Blood Chemistry and Hematology Tests Manual For Clinicians of Biological Regulatory Medicine

The information in this text is intended for informational purposes only, and is designed to assist users better understand clinical laboratory tests. Information is based on review of scientific research data, historical practice patterns, and clinical experience. This information should not be interpreted as specific medical advice. Users should consult with a qualified healthcare provider for specific questions regarding therapies, diagnosis and/or health conditions, prior to making therapeutic decisions.

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Laboratory Normals and Optimum Values

Laboratory tests are monitors of physiologic processes; much as are pulse rate, respiratory rate, and blood pressure. Practitioners have become accustomed to accepting a certain range of values established by laboratories over the years as indicative of health; such results are commonly termed “normal”. They serve as a basis of comparison of each patient encounter, to assess patient status; in this sense, they form a reference for comparison. If such values were not available, it would be impossible for clinicians to determine whether the values obtained in each patient were indicative of a healthy state or not. With time and experience, the practitioner would gain a general sense of what values to expect, recognizing that those far from these typical values are indicative of disease. However, due to factors of biochemical individuality, it is not possible simply and empirically to determine exact cutoff values to distinguish heath and disease.

While many differing definitions of normal can be found in dictionaries, two major ones apply to physiologic parameters. The definition most commonly considered in the medical sense is “free from disease; healthy.” A second, perhaps easier to determine, definition is “average, typical, or usual”. These two definitions do not always produce the same results. For example, while “typical” American diets contain over 45% fat, few practitioners would consider this “healthy”.

To determine “typical” results, a common approach is to take a random sample of “typical” members of a population, and to consider the central 95% of results as allowable limits for “normal”; in a Gaussian distribution this is calculated as the average result + or – 1.96 standard deviations (SD). This Gaussian “normal” distribution is used to establish reference normal for laboratories. This is in effect a randomized approach in which an arbitrary percentage of the population is considered to be “normal”. Considering that most blood chemistries are usually done on two groups, hospital patients and those individuals having a physical exam, this population may be average, but dubiously free from disease. And to randomly assess *this* population’s blood chemistry on a Gaussian curve and proclaim that 95% is “normal” is questionable at best, in a “healthy” sense. Some laboratories attempt to randomly sample a cross-section of the population that they serve. This is often accomplished by conducting health fairs and/or using random mailings to the community offering free blood screening for cholesterol in return for donating samples for reference value testing. If the persons recruited are truly representative of the healthy population, this approach is probably more valid than using hospitalize patients and those seeking physicals. Often, however, those responding to the campaign are more likely to have some signs or symptoms of illness, and thus are more interested in learning the results of their blood tests than asymptomatic individuals. The truth is that the current “typical” population is becoming more ill and more environmental toxic. This has created a constant change in the “normal values”. The laboratory ranges have become broader to allow for this unfortunate change in biochemistry.

While we need a frame of reference for comparing results in an individual, reference values often have limited usefulness. Most importantly, practitioners need to understand that results within the reference values do not exclude disease, nor do results outside reference limits always indicate pathology. Standard reference values indicate typical or usual values for persons in the population tested; however, they fail to recognize that populations are composed of unique individuals whose results often vary less than those of the population as a whole. For example, a distance runner may have a resting pulse rate of 45, which is below the reference range for the general population; a pulse rate of 80 in this individual may be highly abnormal, yet fall within the reference range. Practitioners must be aware of which commonly measured test may be subject to this problem. It is paramount to consider first the individual’s health history and presenting symptomology and second the test result.

It is vital that practitioners develop good working relationships with the laboratory where most patients’ testing is performed. While many practitioners will develop familiarity with the limitations of reference values for tests which they commonly order, the same may not hold true for tests which they occasionally or seldom order. Most laboratories have consultants available who can provide additional information that the practitioner can use to interpret unusual test results.

It should not be surprising that factors such as age, gender, cultural origin, diet, stress, patient preparation, exercise, diurnal variation, and physiologic states such as pregnancy or menses could affect the results of laboratory tests as well as other physiologic variables.

Sex-related differences in the mean serum concentrations of lipids, enzymes, and other analytes do exist. Blood hemoglobin and ferritin concentrations are lower in adult females. Serum iron is lower in females, generally attributable to blood loss during menstruation. The serum concentrations of creatinine, urea nitrogen, and uric acid, as well as the enzyme activities of aldolase, aspartate aminotransferase, and creatine kinase are all greater in males than in females. Many of these differences result from the greater muscle mass in the male group. Alkaline phosphatase activities are lower in the younger adult female population, but they increase after menopause to levels equal to or greater than those do for men.

Prior to age 50, males have higher serum levels of calcium, phosphorus, triglycerides, and cholesterol. However, after this age, females generally have higher serum levels for calcium, phosphorus, and cholesterol. Sex-specific hormone levels will quite naturally differ between males and females and at different ages. Post-pubertal testosterone levels are higher in males, while higher levels of estrogens, follicle-stimulating hormone, and luteinizing hormone can be expected in females.

The effects of race or nationality on the basal concentrations of blood analytes can be considered small. More important are the effects of environmental and socioeconomic factors (e.g., diet, nutrition, exercise, and habits) and the known higher incidence of genetic diseases (e.g., sickle cell anemia, thalassemia) in certain ethnic groups. The amount of muscle mass is generally greater in certain African descent individuals. As a result, the observed activity of the muscle enzymes creatine kinase and lactate dehydrogenase are often higher in this group.

The blood groups ABO predispose individuals to exhibit metabolic and immunologic expression characteristic of that type that have small influences on chemistry and hematology normals.

Following ingestion of a meal, the concentrations of several plasma constituents change noticeably. Potassium and phosphorus may increase or decrease depending on the type of meal ingested. Plasma glucose and triglycerides rise after the ingestion of food. The postprandial elevation in triglycerides may be present for up to 8 hours. The increased triglycerides may produce a lactescent serum, which has the potential to interfere with some clinical assays. Plasma alkaline phosphatase activities may increase in some individuals, especially after eating a meal high in fat content. The increase in alkaline phosphatase results primarily from an increase in the intestinal isoenzyme and is dependent on the blood group of the individual as well as the substrate used in the selected assay.

One factor not controllable by either physician or laboratory is the degree of normal diurnal, day-to-day, and seasonal variation in the concentration of measured components in biological fluids. While the intra-individual variation of analytes such as electrolytes and serum proteins is generally considered to be small, the serum or urinary content of other biological components such as enzymes, lipids, hormones, and iron may change considerably over both shorter and longer time intervals.

Many hormones show both a diurnal variation and random biological variation during each 24-hour period. The diurnal cycles are often regulated by day/night and wake/sleep cycles, and having a knowledge of the daily habits of the patient will help in the proper interpretation of results as well as guide in the decision of when to obtain a specimen for testing. Insulin levels in blood are higher in the morning than later in the day. The serum levels of growth hormone (GH) are normally low during the daytime, although short bursts of GH may be observed 3 to 4 hours after meals. The secretion of growth hormone increases significantly during the first 2 hours of sleep, and concentrations of GH are highest during the initial period of deep sleep. Adrenocorticotropic hormone (ACTH) (corticotropin) and cortisol secretion curves also show diurnal variation, with peak blood concentrations normally observed between 6 and 8 AM (1). Levels gradually fall throughout the day, reaching a nadir between 9 PM and 3 AM. As with many hormones, secretory

spikes occur throughout the day, introducing another component to specimen timing and the interpretation of results. Stress will also cause a sharp rise in the secretion of ACTH and cortisol.

Thyroid-stimulating hormone (TSH) shows a circadian variation in which serum levels are highest around or after midnight and lowest at midday. TSH is also secreted in multiple pulses that occur throughout the 24-hour period. These pulses may result in measured TSH levels that are slightly outside of the reference range, especially if blood samples are obtained at night when the amplitude of these pulses tends to be greater. A recognition that such hormone pulses occur allows a more cautious interpretation of borderline abnormal results.

A diurnal variation can be observed for serum iron and potassium. Iron and potassium levels are higher during the morning hours than during the afternoon and evening hours. For serum iron, the difference can be as great as 30 to 50%. Serum iron levels also show intra-individual day-to-day variation of the same magnitude.

In some cases, diurnal and seasonal variations can coexist. The pineal gland hormone melatonin is purported to play a role in both sexual development and the etiology of affective disorders. The production of this hormone is regulated by light-dark cycles, with secretion occurring principally during the hours of darkness. The length of seasonal daily light exposure, which differs between winter and summer seasons, creates an additional circannual variation in the production of melatonin. Melatonin levels have been shown to be erratic in patients suffering from a psychological depression called seasonal affective disorder.

In biologically oriented treatment, it is necessary to follow the course of disease and to judge the progress or efficacy of a chosen mode of therapy. This is accomplished in part by evaluating the results of laboratory tests designed to monitor specific physiological parameters. Some changes may be the result of the analytical variation of the test (discussed later). Additionally, proper interpretation of a change between two consecutive determinations requires a consideration of the contribution that intra-individual day-to-day variation may play in the observed magnitude of the observed change. In other words, the patient is used as his or her own referent.

Bear in mind that blood chemistry and hematologies are *static* tests. Though they somewhat *reflect* regulatory, dynamic mechanisms, they are not true functional tests which usually involve giving challenge substances and assessing the chemical changes. Functional testing should be preformed when the patient history, symptomology, and blood chemistry and hematology warrants further investigation to determine causes and causal chains of pathophysiology. In addition, serial laboratory measurements over time should be compared to gain better understanding of the individual's overall pattern and normal variance.

Even in full acknowledgement of the above information, there is currently a great deal of resistance by conventional medicine to the concepts of individual variance and "optimum or healthy" ranges on blood chemistry. No diagnostic procedure is absolute, nor should any one be used as a sole means of making a diagnosis. Biological Medicine always uses multi-diagnostic methodology to further determine the causes of an illness. Structural, functional, regulatory, energetic and informational diagnostics must be fully utilized to complete to picture of causes. Blood chemistry, irrespective of the ranges used (optimal or laboratory normals), is yet another tool to be utilized together with the other diagnostic approaches available to the practitioner.

The "optimum normals" presented in this paper are the result of information gathered between 1980 and the present on over 10,000 people using what was known as the Biochemical Biopsy. (See bibliography) This was testing that utilized electrophoretic methods, colorimetric studies, atomic absorption spectroscopy and standard hematological studies, together with the physical findings and the patient's subjective information. These test results were integrated into additional information developed from physical examination, patient histories, urinalysis, hair mineral analysis and other diagnostic criteria available to the physicians who were members of the Balancing Body Chemistry group. Although the optimum values given in this manual cannot be considered absolute, when the optimum guidelines are not present, further investigation is warranted. Always consider biochemical individuality.

The use of clinical laboratory test results in diagnostic decision making is an integral part of clinical medicine. The menu of laboratory tests available to clinicians constitutes an impressive array that has expanded exponentially since 1920 when Folin and Wu devised the first useful test for the quantification of serum glucose concentration. The current list of tests offered by one major reference laboratory includes nearly 3,000 analytes, which does not include the additional array of more commonly ordered tests (eg, complete blood count, electrolytes [sodium, potassium, chloride, carbon dioxide], thyroid stimulating hormone, glucose, etc.) routinely performed on site by most hospital-based clinical laboratories. The following text outlines some of the more common tests and their clinical and nutritional implications.

In the final analysis, it is important for clinicians and laboratorians to recognize that laboratory data, although potentially extremely useful in diagnostic decision making, should be used as an aid and adjunct to the constellation of findings (eg, history, physical exam, functional testing, etc.) relevant to the patient.

Glucose, Serum

Laboratory Range Adult: **65 to 115 mg/dL** **3.6 to 6.4 mmol/L**
> Age 60: **80 to 115 mg/dL**
Child: **60 to 100 mg/dL**

Critical Level: **<40 mg/dL**
Or >700 mg/dL
Optimum Range Adult: **80 to 100 mg/dL**
Method: Enzymatic Colorimetry
Interday variation: 5 to 10% Higher for post-prandial glucose

General Comment: Glucose is the most frequently ordered of all clinical chemistry tests. This has been the case for many decades and will likely continue to be true for many years to come. In most present-day situations, the term "blood glucose" or "blood sugar" is not technically accurate. Although at one time the laboratory used whole blood for this assay, it is rarely used today. What is usually measured is serum or plasma glucose. The serum glucose concentration is higher than whole blood glucose. Glucose is uniformly distributed in the water of whole blood, but due to the large volume of hemoglobin inside the red cells, there is relatively less water, thus less glucose, inside the red cells. Blood glucose is of course a weighted average of the intracellular and plasma glucose levels.

Since red and white blood cells continue to metabolize glucose after they have been removed from the body, it is important to separate the serum and cells within a reasonably short time. After the cells have been removed, the serum can be stored at room temperature for hours, and at refrigerator temperature (4ºC) for days, before the analysis is done. Plasma has a distinct advantage over serum for rapid glucose analysis in that there is no delay while the blood clots: the cells can be immediately separated from the plasma by centrifugation.

Physiology: Glucose is the transport form of carbohydrate in the body. Ingested starches and sugars are converted to glucose by digestive enzymes and by the liver. When dietary sources of glucose are not available, the liver synthesizes glucose from glycogen (glycogenolysis) or protein (gluconeogenesis).

All cells use glucose as an important energy source, some (e.g., the central nervous system - the brain and spinal cord) depend entirely upon it. Insulin, produced by special cells in the pancreas, reduces blood glucose by facilitating its entry into the tissue cells (at least muscle and fat cells) by a mechanism not yet understood. A deficiency of insulin, as in "juvenile" diabetes (onset in childhood), increases blood glucose by reducing the tissue utilization of glucose. In many patients with "adult" diabetes (onset in middle or later years), antibodies develop against insulin that reduce its effectiveness even though it is present in large quantities. Other hormones raise blood sugar. These include adrenaline, hydrocortisone, glucagon, thyroxine, and growth hormone.

As is the case with most of the substances measured in the laboratory, blood glucose is a continuous variable. The point at which gradually increasing blood glucose can be regarded as abnormally high has to be arbitrarily assigned. The blood glucose value is subject to many variables, both methodological and biological. Progressive improvement and standardization in analytical procedures have resulted in substantial reduction in method variance. The method used in the aca should come as close as, or closer than, any other method to measuring what is the "true" glucose.

It is common practice to examine blood before breakfast: this is called the "fasting" blood sugar, or FBS. FBS is the measurement usually studied in clinical practice. There is general agreement that the two-standard deviation "laboratory normal range" (mean ± 2 S.D.) for serum glucose is 65-115 mg/dL. “Optimum range for FBS is 80 to 100 mg/dL. The fasting level of blood glucose expected in a normal individual depends upon a large number of factors that are regarded as physiologic (not disease). This include his age, how long it has been since his last meal, what sort of diet the patient has eaten for several days before the test, whether he has exercised just before the test, whether he is fearful or anxious, etc. Roughly speaking, the older the patient, the higher the blood glucose will be. Fasts longer than 48 hours reduce the blood glucose

concentration (but not to dangerous levels). Diets low in carbohydrate reduce the response of the insulin-producing cells to a recent glucose load. Exercise tends to lower blood glucose somewhat, but if accompanied by excitement may raise the glucose levels. In the fasting state the glucose values obtained on venous and capillary blood samples are for all practical purposes equal.

It is common practice to try to standardize the conditions of the test as much as possible. For this reason, fasting levels are ordinarily measured taking all things into account and allowing a little leeway above the usual 2 standard deviation limit, 120 mg/dL is usually regarded as the dividing point between normal and high glucose concentrations in the fasting state.

Any patient with fasting blood glucose concentration of more than 120 mg/dL has hyperglycemia and the cause should be investigated. This is most often done by determining the patient's response to the oral administration of a standard amount of glucose. This test is called the glucose tolerance test, or GTT. For an adult, 100 grams of glucose are given. The blood glucose is measured immediately before, and at intervals of 0.5, 1, 2 and 3 hours after the glucose solution is ingested. This is the standard GTT procedure. There are a number of ways in which this test can be modified. A more palatable carbohydrate mixture may be used, the glucose can be given intravenously, the glucose levels may be measured for four to six hours, or drugs such as cortisone can be given. In an adult the glucose is measured in venous blood. [After an oral glucose "load" the capillary level of glucose rises more promptly and to higher levels than the venous level. After the peak concentration has been reached and the level begins to fall, the two concentrations again converge.] Normally the glucose raises sharply, peaks at 0.5 to 1 hour, and falls to less than 120 mg/dL by 2 hours and returns to the fasting level by 3 hours. Under these conditions the peak value should not exceed 160 mg/dL. There is no universal agreement as to exactly how the glucose curve should be interpreted, especially in borderline or atypical cases. The initial value, the peak value, and the two-hour value are all considered.

Clinical Significance: Blood glucose may be abnormally high (hyperglycemia) or abnormally low (hypoglycemia).

Hyperglycemia: Diabetes Mellitus is the most important cause of elevated blood glucose since it is both a common and a serious disease. In general terms, diabetes can be regarded as a relative or absolute deficiency of insulin. Insulin is necessary for the utilization of glucose by tissue cells. Deficiency of insulin results in under-utilization and backup in the extracellular fluids, including the blood. Blood glucose measurement is useful in the diagnosis of diabetes and in monitoring treatment, whether this is by diet, hypoglycemic drugs, or insulin.

Cushing's Syndrome: There are many diseases in which excess amounts of cortisol are produced. One of the abnormalities produced by an excess of this hormone is increase gluconeogenesis. As a result, more glucose is generated than consumed, which leads to an increase in the blood glucose concentration. Cushing's syndrome is not common, but is mentioned here as an example.

Hyperadrenalism: Any disease or condition that results in an excess production of adrenaline will cause a rise in blood glucose (usually transient). These include fear, strenuous physical exercise, shock, and a rare disease, pheochromocytoma, which is an adrenaline-producing tumor of the adrenal gland. Adrenaline causes the conversion of glycogen to glucose by the liver.

Hypoglycemia: There is much controversy regarding what should be considered "hypoglycemia". It appears that most people can tolerate levels lower than 70 mg/dL. A value below which patients are considered to have "clinical", that is "sick with", hypoglycemia is, at this writing, difficult to fix. Various authors have given values of 50 or 40 mg/dL, but values below 65 should be further evaluated. The lack of agreement arises from the great variation in the ability of patients to tolerate low glucose levels. A wise physician considers the blood glucose concentration in the context of other observations. Hypoglycemia does occur, and it can be most dangerous. Several causes are recognized. Very low glucose levels result in coma and convulsions and can be generally degenerative to cellular metabolism.

Islet Cell Adenoma (Insuloma) of the Pancreas: The insulin-producing cells of the pancreas are grouped in clusters known as islets that are distributed throughout the main part of the pancreas. These beta cells occasionally undergo uncontrolled growth (tumor formation) and produce more insulin than the body requires. This results in a reduction of the fasting blood glucose levels that becomes progressively more severe as the tumor enlarges. The diagnosis of

this tumor is usually made by fasting the patient for long periods (24-72 hours) and observing a progressive fall in blood glucose. Usually the glucose falls to 30 mg/dL (or lower) within 24 hours. The measurement of blood insulin levels may be of some additional help in diagnosis, but in some cases insulin levels are only in the "high normal" range.

Other Tumors: Tumors of non-pancreatic origin may cause severe hypoglycemia. They are usually large, slow-growing tumors that occur within the abdomen. The reason they cause hypoglycemia remains controversial. One plausible, but unconfirmed explanation is that these tumors elaborate a polypeptide that has insulin-like activity. Removal of the tumor corrects the hypoglycemia.

Hypoadrenalism: Reduced activity of the adrenal cortex, which produces cortisol, removes an important source of control of the blood glucose level. Cortisol is needed to induce gluconeogenesis when insufficient glucose is available either from recently ingested food or from liver glycogen stores. The adrenal gland may become inactive as the result of its destruction (it is unusually susceptible to certain prescription medications, heavy metals, xenobiotics and certain fungal diseases), or because of reduced stimulation by the hormone ACTH (adreno cortico tropic [stimulating] hormone) produced in the pituitary gland. When the output of cortisol is low because of local destruction of the adrenal gland, it is known as Addison's disease, or primary hypo-adrenalism. When it is due to lack of ACTH production, it is known as secondary hypo-adrenalism. Although both conditions reduce the blood glucose, hypoglycemia is more severe in Addison's disease.

Liver Disease: Since the metabolism of glucose centers in a major degree upon the liver, it is not unexpected to find disorders of glucose metabolism in liver disease. It is more of a surprise to find that hypoglycemia occurs only with very marked liver disease, such as in cirrhosis. In liver disease the blood glucose level after a meal reaches higher than normal levels and remains higher longer because the liver has a reduced capacity to metabolize glucose and store it as glycogen. During the fasting period the blood glucose falls to low levels due to the unavailability of liver glycogen and the inability of the liver the carry out gluconeogenesis. Hypoglycemia in liver disease is not regarded as a matter of great consequence in view of the many more serious alterations in metabolism that are present.

Reactive Hypoglycemia: This is a descriptive term applied to many individuals who, for a variety of reasons, develop low glucose levels two to four hours after eating high carbohydrate meals. In most patients this is believed to be due to over-response by the insulin-producing beta cells. A similar hypoglycemic state may appear in early diabetes. In these patients the insulin response to food ingestion may be normal in degree, but is delayed.

Hormonal Influences on Glucose:

Hormones that facilitate its entry to or removal from the circulation influence the blood glucose concentration. The hormones alter glucose uptake by cells (for energy production), and influence blood glucose by promoting or inhibiting glucose production and glycogen production and breakdown. The most important hormone causing a decrease in blood glucose is insulin. Insulin is produced by beta cells in the pancreatic islets. The release of insulin is stimulated by glucose, amino acids, and hormones (glucagon and gastrin). Corticosteroids and growth hormone inhibit insulin by causing peripheral insulin resistance. Insulin decreases blood glucose by promoting glucose uptake and use by liver, muscle and tissue cells. It also enhances triglyceride formation (by stimulating lipoprotein lipase). It promotes uptake of K+, phosphate and Mg+ by cells. It also inhibits glucose production by inhibiting gluconeogenesis and glycogenolysis. The main hormones opposing the action of insulin (and increasing blood glucose) are glucagon, epinephrine, growth hormone, corticosteroids, somatostatin (by inhibiting secretion of insulin) and thyroxine (by increasing sensitivity of cells to epinephrine). The following table demonstrates the most common hormonal affects on glucose.

Hormone	Glycogen	Glucose Production	Glucose Uptake	[Glucose]
Insulin	Synthesis	Decrease	Promotes	Decrease
Corticosteroids	No effect	Increase	Decrease	Increase
Catecholamines	Breakdown	Increase	Decrease	Transient increase
Growth hormone	Breakdown	Increase	Decrease	Increase
Glucagon	Breakdown	Increase	No effect	Increase

Other Relevant Tests:

Glycohemoglobin
The glycohemoglobin or the glycosylated hemoglobin test was developed in the late 1970's. Hemoglobin (Hb) is the compound in the red blood cells that transports oxygen. One of the types of hemoglobin (Hb) is called HbA and HbA1c is a specific subtype of HbA. Glucose binds slowly to Hb and produces glycosylated Hb. There are several types of glycosylated hemoglobin measures (including total glycosylated Hb and HbA1), but HbA1c, which is formed when HbA is glycosylated, is now considered the best and standard measure.

The higher the blood sugar, the faster HbA1c will be formed, resulting in higher HbA1c levels. Red blood cells circulate 90 -120 days, and the HbA1c level is in part affected by blood sugar levels over a 3-month period. However, it mainly represents levels over the past month and is heavily weighted to the past 2 weeks. The normal values for this test vary depending upon the laboratory. Generally, it is a simple way to evaluate average glucose levels over the past 2 - 4 weeks. It is also the best single test for evaluating the risk for glycemic damage to tissues (e.g., nerves, and small blood vessels in the eyes and kidneys) and thus, risk of complications of diabetes.

Fructosamine
The fructosamine test has been developed more recently. Fructosamine is a term referring to the linking of blood sugar onto protein molecules in the bloodstream. Fructosamine levels have been shown to change more rapidly than glycohemoglobin. The fructosamine value depends upon the average blood sugar level over a three-week period. Therefore, it might be able to detect changes in diabetic control earlier than the glycohemoglobin.
The fructosamine test could be viewed as complementary to the glycohemoglobin, since the two tests are different reflections of diabetes control: glycohemoglobin looks back approximately two to eight weeks, and the fructosamine test looks back about three weeks.

Fructosamine is also useful for monitoring response to therapy in diabetic patients, as persistent changes in glucose will be reflected more rapidly in fructosamine, compared to glycosylated hemoglobin, because serum proteins have a shorter half-life than hemoglobin. Indeed, fructosamine is lower (and often within reference intervals) in well controlled versus poorly controlled diabetic patients.

Clinical Pearls:

If glucose is increased or decreased, conduct a glucose tolerance test.
If a “flat curve” on a glucose tolerance test is found, a hair element analysis for toxic elements should be conducted. A toxic element urine provocation with DMPS is a more accurate utility for determining heavy metal body burden. A flat curve on a glucose tolerance test is often found with heavy metal body burden.

If glucose is increased with increased serum cholesterol and triglycerides, carbohydrate sensitivity is possible. If triglycerides are elevated above total cholesterol, carbohydrate sensitivity is probable.

If glucose is increased with a decreased total CO2 and an increased anion gap, thiamine need is probable.

If fasting glucose is decreased, hypoglycemia is possible. If decreased with a decreased LDH, hypoglycemia is probable. LDH activity, among other things, represents active exchange of chloride with glucose and glucose with zinc and sodium (glycolysis). Therefore, LDH activity is associated with pancreatic function and glucose metabolism. (See LDH) Fasting hypoglycemia is rare, reactive hypoglycemia is more common, and is often found in blood type O individuals.

If hypoglycemia is suspect, consider performing a 5 to 6 hour glucose tolerance test. Always have the patient write down their subjective feelings each time the blood is drawn during the test. The subjective feelings of the patient may prove to be a valuable complement to the test results.

Blood Urea Nitrogen (BUN)

Laboratory Range: 8 to 22 mg/dL 2.9 to 7.9 mmol/L
Critical Level: >100 mg/dL
Alarm Range: >50 mg/dL
Optimum Range: 10 to 18 mg/dL
Method: Colorimetry
Interday variation: 5 to 15% Highest at midnight lowest at 5 PM

Physiology: Urea is the detoxification product of the ammonia derived from the de-amination of amino acids. Urea is therefore the most common nitrogen-containing end product of protein catabolism. Its synthesis from ammonia occurs in the liver (via the Krebs-Henseleit cycle), and the kidneys then excrete it. Urea production is increased when excess protein is ingested or when body protein is catabolized, regardless of cause.

Urea is free to pass through all membranes of the body and is equally distributed in the body water. The concentration of urea inside red cells is slightly less than in plasma due to the presence of large amounts of hemoglobin inside the cells. Whole blood urea concentration is therefore slightly less than plasma (or serum) urea. Usually serum is used instead of whole blood for the determination of urea, and the amount of urea is expressed in terms of its nitrogen content. The familiar term BUN (blood urea nitrogen) persists in spite of the fact that it is serum urea nitrogen that is usually measured.

The concentration of urea in the body water depends upon the rate of production by the liver and the rate of removal by the kidneys. In most patients the rate of production is a reflection of the protein intake and the rate of degradation of cell proteins. In some patients, liver function may be rate limiting; in sever liver disease the ability of the liver cells to form urea from ammonia is impaired; ammonia accumulates, and urea levels fall. The rate of removal depends upon the concentration in the plasma, the amount of plasma that passes through the kidney per unit time (the rate of renal perfusion) and the capacity of the kidney to remove the urea from the plasma (kidney function).

In greatly simplified terms, the kidney produces urine by three mechanisms. These are: an initial filtration of the plasma, which occurs in tufts of tissue in the kidney called glomeruli; a selective re-absorption of certain of the materials in the filtrate by the cells that line the renal tubules and active secretion by the tubular cells. Blood cells and proteins do not normally pass through the filter. Small molecular substances, unless they are protein-bound, are filtered and appear in the glomerular filtrate. Selective re-absorption may then occur; some substances are completely reabsorbed, some only partially, and some not at all. In some cases, an additional amount of the substance may be added to the filtrate by the process of excretion by the tubular cells. The process of tubular excretion handles protein-bound small molecules. Urea is excreted by the first two mechanisms: filtration and re-absorption. It is filtered by the glomeruli and then reabsorbed by the tubule to a variable degree that is inversely proportional to the rate of urine flow through the tubule. [In contrast, creatinine is filtered by the glomeruli, not reabsorbed, but an additional small amount is added to the urine by tubular excretion.]

In most clinical situations, changes in urea levels are more dependent upon kidney function than upon liver function. Most commonly the BUN is measured as a screening test for renal disease, more specifically glomerular filtration. If the BUN is normal, it is usually assumed that kidney function is normal. However, the functionality of the kidney may be impaired without elevations in BUN. If the BUN is elevated, or if more precise information is needed, a more quantitative technique, the "urea clearance test", may be employed. This procedure considers the amount of urea being produced (by measuring the amount excreted in the urine over a prescribed period, such as 24 hours), as well as the plasma urea concentration. The "clearance" is expressed in terms of the volume (in ml) of plasma that would be completely "cleared" of urea for one minute as calculated from the plasma urea concentration and the amount of urea excreted in the urine. The clearance value in normal men is about 75 ml/min (if the rate of urine flow is at least 2 ml/min). Values less than 10 indicate severe kidney disease. The urea clearance is seldom used today because the plasma urea level is not constant throughout the period of urine collection, and

the amount of urea that finally appears in the urine is dependent upon the rate of urine production (i.e., the rate of water excretion). These two sources of error do not affect the creatinine clearance, which is therefore a superior procedure for this purpose. Another valuable test to perform is a 24-hour urine organic acid analysis. This test will reveal important information about kidney function when elevated urea or borderline urea is detected.

Clinical Significance: BUN can be either abnormally high or abnormally low (rarely).

Increased Urea Levels: Urea itself is relatively nontoxic, but other substances that are also retained when kidney function fails (a condition called uremia) are toxic. Urea levels rise under the following circumstances.

1. Increased production, due to either a high protein diet, or to excessive destruction of cellular proteins of the body (as in fever, massive infections, etc.).

2. Reduced renal perfusion (blood flow through the kidneys). If the circulation of blood to the kidneys is reduced, this may become the limiting factor in removal of urea from the blood. This may occur in dehydration (insufficient water intake and consequent decrease in the blood volume) or heart failure in which the capacity of the heart to pump blood is compromised.

3. Kidney disease: nearly all types of kidney disease (of which there are a large number) will result in urea retention.

4. Mechanical obstruction to urine excretion, such as may occur in diseases of the ureters (the tubes which connect the kidney to the urinary bladder), bladder or urethra. Stones, tumor, infection, or stricture may cause the obstruction.

Decreased Urea Levels: A pathological clinical condition in which the urea level is significantly lower than normal exist in certain advanced liver disease that disrupt the urea cycle. Blood urea can also be reduced to very low levels by artificial means, as in hemodialysis (dialysis of the blood by the "artificial kidney").

Other Causes of Decreased BUN:

1. Decreased protein intake or protein anabolism: Severe dietary restriction of protein, with increased anabolic rate.

2. If decreased with an increased or decreased total serum globulin, amino acid need is probable, or protein maldigestion secondary to HCL deficiency or a pancreatic insufficiency of proteolytic enzymes.

3. If decreased in pregnancy, need for amino acids are probable.

4. Increased excretion: Any cause of polyuria, e.g. hyperadrenocorticism, diabetes mellitus.

5. Decreased production: Consider advanced liver disease.

Other Complementary Testing for Abnormalities in Blood Urea Nitrogen:

1. Creatinine Clearance and Serum Creatinine

2. Amino Acid Analysis

3. Organic Acid Analysis

Creatinine, Serum

Laboratory Range: 0.7 to 1.5mg/dL 62 to 133 micromole/L
Optimum Range: 0.8 to 1.1 mg/dL
Method: Enzymatic Colorimetry
Interday variation: 15 to 20% Up to 30% higher in PM than AM
Often lower in children and in pregnancy

Physiology: Creatine is involved in energy storage in skeletal muscle and other tissues. Creatine is synthesized in the liver from amino acids and then transported by the blood to muscle. There the enzyme creatine phosphokinase (CPK, q.v.) catalyzes the reaction of creatine with ATP to form phosphocreatine. Phosphocreatine contains a high-energy phosphate bond and serves as an energy storage mechanism. Creatinine is a catabolic end-product, an anhydride of creatine (or phosphocreatine) produced by loss of water (or phosphoric acid) from the molecule in an irreversible reaction. Creatinine is not reutilized, but rather is excreted from the body via the urine. It is formed at a nearly constant rate that is proportional to the body muscle mass. Because of the way in which creatinine is excreted by the kidney, creatinine measurement is used almost exclusively in the assessment of kidney function. In fact, creatinine is generally regarded as the most useful naturally occurring substance to measure in the diagnosis and follow-up of kidney disease.

