A Textbook of Clinical Biochemistry

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CHAPTER 1

INTRODUCTION TO LABORATORY PRINCIPLES AND INTERPRETATION IN CLINICAL BIOCHEMISTRY: AUTOMATION IN CLINICAL BIOCHEMISTRY

Introduction to Laboratory Principles and Interpretation in Clinical Biochemistry

Over the years, clinical laboratories have started playing a very important role in the treatment of patients. From being used as a secondary branch in the treatment, clinical biochemistry is being looked upon as one of the most important bases of overall healthcare management of patients. Its role in the final diagnosis of any medical condition cannot be exaggerated. Around 70% of medical decisions are based on laboratory test results. Diagnostic testing is a critically important step toward improving healthcare outcomes. Thus, it is imperative for any laboratory to maintain the highest of quality standards at all times.

To ensure that a very high grade of quality is maintained in all clinical assessments, it is necessary to follow certain principles.

The first among them is the use of reagent grade water, which comes in three types according to the criteria set by the National Committee for Clinical Laboratory Standards (NCCLS). Reagent grade water is prepared by filtration followed by either distillation, ion exchange or reverse osmosis. Type III reagent water is used for glassware washing and some qualitative estimations like urinalysis. Type II is used for general lab tests; it is not to be stored for long and all precautions are taken to minimize chemical or bacterial contamination. Those tests that require maximum precision and accuracy make use of Type I reagent grade water. Enzyme determinations, electrolyte determinations, and preparation of solutions of reference materials are done with Type I water.

Chemicals that meet the specifications of American Chemical Society are known as reagent or analytical reagent grade chemicals. There are

some selected chemicals that are especially purified to meet the specifications of certain procedures, like HPLC. These are known as ultra-pure reagents. USP and NF grade chemicals are pure chemicals that are produced following the specifications of United States Pharmacopeia (USP) or the National Formulary (NF). These are used in chemical analysis and for the preparation of different reagents.

Those materials whose physical and /or chemical properties are sufficiently established can be used as reference materials. They are used as calibrators, for verification of a measurement method or to assign values. Reference materials are classified as primary, secondary and certified reference materials. A primary reference material is a highly purified chemical that is weighed out directly for the preparation of solutions of specified concentration, or for the calibration of solutions of unknown strength. IUPAC recommends that a primary reference material should be 99.98% pure. A secondary reference material has values assigned to it by a process of value transferred from a primary reference material. A certified reference material (CRM) has one or more of its values certified by a technically valid procedure.

Solutions of different types are used in clinical estimations. A solution is a homogeneous mixture of one or more solutes dispersed molecularly in a sufficient quantity of solvent. Here, the particles of one or more substances (the solute) are distributed uniformly throughout another substance (the solvent), so that the mixture is at the molecular or ionic level. The particles in a homogeneous solution are smaller than those in either a colloid or a suspension. The number of molecules present in a given space determines the colligative properties of the solution. When a solution holds as much of a dissolved solute as it can at a particular temperature, it is called a saturated solution. In many laboratories data is reported as mass of solute per unit volume of solution. Mass concentrations are also reported in terms of gram percent or percent. Some concentrations are expressed through the equations given below:

Mole = Mass / Gram molecular weight

$$\text{Molarity of a solution} = \frac{\text{Number of moles of solute}}{\text{Number of liters of solution}}$$

$$\text{Molality of a solution} = \frac{\text{Number of moles of solute}}{\text{Number of kilograms of solvent}}$$

$$\text{Normality of a solution} = \frac{\text{Number of gram equivalents of solute}}{\text{Number of liters of solution}}$$

Normality (in oxidation-reduction reaction) = molarity x difference in oxidation state.

$$\text{Gram equivalent weight (As oxidant or reductant)} = \frac{\text{Formula weight (g)}}{\text{Difference in oxidation state}}$$

A number of different kinds of hazards may be present at any given time in a biochemistry lab which should be dealt with immediately. There may be biological or chemical hazards, hazards from volatile chemicals or compressed gases, electrical and fire hazards. Hence a safe operation of all clinical labs makes it mandatory to see to the safety of all people working in the lab.

