### **COURSE OBJECTIVES**

To equip the students to acquire knowledge in clinical biochemistry enabling them to understand the basics of life processes and function of the human body in health and disease with special reference to hepato, cardio and renal functions.

## **COURSE OUTCOME**

Students acquire the basis behind the assessment of vital organ functioning through liver function, kidney function and cardiomarker assessment

## Unit 1

## Introduction

Organization of clinical laboratory, Introduction to instrumentation and automation in clinical biochemistry laboratories safety regulations and first aid. General comments on specimen collection, types of specimen for biochemical analysis. Precision, accuracy, quality control, precautions and limitations.

## Unit 2

## Evaluation of biochemical changes in diseases

Basic hepatic, renal and cardiovascular physiology. Biochemical symptoms associated with disease and their evaluation. Diagnostic biochemical profile.

### Unit 3

### Assessment of glucose metabolism in blood

Clinical significance of variations in blood glucose. Diabetes mellitus.

### Lipid profile

Composition and functions of lipoproteins. Clinical significance of elevated lipoprotein.

Unit 4

Liver function tests - Serum enzymes in liver disease- Serum transaminases (SGOT and SGPT), and phosphatases.

**Renal function tests** - Introduction, clinical significance of GGT, LDH and creatine phosphokinase in kidney function.

Urine analysis - Physical examination of urine

### Unit 5

**Tests for cardiovascular diseases** – ECG, Involvement of enzymes in diagnostics of heart disease including aspartate transaminase, isoenzymes of creatine kinase and lactate dehydrogenase and troponin.

Tumour markers for diagnosing various cancers.

## REFERENCES

Mukherjee, K.L., (2010). Medical Laboratory Technology - a Procedure Manual for Routine Diagnostic Tests Vol. I (2010), Tata Mc Graw–Hill Publishing Company Limited (New Delhi). ISBN:9780070076594 / ISBN:9780070076631

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LECTURE PLAN BATCH



## **KARPAGAM ACADEMY OF HIGHER EDUCATION**

(Deemed to be University) (Established Under Section 3 of UGC Act 1956) Coimbatore - 641021. (For the candidates admitted from 2016 onwards) **DEPARTMENT OF BIOCHEMISTRTY** 

## SUBJECT : CLINICAL BIOCHEMISTRY

SEMESTER : V SUBJECT CODE: 16BCU501A

CLASS : III B. Sc. BC

## LECTURE PLAN DEPARTMENT OF BIOCHEMISTRTY

S.No	Lecture	Topics to be Covered	Support		
	Duration		Material/Page		
	Hour		Nos		
		UNIT-I			
1	1	Organization of clinical laboratory, Introduction to instrumentation and automation in clinical biochemistry laboratories safety regulations and first aid	T1: 4 - 5		
2	1	Specimen collection, types of specimen for biochemical analysis	T1: 5 - 7		
3	1	Precision in clinical laboratory	T1: 9 - 10		
4	1	Accuracy in biochemical laboratory	T1: 10 - 15		
5	1	Quality control of biochemical laboratory	T1: 28 - 529		
6	1	Precautions in biochemical laboratory	T1: 34 - 38		
7	1	Limitations of clinical laboratory	T1: 39 - 42		
	Tota	l No Of Hours Planned For Unit 1=07			
		UNIT-II			
1	1	Basic hepatic evaluation of biochemical changes in diseases	T1:222-224		
2	1	Renal changes occurring during disease conditions	T1:224-226		
3	1	Cardiovascular physiology during diseases	T1:34-35		
4	1	Biochemical symptoms associated with diseases	T1:35-38		
5	1	Evaluation associated with diseases	T1:35-36		

## LECTURE PLAN

6	1	Diagnostic biochemical profile	T1: 39 - 42							
7	1									
	Tota	No Of Hours Planned For Unit II=07								
		UNIT-III								
1	1	T1:271-273								
2	1	Clinical significance of variations in blood glucose	T1:270-2767							
3	1	Diabetes mellitus conditions and clinical symptoms	T1:271-280							
4	1	Composition of lipoproteins	T1:262-264							
5	1	Lipoproteins functions	T1:264-266							
6	1	Clinical significance of elevated lipoprotein	T1:266-271							
7	1	Revision of Unit III								
Total No Of Hours Planned For Unit III= 07										
	UNIT-IV									
1	1	Liver function tests - Overview	R1:579-580							
2	1	Serum enzymes in liver disease	R1:588-590							
3	1	Role of SGOT in liver function tests.	R1:589590							
4	1	Role of SGPT in liver function tests.	R1:590-592							
5	1	Clinical significance of GGT	R1:590-591							
6	1	LDH and creatine phosphokinase in kidney function	T1:589-590							
7	1	Physical examination of urine	T1:588-589							
	Tota	No Of Hours Planned For Unit IV=07								
		UNIT-V								
1	1	Role of ECG in cardiovascular disease diagnosis	T1: 283-284							
2	1	Functions of Aspartate transaminase in diagnosis of heart diseases.	T1: 284-286							
3	1	Isoenzymes of creatine kinase	T1: 289-290							
4	1	Functions of lactate dehydrogenase in cardiovascular disease	T1: 290-292							
5	1	Role of troponin to test for cardiovascular diseases	T1: 292-294							
6	1	Tumour markers for diagnosing various cancers.	T1: 297-304							

	Tota		
Tota	al Planned	34	
Hours			

## **References:**

T1: Medical Laboratory Technology - a Procedure Manual for Routine Diagnostic Tests VoI. II (2010), Mukherjee, K.L., Tata Mc Graw – Hill Publishing Company Ltd. (New Delhi), ISBN: 9780070076648.

R1: Baynes, J.W. and Dominiczak, M.H., (2005). Medical Biochemistry 2<sup>nd</sup> ed., Elsevier Mosby Ltd. (Philadelphia), ISBN:0-7234-3341-0.

Signature of the Staff



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## <u>UNIT-I</u>

## SYLLABUS

Organization of clinical laboratory, Introduction to instrumentation and automation in clinical biochemistry laboratories safety regulations and first aid. General comments on specimen collection, types of specimen for biochemical analysis. Precision, accuracy, quality control, precautions and limitations.

## **GENERAL LABORATORY TECHNIQUES**

Laboratory services are an integral part of disease diagnosis, treatment, monitoring response to treatment, disease surveillance programmes and clinical research. Essential Health Technology as an important ingredient of Essential Clinical Services. Use of diagnostic techniques aid early diagnosis enabling appropriate and prompt intervention thereby reducing overall disease burden and promoting health. All laboratories are not equipped with facilities for carrying out complex investigations. The structure and function of a clinical laboratory varies according to the level of health care facility. Peripheral laboratories carry out simple tests like urine analysis and haemoglobin estimation whereas higher centers are equipped with sophisticated technology and trained manpower to carry out complex investigations. Establishing a network between peripheral and higher laboratories allows collection of specimen at periphery and their storage and transport for testing at higher centers and communicating report to the peripheral center efficiently without actually having to transfer the patient. In the event of patient transfer, the higher centers do not need to repeat investigations carried out at the peripheral health center, thereby saving crucial time as well as cost and providing continuity in patient care. Networking between laboratories is also essential in disease surveillance programmes and outbreak investigations in order to obtain quick and reliable results.

## Scope

Good Clinical Laboratory Practices should be used by all laboratories where tests are done on biological specimens for diagnosis, patient care, disease control and research such as:

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- $\Box$  Microbiology & Serology
- □ Hematology & Blood Banking
- $\hfill\square$  Molecular Biology and Molecular Pathology
- □ Clinical Pathology
- □ Clinical Biochemistry
- □ Immunology (Immunohematology and Immunobiochemistry)
- $\hfill\square$  Histopathology/Pathology and Cytology

## Equipment

□ Each laboratory should prepare an exhaustive list of equipment and consumables required and available for general functioning of the laboratory and specialized equipment for special tests.

□ Laboratory equipment should be of adequate capacity to meet work load requirement.

□ Equipment should be suitably located in the laboratory so as to allow accessibility and sequential utilization thus minimizing the need for frequent movement of specimens or reagents.

□ All equipment should be in good working condition at all times. Periodic inspection, cleaning, maintenance of equipment should be done. An equipment log book should be maintained for all major equipment.

Laboratories should maintain necessary instructions for operation and maintenance of equipment in the form of Standard Operating Procedures (SOPs). A copy of SOP should be readily available.

□ Maintenance contracts including warranty cards, telephone numbers of staff to be contacted in case of equipment malfunction should be kept safely. User manual should be available readily for reference. The staff should be aware of trouble shooting measures to be adopted for preventing equipment malfunction. A format of the equipment log book provided in Annexure 1 can be used.

□ New equipment should be calibrated and validated before routine use. AMR (Analytical Measurement range) should be verified, manufacturer can be consulted for verification and selection of range.

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□ Periodic performance check/calibration check for all equipment should be done using reference standard/reference material. The frequency of performance check should be based on the day-to-day performance of the equipment.

□ Equipment performance should be verified from Internal Quality Control results and External Quality Assessment results. Outlier parameter trend analysis record should be maintained in respect of its effect on the equipment.

□ All analytical equipment should be calibrated and calibration certificate provided by equipment company. Non-analytical equipment such as pipette, thermometer, weighing balance and centrifuge should be calibrated by accredited calibration laboratory or done in-house with traceability to National Physical Laboratory (NPL). For in-house calibration, laboratories should use :

□ Calibrated tachometer - for centrifuge

□ Calibrated digital temperature sensor - for checking temperature of refrigerator, incubator etc.

□ Calibrated glass thermometer- for temperature checking of oven, water bath etc.

□ Calibrated weights - for balance

□ National Institute of Science and Technology (NIST) buffer – for pH meter.Standard buffer solutions bought from reputed manufacturers with certifiable traceability can be used as alternative.

## **Standard Operating Procedure (SOP)**

□ SOP is a document, which contains detailed, written instructions describing the stepwise process and technique of performing a test or procedure in the laboratory.

 $\Box$  SOP helps to ensure uniformity, consistency and control over the processes carried out. It ensures that the procedures are done in exactly the same way each time irrespective of the operator.

□ SOP should contain information on who can perform the test, their qualification and training, how to carry out the test including pre-analytical, analytical and post-analytical stages of test/procedure, laboratory conditions required for the test/ procedure, routine care and maintenance of equipment, precautions and safety instructions, trouble shooting measures, waste disposal and linkage with reference laboratories.

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 $\Box$  SOP should be simple and written in an easy to understand language.

□ The procedure described in the SOP must be followed exactly by all staff members to ensure high quality results.

□ It should be titled along with version number, dated and signed by an authorized person and updated regularly.

□ It is important for the SOP document to be readily available in the working area and is therefore also referred to as **'laboratory bench work manual'**.

□ SOPs are **controlled documents** and can be changed only with approval of the laboratory quality manager and/or Head of the laboratory.

## Safety in Laboratories

Personnel working in laboratories may be exposed to risks from various chemicals, infectious materials, fire hazard, gas leak etc. The environment is also at risk of being contaminated by hazardous materials used and wastes generated in the laboratory. Safety in laboratories therefore

includes protection of both the staff and the environment from hazardous materials.

## **General Safety Measures**

□ Documentation of Laboratory Safety Policies and Procedures.

□ All laboratory personnel should be aware about the laboratory safety policies and procedures and follow these at all times.

□ List of hazardous materials used in the laboratory should be prepared. All hazardous materials should be accounted for on a continuous basis.

□ Laboratory personnel should follow safe hygienic practices which include hand washing, wearing protective clothing, gloves, eye protection etc.

□ Eye wash facility should be available as "stand-alone" facility or attached to sink. Portable, sealed, refillable bottles should also be available.

□ Biohazard symbol should be used on all container/equipment containing biohazardous material.

 $\hfill\square$  Laboratories should ensure proper preservation and security of specimens

□ Destruction/disposal of hazardous material should be authorized, supervised and handled according to standard procedures.

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□ Laboratory personnel should be thoroughly trained in managing fire, and nonfire emergencies such as large spillage, gas leakage etc.

□ Adequate fire extinguishers should be readily available in the laboratory

□ Periodic checking of all safety equipment and accessories should be ensured.

□ Accident/incident/injuries record of laboratory personnel should be maintained and reported to the designated authority. The report should include description of the event, factors contributing to the event and information on first aid or other health care provided. This information can be analyzed periodically towards effectively controlling and preventing future events. The records should be checked periodically by the laboratory safety officer even in the absence of fresh entries.

The laboratory diagnostic process to obtain a result can be divided into three phases: the preanalytical, analytical and post-analytical phases

The pre-analytical phase is defined as the period from the physician's indication of the test up to the laboratory analysis of the biological material. In other words, this phase involves an individual's preparation for collection of the biological material, the collection itself, storage of the collected sample and its transport to the laboratory, and preparation of the sample for the assay. The importance of this phase is also supported by many publications mentioning the fact that up to 46 - 68 % of erroneous results are caused by failure to follow or respect the pre-analytical phase rules.

That is why the primary task of the laboratory is to provide clients with all necessary instructions (on patient preparation, sample collection, biological material storage and transport, pre-analytical sample treatment) so as to minimize the risk of errors that could consequently cause harm to the patient. All this information is summarized in the manuals of testing laboratories.

The pre-analytical phase is followed by the analytical phase, involving the sample analysis itself. Each laboratory must have an established quality control system to ensure the validity of the issued results. The analytical phase ends with the post-analytical phase, defined as the period from obtaining the lab result to its hand-over to the physician.

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It is necessary to keep in mind that biological samples constitute a risk of infection, and therefore personal protective equipment (rubber gloves, protective coat) should be used for work with biological material (material collection, lab work with the sample). In addition, a face mask and safety goggles must be used for highly infectious samples such as HIV or hepatitis C. If clothes or skin is contaminated by the biological material, the affected area should be washed and then disinfected. In the event of injury, the wound must be treated (let it bleed for several minutes, wash with soap, disinfect) and medical attention sought.

## **Pre-Analytical Phase and Its Sources of Variability**

As mentioned in the introduction, the pre-analytical phase, i.e. before the analysis of the sample (specified parameter) in the laboratory, can be a source of many errors. Therefore, it is necessary to explain what factors affect the pre-analytical phase most.

## **Before the Biological Material Collection**

Factors affecting the pre-analytical phase before the biological material collection can be further divided into controllable and uncontrollable factors. Controllable factors include, for example, adherence to some daily regimen, dietary habits, etc. Uncontrollable factors are variables such as age, gender, race, etc.

## **Controllable Factors before the Biological Material Collection**

Food is an important controllable factor before the biological material collection. Blood should ALWAYS be collected after a fasting period. Where this is not the case, increased levels of some metabolites can be observed due to ingested nutrient metabolism. Glucose, triacylglycerol, free fatty acid and lipoprotein levels are elevated. People whose diet is rich in fats will primarily have an elevated serum triacylglycerol concentration on the one hand, and a decreased serum nitrogen substance concentration on the other. Protein-rich food leads to increased ammonia and urea levels.

At the same time, postprandial hormones (e.g. insulin, which reduces potassium and phosphate levels) are released. Food composition may also affect the pH of urine. For example, vegetable and fruit consumption makes urine more alkaline, while meat, fat and protein-rich food makes it more acidic. Some metabolite levels may also be influenced by the consumption of certain beverages (caffeine increases the glucose level in blood). Alcohol also significantly

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affects biochemical assays. After alcohol ingestion, the blood lactate concentration increases almost immediately, while hydrogencarbonate and glucose levels go down. Long-term alcohol burden in the body leads to liver damage, which is manifested by increased alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma-glutamyltransferase (GGT) levels. Triacylglycerol and cholesterol concentrations are also elevated.

Another factor that may affect the final result is physical strain before the collection. The impact on the result will depend on the type of physical activity: either a short-term activity, with high-intensity anaerobic metabolism of the body, or a long-term (endurance) activity where the body predominantly employs aerobic metabolism. Medium physical exertion increases the glucose level and insulin secretion is stimulated. Muscular activity also increases levels of AST, lactate dehydrogenase (LD) and creatine kinase (CK) enzymes as well lactate and fatty acid levels. Long-term strenuous activity results in a decrease in blood sugar, an increase in creatinine, and multiple-fold increase in lactate levels. Cholesterol and triacylglycerol levels are also reduced.

Another controllable factor before the biological material collection is mechanical trauma; for example, muscle trauma, including intramuscular injections, causes the release of enzymes (CK, ALT, AST) and muscle tissue proteins (e.g. myoglobin). Cycling may cause mechanical trauma to the prostate, which may manifest itself by the release of prostatic serum antigen leading to a false positive result for this test. Marathon running and heart valve defects lead to the mechanical haemolysis of erythrocytes.

A very common problem, which is very difficult to control, is the effect of drugs. Drugs may affect the level of some monitored analytes; for example, acetylsalicylic acid (aspirin) increases serum AST and ALT and urine protein levels, furosemide increases serum glucose, amylase (AMS) and alkaline phosphatase (ALP), and decreases sodium cation levels. Drugs may also interfere with the analytical assay procedure. For example, since vitamin C has strong reduction properties, it causes a false decrease in the level of analytes detected using peroxide. Drugs may also affect the rate of metabolism or monitored analyte elimination, or damage certain organs – the hepatotoxicity of narcotic agents being an example.

Stress is also a major factor. Stress situations cause the release of stress hormones such as



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renin, aldosterone, somatotropin, catecholamines, cortisol, glucagon and prolactin. This is why blood collection for prolactin assays should be performed within three hours after waking up. Another example might be the 60% drop in cholesterol compared with the initial level within 24 hours after acute myocardial infarction. It takes many weeks before its concentration reverts to normal. For this reason, blood collection for cholesterol, HDL and LDL cholesterol assays is not recommended when patients with suspected acute myocardial infarction are being hospitalized. In contrast, slight stress may increase cholesterol concentration. Post-operative stress decreases the concentration of thyroidal hormones and transferrin, and secondarily increases the concentration.

## **Uncontrollable Factors before the Biological Material Collection**

Uncontrollable factors before biological material collection include age, gender, race and biological rhythms. A further uncontrollable factor which might be included here is pregnancy. However, since this example of influence on the pre-analytical phase is too specific, it will not be described in this communication. Except for biological rhythms and pregnancy, these effects do not require any special attention as they are beyond our control and are considered through reference limits for the relevant analyte.

Age is a very important uncontrollable factor, since most monitored analytes are age related. An older person will have higher cholesterol levels than a younger person. Children and adolescents exhibit higher total alkaline phosphatase activity than adults due to a higher production of the bone isoform of this enzyme as the body grows. The reason is that the assay includes total alkaline phosphatase activity, including the bone isoform. Attention must also be paid to the higher total ALP level in pregnant women due to the higher production of the enzyme.

Gender also has a major influence on the result of the assay. It is commonly known that many parameters depend on the hormone set and physical constitution. For example, men have higher levels of creatine kinase (CK), ALT, AST, ALP, uric acid, urea, haemoglobin, ferritin, iron and cholesterol than women. Furthermore, the non-Caucasian population is increasing in the Czech Republic. For example, the CK and AMS activity or the granulocyte count rise in ascending order from Caucasian through Asian to African-American populations (African-

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Americans have up to twice as much CK activity and Asians have a higher salivary amylase activity and a higher total bilirubin concentration).

Other effects that should be considered are biological rhythms with their different time periods, either occurring within a single day (circadian) or cycles taking roughly a year to complete (circannual). Circadian changes vary for different parameters; for example, there is up to 50 % change in iron levels during the day. Other parameters such as AST, ALT, LD and ALP show changes in the range of tens of percent. Maybe the most notable circadian change occurs in cortisol – about 250 % with minimal levels in the evening. An example of circannual rhythm is the change in vitamin D concentration, with maximum levels in summer months due to skin exposure to intense sunlight.

## **During the Biological Material Collection**

Factors influencing the pre-analytical phase during the biological material collection are primarily related to the work of the sample-collecting nurse, who has to keep in mind the basic sampling principles that may affect the result of the test. In particular, such principles include collection timing, selecting the appropriate collection set, the patient position during the collection, venostasis and local metabolism effects, as well as the effect of infusion and transfusion in the hospital environment.

## **Collection Timing**

Collection timing plays a very important part in the strategy to obtain valid results. Most often, collections take place in the morning when we can be sure that the patient has fasted (provided the patient respects general pre-collection recommendations) and the circadian rhythm effect mentioned in the chapter above is limited. A different example is blood sugar monitoring (blood sugar profile) or pharmacotherapy monitoring, where samples are taken based on drug elimination half-life.

## **Patient Position during the Collection**

Patient position during the collection is also important. It must be kept in mind that the difference in protein concentration when comparing a standing vs. sitting position for 15 minutes is 5 - 8 %, and about 10 - 20 % for a standing vs. recumbent position. In the standing position, water transfers from the intravasal to the interstitial space, which subsequently leads to a rise in



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high-molecular substances, primarily proteins, lipoproteins and protein-bound substances such as calcium cation and hormones (cortisol, thyroxin), or some drugs. In general, biological material should always be collected in the same position, preferably the standard sitting position, which is not always possible in hospitalized patients, though.

## Use of Tourniquet and Local Metabolism Effect

The effect of local metabolism when a tourniquet is used for collection is also interesting. The evidence shows that one minute after constricting the arm with a tourniquet there is already a significant transfer of water and ions from the vessel to the interstitium, with a subsequent rise in protein and blood protein-bound substance concentration. Long-term constriction or overcooling of the arm leads to a change in local metabolism due to hypoxia, which results in a rise in partial carbon dioxide pressure and potassium and lactate concentration, which in turn results in a drop in pH. In addition, there are homeostasis changes connected with the release of the tissue factor. Exercising the arm is not recommended, or it is even forbidden, as it primarily causes an increase in potassium concentration. For these reasons, the period for which the arm is constricted should not exceed one minute, and the tourniquet should be released immediately after the venipuncture.

## **Choosing the Collection System and the Effect of Anticoagulants**

The choice of the collection system is also very important. Options include a closed or an open sample collection system. The open collection system consists of a classical needle and a Luer-taper syringe. Following venipuncture, freely flowing blood is taken directly into the test tube or by gently pulling the plunger. Collection into a closed system is the preferred option today as it minimizes the risk of contaminating the collecting person through the blood, and collection tubes are colour coded depending on the added preservative or anticoagulant. Another advantage of the closed system is that the ratio of anticoagulant (preservative) to collected blood is maintained.

As mentioned above, anticoagulant (heparinate, citrate, oxalate, etc.) can be chosen depending on the required test. Nevertheless, attention needs to be paid when choosing the anticoagulant for cation tests, since the anticoagulant must not contain the cation being determined. For example, the use of EDTA with potassium will lead to highly pathological potassium concentrations in the sample! EDTA is not suitable for determining bivalent cation concentration as it acts as a chelating agent, binding these cations to form a complex, and it

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results in finding a falsely low concentration of these ions. In some cases, another substance (preservative) such as sodium fluoride is added to the anticoagulant in order to determine glucose concentration. The addition of sodium fluoride will cause glycolysis inhibition in red blood cells, thus preventing a drop in glucose concentration over time.

In addition, we must keep in mind that if a collection set containing an anticoagulant is used, we should gently mix the collected blood immediately after the collection. Without mixing, the anticoagulant effect is limited and undesired blood clotting will occur. A suitable needle lumen should be selected for blood collection to avoid red blood cell haemolysis.

## **Effect of Infusion and Transfusion**

Patients in a critical condition have to receive transfusion and infusion products containing high concentrations of selected substances and low concentrations of others. Infusion may therefore affect the determination of some substances, usually by direct contamination during collection or just due to their properties. For example, the infusion of glucose with potassium results in a false increase in glucose and potassium levels. The infusion of lipid emulsion causes serum chylosis and Hartmann infusions containing high lactate concentration (>15 mmol/l) cause a false increase in lactate concentration. On the other hand, Plasmalyte infusion causes a false normalisation of ion concentration in the collected sample. This is why certain rules should be followed during the sample collection following an infusion.

Ideally, collect blood from the other arm, i.e. where the infusion was not applied, or stop the infusion for 15 minutes and then take the sample. With respect to the pre-analytical phase, the age of transfusion must be taken into account. With the growing age of the erythrocyte concentrate, sodium and glucose concentrations decrease due to red erythrocyte metabolism, whereas, in contrast, potassium and lactate concentrations increase.

## **Between Biological Material Collection and Analysis**

This period includes the time from the collection of biological material until its analysis in the laboratory, and involves handling the sample following the collection, its subsequent transport to the laboratory, and centrifuging or pre-treatment before the analysis. In general, if anticoagulated blood is taken (collection container with anticoagulant), the test tube should gently be shaken immediately after the collection. If non-anticoagulated blood is taken, wait about 30 minutes



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before transporting the sample to allow sample clotting (exact time required for clotting is indicated by the manufacturer of the collection set). Immediate transport of the biological material after the collection may cause haemolysis and sample deterioration. The problem of haemolysis interfering with the assay is not only related to the release of erythrocyte content into the serum or plasma with a subsequent increase in the concentration of these substances in the tested material, but also to the release of haemoglobin, whose colour interferes directly with a photometric assay or with the agent used for the assay. Take care – haemolysis may also occur due to sample overcooling, high centrifuge speed or a narrow sampling needle. The following table describes the effect of haemolysis on selected biochemical assays.

Storage of the sample before the transport and the very transport of the biological material are very important and must be given adequate attention, especially if samples are transported from practitioners in the periphery and brought to a specialized laboratory. The transport time will vary; however, always avoid exposing the sample to extreme conditions (heat/freezing) during the transport, minimize shaking the sample and avoid complete deterioration which will occur if the sample is spilled. This is why samples have to be transported in temperature-controlled transport boxes protected against spillage. Some samples (tissues) must be transported frozen even at very low temperatures (-80°C) on dry ice. If the maximum time before sample processing is exceeded or transport conditions are not adhered to, some substance concentrations in the material for testing will change. One example is a decrease in glucose concentration or an increase in lactate concentration due to the anaerobic glycolysis of blood elements. Some analytes in biological material are thermolabile at room temperature (most parameters) and some, paradoxically, at 4°C (e.g. ALT activity decreases or potassium concentration increases due to the ATPase inhibition in the erythrocyte). Some analytes are photo-sensitive (e.g. bilirubin and porphyrins), and their amount drops unless transported and stored in the dark. For these reasons, some analytes have specific recommendations for storage and transport. For example, the recommendations for a plasma ammonia assay are as follows: carry out the anaerobic collection, prevent haemolysis, maintain the anticoagulant to blood ratio and transport in a transport container or on melting ice; analyse within 20 minutes after the collection.

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As soon as the samples are delivered to and received by the laboratory, they are either analysed directly (when whole blood is used), or must be centrifuged to obtain serum or plasma. The required conditions must be adhered to during centrifuging to achieve perfect serum (plasma) separation from erythrocytes and perfect leukocyte sedimentation in the plasma. If the speed (relative centrifugal force) is too high during centrifuging, the cells may break and their content may get released. Many analytes require centrifuging at lower ambient temperatures (cooled centrifuges), for hormone assays, for example.

Urine analysis requires a chapter to itself, since it requires the use of collected, first morning or single random specimens. Very often, patients are not instructed about the collection rules; they typically collect urine for a longer or a shorter time than required; moreover, obtaining an exact reading of the quantity of urine collected over the collection period, usually 24 hours, is always problematic. Nor it is possible to ensure the required storage of the collected urine in the fridge or the urine pre-treatment needed to stabilize the tested parameter. First morning urine collection poses a similar problem, since it has to be delivered for sediment analysis within one hour of collection. There is often a delay in delivering the collected urine to the laboratory, which leads to false negative or false positive results (increase in the bacteria count, increase in the pH value due to the urease of bacteria and cell element degradation). In general, the transport and storage conditions required for transported samples/material must be followed. Material transport in extreme (very hot, very cold) conditions requires special care.

## Ten pieces of advice for obtaining correct results

- Instruct the patient (why they are being tested, diet, physical strain)
- Time the collection correctly
- Fill in the order slip correctly
- Choose the right collection procedure
- Choose the right test tube
- Take the recommended amount of material
- Do not spill any biological material
- Label the test tubes correctly
- Ensure appropriate storage for biological material before transport

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• Ensure appropriate transport to the lab

## Analytical Properties of the Laboratory Method, Quality Control

Inherent in every measurement, and therefore in every method used in biochemistry laboratories, is a typical set of properties, generally referred to as the performance characteristics of the method. Their level indicates the options for measurement made using this method, which is why they are a determining factor in the usability of the method for the required application.

The level of analytical properties of the method is also a determining factor in the use of the method for clinical purposes. Therefore, every physician who uses measurement results should be aware of the basic analytical properties of the method used. The process, the aim of which is, besides determining the functional characteristics of the method, to comprehensively evaluate the suitability of the method for the intended clinical purpose, is called method validation. Realization of this process is an integral part of method development. Basic analytical properties of the method are also checked before the method is first used in the laboratory, and they are likewise regularly checked during routine use of the method. This process is known as verification of the method.

The set of operations carried out in the laboratory aimed at ensuring the adequate likelihood of measurement results is wider in scope. These activities are generally referred to as **quality control**. This set of activities is primarily intended to assure the quality of the analytical process in the clinical laboratory. In line with general trends, even medical laboratories implement comprehensive quality management systems to manage all laboratory operations; their aim is not only to maintain but also gradually improve the quality of laboratory services provided. The implementation of these mechanisms in laboratory management is inspected and certified by independent bodies in certification or accreditation processes, and is also increasingly required by healthcare payers.

## Performance Characteristics of the Analytical Method

The basic analytical properties of the method undoubtedly include two terms referred to as **precision** (in Czech: preciznost) and **trueness** (in Czech: pravdivost). Their combined projection in a specific measurement result then constitutes the property of the result referred to as **accuracy** (in Czech: přesnost).

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## Precision

Precision is the closeness of agreement between independent measurement results obtained under pre-specified conditions. Measurement precision is expressed numerically by measures of imprecision, which define dispersion between independently obtained results, such as standard deviation, variance, or coefficient of variation. Precision is the evaluation of the impact of random errors in measurement that can never be eliminated and whose magnitude is inherent in a certain method or specific measurement procedure. These errors are caused by accidental effects (instability of instruments, fluctuation of ambient measurement conditions such as temperature, variations in the operator's actions, etc.). Their action results in differences between repeated measurement results. If there is a sufficient number of repetitions, they are uniformly dispersed around their average value due to the randomness of their origin. Minimum deviations are most frequent and their number decreases with the increasing value of deviations. The distribution of deviation frequency corresponds to the normal (Gaussian) distribution. The measure of dispersion, i.e. the imprecision of results, is the standard deviation s. Since it is expressed in units of the measured quantity (measurand) and depends on its magnitude, use of a relative expression of standard deviation, i.e. the coefficient of variation CV expressed in % (sometimes also referred to as the relative standard deviation), is preferred.

The precision of the method is not the same throughout the method's working range. The dependence of precision on the measurand magnitude is called *precision profile*, and is an important scale of quality of the measurement method. The *CV* reaches lowest values in the middle area of the measurement range, whereas values grow towards the ends (growth is significant especially in the area of very low values of the measurand). The magnitude of imprecision is affected by the actual working range of the method. One condition for its selection might be that *CV* should not exceed the required level, 10 % for example.

The specified measurement conditions in the precision definition could be conditions of repeatability or reproducibility. The condition of **measurement repeatability** covers the same measurement procedure, operating staff, measuring system, working conditions, the same site and repeating the measurement on the same or similar object over a short period of time. The condition of **measurement reproducibility** covers different sites, operating staff, measuring



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systems, and repeating the measurement on the same or similar object. The relatively large freedom in the setting of these conditions always requires detailed specification as to which factors were variable. Specific conditions of reproducibility involving measurements made using the same procedure, on the same site, and by repeating the measurements on the same or similar objects, but over a more extended period of time, are referred to as conditions of **intermediate measurement precision**. Measurement over a longer period of time may involve the effect of other variable factors such as changes in calibration or calibrators, use of a different lot of reagents or a change of operators. Precision determined under these conditions is the best measure of the quality of method execution in specific laboratory conditions.

## Trueness

Trueness is the closeness of agreement between the average value obtained from a large series of measurement results and either the actual value or an accepted reference value x0. The measure of method trueness is its bias b: or, in the relative expression: The actual (true) value of the measured quantity (measurand) is in practice inaccessible on principle, and could only be obtained by perfect measurement. Therefore, it is replaced by an accepted reference value as the best practical approximation of the actual value of the quantity. The reference value is usually obtained based on a quantity measurement using a generally accepted reference method or other generally recognized process (by measurement in selected reference laboratories, etc.).

Method trueness is determined by the existence of a systematic error incidental to measurement. This kind of error may affect the measurement result either in a constant way (results are shifted always by the same value), proportionally (always by the same multiple), or in a combination of these two ways. In this connection, we speak of the constant and the proportional components of systematic error. While random errors cannot be avoided during measurement (only their magnitude can be influenced), systematic measurement errors can sometimes be eliminated or at least partially corrected by appropriate adjustment.

