of the same vector in the three monopolar limb leads is zero)

Standard leads and monopolar limb leads may be graphically represented in several ways, namely by using Einthoven's triangle or Bailey's triaxial and hexaxial system.

The triaxial standard leads system consists of shifting Einthoven's triangle lines until they all intersect in a central point. Thus, the axes form 60^{o} angles.

The hexaxial system is possible by joining the standard leads and monopolar limb leads axes in the triaxial system.

In the triaxial and hexaxial system, all the frontal cardiac vectors and all their standard leads and monopolar limb leads projections originate in the electric center (CE). standard leads and monopolar limb leads explore the frontal electric activity of the heart

Thoracic (precordial) leads

These are monopolar leads and explore the electric activity of the heart on horizontal plane. They are marked with the letter "V" and with a number from 1 to 8, which indicates the electrode location (figure 1.21).

The recording axis links the exploration electrode to the electric centre of the heart. For each lead, the positive direction points to the spot where the exploration electrode is placed.

- V1 - is placed on the fourth intercostal space at the right sternal border
- V2 - is placed on the fourth intercostal space at the left sternal border
- V3 - is placed halfway between V2 and V4
- V4 - is placed on the fifth intercostal space at the left midclavicular line
- V5 - is placed on the same level as V4 at the left anterior axillary line
- V6 - is placed on the same level as V4 at the left midaxillary line
-

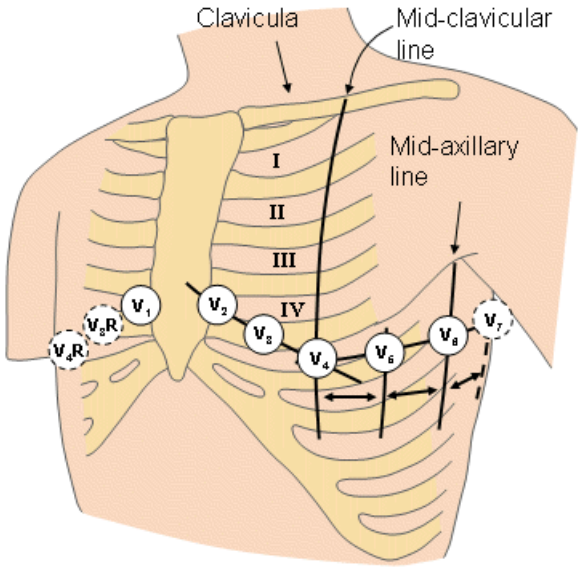


Figure 1.21. Thoracic (precordial) leads

Characteristics:

- no mathematical relations of the type established in standard leads and monopolar limb leads may be defined between these precordial leads and their projections on the axes
- even the slightest displacement of the exploration electrode on the thorax alters the appearance of the path. Consequently, identical records are very rarely achieved.
- the electrode is placed closer to the heart and therefore it is thought to be able to differentially record cardiac potentials

Exploration possibilities

- V_1 – explores mainly the activity of the right ventricle
- V_1, V_2, V_3 – explores the electric activity of the anterior side of the septum
- V_5, V_6 – explores the electric activity of the left ventricle
- V_4 – explores the activity of the apex of the heart

In thoracic leads, QRS is normal if**:**

- R increases progressively from V1 to V5 (16 - 24 mm), then falls to V6
- S reaches its peak in V2 (16- 23 mm) and decreases progressively to the left and may even disappear in V6, and Q occurs to the left of V4 (figure 1.22).

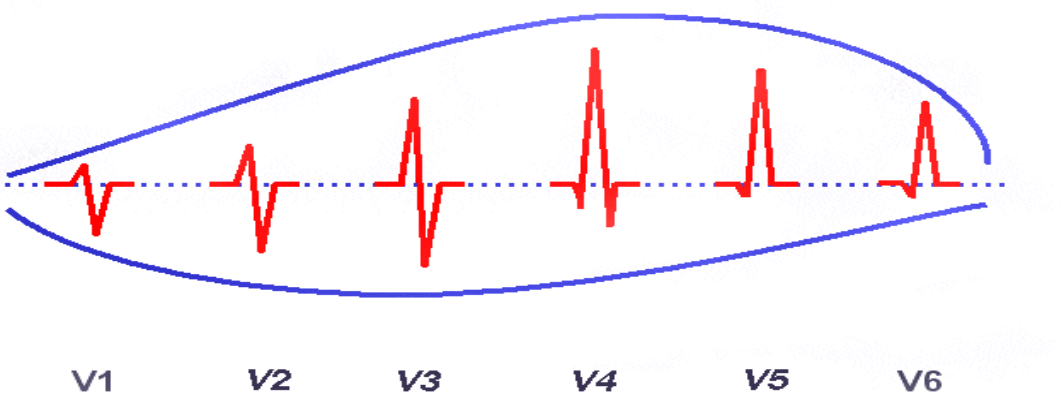


Figure 1.22. Top blue line indicates the R wave; Bottom blue line indicates the S wave

The normal electrocardiogram is illustrated in Figure 1.23. The figure also includes definitions for various segments and intervals in the ECG. The deflections in this signal are denoted in alphabetic order starting with the letter P, which represents atrial depolarization. The ventricular depolarization causes the QRS complex, and repolarization is responsible for the T-wave. Atrial repolarization occurs during the QRS complex and produces such low signal amplitude that it cannot be seen apart from the normal ECG.

There are twelve leads in total, each recording the electrical activity of the heart from a different perspective, which also correlate to different anatomical areas of the heart for the purpose of identifying acute coronary ischemia or injury. Two leads that look at neighbouring anatomical areas of the heart are said to be contiguous (see color coded chart). The relevance of this is in determining whether an abnormality on the ECG is likely to represent true disease or a spurious finding.

In addition, any two precordial leads that are next to one another are considered to be contiguous. For example, even though V4 is an anterior lead and V5 is a lateral lead, they are contiguous because they are next to one another.

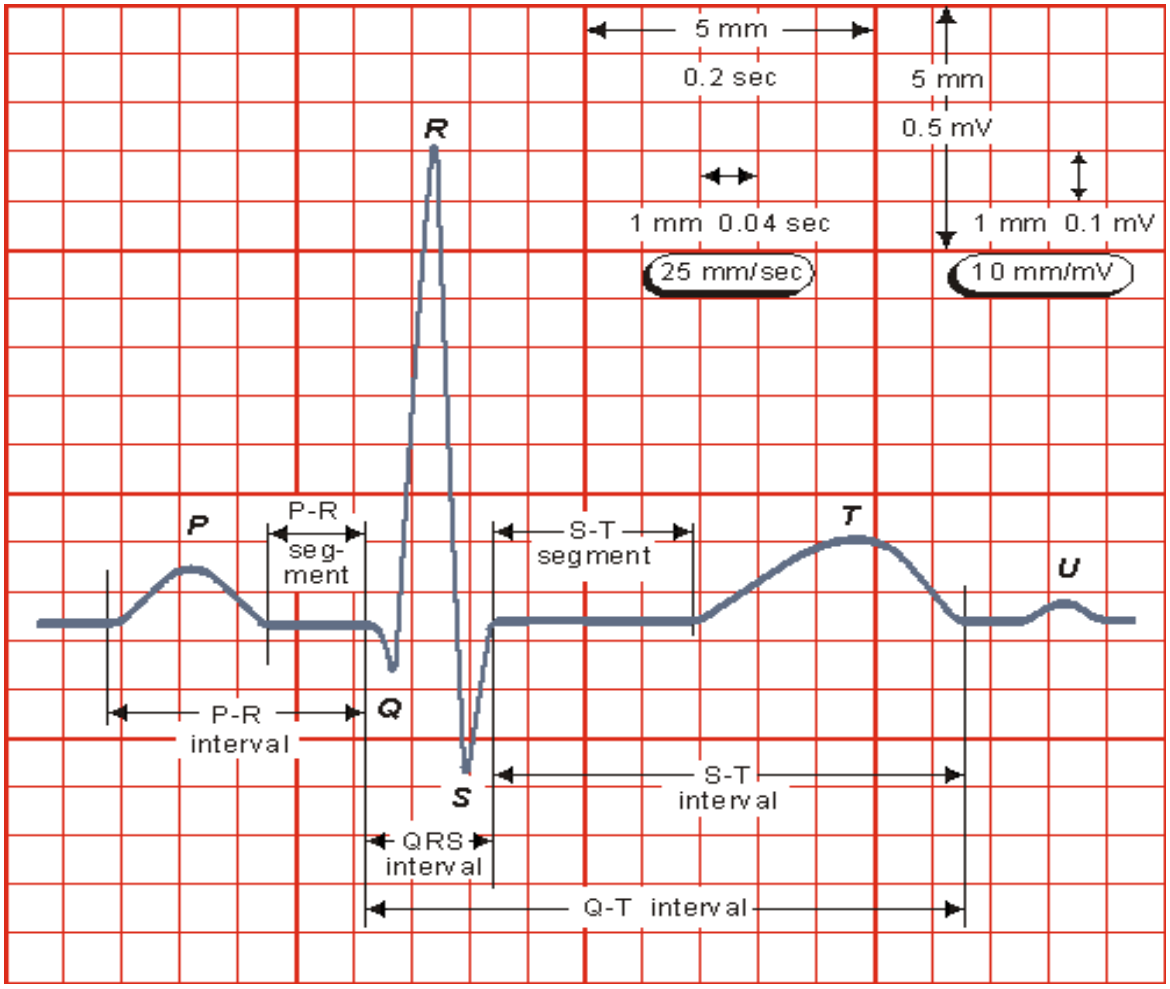


Figure 1.23. Waves and intervals of normal ECG

Category	**Color on chart**	**Leads**	**Activity**
Inferior leads	Yellow	Leads II, III and aVF	Look at electrical activity from the vantage point of the inferior surface (diaphragmatic surface of heart).
Lateral leads	Green	I, aVL, V_5 and V_6	Look at the electrical activity from the vantage point of the lateral wall of left ventricle. • The positive electrode for leads I and aVL should be located distally on the left arm and because of which, leads I and aVL are sometimes referred to as the high lateral leads. • Because the positive electrodes for leads V5 and V6 are on the patient's chest, they are sometimes referred to as the low lateral leads.
Septal leads	Orange	V_1 and V_2	Look at electrical activity from the vantage point of the septal wall of the ventricles (interventricular septum).
Anterior leads	Blue	V_3 and V_4	Look at electrical activity from the vantage point of the anterior surface of the heart (sternocostal surface of heart).

Electrocardiogram Analysis*: rhythm, rate, electric axis, morphological analysis*
Sinus rhythm: the P wave is found in at least two standard leads and it has normal morphology: positive wave with normal duration and amplitude, constant PQ interval (0.12-0.21 sec) and it precedes the QRS complex (fig 1.24).

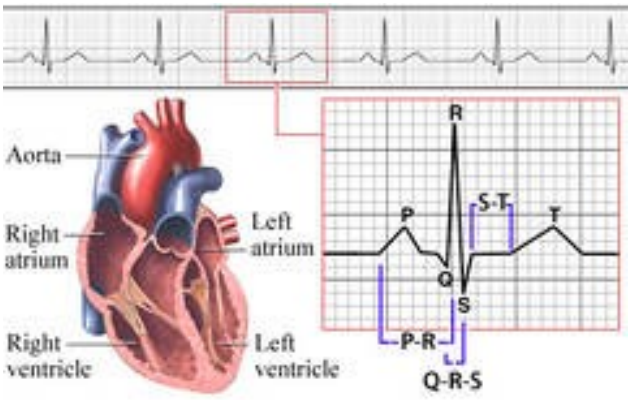


Figure 1.24. Sinus rhythm

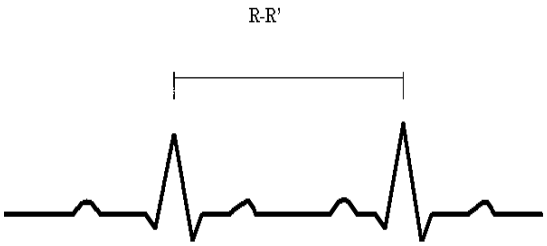


Figure 1.25. Interval R-R' on ECG tracing

Heart rate determination
Direct (precise) method (figure 1.25)
- One always bears in mind that the ECG signal is a periodical signal:
  - the R-R` distance is determined in mm
  - the heart rate (HR) is calculated using the rule of three:

$$HR = \frac{60(\text{sec})}{R-R'(mm) \bullet 0.04(\text{sec})},$$

where the conversion factor is - 0.04 at a paper speed of 25 mm/sec
- 0.02 at a paper speed of 50 mm/sec

*Indirect method (*figure 1.26*)*
The number of **0.20 sec.** intervals (marked by thick lines on the path) occurring between two successive QRS complexes is determined:
- one interval → the heart rate is 300/min
- two intervals → the heart rate is 150/min
- three intervals → the heart rate is 100/min
- four intervals → the heart rate is 75/min
- five intervals → the heart rate is 60/min

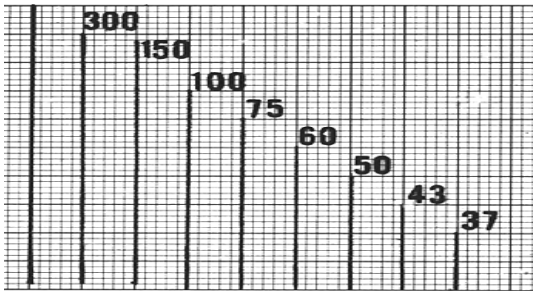


Figure 1.26. Indirect method for heart rate determination

Remarks
- if the heart rhythm is irregular, an average of at least 5-6 cardiac cycles will be calculated
- any possible extrasystoles are revealed and if they are numbered they will be included in the heart rate calculation
- if the atriums and ventricles have different rates, they are calculated separately and then a sequencing relation is set between the atrial and ventricular activities

Heart rate interpretation:
Normal heart rate : 70-80 beats/min
Sinus tahycardia: > 100 beats/min on sinus rhythm
Sinus bradycardia : < 60 beats/min on sinus rhythm
Respiratory sinus arrhythmia: the heart rate increases during inspiration and decreases during expiration (it is a physiological arrhythmia in children and young adults)

Determination of electrical axis

Diagram showing how the polarity of the QRS complex in leads I, II, and III can be used to estimate the heart's electrical axis in the frontal plane.

The heart's *electrical axis* refers to the general direction of the heart's depolarization wavefront (or *mean electrical vector*) in the frontal plane. With a healthy conducting system the cardiac axis is related to where the major muscle bulk of the heart lies.

Normally this is the left ventricle with some contribution from the right ventricle. It is usually oriented in a right shoulder to left leg direction, which corresponds to the left inferior quadrant of the hexaxial system, although -30° to +90° is considered to be normal (figure 1.27).

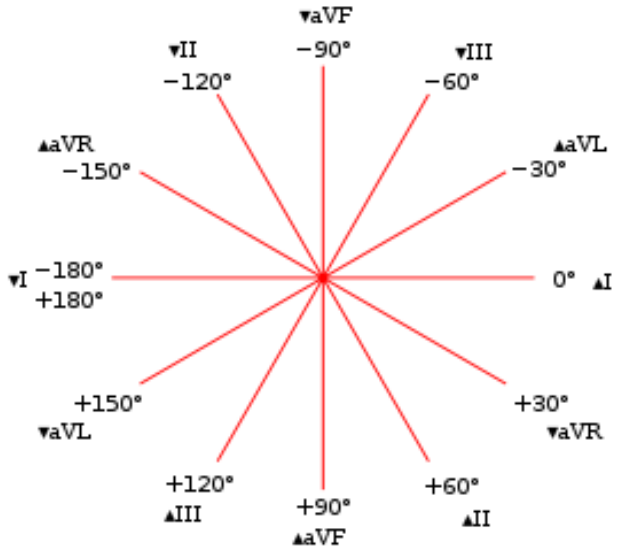


Figure 1.27. Hexaxial system

If the left ventricle increases its activity or its walls become thicker, then there is said to be "*left axis deviation*" as the axis swings round to the left beyond -30°, alternatively in conditions where the right ventricle is strained or hypertrophied then the axis swings round beyond +90° and "*right axis deviation"* is said to exist. Disorders of the conduction system of the heart can disturb the electrical axis without necessarily reflecting changes in muscle bulk.

The hexaxial reference system shows the orientation of each lead. For example, if the depolarization vector of heart muscle is oriented at +60 degrees with respect to the SA node, lead II will show the greatest deflection and aVL the least.

In the setting of right bundle branch block, right or left axis deviation may indicate bifascicular block.

Steps in determination of electrical axis:

- Look at Lead I and aVF or Lead I and Lead III
- Determine if the overall QRS complex deflection is grossly positive or grossly negative in Leads I and aVF
- If the QRS are both positively deflected, then the electrical axis is in the normal quadrant (up, up)

- If the QRS complex for Lead I is positively deflected but the QRS in avF is negatively deflected, then the electrical axis is said to be left axis deviated (up, down)
- If the QRS complex in Lead I is negatively deflected but the QRS in aVF is positively deflected, then the electrical axis is said to be right axis deviated (down, up)
- If the QRS in Lead I and in aVF are both negatively deflected, the electrical axis is said to be extreme right axis deviated (down, down)
- It is not enough to merely determine the quadrant. It is better to be able to determine where in the quadrant the axis is located.

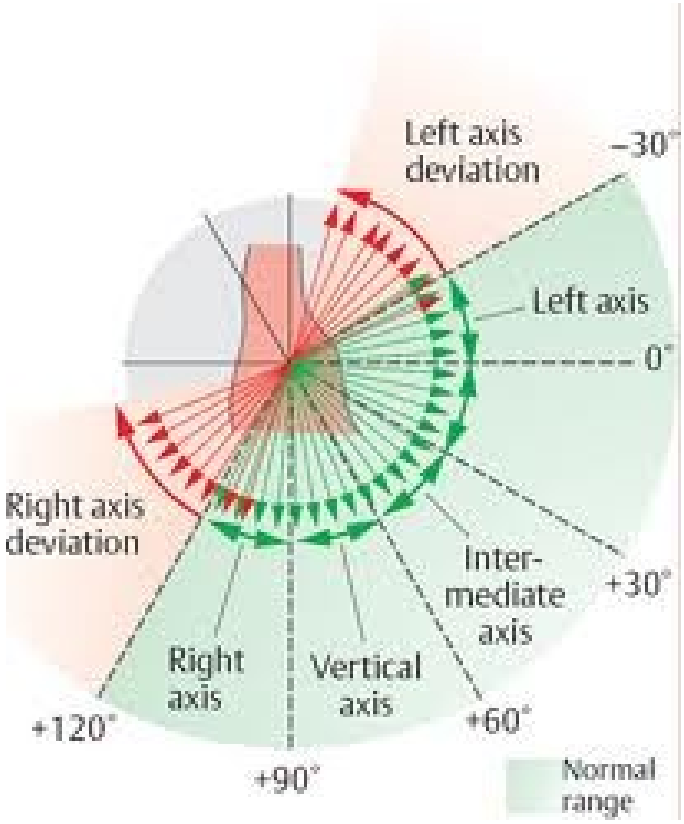


Figure 1.28. Electrical axis of the heart

Normal	-30° to 90°	Normal	Normal
Left axis deviation	-30° to -90°	May indicate left anterior fascicular block or Q waves from inferior MI.	Left axis deviation is considered normal in pregnant women and those with emphysema.
Right axis deviation	+90° to +180°	May indicate left posterior fascicular block, Q waves from high lateral MI, or a right ventricular strain pattern.	Right deviation is considered normal in children and is a standard effect of dextrocardia.
Extreme right axis deviation	+180° to -90°	Is rare, and considered an 'electrical no-man's land'.	

Morphological analysis of ECG tracing

RR interval

- Normal resting heart rate is between 60-80 bpm
- The interval between an R wave and the next R wave.
- Duration = 0,6-1,2 s

P wave

- During normal atrial depolarization, the main electrical vector is directed from the SA node towards the AV node, and spreads from the right atrium to the left atrium. This turns into the Pwave on the ECG.
- Duration 0.08-0.10 sec.

PR interval

- The PR interval is measured from the beginning of the P wave to the beginning of the QRS complex.
- The PR interval reflects the time the electrical impulse takes to travel from the sinus through the AV node and entering the ventricles.
- The PR interval is therefore a good estimate of AV node function
- Duration = 120 to 200ms

PR segment

- The PR segment connects the P wave and the QRS complex. The impulse vector is from the AV node to the bundle of Hiss to the bundle branches and then to the Purkinje Fibres.
- This electrical activity does not produce a contraction directly and is merely traveling down towards the ventricles and this shows up flat on the ECG.
- Duration = 50-120 ms

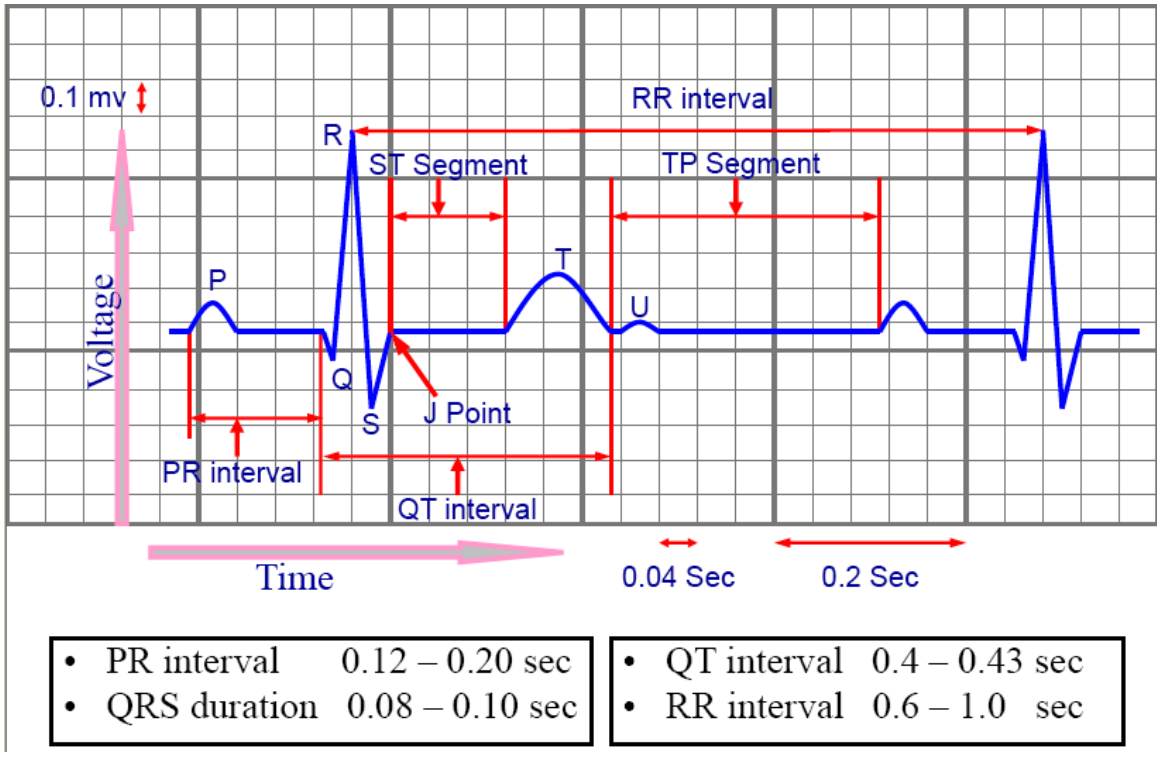


Figure 1.29. ECG tracing.

QRS complex

- The QRS complex reflects the rapid depolarization of the right and left ventricles. They have a large muscle mass compared to the atria and so the QRS complex usually has much larger amplitude than the P-wave.
- Duration = 80 to 120ms

ST segment

- The ST segment connects the QRS complex and the T wave. The ST segment represents the period when the ventricles are depolarized. It is isoelectric.
- Duration = 80-120 ms

T wave

- The T wave represents the repolarization (or recovery) of the ventricles. The interval from the beginning of the QRS complex to the apex of the T wave is referred to as the *absolute refractory period*. The last half of the T wave is referred to as the *relative refractory period* (or vulnerable period).
- Duration = 160 ms

ST interval

- The ST interval is measured from the J point to the end of the T wave
- Duration = 320 ms

QT interval

- The QT interval is measured from the beginning of the QRS complex to the end of the T wave. A prolonged QT interval is a risk factor for ventricular tachyarrhythmias and sudden death. It varies with heart rate and for clinical relevance requires a correction for this, giving the QTcorrected
- Duration = 300-430 ms

Some pathological entities which can be seen on the ECG:

Shortened QT interval	**Hypercalcemia, some drugs, certain genetic abnormalities.**
Prolonged QT interval	Hypocalcemia, some drugs, certain genetic abnormalities.
Flattened or inverted T waves	Coronary ischemia, hypokalemia, left ventricular hypertrophy, digoxin effect, some drugs.
Hyperacute T waves	Possibly the first manifestation of acute myocardial infarction, where T waves become more prominent, symmetrical, and pointed.
Prominent U waves	Hypokalemia.

I.2. Vascular physiology

I.2.1. Central mechanical events during the cardiac cycle

I.2.1.1. Auscultation of heart sounds

Definition: listening for sounds produced by the heart to evaluate its condition. Auscultation may be performed *directly* with the unaided ear or *indirectly* (most common) using a stethoscope to amplify the sounds.
Principle: assessment of acoustic activity of the heart with a stethoscope
Materials required: stethoscope consists of 2 parts: (1) headset (eartips and eartube) connected by tubing with the (ii) chestpiece (diaphragm and bell) (figure 1.30).

The bell is used for light skin contact to hear low frequency sounds.
The diaphragm is used with firm skin contact to hear high frequency sounds.

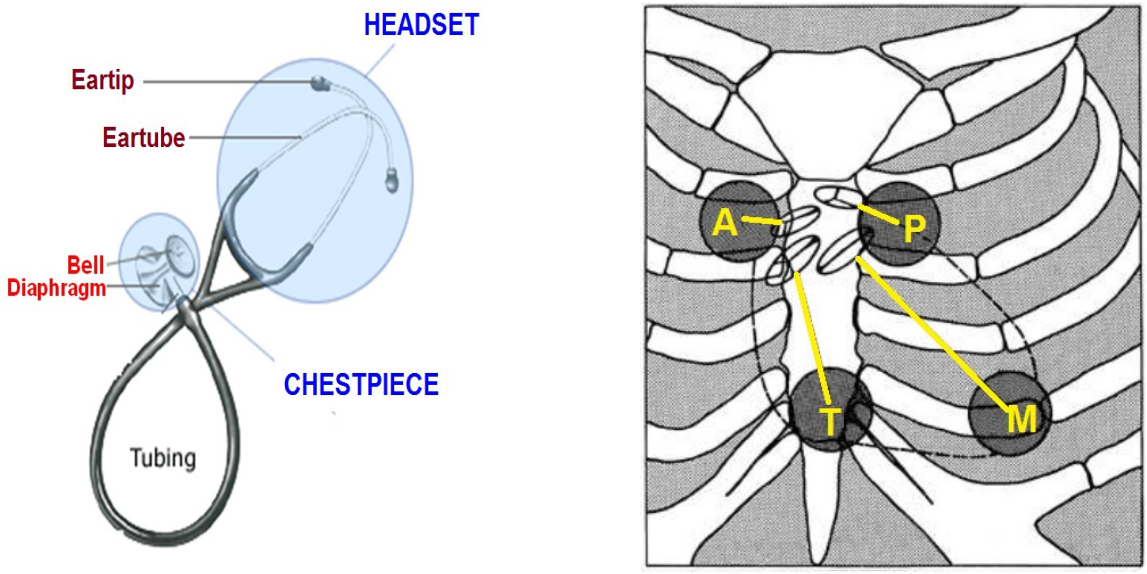


Figure 1.30. Stethoscope (left) and diagram with cardiac area (right); maximum auscultation point for A: aortic valve, P: pulmonary valve, M: mitral valve, T: tricuspid valve.

Technique: place the stethoscope on anterior thoracic wall on **cardiac area** (delimited by 4 maximum ascultatory points corresponding to the heart valves: 2 atrioventricular - mitral and tricuspid; 2 semilunar or sigmoid - aortic, pulmonary) (figure 1.30)

Results and discussions:
Usually with the stethoscope it can be heard two, maximum thirds sounds:
1. FIRST HEART SOUND or "lubb"

- *auscultation area:*
  - heard all over **cardiac area**
  - maximum intensity in the
    - *5th left intercostals space, medial clavicular line for the* ***bicuspid valve***
    - *the base of the xiphoid process for the* ***tricuspid valve.***
- *characteristics:*
  - long, brave and strong;
  - low pitched; loud sound.
- *cause:* vibrations associated with **closure of AV valves**

- *clinical identification:* coincide with apex beat and carotid pulse
- denotes onset of ventricular systole
- duration and intensity indicates the condition of the myocardium (in hypertrophied heart it is more accentuated)
- a clear 1st heart sound indicates properly closure of the AV valves

2. SECOND HEART SOUND or "dupp"

- *auscultation area:* .
  - Heard all over **cardiac area**
  - maximum intensity in the 2nd intercostal space 2 cm of sternum border:
    - right side for **aortic valve**
    - left side for **pulmonary valve**
- *characteristics:*
  - it is short, sharp, high pitched;
- *cause:* vibrations associated with **closure of semilunar valves**
- signifies end of systole and beginning of ventricular diastole.
- *Clinical identification:* occurs just after carotid pulse; it is preceded by the 1st sound and followed by a pause;

The interval between 1st and 2nd sounds mark the duration of ventricular systole (short pause) and the duration between 2nd and 1st sounds mark the duration of the ventricular diastole (long pause).

3. THIRD HEART SOUND

- diastolic sound
- occurs during rapid filling phase of the ventricles
- short, soft, low pitched
- in physiological conditions it is heard only in young people and children because of their thin chest wall
- in pathological conditions it is heard in patients left ventricular failure (atrioventricular valve regurgitation)

Clinical significance: heart auscultation offers significant information regarding the functional integrity of the heart and constitutes an important phase of cardiac physical examination.

I.2.1.2. Phonocardiogram

Definition: Phonocardiogram represents the graphic record plotted against time of heart sounds and other vibrations initiate in the heart and blood vessels.

Principle: obtaining the graph of acoustic activity of the heart. The method is called phonocardiography, a non-invasive procedure and the device which picks up the sounds is named phonocardiograph.

Materials required: phonocardiograph machine consists of:

- microphone is a transducer that convert cardiac acoustic activity into electrical current;
- filtering and processing unit
- display - oscilloscope, PC monitor, recording paper, compact disc, etc

Technique: place the microphones on the cardiac area in the maximum auscultation points for heart valves (figure 1.13)

Results:
- acoustic activity of the heart recorded on phonocardiogram consists of heart sounds, murmurs, cliks, opening snap, gallop rhythms.
- the heart sounds are produced by the vibrations of the heart valves after closure and/ or by vibration of cardiac muscle during abrupt acceleration or deceleration of blood within the heart (turbulence);
- physiologically, the phonocardiogram record all the four heart sounds (figure 1.31);

1. FIRST HEART SOUND
- is a *systolic sound:* occurs in the beginning of ventricular systole
- has 3 components:
    - *muscular* due to myocardium vibrations during isovolumetric contraction;
    - *valvular* (the major component) due to closure of atrio-ventricular valves: mitral valve closes first (M1), tricuspid valve closes after that (T1). The closure of the mitral valve is much louder (revealing the high pressure on the left heart) and usually mask the tricuspid sound; M1 occurs slightly before T1. Since the mitral and tricuspd valves normally close almost simultaneously, only one single heart sound is usually heard;
    - *vascular* due to opening and vibration of the ascending aorta during rapid ejection;
- duration: 0.08 – 0.15 seconds
- frequency: 35 – 100 Hz
- on phonocardiogram 1st heart sound is recorded as a single group of 9-13 waves (the middle group has the highest amplitude corresponding to the valvular component of the 1st sound)
- on electrocardiogram 1st heart sound is positioned at 0.02 – 0.04 second after wave Q or R (approximately in the second half of QRS complex)

2. SECOND HEART SOUND
- is a *diastolic sound:* occurs at the end of ventricular systole and on the beginning of the ventricular diastole
- has 3 components determined by vibrations of:
    - blood (turbulences) of low amplitude which precede closure of the sigmoid valves;
    - high amplitude produced by closing of the semilunar valves;
    - opening of the atrioventricular valves;
- duration: 0.07 – 0.1 seconds
- frequency of vibrations: 100 – 150 Hz
- on phonocardiogram 2nd heart sound is recorded as a single group of 4-6 waves;
- on electrocardiogram 2nd heart sound is placed on the last part of T wave;

3. THIRD HEART SOUND
- is a diastolic sound: occurs during ventricular diastole, specifically during rapid ventricular filling
- is produced by ventricular wall vibrations during rapid filling
- duration: 0.04 – 0.06 seconds
- frequency of vibrations: 25-50 Hz
- on phonocardiogram 3rd heart sound is recorded as a single group of 1-4 vibrations;
- on electrocardiogram is placed between T and P wave;
- occurs at 0.12 – 0.18 seconds after 2nd heart sound;

4. FORTH HEART SOUND

- is a diastolic or presystolic sound: occurs during ventricular filling associated with an effective atrial systole;
- is produced by ventricular wall vibrations during atrial systole
- cannot be heard with stethoscope, but always is recorded on phonocardiogram
- is absent in patients with atrial fibrillation (due to absence of atrial systole)
- frequency of vibrations: 25-50 Hz
- on phonocardiogram 3rd heart sound is recorded as a single group of 1-2 vibrations;
- on electrocardiogram is placed 0.04 – 0.05 seconds after the beginning of P wave;

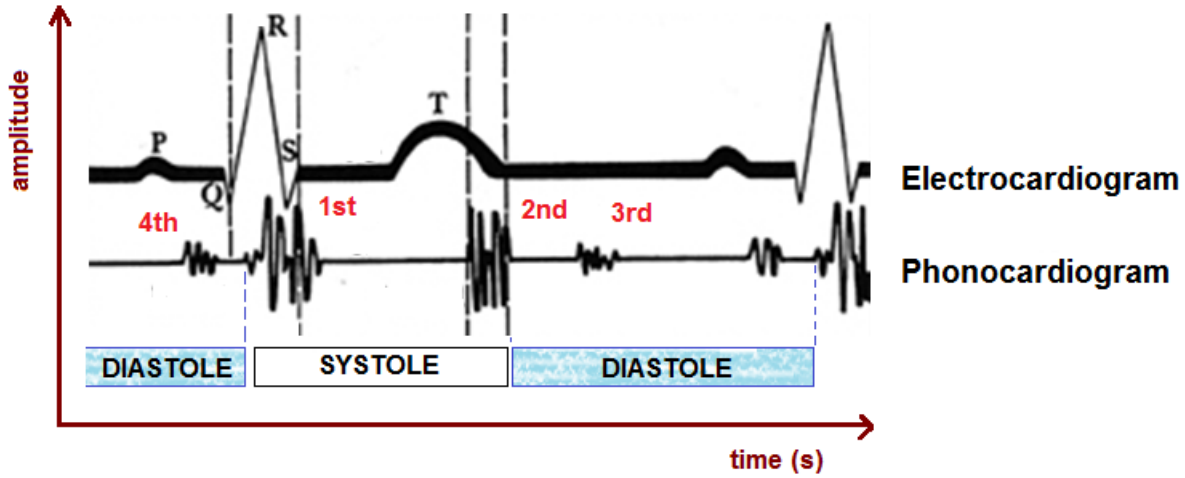


Figure 1.31. Phonocardiogram

Discussions and clinical significance

- 1st sound is softer than 2nd sound at the heart base, and louder than 2nd sound at the apex;

Variations in the 1st heart sound:

- *an accentuated 1st sound* is recoded in tachycardia, extrasystoles, high cardiac output state (exercise, anemia, hyperthyroidism) because in these situations the mitral valve is still open wide at the onset of ventricular systole and then close quickly; this phenomenon is due to to a diminished diastolic ventricular filling or to a short diastole. If atrial contraction precedes ventricular contraction by a short PR interval, S1 is louder also.
- *a diminished 1st heart sound* is recorded in bradycardia or a contraction following an extrasystole because a long diastole causes a long ventricular filling and the mitral valve has time to float back into a closed position before onset of ventricular systole. Mitral valve closes less loudly. Other situations when the mitral valve closes imperfect is mitral insufficiency, congestive heart failure, coronary heart disease when the left ventricular contractility is markedly reduced.
- *1st sound could vary in intensity* in the complete atrioventricular block (3rd degree, when atria and ventricles are contracting independently of each other) or atrial fibrillation when mitral valve is in different positions before being close by ventricular systole.
- when the mitral valve closes significantly before the tricuspid valve results a *"split 1st* sound"; occurs during inspiration when the closure of the tricuspid valve is delayed (due to increased venous return) and in right bundle branch block (left ventricle

depolarizes before the right ventricle). So, the left ventricle contracts before the right ventricle thus the pressure in the left ventricle rises before that of the right ventricle. This increased pressure forces the mitral valve closed before the tricuspid valve resulting in a split 1st sound.

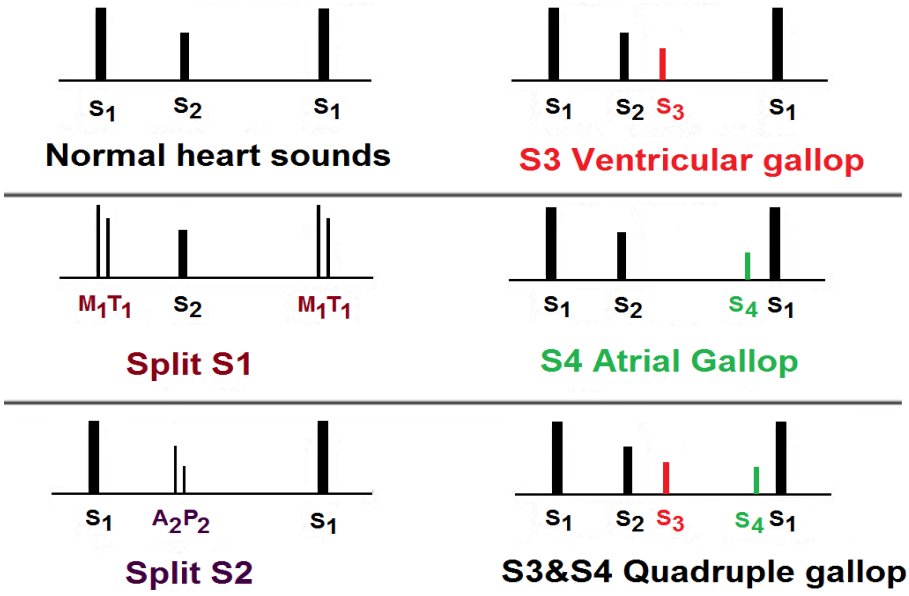


Figure 1.32. Graphical representation of heart sounds
(S1 = 1st sound; S2 = 2nd sound; S3 == 3rd sound; S4 = 4th sound)

- *Physiological variation in the 2nd heart sound*: the aortic valve closes before the pulmonary valve resulting in asynchronous valve closures which produce a normal *physiologic splitting of the 2nd heart sound*. This phenomenon takes place because right ventricular ejection lasts longer than does left ventricular ejection. Inspiration accentuates physiologic splitting (because of an increased venous return to the right heart which produces an increase in volume of right ventricular ejection). During expiration, 2nd heart sound splitting become less distinct or disappears (figure 1.32).
- *an accentuated 3rd heart sound* is referred to as a *ventricular / protodiastolic gallop rhythm* when occurs triplets of sound (the term suggests the addition of another heart sound which simulates the rhythm of a horse gallop) (figure 1.32).
- *an accentuated 4th heart sound* is referred to as a *atrial / presystolic gallop rhythm* (figure 1.32).
- the other forms of acoustic activity of the heart (murmurs, cliks, etc) recorded on phocardiogram will be discussed on physiopathology section of the heart.

Phocardiogram constitutes a useful teaching tool for cardiac auscultation and also it provides information in evaluate the uncertain auscultatory results

I.2.1.3. Apexcardiogram (human cardiogram)

Definition: is a graphic recording of low-frequency vibrations on the surface over the region of the cardiac apex; the motion of the left ventricle causes low-frequency deflections on the chest wall in this region correlated with the hemodynamic events within the left side of the heart.

Principle: obtaining a graphic recording of the chest wall movement towards a transducer placed in the 5th intercostal space, mid clavicular line.

Materials required: apexcardiograph with peripheral mechanical transducer, data acquisition unit and a display to visualise the graph.
Technique:
- on the 5th intercostal space, mid clavicular line palpate the apex beat;
- place the mechanical transducer over the apex beat;
- record with the apexcardiograph the mechanical activity of the heart apex;

Result, discussions and clinical significance:
- the graph obtained is called apexcardiogram (human cardiogram) and it has following components (figure 1.33):

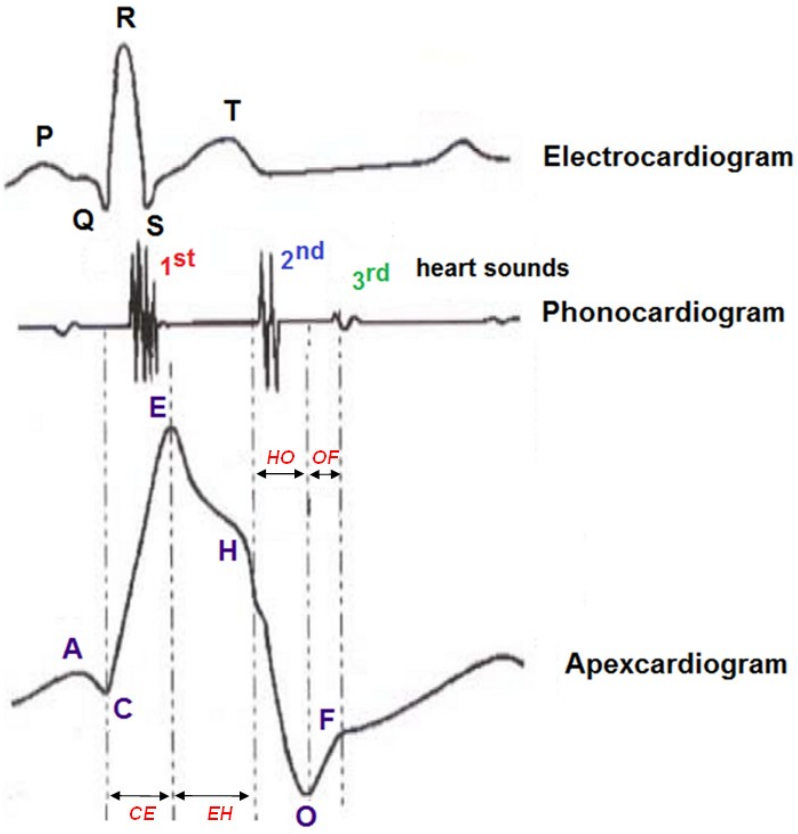


Figure 1.33. Apexcardiogram

A wave = atrial systole, is synchronous with the 4th heart sound and begins after 0.08 - 0.12 s after the beginning of P wave;
C point = beginning of isovolumetric left ventricle contraction;
CE period = isovolumetric systole of left ventricle; coincides with the 1st heart sound;
E peak = systolic peak, is simultaneous with the opening of the aortic valve, it marks onset of ejection of blood from the left ventricle into the aorta;
H point = closure of the aortic valve, coincides with 2nd heart sound and marks the end of ejection/the beginning of the isovolumetric relaxation of the left ventricle;
EH period = ejection of left ventricle;
O point = diastolic peak, corresponds to opening of the mitral valve/onset of the rapid ventricular filling;
HO period = isovolumetric relaxation of left ventricle;
OF period = rapid filling of left ventricle, it matches with 3rd heart sound;
FA period = slow filling of left ventricle;
OC period = Total ventricular filling (rapid, slow filling and filling during atrial systole)

Apexcardiogram offers information about left ventricular function.

I.2.2. Peripheral mechanical events during the cardiac cycle

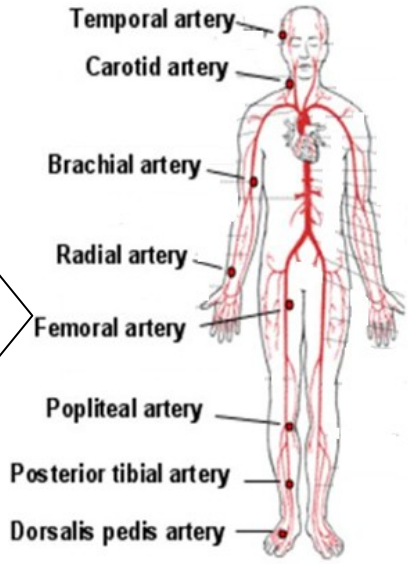
I.2.2.1. Arterial pulse, sphygmogram

Definition: pulse is the rhythmic expansion of arterial walls caused by the increased of blood pressure due to left ventricular systole

Examination of arterial pulse:

There are eight common arterial pulse sites:

- Radial
- Temporal
- Carotid
- Apical-precordial
  (listening to the heart directly).
- Brachial
- Femoral
- Popliteal
- Pedal (dorsalis pedis, posterior tibialis artery)



The blood ejected into aorta during ventricular systole expands the aorta and a pressure wave or pulse travels along its wall to the periphery. This expansion of the arterial wall when the artery is palpated against a bone is called wave pulse. The expansion of aorta during ventricular systole is followed by elastic recoil, so the blood will move forward in the circulatory system.

Velocity pulse wave transmission is higher than the velocity of blood flow.

In people with normal arterial walls elasticity and normal blood pressure, pulse wave spreads in the arteries walls at a speed of:

- 3-5 m/s in normal aorta;
- 7-10 m/s in large arterial branches;
- 15-35 m/s in peripherals arteries.

The velocity of the blood flow in larger arteries is about 0.5 m/s.

Thus, the pulse wave is synchronous with left ventricle systole (palpate as *apex beat* on the 5th intercostal space, mid clavicular line).

With age the arteries become more rigid and velocity of pulse wave transmission is higher.

Examination of radial pulse:

- supporting the patient's arm and hand with the palm down, press the first, second, and third finger of your dominant hand gently against the radius bone until you feel the contraction and expansion of the artery with each heartbeat;
- do not use your thumb; it has a strong pulse of its own and you may be counting your pulse;
- count the pulsations for 30 seconds using a timer;
- multiply the count by 2 to determine the rate of pulse for 1 minute;
- if the pulse is abnormal in any way, count for a full minute to get a more accurate reading;
- it is recommended to palpate the radial pulse in the same time in both arms, because we have to verify the synchronism and the symmetry of the pulse wave.

Characteristics of the arterial pulse:
1. Pulse rate:

- is the number of pulses per minute and it is a suitable method to determine heart rate
- normal: 60-90/min; bradycardia: < 60/min; tachycardia: >90/min

2. Rhythm:

- the interval between two consecutive pulsations
- can be regular or irregular (arrhythmia).

3. Velocity: the speed of the wave ‘s start and disappearance
4. Amplitude : the magnitude of the pulse wave
5. Tension: the force used for artery complete compressing in order to obtain the disappearance of the pulse wave

Characteristics of the arterial pulse change significantly in certain heart diseases and valvular defects.

Examination of arterial pulse by palpation is an important parte of physical exam of cardiovascular system.

Sphygmogram

Principle: the arterial pulse is recorded with a device called sphygmograph/ plethysmograph and the resulted graph is named sphygmogram.
Materials required:

- sphygmograph (Marey) – mechanical device for recording the movements of the arterial wall during and between the pulse beats.
- infra-red photoelectric pulse plethysmograph - record changes in pulsatile blood flow from fingers, toes, ears, the forehead, and other places by sensing the light reflected back from the skin

Technique:
- the devices for recording the pulse are placed on the skin over an artery
Results and interpretation:
The pulse tracing recorded from radial artery reveals (figure 1.34):

- an ascending limb named *anacrotic limb* corresponds to systolic rise of blood pressure during rapid ejection phase of ventricular systole;
- descending limb named *catacrotic limb* (from the systolic peak till the baseline) represents the decrease of blood pressure during slow ejection and ventricular relaxation .
- *dicrotic notch* is due to the closure of aortic valve and corresponds to end of ventricular ejection
- *dicrotic wave appears* due to rebound of blood column from the closed aortic valve because of elastic recoil of arterial wall.

Discussions and clinical significance

Progressive diminution of the pulsations in the periphery is called damping of the *pressure pulses* (difference between systolic and diastolic pressure) (figure 1.34).
Causes are:

(1) increased resistance to blood movement in the vessels and
(2) increased compliance of the vessels.

The *pulse deficit* represents the difference between the apical and radial pulse rates. Apical pulse rate is similar to the apical heartbeat assessed by palpation or auscultation with a stethoscope in the 5th intercostal space, mid clavicular line. For precision, both pulses should be taken at the same time. Normally, there is no pulse deficit. However, in conditions of irregular rhythm (extrasystoles, atrial fibrillation) some of the heart beats are weak, left ventricular filling is decreased, so the ventricle cannot eject enough volume of blood to initiate a peripheral pulse wave.