A single, random measurement of serum creatinine may be used as a qualitative and semi-qualitative indicator of impaired kidney function. More quantitative information regarding the extent of kidney damage, specifically damage to the filtration mechanism, can be gained through use of the "creatinine clearance test". (See section on BUN for a detailed discussion of the mechanisms used by the kidney in the formation of urine.) Creatinine is present in the ultra-filtrate of plasma that is formed by the glomerulus of the kidney. The filtered creatinine is not reabsorbed by the kidney tubules. A small amount of creatinine is added to the urine by the process of tubular secretion. Thus, the amount of creatinine excreted in the urine is primarily a function of glomerular filtration. Measurement of the amount of creatinine in the urine produced during a specified period (urine creatinine concentration X urine volume) and the plasma creatinine level allows one to calculate a value called the creatinine clearance. This is measured in milliliters of plasma per minute, and represents the volume of plasma that must be filtered by the kidney to account for the amount of creatinine found in the urine. The value obtained for creatinine clearance correlates fairly well with more exact measures of glomerular filtration rate (GFR). This is, of course, a slight error since some of the creatinine finds its way into the urine through tubular secretion rather than filtration.

Clinical Significance: As noted above, creatinine is measured primarily to assess kidney function. In this regard, it has certain advantages over the measurements of urea (BUN): the plasma creatinine level is relatively independent of protein ingestion, water intake, rate of urine production, and exercise. Its rate of production is constant in each individual. Therefore, an elevation of plasma creatinine level must represent under-excretion, i.e., kidney impairment.

Although the measurement of glomerular filtration is only approximate, it is sufficient for most clinical purposes (e.g., original estimate of the extent of damage to the kidney and evaluating the changes in kidney function as the disease progresses and more glomeruli are destroyed).

Causes of Increased Creatinine:

1. Decreased GFR: Due to pre-renal, renal or post-renal causes.
2. Prostate pathology (BPH); conduct PSA if elevated

3. Increased production: A mild increase (< 1 mg/dL) may be seen after ingestion of a recent meat meal. Acute myositis does not consistently increase creatinine itself (although severe myositis or myopathy can produce renal azotemia from myoglobinuric nephrosis).

Clinical Pearls:

Increased with normal BUN and normal electrolyte values, suspect prostate hypertrophy. If the patient is 45 years of age, prostate hypertrophy is probable. A physical examination of the prostate and a prostate specific antigen (PSA) should be conducted. LDH isoenzyme #4 and monocytes also will be elevated in many cases of prostate hypertrophy.

With an increase in urinary WBC with bacteria visible in urine, a prostate infection is probable.

Artifact: When measured by the Jaffe method, both creatinine and non-creatinine chromogens react with the reagent. Non-creatinine chromogens include acetoacetate, glucose, vitamin C, uric acid, pyruvate, cephalosporins and amino acids. When present in high concentrations, these can artefactually elevate creatinine values.

Causes of Decreased Creatinine:

1. Decreased production: Loss of muscle mass, muscular dystrophy. Severe liver disease from cirrhosis may result in decreased creatinine values from decreased creatine production.

2. Increased GFR: This occurs with portosystemic shunts and during pregnancy (due to increased cardiac output).

3. Drugs Include: Cefoxitin sodium, cimetidine, chlorpromazine, chlorprothixene, marijuana, thiazide diuretics, and vancomycin.

4. Artifact: With the Jaffe reaction, severe icterus can artefactually decrease creatinine concentrations. Utilizing a reaction blank can minimize this effect.

The daily excretion of creatinine in the urine is relatively constant and averages 10 to 20 mg/kg a day. If in a 24-hour urine collection the creatinine excretion deviates significantly from these values, the collection may not be accurate. The creatinine clearance is elevated 30% to 50% over normal values in pregnancy. During the middle to later years, there is a reduction in renal size and in the number of functional nephrons. Both the glomerular filtration rate (GFR) and tubular secretory capacity decline steadily after the age of 30. Creatinine clearance values decrease with age in a manner paralleling changes in the GFR. The accurate determination of creatinine clearance relies on the proper collection of a 24-hour urine specimen, and this may be difficult to control in the elderly patient.

Urea Nitrogen and Creatinine

BUN/Creatinine Ratio: This is a valuable utility when assessing individuals with chronic renal dysfunction. It is of primary value when the decreased ratio is less than 10 as seen in an antidiuretic hormone deficiency.

Laboratory Range: 10 to 20 Optimum Range: 12 to 16

As previously mentioned, the BUN and Creatinine are used as indicators of glomerular filtration rate (GFR). Neither is perfect in this regard, but they are clinically useful nonetheless. Decreases in GFR are generally due to one of two main causes:

1. Decreased renal perfusion due to hypovolemia or cardiac dysfunction (prerenal causes)
2. Loss of functional nephrons (renal causes)
3. Combination of the two above causes

Azotemia:

Azotemia is defined as an increase in BUN and creatinine and can result form a variety of disorders including, but not limited to, renal failure. Uremia is the term for the clinical syndrome of renal failure with azotemia and multi-systemic problems such as polyuria, mild non-regenerative anemia (in chronic renal failure), vomiting, weight loss, depression, and other sequelae of inadequate renal function. Azotemia can be due to prerenal, renal or post-renal causes. Differentiation of the causes of azotemia requires urinalysis (especially assessment of urine specific gravity), biological terrain assessment BTA™, and evaluation of clinical signs and results of other diagnostic tests (e.g. radiographic evidence of urinary tract obstruction). Remember that the kidney is essential to acid-base and electrolyte homeostasis. Any cause of azotemia will result in retention of organic acids normally excreted by the kidney (i.e. a high anion gap metabolic acidosis) and hyper-phosphatemia.

Prerenal Azotemia:

Prerenal azotemia is due to a decrease in GFR from circulatory disturbances causing decreased renal perfusion, such as hypovolemia (shock, hemorrhage, Addison's disease, vomiting), cardiac disease or renal vasoconstriction. Prerenal azotemia can usually be distinguished from renal azotemia by clinical signs (evidence of dehydration or hypovolemia), and there should be no other evidence of renal tubule dysfunction such as proteinuria) and response to therapy. Urine specific gravity may be decreased (despite a prerenal azotemia) if there are other factors reducing the concentrating ability of the kidney. Therefore, often a response to therapy (fluid administration) is required to differentiate between a primary renal and prerenal azotemia. Note that many causes of a prerenal azotemia will result in renal hypoxia and ischemia. If this is severe or chronic enough, a primary renal azotemia may result, and may co-exist with a renal azotemia. As BUN levels in blood are dependent on flow rate through the renal tubules (decreased flow rate in prerenal azotemia enhances renal absorption of BUN, and increases blood BUN levels), BUN may increase without any increase in creatinine in early pre-renal azotemia.

Renal Azotemia:

Renal azotemia results from a decreased GFR when more than 3/4 of the nephrons are non-functional. Renal azotemia may be due to primary intrinsic renal disease (glomerulonephritis, heavy metal toxicity, ethylene glycol toxicity) or may be secondary to renal ischemia from prerenal causes or from kidney damage from urinary tract obstruction (post-renal azotemia). Loss of 3/4 of kidney function usually follows concentrating defects (requires loss of 2/3 of the kidney). Therefore, isosthenuric urine (usg 1.008-1.012) is common in renal azotemia. In addition, there may be other evidence of renal tubular dysfunction in the urinalysis, such as proteinuria, granular or cellular casts, and glucosuria without hyperglycemia. Azotemia with a urine specific gravity less than those values stated above is presumptive evidence of renal azotemia or renal failure, unless there is also evidence of other diseases or conditions affecting urine-concentrating ability independently of renal failure. As mentioned above, a high anion gap metabolic acidosis is common with renal failure. Hypermagnesemia and hyperkalemia are features of oliguric or anuric renal failure.

Post-renal Azotemia:

Post-renal azotemia results from obstruction (urolithiasis) or rupture (uroabdomen) of urinary outflow tracts. This condition is best diagnosed by clinical signs (e.g. frequent attempts to urinate without success), and ancillary diagnostic tests (e.g. inability to pass a urinary catheter), as urine specific gravity results are quite variable. Individuals with post-renal azotemia are markedly hyperkalemic and hypermagnesemic. Uroperitoneum can be confirmed by comparing the concentration of creatinine in the fluid to that in serum or plasma; leakage of urine is indicated by a higher creatinine in fluid than in serum. Post-renal azotemia can result in primary renal azotemia (failure) due to tubule dysfunction from impaired renal flow.

Cholesterol, Total

Laboratory Range: 100 to 200 mg/dL
Optimum Range: 150 to 220 mg/dL
Method: Colorimetry
Interday variation: 5 to 7%. Higher in winter by 3 to 5%
In females, 10 to 20 % lower in luteal phase of cycle, lowest during menstruation. In pregnancy increases by 75% by the third trimester; may rise sharply after menopause.

Physiology: Steroids are cyclic aliphatic hydrocarbons with solubility characteristics like those of lipids. The basic chemical structure is a cyclopentanophenanthrene nucleus composed of three-fussed cyclohexane rings and one five-membered ring. Aliphatic side chains are usually present, and many natural steroids have one or more hydroxyl groups on the ring. These secondary alcohols are known as sterols. In animals, including man, the most abundant sterol is cholesterol, named from the site of its discovery, gallstones. Gallstones may contain more than 90% cholesterol.

Cholesterol is found in all cells of the body. The adrenal gland contains 6% cholesterol (by wet weight). Cholesterol is a vital building block for the formation of pregnenolone and other related steroidal hormones such as progesterone, cortisol, the estrogens and androgens. The brain and spinal cord contain 2% cholesterol; in these tissues cholesterol forms part of the lipid "insulation" which separates individual nerve fibers. The precise function of cholesterol in other cells is not known, but it is probably related to the structure and permeability of the cell membrane.

Cholesterol may be synthesized from two-carbon fragments in many body tissues, particularly liver, intestine, and skin. Most cholesterol is excreted from the body via the bile. Liver cells oxidize the molecule by adding hydroxyl and carboxyl groups (the ring structure remains intact) to form cholic acids. These are excreted in the bile where they are instrumental in the absorption of fats, including cholesterol itself, from the diet.

Plasma cholesterol is a structural part of the macromolecular aggregates of protein and lipids that are needed to solubilize and transport triglycerides. These complexes are called lipoproteins. All lipoprotein fractions contain some cholesterol, but 50-75% is found in the beta-lipoprotein fraction (low density) and 25-45% in the alpha fraction (high density). [See the section of triglycerides for discussion of function and classification of plasma lipoproteins]. The amount of cholesterol in any given fraction is inversely proportional to the number of triglycerides. The alcohol nature of cholesterol permits its esterification (by the liver) with fatty acids. These esters are widespread in tissues and blood; about two-thirds of plasma cholesterol is esterified. The ratio of esterified to free cholesterol was once considered to be important, particularly in the diagnosis of liver disease, but this is no longer the case. In nearly all present-day cholesterol assays only the total cholesterol is measured.

Total serum cholesterol levels should always be interpreted in relationship to HDL and LDL. In order to accurately interpret the HDL and LDL, levels should be fractionated. Truth is, these parameters most accurately reflect cardiovascular risk when fractionated. *A total serum cholesterol level without these other values lacks biochemical meaning.*

Clinical Significance - Atherosclerosis: The biological physician is both interested in abnormally high and abnormally low cholesterol values. High LDL levels may reflect risk for atherosclerosis. Low-density lipoproteins deliver cholesterol to cells for membrane synthesis and steroid hormone synthesis, via LDL receptors. In healthy humans, more than 70 percent of the LDL circulating in plasma is removed each day through LDL receptors. Diets high in saturated fats and cholesterol decrease the liver's endogenous synthesis of cholesterol and can cause chronic suppression of the LDL receptors, resulting in elevated circulatory levels of LDL.

Excess LDL is removed from the bloodstream by cells of the reticuloendothelial system, via the scavenger-cell pathway. This removal results in the peroxidation of LDL, and may promote the

cholesterol and cholesteryl ester accumulations in macrophages and smooth muscle cells that lead to the development of atherosclerotic plaque.

An abundance of research has established that elevated serum levels of LDL are a major cause of coronary heart disease. Thus, measuring LDL is critical for complete interpretation of total cholesterol level. A long-term epidemiological research project of incredible magnitude, the Güttinggen Risk, Incidence and Prevalence study investigated the relationship of lipid factors with the development of cardiovascular disease in approximately 6000 middle aged men, and found that LDL cholesterol, followed by plasma concentration of total cholesterol and apolipoprotein B, were the predominant markers associated with coronary artery disease. Other independent factors involved in cardiovascular risk are homocystine and C-reactive protein; also see the section on triglycerides.)

Other Associated Conditions Related to Elevated Serum Cholesterol: High serum cholesterol may be incidental to the presence of another disease. Some of these are mentioned below. However, in most instances, the increase in cholesterol is due to the excess fat (including cholesterol itself) in the diet and a substantial lowering of cholesterol levels can be accomplished by a change in diet; especially a reduction in the grams of carbohydrate.

1. Hypothyroidism: An elevation of cholesterol is common in patients with a marked reduction in thyroid hormone levels (unless they are undernourished). This observation may be of value in explaining an unexpectedly high cholesterol value or in following the course of treatment of the thyroid disease.

2. Liver Disease: Serum cholesterol is increased in patients who have liver diseases that are accompanied by obstruction of the flow of bile from the liver. However, in hepatitis, cirrhosis, and cancer of the liver serum, cholesterol *is usually not* increased.

3. Nephrosis: In certain kidney diseases in which large amounts of protein are lost in the urine, the cholesterol levels may reach very high values.

4. Diabetes Mellitus: Patients with this disease often have an increase in cholesterol level, but it is not as marked as the increase in serum triglycerides. Whenever the triglycerides are greater than the total cholesterol consider carbohydrate sensitivity, insulin resistance or so called “syndrome X”.

Disease Associated with Low Cholesterol Levels: Cholesterol in the plasma tends to fall during starvation and as the result of prolonged debilitating illness. Hyperthyroidism (excess thyroid gland activity) also reduces serum cholesterol, but this change is not of diagnostic significance. In one inherited disorder characterized by an absence of beta-lipoprotein, the cholesterol level is extremely low and can be regarded as pathological. This disease is accompanied by neurological abnormalities and abnormalities in the absorption of fats from the intestine.

Clinical Pearl:

If cholesterol is decreased with a normal or low total WBC, a lymph count below 20%, and an albumin value below 3.9, rule-out free radical pathology due to neoplasm or severe immunological deficiency. Find the locus of the free radical pathology and causative factors.

High Density Lipoprotein Cholesterol

Laboratory Range: High Risk: <35 mg/dL <0.9 mmol/L
Low Risk: >60 mg/dL >1.6 mmol/L
Method: Immunoseparation/Colorimetry
Interday variation: 5 to 8%

Physiology: The fatty substances (lipids) present in plasma include triglycerides or neutral fat, cholesterol, cholesterol esters, and phospho-lipids. Lipids are hydrophobic and not water-soluble. Since plasma is an aqueous solution, essentially none of the plasma lipids is in simple solution. They are present as macromolecules - complex mixtures of lipid and protein - called lipoproteins. Lipoproteins can form a colloidal suspension in plasma and thereby permit the transport of large quantities of lipid from one part of the body to another.

Lipoproteins have a relatively low density compared with other plasma proteins. Using flotation ultracentrifugation in salt solutions to separate them based on their density, they have been classified into at least four groups. These are called high-density lipoproteins (HDL), low-density lipoproteins (LDL), very low-density lipoproteins (VLDL), and chylomicrons. Lipoproteins also differ in their electric charge and can be separated by electrophoresis. Since this technique is readily available in most hospital laboratories, most clinical reports of plasma lipoprotein levels use terminology based on this method of separation. In the standard electrophoresis procedure chylomicrons remain at the origin; VLDL moves (toward the anode) slightly faster than beta globulins; LDL moves with beta globulins; and HDL moves with alpha1 globulins.

The variation in the densities of the lipoprotein fractions is due to differences in the proportions of lipid and protein. Chylomicrons and VLDL are mostly lipid, whereas HDL is only about 50% lipid. Conversely, the protein content is lowest in chylomicrons and highest in HDL. The nature of the lipids also varies from one fraction to another. Chylomicrons and VLDL contain predominantly triglyceride, while the lipid in LDL and HDL is mainly cholesterol and phospholipid (A more extensive discussion of lipoproteins is included in the sections on triglycerides and cholesterol.) High-density lipoproteins have a density between 1.063 and 1.21. They are the smallest of the lipoprotein particles, having a diameter of 50-120 Angstrom units (A). The dry weight of HDL is approximately 50% protein, 28% phospholipid, and 20% cholesterol. Most of the cholesterol is esterified. Only a small amount of triglyceride is present. The precursors of HDL probably arise from direct de novo synthesis by the liver and intestine, and are also a catabolic produce of chylomicrons and VLDL.

High-density lipoproteins have been further divided into two subgroups, HDL_2 and HDL_3, based on centrifugal analysis. HDL_2 has a density of 1.063 to 1.120, a diameter of 100 A, and molecular weight of about 375,000. HDL_3 has a density of 1.120 to 1.210, a diameter of about 70 A, and molecular weight of 200,000. There is evidence that the two subtypes have different biologic functions.

Lipoproteins can be disrupted, dissociating the lipidprotein bonds, and the lipid removed. The remainder of the molecule is called an apolipoprotein (or apoprotein). There are several different apoproteins. Two types, apoprotein A-I and A-II, predominate in HDL. They are normally present in a ratio of three to one.

Most lipoproteins transport lipids, including cholesterol, from one body cell to another where they are stored or metabolized. In contrast, there is evidence that HDL functions in the removal of cholesterol from body cells and its transportation to the liver. In the liver cholesterol is catabolized and excreted in the bile. Of particular importance is the removal of cholesterol from the cells of blood vessel walls, including those of the heart (coronary arteries) and the brain (cerebral arteries). When lipid deposits, especially cholesterol, accumulate in vessel walls, a condition called atherosclerosis is produced. The lipid deposits narrow the lumen of the artery and interfere with blood flow. Atherosclerosis is the basis of many important diseases such as myocardial infarction (the usual type of "heart attack"), and cerebrovascular "accidents" (strokes). The mechanism by which HDL removes cholesterol from cells involves activation of the enzyme lecithin-cholesterol acyltransferase (LCAT) by one or more of the apoproteins of HDL. LCAL catalyzes the esterification of free cholesterol in the cell, an essential step in the removal process.

HDL is usually measured by first separating it from other lipoproteins and then determining its cholesterol content. Ordinarily when the term HDL is used, what is meant is HDL cholesterol. Under normal fasting conditions about 17% of plasma HDL carries cholesterol.

Clinical Significance: Total serum cholesterol is known to be useful in predicting the risk of developing atherosclerosis, especially coronary artery disease (CAD). Coronary atherosclerosis may lead to myocardial infarction, which is the leading cause of death in the United States and most European nations. Increases in total cholesterol are associated with increased risk of atherosclerosis. The total cholesterol is a better indicator in persons under age 50 than it is in older age groups. In persons over 50 years of age, HDL is a much better predictor of CAD than is total serum cholesterol. High HDL levels tend to protect against atherosclerosis. The best predictive power is obtained by calculating the ratio of HDL to total cholesterol.

Concentrations of HDL vary from one person to another and from time to time in a given individual. Inheritance seems to be important. It is known that persons with familial hyperalphalipoproteinemia, who have high HDL values, have greater than normal life expectancy. Children of persons who have had coronary artery disease (and presumably low HDL levels) have lower than normal HDL values. Sex is also a determinant. After puberty, males have substantially lower HDL levels than females. HDL decreases with advancing age. Physical activity raises HDL, while inactivity lowers it. Obese persons tend to have lower than normal HDL. Smoking decreases HDL and alcohol increases it.

Low HDL levels predispose the individual to the development of atherosclerosis and the diseases that result from it. As more is learned about the functions of HDL in the metabolism of cholesterol, it is likely that it will become more and more useful in clinical medicine.

Clinical Pearls:

If decreased with elevated cholesterol, suspect a diet high in refined carbohydrate, or a lack of exercise.

If decreased with normal cholesterol, suspect a lack of exercise, and or a need for magnesium, zinc, chromium, trace minerals and essential fatty acids; consider red blood cell lipid membrane analysis for fatty acid determination.

If normal with a decrease cholesterol and triglyceride level, suspect an autoimmune problem secondary to steroidal hormone imbalance.

Triglycerides, Serum

Normal Laboratory Range: < 200 mg/dL <2.26 mmol/L
Borderline Range: 200 to 400 mg/dL 2.26 to 4.52 mmol/L
High: 400 to 1000 mg/dL 4.52 to 11.3 mmol/L
Very High: > 1000 mg/dL > 11.8 mmol/L
Optimum Range: 70 to 110mg/dL
Method: Enzymatic Colorimetry
Interday variation: 30 to 50%. Highest in winter lowest in fall

Chemistry and Physiology: Triglycerides, also known as neutral fats, are a family of esters of fatty acids and glycerol (a trihydroxy alcohol). The word is used in the plural because a number of different fatty acids are involved, and three different fatty acids may be present in a single molecule. The fatty acids are usually long-chained and have an even number of carbon atoms. They may be saturated or unsaturated; stearic acid, with 18 carbon atoms, and palmitic acids, with 16, are saturated. Oleic acid, which has one double bond, is unsaturated. Several other fatty acids with more unsaturated double bonds exist in small amounts. Some of these are essential for normal metabolism. The first three fatty acids mentioned, esterified with glycerol, make up most of the stored fat in human adipose tissue. Synthesis of triglyceride (from fatty acids or from glucose of protein) is the body's main mechanism for storing energy. Triglycerides, when burned, are completely oxidized to carbon dioxide and water, yielding more than twice as much energy per gram as carbohydrate or protein. Body fat is in a state of dynamic equilibrium. Triglycerides are constantly being hydrolyzed and re-synthesized. After a meal, when sources of energy derived from foods are abundant, triglycerides are synthesized and stored in adipose tissue. Fat cells are able to remove ingested triglyceride from the circulating blood and store it; they may also remove glucose from the blood and synthesize fatty acids, glycerol, and then triglyceride. In addition, the liver may convert some glucose and protein to triglyceride that is then transported to adipose tissue for storage. In the fasting state, stored triglyceride is broken down to fatty acids and glycerol that serve as energy sources for other tissues. Fatty acids derived from ingested or stored triglycerides are one of the most important fuels available for oxidative metabolism. Tissues with very high-energy demands, such as heart muscle and kidney, preferentially burn fatty acids rather than glucose. Fatty acids also serve as a major energy source for skeletal muscle.

Lipid Transport in Plasma and Classification of Lipoproteins: Triglyceride and fatty acids are transported from one body site to another by way of the plasma. Since they are essentially insoluble in water, they do not exist in free solution. Fatty acids are bound to albumin; triglyceride is complexed with special carrier proteins and other lipids such cholesterol and phospholipids. In fact, the sole function of the latter two lipids in plasma is probably the solubilization of triglyceride. These macromolecular lipid-protein complexes are called lipoproteins. They have been studied and crudely fractionated using the techniques of ultra-centrifugation and electrophoresis. About 350-800 mg/dL of lipid is present in the plasma of a fasting normal adult. It is distributed in the following fractions:

1. Very large aggregates called chylomicrons were originally found in the lymph (chyle) draining from the small intestine. They are also found in the blood for a few hours after eating. In a fasting individual they make up less than 5% of the total plasma lipid. They consist of triglyceride (90%) complexed with protein (1%) plus small amounts of cholesterol and phospholipids. They are insoluble in water and do not migrate on electrophoresis. Chylomicrons are the vehicles that transport the triglycerides absorbed from foods in the diet. They are synthesized in the cells that line the small intestine, perhaps by the addition of triglyceride to circulating high-density lipoproteins (see below). They leave the bowel via the lymphatics and then enter the blood stream. They are removed from the blood by carious tissues that use the triglyceride as an energy source, or by fat cells.

2. In the fasting state, more than 80% of the plasma lipid is found in the beta (70%) and pre-beta (13%) fractions. These lipoproteins are composed of carrier protein, cholesterol, phospholipid, and a varying amount of triglyceride that has been synthesized by the liver from fatty acids, carbohydrate, or protein. When carbohydrate is present in amounts greater than that needed for immediate energy requirements, it is converted by the liver to triglycerides. This is packaged as pre-beta or very-low-density lipoprotein (LDL) containing only 10% triglyceride. The LDL returns to the liver to pick up another load of newly synthesized triglyceride.

3. High-density (HDL) or alpha lipoprotein comprises about 17% of normal plasma lipids in a fasting individual. These particles contain about 50% protein, 30% phospholipid, and 18% cholesterol. Their function is not well understood. They may be the "building blocks" from which chylomicrons are produced in the intestinal mucosa by the addition of triglyceride. According to this theory, they are "regenerated" at peripheral sites when the triglyceride is removed from the chylomicron by tissue cells.

4. Fatty acids are present in plasma in very small amounts - only a few milligrams per deciliter. In the course of normal metabolism (and at an increased rate during fasting), stored fat is hydrolyzed to release fatty acids and glycerol. The fatty acids are bound to plasma albumin and transported to sites of utilization. The turnover of plasma fatty acids is very high even though the total amount in the plasma at any one time is very small. The total daily transport of fatty acids may at times equal about 3,000 calories.

Analytic Techniques and Normal Values: The extensive research on serum lipids using the techniques of ultra-centrifugation and electrophoresis has lead to the description of several lipoprotein abnormalities which seem to be related to the likelihood of developing atherosclerosis with myocardial infarction and stroke. Only two patterns of lipoprotein abnormality are common. The first is an increase in LDL or beta lipoprotein, which produces an elevation of cholesterol with normal triglyceride levels. The second is an increase in VLDL or pre-beta lipoprotein with a large increase in triglycerides and a minimal increase in cholesterol. Thus, screening for lipid abnormalities can be accomplished by measuring total cholesterol and triglycerides and visually examining the serum for turbidity. The "high normal" level for triglycerides in middle-aged US adults is 140-160 mg/dL. In the following discussion "increased triglycerides" should be interpreted to mean an elevation in the VLDL or pre-beta lipoprotein synthesized by the liver (rather than as increase in triglycerides as chylomicrons).

Clinical Significance: The physician is usually interested in increases in serum triglycerides; however, abnormally low values may reflect abnormalities in lipid metabolism and or essential fatty acid deficiencies and should also be further investigated.

Influence of diet: Population surveys show that about 5% of women and 13% of men who are apparently “healthy” have elevated triglyceride levels. This increase may be a response to a diet high in carbohydrate (the liver converts the excess carbohydrate to triglyceride for storage). Some of these persons will revert to a normal serum lipid pattern if placed on a low carbohydrate diet. In others, the increase in triglycerides is related to alcohol intake, and the abnormality disappears if the person eliminates all alcoholic beverages from his diet. It is not clear whether these two types of individuals have an underlying genetic abnormality in the metabolism of carbohydrate and/or fats. Carbohydrate sensitivity has been observed more common to blood type O individuals. However, liver and pancreas toxicity may impair the ability to process carbohydrate and result in elevated lipids.

Atherosclerosis, coronary heart disease, and stroke: The correlation between premature (before age 50) coronary heart disease and elevated serum cholesterol has been known for decades. Recently it has become evident that an elevated triglyceride level may also predispose to coronary atherosclerosis. Both the absolute quantity of triglycerides and the nature of the carrier protein seem to be important. In a recent study of patients with stroke due to

atherosclerosis of the large arteries supplying blood to the brain, 45-50% had elevated serum lipids, most commonly an increase in triglyceride.

Other diseases: There are many other diseases in which serum triglycerides may be elevated, but the increase is not of major diagnostic importance. The physician must be aware of these associations to avoid making an incorrect diagnosis of a "primary" (possibly genetic) lipid disorder. Nephrosis, diabetes mellitus, and hypothyroidism are three such diseases. Nephrosis is a syndrome, frequently the end-stage of kidney diseases, in which there is an abnormally low level of serum albumin (due to loss in the urine), and evidence of a variable degree of kidney failure. All serum lipids, including triglycerides, cholesterol, and phospholipid, are elevated. The mechanism of the lipid increase is not known. In diabetes mellitus, body fat may be hydrolyzed to release fatty acids when glucose is not available. Some of the fatty acids are removed from the blood by the liver and re-synthesized into triglyceride. Hypothyroidism (decreased out-put of thyroid hormone), when severe and prolonged, is accompanied by increases in serum cholesterol and triglycerides. Again, the mechanism of this lipid increase is unknown, but it probably reflects a reduced rate of breakdown of lipoproteins by the body tissues.

Clinical Pearls:

In general, total cholesterol is increased in most endocrine hypo-function and decreased in endocrine hyperfunction.

If decreased with a TSH below 0.5, suspect thyroid hyperfunction if symptoms are evident.

If increased with a Thyroid Stimulating Hormone (TSH) above 5.5, suspect thyroid hypo-function. If increased with TSH below 1.5 suspect hypo-function of the anterior pituitary. See TSH.

A patient who is correctly metabolizing their fats, proteins, and carbohydrates will generally have about half as much triglyceride as total cholesterol.

If increased with an increased GGT, suspect alcohol abuse.

If decreased with a normal or low total WBC, a lymph count below 20, and an albumin value below 3.9, rule out free radical pathology. If the alpha 1 fraction (serum electrophoresis) is elevated with an elevation of the alpha 1 acid or alpha 1 HS glycoproteins, free radical (tissue destruction) pathology is probable. Consider Computerized Regulation Thermography™ to further determine site or locus of free radical pathology. Consider essential and metabolic fatty acid analysis to determine fatty acid imbalances and or deficiencies.

Calcium, Total

Laboratory Range: 8.9 to 10.5 mg/dL 2.23 to 2.63 mmol/L
Critical Levels:
Tetany: < 7 mg/dL < 1.75 mmol/L
Coma: >12 mg/dL > 2.99 mmol/L
Possible Death: < 6 mg/dL < 1.50 mmol/L
> 14 mg/dL > 3.49 mmol/L
Optimum Range: 9.4 to 10 mg/dL
Alarm Range: < 7.0 mg/dL or >14.0 mg/dL
Method: Colorimetry
Interday variation: 1 to 3% (serum), 20 to 30% (urine).
Highest at 8 PM, lowest at 4 PM

Physiology: Although 99% of the calcium of the body is contained in the bones and teeth, it is the calcium content of blood that is of most importance to the physician. In the blood, essentially, all the calcium exists in one of three forms: 1) as free calcium ion, Ca++ 50%; 2) bound to protein (primarily albumin), 45%; and 3) complexed with certain organic compounds, mainly citrate, 5%. The ionized fraction is the most important from a physiologic standpoint, but direct measurement has proved to be difficult. The percentage of total calcium that is in the ionized form is known to depend upon the amount of protein present and the pH of the blood. High proteins level and higher than normal pH tends to reduce the relative amount of ionized calcium. The amount of ionized calcium is usually estimated from the total calcium plus the knowledge of these latter variables. Within limits, the serum calcium varies inversely with the phosphorus concentration in a relationship expected of a saturated solution of a slightly soluble inorganic compound. It is a far from perfect relationship, however, because of the different forms in which both calcium and phosphorous exist in plasma.

Calcium ion is important in the transmission of nerve impulses, in the maintenance of normal muscle contractility, as a cofactor in certain enzyme reactions, and in the coagulation of the blood. A substantial reduction in calcium ion concentration results in a state of neuromuscular excitability known as tetany. In the extreme state, the muscles are contracted continuously. Higher than normal concentrations of calcium, on the other hand, result in loss of normal neuromuscular excitability and muscle weakness (as well as many other less easily explained symptoms). Relative constancy of serum calcium concentration must be maintained. Bone serves as a reservoir for this purpose, releasing calcium when required to prevent hypocalcemia and trapping calcium to prevent excessively high levels (assisting the kidney in this respect). The uptake and release of calcium from the bone is under the control of the parathyroid hormone. Calcium is absorbed from the upper part of the small intestine. The amount absorbed depends in part on adequate stomach acidity and the amount of phosphate present. Hence, hypochlorhydria or achlorhydria will severely limit calcium absorption.

Clinical Significance: Calcium concentrations in disease may be either lower or higher than normal. Normal, as judged by what is found among presumably normal individuals, is highest in children and falls slightly throughout life. Variations in serum calcium may be due to disease of the parathyroid glands, disease of bone, defective absorption of calcium from the intestine, kidney disease, and various other causes.

Hypercalcemia: (High Serum Calcium)

1. Hyperparathyroidism: Excessive and uncontrolled secretion of parathyroid hormone of varying degree may occur in tumors of the parathyroid glands. This is one of the most important diseases affecting calcium metabolism. Abnormally high levels of this hormone result in excessive release of calcium from the bones and elevated serum calcium levels. Deficiency of parathyroid hormone produces the opposite effect: reduced calcium release

from bone and low serum calcium. Phosphorus metabolism generally varies inversely in its serum concentration with calcium.