The use of Biochemistry is fundamental to practice clinical medicine. Biochemical principles and techniques are applied to the analysis of body fluids and tissue, while biochemical investigations aid in clinical decision making. These investigations help in the **diagnosis** and **management** of many conditions. There are conditions with a metabolic basis which is very obvious (like diabetes mellitus), and there are conditions where metabolic disturbance is a consequence of a disease (e.g., renal failure). There are some conditions that can be diagnosed without a biochemical test and can also be treated successfully. However, till now there are no practical biochemical investigations that can assist the diagnosis and management of psychiatric disorders. At the same time, there is evidence that biochemical disturbances are involved in the pathogenesis of these psychiatric conditions. The potential range of biochemical investigations is high, and range from dip-stick tests on urine (which are very cheap) to magnetic resonance tests (that are quite expensive). Generation of biochemical data is not as easy as it sounds. It is very complex to acquire, interpret and use biochemical data. A biochemical result can be acquired but by itself is of very less value. It requires to be interpreted correctly, and correct interpretation requires that the one understands very clearly why the test has been requested.

Specific Uses of Biochemical Tests

1. Diagnosis. To arrive at a diagnosis a patient's history has to be taken and clinical examination done. It may be further extended by investigations to determine the pathogenesis of the condition, and thus its underlying cause. An ideal diagnostic condition would be 100% sensitive, which means that all cases of the condition in question would be correctly diagnosed using it, and it would also be 100% specific, that is, no individual without the condition would be wrongly diagnosed as having it.

Whether or not a biochemical investigation can provide precise diagnostic information is extremely variable, and spans over a wide spectrum. At one end of the spectrum is genetic analysis, which can reliably diagnose inherited metabolic diseases even *in utero*. On the other end are investigations that are not diagnostic for any particular condition. An example of the latter case is a decrease in plasma Na^+ concentration, which can occur in many conditions and, on its own particularly in a pathogenic situation, is not diagnostic for any of them. Sometimes molecular genetic analysis is also done for diagnosis. Such analyses detect the presence of a mutation responsible for a specific disease. It is not necessary that the mutation results in the development of disease; however, its presence can indicate increased susceptibility to a condition. It is also true that individuals with the same genotype for a characteristic may differ in their phenotypes.

It is a very small number of conditions that only a biochemical test will provide a precise diagnosis. Except for genetically determined diseases, it is very difficult for biochemical investigations alone to provide a precise diagnosis.

This is because

- Biochemical changes are often a consequence of a pathological process. This change can be common to many conditions and hence pose difficulties in diagnosis.
- Frequently, a biochemical variation can be influenced by more than one type of process. For example, the concentration of plasma albumin can be influenced by its changes in rates of synthesis and degradation, by changes in its volume of distribution, supply of substrate and hepatic function, as it is synthesized in the liver.
- Even when a biochemical change is specific to one condition, one may not get to know its cause. To know the cause of a biochemical change is very helpful for appropriate treatment. During hyperthyroidism, an increased concentration of the thyroid hormone iodo-

thyronine is seen in the plasma. But, an increased concentration of iodothyronine can be a result of several different thyroid diseases, and thus treatment appropriate for one of these diseases may not be appropriate for another disease.

Once the result of a biochemical investigation has been obtained, it has also to be compared with a reference range. **Reference range** is a range of values that can be expected in comparable apparently healthy individuals of the same sex and about the same age. The natural variation of biochemical parameters is such that the ranges of concentrations of constituents of the plasma are likely to be less in an individual than in a group. For many biochemical variables there is often considerable overlap, between the range of values seen in healthy individuals and that characteristic of disease. Thus, a test result in a patient with a disease may fall into the range typical of healthy people and vice versa. This overlap is generally seen because some organs have considerable reserve capacity. The organs keep on working even after part of their functional ability is lost. Examples of such organs are liver, kidneys, pancreas and small intestine. A person afflicted with renal failure still has enough renal function to maintain normal homeostasis with respect to body fluid composition. This is true even when the kidneys are left with only fifty percent of their functionality. So if a single test/ measurement is done on the person with renal disease, one can still get normal results. Chronic pancreatitis is demonstrated by malabsorption. However, this does not become evident by biochemical testing even when the body has lost 70% of its pancreatic activity. Loss of pancreatic malabsorption only becomes evident biochemically when at least 80 per cent of the functional capacity of pancreas is lost, although severe pain often occurs at an earlier stage.

If previous measurements are available for an individual, test results are / should be compared with these rather than with a reference range. This is the “Baseline” against which to assess further results, particularly if there is a risk of some complication. This is a better parameter of measuring biochemical changes in an individual.