## Accuracy

Accuracy is the closeness of agreement between the result of a measurement and the true value of a measurand. This property applies to one measurement result and is actually the current expression of the combination of the precision and trueness of a method. It is the contribution of

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the Random Error (RE) and Systematic Error (SE) that occurred at the moment of a specific measurement. The sum of these contributions is referred to as the Total Error (TE) of measurement. As mentioned above, the measure of the contribution of the random error component in a given method is expressed by an estimate of standard deviation s; the measure of the contribution of the systematic error component is the deviation b. By using these two parameters, the method can be characterized by estimating the total analytical error TEA occurring in the measurement: The estimate confidence level is expressed by the coefficient kthat is equal to the corresponding quantile of the selected one-sided confidence interval (1.65 for 95 % and 2.33 for 99 %). Besides the two aforementioned characteristics, the clinical applicability of the method is also affected by the natural diversity of the observed parameter in the normal population, called the biological variability of the parameter (tested analyte). With respect to the origin of the contribution to overall biological variability, interindividual (among different individuals of the population) and intraindividual (within the same biological individual) variability is distinguished, and if coefficients of variation are used for their relative expression, they are usually denoted as CVG or CVI, respectively. The specific values of both contributions for many significant biological parameters have been monitored and published in the technical literature. In terms of assessing a method's clinical applicability, it is desirable that the relative analytical precision CVA of the method should be optimally better than a half of intraindividual biological variability, i.e. It is required for the acceptable relative analytical trueness BA of the method that it should be better than a quarter of the total biological variability, i.e. Thus, for the total analytical error TEA of the method to be acceptable there follows the requirement that: The values of the analytical precision of the method and the intraindividual variability of the analyte are used to compute the Critical Difference CD (sometimes also referred to as the Least Significant Change LSC) between two consecutive patient results. This is the difference between two measurement results that can be, depending on the aforementioned characteristics, indicated as significant at the selected confidence level with certain probability, usually 95 %. It is a parameter that indisputably plays an important role in the clinician's decision-making process when a laboratory result changes over time.

## Relationship between the Precision and Trueness of a Method

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As mentioned above, the mutual interaction of the two characteristics over a certain period of measurement results in a specific accuracy level of the measurement result (see Fig 1).

## **Measurement Result Uncertainty**

Uncertainty is a parameter associated with the result of measurement which characterizes the measure of dispersion of values that could reasonably be attributed to the measurand. The concept of uncertainty has replaced the previous concept of error in contemporary metrology. Unlike the



Figure 3.1. The relationship between the terms of precision, trueness and accuracy of measurement

The overall quality of a method can be evaluated in relation to the levels of both characteristics. This relationship is illustrated using the example of target shooting shown in Figure 2. As the quality of the method improves, so another property of the method namely uncertainty (see below), also improves.



Figure 3.2. Relationship between precision and trueness of the method and their effect on the uncertainty of the method

previous concept, this latest better describes the fact that a measurement result is just an estimate of reality with a degree of uncertainty. This means that it is not a point estimate differing from

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reality by a specified error; rather that the measurement result lies with certain confidence within a bounded interval of possible values. Knowing the uncertainty allows measurement results to be better compared between each other or with reference intervals, for example. An estimate of the measurement result uncertainty obtained using a method is also part of its validation, where it is used to assess whether the method is adequate enough for the required purpose.

The principle of determining the uncertainty consists in evaluating effects that can affect the measurement result, and subsequently in estimating an interval for which we can state with a specified measure of confidence that it contains the actual measurand value. The total uncertainty is the result of the composite action of many sources. The effect of each one is their individual contributions to the resulting uncertainty. This contribution is referred to as a component of uncertainty. The quantification of the contribution of certain components can be obtained from statistical distributions of measurement series results characterized by their experimental standard deviation (A-type uncertainty components). The quantification of the contribution of other components is obtained from probability functions based on experience or other information (B-type uncertainty components). The numerical value of each uncertainty component transformed into the standard deviation is called **standard uncertainty** and denoted as *ux* (the index *x* expresses its relation to the component *x*). Insignificant standard uncertainty contributions are ignored, and the rest are aggregated under the propagation of uncertainty rule

into the combined uncertainty *uC*: Note: The above algorithm is a very simplified method of measurement uncertainty estimation and does not constitute the only possible procedure.

In practice, measurement results are accompanied by the **expanded** 



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**uncertainty** *UC*, which is actually a combined uncertainty multiplied by the expansion coefficient (coverage factor) k so that the uncertainty estimate corresponds to the required confidence level. The coefficient value for 95% probability is 1.96, but the rounded value 2 is usually used in practice:

The specific measurement result is then presented as a value determined by measurement and is accompanied by expanded combined uncertainty, for example:  $132 \pm 6$  nmol/l. In addition to uncertainty sources associated with the analytical method itself, the resulting uncertainty of a measurement result also includes contributions from sources inherent primarily in the pre-analytical phase of the test. See Figure 3.3.

The importance of indicating uncertainty consists not only in the expression of a certain measure of the indeterminacy of a result, which may occur with a given probability within the entire bounded interval, but also the result uncertainty has to be taken into account when interpreting the result against decision limits, especially if the result is close to these limits.

## **Traceability of the Method**

The traceability of a method is the property of a measurement result whereby the result can be related to a reference through a documented unbroken chain of calibrations, each contributing to the measurement uncertainty. This is a very important property of the method in respect of reaching its optimum trueness. If within the method each of the quantity inputs included in the measurement model is traceable to a basic SI unit, then the traceability of the method can be considered optimally ensured and the results of measurement are qualified as comparable on a worldwide scale. This means in practice that particularly the calibrators used in the measurement process are required to be traceable to standards of higher metrological quality. This principle is ensured by the existence of a hierarchical structure of reference materials and methods, through which the working calibrator is gradually traced to the very highest standard, to an SI unit in optimal cases. This hierarchy also comprises different entities responsible for performing individual steps.

## **COLLECTION OF SPECIMENS**

**Collection of Blood sample** 

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Blood and separated serum are the most common specimens taken to investigate outbreaks of communicable disease. Venous blood can be used for isolation and identification of the pathogen in culture.

## Venous blood samples

## Materials for collection

□ Skin disinfection: 70% alcohol (isopropyl alcohol, ethanol) or 10% povidone iodine, swabs, gauze pads, band aid

 $\Box$  Disposable latex or vinyl gloves

□ Tourniquet, Vacutainer, Monovette, or similar vacuum blood collection devices, or disposable syringes and needles

□ Vacutainer or sterile screw-cap tubes (or cryotubes if indicated), blood culture bottles (50ml for adults, 25ml for children) with appropriate media

 $\hfill\square$  Labels and indelible marker pen.

## Method of collection

 $\hfill\square$  Place a tourniquet above the venepuncture site.

□ Palpate and locate the vein. It is critical to disinfect the venepuncture site meticulously with 10% povidone iodine or 70% isopropyl alcohol by swabbing the skin concentrically from the centre of the venepuncture site outwards. Let the disinfectant evaporate. Do not repalpate the vein again. Perform venepuncture.

□ If withdrawing with conventional disposable syringes, withdraw 5-10 ml of whole blood from adults, 2-5ml from children and 0.5-2ml for infants.

 $\Box$  If withdrawing with vacuum systems, withdraw the desired amount of blood

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adults, 2-5ml from children and 0.5-2ml for infants.

 $\Box$  If withdrawing with vacuum systems, withdraw the desired amount of blood directly into each transport tube and culture bottle.

□ Remove the tourniquet. Apply pressure to site until bleeding stops, and apply sticking plaster (if desired).

□ Using aseptic technique, transfer the specimen to relevant cap transport tubes and culture bottles. Secure caps tightly.

□ Label the tube, including the unique patient identification number, using indelible marker pen.

□ Discard the sharps into disposal container without recaping.

□ Complete the case investigation and the laboratory request forms using the same identification number.

## Handling and transport

□ Blood culture bottles and blood sample tubes should be transported upright and secured in a screw cap container or in a rack in a transport box.

□ Cushion or suspend bottles during transport over rough terrain to prevent lysis of red cells. They should have enough absorbent paper around them to soak up all the liquid in case of a spill.

□ If the specimen will reach the laboratory within 24 hours, most bacterial pathogens can be recovered from blood cultures transported at ambient temperature



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## **POSSIBLE QUESTIONS**

## UNIT-I

PART-A (20 MARKS)

## (Q.NO 1 TO 20 Online Examination)

## PART-B (2 MARKS)

1. Define the term precision.

2. Define the term accuracy.

3. Write a note on Quality Assurance.

4. Define Quality control.

5. Define Trueness.

## PART-C (6 MARKS)

1. Explain about the Quality control in clinical biochemistry and its classifications.

2. What are the safety regulations carried out in biochemistry laboratories.

3. Write about the various different methods for collection of blood and how they are preserved.

- 4. Explain the pre-analytical phase of laboratory diagnostic process.
- 5. Describe about the analytical phase of laboratory diagnostic process
- 6. Derive the relationship between precision and Trueness of analytical methods.
- 7. Write a short note on spectrophotometry?
- 8. Write about the differences between Quality control and Quality assurance.
- 9. Explain in detail about the Diagnostic sensitivity and specificity methods for laboratory screening.
- 10. Write about the post-analytical phase of laboratory diagnostic process.

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Questions	opt1	opt2	opt3	opt4	opt5	opt6	Answer
The desire to maintain a safe	prevention	ubiquity	microbiolo	accidents			prevention
laboratory environment for all			gy				
When a chemical splashes in	10 seconds	30 seconds	5 minutes	15 minutes			15 minutes
Which of the following types	A. safety	B. lab coats	C. face	D. all of the			all of the above
of personal protective	glasses		shields	above			
equipment (PPE) is frequently							
used			•				•
Chemical, reagents, broth	mouth	ear	pipette	nose			pipette
cultures should be pippeted by							
Good work practices include	smelling and	not washing	confining	using damaged			C confining
Good work practices include	tasting	hand before	long hair	equipment and			long hair and
	chemicals	and after lab	and loose	glassware			loose clothing
			clothing	-			0
What is the name of	A. sterilization	B. aseptic	C.	D. pathogen			B. aseptic
procedure performed under	technique	technique	disinfectal	technique			technique
sterile conditions to eliminate			technique				
contamination in hopes to							
obtain a pure culture of one							
type of microorganism							
After a biohogend anill in	A 5 minutes	P 20 minutes	C 60	D 20 minutes			<b>D</b> 20 minutes
After a bionazard spill is	A. 5 minutes	B. 50 minutes	C. 00	D. 20 minutes			B. 30 minutes
disinfectant solution, it must			minutes				
sit for							
What is needed for the source	A. pathogens	B. bacteria	C. reagents	D. media			D. media
of nutrient for the growth and	1						
reproduction of microbes							
To prevent the contamination	A. 70%	B. acetone and	C. 5%	D. water and			A. 70% ethanol
of microscopes and	ethanol and	lens paper	methylene	lens paper			and lens paper
surrounding areas disinfect	lens paper		blue and				
/clean used slides prepared by			lens paper				
students with							
Which of the following	A carbon	B powder	C foam	D. polka dot			A carbon
extinguishers is suitable for a	dioxide	extinguisher	extinguishe	extinguisher			dioxide
fire involving flammable	extinguisher	(blue)	r (cream)	(dotty)			extinguisher
liquids	(black)						(black)
GLP is an	A. Glass ware	B. FDA	C.	D. Safety rules			B. FDA
		regulation	Analytical				regulation
			laboratory				
Which of the following is the	A. Test systems	B. Reporting of	C. Test	D. All the above			D. All the above
principles of GLP?		study results	and				
			reference				
			substances				
How many types of inspection	A 2	В Л	C 3	D 5			C 3
now many types of inspection	A. 2	D. <del>4</del>	0.5	<b>D</b> .5			0.5
SOP is otherwise known as	A. Standard	B. System	C. Safety	D. Stationary			A. Standard
	operating	operating	operating	operating			operating
	procedures	procedures	procedures	procedures			procedures
		<u> </u>					
What good laboratory must	A. Area should	B. Maintenance	C. Air	D. Both A and C			D. Both A and C
contain?	be free from	and calibration	conditional				
	smoke, smell,	data	the lab				
	aust		witti humidity				
			control				
			control				

The prevention of large scale loss of biological intergrity is	A. Fire safety	B. Bio safety	C. Chemical safety	D. Test systems	B. Bio safety
Which of the following is not a laboratory safety rule?	A. You should never mix acids with bases	B. You should tie back your long hair	C. You should never add water to acid	D. All the above	A. You should never mix acid with bases
Which piece of laboratory equipment is best-suited for accurately measuring the volume of a liquid?	A. Graduated cylinder	B. Beaker	C. Erlenmeyer flask	D. More than one of the above	A. Graduated cylinder
Which piece of laboratory equipment can be used to store chemicals for long	A. Burette	B. Evaporating dish	C. Beaker	D. More than one of the above	C. Beaker
The independent variable in an experiment is:	A. The variable you hope to observe in an experiment	B. The variable you change in an experiment	C. The variable that isn't changed in an experiment	D. None of these is correct	B. The variable you change in an experiment
Qualitative results refers to	A. Results that can be observed during an experiment	B. Results those are difficult to observe during an experiment	C. Results that require numerical data	D. None of these is correct	D. None of these correct
. When drawing a graph that measures family average income over a period of 50 years, the independent variable is	A. Income	B. Average	C. Years	D. It is impossible to say	C. Years
Accuracy is defined as	A. A measure of how often an experimental value can be repeated	B. The closeness of a measure value to the real value	C. The number of significant figures used in a measureme nt	D. None of these	B. The closeness of a measure value to the real value
How many significant figures are present in the number 10,450?	A. Three	B. Four	C. Five	D. None of these	B. Four
. The key component of GLP system of quality is	A. Quality unit	B. Quantity unit	C. Quality reading unit	D. Quality assurance unit	D. Quality assurance unit
Microscope is wiped by using	A. 90% isopropyl alcohol +30% water	B. distilled water	C. 75 % ethanol	D. only with water	A. 90% isopropyl alcohol +30% water
Which one of the following is correct?	A. acid can be added to water	B. water can be added to acid	C. both a and b	D. none of these	A. acid can be added to water
. Before operating inoculation chamber the palm should be wiped with	A. Ethanol	B. distilled water	C. sanitizer	D. all of the above	A. Ethanol
Which one of the following are GLP regulations on requirements	A. 21CFR58	B. 40CFR160	C. 21CFR211	D. a and b only	D. a and b only

A "class –D" fire extinguisher can be used to treat fires involving which as fuel sources	A. ordinary combustibles (wood and plastics)	B. electrical equipment	C. combustibl e metals	D. flammable to combustible liquids		C. combustible metals
Which of the following id not a type of firefighting equipment	A. fire blanket	B. hose reel	C. sprinkler	D. ice cubes		D. ice cubes
Why shouldn't carbon dioxide extinguishers be used in confined spaces	A. they might explode	B. harmful fumes may be inhaled	C. they could cause claustropho bia	D. they might not show up if its dark		B. harmful fumes may be inhaled
What is the correct definition of fire	A. a chemical reaction from which heat and light are emitted	B. hot orange stuff	C. mixture of carbon dioxide and nitrogen	D. a yellow coloured solution		A. a chemical reaction from which heat and light are emitted
What is the extraction as practiced in the organic chemistry laboratory	A. the removal of one solid material from other	B. the separation of one substance from the another based on solubility	C. the removal of painful or impacted teeth	D. none of these		B. the removal of one substance from the another based on solubility
Latex gloves	A. may be reused only if they have not be been permeated	B. may be reused as long as they are clean	C. should never be reused	D. both a and b only		C. should never be reused
What is distillation?	A. distillation is when a liquid is evaporated and then recondensed in another container	B. distillation is when material heated to melting and then separated	C. distillation is when a substance is dissolved, heated and then precipitated	D. none of these		A. distillation is when a liquid is evaporated and then recondensed in another container
. What piece of laboratory equipment is best suited for accurately measuring the volume of a liquid	A. graduated cylinder	B. beaker	C. Erlenmeyer flask	D. more than one of the above		A. graduated cylinder
What piece of laboratory equipment can be used to store chemical for log periods of time	A. burette	B. evaporating dish	C. beaker	D. more than one of the above		C. beaker
Qualitative results refer to	A. results that can be observed during an experiment	B. results that is difficult to observe during an experiment	C. results that require numerical data	D. none of these is correct		D. none of these is correct
. Accuracy is defined as	A. a measure of how often an experimental	B. the closeness of a measured value to the real value	C. the number of significant figures	D. none of these		B. the closeness of a measured value to the real value
Glassware used to measure 24- hour urine volumes is a:	volumetric flask	beaker	Erlenmeyer cylinder	graduated cylinder		graduated cylinder

The durable material used to make heat resistant glassware is:	polyethylene	soda lime	polystyrene	borosilicate		borosilicate
The destruction of all micro- organisms including spores is called:	sanitation	antisepsis	sterilization	disinfection		sterilization
Cells in a hypertonic solution will:	swell and burst	dehydrate	hemolyze	not be affected		dehydrate
Which reagent is not routinely used to preserve tissue in a life-like manner:	formic acid	Zenker's fluid	40% formaldehy de dissolved in water	Bouin's fluid		formic acid
Which piece of histology equipment is not temperature dependent:	tissue processor	microtome	embedding center	water bath		microtome
A biopsy is:	a removal of biological fluid	the removal of an organ	a post mortem examinatio n	excision of a representative tissue sample		excision of a representative tissue sample
The liquid portion of blood remaining after a clot has formed is called:	the buffy coat	serum	plasma	lymph		serum
The shape of a normal erythrocyte is described as:	biconcave disc	spherocyte	polymorph onucleocyt e	thin column		biconcave disc
The tourniquet is:	applied very tightly to the arm	used to increase venous fill	applied about 6-8" above the elbow	tied in a knot to keep it on securely		used to increase venous fill
What vein/veins is not used to obtain a venous blood sample:	basilica vein	cephalic vein	medial cubital vein	femoral vein		femoral vein
The test procedure that uses a Westergren tube is:	erythrocyte sedimentation rate	hematocrit	reticulocyte count	microhematocrit		erythrocyte sedimentation rate
What areas on an infant are suitable for skin puncture:	any calloused areas of the foot	the second or third finger on either hand	the posterior curvature of the heel	the lateral, flat portion of the heel		the lateral, flat portion of the heel
A disinfectant used on metal surface is:	10% formalin	2% glutaraldehyde	1% hypochlorit e	70% isopropyl alcohol		2% glutaraldehyde
The purpose of heat fixing a bacterial smear is to:	prevent cells from being washed off during staining	causes the cells to absorb the stain more easily	provide a warm temperatur e for the bacteria to grow	make the cells visible under the microscope		prevent cells from being washed off during staining

Which Gram stain reagent	Lugol's iodine	safranin	acetone-	Gram's iodine		Gram's iodine
acts as a mordant to bind the			alcohol			
stain to the bacteria:						
The autoclave is set at	121*C for	130*C for	121*C for	121*C for		121*C for 15min
for small loads:	50min at 6	30min at 30	15min at	45min at 15 p.s.i.		at 15 p.s.i.
	p.s.i.	p.s.i.	15 p.s.i.			
The universally accepted	2%	1% hypochlorite	10%	70% isopropyl		1% hypochlorite
disinfectant for the medical	glutaraldehyde		formalin	alcohol		
workplace is:						
A patient's health card #	4	6	8	10		10
consists of digits:						
Xylene is used in:	dehydration of	histology as a	attaching	paraffin wax		histology as a
	tissues	clearing agent	cover slips	embedding		clearing agent
			to slides	process		
				-		



## **KARPAGAM ACADEMY OF HIGHER EDUCATION**

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## <u>UNIT-II</u>

## SYLLABUS

## Evaluation of biochemical changes in diseases

Basic hepatic, renal and cardiovascular physiology. Biochemical symptoms associated with disease and their evaluation. Diagnostic biochemical profile.

## Liver Physiology

## **1. BILE ACIDS AND BILE**

In the terminal ileum, bile acids present in the lumen are recuperated and returned to the liver where they are taken up into hepatocytes and excreted into the bile again. This enterohepatic circulation retains over 95% of the bile acids. Each day, only 400–500 mg of bile acids are produced, balancing the small physiologic fecal loss (excretion into urine is normally negligible). In 24 h, approximately 12–25 g of bile acids are secreted into the intestine, turning the whole pool over up to 10 times a day. Cholesterol is the starting molecule in the synthesis of bile acids. Conversion of cholesterol into bile acids occurs via two pathways: the classical (or neutral) pathway and the alternative (or acidic) pathway. The classical pathway contributes 75% of the bile acid pool. Reactions leading to primary bile acids, cholic acid and chenodeoxycholic acid, include initiation (hydroxylation in position 7), modification of the sterol ring, oxidation, shortening of the side chain, and conjugation with glycine or taurine. Once secreted into the intestinal lumen, the anaerobic flora metabolizes the primary bile acids into secondary bile acids. The major reaction is  $7\alpha$ -dehydroxylation to give deoxycholic acid from cholic acid and lithocholic acid from chenodeoxycholic acid.

Secondary bile acids are reabsorbed by the enterohepatic circulation and reconjugated within the hepatocytes before they are secreted into the bile system. Once transported back to the

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liver, secondary bile acids can be further processed to form tertiary bile acids such as sulfolithocholic acid and ursodeoxycholic acid, which normally contribute marginally to the bile acid pool. Bile acids are derived from cholesterol and their excretion facilitates biliary cholesterol excretion, influencing cholesterol homeostasis. Resins binding bile acids in the intestinal lumen increase their fecal output, stimulate synthesis of bile acids, and, indirectly, act as hypocholesterolemic agents. In contrast, cholestatic liver diseases are characterized by hypercholesterolemia.

Conjugated bile acids have powerful detergent-like properties that are important in stabilizing the physical state of bile and in promoting fat digestion and absorption. Bile acids support digestion of nutritional components by formation of micelles and activation/stabilization of enzymes such as pancreatic lipase, phospholipase A, and Pancreatic cholesterol esterase. Micelle formation relies on the amphiphilic nature of bile acids, which are hydrophile on one end while lipophile on the other. This mechanism allows biliary excretion of lipophilic compounds such as cholesterol. To prevent cell damage by formation of micelles while transporting bile acids inside the cell, bile acids bind to specific intracellular transport proteins.

Physiologically 600-ml bile is produced daily. It consists of 400-ml canalicular bile formed in the bile canaliculi between hepatocytes and 200-ml ductular bile collected in the bile ducts lined up by cholangiocytes. Hepatocytes and cholangiocytes are polarized cells with basolateral sides and an apical side. Several ATP-dependent pumps are embedded into the canalicular membrane of the hepatocytes at their apical side. These pumps accumulate bile acids, phospholipids, and organic anions in the canalicular bile. Bile salt export pump (BSEP) is one of them, permitting the excretion of conjugated bile acids against a concentration gradient (1). Intestinal recycling of bile acids occurs via a Na+-dependent carrier (apical sodium bile acid transporter (ASBT)) located on the apical side of enterocytes in the terminal ileum as well as on



the apical side of hepatocytes and cholangiocytes. Organic solute and steroid transporters (Osta, Ost $\beta$ ) have been shown to be essential transporters on the basolateral side of enterocytes and cholangiocytes. These bile acids are taken up back into the hepatocytes by another Na+-dependent transporter, Na+-Taurocholate cotransporting polypeptide (NTCP). This system avoids precious cholesterol metabolites to be lost with feces and also permits a cross talk between the intestine and the liver.

Bile acids are now recognized to be important signaling molecules linking feeding to metabolism regulation (2). Their increased intestinal presence postprandially informs adjacent transmitters and metabolic pathways of the availability of nutrients. Bile acids bind and activate a specific G-protein-coupled receptor, TGR5 (also called GPBAR1, membrane bile-acid receptor or BG37) as well as an intracellular receptor, FXR (farnesoid X receptor). FXR belongs to the group of nuclear hormone receptors and functions as a transcription factor. FXR affects not only bile acid metabolism, but also cholesterol metabolism, triglyceride metabolism, and glucose metabolism. In liver, kidney, and intestinal tissues, FXR hinders accumulation of bile acids and thereby prevents toxic damage. In the liver, FXR intensifies bile acid conjugation which consecutively increases bile flow by enhanced excretion of bile acids from hepatocytes into bile canaliculi. In the intestine, FXR activation leads to increased expression of the ileal bile acid binding proteins (I-BABP, FABP6), of the basolateral bile acid transporters and of the secreted growth factor, fibroblast growth factor 19 (FGF19). Bile acids influence energy homeostasis via the TGR5 pathway. Furthermore, after cellular uptake bile acids exert direct signaling functions in cholangiocytes and hepatocytes via calcium, PKC, MEK, ERK, and PI3K pathways, altering gene expression, cell proliferation, apoptosis, and secretion.

## 2. THE LIVER AS A FACTORY

Protein metabolism. In contrast to muscle cells, which synthesize protein for their own use,


hepatocytes synthesize proteins of importance altruistically for the whole organism. The majority of the circulating proteins are synthesized by hepatocytes. These proteins comprise cargo proteins (e.g., albumin, transferrin, ceruloplasmin, haptoglobin, lipoproteins), immune-related proteins (proteins of the complement system, acute-phase proteins), and coagulation factors. Creactive protein is an acute-phase protein, whose hepatocellular production is massively stimulated by cytokines such as IL-6 and IL-1. Albumin is the most abundant plasma protein maintaining intravascular oncotic pressure; its determination reflects the synthesis capacity of the liver over the past few weeks since its half-life is 21 days. To assess the hepatocellular synthesis capacity for a shorter time (hours), the determination of the coagulation factors is appropriate. Aminotransferases transfer an amino group from a donor molecule to a recipient molecule. Aspartate aminotransferase facilitates the conversion of aspartate and  $\alpha$ -ketoglutarate to oxaloacetate and glutamate, and vice versa, whereas alanine aminotransferase facilitates the conversion of alanine and  $\alpha$ -ketoglutarate to pyruvate and glutamate, and vice versa. AST can be cytosolic and mitochondrial, whereas ALT is strictly cytosolic. These enzymes are intensively expressed in cells involved in physiologic protein metabolism, particularly hepatocytes and muscle cells. Elevated serum aminotransferase levels are nonspecific markers for hepatocellular damage. Proteins are degraded by two major pathways: the autophagic lysosomal pathway and the ubiquitin-proteasome-related pathway. Autophagy engulfs part of the cytoplasm in vacuoles whose content is digested by lysosomal enzymes after fusion with lysosomes. In the ubiquitinproteasome pathway, proteins are tagged for degradation by enzymatic linkage with ubiquitin residues.

*Carbohydrate metabolism.* To maintain blood glucose levels within physiologic range, the liver functions as recipient, store, donator, and creator. Up to 90% of the intestinally absorbed glucose is taken up by the liver. Glucose passes membranes via glucose transporters (GLUT family of



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transporters; GLUT-2, 9, and 10 are expressed in the liver). Once in the cytoplasm, glucose is phosphorylated by hexokinase or glucokinase to access cellular metabolism. Glucokinase is expressed only in the liver and phosphorylates only glucose. Glucokinase activity is particularly important postprandially since its velocity is maximal at much higher concentrations of glucose than hexokinase. Glucose-6-phosphate is sequentially transformed into glucose-1-phosphate by phosphoglucomutase and uridine-diphosphate-glucose glucose-1into by phosphouridyltransferase to be finally stored as glycogen. The arborescent structure of glycogen with a central anchor protein termed glycogenin links up to 50,000 molecules of glucose while keeping them easily accessible for reintegration into metabolism. Glucose-6-phosphate is not solely the initial compound for glycolysis; it can also enter the pentose phosphate pathways via glucose-6-phosphate dehydrogenase to produce NADPH and precursors for nucleotides. Other carbohydrates like fructose and galactose are enzymatically transformed to join the glycolysis pathway. When glucose blood levels drop, glucagon and adrenaline stimulate via cAMP a protein phosphorylase reverting glycogen to glucose-1-phosphate ( $\alpha$ -glycanphosphorylase) and to glucose-6-phosphate (phosphoglucomutase). G-6-P is converted to glucose by glucose-6phosphatase. Once glycogen storage has been emptied, glucose needs to be synthesized from other sources. Two third of the glucose derived from neoglucogenesis is synthesized from lactate, which results from anaerobic metabolism and can be supplied to the liver by the muscles. Glucose can also be produced from amino acids, mostly alanine, and from glycerine which is a degradation product of triglycerides. Gluconeogenesis is triggered by hormonal signals. Glucagon increases gluconeogenesis in the short term, while glucosteroids enhance gluconeogenesis in the long term. Insulin inhibits gluconeogenesis. A hallmark of hepatic insulin resistance is the failure of insulin to inhibit hepatic glucose output.

Lipid metabolism. Within each liver lobule, there is zonation of the metabolic functions. The

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periportal zone is where oxidative energy metabolism, amino acid catabolism, cholesterol metabolism, and fatty acid  $\beta$ -oxidation take place, whereas the perivenous zone is where de novo lipid synthesis, ketogenesis and xenobiotic metabolism occur. Liposynthesis occurs by esterification of free fatty acids via acetyl-CoA and glycerol and is driven by glycerophosphate acyltransferase (GPAT), which is activated by nutritional status and insulin and inhibited by glucagon. De novo lipogenesis of free fatty acids from acetyl-CoA is regulated by insulin via activation of sterol regulatory element binding protein-1c (SREBP-1c), which controls the transcription of lipogenic enzymes such as fatty acid synthase. Insulin stimulates the conversion of carboxyl-CoA to malonyl-CoA, a key regulator for the distribution of free fatty acids toward esterification or oxidation. Low levels of malonyl-CoA direct free fatty acids to the mitochondriae and  $\beta$ -oxidation via carnitine palmitoyltransferase-1 (CPT-1), an outer mitochondrial membrane enzyme. High levels of malonyl-CoA inhibit CPT-1, thus enhancing esterification of free fatty acid into triglycerides. Fatty acids can also be oxidized in peroxisomes ( $\beta$ -oxidation) and microsomes ( $\omega$ -oxidation). Triglycerides stimulate apolipoprotein B (Apo-B) synthesis and are secreted as VLDL-Apo-B. Insulin inhibits Apo-B synthesis and impairs secretion of triglycerides as VLDL.

*The regulators*. AMP-dependent protein kinase (AMPK) and mammalian target of rapamycin (mTOR) adapt hepatocellular metabolism to energy status. Activated AMPK switches energy-consuming anabolic lipogenic pathways to ATP-producing catabolic pathways (3). Multiple cues activate AMPK; hypoxia, ATP depletion, starving, chronic alcohol consumption, oxidative stress, adiponectin, leptin, and drugs such as metformin or thiazolinediones. AMPK controls acetyl-CoA-carboxylase 1 reducing lipogenesis, acetyl-CoAcarboxylase 2 increasing fat oxidation, HMG-CoA-reductase lowering cholesterol synthesis, or mTOR lowering protein synthesis. Peroxisome proliferator-activated receptors (PPARs) are transcription



factors essential for the regulation of cell differentiation and metabolism (4). PPARs sense lipid signals and are to be considered "lipostats": endogenous fatty acids activate PPAR- $\alpha$ , while leukotrienes and prostaglandins activate PPAR- $\gamma$ . They are also the targets of several metabolic drugs. Fibrates activate PPAR- $\alpha$  and glitazones activate PPAR- $\gamma$ . PPAR- $\alpha$  stimulates hepatocellular fatty acid uptake and catabolism. PPAR- $\gamma$  is highly expressed in adipose tissue, where it regulates adipogenesis and adipose tissue integrity. PPAR- $\gamma$  is usually poorly expressed in the liver, but its levels increase significantly during lipid accumulation in both hepatocytes and stellate cells. Activation of hepatic PPAR- $\gamma$  decreases steatosis and reduces profibrogenic processes. LXR is a nuclear receptor whose ligands are oxysterols. LXR is involved in the regulation of cholesterol, bile acid, and triglyceride metabolism as well as in inflammatory response and energy balance. LXR stimulates cholesterol synthesis and biliary secretion. LXR activates SREBP-1c inducing lipogenesis. LXR promotes glucose utilization by inhibiting expression of glucose-6-phosphatase and induction of glucokinase expression.