2. Carcinoma (Cancer) Metastatic to Bone: Some forms of cancer spread from the primary site to bone and cause extensive bone destruction. This results in the release of large quantities of calcium into the serum.
3. Multiple Myeloma is a tumor of the "plasma cells" which produce gamma globulin (antibody proteins). (See also the total protein and albumin papers.) The tumor exists as multiple nodules scattered through the bones and is often accompanied by elevated serum calcium levels. This is due in part to destruction of bone by the tumor with release of calcium in the blood secondary to elevated plasma protein levels (protein produced by the tumor itself) and increased binding of calcium to protein.
4. Calcium is considered to be a secondary body buffer and may be utilized to compensate for a lack of primary buffers (magnesium, potassium, and bicarbonate). Hypercalcemia may be due to an overly acidic mesenchyme in the absence of intercellular buffers. This in turn causes calcium to be shifted out of the skeletal system into the circulation to compensate for the pH acidity.
5. Excess exogenous intake with calcium food supplementation may result in hypercalcemia. This is correctable with reduction of excess calcium and inclusion of calcium synergist.

Hypocalcemia: (Low Serum Calcium)

1. Malabsorption: A variety of diseases of the wall of the small intestine may block calcium absorption (and Vitamin D absorption). Among these is celiac disease or sprue. Intestinal damage from infection will often result in diarrhea and malabsorption of nutrients such as calcium. Also, low hydrochloric acid secretion of the stomach (hypochlorhydria or achlorhydria) will block calcium absorption. Hypochlorhydria is most common in the elderly. To rule out malabsorption consider intestinal permeability testing with a lactolose and mannitol oral challenge and urine recovery.
2. Magnesium deficiencies will result in calcium metabolism problems.
3. Hypoparathyroidism: The absence of or destruction of the parathyroid glands produces a marked reduction in serum calcium. This occurs often due to toxic exposure to heavy metals and or certain xenobiotics. This is often correctable by the administration of parathyroid hormone. A chemistry pattern to watch for with parathyroid hypofunction is parathyroid hormone decreased, alkaline phosphatase normal to decreased, serum calcium normal to decreased, and serum phosphorus increased.

Clinical Pearls:

If decreased with a decrease in serum phosphorus, decreased BUN and/or increase or decrease total globulin, protein malnutrition is possible. Conduct urine amino acid analysis. Although many physicians feel that calcium need is wide-spread, research indicates that the need for calcium metabolizing agents and synergist (essential fatty acids, iodine, vitamins A, C, and E, manganese, magnesium, boron, folic acid) and/or a need for digestive correction (need for HCL, biliary dysfunction and pancreatic insufficiency of enzymes) are far more wide-spread than simple calcium deficiency. Common subjective indications of calcium need are: frequent skin rashes or hives, muscle cramps at rest (especially leg or toe cramps while sleeping), soft fingernails frequent nose bleeds, frequent hoarseness, irritability and blood pressure fluctuations – high or low.

Measure urinary calcium levels anytime serum calcium levels are increased or decreased.

Phosphorus, Serum; PO4; Inorganic Phosphate; HPO4-2

Laboratory Range: 2.5 to 4.5 mg/dL 0.8 to 1.45 mmol/L
Optimum Range: 3.0 to 4.0 mg/dL
Alarm Range: < 2.0 mg/dL or > 5.0 mg/dL
Method: Colorimetry
Interday variation 5 to 10% (serum), 15 to 20% (urine)
Usually highest at 8 AM, falls during day. Highest in summer, lowest in winter
Normal increase is seen during bone growth and in the healing of fractures
In children up to 6 mg/dL normal throughout growth, reaches adult levels by late adolescence.

Physiology: The human body contains nearly a kilogram of phosphorus. Approximately 80% is found in the calcium phosphate salts that make up the inorganic substance of bone. The remainder is distributed throughout all of the other cells of the body. Practically all body phosphorus is present as the phosphate radical.

The calcium phosphate salts of bone give them rigidity but also serve as a large storage depot for phosphate. The role of phosphates in other cells is important and complex. Much of the metabolism of carbohydrate involves esterification of the intermediary metabolites with phosphate. One organic phosphate compound, adenosine triphosphate (ATP), plays an important role in energy transfer. The metabolism of all food, whether carbohydrate, protein, or fat, ultimately terminates in the formation of ATP, or in similar compounds. The breakdown of ATP to ADP (adenosine diphosphate) is an energy-producing reaction. This reaction provides the energy needed for other chemical reaction involved in cell function, maintenance and growth. Phosphate esters of fats (phospholipids or phosphatides) are found in every living cell, particularly in cell membranes. The exact role of these compounds in the structure and function of the membrane is not entirely understood, but they are known to participate in transfer of various substances in and out of the cell. Phosphate is also an integral part of the structure of the nucleic acids, ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) that transfer genetic information. Phosphoric acid also makes up part of the prosthetic group of certain proteins.

Phosphate is found in blood plasma as phosphate ion. Most of this, about 95%, is the free ion; the remainder is bound to protein. Since plasma also contains calcium ions, and calcium phosphate is poorly soluble, the concentrations of calcium and phosphate ions must follow the solubility rule. That is, the product of the calcium and the phosphate concentrations is a constant. Therefore, when the level of one of these ions rises, the other must fall. In fact, one of the ways in which the body controls the plasma calcium level, which must be maintained within very narrow limits, is by changing the rate of renal excretion of phosphate. If plasma calcium falls below normal, the parathyroid gland secreted more of its hormone. This hormone causes the kidney to excrete more phosphate into the urine. The resulting fall in plasma phosphate allows calcium ion to move from storage depots in bone into the plasma.

Clinical Significance: Phosphate is usually measured in serum and it is the phosphate ion that is assayed, but the results are reported as milligrams of elemental phosphorus. Serum phosphate should be measured during a period of fasting. After a carbohydrate meal, serum phosphate falls because it moves into cells along with glucose. Normal levels vary with age. Serum phosphate is increased during periods of growth so the normal range for children is higher than the adult normal range. The highest levels are seen in newborn infants. Spuriously high values may be seen if the blood sample is not handled properly. Red blood cells contain high levels of organic phosphates and phosphatase enzymes that can split phosphate from these compounds, so the serum should be quickly separated from the cells with care to avoid hemolysis.

Despite the ubiquitous nature and essential role of phosphate in body structure and metabolism, serum phosphate has a relatively limited application in clinical diagnosis. Generally, serum phosphate levels may assist in the interpretation of studies investigating parathyroid and calcium abnormalities. However, phosphorus levels may be used as a general indicator of digestive

function in reference with serum globulin. (see clinical pearl) Also, there are a few rare congenital diseases that affect phosphate metabolism. Abnormal serum phosphate levels are most commonly seen in kidney, bone and parathyroid diseases. Phosphate is usually measured along with serum calcium since each measurement is useful in the interpretation of the other.

Increased Serum Phosphate:

Kidney Disease: Since phosphates are present in large amounts in all animal and plant cells, the ordinary diet contains far more phosphate than is required to maintain body structure and function. Normally the excess is excreted, mainly as the acid salts of sodium and potassium, into the urine. Kidney disease from almost any cause impairs its ability to remove phosphate from plasma, so there is a gradual rise in serum phosphate levels and a secondary fall in serum calcium. The low calcium level stimulates the release of more hormones from the parathyroid gland, but the damaged kidney is unable to respond to the increased hormone level. The parathyroid hormone continues to promote mobilization of calcium and phosphate from bone, so eventually the serum calcium is normal and the phosphate high. Loss of calcium phosphates from bone leads to a type of bone disease known as renal rickets.

Hypoparathyroidism (abnormally low levels of parathyroid hormone): The parathyroid glands are usually embedded in the substance of the thyroid gland. Therefore, accidental removal of these glands is an occasional complication of thyroid surgery. In the absence of sufficient parathyroid hormone, the kidney tends to retain phosphate ion, so the serum level is high. The serum calcium is low.

Pseudo (false) Hypoparathyroidism: This is a rare, multifaceted disease that includes the inability of the kidney to response to parathyroid hormone. As in true hypoparathyroidism (insufficient hormone), the serum phosphate is high and the calcium low. The two diseases can be distinguished in several ways, one of which is administration of parathyroid hormone. In true hypoparathyroidism the serum phosphate will fall. No change is seen in pseudo-hypoparathyroidism.

Hyperthyroidism (Increased levels of thyroid hormone): Serum phosphate will occasionally rise in this disease. The cause is not well understood, but it may be due to excessive mobilization of calcium phosphate from bone.

Other Causes: Elevated serum phosphate may be seen in diabetic acidosis, acromegaly (increased secretion of growth hormone by the pituitary gland during adulthood), excess vitamin D intake, and acute osteoporosis (a decrease in the mass of bone due to various causes).

Decreased Serum Phosphate:

Hyperparathyroidism (increased levels of parathyroid hormone): As described above, one of the effects of this hormone is to increase the excretion of phosphate by the kidney. This fall in phosphate produces a secondary increase in serum calcium. The rise in calcium "shuts off" the production of parathyroid hormone. This "negative feedback" mechanism operates like a thermostat to control serum phosphate and calcium levels. Benign tumors of one of the parathyroid glands may produce abnormally high amounts of parathyroid hormone, so in this condition the serum calcium is high and the phosphate low. Removal of the tumor results in a return to normal levels of both ions. Occasionally the parathyroid glands may lose their responsiveness to serum calcium levels, or shut off hormone secretion at a higher calcium concentration. In this condition there is a low serum phosphorus and high calcium.

Malabsorption Syndromes: It is difficult to conceive of a diet deficient in phosphorus, but there are diseases in which the bowel is unable to absorb sufficient calcium and phosphate from the ingested food. A lack of HCL, common in elderly, may cause low phosphorus levels. This may be checked with a serum gastrin level; if low, HCL secretion is probably impaired. This eventually results in reduced amounts of calcium phosphate in bone, a condition known as osteomalacia (soft bones). Both the serum calcium and phosphate tend to be low.

Pharmaceuticals: Chronic use of antacids will significantly decrease both phosphorus and calcium absorption. Oral contraceptives and horse derived and conjugated estrogens that are not biologically identical will decrease phosphorus levels through the inhibition of phosphorus metabolism governed in part by normal progesterone and estrogen levels.

Clinical Pearl: If serum phosphorus is decreased with a total globulin above 2.8 and/ or subjective indications of HCL need present, HCL need is possible. If the total protein is increased or decreased with a total globulin above 2.8 and serum phosphorus decreased, HCL need is probable. If the serum gastrin is below 50 with these indications, HCL need is almost certain.

Unusual Causes: During recovery from diabetic acidosis (coma) the serum phosphate may fall below normal. The mechanism is movement of glucose and phosphate from the blood into cells. Hypopituitarism (insufficient production of growth hormone) in children results in lower than normal phosphate levels for a child of the same age. There are several rare, inherited disorders such as Fanconi's syndrome and Albright's renal acidosis in which the kidney loses its ability to retain phosphate.

Sodium, Serum

Laboratory Range: 135 to 143 mEq/L 135 to 143 mmol/L
Optimum Range: 135 to 142 mEq/L 135 to 142 mmol/L
Alarm Range: < 125 mEq/L or > 155 mEq/L
Method: ISE
Interday variation: 1%. Highest at noon, falls 1 to 2% by evening. Highest in summer, lowest in winter

Physiology: Sodium is the most abundant cation in the extra-cellular fluid. It is of greatest importance in osmotic regulation of extra- cellular fluid balance, acid base balance, and renal, cardiac and adrenal functions. Sodium concentration is an indication of the amount of Na+ relative to the amount of water in extracellular fluid (free water). Measurement in serum, plasma, or whole blood is usually accomplished using potentiometry. Na+ concentration is inextricably linked with extracellular fluid (ECF) concentration; therefore, interpretation of sodium levels should always include consideration of the hydration status of the patient (and therefore, changes in free water). Sodium is the major extracellular cation and is a primary determinant of plasma osmolality and ECF volume. The body attempts to maintain a constant ECF volume, as major changes in ECF volume can have profound effects on the cell. The kidney plays a critical role in maintenance of ECF volume, via sodium and water retention. Regulation of body water is accomplished by monitoring of plasma osmolality (determined primarily by sodium concentration) and blood volume. This is achieved by osmoreceptors and baroreceptors.

Osmoreceptors sense changes in osmolality. With hyperosmolality (hypernatremia), osmoreceptors stimulate vasopressin or ADH secretion from the pituitary gland and stimulate thirst. Thirst is stimulated by as little as a 1-2% decrease in osmolality. The result is water retention by the kidney and increased water intake. Increases in free water will thus reduce sodium concentration. Opposite changes occur with hypoosmolality.

Baroreceptors are sensitive to changes in effective circulating volume (ECV). The ECV is that part of the extracellular fluid that is in the arterial system and is effectively perfusing the tissues. It usually varies directly with ECF volume. With hypovolemia (decreased ECV), baroreceptors stimulate the renin-angiotensin system, the result being mineral corticoid (aldosterone) release from the adrenal cortex. Aldosterone stimulates increased absorption of NaCl and promotes the excretion of potassium and hydrogen in the distal tubules of the nephron. NaCl retention promotes water re-absorption, thus correcting the hypovolemia. Hypovolemia also stimulates thirst (a decrease in ECV of 7-10% is required for thirst stimulation). Opposite changes occur with hypervolemia.

Clinical Significance: Serum Sodium level may be either increased or decreased.

Clinical Pearls:

To assess good electrolyte balance, add chloride and Total Co2, subtract this number from sodium. (See Anion gap) The result should be between 9 and 18 for optimum balance.

If increased with increased BUN or Creatinine, renal dysfunction is probable.

If decreased with increased serum potassium, adrenal hypo-function is probable. Consider a salivary adrenal-cortical stress profile with AM, noon, afternoon and bedtime measurements, comparing cortisol levels with DHEA levels.

If decreased with an increased or decreased chloride, bowel dysfunction is possible (diarrhea or constipation).

Potassium, Serum

Laboratory Range: 3.5 to 5.0 mEq/L 3.5 to 5.0 mmol/L
Optimum Range: 4.0 to 4.5 mEq/L 4.0 to 4.5 mmol/L
Child: 4.4 to 4.7 mEq/L
Alarm Ranges: <3.0 mEq/L or > 6.0 mEq/L
Method: ISE
Interday variation: 1 to 2%. Highest at 8 AM, 20% lower at night

Physiology: Potassium is the major intracellular cation (intracellular K+ concentration is approximately 140 mEq/L) and is important for maintaining resting membrane potential of cells. 60-75% of total body potassium is found within muscle cells, with the remainder in bone. Only 5% of potassium is in the ECF, therefore *serum* potassium concentration in blood is not always a reflection of total body potassium levels. Plasma (ECF) K+ concentration is tightly regulated, whereas, fairly small changes can have marked effects on organ function. Ingested K+ is absorbed non-selectively in the stomach and small intestine. Regulation of plasma K+ is by renal excretion and movement of K+ from extracellular fluid to intracellular fluid. If these mechanisms are functioning normally, the amount of K+ ingested has little affect on plasma K+. However, if one or more of the regulatory mechanisms is faulty, then the amount of K+ ingested can exacerbate abnormalities in plasma K+. Urinary excretion of K+ is largely by secretion of K+ into the urine by the distal tubules. 70% of filtered K+ is absorbed in the PCT of the kidney regardless of K+ balance. 20% is absorbed in the ascending limb of the loop of Henle and the remaining 10% is delivered to the distal nephron. Principle cells in the distal nephron secrete K+ and absorb Na+ under the influence of aldosterone. Intercalated cells in the distal nephron absorb K+ in exchange for H+. Secretion of K+ by the distal nephron is governed by: the extracellular concentration of K+, aldosterone, distal tubule flow rate (increases in flow rate enhances K+ secretion), lumen electro-negativity (increased negativity enhances secretion), concentration of NaCl in the distal tubule lumen (low Na+ decreases secretion whilst low CL enhances secretion) and ADH (stimulates secretion). K+ is also excreted in the colon, which is also influenced by aldosterone. Translocation of K+ is largely dependent on insulin and catecholamines. Shifts of K+ in and out of cells can also occur with changes in the pH of ECF.

Clinical Significance: Serum Potassium level may be either increased or decreased. Important to remember that only 2% of total body K+ is distributed in the extracellular compartment, hence serum potassium measurements may not accurately reflect the total body stores. In fact, hypokalemia can occur in the presence of normal serum values. When in doubt, consider whole blood electrolyte analysis. Again, the amount inside the cell is at least 15 to 20 times that in the serum. The normal total body stores of potassium and intercellular and extracellular balance is critical to normal physiology, especially heart, renal and adrenal functions. Potassium is essential in maintenance of pH of both blood and urine.

Whenever potassium is out of the normal range, a careful history with emphasis on the diet and the use of supplements, medications and laxatives should be obtained. Spurious hyper- and hypokalemia must be excluded. In addition to the serum electrolytes, the urine electrolytes, urine osmolality and red blood cell potassium and magnesium levels and should prove illuminating.

Severe abnormalities of plasma K+ are life-threatening situations.

Increased:

Potassium Retention:
Glomerular filtration rate (GFR) < 3 to 5 ml/minute
GFR rate > 20 ml/minute: Addison's disease decreased aldosterone activity, inhibition of tubular secretion of potassium

Potassium Redistribution:
Tissue damage, especially crush injury to tissues
Necrotic infections
Acute acidosis – To maintain physiologic pH during acidosis, potassium is redistributed into the plasma

Increased Potassium Supply:
Excess oral intake – may occur with daily supplementation more than 500 mg
Laboratory Artifacts

Clinical Pearls:

Increased:
If increased with an increased alpha 1 or 2 globulin, tissue destruction is possible. (Potassium coming out of injured cells into the serum.)
If increased with decreased serum sodium, adrenal hypo-function is probable. Consider performing a salivary cortisol DHEA level with AM, noon, afternoon and bedtime measurements of cortisol.
If increased with BUN or creatine and/or other electrolytes increased or decreased, renal dysfunction is probable.

Clinical Pearls:

Decreased:
If decreased with a BUN or creatine increase, diuretic use is the probable cause.
If decreased with an increased chloride or increased sodium, adrenal hyper-function is probable. Consider performing a salivary cortisol DHEA level with AM, noon, afternoon and bedtime measurements of cortisol.
If decreased, always consider accompanying magnesium and trace mineral depletion.
Excess licorice ingestion may cause a potassium deficiency.

Chloride, Serum

Laboratory Range: 90 to 110 mEq/L
Optimum Range: 100 to 106 mEq/L
Alarm Range: < 90 mEq/L or > 115 mEq/L
Method: ISE
Interday variation: 1%

Physiology: The chloride ion is one of four ions commonly measured in serum. The other three are sodium, potassium, and bicarbonate as total CO2. Ordinarily all four are measured together because of important interrelationships with respect to acid-base and cation-anion balance. It is these four ions that are included in the term "serum electrolytes". Although there are many other electrolytes in plasma (such as calcium, magnesium, phosphate, and sulfate), these are present in much lower concentrations and serve different functions. The chloride ion, like the sodium ion, is largely limited to extracellular fluids ("spaces"). There is very little of either of these ions inside body cells. Of the four "serum electrolytes", chloride is often regarded as the least important even though it is the most abundant anion in serum. Under physiological circumstances, changes in serum chloride concentration are believed to be a secondary response to changes in the other electrolytes. The presence of chloride in extracellular fluid is regarded as necessary for maintenance of electrical neutrality of the fluid. Thus, if excessive amounts of bicarbonate ion accumulate in plasma, the chloride concentration falls. Likewise, when the sodium ion increases, the chloride concentration also increases. (These changes are affected through the mechanism of excretion of chloride into the urine.) Most plasma proteins carry a negative charge and serve as anions. Extracellular fluids other than plasma (e.g., cerebrospinal fluid) are generally protein-free. In these fluids the anionic contribution of protein is replaced by chloride ion. Thus, the chloride concentration of cerebrospinal fluid is higher than plasma. At times other anions (usually organic acid anions) appear in serum in quantities sufficient to displace substantial amounts of chloride ion. The presence of such ions is suggested by a low chloride concentration that cannot be accounted for by an increase in bicarbonate or decrease in total cations. This situation is referred to as an "anionic gap". A larger than normal gap prompts the physician to determine the nature of the anion that fills the gap. Commonly it is beta-hydroxybutyric or acetoacetic acid or lactic acid (see below).

Clinical Significance: Serum chloride ion may either be increased or decreased.

Decreases: Hypochloremia:

Hypoventilation: Inadequate removal of carbon dioxide from the blood by the lungs, regardless of cause, results in the accumulation of ionized carbonic acid in the blood. The increase in the bicarbonate ion causes a reciprocal fall in serum chloride (it is excreted into the urine).

Protracted Vomiting: Gastric secretions are high in chloride concentration (about 140 mEg/L) and large volumes (over two liters) are produced and reabsorbed daily. Continuous vomiting over long periods results in the loss of chloride from the body and fall in serum chloride.

Chronic Diarrhea or Loss of Small Intestinal Contents through Fistula: Intestinal secretions are also high in chloride ion concentration. Since large amounts of intestinal secretions are produced and reabsorbed continuously, a substantial loss of these fluids over long periods will reduce the total body chloride and produce hypochloremia.

Diabetic Ketoacidosis: Uncontrolled diabetes results in the accumulation of organic acids (beta-hydroxybutyric and acetoacetic acids) in serum. These are relatively strong acids, so there is a displacement of both bicarbonate and chloride ions.

Adrenal Disease: The adrenal gland produces hormones that control fluid and electrolyte balance. Adrenal hypofunction is associated with decreases in serum chloride and increases in the serum potassium concentration.

Renal Failure: Failing kidneys result in the accumulation of phosphate and sulfate anions that displace serum chloride.

Increases: Hyperchloremia:

Hyperventilation: Excess breathing results in the reduction of carbonic acid content of plasma and therefore a fall in bicarbonate ion concentration. There are many causes of excess ventilation: they include many diverse diseases, drugs which stimulate the respiratory center, anxiety, fear, and decreased oxygen tension or increased CO_2 tension in the blood.

Pharmacological influences: Excess use of salicylates or table salt should be ruled out. Large doses of ammonium or potassium chloride may produce hyperchloremia.

Dehydration: A decrease in plasma water will necessarily result in an increase in the chloride concentration.

Carbon Dioxide, Total CO_2

Laboratory Range: 22 to 28 mEq/L 22 to 28 mmol/L
Optimum Range: 25 to 30 mEq/L
Alarm Range: < 18 mEq/L or > 38 mEq/L
Interday Variation: 5%, highest in AM, 30% lower in PM; should agree closely with calculated bicarbonate from blood gas analysis.
Method: Colorimetry

Physiology: The major end products of the metabolism of most foodstuffs are water and carbon dioxide (CO_2). Approximately 20 moles of CO_2 are produced each day (the exact amount varies with body size and physical activity) and excreted by the lungs. The CO_2 formed in body cells promptly dissolves in water and combines it to form carbonic acid. The latter process is a slow one in a simple aqueous solution in a test tube, but in the body, it is markedly accelerated by the ubiquitous enzyme, carbonic anhydrase. The carbonic acid partially dissociates into hydrogen ion ($H+$) and bicarbonate ion (HCO_3). These reactions are summarized in the following equations.

Thus carbon dioxide is transported from the tissues to the lungs in three forms: dissolved CO_2, un-dissociated or molecular H_2CO_3, and bicarbonate ion (HCO_3). At normal plasma pH (7.4), the amounts of each of these are as follows: hydrogen ion, 4×10^{-8} M; dissolved CO_2, 1×10^{-3} M; molecular carbonic acid, 5×10^{-6} M; bicarbonate ion, 25×10^{-3} M. These relative amounts are determined by the equilibrium constants for the equations above.

The role of bicarbonate ion as the major transport form of CO_2 is ordinarily of only passing interest to the physician; of much greater importance is its role in control of plasma pH, which normally lies within the narrow range of 7.35 to 7.45. The concentration of bicarbonate ion can and does vary more with alteration in acid-base balance than do chloride, sodium, or potassium ion concentrations. The body has control of the excretion of CO_2 through changes in the rate and depth of respiration. Because the various forms of CO_2 in plasma are in equilibrium, a change in CO_2 concentration will result in a concomitant change in hydrogen ion and bicarbonate ion concentrations. The lungs are therefore able to compensate to some degree for abnormal production or loss of hydrogen ion.

The importance of CO_2 derives also from that bicarbonate ion buffering capacity, that is, its effectiveness in limiting changes in pH when acid or base enters the plasma. At first glance, the bicarbonate-carbonic acid buffer system would not appear to be particularly effective at the usual plasma pH of 7.4 since the effective pK* of this system in plasma is 6.1 (buffers work best at pH values close to their pK). Buffering is in fact remarkably effective because the concentration of carbonic acid can be changed by changes in respiration. In brief, the addition of a strong acid to plasma (the usual direction of pH change in the body) results in excretion of the lungs of enough carbonic acid (in the form of CO_2 and water) to partially correct the fall in pH. At the same time, the bicarbonate-carbonic acid buffer system becomes more effective at this lower pH.

Clinical Significance: Knowledge of the CO_2 concentration permits a first approximation of the acid-base balance and goes at least part of the way toward elucidating the cause when an abnormality exists. Full appreciation of the acid-base status also requires knowledge of plasma pH, buffering capacity, hemoglobin concentration, pO_2 and pCO_2. Nevertheless, at the present time, the determination of CO_2 concentration remains the most commonly performed initial test in the evaluation of the body's ability to control pH. As tissue mesenchyme becomes more acidic with metabolic metabolites, the body will utilize bicarbonate to buffer the blood by raising serum levels of pH to compensate for tissue acidity.

Decreased Bicarbonate with Elevated pH – Respiratory Alkalosis: If the respiratory rate is increased, there is an increase in excretion of carbon dioxide, and levels of plasma carbonic acid and dissolved CO_2 fall. The plasma pH rises, hence the name respiratory alkalosis. The causes

of this condition are varied, but they have in common the over-stimulation of that part of the brain that controls breathing (the “respiratory center”). The most common cause is simple anxiety with increased rate and depth of breathing – the so called “hyperventilation syndrome”. Other causes include toxic substances (e.g., salicylates, as in aspirin over-dosage) that stimulate the respiratory center, and central nervous system lesions such as tumors located in this part of the brain.

Decreased Bicarbonate with Decreased pH – Metabolic Acidosis: The condition usually results from the addition of excess amounts of acid to the plasma. Usually these acids are products of normal metabolic reactions, but they are being formed at a faster rate that they can be degraded or excreted. For example, in diabetic coma, increased amounts of acetoacetic acid are produced from the oxidation of fatty acids. It accumulates in the plasma because of the decreased ability to further metabolize it via the citric acid cycle. Most of the acetoacetic acid is reduced to beta-hydroxybutyric acid. Both of these acids are increased in the plasma in diabetic coma. If insulin is not given to correct the metabolic abnormalities, coma and death occur. Another example of metabolic acidosis is the accumulation of lactic acid in the clinical condition called “shock”. The blood pressure is low and the blood supply to muscles therefore decreased. The muscles received insufficient oxygen to completely burn glucose; it is metabolized only to the point of formation of pyruvic and then lactic acids. Lactic acid accumulates in the plasma. In both diabetic coma and shock the bicarbonate level may be very low, and the pH may fall below 7.0. Some of the hydrogen ions from these acids combine with bicarbonate ion to form carbonic acid and then CO_2 and water. The lungs excrete the CO2. Thus the level of plasma bicarbonate is decreased. There is still an excess of hydrogen ion in the plasma, so the pH is low. In fact the changes in this condition can be summarized as the substitution of a strong acid (lactic or betahydroxybutyric) for a weak acid (carbonic). Metabolic acidosis also occurs in patients with kidney disease: phosphoric and sulfuric acids (produced from the breakdown of phosphate and sulfate-containing proteins) cannot be removed from the plasma at the normal rate because the kidneys are damaged.

Increase Bicarbonate with Decreased pH-Respiratory Acidosis: The condition usually results from diseases of the lungs that impede the excretion of carbon dioxide. A typical example is emphysema, a disease in which there is widespread destruction of lung tissue. Carbon dioxide, carbonic acid, and hydrogen and bicarbonate ions accumulate in the plasma.

Increased Bicarbonate with Elevated pH-Metabolic Alkalosis: This condition can result from excess intake of sodium bicarbonate (common baking soda), a commonly used remedy for abdominal pain such as “heartburn” or pain of peptic ulcer. Metabolic alkalosis can also result from prolonged vomiting or, in the hospitalized patient, over-use of gastric suction with loss of the acid stomach contents. There is a net loss of a strong acid, HCl, and a rise in plasma pH. The body attempts to replace the lost HCl by decreasing the excretion of CO_2 by the lungs. Retention of CO_2 will produce an increase in concentrations of hydrogen and bicarbonate ions, thus correcting the pH change to some extent. The net effect is to replace a strong acid, HCl, with a weak acid, carbonic acid; therefore, the pH remains abnormally high.

Clinical Pearls:

Decreased:

If Co2 is decreased with anion gap increased, thiamin need is probable. The need for magnesium and potassium is also probable. (Lactic and pyruvic acidosis) If chronic fatigue is present consider performing a urinary organic acid analysis for cellular energy status.

Anion Gap

Calculated by using formulas:
(Na) – (Cl + Total Co2) Laboratory Range: 8 to 12 mEq/L 8 to 12 mmol/L

Or:
(Better to configure with potassium)
(Na + K) – (Cl + Total Co2) Laboratory Range: 8 to 16 mEq/L 8 to 16 mmol/L
Optimum Range: 7 to 12 mEq/l
Alarm Range: <4 or > 25 mEq/L
Interday variation: 10%. "Normal values differ depending on method used to measure electrolytes; check with laboratory; Increases during pregnancy 1 to 2 mmol/L.

Anion Gap is a mathematical approximation of the difference between the measured anions and cations in the serum. Electrolyte measurements routinely include sodium, chloride, and total Co2. The un-measured cations (calcium and magnesium) average 7 mmol/L and the un-measured anions (protein) average 22 mmol/L. Therefore, there is normally a 15-mmol/L difference in un-measured anions in the serum. Also if the chloride and Total Co2 concentrations are added together and subtracted from the total of the sodium and potassium concentrations, the difference should be equal to or less than 15 mmol/L.

Clinical Pearls:

Increased:

If increased a diet high in refined carbohydrate with B vitamin need (especially thiamine) should be ruled-out.

If anion gap is increased with Total Co2 decreased, thiamine need, and lactic/pyruvic acidosis is probable. Magnesium and potassium need is also probable. Although an increase anion gap in conjunction with a decreased Total Co2 is an excellent indicator for thiamine need, a more specific test for thiamine is RBC transketolase (expensive, but excellent). Most laboratories do not actually measure thiamine, rather measuring the activity of transketolase both before and after thiamine addition to the sample. Thiamine deficiency is usually secondary to alcohol abuse, where nutritional deficiency is accompanied by defects in thiamine absorption and storage.

A need for manganese, zinc, magnesium, potassium, iodine and omega 3 fatty acids are often present with thiamine need.

With the presence of a gastric HCL deficiency, the need for thiamine and zinc is almost certain.

Decreased:

If anion gap is decreased check BUN and creatinine for renal impairment. Though less common, lithium toxicity may abnormally decrease the anion gap.

Uric Acid, Serum

Laboratory Range: M: 4 to 8 mg/dL 0.24 to 0.47 mmol/L
F: 3 to 7 mg/dL 0.18 to 0.41 mmol/L
Optimum Range: M: 3.5 to 5.9 mg/dL
F: 3.0 to 5.5 mg/dL
Alarm Ranges: < 2.0 mg/dL or > 9.0 mg/dL
Interday variation: 10%. Highest in AM, 5% lower in afternoon; higher in summer than in winter.

Physiology: Uric acid is formed from the catabolism of the nucleic acids, adenine and guanine. Both of these nucleic acids are converted to xanthine, guanine directly and adenine after being converted to hypoxanthine first. Xanthine is converted to uric acid via the action of xanthine oxidase. Uric acid is usually converted by hepatic uricase to allantion, which is excreted in the urine.

Clinical Significance: Elevated amounts of uric acid (uricemia) become deposited in the mesenchyme and joints and cause gout an inflammatory reaction to the urate crystal deposition. A condition of fast cell turnover as well as slowed renal excretion of uric acid may cause uricemia. Elevated amounts of urinary uric acid precipitate into urate stones in the kidneys.

Clinical Pearls:
Increased:
If increased suspect stress or a diet high in purines. If increased with decreased serum phosphorus and an increased or decreased total globulin, gout is probable. Consider biological terrain assessment to confirm acidic mesenchyme. Folic acid inhibits the formation of uric acid and may be used therapeutically for gout.

If increased with a decreased HDL and increased total cholesterol or triglycerides, suspect atherosclerosis. Consider homocysteine, C-reactive protein and fibrinogen assessment. Also, consider darkfield examination of live blood for excess “symplast” formation, lipid crystallization and RBC agglutination.