A majority of biochemical investigations that are needed for diagnostic purposes involve analysis of plasma or serum, but changes in the concentration of analytes in these fluids are not necessarily comparable to changes in intracellular or whole-body content, either in time course or in quantity. Yet it may be these quantities that are more relevant to the underlying pathology. Furthermore, if a single measurement is done, it may not provide reliable information in non-steady state situations, or if a change is anticipated in the natural progression of disease, or if one wishes to follow the response to treatment. So at times multiple measurements are required.

At other times, results are needed immediately. So, some quality is sacrificed in order to obtain results rapidly, but all attempts should be made to minimize the influence of both analytical and pre-analytical factors on accuracy and precision of data. If the biochemical data is being generated for diagnosis, it is imperative that the results are available in time and are reliable, to be useful.

2. Management. The management of a disease and the follow-up of the treatment being given needs biochemical investigations regularly.

- *Assessment of disease severity* is required to manage the disease. Most biochemical investigations are quantitative. If it is a pathological disturbance that is causing a biochemical change, we get a more abnormal result. Often, the extent to which a result is abnormal correlates well with the severity of a condition, but it is not always so. In cases of hepatitis the activity of the enzyme aminotransferase is elevated. It may be possible that two patients with hepatitis may have equally increased plasma aminotransferase activities. But to assess the severity of hepatic dysfunction, the prothrombin time has also to be calculated in both of them. The condition of the patient having a longer prothrombin time is judged more severe, as a prolonged prothrombin time indicates impaired hepatic function capacity. Furthermore, overall disease severity depends on other factors also, like the patient's age, previous state of health, existence of other illnesses etc.
- *Prognosis* is the opinion based on medical experience, of the likely development of a disease or illness, the prospect of recovery as anticipated from the usual course of disease or peculiarities of the case. Biochemical tests are generally poor indicators of prognosis. However, this is not a rule. For instance, a person having a high concentration of alpha fetoprotein may be prognostic of testicular cancer. Prognosis is done for the assessment of the benefits and risks of treatment, and it has proven to be good for this purpose. Different patients do not necessarily respond identically to the same drug because of many factors like nutrition, collateral use of other drugs, genetics etc. Genes of a person also affect the response to drugs.
- *Monitoring the progression of disease* is done through biochemical tests. Serial measurements are of value in monitoring the natural history of a disease, or how it is responding to treatment. If an expected change does not occur, it may suggest that the treatment is inadequate or inappropriate, or even that the diagnosis is incorrect.

Biochemical investigations can also be used to detect the development of complications of diseases or their treatment before they become clinically obvious. In other words, we can use biochemical investigations to prevent complications. Therapeutic drug monitoring (TDM) is also done. This helps to determine the dose of the drug so that the patient gets optimum treatment and also that there is no iatrogenic toxicity. TDM is also done to establish a baseline.

3. Screening. In medicine, screening is a strategy used in a population to identify the possible presence of an as-yet-undiagnosed disease in individuals without signs or symptoms. This can include individuals with presymptomatic or unrecognized symptomatic disease. As such, screening tests are somewhat unusual in that they are performed on persons apparently in good health.

Thus, screening is the endeavor to detect disease before it becomes manifest through the development of a clinical disturbance and symptoms. Screening can be done via biochemical clinical tests. If a screening test yields a positive result, it should not be taken as diagnostic on its own. There should always be another investigation done to confirm the first finding obtained through screening tests. Screening may be applied at different levels, like to a population, to groups that have common characteristic within a population or to individuals. Screening may be carried out at different times, like antenatally, shortly after birth, during childhood or during adult life, depending upon the nature of the condition. The strategy of screening that has to be adopted should depend upon factors like the risk of the condition, the probability of its presence, the availability of suitable screening tests, and cost (of the programme), and should be particularly careful in people with inherited diseases.

Screening interventions are designed to identify disease in a community early, thus enabling earlier intervention and management in the hope to reduce mortality and suffering from a disease.

- When screening of whole populations for disease is to be done it has to be precluded by economic and logistic considerations. WHO advocates that all adults should be screened for hypercholesterolemia. There are many people who have severe but asymptomatic hypercholesterolemia who are at increased risk of coronary heart disease. Such individuals can be identified by being screened for hypercholesterolemia. This program is very desirable but is also quite costly. Hence a healthy diet and lifestyle is encouraged.
- Selective screening. Since screening programs are expensive, the screening of a disease is sometimes restricted to the high-risk

groups of the population. In principle, different methods of finding a high-risk group are available. When screening is done for prevention or treatment, then any reduction in the expenditure should be weighed against the outcome of the screening. The process of selection of a highrisk group for selective screening should be such that a substantial proportion of all cases of the disease in the total population is detected. Generally, the screening of those with a high risk of disease only is recommended because of the reduction in cost, or because this helps to avoid the adverse effects of screening. Successful selective screening is done when there is a subpopulation with a high risk of the disease and that these people can be identified. Furthermore, the subpopulation should be small enough to make the cost of the program less than that for blanket screening. Thus, the definition of a high-risk group for the purposes of selective screening takes into account not only a group with a high incidence of the disease, but also the size of the group; in other words, the reduction in cost, and the proportion of cases originating in the high-risk group, that is the yield, should be considered.