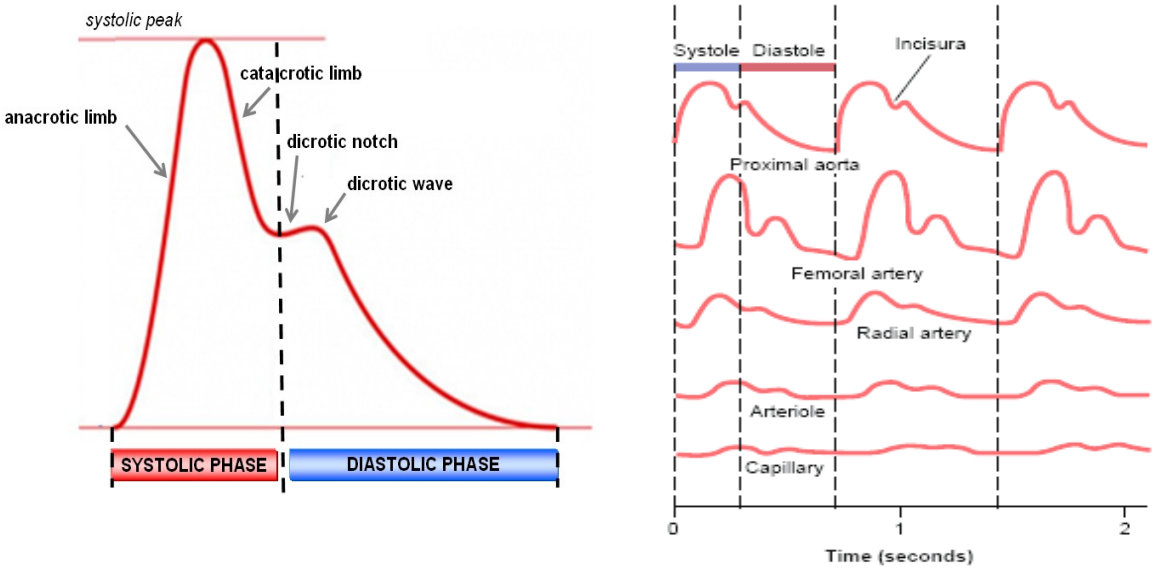


Figure 1.34 . Sphygmogram tracing on radial artery (left)
and tracings at different artery levels (right)

I.2.2.2. Arterial pressure: measuring and recording methods

Definition: arterial pressure is the force of blood applied against the walls of the arteries induced by the contractile activity of the heart.

Arterial pressure parameters:

1. *Systolic blood pressure (SBP)*
   - maximum value of blood pressure during ventricular systole
   - depends mainly on cardiac output
   - value: 120-140 mmHg
2. *Systolic blood pressure (DBP)*
   - minimum value of blood pressure during ventricular diastole
   - depends on arterial wall elasticity (peripheral resistance which depends on diameter of the blood vessels and viscosity of the blood)
   - show the constant load against heart has to work
   - value: 70-90 mmHg
3. *Mean arterial pressure (MAP)*
   - average of pressure during cardiac cycle
   - since the duration of heart systole is shorter than the duration of diastole, the MAP is not equal with arithmetic average of SBP and DBP.
   - MAP = (SBP-DPB)/3 + DBP
   - 90-100 mmHg
   - represents the perfusion pressure of the tissues.
4. *Pulse pressure*
   - difference between systolic and diastolic blood pressure
   - 40mmHg
   - determines the pulse volume
   - high value is a evidence for systolic hypertension

Determinants of (factors affecting) arterial blood pressure (see the lecture)

1. cardiac factors
    - cardiac output = stroke volume x heart rate
    - heart rate
2. vascular factors
    - peripheral resistance
    - elasticity of blood vessels
3. blood factors
    - blood volume
    - blood viscosity

Methods of measurement:

1. Direct method

Blood pressure is measured directly by cannulating an artery and connecting the cannula to mercury manometer or by introducing a catheter in the artery and connecting it to a device that measures and record blood pressure.

This method is usually used to record blood pressure on experimental animals.

The graphic recording shows in figure 1.35 three types of oscillations depending on speed of the paper: vasomotor oscillations appear at a low speed of paper, respiratory oscillations appear at a intermediary speed of paper and systole-diastolic oscillations appear on high speed of paper.

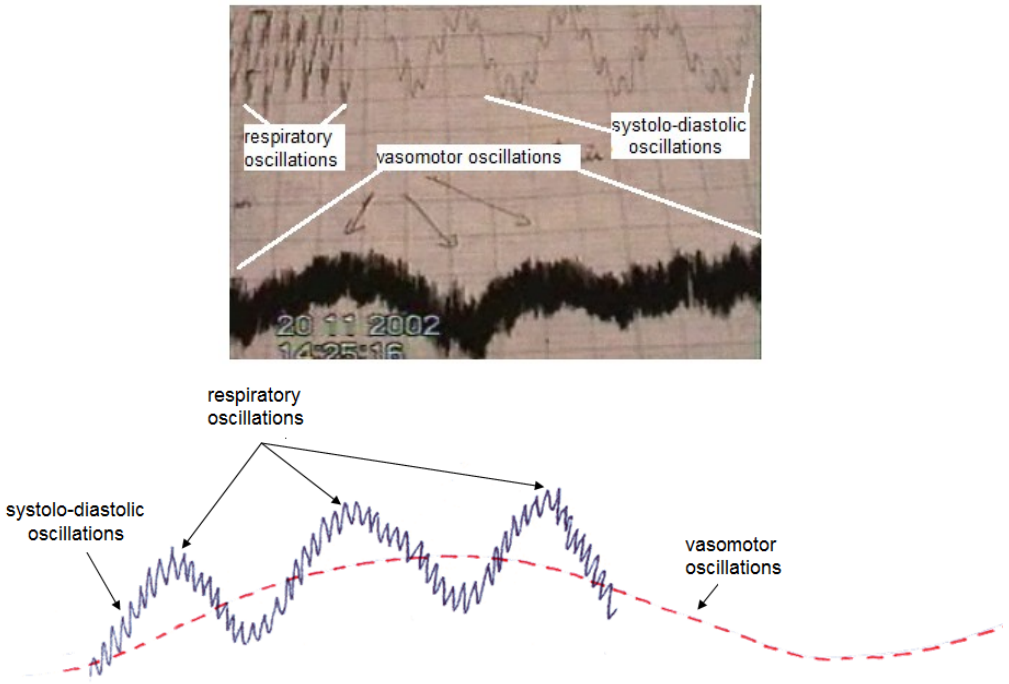


Figure 1.35. Original traces (above) and diagram (below) of direct recording of blood pressure.

2. Indirect method (sphyngomanometry) is a non-invasive method of blood pressure measurement based on use of sphyngomanometer.

Principle: constrict the artery with a cuff which is inflated with air at a pressure that can be monitored and counterregulate the pressure of blood within artery.

Materials required: **Sphyngomanometer** is the most used instrument to measure blood pressure. There are 3 types of sphyngomanometers: mercury sphyngomanometer, aneroid sphyngomanomete and digital sphyngomanometer (figure 1.36).

The main parts of a sphyngomanometer are:

- *manometer*: three types of manometer are used (figure 1.36):
  - *mercury manometer* is used in classical sphyngomanometer and it comprises a graduated narrow glass tube from 0 to 300 mm. Top of the meniscus should be at 0. Upper end of this tube is closed and lower end is linked with a mercury reservoir. The last is connected through a rubber tube with the cuff; mercury manometers are considered to be the gold standard and cannot be decalibrated, they are consistently accurate; a mercury manometer is calibrated if the mercury is at "0" on the scale when the device is not under pressure and the mercury is not fragmented.
  - *aneroid manometer* consists of a metal bellows, which expands as the pressure in the cuff increases, and a mechanical amplifier that transmits this expansion through a lever to the indicator needle, which rotates around a circular, calibrated scale. The needle should rest at the zero point before the cuff is inflated and return to that point after the cycle of inflation and deflation. Aneroid manometers require maintenance every 6 months. Recalibration is required when the readings differ from the standard mercury manometer by more than 4 mm Hg. (AHA Medical/Scientific Statement "Human Blood Pressure Determination by Sphygmomanometry").
  - *digital manometers* are electronic devices; are growing in popularity, particularly for patient's self-monitoring of blood pressure.; they measure mean arterial pressure and use oscillometric detection to calculate systolic and diastolic values, so should not be used by patients with arrhythmias. These devices must be checked regularly for accuracy against a calibrated manometer.

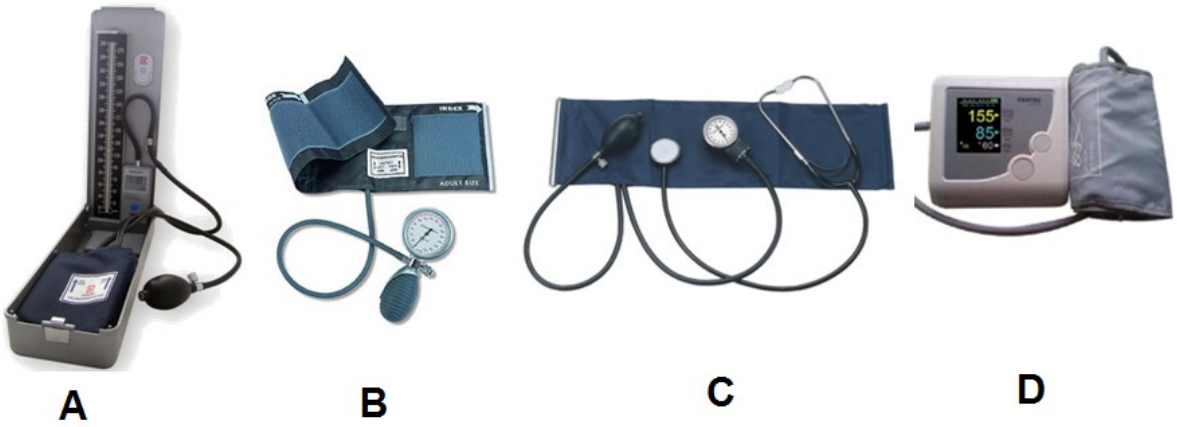


Figure 1.36. Different types of sphyngomanometers
(A = mercury, B and C = aneroid, D = digital)

- *cuff* is an expandable, rectangular rubber bag (or bladder) encased in a nondistensible cuff. The cuff is wrapped around the arm so the non inflatable part overlies that containing the bag and is secured with a self adhesive material (e.g. Velcro). The bag is connected by rubber tube(s) to the manometer and to the air pump. The length and width of the cuff varies with age groups and body build. It is recommended that the width of the rubber bag be 40% of the arm circumference, and the length of the rubber bag be long enough to encircle at least 80% of the arm in adults / 100% of the arm in children.
- *air pump* it is a rubber bulb with one-way valve at its free end and a release valve with a screw at the other end where the rubber tube leading to the cuff is attached. The cuff is inflated by turning the screw clockwise and repeatedly compressing the bulb.

Stethoscope consists of 2 parts: (1) headset (eartips and eartube) connected by tubing with the (ii) chestpiece (diaphragm and bell) (figure 1.30). The diaphragm is used to measure blood pressure.

Before blood pressure measurement follow next rules:

- the subject should be physically and mentally relaxed, should be seated for 5 minutes before measurement;
- the subject should refrain from ingestiong caffeine, alcohol, smoking during the 30 minutes prior to the blood pressure measurement;
- the subject should be sitting in a comfortable position, legs and ankles uncrossed and back supported as the procedure is explained and conducted;
- the arm should be placed at heart level, elbow slightly flexed, forearm with the palm facing upwards and supported on a flat surface.
- the blood pressure cuff should be applied smoothly and snugly around an upper arm, so the cuff is one-inch (about the width of two fingers) above the antecubital fossa. The inflatable rubber bag is centered over the brachial artery (cuffs are usually marked appropriately).

There are **three indirect methods** for measuring blood pressure:

a. palpatory method (Riva Rocci or radial pulse method)

- place the cuff of the sphygmomanometer smoothly and snugly around an upper arm, so the cuff is one-inch (about the width of two fingers) above the antecubital fossa. The inflatable rubber bag is centered over the brachial artery (cuffs are usually marked appropriately).
- feel the radial pulse by placing middle three fingers over it, before inflating the cuff
- inflate the cuff slowly while palpating the radial artery pulse to the point where the pulse disappears. This is the level of systolic blood pressure and you should read it on the aneroid dial or mercury manometer (which should be clearly visible and facing you).
- then inflate the cuff to 20-30 mmHg above the point of disappearing of the pulse.
- deflate the cuff gradually by 2 to 3 mmHg/sec and note the pressure level where the radial pulse reappear. The pressure level where the pulse disappears or reappears correspond to the **systolic blood pressure**.
- after that deflate rapidly the cuff to 0 pressure.
- take 3 readings in each arm, fully deflating the cuff between each reading. Average of these 3 readings is the systolic blood pressure.
- *disadvantage* of the palpatory method:
    - usually, the systolic pressure measured is with 2-5 mmHg higher than the pressure recorded by other methods because initial 2-3 pulsations of radial artery being thin are often missed.
    - this method estimates only the systolic blood pressure.

b. auscultatory method (figure 1.37)

- estimate the systolic blood pressure by palpatory method;
- inflate the cuff to 20-30 mmHg above the point of level at which radial pulse is no longer felt;

- place the diaphragm of the stethoscope over the brachial artery with the thumb and fingers of left hand; apply as little pressure on the head of the stethoscope as possible;
- open the adjusting screw and deflate the cuff gradually by 2 to 3 mmHg/sec (by opening the release valve of air pump with right hand).
- listen for the onset and disappearance of Korotkoff sounds. Note the *systolic pressure - the first Korotkoff sound (phase 1)* and *diastolic pressure - the last soft, rhythmic sound (Korotkoff sound phase 5).* The absence of a tapping sound after the initial sound is known as an *auscultatory gap*. If this occurs, elevate subject's arm overhead for 30 seconds then bring arm to usual supported position to remeasure.
- listen for another 10 to 20 mmHg beyond the last sound heard, then quickly deflate cuff to zero;
- brachial artery pressure may normally differ between left and right arm measurements by as much as 10 mm Hg and is usually higher in the right arm;
- *advantage:* auscultatory method give the value of both systolic and diastolic blod pressure;
- *disadvantage:*
  - unless followed by the palpatory method, the auscultatory method may miss the auscultatory gap
  - adequate experience is required to detect Korotkoff sounds.

– *Korotkoff sounds phases* (figure 1.37):
  - Phase I: sudden appearance of faint tapping sounds which become progressively louder, clearer during the next 10mmHg fall in pressure;
  - Phase II: in the next 10 mmHg fall in pressure the sound become murmurish, i.e. soft and swishing;
  - Phase III: the sound becomes clearer, louder, knocking, banging in the next 15mmHg drop in pressure
  - Phase IV: sounds become muffled for the next 5mmHg fall in pressure ;
  - Phase V: silence; no sound can be heard.

  Since the beginners may not heard muffling of sounds in phase III, disappearing of sounds (phase V) is considered as diastolic blood pressure. In some heart diseases (aortic valve insuffiency, hyperthyroidism, and severe hypertension) the sounds continue to be heard even in phase V when the pressure is reduced. In this case muffling rather than disappearance of sounds is considered as diastolic blood pressure.
  - *mechanism of Korotkoff sounds* (figure 1.37; *see the lecture*)

– The *auscultatory gap* is found in patients with severe hypertension when after the 1st Korotkoff, the sounds disappear (e.g. at a point below 200mmHg for a range of 20-50 mmHg) and then reappear to a much lower pressure and finally disappear at the level of diastolic pressure. This brief interruption is called *auscultatory gap.* If blood pressure is measured only ausculator (without radial pulse palpation), you may inflate the cuff to this gap only and you may miss the 1st Korotkoff sound appearance (systolic pressure) and thus you estimate a false low systolic pressure. To avoid this mistake the cuff must be inflated with 50-60 mmHg above level at which radial pulse is no longer felt.

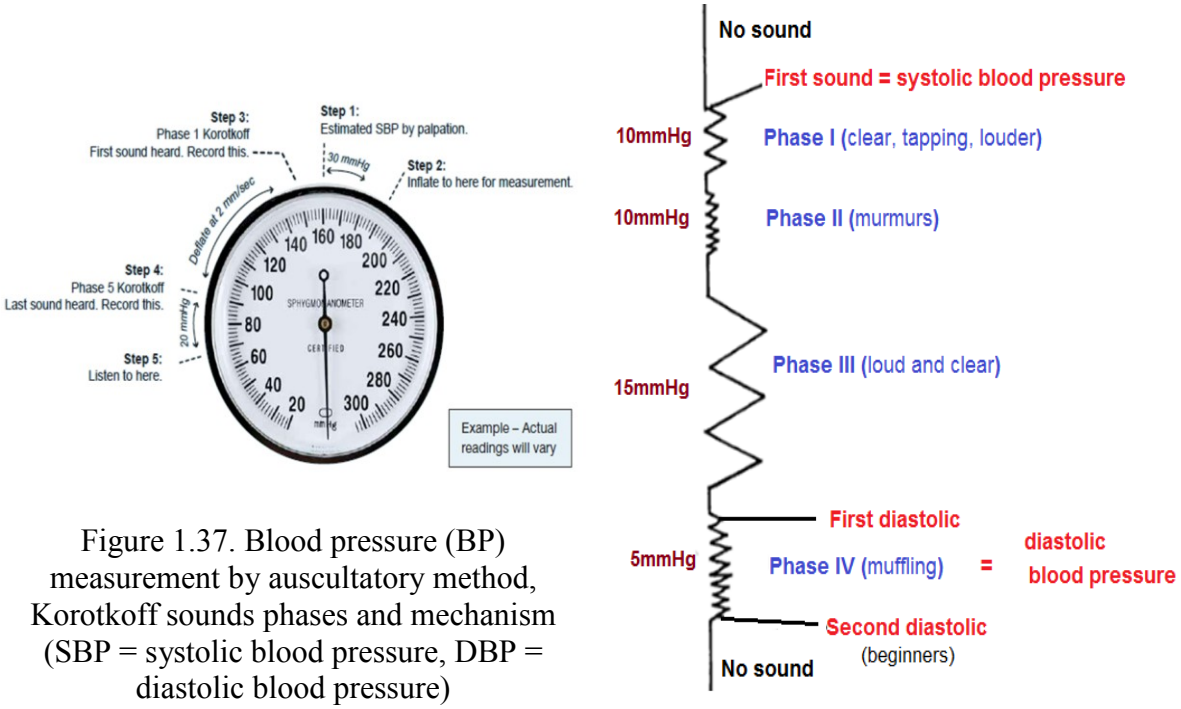
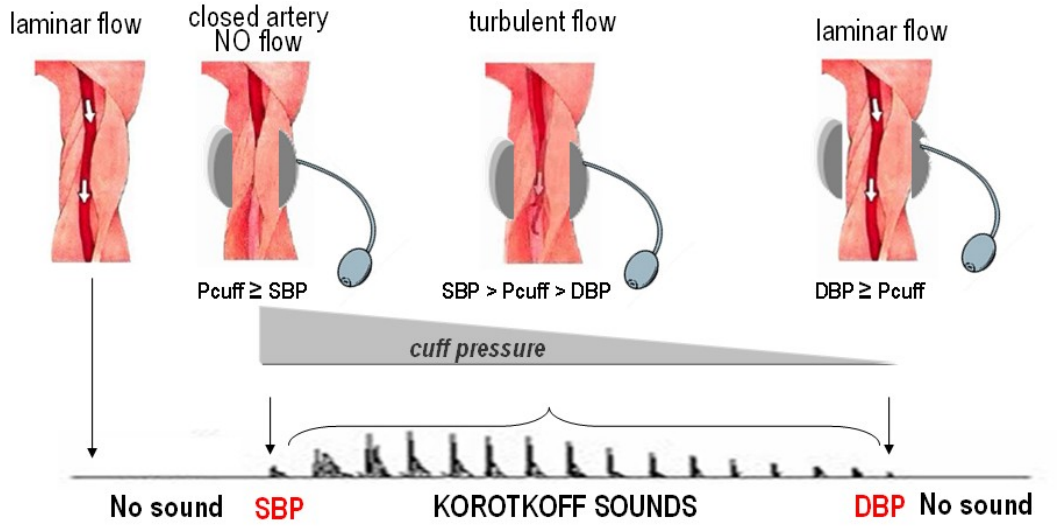


Figure 1.37. Blood pressure (BP) measurement by auscultatory method, Korotkoff sounds phases and mechanism (SBP = systolic blood pressure, DBP = diastolic blood pressure)



- measure blood pressure both in supine and standing position, and on both arms; communicate to patient verbally and in writing the blood pressure goal for their treatment, their specific blood pressure numbers, and when they should be monitored next.

c. oscillometric method (figure 1.38)

- is based on the following principle: oscillations amplitude of an elastic membrane (when is removed from the rest positions by some force) is even greater as the pressure on both sides of the membrane is closer to equality and the amplitude will be maximum for a given force when the pressure on both sides of the membrane is the same. For example, the tympani membrane vibrates with maximum amplitude only when outside pressure is equal to the middle ear pressure. This principle was applied by Marey to the elastic arterial wall.

- Physiologically, the arterial wall oscillates very little under the influence of systolic wave, because the pressure on both sides of the wall varies much. When is applied an external counter pressure that try to equalise the pressure on both sides of the wall, the arterial wall performs oscillations of greater amplitude, the more the more pressure on both sides of the wall is closer to equality.
- Device based on this principle is Pachon oscillometer (figure 1.38) which consist of an aneroid sphyngomanometer (M1) and another more sensitive manometer (M2) with a large dial that indicates the amplitude of the oscillations of pressure in the vessel. The device has two rubber bladder (bag) encased in the same cuff. The rubber bags, C1 and C2 are interconnected by a tap R, also communicate with air pump and with oscillometer box that actually contains two chambers A and B separated by a very smooth elastic membrane (m), vibrating at pressure differences between the two chambers. Vibrations are taken by a pointer moving on the scale of the M2 dial.
- the cuff of the oscillometer is wrapped around the studied segment of the limb, tap R is open, cuff pressure is increased till the peripheral pulse disappears. When pressure on M1 is constant, close the tap R and read the amplitude of oscillations on M2;
- when the artery is a little decompressed, the first flow of blood passes, it hits the vessel wall, the wall vibrates. This vibration is taken by rubber bag C2 and sent to chamber B causing the vibration of membrane m. Membrane vibrations will determine the pointer oscillations on the dial of M2 (when the tap R is closed, so the connection between rubber bag C1 and chamber A is interrupted, leaving any change of pressure to be taken by C2 and sent to chamber B).
- open the tap R, reduce the pressure in the cuff by 10 mmHg, then close the tap and read again arterial wall oscillations on M2. As cuff pressure decreases, the oscillation amplitude of the arterial wall increases to a maximum which correspond to the ***oscillometric index (OI).***
- the first oscillation that appears (called *maximal oscillation*) corresponds to the maximum systolic pressure. Sometimes, before the maximal oscillations, another oscillations (called *supramaximal oscillations*) occur due to systolic oscillations of arterial pressure which hit the upstream top edge of the cuff. These type of oscillations are suppressed on some modern oscillometers with double cuff.
- then progressing in slowly deflating the cuff, observe that the amplitude of the oscillations increase till a maximum (the maximal oscillation - OI corresponds to the mean arterial pressure), followed by a decreasing in oscillation amplitude till some point where this amplitude decreases abruptly (without disappearing completely) which correspond to the diastolic blood pressure. Oscillations persist even after the cuff pressure is below diastolic pressure (*inframinimale oscillations*).
- OI is determined on arm, forearm, thigh, calf (on both sides; in upper, medium or low third limb) and is noted on a diagram of main body limbs (figure 1.38 B). Amplitude of the oscillations diminishes from the thigh/arm to distal segments. OI_{thigh} = 3-6, OI_{calf} = 2-4, OI_{arms} = 3-4.
- pathological values of OI are considered 0 or near 1. when explored artery is blocked or obstructed. Also pathological is when there are differences over 2 oscillations between two symmetrical points or between two nearby points on the same artery.

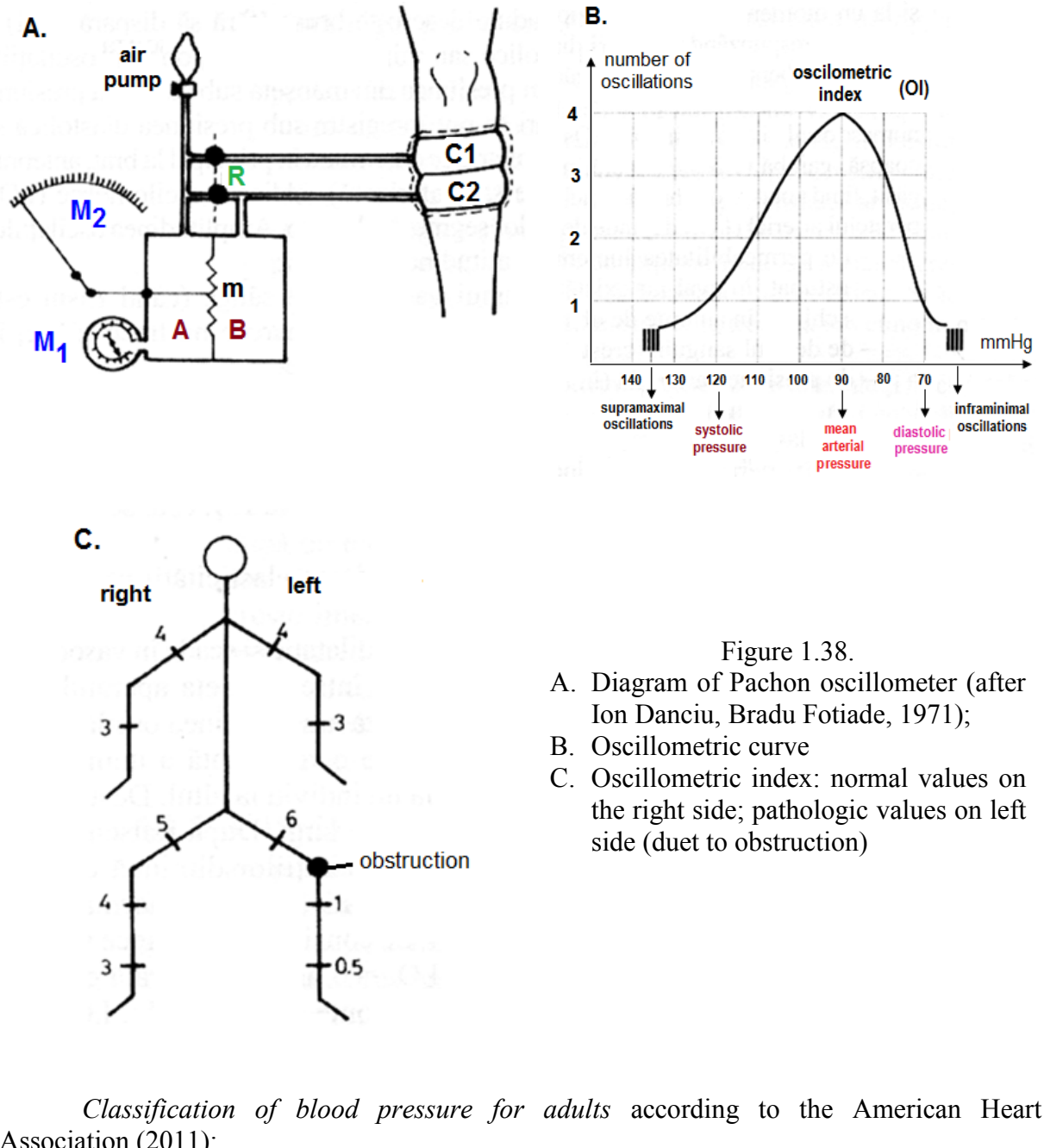


Figure 1.38.
A. Diagram of Pachon oscillometer (after Ion Danciu, Bradu Fotiade, 1971);
B. Oscillometric curve
C. Oscillometric index: normal values on the right side; pathologic values on left side (duet to obstruction)

Classification of blood pressure for adults according to the American Heart Association (2011):

Category	systolic, mmHg	diastolic, mmHg
Hypotension	< 90	< 60
Normal values	**90–119**	**60–79**
Prehypertension	120–139	or 80–89
Stage 1 Hypertension	140–159	or 90–99
Stage 2 Hypertension	160–179	or 100–119
Hypertensive crisis	≥ 180	or ≥ 120

I.2.2.3.Circulation time

Definition: time taken by the blood to flow through a segment of the cardiovascular system
Principle: is based on marking the blood into a segment of circulatory system by means detectable in another segment.

Total circulation time is time taken by substances to pass the entire circulatory system.

Partial circulation time is the circulation time between 2 points and can be estimated by injecting a substance into a segment of the circulation and marking the time lapse when it can be detected at another point. For this purpose it is used substances that are injected into the blood and produce subjective sensations (reported by the patient) or objectives changes that are followed by the doctor or detected by some devices; a timer to count the time from the moment of injection till detection of the substance is required.

Technique:

Method	Substance used	End point	Time	Variations
With Decholin	Sol. 20%, 3-5 ml i.v. antecubital vein	Bitter taste	Arm-to-tongue circulation time = 11" (8-16")	Increase in cardiac failure, policitemia, hypothyroidism; Decrease in anemia, fever, hyperthyroidism, physical effort.
With saccarin	Sol. 25%, 3-5 ml i.v. antecubital vein	Sweet taste	Arm-to-tongue circulation time = 11" (8-16")	Similar as above
With calcium gluconate	Sol. 20%, 3-5 ml i.v. antecubital vein	Hot sensation in the throat	Arm-to-tongue circulation time = 11" (8-16")	Similar as above
With histamine	Sol. Histamine, 3-5 ml i.v. antecubital vein	Flushing of face	Arm to face circulation time = 24"	Similar as above
With ether	1 ml ether i.v. antecubital vein	Characteristic odour of exhalation	Arm to lung circulation time = 6" (explore pulmonary circulation)	Increase when respiratory mechaniscs or hematosis is affected
With fluorescein	2-3 ml fluorescein i.v. antecubital vein	Detection of greenish fluorescence on the opposite arm	Arm to arm circulation time = 20" (explore systemic circulation)	Increase in hypothyroidism, heart failure; decrease in hypertiroidism, anemia, physical effort
With radioactive substance	Na^{24} as NaCl I^{131} (se detectează cu contor Geiger-Müller radioactivitatea la braţul opus)	Detection of radioactivity on opposite arm	Arm to arm circulation time = 15-20" (explore systemic circulation)	Similar as above

I.2.3. Venous circulation - Jugulogram

Definition: the graphic recording of volume variations of internal jugular vein due to pressure changes transmitted retrograde from right atrium

Internal jugular vein being valveless reflects right atrial pressure variations during cardiac cycle

Jugular venous pulse can be estimated by physical examination as an integral part of cardiovascular examination and provides reliable information about jugular venous pressure to reach diagnosis and monitor therapy for heart diseases. Jugular venous pulse is visible, but is not palpable at the jugular vein surface. Main steps in estimation of jugular venous pressure:

- with the patient is in the sitting position look for pulsation of external jugular vein. If no venous pulsation is visible, then lover progressively the head of the examination table by 10-15 degrees, each time waiting for 10-15 seconds and inspecting again the pulse. If the pulsation is not visible at 45 degrees, apply a pressure on the abdomen to cause a venous distension and hence a visible pulsation. Then continue to incline the head gradually to zero degrees until the venous pulsation is visible;
- identify the top of the pulsation, and the vertical distance of that point from the middle of the right atrium should be estimated. It is considered that sternal angle is about 5 cm H2O from the middle of the right atrium in supine position, 9cm H2O at 45 degrees and 10 cm H2O in sitting position.
- so, if the top of jugular venous pulsation in a patient is 3 cm above the sternal angle in supine position, the estimated jugular venous pressure for that patient would be 8 (5 + 3 = 8) cm of water (see examples on figure 1.39).

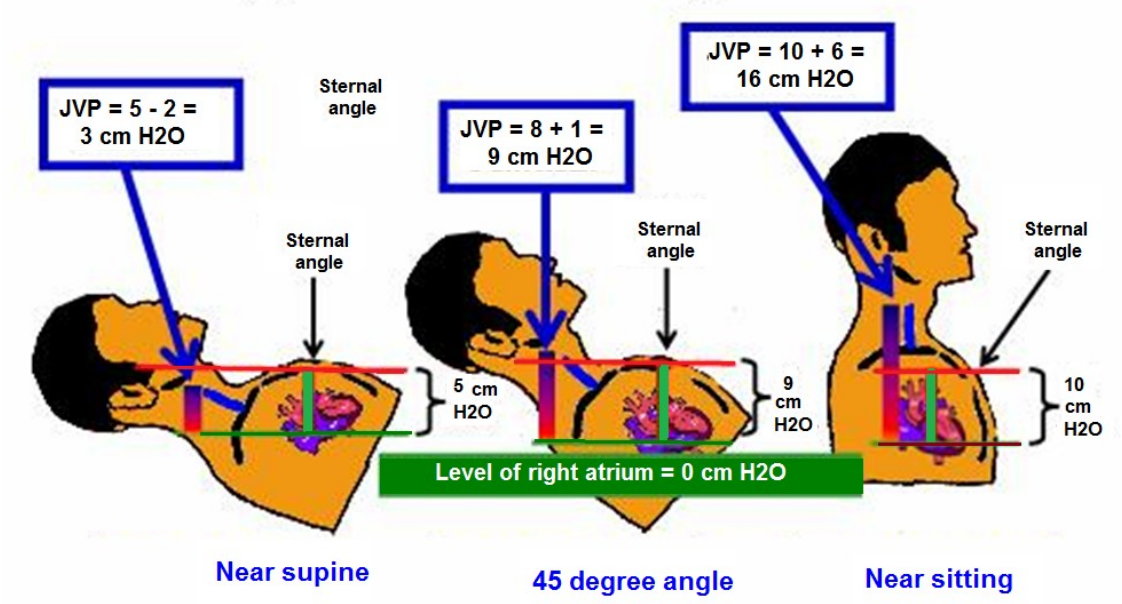


Figure 1.39. Estimation of jugular venous pressure (JVP) using external jugular vein at different body positions

The tracing of jugular venous pulse is almost similar to the intra-atrial pressure curve and it consists of 3 positive waves (a, c, v) and 2 negative waves (x, y) (figure 1.40) (see the lecture):

a wave = venous distension due to atrial contraction systole, before 1st heart sound;

z point = depending on the PR interval, the decent of venous pulse may continue until a plateau ("z" point) is reached just prior to right ventricular systole

c wave = is due to bulging of the tricuspid valve into the right atrium during right

ventricular isovolumic systole as the right ventricular pressure rises

x descent = is due to a combination of atrial relaxation, the downward displacement of the tricuspid valve during right ventricular systole, and the ejection of blood from both the ventricles.

v wave = is due to passive rise in pressure in the right atrium as a result of increased blood volume in the venae cavae and the right atrium (due venous return) during ventricular systole and isovolumetric ventricular relaxation (*see the lecture)*;

y descent = is due to the fall in right atrial pressure as tricuspid valve opens and rapid ventricular filling occurs.

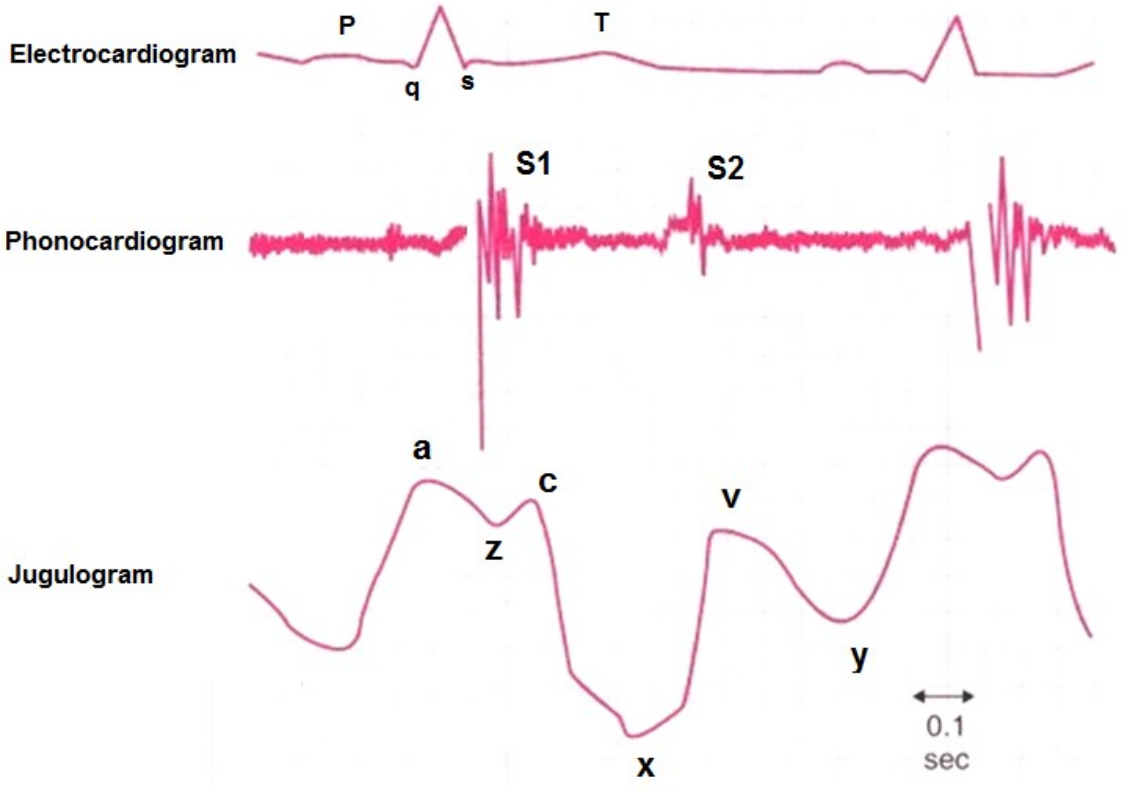


Figure 1.40. Jugulogram recorded simultaneus with electrocardiogram and phonocardiogram

I.2.4. Microcirculation - Intravital microscopy. Capillary permeability evaluation.

Definition: capillaries are the smallest vessels which connect arterioles and venules, and are enable the exchange of water, oxygen, carbon dioxide, and many other nutrient and waste chemical substances between blood and surrounding tissues.

Intravital microscopy (direct in vivo microscopy) is the observation of biological phenomena inside physiological context of a living organism using a microscope and it offers dynamic information.

Principle: application of warn/cold Ringer solution or other reagents change the size of the capillary bed on the webs of the frog studied under light microscopy.

Materials required: microscope, Ringer solution at different temperatures, adrenaline (1:10000), histamine, dropper, living frog, board with a hole in the centre

Technique:

- pith the frog (see section I.1.1)
- lie the frog on the board with the web of one foot spread over the hole

- place the web under the low-power objective of a microscope
- keep the frog moist by applying Ringer solution with the dropper
- observe capillary circulation
- *identify the arterioles, venules and capillaries* by observing the *branching, diameter of blood vessels and direction of flow*
- with the dropper apply warm/cold Ringer solution, adrenaline, histamine to observe changes in capillary circulation

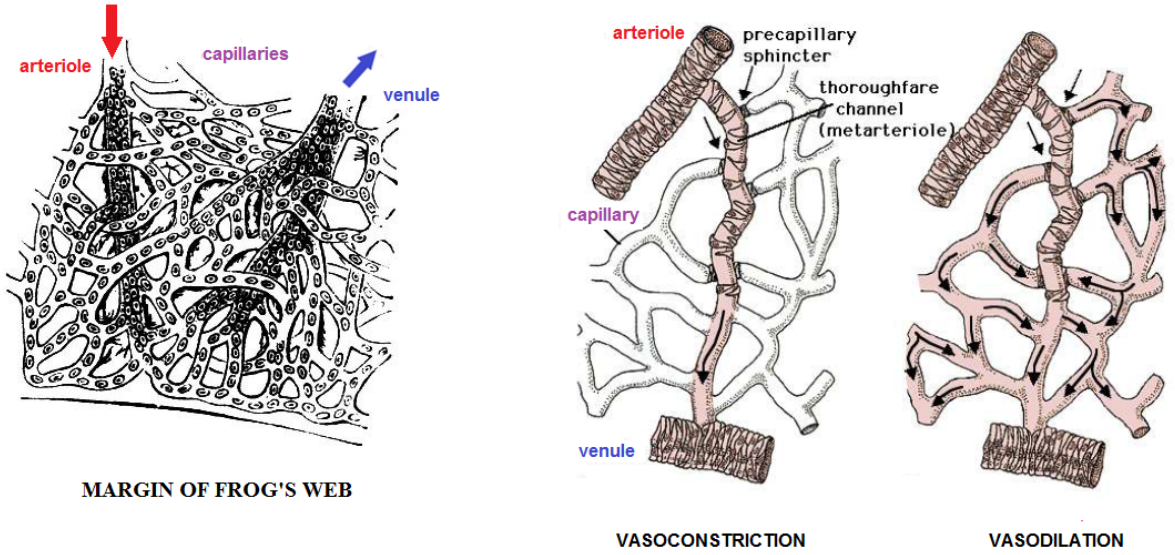


Figure 1.41. Microcirculation of the frog interdigital web (left) and diagram of microcirculation (right)

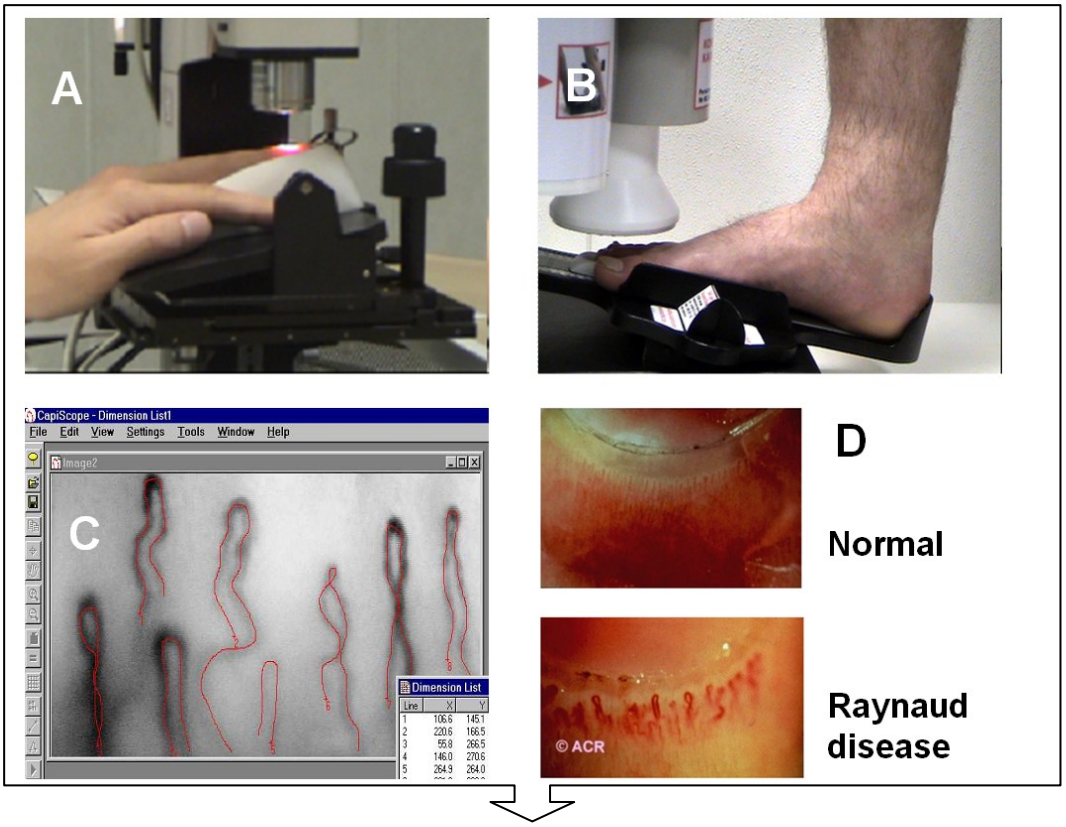
Results:

- application of *cold Ringer solution* decrease the size of the capillary vascular bed and reduces the blood flow through the capillaries (vasoconstriction due to capillary sphincters); *warm Ringer solution* has opposite effect;
- *histamine* is a vasodilator and increases the blood flow through capillary bed (vasodilation due to capillary sphincters)
- *adrenaline* reduces the diameter of the capillaries and decreases the blood flow in the capillaries

In clinic is used a method called ***nailfold capillaroscopy***, which is

- a very sensitive, non-invasive microscopy test
- visualises the hairpin-shaped capillaries in the nail folds

Indication of ***nailfold capillaroscopy*** comprises angiology, dermatology and rheumatology diseases like Raynaud's disease characterized through brief episodes of peripheral vasospasm (narrowing of the blood vessels). Triggers could be cold temperatures or stressful emotions. Prolonged or repeated episodes can cause sores or tissue death (gangrene).



The capillariscope captures images of capillaries of hand finger (picture A) and toe (picture B) and sent these images into a computer where a special soft (picture C) analises them (e.g. calculate capillary density and length per mm2 for any rectangular region). In picture D are presented two aspects of capillaries: normal and distorded capillaries (hairpin-shaped or twisted)

Discussions and clinical significance

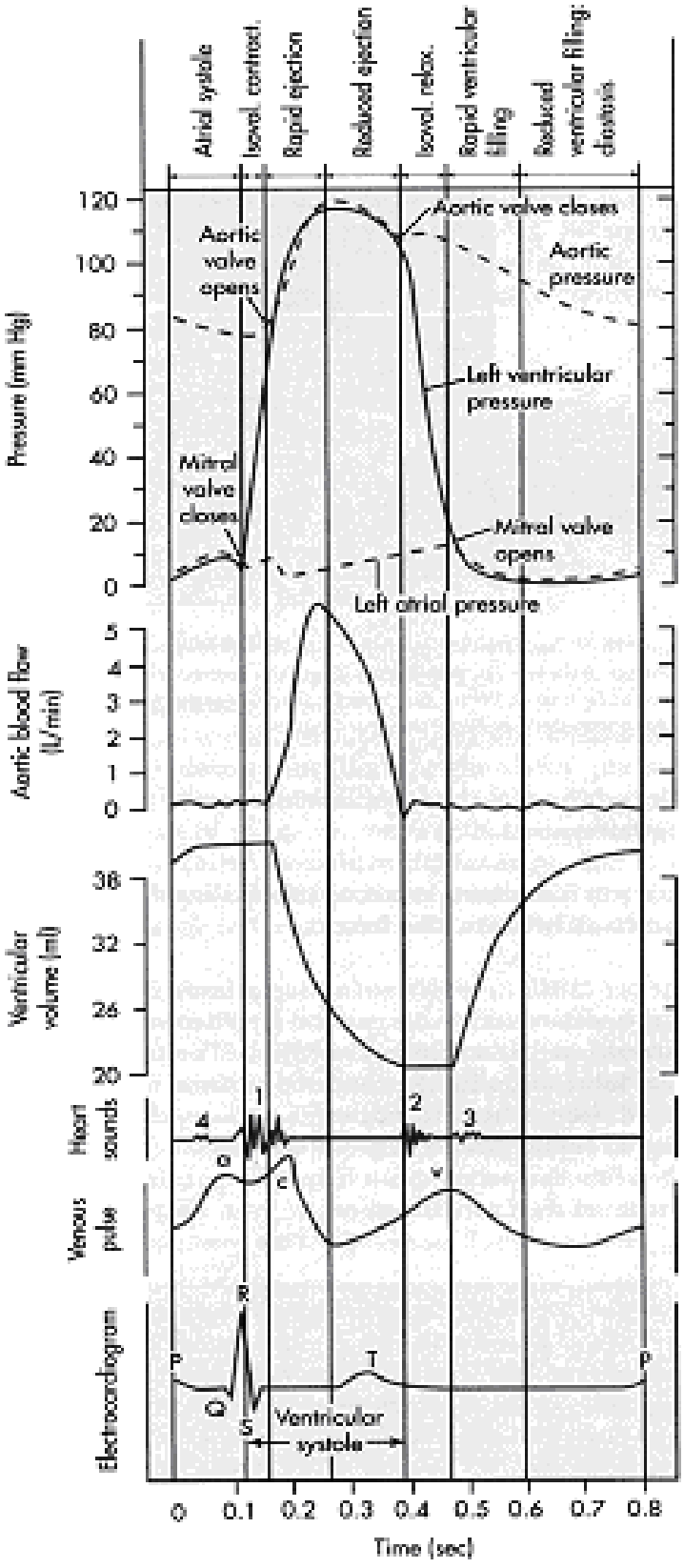
The capillary wall is a one-layer endothelium that permits bidirectional exchanges explained by the Starling equation (*see the lecture*).

If capillary permeability increases (*see the lecture*), edema is formed. Edema is defined as excess of free fluid in the interstitial space. Factors that cause the edema formation:

1. increased filtration pressure
   - arteriolar dilation
   - venular constriction
   - increased venous pressure (congestive heart failure, increased ECF volume, etc)
2. decreased oncotic pressure
   - ↓ protein synthesis from the liver;
   - proteinuria;
   - ↓ protein intake or ↓ intestinal absorbtion (malabsorption syndrome)
3. increased capillary permeability due to various substances like substance P, histamine, toxins (inflammatory conditions, allergic diseases)
4. lymph return is blocked e.g. by an obstruction – filariasis

I.2.5. Exploration of circulation

I.2.5.1. Analysis and interpretation of polygraphic records (see the lecture)



I.2.5.2. Radiologic, radioisotopic, echographic methods

ARTERIOGRAPHY

The arteriography is a radiological exam whose aim is to display the arteries. This exam requires the injection into the arteries of an impervious to X-rays product so as to really display the whole arterial network.

In order to inject this product into the arteries, it is imperative to introduce a hollow hose up to their level, so that the impervious to X-rays product can be injected. Thus, this hose is introduced through an artery, then moves along the arterial network up to the level where the physicians want to achieve an exploration.

Indications of the arteriography:

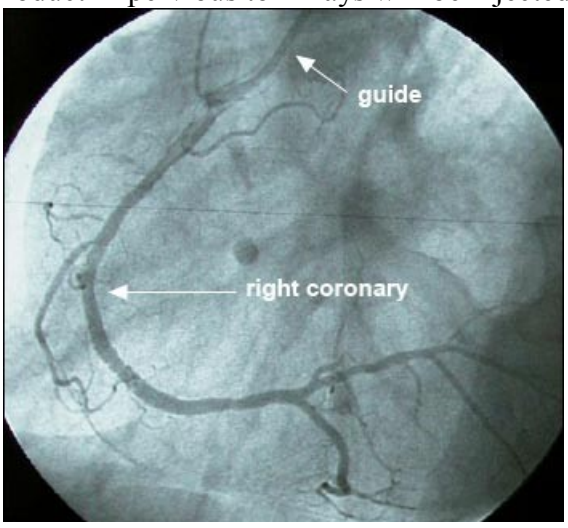
The physician asks for the achievement of an arteriography in the following indications:

- obstruction of an artery;
- obstacle in the artery, such as a clot of blood;
- before implementing a bypass.