If increased with an increased sedimentation rate or basophil count, inflammatory arthritis may be present.

If increased with an increased BUN, creatinine or electrolyte imbalance, renal dysfunction is probable.

If increased always rule out drugs (pharmaceutical or recreational)

Decreased:
If uric acid is decreased with a normal MCV and MCH, suspect molybdenum need. Xanthine oxidase, the enzyme that immediately produces uric acid, uses molybdenum as a cofactor. Molybdenum is known to raise uric acid levels, which is why people with gout are told to avoid molybdenum supplements. Molybdenum deficiency is common in people with sensitivity to perfume, exhaust and other gases and sulfites used in food preservation.

If decreased with a MCV at 90 or higher and a MCH at 32 or higher, suspect a need for B12 and/or folic acid. Confirm B12 need with a serum methyl malonic acid.

If decreased in a child with cognitive dysfunction, suspect low I.Q secondary to B12 need.

Decreased uric acid is a common finding with healthy pregnancy.

Amylase, Serum

Laboratory Range: 44 to 128 U/L
Optimum Range: As above
Interday variation: 5 to 10%.
Method: Enzymatic Colorimetry

Physiology: Amylase is a hydrolytic enzyme which catalyzes the conversion of starch to the disaccharide, maltose. Maltose is further hydrolyzed to the monosaccharide glucose by an enzyme in the intestinal secretions, maltase. Both amylase and maltase, working in sequence, are needed for the absorption of starch from foods. In man, amylase is produced mainly by the pancreas, from which it is released on demand into the upper part of the small intestine. Appreciable quantities of amylase are also produced by the salivary glands and released into saliva. Very small quantities are present in liver and muscle and perhaps in other tissues as well.

Blood plasma contains low but constant levels of amylase activity. The source of this plasma enzyme is unknown. Only a small fraction appears to originate in the pancreas or salivary glands. Amylase is removed from plasma by the kidney and excreted into the urine. The measurement of amylase activity in urine has essentially the same diagnostic implications as that in plasma. Kidney function must be considered in the evaluation of either.

Isoenzymes of amylase probably exist, but so far, they have not been adequately defined nor studied in clinical context so as to be diagnostically useful. Many large molecules with amylase activity (macroamylase) have been described. They appear to be amylase bound to an antibody and may therefore be an indicator of autoimmune disease.

Clinical Significance: Both blood and urine amylase levels may be helpful in diagnosis. For a variety of reasons, blood (i.e., serum) is tested far more commonly than urine. The comments about clinical significance that follow refer to serum amylase levels. Serum amylase may be abnormally high or abnormally low. Low values may be seen in such a wide variety of diseases that they are not diagnostically helpful. The degree of suppression is usually modest. For practical purposes the physician is interested only in abnormally high values.

Acute Pancreatitis: By far the most important use of the serum amylase assay is in the elucidation of the cause of acute abdominal pain. One possible cause is acute pancreatitis, a disease in which the pancreatic enzymes leak out of the cells into the gland itself, causing varying degrees of self-digestion accompanied by inflammation and hemorrhage. Though amylase is used to help diagnose pancreatitis, it is less specific than lipase. Amylase may remain elevated as little as 36 to 48 hours after onset of pancreatitis. When amylase is elevated but lipase normal, amylase isoenzymes may be helpful in determining reason of enzyme increase. There are many differences "causes" of acute pancreatitis, including ingestion of large amounts of alcohol and sudden obstruction of the duct through which pancreatic secretions pass on their way to the small intestine. The most common manifestations of this disease are severe abdominal pain and shock (fall in blood pressure). Acute pancreatitis from any cause is a serious disease with a high mortality rate. Treatment, which is often ineffective, is to minimize secretion of pancreatic juice and maintain the blood pressure. Any kind of stress, including surgery, is to be avoided. The physician uses the serum amylase value to help in differentiating this problem from the more common bowel disorders which may produce the same symptoms, but which require immediate surgery (example - acute appendicitis). In acute pancreatitis, the serum amylase rises within a few hours after the onset of the pain. Typically, peak activity is reached within twelve hours, and there is a rapid fall to normal levels within two to five days. Elevated amylase values persisting more than five days suggest extension of the inflammation.

Other Diseases of the Pancreas: Transient rises in serum amylase may occur during chronic pancreatitis. Opiates, which cause constriction of the pancreatic duct, also may cause a slight rise in serum amylase. The physician must keep these and other causes of temporary or modest rises in serum amylase in mind, but they are not of diagnostic importance.

Salivary Gland Disease: As noted above, amylase is also produced in large amounts by the salivary glands. Consequently, as with the pancreas, acute inflammation of the salivary glands, such as that produced by the mumps virus, or obstruction of the salivary gland ducts will produce a rise in serum amylase activity. Since the diagnosis of both of these problems is rarely difficult, serum amylase levels are not often needed.

Lipase, Serum

Laboratory Range: 7 to 60 U/L
Optimum Range: As above
Method: Enzymatic Colorimetry
Interday Variation 10%; Hepatitis, obstructive jaundice (falsely low); in children, 2X higher in neonates, reaches adult levels by one month; increases slightly after age 60 in females.

Physiology: Lipases are enzymes that split (hydrolyze) the ester linkage of fats to yield alcohols and fatty acids. They are, therefore, esterases and are sometimes referred to as such. Many, if not all, cells in the body contain lipase; but the lipase that is of interest in medical diagnosis comes from the pancreas. Pancreatic lipase is synthesized in the cells of the pancreas and excreted via the pancreatic duct that joins the common bile duct just before it enters the small intestine. Bile contains salts of cholic acid that are potent detergents. The detergent action of bile salts plus the churning action of the small bowel emulsify the fat globules in foods, thus greatly expanding the surface area of the fat that is available for attack by the water-soluble lipase.

Most natural fats are triglycerides - esters of glycerol and three fatty acids. Most of the fatty acids are "long chained" (more than ten carbon atoms in length). In the small intestine pancreatic lipase splits off the two terminal fatty acids leaving a monoglyceride with the center hydroxyl group still esterified. The fatty acids and monoglycerides enter the cells lining the small bowel where they are resynthesized to triglycerides before they move into the lymph system. The fat-laden lymph from the intestine eventually mixes with the blood. Triglycerides containing "short" chain fatty acids are completely hydrolyzed by lipase to form glycerol and three fatty acids. The glycerol and short-chain fatty acids enter the cells and are delivered directly to the blood stream, via the portal venous system.

Trace amounts of lipase are present in normal serum, but this enzyme evidently does not come from the pancreas because removal of the pancreas does not appreciably increase serum lipase activity.

Clinical Significance: Only increases in serum lipase are of concern to physicians. As with serum amylase, serum lipase activity increases as the result of acute inflammatory disease of the cells of the pancreas, blockage of the pancreatic duct system, or blockage of the common bile duct. The measurement of lipase has proven more difficult technically than amylase measurement; and, therefore, much more is known about the relationship of amylase to pancreatic disease.

Acute Pancreatitis: By far the most important use of the serum amylase and lipase assays is the elucidation of the cause of acute abdominal pain. One possible cause is acute pancreatitis, a disease in which the pancreatic enzymes leak out of the cells into the gland itself, causing varying degrees of self-digestion accompanied by inflammation and hemorrhage. There are a number of different "causes" of acute pancreatitis, including ingestion of large amounts of alcohol and sudden obstruction of the duct through which pancreatic secretions pass on their way to the small intestine. The most common manifestations of this disease are severe abdominal pain and shock (fall in blood pressure). Acute pancreatitis from any cause is a serious disease with a high mortality rate. Treatment, which is often ineffective, is to minimize the secretion of pancreatic juice and maintain the blood pressure. Any kind of stress, including surgery is to be avoided. The physician uses serum lipase and amylase values to help in differentiating this problem from the more common bowel disorders which may produce the same symptoms, but which require immediate surgery (example - acute appendicitis). In acute pancreatitis the serum amylase rises within a few hours after the onset of pain. Typically, peak activity is reached within twelve hours, and there is a rapid fall to normal levels within two to five days, In general, increases in amylase and lipase run a parallel course in acute pancreatitis, but the elevation of lipase persists for a

longer time. This permit a diagnosis of acute pancreatitis to be made several days after amylase activity has returned to normal. Elevated amylase and lipase values persisting more than five days suggest extension of the inflammation.

Other Diseases Of The Pancreas: Transient rises in serum amylase may occur during the course of chronic pancreatitis, but lipase is only occasionally elevated. In carcinoma of the pancreas, lipase may be elevated because the tumor obstructs the common bile duct or pancreatic duct.

Salivary Gland Disease: It is of some importance that serum amylase may be substantially elevated in diseases of the salivary glands as well as in pancreatic disease. The two diseases can often be distinguished by the fact that lipase does not rise in diseases of the salivary glands. For example, in a patient with mumps, the presence of an elevated lipase level suggests that there is a concomitant inflammation of the pancreas.

Protein, Total

Laboratory Range: 6.0 to 8.5 g/dL 60 to 85 g/L
Optimum Range: 6.9 to 7.4 g/dL
Alarm Range: < 5.9 g/dL or > 8.5 g/dL
Method: Colorimetry

Nomenclature and Methods: Blood plasma contains at least 100 individual proteins. Serum (as contrasted with plasma) is deficient in those coagulation proteins that are consumed or altered during the process of blood coagulation. The value for total serum protein will be approximately 0.25 g/dL lower than for total plasma protein because of the absence of fibrinogen (which was converted to insoluble fibrin that makes up the blood clot). Only a few of the plasma proteins have been isolated, characterized, and their function determined.

Most of the present methods of classification are based on the general physical or chemical properties of a large and heterogeneous group of proteins. There is much overlap between different systems, and a given protein may be classified in different groups depending upon the method used. The plasma proteins were originally subdivided (by their solubility in water and in varying concentrations of salts such as ammonium sulfate, i.e., "salting out" techniques) into three groups: albumin, euglobulin (eu = true) and pseudoglobulin (pseudo = false). Many more groups were separated by the technique for precipitation from cold alcohol solutions. Variation in electric charge has allowed separation in an electric field (electrophoresis) into seven groups: albumin, alpha$_1$–, alpha$_2$–, beta$_1$–, beta$_2$–, and gamma globulin, and fibrinogen. This classification is probably the most widely used. Electrophoresis is usually performed on serum rather than plasma, so the seventh fraction, fibrinogen is not found.

Simple proteins contain only amino acids. The complex proteins may be further described with respect to the nature of the non-protein moiety. Thus, there are lipoproteins, glycoproteins, metallo-proteins, etc. An ideal classification would require identification of every individual protein and determination of its function. This has been achieved for only a few of the proteins, namely albumin, some of the transport proteins, the antibody proteins, and some of the coagulation proteins.

In comparing methods for measuring total protein and albumin it should be kept in mind that the quantity detected is highly dependent upon the technique used. The most respected method is the Kjeldahl procedure in which the nitrogen content is determined and is multiplied by an appropriate factor to convert grams of nitrogen to grams of protein. This factor, of course, represents the average nitrogen content of this large group of proteins. Unfortunately, but not unexpectedly, the nitrogen content varies from one protein to another. The factor of 6.25 represents an average value for normal serum proteins, present in normal proportions. Another commonly used method is the biuret reaction. This is an alkaline copper reagent that gives a blue-violet color with proteins. Again, the intensity of color is not constant from one protein to another. Quantitation by electrophoresis involves the absorption of dyes, which likewise varies with the protein and with the dye.

Many proteins are present in such minute quantities that they are measured not in milli- or micrograms of protein, but in terms of their enzymatic activity or transport function. The coagulation factors (present in plasma as inactive precursors of enzymes) are assayed in systems that measure the time required for a visible clot to form. The fibrin clot is the end product of a series of reactions that are catalyzed by the various clotting factors. The speed of these reactions is dependent upon the concentrations of the coagulation factors. Transferrin, the protein that transports iron, is measured by the ability of the plasma or serum to complex iron. The result is expressed not in milligrams or protein but in micrograms of iron. It is usually referred to as the iron binding capacity rather than the transferrin level.

The tests with which we are concerned here - total protein and albumin - are crude measurements which in general are intended to assay liver function and malfunction of the immune system (see below).

Physiology: For the most part, the plasma proteins are found only in the blood plasma and not in the tissues. Albumin, the most prevalent protein (about 60% by weight), has a low molecular weight and has an important function in the regulation of the osmotic pressure of the plasma and in distribution of water between the blood plasma and the tissues. Albumin is also important as a relatively nonspecific (with respect to substrate) transport mechanism for many physiologic substances (See bilirubin) as well as drugs, antibiotics, etc. Albumin also serves as a precursor for tissue proteins.

There are many proteins which are recognized to have specific transport functions, and which exist presumably only for this purpose. Thyroxine-binding-globulin complexes with the thyroid hormone, thyroxine, and transports it from its site of synthesis to the other tissues. Transferrin has been mentioned above. Haptoglobin complexes with any free hemoglobin in the plasma to form a complex that cannot be excreted by the kidney. This is of some importance in the body's conservation of iron.

The immunoglobulins, found in the gamma globulin fractions, are antibodies that provide an important defense mechanism against bacterial and viral infections. The role of the coagulation or clotting factors in the arrest of hemorrhage is obvious. Most of these factors are identified by a number (e.g., Factor VIII, the factor that is deficient in hemophilia). The proteins in general also serve as a buffer system to maintain constant pH. The effect of the plasma proteins plus hemoglobin in this respect is approximately equal to the capacity of the bicarbonate buffer system.

The liver synthesizes most of the plasma proteins. The major exception to this is the antibody proteins or immunoglobulins, in the gamma globulin fraction. They are produced by "plasma" cells found in the spleen, lymph nodes, bone marrow, and other organs (but not in the plasma in any significant numbers).

Plasma Proteins In Relation To Water Balance:

If the intake of water is less than the excretion, a state of dehydration will develop. A decrease in plasma water will be reflected by an increase in the concentration of all the plasma proteins. There is, of course, no increase in the absolute amounts of these proteins. Liver dysfunction and/or dehydration are the only clinical situations that may produce an increase in albumin concentration. Conversely, when more water is ingested than is excreted, water intoxication occurs. The measured concentrations of total proteins and albumin are low because of simple dilution.

Plasma Proteins In Relation To Protein:

Considering the many forms and functions of the plasma proteins, it would be reasonable to expect that measurement of total protein by itself would be of little value. It is not a sensitive or specific indicator of disease. It was observed many years ago that the ratio of albumin to globulin (the A/G ratio) was altered in various diseases: some diseases are accompanied by a decrease in albumin; others produce an increase in beta or gamma globulins; there are a few rare diseases in which gamma globulins are decreased. In these situations, there is an abnormal A/G ratio. Determination of the total protein and albumin and calculation of the A/G ratio (for this purpose globulin is equal to total protein minus albumin) serves as a screening test for such diseases. Abnormalities are studied by electrophoresis, immunoelectrophoresis, or other appropriate techniques. Calculation of the absolute concentrations of the various fractions separated by electrophoresis requires knowledge of the total protein level.

Albumin

Laboratory Range: 3.7 to 5.0 g/dL 37 to 50 g/L
Optimum Range: 4.0 to 5.0 g/dL 40 to 50 g/L
Alarm Range: <4.0 g/dL
Interday Variation: 2 to 4%
Method: Colorimetry

Physiology: Albumin is produced almost entirely by the liver; therefore, a decreased albumin level will often reflect liver dysfunction. Since albumin level is responsible for about 80% of the colloid-osmotic pressure between blood and tissue fluids, a decrease in albumin will be reflected in osmotic pressure disturbance, a will in turn stagnate lymphatic flow.

Decreased Albumin: This situation may result from decreased synthesis or abnormal losses. Decreased synthesis may occur because of general nutritional deficiency or specific protein starvation, or because of chronic liver disease. The liver synthesizes albumin. Accelerated loss of albumin occurs in those chronic kidney diseases characterized by large amounts of protein in the urine. In some diseases of the bowel there is "weeping" of plasma into the lumen and loss of protein in the stool. Patients with extensive burns lose large quantities of protein from the burned areas. If albumin is decreased and there is no increase in globulins, the total protein will also be decreased.

Clinical Pearls:

Decreased:

If albumin is decreased with a normal or low total WBC, and a lymphocyte count below 20%, free radical pathology is probable. Consider possible neoplasm and seek its locus and causes of the pathology.

If albumin is decreased with a decreased alkaline phosphatase, HGB or HCT, vitamin C need is probable. If the MCV is also increased with a decreased MCH, the need for vitamin C is highly probable. Consider oxidative stress testing to determine oxidation – reduction balance.

If albumin is decreased, consider that some degree of lymphatic stagnancy is probable due to lack of adequate osmotic pressure.

A decreased albumin in conjunction with decreased serum phosphorus indicates probable digestive dysfunction secondary to HCL need. This may reflect an inflammatory upper GI pattern that cannot be addressed with an initial therapy of HCL supplementation. Specific enzymes, probiotics and mucus membrane regenerative factors must be implemented prior to HCL therapy.

Globulin, Total

Laboratory Range: 1.9 to 3.5 g/dL
Optimum Range: 2.4 to 2.8 g/dL

Physiology: Total Globulin is a combination of the alpha1, alpha 2, beta and gamma fractions. An increase or decrease in any of these fractions can cause an increase or decrease in total globulin. For this reason, care must be exercised in making a determination based upon total globulin alone. Anytime a total globulin is below 2.0 to or above 3.5 g/dL a serum protein electrophoresis should be considered.

Increased Globulins: The increase usually occurs in the gamma globulin fraction and there is a corresponding increase in the total protein concentration. The most common causes of hyper-gammaglobulinemia are chronic infections, "connective tissue diseases" such as rheumatoid arthritis, lupus erythematosus, and certain tumors of the antibody producing plasma cells (called multiple myeloma).

Decreased Globulins may be due to an inherited inability to synthesize gamma globulins. This is called agammaglobulinemia (a = without). These children are very susceptible to infections. In adults, decreased gamma globulins may be found in association with a tumor of the thymus gland. This is a very rare tumor

Clinical Pearls:

Increased:

Total globulin helps to confirm need for HCl supplementation. If the serum phosphorus is below 3.0, and the total globulin is above 3.5, with other confirming GI symptoms of HCl need, HCl need is probable. If the serum phosphorus is below 3.0, with elevated total globulin, and a serum gastrin below 50, the need for HCl is highly probable. Although a total globulin between 2.4 and 2.8 is an optimum normal, individuals with values at 2.8 or higher may also manifest a HCl need.

If total globulin is increased above 3.5, also rule out inflammation and/or tissue destruction as well as bacterial or viral infections. Confirm with a white blood count with differential, and consider serum protein electrophoresis.

Decreased:

If the total globulin is below 2.4, suspect digestive inflammation. However, anemia, chronic viral or bacterial infections, or liver dysfunction will also present with decreased total globulin.

Whenever Total Protein is decreased with or without a decreased in albumin and globulin it is best to conduct a plasma amino acid analysis.

C-Reactive Protein

Laboratory Range: < 0.8-mg/dL High sensitivity Assay is also available
Optimum Range: Same
Interday Variation: 2 to 4%
Method: Nephelometry

Physiology: C-Reactive protein (CRP) is an acute-phase reactant protein used to indicate an inflammatory illness. CRP (MW 115,000 to 140,000) was first identified in 1930 as a substance present in the sera of patients with pneumococcal pneumonia that could bind to C-polysaccharide isolated from Streptococcus pneumonia, producing a flocculation reaction. Subsequently it was found that CRP is elevated in a variety of other acute inflammatory diseases. Thus, CRP was the first recognized acute phase reactant. CRP can bind to many molecules, including phosphate esters, lipids, polyanions (DNA, polylysine), polycations (histones, protamine), and a variety of polysaccharides.

CRP is synthesized by the liver and released into the plasma. Small amounts are also made by a subset of peripheral lymphocytes, but this remains bound to the surface of the cells. The intact molecule is a pentameric protein with five identical subunits arranged in a doughnut-shaped polymer.

A number of functions have been ascribed to CRP, including initiation of opsinization and phagocytosis and activation of complement, neutrophils, and monocyte-macrophages. Collectively these properties imply an important role for CRP in the recognition of microbial organisms and as an immunomodulator in host defense. CRP may also be important to the recognition of necrotic tissues.

Small amounts of CRP are normally found in plasma at levels less than 0.8 mg/dl. Levels may increase markedly during inflammation in an acute phase response (reactive Reckeweg phase). CRP is a substance associated with production of inflammatory cytokines. These cytokines appear to encourage coagulation and damage to the vascular endothelium, increasing the potential threat to cardiovascular health. Hence, there is a growing popularity of C reactive protein measurement as an independent cardiovascular risk marker. Measurement of CRP is superior to the erythrocyte sedimentation rate in determining general inflammation and may someday replace it. Because ESR is inexpensive and quick and easy to do, it is still more commonly used to detect inflammation than CRP, even though the later is more accurate.

Numerous studies now implicate plasma C-reactive protein (CRP) as a marker for systemic inflammation, as well as a strong predictor of myocardial infarction and stroke. Men with CRP values in the highest quartile had three times the incidence of myocardial infarction and two times the incidence of ischemic stroke. Significantly, these relationships remained steady over long periods, and were independent of other lipid and non-lipid factors, including smoking. Evidence suggests that previous infection with pathogens such as *Chlamydia pneumoniae* or *Helicobacter pylori* may initially trigger the chronic inflammation detected by CRP. Researchers thus theorize that one-way aspirin improves cardiovascular function is through its anti-inflammatory effect, and the subsequent lowering of C-reactive protein levels.

Ceruloplasmin (CER)

Laboratory Range: 25 to 63 mg/dL (Adult)
Optimum Range: 27 to 37 mg/dL
Values are slightly lower in children;
Values are increased in pregnancy, with oral contraceptive use
Method: Nephelometry

Physiology: Ceruloplasmin (CER) (MW 132,000) is a glycoprotein synthesized by the liver as a single polypeptide chain to which six copper atoms are attached. CER migrates as an a2-globulin on protein electrophoresis. The principal importance of CER in laboratory medicine is in the diagnosis of Wilson's disease, which typically is associated with low plasma CER levels. Historically it was thought that CER played a central role in copper transport since CER-bound copper normally constitutes 90% of the total plasma copper. However, radioisotope studies have shown a negligible turnover of CER-bound copper, indicating that copper is neither lost nor gained from the molecule in the circulation. Furthermore, the protein deficiency state of hypo-ceruloplasminemia, which is dominantly inherited, produces no symptoms of copper deficiency. It is now thought that CER may play a role in copper metabolism by acting as a donor of copper to certain key copper-containing enzymes. This is accomplished through uptake and degradation of CER by a number of cells but does not involve transfer of copper from intact CER molecules.

This protein's other utility lies in that both CER and transferrin are antioxidants, together accounting for most of the antioxidant activity of plasma. In this capacity CER may play a role in preventing lipid oxidation and free radical formation, both of which are damaging to cells.

While copper is an essential nutrient, in elevate levels, it is also highly toxic to cells. Consequently, efficient means of maintaining copper homeostasis are required. After absorption in the intestine, dietary copper is bound to albumin and then is taken up by the liver and to a lesser extent by all cells. The liver functions as a storage site. Copper is incorporated into the CER apoprotein in the liver and is released into the plasma. Roughly 90% of plasma copper is bound to CER, the remainder being found in a dialyzable fraction bound to albumin and histidine. However, the mechanism by which copper is transported to cells is unknown. The principal route of copper excretion is through the biliary tract, with a small amount being excreted in the urine. Copper homeostasis reflects the balance between intestinal absorption and biliary excretion. The liver is the main recipient of dietary copper and the primary route of excretion.

Wilson's disease (hepatolenticular degeneration) is an autosomal recessive disorder associated with toxic accumulation of copper, particularly in the liver and brain. Hepatic involvement in Wilson's disease produces variable degrees of liver dysfunction. Some patients exhibit only minor impairment in liver function, while others develop severe liver damage as early as age 8. Consequently, Wilson's disease should be considered in all patients with chronic liver disease, and in all patients above 12 years of age with relevant neurological findings.

Clinical Pearls:

Decreased:
Deficiency of CER of less than 20 mg/dL is may be accompanied by histopathologic changes compatible with Wilson's disease. Decreased levels are also seen in malnutrition and protein-losing states. Copper and zinc compete for the same enzymes; excesses of copper will impair zinc metabolism and excess zinc intake may impair copper metabolism. Consider zinc imbalances with abnormal CER levels.

Increased:

Consider copper excess. Biliary tract obstruction may also result with elevated plasma CER. Any condition associated with severe liver dysfunction (particularly primary biliary cirrhosis) will impair CER synthesis.

Bilirubin, Total

Laboratory Range: 0.1 to 1.299 mg/dL
Total Bilirubin Optimum Range: 0.1 to 1.2 mg/dL
Direct Bilirubin Optimum: 0 to 0.2 mg/dL
Indirect Bilirubin Optimum: 0.1 to 1.0 mg/dL
Method: Colorimetry

Physiology: Bilirubin is a waste product derived from the breakdown of hemoglobin, the red oxygen-carrying pigment in red blood cells. Senescent or damaged red cells are destroyed primarily in the spleen where they are engulfed by macrophages (macro=large; phage=eat). The hemoglobin is not "recycled" as such, but enzymes in the cytoplasm of the macrophages break it down to amino acids, iron, and porphyrin. The amino acids go into the general metabolic pool and are reused for protein synthesis. The iron may be immediately reused for synthesis of hemoglobin, myoglobin, or heme-containing enzymes, or it may be temporarily stored in the bone marrow or liver. The porphyrin ring is not recycled at all, but is further degraded to bilirubin and then excreted from the body via the bile. (The name bilirubin, of course, refers to the fact that it is a red-yellow pigment found in bile.)

Bilirubin is composed of four pyrrole rings joined by methine bridges. The bilirubin molecule formed in the macrophages of the spleen is non-polar and only slightly soluble in water at body pH. It is called free, unconjugated, or "indirect reacting"* bilirubin. This insoluble bilirubin must be transported from the spleen to the liver by way of the blood stream. To accomplish this, free bilirubin is solubilized by formation of a complex with albumin, one of the plasma proteins. In the liver the bilirubin-albumin complex dissociates, and bilirubin is actively transported into the liver cells where it is conjugated with glucoronic acid. (The body as a detoxification mechanism commonly uses Conjugation with glucoronic acid. In this case, the enzyme glucuronyl transferase catalyzes the formation of an ester linkage between glucoronic acid and the propionic acid side chains of bilirubin.) The resulting polar, water-soluble bilirubin glucuronide is also known as conjugated bilirubin or "direct reacting" bilirubin. Most of the conjugated bilirubin is actively excreted from the liver cells into the bile ducts. Bacteria in the colon reduce bilirubin to urobilinogen and urobilin. Urobilin is the brown pigment largely responsible for the normal color of feces. Very small amounts of conjugated bilirubin reenter the blood from the liver cells.

- Total Bilirubin - A combination of Direct and Indirect Bilirubin
- Direct Bilirubin - Post-hepatic, water-soluble
- Indirect Bilirubin - Pre-hepatic water-insoluble

Methods: The diazo color reaction was first used by Ehrlich in 1883 for the detection of water-soluble (conjugated) bilirubin in the urine of patients with jaundice. When this method was later applied to normal serum, it was discovered that a small amount of color developed immediately, but if a lipid solvent such as alcohol was added, there was a dramatic increase in the color. The immediate reaction was said to be due to "indirect reacting" bilirubin. It is now known that the bilirubin fraction that reacts initially in the aqueous system is primarily the water-soluble conjugate, bilirubin glucuronide. The fraction which is reactive only in the presence of alcohol is free or unconjugated bilirubin. The terms "direct" and "indirect reacting" therefore correspond to conjugated and free bilirubin, respectively.

Clinical Significance: In a normal adult, about 22 ml of red blood cells are destroyed each day, producing about 250 mg of bilirubin. If liver conjugation and excretion are normal, the serum level of total bilirubin is about 1 mg/dL of which 0.1 mg is measurable as conjugated bilirubin. Increased red cell destruction or defective bilirubin conjugation by the liver will increase the amount of free bilirubin in the body. Levels of conjugated bilirubin will rise if there is decreased excretion of bile. If the accumulation of bilirubin in the body is small, it will be detected only by measurement of serum bilirubin levels. With greater bilirubin retention, some of the pigment will

accumulate in the tissues, producing a yellow discoloration called jaundice. Free bilirubin will accumulate in the body fat, while the conjugated bilirubin accumulates in elastic tissues such as the eyeballs, the mucous membranes of the mouth, noses, etc., and the skin of the abdomen and chest. Thus, jaundice may be due to diseases of the blood, of the liver, or of the bile ducts or pancreas.

Increase Bilirubin Production: There are many diseases in which the life span of red blood cells is less than the normal 120 days. In hereditary spherocytosis the red cell membrane is defective, and the cells have an abnormal spherical shape. In deficiency of the enzyme glucose-6-phosphate dehydrogenase the cells are destroyed when exposed to oxidant drugs. In sickle cell anemia the hemoglobin molecule has an abnormal structure due to an amino acid substitution. When the oxygen tension is low, sickle hemoglobin polymerizes inside the red cells to form tactoids, and the cells assume a sickle shape. These cells are rigid and so are trapped in small capillaries. There are other defects of hemoglobin, red cell membrane structure, and red cell enzymes that also cause a shortened red cell life span. They are due to gene mutations, and are passed from one generation to the next.

Red blood cells may also be prematurely destroyed due to external factors. If antibodies react with structures on the red cell membrane, there is membrane damage and destruction of the cell. An example of this mechanism is the anemia and jaundice of the "Rh" baby whose red cells are destroyed by the Rh antibodies produced by its mother.

Patients who have an artificial mechanical heart valve may have anemia and jaundice because their red cells are subjected to increased mechanical trauma as they pass through this valve.

High levels of free bilirubin characterize hemolytic diseases. Thus, the total bilirubin is high, while the level of conjugated bilirubin is nearly normal. Because indirect bilirubin is not water-soluble the kidneys cannot have excreted it directly into the urine.

Inherited Defects in the Metabolism of Bilirubin: Gilbert's disease. In this disorder there is impairment in the ability of the liver cells to take up free bilirubin from the blood. The serum level of free bilirubin is slightly elevated (but not usually less than 5 mg/dL) and the conjugated bilirubin is normal. The patient is slightly jaundiced, but all other liver function tests are normal (enzymes, etc.). This metabolic abnormality is found in approximately 1 in 200 persons in this country.

A much rarer disorder is the absence of the enzyme glucuronyl transferase. This is called the Crigler-Najjar syndrome. Infants with this defect become deeply jaundiced shortly after birth. The free bilirubin level is high, and there is no conjugated bilirubin. In infants, free bilirubin can enter the brain. It is toxic to nerve cells. These children usually die within the first year of life from the resulting brain damage.

The Dubin-Johnson syndrome is also a rare disease in which the transport mechanism for the excretion of conjugated bilirubin from the liver cell into the bile duct is abnormal. Conjugated bilirubin accumulates in the liver cells and in the blood and is excreted in the urine. There is also an abnormality in the excretion of some organic anions by the liver. Patients with this disease are not usually sick except for occasional episodes of jaundice and abdominal pain. Their life span is normal.

Decreased Excretion of Bile: Bile is excreted from the liver cells into tiny vessels called bile canaliculi (=little channels). These merge into larger and larger ducts, and finally form the common bile duct that carries bile to the small intestine. Obstruction at any point will cause bile to back up. Some of the conjugated bilirubin is reabsorbed into the blood, and jaundice results. Obstruction of the smallest branches of the bile ducts may follow treatment with drugs such as conjugated estrogens, horse derived estrogens, and oral contraceptives. The mechanism of the bile duct obstruction has not been elucidated. Cancer that has spread from the liver to another organ, such as the lung or breast, may cause obstruction of the larger branches of the bile ducts within the substance of the liver through simple mechanical pressure.

Concretions of cholesterol, bilirubin, or other substances excreted in the bile (gallstones) are usually formed in the gall bladder, but they may be expelled into the common bile duct and obstruct it. The bile duct may also be compressed from the outside, usually by a cancer of the head of the pancreas, through which the duct passes before it reaches the intestine. In these cases, the elevation of bilirubin in the serum is due to increases in conjugated bilirubin.

Diseases of The Liver: Any disease that destroys or damages a large number of hepatocytes will decrease the capacity of the liver to convert free bilirubin-to-bilirubin glucuronide, and jaundice will result. In acute viral hepatitis there is an increase in free bilirubin due to fewer functioning liver cells. There is also an increase in the serum level of conjugated bilirubin due to disruption of the bile canaliculi by the inflammatory reaction and stasis of bile within the liver.

Cirrhosis of the liver is a chronic disease characterized by death (necrosis) of hepatocytes followed by regeneration and scarring. The normal anatomic relationships between the newly regenerated liver cells and the bile ducts and blood vessels are not restored. The liver is nodular; there are clumps of liver cells separated by bands of scar tissue. In this country cirrhosis is most often a consequence of chronic alcoholism although it may also follow viral hepatitis. The jaundice that is seen in these disorders is due to elevations of free bilirubin (implying a decreased number of functioning hepatocytes cells) and to increases in conjugated bilirubin (the remaining liver cells do not communicate normally with the bile duct system).