- Selective screening is done where a condition is particularly common in a defined group. Antenatal screening for Tay Sachs disease in Ashkenazi Jews is an example. (Tay Sachs disease is an absence of hexoseaminidase A (αβ) and hexoseaminidase S (α2). Abnormality lies in α subunit, possibly because of a mutation at the α locus on chromosome 15. The incidence of this disease is 1 in 4000 among Jews. Its effects are quite severe and often result in death during infancy. There is no effective treatment for this disease). Selective screening is also generally done for hypercholesterolemia in members of families where there is a history of this condition or premature coronary heart disease or for smokers who comprise a high-risk group. In developed countries, it is a regular practice to screen neonatals for congenital hypothyroidism and phenylketonuria. Pilot screening programs are underway to screen people for congenital hyperplasia and medium chain fatty acid oxidation disorders, diabetes etc.
- Individual screening is done usually as antenatal screening of a fetus for an inherited disease when a previous child of the parents has the condition or when there is a strong family history of the condition. A number of inherited disorders have a mutation responsible for the disease. When a condition with severe consequences is detected, termination of the pregnancy with the genetic abnormality is considered.

4.Provision of information for teaching or research. Research-led teaching encourages reflective involvement in enhancing the student learning experience. The opportunity to experience research-led learning enriches the students. They are introduced to the principles of research methodologies as tools for knowledge creation and development. It raises cognizance of research, understanding why research methods exist and why they are important. Students gain experience in using research methods as a means of studying in order to develop research skills. Therefore, research and evaluation methodologies should be utilized for every student's learning. One can then compare and contrast several different research methods. Every student gains experience of using research and evaluation methods to address an original question, for example through a dissertation, project or design innovation activity. It encourages students to be able to select, justify and use a research method of their own choice from the array of methods available.

Students also gain experience of integrating original knowledge. They can select and justify an appropriate combination of several research methods and apply them to a question in hand. If students are engaged increasingly with research and research methods of their discipline, they would be able to contribute towards the development of evidence based knowledge. Modules can be made making use of topical findings from original research, to draw attention to the latest findings in an area.

5. To assess organ function in potential transplant donors. For transplants to be put in place, a number of biochemical tests need to done.

There are **pre-transplant tests** that are designed to provide a good overall picture of a transplant patient's health.

They also help identify other issues which may cause complications during the process.

The following Table 1.1 outlines many of the tests that are used to test a patient pre-transplant, and what they help to measure:

Table 1-1 Tests and What They Assess

Tests	What they assess
Physical examination	General overview of the patient's various conditions
Chest X-ray	Lungs and lower respiratory tract
Electrocardiogram (EKG or ECG)	Heart health and past undetected heart damage
Ultrasound with Doppler examination	Iliac vessel's function
Blood tests	Blood count, blood and tissue type, blood chemistry, immune system function and blood tests for certain infectious diseases
Blood typing	To determine blood type A, B, AB or O
Pulmonary function	Lung function and the blood's capacity to carry oxygen
Upper gastrointestinal (GI) series	Esophagus and stomach checks for disease
Lower GI series	Intestinal problems
Renal function studies	How well the kidneys are working; kidney function can also be measured with the serum creatinine test which is a blood test
Tissue typing	Markers on white blood cells which determine tissue type used to find an appropriate transplant match
Panel Reactive Antibody (PRA)	Immune system activity -- higher PRA means more antibodies are being made; the less activity here, the better chance the body will not reject the transplanted organ
Viral testing	Test for exposure to viruses such as hepatitis virus, cytomegalovirus, Epstein-Barr, and HIV
Mammogram	Presence/absence of breast cancer
Pap smear	Presence/absence of cervical cancer
Dental Evaluations	Healthy teeth and gums (regular dental check-ups are necessary while waiting for a transplant)

Histocompatibility Laboratory Tests are also performed to aid the transplantation procedure. Histocompatibility is compatibility between the tissues of different individuals, so that one accepts a graft from the other

without giving an immune reaction. There are three different kinds of histocompatibility tests done in a biochemical lab:

1. **Tissue Typing** which ensures proper organ match. This test is done on white blood cells which have special "markers" which gives a "tissue type". Tissue type is inherited from parents.
2. **Panel Reactive Antibody (PRA)** is an immune system activity test. A rigorous immune activity means that the body fights foreign objects (like a transplanted organ) more vigorously. Blood transfusions, pregnancy, previous transplant(s) or a current infection can cause the immune system to be more aggressive. The quieter one's immune system is, the easier it will be to get a transplant.
3. **Crossmatch Testing** –The crossmatch is the final step of pre-transfusion testing as a routine procedure. A portion of donor blood is combined with patient plasma or serum and is checked for agglutination, which would signify incompatible blood. This important step, also known as major crossmatch, serves as the last guard to ensure a safe transfusion. Crossmatch testing and its interpretation is shown in Table 1.2.

Table 1-2 Crossmatch Testing and its Interpretation

Crossmatch	Result	Interpretation
Major	Compatible	The transfusion can be given. The crossmatch does not detect very low titer antibodies.
Major	Agglutinins and/or hemolysins	The crossmatch is incompatible and the donor should **not** be used.
Minor	Compatible	The transfusion can be given.
Minor	Agglutinins and/or hemolysins	Preferably, washed or packed red cells from the donor should be administered. Dilution of the transfusion in the recipient usually eliminates any likelihood of antibodies from the donor affecting the recipient's red cells.
Autocontrol	Agglutinins and/or hemolysins	This reaction is usually seen in animals with immune-mediated hemolytic anemia. In these, interpretation of incompatible crossmatches is very difficult and a compatible donor may not be found.

Other **Clinical Laboratory Tests** are also performed. Some of them are mentioned below:

Blood Typing - There are four main blood types. (A, B, AB and O). The donor's blood type does not have to be the same as the recipient. However, it must be "compatible" with the recipient's blood type for him to receive an organ.

Blood type compatibility chart is given in Table 1.3

Table 1-3 Blood type Compatibility Chart

If one has blood type	One can receive an organ from a donor with blood type	One can donate an organ to a recipient with blood type
O	O	O, A, B, AB
A	A, O	A, AB
B	B, O	B, AB
AB	O, A, B, AB	AB

Viral Testing – For an organ transplant to be successful, it is important to know if the patient has ever been exposed to hepatitis virus, cytomegalovirus (CMV), Epstein-Barr virus (EBV), human immunodeficiency virus (HIV). Hence tests for these viruses are done before a transplant is put in place.

Post-transplant, a patient receives a schedule of follow-up clinic visits for lab tests and checkups when they leave sthe hospital. This is done to detect possible complications as quickly as possible and ensure overall health and wellbeing is good after the transplant.

Lab Tests like Kidney function, blood count, electrolytes and levels of medication in the patient's blood are all monitored in biochemical labs-. However, other tests may be ordered if needed, like:

Blood Count Tests:

- WBC – White Blood Cells count monitors the increase or decrease of white blood cells to determine if there could be an infection, or if the body has a lower defense against infection.
- HCT – Hematocrit test determines the percent of red cells within the blood; red blood cells carry oxygen to the body so when hema-

tocrit is low, it could cause the feeling of tiredness or having no energy.
- PLT – Platelets help the blood clot after an injury - low platelet levels can cause easy bruising and longer bleeding time after an injury or operation.

Kidney Function Tests (KFT):

- Creatinine and blood urea nitrogen (BUN) are waste products that are normally removed by the kidneys. KFT measure the amount of creatinine and blood urea nitrogen to determine how well the kidneys are carrying out their function.

Electrolyte Tests:

Electrolytes are dissolved minerals present in the human body. The balance of the electrolytes in our bodies is essential for normal function of our cells and our organs. Electrolytes that are routinely tested and their function in the body are shown in Table 1.4

Table 1-4 Electrolytes and Their Functions

Electrolytes	Function in the body
Ca (Calcium)	Strong bones, teeth, blood clotting, and heart and nerve function
PO_4 (Phosphate)	Strong bones (works with calcium)
Mg (Magnesium)	Muscle function and blood clotting
K (Potassium)	Heart and muscle function
Na (Sodium)	Salt and water balance
HCO_3 (Bicarbonate)	Acid balance
Cl (Chloride)	Salt and water balance

Other blood tests are done, like the **Glucose test** that measures sugar levels in the blood. For some patients, certain medications can produce a diabetes-like condition in which blood-sugar levels are too high (examples of such medicines are barbiturates, thiazide diuretics, corticosteroids, catecholamines, oral contraceptives, etc.). It is therefore imperative for the blood sugar of such patients be tested regularly post a transplantation.