*Iron.* The liver regulates iron homeostasis and is the main body store for iron. Iron is taken up by enterocytes in a highly regulated manner, since it is not excreted and loss of iron is not controlled. Intestinal iron absorption is regulated by hepcidine, which is mainly produced by hepatocytes and to a lesser amount by adipocytes and macrophages. Hepcidine concentrations increase under inflammatory conditions or iron overload and decrease in case of anemia or hypoxic conditions (5). Expression of hepcidine is activated by bone morphogenic protein, which is controlled by hemojuvelin (HJV), HFE, and transferrin receptor 2 (Tfr-2) proteins. Hepcidine inhibits the expression of the ferroportin transporter, a membrane transporter protein releasing iron from the enterocyte. Once released from the enterocyte, iron binds to transferrin, the main iron transport protein of the body. Iron uptake into the hepatocytes is mediated by transferrin receptor 1 (Tfr-1). Tfr-1 is upregulated by hypoxia-inducible factor, IL-2, mitogens, growth



factors, or other cytokines. Proliferating cells, in need of iron for growth, express more Tfr-1. HFE, the defective protein in hereditary hemochromatosis, competes with transferrin for binding to Tfr-1. Transferrin is also endocytosed via Trf-2, but with an affinity 25–30 times lower; Trf-2 seems to act as a transferrin saturation sensor.

*Copper*. Copper is essential for life as it plays a key role as a cofactor for various enzymes. As copper is cytotoxic, it is accompanied by specific protector proteins, which carry and transfer copper to its intracellular destination. At the level of the plasma membrane, copper-transporting ATPases (Cu-ATPases) with two isoforms (ATP7A and ATP7B) play a central role in copper homeostasis by supporting transmembranous copper exchange. ATP7A is responsible for copper transport across the basolateral membrane of enterocytes into the circulation. ATP7B expressed in hepatocytes is responsible for copper excretion into bile. ATP7B deficiency leads to Wilson's disease with intracellular copper accumulation (5).

### **3. THE LIVER AS A DETOXIFIER**

The liver is the central organ for detoxification of exo- and endogenous substances. While watersoluble substances can be excreted by the kidneys, lipophilic substances have to be transformed in the hepatocytes before excretion. Biotransformations within the liver include not only detoxification, but also activation of certain compounds (e.g., prodrugs). Detoxification processing can be divided into three phases. In a first phase, lipophilic substances are conjugated with an additional reactive group enhancing the polarity of the molecule. These groups most often consist of either –NH2, –COOH, –OH, or –SH groups. Conjugation is achieved by oxidation/hydroxylation, reduction, or hydrolysis, depending on the group to be added. Clinical importance of these processes has been shown best for the microsomal mixed-functional monooxygenases, which contain the cytochromes P450. Cytochromes P450 consist of several dozens of enzymes—among others those metabolizing drugs such as the CYP3A4, which



influences pharmacokinetics and interactions of many drugs. The large number of cytochrome isoenzymes explains the stunning diversity in individual drug metabolization. Phase I reaction may be sufficient to render substances hydrophilic and enhance kidney excretion. The second phase conjugates phase I products with other liver derived substances such as glucuronic acid, amino acids, activated sulfuric acid or mercapturic acid. The newly generated conjugate provides an increased hydrophilicity due to its most often acid characteristics and therefore can be excreted more easily by the kidneys or into the intestinal lumen by bile excretion. The third phase consists of transmembrane transporters. Noxious compounds conjugated with charged moieties such as glucuronide, glutathione, and sulfate are subsequently pumped into bile across the canalicular membrane by different ATP-binding cassette (ABC) transporters. These involve ABCC2 (MRP2), which largely transports organic anions; ABCG2 (breast cancer-related protein (BCRP)), which transports many charged and uncharged compounds; and ABCB1 (MDR1 Pglycoprotein), which mainly transports uncharged or cationic amphiphilic compounds. Conjugated compounds can also be transported back into the blood by pumps such as ABCC3, ABCC4, and ABCC5, resulting in urinary excretion after filtration or active excretion in the kidney.

### 4. Specific Detoxification Pathways

*Bilirubin*. Bilirubin concentration in the serum consists of a balance of pigment production and elimination. An end product of heme and hemoproteins, most bilirubin reaches the bloodstream from the spleen, entering the liver via the portal vein. Hepatocyte uptake happens Na+ independent, by organic anion transporter proteins (OATPs) in a glutathione countertransport manner at the sinusoidal surface of the hepatocyte. Intracellular bilirubin is linked to ligandin and Z-protein, specific cytosolic proteins, thus preventing intracellular toxicity. Glucuronidation for excretion takes place in the smooth endoplasmic reticulum by the rate-limiting enzyme uridine



diphosphoglucuronateglucuronosyl transferase (UDP-GT), resulting in hydrophilic bilirubin glucuronide. Excretion into the bile is ATP-dependent as transmembrane efflux is provided by conjugated export pump MRP2 (see above).

Small amounts of bilirubin are secreted to the plasma via MRP3. Within the intestinal tract, bile-derived bilirubin is metabolized by gut bacteria via  $\beta$ -glucuronidase for oxidation to stercobilin, which is excreted within feces or in small amounts by the kidneys after reuptake by small intestinal endothelium and further metabolization to urobilirubin (6).

Alcohol. The mainstay of alcohol degradation consists of the alcohol dehydrogenase enzyme, though hepatocytes own a microsomal oxidative system located within the ER and catalase within the peroxisomes. The presence of different isoenzymes of ADH explains the individually different capability to cope with ingested alcohol, furthermore, as ADH activity is maximally saturated from 0.3 to 0.5‰ and cannot be upregulated or induced by chronic exposition. ADH metabolizes alcohol to aldehyde acetate, which is highly toxic and has to be further degraded within the microsomes by aldehyde dehydrogenase to acetate acid. Acetate acid is then integrated as acetyl-CoA into the citric acid cycle as well as into the lipid acid cycle and the cholesterin synthesis. ADH is a zinc-depending enzyme, a feature relevant in chronic alcohol abuse, as chronic alcohol consumption most often leads to zinc deficiency. The degradation of alcohol is highly oxygen-dependent and may consume up to 90% of the whole hepatocellular oxygen uptake, meanwhile inhibiting or affecting other oxygen-dependent processes. In chronic alcohol consumption, alcohol specific ADH cannot be induced, whereas the microsomal oxidative system in the ER consisting of cytochrome P450 isoenzymes, primarily unspecific for alcohol, can be upregulated and therefore becomes more and more important as consumption of higher amounts endures. Alcohol induces CYP2E1 subtype, which releases reactive oxygen species and contributes to oxidative stress. Finally, alcohol can also be degraded by catalase, a peroxisomal



enzyme degrading H2O2 into water and O2 and reducing alcohol to acetaldehyde only if higher concentrations occur (>1%) (7).

Ammonium. Ammonium (NH4 +) derives mainly from the colonic bacterial flora by degradation of proteins and urea. The liver produces and metabolizes ammonium within the urea/ornithine cycle. Urinary ammonium excretion amounts to approximately 20-40 mmol/l urine. Ammonium detoxification in the liver is dependent on two systems: the urea/ornithine cycle, which is the mainstay of ammonium detoxification, and the glutamate cycle, which is not liver-specific. In the urea/ornithine cycle, which is liver-specific, ammonium and bicarbonate are conjugated into the mitochondria by carbamylphosphate synthetase to form carbamylphosphate. Carbamylphosphate is transformed to citrulline via the ornithine carbamylphosphate transferase. Citrulline is further metabolized within the cytoplasm via arginine for urea production providing ornithine as a spinoff. The glutamate cycle conjugates ammonium with  $\alpha$ -ketoglutaric acid to produce glutamine, which represents the nontoxic transport form of ammonium. The urea/ornithine cycle depends on high ammonium concentrations and is therefore located in the periportal area and detoxifies the bulk of the portal venous ammonium load. It is vulnerable to exogenous/intestinal toxic substances. The glutamine synthesis is located perivenously and due to its high affinity is less dependent on ammonium concentrations. Importantly, the urea/ornithine cycle and the glutamate cycle are linked to the plasma bicarbonate level s bicarbonate acts as substrate for urea production and glutamine synthesis is dependent on plasma pH levels. Hepatic urea synthesis is a major pathway for the removal of metabolically generated bicarbonate (8).

#### THE LIVER AS A FILTER

The liver is receiving two third of its blood supply from the intestine. This blood full of nutrients contains many antigens, which are filtered through the hepatic sinusoids by cells of the innate immunity system. The innate immunity system is the first line of defense against pathogens



recognizing them via pattern recognition receptors such as the toll-like receptors. The liver is enriched with cells of the innate immune system including Kupffer cells (KCs), dendritic cells (DCs), and natural killer (NK) cells (9). Lipopolysaccharides (LPS), which derive from the cell wall of gram-negative bacteria, are present in concentrations up to 1 ng/ml in the portal blood, whereas LPSs are not detectable in the peripheral blood because they have been cleared in the liver. Liver sinusoidal endothelial cells (LSECs), KCs, and DCs function as antigen-presenting cells (APCs). The KCs are mobile macrophages which position themselves within the sinusoids to contact circulating lymphocytes and engage antigens. KCs are activated by various bacterial antigen stimuli such as LPS and bacterial superantigens. Once activated, KCs produce cytokines (IL-6, TNF, IL-12, and IL-18), influencing the function of other cell types present in their vicinity (hepatocytes, LSECs, and NKs). IL-1 $\beta$ , IL-6, TNF- $\alpha$ , and leukotrienes recruit neutrophils. Neutrophils phagocyte bacterial antigens presented by APCs and secrete cytokines to stimulate other innate immune cells and promote attraction and activation of CD4+ and CD8+ cells. Neutrophil recruitment can significantly contribute to liver injury (10).

LSECs express mannose and scavenger receptors and antigenuptake molecules. LSECs also support immune pathways by expressing costimulatory CD 40, CD 80, and CD86, similar to mature DCs. Receptor-mediated uptake of antigens and MHC class II expression is downregulated by TNF- $\alpha$  and IL-10, while activation of the mannose receptor (e.g., by bacterial walls) induces expression of IL-12, IL-1 $\beta$ , IL-6, und TNF- $\alpha$ . LSECs are affected by aging, leading to agerelated pseudocapillarization of the sinusoids which is characterized by the loss of fenestration and deposition of collagen in the space of Dissé. NK and NKT cells, which are identified by expression of CD56, have the ability to quickly produce high amounts of cytokines. Their strategic localization in the sinusoids enables NK and NKT cells together with KCs and LSECs to provide an effective first-line innate immune defense against invading pathogens,



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toxins, food antigens, and circulating tumor cells (11). The liver is exposed to millions of antigens and exobiotics. If every contact would stimulate the immune system, the liver would be in a permanent state of inflammation. Therefore, one of the important functions of the hepatic immune system is the promotion of active tolerance. KCs are crucial for the development of hepatic antigen tolerance. Depletion of KCs impairs antigen tolerance leading to upregulation of T cells (12). Transformation of CD4 T cells to different T-helper (Th) cells or regulatory T (Treg) cells expressing different chemokines (Th1: IFN- $\gamma$ , Th2: IL-4, IL-10, Th17: IL-17) plays a key role in liver immunotolerance. Short-term inhibition of T-cell stimulation by CTLA-4 and long-term inhibition by PD-1 are nonredundant mechanisms of enduring hepatic immunotolerance (13).

### **RENAL PHYSIOLOGY**

### THE URINARY SYSTEM

**Components and function** 



The urinary system is composed of two kidneys, the functionally filtering apparatus, which connect through two tubular structures called ureters to a urinary bladder, which serve as a reservoir for urine. The bladder, controlled by a sphincter, empties into the urethra to eliminate



the urine from the body.

Since the organ of most interest in the urinary system is the kidney we are going to concentrate in its structure and function. The working capacity of these organs far exceeds the need of a normal organism to the extent that an animal can function absolutely normal with only one quarter of the renal capacity and can survive with only one tenth.

The main role of the kidneys is to filter the circulating blood in order to remove from the body waste products acquired through direct ingestion or resulting from catabolism of the organism (Fig. 6-2). The removal of these products is meant to avoid their accumulation to toxic levels. A second critical role of the kidneys is to regulate and try to maintain within normal levels the extracellular fluid, circulating blood volume and, as a consequence, the blood pressure. This is achieved by regulating the volume of electrolytes and fluid which is excreted in urine and also through the production and release of enzymes by the rennin angiotensin system, leading to the production of vasoactive compounds.



In the process of filtering blood, the kidneys regulate the ionic concentration in circulation

by either retaining or excreting, depending on the needs, ions such as Na<sup>+</sup>, K<sup>+</sup>, Cl<sup>-</sup>, Ca<sup>2+</sup>, HCO

Prepared by Dr. D. Selvakumar, Assistant Professor, Deptartment of Biochemistry, KAHE 14/57



-, HPO <sup>2-</sup>. In order to maintain a narrow physiological intercellular fluid pH the kidney controls the excretion of H<sup>+</sup>. The kidney also has an endocrine role which contributes to several rather important physiological activities. It contributes to the regulation of red blood cell through production or erythropoietin. Regulates diuresis through increased renal blood flow as a result of production urodilatin and. calcium absorption through conversion 25of of hydroxycholecalciferol to 1,25-dihydroxycholecalciferol, the active form of Vitamin D<sub>3</sub>. The kidneys also secrete renin, an enzyme involved in the production of angiotensin II, leading to synthesis and release of aldosterone.

The final role attributed to the kidney is in gluconeogenesis. Tubular cells of the kidney are capable of using amino acids from circulation to make glucose and export it to circulation as the liver does. The main difference appears to be that liver operates more in a circadian rhythm according to food intake, while kidneys produce a continuous supply of glucose.

### Anatomy of the kidney

Depending on the species the kidney can be composed of a single smooth continuous surface structure as it is the case in humans, pigs, dogs, sheep, cats, or a multi-lobulated structure as it is in the case of cattle. In the ventral aspect, the kidney has the renal sinus which has an accumulation of adipose tissue to provide a soft buffer against bumps when the animal makes fast movements.**THE KIDNEY** 



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#### Basic structure of a kidney

The kidney is covered by the renal capsule, a tough, thin connective tissue that contains all other tissue. The second layer towards the center is the cortex where most of the renal corpuscles and convoluted tubules of the nephrons are located. Below the cortex is located the medulla which is made of the renal pyramids, where most of the loops of Henle of the nephrons are located and, towards the tip of the pyramid, the renal papilla which leads to the formation of the minor and major calyces.



Several minor calyces converge to form a major caylx and several major calyces join in the smooth kidney to form a cavity called the renal pelvis which then narrows into a single tube

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called the ureter. The ureter exits the kidney through at the central hilum and empties into the urinary bladder. There are many pyramids in each smooth kidney but in the lobulated kidney there is normally one pyramid in each lobe. In the lobulated kidney there is no renal pelvis. Each pyramid lead to minor and major calyces and these connect directly into the ureter.



Transversal cut of a bovine kidney lobe showing the main compartments



Electron microscope of the Glomerular capillaries (A), Transversal cut of the renal corpuscles capsule showing the glomerular capillaries, the capsular space

### Blood supply and innervations of the kidney

Given that the function of the kidney is to filter the blood, this organ receives a disproportionally high supply of blood in comparison to other parts of the organism. For an organ that accounts for only about 0.4 % of the body weight it receives between 20 and 25 % of the cardiac output. The



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kidney is irrigated through the renal artery which branches of the abdominal aorta. The renal artery enters through the hilus of the kidney into the renal sinus and divides into several segmental arteries which in turn give rise to the interlobar and arcuate arteries. These travel through the renal column towards the cortex of the kidney and upon reaching the base of the pyramids they follow the base projecting interlobular arteries towards the cortex and these, in turn divide into the afferent arterioles that brings blood to each glomerulus forming the glomerular capillaries within each renal corpuscle. Exiting the renal corpuscle the glomerular capillaries coalesce into the efferent arteriole which, intimately associated with each nephron, form a plexus named peritubular capillaries in close apposition with the proximal and distal convoluted tubules of each nephron. The peritubular capillaries then travel into the medulla where it becomes the vasa recta which are in close contact with the loop of Henle of the juxtamedullary nephrons. Finally, the vasa recta join and become the interlobar vein which travels through the renal column towards the renal vein. This in turn leaves the kidney through the renal sinus and joins general circulation through a connexion to the inferior vena cava.

The main innervation of the kidney is sympathetic derived from celiac ganglion. They connect mainly with small arteries and afferent arterioles, the juxtaglomerular apparatus and the tubules. The main purpose of sympathetic stimulation is to cause vasoconstriction of these vessels, thus reducing the formation of filtrate and urine when the animal is under severe stress. Under mild



sympathetic stimulation the change in filtrate volume is negligible.



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### Nephron, the functional filtering unit of the kidney

The nephron is the functional unit of the kidney. There are two types of nephrons, cortical and

juxtaglomerular nephrons (Fig. 6- 9). I domestic animals approximately 25 % of the nephrons are juxtaglomerular and the majority are cortical. Each nephron consists of a renal corpuscle, which is located in the cortex of the kidney, and a tubular component, which, in juxtaglomerular nephrons, extends deep into the medulla of the



kidney, towards the tip of the renal pyramid. In the cortical nephrons most of the tubular component resides in the cortex of the kidney. The renal capsule is made by the glomerulus surrounded by the Bowman's capsule. The tubular component starts with the proximal convoluted tubule followed by the loop of Henle which in turn can be divided in three sections with self explanatory names. These are: the thin descending limb, (descending towards the medulla) the thin ascending limb and the thick ascending limbs (ascending towards the cortex). Once in the cortex the loop of Henle becomes the distal convoluted tubule which travels through the entrance of the renal capsule in very close contact with both, the afferent and efferent arterioles. Then this becomes the collecting duct which, as it travels through the medulla towards the tip of the renal pyramid, it receives the content of many nephrons and increasing in diameter (Fig. 6-10). At this point the collecting duct becomes the papillary duct which then empties in the renal papilla and this into a minor calyx.

The histology of the contents of the Bowman's capsule is of special functional importance. The outside of the renal corpuscle is the Bowman's capsule or capsula glomeruli.



This structure is like a spherical funnel with the mouth in what is also known as the vascular pole, where the afferent arteriole enters and the efferent arteriole leaves the renal corpuscle. The exit of the funnel connects with the proximal convoluted tubule. The external wall of the Bowman's capsule serves as a retention wall to guide the filtrate towards the tubular end. Then we find a urinary space or capsular space which collects the filtrate. The internal wall of the Bowman's capsule is made of very specialized cells called podocytes. These cells are made of finger-like projections which lay on top of a glomerular basement membrane which in turn covers the outside of the glomerular capillaries. The podocytes make a thick cellular layer leaving filtration slits in between fingers. These slits permit the passage of filtrate from the glomerular capillaries towards the capsular space (Fig. 6-7).



The glomerular capillaries constitute the vascular component of the renal corpuscle. At the entrance we find the afferent capillary which then divides into multiple vessels making a plexus which then join at the exit of the capsule to form the efferent capillary. The capillaries in the glomerulus are all fenestrated, that is, they have multiple perforations which permit the passage

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of all of the molecules which will make the filtrate. Each capillary vessel is covered by the basement membrane before being covered by the podocytes.

**Filtration barrier**. All products and materials that form the filtrate have to cross a filtration barrier. This functional structure is composed of the fenestrated endothelial cells of the capillary vessels, the basement membrane and the filtrations slits created by the podocytes covering all the vessels and forming the internal wall of the Bowman's capsule (Fig. 6-11).



The other structure of functional importance in the nephron is the Juxtaglomerular apparatus (JGA) (Fig. 6-12).

The JGA is a collection of cells located at the entrance of the renal corpuscle and inside the distal convoluted tubule. The cells of the JGA physically connect the distal convoluted tubule and the afferent and efferent arterioles. The juxtaglomerular or granular cells which are located in direct contact with the arterioles are capable of sensing the intra-renal pressure to determine if they need to release the enzyme renin. The role of renin is to increase systemic blood pressure through the renin-angiotensin system. The other type of cells in the JGA, those making the macula densa, are located in the distal convoluted tubule and are capable of sensing the sodium



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chloride concentration of the filtrate. If the concentration of NaCl is higher than normal the cells in the macula densa send paracrine signals to the afferent arteriole to reduce the glomerular filtration rate. In this manner the loop of Henle has more time to reabsorb Na<sup>+</sup> from the filtrate. The exact mechanisms of these signals will be discussed later.

### PHYSIOLOGY OF CARDIOVASCULAR SYSTEM

Physiology of the Heart

Heart rate = 70/min, 100 000/day, 5 1/min, 4 500 l/day

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Morphology of the heart:

2 separate pumps – right/left

 $Each-from \ 2 \ pumps-atria/vetricle$ 

Endocardium

Myocardium - heart muscle Pericardium

<u>Histology:</u>

Arrangement of the cardiac muscle fibers (lattice-work) Cardiac muscle - sui generis -

- striated as skeletal muscle
- syncytium as smooth muscle

Cells – cylindric, length 50-100  $\mu$ m , thickness 10-20 microns, intercalated discs (mechanical + electrical connection = functional syncytium).

Physiological Properties of the Heart

- 1) Automatic (autonomic) function
- 2) Conductivity
- 3) Excitability
- 4) Contractility
- 5) Rhytmicity

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### 1) <u>Automatic Function</u>

= ability to work also after an isolation

Principle - existence of primary centre of automatic function – the sino-atrial node – special excitatory system of the heart Necessity to fulfil some condition (temperature, humidity, supply –  $O_2$ , energ. substances, transport away- metabolites...)

### 2) <u>Conductivity</u>

The special conductive system of the heart: SA node - Keith-Flack's node (1907) - pacemaker

3 mm wide, 1 cm long – in the posterior wall of the right atrium (at the junction of v. cava sup. with RA). The fibers are only 3-5 microns in diameter.

In atria – conductive tissue – atrial muscle cells. Velocity – 1 m/s

+ 3 bundles of atrial fibers conducting SA-AV node imp.

Internodal tracts of: 1. Bachman

- 2. Wenckebacnh
- 3. Thorel

AV node – the atrioventricular node. Conduction in AV node (secondary centre of automatic function) is slow – delay of 0.1 s), velocity of conduction 20 mm/s.

Principle: Existence of junctional fibers and transitional fibers. Principle of a convergence ansd divergence. Reverberation circuits.

Physiological role: It allows time for the atria to empty their contents into the ventricles before ventricle contraction begins. His bundle (v- 4-5 m/s), right/left bundle branches, Purkinje system

Very large fibers. This allows quick - immediate transmission of the cardiac impulse throughout the entire ventricular system. Excitation of the myocardium from endocardium to epicardium.

3) <u>The excitability</u>

= ability to react to a stimulus

### Phases:

1. Normal



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- 2. Absolute refractory period
- 3. Relative refractory period
- 4. Supranormal excitability

Refractory phases – condition for alternation systole – diastole – against tetanization Extrasystoles - interpolated

- compensated

Vulnerable period – just at the end of the action potential, because stimulation at this time will sometimes initiate flutter or fibrillation.

Flutter/fibrillation - atrial

- ventricular fatal Defibrillation defibrillator 5-7 kV
- 4) <u>The contractility</u>
- = ability of the myocardial fibres to contract Myosin actin filaments

Tropomyosin, troponin

Excitation – Contraction Coupling:

Depolarization - electrical charges, T-tubules, release of calcium ions from the longitudinal sarcoplasmatic reticulum – to promote sliding of the actin and myosin filaments along each other

- muscle contraction

### All or Nothing Principle of the Heart

= stimulation of any single atrial muscle fiber causes the action potential in entire atrial muscle mass. The same in ventricles. Syncytial nature of cardiac muscle.

5) <u>The Rhythmicity</u>

= regular alternation of contraction and relaxation

HR - reflects metabolic rate/weight birds 800/min

mice 500

men 70

### elephant 25-30 whale 10/min

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Required conditions for the heart activity

- 1) <u>Temperature</u> optimal for humans 37°
  - lower: decreasing of activity
  - -higher: increasing of activity + metabolic needs
- 2) <u>Metabolism</u> of the heart:

Aerobic – without possibility to cover energy demands of anaerobic pathway (only 1% of the total energy is provided by anaerobic metabolism).Lack of the  $O_2$  debt. Sources of energy for heart: Lactate, pyruvate, fat, FFA, AA, ketones.

3) <u>Oxygen consumption:</u> 10 ml/100 g/min, 35 ml/350 g/min = 10% of total  $O_2$  consumption (250 ml/min). During physical work, 5x more

4) <u>Isoionia:</u>

Isoionic - environment (including perfusion fluid)

Balance between: Calcium and potassium During Ca abundance – rigor During K abundance - inhibition

5) <u>pH: acidosis</u> inhibition of the heart activity – heart stops in diastole

alkalosis - heart stops in systole - rigor

### The Cardiac Cycle

- the period from the end of one cardiac systole to the end of the next heart contraction.
- 1) Electrical cycle depolarisation/repolarisation
- 2) Mechanical cycle contraction/relaxation of cardiac muscle <u>Periods of the cardiac cycle</u>
- 1) Filling of the atria during diastole = <u>venous return</u>

### Mechanisms of the filling:

- a) "Vis a tergo" residual energy from the left ventricle
- b) "Negative" intrathoracic (interpleural) pressure:

### Quiet breathing:

expiration:  $P_{pl} = -2.5$  mm Hg (relative to atmospheric)

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- inspiration: -6 mm Hg during = thoracic pump.

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Pressure is transmitted to the great veins and atria – aids venous return. The movement of diaphragm – rise of  $P_{abd}$ .

- c) Shifting down of the A-V ring by contraction of ventricles "vis a fronte".
- d) Gravity from head and vessels above cardiac level
- e) Muscular pump

### Filling of the ventricles

Accumulation of blood in the atria – elevated atrial pressure - decrease of ventricular pressure to diastolic value (near  $\emptyset$ )  $\rightarrow$  pressure in the atria push open the A-V valves – blood flows

### into the ventricles

- 1) Period of rapid filling (first 1/3 of the diastolic time)
- 2) Period of slow filling diastasis (next 1/3)
- 3) Atrial systoly (last 1/3) + 20-30 % of the filling of the ventricles

#### Ventricular systole

1) <u>Period of isovolumic .- isometric contraction</u>

At the start of ventricular contraction, the ventricular pressure rises – causing the A-V valves to close. Also semilunar valves are closed – during first about 0.05 s – until the pressures in LV and RV and RV exceed the pressures in the aorta and pulmonary artery – opening of the semilunar valves  $\rightarrow$ 

- 2) <u>Period of ventricular ejection</u>
- a) Phase of the rapid ejection  $(1/2 \text{ of V is ejected in the first } \frac{1}{4} \text{ of the ventricular systole})$
- b) Phase of the slow ejection (remaining  $\frac{1}{2}$  of V-during next  $\frac{2}{4}$  of the ventricular systole)
- 3) <u>Protodiastole</u> (last ¼ of the ventricular systole)

The ventricular pressure ralls to a value below that in aorta, closing of the semilunar valves

- early diastole.

#### Ventricular diastole

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1) Period of isovolumic (isometric) relaxation – the valves are closed,V pressures continues to drop

2) Filling of the ventricles:

Period of the rapid ventricular filling – when the ventricular pressure falls below atrial pressure and the AV valves open a new cycle.

HR = 72/min - 1 cycle lasts 0.83 s. <u>Length of Systole/Diastole</u>

HR - 65/min: S - 0.3 s

D - 0.6 s 1 : 2

HR – 200/min: S - 0.16 s

D - 0.14 s 1 : 1

The duration of systole is more fixed. Tachycardia is accompanied mainly by shortening of the diastole – if more than 180/min – insufficient filling – <u>critical frequency (HR)</u> for adults.

Functions of the valves

<u>The AV – valves</u> prevent backflow of blood from V to A during systole. <u>The semilunar valves</u> prevent backflow from the aorta and pulmonary artery to V during diastole. All valves close and open passively – by pressure gradient.

The Electrical Activity of the Heart

Resting membrane potential (RMP): myocardial fibers

approximately - 90 mV SA node: -55 to -60 mV

Conductive tissue: - 90 to -100 mV

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RMP depends on differences in concentration of  $K^+ K_i^+ 150 \text{ mmol/l}$ ;  $K^+ - 5 \text{ mmol/l} = 30 \text{ x}$ 

RMP don't allow to  $K^+$  to equalize concentrations. Na<sub>i</sub><sup>+</sup> = 5-10 mmol/l; Na <sup>+</sup> = 140 mmol/l Depolarization: Firing level -65 mV

<u>Initial</u> – is due to an increase in Na<sup>+</sup> permeability (through fast Na<sup>+</sup> channels)

<u>Following</u> – a slower increase in  $Ca^{2+}$  permeability (through slow  $Ca^{2+}$  channels) – <u>plateau</u> (!)

<u>Repolarization</u> is due to a delayed increase in  $K^+$  permeability.

#### The excitation in the conductive system cells

Lower RMP (-60 mV) firing level -35 mV Fast Na<sup>+</sup> channel is not activated.

Unstable RMP – open slow (nonspecific channel) – pacemaker potential = prepotential – due to a steady decrease in  $K^+$  permeability

Effect of <u>heart nerves</u> on prepotential:

- vagus – acetylcholine – increase in  $K^+$  permeability – the slope of prepotentials in decreased

- sympathetic nerves – opposite effect - decrease in K<sup>+</sup> permeability ...

Prepotential in SA node has the slope increased in comparison to one in AV node -

- primary center. Gradient of automaticity. The slope of the prepotential determines HR.

### Electrocardiography (ECG)

Registration of electrical potentials from the heart also from body surface (the tissues of the body contain electrolytes – are conductive).

W. Einthoven (1903) - string galvanometer

<u>A three - lead system:</u> Leads <u>I. II. III. - standard</u> - frontal plane ECG - equilateral triangle

<u>Unipolar leads</u> – V – Wilson – potential of one site is 0. Transverse plane, precordial leads  $V_{1-6}$ 

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(12)

Augmented unipolar limb leads - aVR, aVL, aVF.

Form of the ECG Isoelectric line Waves:

P – atrial depolarization (0.1 – 0.3 mV, 0.1 s)

QRS – ventricular depolarization (atrial repolarization) Q – initial depolarization (His bundle, branches)

R – activation of major portion of ventricular myocardium

S - late activation of posterobasal portion of the LV mass and the pulmonary conus T – ventricular repolarization

U - repolarization of the papillary muscles

The duration of the waves, intervals and segments P-wave0.1 s

PQ-interval 0.16 s

PQ – segment 0.06 - 0.1 s

QRS complex 0.05 - 0.1 s

QT interval 0.2 - 0.4

QT segment 0.12

T wave0.16

<u>The voltage of ECG curve</u> P - 0.1 - 0.3 mV

- R 0.7 1.5 mV
- T 0.3 0.5 mV
- Q, S -0.3 -0.5 mV

Intracellular potential 100 mV

Explanation:

1) ECG potential represents an algebraic sum of the action potentials of myocardial fibres

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2) loss of voltage during spreading of potential
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Special use of ECG

Esophageal leads (e.g. E-30)

Intracardial leads - RA, RV - His bundle electrogram

### Monitoring:

- permanent in UIC
- Holter's monitoring (tape recorder) diagnosis of arrhytmias

### Cardiac Output

- of the LV = the quantity of blood pumped by the LV/min
- of the RV = the quantity of blood pumped by the RV/min

 $CO_{LV} = CO_{RV} !!!$ 

CO = SV x f = 70 ml x 72 = 5000 ml/min Normal values - 5-6 l/min

<u>Cardiac Index</u> (CI) =  $CO/m^2 = 3 - 3.2 l/min/m^2$ 

<u>Stroke volume</u> = volume of blood ejected per systole

Changes in CO

by changes in SV, or f, or both

Change in SV

End diastolic volume (EDV) – approx. 150 ml End systolic volume (ESV) - 70 ml

More effective contractions – positive inotropic effect – ESV – functional reserve for the SV and CO increase

Change in f – heart rate

up to a critical level (180/min - in adults)

Cardiac Output Changes

Effect of age – CI in 10 years –  $4 l/min/m^2$ 

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 $80 - 2 \frac{1}{\min}^2$  Effect of exercise – CO can rise to 30-35 l/min Effects of metabolism – CO proportional to M Effect of gravity - +40 - +80 %

Effect of posture – Co falls about 20 %

Measurement of Cardiac Output

- 1) Direct method: electromagnetic flowmeter
- 2) Indirect methods:
- a) Fick's method
- b) Indicator dilution methods
- using dyes
- thermal dilution intermittent
- continuous infusion
- c) Doppler's method
- d) echocardiography
- e) bioimpendance method

### Methods for heart examination

- 1) <u>Invasive</u> cardiac catheterization (Forssmann 1929):
- Measurement of pressure in atria, ventricles, aorta, pulmonary artery
- application of dyes ...
- angiocardiography, coronarography application of X-ray contrast material
- 2) <u>Noninvasive methods:</u>
- Electrocardiography
- Heart rate variability evaluation
- Auscultation of heart sounds
- Phonocardiography
- Echocardiography

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- Polycardiography

Auscultation/registration of heart sounds 1st heart sound – associated with:

- 1) closure of the AV valves at the beginning of systole
- 2) vibration of the walls of the heart and ejected blood

2nd heart sound – result from closure of the semilunar valves and from reverberation of blood back

3rd heart sound – occasionally – at the beginning of the middle third of diastole – period of the rapid filling of ventricles

4th heart sound – during atrial systole

The Phonocardiography Recording of the heart sounds simultaneously with ECG Advantages:

- 1) More exact analysis of the heart sounds and murmurs
- 2) A writing evidence

The time relationships between ECG and PhCG

### Echocardiography

Pulses of ultrasonic waves are emitted and received by transducer. Reflected ultrasound form the structures with different densities = echo f of ultrasound > 20 kHz - in EchCG

2.25 MHz - in adults

4.4 MHz - in children

7MHz - in newborns

Evaluation of the thickness and motions of the heart walls, septum and function of the valves (mainly mitral) during the cardiac cycle. Valvular lesions.