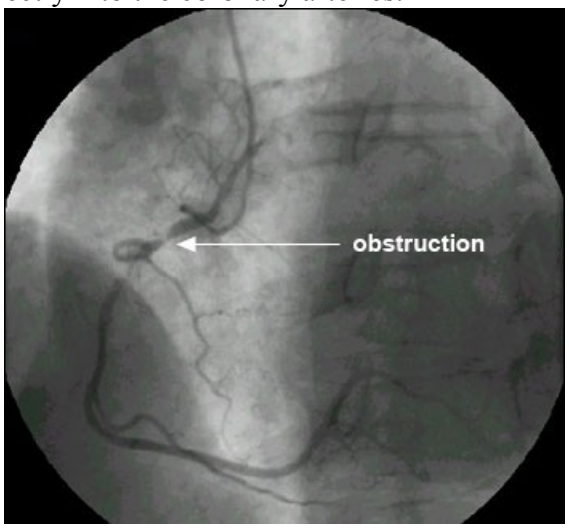
CORONAROGRAPHY

In order to display the obstacle localized on the artery of the heart responsible for the infarct for example, it is necessary to analyse this artery but also the whole coronary arteries (heart arteries) during a radiological exam whose principle is to inject directly into the heart arteries a radiological product making a contrast visible on a x-ray.

The coronarography, literally meaning the "x-ray of the coronary arteries", is an exam requiring to puncture an artery of a member in order to introduce a hose through which a product impervious to X-rays will be injected, directly into the coronary arteries.



Normal coronarography



The complete occlusion of an anterior interventricular artery

The coronarography is abnormal when a reduction of the internal diameter of the artery, responsible for a reduction of the blood outflow, exists. Below, strictures of the interior of a heart artery are represented, called "stenoses".

Indications of the coronarography

- a pain in the chest, evocative of an angina pectoris;
- an electrocardiogram of rest or exercise which is not normal;
- a myocardial infarction;
- before a surgical intervention especially when the patient presents cardiovascular factors of risk.

DOPPLER ULTRASOUND OF THE ARTERIES

The arteries are vessels bringing the blood from the heart to the organs. Their exploration with the help of the Doppler ultrasound permits to analyse their structure and the blood flow present in the arteries.It is a simple, completely painless exam, lasting 10 to 50 minutes according to the localization and the indication. Ultrasound and Doppler are based on the use of the ultrasounds. An ultrasound is emitted by the ultrasound probe then it comes up against the level of the artery which sends back the ultrasound. This one will then be perceived by the probe which then transmits a picture.

The Doppler permits to analyse the blood flow in the artery following:

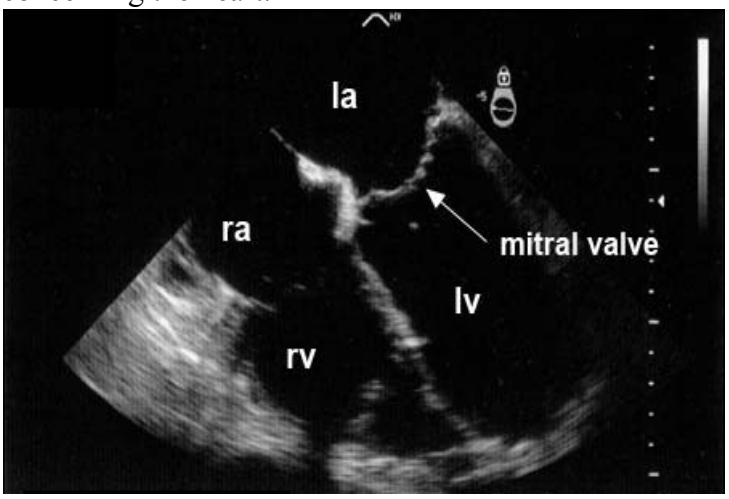
- presence of plates on the walls of the arteries;
- analysis of the wall of the artery;
- measure of the flow in the different arteries;
- research of a stricture of the diameter of the artery
- research of an obstacle

Indications of the arteries Doppler ultrasound:

- research of a stricture;
- research of an obstacle preventing the passage of blood;
- analysis of the walls of the arteries;
- implementation of a preoperative check-up before a by-pass for example;

TRANS-OESOPHAGUS DOPPLER ULTRASOUND OF THE HEART

An ultrasound of the heart allows the cardiologist to perfectly see the whole tissues which constitutes the heart as well as its kinetics. The Doppler, frequently coupled to the ultrasound, permits to measure the flows of blood in the different cavities of the heart. The trans-oesophagus Doppler ultrasound of the heart consists in placing a probe into the oesophagus and to look at the heart. This technique permits to get high quality pictures. It is a laborious but non painful exam, permitting the cardiologist to give you a lot of information concerning the heart.



View of the 4 cavities of the heart with a trans-oesophagus ultrasound: lv: left ventricle, rv: right ventricle, la: left atrium, ra: right atrium.

Indications the trans-oesophagus Doppler ultrasound of the heart

The trans-oesophagus Doppler ultrasound of the heart is achieved by the cardiologist according to the following indications:

- valvular heart disease;
- infectious endocarditis;
- atrial septal defect (ASD) our ventricular septal defect(VSD)
- research of a blood clot in a cavity of the heart, especially the left atrium and in an area situated right beside, named the left auricle.

THE DOPPLER ULTRASOUND OF THE VEINS

Ultrasound and Doppler are based on the use of the ultrasounds.

An ultrasound is given out by the probe then it comes up against the level of the vein which sends back the ultrasound. This one will then be perceived by the probe which then transmits a picture.

As the speed of the ultrasounds is very high, it is possible to get several pictures per second (about 24) which permits to have mobile pictures of the vein.

The Doppler is also based on the same principle, and can function with colour (colour Doppler) or not (pulsed or continuous Doppler).

Thus, the Doppler permits to analyse the flow of blood in the vein.

Indications the doppler ultrasound of the veins

- research of a phlebitis;
- research of a venous insufficiency and varicose veins;
- analysis of the quality of the veins before their removal.

THE EXERCISE TEST

` An exercise test consists in recording cardiology parameters during an exercise. The measured parameters are the pulse, the blood pressure, and especially the electrocardiogram.

This exam is very frequently carried out in cardiology.

The blood pressure is measured at the arm by the nurse, every 2 minutes on average, with the help of an armband and a stethoscope.

The cardiac frequency is measured thanks to the electrocardiogram.

According to the patients' parameters and their physical condition, a procedure of exercise is defined by the physician in charge of the exam. In the most classic situations, this procedure corresponds to an exercise increasing of 30 watts every 3 minutes. The duration of this exam therefore depends on the patient's capacity to achieve the exercise, but as a rule an exercise test lasts 9 to 15 minutes. The physician remains very attentive to the whole parameters during the exercise test.

Indications of the exercise test

The exercise test can be prescribed by your physician to research:

- an illness of the heart arteries;
- a disorder of the heart rhythm;
- the assessment of the efficacy of your treatment;
- the assessment of the exercise capacity.

THE ELECTROCARDIOGRAM HOLTER

The heart is constituted of several tissues, which are from in to outside the pericardium, the myocardium and the endocardium.

The myocardium corresponds to the heart muscle and represents the tissue taking the biggest space in the heart. This tissue is constituted of muscle whose contraction is responsible for the ejection of blood by the heart.

As every muscle, the heart is capable of giving out a certain quantity of electricity when it is in action. This electricity issued can be recorded by a device called an electrocardiograph.

The recording of this electric activity of the heart is called an electrocardiogram.

The measure of the electrocardiogram can be achieved during a 24 hours period, or even more, with the help of an automatic measuring equipment called a holter.

Indications of the electrocardiogram holter

- this exam permits to detect very many illnesses of the heart, first of all the illness of the coronary arteries (angina pectoris, infarct).
- the disorders of the heart rhythm
- the disorders of the cardiac conduction

The practice of a 24 hours EKG holter can permit to find a disorder of the electric conduction between the atria and the ventricles or within the ventricle.

AMBULATORY BLOOD PRESSURE MONITORING

The blood pressure (B.P.) can be monitored for 24 hours with an automatic device. As a rule, this device takes the B.P. every 15 minutes in the day and every 30 minutes at night, knowing that these intervals can be adjusted differently. Besides, the physician or the technician who places the device must clearly explain to his patient that it is possible at any moment to activate a measure of the B.P. by pressing a button to that end.

It is also very important to note down the variation of the B.P. between the day and the night period. Normally, the B.P. is lower during the night period compared to the day period, and this variation must be equal to at least 10%. But sometimes, this cycle is not respected, which reveals the severity of the HBP or the existence of an illness.

THE SCINTIGRAPHY OF THE HEART

The scintigraphy of the heart permits to value the function of the cardiac muscle very well, and give information on its contraction capacities. This exam consists in injecting by i.v. a compound radioactive, which settles down at the heart level. X-rays are then made with the help of a special device. It is a painless exam, with an average length of 15 to 30 minutes.

The radioactive product settles down on the cells of the cardiac muscle when this one is living and does not if the muscle is dead. Thus, the living areas of the muscle are coloured red whereas the dead zones are coloured blue or green. The physician can also assess the capacity of the cardiac muscle to relive after a myocardial infarction or a threat of infarct("myocardial viability").

Indications the scintigraphy of the heart

- assessment of the viability of the heart muscle. This indication before the implementation of a revascularization of a coronary artery (by setting up a prosthesis into the artery or achieving an aorto-coronary bypass);
- assessment of the contraction capacity of the heart muscle (calculation of the ejection fraction).

II. PHYSIOLOGY OF RESPIRATION

In physiology, respiration (often confused with breathing) is defined as the exchanges oxygen and carbon dioxide between organism and the external environment.

The respiration has 4 distinct phases:

1. *pulmonary ventilation* – called breathing – air moves in (inhale / inspiration) and out of lungs (exhale / expiration);
2. *external respiration (hematosis, pulmonary gas exhange)* – gas exchange between alveolar air and pulmonary capillaries by alveolo-capillary membrane;
3. *blood transport of respiratory gases* – O2 from lungs to tissue cells; CO2 from cells to blood;
4. *internal respiration (cellular respiration)* - gas exchange between systemic capillaries and tissue cells.

Functional exploration of respiration comprises *(see the lecture):*

- *Exploration of ventilation*
  - assessment of mechanical properties of thoraco-pulmonary system by
    - changes of thoracic cage diameters during ventilation – thoracometry
    - pneumography
    - assessment of negative intrapleural pressure
    - assessment of elastic recoil: pulmonary pressure – volume curve; compliance; elastance
    - assessment of airway resistance
  - measurement of static and dynamic pulmonary volumes
  - measurement of dead space
- *Exploration of hematosis*
  - Alveolar ventilation – perfusion ratio
  - Measurement of composition (CO2 and O2) of exhaled air
  - Test of difusion
- *Exploration of blood gases trasport*
  - respiratory blood gases (CO2 and O2) concentration – O2 saturation (SaO2) by pulse-oxymetry
- *Test during exercise; bronchoprovocation test with bronchodilators and bronchoconstrictors*
- *Imaging tests*

II.1. Thoracometry

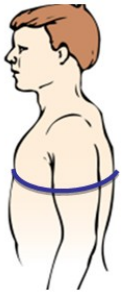
Definition: Measurement of thorax or hemithorax perimeter of with a metre during inhale and exhale

Principle: assessment of thorax expansion during ventilation

Materials required: metre

metre

thorax perimeter



left hemi-thorax = left hemiperimeter of the thorax

Figure 2.1. Thoracometry

Technique (figure 2.1):

- measure perimeter of the thorax superior (axilary level) and inferior (xiphoid appendix)
- measure hemi-perimeter from sternum to the vertebral column

Results and discussions:

- chest perimeter: difference between inhale and exhale
  - 8-10 cm *(normal value);* this difference is increase on athletes and decreased e.g. on emphisema
  - offer informations about
    - chest expansion,
    - excursions of the ribs,
    - respiratory type (costal superior, costal inferior or abdominal)
- Difference between left and right hemiperimeter of the thorax
  - right hemi-thorax > left hemi-thorax with 0.5 – 1cm *(normal value)*
  - detect asymmetrical chest expansion (pneumothorax, ribs fracture, pleural effusion, etc)

II.2. Pneumoghraphy

Definition: graphical recording of thorax movements during ventilation with a device called penumograph

Principle:

- the movement of the chest causes a change in air pressure of the pneumograph which is recorded on a moving drum

Materials required: Marey pneumograph (figure 2.1) consists of:

1.Pneumograph:
- *corugated flexible tube* with a stopper at each end
- air outlet pipe connected to the tambour

2. Marey tambour
- *metallic cup* is attached to a small metal disc, with a side tube and a elastic diaphragm mounted on the top
- *a writing lever* which rests on a rubber diaphragm
- the side tube of the metal cup is connected to the pneumograph by a rubber tube

3. Kymograph with a rotating drum (see section I.1.1)

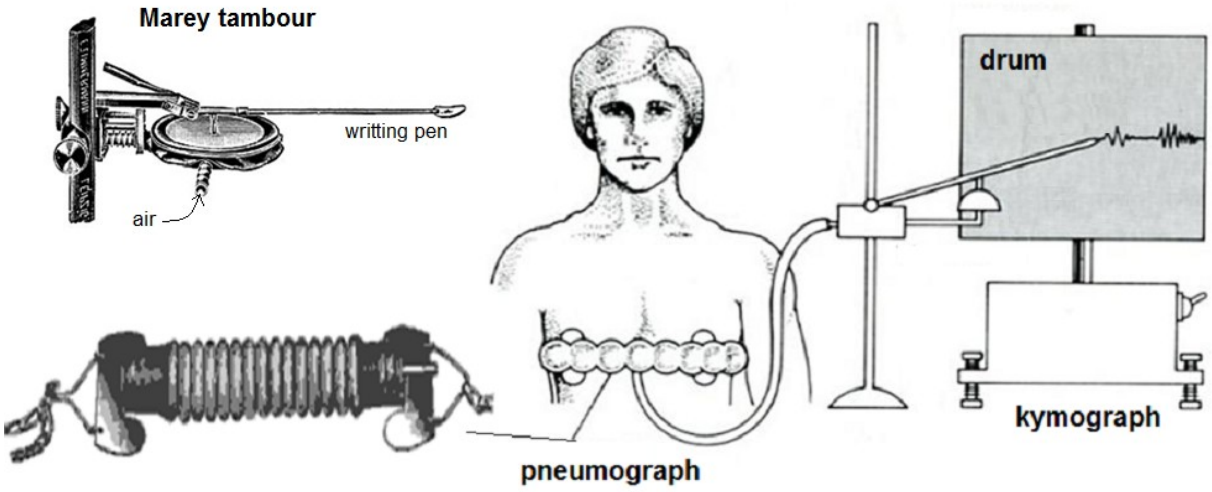


Figure 2.2. Pneumography

Technique:

- the pneumograph it is tied around the chest of the subject on the superior and inferior part;
- chest movements during ventilation (inhale / exhale) cause expansion / recoil of corugated flexible tube of pneumograph and the air will be inhale suck ed or

Results:

The graph recorded is called pneumogram.

Pneumogram offers informations about

- amplitude of ventilation (inhale and exhale)
- rate of ventilation = 15-18/min
- inhale / exhale ratio = ½
- respiratory type
  - *abdominal breathing:* children; based on diaphragm movements
  - *costal inferior breathing* : men
  - *costal inferior breathing* : women

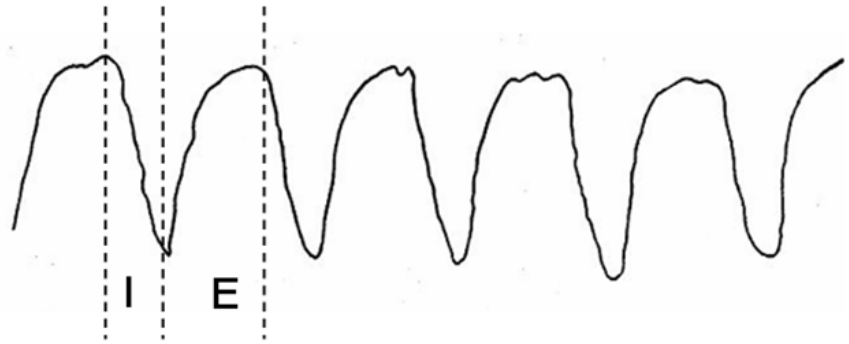


Figure 2.3 Pneumogram (I = inhale; E = exhale)

Discussions and clinical significance:

Inhale has one phase, appears like a vertical descending deflection on pneumogram and is an active process.

Exhale is a passive process and has 2 phases:

- first phase is a vertical ascending deflection and is due to abrupt elastic recoil of the thoracic cage (energy release from bone, condrocostal joints, ligaments);
- second phase is a plateau and is due to elastic recoil of the lungs.

II.3. Auscultation of respiratory sounds.

Principle: listening to breath sounds at the surface of the body with a stethoscope

Materials required: stethoscope consists of 2 parts: (1) headset (eartips and eartube) connected by tubing with the (ii) chestpiece (diaphragm and bell) (figure 1.30).

Technique: place the stethoscope on thoracic wall

Results: usually with the stethoscope it can be heard two sounds:

1. THE LARYNGO-TRACHEAL SOUND

- *characteristics:* strong and stiffing sound
- *cause:* produced by the air passage through the glottis during both expiration and inspiration
- *auscultation area:* the stethoscope is placed on the
  - anterior surface of the thorax over the upper half of the sternum
  - and posterior in the parascapular region (T1 to T 4)

- abolished after section of the trachea below larynx (on animal of experience) or after tracheotomia on humans.

2. THE VESICULAR MURMUR

- *characteristics:* the sound is weak and sweet aspirative.
- *cause:* it's produced by the air passage through the communication between the bronchioles and the alveolar ducts during inhale and at the beginning of the exhale
- *auscultation area:* the stethoscope is placed systematically on the entire thoracic surface to detect pathological changes
- abolished after section of the vagi nerves

Discussions and clinical significance: pulmonary auscultation offers significant information regarding the functional integrity of the lungs and constitutes an important phase of lungs physical examination.

II.4. Pulmonary flows and volumes. Spirometry. Spirography.

Definition: The volume of air that moves in and out of the lungs during breathing is measured with spirometer which records a graph called *spirogram* (whivh show flow in liters over time in seconds).

Principle: air, exhaled from the lungs, will cause displacement of a closed chamber that is partially submerged in water.

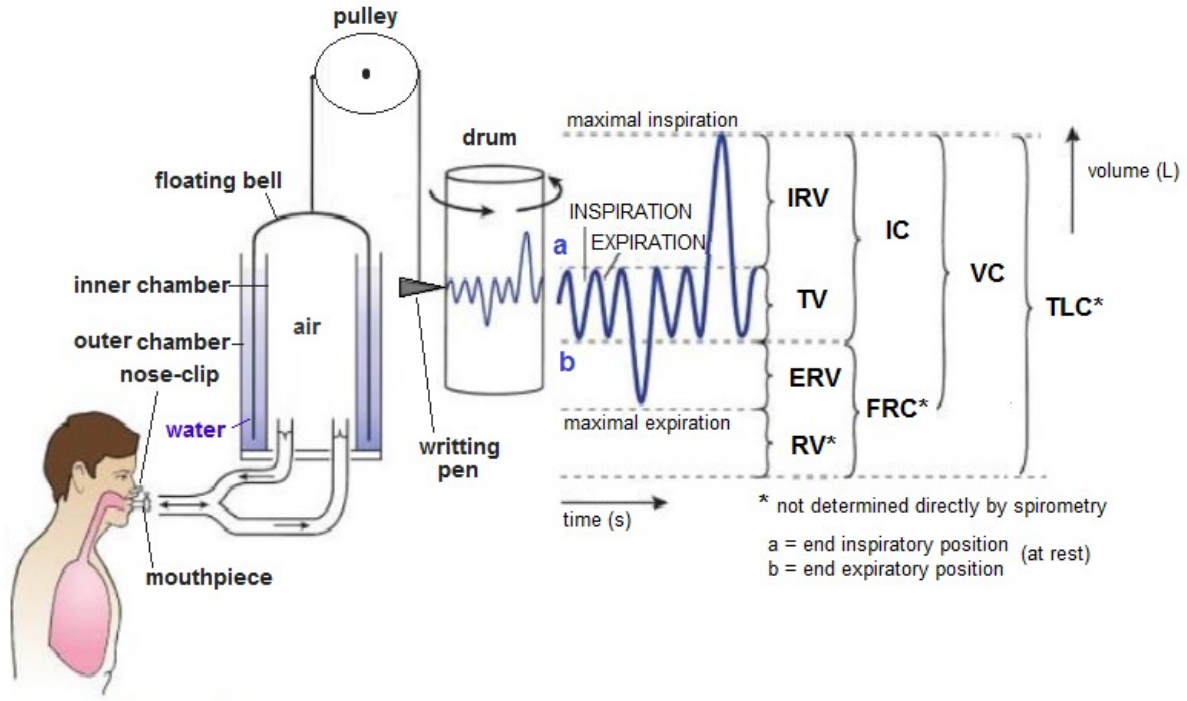


Figure 2.4. Spirometry and spirogram diagram

Materials required: wet spirometer consists of two chylinders (figure 2.4):

- a larger cylinder (or outer chamber) filled with water;
- a smaller cylinder (floating bell) which is inverted inside the first cylinder and immersed in water to form a seal. It is connected by a cord which passes over a pulley bearing a counterweight and a writing pen. The movements of the floating bell is transmited to the pen which record the spirogram on a moving drum;

- inner chamber is opened at the top end which is positioned above the water level inside the outer chamber; at the bottom end a tube is attached which is connected to a mouthpiece. The nose of the subject is clipped and through the mouthpiece ventilates into the spirometer.;
- when the subject exhales the floating bell moves up and the writing pen moves down, so the expiration is recorded as a downward deflection; when the subject inhale, the upward deflection is recorded.

Technique:

- is called *spirometry* (figure 2.4);
- the mouthpiece is disinfected;
- the subject is instructed to breathe normally for a few breat (tidal volume is recorded), then to inhale as deeply as possible (inspiratory reserve volume) and then exhale as deeply as possible (expiratory reserve volume).

Results (figure 2.4):

A. STATIC PULMONARY VOLUMES

There are lung volumes that are not affected by the rate of air movement in and out of the lungs. The static lung volumes are:

- *4 Volumes:* tidal volume (TV), inspiratory reserve volume (IRV), expiratory reserve volume (ERV), residual volume (RV)
- *4 Capacities:* sum of 2 or more lung volumes: vital capacity (VC), inspiratory capacity (IC), functional residual capacity (FRC), total lung capacity (TLC)

Three static pulmonary volumes (RV, FRC, TLC) cannot be measured directly by spirometry, they can be determined indirectly using helium gas dilution techniques or body plethysmography.

The 4 volumes are:

1. **Tidal volume (TV) ~ 0.5 L**
   - volume of air inhaled or exhaled with each breath
   - volume of air inspired and expired during normal quiet breathing
   - the amount of air that moves in and out of the lungs during a normal respiratory cycle
   - values given are for adult males, 70 kg

2. **Inspiratory reserve volume (IRV) ~ 3 L**
   - the maximum volume of air that can be inhaled after a normal tidal volume inhalation

3. Expiratory reserve volume (ERV) ~ 1.2 L
   - the maximum volume of air that can be exhaled after a normal tidal volume exhalation
   - maximum amount of air that can be exhaled beyond the tidal volume (from the resting expiratory level)

4. Residual Volume (RV) ~ 1.2 L
   - Volume of air remaining in the lungs at the end of maximum expiration
   - The volume of air in the lungs that cannot be forcedly expelled

The 4 capacities are:

1. Vital capacity (VC)
   - volume of air that can be exhaled from the lungs after a maximum inspiration
   - VC = IRV + TV + ERV

2. Inspiratory capacity (IC)
- Maximum amount of air that can be inhaled from the end of a tidal volume
- IC = IRV + TV

3. Functional residual capacity (FRC)
- volume of air remaining in the lungs at the end of a TV expiration
- the elastic force of the chest wall is exactly balanced by the elastic force of the lungs
- FRC = ERV + RV

4. Total lung capacity (TLC)
- volume of air in the lungs after a maximum inspiration
- TLC = IRV + TV + ERV + RV

Observation: Functional residual capacity (FRC), residual volume (RV) and total lung capacity (TLC) cannot be measured directly with spirometer. They are measured by:

- *Gas dilution method*
  - comprises closed-circuit helium dilution are based on the inhalation of a known concentration and volume of an inert tracer gas, such as helium, followed by equilibration of 7 to 10 minutes in the closed-circuit helium dilution technique. The closed circuit contain a mixture helium (in known concentration, 10%), O2 and air. The patient breathes into a closed circuit till the concentrion of helium in the lung and in the bag becomes equal. The helium concentration is measured at the end of the normal exhale (because in the lung remain FRC). The final exhaled helium concentration is diluted in proportion to the unknown volume of air in the patient's chest (residual volume). Usually, the patient is connected at the end-tidal position of the spirometer; therefore, the lung volume measured is FRC.
  - open-circuit nitrogen washout technique is based on the assumption that alveolar air has 80% nitrogen. The patient breathes 100% oxygen for 5 minutes, and all the nitrogen in the lungs is washed out. The exhaled volume of gas is collected into a large bag called Douglas and it is measured together with the nitrogen concentration. The difference in nitrogen volume at the initial concentration and at the final exhaled concentration allows a calculation of intrathoracic volume, usually FRC.

- *Body plethysmography*
  - is based on the principle of Boyle's law (volume of gas at a constant temperature varies inversely with the pressure applied to it);
  - advantages of this method are: (i) it can measure the total volume of air in the chest, including gas trapped in bullae; (ii) it can be performed quickly;
  - disadvantages include the complexity of the equipment as well as the need for a patient to sit in a small enclosed space. A patient is placed in a sitting position in a closed body box with a known volume. From the FRC, the patient pants with an open glottis against a closed shutter to produce changes in the box pressure proportionate to the volume of air in the chest. The volume measured by this technique is referred to as *thoracic gas volume* (TGV) and represents the lung volume at which the shutter was closed, typically FRC.

B. DYNAMIC PULMONARY VOLUMES

They are lung volumes that depend upon the rate at which air flows out of the lungs.

1. Pulmonary or minute ventilation

- volume of air breathed in or out per minute
- tidal volume (TV) x respiratory rate (breathing frequency)
- normal respiratory rate ~ 10 -18 / min
- at rest, pulmonary ventilation ~ 6 -7 L/min (~ 0.5L x 12)

2. Maximal voluntary ventilation (MVV)

- the amount of air that passes in and out of the lungs in one minute during maximal ventilation (hyperventilation).
- usually approximated by FEV1 x 30
- 60-90 l/min (females)
- 110-140 l/min (males)

3. Forced vital capacity (FVC) (figure 2.5)

Technique:

- Have patient seated comfortably; Closed-circuit technique:
  - place nose clip on; have patient breathe on mouthpiece;
  - have patient take a deep breath as fast as possible
  - blow out as hard as they can until you tell them to stop

Results:

- forced vital capacity (FVC) represents total volume of air that can be exhaled forcefully from TLC
- the majority of FVC can be exhaled in < 3 seconds in normal people, but often is much more prolonged in obstructive diseases
- measured in liters (L)

4. Forced expiratory volume in first second: (FEV1) (figure 2.5)

- measured with wet spirometer (Volutest) or dry spirometer (Eutest; this device has an outer cylinder that contains a corrugated tube attached at bottom end by a tube to a mouthpiece; at the upper end is connected to a metal stick and a writing pen; !! do not inhale from the spirometer - only exhale into the spirometer);
- technique: inhale as deeply as possible, and then exhale into the mouthpiece as completely and rapidly as possible;

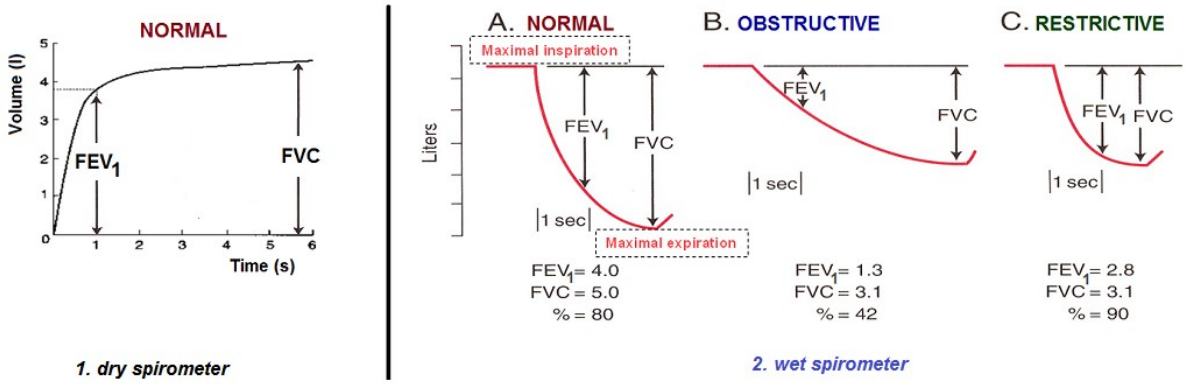


Figure 2.5. Forced expirogram. Measurement of forced expiratory volume in 1 second (FEV1) and forced vital capacity (FVC) with Eutest (dry spirometer) in the left; wet spirometer in the right

- the percentage of FVC that is expelled during the first second measured in liters (L) is named FEV1
- normal people can exhale more than 75-80% of their FVC in the first second
- ***Permeability index (PI) or Tiffeneau index = FEV1/FVC x 100***
  - percentage of FVC that is expelled during the first second of expiration.
  - **normal value: at least 80%**

< 80% - obstructive lung disease

Observation:

- Lung volumes measured by spirometer usually indicate the volume at ambient temperature and pressure, saturated with water vapor (*ATPS*).
- The real volumes are only that measured at 37^0C (body temperature) and that's why we need to correct the volumes measured at room temperature using a correction factor (f_{BTPS}) *taken from special tables*. The result is the corresponding volume according to the body temperature, to athmospheric pressure and saturated with water vapor at 37^0C (*BTPS*).
- The *theoretic (predicted) values* for lung volumes are obtained using the following formula: Predicted volume = H^3 x K, where H = heighr, k = coefficient age dependent *taken from special tables*.
- Finally the real volumes are compared with the predicted volumes. *Normally, the real volumes have to be more than 75% of predicted volume.*
- *Volume variations:* the lung volumes vary with:
  a. body size: all volumes are larger in larger people;
  b. age: the volumes are smaller in children, only partially because of body size, and in old age due to degenerative changes TV tend to be decreased and RV tend to be increased;
  c. sex:: all volumes are slightly smaller in females, not merely due to the difference in body size; the lung volumes in women are 90% of men volmes.
  d. muscular trainng: increase all the lung volumens and allows greater maximal lung ventilation in exercise;
  e. many diseases of the lung and respiratory system
- *Volume variations:* the lung volumes vary with:

FLOW-VOLUME LOOP (figure 2.6)

- displays flow (in L/sec) as it relates to lung volume (in L) during maximal inspiration from complete exhalation (residual volume [RV]) and during maximum expiration from complete inhalation (TLC);

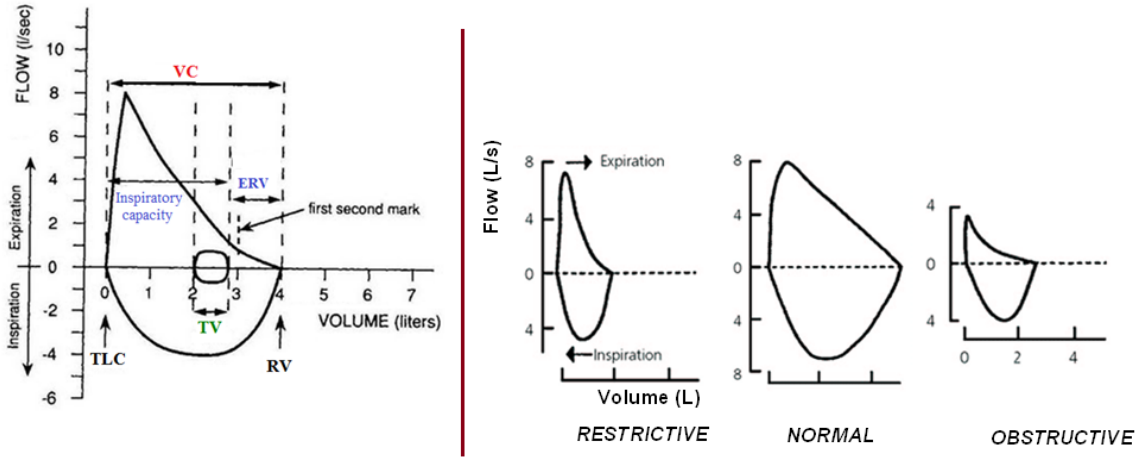


Figure 2.6. Normal flow-volume loop (left) and flow-volume loops of various pulmonary disorders compared with normal (right)

- illustrates maximum expiratory and inspiratory flow-volume curves
- advantage of the flow-volume loop is that it can show whether flows are appropriate for a particular lung volume.
- useful to help characterize disease states (e.g. obstructive vs. restrictive), to characterize disease severity, and to measure responses to therapy.
- *Obstructive disorders* (asthma, COPD)
  - Characterized by a limitation of expiratory airflow
  - Decreased: FEV1,
  - Normal: FVC (forced vital capacity), TLC
- *Restrictive lung disease*
  - Characterized by diminished lung volume due to:
    - change in alteration in lung parenchyma (interstitial lung disease)
    - disease of pleura, chest wall (e.g. scoliosis), or neuromuscular apparatus (e.g. muscular dystrophy)
  - Decreased TLC, FVC
  - Normal or increased: FEV1/FVC ratio

Discussions and clinical significance:

- provides objective evidence in identifying severity of airflow obstruction and/or severity of lung disease;
- factors that affect lung volumes: age, sex, height, weight, race, disease (see the lecture)
- there are 2 types of lung / pulmonary diseases :
  - *restrictive pulmonary/lung diseases*
    - static lung volumes are decreased and compliance of lungs decreased which increases the stiffness of the lung and limits expansion e.g. :
      - a loss in lung volume (eg, lobectomy),
      - abnormalities of structures surrounding the lung (eg, pleural disease, kyphosis, obesity),
      - weakness of the inspiratory muscles of respiration (eg, neuromuscular disease), or abnormalities of the lung
      - parenchyma (eg, pulmonary fibrosis, pulmonary edema, pneumonia)
  - *obstructive pulmonary diseases*
    - characterized by airflow limitation (airway resistance is high);
    - expiratory times are longer than usual, and air may become trapped in the lungs from incomplete emptying and increased lung volumes (eg, TLC, RV);
    - obstructive defects are caused by increased resistance to flow from abnormalities within the airway lumen (eg, tumors, secretions, mucosal thickening); changes in the wall of the airway (eg, contraction of smooth muscle, edema); or elastic recoil (eg, the parenchymal destruction that occurs in emphysema);
    - *the most common obstructive diseases are*
      - *chronic obstructive pulmonary diseases (COPD)*
        - related to cigarette smoking, airflow limitation – not fully reversible, progressive
        - caused by emphysema (destruction of the alveolar walls → permanent enlargement of the airspaces distal to the terminal bronchioles and loss of pulmonary capillaries, collapse of small airways) or chronic bronchitis (excessive mucus production & chronic inflammation of airways) or a combination of the two;

- *asthma* is a chronic inflammatory disorder of the airways, usually associated with airway hyper-responsiveness (airway smooth muscles contract strongly when exposed to a variety of triggers) and variable airflow obstruction, that is often reversible spontaneously or under treatment.

II.3. Functional exploration

II.3.1. Arterial blood gas assesment

The main role of the lung is to uptake oxygen (VO2) and remove carbon dioxid (VCO2). Ensure homeostasis of arterial gases is essential for maintaining life. Measurements of partial pressure of oxygen (PaO2), carbon dioxid (PaCO2) and pH is possible using blood gas analyzers. In addition, the equipment provides other parameters like bicarbonates, base excess (BE).

- **Partial pressure of oxygen (PaO2).** This measures the pressure of oxygen dissolved in the blood and how well oxygen is able to move from the airspace of the lungs into the blood.
  - Normaly PaO2 is 90-95 mmHg.
  - PaO2 < 80 mmHg is defined like hypoxaemia.
  - PaO2 < 60 mmHg means hypoxaemic respiratory failure.
  - PaCO2 = 35-45 mmHg
  - PaCO2 > 50 mmHg diagnosed hypercapnic respiratory failure
- **Partial pressure of carbon dioxide (PaCO2).** This measures how much carbon dioxide is dissolved in the blood and how well carbon dioxide is able to move out of the body.
- **pH.** The pH measures hydrogen ions (H+) in blood. The pH of blood is usually between 7.38 and 7.42.
- **Bicarbonate (HCO3).** Bicarbonate is a chemical (buffer) that keeps the pH of blood from becoming too acidic or too basic.
- **Oxygen content (O2CT)** and oxygen saturation (O2Sat) values. O2 content measures the amount of oxygen in the blood. Oxygen saturation measures how much of the hemoglobin in the red blood cells is carrying oxygen (O2).
- Base on PaCO2, HCO3- and pH acid-base disorders are classified:
  - respiratory acidosis: pH decrease, PaCO2 increase, HCO3- normal
  - respiratory alkalosis: pH increase, PaCO2 decrease, HCO3- normal
  - metabolic acidosis: pH decrease, PaCO2 decrease, HCO3- decrease
  - metabolic alkalosis: pH increase, PaCO2 increase, HCO3- increase

In acute respiratory failure for each 10 mmHg variation in PaCO2, pH is change with 0.07 in acidosis, respectively 0.08 in acidosis. For chronic disorders pH variation is reduced to 0.03. In practice, usually mixed acid-base disorders are mixed.

Indications:

- severe breathing problems and lung diseases, such as asthma, cystic fibrosis, or chronic obstructive pulmonary disease (COPD).
- if you need extra oxygen or help with breathing (mechanical ventilation).

II.3.2. Exhaled Nitric Oxide

The measurement of exhaled nitric oxide is a reflection of airway inflammation. Patients are asked to inspire to total lung capacity and then exhale into an analyzer using a steady, controlled exhaled flow rate. The test is rapid and safe and can be performed by most patients. The normal values are calculated for a measurement flow rate of 50 mL/sec.14:

	Volume (ppb)		
Category	**Adult**	**Child**	**Interpretation**
Normal	5-20	5-15	Normal
High normal or increased	20-35	15-25	Moderately raised exhaled NO can indicate underlying airway inflammation; Colds and influenza can transiently raise exhaled NO and some patients have higher baseline exhaled NO levels than others
Elevated	>35	>25	Indicates ongoing eosinophilic inflammation; Symptomatic patients are likely to respond to steroids; Possible causes (if already on steroids) include poor compliance, recent allergen exposure, inadequate steroid dose, and poor steroid response; Not all patients with ↑ exhaled NO levels experience symptoms.

II.3.3. Thoracic radiography

Radiography is the use of X-rays to view a non-uniformly composed material. By using the physical properties of the ray an image can be developed which displays areas of different density and composition. X-ray visualization of the chest and organs of the thoracic cavity. It is not restricted to visualization of the lungs. The standard chest examination consists of a PA (posterioranterior) and lateral chest x-ray.

Chest radiographs are used to diagnose many conditions involving the chest wall, bones of the thorax, and structures contained within the thoracic cavity including the lungs, heart, and great vessels.

Indications: a persistent cough, chest injury; chest pain, coughing up blood, difficult breathing

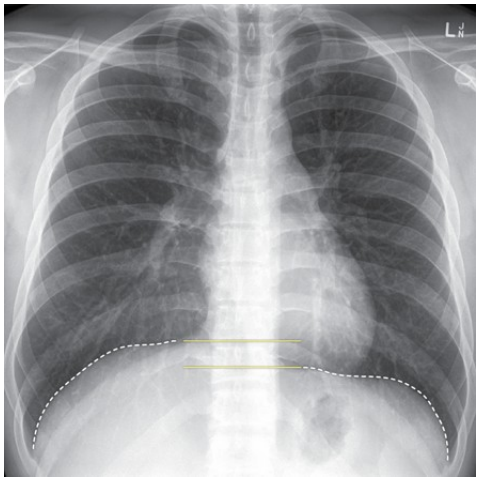


Figure 2.7. Chest radiography

II.3.4. Lung scintigraphy

A diagnostic test in which a two-dimensional picture of a body radiation source is obtained through the use of radioisotopes.

This test is most commonly done in order to check for the presence of a blood clot or abnormal blood flow inside the lungs (such as a pulmonary embolism or PE), although computed tomography with radiocontrast is now more commonly used for this purpose. The V/Q scan may be used in some circumstances where radiocontrast would be inappropriate.

Indications:

- pulmonary embolism, chronic obstructive pulmonary disease
- performance quantification tool pre- and post-lung lobectomy surgery.

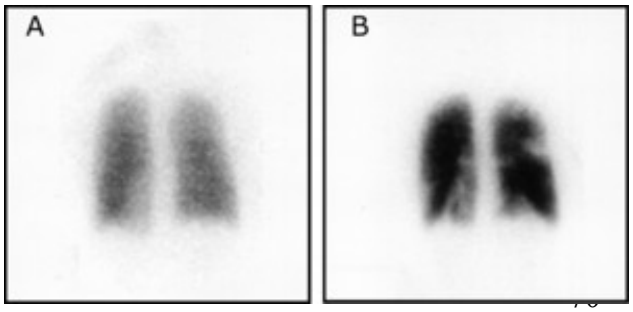


Figure 2.8. Lung scintigraphy
A. After inhalation of Xenon-133 gas;
B. After intravenous injection of Tc-99m

II.3.5. Bronchoscopy

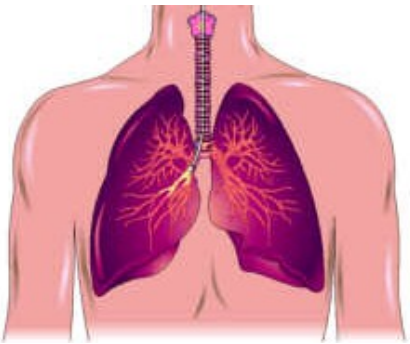
Bronchial fibroscopy (bronchoscopy) is a recent investigation method. Bronchoscopy is a technique of visualizing the inside of the airways for diagnostic and therapeutic purposes. The bronchoscope is inserted into the airways, usually through the nose or occasionally through a tracheostomy. This technique allows to examine the patient's airways for abnormalities such as foreign bodies, bleeding, tumors, or inflammation.

Bronchoscopy can be used for diagnosis or treatment.

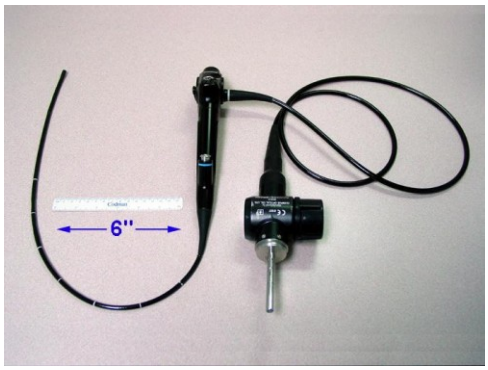
Although a bronchoscope does not allow for direct viewing and inspection of the lung tissue itself, samples of the lung tissue can be biopsied through the bronchoscope for examination in the laboratory.

Indications:

Bronchoscopy is used to make a diagnosis	*Bronchoscopy is used for treatment:*
- persistent or unexplained cough; - blood in the sputum (coughed up mucus material from the lungs); - abnormal chest x-ray such as a mass, nodule, or inflammation in the lung; or - evaluation of a possible lung infection.	- to remove foreign bodies in the airway; - to place a stent (a tiny tube) to open a collapsed airway due to pressure by a mass or tumor; or - to remove a mass or growth that is blocking the airway.



A. Bronchoscopy



B. Bronchoscope

Figure 2.9. Bronchoscopy

II.3.6. Pulmonary gas exchange

In adition of spirometry another method for evaluated pulmonary function is to measure integrity of the alveolar membrane. This method is focused on transfer factor of the lung for carbon monoxide (TL,CO). Due to this method is possible to differentiate between causes of decrease PaO2 due to anatomy of alveolar region to airways and larger blood vessels. TL,CO is the quantity of inhaled CO absorbed, per unit time and per unit CO partial pressure. CO is chosen because it binds to haemoglobin (Hb) as carboxy-Hb, but at an extremely low partial pressure (PCO).

Test procedure

Patient breaths a mixed gas (He and CO) in follow maneuver: exhale slowly to residual volume; inspire rapidly to full inflation; stop breathing.

$\mathbf{T_{L,CO} = K_{CO} \times V_A}$, where, K_{CO} – is the rate of alveolar uptake of CO, V_A – alveolar volume.

Causes of a low $T_{L,CO}$: diffuse alveolar damage (fibrosis, emphysema); pulmonary vascular diseases; chronic heart failure; anaemia.

*Causes of a high TL,CO:*extrapulmonary restriction; loss of aerated units; increased pulmonary blood flow; acute alveolar haemorrhage; polycythaemia.

III. PHYSIOLOGY OF DIGESTION

III.1. Oral digestion

III.1.1. Sampling of saliva

- Sample collection should be made at standardized time, according to the diurnal cycle (and/or the response and recovery time) of the analyte.
- Subjects should not eat within 60 minutes prior to sample collection.
- For recovery of salivary glands, alcohol, caffeine, and dairy products should also be avoided.
- *Resting saliva* can be collected avoiding any stimulation.
- *Stimulated saliva* is collected most widely with chewing stimulation (i.e., parafin wax), and/or with taste stimulation (i.e., candy, lemon).
- *Whole saliva* can be collected by allowing the saliva to accumulate in the mouth and then expectorate it into a vial. Parotid saliva and mixed saliva of submandibular & sublingual glands may be collected with *direct cannulation* of the parotid duct or submandibular duct, respectively or by suction using a Lashley / Carlson–Crittenden cup

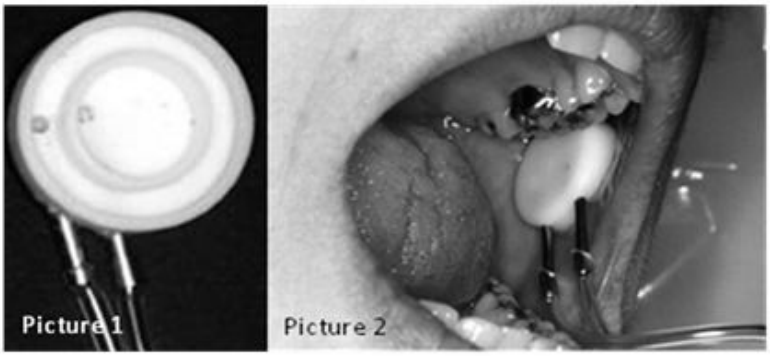


Figure 3.1. The collection of saliva of parotid gland with Lashley cup

Lashley cup (figure 3.1) has an inner circular chamber connected to a plastic tube for the collection of parotid saliva. The outer chamber is connected to a rubber bulb which is squeezed to create a negative pressure that maintains the cup in place without movement. The inner chamber is placed over the exit of Stenon's duct (on the buccal mucosa in the area of the first maxillary molar).

- The collected saliva sample is suitable for areas of applications such as:
  - *Drug detection* like narcotics (cocaine, amphetamines, cannabis etc.) and psychotropic substances is widely used because the concentration of these substances in saliva is is comparable with blood and conclusions can be drawn on current drug consumption;
  - *Tumour diagnostics, human genetics* - DNA and RNA from saliva enable early detection of oral cancer, for example.
  - *Infectious disease diagnostics* refers to screening opportunities for detection of viral or bacterial pathogens - e.g. Herpes simplex or the Varicella zoster viruses;
  - *Hormone analytics* - the determination of steroid hormones, e.g. cortisol, progesterone, or testosterone and biogenic amines such as melatonin from saliva for hormonal profile of a person.
  - *Clinical chemistry parameters* - The determination of saliva electrolytes, saliva enzymes (α-Amylase), saliva proteins (total protein and creatinin) can be used as markers for an indication of a person's state of health.

III.1.2. Chemical examination of saliva

Reaction for recognition of salivary mucine

Requirements:

- test tubes
- glacial acetic acid 20%
- saliva sample

Procedure:

- put in a test tube 2-3 ml of saliva
- add 3-4 drops of glacial acetic acid 20%

Result: filamentous precipitate which attest the presence of mucine

The presence of a sulphocyanates in the saliva

Requirements:

- test tubes, saliva sample
- HCl 5%, FeCl3 3%

Procedure:

- put in a test tube 2-3 ml of saliva
- add 3-4 drops of HCl 5% + 1-2 drops FeCl3 3%

Result: red filamentous precipitate of ferric rhodonite.

The reaction is more intense on smokers.

III.1.3. Digestive role and thermolability of the salivary amylase

- Salivary amylase (or ptialine, from the Greek word *ptyalon*, meaning *saliva*) is a starch digesting enzyme produced by salivary glands;
- Salivary amylase functions best at pH 6.8 to 7.0, typical of the oral cavity. It is denatured upon contact with stomach acid, but it can digest starch for as long as 1 to 2 hours in the stomach as long as it is in the middle of a food mass and escapes contact with the HCl. Being a protein, amylase is then digested by pepsin along with the dietary proteins.
- Starch constitutes major storage of carbohydrate in plant seeds and tubers. Important sources of starch include corn, potato and rice. It supplies 80% of dietary calories in humans worldwide.
- Starch consists of granules (figure 3.2); each of them is formed by several million *amylopectin molecules* (branched every 24-30 glucose residues by alpha 1-6 bonds) together with an even larger number of *amylose molecules* (long straight chain of glucose molecules linked by alpha 1-4 bonds). Usually, starch granules are covered with a thin protective cellulose layer destroyed by cooking.

