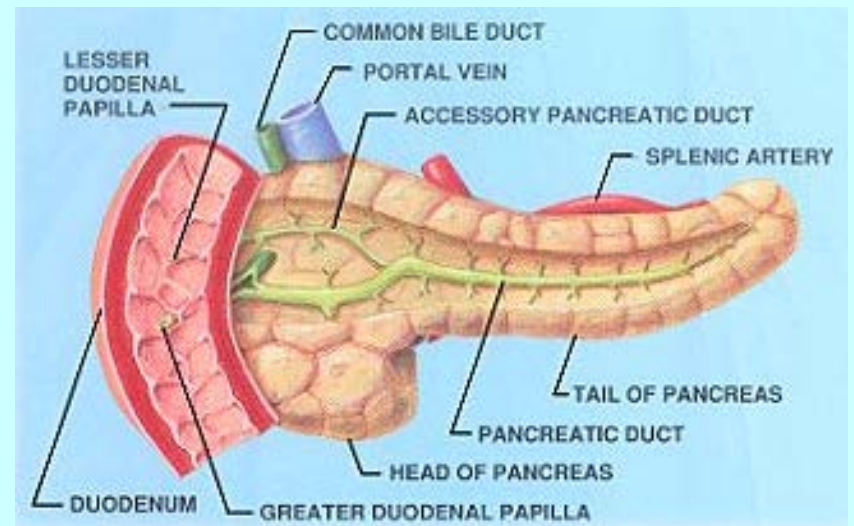
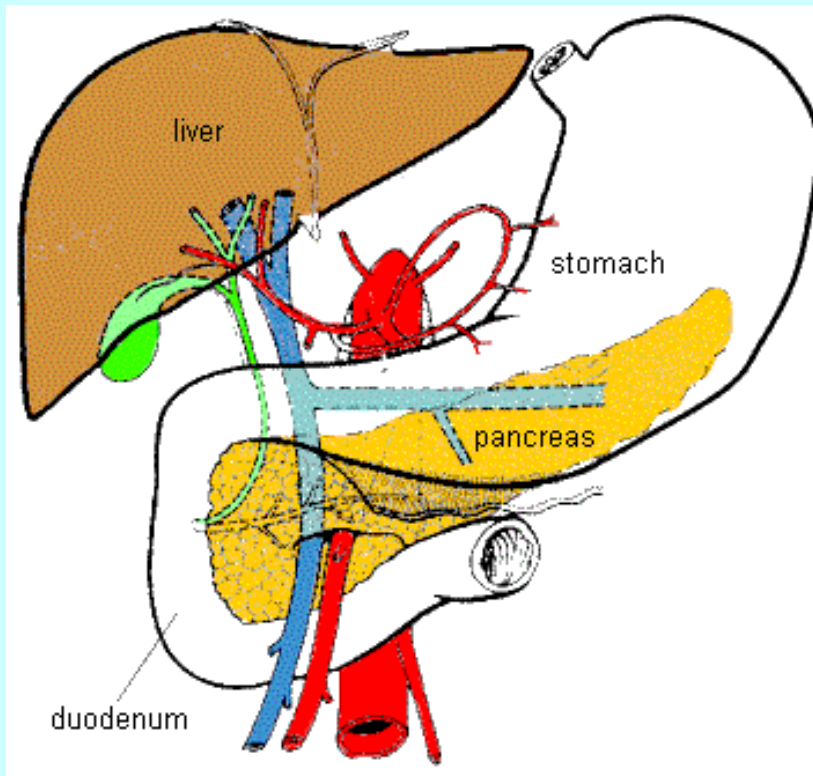
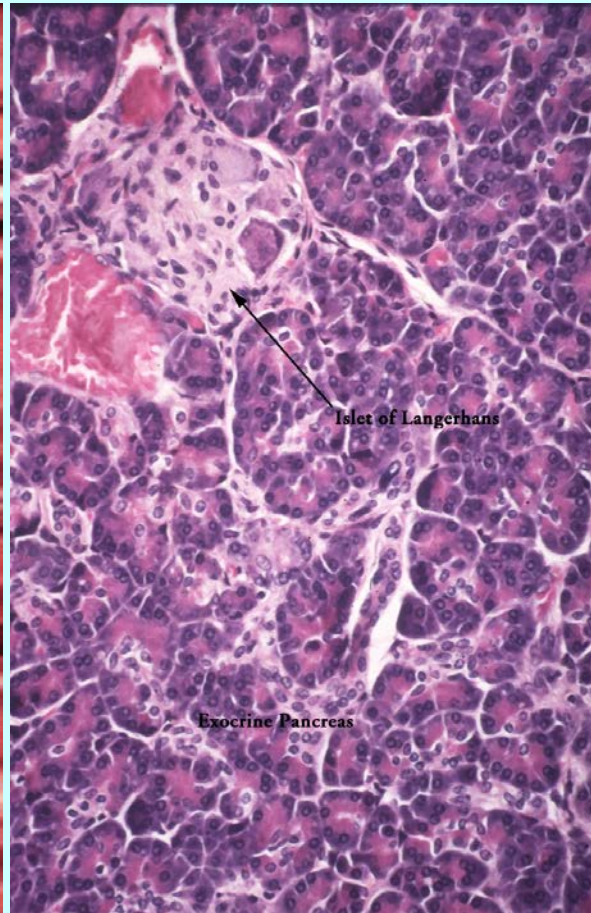
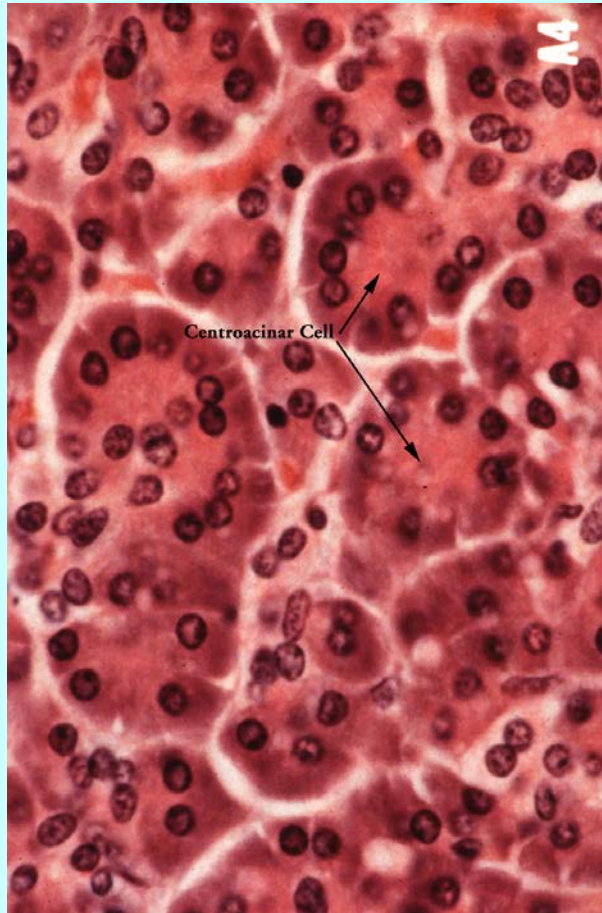


Pancreas



Histology of the normal pancreas



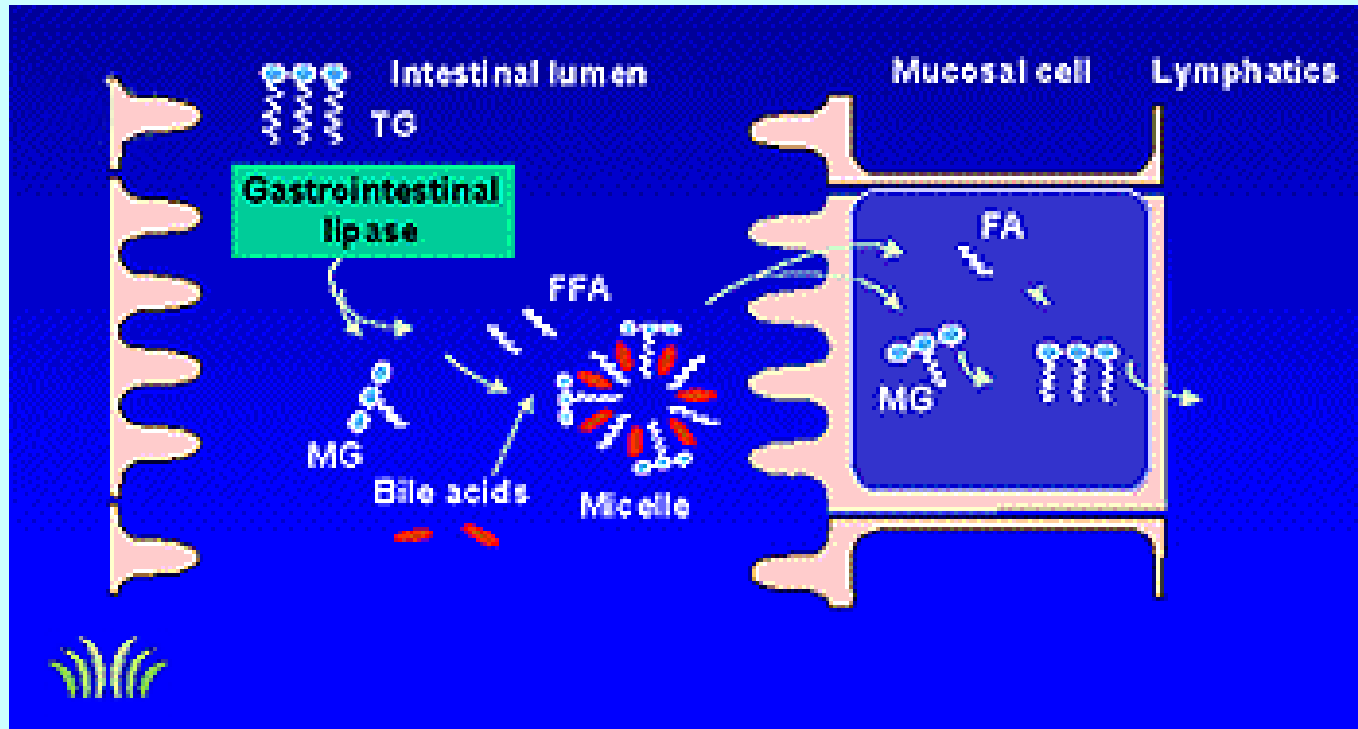
Left: Centroacinar cells. These cells play a role in the exocrine function of the organ (Secretion of enzymes like trypsin, chymotrypsin).

Right: Langerhans' islets. α - and β -cells of these islets play a role in the endocrine secretion of hormones (glucagon, insulin).

Function of digestive enzymes

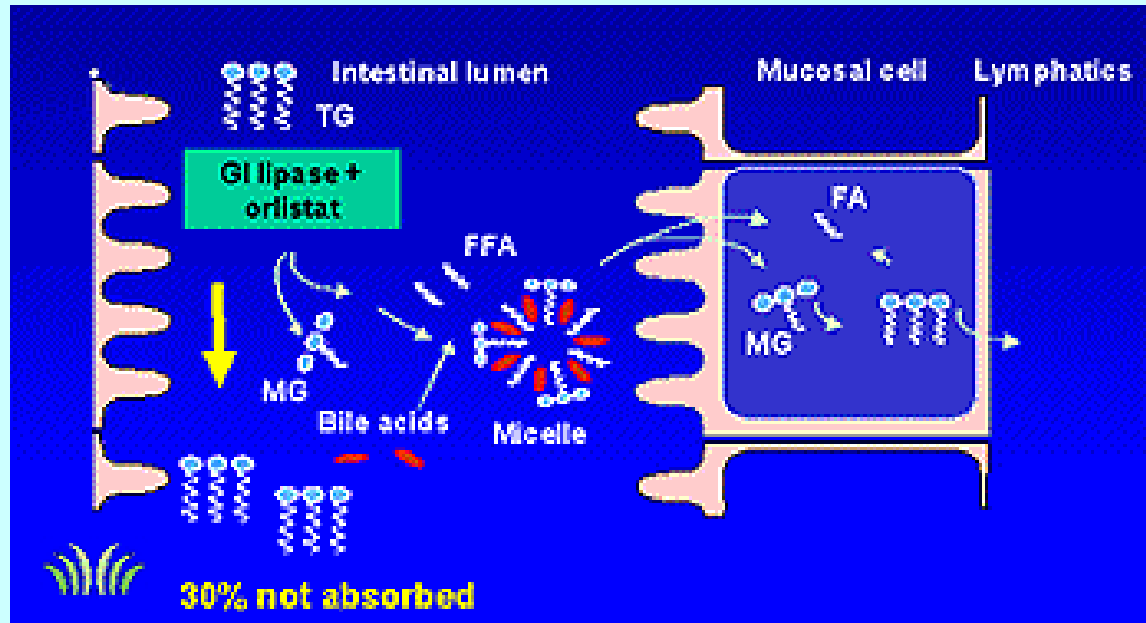
Food type	Enzyme	Source	Products
Carbohydrates	Salivary amylase	Salivary glands	Maltose
	Pancreatic amylase	Pancreas	Maltose
	Maltase	Small intestine	Glucose
Proteins	Pepsin	Stomach mucosa	Peptides
	Trypsin	Pancreas	Peptides
	Peptidases	Intestinal mucosa	Amino acids
Fats	Lipase	Pancreas	Fatty acids and glycerol

Physiology of fat absorption - I



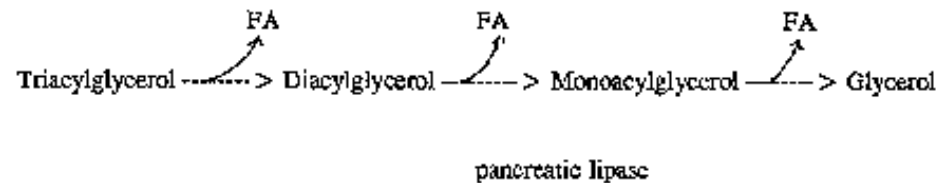
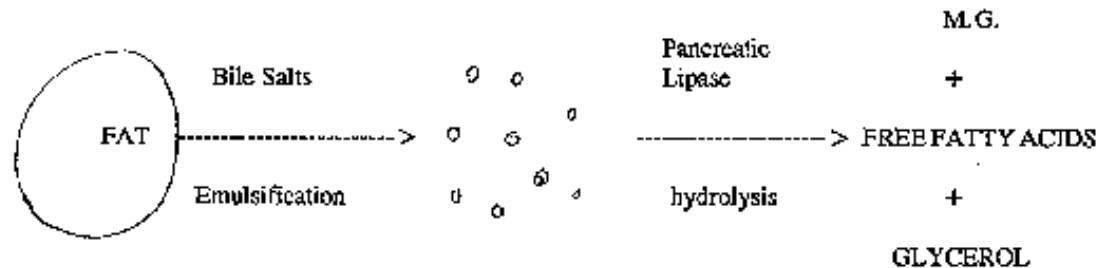
Virtually all dietary fat is in the form of triglycerides which consist of a molecule of glycerol to which three fatty acid chains are attached. Fat digestion mainly occurs in the first part of the small intestine, the duodenum, into which the enzyme-rich pancreatic juices are secreted. Dietary fat is emulsified (broken up into small droplets) resulting in the formation of tiny fat globules measuring 200 to 5000 nm in diameter. Gastrointestinal lipase acts on these emulsified fats producing a mixture of free fatty acids and monoglycerides which bind with bile salts to form micelles. These micelles are then able to cross the intestinal cell membrane and thus carry the lipids into the gut cells. The inner lining of the small intestine is covered in tiny projections called villi which increase the surface area to speed up absorption of digestive products. Once fat is absorbed, it passes into small lymph vessels, called lacteals, for distribution into the lymph system and hence to the circulation.

Physiology of fat absorption - II

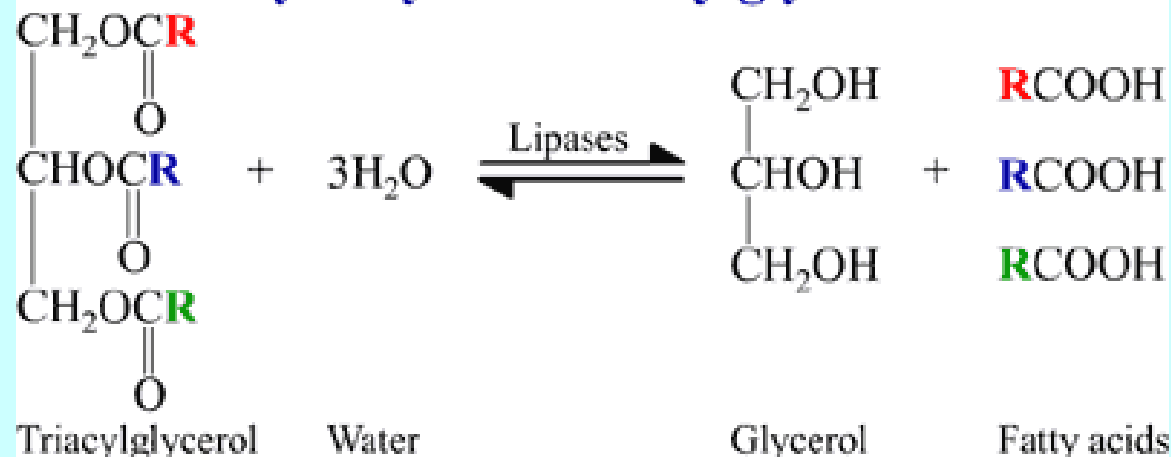


Orlistat acts locally in the gastrointestinal (GI) tract, inhibiting the action of GI lipase and preventing absorption of up to 30 % of ingested dietary fat. Orlistat has a unique molecular structure which allows it to bind to the active site of GI lipase and blocks its activity. The enzyme is thus unable to break triglycerides down into their component parts. A significant proportion of dietary fat therefore remains undigested and unabsorbed, passing through the GI tract, unchanged. 70 % of ingested fat is however absorbed in the normal fashion, ensuring sufficient absorption of fat soluble vitamins. On average, dietary fat accounts for 40 % of daily energy intake, although healthy eating guidelines recommend that it should make up no more than 30 % of calorie intake. Orlistat has been shown to reduce absorption of dietary fat by an average of 30 % at a dose of 120 mg three times daily. By blocking the digestion and absorption of some, but not all, dietary fat, calorie intake is significantly reduced while still allowing the absorption of necessary fat soluble dietary constituents that ensure a healthy, nutritious diet. Less absorption of dietary fat effectively reduces energy intake so that a significant weight loss may occur.

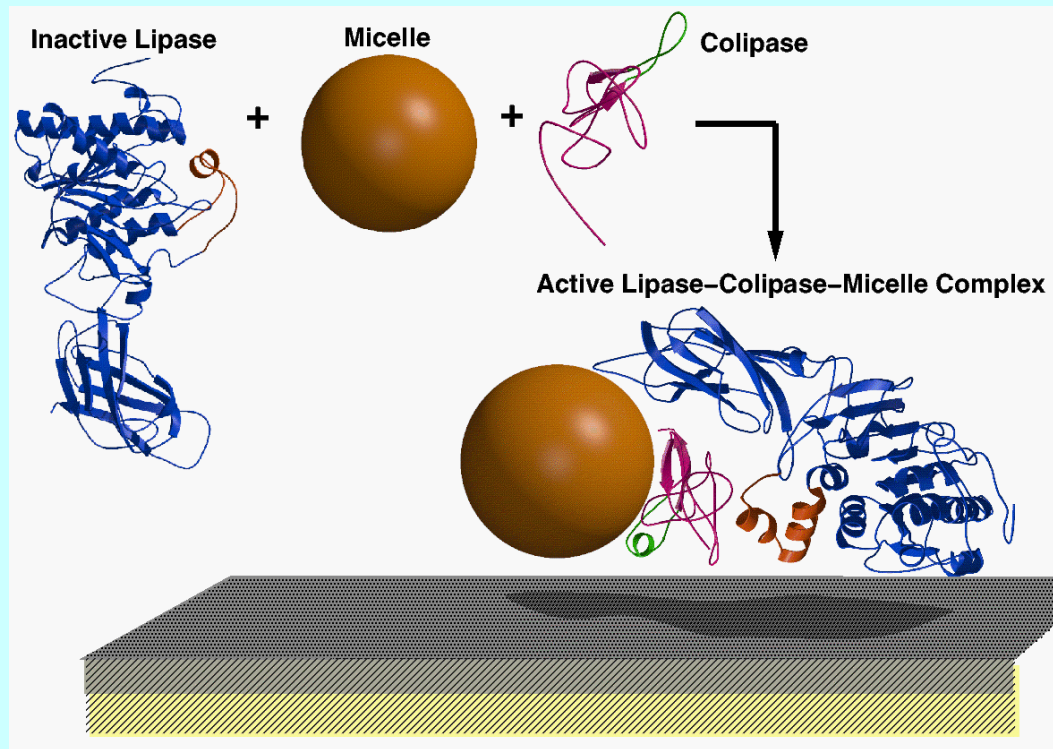
Biochemistry of lipase - I



Hydrolysis of Triacylglycerols

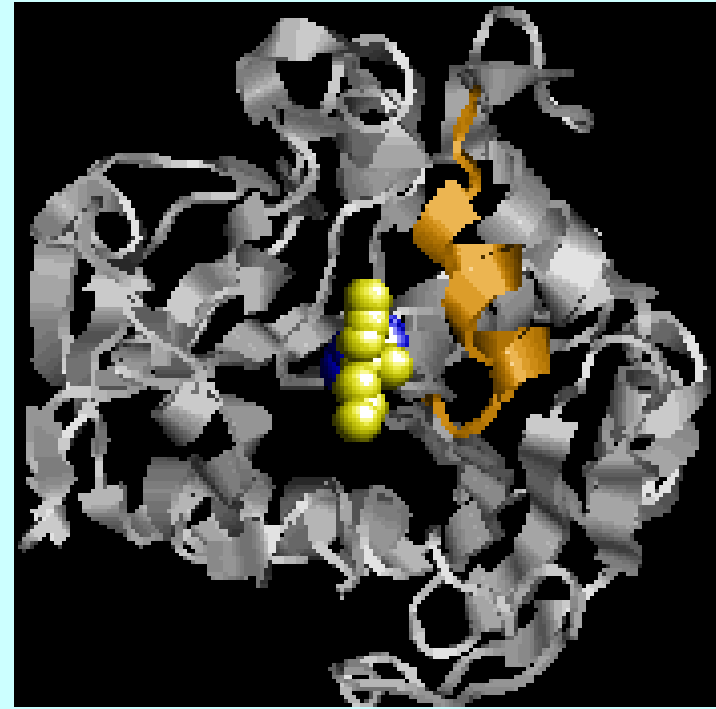
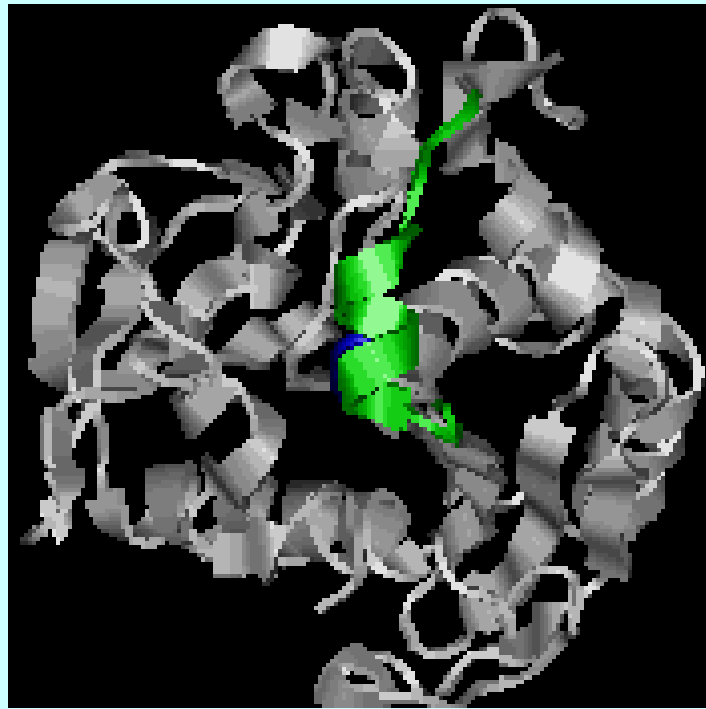


Biochemistry of lipase - II



Activation of lipase: The catalytic activity of most lipases depends on the aggregation state of their substrates. It is thought that activation involves the unmasking and structuring of the enzyme's active site through conformational changes requiring the presence of oil-in-water droplets. Since the micelle and substrate binding sites concern different regions of the protein complex, it is mediated by colipase and a micelle. In vivo, the formation of a complex between inactive pancreatic lipase, colipase and a mixed micelle activates the enzyme by stabilizing the open conformation and exposing a large hydrophobic surface. This surface should facilitate complex binding to the underlying triglycerides of the emulsified duodenal oil particle.