### Polycardiography

Simultaneous registration of: 1) ECG

### 2) PhCG

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- 3) Arterial pulse
- 4) Venous pulse
- 5) Pressure curves (ao, LV, RV, PA ...)

### Hemodynamics – Dynamics of Blood Circulation

- A) General Hemodynamics
- B) Special Hemodynamics
- A) <u>General biophysical considerations: Parameters:</u>
- 1) Flow
- 2) Velocity
- 3) Pressure
- 4) Resistance
- 1) <u>Blood Flow</u>
- V
- $F = ---- (m^3/s; 1/s; ml/min ...) t$

Flow: – luminar (streamline)

- turbulent

dens.x diam. x velocity Reynolds number = \_\_\_\_\_

Viscosity

Critical velocity - in ascending aorta

- in anemia Systolic murmurs

Dif.P F = ------

### R

Critical closing pressure = P at which flow ceases.

Velocity of Blood

1

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v = — (m/s;	cm/s) t		
F = flo	$w \text{ cm}^3 \text{ x s}^{-1}$		
v =	== cm/s		
A = cro	ss–sectional area cm <sup>2</sup>		
d v			
Aorta A.a.	$4.5 \text{ cm}^2 20$	30-40 cm/s	(120)
		20-30 cm/s	
Arterioles	400	3 mm/s	
~			
Capillaries	4500	0.5-1 mm/s	
Venules	4000	0.5-1 cm/s	
Veins	40	1-5 cm/s	
v.cava	5	8 cm/s	

- 1) Electromagnetic flowmeter
- 2) Plethysmography
- 3) Venous occlusion plethysmography

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## **KARPAGAM ACADEMY OF HIGHER EDUCATION**

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- 3) Rheoplethysmography
- 4) Radioactive methods
- 5) Measurement of circulatory time
- 6) Ultrasonic flowmeter (Doppler)

#### **Blood Pressure**

- Frontal
- Lateral
- Systolic
- Diastolic
- Mean
- Pulse
- amplitude

Normal values	<u>mm Hg</u>	<u>kPa</u>
LV	125/0	12-20/0
A.a.	120	12-20/8-13
Arterioles	40-50	4-6
Capillaries	30-15	4-2
Veins	4-7	0.5-1
CVP (RA)	-5+5	-0.4+0.4
RV	30/0	3.5/0
РА	27/10	3.5/1.2
Pcap.	10	1.3

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LA	-5+5	0.4 + 0.4

Measurement of BP

Methods: - direct

- indirect

Direct methods: Hales 1773

Catheterization - electronic manometers with transducers

Indirect methods:

- Palpation (Riva Rocci)
- Auscultatory (Korotkoff)
- Oscillometric (Pachon, electronic-digital)

### Principles for accurate measurement of BP

<u>Patient:</u> should rest undisturbed in a quiet, comfortable setting at room temperature for at least 5-15 minutes. To avoid physical activity, food consumption, smoking, caffeine ingestion and emotional stress for at least half an hour before measurement.Full bladder or bowel can cause an increase in BP. Nonconstring clothing – with no sleeves. Children – should be given sufficient time to recover from crying.

"White – coat hypertension" – physicians cause + 27/15. Measurement at home.

Recommendations for observer measuring BP

- Have normal hearing and vision, be trained in the technique for measurement BP
- Support the patient's arm the antecubital fossea at heart luvel
- Chair with back and arm support when the patient is sitting
- Use an appropriately sized cuff
- Check the BP by palpation before auscultation
- Deflate the cuff 2-3 mmHg/s
- Use the 1st and 5th Korotkoff sounds to determine BP syst. and BP diast.

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### **KARPAGAM ACADEMY OF HIGHER EDUCATION**

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- Allow 1-2 min. between readings
- Take readings with the patient in the lying or sitting position and in the standing position
- Assess the BP at least 3x over 3-6 months among patients with midly elevated BP

### BP depends on:

- 1) Heart activity
- 2) Vascular resistance
- 3) Volume and viscosity of blood
- 4) Compression vessels by different organs and pressures (e.g. intraabdominal)
- 5) Hydrostatic pressure effect of gravity 1)<u>Heart activity</u>

 $CO = SV \ge f$ 

Increase in  $SV \rightarrow$  increase mainly BP syst. Increase in  $f(HR) \rightarrow$  increase mainly BP diast.

### 2) <u>Vascular resistance</u>

increase  $\rightarrow$  rise mainly BP diast.

3) Volume and viscosity of blood

a) Volume – hypovolemia/hypervolemia: hypotension/hypertension

b) Viscosity – the greater the viscosity, the less the flow in a vessel.

The viscosity of blood at normal hematocrit is about 3 (R for blood is 3x the R for water flow), at Ht 60-70 is viscosity about 10x that – of water – slow and difficult flow perfusion.

4) <u>Compression of vessels by different organs and pressures</u> Compression by skeletal muscles, intraabdominal pressure ...

Coughing, defecation, delivery ... Transmission of the pressure to vessels.

5) Effect of gravity – hydrostatic pressure

In standing person the magnitude of the gravitional effect is 0.77 mmHg of height.



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BP is increased by 0.77 mmHg for each cm below the RA and decreased by 0.77 mmHg for each cm above the RA.

Arterial BP in the foot = 100 + (0.77 x 105 cm) = 180 mmHg Venous BP - `` - = 7 + 100 mmHg Venous BP

- " - = 87 mmHg

Physiological Changes in BP

1) Effect of age and sexual differences

Newborns, children, sexual differentiation in the pubertal age, BP in old subjects.

- 2) <u>Postural effects</u> Orthostasis, klinostasis
- 3) <u>Effects of organ activities</u>
- a) breathing fluctuation of BP
- b) GIT food consumption, increase in BP syst., BP diast. unchanged, or decreased
- c) CNS sleep (REM/non REM)
- d) skeletal muscles exercise, physical work

### Vascular Resistance

- the impediment to blood flow in a vessel
- P BP R = ---= = ---
- F F

 $BP_{ao.mean} - BP_{RA} \qquad 100 - \emptyset \text{ mmHg Total SVR} = -----= = -------=$ 

F<sub>ao</sub> 5 l/min

20 mmHg/l/min

 $BP_{PA mean} - BP_{LA}$  20-Ø Pulmonary VR = -----=

FPA 5

= 4 mmHg/l/min Poiseuille – Hagen Formula

8 x vi x 1

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R = \_\_\_\_\_

 $\pi x r^4$ 

BF and R are markedly affected by changes in r (caliber of the vessels).

Two components of R:

- 1) Characteristics of wall vessel reciprocal to elasticity
- 2) Caliber of vessels mainly arterioles

Elasticity - arterial compliance

Importance in maintaining of: - BP diast.

- F diast.

"Secondary hearts"

Deterioration of the arterial elasticity  $\rightarrow$  increased BP syst. and the pulse pressure (arteriosclerosis – <u>systolic hypertension</u>).

Changes of elasticity – changes in quality and velocity of the arterial pulse velocity.

### **REGULATION OF THE CIRCULATION**

The regulation of action of the heart Regulation of circulation/

\ regulation of diameter of the vessels

**REGULATIO OF THE CARDIAC ACTIVITY** 

Autoregulation

Regulation of the heart activity <u>Nervous</u>

Humoral regulation

### 1) <u>Intracardiac regulation - AUTOREGULATION:</u>

a) <u>Heterometric autoregulation</u> – FRANK-STARLING LAW:,,the energy of contraction is proportional to the initial length of the cardiac muscle fiber – to the end diastolic volume"


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Relation between muscle fiber length and tension. Diastolic filling = end diastolic volume

As the diastolic filling increases, the forces of contraction of the ventricles is increased. Principle of this law is in ultrastructure of the cardiac muscle.

Physiological roles of the FS law:

1/ maintaining the equal  $\mbox{CO}_{s}$  of RV and LV

2/ compensation of the law of Laplace "pressure evoked by wall of a cavity is reciprocal to its diameter"

or: the distending pressure in a distensible hollow object is equal to the tension in the wall

(T) divided by the radius

3/ regulation of the CO<sub>s</sub> during venous return changes

b) <u>Homeometric regulation – "Bowditch's stairs"</u>

- effect of heart rate on the force of contraction

Regulation due to changes in contractility independent on length - "force - frequency relation" Principle - increased availability of intracellular Ca<sup>+2</sup>.

<u>Physiological role:</u> better emptying of the ventricles during tachycardia Optimal and critical frequency.

#### NEURAL CONTROL:

The autonomic nervous system:

1) <u>Parasympathetic</u> nervous system (craniosacral division) = cranial nerves: III, VII, IX, X, sacral.  $S_2 - S_4$  Cholinergic system, receptors of muscarin type (M-receptors), blockade by the atropine. Tonic discharge in vagus = <u>vagal tone</u>

After blockade (cutting X./atropine) – tachycardia (of 70 to 150/min)

<u>Development</u> of the vagal tone in ontogeny: Newborns – weak tone – HR = 120/min.

During infancy - the tone rises - mainly in pubertal age Sportsmen - stronger vagal tone

2) <u>Sympathetic</u> nervous system (thoracico – lumbar) =  $Th_1 - L_{3-4}$  Noradrenergic system, receptors NA(A)

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Receptors - alpha - mainly in vessels - vasoconstriction

- beta (heart-beta<sub>1</sub>) – positive tropic effects

Tonic discharge in the cardiac sympathetic nerves =  $\underline{sympathetic tone}$  After blockade – beta<sub>1</sub> sympatholytics – bradycardia (of 70 to 55-60/min).

Vagal tone in humans - dominant. Cardiomotoric center

Located bilaterally in the reticular substance of the medulla and in the lower third of the pons.

The lateral portions transmit excitatory impulses through the sympathetic nerve fibers to the heart with <u>positive</u> tropic effects = cardioexcitatory part.

The *medial portion*, which has in immediate apposition to the dorsal motor nucleus of the vagus nerve, transmits impulses through the vagus nerve to the heart – with negative tropic effects = cardioinhibitory center.

#### HUMORAL REGULATION

Catecholamines (E, NE) - adrenal medulla

 $T_3$ ,  $T_4$  - *thyroid gland* 

Glucagon - pancreas

all = positive tropic effects

#### **REGULATION OF DIAMETER OF THE VESSELS**

autoregulation

- nervous
- humoral

#### 1)Autoregulation Myogenic:

Compensation of pressure changes by changes in diameter (vascular resistance) for remaining constant blood flow:

The vascular smooth muscle in the vessels contract – in response to the tension of the vessel wall. Stimuli from inside.

Autoregulation of renal and cerebral BF.

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Stimuli outside - mechanical - massage - vasodilatation

Humoral:

Vasodilator agents:

Metabolites – lactate, PCO<sub>2</sub>, increase in temperature (working skeletal muscle), adenosine (from ATP) – in cardiac muscle

Bradykinin, lysylbradykinin (kalidin) in sweat and salivary glands Histamine – in skin circulation

NO

Vasoconstrictors:

Serotonin, endothelins (ET1),  $PO_2$  – different reactions in regional circulations (hypoxic pulmonary vasoconstriction, cerebral vasodilatation)

2/Neural regulation

<u>Parasympathetic division</u> – cholinergic vasodilatory fibres – only in some regions of circulation (genital)

Sympathetic division - adrenergic postggl. fibers, alpha1,2 receptors, vasoconstriction

*Exception – cholinergic sympathetic system in vessels in skeletal muscles.* 

Permanent vasoconstrictory tone.

Vasomotor Centre

in medulla oblongata

<u>Pressoric area</u> – tonic activity – in rostral ventrolateral reticular area in medulla <u>Depressoric area</u>

- in the medial and caudal area

Afferents to the vasomotor centre:

- Direct stimulation by  $\uparrow CO_2, \downarrow O_2$
- Excitatory impulses:

cortex – hypothalamus pain pathways

peripheral chemoreceptors

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# **KARPAGAM ACADEMY OF HIGHER EDUCATION**

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- Inhibitory impulses:
- cortex hypothalamus lung receptors baroreceptors
- Hormonal control of vessels
- Vasodilatatory hormones:
- VIP in splanchnic circulation
- ANP secreted by heart in renal ...
- Vasoconstrictory hormones:
- norepinephrine, angiotensin II, vasopressin (ADH)
- Short review of the Autonomic Nervous System Pharmacology
- Sympathomimetic drugs:
- alpha: Norepinephrine
- beta: Isoproterenol (Isuprel) Epinephrine (adrenaline)
- Miscellaneous: the precursors of E, NE:
- Dopamine (Intropin) Dobutamine (Dobutrex)
- Amphetamine (by releasing endogenous catecholamines)
- Blocking agents:
- Anti alpha phenoxybenzamine (Dibenzyline)
- tolazoline, phentolamine (Regitine)
- anti beta<sub>1</sub> propranolol (Inderal)
- metipranolol (Trimepranol)
- pindolol (Visken).....
- Parasympathomimetic drugs: Acetylcholine Pilocarpine Methacholine
- Muscarine (in Amanita muscaria)
- Anticholinesterases:
- Physostigmine Neostigmine
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#### Blocking agents:

- Anticholinergic atropine
- scopolamine

#### CARDIOVASCULAR REFLEXES (CVR)

<u>Reflex</u> = regular response of the organism to stimulation mediated through central nervous systém

#### Reflex arc:

- 1) receptor (sense organ);
- 2) afferent neuron;
- 3) center;
- 4) efferent neuron;
- 5) effector (in CVR heart and vessels)

#### Receptors (for CVR):

- 1) <u>Interoceptors</u> for perception of the internal environment:
- a) Baroreceptors
- b) Chemoreceptors

Baroreceptors - low - pressure receptors

- high pressure receptors
- a) Low pressure receptors atrial stretch receptors:
- 2 types "A" they discharge primarily during atrial systole

- "B" - - " - atrial filling, peak – at the end of atrial diastole.

The discharge is increased when venous return is increased =  $\underline{volumoreceptors}$  for monitoring venous return.

Effects of the increased stimulation of the atrial baroreceptors:

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<u>Vasodilatation</u> – an accumulation of the blood in the periphery – <u>a fall in BP</u>. <u>Bainbridge Reflex</u>

- rapid infusion to the right atrium – changes in HR.

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Initial basal increased HR - effect: bradycardia

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- " - decreased HR – effect: tachycardia

Two components:- reflex - through atrial baroreceptors

- mechanical local stretch effect on the SA node
- b) High pressure receptors
- carotid sinus
- aortic arch
- LV

<u>Carotid sinus receptors</u>: spray type nerve endings - in the adventitia of the carotid bifurcation and the internal carotid artery.

Afferent neurons: Innervation – through the branch of n. IX. – Hering's nerve. Center of the carotid sinus reflex – tractus solitarius in the medulla,

Efferent neurons: n.X and parasympathetic + sympathetic nerves to vessels. Effectors: Heart, smooth muscles in the vessels wall.

Stimulation: The baroreceptors are stimulated by distension of the vessels wall – stretch receptors.

<u>Aortic arch receptors:</u> in the wall of the aorta. Affferent pathway: Innervation – through the vagus)

Center, efferent pathway, effectors and effects - the same as in CSR

#### Left ventricular receptors:

Afferent and efferent pathways: n. vagus.

<u>Activity and effects of the HP baroreceptors</u> Normal BP - r. discharge at a slow rate Increased BP - the discharge rate increases Decreased <math>BP - - " - declines Effects:

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<u>BP increase (hypertension)</u>: The increased discharge rate evokes rising activity in cardioinhibitory center and depressoric area of the vasomotor center - bradycardia, vasodilatation – a fall in BP to the normal level

<u>BP decrease (hypotension)</u>: The decreased discharge ... vice versa. Normalization of the BP in different situations.

#### Baroreceptor Testing

- <u>Pressure on the SC region</u> – unconstant stimulus

Syndrome of the hypersensitive SC (sy HSC)

- <u>Carotid Clamping</u> proximal or distal to the CS on experimental animals
- Cutting the CS nerves on experiments/ treatment sy HSC
- Application of the drugs eliciting an increase in BP (NE,dopamine)
- Different maneuvers (orthostasis, klinostasis, Valsalva ...)

#### Orthostatic Reflex

When a person stands – venous return decreases due to hydrostatic pressure of the blood. A decrease in CO and systemic BP occurs.

Falling BP at the baroreceptors elicits an immediate reflex, with strong sympathetic activity – vasoconstriction, increase in BP diast., tachycardia.

The aim – to maintain an adequate perfusion of organs.

Valsalva maneuver

Forced expiratory effort against a closed glottis  $-P_{pl} + 30-50$  mmHg.

### <u>5 phases:</u>

1st. - at the onset of straining, the BP rises

2nd - a decrease of venous return, CO, BP; HR increases

3rd - reflex vasoconstriction – stop of the fall of BP, HR increases 4th - after the first inspirium, start of the breathing – decrease in BP –

- filling of the pulmonary circulation with blood

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5th - an increase in BP – vasoconstricton persists, an excessive venous return – stimulation of baroreceptors, causing bradycardia and a drop in BP to normal values.

Chemoreceptors - peripheral

- central

Peripheral chemoreceptors:

Carotid and aortic bodies – near the carotid bifurcation, and the arch of the aorta. The highest BF/g.

Stimulation – low PaO<sub>2</sub> (high PaCO<sub>2</sub>, acidosis)

Effects – an increasing of pressoric area of vasomotor centre, vasoconstriction (splanchnic), redistribution of the blood, an increase in BP.

HR - primarily tachycardia, secondary bradycardia through baroreceptors

<u>Central chemoreceptors</u>: in the brain stem, on the ventral surface,  $H^+$  zone. Stimulation: a decrease in pH - acidosis.

Effects - changes in respiration, without any direct effect on cardiovascular system.

- 2) <u>Nonspecific receptors</u>
- A) <u>Trigeminal endings:</u>
- 1) <u>Oculocardiac reflex:</u>

Stimulation: pressure on the mechanoreceptors of eye and orbit -

Effects: Depressoric effects on vessels and breathing - a decrease in HR by 5-14 / min.

2) <u>Kratschmer apnoeic reflex:</u>

Stimulation: Intranasal insufflation of various irritant gases (smoke, ammonia ..)

*Effects:* reflex respiratory arrest + laryngoconstriction + bradycardia

+ redistribution of the blood to the vital most important organs (from the splanchnic circulation).

3) <u>The diving reflex:</u>

Stimulation of the trigeminal region with cold (water).

#### *Effects:* the same as in Kratschmer.

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Oxygen conserving reflex – the changes primarily safeguard the blood and oxygen supply of the heart, the brain.

B) <u>Vagal receptors</u>

Stretch receptors - localized in the smooth muscles of the airways -

- the Hering-Breuer inflation reflex.

Stimulation: lung inflation

*Effects:* termination of inspiration, systemic hypotension (mechanical limitation of the venous return + reflex vasodilatation), tachycardia.

Juxtapulmonary capillary receptors – the pulmonary chemoreflex.

*Stimulation* by interstitial pressure ( pulmonary edema), chemical substances (PDG, NaS, hypertonic solution)...

*Effects:* biphasic – inhibition/stimulation reaction: apnoe/hyperpnoe; hypotension/hypertension + bradycardia (through baroreceptors).

<u>C-fiber endings in the coronary circulation</u> – the coronary chemoreflex

Stimulation: by chemical substances (veratridine, serotonin, capsaicin, metabolites ...)

*Effects:* rapid shallow breathing, hypotension, bradycardia = depressor reflex (during myocardial infarct?)

### The Coronary Circulation

Anatomy:

The right and the left coronary artery

- the anterior descending
- the circumflex branch

Smaller branches (epicardial arteries) give off tiny arteries that course at right angles through the myocardium = <u>intramural vessels</u>

Capillary number: 1 capillary/1 myofiber (3000/mm<sup>3</sup>)

Venules converge to the coronary sinus - into the RA

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The veins from the right side of the heart drain directly at multiple site within the RA and RV.

**Blood Flow** 

 $BF = 250 \text{ ml/min} \square$  (60 ml/min/100 g heart w.) = 5% of CO

<u>Pressure – Flow Paradox:</u>

*BF falls during systolic BP and it rises with the onset of diastole when BP is low – mainly in LV coronary intramural vessels* 

Explanation: Contraction of the LV squeezes the intramural vessels.

The flow dependence on the cardiac cycle phases – on differential pressure between  $BP_{ao} - P_{in}$  myocardial ventricular wal (intraventricular P).

Pressure	Grad	ient (d	if. P)				
Aorta LV	RV	Ao	– LV	Ao – RV			
Systole 120		121	25		-1	95	
Diastole	80		Ø	ø		80	
						80	



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#### Regulation of the Coronary Blood Flow

1) <u>Autoregulation:</u>

When BP is elevated, BF initially rises – then returns toward the control level and vice versa. Functioning mechanism in the range 70-170 mmHg.

#### Explanation:

Myogenic hypothesis: the response is due to altered stretch of the smooth muscle in the wall of CA.

- 2) <u>Neural:</u> ANS
- a) The sympathetic nerve fibres NE alpha adrenergic receptors vasoconstriction.
- b) The parasympathetic n. vagus Ach mild vasodilatation.
- 3) <u>Humoral:</u>

a) Oxygen – extraction of  $O_2$  in coronary bed is nearly complete. Dif. a- $v_{O2} = 12$  Vol. % - the highest in the body.

A decrease in  $PaO_2$  – vasodilation (adenosine)

b) An increase in PaCO<sub>2</sub> and decrease in pH – vasodilatation <u>Vasodilators</u>:

- Adrenaline (epinephrine) (beta 2 receptors)
- Adenosine a metabolic product of ATP breakdown
- Prostaglandins: Prostacyclin (PGI<sub>2</sub>) and PGE<sub>2</sub>
- Calcium antagonists (e.g. Verapamil)

- NO -EDRF – endothelial derived relaxing factor – a substance released by endothelial cells – in response to increasing BF

Nitroglycerine

#### Vasoconstrictors

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- Noradrenaline (norepinephrine)
- Vasopressin
- Angiotensin II.

- Ergonovine – used for provocation f coronary spasm in dg. of insufficiency coronary bed.

#### Inadequate coronary BF and coronary heart diseases

<u>1-</u> <u>st situation:</u> coronary arteries are narrowed but not completely occluded. Coronary BF is adequate to supply the resting metabolic needs of the myocardium but when  $O_2$  demands are increased (exercise) – the blood supply becomes insufficient = ischemia – with a clinical syndrome – <u>angina pectoris.</u>

<u>2-</u> <u>nd situation:</u> Abrupt obstruction of a coronary artery produces within 1-2 min loss of contraction in the involved region. If sustained beyond 40 min – it produces necrosis = <u>acute</u> <u>myocardial infarction</u>.

#### Cerebral Circulation

#### Anatomy:

Arterial inflow through 2 internal carotids + 2 vertebrals. Circle of Willis. Venous drainage by way of the deep veins and dural sinuses – into internal jugular veins.

#### Capillaries:

Number of capillaries of the brain gray matter is about 4x as great as that of white matter. Capillaries are supported on alal sides by "glial feet" – providing physical support to prevent overstretching of the capillaries in case of high pressure and to prevent transudation of fluid into the brain – against brain edema. Difficult penetration – <u>the blood – brain barrier</u>

(except of some areas of the hypothalamus, the pineal gland and the area postrema).

#### Function of the B-B barrier

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Barrier is highly permeable to  $H_2O$ ,  $CO_2$ ,  $O_2$  and lipid soluble substances (alcohol, anesthetics).

<u>Slightly</u> permeable to the electrolytes  $(Na^+, Cl^-, K^+)$ 

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<u>Totally</u> impermeable to plasma proteins and large organic molecules.

Importance: The BB barrier – maintains the constancy of the environment of the neurons in CNS.

- protects of the brain from toxins in the blood
- prevents neurotransmitters against the escape into the circulation

Ontogeny: BB barrier develops postnatal (jaundiced newborns – penetration of the bile pigments into CNS – kernicterus).

Clinical implication: Application of the drugs (ATB) penetrating in the BB barrier for treatment of cerebral diseases.

Cerebral Blood Flow

CBF = Adults: 750 ml/min = 54 ml/min/100 g of brain w. = approx. 15% of CO Children: 105 ml/min/100 g

A decrease of CBF to "adult value" in puabertal age (sex hormones)

BF in various parts of the brain:

BF in gray matter is about 6 times that in white matter.

A marked fluctuation in regional BF with changes in activity (the movements – motor area, the speech – sensory + motor area).

#### Regulation of CBF

Monro-Kellie doctrine: The sum of the volume of blood (75 ml), cerebrospinal fluid (75 ml) and brain (1400 g) in the cranium must be relatively constant.

1) <u>utoregulation:</u>

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The <u>intracranial pressure</u> (ICP – CSF pressure) = 10 mm Hg. When ICP > 33 mm Hg – CBF is reduced – ischemia – stimulation of the vasomotor and cardioinhibitory centers – hypertension, bradycardia = <u>Cushing reflex</u> – helps to maintain CBF and to preserve  $O_2$  for brain and coronary circulation.

<u>The mogenic autoregulation</u> – in the range 65 – 140 mm Hg.

2) H<u>umoral regulation:</u>

Increase in PaCO<sub>2</sub> decreases in PaO<sub>2</sub>, pH – vasodilatation

Cerebral tissue  $PO_2$  – normal 35-40 mm Hg at  $PO_2$  below 20 mm Hg – coma in 5 – 10 s.

Inhalation of  $CO_2$  – increase in CBF by 75%

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Inhalation of  $O_2$  – decrease in CBF by 15 %

#### 3) <u>Nervous regulation:</u>

Sympathetic innervation from the superior cervical sympathetic ganglia.

Vasoconstriction. During streuous exercise – prevention againsthigh pressure and cerebral stroke (a vascular hemorrhage into the brain).

Parasympathetic innervation: n. facialis. Vasodilatation. Mild importance.

Circulation in Skeletal Muscles

<u>Flow</u> – during rest 3-4 ml/min/100g

- during exercise - the increase more than 20-fold

**Regulation** 

1) Local:

Mechanical: Muscle contractions  $\rightarrow$  the decrease in BF (the importance rhythmic contractions). Between contractions – BF is increased.

Temperature: the increase - vasodilation



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#### 2) <u>Humoral:</u>

Vasodilators: Hypoxia, hypercapnia, lactic acid,  $K^+$ , acetylcholine, epinephrine Vasoconstrictor: Norepinephrine

- 3) <u>Nervous control</u> of muscle blood flow
- Sympathetic NA system vasoconstriction

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- Special – Sympathetic cholinergic system – vasodilation (activation before the

start of muscular exercise)

The Skin Circulation

Anatomy: Blood vessels in the fatty subcutaneous tissue.- Important for the thermoregulation:

- venous plexus supplied by inflow of blood from the skin capillaries
- arteriovenous anastomoses (in hands, feet, ears)

#### The Skin Blood Flow

F = 250 ml/min = 1-3 ml/min/100 g = 5% of CO - at rest F = 150 ml/min/100 g - in response to thermal stimuli

Blood is shunted through the anastomoses.

Higher skin BF causes the conduction of the heat from the core to the skin – higher radiation of the heat.

Flow of blood to the skin is a most effective heat transfer from the body core to the skin.

#### Regulation of the skin BF Autoregulation

Nervous: Sympathetic nerves: The increase of the sympathetic nerve traffic -

- vasoconstriction and vice versa. Vasoconstrictory tone.

Local axon reflexes:

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Impulses initiated in sensory nerves (by injury) are relayed <u>antidromically</u> down by other branches of the sensory nerve fiber. The only one situation – antidromic conduction.

Humoral: - Histamine and H-like substances - H1 - receptors - vasodilatatioin

- Bradykinin – sweat glands – kalikrein – effects on plasma proteins – bradykinin –

vasodilatation

- Serotonin, NE – vasoconstriction

### Tests of the skin vascular reactibility

A) White reaction: the mechanical stimulation (pointed object is drawn lightly over the skin) – a pale line – due to contractions of the precapillary sphincters (in 10-15 s).

B) Tripple response: the skin attacked more strongly

1) Red reaction (in 10 s) – capillary dilatation

2) Swelling (local edema) increased permeability of the capillaries – histamine, H-

substances

3) Difuse reddening around the injury – arterial dilatation – axon reflexes.

### Pulmonary Circulation

### Morphology:

Circulation in series to the systemic circulation. The pulmonary vessels are short and have large diameter. The walls are thin and distensible.

### Physiology

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Flow: CO RV – 5.5 l/min; Velocity – 40 cm/s;

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BF – is much more pulsatile than is systemic circulation (low arteriolar resistance)

Ventilation/Perfusion Ratio

Differences in various parts of the lungs.

<u>Pressure:</u> PA = 25/10 mm Hg. Mean = 15 mm Hg.

Pulmonary capillary pressure = 7-10 mm Hg.

Resistance: low - 2-3 mm/l/min

<u>Volume of blood</u>: 1 l of the blood in pulmonary bed – only about 75-100 ml is in the pulmonary capillaries. SV = 70 ml – all the capillary blood is replaced at each heart beat.

The increase of the volume after deep inspirium, in horizontal position. Reservoir function.

Distribution of blood flow in pulmonary circulation

The hydrostatic pressure of the blood within the pulmonary capillaries influences BF in different regions of the lungs. At the top of the lungs is little flow – in the lowest point in the lungs is max.



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#### **POSSIBLE QUESTIONS**

#### UNITII

PART-A (20 MARKS)

#### (Q.NO 1 TO 20 Online Examination)

#### PART-B (2 MARKS)

- 1. Write short note on Lipid profile.
- 2. Write about the condition of Glycosuria.
- 3. Write about the Urea Clearance Test
- 4. Write about the limit of Quantification.
- 5. Define prothrombin index.

#### PART-C (8 MARKS)

- 1. Explain the clinical manifestations of liver disease
- 2. Write the basic defects and consequences of diabetes mellitus.
- 3. Explain in detail about the clinical significance of Serum creatine phosphokinase in heart diseases.
- 4. Write briefly on various laboratory tests of Blood glucose.
- 5. Explain in detail about the clinical significance of Serum glutamate oxaloacetate transaminase (SGOT) in heart diseases.
- 6. Explain in detail about the clinical significance of Lactate dehydrogenase (LDH) in heart disease.
- 7. Explain the diagnosis of liver diseases using enzymes.
- 8. Explain about the Hepatobiliary disorders.
- 9. Explain in detail about the various Glomerular filtration tests.