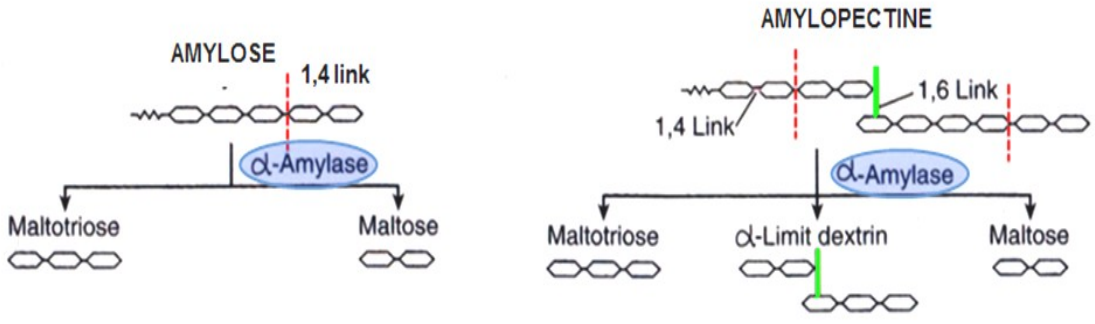


Figure 3.2. Structure of starch gramule.

- Starch is digested to glucose in two phases:

- *first*, salivary amylase in the mouth hydrolyzes only alpha (1-4) glycosidic bonds of cooked starch into three oligosaccharides: maltose (disaccharide), maltotriose (trisaccharide), and a group of alpha-limit dextrins which contain branch points from amylopectin;
- *Second phase* consists of hydrolisation of these oligosaccharides into glucose monomers by the action of maltase, digestive enzyme located on the brush border of the luminal surface of the small intestine. Finally, glucose monomers are absorbed into the small intestinal enterocyte by co-transport with sodium ions.

– Digestive role of salivary amylase is illustrated in figure 3.3.

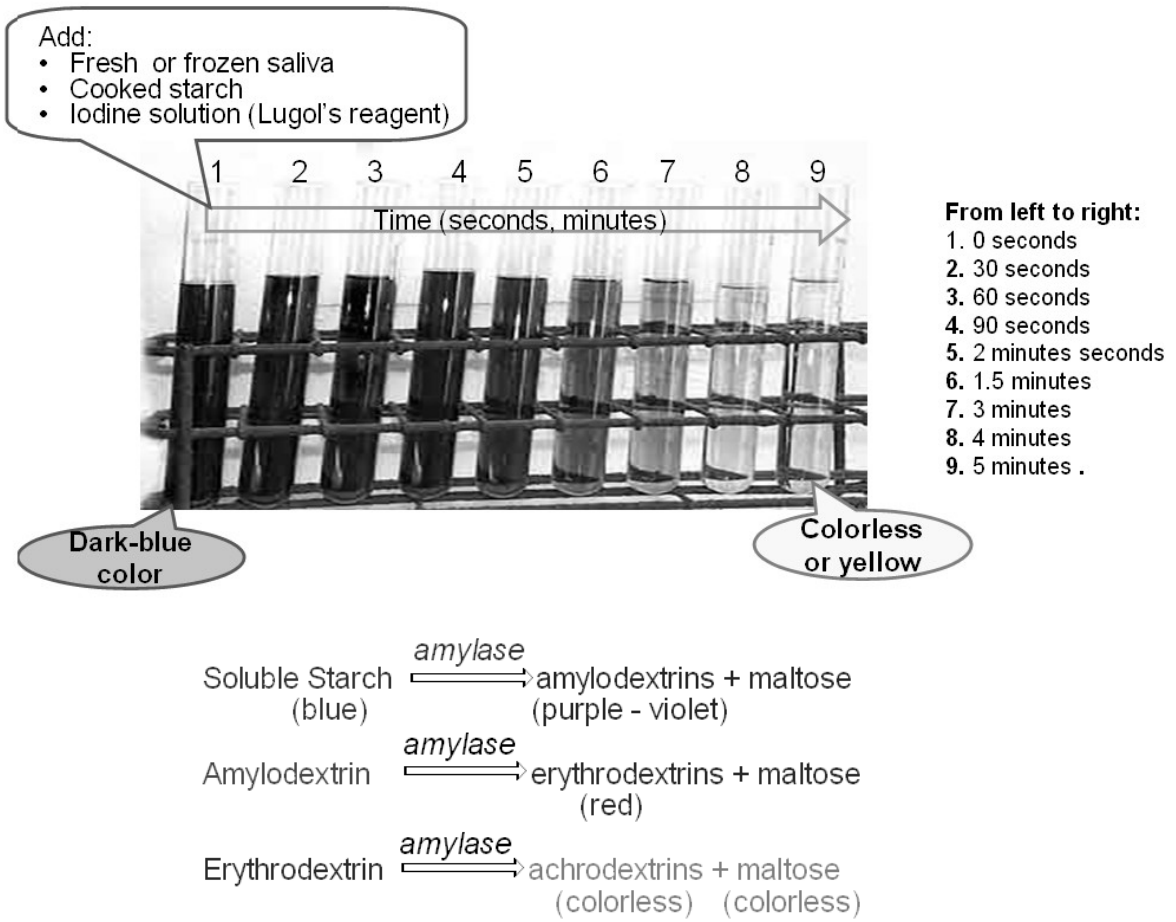


Figure 3.3. Steps of hydrolysis of starch by salivary amylase. The term, amylo-, erythro- and achro indicate the color **with iodine**, i.e**.** violet, red and colorless (or yellow) respectively

Digestive role and thermolability of the salivary amylase:
Principle: Demonstration of the chemical breakdown, disappearance of a substrate (starch) and the appearance of a product (maltose) by action of the enzyme salivary amylase and the effects of temperature on this enzyme activity.
Materials required: test tubes, saliva samples (contain salivary amylase or ptialine), water bath, starch solution, iodine solution
Technique:

– prepare 3 test tubes: in each place 1ml of starch solution + 1ml of saliva sample and mix well;
– put the tubes immediately in the following temperatures:
  - tube no.1: at 37^{o}C in water bath

- tube no.2: at room temperature (leave in the test-tube rack).
- tube no.3: at boiling water bath (boiled salivary amylase)

– leave the tubes for 5 minutes;
– cool the tubes under running tap water;
– add 2 drops of iodine to each of the 3 tubes.
  - if the saliva is boiled the salivary amylase is inactivated
  - if the saliva is frozen the salivary amylase keeps unaltered the enzymatic activity

Results and discussions:

– tube no.1: **yellow or colorless (**because of **achrodextrin** product of starch digestion, salivary amylase is active, and 37^{o}C is optimum for its maximal activity).
– tube no.2: **reddish or violet (**because of **erythrodextrin or amylodextrin -** product of the starch digestion which is incomplete by the end of 5 minutes time. Thus, room temperature is not optimum for the enzyme activity).
– tube no.3: **blue (**that means **starch** is not digested, so salivary amylase is inactive because boiling causes denaturation of the enzyme).

III.2. Gastric digestion

III.2.1. Gastric juice sampling

A piece of special rubber tubing is introduced into digestive tract:

- Faucher: thick, 1cm diameter / 75 cm length
- Einhorn: thin, 0.5 cm diameter / 150 cm length

via the oral (Faucher / Einhorn) or nasal pathway (Einhorn). The presence in the stomach of the distal end of the tubing allows sampling of the gastric content with a syringe attached to the proximal one.

III.2.2. HCl and lactic acid detection in gastric juice

A. Free HCl detection in gastric juice

Principle: HCl reacts with of phloroglucinol, resulting a rose-red color.

Materials required: test tubes, glass slide, Bunsen burner, Günzburg reagent (prepare as needed a solution containing 4 g of phloroglucinol and 2 g of vanillin in 100 ml of absolute ethyl alcohol), gastric juice sample

Technique:

– on a glass slide put 1 drop of gastric juice + 1 drop of Günzburg reagent
– evaporate on a Bunsen burner

Results: In the presence of free HCl a rose-red color is developed.
This method can detect 0.005% HCl.

Discussions and clinical significance:

Functional roles of HCl:

1. It activates the enzymes pepsin (by cleavage of inactive pepsinogen) and lingual lipase
2. HCl provides a low pH environment, which is required for the action of pepsins in digesting proteins and peptides.
3. Stops carbohydrate digestion by inactivating salivary amylase
4. It breaks up connective tissues and plant cell walls, helping to liquefy food and form chyme.

5. It converts ingested ferric ions (Fe3) to ferrous ions (Fe2), a form of iron that can be absorbed and used for hemoglobin synthesis.
6. It contributes to nonspecific disease resistance by destroying ingested bacteria and other pathogens.In pathological conditions, when there is no HCl in the gastric juice, bacterial flora causes fermentation processes in the stomach, which results in small quantities of organic acids (lactic acid, butiric acid).

In pathological conditions, when there is no HCl in the gastric juice, bacterial flora causes fermentation processes in the stomach, which results in small quantities of organic acids (lactic acid, butiric acid).

B. Lactic acid detection in gastric juice

Principle: lactic acid reacts with iron salts, resulting yellow green iron lactate

B1.

Materials required: test tubes, Fe Cl3 10%, distilled water, gastric juice sample

Technique:

- in a test tube add 20 ml distilled water + 2-5 drops of Fe Cl3 10%
- split the content in 2 test tubes:
  - 1st test tube will contain 10 ml distilled water + Fe Cl3 10%
  - 2nd test tube will contain 10 ml distilled water + Fe Cl3 10% + gastric juice (10 ml)

Results: is formed in presence of. In the presence of lactic acid a yellow green color is developed due to iron lactate.

B2.

Materials required: test tubes, gastric juice sample, Uffelman's reagent (prepared by adding 2 drops of 1N ferric chloride to 10 ml of 1% phenol solution)

Technique:

- in 1st test tube add 2 ml of gastric juice – control test tube
- in 2nd test tube add 2 ml gastric juice + Uffelman's reagent

Results: the color of the solution in the 2nd test tube turns from bluish violet to yellow green, indicating the presence of lactic acid

Discussions and clinical significance:

- in conditions of hypoacidity and stasis, when gastric emptying is delayed, the fermentation of glucidic compounds produces lactic acid.
- in humans, the presence of lactic acid in gastric juice is associated with gastric cancer or pyloric stenosis.

III.2.3. Measurement of the gastric juice acidity

Principle: Free and total acidity of gastric juice is measured by titrating against sodium hydroxide 0.1N using two successive indicators.

Materials required: test tubes, 0.1N NaOH solution, gastric juice sample, Töpfer-Linossier reagent, biurette for titration.

- Töpfer-Linossier reagent's double indicator has this formula:

  Dimethylamidoazobenzol . . 0.25 g (color change at pH = 3.1 – 4.4)
  Phenolphthalein . . . . . 120 g (color change at pH = 8.2 -10)
  Alcohol (90%) 100 g.

Technique:

- Take 10 ml of gastric juice and add 1 drop of Töpfer-Linossier indicator.
- Titrate this solution with 0.1N NaOH until yellow - orange colour (pH ≈ 3.5) appears.

- Read in and write down volume of used NaOH solution (n1 ml used to neutralise free HCl).
- Then titrate further until pink colour (pH ≈ 8.2) appears.
- Read in and write down volume of used NaOH solution (n2 ml used to neutralise combined HCl).
- Calculate free, related and total acidity by reading the used volume of 0.1M NaOH from biurette and multiplying it by 10

Calculation and results:

- For titration process we use 10 ml of gastric juice, but we calculate acidities for 100 ml of gastric juice.
- The values of gastric juice acidity can be expressed in 3 ways:

1. in clinical units:

- One clinical unit represents the volume of 0.1 N NaOH solution used for neutralise free or combined HCl from 100 ml gastric juice
- free, related and total acidity it can be calculated by reading the used volume of 0.1M NaOH from biurette and multiplying it by 10:

	Normal values (clinical units HCl)
Free HCl = n1 X 10	15
Combined HCl = n2 X 10	25
Total HCl = (n1 +n2)	40

2. g/l:

- 1ml NaOH 0.1 N…….neutralise..... 0.00365 g HCl
  n1X100ml NaOH 0.1 N …….... y g HCl

y = (n1 X 100 X 0.00365) / 1
So Free HCl = n1 X 0.365 g ‰ (g/l) HCl

	Normal values (‰ or g/l HCl)
Free HCl = n1 X 0.365	0.9 – 1
Combined HCl = n2 X 0.365	1 - 2.5
Total HCl = (n1 +n2) X 0.365	2.5 – 3.5

3. mEq/l:

- 1Eq/l HCl……36.5 g HCl
- 1mEq/l HCl …….... 0.0365 g HCl
- So Free HCl = n1 X 10 mEq/l HCl

Discussions and clinical significance: sees the lecture

III.2.4. Action of labferment

Definition: Gastric rennin (labferment) from the gastric secretion of newborn babies is involved in casein coagulation. Optimal pH of action = 4.5 – 5.5
Principle: calcium dependent milk coagulation
Materials required: CaCl2 sol. 10%; gastric rennin (Labferment) sol. 1%; milk; water bath; test tubes.

Technique and results:

Content	**Test tube 1**	**Test tube 2**	**Test tube 3**	**Test tube 4**
Milk	5 mL	5 mL	5 mL	5 mL
Labferment solution 1%	2-3 drops	2-3 drops (the enzyme was previously inactivated, by boiling process)	2-3 drops	2-3 drops
Potassium oxalate solution 1%0			+ 1 ml sol *- to block Ca – ions*	+ 1 ml sol *- to block Ca – ions*
	Water bath 40^0 for a few minutes			
Results	Casein coagulation (a coagulum is formed in the glass tube)	Coagulation doesn't occur	Coagulation doesn't occur	Coagulation doesn't occur
				+ 1 ml CaCl2 Instantly, coagulation process occurs

Discussions and clinical significance:

The experiments explain the two phases that took place in the process of casein coagulation:

- Phase I: Casein from milk + Labferment → Paracasein (soluble)
  The reaction doesn't require Ca-ions.
- Phase II: Paracasein + (Ca^{2+}) → Ca^{2+}-paracaseinate (insoluble).

III.3. Intestinal digestion

III.3.1. Bile, pancreatic and intestinal juice sampling

Duodenal sampling technique

A piece of special rubber tubing is introduced into the digestive tract; Einhorn type, thin, 0.5 cm diameter/150 cm length. The presence in the duodenum of the distal end of the tubing allows sampling of the duodenal content, with a syringe attached to the proximal one.

Bile sampling by duodenal probing

A piece of special rubber tubing is introduced into the digestive tract; Einhorn type, thin, 0.5 cm diameter/150 cm lengths. The presence in the duodenum of the distal end of the tubing allows sampling of the duodenal content, with a syringe attached to the proximal one.

- *bile A*: coledocian, dark yellow, viscous
- *bile B*: vesicular, green yellow
  - *via the tubing a colagogue substance is administered (for example 20mlMgSO4 33%) to stimulates the gallbladder contraction, with bile elimination to the duodenum*
- *bile C*: hepatic: golden yellow

Pancreatic juice collection

A piece of special rubber tubing is introduced into the digestive tract; Einhorn type, thin, 0.5 cm diameter/150 cm lengths. The presence in the duodenum of the distal end of the tubing allows sampling of the duodenal content, with a syringe attached to the proximal one. Biliary secretion should be evacuated with MgSO4 33% before pancreatic juice is collected. Then, a stimulatory substance for pancreatic juice is administered:

- secretin (1-2 u/kgc i.v); the pancreatic juice is collected for 30-40 minutes;
- pancreosimine - 100 u in a single dose;); the pancreatic juice is collected for 20 minutes;

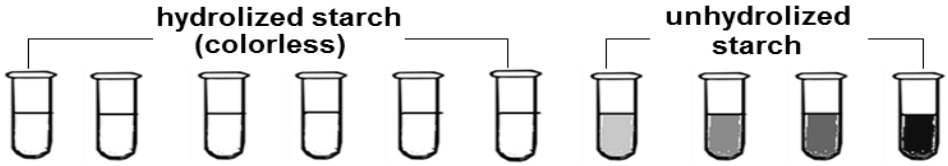
III.3.2. Pancreatic amylase assessment by Wolgemuyh method

Principle: the method is base upon the ability of pancreatic amylase (diastase) to hydrolise starch up to dextrins and maltose.

Materials required: rack with test tubes; thermostat; NaCl solution 0,9%; urine sample; starch solution 1‰ ; iodine solution

Technique:

- a rack with 10 test tubes is taken in order to realize increasing urine dilutions (more specifically, urinary amylase dilutions);
- in the first test tube is added 2 ml of urine;
- in the rest of test tubes (9 in total) is added 1 ml NaCl solution 0.9%;
- is taken 1 ml of urine from the first test tube and is added it in the second test tube and it is mixet well. From this mixture is taken 1 ml and is added it in the third glass tube and so on. Using this technique it is obtained the following dilutions: 1/1, 1/2, 1/4, 1/8, 1/16, 1/32, 1/64, 1/128, 1/256, 1/512;
- in every glass tube is added 2 ml starch solution 1‰ (which means 2 mg starch) and shaked well;
- the rack with tubes is put in a thermostat at 37°C for 30 minutes (a time designated for optimal activity of enzyme);
- than the tubes are cooled and in each of them is added 3 - 4 drops of iodine solution.



Test tube no.	1	2	3	4	5	6	7	8	9	10
Dilution	-	1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/512
Urine(ml)	1	1	-	-	-	-	-	-	-	-
NaCl sol. 0.9% (ml)	-	1	1	1	1	1	1	1	1	1
Starch sol. (ml)	2	2	2	2	2	2	2	2	2	2

Results and calculation:

- the glass tubes that contain unhydrolezed starch have a blue color, while those with hydrolyzed starch have no color.
- notice the last glass tube with uncolored content (*test tube 6*), where it exists enough amylase to hydrolyze the entire amount of starch (2 ml).
- the results are expressed in *Wolgemuth units(WU)*: 1 Wolgemuth unit represents the amount of amylase necessary to hydrolyze 1 ml of starch in 30 minutes at 37°C;
- calculation:
  1 ml of urine hydrolize 32 X 2 = 64 ml of starch
  So, number of Wolgemuth units (WU) = 64
- number of WUs correspond to denominator of the first test tube dilution with colored content (denominator is the bottom part of a fraction)

Normal Values

- Concentration of urinary amylase is 32 - 64 Wolgemuth units.
- Concentration of serum amylase is 16 – 32 Wolgemuth units

Pathological results are present in:

- hemorrhagic acute pancreatitis, disease marked by rapid and very high increases of serum and urinary amylase up to 1000 - 3000 WU
- chronic pancreatitis with a very low value for amylase due to glandular hypofunction.

Discussions and clinical significance: Among the pancreatic enzymes amylase is the most widely used a a screening test for acute pancreatitis in the patient with acute abdominal back pain.The necrosis of pancreatic cells is responsible for the release of the pancreatic enzymes in the blood. Urine amylase is increased in acute pancreatitis and may be elevated after serum values have retured tor normal.

III.3.3. Differentiation of gastric and intestinal proteolysis products

A. Differentiation of gastric proteolysis products

Principle: in the digestion of proteins by pepsin-hydrochloric acid results three well-defined classes of products: acid-albumin, albumoses and peptones identified by precipitation and neutralisation.

Materials required: test tubes, filter paper, filter funnel, pipettes, gastric juice sample, reagents: NaOH 4%, 10% solutions, ammonium sulfate (NH4)2SO4: solution and crystals, CuSO4 1% solution

Technique: see figure 3.4

- acid-albumins, as the initial product of gastric digestion are identified by its wellknown precipitation by neutralization;
- in the filtrate, the albumoses can be detected by saturation of the fluid with sodium chloride and addition of acetic acid, or best by saturation with ammonium sulphate, while the true peptones are found in the filtrate from the latter precipitate, after dilution with water, by testing with cupric sulphate and potassium hydroxide;
- true peptones are bodies not precipitated by saturation with ammonium sulphate, and are the final products of pepsin digestion.

The biuret test is a chemical test used for detecting the presence of peptide bonds. In a positive test, a copper (II) ion is reduced to copper (I), which forms a complex with the nitrogens and carbons of the peptide bonds in an alkaline solution. A violet color indicates the presence of proteins. The test can be done by adding an equal volume of 1 % of NaOH

(sodium hydroxide) and then a few drops of 1 % of CuSO4 (copper(II) sulfate) to the sample solution. If the solution turns purple, protein is present; 5-160 mg/ml can be determined.

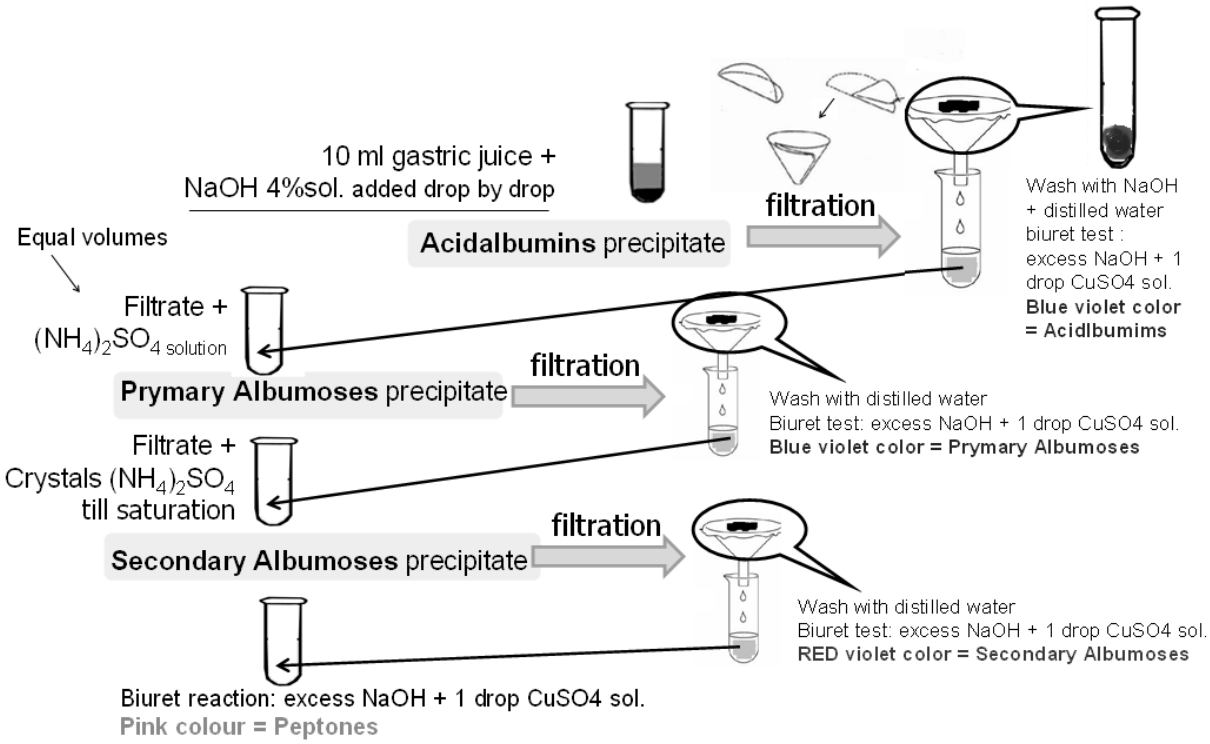


Figure 3.4 Differentiation of gastric proteolysis products

Results: Gastric proteolysis products detected are albumins, albumoses and peptones.
The albumoses are to be considered as the primary products of gastric digestion, while peptones are the end products of proteolytic action, the latter being formed by the gradual hydration of the primary products.

B. Differentiation of intestinal proteolysis products
Principle: in the digestion of proteins by pancreatic juice'enzymes results three well-defined classes of products: alkali-albumin, primary and secondary albumoses.

Materials required: test tubes, filter paper, filter funnel, pipettes, pancreatic juice sample, reagents: HCl 1‰ solution, NaOH 10% solutions, ammonium sulfate (NH4)2SO4: solution and crystals, CuSO4 1% solution

Technique: see figure 3.5.

Results: Intestinal proteolysis products detected are alkali-albumins, primary and secondary albumoses.

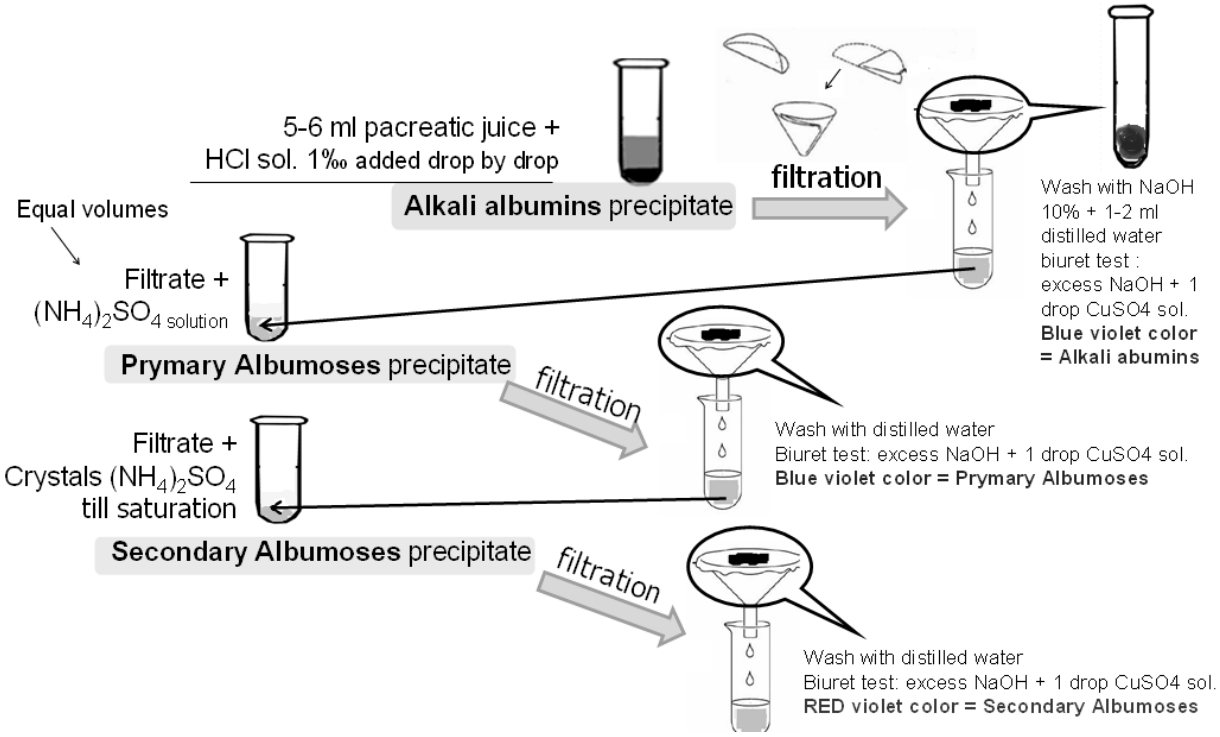


Figure 3.5. Differentiation of pancreatic proteolysis products

III.3.4. *In vitro* intestinal motility

Experimental, i*n vitro* intestinal motility can be demonstrated *in vitro* using organ bath.
Principle: experimental analysis of intestinal smooth muscle contraction in organ bath.
Materials required: small intestine of rat (smooth muscle), organ bath assembly, lever, and kymograph
Technique (figure 3.6): anesthetize the rat, open abdominal cavity, take a ring of small intestine (2 mm), rinse with Krebs-Henseleit solution and tie both the ends of the intestine with threads and leave it for 10 minute in buffer solution. Than tie the free end of one thread in the "L" shaped bent end a glass tube (to be place in the organ bath chamber) and the free end of the other thread to long arm of the leaver (or an isometric force transducer connected to a computer). Fill the chamber with Krebs-Henseleit solution (isotonic with internal environment of the rat) at 37^0C and bubbles air (O2) through glass tube L shaped. Attach the writing pen to a smoked drum or other recording device and observe after 5-10 minutes spontaneous contractions of small intestine. Note the rate and the amplitude of the contractions.

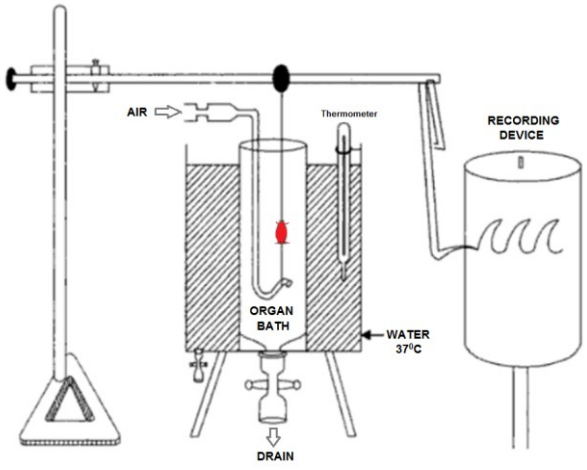


Figure 3.6. Experimental set up for recording of smooth muscle contraction *(after Rastogi Sc, 2005)*

In order to study the drug action, add them to the organ bath and after recording the effect of each drug, the Krebs Henseleit solution should be drained and replaced. The effect of the next drug should be recorded after the intestine is allowed too equilibrate, means it shows normal spontaneous movements.

Interpretation:

Two drugs are choosing for study: acetylcholine and epinephrine, because smooth muscle has dual innervations: postganglionic sympathetic fibers discharge epinephrine and parasympathetic fibers release acetylcholine. Acetylcholine accelerates contraction and adrenaline inhibits smooth muscle and the resting potential is made more negative by making the muscle less active (figure 3.7).

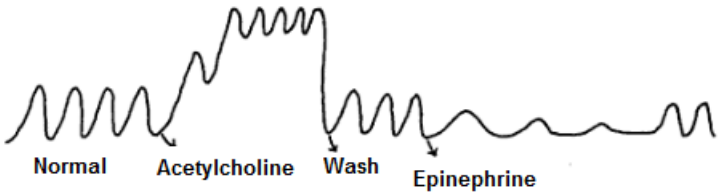


Figure 3.7. Effects of drugs on intestine smooth muscle.

III.4. Physiology of the liver

III.4.1. Chemical and emulsifying properties of bile

III.4.1.1. Tensio-active role of biliary salts

A. Lipid emulsification

Definition: process based on the biliary salts propriety to decrease the superficial tension at water-lipid interface (emulsion).

Materials required: test tubes; oil; biliary salts solution

Technique:

- put in a test tube a 2 ml of biliary salts solution
- add 1 ml of oil;
- stir the mixture

Result: it can be observed the dissolution of the 2 different layers (oil and biliary salts) and the presence of an emulsion.

B. Hay Reaction

Principle: Sulfur powder, spread over the water or normal urine surface, is kept on the surface. If the liquid contains biliary salts, sulfur powder falls at the bottom of the test tube in 2-3 minutes.

Materials required: test tubes; biliary salts solution; urine with biliary salts; sulfur powder.

Technique: in test tube put 3-4 ml sample containing biliary salts, and then spread sulphur powder

Result:

- positive reaction: sulphur powder falls at the bottom of the test tube (the sample contains biliary salts)
- negative reaction: sulphur powder remains on the surface of the liquid (the sample does not contain biliary salts).

III.4.1.2. Recognizing reaction for cholesterol - *Salcowschi reaction*

Materials required: test tube; thin pipette; bile solution, chloroform solution

Technique:

- in a test tube add 4 ml chloroform solution + 3 ml bile solution in a test tube

- stir and leave it for 5 min
- observe the dissolution of the 2 different layers
    - inferior layer which contain soluble cholesterol
    - superior layer
- drop 1 ml H2SO4 solution into the test tube wall, and let it fall until the tube bottom.

Results: a red ring is formed at the interface between bile and H2SO4.

III.4.1.3. Reaction for recognizing biliary salts - Pettenkofer reaction

Principle: Biliary salts react with oximethylfurufural and a violet product is obtained.

Materials required: test tubes; saccharose sol. 10 %; bile or biliary salts solution; H2SO4 concentrated solution.

Technique:

- add 1 ml saccharine in a test tube, then add 3-4 ml biliary salts solution
- stir and let the test tube inclined;
- drop 1 ml H2SO4 into the test tube wall, and let it fall until the tube bottom.

Results: a red-violet ring is formed at the interface between bile and H2SO4.

III.4.1.4. Recognizing reaction for biliary pigments in biological fluids

1. Gmelin reaction

Materials required: test tube; thin pipette; biological sample fluid with biliary pigments, HNO3 concentrated solution with NaNO3 crystals.

Technique:

- add 5- 10 ml from biological sample fluid in a test tube;
- add HNO3 concentrated solution on the bottom of the test tube using a Pasteur pipette so that the liquids will not fuse ;

Results: at the liquids interface we will notice the *appearance of rings of different colors:* yellow-red, blue-violet, green (indicates the presence of biliverdin), due to different oxidative stages of bilirubin.

2. Trousseau reaction

Materials required: test tube; thin pipette; biological sample fluid with biliary pigments, concentrated iodine solution

Technique:

- add 5- 10 ml from from biological sample fluid in a test tube
- add concentrated iodine solution and let it fall until the tube bottom

Results: a concentric green ring at the liquids interface indicates the presence of biliverdin

3. Methylene Blue reaction

Materials required: test tube; methylene blue solution, biological sample fluid with biliary pigments,

Technique:

- in a test tube add 3-4 ml of biological sample fluid with biliary pigments
- add 1-3 drops methylene blue solution

Results: a green color (due to biliverdin) is obtained.

III.5. Functional exploration of digestive system

III.5.1. Superior digestive endoscopy

Superior digestive endoscopy is a procedure that allows the doctor to examine directly, visually, the superior part of the digestive tract: esophagus, stomach and the first part of the thin intestine.

The visualization is done through a video camera that shows, in real time, the mucous membrane of the digestive tract. The video camera is situated at the end of a very flexible tube, 8-9 millimeters thick, called endoscope (or gastroscope). The endoscope enters the body via the mouth, with no special swallowing efforts needed from the patient.

Indications

Diagnostic	**Therapeutic**
• Hiatus hernia. • Reflux esophagitis. • Barrett's esophagus. • Webs, strictures and carcinoma esophagus. • Infective esophagitis. • Varices or micro bleeding (esophageal). • Mallory-Weiss syndrome. • Gastritis. • Tumors and polyps in stomach and duodenum. • Ulcer (including biopsy for H. pylori infection).	**Esophagus** • Dilatation of strictures. • Palliation of carcinoma. • Variceal therapy to stop bleeding (scleroplasty and banding). **Stomach** • Controls of ulcer bleed (injection and coagulation). • Foreign body extraction. • Polypectomy. • Dilatation of pyloric stenosis. • Percutaneous endoscopic gastrotomy. **Duodenum** • Control of ulcer bleed

Figure 3.21. Endoscop

III.5.2. Radiology of the esophago-gastrointestinal tract

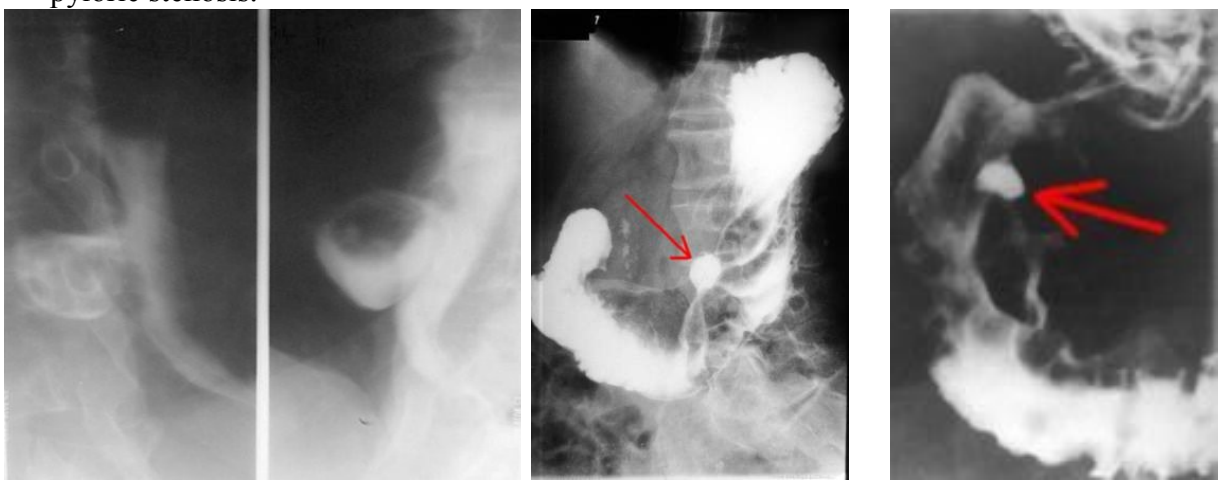
Radiological procedures played a leading role in the visualization of anatomy and diseases of the esophago-gastrointestinal tract for a long time.

Upper gastrointestinal tract radiography is an x-ray examination of the pharynx, esophagus, stomach and first part of the small intestine (duodenum). Images are produced using a special form of x-ray called fluoroscopy and an orally ingested contrast material (barium).

Indications

- *Esophagus:* reflux, peptic ulcer, diverticulum, achalasia, hiatus hernia, varicosity, tumor;

- *Stomach-duodenum:* gastritis, peptic ulcer, strictures, tumor, external compression and pyloric stenosis.



Esophagus diverticul Gastric ulcer Duodenal ulcer

Figure 2.22. Radiology of the esophago-gastrointestinal tract

III.5.3. Endoscopic Retrograde Cholangiopancreatography (ERCP)

Endoscopic retrograde cholangiopancreatography is a procedure that combines upper gastrointestinal (GI) endoscopy and x rays to treat problems of the bile and pancreatic ducts. ERCP is used when it is suspected a person's bile or pancreatic ducts may be narrowed or blocked due to

- Tumors; gallstones that form in the gallbladder and become stuck in the ducts
- inflammation due to trauma or illness, such as pancreatitis—inflammation of the pancreas;
- infection
- valves in the ducts, called sphincters, that won't open properly
- scarring of the ducts, called sclerosis
- pseudocysts - accumulations of fluid and tissue debris

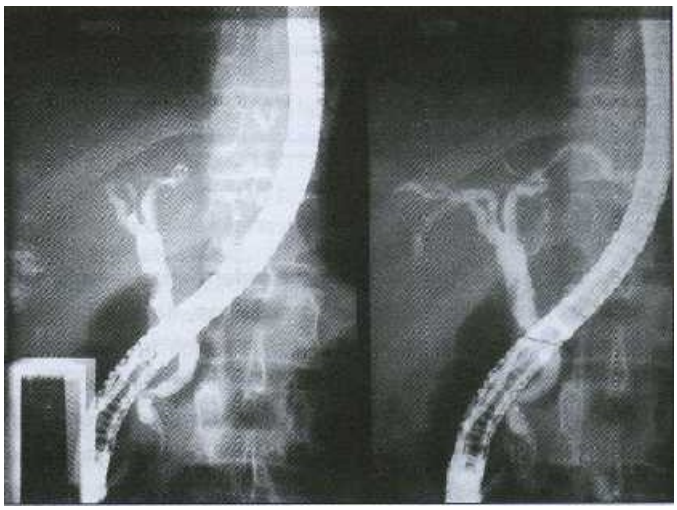


Figure 2.23. Endoscopic Retrograde Cholangiopancreatography (ERCP)

III.5.4. Colonoscopy

Colonoscopy is an investigation used to look at the lining of your large intestine. It is performed using a narrow, flexible, telescopic camera called a colonoscope. Colonoscopy can

detect inflamed tissue, ulcers, and abnormal growths. The procedure is used to look for early signs of colorectal cancer and can help doctors diagnose unexplained changes in bowel habits, abdominal pain, bleeding from the anus, and weight loss.
Indications: persistent diarrhoea or a change in your bowel habit; anaemia

Figure 2.24. Colonoscop used for colonoscopy

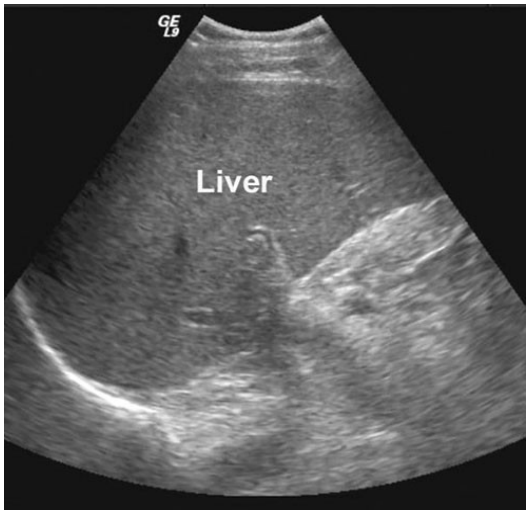
III.5.4. Abdominal ultrasonography

Medical sonography (ultrasonography) is an ultrasound-based diagnostic medical imaging technique used to visualize muscles, tendons, and many internal organs, to capture their size, structure and any pathological lesions with real time tomographic images. The technology is relatively inexpensive and portable, especially when compared with other techniques, such as magnetic resonance imaging (MRI) and computed tomography (CT). Unlike with an x-ray or CT scan, there is no ionizing radiation exposure with this test.

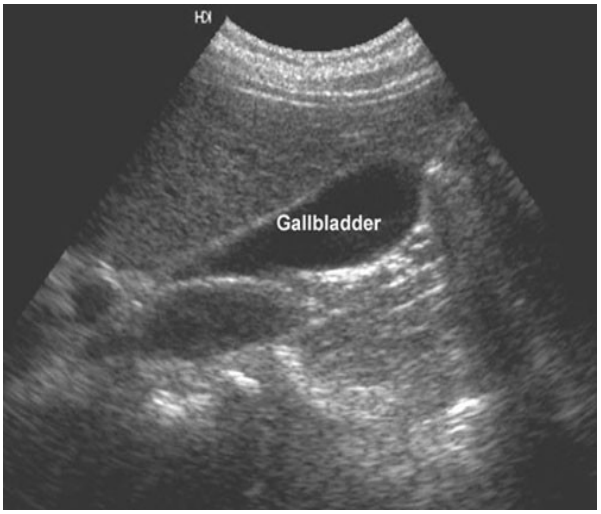
The ultrasound can evaluate most of the solid structures in the abdominal cavity. This includes the liver, gallbladder, pancreas, kidneys, bladder, prostate, testicles, uterus, and ovaries. The ultrasound can reveal the stones as well as signs of infection, including thickening of the gallbladder wall and fluid surrounding the gallbladder. The ultrasound may find blockage in the bile ducts.

Indications

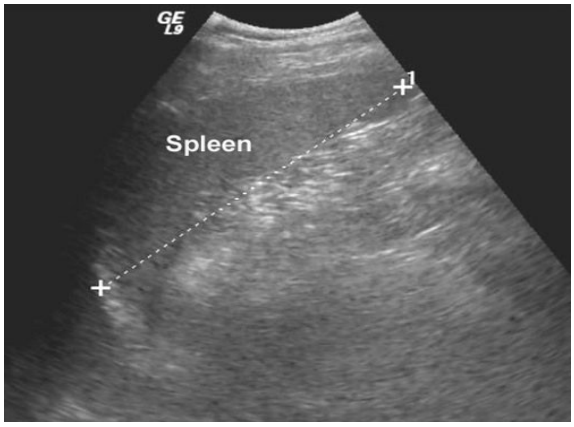
- the cause of abdominal pain; diagnose and monitor tumors and cancers
- diagnose or treat ascites; stones in the gallbladder or kidney



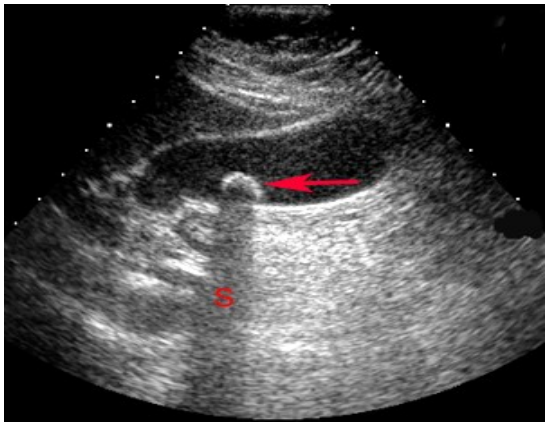
Normal liver



Ultrasound normal gallbladder



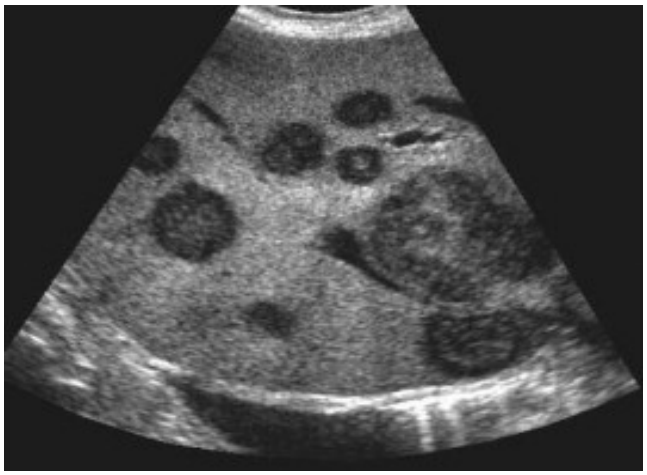
Ultrasound spleen



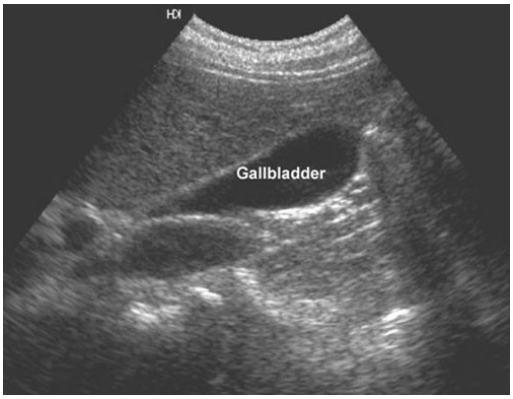
Ultrasound pictures of gall stone

Figure 2.25. Abdominal ultrasonography

Transabdominal ultrasound can detect focused liver lesions (> 1 cm in diameter) more accurately than diffuse diseases (eg, fatty liver, cirrhosis). Generally, cysts are echo-free; solid lesions (eg, tumors, abscesses) which are echogenic (can reflect a high-frequency sound wave that allows imaging by ultrasound techniques).



Multiple liver masses



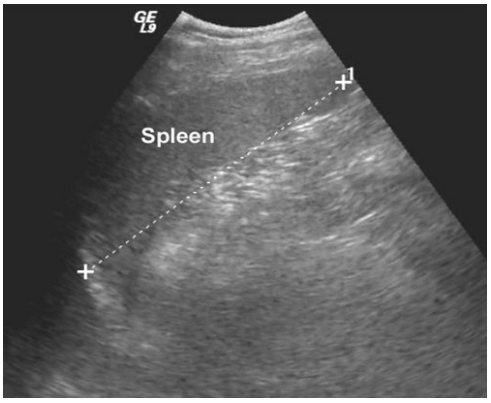


Figure 2.26. Abdominal ultrasonography

IV. PHYSIOLOGY OF THE ENDOCRINE GLANDS

IV.1. Physiology of the pituitary gland

IV.1.1.Radioimmunoassay of pituitary hormones

Definition: Radioimmunoassay (RIA) involves the separation of a protein (from a mixture) using the specificity of antibody - antigen binding and quantitation using radioactivity.

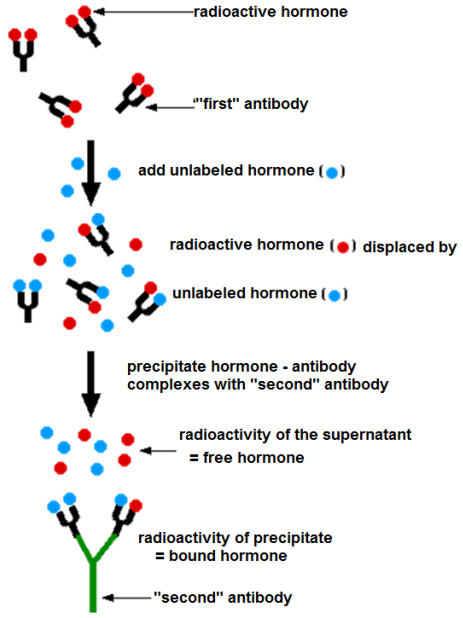
The technique was introduced in 1960 by Berson and Yalow as an assay for the concentration of insulin in plasma. It represented the first time that hormone levels in the blood could be detected by an in vitro assay.

Principle: it is based on the competition between the hormones to be measured (unlabeled) and the labeled hormone in the reaction with the specific antibody.

Materials required: antibody specific for the hormone, radioactive-labeled hormone, plasma sample to be assayed (the unknowns)

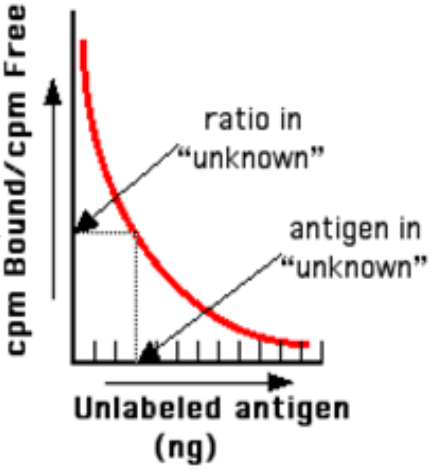
Technique:

- a mixture is prepared of known amounts of
  - *radioactive - labeled hormone* (radioactive antigen, ligand)
  - *antibody* specific for the hormone
  - *unlabeled ("cold") hormone* are added to samples of the mixture;
- these compete for the binding sites of the antibodies;
- at increasing concentrations of unlabeled hormone, an increasing amount of radioactive hormone is displaced from the antibody molecules;
- the antibody-bound hormone is separated from the free hormone in the supernatant fluid, and the radioactivity of each is measured with a gamma counter.