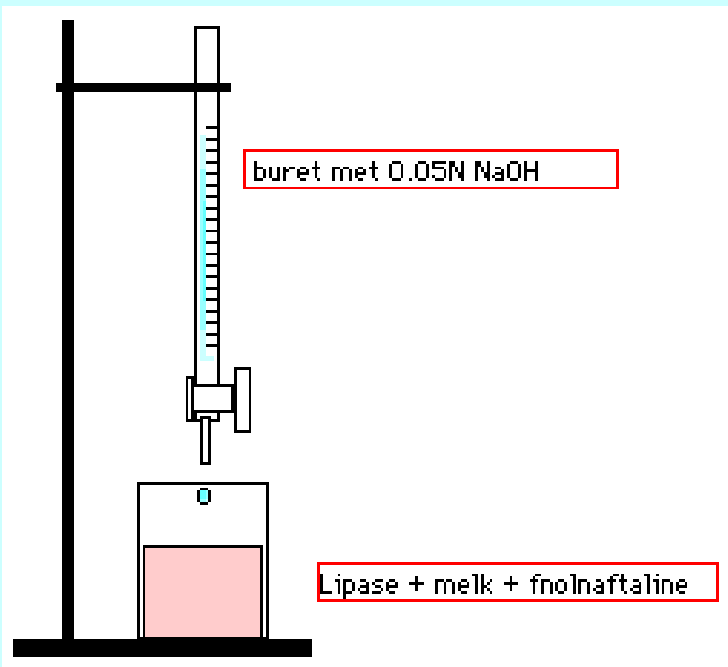
Biochemistry of lipase - III



Left: This is lipase. This enzyme hydrolyzes esters of lipids which are found in the interface between lipid and water. In water, access to the active site serine is blocked by a helix.

Right: When lipase binds to the the interface created by its substrate, the helix moves allowing the substrate to bind at the active site.

Measurement of pancreatic lipase - I



pH-stat method:

This method serves as reference method. Trioleine is hydrolyzed enzymatically and the released oleic acid is continuously titrated to $\text{pH}=9.0$. Hydrolysis of one ester bond produces 1 H^+ which is titrated.

However, this method is tedious and cannot be used in clinical routine.

Measurement of pancreatic lipase - II

Hydrolysis of tri- and diglycerides:

The enzymatic release of fatty acids from glycerol carbonic atoms C1 or C3 causes a decrease of sample turbidity which can be directly determined. The emulsion is stabilized by means of sodiumdesoxycholate.

Some methods are based on an additional enzymatic reaction. The monoglyceride produced by lipase is cleaved by monoglycerolhydrolase into fatty acid and glycerol. The latter serves for a further detection reaction. In detail, glycerol is phosphorylated to glycerol-3-phosphate. The latter is oxidized and the produced H_2O_2 is determined by a Trinder reaction.

The precision of these methods in clinical routine is well.

Measurement of pancreatic lipase - III

Reference values:

Method	Temperature	Adults [U/l]	Children [U/l]	Infants [U/l]
pH-stat with olive oil	30°C	30-235		
Turbidimetry	25°C	<190	2-78	5-27
Photometry with 1,2 diglycerol	37°C	7-59		

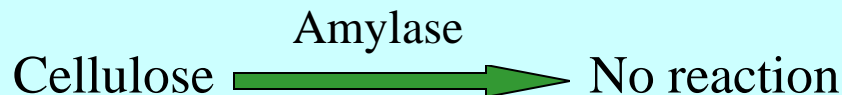
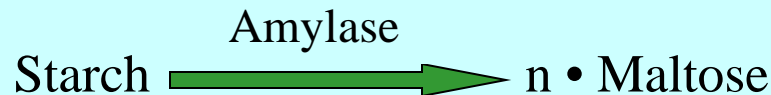
Amylases - I

Amylases:

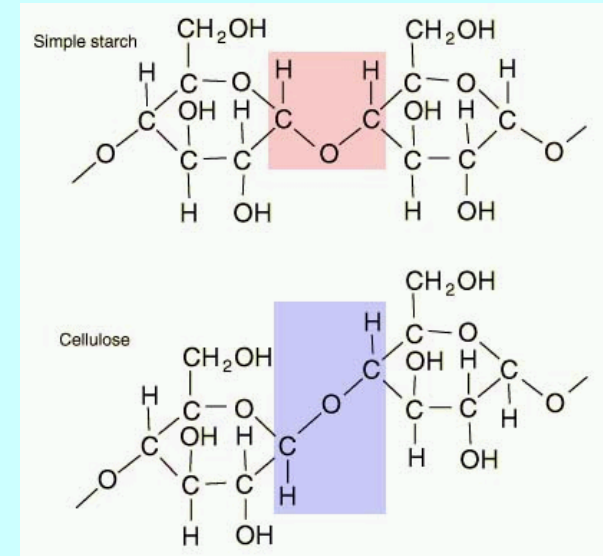
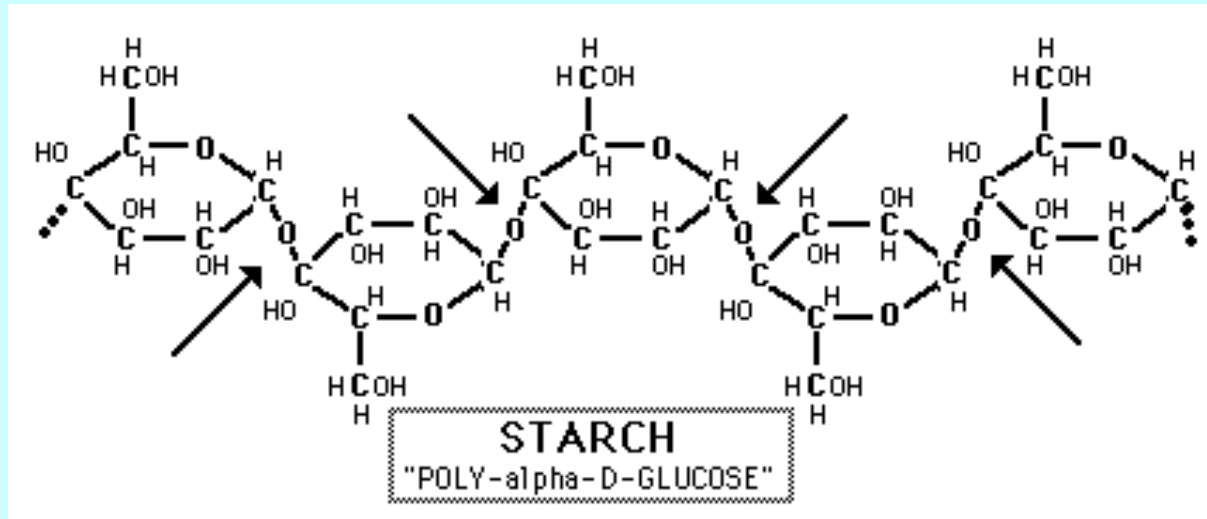
Molecular weight: about 50 kD.

The enzymes consist of only 1 protein chain and contains Ca^{++} as a cofactor. It is inactivated by heavy metals and Ca^{++} chelating agents and activated by Cl^- .

Relevant, amounts are produced in salivary glands and pancreas. The enzyme catalyzes the cleavage of starch but not cellulose although both carbohydrates consist of glucose; however, the glycosidic binding is different.



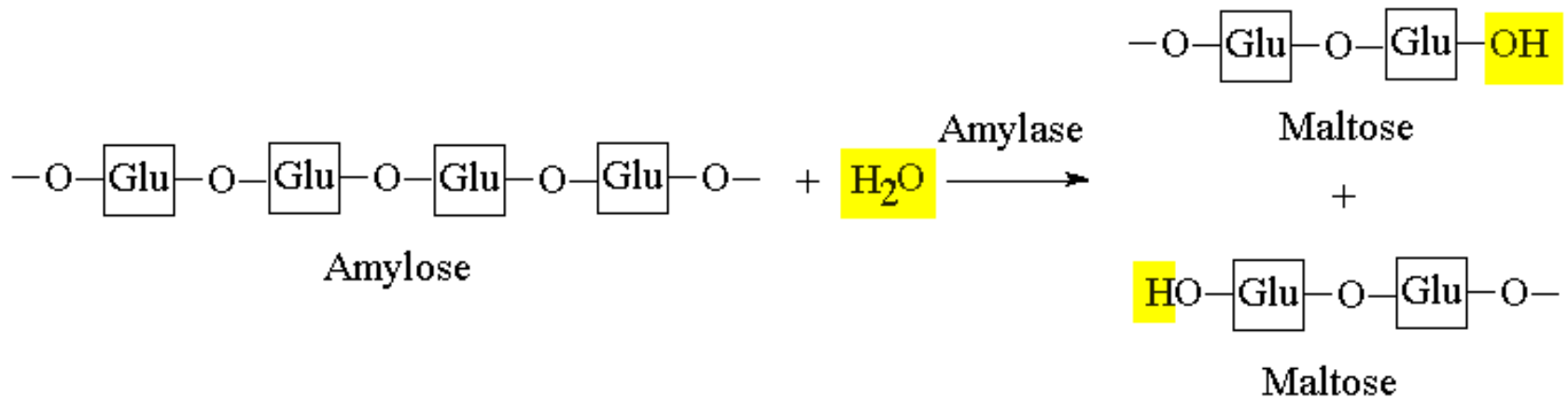
Amylases - II



α -amylase hydrolyzes saccharide bonds, which are those pointed at by the arrows in the figure. So imagine, a bunch of α -amylases randomly bouncing around (Brownian motion!) in among some extremely long starch molecules. Whenever a "mouth" - the enzymatic or active site - bounces against a saccharide bond, "snip", and the bond is broken (hydrolyzed as a water molecule is added "across" the saccharide bond). With that bond broken, the whole starch molecule is now in two pieces. The more "bites," the more and smaller pieces.

The **β -amylase** can only "chew" on the ends of a starch molecule, and only on one end and not both. It can only chew on the "reducing" end of starch, and that is the end far off the righthand side of the figure above. When β -amylase does its job, it bites off maltose units - in other words, two glucose units at a time.

Amylases - III



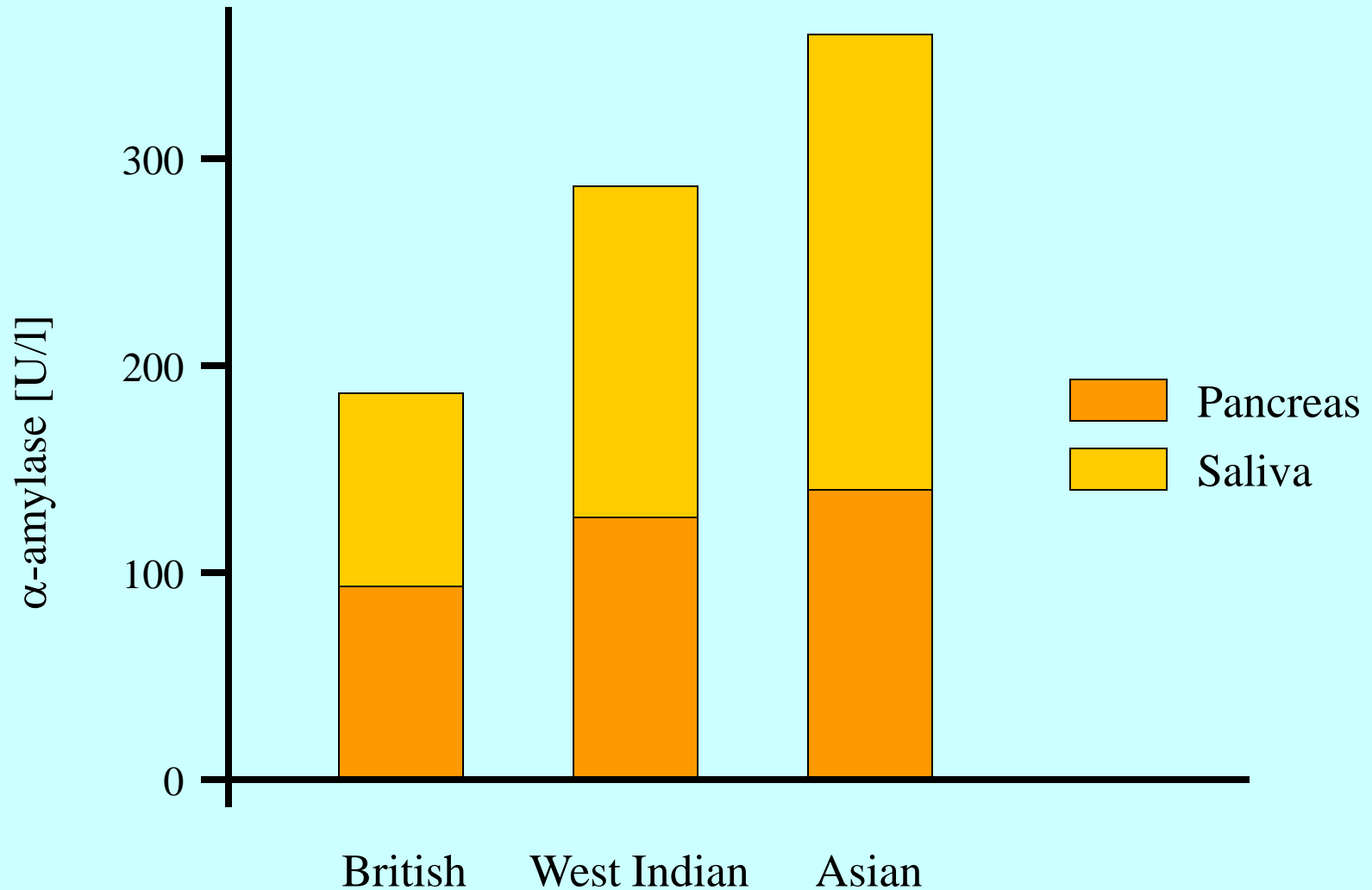
Cleavage of carbohydrate bonds by amylase

Amylases - IV

Types of amylases:

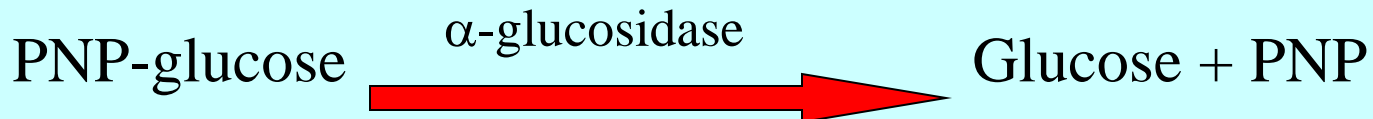
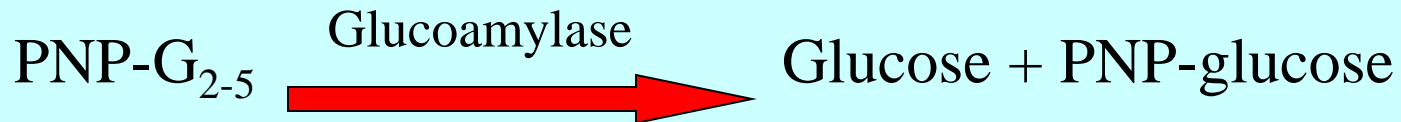
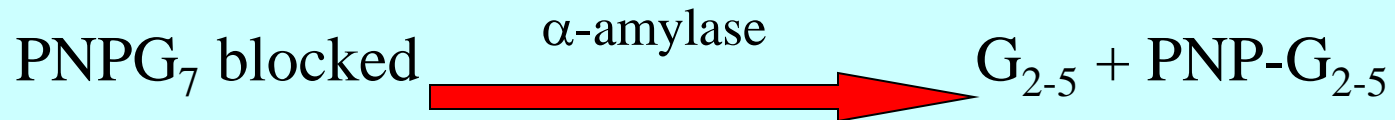
- Pancreas amylase: P-type α -amylase; 50 kD
- Salivary gland amylase: S-type α -amylase; 51 kD. Amino acid sequences of salivary gland amylase and pancreas amylase are 97 % homologous. Combination of two different monoclonal antibodies against the S-type inhibits ≥ 97 % of enzyme activity.
- S-type and P-type α -amylase are filtered in the renal glomeruli and reabsorbed in the tubuli to 50 %. The serum half-life time is 9.3-17.7 hours. Intact amylase may be determined in urine.
- Tumor amylase: Intestinal tumors are able to produce many isoforms of α -amylase.
- Macroamylases: Ig- α -amylase complexes. Because of their high molecular weight these complexes are not filtered in urine and have a longer serum half-life time.

Pancreatic and salivary α -amylase



Test for α -amylase

Kinetic colorimetric determination of α -amylase activity according to the following reaction:



PNPG_7 : p-nitrophenyl(G1)- α -D-maltoheptaoside

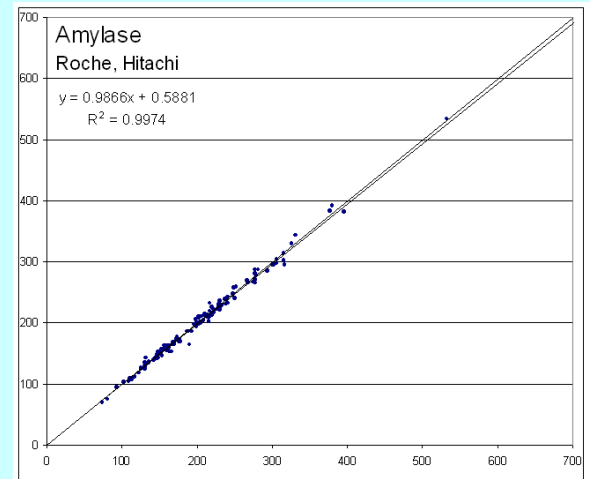
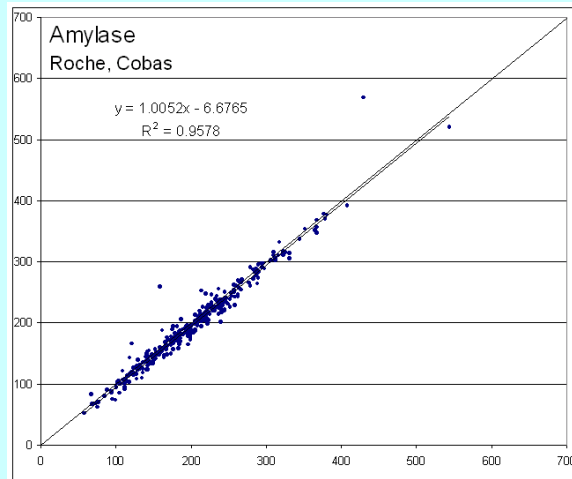
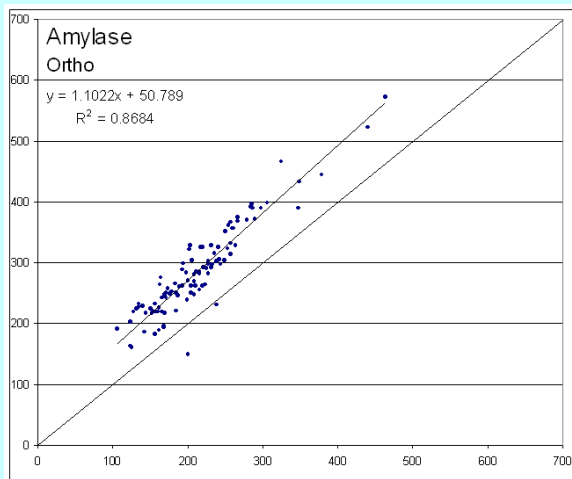
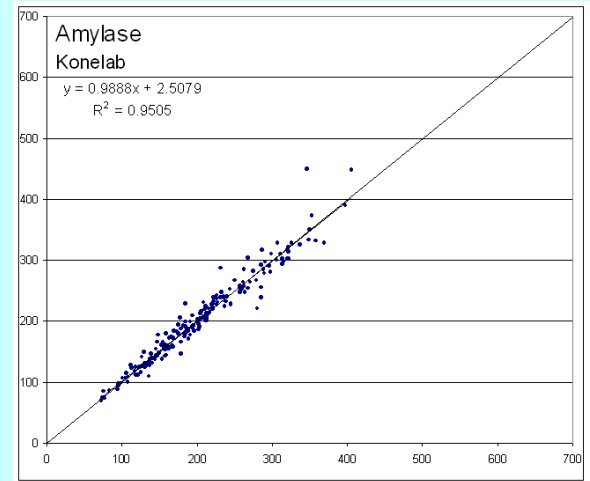
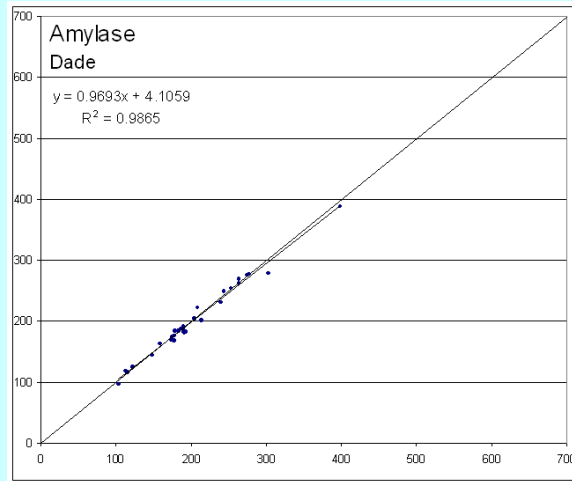
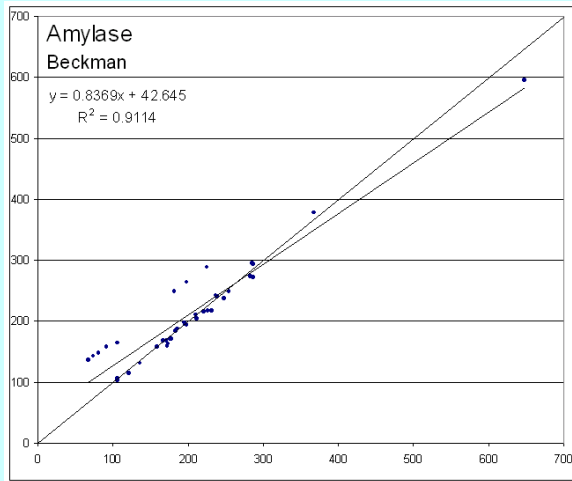
The rate of production of PNP is proportional to α -amylase activity in the sample.