“Liver Function Test”

The term "liver function tests" and its abbreviated form "LFTs" is a commonly mis-used term that is applied to a variety of blood tests that assess the general state of the liver and biliary system usually through enzyme levels. A true functional liver test utilizes oral challenge substances (such as caffeine, acetaminophen, salicylate), and then measures their metabolic byproducts in the urine, saliva and blood. Through such a challenge, the functional liver detoxification pathways (cytochrome p-450 and phase two conjugation pathways) may be determined.

Routine liver blood tests can be divided into those tests that are *reflective* of liver function, such as serum albumin or prothrombin time, and those tests that are simply markers of liver or biliary tract disease, such as the various liver enzymes. In addition to the usual liver tests obtained on routine automated chemistry panels, physicians may order more specific liver tests such as viral serologic tests or autoimmune tests that, if positive, can determine the specific cause of a liver disease.

There are two general categories of "liver enzymes." The first group includes the alanine aminotransferase (ALT) and the aspartate aminotransferase (AST), formerly referred to as the SGPT and SGOT. These are enzymes that are indicators of liver cell damage. The other frequently used liver enzymes are the alkaline phosphatase (alk. phos.) and gamma-glutamyl transferase (GGT) that indicate obstruction to the biliary system, either within the liver or in the larger bile channels outside the liver.

The ALT and AST are enzymes that are located in liver cells and leak out and make their way into the general circulation when liver cells are injured. The ALT is thought to be a more specific indicator of liver inflammation, since the AST may be elevated in diseases of other organs such as the heart or muscle. In acute liver injury, such as acute viral hepatitis, the ALT and AST may be elevated to the high 100s or over 1,000 U/L. In chronic hepatitis or cirrhosis, the elevation of these enzymes may be minimal (less than 2-3 times normal) or moderate (100-300 U/L). Mild or moderate elevations of ALT or AST are nonspecific and may be caused by a wide range of liver diseases. ALT and AST are often used to monitor the course of chronic hepatitis and the response to treatments, such as prednisone and interferon.

The alkaline phosphatase and the GGT are elevated in many disorders that affect the drainage of bile, such as a gallstone or tumor blocking the common bile duct, or alcoholic liver disease or drug-induced hepatitis, blocking the flow of bile in smaller bile channels within the liver. The alkaline phosphatase is also found in other organs, such as bone, placenta, and intestine. For this reason, the GGT is utilized as a supplementary test to be sure that the elevation of alkaline phosphatase is indeed coming from the liver or the biliary tract. In contrast to the alkaline phosphatase, the GGT is not elevated in diseases of bone, placenta, or intestine. Mild or moderate elevation of GGT in the presence of a normal alkaline phosphatase is difficult to interpret and is often caused by changes in the liver cell enzymes induced by alcohol or medications, but without causing injury to the liver.

Bilirubin is the main bile pigment in humans which, when elevated, causes the yellow discoloration of the skin and eyes called jaundice. Bilirubin is formed primarily from the breakdown of a substance in red blood cells called "heme." It is taken up from blood processed through the liver, and then secreted into the bile by the liver. Normal individuals have only a small amount of bilirubin circulating in blood (less than 1.2 mg/dL). Conditions that cause increased formation of bilirubin, such as destruction of red blood cells, or decrease its removal from the blood stream, such as liver disease may result in an increase in the level of serum bilirubin. Levels greater than 3 mg/dL are usually noticeable as jaundice. The bilirubin may be elevated in many forms of liver or biliary tract disease, and thus it is also relatively nonspecific. However, serum bilirubin is generally considered a true test of liver function (LFT), since it reflects the liver's ability to take up, process, and secrete bilirubin into the bile.

Two other commonly used indicators of liver function are the serum albumin and prothrombin time. Albumin is a major protein that is formed by the liver, and chronic liver disease causes a decrease in the amount of albumin produced. Therefore, in more advanced liver disease, the level of the serum albumin is reduced (less than 3.5 mg/dL). The prothrombin time, which is also called protime or PT, is a test that is used to assess blood clotting. Blood clotting factors are proteins made primarily by the liver. When the liver is significantly injured, these proteins are not

normally produced. The prothrombin time is also a useful test of liver function, since there is a good correlation between abnormalities in coagulation measured by the prothrombin time and the degree of liver dysfunction. Prothrombin time is usually expressed in seconds and compared to a normal control patient's blood.

Finally, specific and specialized tests may be used to make a precise diagnosis of the cause of liver disease. Elevations in serum iron, the percent of iron saturated in blood, or the iron storage protein ferritin may indicate the presence of hemochromatosis, a liver disease associated with excess iron storage. In another disease involving abnormal metabolism of metals, Wilson's disease, there is an accumulation of copper in the liver, a deficiency of serum ceruloplasmin and excessive excretion of copper into the urine. Low levels of serum alpha1-antitrypsin may indicate the presence of lung and/or liver disease in children or adults with alpha1-antitrypsin deficiency. A positive antimitochondrial antibody indicates the underlying condition of primary biliary cirrhosis. Striking elevations of serum globulin, another protein in blood, and the presence of antinuclear antibodies or anti-smooth muscle antibodies are clues to the diagnosis of autoimmune hepatitis. Finally, there are specific blood tests that allow the precise diagnosis of hepatitis A, hepatitis B, hepatitis C, and hepatitis D.

Urinary organic acid analysis is an excellent adjunct in assessing liver detoxification. Specifically: Glucaric acid (glucarate) is the oxidation product of glucuronic acid. It is a by-product of the predominant liver Phase II detoxification reactions involving glucuronic acid conjugation and decreased urinary glucarate is an indicator of reduced overall hepatic function. Orotic acid accumulation is a sensitive marker of ammonia build-up. Ammonia (via glutamine) is normally disposed of by forming carbamoyl phosphate which enters the urea cycle. When there is insufficient capacity for detoxifying the load of ammonia, carbamoyl phosphate leaves the mitochondria and stimulates the synthesis of orotic acid. 2-Methylhippurate is a metabolite of the detoxification of the common solvent, xylene. Elevations of this organic acid in the urine indicate an exposure to this potentially toxic compound. The ratio of sulfate to creatinine has been used to assess the body’s reserve of sulfur-containing compounds (especially glutathione) used in Phase II pathways. When the ratio of sulfate to creatinine is low, these stores need replenishment. Glutathione administration with oral cysteine and taurine and salts of sulfate are used in combinations to replenish sulfur pathways and restore the hepatic supply of inorganic sulfate.

In summary, numerous blood tests are used to diagnose or monitor liver disease. They may be simply markers of disease (e.g., ALT, AST, alkaline phosphatase, and GGT), more valued indicators of overall liver function (serum bilirubin, serum albumin, and prothrombin time) or specific tests that allow the diagnosis of an underlying cause of liver disease. Often liver function may be impaired in the absence of abnormal enzyme studies. Hence, interpretation of these liver tests is a sophisticated process that is best used in the context of medical history, physical examination, and other tests such as oral tests challenges for detoxification determination, Computerized Regulation Thermography, radiographic or sonic imaging studies of the liver, and liver biopsy.

γ-Glutamyl Transferase (GGT)

Laboratory Range: M: 11 to 49 U/L
F: 7 to 32 U/L
Optimum Range: 10 to 30 U/L
Method: Enzymatic Colorimetry
Interday variation: 8 to 15%

Physiology: Gamma glutamyl transferase (GGT) is an enzyme that catalyzes the transfer of the gamma glutamyl group from one peptide to another or to an amino acid. Recent studies have led to the hypothesis that the specific function of this enzyme relates to the transport of amino acids through cell membranes. According to this theory, glutathione serves as a donor of the glutamyl group that is transferred to any one of a number of amino acids. The glutamyl amino acid traverses the cell membrane where it is converted to the amino acid and 5-oxoproline, a reaction that is catalyzed by the enzyme gamma glutamyl cyclotransferase. GGT is found in highest concentration in cells that are very active in amino acid transfer, such as the renal tubules and intestines. The presence of the enzyme in high concentrations in renal tubules suggests that these cells may serve to recover amino acids from the urine. GGT has been found in a wide variety of other tissues including the pancreas, prostate and salivary glands, seminal vesicles, brain, and heart. In support of the amino acid transport hypothesis is the finding that GGT is bound to the cell membrane. It is known to have at least five isoenzymes, but the diagnostic significance of these is not known.

Clinical Significance: In clinical applications, GGT is measured in serum. Normally only small amounts are present. Diagnostic significance is assigned only in increases in activity. As is the case with most enzymes, the interpretation of an elevated GTT level is greatly aided by comparing it with other serum enzyme activities.

Diseases of The Liver: Despite the widespread distribution of GGT in tissues, increases in serum GGT activity usually result from diseases of the liver, particularly those diseases associated with obstruction of the flow of bile. The system of small and large ducts that collect bile from the individual liver cells and transport it to the gall bladder and then to the duodenum (first part of the small intestine) is known as the biliary tract or tree. Obstruction of the bile flow may be due to disease of the duct itself, of the liver cells, or of adjacent organs. These so-called "obstructive liver diseases" are associated with the greatest increases in serum GGT levels. Bile is very high in GGT activity. The enzyme probably comes from the cells that line the bile ducts.

1. Obstructive Liver Diseases: There are many diseases of the biliary tree that may be associated with obstruction of the flow of bile. These include inflammation of the walls of the bile ducts (cholangitis); inflammation of the gall bladder (cholecystitis); precipitation of bile within the smallest ducts (cholestasis) in cases of viral hepatitis; gall stones of precipitated cholesterol or bilirubin blocking the larger ducts; tumors within or outside the liver which compress and block the ducts, such as spread of cancer from other organs to the liver, tumor of the biliary ducts themselves, or cancer of the pancreas - through which the main bile duct passes on its way to the small intestine. The elevation of serum GGT is highest when the obstruction is lower down in the biliary tree. The highest levels have been seen in cancers of the bile duct at the point where it enters the wall of the small intestine. In these diseases the GGT levels tend to parallel the elevation of serum alkaline phosphatase. The elevation of GGT is usually greater and persists for a longer time. The primary advantage of measurement of GGT rather than ALK P is that ALK P is frequently elevated in diseases of other organs, particularly bones, while the GGT is normal in such diseases.

2. Acute Hepatitis: Serum GGT is elevated in nearly all patients with acute viral (infectious and serum) hepatitis. The increase usually does not begin until the first week of actual illness, and the highest levels are reached during the second or third week. The abnormal GGT levels persist for several weeks longer than elevations of other serum enzymes such as LDH, GOT and GPT. The increases in the latter enzymes are probably due to necrosis (death) of liver cells, while the delayed elevation of GGT may reflect a reparative or healing process. In toxic hepatitis (due to chemicals, drugs, etc.), the small ducts that gather bile from the individual liver cells, become plugged by precipitates from bile. This is called cholestasis ("sitting" of the bile). Significant degrees of cholestasis produce very high levels of serum GGT.

3. Cirrhosis of the Liver: In the usual type of cirrhosis (scarring), Laennec's cirrhosis, due to prolonged excessive alcohol consumption, GGT is often elevated. Some elevation is found in 90% of patients. The levels average about four times the upper limit of normal. Alcoholism itself induces a rise in serum GGT. In fact the correlation between alcohol ingestion and serum GGT is so good that is has been suggested that GGT screening might be useful in seeking out occult alcoholics. In another type of cirrhosis, biliary cirrhosis, the walls of the bile ducts obstruct the bile flow and cause very great elevations of GGT averaging 25 times the upper limit of normal.

4. Metastatic Carcinoma of the Liver: With few exceptions, cancer of organs other than the liver generally does not cause an increase in serum GGT unless they have spread (metastasized) to the liver. The GGT increase may be due to obstruction of the biliary tract by tumor nodules or to increased synthesis of GGT, or both. GGT is increased more often than any of the other “liver” enzymes, but the true incidence of elevated GGT in metastatic carcinoma is not known. Normal GGT levels may be seen when the metastatic tumor nodules are very small.

Diseases of the Pancreas: There is twice as much GGT (per gram of tissue) in the pancreas than the liver. The enzyme is located in the acinar cells that produce the pancreatic juice and in the cells that line the pancreatic ducts. Diseases of the pancreas therefore, like liver diseases, result in an increase in serum GGT activity. The reason for the greater clinical significance of GGT in liver disease is simply that liver disease is much more common than diseases of the pancreas.

1. Pancreatitis: In acute inflammation of the pancreas, serum GGT is almost always above normal. The levels do not rise as promptly as amylase levels, but they remain high for a longer time (2-6 weeks). In chronic inflammation of the pancreas the GGT levels are usually normal.
2. Cancer of The Pancreas: Cancer of the “head” of the pancreas, through which the common bile duct passes, usually results in very high GGT levels because it obstructs the flow of bile. Carcinoma of the “body” and “tail” of the pancreas may not be associated with increases in GGT.

Heart Disease: Normal heart muscle contains very little GGT. However, cardiac muscle in the process of healing has many times the normal amount of GGT. Because of this, GGT may be elevated in heart disease.

1. Myocardial Infarction: Moderate elevation in serum GGT often follows death of heart muscle due to lack of oxygen. CPK, GOT, and LDH levels rise promptly when the blood supply to a part of the heart muscle is cut-off. But GGT levels remain normal for the first three or four days and then rise to a peak at ten days. The elevation of GGT persists for about a month. The increases in CPK, GOT, and LDH reflect the death of muscle cells, while the increase in GGT reflects the healing process. GGT is not elevated in angina pectoris – chest pain due to a poor blood supply to the heart without actual death of cardiac muscle cells.
2. Congestive Heart Failure: A moderate elevation of GGT is common in congestive heart failure, probably due to the accompanying impairment of liver function.

Diseases of The Nervous System: Nerve tissue itself contains very little GGT. The small amount of the enzyme that is found in the brain is probably located in the cells lining the small blood vessels. In most diseases of the nervous system the GGT levels are normal. The increases found in epilepsy are probably due to the drugs used to control the seizures. Increases in GT have been seen in about half of the patients with brain tumors.

Diseases of The Kidney: GGT is found in highest concentration in the kidney, yet kidney disease is not usually associated with elevated serum GGT.

Other Causes of Elevated GT: Certain drugs (alcohol, barbiturates, glutethimide, methaqualone) increase serum GGT levels, presumably because they increase the synthesis of this enzyme.

Diseases and Physiologic States That Do Not Increase GT: Most of the diseases in which GGT is elevated are also accompanied by an elevation in alkaline phosphatase. It is important for the physician to be aware of those diseases (and physiologic states) in which the ALK P is increased but GGT is normal. A normal GGT level is helpful in interpreting an increased ALK P.

1. Pregnancy: Alkaline phosphatase is usually slightly elevated at the end of pregnancy but GGT is not.
2. Childhood and Adolescence: Active bone growth produces an increase in ALK P. Children often have elevated ALK P levels, while their GGT levels are the same as in adults.
3. Hyperthyroidism: ALK P is usually increased in this disease in which there is an increased production of the thyroid hormone. The mechanism of the ALK P increase is rapid turnover of bone tissue. GGT levels are normal.
4. Bone Diseases: Hyperparathyroidism, bone cancer, and Paget's disease of bone all produce substantial increases in ALK P but rarely cause an increase in GGT levels.

Clinical Pearls:

If the GGT is increased more than the AST and the ALT, the site of involvement is probably outside the liver in the biliary tree.

GGT may be used to determine the source of elevated Alkaline Phosphatase; less expensive, but not as accurate as Alkaline Phosphatase Isoenzymes. If the GGT is increased with an increase in Alkaline Phosphatase, a common bile duct involvement is probable.

GGT is useful to detect ethanol abuse.

If GGT is below 10, vitamin B6 need is possible. If the AST and ALT are also below 10, vitamin B6 need is probable.

Glutamic Pyruvic Transaminase (SGPT)
Alanine Aminotransferase (ALT)

Laboratory Range: M: 8 to 45 U/L
F: 6 to 38 U/L
Optimal Range: 10 to 30 U/L
Interday variation: 5 to 30%; slightly higher in males than females

Physiology: This enzyme catalyzes the formation of glutamic acid from ketoglutarate through transfer of the alanine amino group. There is only one molecular species known at present.

Clinical Significance: The SGPT activity in tissues is generally much less than GOT. It is found in highest concentration in the liver. Significant elevations of SGPT occur only in diseases of the liver. SGPT is often measured in conjunction with GOT to determine whether the source of the GOT is the liver or the heart. Some of the liver disorders which produce SGPT elevations are described below.

Infectious Hepatitis: Large amounts of SGPT (and GOT) are released into the circulation in severe acute hepatitis. The SGPT level is usually equal to or slightly higher than the GOT level. When both enzyme levels are found to be elevated, there is no advantage in repeatedly measuring both to diagnose and follow the course of the disease. Most physicians elect to follow only the GOT level. In mild cases of hepatitis, the symptoms and signs may not be completely diagnostic and the GOT may only be moderately elevated. In this case, if there are reasons to question the hepatic source of the GOT, a concomitant elevation of SGPT will resolve the problem.

Obstructive Jaundice: There is many diseases in which the outflow of bile from the liver into the duodenum (the upper small intestine) is blocked. This obstruction causes the retention of all substances that are normally excreted from the body by the liver via the bile. Among these is bilirubin (see paper on bilirubin). The accumulation of bilirubin in the body imparts a yellow color called jaundice. Transaminases are also excreted into the bile, and therefore also "back up" in the blood in obstructive jaundice. Both GOT and SGPT are moderately increased. The levels of GOT in obstructive jaundice are of about the same magnitude as those seen after a myocardial infarct ("coronary" or "heart attach"), so an associated rise in SGPT may help to differentiate these two conditions.

Cirrhosis and Other Liver Diseases: In cirrhosis (disease described in section on GOT) the SGPT is usually elevated to a lesser extent than the GOT. Some physicians use a ratio of GOT: SGPT to aid in diagnosing the nature of the liver disease. In acute hepatitis, the ratio is usually 1 or less, while in cirrhosis it is usually about 2.5. Fatty necrosis of the liver, another chronic liver disease also associated with chronic alcoholism, behaves much like cirrhosis with respect to GOT and SGPT levels.

Myocardial Infarction: The importance of measuring SGPT in the case of suspected myocardial infarction lies in the fact that SGPT is not ordinarily elevated. A normal SGPT level would confirm the hypothesis that an elevated GOT level is due to cardiac disease. Slight elevations of SGPT may occur if the infarct destroys a very large volume of heart muscle.

Clinical Pearls:

If increased with the SGPT/ALT increase greater than the GOT/AST and GGTP increase, the area of involvement is probably inside the liver.

If decreased below 10, a vitamin B 6 need is possible. If the GOT/AST and/or the GGT are also decreased below 10, a vitamin B 6 need is probable.

Aspartate aminotransferase (AST)
Serum Glutamic oxaloacetic transaminase (SGOT)

Laboratory Ranges: 8 to 40 U/L
Interday Variation: 8 to 12%
Method: Enzymatic Colorimetry

Nomenclature: Aspartate aminotransferase is the name recommended by the IUM, but among physicians this enzyme is almost always referred to as glutamic oxalacetic transaminase, or more simply, GOT.

Physiology: This enzyme catalyzes formation of glutamic acid from d-ketoglutarate through transfer of the aspartate amino group. There are different molecular species separable by electrophoresis or chromatography, with the same substrate specificity. These are called isoenzymes. Measurement of GOT isoenzymes has not yet proved clinically useful.

Clinical Significance: GOT is a ubiquitous enzyme. It is found in highest concentrations in the liver and heart muscle, and it is also abundant in skeletal muscle, kidney, and pancreas. Its clinical usefulness is largely restricted to the diagnosis of diseases of the liver and heart. The most information is gained if this enzyme is measured simultaneously with other enzymes, particularly GPT, CPK, LDH, and alkaline phosphatase.

GOT In Liver Disease: The liver (Latin: hepar) is a large organ with a high concentration of GOT. Diseases of the liver are usually diffuse: that is, all of the liver cells are involved. As a result, large amounts of GOT may be released into the blood. Very high levels are observed in acute diseases, while lesser elevations are seen in chronic liver diseases. The most common disease entities are listed below.

1. Infectious Hepatitis (Latin: - itis = inflammation of): This is a virus disease that affects the liver uniformly and often severely. It is a relatively common disease. The increase in GOT occurs early in the illness, reaches very high levels during the height of the disease, and remains elevated throughout the course of illness. Peak levels are usually 10 to 100 times the normal levels. [GOT levels are not of help in distinguishing viral hepatitis acquired by ingestion of the virus (infectious hepatitis, hepatitis A) from that acquired from blood transfusion or injection (serum hepatitis, hepatitis B).]

2. Toxic Hepatitis: Certain chemicals, particularly chlorine-containing compounds, are toxic to the liver. Carbon tetrachloride is a "classic" hepatotoxin. Chloroform, though far less toxic, may also produce diffuse liver damage. Many drugs produce damage to the liver, though rarely, and not to such a severe degree. These chemicals produce severe, generalized destruction of liver cells with the consequent release into the blood of many intracellular enzymes, including GOT. GOT is greatly elevated in toxic hepatitis. It reaches about the same levels that are seen in infectious hepatitis. Toxic hepatitis is far less common than infectious hepatitis.

3. Infectious Mononucleosis: This is probably a virus disease although the virus has not as yet been identified. It is quite common among young adults. The most characteristic features of the disease are a "sore throat" with enlarged lymph noted in the neck, and fever. The liver is also involved in most, if not all cases, but symptoms (such as jaundice, etc.) of liver insufficiency are uncommon. GOT levels are elevated in 80% of the patients. The degree of elevation is less than in infectious or toxic hepatitis, but it may persist for a longer time.

4. Cirrhosis of The Liver (Greek: kirrhos = orange yellow): This is a chronic disease in which there is gradual, progressive destruction of the liver cells and replacement by connective (scar) tissue. The liver is very hard, nodular, and pale yellow (due to retention of bile pigments). There are several different types of cirrhosis with different causes. The most common type, alcoholic cirrhosis (or Laennec's or portal cirrhosis) occurs after prolonged and excessive alcohol ingestion that occurs in chronic alcoholics. Since the disease develops slowly and the cells are not uniformly involved, there is no marked increase in enzyme levels in the serum. GOT is usually only moderately elevated and may even be normal. However, if GOT is elevated with elevated triglycerides, suspect alcoholism.

5. Other Diseases of the Liver: Some other diseases in which elevations of GOT may occur are a) obstruction of the bile ducts (due either to gall stones or to cancer of the pancreas), b) spread of carcinoma (cancer) from another organ, usually the bowel or lung, to the liver (called metastatic carcinoma of the liver), c) cholangitis (Greek Chole = bile + angeion = vessel + itis) or inflammation of the bile ducts with swelling and closure of the lumen, d) abscesses of the liver, e) amoeba infection. The GOT elevation in these and other diseases is usually modest or slight and may not be present at all. At any rate, the elevation is not usually of diagnostic importance.

GOT in Heart Disease: The concentration of GOT in heart muscle is equal to or slightly higher than in liver cells. The heart, however, is a much smaller organ than the liver (250 g vs. 1300 g), and in addition, cardiac diseases that result in leakage of GOT into the blood are usually localized to a small area of the heart muscle rather than diffuse. As a result, certain diseases of the heart produce increases of GOT, but never to the degree seen in acute hepatitis.

Myocardial Infarction: this is a disease due to occlusion on one of the arteries (coronary arteries) that provide blood to the heart muscle itself (myocardium). The part of the heart muscle that depended upon the occluded vessel for its blood (and oxygen) supply undergoes necrosis (death - localized "gangrene"). Localized necrosis due to an occluded coronary artery is called infarction. As the heart tissue dies, it releases its enzymes (as well as other intracellular substances not now measured or measurable) into the veins that drain the area. GOT begins to rise 6 - 8 hours after a myocardial infarction occurs. Peak activity occurs at 24 - 36 hours, and a return to normal levels usually follows in 4 - 5 days. There is a rough correlation between the degree of GOT rise and its duration with the extent of the infarction. If the GOT measurement is made at the optimal time, i.e., 12 - 48 hours after onset, nearly every patient will have an increase in GOT. This test serves to confirm the diagnosis suggested by the electrocardiogram (ECG or EKG) and to assist in judging the extent of the myocardial damage. As with liver disease, GOT elevations are most helpful when interpreted in conjunction with other enzyme levels, e.g., CPK, LDH, and GPT.

Although the diagnosis of infarction can in most cases be made from clinical evidence (symptoms and signs) without resorting to enzyme studies the ECG may at times be confusing or equivocal, and the symptoms are not always characteristic. In such cases enzyme levels are very helpful. GOT levels are also useful in separating true infarction from other diseases if the heart which may have similar symptoms (angina pectoris - chest pain due to inadequate blood supply to the myocardium but without death of the muscle; dissecting aneurysm of the aorta: and pericarditis - inflammation of the sac which encloses the heart) since these other conditions do not result in elevated GOT levels. GOT is also normal in heart disease due to abnormalities of the valves (valvular disease) and in (congestive) heart failure unless there is associated liver disease.

Other diseases in which GOT might be measured:

Skeletal Muscle Disease: Since skeletal muscle (the muscles which move the arms and legs, etc.) contains appreciable amounts of GOT, increases in serum GOT follow extensive damage of this tissue, e.g., in accident victims. It is important for the physician to keep in mind the possibility of muscle damage as the cause of an elevated GOT in such a patient and thus to avoid an incorrect inference of heart or liver disease. GOT is also elevated in certain inherited diseases of muscle (some of the muscular dystrophies), but other enzymes are more useful in diagnosis of these conditions.

Central Nervous System Diseases: Brain tissue also contains a high concentration of GOT; yet brain damage rarely causes an increase in serum GOT. GOT is unable to diffuse across capillaries of the brain.

Diseases of the Kidney: Despite the high concentrations of GOT in the kidney tissue, renal diseases do not significantly alter the serum GOT but may instead produce an increase in GOT levels in the urine.

Clinical Pearls:

Numerous pharmaceutical drugs and environmental toxins may damage the liver and cause elevations in the AST.

Parasitic infestations of the liver and/or muscle will elevate the AST.

Mild increases are usually typical of chronic hepatitis and alcoholic hepatitis; in the former, AST is typically greater than ALT. With acute hepatitis of most types, AST is elevated to a lesser degree than ALT. Very high values occur with ischemic and toxic hepatitis, and AST is transiently higher than ALT. With muscle injury, AST is usually 3 to 5x higher than ALT.

Alkaline Phosphatase

(Laboratory normals vary for this enzyme depending on the temperature the test is run at) Most now use:
Laboratory Ranges: 42 to 138 U/L
Optimum Range: 60 to 120 U/L
Ranges will be higher for children during bone growth.
Interday variation: 5 to 10%

Physiology: There are a very large number of enzymes that catalyze the hydrolysis of most organic phosphate monoesters. Some of these are normally present in serum. Two groups have been studied for several decades. They are distinguished by their pH optima as acid phosphatase (ACP) and alkaline phosphatase (ALK P). ALK P is present in high concentration in bone-forming cells or osteoblasts (osteo = bone; blas = germ), liver, kidney, the lining cells of the intestine, and placenta. Electrophoresis of serum ALK P has suggested that there are numerous isoenzymes, but this technique has not proved useful in clinical studies, largely because of lack of discrete separation of many of the molecular species. Inactivation by heat has been proposed as a substitute for electrophoresis in distinguishing isoenzymes. Bone ALK P is most sensitive to heat inactivation, liver ALK P intermediate, and placental ALK P least sensitive. Inhibition with L-phenylalanine is also of some use in isoenzyme investigation. Intestinal and placental ALK P is strongly inhibited by this amino acid, while bone and liver ALK P are not. Recently an alkaline phosphatase has been described which is produced by malignant tumors and has properties closely resembling placental enzyme. This enzyme is known as the Regan isoenzyme.

Normal Variation in Serum ALK P Levels with Age: Bone growth is associated with an increase in osteoblastic activity and therefore with an increase in ALK P. Therefore, serum ALK P is elevated in infancy and childhood. The highest levels occur within a few weeks after birth. The levels fall during the first year of life to values that are 2 to 3 times the normal adult levels. A rise may occur at puberty, followed by a rapid fall to the adult level. Elevations also occur during the last trimester of pregnancy. These normal (physiologic) variations must be kept in mind when interpreting ALK P levels in children and women.

Clinical Significance: Alkaline phosphatase was one of the first enzymes that were studied in an attempt to correlate serum levels with disease. Its use has been rewarding, primarily because elevations are largely limited to diseases of two organs: bone and liver. As with most serum enzymes, it is an increase in activity that is usually of diagnostic importance (abnormally low ALK P levels occur only in a rare bone disorder called hypophosphatasia). The increase in ALK P in bone disease has been related to an increase in osteoblast activity (i.e., formation of new bone). The increase in liver disease has been related to obstruction of the ducts through which bile is discharged into the small intestine.

Diseases of Bone Hyperparathyroidism: Calcium metabolism is controlled by the parathyroid hormone produced by these four small glands in the neck. Because these glands are located behind the thyroid, they are called the parathyroid glands (para = beside). Increases in secretion of parathyroid hormone, usually due to a benign tumor, cause loss of calcium from bone. This in turn causes an increase in new bone formation by the osteoblasts. Therefore, we see an increase in serum ALK P. ALK P levels vary a great deal in this disease and are modified by calcium intake. If dietary calcium is adequate, there may be no ALK P increase. Marked elevations of ALK P occur when dietary calcium is deficient. Removal of the parathyroid gland tumor restores normal calcium balance and the ALK P levels return to normal.

Paget's Disease: This is bone disease in which there is marked proliferation of blood vessels within the bone which destroys the bone. The bone destruction stimulates bone regeneration. The disease may be localized to small areas, in which case there is no elevation of ALK P. As the size of the affected areas increases, the ALK P levels also increase. When the disease is widespread, ALK P levels may be extremely high - in fact the highest levels of ALK P that occur in conjunction with bone disease are found in Paget's disease.

Bone Tumors: Primary tumors of bone (i.e. cancer of the bone itself) that destroy bone without causing new bone formation are called osteolytic (lysis = dissolve) tumors. Those that cause the

production of bone are called osteogenic (or osteoblastic) tumors. ALK P, as one might expect, is normal in osteolytic tumors and elevated in osteogenic tumors. Most tumors, however, are mixtures of both types, so some degree of ALK P elevation is usual. Carcinoma that has spread to bone from some other organ (such as lung, breast, or prostate) usually causes an osteoblastic lesion.

Osteomalacia (malacia = softening): This is a bone disease in which there is an abundant amount of protein bone matrix but an inadequate amount of calcium hydroxyphosphate (hydroxyapatite) deposited in the matrix. In children it is known as rickets. Rickets may be due to simple vitamin D deficiency, a failure to absorb calcium because of disease of the intestine, or calcium loss due to kidney disease. In such children the ALK P is usually several times the normal value for their age group. In adults osteomalacia is usually due to diseases of the small bowel, such as sprue, which lead to failure to absorb sufficient calcium. The ALK P in these patients in only slightly elevated.

Bone Fractures: ALK P may be slightly elevated as the fracture heals.

Disease of the Liver: The liver excretes ALK P into the bile. Bile is carried from the liver cells to the small intestine by the bile ducts. Obstruction of these ducts therefore results in the damming up of substances normally excreted in the bile, including ALK P. These substances therefore increase in the blood. Most of the ALK P found in the plasma of patients with bile duct obstruction is believed to be of osseous (bone) origin. A small portion is of hepatic (liver) origin.

Obstructive Jaundice: Jaundice is a yellow discoloration of the skin and other organs due to retention of bilirubin that is normally excreted in the bile. The adjective "obstructive" implies that this retention of bile contents is due to mechanical blockage of the bile ducts. The most common causes of bile duct obstruction are 1) concretions of substances usually dissolved in the bile, such as cholesterol and bilirubin (i.e., "gall stones"); 2) cancer - of the pancreas (through which the bile duct passes on its way to the intestine), of the bile duct itself, of the gall bladder, or of the small intestine at the point where the duct enters; 3) metastatic cancer of the liver - cancer of another organ (such as bowel, breast, lung, etc.) which has spread to the liver; and 4) inflammation or other damage to the bile canaliculi (the smallest branches of the duct system which collect the bile from the liver cells themselves), usually due to drugs.

Infectious Hepatitis: ALK P may be slightly elevated in infectious hepatitis. Isoenzyme studies suggest that, contrary to the situation in bile duct obstruction, in hepatitis this ALK P is of liver origin. The important observation is that ALK P is only slightly elevated in comparison with the transaminase enzymes.

Clinical Pearls:

Increased:
If increased with an increase in GGT, common bile duct problems are probable.
If increased with an increase in ALT (GPT), liver dysfunction is probable.
With a normal ALT, AST (GOT), and GGT, bone problems are probable.