Questions	opt1	opt2	opt3 opt4	opt4	opt5	opt6	Answer
Which type of gallbladder	Cholesterol	Pigment	Bilirubin stones	Gall stones			Pigment
stones can be present due	stones	stones					stones
Pigment gallstones that	yellow	green	brown	black			black
Colesterol stones usually	radio-opaque	radio-opaque\	radiolucent\radio-	radiolucent\ra			radioluc
appear on x-ray as? While	\ radio-opaque	radiolucent	opaque	diolucent			ent∖radi
pigment stones most of the							0-
time is?							opaque
The main complication of	pancreatitis	empyema	Cholesystitis	Chirosis			Cholesys
cholelithiasis is?							titis
The most common site of	neck or cystic	intestinal	liver ducts	biliary tree			neck or
obstruction by gallstones	duct	obstruction					cystic
in case of acute calculous							duct
Cholesystitis is?							
What is the type of	Acalculous	Chronic	Acute calculous	Calculous			Acute
Cholesystitis that requires	cholecystisis	Cholesystitis	Cholesystitis	Cholesystitis			calculou
a quick cholecystectomy?							s
							Cholesys
							titis
A 45-year-old obese	Increased	Decreased	Increased bilirubin	Increased			Increase
woman suffers from	hepatic	serum albumin	uptake by the liver	hepatic			d
abdominal pain after fatty	cholesterol			calcium			hepatic
meals, some abdominal	secretion			secretion			cholester
distension, and frequent							ol
indigestion. An ultrasound							secretion
examination discloses							
multiple echogenic objects							
in the gallbladder. Which							
of the following metabolic							
changes is most likely							
associated with the							
formation of gallstones in							
this patient?							
Which ONE of the	Hepatocellular	Cholangiocarc	Hepatoblastoma	Angiosarcoma			Hepatob
following is a primary	Carcinoma	inoma					lastoma
liver cancer that occurs in							
childhood?							
Which of the following	Kidney	Breast	Bone	Brain			Breast
primary sites has highest							
incidence to spread to the							
liver?							
53-year-old male came	alpha-	Alanine	Bilirubin	Albumin			alpha-
with abdominal pain,	fetoprotein	aminotransfera					fetoprot
fatigue, weight loss and		se					ein
abdominal swelling.							
Histological findings of							
the liver show well-							
differentiated cells with							
bile pigments. Which of							
the following most likely							
to be elevated in serum?							
Pancreatic carcinoma	Ductal	Acinar cells	Islets of Langerhans	Pancreatic			Ductal
arises from which of the	epithelial cells			blood vessels			epithelia
following cells?							l cells

An autopsy was performed on a 48 years old patient who has died from cachexia, the pathologist noticed enlargement of the liver, the following morphologies were written in the report: diffusely infiltrative liver cancer with evidence of invasion the portal vein, bile pigment is present and tumor cells were positive for $\alpha$ -fetoprotein, which one of these conditions was the reason of the death?	metastasis	poorly differentiated Hepatocellular carcinoma	hemangioma	Well differentiated Hepatocellular carcinoma		Well different iated Hepatoc ellular carcino ma
Which one of these tumors has the worst prognosis (high mortality rate)?	Pancreatic carcinoma	Fibrolamellar carcinoma	Angiosarcoma	Hepatocellular carcinoma		Pancreat ic carcino ma
Which one of these tumors is considered as the most frequent PRIMARY malignant tumor of the liver?	Acinar cell carcinoma	hepatocellular carcinoma	Angiosarcoma	Cholangiocarc inoma		hepatoce llular carcino ma
A patient is known to have chronic liver cirrhosis came to the ER because of hematemesis. The most common mechanism of esophageal varices in this patient is?	Inflammatory erosion	Increased vascular hydrostatic pressure	Increased tension in progressively dilated veins	both a and b		Increase d vascular hydrosta tic pressure
Hematological abnormalities such as thrombocytopenia or pancytopenia can be found in liver cirrhosis due to?	Renal failure	Splenomegaly	Bacterial peritonitis	Heptomegaly		Splenom egaly
The dominant intrahepatic cause of portal hypertension is?	Ascites	Bacterial infection	Cirrhosis	Fungal infection		Cirrhosi s
Cryptogenic cirrhosis means?	Cardiac cirrhosis	drug-induced cirrhosis	primary cirrhosis due to unknown cause	secondary cirrhosis due to unknown cause		primary cirrhosis due to unknow n cause
Alcoholic cirrhosis is classified depending on the size as (less than 3 mm	Micronodular	Macronodular	Nanonodular	Meganodular		Microno dular
Which one of the following is not a characteristics of liver cirrhosis?	Fibrosis	Nodules	Cyst	Thread like		Cyst

Inflammatory howl diseases are more common in?females femalesmalesinfantselderlyoediseases are more common in?irritable bowel bowel bowel only affect the mucosa?irritable bowel polypsjuvenile diseaseCrohn's diseaseulcerative colitisulcerative e colitisulcerative e colitisA biopsy of the large infortance of non-caseating granulomas with thickening of the bowel wal and presence of structures. Which of the following is the mostCrohn's diseaseadenoma neoplasti presence of syndromeBSCrohn's diseaseGibert syndrom eWhich of the following perbicibiotical coll granulomas with has increased excretion of syndromeGold syndrome syndrom eGall stones syndrom eGibert syndrom eGall stones syndrom eGibert syndrom eGall stones syndrom eBiliary circhosis spherocytBiliary circhosisBiliary circhosisWhich of the following torolition is suscicted with plorebiribrinemia ?Go PD syndrome syndromHereduta sisBiliary circhosisBiliary circhosisBiliary circhosisA patient with Na copies are ike following iscense in all of word inserved structureWild type spherocytPreCore insective HBV carrier mutantPreCore informia tableDeferriox sisBiliary circhosisJOU000/mL bil tickely diagnosis ?Wild type sis the treatment of choiceArthropalhy diseaseCronic alcoholism circhosisCronoic alcoholis 	Which of the following present with red blood in stool?	Peutz-Jehgers syndrome	Juvenile polyps	Inflamma polyps	tory Hyperplasti polyps			Peutz- Jehgers syndrom
Inflammatory bowl diseases at more common in? which of the following diseases is only limited to be order and rectum and syndrome only affect the mucosa? A biopy of the large inflammation and presence of non-cascating presence of non-cascating difficiency has increased excretion of uncojugated bilinvinemia ? A 20 ye od man with Has Nila Y No duam with Has Nila Y No duam vith Has Nila Y No duam vith Pas No dual regenerative characteristically cause hard the following casea characteristically cause hard the presence of site the treatment of choice Nichoice No dual regenerative characteristically cause characteristically cause characteristically cause characteristically cause characteristically cause characteristically cause characteristically cause characteristically cause characteris					-			e
diseases are more common in? which of the following sing in extine and syndrome only affect the mucosa? A biopsy of the most infarmation and presence of non-casating granulomas with thickening of the bowel wall and presence of sritcurus. Which of the following is the most Which of the following syndrome syndr	Inflammatory bowl	females	males	infants	elderly			females
in?     irritable     juvenile     Crohn's     ulcerative colitis     ulcerative colitis       disease is only limited to bowel     syndrome     polyps     disease     e colitis     e colitis       A biopsy of the large     Crohn's     adenoma     neoplasti     IBS     Crohn's       inststine is taken and shear and presence of non-cascating epithelioi dcell     sicease     c polyps     Gilbert     Gall stones     Gilbert       granulomas with thickening of the bowel wall and presence of strictures. Which of the following is the most     polin-     Rotor     Gilbert     Gall stones     Gilbert       Yhich of the following is the most     johnson     syndrome     g     e     gilfary     Gilbert       Yagient With Gf the following is the most     GGP D     Hemolytic     Hereduta     Biliary cirrhosis     Biliary       uncojugated biltrubinemia ?     GfP D     Hemolytic     Hereduta     Biliary cirrhosis     Biliary       syndrom e     syndrome     spherocytic     active HBV carrier     PreCore     PreCore       10x0 (si the following is the torone of site interversion of urboditions evention eventin all of the following disease     <	diseases are more common							
which of the following only affect the merosa?       interative polyps       Crohn's diseases       ulcerative colitis       e colitis         A biopsy of the large only affect the merosa?       Crohn's diseases       adenoma       neoplasti       IBS       Crohn's diseases         A biopsy of the large pribelioid cell granulomas with thickening of the bovel wall and presence of non-caseating epithelioid cell granulomas with thickening of the bovel wall and presence of strictures. Which of the following is the most       Crohn's disease       adenoma       neoplasti       IBS       Crohn's disease       Gilbert syndrom         Which of the following condition is associated with playerbilitybinemia?       Dubin- syndrom       Rotor syndrom       Gilbert syndrom       Gilbert syndrom       Biliary cirrhosis       Biliary cirrhosis       Biliary cirrhosis         A patient with uncojugated biirubinemia?       Go PD uncojugated biirubinemia deficiency has increased excretion of urobilingen in his urine. This can be seen in all of the following conditions e2 -Ve with SGOT and USOFT and 25 firms the normal value. The HBV DNA copies are 1.00.000/ml. Which is the the morthal value. The HBV hemochromatosis is : m       Milson's m       PreCore mutant HBV HBV       Inactive HBV carrier mutant HBV       Deferrioxamine is the diabetes       Deferrioxamine is the diabetes       Deferrioxamine is the treatment of choice       Deferrioxamine sis the treatment of choice         Which one of the charace risk charace risk condition that change in liver ?       Hapathis m       Wilson's di	in?							
disease is only limited to he colon and return and only affect the mucosa?       Crohn's adenoma       meoplasti       IBS       Crohn's disease         A biopsy of the large inflation and presence of non-cascuting epithelioid cell granulonas with thickening of the bowel wall and presence of strictures. Which of the following is the most       Crohn's disease       Crohn's adenoma       IBS       Crohn's disease         Which of the following condition is associted with following is the most       Dubin- syndrome       Rotor       Gilbert       Gall stones       Gilbert         A patient with uncojugated bilinubinemia following conditions except :-       Dubin- syndrome       Rotor       Gilbert       Gall stones       Billary cirrhosis         A patient with uncojugated bilinubinemia following conditions except :-       G6 PD       Hemolytic       Hereduta anemia       Billary cirrhosis       Billary cirrhosis         A 20 ye old man with HBS VIAC oppies are 1.00.000/ml. Which is the romanu value. The HBV       Surface mutant HBV       PreCore mutant HBV       Deferrio mutant HBV       Deferrio calculated and the diabetes       Deferrio calculated alcoholism       Deferrio calculated alcoholism       Deferrio calculated alcoholism       Deferrio calculated alcoholism       Chronic alcoholism       Chronic alcoholism       Chronic calcoholism         SCPT raised 5 times the following disease characteristically cause following disease       Hepatitis B wins infection       Wilson's wins infection <td< td=""><td>which of the following</td><td>irritable</td><td>juvenile</td><td>Crohn's</td><td>ulcerative</td><td>colitis</td><td></td><td>ulcerativ</td></td<>	which of the following	irritable	juvenile	Crohn's	ulcerative	colitis		ulcerativ
the colon and rectum and syndrome only affect the macosa? A biopsy of the large interstance of	diseases is only limited to	bowel	polyps	disease				e colitis
only affect the mucoss?     Crohn's     adenoma     neoplasti       a biopsy of the large inflammation and presence of non-caseating epithelioid cell granulomas with thickening of the bowel wall and presence of strictures. Which of the following is the most     Crohn's adenoma     neoplasti c polyps     IBS     Crohn's disease       Which of the following condition is associed with procongued associed with associed with solution associed with SGOT and SGPT raised 5 times the following is its in the following is its in the following disease     Dubin- syndrom     Gilbert     Gilbert syndrom       Which of the following conditions is associed with syndrome apperbilimbinemia of the following is the following disease     Dubin- syndrome     Rotor     Gilbert       Which of the following conditions except :-     C6 PD     Hemolytic anemia     Hereduta ry spherocyt osis     Biliary cirrhosis     Biliary cirrhosis       A 20 ye old man with HBs GOT raised 5 times the following disease rhave with SGOT and SGPT raised 5 times the following disease characteristically cause farty charge in live ?     Surface mutant HBV     PreCore mutant HBV     Deferrioxamine is the treatment of choice     Deferrio xamine is the treatment of choice       Which one of the following disease characteristically cause farty charge in live ?     Hepatitis B. Virus infection     Wilson's disease     Hepatitis tice infection     Chronic alcoholism m     Chronic alcoholism m       Which one of the following disease characteristically cause	the colon and rectum and	syndrome						
A biopsy of the large intestine is taken and showed transmural inflammation and presence of non-caseating epithelioid cell granulomas with thickening of the bowel wall and presence of strictures. Which of the following condition is associted with johnson syndromeRotor syndrome eGilbert syndrome eGall stones syndrom eGilbert syndrom eWhich of the following condition is associted with has increased exerction of the following conditionsDubin- syndromeGilbert syndrome eGall stonesGilbert syndrom eA patient with unconjugated byperbilimbinemia ?Gf PD deficiency has increased exerction of the following conditionsHemolytic anemia syndromeHereduta syndrome eBiliary cirrhosis syndrom eBiliary cirrhosis syndrom eA patient with unconjugated byperbilimbinemia file can be secretion of the following conditionsSurface mutant HBVPreCore mutant HBVBiliary cirrhosis syndrom eBiliary cirrhosis syndrom eA 20 ye old man with HBs SGPT raised 5 times the hemochromatosis is :Wild type mSurface mutant HBVPreCore mutant HBVInactive HBV carrier mutant HBVPreCore mutant HBVMich of the bilkely diagnosis ?Hypogonadis rise and stress defined wirus infectionDeferrio stamine to choiceWhich one of the following disease characteristically cause faluy charge in liver ?Hepatitis B virus infectionWilson's diseaseHepatitis sisChronic alcoholism m mChronic alcoholism m <br< td=""><td>only affect the mucosa?</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></br<>	only affect the mucosa?							
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following is the most       Gilbert       Gall stones       Gilbert       Syndrome       Syndrom	strictures. Which of the							
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condition is associted with unconjugated syndrome synd	Which of the following	Dubin-	Rotor	Gilbert	Gall stone	es		Gilbert
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tumor of heart is :-	a				
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In controlling the synthesis and secretion of aldosterone, which of the following factors is least important ?	angiotensin II	concentration of plasma Na+	concentr ation of plasma K+	N- Acetyl galactose mine	adrenocorticot ropic hormone ( ACTH )	adrenc orticot pic hormo ( ACTH
Renal correction of acute hyperkalemia will result in	acidosis	increased secretion of HCO3	increased secretion of H+	dermatin sulphate	increased secretion of Na+	acidosi
Most of the glucose that is filtered through the glomerulus undergoes reabsorption in the :	proximal tubule	descending limp of the loop of Henle	ascendin g limb of the loop of Henle	heating with con.HCl	distal tubule	proxin tubule
In the presence of ADH, The distal nephron is least permeable to :	water .	ammonia .	urea	phenyl hydrazin e	sodium	urea
When a person is dehydrated, hypotonic fluid will be found in the:	loop of Henle	distal convoluted tubule	collectin g duct .	proximal	tubule .	loop of Henle
The ability of the kidney to excrete a concentrated urine will increase if :	the permeability of the proximal tubule to water decreases .	the rate of blood flow through the medulla decreases .	the rate of flow through the loop of Henle increases		the activity of the Na-K pump in the loop of Henle decreases	the rat of bloo flow throug the medull decrea s.
The glomerular filtration rate will increase if :	circulating blood volume increase	the afferent arteriolar resistance increases.	the efferent arteriolar resistanc e decreases		the plasma protein concentration decreases	the plasma protein concen ation decrea s
Reabsorption of Na+ :	takes place in association with CL- & HCO3	occurs only in PT	is under control of parathor mone hormone		is a passive process .	takes place i associa on witl CL- & HCO3
Diamox causes :	water diuresis	hypokalaemia	alkalosis		hyperkalaemia	hypoka aemia
K+ excretion is markedly influenced by :	aldosterone	amount of Na+ delivered to tubules	rate of tubular secretion of H+		Ketosterone	aldoste one
More hydrogen is secreted in : Urinary volume is increased by all the following except :	alkalosis diabetes insipidus	administration of diamox diabetes mellitus	hypokala emia sympathe tic stimulati on		hyperventilatio n. increased renal arterial pressure	hypoka aemia sympa etic stimula on

Extracellular bicarbonate ions serve as effective buffer for all the following except :	sulfuric acid	phosphate acid	lactic acid	carbonic acid		carbonic acid
The glomerular filtration barrier is composed of all the following except :	fenestrated capillary endothelium	macula densa	basement membran e	podocytes		macula densa
The hypothalamus will effect the release of ADH in response to all the following stimuli except :	severe hemorrhage	decreased blood osmolarity	pain, anxiety, or surgical stress	nicotine		nicotine



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#### UNIT-III

#### SYLLABUS

#### Assessment of glucose metabolism in blood

Clinical significance of variations in blood glucose. Diabetes mellitus.

#### Lipid profile

Composition and functions of lipoproteins. Clinical significance of elevated lipoprotein.

### **BLOOD SUGAR**

The blood sugar concentration or blood glucose level is the amount of glucose (sugar) present in the blood of a human or animal. The body naturally tightly regulates blood glucose levels as a part of metabolic homeostasis. Glucose is the primary source of energy for the body's cells. Glucose is transported from the intestines or liver to body cells via the bloodstream, and is made available for cell absorption via the hormone insulin, produced by the body primarily in the pancreas.

The mean normal blood glucose level in humans is about 100 mg/dL; however, this level fluctuates throughout the day. Glucose levels are usually lowest in the morning, before the first meal of the day (termed "the fasting level"), and rise after meals for an hour or two by a few milligram. The normal blood glucose level (tested while fasting) for non-diabetics, should be between 70 and 100 milligrams per deciliter (mg/dL). Blood sugar levels for those without diabetes and who are not fasting should be below 125 mg/dL. The blood glucose target range for diabetics, according to the American Diabetes Association, should be 70–130 (mg/dL) before meal, and less than 180 mg/dL after meals (as measured by a blood glucose monitor).

Blood sugar levels outside the normal range may be an indicator of a medical condition. A persistently high level is referred to as hyperglycemia; low levels are referred to as hypoglycemia. Diabetes mellitus is characterized by persistent hyperglycemia from any of several causes, and is the most prominent disease related to failure of blood sugar regulation. Intake of alcohol causes an initial surge in blood sugar, and later tends to cause levels to fall.

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Also, certain drugs can increase or decrease glucose levels.

#### Regulation

The body's homeostatic mechanism keeps blood glucose levels within a narrow range. It is composed of several interacting systems, of which hormone regulation is the most important.

There are two types of mutually antagonistic metabolic hormones affecting blood glucose levels:

 $\hfill\square$  catabolic hormones (such as glucagon, cortisol and catecholamines) which increase blood glucose

 $\Box$  anabolic hormone (insulin), which decreases blood glucose.

#### Abnormality in blood sugar levels

#### High blood sugar

If blood sugar levels remain too high the body suppresses appetite over the short term. Longterm hyperglycemia causes many of the long-term health problems including heart disease, eye, kidney, and nerve damage. The most common cause of hyperglycemia is diabetes. When diabetes is the cause, physicians typically recommend an anti-diabetic medication as treatment. From the perspective the majority of patients, treatment with an old, well-understood diabetes drug such as metformin will be the safest, most effective, least expensive, most comfortable route to managing the condition. Diet changes and exercise implementation may also be part of a treatment plan for diabetes.

#### Low blood sugar

If blood sugar levels drop too low, a potentially fatal condition called hypoglycemia develops. Symptoms may include lethargy, impaired mental functioning; irritability; shaking, twitching, weakness in arm and leg muscles; pale complexion; sweating; paranoid or aggressive mentality and loss of consciousness.

#### **Glucose measurement**

#### Sample type

Glucose is measured in whole blood, plasma or serum. Historically, blood glucose values were given in terms of whole blood, but most laboratories now measure and report plasma or serum

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glucose levels. Because red blood cells (erythrocytes) have a higher concentration of protein (e.g., hemoglobin) than serum, serum has a higher water content and consequently more dissolved glucose than does whole blood. Collection of blood in clot tubes for serum chemistry analysis permits the metabolism of glucose in the sample by blood cells until separated by centrifugation. Red blood cells, for instance, do not require insulin to intake glucose from the blood. Higher than normal amounts of white or red blood cell counts can lead to excessive glycolysis in the sample, with substantial reduction of glucose level if the sample is not processed quickly. Ambient temperature at which the blood sample is kept prior to centrifuging and separation of plasma/ serum also affects glucose levels. At refrigerator temperatures, glucose remains relatively stable for several hours in a blood sample.

Loss of glucose can be prevented by using Fluoride tubes since fluoride inhibits glycolysis. However, these should only be used when blood will be transported from one hospital laboratory to another for glucose measurement. Red-top serum separator tubes also preserve glucose in samples after being centrifuged isolating the serum from cells. Arterial, capillary and venous blood has comparable glucose levels in a fasting individual. Following meals, venous levels are somewhat lower than those in capillary or arterial blood; a common estimate is about 10%.

#### **Measurement techniques**

Two major methods have been used to measure glucose. The first, still in use in some places, is a chemical method exploiting the nonspecific reducing property of glucose in a reaction with an indicator substance that changes color when reduced. The more recent technique, using enzymes specific to glucose, is less susceptible to this kind of error. The two most common employed enzymes are glucose oxidase and hexokinase. In either case, the chemical system is commonly contained on a test strip which is inserted into a meter, and then has a blood sample applied. Test-strip shapes and their exact chemical composition vary between meter systems and cannot be interchanged. More precise blood glucose measurements are performed in a medical laboratory, using hexokinase, glucose oxidase or glucose dehydrogenase enzymes.

#### **Blood glucose laboratory tests**

1. fasting blood sugar (i.e., glucose) test (FBS)

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- 2. two-hr postprandial blood sugar test (2-h PPBS)
- 3. oral glucose tolerance test (OGTT)
- 4. intravenous glucose tolerance test (IVGTT)
- 5. glycosylated hemoglobin (HbA1C)
- 6. self-monitoring of glucose level via patient testing
- 7. Random blood sugar (RBS)
- 8. Average blood glucose may be estimated by measuring glycated hemoglobin (HbA1c)

#### **Clinical Correlation**

ARPAGAM

Y OF HIGHER EDUCATION

The fasting blood glucose level, which is measured after a fast of 8 hours, is the most commonly used indication of overall glucose homeostasis, largely because disturbing events such as food intake are avoided. The metabolic response to a carbohydrate challenge is conveniently assessed by a postprandial glucose level drawn 2 hours after a meal or a glucose load. In addition, the glucose tolerance test, consisting of several timed measurements after a standardized amount of oral glucose intake, is used to aid in the diagnosis of diabetes. Finally, there are several influences on blood glucose level aside from food intake. Infection, for instance, tends to change blood glucose levels, as does stress either physical or psychological. Exercise, especially if prolonged or long after the most recent meal, will have an effect as well.

#### **Definition of Diabetes and Prediabetes**

Diabetes mellitus is a metabolic disorder characterized by the presence of hyperglycemia due to defective insulin secretion, defective insulin action or both. The chronic hyperglycemia of diabetes is associated with relatively specific long-term microvascular complications affecting the eyes, kidneys and nerves, as well as an increased risk for cardiovascular disease (CVD). The diagnostic criteria for diabetes are based on thresholds of glycemia that are associated with microvascular disease, especially retinopathy. "Prediabetes" is a practical and convenient term referring to impaired fasting glucose (IFG), impaired glucose tolerance (IGT) (1) or a glycated hemoglobin (A1C) of 6.0% to 6.4%, each of which places individuals at high risk of developing diabetes and its complications.

#### **Classification of Diabetes**

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The classification of type 1 diabetes, type 2 diabetes and gestational diabetes mellitus (GDM) is

summarized in Table 1.

Table 1

Classification of diabetes (1)

- Type 1 diabetes\* encompasses diabetes that is primarily a result of pancreatic beta cell destruction and is prone to ketoacidosis. This form includes cases due to an autoimmune process and those for which the etiology of beta cell destruction is unknown.
- Type 2 diabetes may range from predominant insulin resistance with relative insulin deficiency to a predominant secretory defect with insulin resistance.
- Gestational diabetes mellitus refers to glucose intolerance with onset or first recognition during pregnancy.
- Other specific types include a wide variety of relatively uncommon conditions, primarily specific genetically defined forms of diabetes or diabetes associated with other diseases or drug use (Appendix 1).

 Includes latent autoimmune diabetes in adults (LADA); the term used to describe the small number of people with apparent type 2 diabetes who appear to have immune-mediated loss of pancreatic beta cells (4).

Appendix 1 addresses the etiologic classification of diabetes. Distinguishing between type 1 and type 2 diabetes is important because management strategies differ, but it may be difficult at the time of diagnosis in certain situations. Physical signs of insulin resistance and autoimmune markers, such as anti-glutamic acid decarboxylase (GAD) or anti-islet cell antibody (ICA) antibodies, may be helpful, but have not been adequately studied as diagnostic tests in this setting. While very low C-peptide levels measured aftermonths of clinical stabilization may favour type 1 diabetes (2), they are not helpful in acute hyperglycemia (3). Clinical judgement with safe management and ongoing follow-up is a prudent approach.

#### **Diagnostic Criteria**

#### Diabetes

The diagnostic criteria for diabetes are summarized in Table 2 (1). These criteria are based on venous samples and laboratory methods.

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Table 2 Diagnosis of diabetes

#### FPG ≥7.0 mmol/L

Fasting = no caloric intake for at least 8 hours

ог

#### A1C 26.5% (in adults)

Using a standardized, validated assay in the absence of factors that affect the accuracy of the A1C and not for suspected type 1 diabetes (see text)

ОΓ

#### 2hPG in a 75 g OGTT ≥11.1 mmol/L

OL

#### Random PG≥11.1 mmol/L

Random = any time of the day, without regard to the interval since the last meal

In the absence of symptomatic hyperglycemia, if a single laboratory test result is in the diabetes range, a repeat confirmatory laboratory test (FPG, A1C, 2hPG in a 75 g OGTT) must be done on another day. It is preferable that the same test be repeated (in a timely fashion) for confirmation, but a random PG in the diabetes range in an asymptomatic individual should be confirmed with an alternate test. In the case of symptomatic hyperglycemia, the diagnosis has been made and a confirmatory test is not required before treatment is initiated. In individuals in whom type 1 diabetes is likely (younger or lean or symptomatic hyperglycemia, especially with ketonuria or ketonemia), confirmatory testing should not delay initiation of treatment to avoid rapid deterioration. If results of 2 different tests are available and both are above the diagnostic cutpoints, the diagnosis of diabetes is confirmed.

2hPG, 2-hour plasma glucose; A1C, glycated hemoglobin; FPG, fasting plasma glucose; OGTT, oral glucose tolerance test; PG, plasma glucose.

A fasting plasma glucose (FPG) level of 7.0 mmol/L correlates most closely with a 2hour plasma glucose (2hPG) value of \_11.1 mmol/L in a 75 g oral glucose tolerance test (OGTT), and each predicts the development of retinopathy (5e11). The relationship between A1C and retinopathy is similar to that of FPG or 2hPG with a threshold at around 6.5% (5e7,11,12). Although the diagnosis of diabetes is based on an A1C threshold for developing microvascular disease, A1C is also a continuous cardiovascular (CV) risk factor and a better predictor of macrovascular events than FPG or 2hPG (13,14). Although many people identified

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by A1C as having diabetes will not have diabetes by traditional glucose criteria and vice versa, there are several advantages to using A1C for diabetes diagnosis (15). A1C can be measured at any time of day and is more convenient than FPG or 2hPG in a 75 g OGTT. A1C testing also avoids the problem of day-to-day variability of glucose values as it reflects the average plasma glucose (PG) over the previous 2 to 3 months (1). In order to use A1C as a diagnostic criterion, A1C must be measured using a validated assay standardized to the National Glycohemoglobin Standardization Program-Diabetes Control and Complications Trial reference. It is important to note that A1C may be misleading in individuals with various hemoglobinopathies, iron deficiency, hemolytic anaemias, and severe hepatic and renal disease (16). In addition, studies of various ethnicities indicate that African Americans, American Indians, Hispanics and Asians have A1C values that are up to 0.4% higher than those of Caucasian patients at similar levels of glycemia (17,18). The frequency of retinopathy begins to increase at lower A1C levels in American blacks than in American whites, which suggests a lower thresholdfor diagnosing diabetes in black persons (19). Research is required to determine if A1C levels differ in African Canadians or Canadian First Nations. A1C values also are affected by age, rising by up to 0.1% per decade of life (20,21). More studies may help to determine if age- or ethnic-specific adjusted A1C thresholds are required for diabetes diagnosis. Also, A1C is not recommended for diagnostic purposes in children, adolescents, pregnant women or those with suspected type 1 diabetes.

The decision of which test to use for diabetes diagnosis (Table 2) is left to clinical judgement. Each diagnostic test has advantages and disadvantages (Table 3). In the absence of symptomatic hyperglycemia, if a single laboratory test result is in the diabetes range, a repeat confirmatory laboratory test (FPG, A1C,2hPGin a 75 gOGTT) must be done on another day. It is preferable that the same test be repeated (in a timely fashion) for confirmation, but a random PG in the diabetes range in an asymptomatic individual should be confirmed with an alternate test. In the case of symptomatic hyperglycemia, the diagnosis has been made and a confirmatory test is not required before treatment is initiated. In individuals in whom type 1 diabetes is likely (younger or lean or symptomatic hyperglycemia, especially with ketonuria or ketonemia), confirmatory testing should not delay initiation of treatment to avoid rapid deterioration. If results of 2 different tests are available and both are above the diagnostic cutpoints, the diagnosis

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Table 3

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of diabetes is confirmed.Whenthe results ofmore than1 test are available(among FPG, A1C,2hPGin a 75 gOGTT) and the results are discordant, the test whose result is above the diagnostic cutpoint should be repeated and the diagnosis made on the basis of the repeat test.

Parameter	Advantages	Disadvantages
FPG	<ul> <li>Established standard</li> <li>Fast and easy</li> <li>Single sample</li> <li>Predicts microvascular complications</li> </ul>	<ul> <li>Sample not stable</li> <li>High day-to-day variability</li> <li>Inconvenient (fasting)</li> <li>Reflects glucose homeostasis at a single point in time</li> </ul>
2hPG in a 75 g OGTT	<ul> <li>Established standard</li> <li>Predicts microvascular complications</li> </ul>	<ul> <li>Sample not stable</li> <li>High day-to-day variability</li> <li>Inconvenient</li> <li>Unpalatable</li> <li>Cost</li> </ul>
AIC	<ul> <li>Convenient (measure any time of day)</li> <li>Single sample</li> <li>Predicts microvascular complications</li> <li>Better predictor of macrovascular disease than FPG or 2hPG in a 75 g OGTT</li> <li>Low day-to-day variability</li> <li>Reflects long-term glucose concentration</li> </ul>	<ul> <li>Cost</li> <li>Misleading in various medical conditions (e.g. hemoglobinopathies, iron deficiency, hemolytic anaemia, severe hepatic or renal disease)</li> <li>Altered by ethnicity and aging</li> <li>Standardized, validated assay required</li> <li>Not for diagnostic use in children, adolescents, preg- nant women or those with suspected type 1 diabetes</li> </ul>

2hPG, 2-hour plasma glucose; A1C, glycated hemoglobin; FPG, fasting plasma glucose; OGTT, oral glucose tolerance test.

 Adapted from Sacks D. A1C versus glucose testing: a comparison. Diabetes Care. 2011;34:518-523.

#### **Prediabetes**

The term "prediabetes" refers to IFG, IGT or an A1C of 6.0% to 6.4% (Table 4), each of which places individuals at high risk of developing diabetes and its complications. Not all individuals with prediabetes will necessarily progress to diabetes. Indeed, a significant proportion of people who are diagnosed with IFG or IGT will revert to normoglycemia. People with prediabetes,

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particularly in the context of the metabolic syndrome, would benefit from CV risk factor modification.

### Table 4

Diagnosis of prediabetes

Test	Result	Prediabetes category
FPG (mmol/L) 2hPG in a 75 g OGTT (mmol/L)	6.1-6.9 7.8-11.0	IRG IGT
A1C (%)	6.0-6.4	Prediabetes

2hPG, 2-hour plasma glucose; A1C, glycated hemoglobin; FPG, fasting plasma glucose; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; OGTT, oral glucose tolerance test.

While people with prediabetes do not have the increased risk for microvascular disease as seen in diabetes, they are at risk for the development of diabetes and CVD (23). IGT is more strongly associated with CVD outcomes than is IFG. Individuals identified as having both IFG and IGT are at higher risk for diabetes as well as CVD. While there is no worldwide consensus on the definition of IFG (24,25), the Canadian Diabetes Association defines IFG as an FPG value of 6.1 to 6.9 mmol/L due to the higher risk of developing diabetes in these individuals compared to defining IFG as an FPG value of 5.6 to 6.9 mmol/L (25). While there is a continuum of risk for diabetes in individuals with A1C levels between 5.5% and 6.4%, population studies demonstrate that A1C levels of 6.0% to 6.4% are associated with a higher risk for diabetes compared to levels between 5.5% and 6.0% (26). While the American Diabetes Association has based the definition on a higher risk group and includes an A1C of 6.0% to 6.4% as a diagnostic criterion for prediabetes (1).

However, A1C levels below 6.0% can indeed be associated with an increased risk for diabetes (26). The combination of an FPG of 6.1 to 6.9 mmol/L and an A1C of 6.0% to 6.4% is predictive of 100% progression to type 2 diabetes over a 5-year period (27). Metabolic syndrome Prediabetes and type 2 diabetes are often manifestations of a much broader underlying disorder (28), including the metabolic syndromeda highly prevalent, m ultifaceted condition characterized

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by a constellation of abnormalities that include abdominal obesity, hypertension, dyslipidemia and elevated blood glucose. Individuals with the metabolic syndrome are at significant risk of developing CVD. While metabolic syndrome and type 2 diabetes often coexist, those with metabolic syndrome without diabetes are at significant risk of developing diabetes. Evidence exists to support an aggressive approach to identifying and treating people, not only those with hyperglycemia but also those with the associated CV risk factors that make up the metabolic syndrome, such as hypertension, dyslipidemia and abdominal obesity, in the hope of significantly reducing CV morbidity and mortality.