Reference range of α -amylase - I

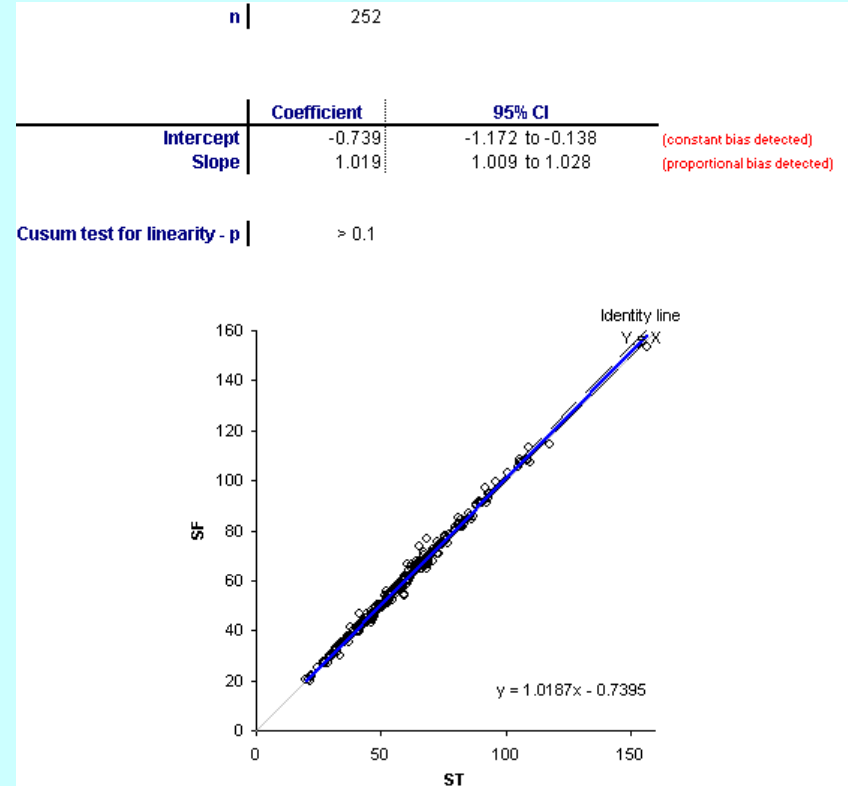
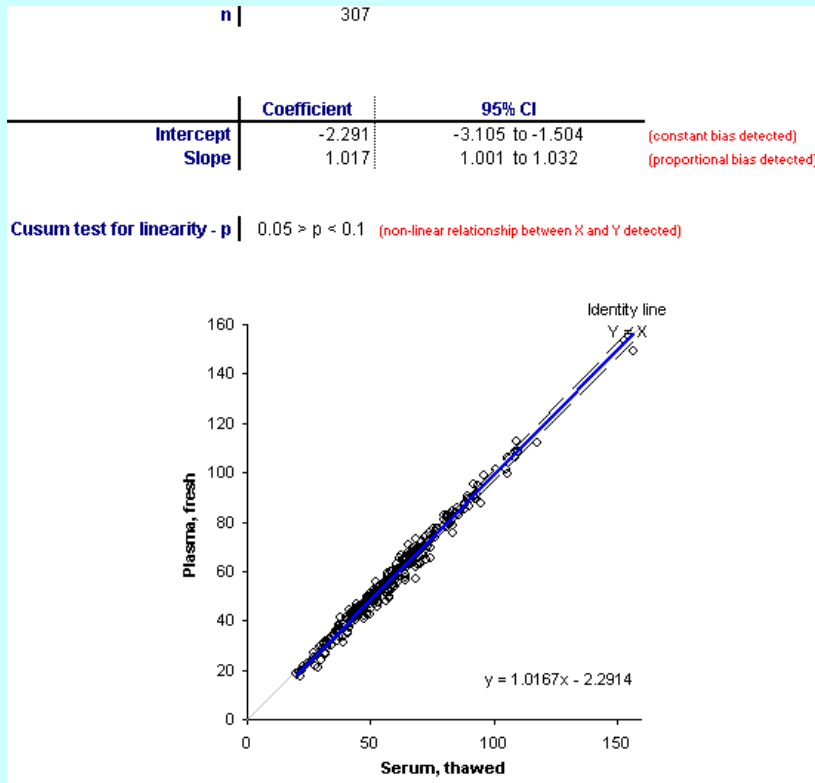
Determined product	Method	Temperature	Reference values	
			serum	urine
NADH	Maltotetraose (G4), maltosephosphorylase, β -phosphoglucomutase, glucose-6-phosphate-dehydrogenase, NAD	25°C	6-34	24-154
		37°C	25-125	
2-chloro-4-nitro-phenol	2-chloro-4-nitrophenyl- α -D-maltotrioside (CNP-G3)	25°C	23-130	<600
		37°C	69-210	<1050
2-chloro-4-nitro-phenol	2-chloro-4-nitrophenyl- β -D-maltoheptoside (CNP- β -G7), α -glucosidase, β -glucosidase	25°C	<120	<600
		37°C	<195	<1050
2-chloro-4-nitro-phenol	3-ketobutylidene(G5)-2-chloro-4-nitrophenyle (G1)- β -D-maltopentaoside(KB-CNP-G5), α -glucosidase, β -glucosidase	37°C	45-136	
		37°C	21-61 ^{#)}	
4-nitrophenol	6-benzyl(G5)-4-nitrophenyl(G1)- α -D-malto-pentaoside (Bz-G5), α -glucosidase, glucoamylase	25°C	17-56	<190
		37°C	42-116	<380
4-nitrophenol	p-nitrophenyl(G1)- α -D-maltoheptaoside (pNP-G7), α -glucosidase (2 monoclonal antibodies against salivary amylase) ^{#)}	25°C	28-141	<600
		37°C	46-244	<1000
		25°C	8-65 ^{#)}	<450
		37°C	17-115 ^{#)}	<800
4-nitrophenol	4,6-ethylidene(G7)-p-nitrophenyl(G1)- α -D-maltoheptaoside (EPS-G7) α -glucosidase (2 monoclonal antibodies against salivary amylase) ^{#)}	25°C	23-120	<753
		37°C	70-220	<1240
		25°C	8-65 ^{#)}	
		37°C	17-115 ^{#)}	
4-nitrophenol	4,6-benzyliden(G7)-p-nitrophenyl(G1)- α -D-maltoheptaoside (Bzn-G7), α -glucosidase, glucoamylase	25°C	<40	
		37°C	<82	

^{#)} pancreas amylase only

Comparison of different methods for determination of amylase - I

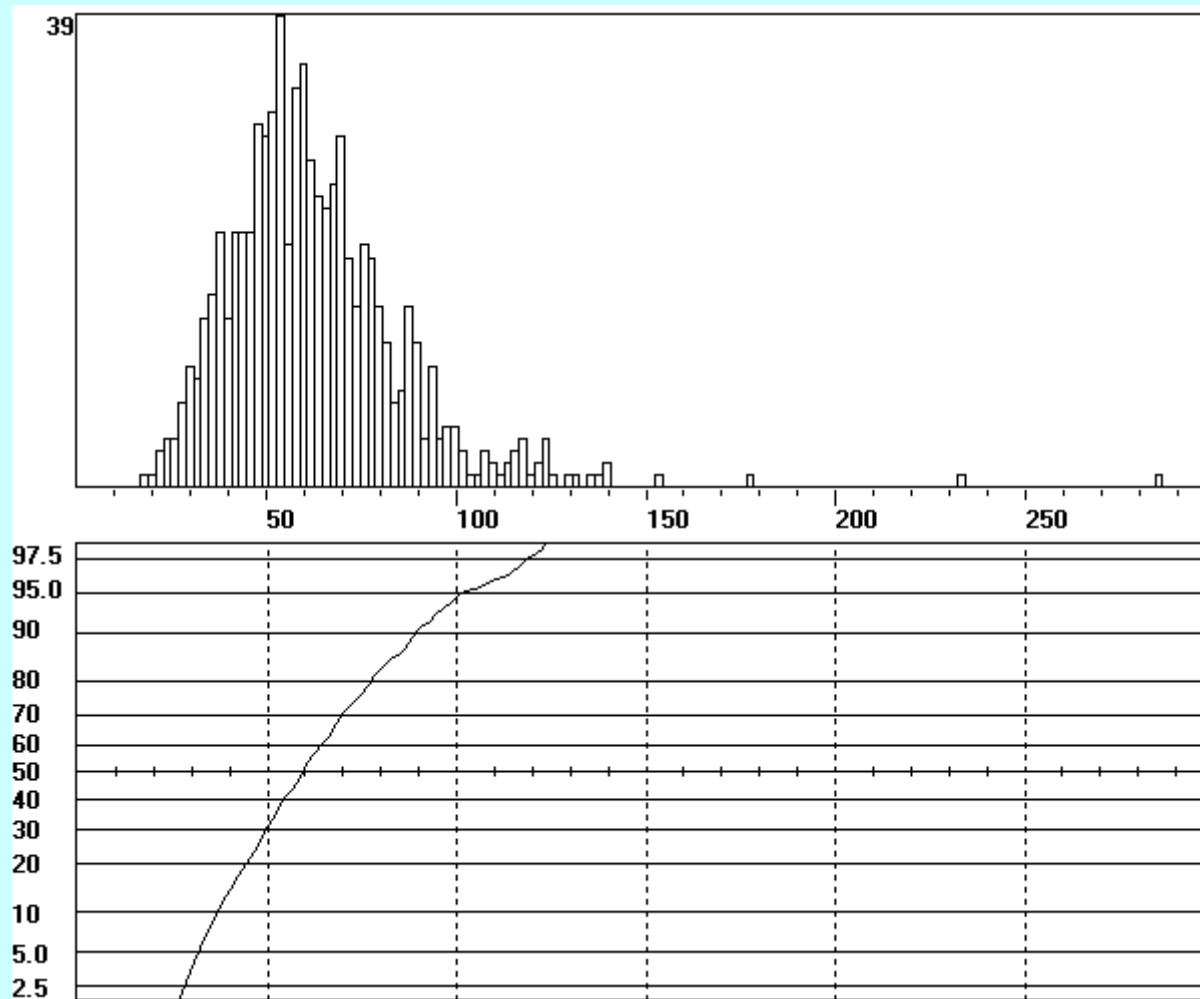


Comparison of different methods for determination of amylase - II



Left: Comparison thawed serum (x)/fresh plasma (y).
 Right: Comparison fresh serum/thawed serum.

Reference values of amylase



Distribution pattern of serum amylase activity

Laboratory findings in acute pancreatitis - I

Increased serum amylase begins after 3-6 hr, peaks at 20-30 hr, persists for 48-72 hr; some patients with severe disease may have normal values; no correlation exists between the severity of pancreatitis and the degree of serum amylase elevation. Amylase passes from the inflamed pancreas directly into the bloodstream or into the peritoneal cavity.

Increased serum lipase peaks at 72-96 hr and persists up to 14 days after serum amylase has returned to normal.

Increased urine amylase occurs 6-10 hr after serum amylase elevation; urine levels are higher and of longer duration than serum levels. This is believed to be due to a reversible renal tubular defect, which results in decreased amylase reabsorption.

Increased amylase-creatinine clearance ratio; amylase clearance by the kidneys is accelerated in acute pancreatitis; this also occurs in nonpancreatic diseases, such as diabetic ketoacidosis and extensive burns.

Decreased serum calcium occurs in severe acute pancreatitis; this is the result of calcium binding to fatty acids in fat, which undergoes from pancreatic enzyme action.

Laboratory findings in acute pancreatitis - II

Leukocytosis ($10.000/\mu\text{l}$ - $20.000/\mu\text{l}$)

Increased hematocrit reflects hemoconcentration.

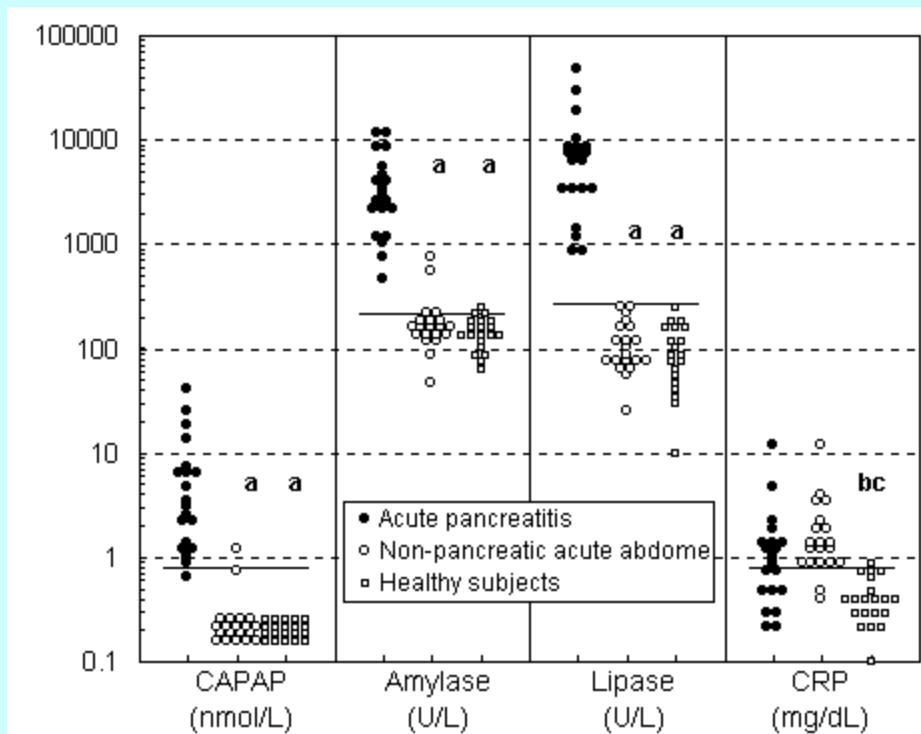
Increased blood glucose, transient-probably due to decreased release of insulin, increased release of glucagon, and increased output of glucocorticoids and catecholamines

Hypertriglyceridemia, usually in association with alcoholism

Increased erythrocyte sedimentation rate reflects acute inflammation.

Pleural or ascitic fluid with increased amylase and albumin; presence of blood in ascitic fluid occurs in haemorrhagic pancreatitis

Laboratory findings in acute pancreatitis - III



Individual values of serum carboxypeptidase B (CAPAP), amylase, lipase and C-reactive protein (CRP) in patients with acute pancreatitis, in those with non-pancreatic acute abdomen and in healthy subjects. The horizontal solid lines indicate the upper reference limit of each protein. Eighteen patients with non-pancreatic acute abdomen and 20 healthy subjects had no detectable serum concentrations of CAPAP (less than 0.63 nmol/L).

^a $P < 0.001$ vs. patients with acute pancreatitis

^b $P < 0.01$ vs. patients with acute pancreatitis

^c $P < 0.001$ vs. patients with non-pancreatic acute abdomen

CAPAP: Carboxypeptidase B

Laboratory findings in acute pancreatitis - IV

Poorer prognosis exists when three or more of the following are present:

Initial WBC > 16,000/ μ l

Initial blood glucose > 200 mg/dl

Decreased serum calcium < 8 mg/dl

Fall in hematocrit > 10 %

Rise in BUN > 5 mg/dl

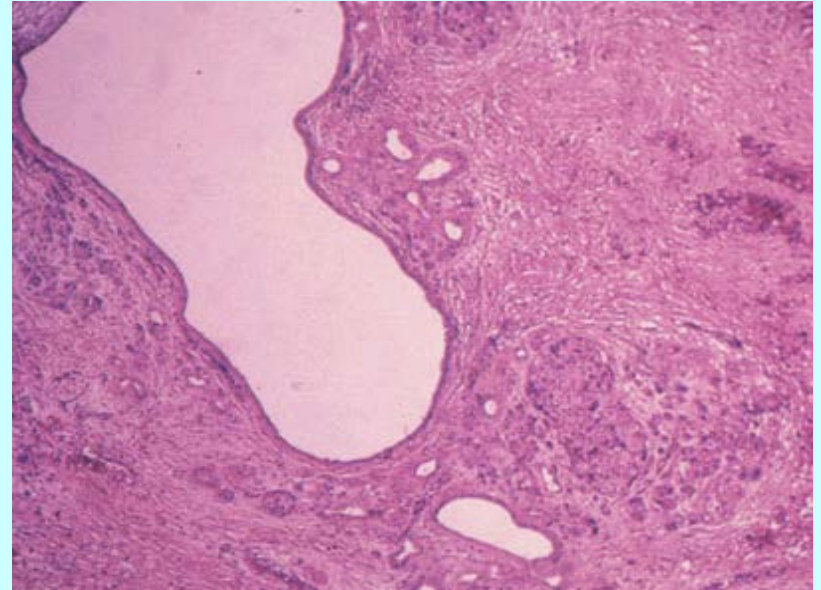
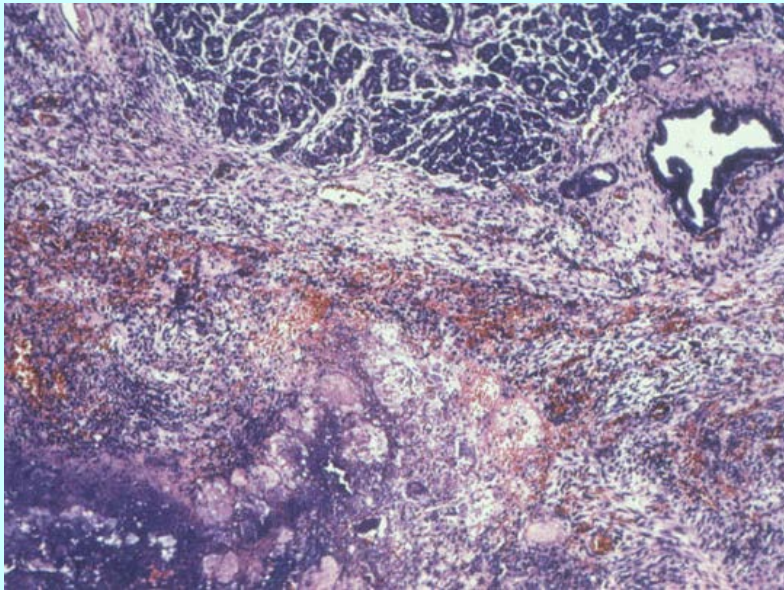
Arterial pO₂ < 60 mm Hg

Metabolic acidosis with base deficit > 4 mEq/l

Initial serum LDH > 350 IU/l

Initial serum ASAT (GOT) > 250 IU/l

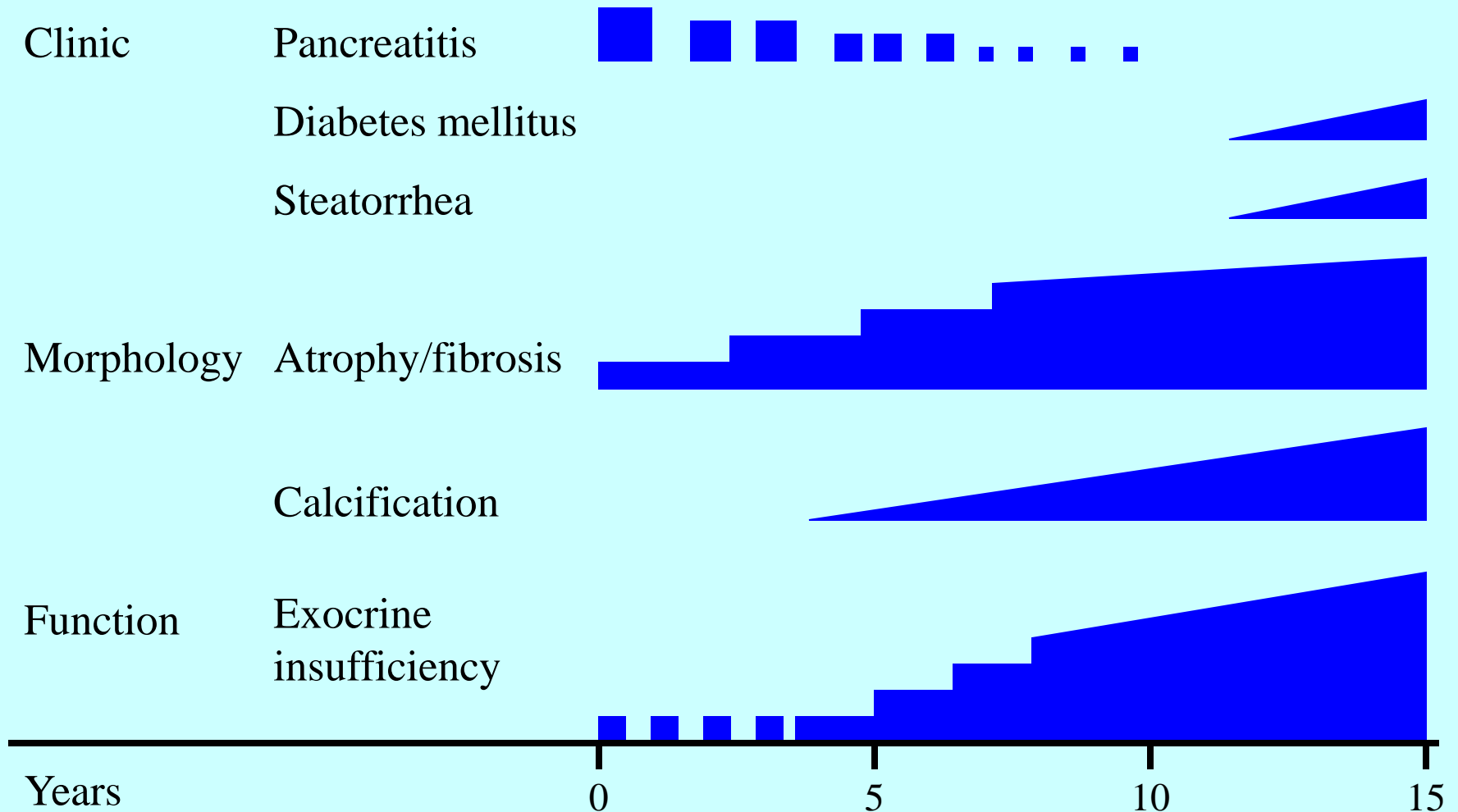
Histology of acute and chronic pancreatitis



Left: Acute pancreatitis. High concentration of inflammatory cells in the tissue.

Right: Chronic pancreatitis. High concentration of fibers in the tissue.

Time course of chronic pancreatitis



Stool fat analysis - I

Principle:	Pancreatic insufficiency correlates with maldigestion of fats because of a low secretion of lipase. In consequence, patients develop an increased fat excretion with stool, an impaired uptake of fat soluble vitamins and diarrhea. Nutritional uptake of fats must be >80 g/day.
Indication:	Exocrine pancreatic insufficiency (chronic pancreatitis)
Contraindication:	None
Pretreatment:	Enzyme supplements (hog elastase) must be discontinued 3 days before the test
Side effects:	None
Parameter(s):	Stool fat in 3 consecutive stools (sampling period 3 days)
Methods:	Titration after ester hydrolysis, near infrared reflectance analysis (NIRA)
Results:	Normal: <7 g/day total excretion; an excretion of 3 g/day is normal and caused by lysis of intestinal bacteria.
Problem:	Other diseases (malabsorption, obstructive biliary disease, bacterial growth in the ileum) cause false positive results.

Stool fat analysis - II

Ester hydrolysis:

A defined mass of a stool sample is hydrolyzed with ethanolic KOH. After boiling with 25 % HCl for 20 minutes fatty acids are released and consecutively extracted by means of ethanol and petrolether. In a next step the extracted fatty acids are titrated with NaOH using thymol blue as pH indicator. The excreted mass of fat can be calculated from the required NaOH volume, the sample volume and the total stool volume.

Near infrared reflectance analysis (NIRA):

The spectrum of light at a wavelength between $\lambda=700$ nm and $\lambda=2500$ nm (near infrared) reflected from a sample surface is characteristically affected by the sample composition. Determinants of the absorption bands are functional organic groups as CH, NH and OH and their surrounding matrix. Concentrations of stool fats, proteins and carbohydrates as well as the water content can be analyzed by means of specific rotation filters.

Clinical significance of ALP - I

Alkaline Phosphatase is of particular value in investigation of hepatobiliary and bone disease.

Liver Disease:

Liver synthesizes ALP in response to biliary tree obstruction (enzyme induction).

Relatively greater increases in serum activity (>3 fold) in extra-hepatic obstruction.

Parenchymal liver diseases show relatively less elevation in general.

Clinical significance of ALP - II

Bone diseases causing elevations of ALP are those with an element of bone remodelling:

Highest elevations are found in Paget's disease (x 10 - 20), reflecting increased activity of Osteoblasts

Osteomalacia / Rickets (x 2 - 4)

Hyperparathyroidism - primary or secondary

Osteogenic bone malignancies

Secondary deposits of malignancy in bone (e. g. breast, prostata)

Healing bone fractures

Physiological growth:

Post illness

Children

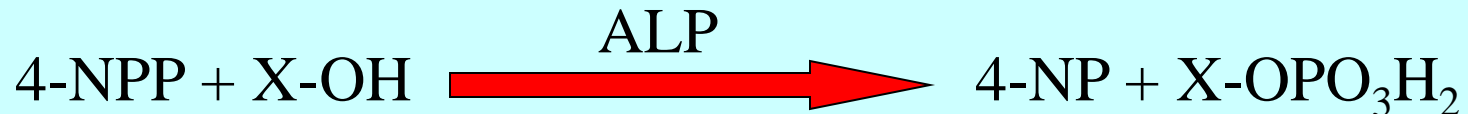
Puberty

Pregnancy

Measurement of total alkaline phosphatase (ALP) - I

Method of the International Federation of Clinical Chemistry (IFCC):

The phosphomonoesterase activity of ALP is determined by the cleavage rate of the substrate 4-nitrophenylphosphate (4-NPP). In presence of an aminoalcohol (X-OH) ALP acts as a phosphotransferase and carries a phosphate-group from 4-NPP to the aminoalcohol. The rate of the reaction is determined as an increase of the absorbance at $\lambda=405$ nm. The method is standardized on a temperature of 30°C.



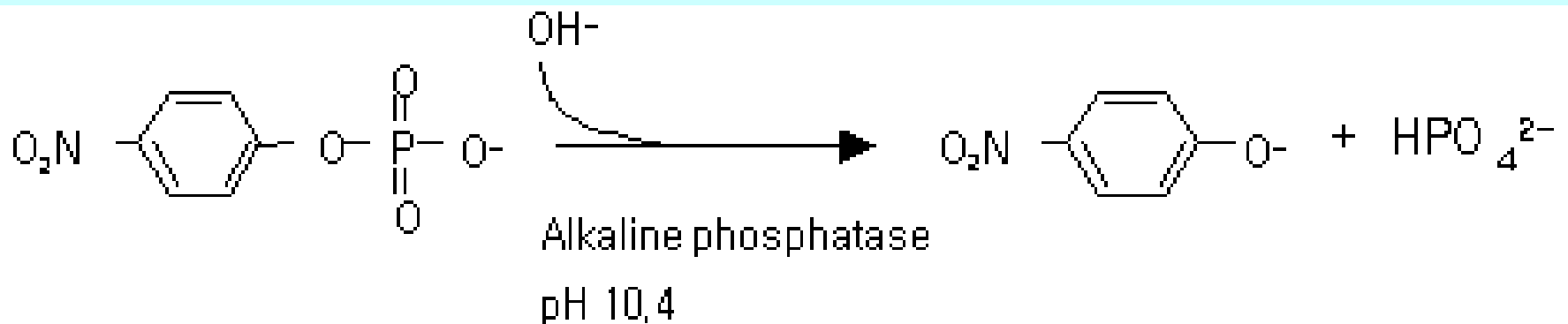
Method of the German Federation of Clinical Chemistry:

ALP catalyzes in a first step the transfer of the phosphate group to Diethanolamine (DEA) which is hydrolyzed in a second step to DEA and inorganic phosphate. The method is optimized on a temperature of 25°C.

Sample material:

Serum or plasma; no anticoagulation with chelating agents (citrate, EDTA, oxalate).

Measurement of total alkaline phosphatase (ALP) - II



Reference values of total ALP in plasma or serum

IFCC method at 30 °C (values given in [U/l]):

Females <60 years:	30 - 80 U/l
Males <60 years:	30 - 90 U/l
Males and females >60 years:	30 - 90 U/l

IFCC method at 37 °C (values given in [U/l]):

	Males	Females
1 - 30 days	75 - 316	48 - 406
1 month - 1 year	82 - 383	124 - 341
1 - 3 years	104 - 345	108 - 317
4 - 6 years	93 - 309	96 - 297
7 - 9 years	86 - 315	69 - 325
10 - 12 years	42 - 362	51 - 332
13 - 15 years	74 - 390	50 - 162
16 - 18 years	52 - 171	47 - 119
Adults	30 - 120	30 - 120

ALP isoenzymes - I

A number of biochemical/biophysical methods allow a differentiation of ALP isoenzymes. They are based on:

Differences in enzyme substrate specificity or physical characteristics (e. g. lectin-precipitation)

Different relative rates of reaction with enzyme substrates or inhibitors

Differences of heat stability

Immunological characteristics (best for placental and intestinal separation).