Decreased:
If decreased with a TSH above 5.5, thyroid hypo-function is probable.
If decreased, consider zinc and/or magnesium deficiency.
If decreased, consider B12 deficiency.

Lactic Dehydrogenase (LDH)

Laboratory Normal: (Laboratory normals vary for this enzyme depending on the temperature the test is run at) Most now use:
Adult/elderly: 45 to 90 U/L (30C°)
Isoenzymes in adult/elderly: LDH-1: 17% - 27%
LDH-2 27% - 37%
LDH-3: 18% - 25%
LDH-4: 3% - 8%
LDH-5: 0% -5%
Child: 60 to 170 U/L (30C°)
Optimum Value: Same
Interday variation 5 – 10%

Physiology: The enzyme lactate dehydrogenase catalyzes the inter-conversion of lactic and pyruvic acids. It is widely distributed in the body, being present in most, if not all, tissues. The relatively high concentration in tissue, as opposed to normal plasma, makes increased plasma LDH a good indicator of tissue damage. Other additional information is needed to decide which tissue is injured. There are quantitative differences in the amount of LDH in various tissues. It is found in highest concentration in the liver, followed by heart and skeletal muscle. Red blood cells contain an appreciable amount (about 200 times the normal plasma level), so care must be exercised in preventing hemolysis (rupture of the red cells) when collecting blood samples for this assay.

The lack of tissue specificity of LDH has been partly circumvented by the discovery that it exists in a variety of molecular forms called isoenzymes. LDH is a tetrameric protein containing two types of monomer sub-units designated as "M" and "H". These monomers combine in various proportions to form five isoenzymes: H_4 (LD1), H_3M_1 (LD2), H_2M_2 (LD3), H_1M_3 (LD4), and M_4 (LD5). These isoenzymes are present in different proportions in various tissues. So far, the most clinical use has been made of the observation that heart tissue produces a proportionately large amount of LD1 and LD2, and liver tissue produces a very high proportion of LD4 and LD5 (also called LLDH).

In general, isoenzyme LDH-1 comes mainly from the heart; LDH-2 from the reticuloendothelial system; LDH-3 from the lungs and other tissues; LDH-4 from the kidney, placenta, and pancreas; and LDH-5 from the liver and striated muscle.

Clinical Significance: This is an intracellular enzyme used to support the diagnosis of injury or disease involving the heart, liver, RBC's, kidneys, skeletal muscle, brain and lungs. Clinically, elevations in one or more of the isoenzymes reveals information concerning specific tissue damage. Increases may be absolute (increased total LDH) or fractional (change in the proportion of the five isoenzymes). Given the above information specific patterns of LDH isoenzymes are considered classic for certain diseases. For example:

Isolated elevation of LDH-1 indicates myocardial injury.
Isolated elevation of LDH-5 indicates hepatocellular injury or disease.
Elevation of LDH-2 and LDH-3 indicates pulmonary injury or disease.
Elevation of all LDH isoenzymes indicates multiorgan injury. Advanced malignancy and diffuse autoimmune inflammation disease such as lupus can also cause this pattern.

Most physicians are taught that only increases in the level of LDH activity are known to have diagnostic importance. However, since LDH is an enzyme necessary for glycolysis, a low LDH may often be seen in reactive hypoglycemia.
A decrease in the 5th isoenzyme of LDH may reflect exposure to heavy metals and/or long-term exposure to noxious gases.

Myocardial Infarction: A rise in LDH in infarction is even more common than elevations of CPK or SGOT. Furthermore, the LDH elevation persists longer, with some degree of elevation remaining for as long as ten days. This is an advantage if the patient is not examined until several days after the infarction has occurred. Assay of the LD1 fraction seems to offer advantages over total LDH in that it is more sensitive and more specific for the isoenzymes from heart muscle. Consequently, LD1 is more consistently elevated, the elevation persists for a longer time after the infarction, and there is less likelihood of confusion in the interpretation of the laboratory results when there is a release of LDH from other organs such as the liver.

Liver Disease: Until the availability of a specific assay for LD5 (or LLDH) the measurement of serum LDH was regarded to be of little diagnostic value in liver disease. Slight elevations in total LDH are common in all types of liver disease; but moderate to marked elevations imply that other organs or tissues must be diseased. The introduction of a specific assay for the liver isoenzyme has substantially improved the usefulness of LDH measurement in liver disease. LLDH is regularly elevated in hepatitis, correlating well with the changes in SGPT. Serum LLDH is also usually elevated in neoplastic disease of the liver, and the LLDH changes correlate well with the changes in serum alkaline phosphatase. Only half of the patients with hepatic cirrhosis have an elevation in serum LLDH, and in these instances the elevation is likely to be modest.

Blood Diseases: Serum LDH activity may be elevated in various diseases of blood cells. The increase is not consistent nor is the magnitude ordinarily very remarkable, so not much diagnostic significance has been given to this assay in the diagnosis of such diseases. Megaloblastic anemia, (Greek megalo = large or giant, blast = germ cell; referring to the very large red blood cell precursor cells in the bone marrow) such as pernicious anemia or anemia due to deficiency of folic acid, is an exception. Very high LDH levels are found in patients with untreated megaloblastic anemia. The increase is due to an increase in LD1. LDH (mainly LD2 and LD3) is commonly elevated in the acute leukemia and in chronic myelocytic (or granulocytic) leukemia, but it is less commonly abnormal in chronic lymphocytic leukemia.

Cancer: When a malignant tumor is localized to its site of origin, it does not usually produce an elevation in serum LDH. With widespread metastasis (spread to other organs, such as liver, lung, bone, brain), some degree of elevation of LDH is common, and very high levels may occur. No definite correlation can be made between the presence or degree of LDH elevation and the organ in which the cancer originated (the primary site).

Muscle Diseases: Since skeletal muscle contains LDH, extensive damage to muscle, whether by physical trauma or disease, causes an increase in the serum LDH activity. The isoenzyme LD5 is more abundant in non-myocardial muscle than LD1 and LD2. Consequently, in muscle disease serum, LLDH values tend to be higher than LD1 values.

Clinical Pearls:

As previously mentioned, a decrease in the 5th isoenzyme of LDH may reflect heavy metal body burden or chronic exposure to noxious gases. If this pattern is present, consider identifying heavy metals with hair element analysis or urine provocation for toxic elements. In addition, have the patient ask the Gas Company to analyze the home for gas leaks.

LDH will often decrease with the use of estrogen containing compounds.

Ferritin

Laboratory Normal: M: 18 to 350 ng/ml
F 15 – 49 yr.: 12 to 156 ng/ml
F >49 yr.: 18 to 204 ng/ml
Optimum Ranges: As above
Interday variation 10 – 20%
Method: Immunoassay

Structure: Ferritin is a soluble ion storing protein present in most animal cells. It consists of a 24-unit protein shell enclosing a core of ferric-hydroxy-phosphate. The iron free apoferritin protein has a molecular mass of about 450,000 daltons. Each ferritin molecule may contain up to 4,500 iron atoms but it is normally only about 20% saturated. Plasma ferritin contains almost no iron. Up to twenty-five isoferritins may exist based on immunological evidence using ferritin preparations originating from various tissues separated on isoelectric focusing. These isoferritins are composed of different proportions of two primary subunits named for heart (H) and liver (L). H-subunit rich isoferritins are found in heart muscle, red blood cells, lymphocytes, monocytes, HeLa cells and several other cultured cells. L-subunit rich isoferritins are found in liver, spleen and placenta. Plasma typically contains mostly L-subunit rich ferritin from the liver and spleen.

Functions: Ferritin serves three major physiological functions. First it acts as the primary storage site for a large proportion of the body's iron, a necessary but potentially toxic compound. Second, ferritin readily releases its store of iron if plasma iron levels fall. Third, there is considerable evidence that ferritin serves as an important antioxidant, protecting cells against ferrous iron-catalyzed oxidative damage. Ferritin is second only to hemoglobin in accounting for the total iron present in the human body as shown in Table I. About 700 mg of iron is available for mobilization from ferritin in the adult male and 250 mg in the adult female. Tissues having the largest amount of iron in ferritin include the liver and bone marrow, each of which holds about one-third of body iron stores. Once released from ferritin, iron may serve as a catalyst in the generation of free radicals that can cause oxidative damage to DNA, proteins, and lipids. Ferritin's dose response antioxidant activity probably depends on both its efficient storage capacity for inorganic iron and the ferroxidase activity of its H-subunit.

Approximate Adult Iron Distribution

	Menstruating Females	**Males, PM Females***
Total Body Iron	3 grams	4 grams
Hemoglobin	2,000-2,500 mg	2,500 mg
Ferritin	50-300 mg	700-1,000 mg
Myoglobin & Enzymes	300 mg	300 mg
Hemosiderin	100-400 mg	100-400 mg
Transferrin	4 mg	4 mg

*PM = Post Menopausal

Clinical Significance

Ferritin is the primary source of reserve iron for hemoglobin synthesis. Increases and decreases in serum ferritin generally parallel changes in tissue ferritin iron stores. Iron deficiency anemia is the most common deficiency disorder in the industrialized world. In the United States 2% of men,

20% of menstruating women and up to 50% of pregnant women have depleted iron stores. Serum ferritin levels in normal adults correlate with total mobilizable body iron stores, with 1 ng/ml serum ferritin corresponding to about 8 mg of iron stores. Measurement of the serum ferritin levels is the most sensitive indicator of tissue iron depletion, the earliest phase of iron deficiency as shown in Table II. Serum ferritin levels are constant over a long period of time in healthy individuals and show none of the diurnal rhythm fluctuations associated with serum iron levels. A low serum ferritin level is virtually diagnostic of iron deficiency.

Iron status is monitored clinically through the measurement of serum iron, total iron binding capacity (TIBC), unsaturated iron binding capacity (TIBC minus serum iron level), percent transferrin saturation (serum iron divided by TIBC), and ferritin. Since the TIBC level is proportional to transferrin level present, direct measurement of transferrin level is seldom needed.

Low Plasma Ferritin Levels - Iron Deficiency Anemia

Clinical Picture: Iron deficiency anemia is a widespread health problem that greatly affects women of childbearing age. The deficiency of available iron retards hemoglobin synthesis, resulting in a lowered hematocrit and a microcytic, hypochromic appearance of circulating red blood cells. The long-term consequences of mild iron deficiency anemia on the patient are controversial. Symptoms of iron deficiency are largely a consequence of the degree of anemia and can include weakness, fatigue, dizziness, and palpitations. Other nonspecific symptoms may include nausea, anorexia, constipation and menstrual irregularities. Pallor and tachycardia are frequent findings on physical examination. Some individuals develop pica, a craving to eat such things as clay or starch.

The general sequence of changes with progressive iron deficiency is shown in Table II. When assessing the significance of low serum ferritin levels there are two points to keep in mind. The first is that lowered serum ferritin levels are highly specific for iron depletion if there are no complications from clinical conditions that might increase red cell volume (pregnancy, polycythemia, infancy) or cause vitamin C deficiency. Second, once serum ferritin levels fall below 12 ng/ml, which might occur even in the early stages of iron depletion in some individuals, the serum ferritin level may no longer correspond to the severity of iron deficiency because the iron stores are essentially exhausted.

Table II

Sequential Changes in the Development of Iron Deficiency Anemia

	Stage I Iron-Depletion	**Stage II Iron-Deficient**	**Stage III Iron-Deficiency**
Serum Ferritin	Decreased	Decreased	Decreased
Bone Marrow Iron	Decreased	Decreased	Decreased
TIBC	Normal	Elevated	Elevated
Serum Iron Protoporphyrin	Normal	Decreased	Decreased
Erythrocyte	Normal	Elevated	Elevated
Hemoglobin	Normal	Normal	Low
Hematocrit	Normal	Normal	Low
MCV	Normal	Normal	Low
RBC Morphology	Normal	Normal	Microcytosis Hypochromia

Ferritin with Blood Donation: The donation of one unit of blood drops serum ferritin levels 40-50% in males and about 20% in females who had not previously donated blood. Less severe ferritin level decreases are observed with subsequent donations. Voluntary donation of about 472 ml of blood represents an iron loss of about 236 mg for males and 213 mg for females. This means that there is approximately 0.5 mg of iron per mL of whole blood in males and in non-menstruating females. Menstrual blood loss, sometimes amounting to 1,600 ml/year, may account for the observation that about 80% of donors rejected for low hemoglobin levels were females.

Decreased Ferritin with Celiac Disease: Celiac sprue is much more common than clinically realized. Wheat gluten is the primary culprit causing inflammatory bowel, malabsorption and altered bowel permeability. Always consider food allergy as a potential cause of low ferritin.

Ferritin Increase: Elevated serum ferritin levels are also typically derived from metabolically released ferritin rather than from acute tissue destruction. Consequently, increases in serum ferritin correlate with increased tissue iron stores. However, acute damage to tissue high in ferritin, like liver, could elevate serum ferritin without an increase in tissue iron stores. For this reason the significance of serum ferritin elevations must be evaluated carefully, considering patient history and other laboratory tests to assess possible liver injury.

Hemochromatosis: Hemochromatosis is a rare hereditary condition characterized by enlarged liver (90%), diabetes (75%), increased skin pigmentation (90%), liver cirrhosis, endocrine failure, heart failure and arthritis (30%). Accumulation of intracellular iron is toxic and will ultimately lead to cell death and organ failure. An autosomal gene associated with HLA-A3 causes hemochromatosis. Homozygosity for hemochromatosis in white males in the United States is approximately 5 per 1000, and should be similar for females since an autosomal gene causes this disease. Nevertheless, about ten times more males than females are diagnosed with hemochromatosis. This prevalence makes hemochromatosis one of the most common hereditary metabolic diseases. Clinical evidence of iron overload is not typically present in the heterozygous individual unless they also have another iron accumulating disorder such as b-thalassemia minor, idiopathic refractory sideroblastic anemia, hereditary spherocytosis or sporadic porphyria cutanea tarda. Screenings have found that transferrin saturation, employing thresholds of 60% saturation for males and 50% for females, may provide the best initial marker for hemochromatosis. It has been suggested that if on the initial screening the percent transferrin saturation is elevated, and ferritin is within normal limits, the patient has generally been caught before significant disease has been manifested. These patients should be monitored with percent transferrin measurements every two years until both percent transferrin and ferritin become elevated. However, if the first screening shows elevation of both percent transferrin and ferritin the patient may already have significant iron deposition and a liver biopsy, considered the definitive test for hemochromatosis, is recommended to assess the deposition extent. In either situation, when ferritin becomes elevated blood removal is initiated from the patient with therapeutic phlebotomy. Initially the phlebotomy is aggressive, and some level of blood removal is generally required at two to six month intervals depending on the serum ferritin level. With successful therapeutic phlebotomy, the serum ferritin level will return to normal before the transferrin saturation.

Acquired Hemochromatosis: Excess intake of iron, through dietary sources or blood transfusion, initially causes increased iron accumulation only in the reticuloendothelial cells of liver, spleen and bone marrow. Tissues and organs remain anatomically and functionally normal during this stage of iron overload called hemosiderosis. If iron overload continues, the iron distribution pattern changes to cytoplasmic ferritin storage areas throughout all body tissues, creating a clinical picture much like hereditary hemochromatosis. Clinical problems associated with iron deposition involve liver, pancreas and heart. Chronic anemias such as b-thalassemia and sideroblastic anemia are often characterized by increased physiological iron uptake and multiple blood transfusions.

Inflammation and Infection: Serum ferritin levels may rise through two different mechanisms. In most situations changes in serum ferritin levels accurately forecast what is happening with tissue iron and what will eventually happen with serum iron and TIBC levels. However, in patients showing inflammation and infection, iron may be diverted from hemoglobin to tissue ferritin and hemosiderin. This iron diversion results in a decreased serum iron level, a normal to low transferrin level, increased serum iron stored, and increased serum ferritin. Further complicating the situation is that serum ferritin may act as an "acute phase response" protein, rising with inflammation and infection even in the presence of iron store depletion.

Iron, Serum

Laboratory Normal: 44 to 136 µg/dL 7.9 to 24.5 µmol/L
Optimum Values: 50 to 100 µg/dL
Interday variation 25% Up to 40% diurnal variation, highest in AM lowest at night
Method: Colorimetry

Physiology: The normal human body contains about four grams of iron, most of which exists as a component of hemoglobin (2.5 gms) or in storage as ferritin and hemosiderin (0.5-1 gms). The remainder is widely distributed throughout the body tissues and plasma. Although the quantity of iron in tissues is small (only a few milligrams), it plays a vital role in metabolism. Many enzymes either contain iron or require its presence. Skeletal and heart muscle are richer in iron than most tissues because of the presence of hemoglobin-like iron containing protein, myoglobin.

Iron is transported from one site to another through blood plasma. To accomplish this task, there is in plasma a protein that binds, carries, and releases iron. This protein is a beta globulin named for the function it performs transferrin. Transferrin contains specific binding sites for iron that under normal circumstances are one third occupied. The amount of iron in normal plasma is very small, but since plasma is the conduit through which iron is moved from one site to another in the body, knowledge of the amount of iron (and transferrin) in the plasma and the rate of iron turnover provides insight into total iron metabolism which is useful in diagnosis.

The total body economy is controlled by the regulation of intake. There is no mechanism for excretion of iron. Iron is lost from the body through the loss of body cells, notably red blood cells.

Clinical Significance: There are essentially no differences between plasma and serum iron. Serum is usually used for assay for reasons of technical convenience. Serum iron may be abnormally high or low. It is often useful to know the concentration of transferrin as well as iron.

Increased Serum Iron: Because of the way iron absorption is controlled, an increase in serum iron content is unusual but sometimes does occur.

Hemochromatosis: This is a disease in which there is excessive absorption of iron from the gastrointestinal tract. Iron is deposited in the pancreas, liver, and other organs and produces scarring. Disorders such as diabetes mellitus and liver cirrhosis may result. In this disease the serum iron is above normal, and transferrin is more saturated with iron than normal (saturation is greater than 60%).

Excessive parenteral administration of iron, either as iron salts or in the form of hemoglobin in transfused red blood cells: The administration of iron in a manner which bypasses the regulatory mechanism in the bowel wall may result in abnormally high and often harmful body iron content. The usual circumstance is the patient with a congenital hemolytic anemia who requires repeated blood transfusions over a period of many years. The overuse of intramuscular or intravenous injections of iron in the treatment of iron deficient anemia may also be an occasional cause of this problem.

Acute Iron Toxicity: The accidental ingestion of very large quantities of iron by infants or young children may result in death.

Liver Disease: In active cirrhosis or acute hepatitis, iron is released from storage sites in the liver, causing a temporary rise in the plasma iron level.

Increased Transferrin Levels: An increase in the serum iron-binding protein, transferrin, will cause an increase in the serum iron level if there is an ample supply of available iron. Transferrin as well as other transport proteins, is increased by the administration of estrogenic substances such as those in oral contraceptives.

Decreased Serum Iron: A decrease in serum iron is much more common than is an increase. Low serum iron may be due either to an absolute, total body deficiency of iron or to a reduction in the quantity of transferrin. The assay for serum iron is of importance in differentiating the various causes of anemia (decreased oxygen-carrying capacity of the blood due to decreased amounts of hemoglobin in red blood cells).

Iron Deficiency: The lack of iron in the diet is an unusual cause of iron deficiency except in very young infants who have been maintained on a milk-only diet for many months. The ability to absorb iron through the small intestine is somewhat more common. This condition may be due to diseases of the bowel wall resulting in "malabsorption" or may result from surgery for peptic ulcer that routes the flow of food around the upper small intestine where iron is normally absorbed. By far the most common cause of iron deficiency is blood loss that is not compensated by adequate iron intake or reserves. In men this is usually due to chronic slow bleeding from a peptic ulcer or other lesion of the gastrointestinal tract. In women the bleeding is associated with heavy menstrual periods or abnormal uterine bleeding. In iron deficiency the saturation of transferrin is low and there is an absolute increase in the transferrin level.

Infection: Serum iron concentration is often low in chronic infections and malignant diseases (cancer). In such cases the transferrin concentration also tends to be low.

Chronic Kidney Disease: Serum iron is often low in chronic renal disease and the patients are anemic. The underlying mechanism is not understood. In some cases, such as "nephrosis", there is a loss of transferrin in the urine because the diseased kidneys have lost the ability to retain this and other proteins in the plasma.

Clinical Pearls:
A common mistake made is to conduct a red blood count and indices without conducting a serum iron. Without the serum iron, the amount of inorganic iron available to convert to heme is unknown. Serum iron is needed to differentiate between iron anemia and anemia from the inability to convert iron to heme (vitamin B12, folic acid, B6, copper, molybdenum).

If increased with increased liver enzymes and/or increased serum ferritin, liver dysfunction is probable.

If increased rule out excess iron intake from drinking water, cooking utensils, and iron containing supplements.

If increased with elevated MCV/MCH, vitamin B12 and/or folic acid need is possible. Conduct a serum methyl malonic acid to confirm.

Iron, zinc, copper and manganese are synergistic, but also oppose each other enzymatically. Therefore, an increase or decrease in any of these minerals is reason to rule out and increase or decrease in the other minerals. These minerals may be determined with hair element analysis.

Thyroid Function Tests:
Thyroxine / Thyronine Uptake / Free Thyroxine Index

Thyroxine, Total Laboratory Normal: 4.5 to 12.5 µg/dL 58 to 161 nmol/L
Thyroxine, Free Laboratory Normal: 0.7 to 1.5 ng/dL 9.0 to 19.4 pmol/L
Thyroxine Index, Free Laboratory Normal 1.0 to 4.3
Triiodothyronine, Total Laboratory Normal: 80 to 220 ng/dL 1.23 to 3.39 nmol/L
Triiodothyronine, Free Laboratory Normal: 1.9 to 5.0 ng/L 2.9 to 7.7 pmol/L
Triiodothyronine Resin Uptake Laboratory Normal 22 to 34% 0.22 to 0.34

Physiology: Thyroid hormones are essential and primary regulators of the body's metabolism. Imbalances can affect virtually every metabolic process in the body, exerting significant effects on mood and energy level. Thyroid function has a profound impact on overall health via its modulation of carbohydrate, protein, and fat metabolism, vitamin utilization, mitochondrial function, digestive process, muscle and nerve activity, blood flow, oxygen utilization, hormone secretion, sexual and reproductive health, and many other physiological parameters.

Thyroxine (T_4) is the principal hormone produced by the thyroid gland. In a normal adult, about 150 mg of T_4 are produced each day. After it is released from the thyroid gland, T_4 enters the blood stream where it is bound to plasma proteins which serve to transport the hormone to body cells. The most important of these transport proteins is thyronine-binding globulin (TBG). T_4 is the most abundant iodine-containing substance in plasma with a normal concentration of about 8 µg (8000 ng)/dL. Almost all this T_4 (99.96%) is bound to plasma proteins, primarily TBG. The concentration of unbound thyroxine (free-T_4) is therefore very low - normally about 0.0024 µg (2.4 ng) per dL. Current findings indicate that the free T_4 (fT4) is a more accurate measurement of available thyroxine.

All other iodinated substances normally found in blood plasma are metabolites of T_4. One of these is T_3 (triiodothyronine). The concentration of T_3 in normal plasma is about 120 ng/dL. T_3 is also bound to TBG, but less tightly than T_4. In a normal person about 99.7% of T_3 is bound and 0.3% (0.36 ng/dL) is free. Again, current findings indicate that the free T_3 is a more accurate measurement of available triiodothyronine. The biologic potency of T_3 is several times greater than that of T_4. In fact, the current belief is that T_3 is the thyroid hormone, and T_4 is its precursor. Only a small proportion of T_3 is synthesized by the thyroid gland. Most of the plasma T_3 is formed in tissues other than the thyroid gland by removal of one iodine atom from the terminal phenolic ring of T_4. Another triiodothyronine, reverse T_3, (rT_3) is found in plasma in amounts of about 40 ng/dL. Reverse T_3 has significantly less biologic activity. Like T_3, reverse T_3 is formed through monodeiodination of T_4 in tissues other than the thyroid gland. Many knowledgeable clinicians will measure both the T_3 and rT_3 to make sure most is in the active form. Current findings indicate that the free T3 (fT3) is a more accurate measurement of available triiodothyronine.

Thyroid hormone has many effects of metabolism, but its exact mechanism of action is not known. There is hardly any metabolic function that is not influenced in some way by this hormone. T_4 is necessary for normal growth and development, it plays a major role in maintaining body temperature; it influences the distribution of ions across cell membranes, et. One popular theory of the mode of action of T_4 is that it (probably in the form of T_3) binds to nuclear proteins and enhances the synthesis of messenger RNA. A net effect in the whole animal in increased oxygen consumption, increased production of heat (thermogenesis), and overall loss of metabolic efficiency. This effect, especially thermogenesis or the lack thereof, accounts for the major symptoms and signs that accompany over- or under-function of the thyroid gland.

Although T_3 appears to be the active thyroid hormone, plasma T_4 is still the hormone most commonly measured in conventional medicine. This fortunately is changing and is being replaced with free T_3, rT_3, and free T_4 levels, so as to get an accurate interpretation of conversion. The concentration of T_4 in plasma depends upon its rate of production by the thyroid gland (or the amount ingested) and the concentration of plasma TBG. Under usual circumstances the amount of TBG is fairly constant from one person to another and from time to time. There are many exceptions to this rule. TBG levels are increased during pregnancy and in women using

anovulatory medication (birth control pills). They are decreased in nephrosis, in persons receiving anabolic steroids, and in chronic liver disease. Genetic disorders occur in which the synthesis of TBG is increased or decreased.

Although the total amount of plasma TBG can be measured, this is usually not done. Instead, it is convenient to estimate the amount of unsaturated thyroxine binding sites on the TBG molecule. This is accomplished by adding to a serum sample a known amount of a substance that attaches to the empty sites on TBG without displacing the bound T_4. Radioactive triiodothyronine is commonly used, and the test is called "T_3 Uptake." In the Thyronine Uptake method, a thyroxine-phosphonate conjugate is used instead of triiodothyronine. The results are reported in terms of the amount of thyroxine-phosphonate that is not bound to TBG. Low levels of unbound thyroxine-phosphonate indicate many empty binding sites, giving low Uptake results as in hypothyroidism, and high levels, few empty sites, giving high Uptake results as in hyperthyroidism.

Again, current research indicates that free-T_4 to be the physiologically active T_4 fractions that correlates best to the actual thyroid status of patients. The concentration of free-T_4 can now be measured directly. In the past the free-T_4 level was evaluated from the total serum T_4 concentration and the TBG level (as estimated by an Uptake method). The two test results are multiplied together to obtain the "Free thyroxine index" (FTI) that is assumed to reflect the concentration of free-T_4.

Clinical Significance: Thyroid function tests are used in clinical medicine both to diagnose diseases of the thyroid gland and to determine the correct dosage when thyroid hormone replacement is required. On rare occasions a physician may order these tests when he suspects a patient has taken the hormone surreptitiously. Diseases of the thyroid may result in overproduction or underproduction of T_4. The rate of hormone production is reflected in the plasma T_4 and T_3 levels, while the degree of saturation of TBG binding sites is reflected in the Thyronine Uptake results.

Hyperthyroidism: Overproduction of T_4 is called hyperthyroidism or thyrotoxicosis. Hyperthyroidism may occur spontaneously in two different forms. The most common, Graves' disease, is characterized by diffuse enlargement of the thyroid gland and an unexplained protrusion of the eyes (exophthalmos). Graves' disease usually occurs in young or middle-aged women. The patient feels excessively warm in environments that are comfortable to others. The blood vessels of the skin dilate to dissipate the excess body heat, causing flushing, and profuse sweating. Catabolism of body protein leads to muscle weakness, especially when the disorder is severe. The appetite is usually increased; nonetheless the patient loses weight. T_4 also potentates the sympathetic nervous system, causing increased heart rate, cardiac arrhythmias, dilated pupils, a sense of anxiety, and tremor.

Hyperthyroidism may also be associated with a benign tumor of the thyroid gland. This condition is known as toxic adenoma. The tumor cells are able to synthesize T_4 but do not respond normally to the negative feedback mechanism from the pituitary gland. T_4 production is therefore uncontrolled, and large tumors may produce more than physiologic amounts of T_4, resulting in symptoms and signs of hyperthyroidism. All of the hypermetabolic symptoms that are seen in Graves' disease may also occur in toxic adenoma, but the cardiovascular symptoms (rapid heart rate and arrhythmias) tend to predominate. Exophthalmos does not occur. Toxic thyroid adenomas also occur more commonly in women than men, but they tend to occur later in life than does Graves' disease.

In hyperthyroidism from most causes, the serum T_4 concentration, the Thyronine Uptake value, and the FTI are nearly always elevated. An exception is T_3 thyrotoxicosis, hyperthyroidism die to excess T_3. The T_4 levels and FTI levels are normal in this rare disease. The serum T_3 level must be measured to confirm the diagnosis.

Hypothyroidism: Reduced thyroid function produces a disease called hypothyroidism. This condition has almost reached epidemic proportions in the United States. It may also be present at birth, in which case it is known as cretinism. Both the physical and mental development of the

child is slowed. Serious mental deficiency and stunting of growth (dwarfism) occur if treatment with thyroid hormone is not begun promptly.

Hypothyroidism in adults is a common result of the treatment of hyperthyroidism. Graves' disease and toxic adenoma are usually treated by either surgical removal of part of the gland (subtotal thyroidectomy) or with radioactive iodine. If too little normal thyroid tissue remains after treatment, hypothyroidism may result. Spontaneously occurring myxedema is not uncommon. A frequent mechanism is selective destruction of the thyroid tissue by an autoimmune process such as Hashimoto's disease, but the exact cause is often not known.

The environmental toxins mercury, chlorine and fluorine also cause hypothyroidism.

The hypothyroid patient is lethargic and mentally dull. Intolerance to cold environments is a common early complaint. The skin is pale, cold, dry and coarse. The heart rate is slow, and the heart sounds reduced in volume. The deep tendon reflexes are sluggish. In the most severe cases, lethargy progresses to coma.

In hypothyroidism the T_4, the Thyronine Uptake value, and the FTI are below normal. T_3 measurements are helpful in determining peripheral conversion of T_4 to T_3. The additional measurement of thyroid stimulating hormone (TSH) will indicate whether hypothyroidism is due to intrinsic disease of the thyroid or whether it is secondary to a deficiency in the hypothalamus or anterior pituitary (a very common problem).

Clinical Pearls

Currently specialized laboratories offer comprehensive thyroid panels, which reveal imbalances that often go undetected with more limited assessments. Unbound levels of T4 and T3 are measured to reflect the bioactive portion of thyroid hormone, increasing clinical insight. This assessment can identify not only overt hyper- and hypothyroidism, but subtle sub-clinical manifestations of thyroid dysfunction, such as auto-immune reactions and altered peripheral conversion into T3 leading to reverse T3 dominance. These metabolic anomalies may trigger chronic symptoms, and promote the gradual development of degenerative disorders.

A comprehensive thyroid assessment usually includes - Hypersensitive thyroid-stimulating hormone (TSH); free thyroxine (fT4); free triiodothyronine (fT3); reverse T3 (rT3); anti-thyroglobulin antibodies (anti-TG); anti-thyroid peroxidase antibodies (anti-TPO) and the ratios - fT4/fT3; fT3/rT3. Such a study can detect metabolic irregularities arising from vitamin and mineral deficiencies, heavy metal toxicity, chronic stress, enzyme dysfunction, and aging even when TSH and T4 levels are normal. Thyroid antibody levels help gauge autoimmune response frequently associated with antigenic cross-reactivity and gastrointestinal dysfunction, which may require additional clinical investigation and focused intervention. Thyroid antibody levels may rise in response to trauma, dysbiosis, inflammation (including thyroiditis) and progressive thyroid degeneration. At high levels, antibodies may block thyroid hormones from attaching to cellular receptors, causing symptoms of hypothyroidism even when thyroid hormone levels are adequate.

Thyroid Stimulating Hormone (TSH), Thyrotropin

Laboratory Normal: 0.3 to 5.0 µIU/ml
Optimum Range: 1.5 to 2.5 µIU/ml

Structure: Thyroid Stimulating Hormone (TSH) or thyrotropin is a complex glycoprotein of about 28,000 daltons molecular weight, making it almost 200 times the size of the thyroid hormones whose secretion it controls. It is produced in specialized basophilic thyrotrope cells of the anterior pituitary, and endocrine gland located near the brain within the sella turcica. TSH is composed of alpha and beta subunits that are produced under separate gene control. These separate subunits combine post-transitionally by non-covalent bonding to form an intact and biologically active molecule. Free alpha, but not beta, subunits of TSH have been measured in serum. Follicle Stimulating Hormone (follitrophin) and Lutinizing Hormone (lutropin) from the anterior pituitary and Human Chorionic Gonadotropin (choriogonadotropin) have virtually identical alpha subunit structures as alpha TSH. The alpha subunit binds to the target tissue receptor. The beta subunit differs in peptide structure between these hormones, permitting specific antibody detection of each hormone, and confers the biological specificity to each intact hormone molecule.