Results:

- from these data, a standard binding curve, can be drawn.
- the samples to be assayed (the unknowns) are run in parallel.
- after determining the ratio of bound to free antigen in each unknown, the antigen concentrations can be read directly from the standard curve.
- separating bound from free antigen: precipitate the antigen-antibody complexes by adding a "second" antibody directed against the first (e.g. if a rabbit IgG is used to bind the antigen, the complex can be precipitated by adding an antirabbit-IgG antiserum - raised by immunizing a goat with rabbit IgG).



Discussions and clinical significance:

The technique of radioimmunoassay has revolutionized research and clinical practice in many areas, e.g.,

- endocrinology
- blood banking
- diagnosis of allergies

Radioimmunoassay is widely-used because of its great sensitivity.

Using antibodies of high affinity, it is possible to detect a few picograms (10−12 g) of antigen in the tube.

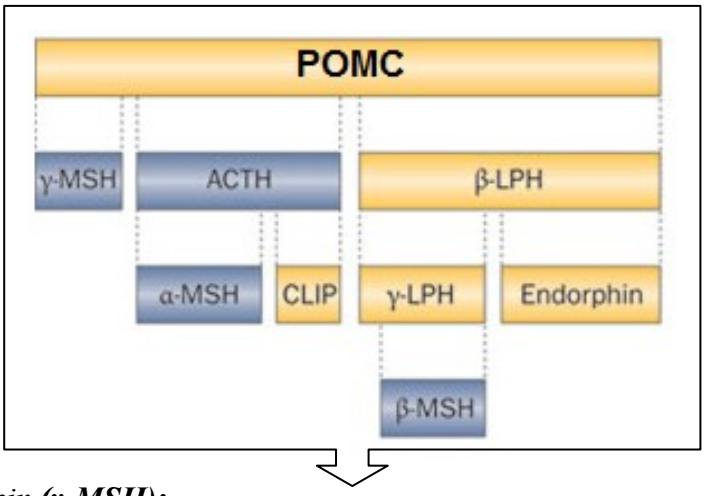
The greater the specificity of the antiserum, the greater the specificity of the assay.

The main drawbacks to radioimmunoassay are the expense and hazards if preparing and handling the radioactive antigen. Despite these drawbacks, RIA has become a major tool in the clinical laboratory where it is used to assay

- plasma levels of: most of our hormones, digitoxin or digoxin in patients receiving these drugs, certain abused drugs;
- for the presence of hepatitis B surface antigen (HBsAg) in donated blood;
- anti-DNA antibodies in systemic lupus erythematosus.

IV.1.2. Role of the pituitary in melanogenesis

Anterior/intermediate lobe of the pituitary gland, neurons in the arcuate nucleus of the hypothalamus, melanocytes in the skin syntesised a precursor peptide called pro-opiomelanocortin (POMC). POMC can be cleaved enzymatically into the following peptides:



- *γ-melanotropin* ***(γ-MSH);***
- *corticotropin (adrenocorticotropic hormone, or ACTH)* - regulates the secretion of glucocorticoids from the adrenal cortex;
- *α-melanotropin (α-melanocyte-stimulating hormone, or* ***α-MSH****)* - produced by neurons in the arcuate nucleus has important roles in the regulation of appetite and sexual behavior, while α-MSH secreted from the intermediate lobe of the pituitary regulates the production of melanin.;
- *corticotropin-like intermediate peptide (CLIP);*
- *β-lipotropin (β-LPH)* **-** weak lipolytic effects, its major importance is as the precursor to *β*-endorphin;
- *γ-lipotropin (γ-LPH);*
- *β-melanotropin* ***(β-MSH);***
- *β-endorphin* - opioid peptides with pain-alleviation and euphoric effects.

Melanocyte-stimulating hormone (MSH) belongs to a group called the melanocortins. α-MSH is the most important melanocortin for pigmentation. MSH stimulates the production and release of melanin (melanogenesis – figure 4.1) by melanocytes in skin, eyes and hair. The melanocyte (pigment cell) resides in the basal layer of the epidermis and supplies melanin (packaged within melanosomes) to surrounding keratinocytes. Following exposure to UV light, melanocytes becomes enlarge, more branched with more MSH receptors. So, there is an increase in the number of melanosomes, their melanin content and their transfer to keratinocytes causing darkening of skin.

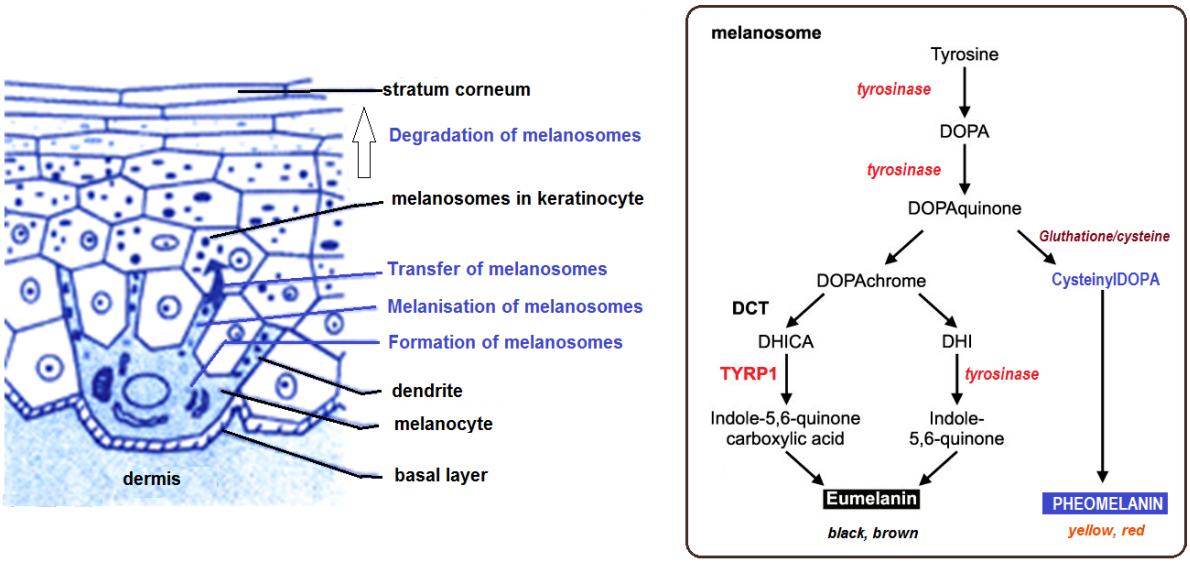


Figure 4.1. Melanin synthesis diagram.

MSH variations:

- *Racial variation* in skin colour does not entirely depend on different levels of MSH. Though, in many red headed people, and other people who do not tan well, there are variations in their hormone receptors, causing them to not respond to MSH in the blood;
- MSH increases during *pregnancy* and along with increased estrogens, causes increased pigmentation in pregnant women;
- *Cushing's syndrome* due to excess adrenocorticotropic hormone (ACTH) may also result in hyperpigmentation, such as *acanthosis nigricans* in the axilla;
- Most people with *primary Addison's* have darkening (hyperpigmentation) of the skin, including areas not exposed to the sun; characteristic sites are skin creases (e.g. of the hands), nipple, and the inside of the cheek (buccal mucosa), also old scars may darken. This occurs because melanocyte-stimulating hormone (MSH) and adrenocorticotropic hormone (ACTH) share the same precursor molecule, pro-opiomelanocortin (POMC);
- *Albinos* - inability to synthesize melanin due to lack of tyrosinase (recessive mutation) causing white hair, pink eyes (due to no melanin in iris or retina);
- *Vitiligo* - a decrease in the number or absence of melanocytes in the epidermis determines areas of skin depigmentation of varying sizes.

Demonstration of role of the pituitary in melanogenesis on frog

Principle: changes in dispersion of pigment melanin within melanophores of skin frog after removing of hypophysis.

Materials required: frog, container, board with a hole in the centre, microscope.

Technique:

- remove the pituitary gland (hyphophysectomy) on a frog (open the mout and through the the cranial floor with a dental drill extract the gland)

- keep the animal in a dark-walled container at room temperature 24 hours
- pith the frog (see section I.1.1)
- lie the frog on the board with the web of one foot spread over the hole
- place the interdigitate membrane of the web under the low-power objective of a microscope
- keep the frog moist by applying Ringer solution with the dropper
- observe the skin of the frog

Results and discussions:

- the dispersion of the melanophore pigment in the skin of the frog is assessed by Hogben index (see figure 4.2);
- production of MSH is decreased when the animal is in a dark location. This causes retraction of melanophores and decoloration of skin.

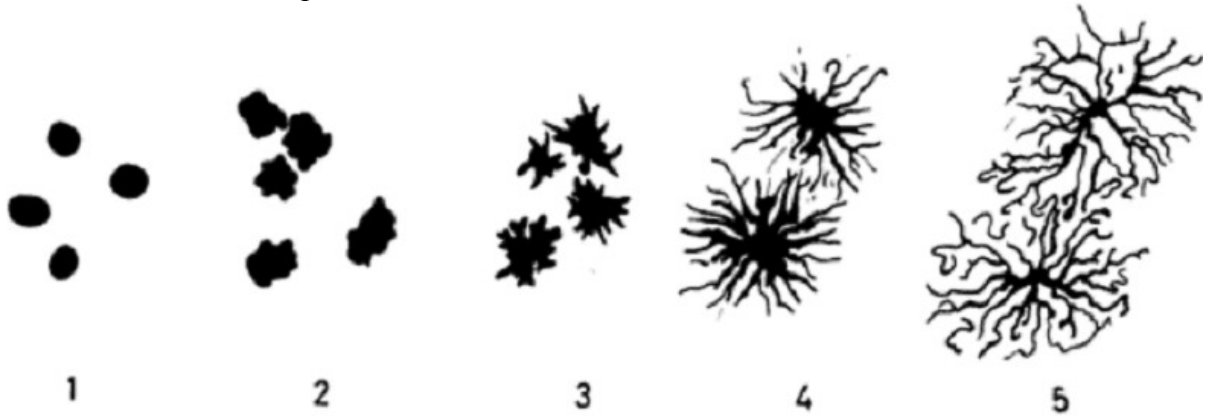


Figure 4.2. The melanophore index (from Hobken and Slome, 1931)
1 = spot melanophores (complete retractions), 2 = short terminations,
3 = expansion of bifurcate terminations, 4 = trifurcate terminations,
5 = intercellulary continuous network (complete expansion of melanophores)

IV.1.3. Action of oxytocin upon the uterine smooth muscle

Experimental, action of oxytocin upon the uterine smooth muscle can be demonstrated *in vitro* using organ bath (see section III.3.4).

Principle: experimental analysis of uterine smooth muscle contraction in organ bath.

Materials required: uterine smooth muscle of rat, organ bath assembly, lever, and kymograph

Technique: see section III.3.4.

Results:

Oxytocin increases the frequency and amplitude of uterine smooth muscle contraction and the tone of the muscle (figure 4.3).

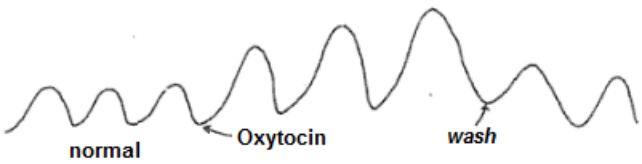


Figure 4.3. Effects of oxytocin on uterine smooth muscle of rat.

Oxytocin acts on the receptors present on the myometrium. It increases intracellular calcium concentration and therefore stimulates the contractions.

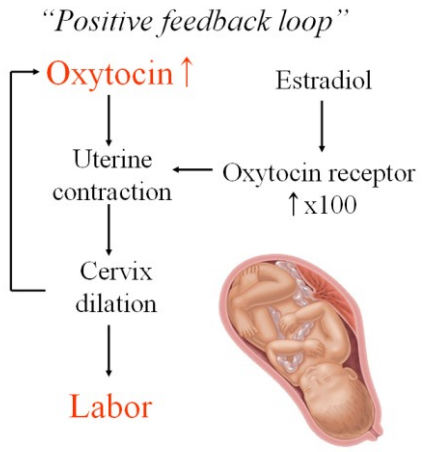
Discussions (also see the lecture):

1. Effect of oxytocin on mammary glands:

- cause the contraction of the myoepithelial cells that surround the outer walls of the alveoli of the mammary glands, press the milk from the alveoli to the duct and make it flow out (milk ejection);

2. Effect of oxytocin on the uterine smooth muscle:

- the sensitivity of the myometrium to exogenous oxytocin during pregnancy increases as pregnancy advances.
- oxytocin plays a role in labor and has been shown to be a useful therapeutic agent in the induction of labor.



IV.2. Physiology of the thyroid and parathyroid glands

IV.2.1. Determination of basal metabolism

Definition:

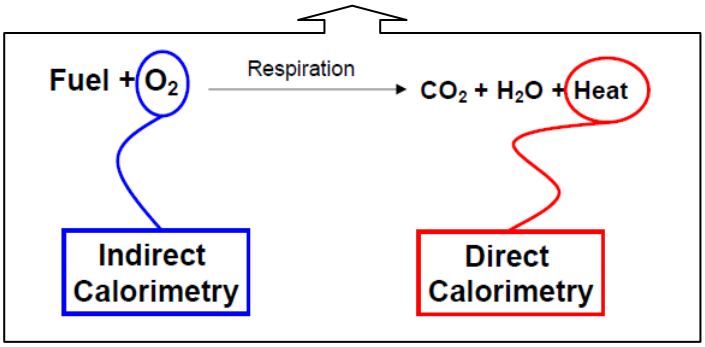
1. The basal metabolism or basal metabolic rate (BMR) represents the lowest power consumption of the waking body, needed to ensure vital functions.
2. Also, BMR can be defined as the amount of heat produced in the body in a given time.

Principle: determination of BMR is carried out in basal conditions:

- in the morning, physically and mentally absolute rest, supine,
- total starvation for 12 hours and protein to 18 hours,
- at the thermal neutral point of 18-22 °C,
- after discontinuation of sedative or stimulant medication.

Technique: is named *calorimetry* and measure the amount of heat produced by the body by two methods:

- *direct calorimetry* – uses a body calorimeter to measure the amount of heat given off by the body;
- *indirect calorimetry* – uses a spirometer to measure the amount of oxygen consumed by the body over a short period of time using closed circuit system (Benedict - Roth spirometer) or open circuit system (Douglas bag or Tissot method)



- in open circuit system both O2 consumption and CO2 output are measured. Method is less rapid, but more acuurate;
- in closed circuit system (Benedict - Roth spirometer – figure 4.4) is measured O2 consumption of the patient for 2 to 6 minutes period under „basal" conditions. The subject with nose clipped breathes in O2 from a metal cylinder of about 6 L capacities and CO2 produced is absorbed by soda-lime present in the tower. The volume of O2 used is recorded on a graph paper attached to a revolving drum by a pen attached to it.

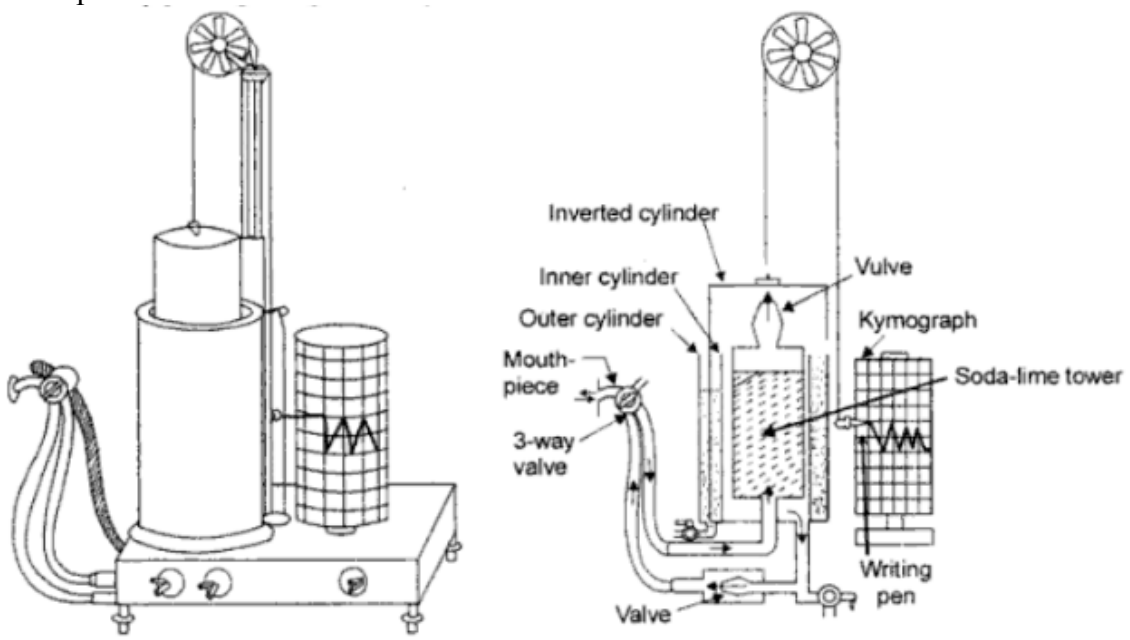


Figure 4.4. Benedict - Roth spirometer

Results:

Calculation of BMR for a male aged 35 years old, height = 170cm and weight = 70kg that consumed 1.2L of O2 (corected to normal temperature and pressure: 0^0C, 760 mmHg) in a 6 minutes period): O2 asumption per hour = 1,2 x 10 x 4,825C = 12L x 4,825C = 58C/hour = heat production in C/hour (10 = conversion factor and 4,825C is heat production represented by each liter of O2 consumed).

Since BMR is expressed in c/sq. meter/ hour, the energy output per hour obtained above has to be divided by the surface area of the subject.

Surface area is calculated after Du Bois formula ($A= H^{0,725} \times W^{0,425} \times 0,007184$, where A= surface area in sq. metre, H = height in cm and W = weight in kg) or more easily the surface area is taken from nomograms (with condition to know height and weight).

Surface area from nomogram in this case is 1,8 sq metre, so BMR = 58C/1,8 = 32 C/sq metre/hour. The normal BMR for this subject by reference to standard table is 39,5 C/sq metre/hour. Hence the subject's BMR which is below normal is presented as (39,5 -32) / 39,5 x 100 = -18,98%. *In terms of percentage difference as compared to standard values, BMR between -15 to 20% is considered normal.*

Normal BMR values: BMR can be expressed as

a) calories per square meter body surface area per hour:

- a *healthy adult male* has a BMR of about *40C/sq m/hour*
- an adult *female about 37 C/ sq m/ hour*

b) in terms of percentage difference between the determined value and the standard value (as calculated from age, sex, weight and height of person): *normal BMR is between -15 to 20%.*

Interpretation and clinical significance:
BMR is affected by various factors as:

- *body size and composition*: lean body mass ans muscular bodies have higher BMR than obese persons, whose higher body weight is duet o adipose tissue;
- *sex*: males have slightly higher BMR than females
- *age*: infants and young children have higher BMT than adults
- *climate*: persons living in temperate zones have 10% more BMR compared to those who live in tropical climate
- *undernutrition and starvation*: reduce BMR by about 20%
- *sleep and physical activity*: BMR decreases up to 5% in sleep and increases to physical activity
- *physiological state:* BMR is higher in pregnancy
- *fever:* BMR increases at a rate of 13% for every degree Celsius rise in body temperature
- *BMR is raised* with nervous tension, when muscles tend to be tense; cold weather, when body temperature is maintained by act of shivering; after food intake; on hospitalized patients.
- *hormones:*
  - hyperthyroidism increases BMR > +15%
  - hypothyroidism, adrenal insufiency decrease BMR >-10%

Importance of noting BMR:
- prescribing a diet of adequate caloric value;
- diagnosis of pathological conditions
- note the effect of differnt types of food and drug on BMR.

IV.2.2. Reflexogram

Principle: use of the Achilles-tendon reflex (ankle-jerk) in thyroid clinical investigation.
Materials required: reflexograph (figure 4.5) consists of a rotating drum, a lever at one end with a leg-piece and at the other end with a writing pen.

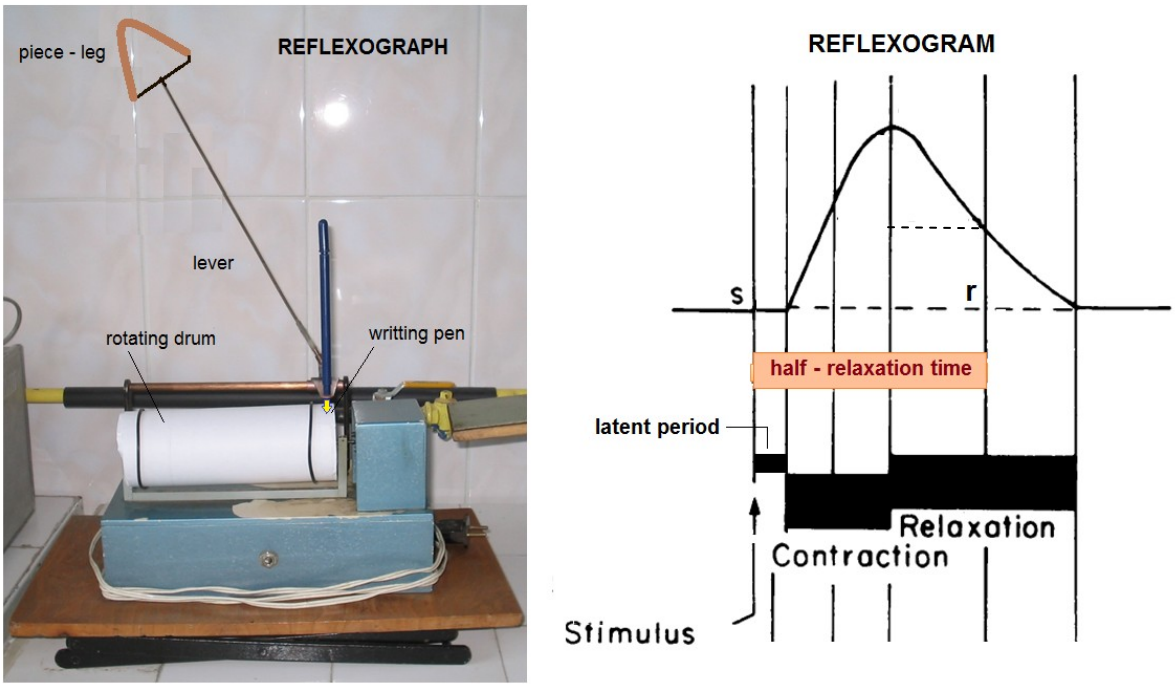


Figure 4.5. Reflexograph and reflexogram for Achilles-tendon reflex (ankle-jerk)

Technique:
- the patient put the kneel on the seat of a wooden chair
- with a reflexes hammer tap the tendon of Achilles-tendon and a plantar flexion is obtained
- movement of the sole of the foot cause the movement of the lever and subsequently of the writing pen on the rotating drum of the reflexograph.

Results and interpretation:
- recorded graph is called reflexogram which consists of three periods: latent period, contraction and relaxation (figure 4.5);
- it is measured half-relaxation time (from point s to point r) expressed in milliseconds;
- half-relaxation time is determined by following method: draw the perpendicular from the highest point of the curve on the baseline. It is measured half of this perpendicular and it is unit it with a horizontal line to the downward slope of the curve. The distance obtained is converted into time, knowing the speed of the paper (60 mm/sec);
- *normal values:*
  - **310 msec on euthyroid patients;**
  - 440 msec on hypothyroid patients;
  - 250 msec on hyperthyroid patients.
- also, in hypothyroid patients there is a delayed Achilles-tendon reflex compared with hyperthyroid patients where the reflex amplitude and duration is increased

Clinical significance:

Because the ankle-jerk, used on a statistical basis, is capable of detecting small clinical differences and because of the simplicityof its assessment, it is suggested that this could be utilizedas *an index of the peripheral thyroid state*, together with the other parameters of thyroid metabolism, for the study of thyroid function as a whole.

IV.3. Physiology of adrenocortical glands: Thorn test

Thorn test is named also adrenal function eosinophil count. This test establishes the patient's total eosinophil count, to which the response of the adrenal cortex to adrenocorticotropic hormone (ACTH) can be judged.

Principle: the test is based on the inability of the diseased adrenal gland to secrete cortisol after corticotrophin (ACTH) is given (figure 4.6).

Technique:
- take a blood sample from the subject and count the eosinophils
- administer to the patient an injection of ACTH as a test of adrenal cortical function
- after 4 hours take again a blood sample and count the eosinophils

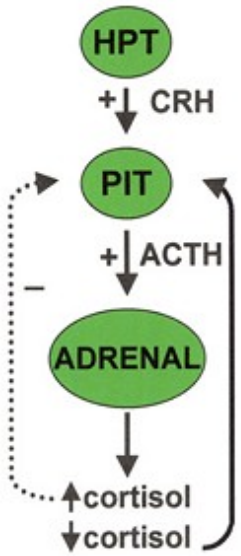


Figure 4.6. Normal adrenal function (HPT = hypothalamus, PIT = pituitary gland)

Results and interpretation:
- if adrenocortical gland function is **normal** eosinophil count before the injection ***will be about twice*** the value of the eosinophil count after the injection;
- if the adrenal cortical function is **decreased,** eosinophil count before the injection ***will be about the same*** as the eosinophil count after the injection. This indicates *hypoadrenalism (Addison's disease)*

Discussions:
- the ACTH stimulation test is recognized as the gold standard assay of adrenal insufficiency;
- the test is extremely sensitive to primary adrenal insufficiency (Addison's disease), but less so to secondary adrenal insufficiency.
- *Addison's disease* occurs when the adrenal glands are damaged and cannot produce enough of the hormone cortisoland often the hormone aldosterone.
- *Secondary adrenal insufficiency* occurs when the pituitary gland fails to produce enough ACTH, a hormone that stimulates the adrenal glands to produce cortisol. If ACTH output is too low, cortisolproduction drops. Eventually, the adrenal glands can shrink (atrophy) due to lack of ACTH stimulation. Secondary adrenal insufficiency is much more common than Addison's disease.
- *for the role of ACTH, feedback mechanisms, roles of cortisol - see the lecture.*

IV.4. Physiology of gonads: early pregnancy test

Definition: a pregnancy test is a test of blood or urine used to determine whether a woman is pregnant or not.
Principle: the placental hormone human chorionic gonadotropin (hCG) is excreted in the urine during pregnancy. its detection in urine is generally a positive test for pregnancy.

There are two types of tests:
1. Quantitative tests (blood tests):
- measures the amount of hCG on your blood in milli-international units per milliliter (mIU/ml)
- range: 5 mlU/ml to 2,000,000 mlU/ml
- less than 5 mlU/ml generally indicates you are not pregnant.
- 10 to 50 mlU in the week following conception.
- the test become positive at 2-3 days after implantation or 8-9 days after fertilization. A maximum is reached by the second to third month, followed by a decrease in the third trimester.

2- Qualitative tests (urine tests)
- only indicate whether you are pregnant or not, yes or no.
- women who are late for their menstrual period (menses) often perform qualitative urine test at home; home pregnancy tests are available at pharmacies and do not required prescriptions;
- detect hCG levels about 25-50 mill international units per milliliter (mlU/ml)
- urine hCG levels are usually lower than serum (blood) hCG levels.

Pregnancy test strip – urine test (qualitative test)

Urine collection:
- for optimal detection of early pregnancy, a "first morning" urine specimen is preferred, (highest concentration of ß hCG);
- randomly collected urine specimens may also be used;
- collect the urine specimen in a clean container.
- if testing is not immediate, the specimen should be refrigerated for up to twenty-four hours. Bring the urine specimen to room temperature prior to testing.

Test procedure:

- remove the test strip from its foil pouch;
- holding the strip vertically, carefully dip it into the specimen. Do not immerse the strip past the maximum line;
- remove the strip after 3 seconds and lay the strip flat on a clean, dry, non-absorbent surface;

Interpretation of results:

- wait for colored bands to appear. Depending on the concentration of the hCG in the test specimen, positive results may be observed in as little as 60 seconds.
- however, to confirm results , the complete reaction time of 5 minutes is required. Interpret the test results at 5 minutes.

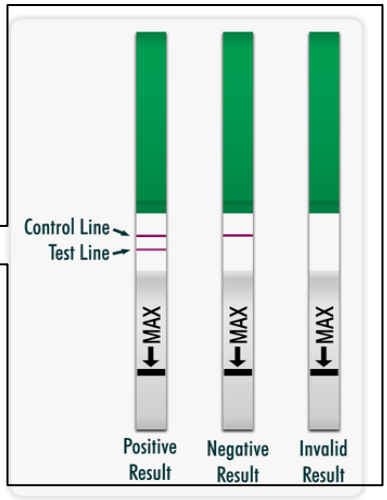
Positive:

2 color bands appear in the result window. The top is the control line; the bottom is the test line. The test line can appear lighter of darker depending on the concentration of hCG in the urine. Positive results should always be verified with a doctor.

Negative:

1 color band appears in the result window. This indicates that only the sample has flowed through the test, but pregnancy has not been detected.

Invalid: if no control line appears, the test was performed improperly or an error occured. Please repeat the test with a new device and follow instructions carefully.



Discussions:

1. Higher than normal levels of human chorionic gonadotropin (hCG) may indicate:

- a normal pregnancy
- multiple pregnancy, such as twins or triplets
- a tumor of the placenta with death of the fetus
- ovarian cancer and other types of cancer in some cases
- blood or protein in the urine, which can interfere with the result
- use of medications to prevent seizures, drugs to treat Parkinson's syndrome, or phenothiazine drugs, such as chlorpromazine, by the mother

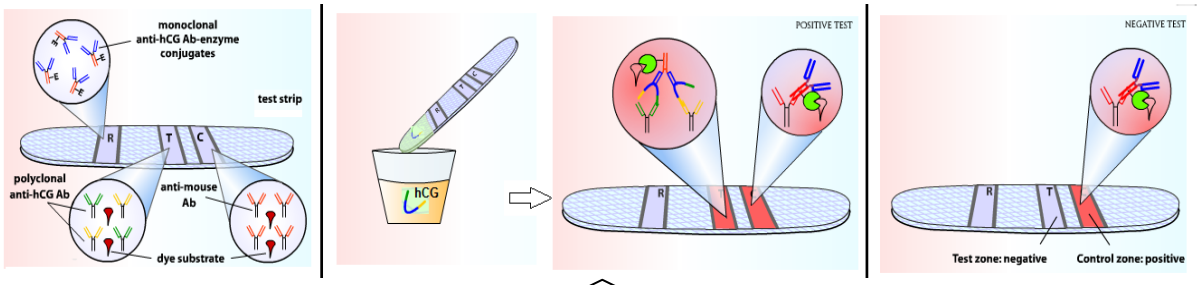
2. Lower-than-normal human chorionic gonadotropin (hCG) levels may indicate:

- ectopic pregnancy
- miscarriage or abortion.

With ***obstetric ultrasonography*** the gestational sac sometimes can be visualized as early *as four and a half weeks of gestation* and the yolk sac at about five weeks' gestation. The embryo can be observed and measured by about five and a half weeks. The heartbeat may be seen as early as six weeks, and is usually visible by seven weeks' gestation.

Traditional obstetric sonograms are done by placing a transducer on the abdomen of the pregnant woman. One variant, a transvaginal sonography, is done with a probe placed in the woman's vagina. Transvaginal scans usually provide clearer pictures during early pregnancy and in obese women. Also used is Doppler sonography which detects the heartbeat of the fetus. Doppler sonography can be used to evaluate the pulsations in the fetal heart and bloods vessels for signs of abnormalities.

Pregnancy test using "sandwich" ELISA method which involv three different antibody preparations:



- *the reaction zone (R)* contains soluble mouse monoclonal anti-hCG antibody linked with an enzyme (E) which recognize a single epitope on hCG.
- *the test zone (T)* contains fixed polyclonal anti-hCG antibodies (which can recognize a variety of epitopes on hCG molecules) and dye molecules which act as a substrates for the enzymes from R zone (catalise a color reaction)
- *the control zone (C)* contains fixed goat anti-mouse antibodies (which can recognize epitopes on mouse monoclonal antibodies that are in the R zone) and dye substrates.
- *if the urine contains hCG*, the sample is drawn up the strip by capillary action and first arrive at the R zone where it binds with mouse monoclonal anti-hCG antibody-E conjugated. The complexes antigen (Ag) – antibody (Ab), as well as unbound Abs dissolve in the fluid and detach from the capillary membrane and carried along in the capillary flow. They will arrive in the T zone, where the immobilised polyclonal hCG Abs bind other epitopes on hCG molecule. The free Abs continue to flow along the strip and they arrive in the C zone. Here, goat anti-mouse Abs that are affixed to the strip bind the monoclonal mouse Abs. In the T and C zones the E catalyse a color reaction with dye substrate, so these zone will become colored – *positive test.*
- *Explain what happens in the strip if the test is negative!*

(see animation on
http://www.whfreeman.com/catalog/static/whf/kuby/content/anm/kb07an01.htm)

Galli-Mainini test (frog test)

Principle: In nature the male frogs and toads release spermatozoa after a prolonged contact with the female, which stimulates the secretion of the gonadotrophic hormone from the hypophysis. A similar effect may be obtained by injecting human chorionic gonadotrophin excreted in urine during pregnancy, or in cases of hormone-secreting neoplasms (chorionic epithelioma, hydatid mole).

Technique:

- the urine sample (1 to 3 ml) of the woman suppose to be pregnant is injected into a male frog through the muscle of the hip in the dorsal lymphatic sacs
- before the injection and 45 minutes after it a specimen of the animal's urine is procured from the cloaca with a capillary pipette and examined directly under the microscope

Results:

- the presence of spermatozoa indicates a positive reaction (pregnancy)
- the absence of spermatozoa indicates a negative reaction

Discussions:

The " frog test " described by Galli-Mainini is a very useful procedure for the diagnosis of pregnancy. It is characterized by technical simplicity and a very high degree of accuracy (the pregnancy was detected 4 days after missing period).

IV.5. Functional explorations of endocrine system

1. PITUITARY IMAGING TESTS

Before the introduction of computed tomography and magnetic resonance (MR) imaging, intracranial imaging relied mostly on skull radiographs, pneumencephalography, and cerebral angiography. To diagnose a pituitary adenoma, several signs had to be present on skull radiographs. The size of the sella often could indicate an intrasellar mass. Frequently, pituitary adenomas tend to grow asymmetrically. The sellar floor is then uneven, reflecting uneven enlargement of the bone sella.

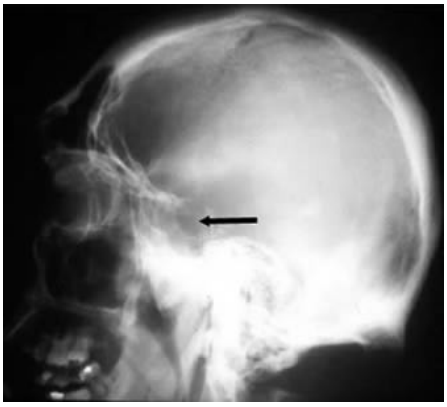


Figure 4.7. X-ray of sella turcica

Radiography of the sella turcica (figure 4.7)
Radiographic changes of the sella turcica or pituitary fossa reflect multiple disease processes of intracranial, cranial and systemic origin. Their value as diagnostic aids is related to the fact that the development of the fossa depends somewhat upon that of the pituitary gland, which it partially encloses.

Conventional single-section CT has a limited role in pituitary imaging, with a sensitivity of 17-22% in detecting microadenomas. Multidetector-row CT with 64 channels may have a role, especially in patients unable to undergo MRI. CT is best for visualizing bony detail and calcification in tumors such as germinomas, craniopharyngiomas, and meningiomas. CTA is excellent for showing the morphology of parasellar aneurysms and for presurgical planning. CT scans are valuable when MRI is contraindicated, as in patients with pacemakers or metallic implants in the brain or eyes.

Pituitary CT can be performed in the axial plane. Images can usefully be reformatted in the coronal and sagittal planes. Alternatively, thin sections in the coronal plane with the patient prone and the neck extended allow pituitary abnormalities to be demonstrated with a lower radiation dose to the lens than is possible with axial sections. For pituitary adenoma imaging, CT and MRI have largely replaced plain radiography because conventional radiography is poor for delineating soft tissues.

Angiography is seldom performed; if indicated, CT angiography (CTA) and MRA have largely replaced conventional angiography. Angiography has a role when intervention is indicated in the cavernous sinus or in the cavernous part of the carotid artery.

MRI is generally preferred over CT for the diagnosis of pituitary adenomas because of its superior definition of small lesions in the pituitary sella and its improved anatomic definition before surgery. MRI is also preferred for postsurgical surveillance

2. THYROID IMAGING TESTS

Thyroid ultrasound is an imaging method usually done when you have a growth on your thyroid gland. The exam can help tell the difference between a sac containing fluid (cyst) and abnormal tissue that may or may not be cancerous (a tumor). Indications of thyroid ultrasound comprise the presence of cysts, enlargement of the thyroid gland (goiter), thyroid nodules, tumors.

Nuclear Scan / Radioactive Iodine Uptake (RAI-U) – which can tell whether a person has Graves' disease, toxic multinodular goiter, or thyroiditis.

CT Scan – to help detect and diagnose a goiter, or larger thyroid nodules.

MRI / Magnetic Resonance Imaging – to evaluate the size and shape of the thyroid.

Thyroid biopsy/aspiration- A needle biopsy, also known as fine needle aspiration (FNA) is used to help evaluate lumps or cold nodules. In a needle biopsy, a thin needle is inserted directly into the lump under ultrasound control, some cells are withdrawn and they are evaluated for cancer. Cancer can be definitively diagnosed about 75 percent of the time from FNA. Evaluation of biopsy results can also show cells indicative of Hashimoto's thyroiditis. Thyroid nodules are common, 4-7% of adults have such palpable formations and 50-70% of adults discover thyroid nodules after a ultrasound examination of the thyroid.

Thyroidscintigraphy (figure 4.8) is a nuclear medicine procedure that produces a visual display of functional thyroid tissue based on the selective uptake of various radionuclides by thyroid tissue. Thyroidscintigraphy provides valuable information regarding both thyroid anatomy and physiology and can play an integral role in the diagnosis. Thyroidscintigraphy provides an additional method for determining the relative severity of thyroid disease that is less affected by the presence of concurrent nonthyroidal illness than laboratory evaluations.

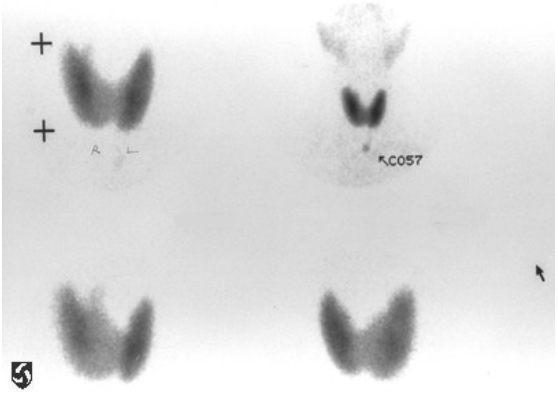


Figure 4.8. Scintigraphy of the thyroid

3. PARATHYROID IMAGING TESTS

CT	Examines head, neck and chest; easy detection of calcification; biopsy capable
MR imaging	Examines head, neck and chest; No iodinated contrast required; Excellent soft tissue discrimination
Nuclearm edicine scintigraphy	Examines head and neck well; Functional, not anatomic imaging; Distinguishes nodes from adenomas; reasonablec cost
Ultrasonograph	Examines neck well; noninvasive; Real-time, biopsy capable

4. PANCREAS IMAGING TESTS

CT scan with contrast dye:. a CT scan can help rule out other causes of abdominal pain, determine whether tissue is dying (pancreatic necrosis), and find complications such as fluid around the pancreas, blocked veins, and obstructed bowels.

MRI. Occasionally an MRI is used to look for signs of pancreatitis.

Computed tomography (CT) and abdominal ultrasound are used especially for diagnosis of insulin secreting tumors (to help locate the tumor in retroperitoneal or abdominal cavity).

Oral Glucose Tolerance Test (OGTT)

Normal value of blood glucose : 70 - 110 mg/dl (venous blood)

Variations of blood glucose: >110 mg/dl(hyperglycaemia); 50-60 mg/dl (hypoglycaemia)

During the oral glucose tolerance test your blood glucose is tested two hours after drinking 75 grams of glucose (1 g glucose/kg body weight) solved in 200-300 ml water.

- *Normal values* : fasting value < 110 mg/dl; 2 hours < 140 mg/dl
- *Diabetes mellitus*: fasting glucose > 110 mg/dl; 2 hours < 140 mg/dl
- *Impaired fasting glycaemia (IFG):* fasting glucose > 110 mg/dl; 2 hours 140 - 200 mg/dl
- *Impaired glucose tolerance (IGT):* fasting glucose > 110 mg/dl; 2 hours > 200 mg/dl

Indications:

- in patients with multiple border-line fasting serum glucose levels (110-125 mg/dl)
- in patients with symptoms that may be clearly related to diabetes mellitus
- in subjects who manifest pathology that may be a complication of diabetes mellitus

V. PHYSIOLOGY OF THE NERVOUS SYSTEM

V.1. Physiology of the somatic effector system

V.1.1. Dynamometry

Principle: the measurement of the maximum voluntary force (strengh) developed during an isometric (static) maximal voluntary contraction.

S*trength* is defined as the force exerted by a muscle or muscle groups during a single contraction.

For example, **handgrip strength test** measures the maximum isometric strength of the hand and forearm muscles.

Materials required: handgrip dynamometer

Technique:

- the subject holds the dynamometer in the hand to be tested, with the arm at right angles and the elbow by the side of the body;
- the handle of the dynamometer is adjusted if required - the base should rest on first metacarpal (heel of palm), while the handle should rest on middle of four fingers;
- when ready the subject squeezes the dynamometer with maximum isometric effort, which is maintained for about 3 seconds;
- no other body movement is allowed; the subject should be strongly encouraged to give a maximum effort;
- the subject repeats the test 3 times with at least 15 seconds recovery between each effort;
- the best of 3 attempts should be recorded on the dominant and non-dominant hands.

Results: on dominant hand the maximum voluntary force is higher compare with non-dominant hand.

Dominant Handgrip Rating (kg)		
Males	**Females**	**Rating**
>56	>36	Excellent
51-56	31-36	Good
45-50	25-30	Average
39-44	19-24	Fair
<39	<19	Poor

Discussion and practical importance:

The importance of hand strength and function is evident in all aspects of our daily living, from eating and maintaining personal hygiene to keyboarding at the computer, performing brain surgery, or playing tennis or the piano. People suffering from arthritis or hand injury quickly appreciate the difficulty of performing even the most mundane tasks with reduced grip strength.

Testing of hand grip strength is used by orthopedic surgeons and physical therapists to evaluate the extent of an injury and the progress of recovery.

Grip strength can also be used to diagnose neuromuscular problems such as stroke, herniated disks in the neck, carpal tunnel syndrome, and elbow tendonitis.

Athletes are interested in grip strength because it relates to performance in many sports, such as tennis, golf, baseball, football, gymnastics, and rock climbing (sports in which the hands are used for catching, throwing or lifting).

V.1.2. Chronaximetry

Rheobase and chronaxie are parameters of neuro-muscular excitability. These two are to compare excitability of different nerves or excitable tissues using electric stimuli (galvanic currents). Not any stimulus at any strength can get a response. For response to take place two factors are necessary: (i) minimum strength, and (ii) adequate duration of the stimulus (these two factors are inversely related to each other). The relationship between strength and duration of a stimulus has been studied by varying the duration of a stimulus and finding out the threshold strength for each duration. The record of results plotted gives the *strength-duration curve (figure 5.1)*:

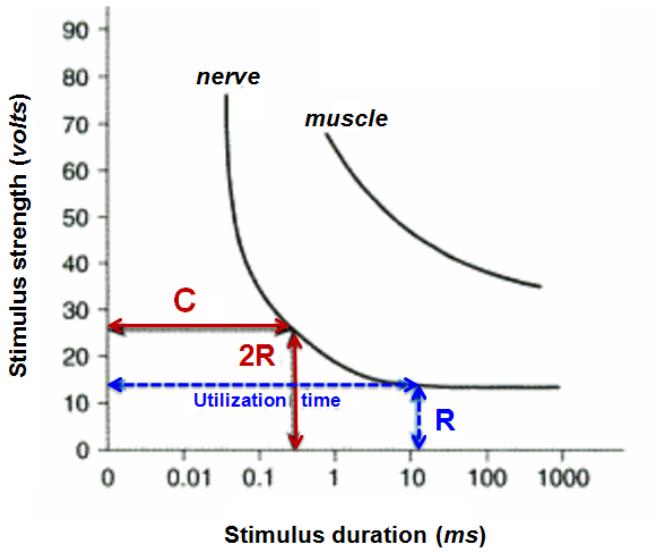


Figure 5.1. The strength-duration curve for the nerve and for the muscle (R = rheobase, C = chronaxie)

- **rheobase** refers to the
  - minimum intensity (strength, amplitude) of the electrical stimulus which if applied for adequate time (utilisation time) produces a response from the tissue, e.g., muscle or nerve. In practice, this minimum intensity it is called threshold, minimal or liminal stimulus.
  - minimum voltage of galvanic current which when allowed to indefinitely, will excite the tissue (by Lapique).
- **utilisation time** - time taken for excite the tissue when rheobase current is applied.
- **chronaxie** *(Greek: chronos, time, + axia, value)* refers to the
  - minimum time taken for response when twice rheobase current is applied
  - minimum needed by a stimulus with an intensity twice as high as the rheobase to elicit a response from an excitable tissue
  - shortest duration of a galvanic current which is twice as great as rheobase to excite the tissue when applied.
  - minimum duration of electric current required to induce muscular contraction at a voltage of two times the threshold voltage
- when a *stimulus of weaker intensity* than rheobase is applied, it will not produce a response whatever may be the duration of the stimulus; stimulus *with extremely short duration* will not elicit any response, no matter how intense it may be; in between, values of strength and duration are inversely related; this means that low intensity stimuli take

longer time to excite an excitable tissue whereas stronger stimuli need shorter duration for the same.

Chronaxie is a measure of excitability; **shorter the chronaxie, more excitable is the tissue.**

Interpretation:

- Chronaxies differ in various species; in different tissues of the same species; in same group of tissues in the same body.
- Comparison of chronaxies of various excitable tissues:
    - in general, muscles have longer chronaxies than nerves
    - myelinated nerves < unmyelinated nerve < skeletal muscle < smooth muscle < cardiac muscle
    - conducting system of the heart < cardiac muscle fibers;
    - nerve fibers of larger diameter have shorter chronaxie than short fiber of a small diameter. Somatic nerves are thick hence have shorter chronaxies. Autonomic nerves are thin hence have longer chronaxies;
    - the chronaxie of corresponding motor and sensory nerves are almost the same;
    - adults have shorter chronaxies than infants ;
    - pale fibers (fast-twitch, glycolitic) have shorter chronaxies than red fibers (slow twitch/ oxidative);
    - in the same limb the muscles with the same function have the same chronaxies;
    - chronaxie of the flexors = ½ chronaxie of the extensors;
    - in the same limb chronaxies of the distal muscles are longer as compare as chronaxies of the proximal muscles (forearm > arm; leg > thigh).
- temperature - cold increases and heat diminishes chronaxie within physiological limits
- tissue injury or degeneration diminishes chronaxie

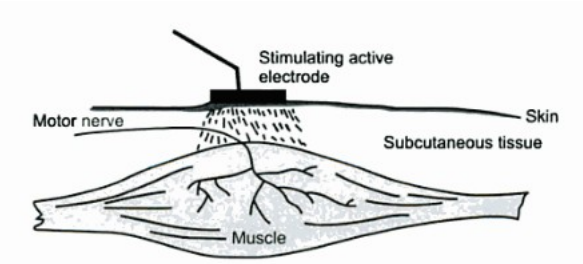
Practical significance:

1. In ***physiotherapy***, the strength / duration (S/d) curves are made for:
    a. *diagnostic* in order to determine the degree of a peripheral nerve lesions and the degree of denervation of the muscle tissue;
    b. *therapy* because the the S/d curve gives information about the parameters for muscle stimulation (pulse duration, frequency, intensity of the current);
    c. monitoring the progress of the lesion and denotes if the lesion is recovering / regressing.
2. In ***sport medicine***, chronaxie determination provides valuable information on their physical fitness, training and state of fatigue.
3. In ***cardiac pacing*** the strength / duration (S/d) curves is used for the proper programming of stimulus amplitude and pulse duration.

Technique:

- With a *neuromuscular stimulator* it is applied on motor points of the muscles rectangular impulses of various duration such as 0.1, 0.3, 1, 3, 10, 30, 100 ms, which may be constant current or constant voltage type.
- *Motor point* is defined as point over the surface of the muscle where the main motor nerve enters it (with great density of terminal endplates). This is an area of low threshold excitability because of low skin resistance and good electrical conductivity. Through these points the muscle can be stimulated optimally at a relatively low intensity. There are charts with the exact location of the motor points in the muscles (figure 5.2).