Electrophoretic mobility

ALP isoenzymes - II

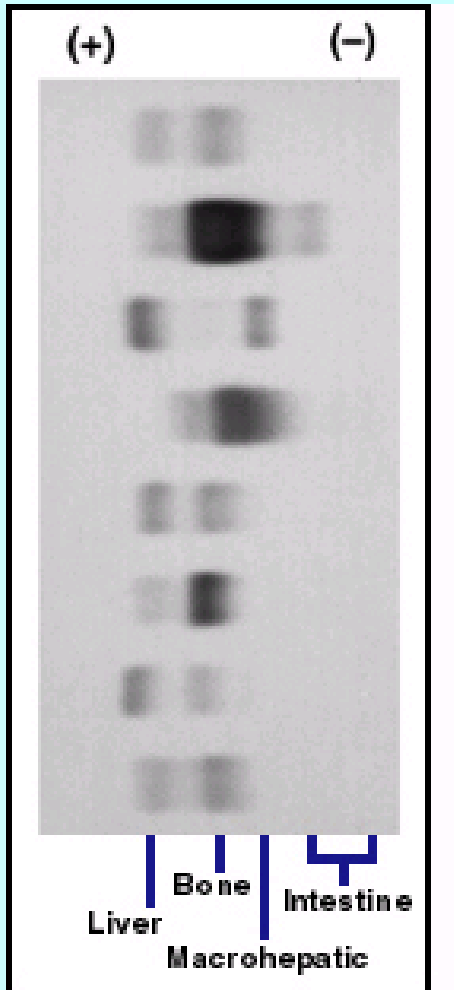
Lectin-precipitation:

After determination of total ALP activity bone ALP is precipitated by a lectin and the ALP activity is determined in the supernate. Bone-ALP can be calculated as the difference.

Chemical inhibition and heat stability:

ALP- isoenzyme	Phenyl- alanine 2.5 mmol/l	Homo- arginine 10 mmol/l	Leva- misole 1 mmol/l	65°C 10 min
Liver-bone- kidney	-	+	+	+
Placenta	+	-	-	-
Intestine	+	-	-	+

ALP isoenzymes - III



The alkaline phosphatase (ALP) isoenzymes found in human serum originate from several sources with the greatest activity occurring in the bone, liver, intestine, and placenta. Because of wide distribution of alkaline phosphatase in tissue, limited information can be obtained from a total ALP assay. Fortunately, the tissue sources of elevated ALP in serum can be determined by identifying the isoenzyme.

The isoenzymes of alkaline phosphatase are unique in that some organs have only one major isoenzyme rather than multiple isoenzyme forms. The isoenzymes of ALP differ in their physicochemical and electrophoretic properties, and it is by taking advantage of these differences that individual isoenzymes can be identified. In addition to the liver, bone, intestinal and placental isoenzymes, macrohepatic, Regan, PA, Nagao, and renal isoenzymes have also been identified in serum.

The resolution, accuracy and convenience of the TITAN GEL Alkaline Phosphatase High Resolution procedure make it better than heat denaturation, isoelectric focusing and wheat germ lectin techniques for separating ALP isoenzymes.

ALP isoenzymes - IV

Characteristics of the electrophoretic mobility of different ALP isoenzymes:

Liver isoenzyme moves more rapidly towards the anode

Bone ALP migrate slower and more diffuse, but overlaps

Intestinal ALP is slower still

Kidney ALP is the slowest

Placenta ALP moves as either liver or bone (pretreatment with neuraminidase improves resolution)

Note: The migration behavior depends on the used gel

ALP isoenzymes – V

Lectin-affinity electrophoresis:

Electrophoresis is performed in a lectin-containing agarose gel. The lectin binds bone alkaline phosphatase. In consequence, bone alkaline phosphatase cannot migrate in the gel whereas the other ALP isoenzymes migrate. The method allows a good differentiation between liver ALP and bone ALP.

Detection of ALP in the gels after electrophoresis:

Irrespective to the type of the electrophoresis technique used for differentiation of ALP isoenzymes a detection reaction based on the enzymatic properties of ALP is used for the detection of ALP in the gels. Densitometric scanning (e. g. by means of a laser densitometer) allows the relative quantification of ALP isoenzymes. If the total activity of ALP is known, the activities of ALP isoenzymes can be calculated.

Clinical indications for the determination of serum phosphatases

Alkaline Phosphatase

Primary bone tumors (e. g. osteosarcoma)

Secondary bone tumors (metastases)

Non-malignant bone diseases

 Paget's disease

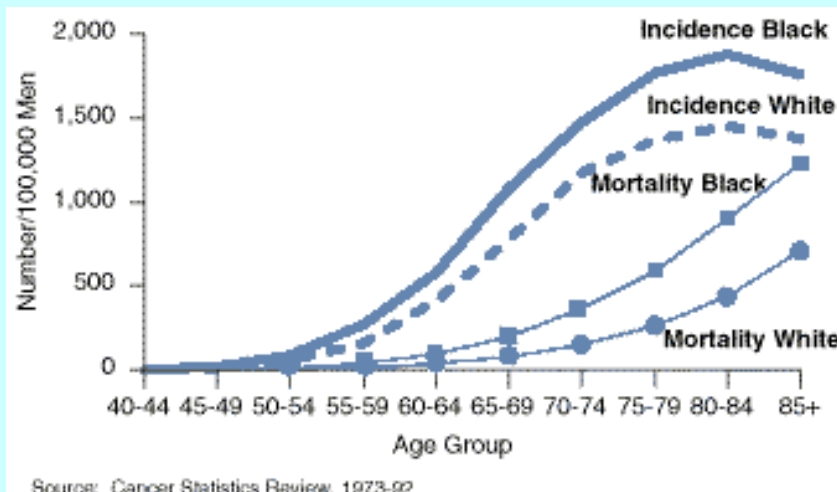
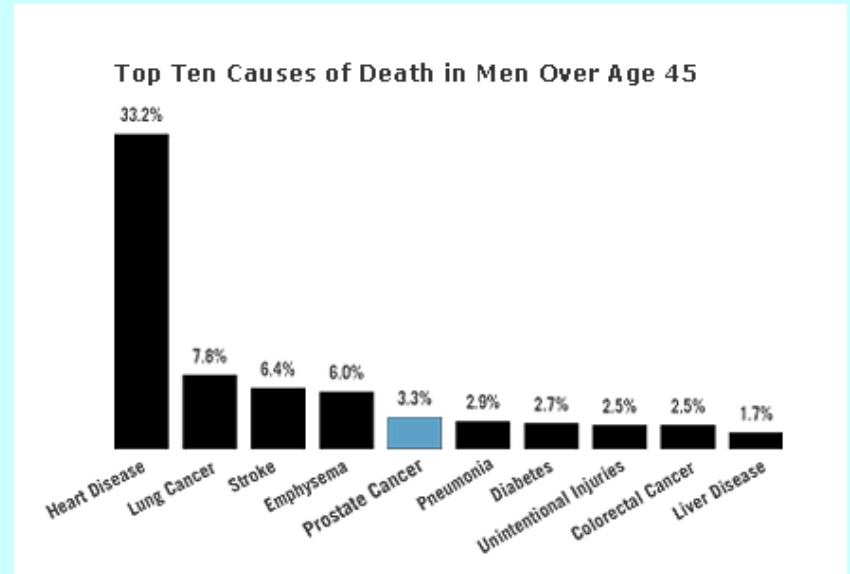
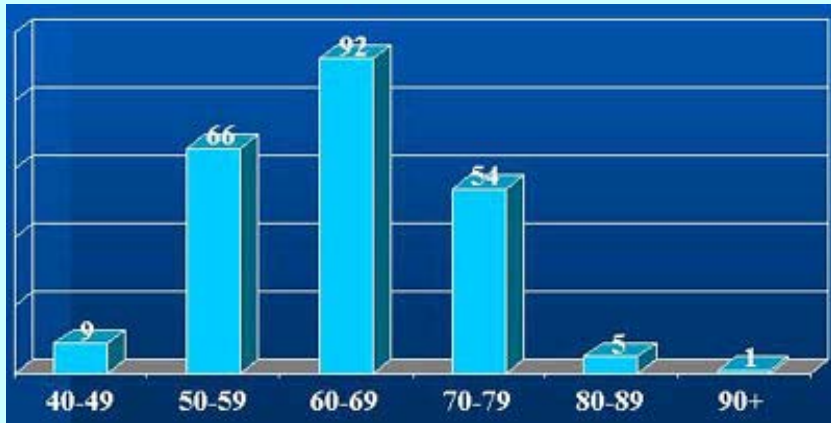
 Rickets and osteomalacia

Cholestatic liver disease

Acid phosphatase

Prostate cancer (and its metastases)

Epidemiology of prostate cancer

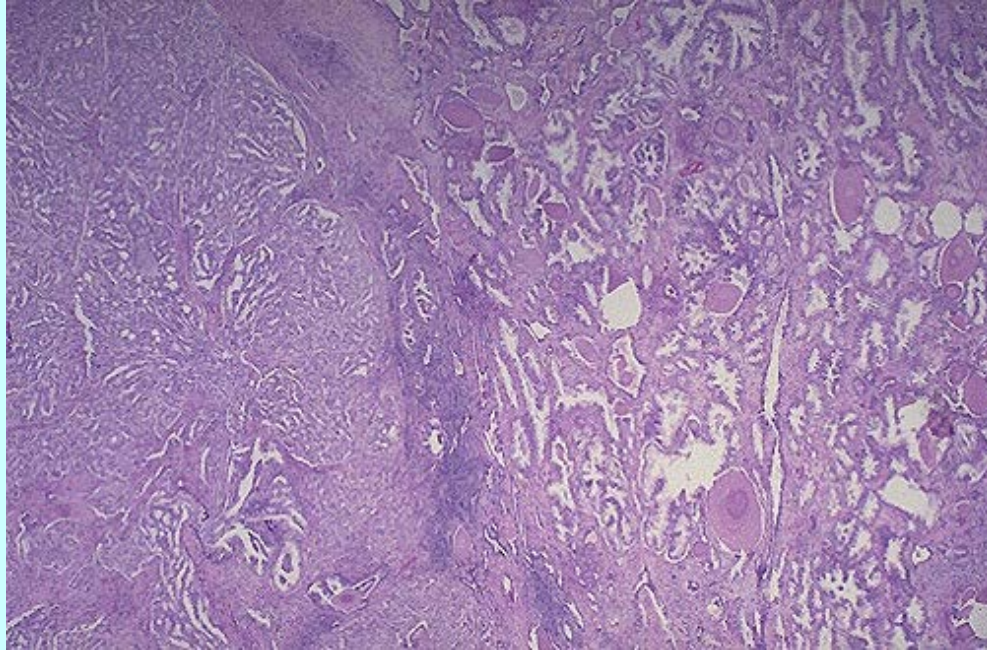


Top left: Age distribution

Bottom left: Effect of age and race

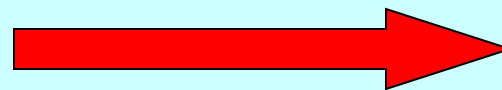
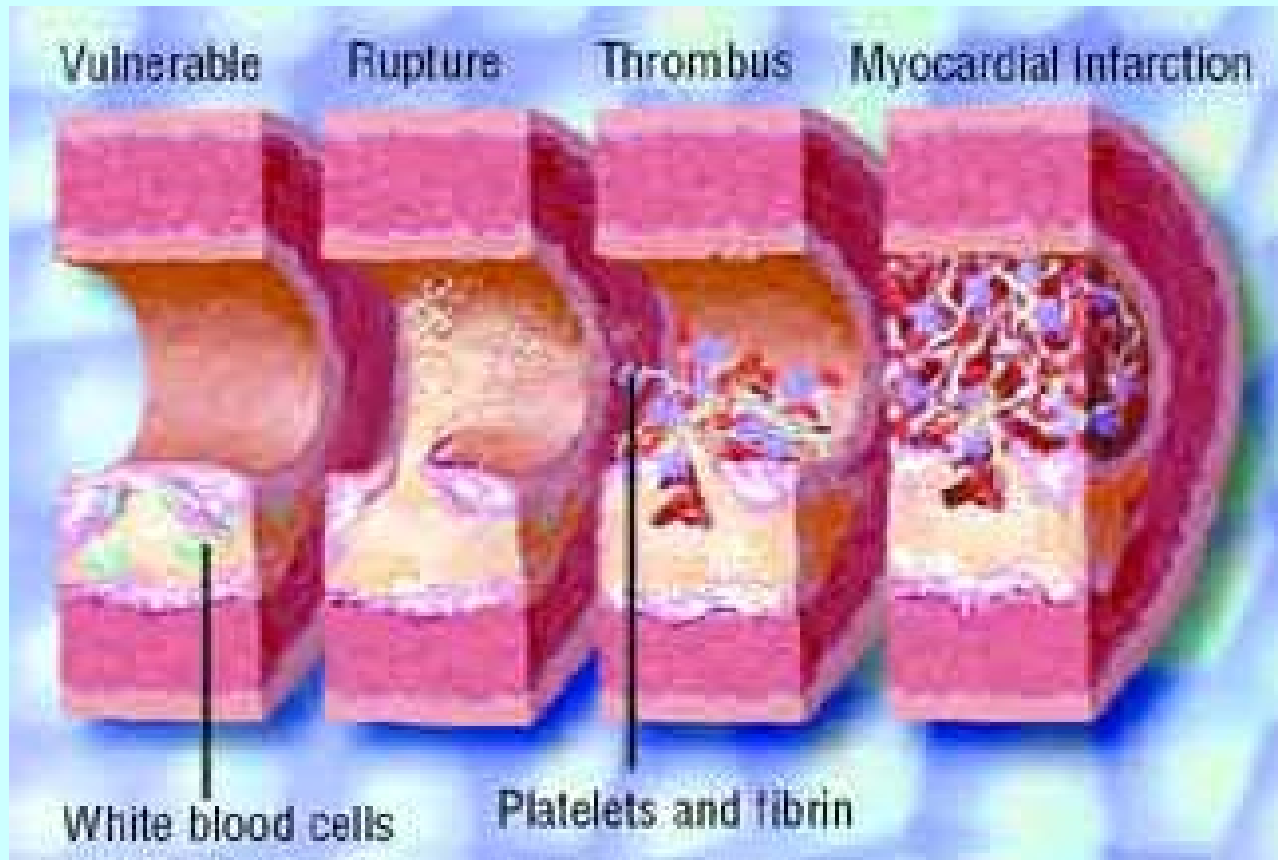
Top right: Mortality from tumors and other diseases in males

Prostate carcinoma



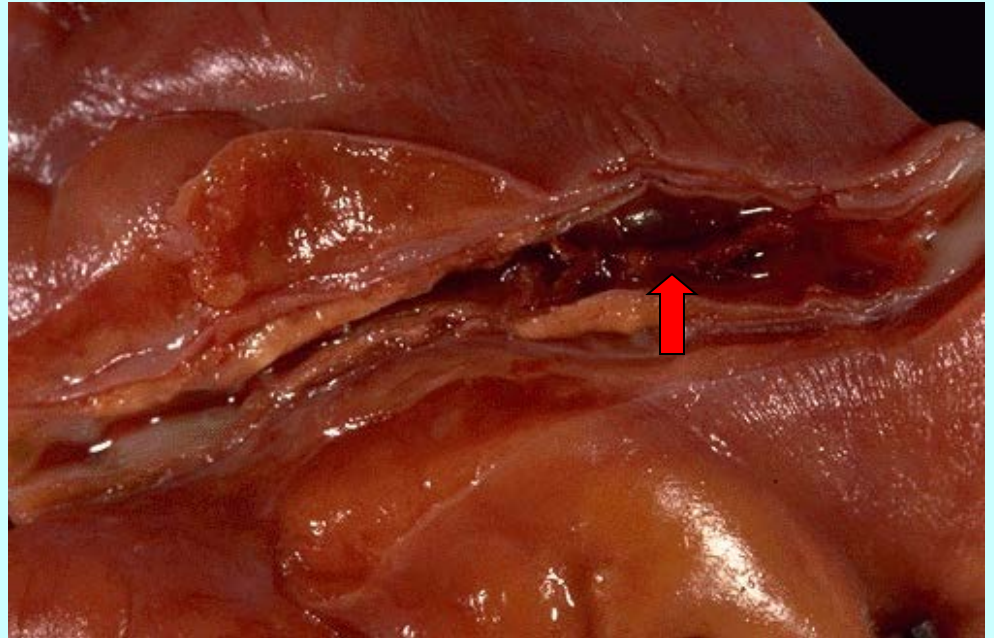
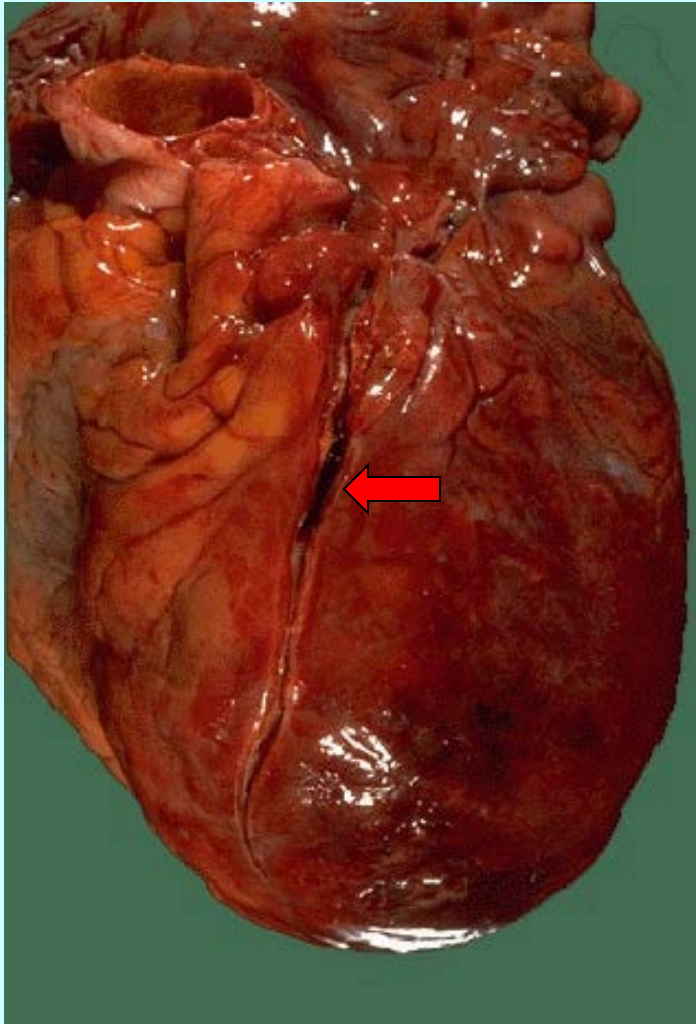
At the right are normal prostatic glands containing scattered corpora amylacea. At the left is prostatic adenocarcinoma. Note how the glands of the carcinoma are small and crowded. Prostatic adenocarcinomas are given a histologic grade.

Plaque rupture



Progression

Morphology of myocardial infarction



Left: Thrombosis in the left anterior descending coronary artery opened longitudinally here over the surface of the heart. This is another complication of atherosclerosis. The purpose of thrombolytic therapy (as with streptokinase or with tissue plasminogen activator, or TPA) is to dissolve recently formed thrombi and reestablish circulation before irreversible myocardial damage has been done, or at least to prevent additional myocardial injury.

Right: Thrombosis in a coronary artery. Such a thrombus severely narrows or occludes the lumen and can produce a sudden ischemic event. "Sudden death" as well as infarction can occur.

Laboratory diagnostics of myocardial infarction - I

Creatine kinase MB (CK-MB):

This is a standard criterion for detection of myocardial necrosis.

Levels begin to rise within 4 hours after injury, peak at 18-24 hours, and subside over 3-4 days.

Upper limit of reference range values for CK-MB is 3-6 % of total CK.

A level within the reference range in the emergency diagnostics does not exclude the possibility of myocardial necrosis.

A single assay in the emergency diagnostics has a sensitivity of 34 %.

Serial sampling over periods of 6-9 hours will increase the sensitivity to nearly 90 %.

Over 24 hours, the sensitivity is near 100 %.

Laboratory diagnostics of myocardial infarction - II

Myoglobin:

Myoglobin (MW: 17800 D) can be detected in plasma 2 - 4 hours after onset of the clinical symptoms. After 10 hours plasma concentrations are again within the reference values.

Myoglobin is not specific for myocardial cell necrosis. False positive test results are observed after intramuscular injection, degenerative muscle disease (e. g. Duchenne) and after physical activity.

Mean values of plasma myoglobin in patients with myocardial infarction are between 600 µg/dl and 1000 µg/dl.

Positive and negative predictive value of plasma myoglobin are 64 % and 98 %, respectively.

The myoglobin/carbonic anhydrase III ratio increases specificity. Myocardial cells do not release carbonic anhydrase.

Strong increases of plasma myoglobin are observed after vascular reperfusion (e. g. thrombolysis, PTCA).

Although myoglobin is filtrated into the urine, urinary myoglobin is usually negative or even slightly positive in patients with myocardial infarction.

Laboratory diagnostics of myocardial infarction - III

Troponin I:

This is a contractile protein that normally is not found in serum. It is released only when myonecrosis occurs.

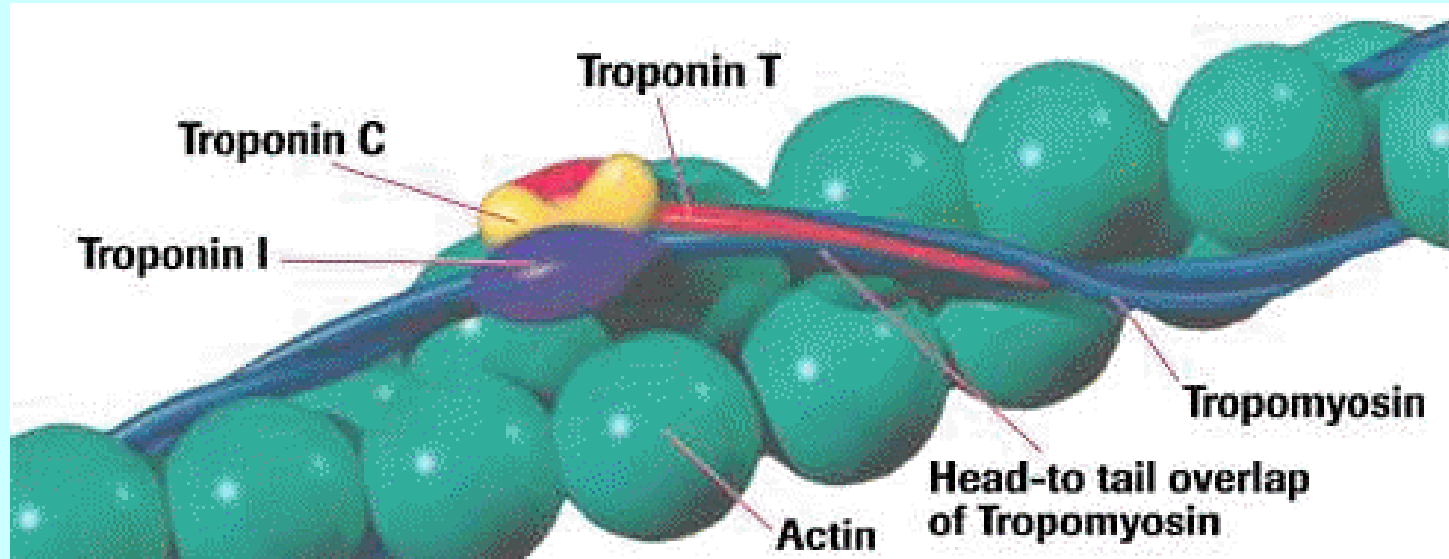
For early detection of myocardial necrosis, sensitivity of this study is superior to that of the CK-MB. Troponin I is detectable in serum 3-6 hours after an AMI, and its level remains elevated for 14 days.

The plasma concentration at days 3 and 4 correlates to the size of the myocardial infarction.

Troponin T:

Troponin T has similar release kinetics and specificity for myocardial necrosis, but it is slightly less sensitive than troponin I within the first 6 hours.

Function of troponins - I



Troponin T is part of the troponin complex which, in addition, comprises the subunit I and C (T for "tropomyosin binding", I for "inhibitory", and C for "calcium binding"). The complex is located on the thin filament of the contractile muscle apparatus and regulates the calcium mediated interaction of myosin and actin. Apart from their synergisms in the complex, troponin T, C and I are otherwise unrelated proteins.

Skeletal and cardiac troponin T isoforms differ from each other in amino acid sequence. Following myocardial damage, cardiac troponin T (cTnT) is released into blood within 2 to 6 hours after the event and can be detected with immunoassays based on specific antibodies.