TSH Function and Production Control: The principal biological role of TSH is to stimulate the production and release of thyroxine (T4) and triiodothyronine (T3) from the thyroid gland. Production of TSH is stimulated by thyroid releasing hormone (TRH), produced in the hypothalamus region of the brain. TSH production in the anterior pituitary is regulated by negative feedback from T4 and T3. Pharmacological levels of dopamine and glucocorticoid hormones like cortisol inhibit TSH secretion, producing a rapid fall in serum TSH levels. Endogenous increases of these two hormones may also decrease serum TSH through direct inhibition of pituitary TSH secretion and possibly by decreasing hypothalamic TRH secretion.

TSH Synthesis: TSH is a complex hormone produced only in the anterior pituitary. Initial TSH synthesis begins with the assemblage of amino acids into the peptide sequences of the alpha and beta subunits as a result of the transcription of information on separate genes. In a cotranscriptional event, mannose rich oligosaccharide sugars are attached to the peptide sequences of the two subunits. This oligosaccharide attachment step plays a key role in the folding of the nascent peptides to facilitate proper disulfide bond formation and to prevent proteolytic destruction of aggregation of subunits. In a series of subsequent enzymatic steps fucose, galactose N-acetylglucosamine and N-acetylgalctosamine, sulfate and sialic acid residues may be incorporated into the mannose rich oligosaccharide portion of the subunits to produce the complex oligosaccharide characteristic of secreted hormone. The sulfate and sialic acid additions have special importance since TSH produced in response to TRH stimulation contains greater levels of both sulfate and sialic acid than does TSH produced without TRH mediation.

TSH Biological Activity and Clearance: Glycation of the peptide subunits with oligosaccharides and the amounts of sulfate and sialic acid added to the oligosaccharide have a profound positive effect on the biological activity of TSH. It is through modulation of this activity that TRH may promote the synthesis of TSH with higher biological activity than the TSH endogenously produced by the pituitary. The metabolic clearance rate of TSH differs between pituitary and serum forms of the hormone and is affected by the physiological state at time of sampling. Deglycosylation and desialyation of the TSH molecule accelerate metabolic clearance and may be one way that biological activity of TSH is controlled by physiological thyroid hormone status.

Clinical Significance: Measurement of serum TSH levels has become increasingly important in the diagnosis of thyroid disorders since its first measurement by radioimmunoassay (RIA) in 1964. Serum TSH is currently the most sensitive and reliable method for assessing circulating thyroid hormone action in healthy, ambulatory patients. Much of the high diagnostic performance of modern serum TSH analyses can be attributed to the progressive ten-fold increases in assay sensitivity that characterized the first three generations of TSH assays.

TSH Measurement: Until recent years, the TSH was measured by RIA, and the normal range was generally 0 to 10 mIU/L. This essentially restricted the clinical utility of TSH measurement to verification of elevated TSH levels in the diagnostic workup for hypothyroidism. With the advent of "sandwich" immunometric assays in the mid-1980's the functional sensitivity of the methods improved to 0.1 and 0.01 µIU/ml of TSH for second and third generation assays respectively. This improved technology has permitted a tightening of the “normal” range to something approximating 0.5 to 4.5 µIU/ml. However, many clinicians believe that low TSH levels with hypothyroidism symptoms mat reflect an anterior pituitary hypofunction that can result in deficient stimulation to the thyroid.

TSH and Free T4: Serum TSH shows proportional and inverse logarithmic changes in response to changes in serum free T4 (FT4). For example, a two-fold decrease in serum FT4 will result in an approximate 100-fold increase in serum TSH. Serum TSH's amplified response to changes in FT4 levels enables TSH to detect subclinical hyper- or hypothyroidism before diagnostically significant changes in other thyroid function evaluations can be detected. However, it must be emphasized that each person has an individualized, relatively constant setpoint for TSH response to FT4 that persists for years or even decades. This individualized setpoint accounts for the fact that when TSH values are within normal limits there is no significant correlation between serum FT4 and TSH levels.

Hypothyroidism:

Hypothyroidism-Clinical Picture: Failure of the thyroid gland itself or primary hypothyroidism is termed myxedema, nearly all cases of which show elevation of TSH above normal limits. The thyroid's failure is most commonly due to destructive autoimmune disease that can be verified by increased serum levels of antimicrosomal and/or antithyroglobulin antibody levels. Hashimoto’s thyroiditis is associated with the presence of goiter. Congenital hypothyroidism shows a frequency of about 1 in 4000 births in the United States. A condition termed cretinism, characterized by abnormal skeleton and mental development, occurs if adequate thyroid hormone levels are not present at birth or cannot be restored within the first month of life. Most developed countries now screen for congenital hypothyroidism by measuring TSH levels in blood samples collected on filter paper between the first and second weeks of life. TSH is considered such a sensitive indicator of hypothyroidism that elevations in association with normal serum thyroid hormone levels and a normal clinical picture suggest subclinical hypothyroidism. The rising TSH levels noted in the elderly and in 10% of females above 45 years of age suggest many individuals may benefit from TSH thyroid function assessments.

Hypothyroidism and Serum TSH: In the absence of adequate free serum levels of the thyroid hormone T4 and T3, TSH production is increased by both endogenous anterior pituitary mechanisms and through TRH stimulation. Primary hypothyroidism, referring to low T4 and T3 output due to dysfunction of the thyroid gland itself, will generally result in high serum TSH levels. If, however, the cause of low thyroid hormone levels is due to dysfunction of the anterior pituitary or hypothalamus, the TSH level will often be normal or low when measured by second or third generation TSH assays. Differentiation between secondary, meaning pituitary, and tertiary, meaning hypothalamic cause of hypothyroidism, can be determined by administration of TRH to the patient. If TSH levels show an approximately ten-fold rise after TRH administration the hypothyroidism is likely due to inappropriately low TRH production in the patient from hypothalamic or tertiary causes. This rise will occur whether the TSH value is initially subnormal, normal, or elevated so long as the pituitary responds normally to the TRH. Therefore, if basal serum TSH levels are detected there is little diagnostic benefit in performing a TRH stimulation test unless secondary hypothyroidism is suspected. With secondary hypothyroidism the TSH increase following TRH administration is blunted, showing less than two-fold instead of tenfold increase.

Hypothyroidism Due to Serum TSH of Low Biological Activity: Normal or even elevated serum TSH levels can occur with secondary and/or tertiary hypothyroidism. While present at measurable levels, the TSH produced due to pituitary and/or hypothalamic dysfunction appears to be of such low biological activity that it is unable to maintain thyroid hormone levels within normal limits. The most plausible explanation for this is found in the role of TRH in modulating synthesis of TSH containing fully developed complex oligosaccharides and proper amounts of sialic acid and sulfate for full biological activity and normal metabolic clearance rate. Since functionally compromised TSH typically reacts normally in immunoassays, the laboratory will measure higher TSH levels than would be expected based on patient clinical status and thyroid hormone levels.

TSH Response to Thyroxine Replacement Therapy: The elevated serum TSH levels typical of primary hypothyroidism require from 4 to 8 weeks to return to stable serum levels following changes in oral T4 dosage. Apparently TSH responds to T4 levels in two phases. The initial fall in TSH is rapid and occurs in about one day. The second phase requires up to 8 weeks. Therefore, when starting oral T4 replacement therapy serum T4 levels should be monitored, usually as the free thyroxine index (FTI), for the first one to two months. After that time, TSH, with or without the FTI, may be monitored if there is no change in dosage. About 10% to 20% of hypothyroid patients on replacement T4 therapy may require serum FTI values to become elevated above normal limits before serum TSH and T3 levels are normalized. This FRI elevation does not reflect over-treatment but rather that oral T4 replacement does not directly supply T3 like the thyroid. Low TSH levels should be investigated to determine if thyroid replacement is excessive.

Hyperthyroidism-Clinical Picture: Hyperthyroidism most often presents as Graves disease, where abnormal thyroid-stimulating immunoglobulins are responsible for elevating serum FT4 and/or FT3 levels. A subnormal serum TSH and diminished TSH response to TRH administration is characteristic of all hyperthyroid cases, except those arising from TSH-secreting pituitary tumor. In those rare cases where clinical hyperthyroidism is accompanied by elevated serum thyroid hormone levels and an elevated serum TSH level, the possibility of a TSH secreting pituitary tumor should be considered. Serum TSH levels may be suppressed, sometimes dramatically, in the first two trimesters of pregnancy and in association with severe illness, severe depression, hypopituitarism (anterior pituitary), and after administration of glucocorticoid or dopamine.

TSH and Hyperthyroidism: Because TSH shows a 100-fold decrease for every two-fold increase in serum FT4, serum TSH levels should be depressed below normal limits due to feedback inhibition of TSH synthesis if thyroid hormones are elevated. The ability of newer "sandwich" immunoassays to measure subnormal hormone levels with relatively good precision offers the prospect of using TSH measurements as a front-line test for detection of hyperthyroidism.

Non-thyroidal Illness: The most common response to severe non-thyroidal illness is a decrease in circulating T3 levels in conjunction with a rise in reverse T3. Reverse T3 is formed when the third iodine is placed on the alternate binding location on the thyroid molecule from that found with T3. Non-thyroidal illness may also be accompanied by increased free T4 and free T3 due to a combination of reduction in circulating binding protein levels and alterations in binding of thyroid hormones to the binding proteins due to endogenous binding inhibitors or drugs. Total T4 and the T-uptake are often decreased because of the lowered binding protein, principally thyroxine binding globulin, levels. TSH levels are often within normal limits, but may also be depressed into the range between normal and hyperthyroid. Reliable evaluation of apparent hypothyroid conditions in severe non-thyroidal illnesses requires accurate estimate of free hormone levels, preferably by a method not inappropriately biased by binding protein related aberrations. The finding of a low FTI and high TSH suggests that a hypothyroid condition is likely present.

Complete Blood Count (CBC)

For the clinician involved in Biological Regulatory Medicine, live blood analysis viewed with the optical modes of darkfield and phase contrast together with the complete blood count (CBC) are the duel backbones of any hematological evaluation. Analysis of living blood helps define the qualitative features, regulatory activities of the cells, integrity of formed structural components, and the overall biological terrain of the blood. The CBC includes a determination of more quantitative values such as: red blood cell data (total red blood cell count - RBC), hemoglobin/ hematocrit, red blood cell indices (including MCV, MCHC, and mean corpuscular hemoglobin MCH), red cell distribution width (RDW), white blood cell data, and usually, platelet count with sometimes mean platelet volume (MPV). A white blood cell differential count absolutely should be ordered and included as part of a routine CBC.

Unfortunately, it is beyond the scope of this manual to include the many varied hematological features seen in live blood. Whenever possible a brief explanation will be given as to some of these features as observed in living blood. The focus here is primarily on the CBC and its reflection to functional biochemical health.

The CBC in the broadest sense can provide important baseline information about the functional state of the bone marrow. Recognition of a “-penia,” or deficiency of a blood component, is indication of either a marrow production problem or a peripheral destruction activity. Likewise, a “-cytosis,” or elevation of a blood component, could indicate either a normal marrow response to a peripheral stimulation or the peripheral manifestation of an uncontrolled malignant proliferation. Thus, in general, the CBC is useful in broadly screening for the hematological regulation mechanisms.

The hemoglobin (Hgb) and/or hematocrit (Hct) quantify the degree of anemia or polycythemia and the MCV/MCHC/RDW can be useful in further subclassifying the type of anemia (e.g., normocytic, microcytic, macrocytic). The differential of a white blood cell count (WBC) can provide diagnostic or follow-up information regarding either a benign response or malignant process. Obtaining a platelet count is the first step in the evaluation of the homeostatic process. Any of the CBC components can be useful in monitoring patient responses to biological therapy (orthomolecular, phytotherapy, homeopathy, isopathy or other types of therapies). A CBC may also serve to monitor the detrimental effects of cytotoxic or marrow toxic drugs like chemotherapy, antibiotics, steroids, etc.). CBC is, however, not to be a substitute for live blood analysis. Both are complementary, whereas the CBC is more quantitative, the live blood analysis is more qualitative and reflects active regulatory mechanisms at work.

Erythrocytes and the Erythrocyte Count (RBC)

Laboratory Range: **Males: 4.2 to 6.0 million per cu. mm**
Females: 3.9 to 5.2 million per cu. mm

Optimum Range: **Males: 4.2 to 5.0 million per cu. mm**
Females: 3.9 to 4.5 million per cu. mm

Laboratory normals vary slightly. The result of the test is expressed as number of cells per unit volume, specifically cells per cu. mm or cells/µL. Women tend to have lower values than men, and RBC counts tend to decrease with age. Normal RBC decreases are seen during pregnancy because of normal body fluid increases that dilute the RBCs. Certain nutritional deficiencies that affect the elderly and pregnant may also play a role in the anemia. When the value is decreased by more than 10% of the expected normal value, the patient is said to be anemic.

Physiology: Structurally erythrocytes are the simplest cell in the body. The basic function of the RBC is the creation and maintenance of an environment salutary to the physical integrity and functionality of hemoglobin, the primary carrier of oxygen. In the normal state, erythrocytes are produced only in the skeleton (in adults only in the axial skeleton), but in certain pathologic states, almost any organ can become the site of erythropoiesis. Numerous substances are necessary for creation of erythrocytes, including metals (iron, cobalt, manganese), vitamins (B_{12}, B_6, C, E, folate, riboflavin, pantothenic acid, thiamin), and amino acids. Regulatory substances necessary for normal erythropoiesis include erythropoietin, thyroid hormones, and androgens. Erythrocytes progress from blast precursors in the marrow over a period of five days. Then they are released into the blood as reticulocytes, distinguishable from regular erythrocytes only with special supravital stains. The reticulocyte changes to an erythrocyte in one day and circulates for 120 days before being destroyed in the reticuloendothelial system.

Clinical laboratories measure several important parameters that reflect RBC structure and function. These measurements are used to 1) evaluate the adequacy of oxygen delivery to the tissues, at least as is related to hematologic (as opposed to cardiopulmonary) factors, and 2) detect abnormalities in RBC size and shape that may provide clues to the diagnosis of a variety of hematologic conditions. Most of these tests are performed using automated equipment to analyze a simple venipuncture sample collected in a universal lavender- (or purple-) top tube containing EDTA as an anticoagulant.

Erythrocyte Count Methodology: Manual methods using the hated hemocytometer have been universally replaced by automated counting. The major source of error in the RBC count is an artificially reduced result that occurs in some conditions where RBC's aggregate or stick together in the sample tubes, with two or more cells being counted as one. This aggregation phenomenon is also easily seen in live blood analysis utilizing either the optical modes or darkfield or phase contrast.

Clinical Pearls:

If increased: with an increased hemoglobin (HGB) and/or hematocrit (HCT) blood stasis is probable. Significant increases indicate polycythemia.

If decreased: with an increased HGB and/or HCT, iron anemia is possible. If serum iron is decreased and ferritin is decreased, iron anemia is probable.

If all of these values are decreased, internal bleeding must be ruled-out.

Hemoglobin Concentration in Whole Blood (HGB)

Laboratory Range:	**Males: 14 to 18 g/dl or 8.7 to 11.2 mmol/L (SI units)**
	Females: 12 to 16 g/dl or 7.4 to 9.9 mmol/L (SI units)
	Pregnant female: > 11 g/dl
	Elderly values are slightly decreased
Optimum Range:	**Males: 14 to 15 g/dl**
	Females: 13.5 to 14.5 g/dl
Critical Values:	**<5.0 or > 20 g/dl**

HGB levels vary considerably in healthy individuals. Caution is advised when interpreting levels somewhat above or below the normal range as pathological.

Indications: Hemoglobin is usually measured together with the hematocrit and then commonly called “H and H”. It is used as a rapid indirect measurement of the RBC count. It is repeated serially in patients with ongoing bleeding or as a routine part of the CBC. It is an integral part of the evaluation of anemic patients. HGB should be evaluated along with HCT, ferritin, serum iron and the RBC Indices when attempting to ascertain any type of anemia or bleeding.

Decisions concerning the need for blood transfusion are usually based on the HGB and HCT. In an otherwise healthy person, transfusion is not considered as long as the HGB is above 8 g/dl and the HCT is above 24. In an older individual with already compromised oxygen-carrying capacity (cardiopulmonary diseases), transfusion may be recommended when the HGB level is below 10.

Physiology: HGB is made up of heme (iron surrounded by protoporphyrin) and globin consisting of an alpha- and beta-polypeptide chain. Interestingly, the chlorophyll molecule is identical to the hemoglobin molecule except for the central element in chlorophyll is manganese not iron. The normal range for hemoglobin is highly age and sex dependent, with men having higher values than women, and adults having higher values than children (except neonates, which have the highest values of all). Abnormalities in the globin structure are called hemoglobinopathies (e.g. sickle cell disease, hemoglobin C disease). Some diseases are caused by abnormalities in globin chain synthesis (such as thalassemia). In these diseases the RBC counts can be low, the RBC survival can be diminished, and the RBC-carrying capacity can be reduced.

Methodology: This test involves lysing the erythrocytes, thus producing an evenly distributed solution of hemoglobin in the sample. The hemoglobin is chemically converted mole-for-mole to the more stable and easily measured cyanmethemoglobin, which is a colored compound that can be measured colorimetrically, its concentration being calculated from its amount of light absorption using Beer's Law.

Through automated cell counters this is an easy test to perform, as hemoglobin is present in the blood in higher concentration than that of any other measured substance in laboratory medicine. There is very little variability (2% to 3%) with well-kept machines. The result is traditionally expressed as unit mass per volume, specifically grams per deciliter (g/dL). Ideologues in lab medicine have been maintaining for years that this unit measurement will be replaced by Système Internationale (SI) units of moles per liter, but this so far has not gained any significant acceptance in clinical medicine.

Clinical Pearl: When HGB levels are too high because of increased number of RBCs, intravascular sludging occurs. This may lead to stroke and other organ infarction.

When the HGB, HCT, serum iron or ferritin are low, a need for hydrochloric acid and digestive enzymes should be ruled-out

Hematocrit (HCT)

Laboratory Normal: **Males: 0.42 to 0.52 L/L**
Females: 0.42 - 0.52 L/L
Pregnant Female: >33%
Elderly: Values may be slightly decreased
Children 1 to 6 years: 0.30 to 0.40 L/L
6 to 18 years: 0.32 to 0.44 L/L
Critical Values: <0.15 or > 0.60 L/L

Methodology: HCT is also called the packed cell volume or PCV. It is a measure of the total volume of the erythrocytes relative to the total volume of whole blood in a sample. The result is expressed as a proportion, either unitless (e.g., 0.42) or with volume units (e.g., 0.42 L/L, or 42 cL/L [centiliters/liter]). An archaic way of expressing hematocrit is "volumes per cent" or just "percent" (42%, in the above illustration). Small office labs and stat labs measure hematocrit simply by spinning down a whole blood sample in a capillary tube and measuring the length of the column of RBC's relative to the length of the column of the whole specimen. Larger labs use automated methods that actually measure the volume individually of each of thousands of red cells in a measured volume of whole blood and add them up. The volume of individual erythrocytes can be electronically determined by measurement of their electrical impedance or their light-scattering properties.

Indications: The HCT is an indirect measurement of RBC's number and volume. It basically is used as a rapid measurement of RBC count. It is repeated serially in patients with ongoing bleeding as a routine part of their complete blood count. It is an integral part of the evaluation of anemic patients.

Decreased levels indicate anemia (reduced numbers of RBC's), whereas, increased levels can indicate erythrocytosis. Like other RBC values, the HCT can be altered by many factors other than RBC production. For instance, in dehydrated patients the total blood volume is contracted. The RBC's make up the greater portion of the blood volume, and the HCT measurement is therefore, falsely high. Conversely, if the patient is over-hydrated (not nearly as common as dehydration, and not likely unless on IV fluids) the HCT value will be decreased. If the RBC is morphologically increased in size (macrocyte), the RBC's will make up the greater portion of the blood volume, and the HCT will again be falsely elevated.

Decisions concerning the need for blood transfusion are usually based on the HGB and HCT. In an otherwise healthy person, transfusion is not considered as long as the HGB is above 8 g/dl and the HCT is above 24. In an older individual with already compromised oxygen-carrying capacity (cardiopulmonary diseases), transfusion may be recommended when the HCT level is below 30.

Erythrocyte Indices

The three cardinal RBC measurements previously described (RBC count, hemoglobin and hematocrit) are used to arithmetically derive the erythrocyte indices - mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration. It is clinically useful to know how to calculate these indices and have some idea of the normal ranges.

Mean Corpuscular Volume (MCV)

Laboratory Normal: Adult/Elderly/Child 80 to 94 fL

This is the mean volume of all the erythrocytes counted in the sample and is used in classifying anemias. The value is expressed in volume units, in this case very small ones - femtoliters (fL, 10-15 liter). The formula for the calculation in general terms is:

MCV = hematocrit ÷ RBC count

When using specific units, decimal fudge factors are required; for example,

MCV (in fL) = (hematocrit [in L/L] x 1000) ÷ (rbc count [in millions/µL])

It is easier to forget the fudge factors and use the first formula. Multiply out the values while ignoring the bothersome decimal, and reposition the decimal in the final result so as to approximate the order of magnitude of the normal range. This is safe, since you will not see an MCV of 8 fL, or one of 800 fL.

When the MCV is increased, the RBC is said to be abnormally large or **macrocytic.** This is frequently seen in megaloblastic anemias (e.g., vitamin B12 or folic acid deficiency). When the MCV is decreased, the RBC is said to be very small or **microcytic**. **Normocytic** refers to blood with a normal MCV. Keep in mind that the MCV measures only average cell volume. The MCV can be normal while the individual red cells of the population vary wildly in volume from one to the next. Such an abnormal variation in cell volume is called **anisocytosis**. Some machines can measure the degree of anisocytosis by use of a parameter called the **red cell distribution width (RDW)**. This is simply a standardized parameter (similar to the standard deviation) for mathematically expressing magnitude of dispersion of a population about a mean.

The normal range for RDW is 11.5 - 14.5 %.

Mean Corpuscular Hemoglobin (MCH)

Laboratory Normal: Adult/Elderly/Child 27 to 31 pg

The MCH represents the mean mass or weight of hemoglobin in the RBC and is expressed in the mass unit, picograms (pg, 10^{-12} gram). The value is determined by the formula,

MCH (in pg) = (hemoglobin [in g/dL] x 10 ÷ (rbc count [in millions/µL])

Again, a fudge factor is required in this equation, so it helps to get some feel for the normal range (27 - 31 pg) and gestalt the decimal point, as described for MCV, above. Since small cells have less hemoglobin than large cells, variation in the MCH tends to track along with that of the MCV. So, the MCH adds little information independent of the MCV.

Mean Corpuscular Hemoglobin Concentration (MCHC)

Laboratory Normal: Adult/Elderly/Child 32 to 36 g/dL

Only 37 g/dL of hemoglobin can fit into a RBC. Alteration in RBC shape (spherocytosis, acute transfusion reactions, erythroblastosis fetalis) may cause automated counting machines to indicate MCHC levels above normal.

This is the mean concentration of hemoglobin in the red cell. Since whole blood is about one-half cells by volume, and all of the hemoglobin is confined to the cells, you would correctly expect the MCHC to be roughly twice the value for hemoglobin in whole blood and to be expressed in the same units; the normal range is 32 - 36 g/dL. The value is calculated using the formula,

MCHC [in g/dL] = hemoglobin [in g/dL] ÷ hematocrit [in L/L]

Cells with normal, high, and low MCHC are referred to as **normochromic, hyperchromic, and hypochromic**, respectively. These terms have importance in anemia classification.

Clinical Pearls for RBC Indices:

If Increased:
If the MCV is above 90 with the MCH increased above 32, a vitamin B 12 and/or folic acid need is possible. If the methyl-malonic acid level (serum or urine) is increased and/or the holo-transcobalamin II level is outside the laboratory range, the need for B 12 and/or folic acid is quite probable.

If the MCV is increased above 97 in conjunction with the MCH increased above 34, oral supplementation with B 12 and/or folic acid may not be completely effective. Injections may be required.

If Decreased:
If MCV is below 82.0 with the MCH below 28, and a decreased HGB or HCT, iron anemia is possible. If the serum iron, RBC and ferritin are also decreased, iron anemia is probable.

If MCV is below 82.0 with the MCH below 28, normal HGB, and the SGOT abnormally decreased, vitamin B 6 need is possible. If the urinary or serum homocystine is increased, the need for vitamin B 6 is probable.

Total Leukocytes (WBC) and the Leukocyte Differential Count

To consider the leukocytes together as a group is somewhat nebulous, because each type of leukocyte has its own function and ontogeny semi-independent of the others. To measure the total leukocyte count and allow this term to mean anything to the doctor is a travesty, yet the "WBC" count has traditionally been considered a cardinal measurement in a routine laboratory workup for just about any condition. To evaluate critically the hematologic status of a patient, one *must* consider the individual absolute counts of each of the leukocyte types rather than the total WBC count. For such a critical evaluation, the first step is to order a WBC count with differential. In many labs, the result will be reported as a **relative differential** as follows; see optimum ranges:

WBC Lab Normals	**6000/µL**	**Optimum Ranges**
Segmented neutrophils	**35 to 65%**	**40 to 60%**
Band neutrophils	**0 to 10%**	**< 5%**
Lymphocytes	**24 to 44%**	**Same**
Monocytes	**0 to 10%**	**< 7%**
Eosinophils	**0 to 7%**	**< 3%**
Basophils	**0 to 2%**	**1 to 2%**

To derive the numbers from percentages, multiply the Total WBC count by each of the percentages given for the cell types; this gives you an **absolute differential**. Thus, the illustration above becomes:

WBC Lab Normals	**6000/µL**
Segmented neutrophils	**3600/µL**
Band neutrophils	**120/µL**
Lymphocytes	**1500/µL**
Monocytes	**480/µL**
Eosinophils	**180/µL**
Basophils	**120/µL**

Laboratory Normal: Adult/Child > 2 years old: 5000 to 10,000 mm^3
Child < 2 years: 6200 to 17,000 mm^3
Optimum Range: 5,000 to 7,500 mm^3
Alarm Range: < 3,000 mm^3 or > 13,000 mm^3

The total WBC values are somewhat age related, whereas newborns and infants tend to have higher values than adults.

The total WBC will frequently be slightly below the optimum range for individuals on a raw food diet and slightly above the optimum range for individuals on a processed, "fast food" diet.

The total WBC value without the differential is not as informative, because it not uncommon for an increase or decrease to be present in one of the fractions with a normal total WBC value. A significant increase or decreased total WBC is justification to conduct a differential if one has not been performed. Depending on the findings, further immune testing may be necessary (T and B Lymphocyte Subset Assay, immunoglobulins, C-Reactive Protein)

Methodology: The total WBC count is invariably done using an automated method. Routinely, the differential count is done "by hand" (i.e., through the microscope) in smaller labs, and by automated methods in larger facilities. The automated methods are amazingly accurate, considering the fine distinctions that must often be made in discerning one type of leukocyte from the other. Current machines can quite reliably pick out one leukemic blast cell in eight hundred or more leukocytes.

Neutrophils

Laboratory Normal: (band + seg) count is 1700 - 8100 /µL
1160 - 8300 /µL for African Type A or O blood group

Reference Range % (Segs):	**35 to 65%**
Optimum Range % (Segs):	**40 to 60%**
Alarm Range % (Segs):	**< 30% or > 80%**
Reference Range % (Bands):	**0 to 10%**
Optimum Range % (Bands):	**< 5%**
Alarm Range % (Bands):	**> 10%**

Keeping in mind the lower expected low-end value for African Type A or O blood group will save you much time (and patients much expense and pain). Obesity and cigarette smoking are associated an increased neutrophil count. It is said that for each pack per day of cigarettes smoked, the granulocyte count may be expected to rise by 1000 /µL.

The absolute neutrophil count is calculated by multiplying the differential count (%) by the total WBC count. ANC = WBC X (% Neutrophils + % Bands) If the ANC is below 1000, the patient is considered immunocompromised and is at great risk for infection.

Non-segmented Neutrophils (Bands) - These are the youngest forms normally found in the peripheral blood. These are increased in acute infection with or without an increase in the total WBC. An increase in bands constitutes a "shift to the left" as described by Shilling.

Physiology: Neutrophils are the most populous of the circulating white cells; they are also the most short-lived in circulation. After production and release by the marrow, they only circulate for about eight hours before proceeding to the tissues (via diapedesis), where they live for about a week, if all goes well. They are produced as a response to acute body stress, whether from infection, infarction, trauma, emotional distress, or other noxious stimuli. When called to a site of injury, they phagocytize invaders and other undesirable substances and usually kill themselves in the action, and thereby become autolytic. Normally, the circulating neutrophil series consists only of band neutrophils and segmented neutrophils, the latter being the most mature type. In stress situations (i.e., the "acute phase reaction"), the immature cells called band or stab cell can be seen in the blood. This picture is called a "left shift." The band count has been used as an indicator of acute stress of a sign of a bacterial terrain. In the acute phase reaction, any of the neutrophil forms may develop deep blue cytoplasmic granules, vacuoles, and vague blue cytoplasmic inclusions called Döhle bodies, which consist of aggregates of ribosomes and endoplasmic reticulum. All of these features are easily seen (except possibly the Döhle bodies). In live blood analysis using the optical modes of darkfield or phase contrast, it is useful to determine how mobile they act and how "loaded" the neutrophils are with toxic waste. When loaded, the segments will enlarge and fill in the entire cell body, eventually becoming autolytic and breaking apart.

Clinical Pearls:
If neutrophils are increased with an increased total WBC and a decreased lymphocyte count, an acute viral or bacterial infection is probable.
If neutrophils are decreased with a decreased total WBC and an increased lymphocyte count, a chronic viral or bacterial infection is probable. The gamma globulin of a protein electrophoresis will also frequently be decreased with a chronic viral or bacterial infection.

Monocytes

Laboratory Range: 200 - 950 /µL Up to 10 % of the Total WBC
Optimum Range: Up to 7% of the Total WBC
Alarm Range: > 15% of total WBC

Physiology: These large cells are more closely related to neutrophils than are the other "granulocytes," the basophil and eosinophil. Monocytes and neutrophils share the same stem cell. Monocytes are histiocytes or macrophages. They are produced by the marrow, circulate for five to eight days, and then enter the tissues where they are mysteriously transformed into histiocytes. Here they serve as the welcome wagon for any outside invaders and are capable of "processing" foreign antigens and "presenting" them to the immunocompetent lymphocytes. They are also very capable of phagocytosis. Unlike neutrophils, histiocytes can usually survive the phagocytosis of microbes. What they trade off is killing power. For instance, mycobacteria can live in histiocytes (following phagocytosis) for years. Unfortunately, when the terrain becomes opportune for mycobacterium to flourish again, bacterial bodies may reenter the circulation and again present their toxic presence. In the inflammatory process, neutrophils predominate for about 72 hours; they then break-up and the monocytes remain to phagocytize the cell fragments. Hence, monocytes are usually increased during the recovery phase of infection.

Clinical Pearls:

Increase:
If increased with patient improvement from an active infection, the patient has probably entered a recovery phase.
If increased rule out liver dysfunction.
If increased with an increased eosinophil (>3%) or basophil count (>1%), parasites are possible. If parasitic symptomology is present, consider a Comprehensive Digestive Stool Analysis with purged Parasitology X 3.
If increased with an increased creatinine (1.2 or higher), prostate hypertrophy is possible. Consider running a PSA.

Eosinophils

Laboratory Range: 0 - 450 /µL. **0 to 7% of the Total WBC**
Optimum Range: **< 3% of the Total WBC**

Physiology: These intriguing cells are traditionally grouped with the neutrophils and basophils as "granulocytes". Given all their unique and dissimilar abilities, this is a rather meaningless terminological grouping. Current thinking is that eosinophils and neutrophils are derived from different stem cells, which are somewhat difficult to distinguish from each other by currently available techniques of examination. Although the hallmark of the eosinophil is the presence of bright orange, large, refractile granules, another feature helpful in identifying them (especially on H&E-stained routine histologic sections) is that they rarely have more than two nuclear lobes (unlike the neutrophil, which usually has three or four).

Eosinophils are capable of ameboid motion (in response to toxic substances released by bacteria and components of the complement system) and phagocytosis. They are often seen at the site of invasive parasitic infestations and allergic (immediate hypersensitivity) responses. Individuals with chronic allergic conditions (such as atopic rhinitis or extrinsic asthma) typically have elevated circulating eosinophil counts. The eosinophils may serve a critical function in mitigating allergic responses, since they can 1) inactivate slow reacting substance of anaphylaxis (SRS-A), 2) neutralize histamine, and 3) inhibit mast cell degranulation. The life span of eos in the peripheral blood is about the same as that of neutrophils. Following a classic acute phase reaction, as the granulocyte count in the peripheral blood drops, the eosinophil count temporarily rises.