Various diagnostic criteria for the metabolic syndrome have been proposed. In 2009, a harmonized definition of the metabolic syndrome was established, with at least 3 or more criteria required for diagnosis (Table 5) (29).

#### Table 5

Harmonized definition of the metabolic syndrome; ≥3 measures to make the diagnosis of metabolic syndrome\* (29)

Measure	Categorical cut points			
	Men	Women		
Elevated waist circumference (population- and country-specific cutpoints);				
Canada, United States	≥102 cm	≥88 cm		
<ul> <li>Europid, Middle Eastern, sub-Saharan African, Mediterranean</li> </ul>	≥94 cm	≥80 cm		
Asian, Japanese, South and Central American	≥90 cm	≥80 cm		
Elevated TG (drug treatment for elevated TG is an alternate indicator <sup>†</sup> )	$\geq$ 1.7 mmol/L			
Reduced HDL-C (drug treatment for reduced HDL-C is an alternate indicator <sup>†</sup> )	<1.0 mmol/L in ma	les,		
	<1,3 mmol/L in fen	nales		
Elevated BP (antihypertensive drug treatment in a patient with a history of hypertension is an alternate indicator)	Systolic ≥130 mm	Hg and/or		
	diastolic ≥85 mm H	tg .		
Elevated FPG (drug treatment of elevated glucose is an alternate indicator)	$\geq$ 5,6 mmol/L			

BP, blood pressure; FPG, fasting plasma glucose; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides.

Three or more criteriaare required for diagnosis.

Adapted from Alberti KGMM, Eckel R, Grundy S, et al. Harmonizing the metabolic syndrome. Circulation, 2009;120:1640-1645.

<sup>†</sup> The most commonly used drugs for elevated TG and reduced HDL-C are fibrates and nicotinic acid. A patient taking 1 of these drugs can be presumed to have high TG and reduced HDL-C. High-dose omega-3 fatty acids presumes high TG.

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### RECOMMENDATIONS

- 1. Diabetes should be diagnosed by any of the following criteria:
  - FPG ≥7.0 mmol/L [Grade B, Level 2 (11)]
  - A1 C ≥6.5% (for use in adults in the absence of factors that affect the accuracy of A1C and not for use in those with suspected type 1 diabetes) [Grade B, Level 2 (11)]
  - 2hPG in a 75 g OGTT ≥11.1 mmol/L [Grade B, Level 2 (11)]
  - Random PG ≥11.1 mmol/L [Grade D, Consensus]
- 2. In the absence of symptomatic hyperglycemia, if a single laboratory test result is in the diabetes range, a repeat confirmatory laboratory test (FPG, A1C, 2hPG in a 75 g OGTT) must be done on another day. It is preferable that the same test be repeated (in a timely fashion) for confirmation, but a random PG in the diabetes range in an asymptomatic individual should be confirmed with an alternate test. In the case of symptomatic hyper-glycemia, the diagnosis has been made and a confirmatory test is not required before treatment is initiated. In individuals in whom type 1 diabetes is likely (younger or lean or symptomatic hyperglycemia, especially with ketonuria or ketonemia), confirmatory testing should not delay initiation of treatment to avoid rapid deterioration. If results of two different tests are available and both are above the diagnostic cutpoints, the diagnosis of diabetes is confirmed [Grade D, Consensus].
- Prediabetes (defined as a state which places individuals at high risk of developing diabetes and its complications) is diagnosed by any of the following criteria:
  - IFG (FPG 6.1–6.9 mmol/L) [Grade A, Level 1 (23)]
  - IGT (2hPG in a 75 g OGTT 7.8-11.0 mmol/L) [Grade A, Level 1 (23)]
  - A1C 6.0%—6.4% (for use in adults in the absence of factors that affect the accuracy of A1C and not for use in suspected type 1 diabetes) [Grade B, Level 2 (26)].

#### Abbreviations:

2hPG, 2-hour plasma glucose; A1C, glycated hemoglobin; FPG, fasting plasma glucose; IFG, impaired fasting glucose; KGT, impaired glucose tolerance; OGTT, oral glucose tolerance test; PG, plasma glucose.



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### LIPID PROFILE

Lipid profile or lipid panel, is a panel of blood tests that serves as an initial broad medical screening tool for abnormalities in lipids, such as cholesterol and triglycerides. The results of this test can identify certain genetic diseases and can determine approximate risks for cardiovascular disease, certain forms of pancreatitis, and other diseases.

### Components

The lipid profile typically includes:

- □ Low-density lipoprotein
- □ High-density lipoprotein
- □ Triglycerides
- □ Total cholesterol

Using these values, a laboratory may also calculate:

- □ Very low-density lipoprotein
- □ Cholesterol:HDL ratio

### Procedure

Traditionally, most laboratories have required patients to fast for 9–12 hours before screening. However, recent studies have questioned the utility of fasting before lipid panels, and some diagnostic labs now routinely accept non-fasting samples.

### Implications

This test is used to identify hyperlipidemia (various disturbances of cholesterol and triglyceride levels), many forms of which are recognized risk factors for cardiovascular disease and rarely pancreatitis. A total cholesterol reading can be used to assess an individual's risk for heart disease; however, it should not be relied upon as the only indicator. The individual components that make up total cholesterol reading LDL, HDL, and VLDL are also important in measuring risk.

The lipid profile includes total cholesterol, HDL-cholesterol (often called good cholesterol), LDL-cholesterol (often called bad cholesterol), and triglycerides. Sometimes the report will include additional calculated values such as the Cholesterol/HDL ratio or a risk score based on lipid profile results, age, sex, and other risk factors. The lipid profile is used to guide

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providers in deciding how a person at risk should be treated. The results of the lipid profile are considered along with other known risk factors of heart disease to develop a plan of treatment and follow-up.

#### Normal range

LDL	:	60-130 mg/dL
HDL	:	> 40  mg/dL
Total cholesterol	:	< 200  mg/dL
Triglycerides	:	10–150 mg/dL
VLDL	:	2–38 mg/dL

#### Clinical Significance of lipoprotein metabolism

Fatty Liver

□is an abnormal accumulation of certain fats (triglycerides) inside liver cells.

□Hepatic triacylglycerol synthesis provides the immediate stimulus for the formation and secretion of VLDL.

□ Impaired VLDL formation or secretion leads to nonmobilization of lipid components from the liver, results in fatty liver.

Fatty livers fall into two main categories

A)More synthesis of Triglycerides

 $\theta$ High carbohydrate diet

θHigh fat feeding θStarvation

θDiabetes mellitus High carbohydrate diet stimulates de novo fatty acid synthesis by providing excess of Acetyl CoA and high fat feeding provides more flux of fatty acids from the diet that can be esterifies to provide excess triglycerides

B) Defective VLDL synthesis –

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The second type of fatty liver is usually due to a metabolic block in the production of plasma lipoproteins, thus allowing triacylglycerol to accumulate. The lesion may be due to -(1)A block in apolipoproteins synthesis a) Protein energy Malnutrition b) Impaired absorption c) Presence of inhibitors of endogenous protein synthesis e.g.- Carbon tetra chloride, Puromycin, Ethionine , Heavy metals etc. d) Hypobetalipoproteinemia- Defective apo B gene can cause impaired synthesis of apo B protein.

(2) A failure in provision of phospholipids that are found in lipoproteins a)A deficiency of choline, a lipotropic factor can cause impaired formation of phosphatidyl choline (Lecithin),a glycerophospholipid. b)Methionine deficiency can also cause impaired choline synthesis c)Inositol deficiency d)Deficiency of essential fatty acids can also cause impaired PL synthesis.

(3) Impaired Glycosylation- Orotic acid also causes fatty liver; it interferes with glycosylation of the lipoprotein, thus inhibiting release, and may also impair the recruitment of triacylglycerol to the particles. In conditions of orotic aciduria(disorder of pyrimidine nucleotide biosynthesis), fatty liver can be observed.

4) Impaired secretion of VLDL- oxidative stress is a common cause for membrane disruption of lipoproteins.

2) Alcoholic fatty liver

θAlcoholism leads to fat accumulation in the liver, hyperlipidemia, and ultimately cirrhosis.

 $\theta$  The fatty liver is caused by a combination of impaired fatty acid oxidation and increased lipogenesis, which is thought to be due to changes in the [NADH]/ [NAD+] redox potential in the liver,

 $\theta$  and also to interference with the action of transcription factors regulating the expression of the enzymes involved in the pathways.

Lipotropic agents- Agents such as-  $\theta$  Choline  $\theta$ Inositol  $\theta$  Methionine and other essential amino acids,  $\theta$  Essential fatty acids,  $\theta$ Anti oxidant vitamins,  $\theta$  Vitamin B12, folic acid and  $\theta$  Synthetic antioxidants which have the apparent effect of removal of fats from the liver cells, and thus prevent the formation of fatty liver are called lipotropic agents.

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Primary Disorders of Plasma Lipoproteins (Dyslipoproteinemias)

CLASS: III BSC BC

Olnherited defects in lipoprotein metabolism lead to the primary condition of either hypo- or hyperlipoproteinemia.

 $\theta$  In addition, diseases such as diabetes mellitus, hypothyroidism, nephrotic syndrome, and atherosclerosis are associated with secondary abnormal lipoprotein patterns that are very similar to one or another of the primary inherited conditions.

 $\theta$  All of the primary conditions are due to a defect at a stage in lipoprotein formation, transport, or degradation.

# Primary Disorders of Plasma Lipoproteins (Dyslipoproteinemias)

Name
------

Defect

Characteristics

Hyperlipoproteinemia		
Familial lipoprotein lipase deficiency (type I)	Hypertriacylglycerolemia due to deficiency of LPL, abnormal LPL, or apo C- II deficiency causing inactive LPL.	Slow clearance of chylomicrons and VLDL. Low levels of LDL and HDL. No increased risk of coronary disease.
Familial hypercholesterolemia (type II a)	Defective LDL receptors or mutation in ligand region of apo B-100.	Elevated LDL levels and hypercholesterolemia, resulting in atherosclerosis and coronary disease.

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#### **POSSIBLE QUESTIONS**

#### UNIT-IV

#### PART-A (20 MARKS)

#### (Q.NO 1 TO 20 Online Examination)

### PART-B (2 MARKS)

1. Give a note on VD Bergh Reaction.

- 2. Define Inulin clearance test.
- 3. Write about the condition of Glycosuria.
- 4. What is meant by Clearance Test?
- 5. Write short note on Lipid profile.

#### PART-C (8 MARKS)

- 1. Describe in detail on the Lipid profile.
- 2. Write briefly on various laboratory tests of Blood glucose.
- 3. Explain in detail about the classification of lipoproteins.
- 4. Describe about the regulations of blood sugar.
- 5. Write the basic defects and consequences of diabetes mellitus.
- 6. Describe about the composition and functions of lipoproteins.

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## KARPAGAM ACADEMY OF HIGHER EDUCATION

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UNIT: IV BATCH-2016-2019

COURSE NAME: CLINICAL BIOCHEMISTRY

#### UNIT-IV

#### SYLLABUS

Liver function tests - Serum enzymes in liver disease- Serum transaminases (SGOT and SGPT), and phosphatases.

**Renal function tests** - Introduction, clinical significance of GGT, LDH and creatine phosphokinase in kidney function.

Urine analysis - Physical examination of urine

#### LIVER FUNCTION TESTS

#### Definition

Liver function tests, or LFTs, include tests that are routinely measured in all clinical laboratories. LFTs include bilirubin, a compound formed by the breakdown of hemoglobin; ammonia, a breakdown product of protein that is normally converted into urea by the liver before being excreted by the kidneys; proteins that are made by the liver including total protein, albumin, prothrombin, and fibrinogen; cholesterol and triglycerides, which are made and excreted via the liver; and the enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), and lactate dehydrogenase (LDH).

Other liver function tests include serological tests (to demonstrate antibodies) and DNA tests for hepatitis and other viruses; and tests for antimitochondrial and smooth muscle antibodies, transthyretin (prealbumin), protein electrophoresis, bile acids, alpha-fetoprotein, and a constellation of other enzymes that help differentiate necrotic (characterized by death of tissues) versus obstructive liver disease.

The hepatic function panel evaluates: Alanine aminotransferase (ALT); Alkaline phosphatase (ALP); Aspartate aminotransferase (AST)

#### Total bilirubin and direct bilirubin

Bilirubin is a byproduct of the normal breakdown of red blood cells. It usually passes through the liver and is excreted from the body. But if that doesn't happen due to a liver disease, bilirubin levels in the blood can rise and the skin can take on the yellow discoloration known as jaundice. Tests for bilirubin may be total (measuring the level of all of the bilirubin in the



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blood) or direct (measuring only bilirubin that has been processed by the liver and attached to other chemicals).

**Albumin and total protein.** Protein is needed to build and maintain muscles, bones, blood, and organ tissue. Sometimes when there's a problem with the liver, it can't make proteins as well, so protein levels decrease. Liver function tests measure albumin specifically (the major blood protein produced by the liver), as well as the total amount of all proteins in the blood.

#### Normal results

Reference ranges vary from laboratory to laboratory and also depend upon the method used. However, normal values are generally framed by the ranges shown below. Values for enzymes are based upon measurement at 37°C.

**ALT**: 5–35 IU/L. (Values for the elderly may be slightly higher, and values also may be higher in men and in African-Americans).

**AST**: 0–35 IU/L

ALP: 30-120 IU/LALP is higher in children, older adults and pregnant females

GGT: males 2–30 U/L; females 1–24 U/L

LDH: 12-60 years: 100-190 U/L

**Bilirubin**: (Adult, elderly, and child)

Total bilirubin: 0.1–1.0 mg/dL

Indirect bilirubin: 0.2–0.8 mg/dL

Direct bilirubin: 0.0–0.3 mg/dL. (Newborn)

Note: Critical values for adult: greater than 1.2 mg/dL

Critical values for newborn (requiring immediate treatment): greater than 15 mg/ dL

**Ammonia**: 10–70 micrograms per dL (heparinized plasma). Normal values for this test vary widely, depending upon the age of the patient and the type of specimen.

**Albumin**: 3.2–5.4 g/L

#### **RENAL FUNCTION TEST**

Kidney function tests are a collective term for a variety of individual tests and procedures that can be done to evaluate how well the kidneys are functioning. A doctor who orders kidney



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function tests and uses the results to assess the functioning of the kidneys is called a nephrologist.

#### Laboratory tests

There are a number of urine tests that can be used to assess kidney function. A simple, inexpensive screening test a routine urinalysis is often the first test conducted if kidney problems are suspected. A small, randomly collected urine sample is examined physically for things like color, odor, appearance, and concentration (specific gravity); chemically, for substances such a protein, glucose, and pH (acidity/alkalinity); and microscopically for the presence of cellular elements (red blood cells [RBCs], white blood cells [WBCs], and epithelial cells), bacteria, crystals, and casts (structures formed by the deposit of protein, cells, and other substances in the kidneys's tubules). If results indicate a possibility of disease or impaired kidney function, one or more of the following additional tests is usually performed to pinpoint the cause and the level of decline in kidney function.

#### **Creatinine clearance test**

This test evaluates how efficiently the kidneys clear a substance called creatinine from the blood. Creatinine, a waste product of muscle energy metabolism, is produced at a constant rate that is proportional to the individual's muscle mass. Because the body does not recycle it, all creatinine filtered by the kidneys in a given amount of time is excreted in the urine, making creatinine clearance a very specific measurement of kidney function. The test is performed on a timed urine specimen - a cumulative sample collected over a two to 24-hour period. Determination of the blood creatinine level is also required to calculate the urine clearance.

#### Urea clearance test

Urea is a waste product that is created by protein metabolism and excreted in the urine. The urea clearance test requires a blood sample to measure the amount of urea in the bloodstream and two urine specimens, collected one hour apart, to determine the amount of urea that is filtered, or cleared, by the kidneys into the urine.

#### Urine osmolality test

Urine osmolality is a measurement of the number of dissolved particles in urine. It is a more precise measurement than specific gravity for evaluating the ability of the kidneys to concentrate or dilute the urine. Kidneys that are functioning normally will excrete more water into the urine as fluid intake is increased, diluting the urine. If fluid intake is decreased, the kidneys excrete less water and the urine becomes more concentrated. The test may be done on a urine sample collected first thing in the morning, on multiple timed samples, or on a cumulative

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sample collected over a 24-hour period. The patient will typically be prescribed a high-protein diet for several days before the test and be asked to drink no fluids the night before the test.

#### Urine protein test

Healthy kidneys filter all proteins from the bloodstream and then reabsorb them, allowing no protein, or only slight amounts of protein, into the urine. The persistent presence of significant amounts of protein in the urine, then, is an important indicator of kidney disease. A positive screening test for protein (included in a routine urinalysis ) on a random urine sample is usually followed up with a test on a 24-hour urine sample that more precisely measures the quantity of protein. There are also several blood tests that can aid in evaluating kidney function.

These include:

### **Creatinine test**

This test measures blood levels of creatinine, a by-product of muscle energy metabolism that, similar to urea, is filtered from the blood by the kidneys and excreted into the urine. Production of creatinine depends on an person's muscle mass, which usually fluctuates very little. With normal kidney function, then, the amount of creatinine in the blood remains relatively constant and normal. For this reason, and because creatinine is affected very little by liver function, an elevated blood creatinine level is a more sensitive indicator of impaired kidney function than the BUN.

#### Other blood tests

Measurement of the blood levels of other elements regulated in part by the kidneys can also be useful in evaluating kidney function. These include sodium, potassium, chloride, bicarbonate, calcium, magnesium, phosphorus, protein, uric acid, and glucose.

### Results

Normal values for many tests are determined by the patient's age and gender. Reference values can also vary by laboratory, but are generally within the following ranges:

### Urine tests

**Creatinine clearance**. For a 24-hour urine collection, normal results are 90 mL/ min–139 mL/min for adult males younger than 40, and 80–125 mL/min for adult females younger than 40. For people over 40, values decrease by 6.5 mL/min for each decade of life.

**Urine osmolality**. With restricted fluid intake (concentration testing), osmolality should be greater than 800 mOsm/kg of water. With increased fluid intake (dilution testing), osmolality should be less than 100 mOsm/kg in at least one of the specimens collected. A 24-hour urine osmolality should average 300–900 mosm/kg. A random urine osmolality should average 500–800 mOsm/kg.

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Urine protein. A 24-hour urine collection should contain not more than 150 mg of protein.

Urine sodium. A 24-hour urine sodium should be within 75–200 mmol/day.

**Blood tests** 

Blood urea nitrogen (BUN) should average 8-20 mg/dL.

Creatinine should be 0.8–1.2 mg/dL for males, and 0.6–0.9 mg/dL for females.

Uric acid levels for males should be 3.5–7.2 mg/dL and for females 2.6–6.0 mg/ dL.

Low clearance values for creatinine indicate a diminished ability of the kidneys to filter waste products from the blood and excrete them in the urine. As clearance levels decrease, blood levels of creatinine, urea, and uric acid increase. Because it can be affected by other factors, an elevated BUN, alone, is suggestive, but not diagnostic for kidney dysfunction The inability of the kidneys to concentrate the urine in response to restricted fluid intake, or to dilute the urine in response to increased fluid intake during osmolality testing, may indicate decreased kidney function. Because the kidneys normally excrete almost no protein in the urine, its persistent presence, in amounts that exceed the normal 24-hour urine value, usually indicates some type of kidney disease.

#### LIVER ENZYMES

Liver enzymes along with bilirubin are the most commonly measured parameter measured in the liver function test. These enzymes are hepatic in origin and they are leaked into the serum with the destruction of hepatic cells. Liver enzymes are measured to get an idea of the cellular insult on the liver and are increased in a wide variety of conditions such as viral hepatitis, toxic hepatitis, cirrhosis of liver etc.

The commonly measured enzymes are:

- (a) Transaminases: AST (SGOT), ALT (SGPT)
- (b) Transpeptidases: GGT
- (c) Phosphatase: ALP.

(a) **Transaminases:** They are a group of enzymes that transfer the amino group from an amino acid to  $\alpha$  keto acid converting the  $\alpha$  keto acid into an amino acid while converting the amino acid into a keto acid. The transaminases that are measured in the liver function test are ALT and

AST. Alanine transaminase (ALT) catalyses the following reaction:

Alanine +  $\alpha$  keto Glutarate -A-LT---> Pyruvate + Glutamate

Aspartate transaminase (AST) catalyses the following reaction:

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Aspartate +  $\alpha$  keto Glutarate -A-ST- $\rightarrow$  Oxaloacetate + Glutamate

 $\Box$  The normal level of ALT in serum is 7 to 40 IU/L.

 $\Box$  The normal level of AST in serum is 8 to 40 IU/L.

An increase in AST or ALT levels hints at an insult to the liver parenchyma tissue. ALT is a more specific marker of hepatic injury than AST as AST elevation is also seen in cardiac tissue injury, haemolysis and muscle tissue. To measure the level of transaminases the reaction catalysed by them is coupled to a reaction in which NADH is used up resulting in change in the

photometric intensity when read in the UV range at 340 nm. It is a UV kinetic method.

#### For ALT (SGPT):

Alanine +  $\alpha$  Keto glutarate -A-LT---> Pyruvate + Glutamate

Pyruvate + NADH + H+ LDH

(Lactate dehydrogenase)  $\longrightarrow$  Lactate + NAD+

#### For AST (SGOT):

Aspartate +  $\alpha$  Keto glutarate -A-ST- $\rightarrow$  Oxaloacetate + Glutamate

Oxaloacetate + NADH + H+ MDH

(Malate dehydrogenase)  $\longrightarrow$  Malate + NAD+

(b) Alkaline Phosphatase: It is a hydrolase that removes phosphates from all kinds of molecules such as proteins, nucleotides etc. It is found in cells lining the billiary system hence a rise in it level is indicative of damage to the billiary tree due to cholestasis. It maybe due to stone blocking the large ducts or intrahepatic obstruction, inflammation of the billiary channels. Alkailine phosphatase is also found in placenta and bones. Hence the level is also increased in growing children in whom bones undergo remodeling and in Paget's disease in adults. Normal level of alkaline phosphatase is between 45 to 115 IU/L. The method for measuring the level of alkaline phosphatase is a kinetic method using p-nitrophenylphosphate as substrate for the enzyme and measuring rate of formation of the colored substrate (p- nitrophenol) formed from the reaction. This measurement of the color intensity is done colorimetrically at a wavelength of 405 nm.

p-Nitrophenylphosphate + H2O  $-A-LP \rightarrow p$ - Nitrophenol + Phosphate

(c) Gamma glutamyl transpeptidase: It is another enzyme specific to the biliary tree and a more specific indicator of cholestasis and damage to the biliary tree. It is also a highly specific marker and is raised in even minute and subclinical damage to the biliary tree. Its normal range is in between 0 to 42 IU/1.

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#### **KIDNEY FUNCTION TEST**

#### **16.1 INTRODUCTION**

The main function of the kidney is excretion of water soluble waste products from our body. The kidney has various filtration, excretion and secretary functions. Derangement of any of these function would result in either decreased excretion of waste products and hence their accumulation in the body or loss of some vital nutrient from the body. Based on the level of these excretory products and nutrients in the urine as well as in blood we can make an accurate calculation to decipher the efficient of the kidney to undertake its various functions.

#### **OBJECTIVES**

After reading this lesson, you will be able to:

□ explain the importance of kidney function test.

 $\Box$  describe the types of lesions detected by the renal function tests.

 $\Box$  describe the various components of the kidney function test.

 $\Box$  explain the importance of various components of the kidney function test.

#### **16.2 THE FUNCTIONAL COMPONENTS OF A KIDNEY**

The functional unit of the kidney is called a nephron. It consists of two main parts, the glomerulus and the tubular system. The glomerulus is composed of a bowman's capsule and a tuft of leaky blood vessels encapsulated by the bowman's capsule. The primary purpose of the glomerulus is filtrate ions. The leaky vessels filter into the glomerulus almost all the water, electrolytes, small proteins, nutrients such as sugar etc and excretory products such as urea etc. The filtrations is dependent on the size and charge of the particles. The average pore size is 8 nm hence particles of only smaller size will pass through. Also the basement membrane carries a negative charge hence preventing negatively charged particles from passing through. The Tubular system is responsible for re absorption of most of the water, electrolytes, nutrients as well as excretion of the remaining nutrients by means of secretion into the tubules. These tubules are responsible for the concentration of urine.



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#### **16.3 COMPONENTS OF KIDNEY FUNCTION TEST**

The components of the Kidney function test can be broadly divided into two categories.



The tests that are part of the Kidney Function test panel are:

- (a) Urine examination
- (b) Serum Urea
- (c) Serum creatinine
- (d) Blood urea nitrogen (BUN)
- (e) Calcium
- (f) Phosphorus
- (g) Protein

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#### (h) Albumin

- (i) Creatinine clearance
- (j) Urea clearance
- (k) Inulin clearance
- (l) Dilution and Concentration test
- (l) Serum electrolyte levels

### URINE EXAMINATION

Before we do a quantitative examination of urine a qualitative examination is necessary as it can provide excellent clues to the nature and location of the lesion in the renal system. This examination consists of a physical examination where the colour, odour, quantity, specifc gravity etc of the urine is noted. Microscopic examination of urine is done to rule out any pus cells, Rbc casts, Crystals.

### SERUM UREA

Urea is the end product of protein catabolism. The urea is produced from the amino group of the amino acids and is produced in the liver by means of the Urea cycle. Urea undergoes filtrations at the glomerulus as well as secretion and reabsorption at the tubular level. The rise in the level of serum urea is generally seen as a marker of renal dysfunction specially glomerular dysfunction. Urea level only rises when the glomerular function is reduced below 50%. he normal serum urea level is between 20-45 mg/dl. But the level may also be affected by diet as well as certain non kidney related disorders. A high protein diet may increase the blood urea level. Similarly a low protein diet may decrease blood urea level. Other causes of protein catabolism such as any hyper metabolic conditions, starvation etc also cause increased blood urea levels. Similarly the level of urea may also be decreased in case of hepatic injury.

So even though blood urea is not an excellent marker of renal dysfunction as it rises quite late in the dysfunction and its rise is also not exclusive to kidney dysfunction, but for practical purposes serum urea level is still one of the most ordered test and forms an important part of the kidney function test. Urea is measured in diagnostic labs either by UV kinetic method using á keto glutarate as an NH3+ acceptor in presence of enzyme glutamate dehydrogenase. It is also measured calorimetrically by Berthelot's end point method and is read in visible range using a calorimeter.

### **BLOOD UREA NITROGEN (BUN)**

Sometimes the Serum urea level is expressed as blood urea nitrogen. BUN can be easily



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calculated from the serum urea level. The molecular weight of urea is 60 and it contains two nitrogen atoms of combined atomic weight of 28. Hence the contribution of nitrogen to the total weight of urea in serum is 28/60 that is equal to 0.47. Hence the serum urea levels can be easily converted to BUN by multiplying it by 0.47. A rise in blood nitrogen level is known as azotemia.

### CALCIUM

This test measures the amount of Calcium in your blood, not the calcium in your bones. The body needs it to build and fix bones and teeth, help nerves work, make muscles contraction, help blood clot, and help the heart to work. The Calcium test screens for problems with the parathyroid glands or kidneys, certain types of cancers and bone problems, inflammation of the pancreas (pancreatitis), and kidney stones. Normal Results: 8.5 to 10.2 mg/dl

### PHOSPHORUS

Phosphorus is a mineral that makes up 1% of a person's total body weight. The body needs phosphorus to build and repair bones and teeth, help nerves function, and make muscles contract. The Kidneys help control the amount of phosphate in the blood. Extra phosphate is filtered by the kidneys and passes out of the body in the urine. It plays an important role in the body's utilization of carbohydrates and fats and in the synthesis of protein for the growth, maintenance, and repair of cells and tissues. High levels of phosphorus in blood only occur in people with severe kidney disease or severe dysfunction of their calcium regulation. Excessively high levels of phosphorus in the blood, although rare, can combine with calcium to

form deposits in soft tissues such as muscle. Normal Results: Standard range not available

### PROTEIN

Protein in urine is noticeably increased in renal disease of any etiology, except obstruction, and is therefore a very sensitive, general screening test for renal disease, though not specific. The extent of proteinuria also provides useful information. The greatest degree of proteinuria is found in the nephrotic syndrome (> 3 - 4 g/day). In renal disease with the nephritic syndrome, the urinary protein excretion rate is usually about 1 - 2 g/day. In tubulo-interstitial disease, urine protein is generally less than 1 g/day. Only in the nephrotic syndrome is the urine protein loss sufficiently great to result in hypoproteinemia. **Protein** in serum can generally be maintained at concentrations above the lower limit of normal by increased hepatic protein synthesis so long as protein loss is less than about 3 g/day

### SERUM CREATININE LEVEL

Creatine is a small tripeptide found in the muscles. It stays in its phosphorylated form and releases energy for any burst of muscular activity. It is released from the muscles during regular wear and tear and is converted to creatinine (its internal anhydride). It is to be remembered that

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unlike urea, creatinine is not a toxic waste. It is simply used as a marker of renal function. Creatinine is freely filtered at the glomerulus and is also to a very small extent secreted into the tubules. So any problem with gromerular filtrations has a significant effect on the excretion of creatinine resulting in a much substantial rise in serum creatinine level.

Normal serum creatinine level is 0.6 to 1.5 mg/dl. Serum creatinine is a better indicator of renal function and more specifically glomerular function than urea. For a particular individual the creatinine level is dependent on the muscle mass and muscle wear and tear. There may be significant difference in creatinine level of individuals with vastly differing muscle mass. For example a body builder or athlete will have higher creatinine levels than a sedentary desk worker. Similarly creatinine level will also increase in case of any muscle trauma or excessive wear and tear as seems in athletes and people involved in hard physical labor. Creatinine is most commonly measured in laboratories calorimetrically by Jaffe's method.

### UREA CLEARANCE

Urea clearance is the hypothetical amount of blood from which kidney clears urea in one minute. This is measured by measuring the concentration of urea in blood, concentration of urea in urine and amount of urine excreted over a one hour interval. Urea clearance is less than its glomerular filtration as some of the urea that is filtered at the glomerulus is reabsorbed at the tubules. To measure urea clearance first the patient is made to void urine and then the made to drink two glasses of water. Then the urine is collected after an hour and a blood specimen is also collected at the same time. Then the patients urine sample is collected after another hour. The urea level in the two urine samples and the blood sample is measured. The urine volume is calculated as urine output per minute.

If the urine output is more than 2 ml/minute then urea clearance (in ml/ minute) is measured as:

If urine output is less than 2 ml/minute then urea clearance (in ml/min) is measured as:

(Urine urea conc. × √urine volume ml/min) Urea conc. in serum

Maximum urea clearance of an average individual or body surface area of 1.73 sq m is 75 ml/ min and a standard urea clearance is 54 ml/min. A urea clearance below 60% of standard is considered impaired.

#### **CREATININE CLEARANCE RATE**

Creatinine is filtered at the glomerulus and its reabsorption at the tubular level is insignificant.

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Because of this creatinine clearance can be used to measure Glomerular Filtration Rate (GFR). It is measured over a period of 24 hrs. For this urine is collected over a 24 hour period and blood sample is also collected. The concentration of creatinine is measured both in the urine as well as the serum sample. Creatinine clearance is measured by the following method:

(Conc. of creatinine in urine × volume of urine) Conc. of creatinine in serum.

The normal range of creatinine clearance is:

Males : 100 – 120 ml/ min

Females : 95 - 105 ml/min

This is very close to the glomerular filtration rate.

### **16.13 INULIN CLEARANCE**

Inulin is a small polysaccharide of low molecular weight made up of fructose. To measure glomerular filtrate the substance used should have the following qualities:

- (a) It should be non toxic.
- (b) Should not be metabolized in the body.
- (c) Should be completely filtered at the glomerulus.
- (d) Should neither be secreted or reabsorbed at the tubules.

Inulin meets all these criteria and hence makes for a suitable candidate to measure GFR. Inulin clearance hence equals to GFR. GFR is the amount of blood that passes though and is filtered through the glomerulus in a minute. To measure Inulin clearance first Inulin is introduced in the blood by means of a slow continuous infusion to maintain a steady conc. of Inulin in the blood. This is done by first infusing 30 ml of 10% inulin in 250 ml of normal saline infused at a rate of 20 ml/ min to achieve desired concentration. Then 70 ml of 10% inulin in 500 ml saline in infused at a rate of 4 ml/ min to maintain the desired concentration.

The patient is asked to micturate 20 minutes after the second infusion and the urine in discarded and the time noted. After exactly 60 minutes, take another sample of urine and blood is collected. Measure the volume of urine and the conc. of inulin in both the serum and urine.

## (Conc. of Inulin in urine × volume of Inulin) Conc. of Inulin in serum

Thereafter the inulin clearance is measured by the formulae:

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Normal inulin clearance is 120 to 130 ml/minute for an average person with a body surface area of 1.73 sq m. This is a close approximation of the GFR. A below normal inulin clearance shows an impaired glomerular function.