Diagnostic value of CK-MB and troponin in myocardial infarction

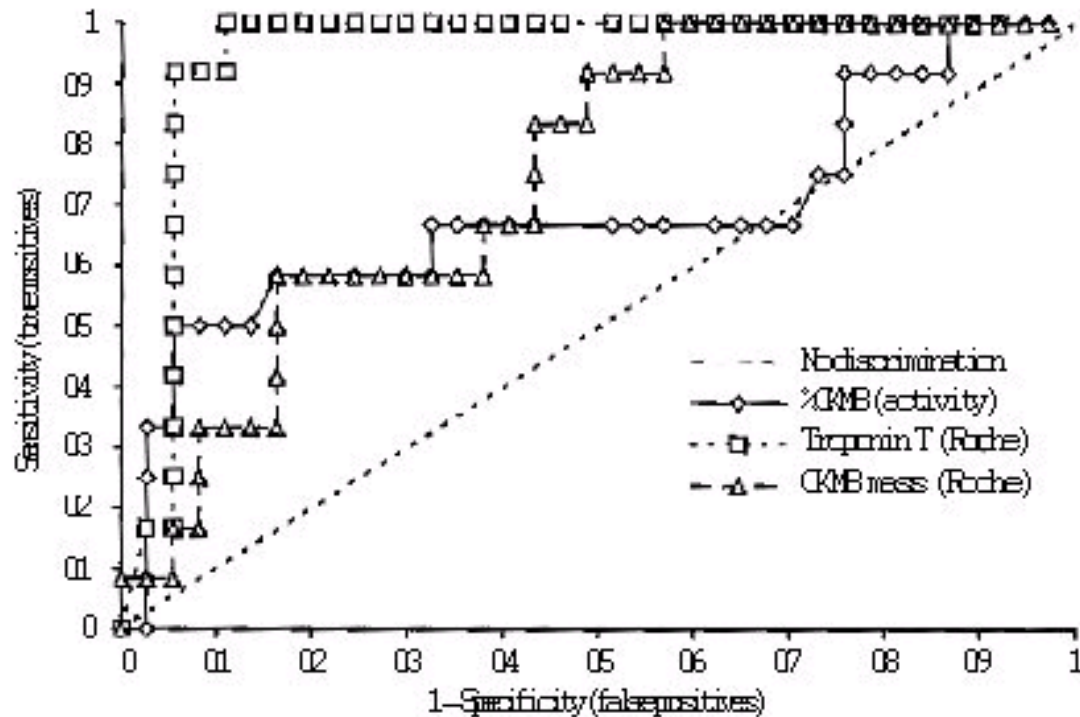
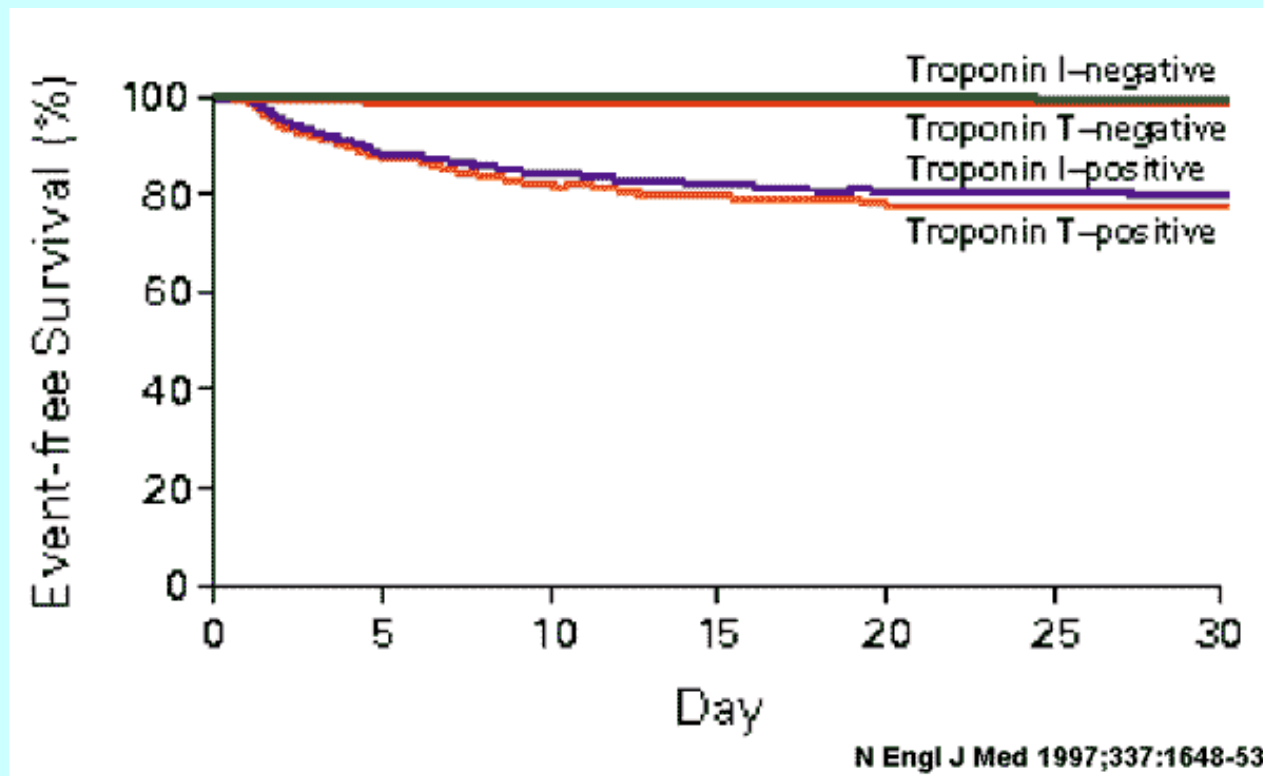
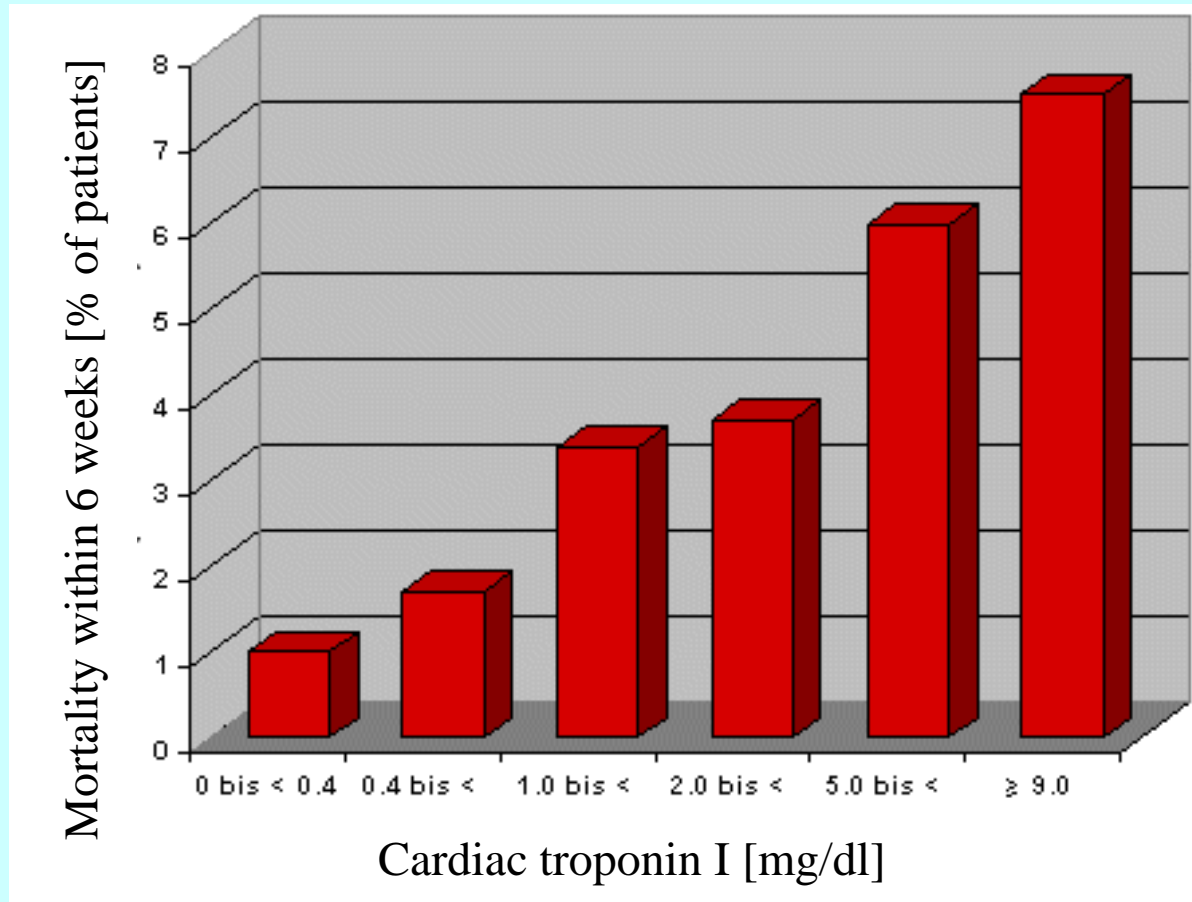


Fig 1 - Receiver Operating Characteristic (ROC) Curve for %CKMB (activity), CKMB mass (Roche) and Troponin T.

Troponin and mortality after myocardial infarction and unstable angina pectoris - I



Troponin and mortality after myocardial infarction and unstable angina pectoris - II



The higher the concentration of troponin the higher the mortality within 6 weeks after the event.

Laboratory diagnostics of myocardial infarction - IV

Lactate dehydrogenase (LDH):

LDH rises above the reference range within 24 hours of an AMI, reaches a peak within 3-6 days, and returns to the baseline within 8-12 days. However, LDH is not specific for myocardial infarction.

α -hydroxybutyrate dehydrogenase (HBDH):

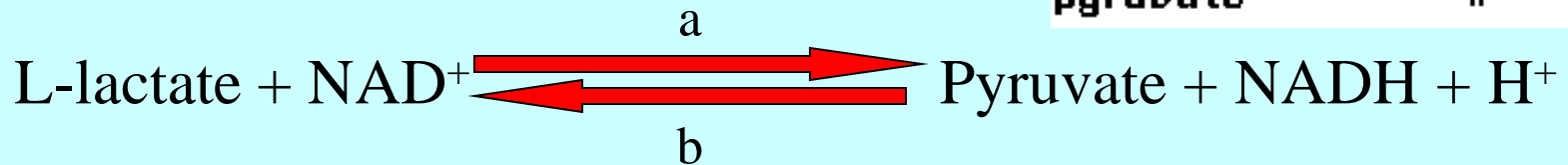
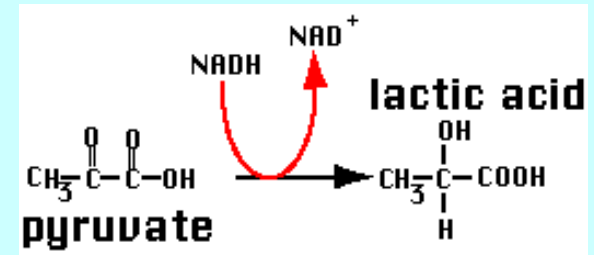
LDH-1 and LDH-2 isoenzymes are able to catalyze the reaction of α -hydroxybutyrate and are therefore also named α -hydroxybutyrate-dehydrogenase. Values increase 6 - 12 hours after infarction and become maximal after 1 - 3 days before getting normal after 7 - 15 days.

Aspartate aminotransferase (ASAT):

ASAT is a cytoplasmatic and mitochondrial enzyme which is found in most cells. High concentrations are observed in heart and skeleton musculature. Infarction causes an increase of enzyme activity in plasma after 4-8 hours. Maxima are achieved after 12 - 48 hours and values are again normal after 3 - 6 days.

Measurement of LDH and HBDH in plasma

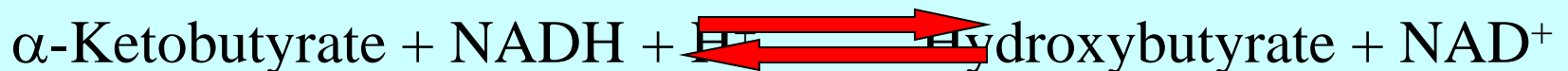
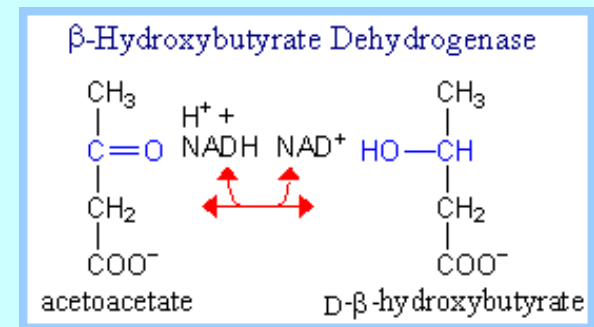
Principle of the LDH reaction:



a: pH 8.8 - 9.9

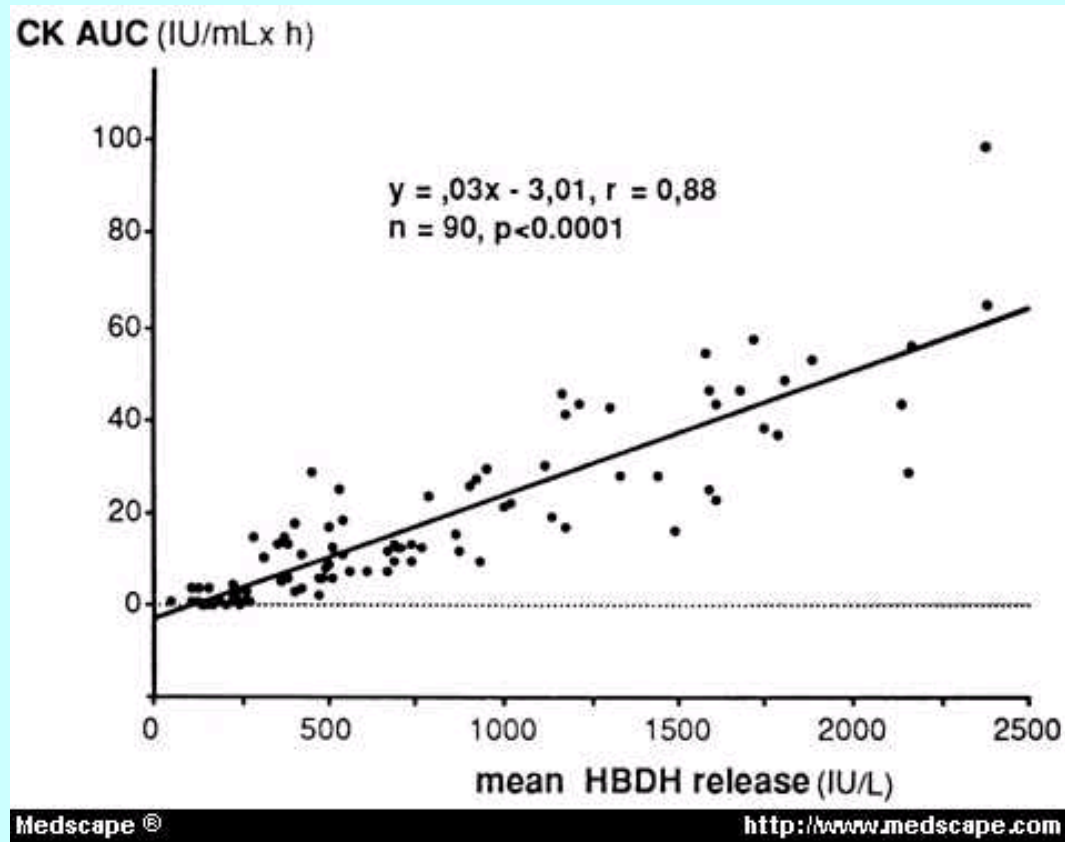
b: pH 7.4 - 7.8

Principle of the HBDH reaction:



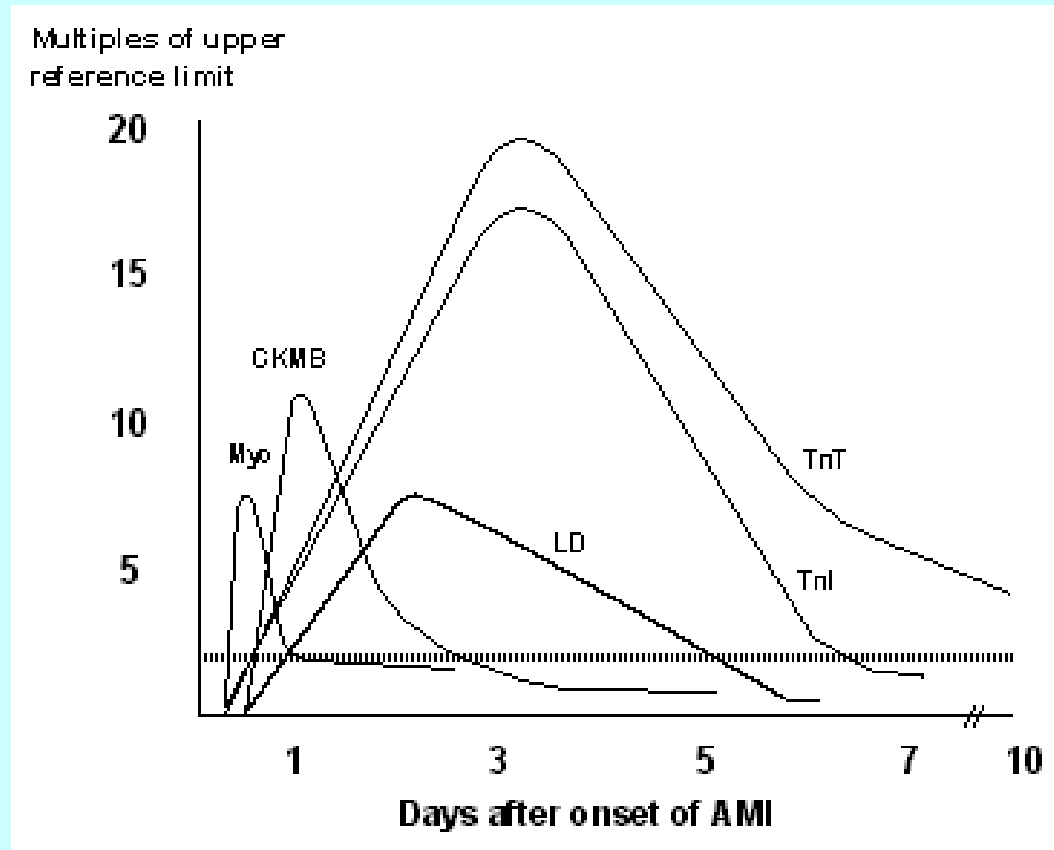
LDH-1 and LDH-2 isoenzymes are able to catalyze the reaction of α -hydroxybutyrate and are therefore also named α -hydroxybutyrate dehydrogenase

α -HBDH and myocardial infarction - I



Correlation between infarct sizes measured by CK AUC and mean HBDH release for 90 study patients.

Release patterns of cardiac markers after acute myocardial infarction (AMI) - I



Myo: Myoglobin

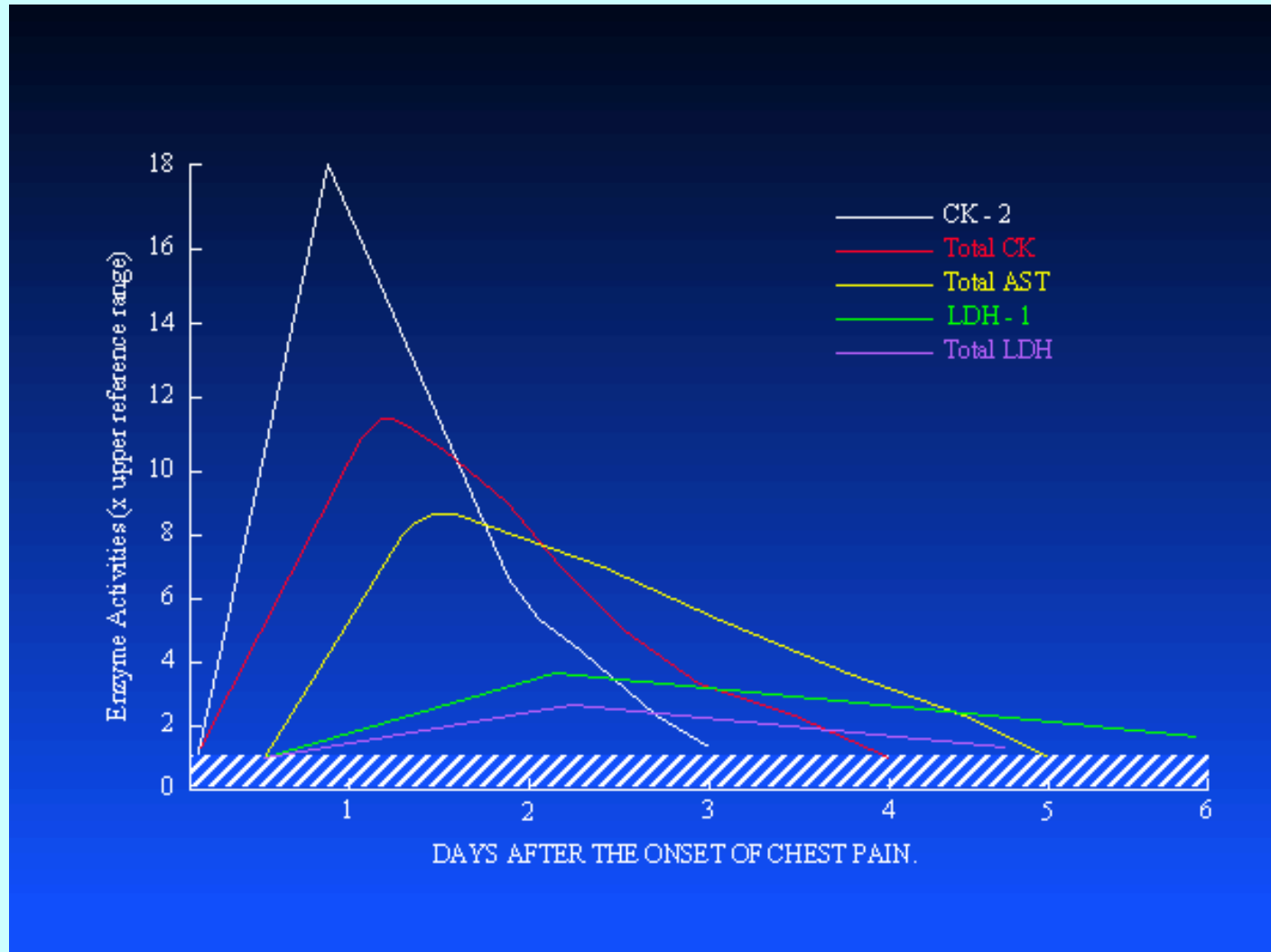
TnT: Troponin T

LD: Lactate dehydrogenase

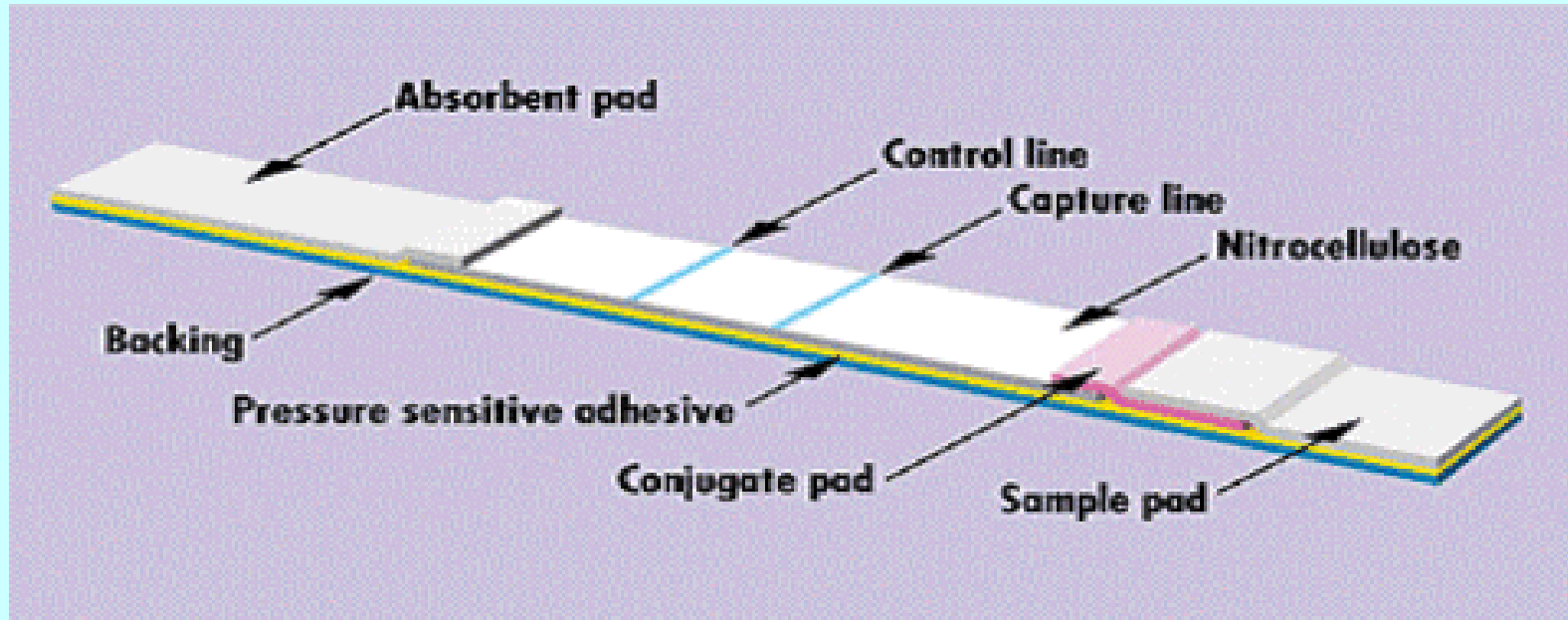
CKMB: Creatine kinase-MB fraction

TnI: Troponin I

Release patterns of cardiac markers after acute myocardial infarction (AMI) - II



Self-tests for myocardial infarction



Self-test for FABP:

Fast, reliable and sensitive immunosensor for the detection of the early infarction marker, fatty acid-binding protein (FABP) which is as easy to use as over-the-counter pregnancy tests and which gives a result within a few minutes. This test, based on antibody recognition, is performed on a membrane-like paper and does not need sample pretreatment. A drop of blood is put onto the device without any additional steps and then the result is a diagnosis of AMI.