Clinical Pearl:

If increased, consider possible **allergy and or food sensitivity**. An ELISA allergy panel for IgE and Total IgG may detect offending intrinsic or extrinsic agent.

Consider possible parasitic infestation: if increased with IgE increased, basophils normal to increased. Monocytes normal to increased, serum iron normal to decreased, HGB and HCT normal or decreased. If parasitic symptomology is present, a Comprehensive Digestive Stool Analysis with purged Parasitology X 3 should be conducted to rule out the presence of parasites.

Less Common Causes of Eosinophil Increase: Neoplasm, polycythemia, hyperthyroidism, anterior pituitary dysfunction, eosinophilia myalgia syndrome, phlebitis.

Basophils

Laboratory Normal: 0 - 200/µL **0 to 2 % of the Total WBC**
Optimum Range %: **0 to 1% of the Total WBC**
Alarm Range %: **> 5% of the Total WBC**

Physiology: The most aesthetically pleasing of all the leukocytes, the basophils are also the least numerous in peripheral blood. They are easily recognized by their very large, deep purple cytoplasmic granules, which overlie, as well as flank, the nucleus (eosinophil granules, by contrast, only flank the nucleus but do not overlie it). It is tempting to assume that the basophil and the mast cell are the blood and tissue versions, respectively, of the same cell type. Actually, it is controversial as to whether this concept is true or whether these are two different cell types.

The table below presents some of the contrasts between mast cells and basophils.

characteristic	Basophils	Mast cells
Nuclear morphology	segmented	round or ovoid
Mitotic potential	no	yes
Peroxidase content	+	-
Acid phosphatase	-	+
Alkaline phosphatase	-	+
PAS reaction	++++	+

In active allergic reactions, blood basophils decrease in number, while tissue mast cells increase. This reciprocal relationship suggests that they represent the same cell type (i.e., an allergen stimulates the passage of the cells from the blood to the site of the allergen in the tissues). Some experiments with animals have also shown that mast cells are marrow-derived and are capable of differentiating into cells that resemble basophils. Conversely, some recent evidence suggests that basophils (as well as eosinophils) can differentiate from metachromatic precursor cells that reside among epithelial cells in the nasal mucosa.

With inflammation, basophils deliver heparin to the affected tissue to prevent clotting.

Clinical Pearls:

If increased with an increased eosinophil count, monocytes count, IgE, parasites are possible.
If increased with an increased eosinophil count, monocytes count, IgE, food allergy is possible.
Anytime Basophils are greater than 2%, inflammation is possible, and its cause and locus must be determined.

Other tissue inflammation markers: C-reactive protein increased, erythrocytic sedimentation rate increased, gamma globulin and beta globulin increased, alpha 1 globulin increased with severe inflammation and subsequent tissue destruction, ALP increased with liver, bone or gastric inflammation.

Lymphocytes

Laboratory Normal: 1000 - 4800/µL. Up to 24 to 44% of the Total WBC
Optimum Range: Same as Laboratory Normal
Alarm Range: < 20% of Total WBC or > 55% of Total WBC

Physiology: In the immune/inflammatory response, if the neutrophils and monocytes are analogous to the “muscle”, then the lymphocytes are the brains. It is possible to observe the horror of life without lymphocyte function by studying the unfortunate few with hereditary, X-linked, severe combined immune deficiency. Such individuals uniformly die of systemic infections at an early age (except for the "bubble boys" of yesteryear, who lived out their short lives in antiseptic prisons). The functions of lymphocytes are so diverse and complex that they are beyond the scope of this text. What follows are a few general remarks concerning examination of lymphocytes in peripheral blood.

After neutrophils, lymphocytes are the most numerous of the circulating leukocytes. Their life span may vary from several days to a lifetime (as for memory lymphocytes). Unlike neutrophils, monocytes, and eosinophils, the lymphocytes 1) can move back and forth between the vessels and the extravascular tissues, 2) are capable of reverting to blast-like cells, and 3) when so transformed, can multiply as the immunologic need arises. Lymphocytes assist in destroying the toxic products of protein metabolism. Lymphocytes originate in the lymphoblast of the bone marrow, spleen, lymph glands, gut associated lymphoid tissue (GALT), tonsils and possibly the appendix.

The two major types of lymphocytes in blood are B cells, which produce specific antibody when activated, and T cells, which have various roles in cell-mediated immunity and in immunoregulation. T and B cells cannot be distinguished from one another in routine smears, so the differential counts the combination of the two. Immunostains for specific membrane antigens must be used to determine the difference. B cells work chiefly by secreting soluble substances called antibodies into the body's fluids, or humors. (This is known as humoral immunity.) Antibodies typically interact with circulating antigens such as bacteria and toxic molecules, but are unable to penetrate living cells. T cells, in contrast, interact directly with their targets, attacking body cells that have been commandeered by viruses or have become neoplastic. (This is cellular immunity.) Although small lymphocytes look identical, even under the microscope, they can be told apart by means of distinctive molecules they carry on their cell surface. Not only do such markers distinguish between B cells and T cells, they distinguish among various subsets of cells that behave differently. Every mature T cell, for instance, carries a marker known as T3 (or CD3); in addition, most helper T cells carry a T4 (CD4) marker, a molecule that recognizes class II MHC antigens. A molecule known as T8 (CD8), which recognizes class I MHC antigens, is found on many suppressor/cytotoxic T cells. In addition, different T cells have different kinds of antigen receptors-either alpha/beta or gamma/delta. When activated by whatever means, lymphocytes can become very large (approaching or exceeding the diameter of monocytes) and basophilic (reflecting the increased amount of synthesized cytoplasmic RNA and protein). The cytoplasm becomes finely granular (reflecting increased numbers of organelles), and the nuclear chromatin becomes less clumped. Such cells are called "transformed lymphocytes," "atypical lymphocytes," or "viral lymphocytes". Although such cells are classically associated with viral infection (particularly infectious mononucleosis), they may also be seen in bacterial and other infections and in allergic conditions

Clinical Pearls:

If the total WBC is deceased with a lymph count below 20%, a low total cholesterol value, and an albumin value below 3.9, rule-out free radical pathology due to neoplasm or severe immunological deficiency. Find the locus of the free radical pathology and causative factors. Always keep in mind Steroids and immunosuppressive therapy may significantly change amounts of various cells in blood and body tissues, especially WBC’s.

T and B Cell Lymphocyte Subset Assay
Lymphocyte Marker Studies, Lymphocyte Receptor Studies, T and B Cell Typing, Lymphocyte Subset Analysis

Laboratory Normal: T Cells 60 to 80% of Total Lymhocytes
B Cells 5 to 15% of Total Lymphocytes

T and B Cell Lymphocytes are divided into several subsets such as CD2, CD3, CD5, CD7, CD8, etc. Absolute levels and ranges are generally provided by laboratory with the T and B Cell Lymphocyte Subset Assay.
IAG should be considered for all cases where the CD4 Lymphocytes are decreased.

T and B Cell Subset Assay Indications:

- To detect immunodeficiency
- To distinguish between inflammatory skin diseases and leukemias or lymphomas that cause rashes
- To diagnose and distinguish between certain cancers, particularly leukemias and lymphomas
- To diagnose skin allergies that may be due to abnormal activity of certain T cells

Interpretation:

- Immunodeficiency is associated with low levels or absence of T or B cells. For example, in congenital agammaglobulinemia, a genetic disease that affects only boys, no B cells are present. In HIV infection, one type of T cell is decreased (CD4)
- An increase in T cells may signal leukemia or lymphoma
- In a person with reddened skin, increased T cell levels may signal cutaneous T-cell lymphoma, a cancer that causes a skin rash, rather than an inflammatory skin condition

Platelets

Laboratory Normal Adult: 150,000 to 400,000/cu mm
Laboratory Normal Child: 150,000 to 400,000/cu mm
Critical Values: 50,000 or >1 million/cu mm
30,000 to 70,000/cu mm: Increased hemorrhage with trauma/procedures
<20,000/cu mm: Increased spontaneous hemorrhage

Methodology: Platelets are counted by machine in most hospital labs and by direct phase microscopy in smaller facilities. Since platelets are easily mistaken for garbage (and vice versa) by both techniques, the platelet count is probably the most inaccurate of all the routinely measured hematologic parameters. Actually, you can estimate the platelet count fairly accurately (up to an absolute value of about 500 x 10^3/µL) by multiplying the average number of platelets per oil immersion field by a factor of 20,000. For instance, an average of ten platelets per oil immersion field (derived from the counting of ten fields) would translate to 200,000/µL (10 x 20,000). Abnormal bleeding generally does not occur unless the platelet count is less than 30,000/µL, if the platelets are functioning properly. Screening for proper platelet function is accomplished by use of the bleeding time test. The central portion of a platelet stains purple with Wright's stain and is referred to as the granulomere. The peripheral portion stains clear and is called the hyalomere.

Physiology: Blood platelets, or thrombocytes, are not true cells, but rather cytoplasmic fragments of a large cell in the bone marrow, the megakaryocyte. Platelets have several types of membrane-bound granules (GR), which contain many constituents including fibrinogen and several growth factors (e.g., PDGF). Most of the platelets exist in the bloodstream. A smaller percentage (25%) exist in the liver and spleen. Survival of platelets is measured in days (average 7 to 9 days).

Platelet activation occurs when injury to the vessel wall exposes sub-endothelial components, especially collagen. Platelets adhere to the damaged area and become cohesive to other platelets. This aggregation leads to the formation of a platelet plug, which prevents further blood loss and allows the repair process to begin.

In the body, 2% of the serotonin, a mood elevating neurotransmitter, is stored in platelets. In addition to serotonin, your platelets also carry its 'parent' or precursory chemical L-tryptophan. While serotonin can't pass through the blood brain barrier, L-tryptophan can. These substances are involved in such processes as sleep/wake cycles, biological rhythms, appetite, and mood regulation.

Quantitative Disorders:

Thrombocytopenia (Decreased Levels)

Decreased Production:
- Marrow Failure: Acquired (aplastic anemia), Congenital (Fanconi's syndrome, congenital intrauterine rubella)
- Marrow Invasion: Tumors, Leukemia, Fibrosis
- Marrow Injury: Drugs (cancer chemotherapeutic agents, ethanol, chloramphenicol, gold sulfonamides)
- Chemicals (Benzene)
- Radiation
- Infection

Increased Destruction:
- Immune: ITP, SLE, Drugs, Transfusion-related, Infections
- Non-Immune: DIC, Thrombotic Thrombocytopenic Purpura

Thrombocytosis (Increased Levels)

Reactive:
- Iron Deficiency
- Malignancy
- Splenectomy
- Inflammatory Diseases, especially Rheumatoid arthritis
- Rebound Thrombocytosis

Autonomous:
- Myeloproliferative Diseases
- Thrombocythemia from drugs such as conjugated estrogen and birth control pills.

Qualitative Disorders:
- Characterized by abnormalities in platelet function; the counts are generally normal. The bleeding time is usually prolonged.
- Clinical features highly variable and consist of mucocutaneous bleeding manifestations.
- May be acquired or congenital.
- Acquired disorders of platelet function are more common than inherited varieties.

Other Tests of Platelet Function:

- **Bleeding Time: Normal Range: 2-7 minutes**

The bleeding time reflects platelet function and platelet-vessel wall interaction. As platelet counts decline below platelet counts of 70,000-80,000/cu mm, the BT gets prolonged. It is prolonged when platelets are functionally abnormal even when platelet counts are normal.

- **Mean Platelet Volume (routinely used especially in thrombocytopenia)**
- **Platelet Aggregation (routinely used in evaluation of bleeding disorders)**
- **Antiplatelet Antibody Detection (routinely used to evaluate thrombocytopenia and exclude immune-associated etiology)**
- **Platelet Secretion (used in many laboratories)**
- **Platelet Adhesion (available only in few research laboratories)**
- **Platelet Thromboxane Synthesis (research laboratories)**
- **Platelet Coagulant Activities (research laboratories)**

Clinical Pearls:
Often the underlying cause of many quantitative and qualitative platelet disorders are associated with heavy metal body burden, xenobiotics exposure and prescription drugs.

Tumor and Neoplastic Laboratory Markers

Cancers are rarely diagnosed at their very early stages. To be detectable by x-rays, computer tomography (CAT scan) or ultrasound, the size of the cancer must reach at least 1 cm or more. With new immunological methods however, the characteristic substances known as cancer markers that are produced as the cancer grows are detectable even before it reaches a size big enough for detection by other methods. This early detection system is vital for early biologically oriented medical intervention that significantly improves the chances of recovery.

It is also important to bear in mind that all results are best interpreted with full history and medical details, and results by themselves should not be taken as indicative of the presence or absence of the diseases. They, however, are useful as perimeters for further investigations. Tumor markers are molecules occurring in blood or tissue that are associated with cancer and whose measurement or identification is useful in diagnosis or clinical management. The ideal marker would be a "blood test" for cancer in which a positive result would occur only in patients with malignancy, one that would correlate with stage and response to treatment and that was easily and reproducibly measured. No tumor marker now available has met this ideal.

It appears that new tests are created daily to identify and monitor cancer. The following is a list of the more common. Included is the biochemical site of production and their clinical interest.

Prostate Specific Antigen (PSA)

Normal Values:
Males: 0 to 39 years <2.0 micrograms/L
> 40 years old <2.8 micrograms/L
Females: <0.5 micrograms/L

This antigen is a glycoprotein produced in the prostate (primarily), breast and parotid glands. Assist in the identification, differentiation, classification, staging, and location of tumor. It is smaller than the prostatic acid phosphatase molecule, more stable, and does not demonstrate diurnal variations. Tumor catabolic activity and accelerated metabolic rate in prostate carcinoma elevate the serum value of PSA. PSA is, therefore, a reliable immunocytochemical marker used in the detection of adenocarcinoma of the prostate. PSA may assist in assessment of tumor response to treatment protocols. This test is recommended for men over 40 years old as part of a routine chemistry screen.

Actually, there are now at least six different ways to look at serum PSA: total PSA, free PSA, age-adjusted PSA, ethnically adjusted PSA, PSA velocity and PSA density. Each of these has unique characteristics. PSA exists in multiple forms in the blood. Most is bound to proteins, but some is free-floating. In the early 1990s, it was discovered that measuring the ratio of "free" to "total" PSA could further help in distinguishing prostate cancer from benign prostate disease.

Interpretation: Values suggestive of Prostate Cancer
PSA 3.0 to 4.0 with Free PSA <19%
PSA 4.0 to 10.0 with Free PSA <24%

Carcinoembryonic Antigen (CEA)

Normal values are less than 3.0 ng/mL, with a gray zone up to 5.0 ng/mL. Smokers have slightly higher CEA values than nonsmokers do.

Carcinoembryonic antigen (CEA) is a glycoprotein that is overexpressed in a wide range of carcinomas, including breast, colorectal, gastric, pancreatic, and non-small cell lung carcinoma. It is an adhesion molecule, and its overexpression in cancer cells where it promotes adhesion and metastasis. CEA involved in cell adhesion normally produced during fetal development; however, the production of CEA stops prior to birth. Therefore, it is not usually present in the blood of healthy adults. CEA measurement is mainly used as a tumor marker to identify recurrences after surgical resection, or identify cancer spread though biological fluids. The CEA blood test is not reliable for diagnosing cancer or as a screening test for early detection of cancer. It is an antigen that can be found in trace amounts in health adults, but is secreted in greater amounts during rapid multiplication of epithelial cells, especially of the gastrointestinal tract.

CEA belongs to a family of cell-surface glycoproteins with increased expression found in a variety of malignancies, including breast cancer. CEA is elevated in 60% of patients with advanced breast cancer, and the level and frequency of elevation correlates with the tumor burden. Because of its low sensitivity in early-stage disease, CEA cannot be used in screening or diagnosis of breast cancer. The two roles that have been most frequently investigated are in monitoring patients following surgery for primary breast cancer, and in monitoring patients for the response to treatment for metastatic disease. Monitoring patients following breast cancer surgery cannot be recommended. Half of patients with recurrences will not be detected, and there is a 12% false-positive rate. CEA monitoring of treatment of metastatic disease may correlate with other markers of disease progression in patients known to have elevated levels. However, with a high error rate, supporting clinical information must be awaited before modifying therapy. Care should be taken to avoid misinterpreting the paradoxical CEA elevation that can occur shortly after institution of therapy for metastatic disease. CEA determination can only be recommended in patients with elevated levels in whom there is no other ready means of following the disease.

The sensitivity and specificity determine whether a marker will be useful in the screening and diagnosis of cancer. Multiple studies have shown that the incidence of CEA level increases with an increasing stage of the disease. Both the incidence and the level are lower in early-stage disease. Merging data from seven studies, 10% of women with stage I, and 19% with stage II breast cancer have elevated CEA levels; the incidence increases to 31% and 64% for patients with stages III and IV disease, respectively.

The data suggest that CEA can detect recurrence of breast cancer before clinical recurrence. However, its routine use in this setting is not recommended because half of all recurrences will be missed; and because the impact of the small percentage of false-positive levels is amplified by the larger number of women who have not recurred and by the repeated testing required in monitoring therapy. Secondly, the short lead-time from marker elevation to clinical recurrence suggests that there will be little biological impact of the earlier diagnosis of recurrence.

In conclusion:

The CEA has been suggested as having prognostic value for patients with colon cancer. Preoperative CEA values have been positively correlated with stage and negatively correlated with disease free survival.

CEA is not recommended for screening, diagnosis, staging, or routine surveillance of breast cancer patients following primary therapy.

The CEA is often positive in malignancies other than colonic. In cancer of the breast, lung, pancreas, stomach, and ovary the CEA may be elevated and can be used to monitor the progress of disease or response to treatment.

Routine use of CEA for monitoring response of metastatic disease to treatment is not recommended. But, in the absence of readily measurable disease, an increasing CEA level may be used to suggest treatment failure.

Most types of cancer do not produce a high CEA. A normal level of CEA is less than or equal to 3 nanograms per milliliter (ng/mL). Most healthy people have levels below this amount. Elevated CEA levels should return to normal after successful surgical resection, or within 6 weeks of starting treatment, if cancer treatment is successful.

Serum Human Chorionic Gonadotrophin (hCG) (CSF, serum, urine)

Normal Values:
Adult males or non-pregnant females:
Serum: <5 IU/L;
Urine: <25 IU/L;
CSF: <5 IU/L.
In the absence of brain metastases, the ratio of hCG concentrations in serum and CSF is >60:1 (mean 280:1). Ratios of <60:1 are highly suggestive of the presence of intracerebral tumor tissue secreting hCG, unless serum hCG is falling rapidly in response to therapy.

hCG is a glycoprotein hormone with alpha and beta-subunits, which are normally produced by a developing placenta and may be produced by some germ cell tumors. The alpha sequence is identical to the follicle stimulating hormone, lutenizing hormone, and thyroid stimulating hormone, which can cause a false-positive pregnancy test if not tested along with the beta-subunit. If the patient is not pregnant, an increase in hCG is abnormal and may indicate a neoplastic process. The beta-subunit is often used to follow the status of neoplasms after surgery or therapy. Specifically, in oncology, hCG is used for the diagnosis and monitoring of patients with trophoblastic tumors, testicular cancer and bladder cancer.

Indications:

1. Diagnosis of choriocarcinoma and germ cell tumors of the ovary, testis or mediastinum and monitoring response to therapy.
2. Monitoring other rare cancers that produce this gonadotrophin ectopically.
3. Detection of metastases to the brain from choriocarcinoma or germ cell tumors.

Applications:

When hCG is used to diagnose early pregnancy, the concentration will double in approximately 48h. In both trophoblastic tumors and ectopic pregnancies, there is a slower rise with hCG values lower than for a comparable stage of normal pregnancy. A fall in concentration will suggest a failing pregnancy or miscarriage.

Trophoblastic disease and germ cell tumors
In these conditions hCG may be secreted as the intact dimer, free b-subunit or fragments such as b-core fragment (which is cleared very rapidly from serum and detectable only in urine). In addition, the b -subunit may be "nicked" i.e. lose specific sections of the peptide chain and the C-terminal may be missing altogether. These changes will affect most commercial assays which were developed principally for measuring hCG in pregnancy and which require two unaltered epitopes to complete the "sandwich".

The antibody used in this radioimmunoassay is directed to a single epitope on the b -subunit, detects both free b, intact hCG and b-core fragment, is not affected by "nicks" and cross-reacts less than 0.25% with luteinizing hormone of pituitary origin.

Secretion of hCG declines during successful therapy; renewed secretion provides evidence of recurrence.

Cerebral metastases
Intracerebral tumors secreting hCG can be detected from comparison of b -hCG concentrations in peripheral serum and cerebro-spinal fluid.

Cancer Antigen 125 (CA 125)

Normal Values: <35 U/ml
Normal: CA-125 less than 35 (Does not exclude cancer)
Increased
CA-125 >35 highly correlated with cancer
CA-125 >65 associated with cancer in 90% pelvic mass

This glycoprotein is present in normal endometrial tissue and in mucinous uterine fluid. It enters the circulation only when natural barriers are destroyed. In the presence of endometrial or ovarian malignancy, a persistently rising CA 125 level may be associated with progression of the disease and poor therapeutic response. Normal levels do not rule out extensive tumor presence or recurrence. Considered to be superior to other markers such as CEA.

Indications and limitations:

Serial monitoring of gynecologic malignancy
Generally, not an accurate screening test for ovarian cancer (too insensitive); Not sensitive in 50% of Stage I ovarian cancer.
This antigen is also expressed by neoplasms of the pancreas, liver, colon, breast and lung in smaller percentages. May be obtained as baseline in high-risk patients.

Increased CA-125 Causes (Non-Cancer Causes)
Endometriosis; Pelvic Inflammatory Disease; Uterine Fibroids
Pregnancy
Liver Cirrhosis
Post-Menopause
Pelvic Irradiation

Malignant Causes:
Ovarian Cancer
Liver Cancer
Lung Cancer
Breast Cancer
Colon Cancer
Pancreatic Cancer
Endometrial Cancer
Cervical Cancer

Cancer Antigen 15-3 (CA 15-3)

Normal Values: <30 U/ml

Cancer antigen 15-3 (CA 15-3) is a glycoprotein produced by normal breast cells, and is increased in many patients with breast tumors. CA 15-3 is shed by the tumor cells and enters the bloodstream, making it useful as a tumor marker to follow the course of the cancer. CA 15-3 is elevated in about 10% of women with early localized breast cancer and in about 70% of those with metastatic breast cancer. CA 15-3 may also be elevated in healthy people and in individuals with other cancers, conditions, or diseases, such as colorectal cancer, lung cancer, cirrhosis,

hepatitis, and benign breast disease. CA 15-3 is a commonly used marker to follow the course of treatment in women diagnosed with advanced breast cancer and metastasis to the bone. Because CA 15-3 levels are rarely elevated in women with early stage breast cancer this test is not specific enough to be used as a solo screening for breast cancer. Rather, it correlates with tumor progression after initial diagnosis and therapy. Hence, the CA 15-3 assay value, regardless of level, should not be interpreted as absolute evidence of either the presence or absence of breast cancer. The CA 15-3 epitope is recognized by two monoclonal antibodies in a double-determinant or sandwich radioimmunoassay. CA 15-3 is a commonly used marker to follow the course of treatment in women diagnosed with advanced breast cancer and metastasis to the bone.

The reference range of serum CA 15-3 is less than 30 U/mL. The upper limit of the range varies depending on the laboratory and kit used for the test. Values obtained with different assay kits, methods, or laboratories cannot be used interchangeably.

CA 15-3 levels are rarely elevated in women with early stage breast cancer. This test is not specific enough to be used in screening for breast cancer, but rather correlates with tumor progression after initial diagnosis and therapy. Hence, the CA 15-3 assay value, regardless of level, should not be interpreted as absolute evidence for the presence or absence of malignant disease. The CA 15-3 assay value should be used in conjunction with information available from clinical evaluation and diagnostic procedure.

HER-2/neu Testing

“Human Epidermal Growth Factor Receptor 2"

Like the hormone receptor test, the HER2/*neu* test looks for a specific kind of protein that is found with certain types of cancer cells and the gene that produces it. The formal name of that gene is the *human epidermal growth factor receptor 2*, and it makes HER2 proteins. These proteins are receptors on breast cells. This proto-oncogene testing is routinely conducted at the time of surgery, using a sample of tumor tissue. Technically speaking, HER2 is a cell membrane surface-bound receptor tyrosine kinase and is normally involved in the signal transduction pathways leading to cell growth and differentiation. HER-2/*neu* proto-oncogene encodes a growth factor receptor that is over-expressed in 25-30% of human breast cancers. Generally, these tumors tend to be aggressive, faster-growing, and generally more likely to recur than those that do not overproduce the oncoprotein. The industry purpose of testing for this proto-oncogene is primarily to determine if a woman is a candidate for the pharmaceutical tratuzumab (Herceptin), a drug that was created to target Her-2/*neu* protein.

There are two main ways to test Her-2/*neu* status: immunohistochemistry (IHC) and fluorescent *in situ* hybridization (FISH). IHC measures the amount of Her-2/*neu* protein present. FISH examines a woman’s genetic level for actual gene amplification – the number of copies of the gene present. IHC is currently the most widely used initial testing method; however, if the IHC test is “indeterminate or negative,” then the FISH method is often performed as a follow-up test.

If an IHC Her-2/*neu* test is positive, then the Her-2/*neu* gene is over-expressing the Her-2/*neu* protein. If a FISH test is conducted, then amplification of the Her-2/*neu* gene can be detected. If either of these tests is positive, then the woman likely has an aggressive tumor that will respond poorly to endocrine treatment, and that will be resistant to chemotherapy. In conventional oncology, these individuals are considered candidates for Herceptin therapy. If the IHC is negative but the FISH is positive, the woman may still be considered a candidate for Herceptin, but if both are negative the treatment will not be useful. If a breast cancer is Her-2/*neu*-positive and the serum Her-2/*neu* is initially elevated, then changes in serum Her-2/*neu* levels can be used to help monitor the effectiveness of Herceptin treatment and detect early disease progression. Decreases indicate a response to treatment; levels that stay the same or increase indicate that the treatment is not effective; and concentrations that fall and then rise may indicate a cancer recurrence. If the serum Her-2/*neu* is initially negative or low, such as may be seen with

early cancers and with Her-2/*neu*-negative cancers, then it may not be useful as a monitoring tool. Some studies have shown that there can be differences in Her-2/*neu* positivity between the primary tumor and tumor that has spread to other parts of the body. If a patient who has metastatic breast cancer is found to be Her-2/*neu* negative when the primary tumor is tested, but then has a serum Her-2/*neu* level greater than 15 ng/mL, then additional tissue testing may be indicated.

A Her2/*neu* blood test is also available. The amount of Her-2/*neu* protein present in the serum is loosely associated with the amount of Her-2/*neu* positive cancer present. This test is not used for screening purposes and does not replace tissue testing, but may be ordered to help assess a person's prognosis and to monitor the effectiveness of treatment. After an initial diagnosis of metastatic breast cancer, this blood test may be performed and, if the initial level is greater than 15 ng/mL, then the test may be used to monitor treatment.

Cancer Antigen 19-9 (Carbohydrate AG 19-9, GICA)

Normal Values: <37 AU/ml
Metastasis: >1000 AU/ml
(One AU equals 0.59 ng/ml of antigen)

This carbohydrate antigen is related to the Lewis blood group antigen. Individuals who are Lewis (a negative, b negative) cannot synthesize CA 19-9; therefore, CA 19-9 is not a useful marker for this group of patients.

CA19-9 is a monoclonal antibody generated against a colon carcinoma cell line to detect a monosialoganglioside found in patients with gastrointestinal adenocarcinoma. It is found it to be elevated in 21 to 42 percent of cases of gastric cancer, 20 to 40 percent of colon cancer, and 71 to 93 percent of pancreatic cancer, and has been proposed to differentiate benign from malignant pancreatic disease, but this capability remains to be established.

Elevated levels can indicate recurrence of cancer before radiographic or clinical findings by 1 to 7 months. It is produced primarily in pancreatic and gastric tissue and is mostly used to identify and monitor intra-abdominal carcinoma, pancreatic carcinoma (most frequently elevated marker; elevated levels found in 80% of patients with pancreatic cancer), and possibly with other adenocarcinomas such as lung, gastric, biliary, and colonic. It has also been used to monitor head and neck cancer and gynecological cancer.

CA 19-9 may also be elevated in pancreatitis and inflammatory bowel disease

Cancer Antigen 50 (Carbohydrate Antigen 50)

Normal Values: <17 U/ml

This carbohydrate antigen is a tumor marker that increases with many malignancies, particularly those of the digestive tract. This test is not specific enough for screening and correlates more with tumor progression than with tumor regression. It is found increased in colorectal adenocarcinomas, digestive tract carcinoma, non-small cell lung carcinoma, pancreatic cancer and transitional cell bladder cancer.

Cancer Antigen 27.29 (CA 27.29)

Normal Values:<38 U/mL

CA 27.29 is another glycoprotein present on the surface of epithelial cells like breast cancer cells. While CA 27.29 is expressed at the apical surface of normal epithelial cells, it is present throughout malignant epithelial cells of the breast, lung, ovary, pancreas, and other sites. The cancer-associated form of the antigen is less extensively glycosylated than the normal form and more specific for tumor cells. Breast cancer cells can shed copies of the CA 27.29 protein into the bloodstream causing an elevation. Like CA 15-3, the CA 27.29 assay value, regardless of level, should not be interpreted as absolute evidence of either the presence or absence of breast cancer.

The reference range of serum CA 27-29 is less than 38 U/mL. The upper limit of the range may vary depending on the laboratory and testing kit used for the test. Values obtained with different assay kits, methods, or laboratories cannot be used interchangeably.

5-Hydroxyindoleacetic acid 5 HIAA

Values >25 mg/24 hours are strong evidence for carcinoid. (higher if the patient has malabsorption) Urine 5-HIAA--Less than 6mg/24 hours

The most frequently used diagnostic test for carcinoid tumors is 5-HIAA, the final metabolite of serotonin This analysis is most frequently performed for the diagnosis of carcinoid tumors of the enterochromaffin (Kultschitzsky) cells of the small intestine. These tumor cells release large amounts of serotonin, which can produce the clinical syndrome of flushing, diarrhea, and right-sided heart failure. Diagnose carcinoid tumors and syndrome; Limitations 5-HIAA may be normal with nonmetastatic carcinoid tumor and may be normal even with the carcinoid syndrome, particularly in subjects without diarrhea. Some patients with the carcinoid syndrome excrete nonhydroxylated indolic acids, not measured as 5-HIAA. Midgut carcinoids are most apt to produce the carcinoid syndrome with 5-HIAA elevation. Patients with renal disease may have falsely low 5-HIAA levels in the urine.[2] 5-HIAA is increased in untreated patients with malabsorption, who have increased urinary tryptophan metabolites. Such patients include those with celiac disease, tropical sprue, Whipple's disease, stasis syndrome, and cystic fibrosis. It is increased in those with chronic intestinal obstruction. However poor correlation exists between 5-HIAA level and the clinical severity of the carcinoid syndrome. Recent studies confirm its use as a prognostic factor in this disease.

The AMAS Test

The AMAS Test measures serum levels of AMA, an antibody found to be elevated in most patients with a wide range of active non-terminal malignancies. AMA is the antibody to Malignin, a 10,000 Dalton polypeptide that has been found to be present in most malignant cells regardless of cell type or location. Unlike tests such as CEA, which measure less well-defined antigens whose serum levels tend to be inconstant but elevated late in the disease, the AMAS test measures a well-defined antibody whose serum levels rise early during the disease. In some cases, the AMAS test has been positive (elevated) early, i.e. 1 to 19 months before clinical detection. On the other hand, since antibody failure often occurs late in malignancy, elevated antibody is then no longer available as evidence of the presence of antigen and therefore, late in the disease, the AMAS test cannot be used as a diagnostic aid, but may be useful for monitoring.

Limitations

1. The low false-positive and false-negative rates (<1 % on repeat determinations of 24-hour sera) have permitted successful screening in selected high-risk populations, as in chemical workers (ref.8) and in the preclinical detection of cancer in 2.3% of medical-surgical cases (ref.4), but the efficacy of screening in larger normal populations has yet to be determined.
2. A normal AMA level can occur in non-cancer, in terminal cancer, and in successfully treated cancer in which there is no further evidence of disease; clinical status must be used to distinguish these states.
3. As in all clinical laboratory tests, the AMAS test is not by itself diagnostic of the presence or absence of disease, and its results can only be assessed as an aid to diagnosis, detection or monitoring of disease in relation to the history, medical signs and symptoms and the overall condition of the patient.

Alpha fetoprotein (AFP)

Alpha fetoprotein (AFP) levels are often elevated in liver cancers (hepatocellular) and testicular cancers (non-seminomatous). Raised levels are also present during pregnancy or some gastrointestinal cancers. AFP is also used in combination with other tests as a screening test for open neural tube defects.

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