### **CONCENTRATION TEST**

In case of water shortage in the body the kidney is able to concentrate urine and conserve water. This is done by increasing the reabsoption of water from the glomerular filtrate at the tubular level. So in effect the measure of the ability of the kidney to conserve water and concentrate urine is a measure of tubular function.

For this test the patient is not allowed to take any food or water after the evening meal. The first three urine samples passed in the morning are collected and their specific gravity measured. In a normal person the specific gravity of atleast one of the samples should be above 1.025 or above. If the specific gravity remains below 1.025 then it is a sign of tubular dysfunction.

### **DILUTION TEST**

Like the concentration test the dilution test is also a measure of functioning of the tubules. In cases of fluid overload of our body the tubules reabsorb lesser amounts of water resulting in excretion of diluted urine. For this test the subject is put on overnight fast and then in the morning the subject is made to drink 1200 ml of water over a time period of 30 minutes. Then

the urine samples are collected every hour for 4 hours. The specific gravity of the samples is measured and atleast one of the samples should have a specific gravity of 1.003 or less. If none of the samples have the specific gravity of 1.003 or less this is a sign of tubular dysfunction.

### ELECTROLYTES

The purpose of the kidney is not just water balance and excretion but also to maintain the electrolyte balance of our body. Kidneys actively reabsorb or excrete electrolytes to maintain the electrolyte balance of the body. Owing to their small size almost all electrolytes are filtered at the glomerulus. After filtration most of the electrolytes are absorbed back at the tubular level but any problem at the tubular level will result in non absorption and excessive loss of electrolytes in urine.

Serum electrolytes that are measured for this purpose are:

Serum Sodium levels (Na+) : 135 to 145 mmols/liter

Serum Pottasium level (K+) : 3.5 to 5 mmols/liter

Serum Chloride level (Cl- ) : 95 to 105 mmols/liter



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## POSSIBLE QUESTIONS

### UNIT-IV

### PART-A (20 MARKS)

### (Q.NO 1 TO 20 Online Examination)

### PART-B (2 MARKS)

- 1. What are isoenzymes. Add a note on its clinical significance.
- 2. Give a note on VD Bergh Reaction.
- 3. Write a detail note on safe handling of radioisotopes.
- 4. Write a note on Serum ornithine carbamoyl transferase (OCT).
- 5. Define prothrombin index.

## PART-C (8 MARKS)

- 1. Comment on the role of Isoenzymes in liver diseases.
- 2. Write a note on the clinical significance of SGOT and SGPT.
- 3. Discuss in detail the biosafety methods in radioactive laboratory.
- 4. Comment on the role of serum enzymes in liver diseases.
- 5. Write about the chemical examination of urine samples
- 6. Describe the clinical significance of urinary components.

Questions	ont1	ont?	ont3	opt4	ont5	ont6	Answer
who was the first to study concentation	opti	0002	0013	оріч	opts		
of urea in blood and its excretion in urine	Louis pasteur	Ambard	Wilson	Edward			Ambard
If the uree volume exceeds $2m/mt$ , the	Louis pasteur	Anibard	vv 115011	Luwalu			Alloalu
in the drea volume exceeds 2111/111, the	minimum	n o mno o l	mowingsyng	standy			movimum
	IIIIIIIIIIIIII	normai	maximum	steady			maximum
volume of blood cleared of urea per							
minute can be calculated by the							
following formula	CX V/A	AXV/B	CXV/B	UxV/B			UxV/B
The clearance which occurs when the							
urinary volume exceeds 2ml/mt is termed	maximum urea	minimum urea	standard urea				maximum urea
as	clearance	clearance	clearance	all the above			clearance
The average normal value for standard							
urea clearance is	54 ml	64 ml	44 ml	33 ml			54 ml
The average normal value for maximum							
urea clearance is	75 ml	85 ml	65 ml	40 ml			75 ml
	surface area of	surface area of	surface area	surface area of			
The urea clearance is proportional to the	head	body	of neck	lungs			surface area of body
urea clearance of % indicates							
normal excretion of kidneys	60	80	70	20			70
values of urea clearance between 20 -		severe	moderate				moderate
40% indicates	mild impairment	impairment	impairment	all the above			impairment
urea clearance values between							
indicate mild impairment of excretion of							
kidneys	40 -70%	20 - 40%	50-60%	20 - 30 %			40 -70%
normal values for creatine clearance							
varies from	95-105 ml/mt	100 -105 ml/mt	10 -150 ml/mt	5-60 ml/mt			95-105 ml/mt
Endogenous creatinine is a			normal				
of body	substrate	product	metabolite	all the above			normal metabolite

creatinine is neither secreted nor				Excretory	
reabsorbed by the tubules. So its			glomerular	function of	glomerular
clearance gives	Renal function	liver function	filteration rate	kidnev	filteration rate
nations with mild renal disease are		moderate	low protein	lituitey	
recommended to take	high protein diet	protein diet	diet	High fat diet	low protein diet
In terminal uraemia, the urea clearance	ingii protein diet		alet	ingh fut thet	
fallsto about of the normal					
values	5%	10%	15%	11%	1%
Crystals of ammonium magnesium	570	1070	1570	Acid and	 170
phosphate found in	acid urine	alkaline urine	neutral urine	alkaline urine	alkaline urine
phosphate crystals of urine deposits are					
in form	amorphous	crystalline	colourless	powder	colourless
name the type of phosphate crystals				r · · · · ·	
which are much commonly seen in urine					
deposits	coffin lid type	feathery	fernlike	needle shape	coffin lid type
calcium hydrogen phosphate most often		<u> </u>	Rosettes and		Rosettes and star
found crystalline in the form of	rosettes	clusters	star shape	star shape	shape
Magnesium phosphate is found as			-	-	
in alkaline to weakly acid			rectangular		
urines	rhombic plates	diagonal shape	shape	needle shape	rhombic plates
Amorphous phosphates are found in the	-			-	-
form of	fine granules	crystals	paste	clusters	fine granules
crystals of uric acid are found frequently			slightly		
in the deposits from urines	alkaline	slightly acidic	alkaline	acid urines	acid urines
pure uric acid crystals are in					
nature	colored	colourless	red color	brown color	colourless
	inclusion of				inclusion of urinary
The pigment found in urine deposit	urinary pigments				pigments in the
containing uric acid crystals is due to the	in the crystals	bile pigments	skin pigments	UTI	crystals

		hydrochloric	sodium		
uric acid crystals dissolve in	acetic acid	acid	hydroxide	ethanol	sodium hydroxide
			After		
uric acid crystals are found in normal			sweating and		After sweating and
people	after sweating	fevers	fever	Cold	fever
				Ammonium,	
				sodium,	Ammonium,
				potassium,	sodium, potassium,
urates are found in	ammonium and		calcium and	calcium and	calcium and
urine deposits	sodium	potassium	magnesium	magnesium	magnesium
Obstructive jaundice is otherwise called	Regurgitation	Retardation	Hemolytic		Regurgitation
as	jaundice	jaundice	jaundice	Hepatic jaundice	jaundice
			Icterus and		Icterus and Jaune
The othername of jaundice is called as	Icterus	jaune - yellow	Jaune yellow	Albinism	yellow
	congenital non-		neo-natal		
crigler - Najjar syndrome type I is also	hemolytic	obstructive	physiologic		congenital non-
known as	jaundice	jaundice	jaundice	All the above	hemolytic jaundice
		UTP -	bilirubin	biliverdin	
crigler - Najjar syndrome type I is caused	udp-glucuronyl	glucuronyl	glucuronyl	glucuronyl	udp-glucuronyl
by the defect of the enzyme	transferase	transferase	transferase	transferase	transferase
The children affected with crigler -					
Najjar syndrome I die within the first					
of life	2	5	4	3	2
crigler najjar syndrome type II is due to	biliverdin	bilirubin	bilirubin		bilirubin
severe defect in the	conjugation	conjugation	diglucuronide	all the above	conjugation
Gilberts disease is a disease with		multiple	tripe		
combination of	single disorder	disorders	disorders	All the above	tripe disorders
crigler najjar syndrome the bilirubin					
concentration falls within	20 mg/dl	50 mg/dl	60 mg/dl	10 mg/dl	20 mg/dl

is the end product of					
purine metabolism in human	urea	creatine	orotic acid	uric acid	uric acid
The normal serum uric acid					
concentration is in the range of	3 - 7 mg/dl	9 - 10mg/dl	11 - 12 mg/dl	1- 2 mg/dl	3 - 7 mg/dl
Hyper uricemia refers to an elevation in					
the serum concentration	ammonia	urea	uric acid	creatinine	uric acid
The excretion of uric acid is otherwise				emotional	
called as	glycosuria	uricosuria	anemia	glycosuria	uricosuria
Deposits of uric acid in the joints is					
called as	uricosuria	goutyarthritis	tophi	arthritis	tophi
				High living,	
				over eating and	High living, over
Historically gout was found to be often			alcohol	alcohol	eating and alcohol
associated with	high living	over eating	consumption	consumption	consumption
			increase in		
primary gout is due to over	in born error of	impairment of	the synthesis		in born error of
production of uric acid	metabolism	kidneys	of uric acid	liver impairment	metabolism
			denovo		
			pathway of		
	purine salvage	pyrimidine	purine		purine salvage
HGPRT is an enzyme of	pathway	biosynthesis	synthesis	All the above	pathway
The end product of purine metabolism in					
humans is	xanthine	uric acid	urea	allantoin	uric acid
An enzyme of purine metabolism					
associated with immunodeficiency	adenosine		PRPP		adenosine
disease	deaminase	xanthine oxidase	synthetase	HGRPT	deaminase
the drug used for					
effective treatment for gout	ibuprofen	paracetamol	avil	amoxilin	ibuprofen
In crigler -Najjar syndrome the bilirubin					
concentration falls within	20 mg/dl	50mg/dl	100 mg/dl	10 mg/dl	20 mg/dl

gilberts syndrome is a					
group of diseases	homogenous	hetrogenous	mixed	single	hetrogenous
gilberts syndrome is associated with	uncojugated hyper bilirubinaemia	hypouricemia	hyper bilirubinaemi a	hyperuricemia	uncojugated hyper bilirubinaemia
		71	defect in	51	
	reduced	defect in hepatic	uptake of		
	glucuronyl	clearance of	bilirubin by		
The causes for gilberts syndrome include	transferase activity	bilirubin	liver cells	All the above	All the above
the bilirubin level in gilberts syndrome is	<3 mg /dl	<6mg/dl	< 4mg/dl	< 5mg/dl	<3 mg /dl
				Conjugated hyper bilirubinaemia, child hood iaundice and	Conjugated hyper bilirubinaemia, child hood jaundice
Dubin - johson syndrome is characterised	conjugated hyper	child hood	adult life	adult life	and adult life
by	bilirubinaemia	jaundice	jaundice	jaundice	jaundice
The symptoms of gilbert syndrome is	fatigue	weakness	abdominal pain	Fatigue, weakness and abdominal pain	Fatigue, weakness and abdominal pain
In gilberts disease there is defect in	unconjugated	conjugated	direct	uo uo ininini puni	
secretion of in bile	bilirubin	bilirubin	bilirubin	total bilirubin	conjugated bilirubin
Hemolysis is the one of the symptom of	gilberts syndrome	jaundice	Dubin - johnson syndrome	gout	gilberts syndrome
therapy has been found useful in curing crigler - NAjjar syndrome	Radiation	photo	chemo	Radiation and chemo	photo
Type II crigler - Najjar syndrome is a		hereditary	rare inherited	none of the	rare inherited
	Mutation	disorder	disorder	above	disorder

Bile of type II crigler - Najjar syndrome		bilirubin				
patients found to contain	bilirubin	monoglucuronid				bilirubin
	diglucuronide	e	biliverdin	bile pigments		monoglucuronide
Patients with crigler Najjar syndrome			phenobarbito			
repond to treatment with large doses of	aspirin	acetamide	ne	chloroform		phenobarbitone
Type II crigler Najjar syndrome is					NADP+	
characterised by in bilirubin			moderate			
conjugating system	chronic defect	severe defect	defect	mild effect		mild effect



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## <u>UNIT-V</u> SYLLABUS

**Tests for cardiovascular diseases** – ECG, Involvement of enzymes in diagnostics of heart disease including aspartate transaminase, isoenzymes of creatine kinase and lactate dehydrogenase and troponin.

Tumour markers for diagnosing various cancers.

Enzymes are catalysts that increase the rate or velocity of physiologic reactions. Each and every reaction in our body takes place with the help of an enzyme. In general, most enzymes are present in cells at much higher concentrations than in plasma. Measurement of their levels in plasma indicates whether their tissue of origin is damaged leading to the release of intracellular components into the blood. This forms the basis of clinical enzymology. Thus clinical enzymology refers to measurement of enzyme activity for the diagnosis and treatment of diseases.

Enzymes present in plasma can be classified into 2 types, they are

□ Functional Plasma enzymes and

□ Non-functional plasma enzymes

Functional plasma enzymes:

□ Present in plasma at higher concentration than tissues

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- □ They function in plasma.
- □ Mostly synthesized by the liver
- Usually decreased in disease conditions
- □ Eg. Clotting enzymes, lipoprotein lipase
- Non-functional plasma enzymes:
- □ Present in plasma at lower concentration than tissues
- □ Do not have any function in plasma
- □ Mostly synthesized by liver, skeletal muscle, heart, brain etc
- □ Usually increased in disease conditions
- □ Eg. Creatine kinase, Alanine transaminase etc
- □ Measurement of these enzymes in plasma can be used to assess cell damage and proliferation
- i.e. diagnosis of disease.

### Assessment of Cell Damage and Proliferation

Plasma enzyme activities can be used in the diagnosis of disease and prognosis of treatment. Plasma enzyme levels depend on balance between the rate of influx of active enzyme into the circulation and its eventual clearance from the blood. The rate of influx is determined by the rate of release from damaged cells and altered rate of enzyme synthesis.

### Localization of Damage

Enzymes used to measure tissue damage are present in nearly all cells with varying concentration. So the measurement may indicate an abnormality, but the specific diagnosis cannot be made. For example if there is circulatory failure after a cardiac arrest very high plasma

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levels of enzymes originating from many tissues may occur because of hypoxic damage to cells and reduced rates of clearance: the raised plasma levels of 'cardiac' enzymes do not necessarily mean that a myocardial infarct caused the arrest. The diagnostic precision of plasma enzyme analysis may be improved by

1. Estimation of more than one enzyme. Many enzymes are widely distributed, but their relative concentrations may vary in different tissues. For eg. Alanine and aspartate transaminases are abundant in the liver, the concentration of aspartate transaminase is much greater than that of alanine transaminase in heart muscle

2. Isoenzyme determination. Some enzymes exist in more than one form: these isoenzymes may be separated by their different physical or chemical properties. If they originate in different tissues such identification will give more information than the measurement of plasma total enzyme activity: for example, creatine kinase may be derived from skeletal or cardiac muscle, but one of its isoenzymes is found predominantly in the myocardium

3. Serial enzyme estimations. The rate of change of plasma enzyme activity is related to a balance between the rate of entry and the rate of removal from the circulation. A persistently raised plasma enzyme activity is suggestive of a chronic disorder or occasionally of impaired clearance. The distribution of enzymes within cells may differ. Alanine transaminase and lactate dehydrogenase are predominantly located in cytoplasm and glutamate dehydrogenase in mitochondria, whereas aspartate transaminase occurs in both these cellular compartments. Different disease processes in the same tissue may affect the cell in different ways, causing alteration in the relative plasma enzyme activities

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#### Isoenzymes

□ Isoenzymes (also known as isozymes) are enzymes that differ in amino acid sequence but

catalyze the same chemical reaction

- □ Believed to be originating from closely linked genes or from multiple gene loci
- □ Evolution from a single form possibly due to long-term mutations
- □ They vary with respect to their kinetic parameters, electrophoretic mobility, and localization
- □ They all have independent action
- Eg.Lactate dehydrogenase have 5 isoenzymes (LDH1, LDH2, LDH3, LDH4 & LDH5)
- □ They can be used to identify the specific affected tissues
- □ They can be differentiated from each other and can be clinically quantified in the lab

## **ENZYMES IN HEALTH AND DISEASES**

Estimation of enzymes activities in the serum has many applications in the diagnosis, differential diagnosis (e.g. in myocardial infarction both AST and LDH are increased in the serum but in case of pulmonary embolism AST is normal but LDH is increased), assessing prognosis of diseases, and early detection of disease (e.g. increase level of ALT in serum in viral hepatitis before the occurrence of jaundice). Some important enzymes of clinical significances are discussed below:

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## Distribution and application of clinically important enzymes

Enzymes	Tissues	Clinical applications
Alanineamino transferase	Liver	Hepato parenchymal diseases
Alkaline phosphatase	Liver, bone, intestinal mucosa, Placenta	Liver and bone diseases
Amylase	Salivary glands, Pancreas	Pancreatic diseases
Aspartate amino transferase	Liver, Skeletal muscle, Heart, Erythrocytes	Hepatic parenchymal disease, Muscle disease
Cholinesterase	Liver	Organophosphorus insecticide poisoning, Hepatic parenchymal diseases
Creatine kinase	Skeletal muscle,Heart	Muscle diseases
Gamma glutamyl transferase	Liver	Hepatobiliary diseases, Marker of alcohol abuse
Lipase	Pancreas	Pancreatic diseases
Lactate dehydrogenase	Heart, liver, skeletal muscle erythrocytes, lymph nodes, Platelets	Hepatic parenchymal diseases, muscle diseases Hemolysis, tumor marker
5'nucleotidase	Liver	Hepatobiliary diseases
Trypsin	Pancreas	Pancreatic diseases

#### **Pancreatic enzymes**

α-Amylase: (EC3.2.1.1; 1,4- a-D-glucan glucanohydrolase; AML) belongs to hydrolyase class that catalyzes the hydrolysis of 1,4- a-glycosidic linkages in polysaccharides. They are low

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molecular weight proteins (54 to 62 kDa) that can pass the glomeruli of the kidneys. It is the only plasma enzyme physiologically found in urine. The AMY activity present in normal serum and urine is of pancreatic (P-AMY) and salivary gland (S-AMY)origin.

## **Clinical Significance**

Normal values of amylase: 28-100 U/L = 0.48-1.7 i kat/L

## Causes of Raised Plasma Amylase Activity

- 1. Marked increase (five to 10 times the upper reference limit):
- □ Acute pancreatitis
- □ Severe glomerular impairment
- 2. Moderate increase (up to five times the upper reference limit):
- □ Perforated peptic ulcer
- □ Acute cholecystitis
- □ Intestinal obstruction
- □ Salivary gland disorders like mumps, salivary calculi

Lipase: (EC 3.1.1.3; triacylglycerol acylhydrolase; LPS) is a single -chain glycoprotein with

molecular weight of 48 kDa.

## **Clinical Significance**

Normal values: 40-200 U/L

□ Plasma lipase levels are elevated in acute pancreatitis and carcinoma of the pancreas.

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□ serum amylase is increased in mumps, pancreatic disease or due to some other cause, whereas lipase is increased only in pancreatitis. Therefore, the determination of both amylase and lipase together helps in the diagnosis of acute pancreatitis.

**Trypsin:** (EC 3.4.21.4; no systemic name; TRY) is a serine proteinase that hydrolyze the peptide bonds formed by the carboxyl groups of lysine arginine with other amino acids.

### **Clinical Significance**

Normal values of trypsin:  $25 \pm 5.3 \ \mu g/L$ . Increased in pancreatic disease. But as there is no distinct role of trypsin estimation in the routine management of patients with acute pancreatitis, this test is therefore considered of limited clinical value.

### Liver enzymes

The assay of serum enzymes is very useful for the differential diagnosis and monitoring of various heptobiliy disorders.

There are three types of enzymes:

1. Enzymes which are normally present inside the hepatocytes released into the blood when there is a hepatocellular damage markers of hepatocellular damage.

2. Enzymes which are primary membrane bound (plasma membrane or side of hepatocytes) = markers of cholestasis

3. Enzymes which are synthesized in the hepatocyte indicates disturbances in the hepatocellular synthesis.

### Markers of hepatocellular damage

### 1. Aminotransferases/Transaminases

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The transaminases are enzymes involved in the transfer of an amino group from a 2-amino- to a 2-oxoacid: they need the cofactor, pyridoxal phosphate for optimal activity. They are widely distributed in the body. The 2-oxoglutarate/L-glutamate couple serves as one amino group acceptor and donor pair in all amino-transfer reactions; the specificity of the individual enzymes derives from the particular amino acid that serves as the other donor of an amino group. Thus AST catalyzes the reaction:



ALT catalyzes the analogous reaction:



The reactions are reversible, but the equilibrium of AST and ALT reactions favor formation of aspartate and alanine respectively.

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□ In the liver, the concentration of ALT per unit weight of the tissue is more than AST.

□ AST and ALT enzymes are more important in assessing and monitoring the degree of liver cell

inflammation and necrosis.

□ Elevated plasma ALT are considered to be relatively specific for liver disease.

□ AST may be elevated in other forms of tissue damage, such as myocardial infarction, muscle necrosis and renal disorders.

□ In liver disease, the ALT level is increased markedly compared to AST. In acute viral hepatitis there is a 100-1000 times increase in both ALT and AST but ALT level is increased more than that of AST

(a) Aspartate Transaminase (EC 2.6.1.1; L-aspartate:2-oxoglutarate aminotransferase; AST)

### **Clinical Significance**

Normal values of AST: Male:  $\langle 35 \text{ U/L} = \langle 0.60 \text{ mkat/L} \rangle$ ; Female:  $\langle 31 \text{ U/L} = \langle 0.53 \text{ mkat/L} \rangle$ 

(b) Alanine Transaminase (EC 2.6.1.2; L-alanine:2-oxoglutarate aminotransferase; ALT)

### **Clinical Significance**

Normal values of ALT: Male: <45 U/L = <0.77 mkat/L ; Female: <34 U/L = <0.58 mkat/L

## Markers of cholestasis

I. Alkaline phosphatase (EC 3.1.3.1; orthophosphoric-monoester phosphohydrolase

[alkaline optimum]; ALP). Half-life= 10 days

## **Clinical Significance**

The alkaline phosphatases are a group of enzymes that hydrolyse organic phosphates at high pH.

They are present in most tissues but are in particularly high concentration in the osteoblasts of

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bone and the cells of the hepatobiliary tract, intestinal wall, renal tubules and placenta. The exact metabolic function of ALP is unknown but it is probably important for calcification of bone In adults plasma ALP is derived mainly from bone and liver in approximately equal proportions: the proportion due to the bone fraction is increased when there is increased osteoblastic activity that may be physiological

### Causes of raised Plasma ALP activity

- 1. Physiological: There is a gradual increase in the proportion of liver ALP with age: in the elderly the plasma bone isoenzyme activity may increase slightly.
- 2. Bone ailment: rickets and osteomalacia
- 3. Liver disease:
- 4. Malignancy bone or liver involvement or direct tumor production.

### Possible Causes of Low Plasma ALP Activity

- □ Arrested bone growth
- □ Hypophosphatasia: an autosomal recessive disorder, associated with rickets or osteomalacia.

### **Isoenzymes of Alkaline Phosphatase**

Bone disease with increased osteoblastic activity, or liver disease with involvement of the biliary tracts, are the commonest causes of an increased total alkaline phosphatase activity. The isoenzymes originating from cells of bone, liver, intestine and placenta may be separated by electrophoresis, but interpretation may be difficult if the total activity is only marginally raised.

Assays for ALP isoenzymes are needed when:

I. The source of an elevated ALP in serum is not obvious and should be clarified.

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II. The main clinical question is concerned with detecting the presence of liver or bone involvement

III. In the case of metabolic bone disorders, to ascertain any modifications in the activity of osteblastes to monitor the disease activity and the effect of appropriate therapies.

2. Gamma-glutamyl-transferase (EC 2.3.2.21;  $\gamma$ -glutamyl-peptide: amino acid  $\gamma$ glutamyletransferase; GGT): catalyzes the transfere of the  $\gamma$ -glutamyl group from peptides and compounds that contain it to an acceptor Gammaglutamyl transferase occurs mainly in the cells of liver, kidneys, pancreas and prostate. Plasma GGT activity is higher in men than in women.

## **Clinical Significance**

Normal values for GGT Male:  $<55 \text{ U/L} = <0.94 \text{ }\mu\text{kat/L}$ ; Female:  $<38 \text{ U/L} = <0.65 \text{ }\mu\text{kat/L}$ 

### Causes of raised plasma GGT activity

□ Induction of enzyme synthesis, without cell damage, by drugs or alcohol.

□ Hepatocellular damage, such as that due to infectious hepatitis:

## Other enzymes

**1.** Cholinesterase (EC 3.1.1.7, acetylecholine acetylhydrolase), which is called true cholinesterase or choline esterase I. found in:

- (a) erythrocytes
- (b) lung and spleen
- (c) nerve endings
- (d) the gray matter of the brain.

Normal values for CHE: 4.9-11.9 U/mL

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Measurements of CHE activity in serum are used:

- 1. as a test of liver function
- 2. as an indicator of possible insecticide poisoning

Causes of decreased plasma cholinesterase activity

- 1. Hepatic parenchymal disease (reduced synthesis)
- 2. Ingestion or absorption through the skin, of such anticholinesterases as organophosphates.

Causes of increased plasma cholinesterase activity

- 1. Recovery from liver damage (actively growing hepatocytes)
- 2. Nephrotic syndrome
- 2. Glutamate dehydrogenase (EC 1.4.1.3; L-glutamate: NAD(P)+ oxidoreductase, deaminating;

GLD) is a mitochondrial enzyme found mainly in the:

- (a) liver
- (b) heart muscle
- (c) kidneys but small amounts occur in other tissue, including
- (d) brain
- (e) skeletal muscle tissue
- (f) leukocytes

### **Clinical significance**

GLD is increased in serum of patients with hepatocellular damage offering differential diagnostic potential in the investigation of liver disease, particularly when interpreted in conjunction with other enzyme test results. The key to this differential diagnostic potential is to be found in the

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intraorgan and intracellular distribution of the enzyme. As an exclusively mitochondrial enzyme, GLD is released from necrotic cells and is of value in estimation of the severity of liver cell damage. GLD activity in serum is stable at 4°C for 48 hours and at -20°C for several weeks. The GLD upper reference limits are 6U/L (women) and 8U/L (men), when a method optimized at 37°C is used.

#### **Muscle enzymes**

**Creatine Kinase** (EC 2.7.3.2; adenosine triphosphate: creatine Nphosphotransferase CK) CK is most abundant in cells of cardiac and skeletal muscle and in brain, but also occurs in other tissues such as smooth muscle. The concentration gradients between some human tissues and serum for creatine kinase. The concentration gradient is logarithmic

#### **Clinical significance**

Normal range for total CK: Male : 46-171 U/L= 0.78-2.90  $\mu$ kat/L ; Female: 34-145 U/L= 0.58-2.47  $\mu$ kat/L Serum CK activity is greatly elevated in all types of muscular dystrophy. In progressive muscular dystrophy (particularly Duchenne sex-linked muscular dystrophy), enzyme activity in serum is highest in infancy and childhood (7-10 years of age) and may increase long before the disease is clinically apparent. Serum CK activity characteristically falls as patients get older and as the mass functioning muscle diminishes with the progression of the disease. About 50%- 80% of the asymptomatic female carriers of Duchenne dystrophy show threefold to sixfold increase of CK activity. Quite high values of Ck are noted in viral myositis, polymyositis and similar muscle disease. However in neurogenic muscle disease, such as:

(a) Myasthenia gravis

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- (b) Multiple sclerosis
- (c) Polimyeltis
- (d) Parkinsonism

Serum enzyme activity is normal

#### Isoenzymes of CK

CK consists of two protein subunits, M (for muscle) and B (for brain), which combine to form three isoenzymes. BB (CK-1), MB (CK-2) and MM (CK-3). CK-MM is the predominant isoenzyme in skeletal and cardiac muscle and is detectable in the plasma of normal subjects. CK-MB accounts for about 35 per cent of the total CK activity in cardiac muscle and less than five per cent in skeletal muscle: its plasma activity is always high after myocardial infarction. It may be detectable in the plasma of patients with a variety of other disorders in whom the total CK activity is raised, but this accounts for less than six per cent of the total. CK-BB is present in high concentrations in the brain and in the smooth muscle of the gastrointestinal and genital tracts. Although they have also been reported after brain damage and in association with malignant tumours of the bronchus, prostate and breast, measurement is not of proven value for diagnosing these conditions. In malignant disease plasma total CK activity is usually normal. Approximate concentrations of tissue CK activity (expressed as multiple activity concentrations in serum and cytoplasmic isoenzyme composition.

**Lactate Dehydrogenase** (EC 1.1.1.27; L-lactate: NAD+ oxidoreductase; LD) catalyses the reversible interconversion of lactate and pyruvate. The enzyme is widely distributed in the body, with high concentrations in cells of cardiac and skeletal muscle, liver, kidney, brain and

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erythrocytes: measurement of plasma total LD activity is therefore a non-specific marker of cell damage. LD has a molecular weight of 134 kDa and is composed of four peptide chains of two types:

M (or A)

H (or B)

Each under separate genetic control The subunit compositions of the five isoenzymes are listed below in order of their decreasing anodal mobility in an alkaline medium.

LD-1 (HHHH; H4) = migrates fastest towards the anode

LD-2 (HHHM; H3M)

LD-3 (HHMM; H2M2)

LD-4 (HMMM; HM3)

LD-5 (MMMM; M4)

Normal range of total LDH: 180-360 U/L=  $3.1-6.1 \mu$ kat/L It is increased in plasma in Myocardial injury, acute leukemias, generalized carcinomatosis and in acute hepatitis. Estimation of its isoenzymes in more useful in clinching diagnosis between hepatic disease and Myocardial.Injury.

### **Causes of Raised Plasma Total LD Activity**

□ Artefactual: Due to in vitro haemolysis or delayed separation of plasma from whole blood.

□ Marked increase (more than 5 times the upper reference limit in adults):

□ Circulatory failure with 'shock' and hypoxia:

□ Myocardial infarction

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□ Some haematological disorders. In blood diseases such as megaloblastic anaemia, acute leukaemias and lymphomas. very high levels (up to 20 times the upper reference limit in adults) may be found.

□ Moderate increase. viral hepatitis: malignancy of any tissue: skeletal muscle disease: pulmonary embolism: infectious mononucleosis.

### **Isoenzymes of LD**

LD1 fraction predominates in cells of cardiac muscle, erythrocytes and kidneys. LD5 is the most abundant form in the liver and in skeletal muscle. Whereas in many conditions there is an increase in all fractions, the finding of certain patterns is of diagnostic value.

□ Predominant elevation of LD1 and LD5. (LD1 greater than LD5 occurs after myocardial infarction, in megaloblastic anaemia and after renal infarction.

□ Predominant elevation of LD2 and LD3 occurs in acute leukaemia: LD3 is the main isoenzyme elevated due to malignancy of many tissues.

□ Elevation of LD5 occurs after damage to the liver or skeletal muscle.

## Other clinically important enzymes

Acid Phosphatase (EC 3.1.3.2; orthophosphoric acid-monoester phosphohydrolase [acid optimum]; ACP) Acid phosphatase is present in lysosomes, which are organelles present in all cells with the possible exception of erythrocytes. Extra lysosomal ACPs are also present in many cells:

(a) prostate,

(b) bone (osteoclasts),

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- (c) spleen
- (d) platelets
- (e) erythrocytes.

The lysosomal and prostatic enzymes are strongly inhibited by d-tartrate ions (tartrate-labile

ACP), whereas the erythrocyte and bone isoenzymes are not (TRACP)

Normal range of TR-ACP: 1.5-4.5 U/L= 0.03-0.08 i kat/L

Elevated TR-ACP

- (a) Paget disease
- (b) Hyperparathyroidism with skeletal involvement
- (c) Presence of malignant invasion of bones by cancers

The only nonbone condition in which elevated activities of TR-ACP are found in serum is Gaucher disease of the spleen, a lysosome storage disease. The main indications for estimation are to help diagnose prostatic carcinoma and to monitor its treatment. The estimation is gradually being replaced by the measurement of plasma prostate specific antigen (PSA) a protein derived from the prostate. This test is more specific and sensitive for diagnosis and monitoring treatment. However, it may be raised in similar circumstances to those affecting prostatic ACP and is more expensive to estimate. ACP is more useful for monitoring the treatment of a known case of disseminated prostatic carcinoma than for making the diagnosis.

**Glucose -6-phosphate Dehydrogenase** (EC 1.1.1.49); D-Glucose -6- phosphate: NADP+ oxidoreductase; G6PD) is expressed in all cells and catalyzes the first step in the hexose monophosphate pathway, the conversion of glucose-6-phosphate to 6-phosphogluconate,

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generating NADPH. G6PD deficiency is the most common ezymeopaty, affecting 400 million people worldwide. More than 400 different types of G6PD variants have been described, leading to different enzyme activities associated with a wide range of biochemical and clinical phenotypes.