Self-test for myocardial infarction from Vitest - I

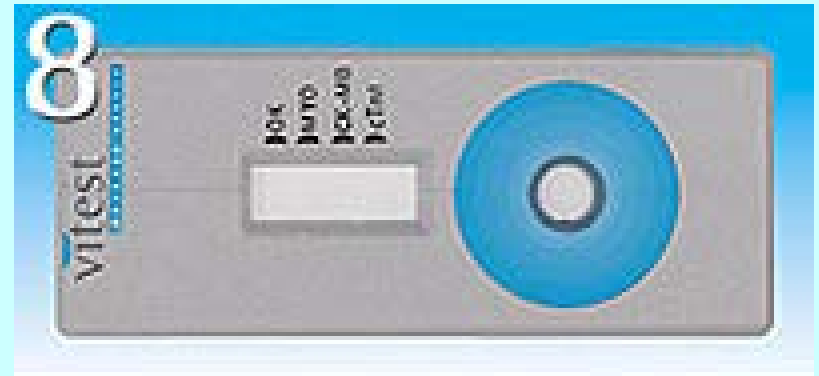


Self-test for myocardial infarction from Vitest - II



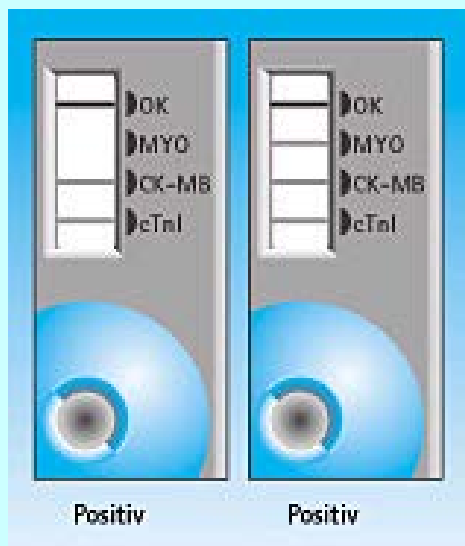
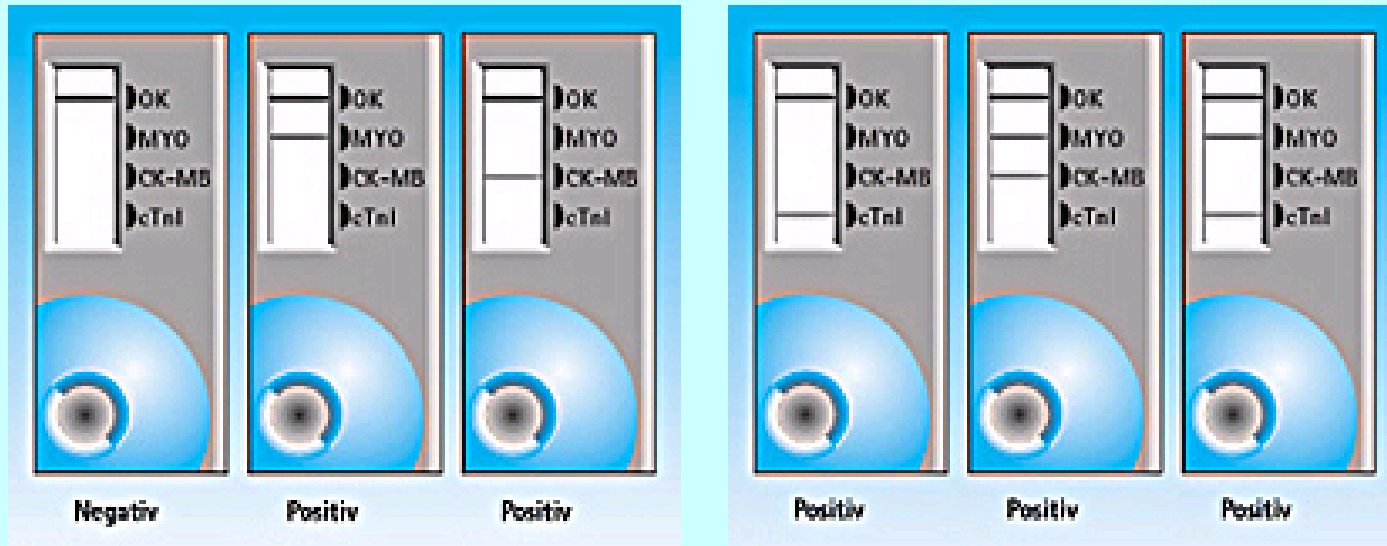
1 - 4: Description of blood sampling

Self-test for myocardial infarction from Vitest - III



5 - 8: 50 μ l blood are sampled by a pipette and transferred to the device. Then 2 drops of the buffer solution are added and the device is incubated at room temperature for 15 minutes.

Self-test for myocardial infarction from Vitest - IV



OK: Control band (must be visible in all test results; if not, the test must be repeated)

MYO: Myoglobin

CK-MB: Creatine kinase MB

cTnI: Cardiac troponin I

Musculature damage

Following markers can be used for diagnostics and monitoring of muscular damage:

Aspartate aminotransferase (ASAT)

Alanineaminotransferase (ALAT)

Lactate dehydrogenase (LDH)

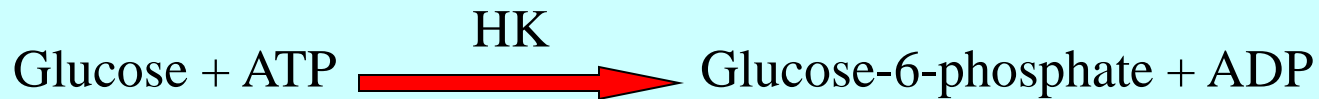
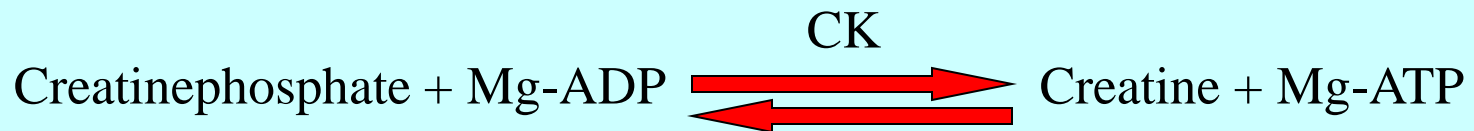
Creatine kinase (CK)

Myoglobin

Except myoglobin these markers are not tissue specific.

However, determination of LDH and CK isoenzymes may help to discriminate between muscular and non-muscular diseases (e. g. liver and musculature for LDH) and different types of musculature (heart and skeleton for CK).

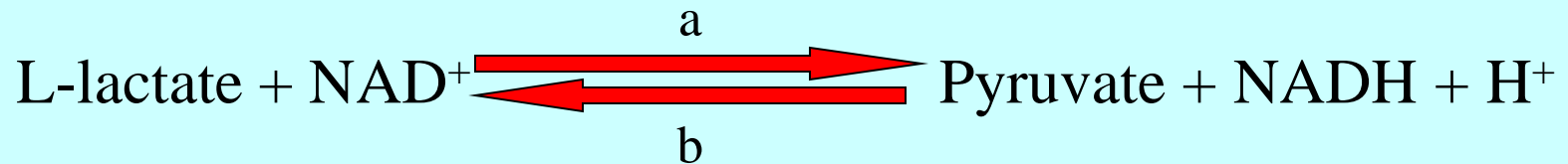
Measurement of total creatine kinase



CK catalyzes the reversible transfer of phosphate from creatinephosphate to Mg-ADP. In the next steps of the reaction the produced Mg-ATP is subject of a combined hexokinase (E.C.2.7.1.1) and glucose-6-phosphatedehydrogenase (G6PDH; E.C.1.1.1.49) reaction. The absorbance of the produced NADH₂ in the latter reaction correlates to the activity of creatine kinase (CK). **The reaction mixture contains N-acetylcysteine for preventing oxidation of CK as well as AMP and diadenosinepentaphosphate for preventing interference by adenylatekinase (E.C.2.7.4.3).** The method is standardized for 25°C, 30°C and 37°C according to the IFCC.

Measurement of LDH in plasma

Principle of the reaction:



a: pH 8.8 - 9.9

b: pH 7.4 - 7.8

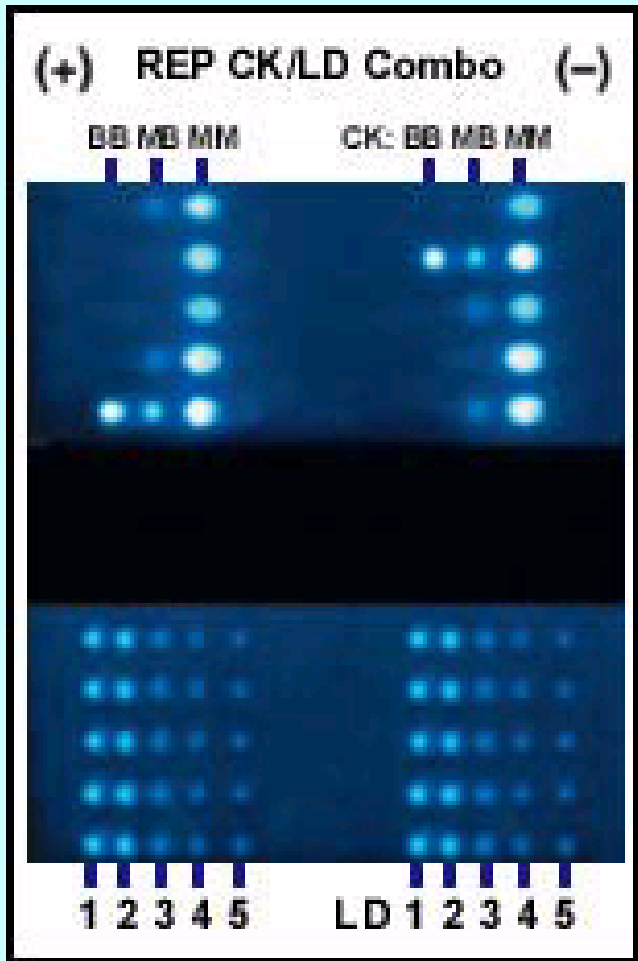
The forward as well as the backward reaction are used in a large number of commercial tests for the determination of LDH activity. The changes of NADH/NAD serve for the detection of the reaction. In detail, the increase of NADH can be determined by measurement of the absorbance at $\lambda=334$ nm, $\lambda=340$ or $\lambda=360$ nm respectively. In the backward reaction the oxidation of NADH causes a decrease of the absorbance at these wavelengths. Reactions are standardized for 25°C, 30°C and 37°C.

Reference values for plasma LDH

Total LDH activity in plasma or serum. IFCC-method, standardized on 37°C. Values are given in [U/l].

Age	Females	Males
1 - 30 days	145 - 765	125 - 735
1 - 12 months	190 - 420	170 - 450
1 - 3 years	165 - 395	155 - 345
4 - 6 years	135 - 345	155 - 345
7 - 9 years	140 - 280	145 - 300
10 - 12 years	120 - 260	120 - 320
13 - 15 years	100 - 275	120 - 290
16 - 18 years	105 - 230	105 - 235
Adults	135 - 215	135 - 225

Isoenzymes of LDH and CK



The REP CK/LD Combo Procedure provides definitive testing for myocardial infarction (MI) with automated, simultaneous separation of both CK and LD isoenzymes.

The CK/LDH isoenzyme combination gel is actually two gel formulations on one support backing. One half is optimized for CK isoenzyme separations, the other for LDH. The gels are designed so that the same electrophoresis parameters are used for both CK and LDH isoenzymes. Appropriate reagents for each test are automatically applied by the REP. The automated densitometer can scan both results without operator intervention.

Up to 10 CK and 10 LDH samples may be assayed on each gel. The procedure requires about 20 minutes for isoenzyme separation and development. Automated densitometric quantitation and printing of customized patient report forms can be completed in a few minutes more. Electrophoresis remains the reference method for CK and LDH isoenzyme analysis because it is the only method to provide definitive separation and quantitation of all the isoenzymes.

Reference values of LDH isoenzymes determined by electrophoresis

Reference values of LDH isoenzymes in plasma depend on the electrophoretic technique which is used.

	CAF [%]	Agarose [%]
LDH-1	18 - 33	15 - 23
LDH-2	28 - 40	30 -39
LDH-3	18 - 30	20 -25
LDH-4	6 - 16	8 - 15
LDH-5	2-13	9 - 14

CAF: Cellulose acetate

Specific determination of LDH isoenzymes

Chemical inhibition of LDH-isoenzymes:

LDH-isoenzymes containing an M subunit are specifically inhibited by 1,6-hexandiol or sodium perchlorate. Therefore, LDH-1 (4H subunits) is specifically determined.

Immunological inhibition of LDH-isoenzymes:

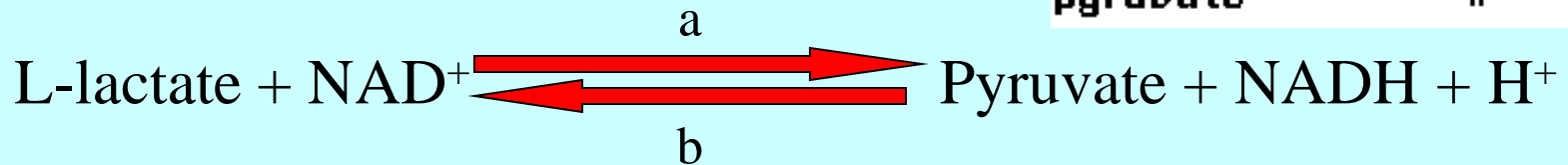
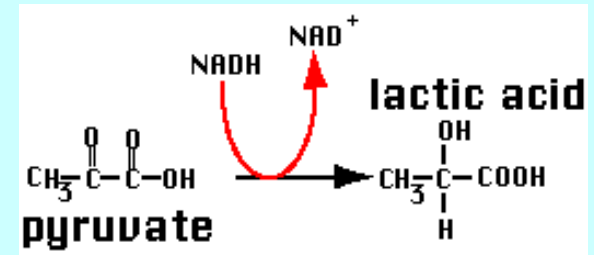
LDH-isoenzymes containing an M subunit are specifically inhibited by a monoclonal antibody. Therefore, LDH-1 (4H subunits) is specifically determined.

Biochemical reaction with α -hydroxybutyrate:

LDH-1 and LDH-2 isoenzymes are able to catalyze the reaction of α -hydroxybutyrate and are therefore also named α -hydroxybutyrate dehydrogenase

Measurement of LDH and HBDH in plasma

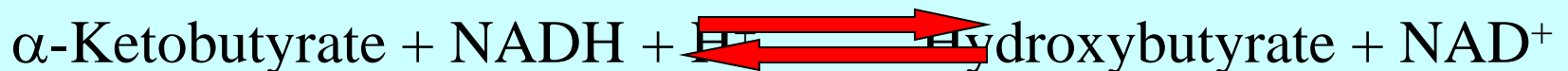
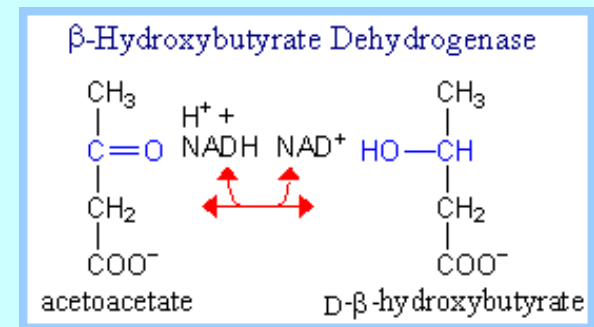
Principle of the LDH reaction:



a: pH 8.8 - 9.9

b: pH 7.4 - 7.8

Principle of the HBDH reaction:



LDH-1 and LDH-2 isoenzymes are able to catalyze the reaction of α -hydroxybutyrate and are therefore also named α -hydroxybutyrate dehydrogenase

Half-life times of plasma enzymes

Enzyme	$t_{1/2}$
ALAT	50 hours
ASAT	12 – 14 hours
Creatine kinase (CK)	12 hours
CK-MM	20 hours
CK-MB	10 hours
CK-BB	3 hours
LDH-1 (heart, erythrocytes)	4 – 5 days
LDH-5 (liver, body musculature)	8 – 10 hours

Determination of myoglobin - I

Turbidimetry:

Antibodies against myoglobin are coated on polystyrol spheres. Sample myoglobin and antibody-coated spheres aggregate and the increasing turbidity of the sample is determined. Values are obtained after 1-2 minutes by means of a photometer.

Nephelometry:

Agglutination reaction between myoglobin and anti-myoglobin antibodies linked to polystyrol particles. The increase of scatterlight is determined after an incubation time of 12 minutes by means of a laser nephelometer.

Immunoassay:

Determination of myoglobin by means of a sandwich ELISA.

Determination of myoglobin - II

Sample material:

Serum, plasma, urine

Analytical sensitivity and variability:

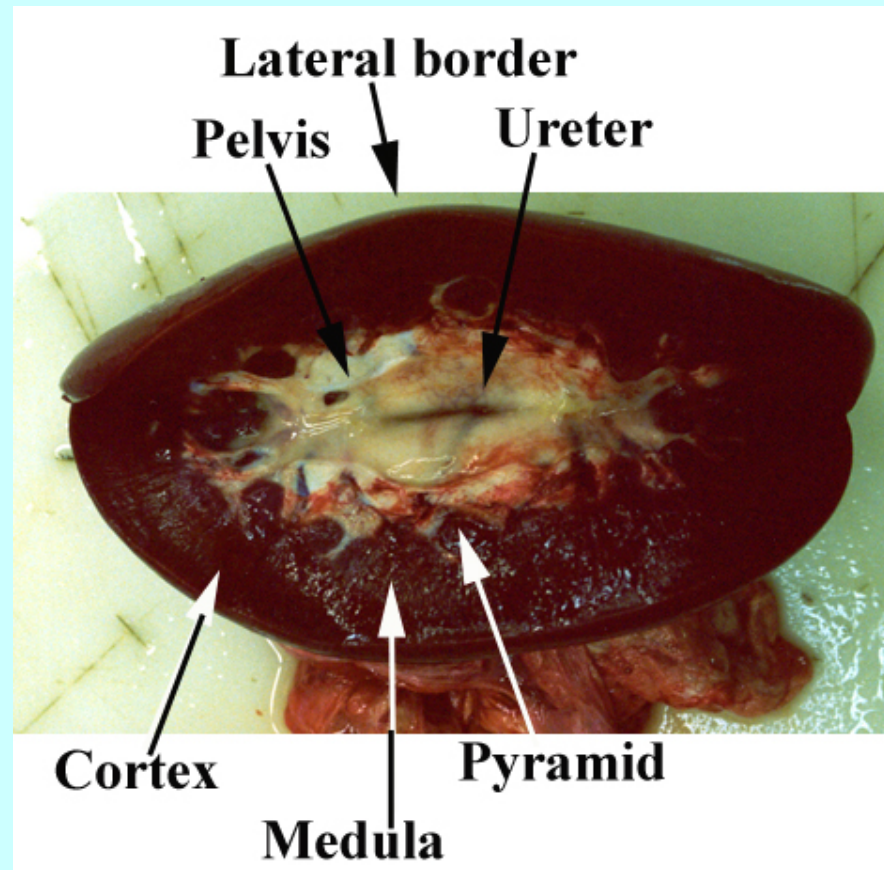
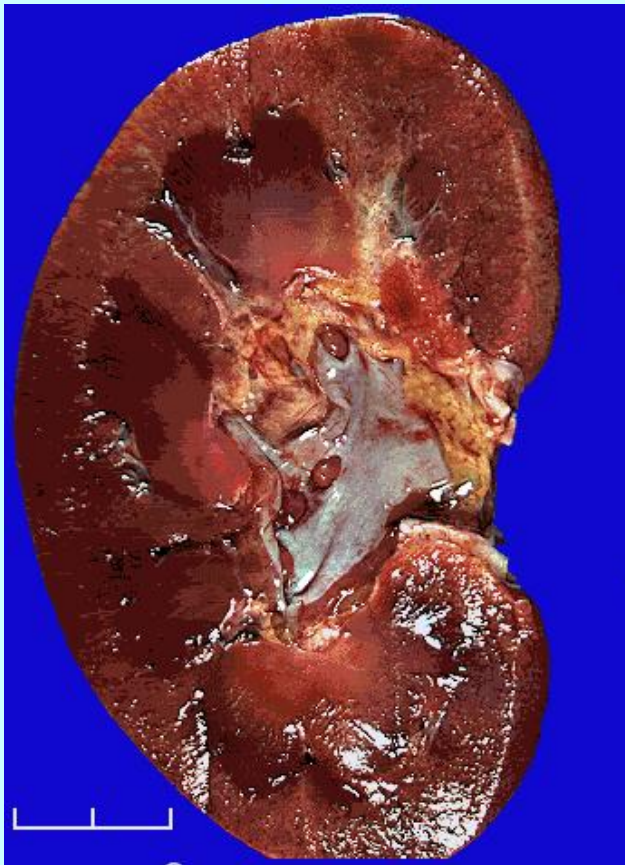
	Sensitivity [$\mu\text{g/l}$]	Intra-assay CV [%]
Immunoassay	1	7.0-8.0
Nephelometry	6	1.5-7.5
Turbidimetry	50	2.5-6.0

Reference values:

Serum, plasma: $<70 \mu\text{g/l}$ (males $>$ females)

Urine: $<17 \mu\text{g/g}$ creatinine

Morphology of the kidney



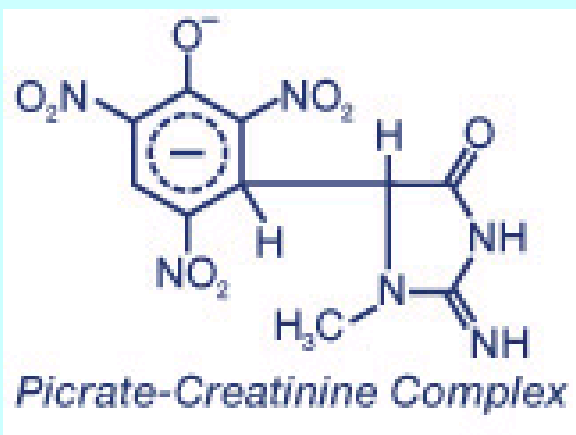
Jaffé test for creatinine - I

Jaffé reaction and non-creatinine Jaffé chromogens

The Jaffé reaction is based on the observation that at an alkaline pH, creatinine reacts with picrate to form a red-orange adduct.

Unfortunately, although the Jaffé is a simple procedure, it is subject to interferences from a number of compounds collectively called "non-creatinine Jaffé chromogens" which can introduce a positive bias of up to 20 %.

Therefore, a number of modifications of the Jaffé reaction have been developed which reduce the interference from the major non-creatinine Jaffé chromogens, including protein and acetoacetate and provide results comparable to enzymatic methods.



Jaffe´test for creatinine - II

Diagnostic chemicals limited (DCL) has modified Jaffé reaction utilizing a combination of surfactants and optimized reaction conditions to significantly reduce interference from many classic non-creatinine Jaffé chromogens. The test is linear up to 25 mg/dl (2210 µmol/l). Analysis is performed at $\lambda=510$ nm.

Precision of the DCL-test.