- The negative electrode is placed on the motor point and positive electrode is placed proximally from the negative electrode.
- First is applied the stimulus with the longest duration and is observed visually the minimum perceptible contraction. It is noted the magnitude of the current / voltage required for the contraction (rheobase).
- (i) Gradually shorten the impulse duration and it is noted the corresponding current (voltage) required to get the same type of muscle contraction (chronaxie).
- *or* (ii) double intensity of the rheobase and set the time scale at the shortest duration (0.1 milliseconds). Gradually lengthen the impulse duration until the same type of muscle contraction is observed and it is noticed the time (chronaxie).
- The active electrode is kept over the same point over the muscle during the test. It is plotted the strength-duration cure from the results of this test by putting the durations (ms) along the abscissa and the corresponding strength (mA or V) along the ordinates.
-



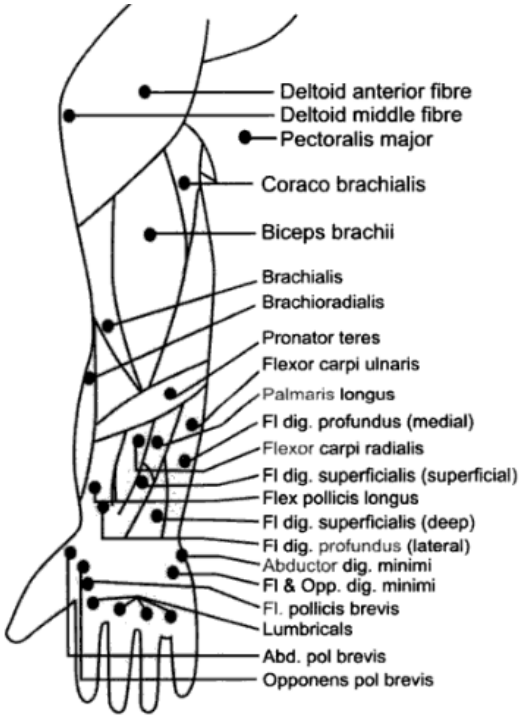


Figure 5.2. The motor point and the chart of motor points in the front of upper limb *(after de Nanda B, 2008)*

Normal values:

- *Rheobase:* 10-20 volts for a constant voltage or 2-10mA for a constant current stimulator
- *Chronaxie*: < 1ms (0.08 – 0.32 ms) for a constant current and < 0.1ms for a constant voltage stimulator

V.1.3. Global and motor unit electromyography

Definition: refers to the technique of recording the electrical activity of the muscle by placing electrodes on the skin or inserting electrodes into the muscle. The recorded graph is called electromyogram (EMG).

Materials required: the electromyography recording set up includes: data collection electrodes, amplifier system and recording display system.

A. The data collection electrodes - 2 different types:

a. surface or skin electrodes are placed at 4 cm between them (one on the muscle surface and the other on the tendon) and they collect data about the potentials of all muscle fibers, thus they record a *global EMG*. These electrodes are flat, they have a button- or plate-like appearance and they are applied directly on the skin.

b. needle electrodes (intramuscular) collect data on the activity of a single motor unit that is almost isolated from the potentials of the neighbouring fibers (*motor unit EMG*).

B. Amplifier system produces an increase in the order of tens of microvolts and is capable of selective amplification, i.e. it only amplifies the electrode current and removes the currents coming from remote elements.

C. Display system: oscilloscope, chart recorder, audio for collected muscle potentials; provides data about shape, amplitude, duration and frequency of biocurrents (figure 5.3).

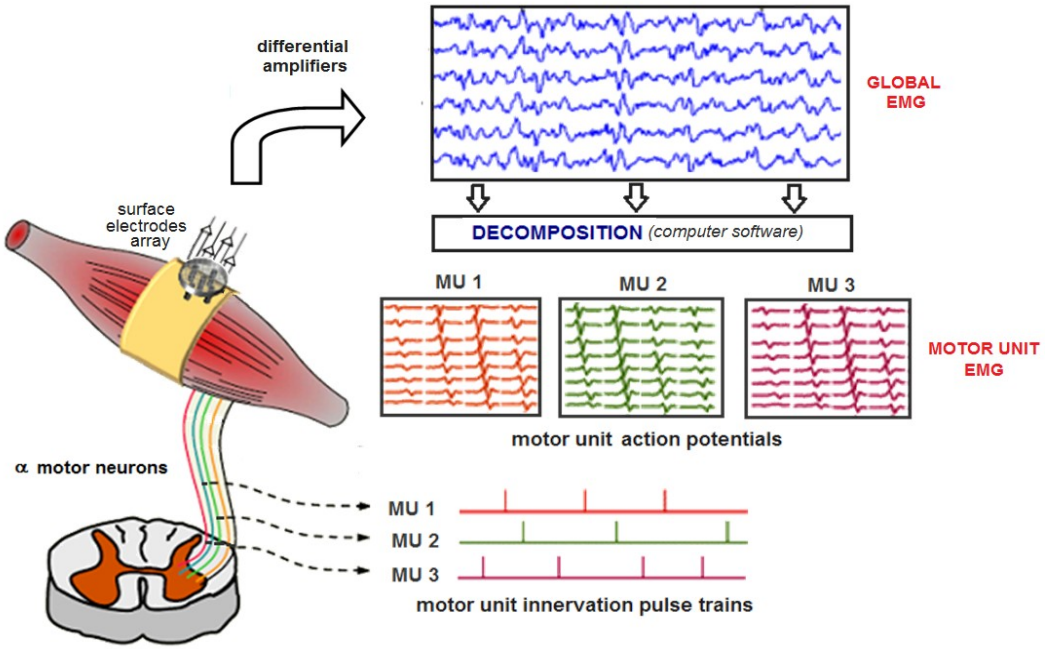


Figure 5.3.
The diagram of generation, detection (left), acquisition (top) and decomposition (bottom right) of multichannel surface electromyography signals.

Technique:

- patient in restful position (spontaneous movements can disturb the measurement) at a comfortable room temperature (23^0, to avoid shivering or sweating), in a faraday room
- the electrodes are placed on the skin / inserted into muscle and then are connected by cables to the myograph
- the muscle electrical activity is recorded in different conditions: during rest or graded voluntary effort in agonist / antagonist muscles (contraresistence)

- the trace obtained depends on the number /size of activated motor units of investigated muscle.

Normal EMG (figure 5.4):

A. at rest *no bioelectric activity* is detected ("electric silentium" state, A), except for:
   - the insertion activity when a needle electrode is inserted into a muscle and it is recorded a discharge of action potentials of short duration / small amplitude. The insertion activity is prolonged in denervated muscles and absent when muscle is not viable.
   - end plate noise is recorded as a negative monophasic potential after the cessation of insertion activity.

B. during minimal voluntary contraction, a few motor units discharge: some action potentials are recorded – *simple recording* (B)

C. with progressive increase of voluntary effort, the firing rate of small units increases until it reaches a certain frequency, when larger units are recruited giving a moderate force

of contraction - *intermediary recording* (C, many peaks, butt distinguishable from one another)

D. during maximal contraction, most of the motor units discharge and a very high number of potentials are recorded; the graphic components belonging to one particular motor unit cannot be distinguish - *interference recording*(D)

E. during maximal contraction against resistance, a sinusoidal interference pattern appears – synchronous activity of spinal motor neurons – Piper rhytm (E)

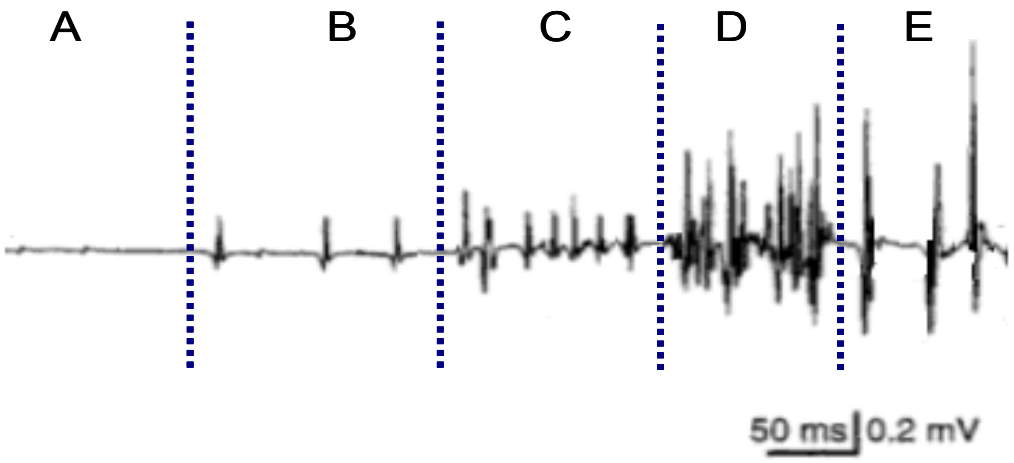


Figure 5.4. Types of electromyographic recording (A - isoelectrical activity, B – simple recording, C – intermediary recording, D – interference recording, E - Piper rhytm)

Clinical significance of EMG:

Electromyography is a useful tool for nerve and muscle function in order to:

- determine extent of disease and monitor progress
- measure dysfunction and propose solutions
- study normal anatomy and physiology

Usually electromyography is used to differentiate myopathies and neuropathies.

Clinical applications of electromyography:

- diagnosis of neuromuscular diseases (neuropathy, myopathy);
- evaluation, diagnosis in work medicine and ergometry studies (to measure muscle force exerted during physical activity);
- in sports medicine to measure muscle strength and onset of fatigue during exercise;
- in rehabilitation and physiotherapy to diagnose and monitor neuro-muscular disease

The parameters of muscles potential could be modified by a primary injury in the muscle fiber, by a motor neuron or motor nerve injury, or by local metabolism alterations. The pathological aspects of the EMG may be classified in myogenic or neurogenic recordings, and those generated by endocrine-metabolic disorders (figure 5.5):

- **neurogenic trace** occurs in case of spinal motor neuron (central neurogenic path) or motor nerve (peripheral neurogenic path) diseases such as radiculitis, neuritis or trauma. It is characterized by: the occurrence of action potential while at rest; the occurrence of greater amplitude and duration potentials both at rest and during contraction.
- **myogenic trace** occurs in primary muscle fiber diseases such as myasthenia, myodystrophy, etc. It is characterized by: the absence of electric activity while at rest or interference trace with polyphasic potentials of lower amplitude and duration, in addition to specific electric manifestations like *myotonic discharges* and *myasthenia*

- EMG trace in **endocrine-metabolic disorders** like spasmophilia. It is characterized by: repetitive double or triple electric potentials at rest, or potentials occurring spontaneously or only after path activation by compressing the limb in question using the sphygmomanometer band at mean blood pressure, for 10 min, in addition to other 5 minutes of hyperpnea.

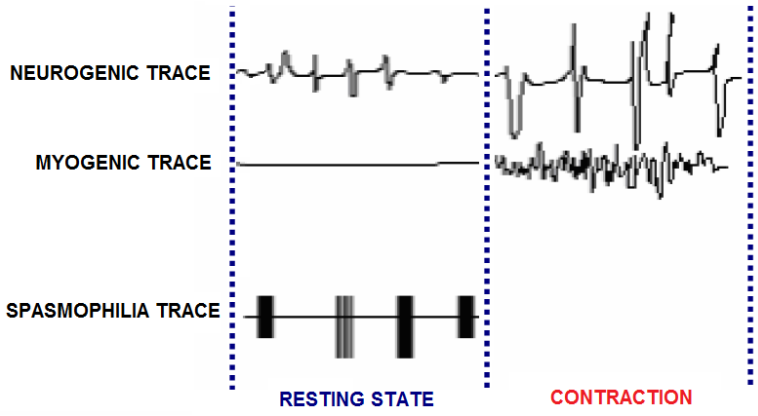


Figure 5.5. Pathological aspects of elecetromyographic recordings

V.2. Physiology of the peripheral nervous system

V.2.1. Relative infatigability of nerve

The nerve is not the first site of the fatigue and this can be demonstrated on the next experiment:

- Two frog nerve muscle preparations (sciatic nerve and gastrocnemian muscle) named N1 and N2 are fixed in such manner that both can be stimulated simultaneously;
- A piece of ice is kept on nerve just before the neuromuscular junction on the N2 preparation: it blocks the conduction of stimulation on the nerve itself;
- Both, N1 and N2 are stimulated till the N1 muscle does not contract i.e. the muscle is fatigue;
- The ice is removed from N2 and again both preparations are stimulated;
- N1 will not respond, but N2 respond.

In intense physical effort muscular fatigue is due to the events at the synapses in the central nervous system. This is considered to be a protective phenomenon. The *central fatigue* is manifested before there is any block at neuro-muscular junction and long before the muscle completes fatigued.

Fatigue: compared to the nerve, when repeatedly stimulated, muscle looses its excitability, become gradually less excitable and ultimately stops to respond (figure 5.6).

Fatigue can be demonstrated on nerve muscle preparation by applying repeated stimuli over a prolonged period. Initially, for the first few contractions, staircase phenomenon (treppe) is observed (curves 1, 2, 3 due to the beneficial effect). Following contractions gradually contracture is observed: the muscle contracts but fail to relax; the contraction period become smaller and the relaxation period are progressively increased (early sign of fatigue).

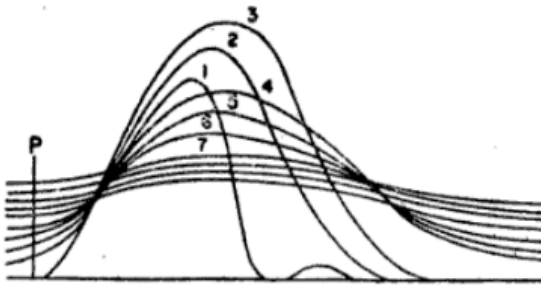


Figure 5.6. Graph showing the effect of the fatigue on frog isotonic muscle twitch

Finally, the tracing fail to return to the baseline during relaxation – this is called contraction reminder (traces 4-7). It occurs due to a decrease in ATP content and accumulation of the metabolites in the muscle.

Causes of fatigue: lack of energy in muscle; accumulation of lactic acid in the muscle; depletion of acetylcholine (neurotransmitter of neuromuscular junction); raised intramuscular tension hampers passage of blood through muscle.

- *The first site of the fatigue in this experiment is neuromuscular junction*.
- *The muscle is not the first site of the fatigue* because the direct stimulation of the muscle gives a response even after the fatigue is obtained when the muscles is stimulated through the nerve.

V.2.2. Electronervogram

Definition: Electronervogram or compound action potential (CAP) is the algebraic sum of individual "all or none" action potentials produced by all the fibers (motor and sensory) from a mixed (large, compound) nerve that were fired up by an electric stimulus.

What is the difference between nerve and neuron? (*see lecture*)

The axons within a mixed nerve include afferent (sensory) fibers and efferent (motor) fibers. Individual axons vary in diameter, myelination, excitability, threshold and conduction speed (Table 3.1.)

The CAP is *not* a naturally occurring phenomenon. It is elicited experimentally or clinically, by stimulating the whole nerve (e.g. the sciatic), with *extracellular stimulating electrodes* and is recorded, using *extracellular recording electrodes*, which measure the summed electrical response of all the excited axons or dendrites in the nerve.

Principle: graphical recording with extracellular electrodes of compound action potential from a mixed nerve (frog sciatic nerve)

Materials required: isolated sciatic nerve preparation, a stimulation set up (stimulator, amplifier, software for data acquisition and processing, lab computer) and a nerve chamber with electrodes, Ringer's solution.

Technique:

- obtain the isolated sciatic nerve using the same procedure from the sciatic nerve preparation (section 3.1.), cut the nerve and mount it into the nerve chamber; prepare the stimulation set-up (figure 5.7)

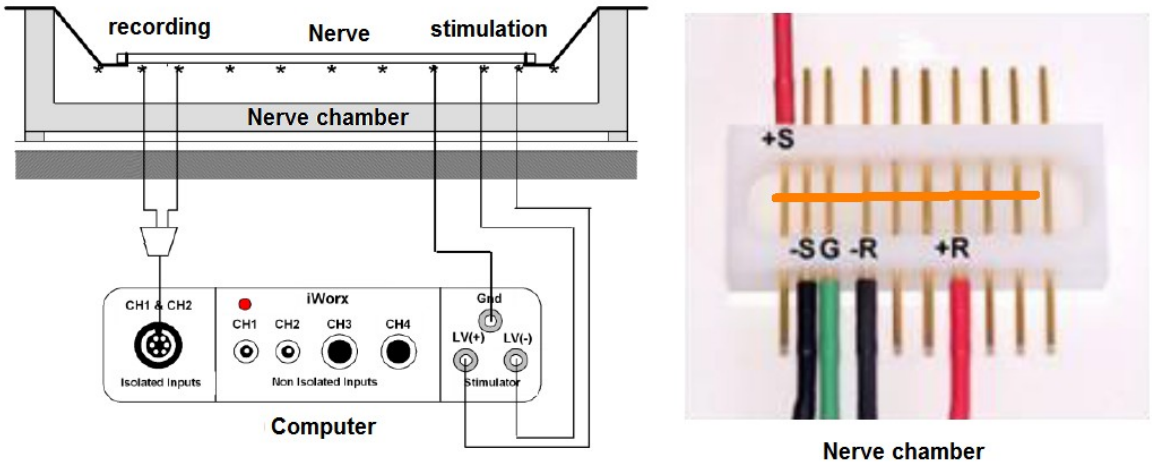


Figure 5.7. Experimental set-up for recording compound action potential on frog sciatic nerve.

- stimulating electrodes are placed to one end of the nerve; recording electrodes are located along the nerve. If the nerve is stimulated with a current pulse of sufficient amplitude, the action potentials produced in the fibers propagate toward the recording electrodes. The cumulative effect of the many action potentials is an extracellular wave of negative potential, moving along the surface of the nerve;
- if for the recording of CAP *two electrodes* are used it will be obtained a positive and negative deflection called *dysphasic CAP* (figure 5.8):
  - when the nerve is inactive there is no potential difference between the electrodes and the trace is at baseline;
  - when the CAP arrives at the proximal electrode it becomes transiently negative (relative to the distal electrode) and the trace is an upward deflection
  - when the CAP is in between the proximal/distal electrodes there is no potential difference and the trace returns to baseline;
  - when the CAP arrives at the distal electrode it becomes negative (relative to the proximal electrode) and the trace is a negative deflection;
- if *one electrode* remain active it will be obtained a positive deflection called *monophasic CAP*. That means the CAP reaches the first recording electrode, but does not reach the second recording electrode because the propagation of CAP was blocked between the two recording electrodes by mechanical methods (pressure applied to the nerve or crushing the nerve with forceps) or chemical methods (applying potassium chloride, local anaesthetics, cocaine, or tetrodotoxin to the nerve) (figure 5.8).

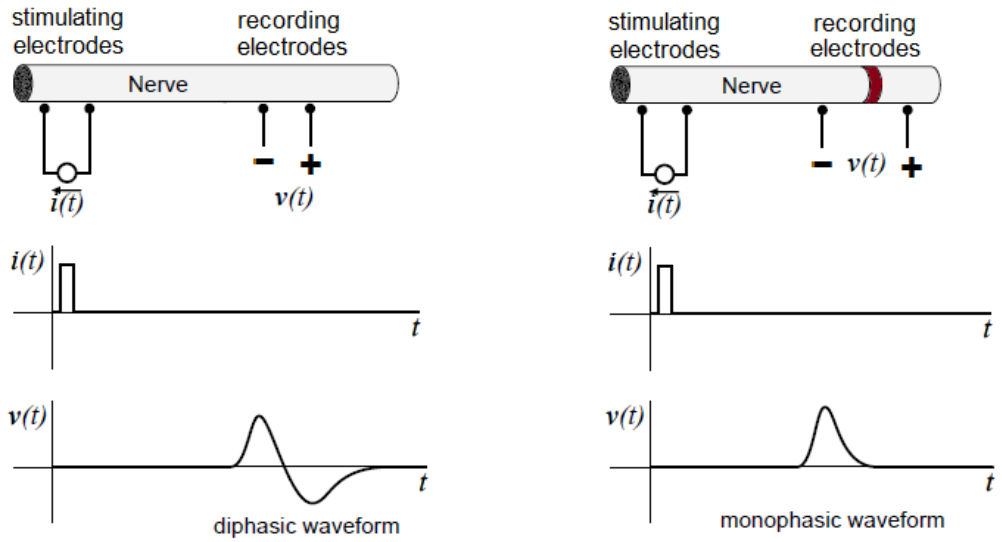


Figure 5.8. Extracellular recording of compound action potential as dysphasic or monophasic wave.

- if a maximal stimulus is applied to the nerve, all the fibers will be depolarized and the CAP will display a number of peaks, each corresponding to the group of axons with different velocities of conduction (figure 5.9).

Interpretation:

The CAP differs from individual AP of a single axon in several ways:

- the individual AP demonstrated by intracellular recording from a single axon obeys "all or none" law;
- the CAP demonstrated by extracellular recording from many axons, is a graded response whose magnitude increase with the intensity of stimulation, because different axons have *different thresholds of excitation.* The largest axons have the lowest threshold, i.e., they are the most excitable ($A\alpha > A\beta > A\gamma > A\delta > B > C$). At low stimulus intensities, only the larges axons are activated, but as the stimulus intensity is raised in steps, many small

axons are progressively recruited and many peaks will appear on CAP trace. The conduction velocity of individual action potentials increases with axon diameter, thus, AP of largest axons will reach the recording electrodes first. (figure 5.9, table 5.1).

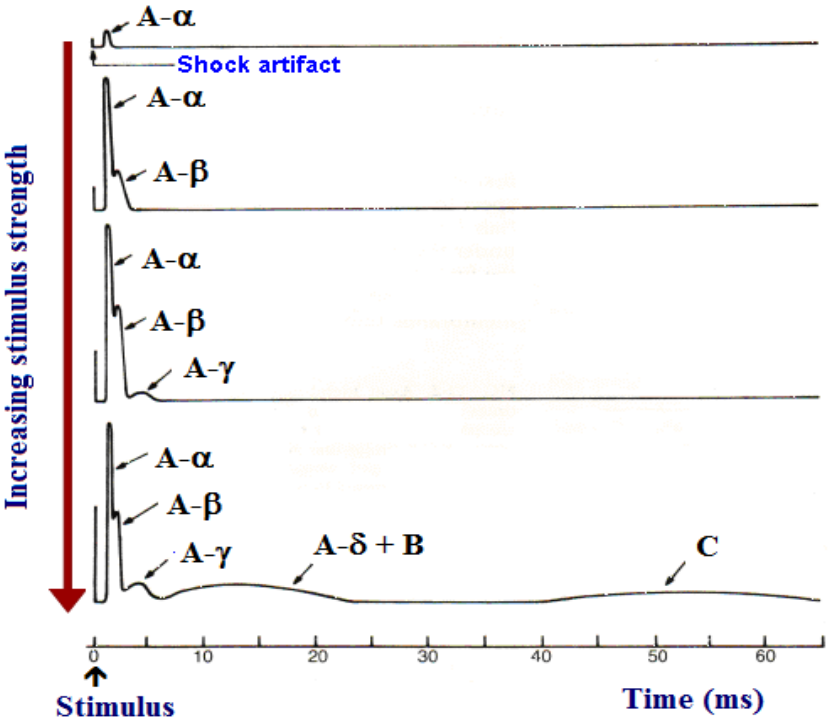


Figure 5.9. Compound action potential (CAP) traces of the sciatic nerve determined electrical stimuli of increasing intensity applied to the nerve. The various components of the CAP are labelled where they appear.

Group / Type		Myelination	Diameter µm	Conduction velocity m/s	Function	Agents to which conduction is most susceptible
Erlanger & Gasser	Lyod & Hunt					
Aα	Ia, Ib	yes	13-20	70-120	Motor supply to skeletal muscles, Proprioception (Ia - sensory fibers, afferents of muscle spindles; Ib – afferents of Golgi)	pressure
A β	II	yes	4-13	25-70	Touch, pressure, kinesthetic sense, (secondary afferents of muscle spindles-innervate nuclear chain only)	pressure
A γ	-	yes	3-6	15-30	Motor supply to intrafusal fibers	pressure
A δ	III	yes	1-5	5-30	Pain, temperature, pressure, touch	pressure
B	-	yes	1-3	3-14	Preganglionic autonomic fibers	hypoxia
C	IV	no	0.2 - 1	0.2 - 2	Pain, temperature, pressure Postganglionic autonomic fibers	Local anaesthetics

Table 5.1. Classification of nerve fibers according to Erlanger & Gasser and the corresponding group in the classification of Lyod & Hunt (Lyod & Hunt consider only sensory fibers).

Practical importance of recording CAP:

- basic science: to find out how our nerves operate
- science applied to clinical problems: in nerve conduction studies, to detect/analyze nerve malfunction in patients suffering from neurological disorders.

V.2.3. Nerve conduction velocity test

Definition: A nerve conduction study (NCS) is a test commonly used to evaluate the conduction of electrical impulses on the motor and sensory nerves of the human body. *Nerve conduction velocity test (NCV)* measures how quickly electrical impulses move along a peripheral nerve. It is often done at the same time as an electromyogram.

The principle of NCV is based on applying an external stimulation at one point of the nerve and record the signal from another. For interpretation of the results it is required to know the anatomical path of the peripheral nerve, the muscle supplied by the nerve, the normal conduction velocity of the nerve, the physiologic basis of the conduction of action potential in the nerves, patophysiologic response of the nerve and muscle to disease.

The NCV depends on the: fibre diameter (varies direct proportional), degree of myelination (myelinated fibers > unmyelinated fibers), and the intermodal distance (varies direct proportional) (see table 3.1).

Factors that affect NCV:

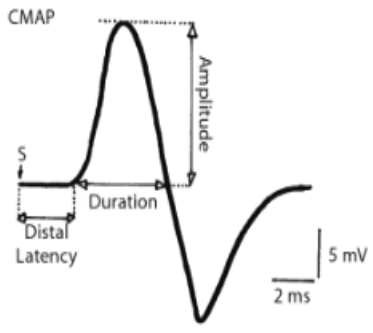
- *temperature*: NCV varies is directly related with the internal body temperature
  - NCV increase with 5% per per degree Celsius rise of body temperature from 30^0 to 40^0C;
  - for each degree Celsius fall in temperature, the latency increases by 0.3ms and velocity decreases by 2.4 m/s;
  - the effect of temperature on NCV is attributed to the sodium channels in the nerve fibres membrane.
- *age:* NCV is low in infants and children up to 2 years. It attains the adult value by 3 to 5 years of age, then remains stable until 60 years of age, after which it starts to turning down at a rate of 1.5 per decade.
- *height:* NCV varies inverse proportional with height of individuals because shorter nerves conduct faster than the longer nerves on the same age of groups.
- *limb:* in the upper limb NCV is higher compare to the lower limb due to shorther intermodal distance, progressive reduction in axonal diameter, lower temperature in the lower limbs.
- *gender*.

V.2.3.1. Motor nerve conduction velocity

Definition: Motor nerve conduction velocity study evaluates the conduction of electrical impulses along the motor fibres in the nerves.

Principle: is based on applying an electric stimulus at one point of the studied nerve that initiates depolarisation of all motor fibres and record the response of the innervated muscles fibers (compund action muscle potential, CMAP).

The measurement of the nerve conduction study includes the onset of latency, duration and amplitude of muscle compund action potential and nerve conduction velocity.



Materials required: stimulator, stimulating and recording electrodes, amplifier, osciloscoppe / PC monitor, electrode jelly, alcohol.

Technique (figure 5.10)*:*

- disinfect the skin with alcohol at proximal and distal end;
- apply the stimulating and the recording electrodes in proper points;
- connect the electrodes with amplifier and oscilloscope;
- stimulate the nerve with a supramaximal stimulus first at the wrist end (1st site of stimulation) and visualise the CMAP; note the latent period (latency 1);
- than stimulate the nerve a the elbow and record CMAP; note the latency 2;
- the difference between the two latent period give the time taken bythe impulse to travel from the elbow to the wrist;
- measure the distance between the two points of stimulation;

Results and discussions:

- calculate the motor conduction velocity in metre per seconds (m/s) by the formula distance / difference in latent period.

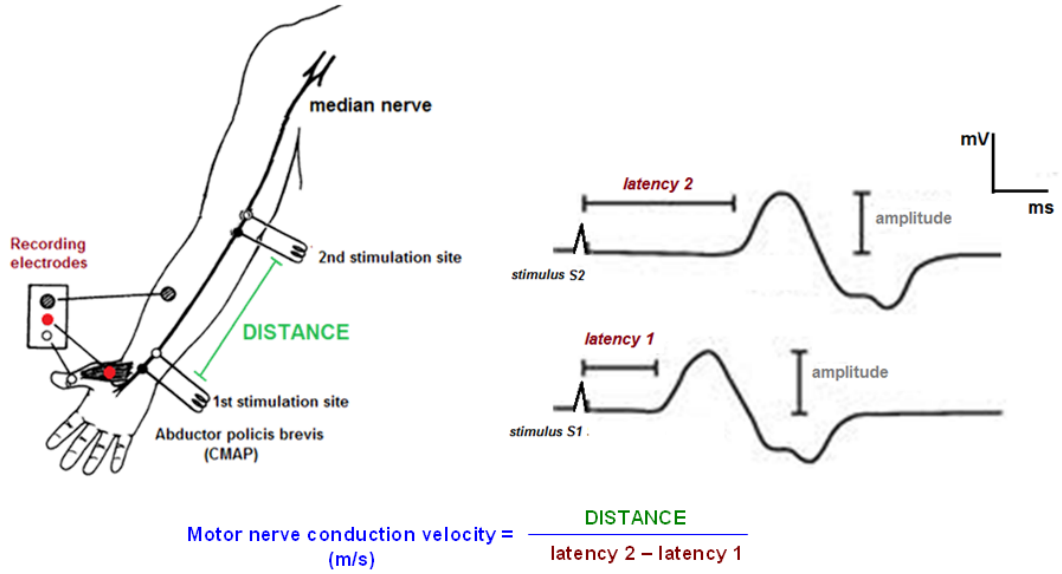


Figure 5.10. Motor nerve conduction velocity diagram.

- *the CMAP onset latency and motor nerve conduction velocity* provides information about myelin sheat surrounding the nerve fibres. With loss of myelin thickness nerve conduction is slowed and, if severe enough, saltatory conduction fails (conduction block). *Demyelinating disorders* determine prolonged motor latencies and slow conduction;
- *the CMAP amplitude and morphology provides inforation on motor nerve axons.* Since myelin is unaffected, the remaining axons conduct normally and one would expect latencies and conduction velocities to remain normal. However, with increasing motor axon loss some of the largest fastest conducting fibres will be lost. Therefore distal motor latency may be slightly prolonged (< 120% of normal limit) and conduction velocity slightly slowed (> 80% of normal limit).

V.2.3.2. Sensory nerve conduction velocity

Definition: Sensory nerve conduction velocity study evaluates the conduction of electrical impulses along the sensory fibres in the nerves.

Principle: Sensory nerve conduction velocity can be measured orthodromically or antidhromically (figure 5.11):

- in *orthodromic conduction*, a distal portion of the nerve is stimulated and sensory nerve action potential (SNAP) is recorded at a proximal point along the nerve (stimulation away from the sensory recptors);
- in *antidromic sensory nerve conduction*, the nerve is stimulated at a proximal point and nerve action potential is recorded distally (stimulation towards sensory receptors);
- Amplitude of the SNAPs are generally higher in the antidromic potentials.

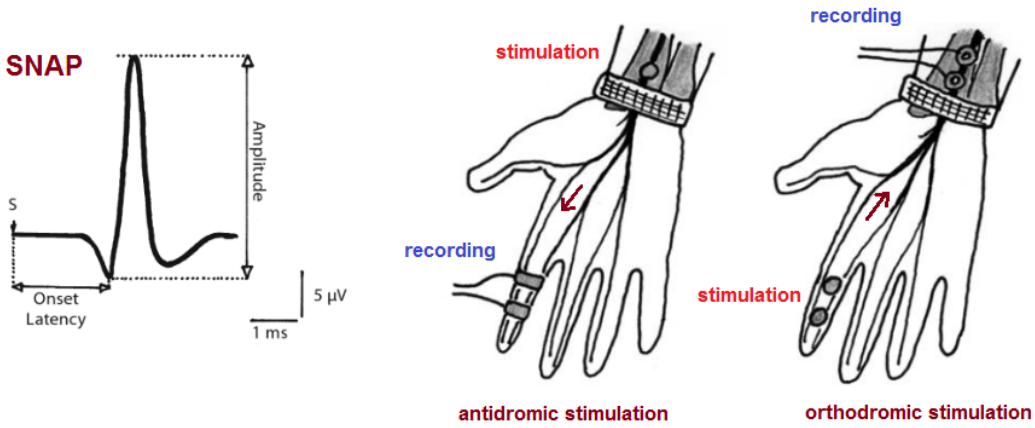


Figure 5.11. Sensory nerve conduction velocity diagram

Technique: the same like in motor nerve conduction studies
Results and discussions:

The sensory nerve is in the dorsal root ganglion. If the lesion is proximal to the sensory ganglion (preganglionic lesion, e.g. radiculophaties), the sensory neurn is not affected and its distal axon is still intact. So, sensory motor ve,ocity is normal even though clinocal sensory may be abnormal.

A lesion distal to the dorsal root ganglion (postganglionic lessions) affect either sensory neuron or distal axon or both (e.g. plexophathy, ulnar neuropathy, peripheral polineuropathy):

- ↓ SNAP amplitude or SNAP absence imply
- ↓ velocity / ↑ latency

V.2.4. Hoffman reflex (H-reflex)

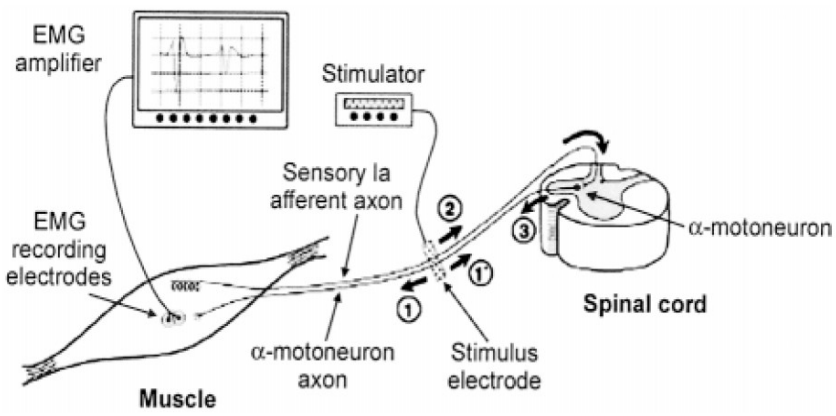
Originally described by Paul Hoffmann in 1910, the Hoffmann reflex (*H-reflex*) is an electrically induced reflex analogous to the mechanically induced spinal stretch reflex.

It is elicited by selectively stimulating the *sensory (Ia) fibres of the posterior tibial or median nerve.* The stimulus travels along the Ia fibers (2 in figure 5.12), through the dorsal root ganglion, and is transmitted across the central synapse to the anterior horn cell which fires it down along the alpha motor axon to the muscle (3 in figure 5.12).

The H-reflex test is performed using an electric stimulator that apllies ***subthreshold electric stimuli***. The response is usually a clear wave, called H-wave or late response (the highest amplitude is obtained with a sub-threshold stimulus).

As previously mentioned, the H-reflex is analogous to the spinal stretch reflex. The only difference between the 2 reflexes is that the spinal stretch reflex is induced after a muscle stretch, whereas the H-reflex is the result of electric stimulation. The pathway for the H-reflex and stretch reflex is the same.

Amplitude may be decresed by afferent or efferent problems: e.g. neuropathy or radiculopathy (e.g. S1 radiculopathies – posterior tibial H reflex).



The H-reflex has been used in athletic training-related research to evaluate musculoskeletal injuries, effects of therapeutic modalities, and pain.

Figure 5.12. Hoffman reflex diagram

If a *mixed nerve* is stimulated with *suprathreshold stimuli* the wave of depolarization spreads in both directions from the site of stimulation (figure 5.13):

- there are orthodromically directed action potentials that may cause a response in the muscle (CMAP – ***M wave***);
- antidromically directed action potentials will pass proximally towards to the motor neurons in the spinal cord where it triggers a backfiring of a small andd variable subpopulation of these cells. So, the second orthodromic directed action potentials will pass down the nerve causing second low- amplitude CMAP called – ***F wave.*** Therefore the F-wave involves only neuromuscular junction synapses and depends on the integrity of the whole axons.

As the stimulus strength increases *(sub-maximal to sura-maximal),* the amplitude of the *M-wave increases*, the *H-wave will disappear*, and *will appear F-wave*.

Thus, F waves allow testing of proximal segments of nerves that would otherwise be inaccessible to routine nerve conduction studies, e.g. brachial plexopathy or thoracic outlet syndrome.. F waves test long lengths of nerves whereas motor studies test shorter segments. In conclusion, F wave abnormalities (delay or absence of F-wave) can be a sensitive indicator of peripheral nerve pathology, particularly if sited proximally.

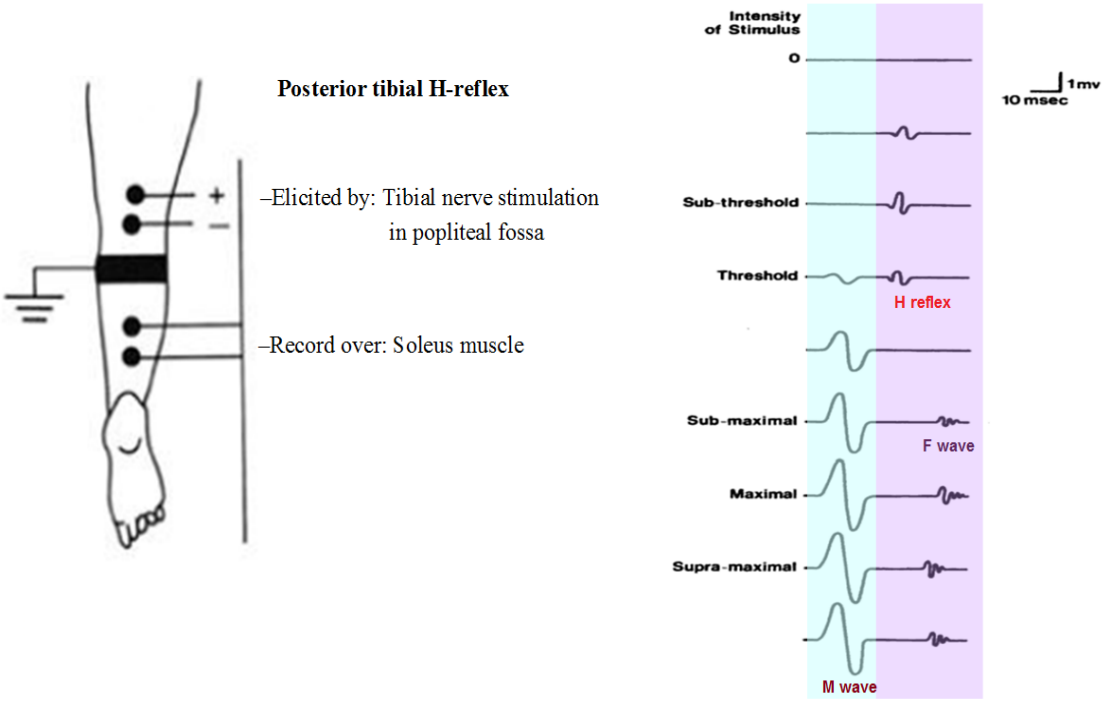


Figure 5.13. M, H, F wave diagram

V.3. Physiology of the central nervous system

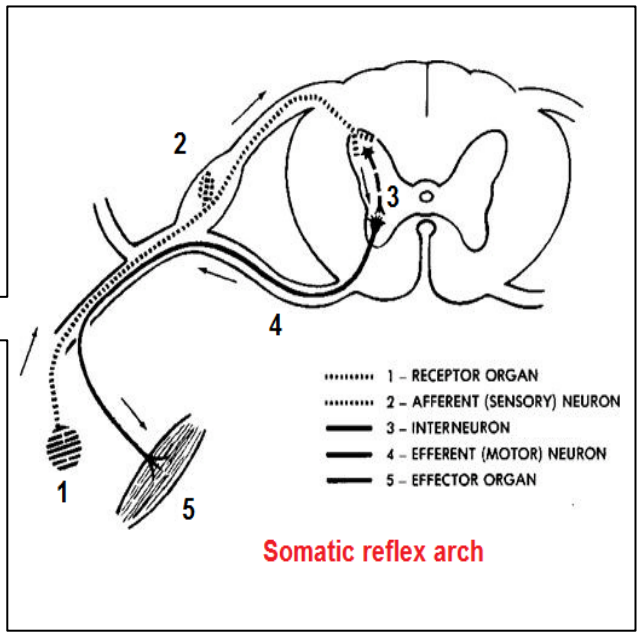
V.3.1. Reflex function of the spinal cord.

Reflex is an automatic response of the body to the reception of a stimulus. Anatomical support of reflex actions is the *reflex arc*.

Reflex arc comprises the neural pathway that mediates a reflex action; most sensory neurons do not pass directly into the brain, but synapse in the spinal cord, this allows quick reactions.

Reflex arc has 5 components:

1. *receptor* organ specific to a certain stimulus;
2. *afferent pathway* of the appropriate peripheral nerve that transmit s information to the central nervous system;
3. *nervous centre* - within the spinal cord, the afferent neuron synapses with a special connecting neuron called interneuron; the information is analysed and a response is elaborated;
4. *efferent pathway* carries the response to the effector organ;
5. *efector* (skeletal muscle, smooth muscle, glands) receives the command to act.



Types of reflexes

Reflexes may be classified in 4 ways:

1. **The complexity of the reflex arc:**
   - *monosynaptic reflex*:
     - sensory neuron synapses directly onto motor neuron;
     - example: peripheral muscle reflexes or deep tendon reflexes (patellar reflex, achilles reflex);
     - high conduction velocity, short latency, without extension (i.e. no irradiation, increase in stimulus intensity increases only the amplitude of the response)
   - *polysynaptic reflex*
     - has at least 1 interneuron between the sensory neuron and the motor neuron;
     - example: tendon reflexes, withdrawal reflexes (flexor reflex), crossed extensor reflexes;
     - longer response time, response proportional with the intensity of stimulus - Pfluger's laws

2. **The site of information processing:**
   - *spinal reflexes:* nervous center is in the spinal cord;
   - *cranial reflexes:* nervous centers are in the brainstem (bulb, pons cerebri), cerebellum, mesencephalon, subcortical or cortical

3. **How the reflex was developed:**

Unconditioned	Conditioned
- Innate reflexes are the basic neural reflexes a person is born with; they are present at birth: swallowing, breathing, etc	- Acquired by organism throughout his life. They are absent at birth.
- Are species characteristics	- Individual one
- Are constant, non-changed	- Changeable, may appear and disappear
- Are realized at the level of spinal cord, brainstem, subcortical nuclei	- Are formed by subcortex,but realized by cortex
- Provide organism's living after birth and then constitutes the base of the developemnt of conditioned reflexes development	- Play a major role in organism adaptation to environemental conditions

Classical conditioning represents a form of associative learning that was first demonstrated by Ivan Pavlov. It involves association of a neutral stimulus along with a stimulus of some significance:

- the neutral stimulus (conditioned stimulus) could be any event that does not result in an clear behavioral response from the organism;
- presentation of the significant stimulus (unconditioned stimulus) necessarily evokes an innate, unconditional response
- if the two are repeatedly paired, eventually the two stimuli become associated and the organism begins to produce a behavioral response to the neutral stimulus.

This is called the conditioned response.

4. **The nature of the resulting motor response:**
   - *somatic reflexes* provide involuntary control of the nervous system and in which the effectors are skeletal muscles
     - *superficial reflexes* of the skin or mucous membranes
     - *stretch reflexes* (deep tendon reflexes) such as the patellar reflex
   - *visceral reflexes (autonomic reflexes)* control systems other than the muscular system.

Pflüger's laws of reflexes

Principle: Pflüger's laws of reflexes demonstrate reflex activity of the spinal cord upon isolation from higher areas of the central nervous system. For this the response to progressively increasing intensity stimuli it is examined on a brainless frog with intact spinal cord.

Materials required: frog, dissection kit, vertical support with a wire and hook, acetic acid solutions (1; 2.5; 5; 15; 96%), timer, Berzelius glasses with Ringer solution.

Technique:

- open the mouth of the frog, introduce a pair of scissors in the mouth and cut the superior maxillary with the tongue and brain;
- put the hook in the inferior maxillary of the frog and suspend it into the vertical support
- apply acetic acid solution 1% on big toe of the left foot and observe the effect;
- wash the foot with Ringer solution to abolish the effect of stimulus;
- then apply the other acetic acid solutions, observe the effect and wash after every concentration.

Results (figure 5.14):

	Acid acetic concentration	**The response**	**Pflüger's law of reflex action**
I	1%	Flexion of stimulated finger	Law of localised reflexes
II	2.5%	Flexion of the foot whose finger was stimulated	Law of unilateral reflexes
III	5%	Flexion of the other foot, too	Law of reflex symmetry
IV	15%	Flexion of all feet	Law of reflex irradiation
V	96%	Contraction of all skeletical muscles of the body	Law of reflex generalisation

Finally if it is applied on the left dorsal region a pacth with acetic acid 15% and the left foot is tied on the vertical support, it is observed thet the frog will remove with right foot the nocive stimulus. This is the *law of reflex coordination (VI)*.

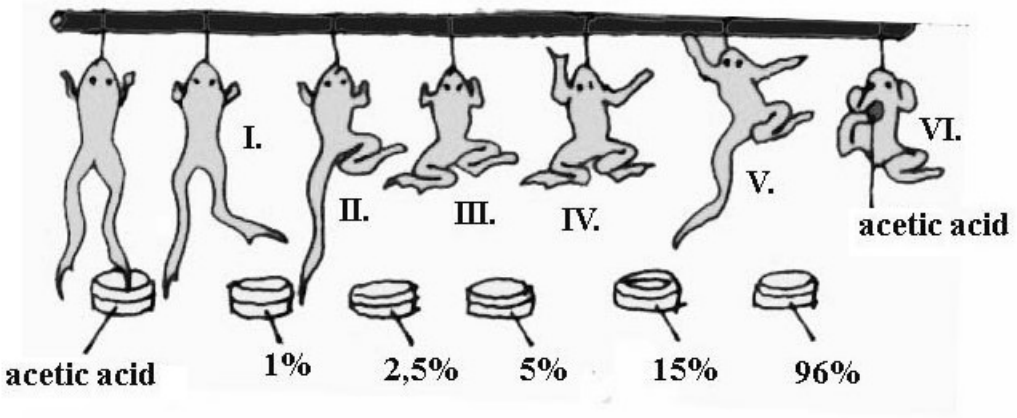


Figure 5.14. Diagram of Pflüger's laws of reflex action on frog

Discussions:

Pflüger's laws of reflex action:

I. **Law of localised reflexes:** a low peripheral stimulation will cause a small contraction limited at a few muscle under the stimulated tegument.
II. **Law of unilateral reflexes:** if a mild peripheral stimulations cause contraction in only half of the body, the contraction always occurs on the same side as the stimulus, and in general those muscles contract whose nerves arise from that segment of the cord, to which the irritated sensory nerve belongs.
III. **Law of reflex symmetry:** if the stimulation is sufficiently increased, motor reaction is manifested, not only on the irritated side, but also in similar muscles on the opposite side of the body.
IV. **Law of reflex irradiation:** if the excitation continues to increase, it is propagated upward, beyond its own segment, and responses take place through centrifugal nerves coming from the cord segments higher up
V. **Law of reflex generalisation:** highest intensity peripheral stimulation it is propagated in the medulla oblongata, which becomes a focus from which stimuli mradiate to all parts of the spinal cord, causing a general contraction of all muscles of the body (convulsive movements).
VI. **Law of reflex coordination:** the nocive stimulus is removed by coordinated movements of limbs

The brainless frog reacts more regularly to this experiment compare to one possessing the brain. This is evidence that the brain is capable of exercising a controlling influence or inhibitory effect over reflex actions.

Pfluger demonstrated the correlation between stimulus intensity and the area on which response is propagated (the number of muscles that respond to the stimulus). Thus, the higher intensity of a stimulus is applied to the receptor, the higher number of medullar nervous centers implied in the response. This is possible due to the existence of the intermediate neurons, which connect different spinal cord nervous centers and transmit the information from one to another, amplifying the response.

Pflüger's laws of reflexes are valid only for exteroceptive reflexes or nociceptive or flexion reflexes (*polysynaptic reflexes)*.

V.3.2. Somatic and vegetative reflexes of clinical relevance

Somatic Reflexes (figure 5.15)	***Autonomic Reflexes***(figure 5.16)
Sensory receptor (transducer) responds to stimuli at an exteroreceptor or proprioceptor	Sensory receptor (tranducer) responds to stimuli at an enteroreceptor or proprioceptor located in or associated with visceral organs.
Motor neuron communicates directly (one cell path) with its effector.	The motor output *two* motor neurons: pre- and post-ganglionic autonomic neurons. Motor neuron communicates indirectly (two cell path) with its effector.
Motor neuron cell body resides in brain stem or ventral = anterior horn of spinal cord.	Pre-ganglionic motor neuron cell body resides in brain stem or lateral horn of spinal cord; post-ganglionic motor neuron cell body resides in autonomic ganglion.
Somatic effector is skeletal muscle (motor units).	Visceral effector is smooth muscle or gland cells
Acetyl choline is the excitatory transmitter at the neuro-muscular junction.	Acetyl choline or norepinephrine are the transmitters at the visceral effectors and their effects may be excitatory or inhibitory, depending on the receptor type at the target tissue.
Sometimes, they are perceived consciously, after the reflex has been initiated, especially if the reflex causes a dramatic movement of a body part, such as when the hand draws back reflexively after experiencing a painful stimulus. Many somatic reflexes are involved in minor postural adjustments and those would not generally be perceived consciously.	Generally, they are not perceived consciously.