The majority of G6PD – deficient individuals develop hemolysis only when oxidative stress occurs, as with infections and after ingestion of certain drugs or fava beans. Outside these periods, they are usually asymptomatic; however, G6PD deficiency also leads to mild to sever chronic hemolysis, exacerbated by oxidative stress. The reference interval for G6PD on erythrocytes is 8-14U/g Hb. Values >18 U/ g Hb are often encountered in any condition associated with younger t han normal RBCs but are of no clinical significance

**Tumour Markers:** Tumour markers are substances that can be found in the body when cancer is present. They are usually found in the blood or urine. They can be products of cancer cells or of the body in response to cancer. Most tumour markers are proteins. For many reasons, tumour marker itself is usually not enough to diagnose or rule out cancer. Most tumour markers can also be made by normal cells as well as by cancer cells. Sometimes, non-cancerous conditions can also cause elevation of some tumour markers to be higher than normal. Besides, not every cancer patient may have raised level of a tumour marker. For these reasons, only a handful of tumour markers are commonly used by most doctors.

How Are Tumour Markers Used?

(I) For Screening and Early Detection of Cancer Screening refers to looking for cancer in people who have no symptoms of the disease, while early detection is finding cancer at an early stage.

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Although tumour markers were first developed to test for cancer in people without symptoms, very few tumour markers have been found to be helpful in this way because most tumour markers have not been shown to detect cancer much earlier than they would have been found otherwise.

- (II) Diagnosing Cancer In most cases, cancer can only be diagnosed by a biopsy and tumour markers are usually not used to diagnose cancer. However tumour markers can help determine if a cancer is likely in some patients. It can also help diagnose the origin of the cancer in patients presenting with advanced widespread disease.
- (III) Determining the Prognosis (Outlook) for Certain Cancers Some newer tumour markers help to assess how aggressive a cancer is likely to be or even how well it might respond to certain drugs.
- (IV) Determining the Effectiveness of Cancer Treatment One of the most important uses for tumour markers is to monitor patients being treated for cancer. If the initially raised tumour marker level goes down with treatment, it indicates that the treatment is working and is having a beneficial effect. On the other hand, if the marker level goes up, then the treatment is probably not working and change of treatment should be considered.
- (V) Detecting Recurrent Cancer Markers are also used to detect cancers that recur after initial treatment. Some tumour markers can be useful once treatment has been completed and with no evidence of residual cancer left. These include PSA (for prostate cancer), HCG (for gestational trophoblastic tumours & germ cell tumours of ovaries & testicles), and CA 125 (for epithelial ovarian cancer).

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### **Tumour Markers in Use:**

Tumour Marker	Comments	
Alpha-fetoprotein (AFP)	<ul> <li>AFP is elevated in hepatocellular carcinoma of liver and is useful to monitor response to treatment.</li> </ul>	
	<ul> <li>AFP is also elevated in certain testicular cancers (embryonal cell &amp; endodermal simus types).</li> </ul>	
Beta-2 microglobulin (B2M)	Elevated in multiple myeloma, chronic lymphocytic leukaemia & some lymphomas.	
	<ul> <li>Patients with higher levels of B2M usually have a worse prognosis.</li> </ul>	
	<ul> <li>Beta-2 microglobulin is often elevated in chronic renal failure and dialysis patients without cancer.</li> </ul>	
Bladder tumour antigen (BTA)	<ul> <li>BTA is found in urine of many bladder cancer patients.</li> </ul>	
	Test results are reported as either positive (BTA present) or negative (BTA not present).	
	<ul> <li>It can be used together with NMP22 (see below) to detect recurrent tumour.</li> </ul>	
	<ul> <li>This test is not widely used and is still being studied.</li> </ul>	
	• It is not certain whether it is as sensitive as cystoscopy for diagnosis & follow-up.	
CA15-3	<ul> <li>CA 15-3 can be used to monitor breast cancer patients.</li> </ul>	
	<ul> <li>Elevated blood levels are found in &lt;10% of patients with early disease and in about 70% of patients with advanced disease.</li> </ul>	
	<ul> <li>CA 15-3 levels usually drop following effective treatment.</li> </ul>	
	<ul> <li>But CA 15-3 can also be elevated in other cancers &amp; in some non-cancerous conditions such as beingin breast conditions &amp; hepatitis.</li> </ul>	
CA27.29	CA 27.29 is another marker to monitor breast cancer patients.	
	<ul> <li>This test measures the same marker as CA 15-3 but in a different way &amp; does not appear to be any better in detecting early or advanced disease.</li> </ul>	
	It can also be raised in other cancers and in some non-cancerous conditions.	
CA125	<ul> <li>CA 125 is the standard tumour marker to follow patients with epithelial ovarian cancer during or after treatment.</li> </ul>	
	<ul> <li>&gt;90% of patients with advanced ovarian cancer have elevated CA 125.</li> </ul>	
	<ul> <li>Because about half of ovarian cancer patients with elevated CA 125 still have tumour confined to the ovary, CA 125 is being studied as screening test for ovarian cancer (See next section for details).</li> </ul>	
	<ul> <li>CA 125 can also be raised in patients with endometrial and pancreatic cancer as well as in being conditions such as endometriosis, pehric inflammatory disease and built provide parts.</li> </ul>	
	where we have a state	

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## **KARPAGAM ACADEMY OF HIGHER EDUCATION** CLASS: III BSC BC COURSE NAME: CLINICAL BIOCHEMIST

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CA72-4	<ul> <li>CA 72-4 is a newer test being studied in ovarian, pancreatic and stomach cancer.</li> <li>Studies of this marker are still in progress.</li> </ul>
CA19-9	<ul> <li>CA 19-9 is considered the best tumour marker for following patients with pancreatic cancer.</li> <li>A high level in a newly diagnosed patient usually means advanced disease.</li> <li>CA 19-9 is not used as a screening test because usually it will not detect early disease.</li> <li>CA 19-9 may also be used to monitor colorectal cancer, but because it is less sensitive than CEA test, most would recommend CEA.</li> <li>CA 19-9 can also be raised in other cancers such as stomach and bile ducts cancer and in some non-cancerous conditions such as pancreatitis.</li> </ul>
Calcitonin	<ul> <li>Calcitonin is a hormone secreted by parafollicular C cells of thyroid.</li> <li>In patients with cancer of parafollicular C cells of thyroid called medullary thyroid carcinoma (MTC), blood levels of calcitonin are raised.</li> <li>Calcitonin is one of the rare tumour markers that can be used to detect early cancer: because MTC is often inherited, measurement of blood calcitonin level can be used to detect cancer at its earliest stages in family members at risk.</li> </ul>
Carcinoembryonic antigen (CEA)	<ul> <li>CEA is the preferred tumour marker to monitor patients with colorectal cancer during treatment, but it is not useful as a screening or diagnostic test.</li> <li>The higher the CEA level at time of diagnostic, the more likely it is that the disease is advanced.</li> <li>CEA can also be raised in cancer of hung, breast, thyroid, pancreas, liver, stomach, ovary and bladder.</li> <li>It can also be elevated in non-cancerous diseases and in chronic smokers.</li> </ul>
Chromogranin A (CgA)	<ul> <li>Blood level of CgA is raised in patients with neuroendocrine tumours such as carcinoid tumours, neuroblastoma, small cell hung cancer and some rare cases of prostate cancer that have neuroendocrine features.</li> <li>CgA is probably the most sensitive tumour marker for carcinoid tumours: level raised in 1/3 of patients with localized disease and 2/3 with metastatic disease.</li> </ul>
Estrogen / Progesterone receptors	<ul> <li>Breast tumour samples (not blood samples) from patients with breast cancer are tested for these markers.</li> </ul>
HER2 (Human Epidermal Growth Factor receptor, also known as EGFR 2)	<ul> <li>About 25% of patients with breast cancer have tumours that overexpress HER2, which is associated with aggressive disease, poor clinical outcomes and shortened overall survival.</li> <li>Samples of tumour tissue (not blood sample) are used to test for HER2 status.</li> </ul>

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**The ECG** - E.C.G stands for Electrocardiogram and represents the electrophysiology of the heart. Cardiac electrophysiology is the science of the mechanisms, functions, and performance of the electrical activities of specific regions of the heart. The ECG is the recording of the heart's electrical activity as a graph. The graph can show the heart's rate and rhythm, it can detect enlargement of the heart, decreased blood flow, or the presence of current or past heart attacks. ECG's are inexpensive, Non-invasive, quick, and painless. Depending on the results, the patient's medical history, and a physical exam; further tests or a combination of medications and lifestyle changes may be ordered.



**Cardiac Muscle Contraction -** After an action potential excites the plasma membrane of the cardiac muscle cell the contraction is due to an increase in the cytoplasmic concentration of Calcium ions. Similar to skeletal muscle, the release of Ca+ ions from the sarcoplasmic

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reticulum binds to troponin which allows actin to bind with myosin. The difference between skeletal muscle and cardiac muscle is that when the action potential opens voltage gated calcium ion channels in the T-tubules. The increase in cytosolic calcium causes calcium ions to bind to receptors on the surface of the sarcoplasmic reticulum. The binding of calcium ions to these receptors causes the opening of more calcium ion channels in the SR membrane. Calcium ions then rush out of the SR and bind to troponin and allow the myosin and actin to bind together which causes contraction. This sequence is called calcium-induced calcium release. Contraction ends when the level of cytosolic calcium returns to normal resting levels.

**Blood Pressure -** Blood pressure is the pressure exerted by the blood on the walls of the blood vessels. Unless indicated otherwise, blood pressure refers to systemic arterial blood pressure, i.e., the pressure in the large arteries delivering blood to body parts other than the lungs, such as the brachial artery (in the arm). The pressure of the blood in other vessels is lower than the arterial pressure. Blood pressure values are universally stated in millimeters of mercury (mmHg). The systolic pressure is defined as the peak pressure in the arteries during the cardiac cycle; the diastolic pressure is the lowest pressure (at the resting phase of the cardiac cycle). The mean arterial pressure and pulse pressure are other important quantities. Typical values for a resting, healthy adult are approximately 120 mmHg systolic and 80mm Hg diastolic (written as 120/80 mmHg), with individual variations. These measures of blood pressure are not static, but undergo natural variations from one heartbeat to another, and throughout the day (in a circadian rhythm); they also change in response to stress, nutritional factors, drugs, or disease.

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#### Systolic Pressure

Systolic Pressure is the highest when the blood is being pumped out of the left ventricle into the aorta during ventricular systole. The average high during systole is 120 mmHg.

**Diastolic Pressure -** Diastolic blood pressure lowers steadily to an average low of 80 mmHg during ventricular diastole.

**Cardiovascular Disease** - Cardiovascular disease refers to the class of diseases that involve the heart and/or blood vessels (arteries and veins). While the term technically refers to any disease that affects the cardiovascular system, it is usually used to refer to those related to atherosclerosis (arterial disease). These conditions have similar causes, mechanisms, and treatments. Over 50 million Americans have cardiovascular problems, and most other Western countries face high and increasing rates of cardiovascular disease. It is the number 1 cause of death and disability in the United States and most European countries. By the time that heart problems are detected, the underlying cause (atherosclerosis) is usually quite advanced, having progressed for decades. There is therefore increased emphasis on preventing atherosclerosis by modifying risk factors, such as healthy eating, exercise and avoidance of smoking.

**Hypertension** - Hypertension or high blood pressure is a medical condition wherein the blood pressure is chronically elevated. Persistent hypertension is one of the risk factors for strokes, heart attacks, heart failure and arterial aneurysm, and is a leading cause of chronic renal failure

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Atherosclerosis - Atherosclerosis is a disease affecting the arterial blood vessel. It is commonly referred to as a "hardening" or "furring" of the arteries. It is caused by the formation of multiple plaques within the arteries. Arteriosclerosis ("hardening of the artery") results from a deposition of tough, rigid collagen inside the vessel wall and around the atheroma. This increases the stiffness, decreases the elasticity of the artery wall. Atherosclerosis typically begins in early adolescence, is usually found in most major arteries, and yet is asymptomatic and not



detected by most diagnostic methods during life. It most commonly becomes seriously symptomatic when interfering with the coronary circulation supplying the heart or cerebral circulation supplying the brain, and is considered the most important underlying cause of strokes, heart attacks, various heart diseases including congestive heart failure and most cardiovascular diseases in general.

**Plaque** - Plaque Atheroma or commonly known as plaque is an abnormal inflammatory accumulation of macrophage white blood cells within the walls of arteries.

**Circulatory Shock -** Circulatory Shock is a severe condition that results from reduced blood circulation.

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**Thrombus** - A thrombus, or blood clot, is the final product of the blood coagulation step in hemostasis. It is achieved via the aggregation of platelets that form a platelet plug, and the activation of the humoral coagulation system (i.e. clotting factors). A thrombus is physiologic in cases of injury, but pathologic in case of thrombosis. Preventing blood clots reduces the risk of stroke, heart attack and pulmonary embolism. Heparin and warfarin are often used to inhibit the formation and growth of existing blood clots, thereby allowing the body to shrink and dissolve the blood clots through normal methods.

**Embolism** - An embolism occurs when an object (the embolus) migrates from one part of the body (through circulation) and causes a blockage (occlusion) of a blood vessel in another part of the body. Blood clots form the most common embolic material by far: other possible embolic materials include fat globules (a fat embolism), air bubbles (an air embolism), septic emboli (containing pus and bacteria), or amniotic fluid.

**Stroke** - A stroke, also known as cerebrovascular accident (CVA), is an acute neurological injury whereby the blood supply to a part of the brain is interrupted. Strokes can be classified into two major categories: ischemic and hemorrhagic. ~80% of strokes are due to ischemia.

**Ischemic Stroke**: In ischemic stroke, which occurs in approximately 85-90% of strokes, a blood vessel becomes occluded and the blood supply to part of the brain is totally or partially blocked. Ischemic stroke is commonly divided into thrombotic stroke, embolic stroke, systemic hypoperfusion (Watershed or Border Zone stroke), or venous thrombosis

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• **Hemorrhagic Stroke**: A hemorrhagic stroke, or cerebral hemorrhage, is a form of stroke that occurs when a blood vessel in the brain ruptures or bleeds. Like ischemic strokes, hemorrhagic strokes interrupt the brain's blood supply because the bleeding vessel can no longer carry the blood to its target tissue. In addition, blood irritates brain tissue, disrupting the delicate chemical balance, and, if the bleeding continues, it can cause increased intracranial pressure which physically impinges on brain tissue and restricts blood flow into the brain. In this respect, hemorrhagic strokes are more dangerous than their more common counterpart, ischemic strokes. There are two types of hemorrhagic stroke: intracerebral hemorrhage, and subarachnoid hemorrhage.

The term "brain attack" is starting to come into use in the United States for stroke, just as the term "heart attack" is used for myocardial infarction, where a cutoff of blood causes necrosis to the tissue of the heart. Many hospitals have "brain attack" teams within their neurology departments specifically for swift treatment of stroke. If symptoms of stroke are detected at early on-set, special "clot busting" drugs may be administered. These clot busters will dissolve clots before they can cause tissue death and restore normal circulation. One of the initial drugs used to dissolve clots was **streptokinase**, although its use creates a possiblity of clot destruction throughout the entire body, leading to serious hemorrhage. There are newer, third generation thrombolytics that are safer.

Heart Attack - Acute myocardial infarction (AMI or MI), commonly known as a heart attack, A heart attack occurs when the supply of blood and oxygen to an area of heart muscle is blocked,

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usually by a clot in a coronary artery. Often, this blockage leads to arrhythmias (irregular heartbeat or rhythm) that cause a severe decrease in the pumping function of the heart and may bring about sudden death. If the blockage is not treated within a few hours, the affected heart muscle will die and be replaced by scar tissue. It is the leading cause of death for both men and women all over the world

**Angina Pectoris -** Angina Pectoris is chest pain due to ischemia (a lack of blood and hence oxygen supply) of the heart muscle, generally due to obstruction or spasm of the coronary arteries (the heart's blood vessels).

**Coronary Bypass -** Coronary artery bypass surgery, coronary artery bypass graft surgery and heart bypass are surgical procedures performed on patients with coronary artery disease for the relief of angina and possible improved heart muscle function. Veins or arteries from elsewhere in the patient's body are grafted from the aorta to the coronary arteries, bypassing coronary artery narrowing caused by atherosclerosis and improves the blood supply to the myocardium (heart muscle).

**Congestive Heart Failure -** Congestive heart failure (CHF), also called congestive cardiac failure (CCF) or just heart failure, is a condition that can result from any structural or functional cardiac disorder that impairs the ability of the heart to fill with or pump a sufficient amount of blood throughout the body. It is not to be confused with "cessation of heartbeat", which is known as asystole, or with cardiac arrest, which is the cessation of normal cardiac function in the face of

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heart disease. Because not all patients have volume overload at the time of initial or subsequent evaluation, the term "heart failure" is preferred over the older term "congestive heart failure". Congestive heart failure is often undiagnosed due to a lack of a universally agreed definition and difficulties in diagnosis, particularly when the condition is considered "mild".

Aneurysm - An aneurysm (or aneurism) is a localized dilation or ballooning of a blood vessel by more than 50% of the diameter of the vessel and can lead to instant death at anytime. Aneurysms most commonly occur in arteries at the base of the brain (the circle of Willis) and in the aorta (the main artery coming out of the heart) - this is an aortic aneurysm. This bulge in a blood vessel, much like a bulge on an over-inflated inner tube, can lead to death at anytime. The larger an aneurysm becomes, the more likely it is to burst. Aneurysms are also described according to their shape: Saccular or fusiform. A saccular aneurysm resembles a small sack; a fusiform aneurysm is shaped like a spindle.

**Dissolving Blood Clots** - To dissolve blood clots you would use a drug that converts plasminogen (molecule found in blood), to plasmin, (enzyme that dissolves blood clots).

**Clearing Clogged Arteries -** One way to unblock a coronary artery (or other blood vessel) is percutaneous transluminal coronary angioplasty (PTCA), which was first performed in 1977. A wire is passed from the femoral artery in the leg or the radial artery in the arm up to the diseased coronary artery, to beyond the area of the coronary artery that is being worked upon. Over this wire, a balloon catheter is passed into the segment that is to be opened up. The end of the

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catheter contains a small folded balloon. When the balloon is hydraulically inflated, it compresses the atheromatous plaque and stretches the artery wall to expand. At the same time, if an expandable wire mesh tube (stent) was on the balloon, then the stent will be implanted (left behind) to support the new stretched open position of the artery from the inside.

#### **Dilated and Inflamed Veins**

**Varicose Veins -** Varicose veins are veins on the leg which are large, twisted, and ropelike, and can cause pain, swelling, or itching. They are an extreme form of telangiectasia, or spider veins. Varicose veins result due to insufficiency of the valves in the communicating veins. These are veins which link the superficial and deep veins of the lower limb. Normally, blood flows from the superficial to the deep veins, facilitating return of blood to the heart. However, when the valve becomes defective, blood is forced into the superficial veins by the action of the muscle pump (which normally aids return of blood to the heart by compressing the deep veins). People who have varicose veins are more at risk of getting a Deep Vein Thrombosis (DVT) and pulmonary embolisms.

**Phlebitis -** Phlebitis is an inflammation of a vein, usually in the legs. This is usually the most serious if found in a deep vein. However, most people with the condition, perhaps 80 to 90 percent, are women. The disease may also have a genetic component, as it is known to run in families.

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#### **Congenital Heart Defects**

Heart defects present at birth are called congenital heart defects. Slightly less than 1% of all newborn infants have congenital heart disease. Eight defects are more common than all others and make up 80% of all congenital heart diseases, whereas the remaining 20% consist of many independently infrequent conditions or combinations of several defects.



**Acyanotic Defects -** Acyanotic heart defects are those in which there is a normal amount of oxygen in the bloodstream. The most common congenital heart defect is a ventral septal defect, which occurs in about 20% of all children with congenital heart disease. In VSD blood from the left ventricle is shunted to the right ventricle, resulting in oxygenated blood returning into pulmonic circulation. One of the potential problems of VSD is pulmonary hypertension.

**Cyanotic Defects -** Cyanotic heart defects refer to defects that result in decreased amounts of oxygen in the blood. In cyanotic heart defects deoxygenated blood from the right ventricle flows into the systemic circulation. Cyanotic defects include tetrogy of fallot and transposition of the great arteries.

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**Homeostasis** - Homeostasis in the body is only possible if the cardiovascular system is working properly. This means that the system needs to deliver oxygen and nutrients to the tissue fluid that surrounds the cells and also take away the metabolic waste. The heart is composed of arteries that take blood from the heart, and vessels that return blood to the heart. Blood is pumped by the heart into two circuits: the pulmonary and systemic circuits. The pulmonary circuit carries blood through the lungs where gas exchange occurs and the systemic system transports blood to all parts of the body where exchange with tissue fluid takes place. The cardiovascular system works together with all other systems to maintain homeostasis.

**The Lymphatic System -** The lymphatic system is closely related to the cardiovascular system. There are three main ways that they work together to maintain homeostasis: the lymphatic system receives the excess tissue fluid and returns it to the bloodstream, lacteals take fat molecules from the intestinal villi and transport them to the bloodstream and both systems work together to defend the body against disease.

#### **Interesting Facts**

- Heart Disease is the number one killer in American women.
- 16.7 million deaths are result forms of cardiovascular disease, heart disease and stroke.
- Stress, eating high fat foods, obesity, tobacco and alcohol use are just some risk factors of developing heart disease.
- Recent research suggests that taking a small dose of aspirin daily may help prevent a heart attack (because aspirin inhibits platelet clumping).

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• The length of all your blood vessels lined up is about 60,000 miles long! To put this in perspective, the Earth's circumference is 40,075.02 kilometres and 60,000 miles is around 96,000 km - so your blood vessels would go twice around the world and still have some to spare!

### Ways to a Healthy Heart

- Eating healthy, good nutrition.
- Fitness and Exercise.
- Having a healthy lifestyle; don't drink, smoke, or do drugs.
- Lowering LDL cholesterol and high blood pressure.
- Reduce the fat, sodium, and calories in your diet.
- The total length of capillaries in an average adult human is approximately 25,000 mi (42,000

km).

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Aging - The heart muscle becomes less efficient with age, and there is a decrease in both maximum cardiac output and heart rate, although resting levels may be more than adequate. The health of the myocardium depends on its blood supply, and with age there is greater likelihood that arthrosclerosis will narrow the coronary arteries. Atherosclerosis is the deposition of cholesterol on and in the walls of the arteries, which decreases blood flow and forms rough surfaces that may cause intravascular clot formation High blood pressure (hypertension) causes the left ventricle to work harder. It may enlarge and outgrow its blood supply, thus becoming weaker. A weak ventricle is not an efficient pump, and may progress to congestive heart failure. This process may be slow or rapid. The heart valves may become thickened by fibrosis, leading to heart murmurs and less efficient pumping. Arrhythmias are also more common with age, as the cells of the conduction pathway become less efficient.

#### Shock

#### Physiological Stress

Physiological stress can be any kind of injury from burns, to broken bones; the body's response to stress is categorized in two phases the ebb phase (early phase) begins immediately after the injury. And the second phase is about 36 to 48 hours after injury is called the flow phase. In the ebb (shock) phase there is Inadequate circulation, decreased insulin level, decreased oxygen consumption, hypothermia (low body temperature), hypovolemia (low blood volume), and hypotension (low blood pressure). In the flow phase there is increased levels of catecholamine, glucocorticoids, and glucagons, normal or elevated insulin levels,

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catabolic (breakdown), hyperglycemic (high blood sugar), increased oxygen consumption/respiratory rate, hyperthermia (high body temperature) fever sets in,

hypermetabolism, increased insulin resistance, increased cardiac output.

**Premature ventricular contractions (PVC's)** - Excitation occurs through the SA node to the AV node if there are abnormalities or drug interference that malfunctions the AV node the ventricles will not receive the initiating stimuli and the autorhythmic cells in the bundle branches begin to initiate actions on their own rate becoming the pacemakers for the ventricles. This in turn will cause conduction disorder. With conduction that causes problems with the bundle branches there is the right and the left premature ventricular contractions. Right is most common and may go untreated. Left is always a serious problem and must be treated.

#### **Intrinsic Control of heartbeat**

- SA node (located in the right atrium near the entrance of the superior vena cava)
- AV node (located at the base of right atrium)

• AV bundle (located in the intraventricular septum between the two ventricles that go in two directions right and left bundle branches that leave the septum to enter the walls of both ventricle)

• Bundle Branches (the branching off the septum to the walls of the ventricles that run into the purkinje fibers that then make contact with ventricular myocardial cells to spread the impulse to the rest of the ventricles)

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#### Electrocardiogram

- The P is the atrial depolarization
- QRS is the ventricular depolarization, as well as atrial repolarization.
- T is the ventricular repolarization

#### **Extrinsic Control of Heartbeat**

Autonomic system with two subdivisions: the sympathetic division and the parasympathetic

division. Hormonal control of blood pressure

- Epinephrine
- Norepinephrine
- ANP : Atrial natriuretic peptide
- ADH: Antidiuretic hormone
- Renin-Angiotension system

## Case Study

An example of the ever expanding technology for the heart is best described in this story: In 1955, when I was five years old, I first learned by my family physician that I had a heart murmur and that it would eventually need attention. By the time I was

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15 in 1965, I had two cardiac catherizations at Rhode Island Hospital. The tests were inconclusive and I was told to go on with my life and wait and see if I had a problem. It wasn't until 1975 that I was told by my family physician that I should have my heart checked again. Dr. David Kitzes of Mariam Hospital performed another catherization. This time, unlike the others, I was told that because of new machine technology, Dr. Kitzes found that I had aortic stenosis, which is a narrowing of the valve passage by build-up of plaque due to the valve being malformed at birth. Dr. Kitzes informed me that I could lead a normal life until I was in my fifties or sixties before I would need corrective surgery. In 1996, I had an echocardiogram and it was determined that my heart was enlarged. My family physician said that I should see a cardiologist. I down played the visit as not being serious after hearing the same thing many times. This time I entered the office of Jon Lambrecht, I had never met him before. Within a few minutes my whole life was turned around. After asking me about my symptoms, which were fatigue, weakness, asthmatic symptoms, as well as ashen skin color and dizziness, he informed me of how serious my condition was and the only salvation was immediate openheart surgery to replace the aortic valve. I began to cry as I thought my life was over. Dr. Lambrecht studied my reaction and told me that this condition is repairable and that I don't have a terminal illness. I didn't have a lot of time to think about it. Within 10 days from that visit, I was the recipient of a Meditronic Hall Prosthetic heart valve. The operation was performed by Dr. Robert Indeglia at Miriam Hospital in Providence,

R.I. on March 20th, 1996. It has been almost 3 years since the surgery and I am doing better than I could have expected. In 1977 my son Kevin was born with Hypoplastic Left-heart

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# **KARPAGAM ACADEMY OF HIGHER EDUCATION**

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Syndrome and only lived for 2 days because heart surgery wasn't performed like today. I am thankful that I lived at a time when medical technology paved the way for a second chance because of my new aortic heart valve. Our goal in this chapter is to take you by the hand and lead you through each part of the cardiovascular system, so that you too may learn and come to respect the greatness of this blood pumping machine we all call the heart.

**Stroke** - Cerebrovascular disease are those that affect blood vessels in the brain and happen to be the third cause of death in the United States only behind heart disease and cancer. Stroke (also called cerebrovascular accident or CVR) is a cerebrovascular disorder caused by a sudden decrease or stoppage of blood flow to a part of the brain. Decreased blood flow also known as ischemia is dangerous to any tissue but brain tissue is even more vulnerable, mainly due to the high rate of its metabolic reactions. In fact if you stopped blood flow for no more than three minutes it may be sufficient enough to cause death of most brain cells. For this reason a stroke can kill people within minutes or leave them with severe brain damage. Strokes may be classified as either occlusive or hemorrhagic and may happen either in the interior of the brain or on its surface. In a occlusive stroke blood flow through a vessel is blocked. In a hemorrhagic stroke a blood vessel ruptures causing a hemorrhage.

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## **KARPAGAM ACADEMY OF HIGHER EDUCATION**

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#### POSSIBLE QUESTIONS

UNIT-I

#### PART-A (20 MARKS)

#### (Q.NO 1 TO 20 Online Examinations)

### PART-B (2 MARKS)

- 1. Write a short note on Tumour markers?
- 2. Write about creatinine clearance.
- 3. What are isoenzymes. Add a note on its clinical significance.
- 4. Expand BUN and its importance.
- 5. Write about Acute myocardial infarction.
- 6. Write the definition of Tumour markers and its classification.
- 7. What makes an importance of ECG in cardiovascular diseases?
- 8. Write about the basic characteristics of selectable tumour markers.

### PART-C (6 MARKS)

- 1. Explain in detail about the role of creatine phosphokinase.
- 2. Explain the defect and diagnosis of myocardial infarction.
- 3. Write in detail on the tests of tumour markers, indications and interpretation.
- 4. Explain the isoenzymes of troponin in diagnosis of heart diseases.
- 5. Give an account on the therapies of cancer.

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Questions	opt1	opt2	opt3	opt4	opt5	opt6	Answer
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substrate							
for							
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phosphok	oxido	creatine	phosphok	hydroper			creatine
inase is	reductase	kinase	inase	oxidase			kinase
Normal							
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is						
slowest						
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				pH, Km		pH, Km
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most	osteoblas					osteoblas
valuable	tic	Hepatic	Renal	None of		tic
index of	activity	activity	activity	the above		activity
What						
tumor						
marker is						
extremely						
elevated						
in a						
patient						
with a				α-		
hydatidifo				fetoprotei		
rm mole?	β-hCG	CA-125	PSA	n		β-hCG

Obstructi						
ve biliary						
disease						
and						
Paget's						
disease						
of the						
bone						
have						
which of						
the		Tartrate-				
following		resistant				
tumor		acid				
markers		phosphat	Alkaline			Alkaline
in		ase	phosphat	Calcitoni		phosphat
common?	S-100	(TRAP)	ase	n		ase
Which						
childhood						
cancer						
has						
bombesin	Acute					
as a	lymphobl					
tumor	astic	Gastric	Lung	Neurobla		Neurobla
marker?	leukemia	cancer	cancer	stoma		stoma
Which of						
the						
following						
tumor						
markers						
is/are						
associate						
d with						
pancreati	CA-19-9			CEA		CEA
С	and CA-			and CA-		and CA-
cancers?	125	CEA	CA - 125	19-9		19-9

Which of						
the						
following						
is found						
in						
patients						
with						
prostate						
carcinom						
a but not						
in						
natients						
that only						
have	Prostatic		Prostate	Prosphori		Prostatic
henion	acid	Prostate	non	c acid		acid
prostatic	nhosnhat	specific	specific	nhosnhat		nhosnhat
hyperplas	ase	antigen	antigen	ase		ase
ia?	$(\mathbf{P} \Delta \mathbf{P})$	$(PS \Delta)$	$(PS\Delta)$	$(\mathbf{P} \Delta \mathbf{P})$		$(\mathbf{P} \Delta \mathbf{P})$
What		(15A)	(15A)	(1 / 1 )		(171)
vv llat						
			Surface			Surface
marker is	Hanataaa		Surface			Surface
a	llulor		tumora			tumora
d with		Malanam	of the	Domonosti		of the
d  with	carcinom	Melanom	of the	Pancreati		or the
CA-125	as	а	ovaries	c cancers		ovaries
What						
tumor						
marker 1s						
associate						
d with	Alkaline					
melanom	phospata			Bombesi		
a?	se	TRAP	S-100	n	 	S-100
Which of						
the						
following						
cancers				Gestation		
is not ass				al		
ociated	Prostate			trophobla		Prostate
with β-	carcinom	Chorioca	Hydatidif	stic		carcinom
hCG?	a	rcinoma	orm mole	tumors		a

The						
enzyme						
assay						
that are						
carried						
out in						
myocardi						
al				CK,		CK,
infarction				AST and		AST and
are	СК	AST	LDH	LDH		LDH
The						
enzyme						
assay						
that are						
carried						
out in				SGOT,		SGOT,
muscle				SGPT,		SGPT,
diseases	SGOT /			aldolase		aldolase
are	SGPT	Aldolase	СРК	and CPK		and CPK
Essential	chloride	calcium	sulphate	phosphat		calcium
element				e		
for blood						
clotting is						
The						
normal						
level of	0.6-3.1	0.2-0.5		0.5-1.0		0.6-3.1
acid	KA	KA	1-5 KA	KA		KA
phosphata	units/100	units/100	units/100	units/100		units/100
se	ml	ml	ml	ml		ml