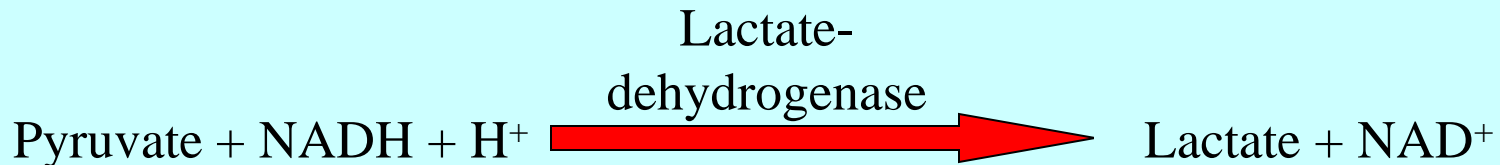
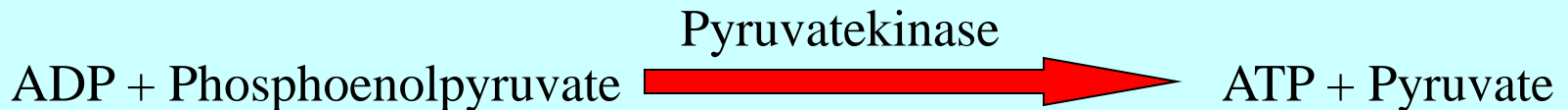
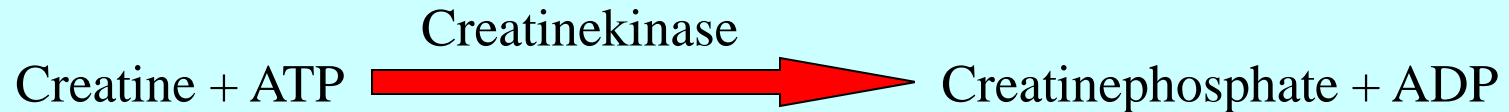
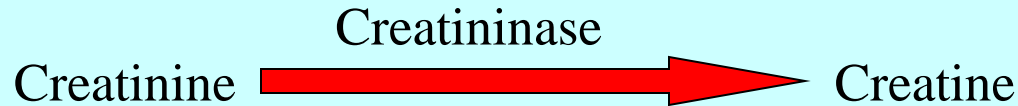
Precision	Mean		CV-value [%]
	[mg/dl]	([µmol/l])	
Day to day	1.09	(96)	2.8
	6.03	(533)	1.3
Within run	1.09	(96)	2.8
	6.03	(533)	1.2

Interferences of the DCL-test.

Interference factor	Level tested	
Ketone (acetoacetate)	40 mg/dl	4 mmol/l
Lipemia (triglyceride)	3000 mg/dl	33.9 mmol/l
Bilirubin	40 mg/dl	684 µmol/l
Hemolysis (hemoglobin)	800 mg/dl	124 µmol/l

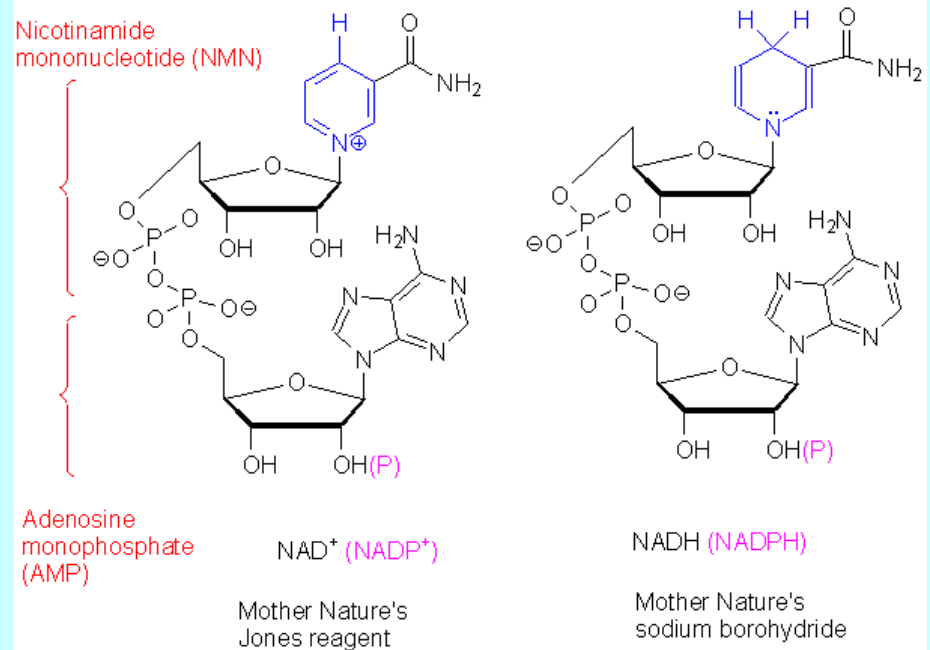
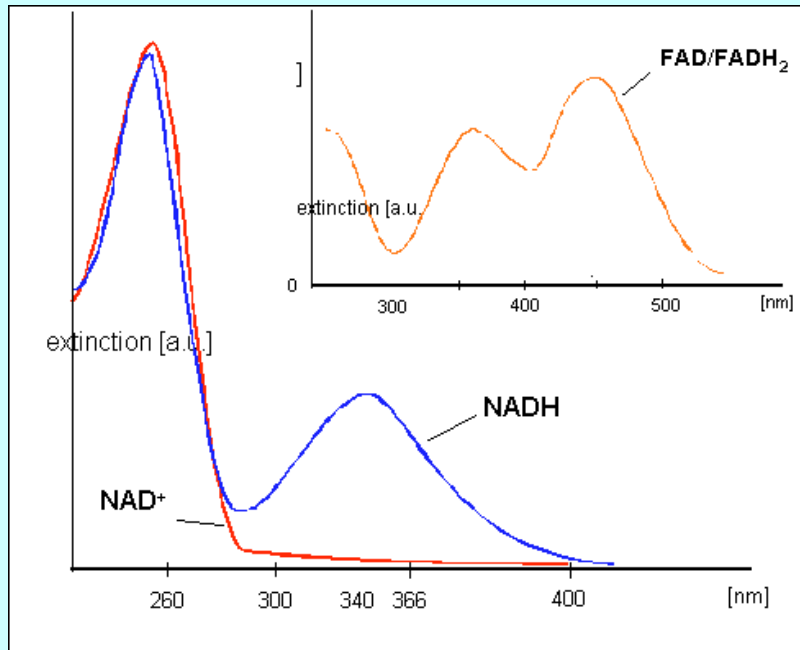
Enzymatic tests for creatinine - I

Reaction of creatinine to creatine by means of creatininase (creatineaminohydrolase; EC 3.5.2.10).



The decrease of the uv absorbance is proportional to the creatinine concentration of the sample.

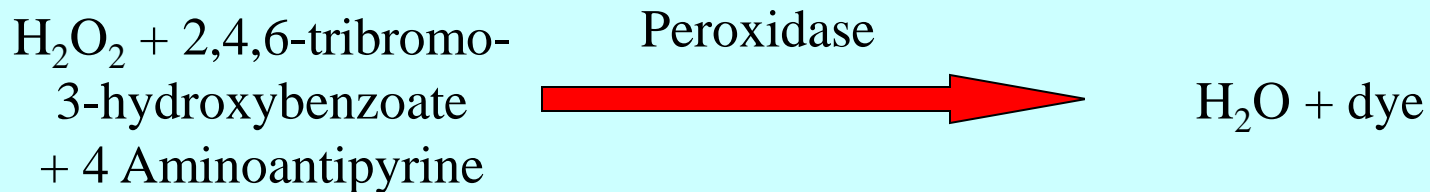
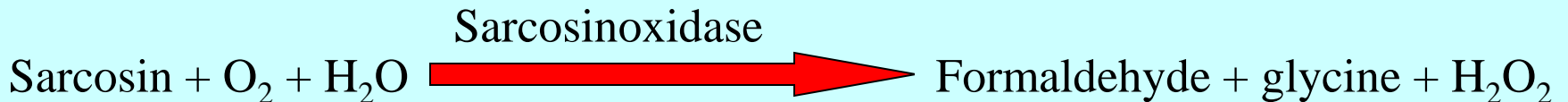
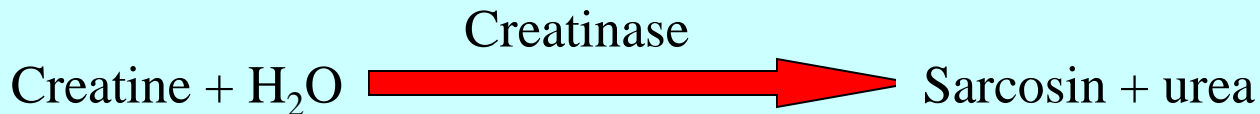
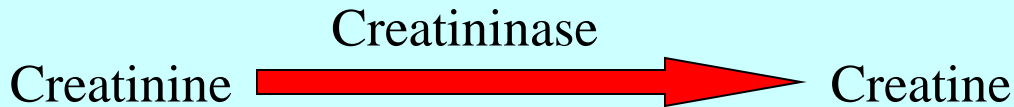
Enzymatic tests for creatinine - II



The redox reaction of the NAD⁺/NADH⁺ and the NADP⁺/NADPH⁺ system is frequently used in clinical chemistry for the determination of substrates and enzyme kinetics.

Enzymatic tests for creatinine - III

Production of sarcosin by combination of creatininase and bacterial creatinase (creatinamidinohydrolase; EC 3.5.3.3.) and consecutive oxidation of sarcosin by means of a sarcosinoxidase (EC 1.5.3.1).

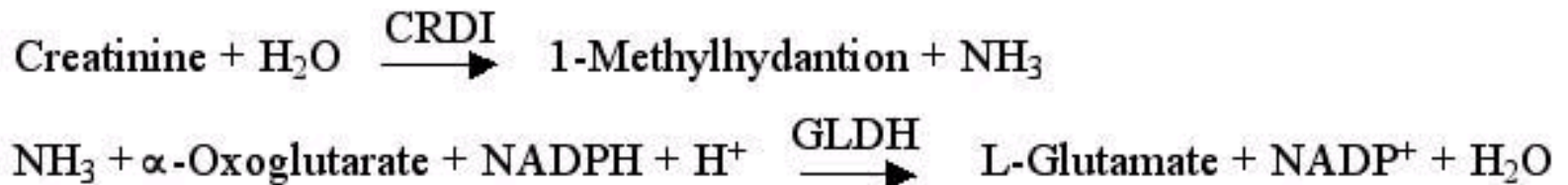


Production of H_2O_2 is determined by the Trinder reaction. The increased absorbance of the produced dye correlates to the creatinine concentration.

Enzymatic tests for creatinine - IV

Assay Principle

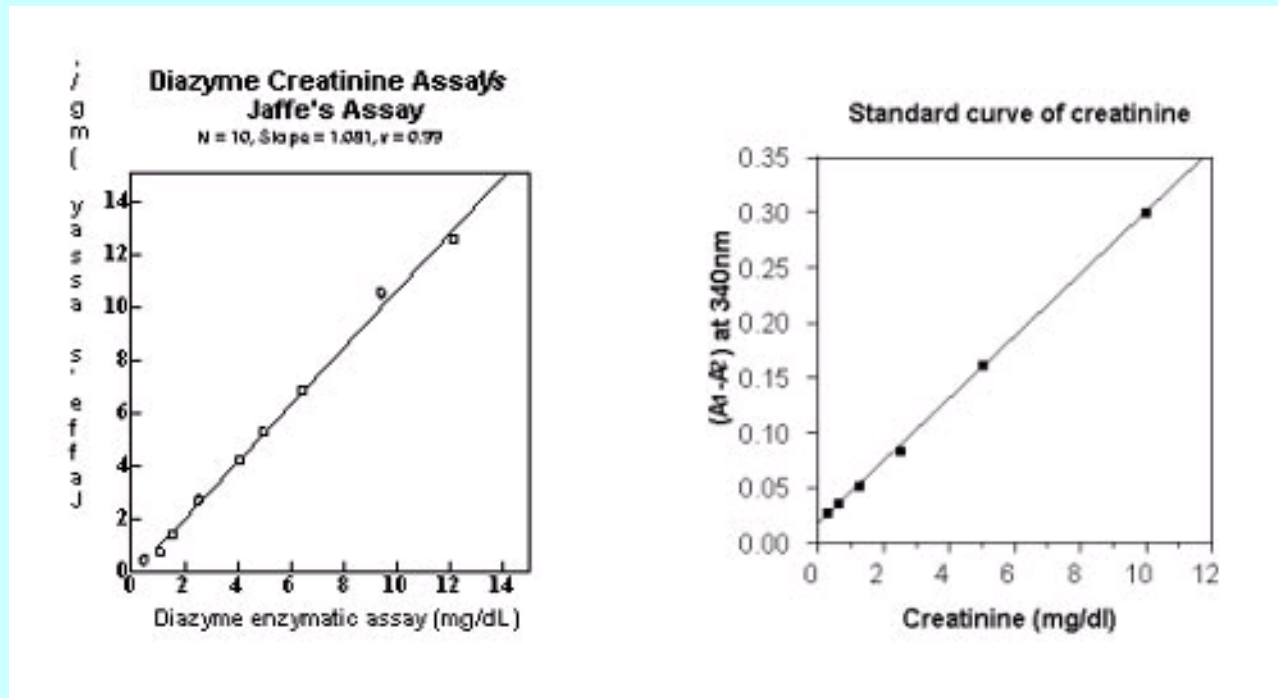
The Dizyme enzymatic assay for creatinine involves conversion of creatinine into ammonia by creatinine deiminase (EC 3.5.4.21). The ammonia is subsequently assayed by glutamate dehydrogenase (EC 1.4.1.3) acting on α -oxoglutarate and NADPH. Use of NADPH as the coenzyme eliminates any endogenous interferences.



Any endogenous ammonia present in the sample is removed by glutamate dehydrogenase (GLDH) during preincubation. The addition of creatinine deiminase (CRDI) subsequently converts creatinine into 1-Methylhydantion + NH_3 . The reduction in absorbance at 340 nm is caused by reduction of NADPH and is proportional to NH_3 concentration released by creatinine.

Enzymatic tests for creatinine - V

Characteristics of the Dizyme test.



Normal values

	Men	Women
Serum	53-123 $\mu\text{mol/L}$	44-106 $\mu\text{mol/L}$
	0.6-1.4 mg/dL	0.5-1.2 mg/dL
Urine	8.84-24.7mmol/L (24hrs)	7.9-14.1mmol/L (24hrs)
	1-2.8 gm/L (24hrs)	0.9-1.6 gm/L (24hrs)

Reference values

Reference values in serum or plasma of adults

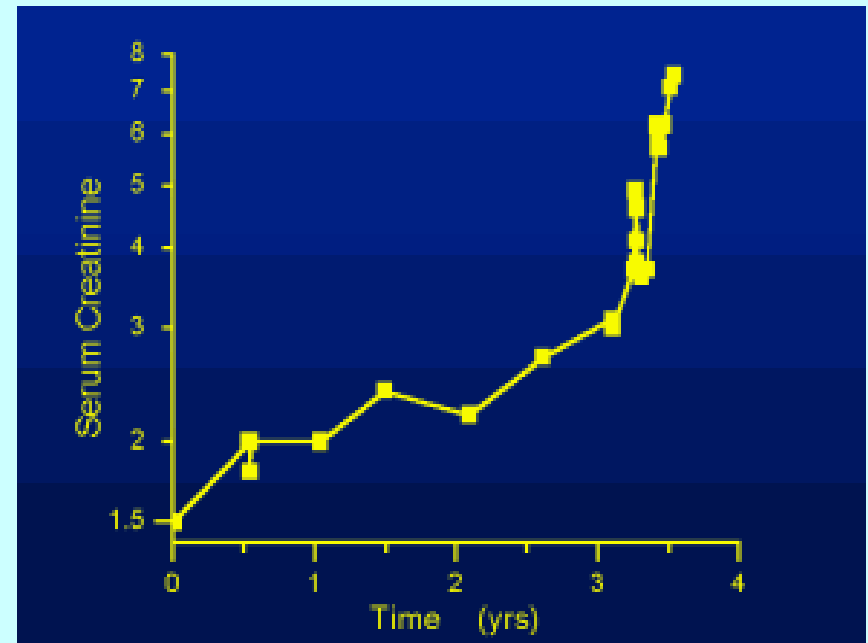
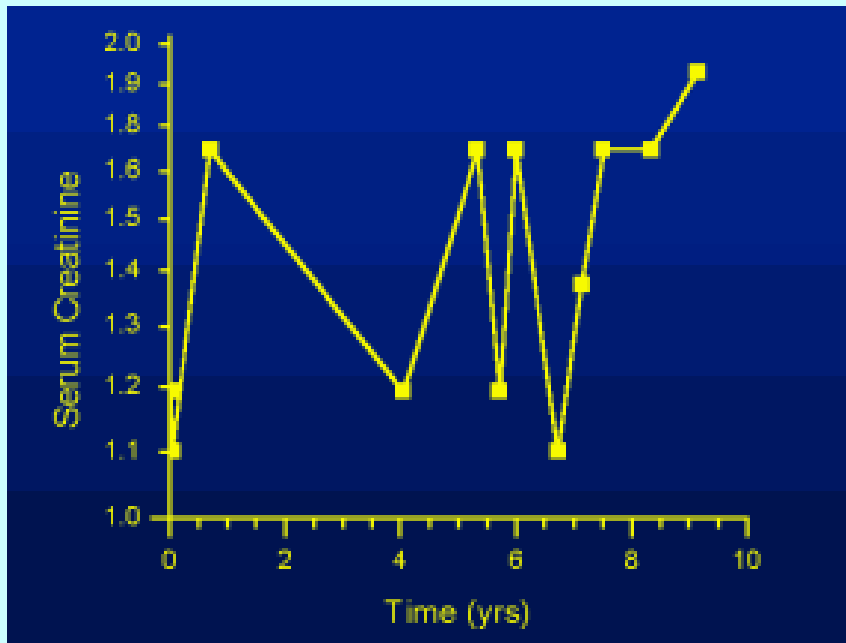
	Females	Males	
Creatinine#)	0.47 – 0.90	0.55 – 1.10	[mg/dl]
Uric acid	2.3 – 6.1	3.6 – 8.2	[mg/dl]
Urea	15 – 43	19 – 55	[mg/dl]

#) determined by the enzymatic method

Reference values in the first morning urine of adults

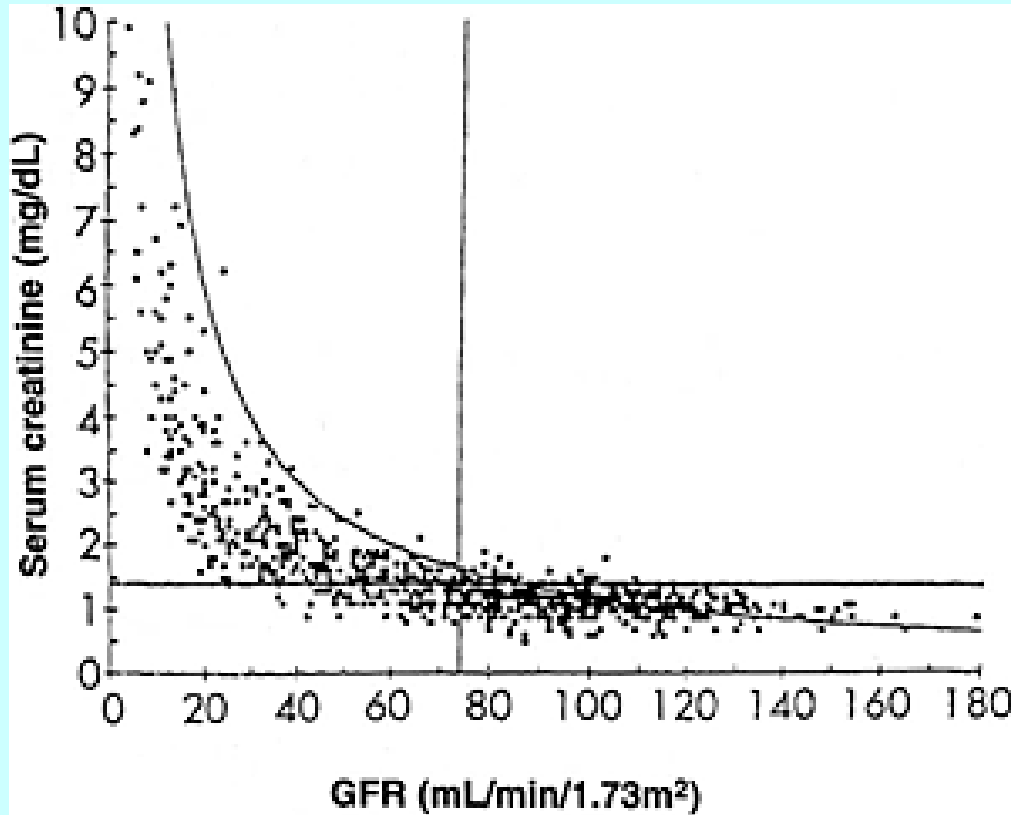
Creatinine	900 – 3100	[mg/dl]
Uric acid	130 – 330	[mg/dl]
Urea	800 – 2000	[mg/dl]

Examples for the time course of kidney failure



The time course of kidney failure depends on the underlying disease and the treatment of the patient. However, the disease is often progressive and ends in kidney failure requiring hemodialysis, peritoneal dialysis or kidney graft transplant.

Creatinine clearance



Relationship of serum creatinine to ^{125}I -iothalamate glomerular filtration rate (GFR) in 823 patients studied at Cleveland Clinic Foundation. The curved line represents the classic, theoretic relationship of serum creatinine concentration to creatinine clearance. The horizontal and vertical lines represent, respectively, the upper limit of normal serum creatinine (1.4 mg/dL) and lower limit of normal GFR (74 mL/min/1.73 m²).

Creatinine clearance - VII

Creatinine clearance (C_{cr}):

$$C_{cr} = \frac{U_{cr} \text{ (mg/dL)} \bullet V \text{ (ml/min)}}{P_{cr} \text{ (mg/dL)}}$$

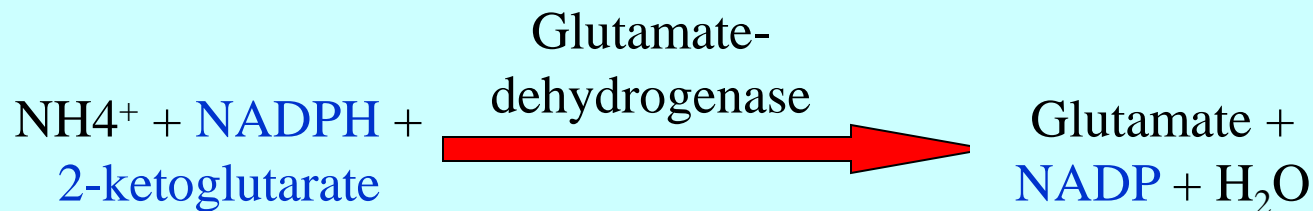
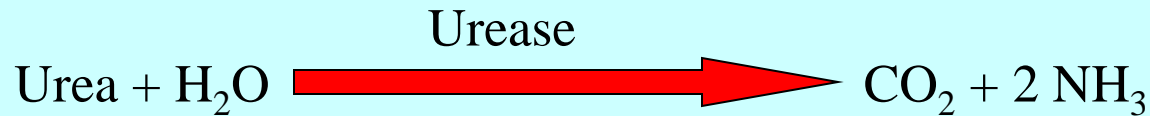
Example: Patient A has an urine volume (V) = 1.2 l/24 hour (must be complete), an urine creatinine concentration (U_{cr}) = 100 mg/dL and a plasma creatinine concentration (P_{cr}) = 1.2 mg/dL

$$C_{cr} = \frac{U_{cr} \bullet V}{P_{cr}} = \frac{100 \text{ mg/dl} \bullet 1.2 \text{ l/24 hr}}{1.2 \text{ mg/dl}}$$

$$\begin{aligned} &= 100 \text{ (l/24 hr)} = 100 \bullet (1000 \text{ ml/1440 min}) = 100 \bullet 0.7 \text{ ml/min} \\ &= 70 \text{ ml/min} \end{aligned}$$

Enzymatic test for urea - I

Ammonium is cleaved from urea by means of the enzyme urease. The produced ammonium is then determined by chemistry or **enzymatically**.



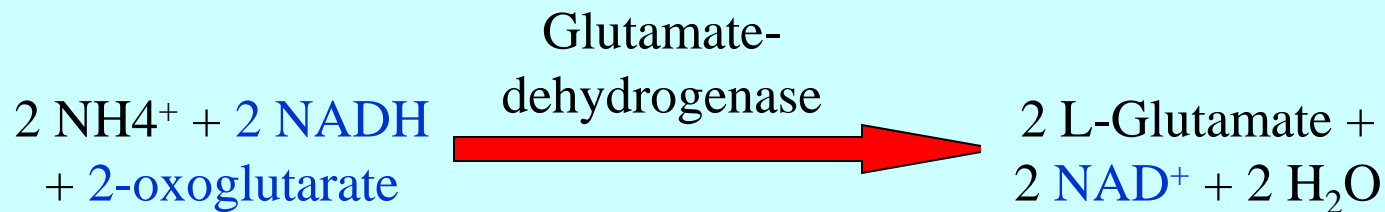
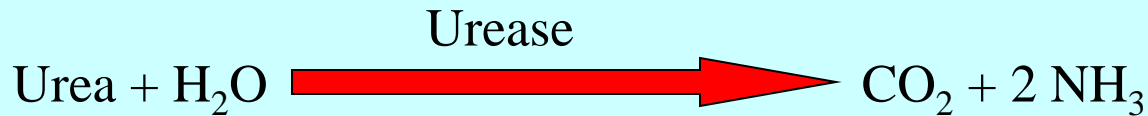
The decrease of the uv absorbance is proportional to the urea concentration of the sample.

The concentration of free ammonium in blood is lower than 1 %; therefore a blank correction is not required for the determination of urea. However, a blank is required in samples with high ammonium concentrations or activity of enzymes producing ammonium (e. g. patients with hepatic failure; γ -glutamyltransferase).

The imprecision of the enzymatic method is low (CV-value < 5 %).

Enzymatic test for urea - II

Ammonium is cleaved from urea by means of the enzyme urease. The produced ammonium is then determined by chemistry or **enzymatically**.