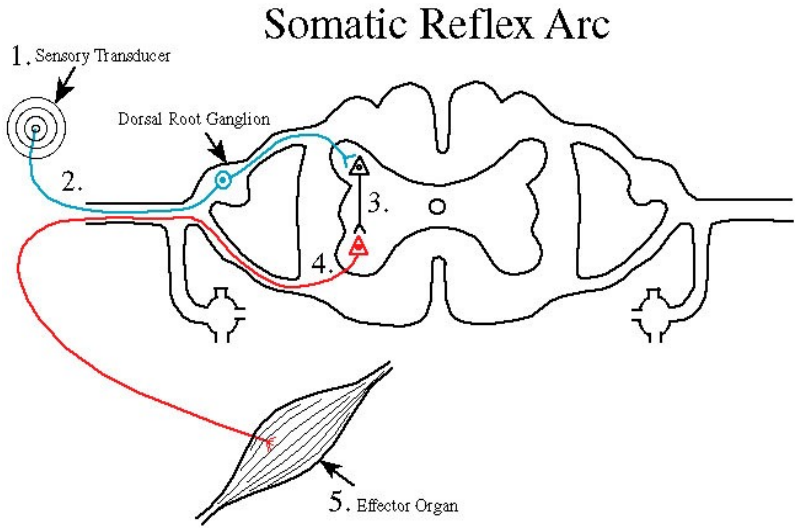


Figure 5.15. Somatic reflex arc
1 = sensory transducer (e.g. Pacinian corpuscle in the skin)
2 = afferent pathway (a dorsal root ganglion, but it could also be on a cranial nerve)
3 = interneuron (CNS)
4 = motor nuron (soma is in the ventral horn of the gray H of the spinal cord)
5 = effector (skeletal muscle)

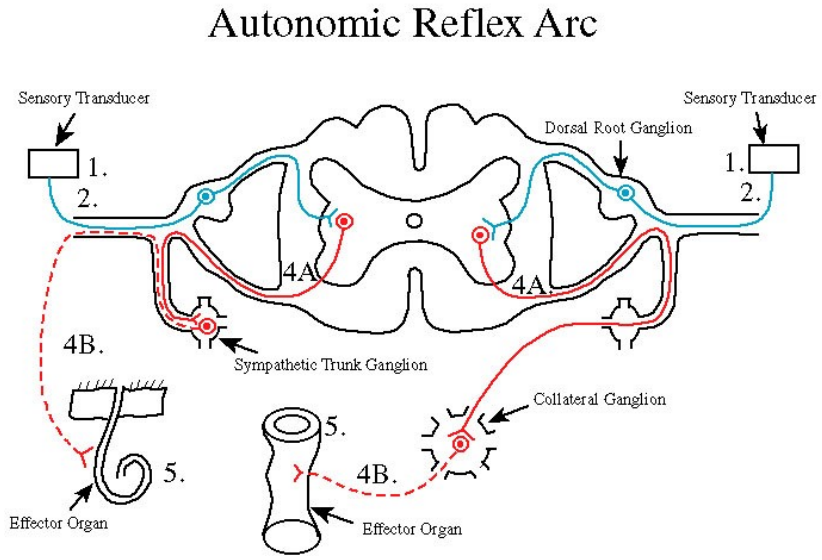


Figure 5.16. Vegetative (autonomic) reflex arc
1 = sensory transducer
2 = afferent pathway
4A = preganglionic autonomic fibres from the the spinal cord (CNS).
4B = post-ganglionic autonomic fibres from an autonomic ganglion (PNS)
5 = effector (sweat gland – left; smooth muscle of gut wall - right)

Reflex Tests

Reflex tests are simple physical tests of nervous system function by assessing the presence and strength of a number of reflexes. So, a reflex test evaluates the integrity of the reflex arc involved. Reflex tests are performed as part of a neurological exam to diagnose the presence and location of various nervous injuries.

Somatic Motor Spinal Reflexes

1. Monosynaptic Reflexes

The best known monosynaptic reflexes are **deep tendon reflexes** (myotatic reflex, stretch reflex, tendon-jerk reflex).

Principle: when you strike a slightly stretched tendon with a reflex hammer, a simple muscle contraction occurs.

Components of the stretch reflex arc (see the lecture):

Receptor	: muscle spindle.
Afferent Nerve	: Ia, II afferent fibers.
Center	: α motor neuron cells in ventral horn.
Efferent nerve	: fast conducting Aα fibers of the motor unit.
Effector organ	: extrafusal muscle fibers.

Materials required: Reflex hammer presented in figure 5.17.

Figure 5.17. Reflex hammer

Technique:

- encourage the patient to relax the arm or leg being tested.
- the tendon attached to the muscle(s) which is/are to be tested must be clearly identified. the extremity should be positioned such that the tendon can be easily struck with the reflex hammer; if you are having trouble locating the tendon, ask the patient to contract the muscle to which it is attached. when the muscle shortens, you should be able to both see and feel the cord like tendon.
- hold the reflex hammer lightly and swing it freely in an arc.
- strike the tendon with a single, quick downward stroke, then lift up on the hammer immediately (leaving the hammer on the tendon reduces the response).
- compare responses from one side to the other for symmetry of movement and muscle strength;
- *to improve leg reflexes* have the patient clench his hands together and tense the arm muscle during the reflex testing;
- *to improve arm reflexes* have the patient clench his teeth or squeeze one thigh with the hand not being evaluated;

These maneuvers force the patient to concentrate on something other then reflexes being tested, which can help to eliminate unnintentional inhibition of the reflexes.

Grade the reflexes in the following manner:

Grade	*Interpretation*	*Indication*
0	**Absent** reflex	**Pathologic;** complete loss of neuromuscular integrity; injury to a nerve root or peripheral nerve; usually indicates an injury of the *lower motor neuron* on the anterior horn of the spinal cord where the peripheral nerve originates (e.g. Guilan Barre sindron, amyotrophic lateral sclerosis); a compressed spinal *nerve root* (e.g. herniated intervertebral disk at L4/L4 cause *a symmetrical hypoactive* knee jerk reflex)
+1	**Diminished** reflex	**May be normal;** reduced neurological function; incomplete injury to a nerve root or peripheral nerve
+2	**Normal** reflex	**Norma**l neurological integrity and function
+3	**Increased** reflex, brisker than normal;	**Not necessarily pathologic;** could indicate an upper motor neuron lesion (brain or spinal cord injury)
+4	**Hyperactive** with sustained *clonus (repetitive shortening of the muscle after a single stimulation);*	**Often pathologic**; indicates an upper motor neuron lesion (damaged CNS neurons in the cerebral cortex or corticopsinal tracts prevent the brain from inhibiting peripheral reflex activity, thereby allow a small stimulus to trigger a hyperactive response). E.g. spasticity associated with spinal cord injuries; multiple sclerosis

Biceps reflex (C5, C6 – musculocutaneous nerve):

- patient's position: with the patient sitting, flex his arm at the elbow and rest his forearm on his thigh with the palm up (figure 5.18).
- identify the location of the biceps tendon in the antecubital fossa.
- place your thumb firmly on the biceps tendon in the antecubital fossa.
- strike your thumb with the hammer.
- *normal response*: the elbow and forearm should flex, and the biceps muscle should contract.

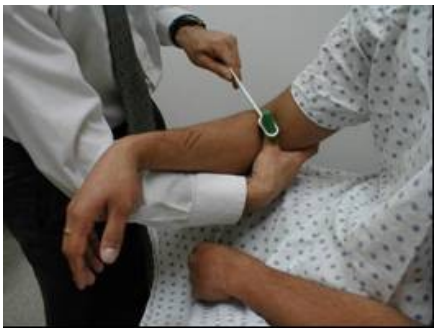


Figure 5.18 Biceps reflex

Triceps reflex (C7, C8 – radial nerve):

- patient's position: the triceps tendon is tested with the patient's arm flexed at a 90° angle.
- identify the triceps tendon, a discrete, broad structure that can be palpated as it extends across the elbow to the body of the muscle, located on the back of the upper arm. Ask the patient to extend their lower arm at the elbow while you observe and palpate in the appropriate region
- supporting the arm with your hand, strike the triceps tendon on the posterior arm just above the elbow (figure 5.19).
- *normal response*: the tendon should contract and the elbow extend (swing away from the body).

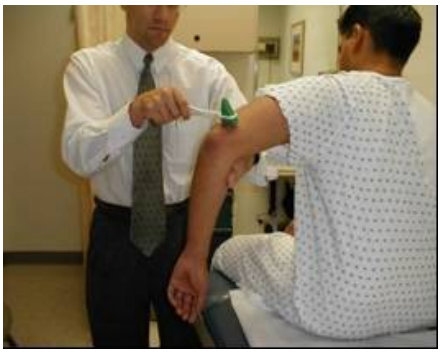


Figure 5.19. Triceps reflex

Brachioradialis reflex (C5, C6 – radial nerve):

- patient's position: slightly flexed arm on his lap with the palm facing downward (figure 5.20).
- the tendon of the brachioradialis muscle cannot be seen or well palpated, which makes this reflex a bit tricky to elicit. The tendon crosses the radius (thumb side of the lower arm) approximately 10 cm proximal to the wrist.
- strike this area with your reflex hammer.
- *normal response*: elbow flexion and supination of the forearm (the forearm should rotate laterally and the palm turn upward).

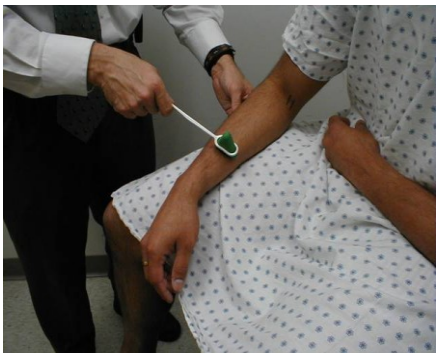
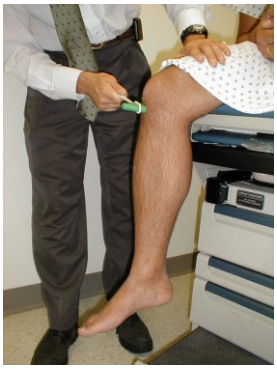


Figure 5.20. Brachioradialis reflex

Patellar reflex (knee jerk reflex, L3, L4 – femoral nerve):

- patient's position: seated, feet dangling over the edge of the exam table; if the patient must remain supine, flex each leg to a 45° angle and place your dominant hand behind his knee to support it (figure 5.21).
- identify the patellar tendon, a thick, broad band of tissue extending down from the lower aspect of the patella (knee cap). If you are not certain where it's located, ask the patient to extend their knee. This causes the quadriceps (thigh muscles) to contract and makes the attached tendon more apparent.
- strike the distal patellar tendon just below the kneecap.
- *normal response:* contraction of the quadriceps muscle with extension of the knee.



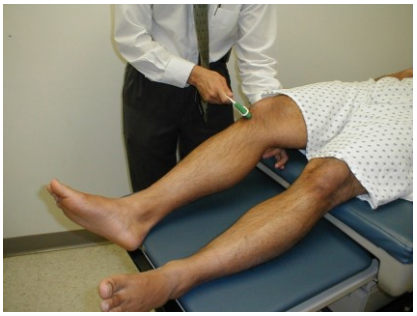


Figure 5.21. Patellar reflex

Achilles reflex (ankle jerk reflex; S1, S2 – sciatic nerve):

- patient's position:
  - seated, *legs dangling* over the edge of the exam table; with your left hand, grasp the patient's foot and pull it into dorsiflexion to find the degree of Achilles stretch that produces the optimal response. Tap the tendon directly (figure 5.22A);
  - *supine*, crossing one leg over the other or a frog-type position; Partially flex the patient's hip and knee while rotating the knee outward as far as comfort permits.With your left hand, grasp the foot and pull it into dorsiflexion, then tap the Achilles tendon directly (figure 5.22B);
  - *kneeling*: Have the patient kneel with the feet hanging over the edge of a chair, table, or bed.With your left hand dorsiflexing the foot, tap the tendon directly. Assess both the contraction and relaxation of the muscle; delayed relaxation (hung-up reflex) is characteristic of hypothyroidism (figure 5.22C).□□□
- *normal response*: plantar flexion of the foot at the ankle and contraction of the gastrocnemius.

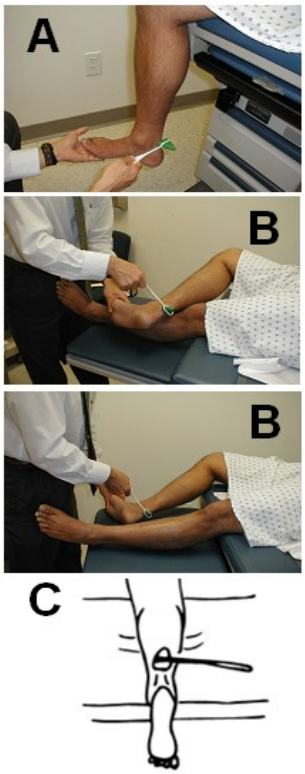


Figure 5.22. Achilles reflex

Other deep tendon reflexes:

Pectoralis Reflex (C5 – T1, medial and lateral anterior thoracic nerves).

- Support the arm in 10 degrees of elevation at 90 degrees of abduction. Place the fingers of your left hand on the patient's shoulder with your thumb extended downward pressing firmly on the tendon of the pectoralis major (figure 5.23).
- *normal response:* muscle contraction can be seen or felt

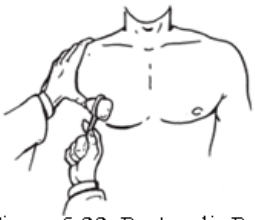


Figure 5.23. Pectoralis Reflex

Pronator Reflex (C6 – C7, median nerve).

- hold the patient's hand vertically so the wrist is suspended
- from the medial side, strike the distal end of the radius directly with a horizontal blow (figure 5.24).
- normal response: pronation of the forearm.

Figure 5.24. Pronator Reflex

Upper Abdominal Muscle Reflex T8 – T9 (figure 5.25A)
- tap the muscles directly near their insertions on the costal margins and xiphoid process

Mid Abdominal Muscle Reflex T9 – T10 (figure 5.25B)
- stimulate themuscles of the mid-abdomen by tapping an overlaid finger or doubled-tongue blades

Lower Abdominal Muscle Reflex T11 – T12 (figure 5.25C)
- tap the muscle insertion directly near the symphysis pubis

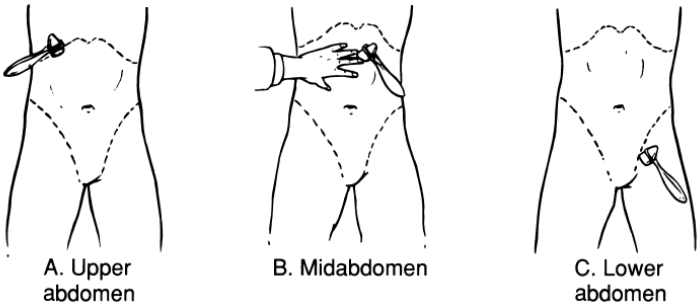


Figure 5.25. Abdominal Muscle Reflex

Adductor Magnus Reflex (L2 – L4, obturator nerve).
- with the patient supine, place the limb in slight abduction.
- directly tap the adductor magnus tendon just proximal to its insertion on the medial epicondyle of the femur (figure 5.26).
- normal response: the thigh adducts
- if the quadriceps reflex is absent and the adductor reflex is present, this indicates a lesion of the femoral nerve.

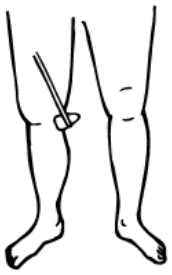


Figure 5.26. Adductor magnus reflex

Hamstring Reflex (L4 – S2, sciatic nerve).
- Have the patient supine with hips and knees flexed at about 90 degrees and the thighs rotated slightly outward.
- Place your left hand under the popliteal fossa so the index finger compresses the medial hamstring tendon (a bundle of tendons fromsemitendinosus, semimembranosus, gracilis, sartorius) (figure 5.27).
- Tap your finger.

Figure 5.27. Hamstring Reflex

- normal response: flexion of the knee and contraction of the medial mass of hamstring muscles.
- Test *the lateral hamstrings* in a similar manner: with your finger compress the lateral hamstring tendon just proximal to the fibular head and tap your finger. The normal response is contraction of the lateral hamstring mass (biceps femoris) and flexion of the knee.

2. Polisynaptic Reflexes

1. The Tendon Reflex (inverse stretch reflex) prevents skeletal muscles from developing enough tension to tear or break the tendon.

Reflex arc:

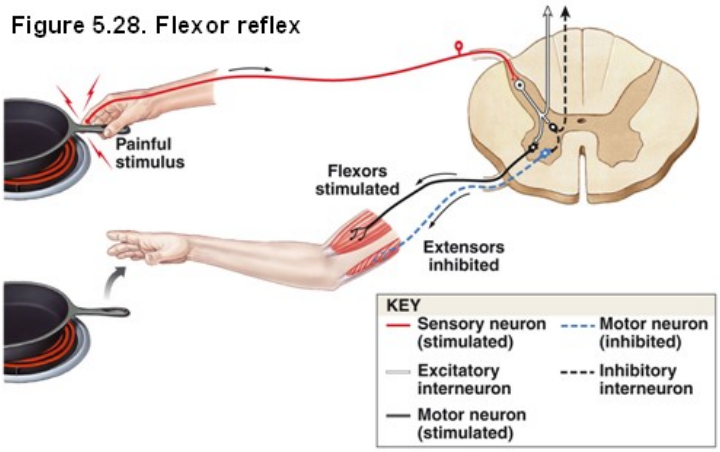
Receptor : Golgi tendon organ.
Afferent : Ib afferent nerve.
Center: Inhibitory interneurons at the same segment of spinal cord.
Efferent: α motor Neuron (inhibition).
Effector organ: stretched skeletal muscle.
!!! Difference between the Golgi tendon organ and the muscle spindle (*see the lecture)*

2. Withdrawal Reflexes move a body part away from a nocive stimulus such as pain or pressure.

2a. Flexor reflex *(see the lecture)*

- elicited by noxious stimulus of skin, subcutaneous tissue or muscles and results in withdrawal of the affected limb away from the stimulus by stimulation of epsilateral flexors and inhibition of extensors; this reflex can be suppressed by conscious effort
- *reflex arc* (figure 5.28):
  - Receptor: Nociceptors (pain receptors in the skin).
  - Afferent pathway: Flexor reflex afferents
  - Center: excitatory interneurons "to anterior horn cells of flexors" and inhibitory interneurons "to those of the extensors".
  - Efferent pathway: α motor neuron to flexor muscle "stimulated" and α motor neuron to extensor muscles "inhibited"

Figure 5.28. Flexor reflex

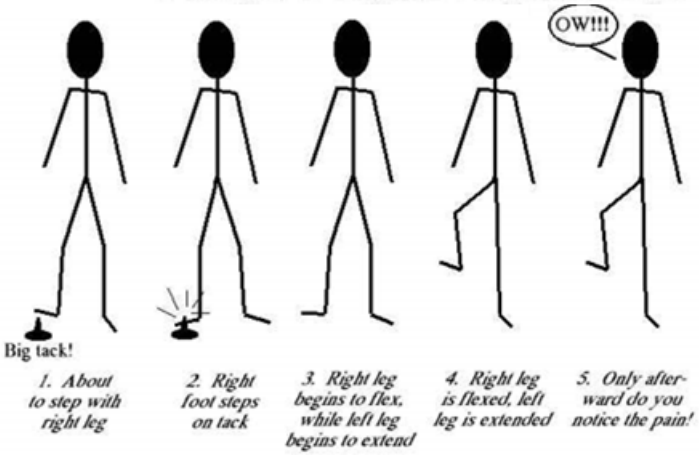


  - Effector: Skeletal muscles "flexors stimulated and extensors inhibited"
- There are 3 possible circuits for signals of the flexor reflex "withdrawal reflex":
  - Diverging circuits spreading the reflex into necessary muscles for withdrawal.
  - A Circuit that inhibits antagonistic muscle "reciprocal inhibitory circuit"
  - A circuit that causes after discharge (continuous discharge after the stimulus is over).

2b. Crossed extensor reflex *(see the lecture)*

- *stretch, tendon and flexor "withdrawal" reflexes* are ipsilateral reflex arcs, occurring on the same side of the body as the stimulus.
- A crossed extensor reflex occurs on the side opposite the stimulus (contralateral reflex arc): when a strong stimulus is applied to the limb, the response includes extension of the contra lateral limb, stimulation of extensors and inhibition of flexors; this results from irradiation of impulses in the spinal cord and serves to support body posture upon retraction of one limb. The flexion-withdrawal reflex is rather similar to stepping (figure 5.29).

Figure 5.29. Function of Crossed Extensor Reflex



- *The crossed extensor reflex* occurs simultaneously and in coordination with a flexor reflex. *E.g.* when the flexor reflex causes one leg to pull up, the crossed extensor reflex straightens the other leg to receive the body's weight, using reverberating circuits to maintain the response (figure 5.30).

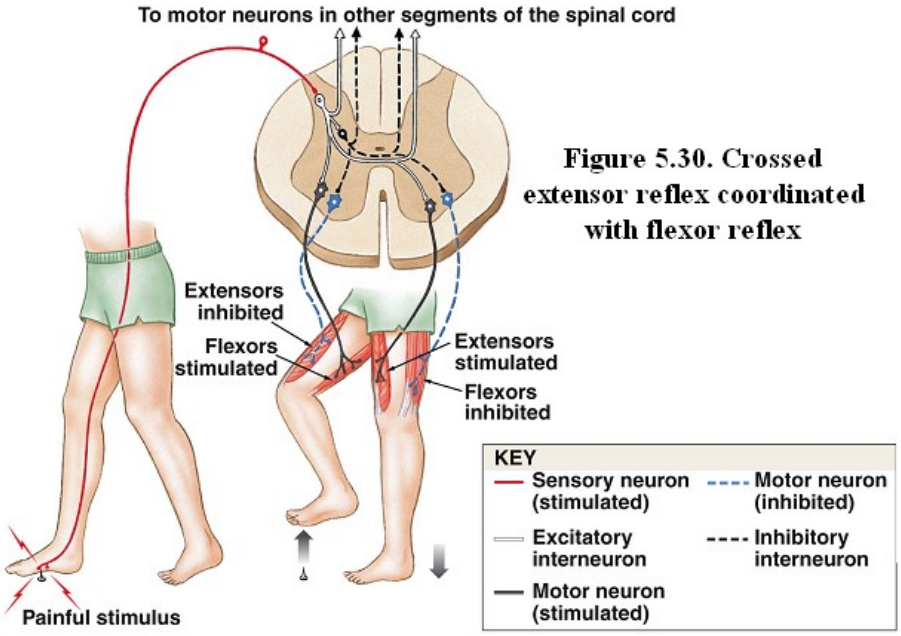


Figure 5.30. Crossed extensor reflex coordinated with flexor reflex

3. Superficial (skin, cutaneous) reflexes

- These *reflex arcs* have receptor organs in the skin or mucous and the *adequate stimulus* is stroking, scratching, or touching. Because are cutaneous reflexes, the more it is tried to elicit them in succession, the less of a response it'll getted. So, must be observed carefully the firt time it is stimulated.
- The sensory signal has to not only reach the spinal cord, but also must ascend the cord to reach the brain. The motor limb then has to descend the spinal cord to reach the motor neurons (*a polysynaptic reflex*). This can be abolished by severe lower motor neuron damage or destruction of the sensory pathways from the skin that is stimulated.
- *The utility of superficial reflexes*: they are decreased or abolished by conditions that interrupt the pathways between the brain and spinal cord (such as with spinal cord damage).
- *Classic examples* of superficial reflexes include the abdominal reflex, the normal plantar response.

Abdominal Reflexes (figure 5.31)

- *upper abdominal skin reflex* (reflex center at T5 to T8):
  - have the patient supine and relaxed, with the arms at the sides and knees slightly flexed.
  - use a pin to stroke the skin over the lower thoracic cage from the midaxillary line towardthe midline
  - watch for ipsilateral contraction of the muscles in the epigastric abdominal wall. When the muscle contractions cannot be seen, observe for umbilical deviation toward the stimulated side. In very obese persons, retract the umbilicus toward the opposite side to feel it pull toward the side of stimulation.

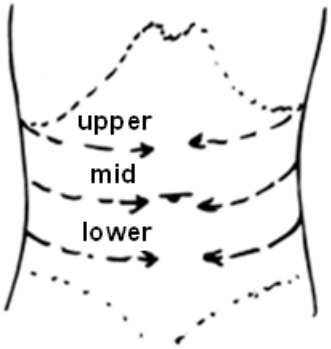


Figure 5.31. Abdominal reflexes

- *mid-abdominal skin reflex* (reflex center at T9 to T11):
  - make similar strokes from the flank toward the midline at the umbilical level.
- *lower abdominal skin reflex* (reflex center at T11 to T12):
  - make similar strokes from the iliac crests toward the midline of the hypogastrium.

Plantar reflex (reflex center at L4 to S2):

- grasp the patient's ankle with your left hand. With a blunt point and moderate pressure, stroke the sole near its lateral border, fromthe heel toward the metatarsal heads, where the course should curve medially following the bases of the toes (figure 5.32) .
- blunt point means a wooden-tip applicator, the end of a split wooden tongue blade, or the dull handle end on a reflex hammer. If no response is observed, a pin should be used as this is a nociceptive reflex.

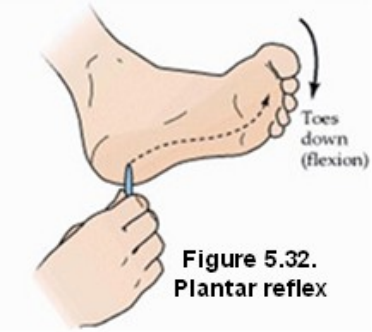


Figure 5.32. Plantar reflex

- *Normal response: plantarflexion of the toes (toes move downward)*
- *Babinski sign* also called *inversed plantar reflex or hallucal dorsiflexion reflex:*
  - the great toe will dorsiflex and the remainder of the other toes will fan out (figure 5.33);
  - withdrawal of the entire foot (due to unpleasant stimulation), is not interpreted as a positive response;
  - newborns normally have a positive Babinksi. It usually goes away after about 6 months
  - in diseases of the pyramidal tract, this reflex results, with some or all of four components: dorsiflexion of the great toe, fanning of all toes, dorsiflexion of the ankle, and flexion of the knee and thigh.

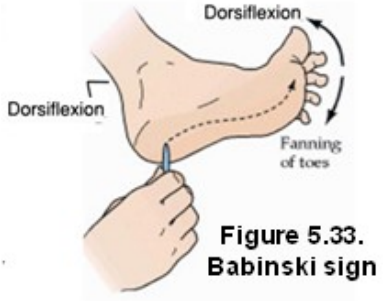


Figure 5.33. Babinski sign

Other superficial reflexes:

- ***Cremasteric reflex*** (reflex center L1 to L2): stroke the inner thigh distal from the pubis and observe promp elevation of the testis on the ipsilateral side
- ***Superficial anal reflex*** (anal wink, reflex center at S1 to S2): stroke the skin or mucosa of the perianal region. Normally, the anal sphincters contract.

Vegetative Spinal Reflexes	*Nervous center*
- cardioaccelerator	cervico-dorsal
- sudoral, pilo-motor, digestive motility	dorso-lumbar
- micturition , defecation, sexuale	lumbo-sacral

BRAINSTEM REFLEXES are tested during cranial nerve examination.

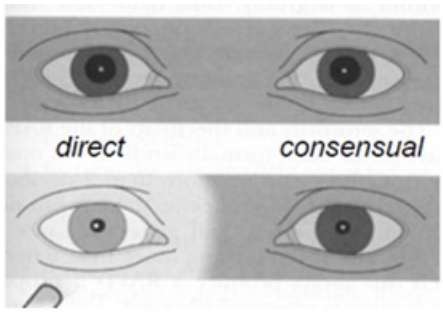
- *Ciliospinal Reflex* - pinching the skin of the back of neck causes pupillary dilatation (afferent cervical somatic nerves; efferent-cervical sympathetic chain).
- *Corneal Reflex* - touching the cornea causes blinking of the eyelids (afferent cranial nerve V; efferent cranial nerve VII). The evolutionary purpose of this reflex is to protect the eyes from foreign bodies and bright lights. The blink reflex also occurs when sounds greater than 40-60 dB are made. The examination of the corneal reflex is a part of some neurological exams, coma. Stimulation of one cornea normally has a consensual response, with both eyelids normally closing.
- *Orbicularis Oculi Reflex* - the eyelids close when the retina isexposed to bright light
- *Auditocephalogyric Reflex* - the head and eyes turn toward the source of a loud sound
- *Vestibulo-ocular reflex* (VOR) - eye movement that functions to stabilize gaze by countering movement of the head; the semicircular canals of the inner ear measure

rotation of the head and provide a signal for the oculomotor nuclei of the brainstem, which innervate the eye muscles; the muscles counter-rotate the eyes in such a way that a rightward head rotation causes an equal leftward rotation of both eyes, with the result that gaze direction stays stationary. VOR works in conjunction with the optokinetic reflex (OKR), which is a feedback mechanism that ensures that the eye moves in the same direction and at almost the same speed as an image (it develops at about 6 months of age). Together, VOR and OKR keep the image stationary on the retina, with VOR compensating for fast movements and OKR for slower ones.

- *Jaw Reflex* - when the mouth is partially opened and the muscles relaxed, tapping the chin causes the jaw to close. The reflex center is in the mid-pons (afferent cranial nerve V; efferent cranial nerve V).
- *Gag (pharyngeal) Reflex* is a reflex contraction of the back of the throat, evoked by touching the soft palate. It prevents something from entering the throat except as part of normal swallowing and helps prevent choking. Triggering the reflex is sometimes done intentionally to induce vomiting, for example by those who suffer from bulimia nervosa. The reflex center is is the medulla (afferent cranial nerve IX,; efferent cranial nerve X).

- ***Pupillary light reflex*** (figure 5.34)

- tests cranial nerves II and III, midbrain function
- pupils modulate amount of light entering eye (like shutter on camera lens); dark conditions➔dilate; bright➔constrict; pupils respond symmetrically to input from either eye



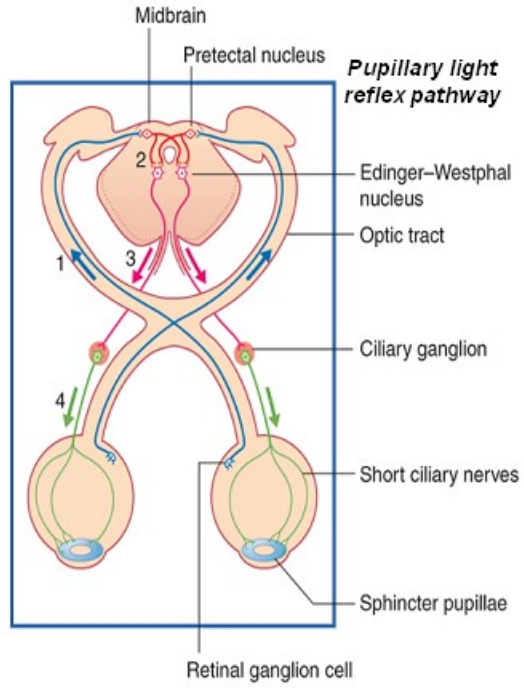


Figure 5.34. Pupillary light reflex

- ❑ *Direct pupillary response:* constriction in response to direct light
  - ◊ *Techique:* darken the room (or use your hand to provide "shade" over eyes), and check your patient's eyes with a penlight. To do this, shine the light directly into one of your patient's pupils, as patient keeps his other eye closed. Note the pupil's reaction. Then, check the other eye;
  - ◊ *normal response:* pupils constrict and remain constricted with light; pupils dilate when light is removed.
  - ◊ *pathway:* afferent cranial nerve II; efferent ipsilateral cranial nerve III
- ❑ *Consensual pupillary response:* constriction in response to light shined in opposite eye
  - ◊ *Techique:* darken the room (or use your hand to provide "shade" over eyes), but make sure your patient keeps both eyes open.
  - ◊ position the penlight directly in front of the patient *right eye*. Turn the penlight ON, and observe the *reaction of the patient left pupil.*
  - ◊ then, check the other eye.
  - ◊ *normal response:* pupils constrict bilaterally and remain constricted with light.
  - ◊ *pathway:* afferent cranial nerve II; efferent controlateral cranial nerve III

- *Pathway* (figure
  Retina → Optic Nerve (CN II) → Optic chiasma → Optic tract → Some optic tract fibres (CN II) branch to → Pretectal area → Bilateral projection to Edinger-Westphal nuclei → CN III → Ciliary ganglion → Short ciliary nerve → Sphincter pupillae
- *Abnormal responses* can be secondary to:
  - direct or indirect damage to either cranial nerves 2 or 3
  - *systemic drugs* e.g. sympathomimetics e.g. adrenaline and antimuscarinics e.g. atropine; tricyclic antidepressants, amphetamines ➔ *dilate*; narcotics (heroin), opiates e.g morphine and organophosphate ➔ *constrict.*
  - *topical drugs:* sympathomemtic e.g. phenylephrine, adrenaline diperavine and antimuscarinics e.g. cyclopentolate, tropicamide , atropine. ➔ *dilate*; muscarinic agonists e.g. pilocarpine ➔ *constrict.*

- ***Pupillary accommodation reflex***

- ask patient to look at a near object and then at a distant object; alternate the gaze from the near to the far object; next, move an object towards the patient's nose ➔ *pupils constrict when looking at a near object, dilate when looking at a distant object, converge when near object is moved towards the nose.*
- *Technique:* hold a pencil approximately 45 cm in front of the patient's nose. Then, ask the patient her to watch the pencil as you move it. Instruct the patient to keep the head and eyes stationary throughout the examination. Then, slowly move the pencil toward the bridge of his nose. If everything's OK, both your patient's eyes will converge on the pencil at the same level and distance. At that point, expect his pupils to constrict and remain constricted. When the pencil's 5 to 7.6 cm from the bridge of his nose, your patient should be able to comfortably hold his gaze.
- *Normal response:* Both eyes converge on pencil at same level and distance. Patient maintains gaze on pencil when it's held 2" to 3" (5 to 7.6 cm.) from the bridge of his nose. When your patient's eyes converge, both of her pupils constrict and remain constricted.
- *Pathway*
  Retina → Optic Nerve (CN II) → Optic chiasma → Optic tract → Lateral geniculate body → Optic radiation → Visual Cortex → Association fibres → Frontal lobes → Descend via anterior limb of internal capsule → Superior colliculus → Edinger-Westphal nuclei → cranial nerve (CN) III → Ciliary ganglion → Muscles of iris and ciliary body
- *Close vision requires:*
  - Accommodation – changing the lens shape by ciliary muscles to increase refractory power
  - Constriction – the pupillary reflex constricts the pupils to prevent divergent light rays from entering the eye
  - Convergence – medial rotation of the eyeballs toward the object being viewed
- Normal recorded as: PERRLA (Pupils Equal, Round, Reactive to Light and Accomodation)

Vegetative reflexes of clinical relevance: **<u>methods of vagal stimulation determine a transient bradycardia</u>**

- coughing
- lying recumbent on the foot with legs elevated against the wall
- Valsava maneuver – deep inspiration followed by expiratory effort against a closed glottis
- carotid sinus massage
- immersion of the face in cold water (the dive reflex)

E.g. carotid sinus massage:

- may be used to interrupt sinus tachycardia or paroxysma atria tachycardia. Massagingt he carotid sinus stimulates the vagus nerve, which inhibits firing of SA node and slows AV conduction. As a result, the SA node can resume its function as primary pacemaker;
- *mechanism:* baroreceptor reflex *(see the lecture)*
- *technique:*
  - locate the carotid artery and listen for carotid bruits;
  - turn the head of the patients to the left to massage right carotid sinus;
  - press the carotid sinus aginst the ateral process of the cervical vertebrae and massage firmly no longer than 5 to 10 seconds; monitor the effect on ECG.
- *contraindications:* presence of carotid bruits, history of transient ischemic attacks, carotid artery stenosis
- *risks of the maneuver:* decreased heart rate, syncope, increased degree of AV block, cerebral emboli, stoke, asystole

V.3.3. Electroencephalogram (EEG) test.

Definition: EEG is the noninvasively recording of the electrical activity of the brain using surface electrodes placed over the scalp.

The main generators of electrical activity are postsynaptic potentials from the cortical pyramidal layers. The ondulations in the recorded electrical activity of the brain are called *brain waves* characterized by :

- *amplitude:* range from 20 to 100uV; EEG activity is quite small measured in microvolts (μV) compare to ECG signal (microV)
- *frequency:* range from 1 – 50 HZ
- *character of the waves* (intensity and patterns) depends on the degree of activity of the cerebral cortex:
  - at the highest state of alertness, when sensory input is greatest, the waves are of high frequency and low amplitude, as many units *discharge asynchronously*.
  - at the opposite end of the alertness scale, when sensory input is at its lowest, in deep sleep, a synchronized EEG has the characteristics of low frequency and high amplitude.
  - an absence of EEG activity is the legal criterion for death

Practical significance of EEG:

- diagnosis of central nervous system disorders (metabolic, degenerative, compressive, neoplasic and vascular diseases)
- monitoring of cerebral development, anesthesia
- investigation of epilepsy: diagnosis, monitoring, testing the effects of antiepileptic medication;
- the study of the sleep stages; investigating sleep disorders
- monitoring coma and diagnosis of brain death

In normal subjects the EEG *wave patterns are classified according to their frequency* in alpha, beta, theta and delta waves (figure 5.35):

Brain waves	*Appearance*	*Voltage (μV)*	*Frequency (Hz)*	*Distribution*
alpha	In all normal, adult persons, when they are awake, but relaxed, with the eyes closed	20-100	7-13	Occurs most intensely in the occipital and parietal regions
beta	When the eyes are open, the added visual input to the cortex (activation of the CNS) determines a faster rhythm to the EEG – beta waves; it is blocked by proprioceptive or tactile stimuli.	5-10	13-27	Occurs mostly in the frontal region
theta	Appears mainly at children(2-5 years); in adult durind first stages of sleep. It is considered pathologic when represent more than 15% of total brain electrical activity on adult; focal in subcortical lesions and diffuse in profound lesions or metabolic encefalopathies	30-70	4-7	Occurs in temporal and frontal region
delta	Occurs in childrens (2-3 years); in adults in deep sleep, considered pathologic if appear while the person is awake.	50-200	0.5-4	Occurs in all regions

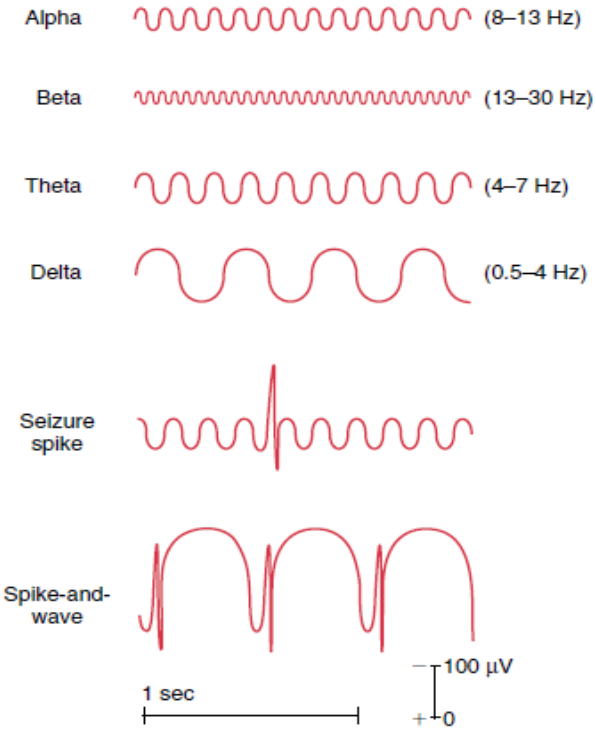
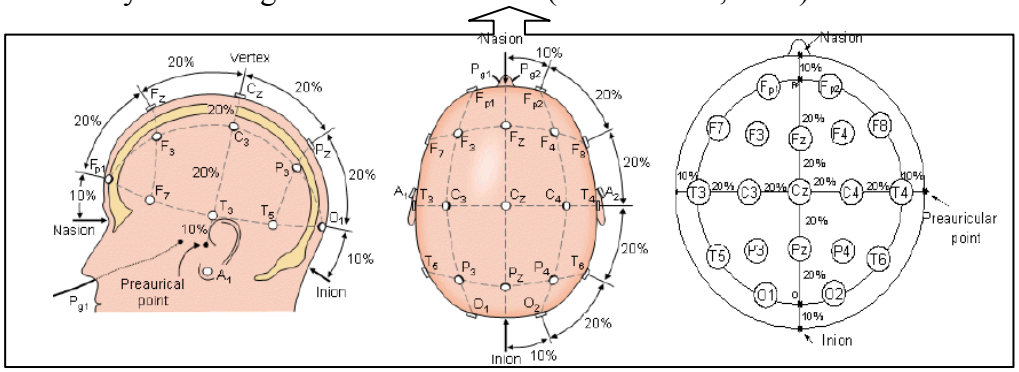


Figure 5.35. Patterns of brain waves recorded on an EEG. In epilepsy, abnormal spikes and large summated waves appear as many neurons are activated simultaneously.

***Recording EEG*:**
The EEG measured directly from the cortical surface is called *electrocortiogram* while when using depth probes it is called *electrogram*.
Materials required: electrodes with conductive media, amplifications and filters, digital convertor, recording machine
Technique:

- electrodes are placed on the scalp degreased with alcohol

- 10-20 system using 21 active electrodes (standardized, 1958)



- method advantage: there is an anatomic correlate for each electrode, which is consistent from patient to patient
- 3 distances are measured: between two preauricular points; between the nasion (nose bridge) and inion (the occipital bone mount), both across vertex; the circumference between nasion and inion. These distances are divided in proportion of 10-20-20-20-20-10% in both orthogonal axes and in circumference, and a net of imaging quadrates is built on head surface. The electrodes are placed in a quadrates angles.
- 3 types of electrodes: active, reference and ground electrode

- the conections with the electroencephalograph are made (with electric wires)
- the signal is amplified (100-100000x) and filtrated (from "background noise", e.g. signals from the electrical network 50-60Hz and from low frequency signals determined by physiological biopotentials, e.g. respiration)in order to be visualized, recorded and digitally converted
- the recordings are made in a quiet room, in resting state of cerebration, awake, whithout visual sensations (closed eyes).
- recording whith activation of the central nervous system are made by: opening the eyes in bright light, intermitent light stimulation with bright light flashes (1-30/sec) delivered for 10 sec. (most effective), hyperventilation for 3-5 min (most sed), acoustic stimulation, etc

Results:

- *Normal EEG*: see figure 5.36
- *Epileptiform EEG discharges*, during epileptic attack, used in epilepsy diagnosis (with activation of the central nervous system techiques):
  1. spikes (20-70ms);
  2. sharp waves (70-200 ms);
  3. a spike and wave complex (spike or sharp wave followed by a slower wave).

  Epilepsy is a neurological disorder of the brain characterized by spontaneous discharges of electrical activity, resulting in abnormalities ranging from momentary lapses of attention, to seizures of varying severity, to loss of consciousness if both brain hemispheres participate in the electrical abnormality.
- *Study of the sleep stages:*
  - *a normal sleep cycle* begins with
    - *slow-wave sleep* that comprise *4 stages of increasingly deep sleep* during which the EEG becomes progressively slower in frequency and higher in amplitude (stage 4 is reached at the end of about an hour, when delta waves are observed - see figure 5.18). The subject then passes through the same stages in reverse order, approaching stage 1 by about 90 minutes, when a
    - REM (rapid eye movements) period begins, followed by a new cycle of slow-wave sleep.
  - *Slowwave sleep* is characterized by decreased heart rate and blood pressure, slow and regular breathing, and relaxed muscle tone. Stages 3 and 4 occur only in the first few sleep cycles of the night
  - *REM periods* increase in duration with each successive cycle, so that the last few cycles consist of approximately equal periods of REM sleep and stage 2 slow-wave sleep. REM sleep (paraadoxical sleep) is characterized by unsynchronized, high-frequency, lowamplitude waves (i.e., a beta rhythm) on ECG, which is more typical of the awake state than sleep; blood pressure and heart rate are increased and breathing is irregular. Also, REM sleep as dream sleep. Most voluntary muscles are temporarily

paralyzed with 3 exceptions: muscles of respiration, extraocular muscles (for REM) and muscles of the middle ear, which protect the inner ear .

- Sleep duration: newborns - 16 hours per day (50% is spent in REM sleep); normal adults sleep 7 to 8 hours per day (25% - REM sleep).
- The percentage of REM sleep declines further with age, together with a loss of the ability to achieve stages 3 and 4 of slow-wave sleep.

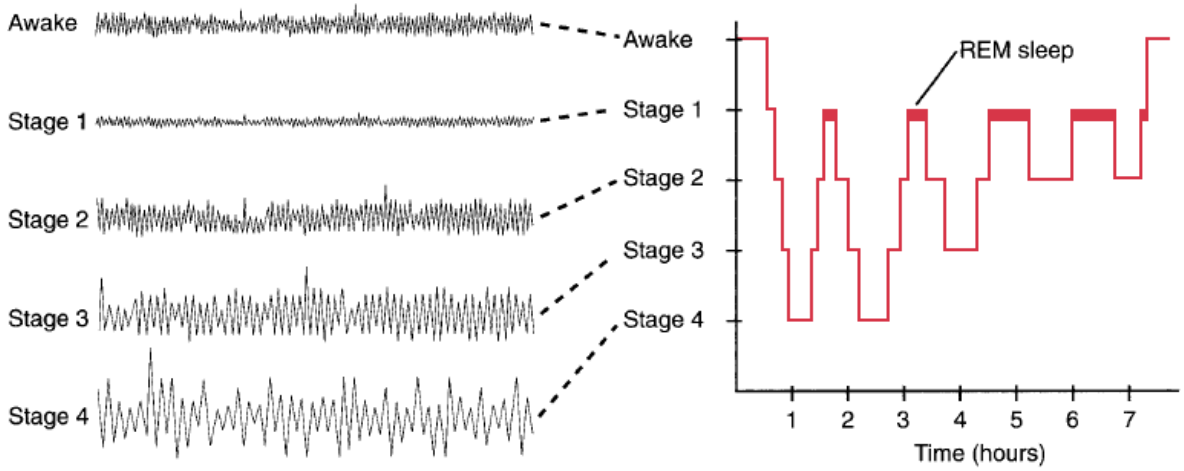


Figure 5.36. The brain wave patterns during a normal sleep cycle *(after from Kandel ER, Schwartz JH, Jessel TM. Principles of Neural Science. 3rd Ed. New York: Elsevier, 1991.)*

Polysomnography (PSG), also known as a sleep study, is a multi-parametric test which record simultaneously electroencephalogram *(EEG),*electrooculogram (eye movements, EOG), electromyogram (EMG), electrocardiogram (ECG), nasal and oral airflow, O2 saturation (peripheral pulse oximetry) during sleep. PSG is used in the study of sleep and as a diagnostic tool in sleep medicine.

V.3.4. Evoked cortical potentials.

Evoked cortical potentials are the electrical signals generated by the brain in response to a stimulus (auditory, visual or somatosensory) and recorded from the surface of the scalp.

There can be describe:

- Primary evoked potentials
  - the initial, short duration and localized respone over the specific sensory cortex;
  - characterized by latency of about 10 ms; first appears a surface positive wave followed by a small negative wave
- diffuse secondary response characterized by
  - latency of about 50ms
  - positive-negative wave sequence is larger and prolonged compare with primary evoked potentials
  - is not highly localized; it can be recorded from most cerebral cortex due

Types of evoked cortical potentials depending on the type of stimulus:

- visual evoked potential
- brain stem auditory evoked potential
- ofactory evoked potentials (lfactory stimulation of a subject's olfactory system)
- somatosensory evoked potential

The visual-evoked potential (VEP) is the potential recorded from the occipital region in response to visual stimuli. VEPs depend on functional integrity of central vision at any level of the visual pathway including the eye, retina, the optic nerve, optic radiations and occipital cortex.
Two types of stimuli are used to record VEPs:

- *Pattern-reversal* is the preferred stimulus for most clinical purposes. Pattern-reversal VEPs are less variable in waveform and timing than the VEPs elicited by other stimuli. A computer screen is placed in front of the patient and an alternating black and white checkerboard pattern is generated (black and white squares reverse color: black becoming white and opposite)
- *Flash VEPs* (flash light) are useful when poor optics, poor cooperation/poor vision, coma, anesthesia makes the use of pattern stimulation inappropriate

The waveforms (figure 5.37) are alternately positive and negative and are designated in accordance with their polarity and latency:

- positive waves are designated P, followed by a number indicating the peak latency in milliseconds (e.g., P60 and PlOO)
- negative waves are designated N, followed by a number indicating the peak latency N70 and NI45

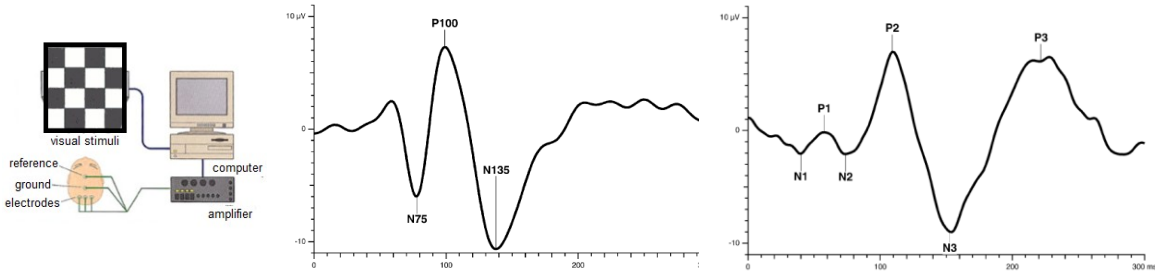


Figure 5.37. Visual evoked potential (VEP): set up diagram,
normal attern reversal VEP and normal Flash VEP (from left to right)

Responses are recorded from electrodes that are placed on the back of your head and are observed as a reading on an electroencephalogram (EEG). These responses usually originate from the occipital cortex, the area of the brain involved in receiving and interpreting visual signals.
Practical importance of VEP:

- diagnosis of optic neuropahty as part as demyelination disease (e.g. multiple sclerosis);
- tumoral compression of optic nerve or optic chiasm (glioma, sellar tumors);
- retrochiasmal disorders;
- metabolic disorders (vitamine B12 deficiency);
- visual resolution and contrast sensitivity in patients who cannot cooperate.