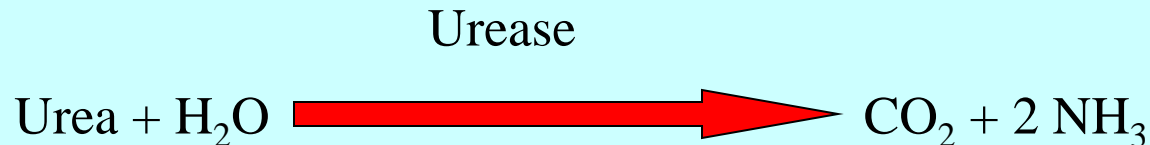
The decrease of the uv absorbance is proportional to the urea concentration of the sample.

The concentration of free ammonium in blood is lower than 1 %; therefore a blank correction is not required for the determination of urea. However, a blank is required in samples with high ammonium concentrations or activity of enzymes producing ammonium (e. g. patients with hepatic failure; γ -glutamyltransferase).

The imprecision of the enzymatic method is low (CV-value < 5 %).

Enzymatic test for urea - III

Ammonium is cleaved from urea by means of the enzyme urease. The produced ammonium is determined by **chemistry** or enzymatically.



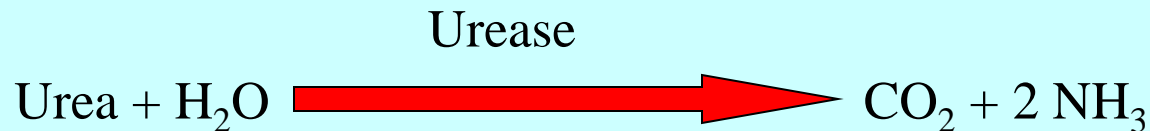
The resulting changes of the pH-value or electrical conductivity can be determined by means of electrodes. Alternatively, NH_4 can be measured by means of a specific ammonium electrode.

The concentration of free ammonium in blood is lower than 1 %; therefore a blank correction is not required for the determination of urea. However, a blank is required in samples with high ammonium concentrations or activity of enzymes producing ammonium (e. g. patients with hepatic failure; γ -glutamyltransferase).

The imprecision of the enzymatic method is low (CV-value < 5 %).

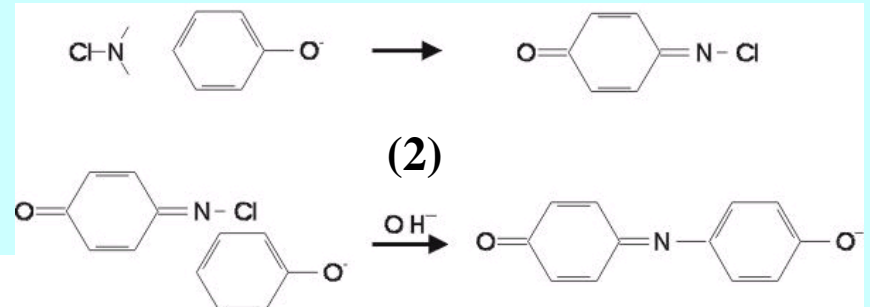
Enzymatic test for urea - IV

Ammonium is cleaved from urea by means of the enzyme urease. The produced ammonium is then determined by **chemistry** or enzymatically.

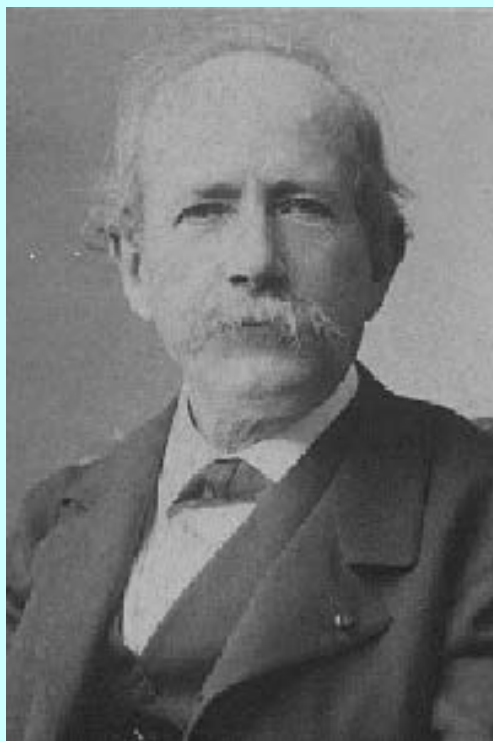


In the following **Berthelot reaction** the combination of ammonia, phenol and hypochlorite resulted in a blue coloration. Nowadays, this reaction is used to determine the concentration of **ammonia in water samples (e. g. urine)**. The reaction consists of two steps. Firstly (1), hypochlorite is added to the ammonia sample resulting in the formation of mono-chloramine. Care has to be taken that pH of the solution is high enough (~11), such that the side products dichloramine or even tri-chloramine are produced in minimal amounts. The second step is the addition of phenol to the sample. The phenol reacts with the mono-chloramine to form indophenol blue (2).

Indophenol blue is a blue dye, with its absorption peak at 600 nm. Therefore, it can be detected using the measurement of the intensity at $\lambda=600$ nm (red light).



Nonenzymatic test for urea - I

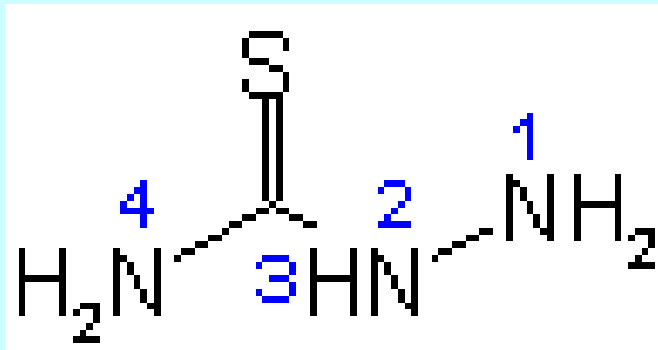


Pierre Eugène
Marcelin Berthelot
(1827-1907)

Pierre Eugène Marcelin Berthelot

After studying under Antoine Jérôme Balard he became professor in 1859, since 1865 he taught at the Collège de France. In 1886/87 Berthelot was Minister of Education, in 1895/96 Foreign Minister. He published many papers on thermochemistry, organic syntheses (sugars, terpenes, glycerides, fats and many others), and the history of alchemy. As one of the foremost chemists of the late 19th century he was much honoured during and after his lifetime.

Nonenzymatic test for urea - II

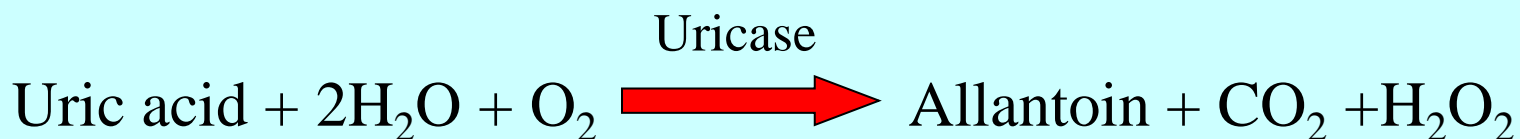


Thiosemicarbazide

Diacetylmonoxim method

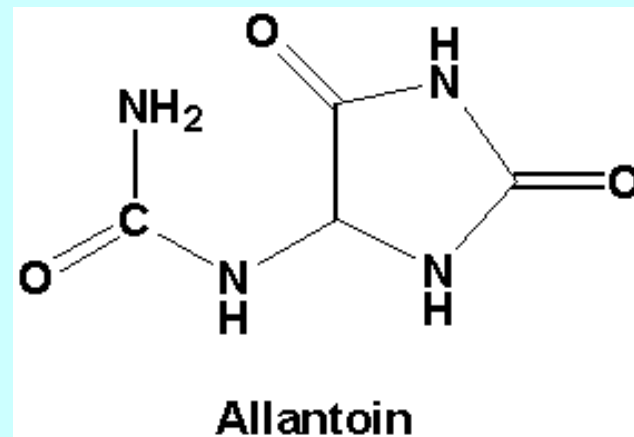
Urea reacts with diacetylmonoxim in presence of thiosemicarbazide and FeCl_3 . This results in the production of a pink dye which can be determined by means of a photometer.

Enzymatic test for uric acid - I



The decrease of the absorption of uric acid at a wavelength of $\lambda=282-292$ nm can be determined directly.

However, a number of methods is based on further reactions of H_2O_2



Enzymatic test for uric acid - II

Uricase-catalase reaction:

In presence of hydrogen peroxide catalase oxidizes methanol to formaldehyde. Formaldehyde reacts with acetylacetone in presence of ammonium to a yellow dye which is determined at 410 nm.

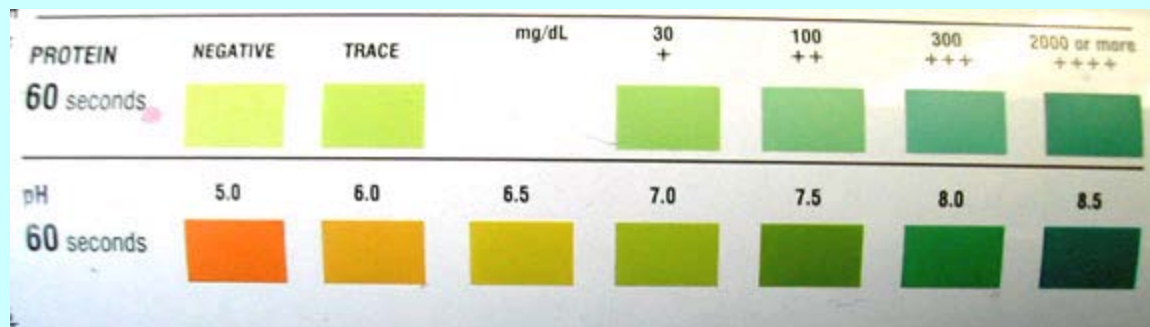
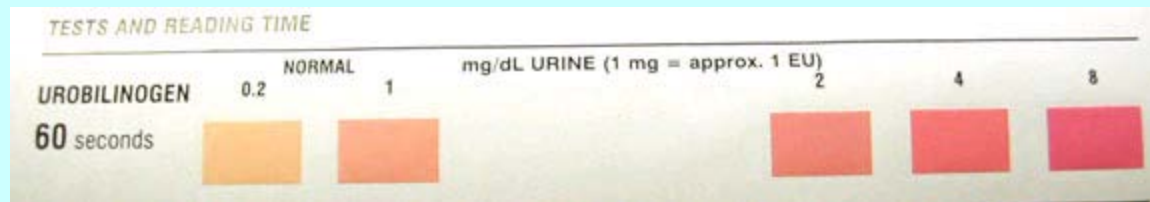
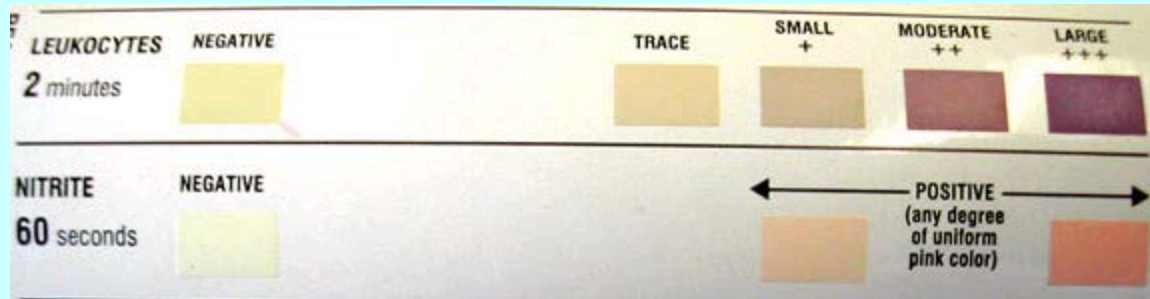
Trinder reaction:

H_2O_2 reacts in presence of peroxidase with a chromogen system of phenol and 4-aminophenazone to red chinonimine which can be determined at 500 nm. Several modifications have been described.

Aldehyde dehydrogenase reaction:

If H_2O_2 is present, catalase oxidizes ethanol to acetaldehyde. The latter is oxidized by acetaldehyde dehydrogenase to acetate and the hydrogen produced in this reaction is transferred to NADP. Finally the produced NADPH_2 is determined e. g. at 340 nm.

Compiled data of dip stick analysis - I



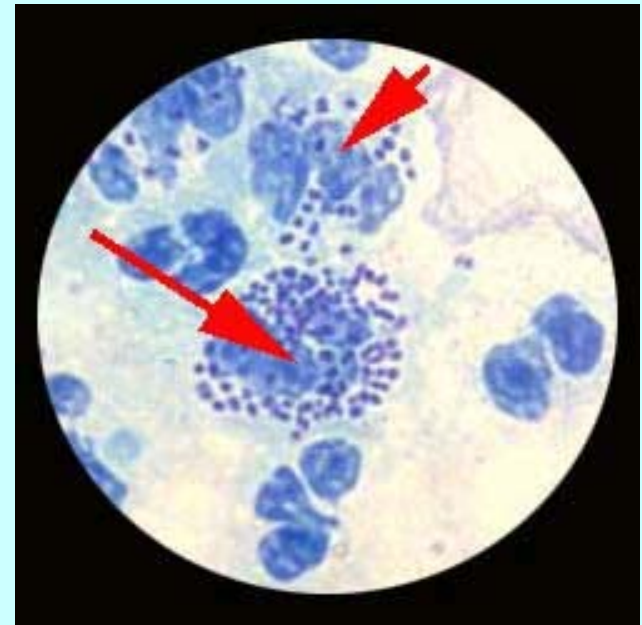
These images show the color indicators for the Baxter urinalysis test strip.

Compiled data of dip stick analysis - II



These images show the color indicators for the Baxter urinalysis test strip.

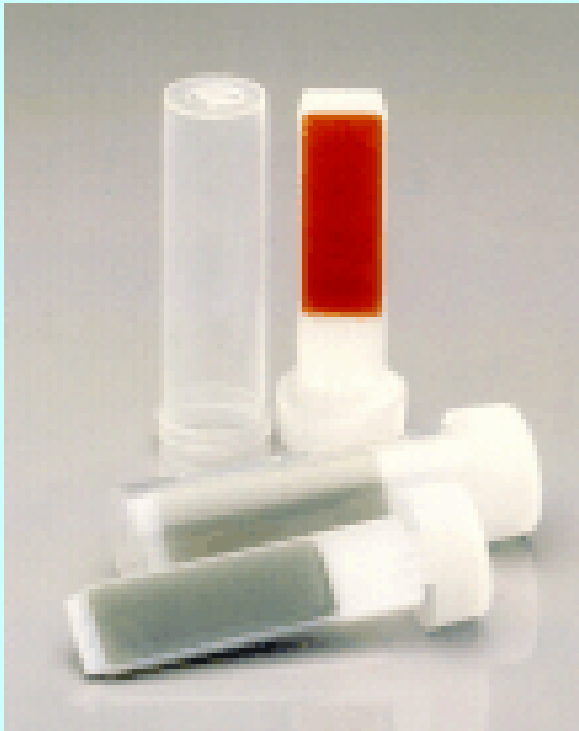
Microbiological testing of urine - I



Urinary tract infections may be diagnosed by dipstick analysis, microscopy and culture of the microbes.

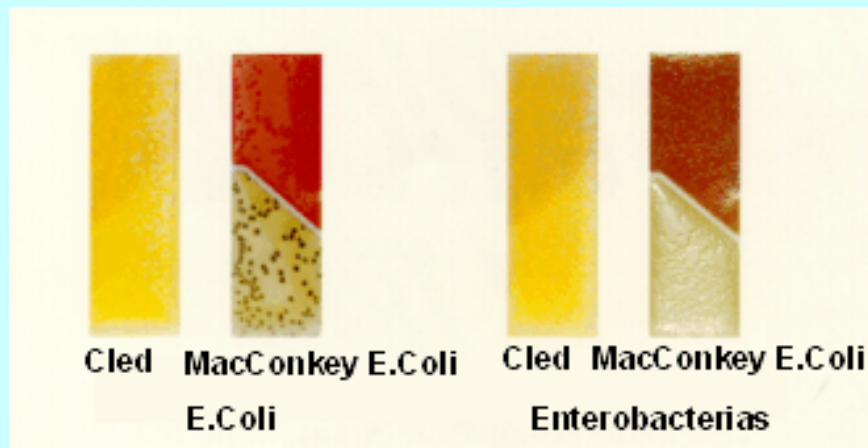
Right: Intracellular bacteria in a patient with urinary tract infection.

Microbiological testing of urine - II

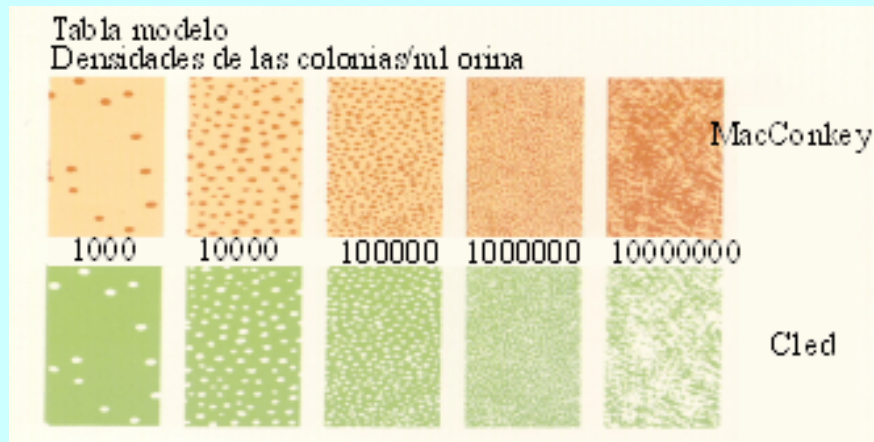


The tests consists of a tube for urine sampling and culture media. Directly after sterile sampling the media are briefly dipped into the urine. The urine is discarded and the culture media are incubated in the tube at 37 °C for 24 hours.

Microbiological testing of urine - III



The different culture media allow a first differentiation of the bacteria and their quantification.



A colony density of $>10^5$ /ml urine indicates an urinary tract infection

Clinical case - I

Urinary tract infection - I

Patient history:

Mrs. JG complains of dysuria and frequency

Mrs. JG is a 28 year old married woman, mother of two children.

She gives a two-day history of burning upon urination.

Describes a feeling of urinary urgency and frequency.

Mild suprapubic pain.

Odd smell to her urine.

Today her urine is cloudy and red.

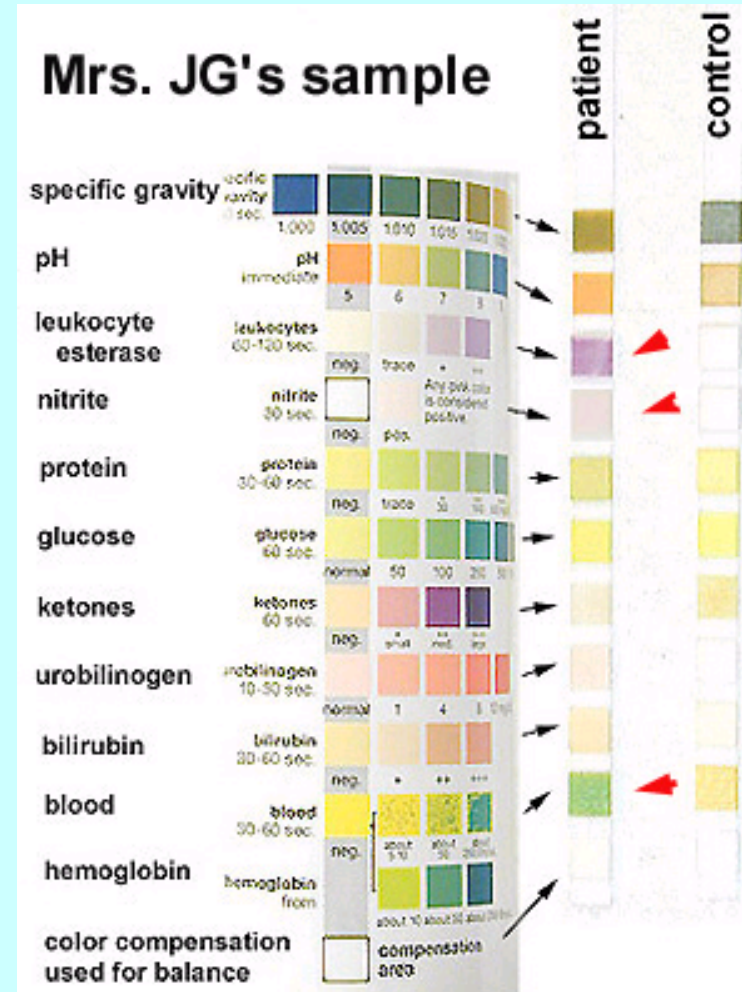
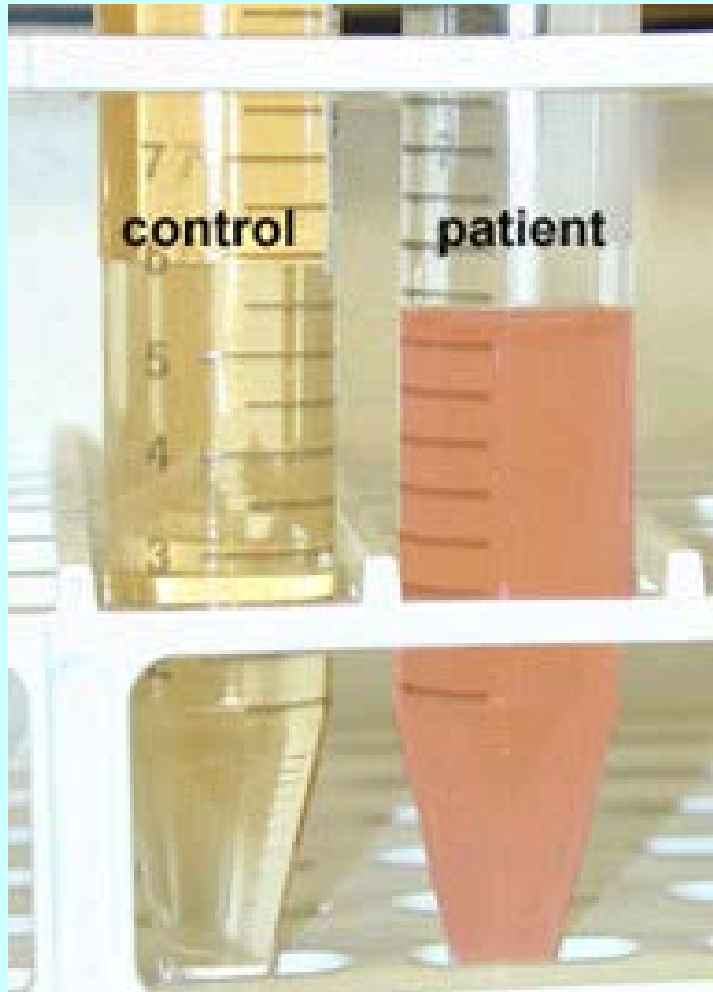
She's worried it may contain blood.

Blood pressure 120/70 mmHg; temperature: 98.8 °F



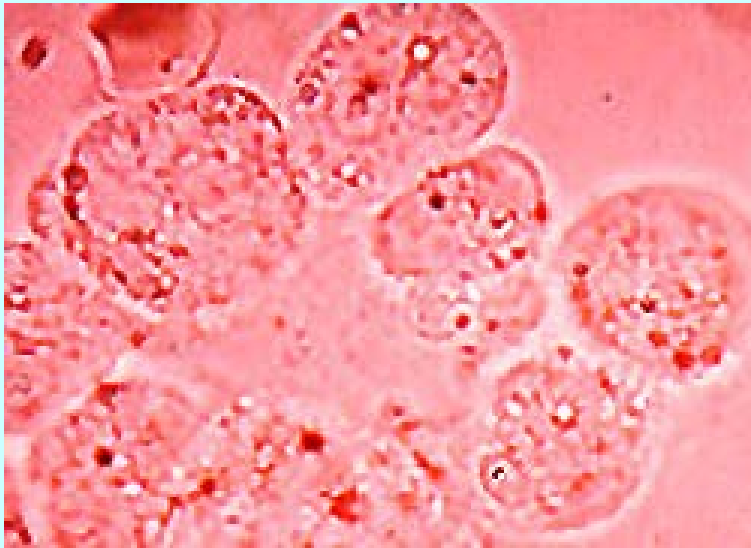
Clinical case - I

Urinary tract infection - II



Clinical case - I

Urinary tract infection - III



Observations from Mrs. JG's urinary sediment:

Left: Numerous acute inflammatory cells. WBCs in clusters, but no casts.

Right: Gram stain reveals numerous gram negative rods.

Clinical case - I

Urinary tract infection - IV

Results of dipstick analysis and urine sediment:

The positive leukocyte esterase indicates WBCs in the urine.

Also, WBCs were seen in the urinary sediment.

Positive nitrite indicates the presence of bacteria.

The blood is positive because there's hemoglobin in the urine.

A little bleeding from the bladder mucosa is not uncommon with a lower urinary tract infection.

Her symptoms indicate irritation of the bladder.

Clinical case - I

Urinary tract infection - V



Culture plate from the urine of Mrs. J.G.

Diagnosis:

Mrs. JG has a bladder infection.

The culture looks like *E. coli*.

She needs an appropriate antibiotic.

She needs something for relief from her bladder pain, like Pyridium.