Auditory evoked potentials (AEP)

This technique isolates the neurophysiological signal generated during stimulation of the 8th cranial nerve using a repetitive auditory stimulus at 6-10 Hz.
The repeated sampling allows the signal to be extracted from the background EEG noise. The signal is acquired using EEG electrodes located on the mastoid processes, a midline reference electrode and a ground electrode.
Over recent years AEP's have been used to develop a monitor to measure anaesthetic depth and to prevent awareness in surgery.
The auditory evoked potential is a composite waveform (figure 5.38) that can be plotted against time. The brainstem AEP (1-10 ms latency) and the late cortical AEP (50-500 ms latency) do not correspond with depth of anaesthesia but the early or mid-cortical AEP does (MCAEP 10-50 ms latency). The mid-latency of the Pa and Nb components is analysed.

Anaesthetic agents decrease the amplitude and increase the latency of the MCAEP in a dose-dependent, but agent-independent, manner.

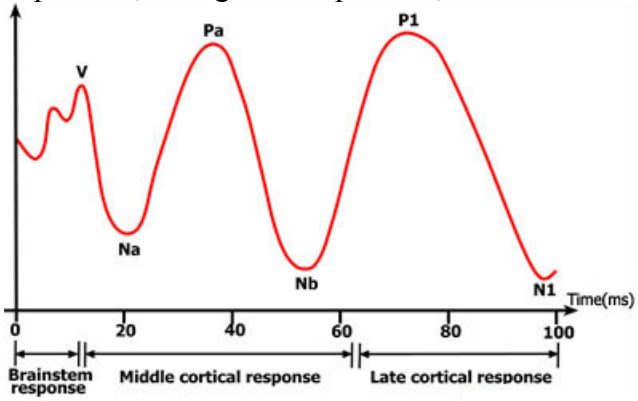


Figure 5.38. Auditory evoked potentials (AEP)

Somatosensory Evoked Potentials (SSEPs) are recorded by *stimulating peripheral nerves*, trancutaneous electrical stimulation applied on the skin over the trajectory of peripheral nerves of the upper limb (e.g., the median nerve) or lower limb (e.g., the posterior tibial nerve), and then recorded with electrodes placed on on the *scalp*, neck or back. (figure 5.39).

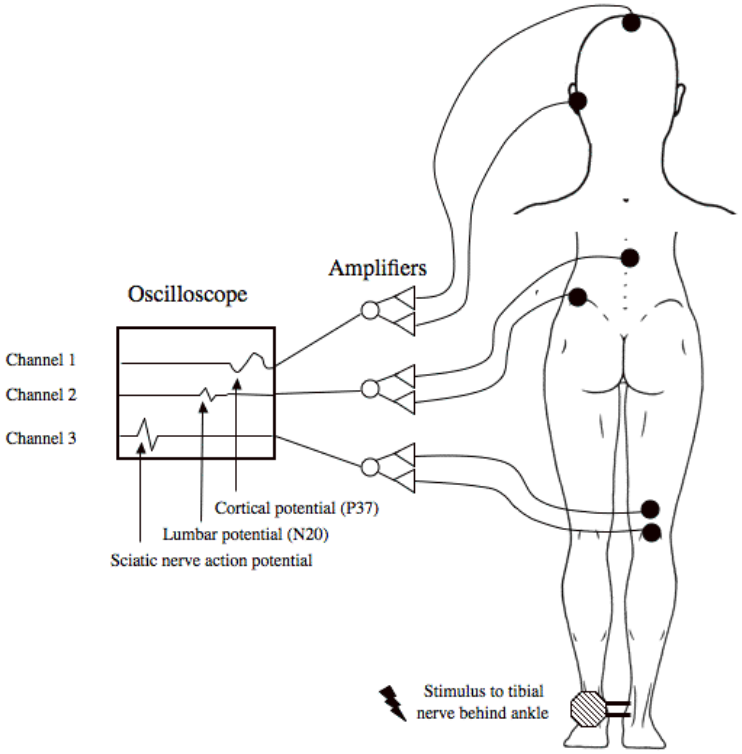


Figure 5.39. Somatosensory evoked potentials (SSEPs) diagram following stimulation of the tibial nerve at the ankle.

Practical importance of SSEPs:

- SSEPs are routinely used in neurology today to confirm and localize sensory abnormalities, to identify silent lesions;
- monitor changes during surgical procedures;
- SSEP analysis can be an accurate technique in measuring sensory conduction;
- it can be used to monitor neurological condition and thus track disease progression;
- less costly than other techniques such as an MRI;
- SSEP analysis is also used to monitor a patient's status during surgery near the spinal cord, or in the intensive care unit (ICU) for brain injury;
- it is often less costly than other techniques such as an MRI.

V.4. Sensorial physiology

V.4.1. Determination of tactile discrimination threshold

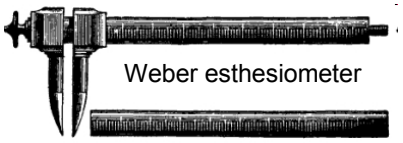
- touch can provide information about an object, such as surface texture, that is not easily detectable by vision.
- touch experiences are triggered by mechanical disturbance of the skin produced by physical contact with an object.
- the human skin contains *mechanoreceptors (see the lecture)*, receptors that are sensitive to mechanical pressure or deformation of the skin. Though, *the concentration of mechanoreceptors within the skin is not uniform*. Rather, the *highly sensitive areas* of skin, such as the *lips and fingertips*, contain densely packed mechanoreceptors, while *insensitive areas*, such as the *stomach and back*, contain lower concentrations of mechanoreceptors.
- *more sensitive areas of the skin also project to a larger proportion of the somatosensory cortex* than less sensitive areas. Thus, the area of the brain which receives touch sensations (for example, from the fingertip) is proportional to the actual sensitivity of the skin area *(see the lecture)*.

Two-point threshold test

Definition: Two-point threshold test measures the density of touch receptors on a specific region of the skin.

Principle: accurately measurement of the innervation density of the skin.

Materials required: Weber's esthesiometer, which consists of two pointed legs, one of which is fixed at the end of a scale graduated in millimetres, along which the other slides.



Technique:

- starting with the esthesiometer or caliper wide apart and the subject's eyes closed, determine the two-point threshold on the back of the hand.
- two points of the Weber's esthesiometer are placed simultaneously on the skin with equal pressure and the subject is asked if 2 separate points of contact are felt.
- if so, the pointed legs of the esthesiometer are brought closer together with 1mm, and the test is repeated until only one point is felt (figure 5.40).

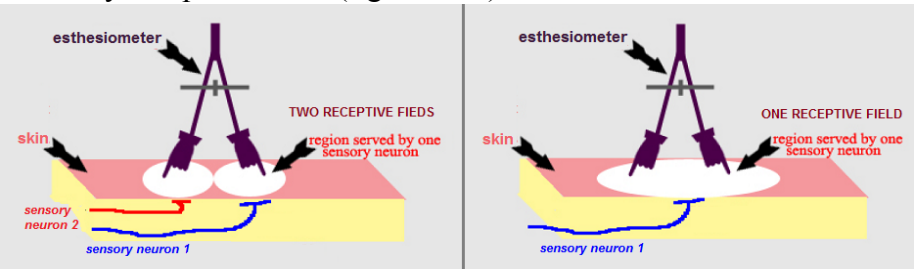


Figure 5.40. Two-point threshold test diagram

- *the minimum distance at which two points can still be felt is the two-point threshold.*
- repeat this procedure with the face, back of hand, palm of hand, fingertips, lips, back of neck and anterior forearm.
- record the results of two-point threshold for every region.

Results :

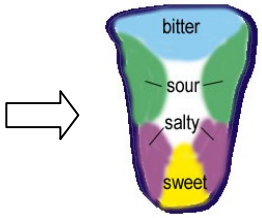
` Normally, a person should be able to recognize two points separated by as little as 2-4 mm on the lips and finger pads, 4.5 mm on middle phalanx of fingers, 8-15 mm on the palms, 31.6 mm on the back of the hand, 67.7 on middle of the back, arms, thighs. The two-point threshold for any part of the body is determined by the size of the receptive fields.

Discussions:
Lesions of the sensory cortex will increase the distance between two recognizable points.

V.4.2. Determination of taste threshold and location of taste

Taste is a chemical sense because the stimuli are various chemicals which on the surface of the tongue mix with saliva, break down into molecules and stimulate the taste buds.
Primary taste sensations:

- *Sweet* - usually indicates energy rich nutrients
- *Umami* - the taste of amino acids (e.g. meat broth or aged cheese)
- *Salty* - allows modulating diet for electrolyte balance
- *Sour* - typically the taste of acids
- *Bitter* - allows sensing of diverse natural toxins



Taste threshold is the lowest concentration of a solution that a person **can still taste**.
Tecnique:

- using volunteers, it will be attempted to determine the taste thresholds for sweet, sour and salty solutions.
- start with a 10% solution, and use the process of *serial dilution* to make a series of solutions, each 10-fold weaker than the preceding one (i.e., 1%, 0.1%, 0.001%, etc.)
- start the experiment with the lowest concentration of substance and than increase the concentration till you can feel the taste.
- continue making serial dilutions, rinsing and drying your tongue, and testing each new solution with the cotton swab procedure.

Results and discussions:

Taste	***Substance***	**Threshold for tasting**
Salty	NaCl	0.01 M
Sour	HCl	0.0009 M
Sweet	Sucrose	0.01 M
Bitter	Quinine	0.000008 M
Umami	Glutamate	0.0007 M

Taste sensitivity shows significant individual differences, some of which are inherited
Gustatory receptors are rapidly adapting (see the lecture).

V.4.3. Determination of visual discrimination ability

The measurement of visual acuity (VA) is the most widely used test for assessing the integrity of the visual function. It forms an essential part of the routine ophthalmological examination and is used in the basic set of exams for the evaluation of ocular pathologic conditions.
Material required, technique, results:
It will be used a standard Snellen hanging wall chart read (optotipe) with the patient standing at a distance of 6 meters (fig 5.41 a), a "tumbling E" chart (fig. 5.41b) or specially designed pocket card held at 36 centimeters (fig. 5.41c). Each eye is tested independently.
You do not need to assess their ability to read every line on the chart.

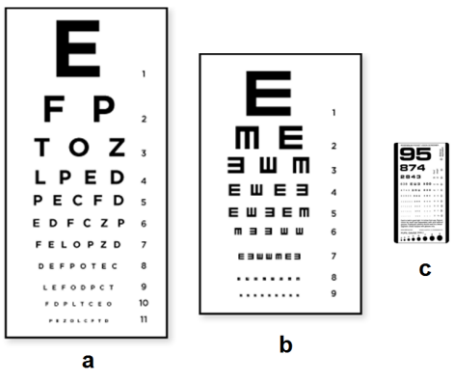


Figure 5.41. Optotipe

The numbers at the end of the line provide an indication of the patient's acuity compared with normal subjects.
If a subject needs letters that are twice as large or twice as close as those needed by a standard eye, that person's visual acuity is said to be 1/2. If the letters needed are five times larger, the acuity is 1/5, if ten times larger: 1/10, etc. The value of those fractions can be expressed in different ways.
E.g. 1/2 = 20/40 = 6/12 = 0.5 or 1/5 = 20/100 = 6/30 = 0.2.
The **MA**gnification **R**equirement is also known as **MAR**, so that MAR = 1/V and V=1/MAR. Snellen expressed this in the well known Snellen formula (here in its metric version): *VA = m/M = wiewing distance (in meters) / letter size (in M-units)*

- In the US the 20/… notation is routinely used, even if the testing distance is not 20 ft.
- In Europe the decimal notation is common;
- in Britain (and former British dominions) the 6/… notation is common.

If the patient is unable to read any of the lines, to estimate what they are capable of seeing, the ability to detect number of fingers placed in front of them, light should be determined .

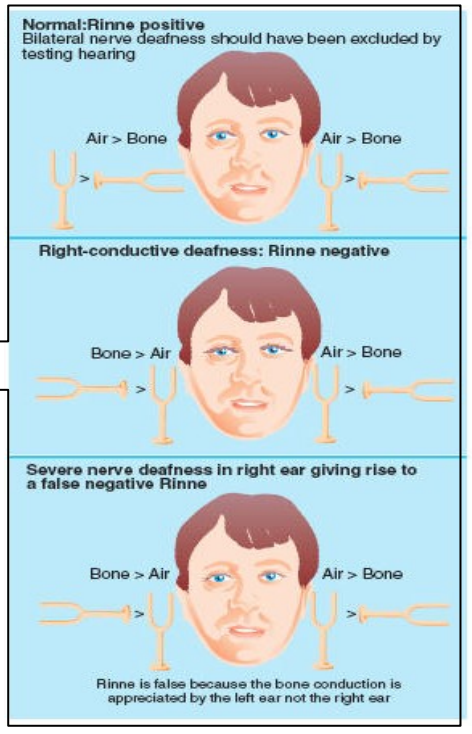
V.4.4. Laboratory Tests of Hearing

Use a rubber mallet or the heal of your hand to vibrate the tuning forks (*do not hit them on the counters*)
Substitute the following tuning forks:

low freq. 128 Hz
medium freq. 512 Hz
high freq. 4096 Hz

1. Rinne Test for comparing bone and air conduction hearing

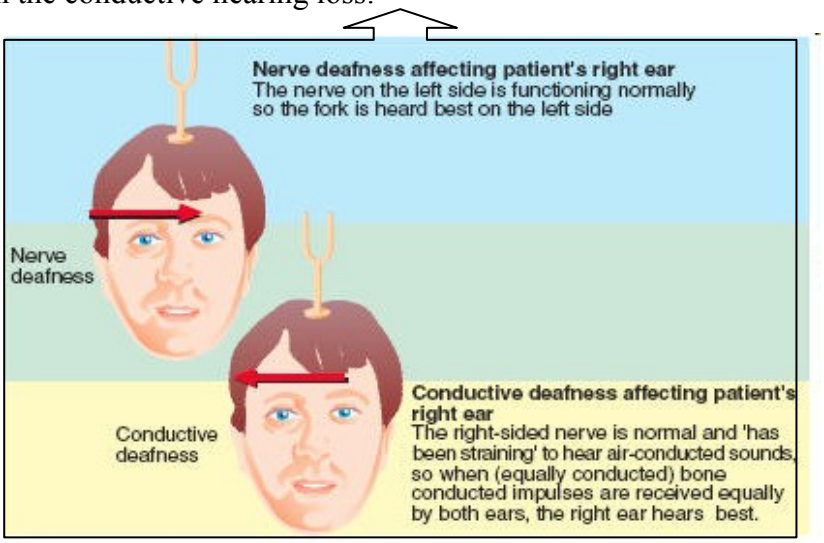
The Rinne test compares air conduction to bone conduction. Hit the tuning fork (512 Hz) firmly on the palm of your hand or the cover of a soft covered book. Place the butt of the tuning fork on the mastoid eminence firmly. Tell the subject to indicate when they can no longer hear the vibration. When that happens, remove the butt of the tuning fork and place the U of the tuning fork approximately 1 inch the ear without touching it. Have the subject tell you when they can no longer hear anything.
Normal: Air Conduction is better than Bone Conduction (air conduction usually persists twice as long as bone); referred to as "positive test"
Abnormal: Bone conduction better than air conduction➔suggests conductive hearing loss; referred to as "negative test"



2. Weber Test for lateralization of sound

Hit the tuning fork (512 Hz) firmly on the palm of your hand or the cover of a soft covered book. Place the butt of the tuning fork on the top of the subject's head in the midline and ask the subject where they hear the sound. Normally the sound is heard in the center of the head

or equally in both ears. If there is conductive hearing loss present, the vibration will be heard louder on the side with the conductive hearing loss.



In the Weber test sound will lateralize to the side away from a sensorineural loss and toward a conductive deficit.

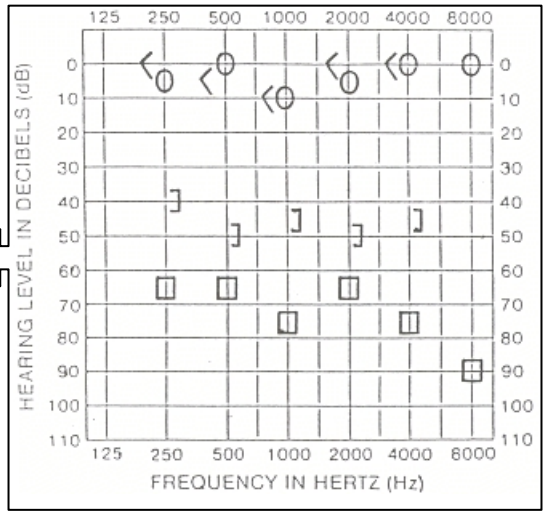
The Rinne test will demonstrate air conduction better than bone conduction if the loss is sensorineural. If available, office tympanometry can also help detect conductive hearing problems.

3. Audiometry

The testing of hearing is most often carried out by establishing the threshold of hearing, the softest sound which can be perceived in a controlled environment. It is typical to do this testing with pure tones by providing calibrated tones to a person via earphones, allowing that person to increase the level until it can just be heard. Various strategies are used, but pure tone audiometry with tones starting at about 125 Hz and increasing by octaves, half-octaves, or third-octaves to about 8000 Hz is typical. Hearing tests of right and left ears are generally done independently. The results of such tests are summarized in *audiograms.*

Screening audiometers for office use generally test at several frequencies in the speech range between 500 and 4,000 Hz. An audiogram hearing threshold level above 20 dB is considered abnormal.

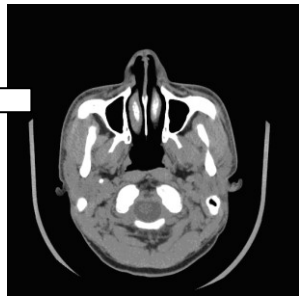
Audiograms compare hearing to the normal threshold of hearing, which varies with frequency as illustrated by the hearing curves. The audiogram is normalized to the hearing curve so that a straight horizontal line at 0 represents normal hearing.



Audiometry results. The right ear shows thresholds that are within normal limits for air and bone conduction. The left ear shows a mixed hearing loss. The bone conduction thresholds show a sensorineural hearing loss. Air conduction thresholds show an additional loss. This difference between air and bone conduction is known as an air-bone gap and signifies a conductive hearing loss. *KEY: < Right ear, bone conduction; ○ Right ear, air conduction;] Left ear. bone conduction; □ Left ear, air conduction.*

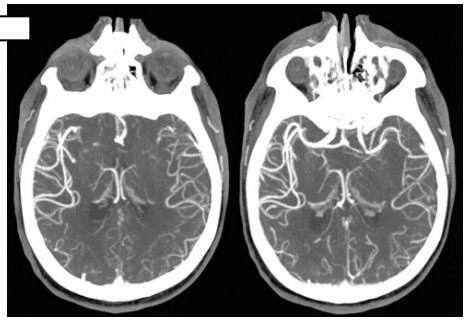
IV.5. Functional exploration of nervous system

Computed tomography (CT) scan (often called an ***axial CT***) can reveal hemorrhagic (bleeding) stroke readily, but early detection of ischemic stroke caused by a blockage in a blood vessel is not always easy to detect.



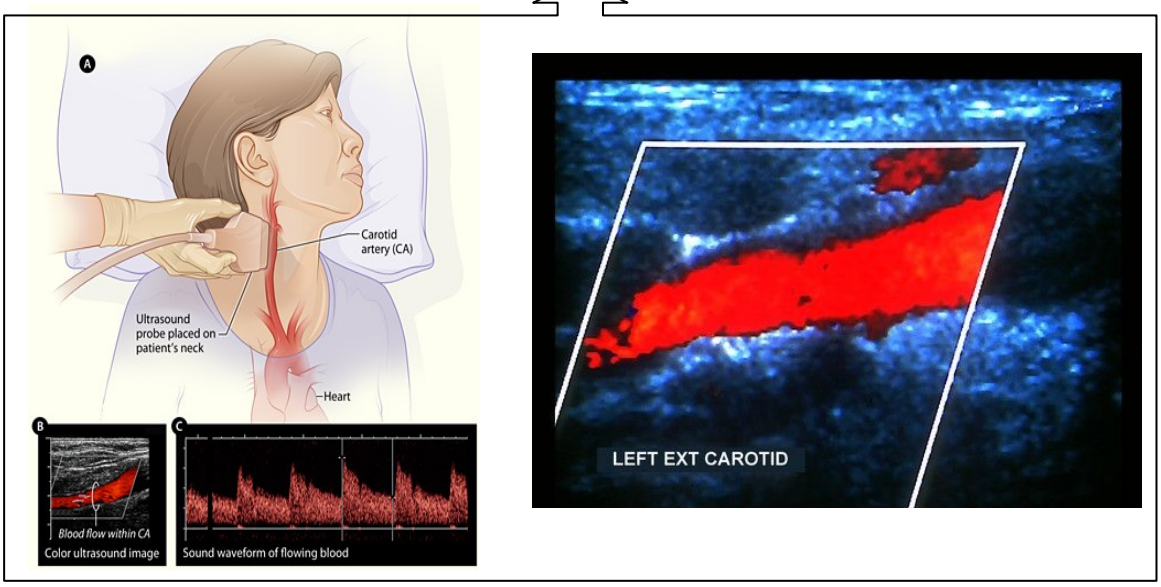
CT scans can immediately determine whether hemorrhage (bleeding) is the cause of a stroke. This is very important because people with a hemorrhagic stroke can die if they are mistakenly given one of the clot-busting drugs known as thrombolytics that are designed to break up small clots in the early stages of an ischemic stroke.

Computed tomography angiography (CTA) is high-speed CT scanning that uses an intravenous injection of contrast dye to differentiate flowing arterial blood from the surrounding tissue. This technique can safely and reliably study the major arteries supplying the brain and detect the type and location of the problem causing a stroke. It can differentiate ischemic from hemorrhagic stroke and help diagnose an aneurysm in an artery at the base of the brain in people with subarachnoid hemorrhage. To some extent, this technology can also distinguish which areas of the brain suffer from reduced blood flow and are at risk of infarction. In addition, this is the best and safest noninvasive method to diagnose a ruptured or unruptured berry aneurysm.



The **ultrasound scan** of the carotid arteries is usually used when the doctor hears a bruit (an abnormal sound in the artery) or when a person has known risk factors for stroke, such as high blood pressure, elevated cholesterol, advanced age, or diabetes.

Doppler ultrasound is a noninvasive method that enables doctors to view blood vessels and analyze blood flow in the neck and at the base of the brain.



Two Doppler ultrasound techniques are used for diagnosis of stroke. **Carotid duplex Doppler scanning** makes images of the carotid arteries. **Transcranial Doppler scanning**

analyzes flow in the major intracranial arteries at the base of the brain. These techniques are essential to the stroke neurologist, as they can provide a minute-to-minute assessment of the adequacy of flow in the damaged artery and the availability of collateral flow from other arteries to help bring blood to threatened brain tissue.

Cerebral angiography (figure 5.42) is also known as intraarterial digital subtraction angiography (IADSA). In cerebral angiography, a catheter (long, thin, flexible tube) is inserted into an artery in the arm or leg. Using the catheter, a technician injects a special dye into the blood vessels that lead to the brain. In cerebral angiography, x-ray images show blood vessel abnormalities in the brain. Results from a cerebral angiogram are more accurate than those produced by carotid Doppler. Usually, cerebral angiography is used after another test has already found an abnormality. Angiography is used to help detect and diagnose acute stroke. The images that result from cerebral angiography are not available from other techniques.

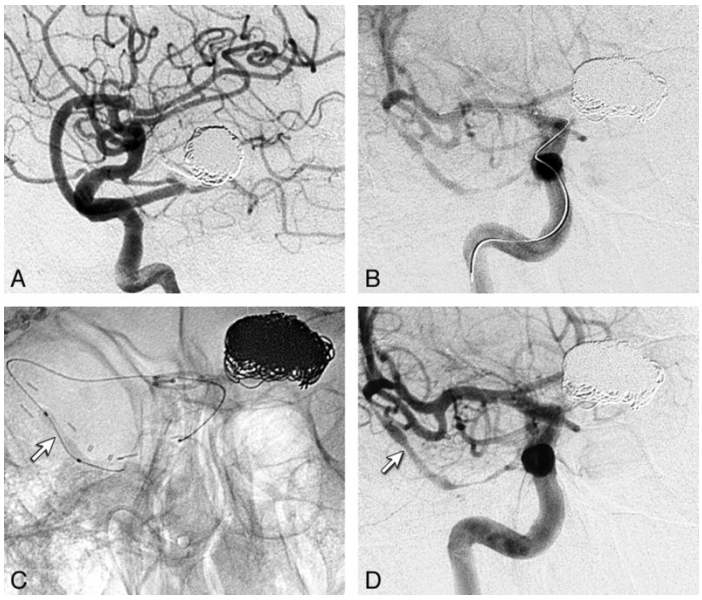


Figure 5.42. Cerebral angiography

Positron emission tomography (PET)

MRI and CT scans render exquisite anatomical detail about the structure of the brain, but are unable to determine anything about its function. PET or PET/CT imaging can help physicians detect functional abnormalities early in the course of the disease, before anatomical changes occur. Brain disorders start with functional abnormalities that result in either an increase or decrease in glucose metabolism at a cellular level. These functional changes precede the formation of an abnormal mass, the shrinkage of brain tissue, or other abnormalities seen on anatomical imaging, sometimes by years. PET and PET/CT imaging (figure 5.43) can show precise areas of increased or decreased glucose metabolism in the brain. In Alzheimer's disease for example, there is a characteristic pattern of decreased biomarker uptake in the posterior parietotemporal cortex, which could be caused by neuronal depletion in that area. Physicians use this type of information to differentiate Alzheimer's disease from fronto-temporal and other dementia.

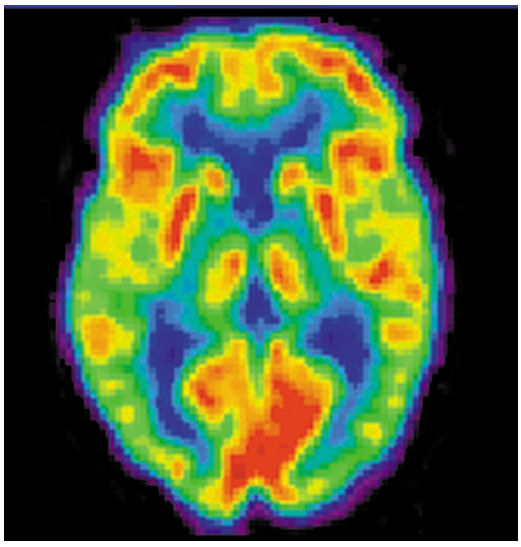


Figure 5.43. PET scan

ANNEXE – MINIMUM KNOWLEDGES
(IInd Year)

1. **Osmolarity of internal environment** = 280 – 300 mOsm

2. **Intracellular pH:** 7 (neutral)
   Normal values range: 6 – 7.5 (depend on cell type)

3. **Extracellular pH**:
   pH = 7.4 (arterial plasma)
   pH = 7.35 (venous blood plasma)
   pH = 7.4 (interstitial fluid)

4. **Sodium concentration** in intra- and extracellular medium:
   Intracellular medium: 10 - 37 mEq/l
   Interstitial fluid: ≈ 136 mEq/l
   Blood plasma: 140 ± 5 mEq/l

5. **Potassium concentration** in intra- and extracellular medium:
   Intracellular medium: ≈ 150 mEq/l
   Blood plasma: 3.5 -5.6 mEq/l

6. **Calcium concentration** in intra- and extracellular medium:
   Intracellular medium: ≈ 10 (-7) M
   Blood plasma: 2.1 – 2.6 mEq/l

7. **Magnesium concentration** in intra- and extracellular medium:
   Intracellular medium: 2.6 mEq/l
   Interstitial fluid: ≈ 136 mEq/l
   Blood plasma: 1.5-2.5 mEq/l

8. **Chloride concentration** in intra- and extracellular medium:
   Intracellular medium: 1-3 mEq/l
   Interstitial fluid: ≈ 117 mEq/l
   Blood plasma: 90-110 mEq/l

9. **Total blood plasma protein**: 6-8 g/dl

10. **Blood plasma protein (proteinogram)**

	g/dl	%
Total protein	6-8	100
Albumins	4.5-5.7	55-70
Globulins	1.46-2.54	30-45
Fibrinogen	0.2-0.4	
Fraction albumins / globulins	1.5-2.5	

11. **Globulin fractions** (% of total plasma proteins)
    α_1: 3-7 %; α_2: 7-10 %; β: 9-17 %; γ: 12-20 %;

12. Hematocrit: Definition and normal values.
Definition: It is defined as the ratio between the volume of blood cells and the volume of blood in which they are contained.
Normal values: Men: 46.5% ± 5 %;
Women: 42% ± 5 %;
New born: 56% ± 5 %;
Children: 40% ± 5 %;
Somatic hematocrite = 0.91 x venous hematocrite
(Therefore, somatic hematocrite is lower than venous hematocrite)

13. Red Blood Cells count. Normal values
Normal Values: Men: 4.5 - 5 millions/mm^3
Women: 4.2 - 4.5 millions/mm^3
Children: 5 - 6 millions/mm^3

14. Hemoglobin concentration in RBC's and blood
Normal Values: Men: 14-18 g/dl
Women: 12 - 16 g/dl
New born: 18 - 27 g/dl
Children: up to 1 year: 10 - 15 g/dl
up to l0 years: 12 - 17 g/dl

15. Erythrocyte Sedimentation Rate (ESR): Definition and normal values.
Definition: It is defined as the height of the supernatant column, separated after 1, 2 or 24 hours from the mixture of blood (venous blood) and anticoagulant (Sodium Citrate 3.8%) in the ratio 4:1.
Normal Values: Men: 2 - 7 mm (after 1 hr); 7 - 15 mm (after 2 hours).
Women: 4 - 9 mm (after 1 hr); 12 - 17 mm (after 2 hours).
In pregnancy: 35 mm (after 1 hr);
Newborn: 0.5 mm (after 1 hr);

16. **White Blood Cells Count. Normal Values**:
Adult: 4.000 - 8.000/mm^3
New born: 15.000 - 30.000/mm^3
Children: up to 1 year: 9.000 - 15.000/mm^3
up to 6 years: 4.000 - 10.000/mm^3

17. Differential white blood cell count (leukocyte formula)

GRANULOCYTES

Neutrophils	60 - 70%	3000 - 8000/mm3
Eosinophils	1 - 4%	300 - 400/mm3
Basophils	0 - 1%	20 - 80/mm3

AGRANULOCYTES

Lymphocytes	25 - 35%	1500 - 2400/mm3
Monocytes	4 - 8%	240 - -600/mm3

18. Bleeding Time: definition and normal values: Duration of bleeding induced after dermal puncture with a Frank needle. *Normal Values*: 2 - 4 minutes

19. Coagulation Time: definition and normal values.
Definition: It's the time interval between harvesting of the venous blood until its complete coagulation (disappearance of blood fluidity). There are 2 techniques:
a) Lee and White technique (on test tubes)
- in the 1st test tube: 6 - 10 minutes
- in the 2nd test tube: 8 - 12 minutes.

b) Bazarov technique (on glass slide):
- 8 - 10 minutes.

20. Platelet count: normal values.
Normal Values: 200.000 - 300.000/mm^3

21. Exploration of the capillary fragility using the Rumpel Leeds test.
Using a pneumatic cuff, a pressure of about 10 mmHg (below the systolic pressure) is applied around the forearm and maintains it for 5 minutes. Then on a circular surface of diameter 2 cm below the edge of the cuff we count the number of red spots:
Simple positive test: 10 - 12 spots (+)
Highly positive test: 20 spots (++)
Pathologic test: 80 spots (+ + +)

21. Blood typing. Determination of blood groups.

There are 2 methods:
a) Determination of agglutinogen (Beth-Vincent method):
On a slide we put 3 drops of serum belonging to groups O, A, B. To each of them add a small quantity of blood that has been obtained from venous or capillary puncture. If there is agglutinogen in the blood and if it corresponds with the agglutinin in the serum, then the RBC's will agglutinate (clot) and then precipitate. The result will be a red precipitate. If there is no agglutinogen then the drops will remain as a homogenous mixture with a reddish yellow color without precipitation.

b) Determination of the agglutinin (Simonin method)
On a glass slide we put 3 drops of the patient's blood. On top of them we put a small quantity of standard erythrocyte (corresponding to the groups O, A, B). Then we read the result like in the 1st determination.

22. Normal urine: aspect, pH and diuresis.
Aspect at emission: pale yellow (but darker in the morning), transparent, clear
pH: - in balanced diet - 6.2 - 6.8
- in predominantly proteic diet - 5.2 - 5.3
- in vegetarian diet - 7 - 7.5

Diuresis: volume of urine in 24 hrs is 900 - 1500ml, with physiological variations according to age, sex, environmental temperature and liquid ingestion, etc

23. Components of standard urine examination (urinalysis).
Density: 1.015 - 1.022
pH: 6.2 - 6.8 (in normal diet)

In the normal urine glucose, albumin, ketone bodies, biliary pigments and salts are absent

There will be normal values for urobilinogen.

In the sediments will be leukocytes, epithelial cells and crystals.

24. Addis test: normal values.

Definition: quantitative examination of blood cells and cylinders in the urinary sediment from the urine in 24 hours.

	Adults
Red blood cells	< 500 000/24 h
White blood cells	1-1.8 millions/24 h
Cylinders (casts)	<5 000/24 h

25. Normal concentration of urea in blood plasma

0.2 - 0.4g/l

26. Normal concentration of urea in final urine.

15 - 35 g/l.

27. Normal concentration of uric acid in blood plasma

2 - 5mg/dl

28. Normal concentration of uric acid in final urine

0,5 - 1 g/l.

29. Normal concentration of creatinine in blood plasma

0.6 - l.3 mg/dl

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30. Normal concentration of creatinine in final urine.

0.75 - 2 g/l.

31. Normal concentration of glucose in blood plasma

0.8 – 1.1 g/l.

32. Normal concentration of glucose in final urine.

0 g/l.

33. Normal concentration of Ca^{2+} in blood plasma

4.3 – 5.2 mg/dl.

34. Normal concentration of Ca^{2+} in final urine

0.14 – 0.20 mg/dl.

35. Normal concentration of PO_4^{3-} in blood plasma

3 - 4.5 mg/dl

36. Normal concentration of PO_4^{3-} in final urine

1.2 – 1.5 mg/dl.

37. Electrode placement in the standard leads.

DI - Right forearm (-)
Left forearm (+)

DII - Right forearm (-)
Left leg (+)

DIII - Left forearm (-)
Left leg (+)

Color code:
Right forearm: Red
Left forearm: Yellow
Left leg: Green
Grounding: Right leg - Black

38. Electrode placement in the precordial (thoracic) leads.

- V1 4th intercostal space right margin sternum
- V2 4th intercostal space left margin sternum
- V4 5th intercostal space left mid-clavicular line
- V3 midway between V2 and V4
- V5 5th intercostal space left anterior-axillary line
- V6 5th intercostal space left mid-axillary line

Color code: V1…V6: red, yellow, green, brown, black, violet

39. Electrode placement in the amplified unipolar leads of the limbs.

Exploratory electrodes (+) aVR, aVL, aVF. In each of the leads the reference electrical potential (0) is obtained by connecting the other 2 leads in the Einthoven's triangle using the 5 KOhm resistance.

40. Duration and amplitude of the P wave

Duration: 0.08 to 0.11 s
Amplitude: 0.1 to 03 mV

41. Duration of PQ segment: 0.04 to 0.12 s

42. Duration of QRS complex: 0.04 to 0.1 s

43. Duration of ST segment: 0.1 to 0.2 s

44. Duration and amplitude of the T wave

Duration: 0.15 to 0.25 s
Amplitude: 0.2 to 0,6mV

45. Determination of the heart rhythm

Normal heart rhythm is sinus rithm.
Each QRS complex is preceded by a single P wave: positive, normal aspect in at the least 2 standard leads. Normal rate of the sinus rhythm is 70/minute.

46. Determination of the heart rate on the ECG

The number of heart beats per minute is determined by dividing 60 by the duration in seconds of the cardiac cycle (R-R interval). If the rate is irregular, then the average for 3 to 4 cycles is calculated. The normal heart rate is 70/minute.

47. First heart sound: auscultation area and characteristics.
Heart all over cardiac area having the maximum intensity in the 5th left intercostals space, medial clavicular line for the bicuspid valve and the base of the xiphoid process: for the tricuspid valve.

- Long, brave and strong
- Duration: 0.08 to 0.15 sec
- Pitch: 35 to 100 Hz
- Timber. duel, stiffened

48. Second heart sound: auscultation area and characteristics.
Heart all over cardiac area having the maximum intensity in the 2^{nd} intercostal space 2 cm parasternal. Right side for aortic valve and left side for pulmonary valve.

- Its short, acute, weak and highly analytic
- Duration: 0.07 to 0.1 sec
- Pitch: 100 to 150 Hz
- Timber: clear, stroke like.

49. Arterial blood pressure: Normal values.
Maximum: 120 to 140 mmHg
Minimum: 60 to 90 mmHg.

50. Measurement of the arterial blood pressure: the auscultatory method.
Auscultatory method is based on the stethacoustic exploration of the sounds determined by the arterial wall vibration (Korotkoff sounds) distally from a compression. The pneumatic cuff of a sphygmomanometer is placed around a patient's upper arm and the bell of the stethoscope should be lightly placed over the brachial artery pulse, proximal and medial to the cubital fossa, and below the bottom edge of the cuff (i.e., about 2 cm above the cubital fossa). The cuff is inflated to a pressure higher than assumed systolic value. The air is slowly released (2 to 3 mm Hg/second). The pressure equal to the appearance of the I^{st} sound is the maximal systolic arterial blood pressure value. The pressure equal to the sudden decrease in sound intensity (or last sound) is the minimal diastolic arterial blood pressure value.

51. The laryngo-tracheal sound.
This sound it is strong and stiffilating. It is produced by the air passage through the glottis during both expiration and inspiration. The stethoscope is placed on the anterior surface of the thorax over the upper half of the sternum and posterior in the infrascapular region (T1 to T 4).

51. The vesicular murmur.
The sound is weak and sweet aspirative. It's produced by the air passage through the communication between the bronchioles and the alveolar channels. The stethoscope is placed systematically on the entire thoracic surface to detect pathological changes.

52. Normal values of the respiratory volumes (ml; ~80% in women)
Tidal volume (normal breath) = 500 ml
Inspiratory reserve volume (IRV): 3000ml.
Expiratory reserve volume (ERV): 1200ml
Residual volume (RV): 1200ml
Functional residual capacity = Expiratory reserve volume + Residual volume
Inspiratory capacity = Tidal volume + Inspiratory reserve volume
Vital capacity = Tidal volume + Inspiratory and Expiratory reserve volume
Total pulmonary capacity = Vital capacity + Residual volume.

53. Determination Forced Expiratory Volume in 1 second (FEV1).
On a spirogram is determined volume exhaled during the first second of a forced exhalation started from the level of total lung capacity (after a maximal inspiration).

Tiffeneau index (airway permeability index)
= FEV1/VC * 100
= 80% ;
↓ (below normal) = obstructive airway disease
↑= restrictive lung disease (VC is lower)

54. Gastric sampling technique
A piece of special rubber tubing is introduced into the digestive tract;
F: Faucher, thick, 1 cm diameter/75 cm lenght,
E: Einhorn, thin, 0.5 cm diameter/150 cm length;
via the nasal (F or E) or the oral pathway (E).
The presence in the stomach of the distal end of the tubing allows sampling of the gastric, content, with a syringe attached to the proximal one.

55. Duodenal sampling technique
A piece of special rubber tubing is introduced into the digestive tract; Einhorn type, thin, 0.5 cm diameter/150 cm length. The presence in the duodenum of the distal end of the tubing allows sampling of the duodenal content, with a syringe attached to the proximal one.

56. Gastric juice acidity: determination; normal values
Filtered gastric juice is neutralized using NaOH 0.1N in the presence of Topffer reagent and phenolphthalein. Color changes along titration:
-initially red
- yellow-orange, at pH-3.5 (free acidity)
- intense pink, at pH ~ 8.2 (total acidity)

Normal values	**Clinical units**	**HCI (g/1)**
free	15	0.9-1
combined.	25	1-2.5
total	40	2.5-3.5

Gastric juice acidity: secretory rates in basal conditions and with maximal stimulation (Kay test):

Parameter	Basal	Maximal
Volume flow rate (ml/h)	60	250
Acid flow rate (mEq/h)	2	18

57. Bile sampling by duodenal probing
A piece of special rubber tubing is introduced into the digestive tract; Einhorn type, thin, 0.5 cm diameter/150 cm lengths. The presence in the duodenum of the distal end of the tubing allows sampling of the duodenal content, with a syringe attached to the proximal one.
- Bile A: coledocian, dark yellow, viscous
- Bile B: vesicular - green yellow

- Via the tubing a colagogue substance is administered (for example 20ml MgSO4 33%; stimulates the gallbladder contraction, with bile elimination to the duodenum).

- Bile C: hepatic - golden yellow

58. Principle of Radioimmunoassay (RIA).

It is based on the competition between the hormones to be measured (not labeled) and the labeled hormone in the reaction with the specific antibody.

59. Presentation of an early pregnancy diagnosis method

It is based on the evidentiation of an increase in the concentration of the human chorionic gonadotrophins in the urine of a pregnant woman, starting from the 7th to the 14th day of pregnancy with the maximum in the 6th week.

The early pregnancy test uses 1 to 3 ml of urine which is injected into a male frog through the muscle of the hip in the dorsal lymphatic sacs. After around 45 minutes urine is sampled and examined directly - under the microscope for the presence of spermatozoa that indicates a positive reaction.

60. Patellar reflex

Location of the center: L2 to L4

Percussion of the patellar tendons determine contraction of the femoral quadriceps with extension of the leg. The subject is sitting cross-legged or dorsal cubitus when me examiner is holding the limbs from the popliteal space.

61. Achillean reflex

Location of center: L5 to S 2

Percussion of the achillean tendon determine contraction of me sural triceps with the plantar flexion of the foot The subject is standing with the respective knee supported by a chain.

62 Biceps reflex

Location of the center: C5 to C 6

Percussion of the distal tendon of the brachial biceps through the finger of the examiner determine contraction of the biceps with the flexion of the forearm on the arm. The subject is holding the forearm in a mild flexion.

63. Triceps reflex

Location of the center: C7 to Tl

Percussion of the distal tendon of the brachial triceps at the level of the olecranon. Determine the contraction of the triceps with the extension of the forearm on the arm. The examiner is holding the subject's arm at the elbow joint with, a mild abduction and backward position.

64. Plantar cutaneous reflex

Location of center: L4 to S2.

Excitation with a blunt object of the external edge of the plantar, from the calcanean towards the toes determines plantar flexion of the big toe and flexion and adduction of the other toes.

- In the inverse response, extension of the hallucis and abduction of the other toes is caused - the Babinski sign.
- Physiologically, it occurs for kids under age of 3 and adults in deep sleep.
- Pathologically, it occurs in lesion of the pyramidal tract.

65. Abdominal cutaneous reflex

Light excitation with a blunt tip on the abdominal skin at the superior, middle and inferior region, determines the contraction of the abdominal muscle. The subject is in dorsal cubitus with the legs mildly flexed on the abdomen for the optimal relaxation of the abdominal muscles.

66. Methods of vagal stimulation determine a transient bradycardia

- coughing
- lying recumbent on the foor with legs elevated against the wall
- valsava maneuver – deep inspiration followed by expiratory effort against a closed glottis
- carotid sinus massage
- immersion of the face in cold water (the dive reflex)

Compression of the carotid sinus unilateral for 20 sec determines bradycardia and hypotension - the Valsalva mannever.

Deep inspiration followed by post-inspiratory apneea results in transient bradycardia.

67. Determination of visual discrimination ability.

Its done with standard optotype for each eye separately, starting with the one that is weak in sight. One side is covered with the operculum (semi-transparent). The subject is placed in condition to the optotype. The subject is asked to read signs in each row, starting with the large one. The value of visual acuity is corresponded to the last row read with less mistakes and it is expressed using formula ratio (D/d)

D= distance (actually determined)

d = distance for the normal eye (emetrope)

If the reading is impossible from that distance then the subject moves closer until he or she can recognize the largest row (if it is impossible, in this case the patient is invited to number the hand fingers, to recognize the movements of the hand and in the last instance it is evaluated light perception).

68. Oscillometric method

The Pachon oscillometer measures the oscillations amplitude of the arterial wall. Oscillometric index (OI) means the maximum amplitude of the oscillations. It is determined the directly systolic pressure (suddenly increase of the oscillations), diastolic pressure (suddenly decrease of the oscillations) and mean arterial pressure (maximum oscillations). Normal values of OI:

- Superior 1/3 of arm: 4-20
- Inferior 1/3 of arm: 2-12
- Inferior 1/3 of forearm: 1-10
- Medium 1/3 of thigh: 4-16
- Superior 1/3 of leg: 3-10

Normal values of mean arterial pressure: 85-100 mmHg. Clinical significance have the comparison of OI values between 2 simetrical sites. The differences bigger than 2 units between points on the same level on the 2 limbs is an evidence for the presence of an injury, but do not give information about the length of the lesion. Oscillometric method assesses the arterial obstructions and arterio-venous aneurysms.

69. Blood circulation time

Inject into a vein in the cubital fossa 5 ml of calcium gluconate (20%).
Measure the time between the moment of injection to the moment when the subject feels the heat in the tongue and pharynx.
Normal values: 8-16 seconds

- shorter circulation time: physical effort, pregnancy, anxiety, increased temperature of external environement
- prolonged time: vagotony
- pathological circulation time: arterio-venous communication (shorter time), arteritis obliterans (prolonged time)
-

70. Cold pressor test: vascular reactivity

The cold pressor test is a cardiovascular test performed by immersing the hand into an ice water container, usually for one minute, and measuring changes in blood pressure. Results:

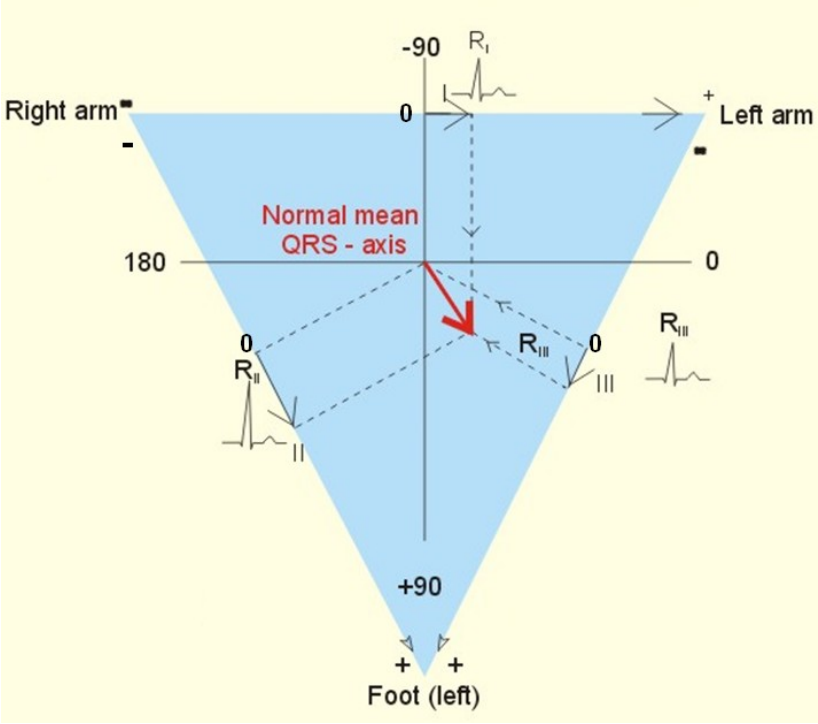
- normal: BP increase by 10-20 mmHg
- hyporeactivity: BP increase less than 10 mmHg
- hyperreactivity: BP increase more than 10 mmHg

71. ECG technique

- Explain the procedure to the patient, as it can be quite daunting
- Patient is lied down in a supine position on a flat surface, relaxed
- Room temperature - 20°C
- The electrode location on body surface must be degreased (with alchool)
- Apply conducting gel on the surface of the electrodes
- Fix the electrodes on specific locations on the body surface
- Do not fix the electrodes on areas with hair / bones
- Before starting the ECG determine first standard reading to be used: 1mV (etalon signal) = 10 mV and speed of the paper – 25 mm/s ⇨ 0.04 mV

72. Determining the mean QRS axis from EKG traces using the triaxial method:

- Isolate Lead I and III. Determine algebraically the sum of QRS complex in mm in lead I and III (the net deflection is the sum of the positive deflections of the QRS complex for Lead I and III)
- Draw the corresponding vectors on each lead (on Einthoven's Triangle). The tail of the vector is placed at the zero point on the appropriate lead axis on the triangle. The vector arrow is drawn with a length corresponding to the net deflection for that vector, as determined above.
- Draw a line perpendicular to each lead at the head of each vector arrow.
- Two (or more) perpendiculars will intersect. While you can do this with only two perpendiculars, it is best to use 3 as a check. If the third perpendicular doesn't come anywhere near the intersection of the other two, something is wrong.
- Draw the mean axis vector by putting the tail of the arrow at the center of Einthoven's Triangle and the head of the arrow at the point of intersection of the parallels determined in anterior step and drawing the complete arrow ⇨ mean QRS axis



73. The exercise stress test -- also called a stress cardiac test -- is a test used to provide information about how the heart responds to exertion. It usually involves walking on a treadmill or pedaling a stationary bike at increasing levels of difficulty, while your electrocardiogram, heart rate, respiratory rate, and blood pressure are monitored before, during and after the effort.

Cycle Ergometer Tests

- intensity of physical effort: 15W on children, 25W on ill adult, 50W on untrained healthy adult, 75 W on trained adults (hard physical workers).
- duration: 6 – 8 minutes
- results: on healthy subjects over 50 years the heart rate <= 150/min
  - heart rate increase with 10 mmHg
  - respiratory rate increase with 3-5 respirations/min
  - ECG remain unchanged
- stop the test if appear intense fatigue, chest pain, dyspnea
- indications: obliterant arteriopathy
- contraindications: cardiac failure, recent myocardial infarct (< 6 months)

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