Drug levels are monitored to measure the amount of immunosuppressant medication (e.g. Tacrolimus or Cyclosporine) in blood. These levels have

to be checked regularly, as high levels could lead to toxicity or over-immunosuppression, and low levels may lead to rejection. A “normal range” varies for every patient based on medications and length of time since transplant.

Some additional tests and procedures may also be performed to keep tabs on a patient's transplant, which are depicted in Table 1.5 below.

Table 1-5 Additional Tests

Test	Utility
Ultrasound	Checks for abnormal collections of fluid (blood) and allows monitoring of the main blood vessels leading to the organ to ensure they are functioning normally.
Biopsy	Used to test for rejection, this test involves the use of a needle to obtain a small piece of the transplanted organ which can be viewed under a microscope and be checked for rejection, or other possible problems.
Computerized tomography (CT) scan	A multi-view X ray which allows a physician to detect possible infections, fluid collections, or other problems in or around the kidney.
Magnetic resonance imaging (MRI)	Similar to a CT scan in terms of producing images, this test also allows a patient's organ to be viewed from different angles and in three-dimensional images. An MRI shows soft tissues, such as the kidney, more clearly than a CT scan.

6. During trials of drugs to check the drug efficacy and possibility of toxicity.

The pre-clinical testing evaluates the efficacy and the toxicity of the product before its possible administration as drugs to human beings.

Assessment of the efficacy. Whatever the drug selected, an analgesic or an antibiotic, it is necessary to thoroughly determine its principal property, to specify by a systematic study any other possible parallel effect on the other systems: cardiovascular, respiratory, renal, etc. A systematic exploration of

all the possible effects of a drug is always necessary. The studies are carried out in live animals, on isolated organs, isolated cells, isolated cellular fractions, enzymes, receptors. They specify the properties and the mechanisms of action. Generally, pharmacokinetic studies are also carried out in parallel to the efficacy ones, particularly to find the main metabolites.

Assessment of toxicity

Acute toxicity is studied by testing mortality after a single administration of the product under well-defined conditions to an animal species. It allows the determination of the lethal dose 50, or LD50, which is the dose which kills 50% of the treated animals in a determined time, for example ten days. LD50 of the same product depends on the animal species and the route of administration; it is generally lower (i.e. toxicity is higher) by parenteral route than by oral route.

Chronic toxicity testing studies the consequences of repeated administrations of the product being investigated. The product is given daily, once or twice a day, for a long time period, three to six months in general, according to the duration of administration envisaged in humans. The experimentation is carried out in two or three different animal species, mouse, rats, rabbits, each one receiving generally three different doses (low, medium, high) of the product.

When the drug is intended for a pediatric use, a complementary experimentation on young animals (a few days old) can be useful to detect a possible particular toxicity in children. Aspects like weight, intake of food and drink, behavior; and biological tests like hematologic, biochemical, pathological parameters are examined clinically as the signs of toxicity.

Toxicity and reproduction

Any molecule that is studied as a potential drug can be suspected to modify sexual activity, fertility and offspring if it is taken during pregnancy. The modifications of the **sexual activity** after administration of the product can be detected by studying couplings and **fertility** (frequency of gestations). A study of the spermatozoa can also be undertaken. A compound can have **toxic effects on the offspring** if it is taken during the gestation period. The term teratogen is used to characterize the toxicity of a compound, if any morphological or functional damage or growth retardation is induced in the offspring by the intake of a drug by the mother during gestation. The word embryotoxic is used to indicate the toxic effects elicited during the period of embryogenesis (the first two months of

the pregnancy in mankind) and the term fetotoxic is used to indicate the alterations induced during the second phase of the pregnancy (starting from the beginning of the third month). The experimentation is carried out on three animal species, in repeated administrations and at several doses. It involves the examination of the animals at birth and possibly later on. If important or frequent anomalies are observed, the product is contraindicated in pregnant women. If the anomalies are not more frequent than those occurring spontaneously, the teratogenic risk is low. Generally, the use of drugs by pregnant women is not advised.

Perinatal effect is also tested biochemically if a mother takes a drug a little before the labor, as it can diffuse through the placenta and affect the neonate. An example is drowsiness of the neonate after the intake of a sedative by the mother. Accidents where the baby can die before or just after birth can also occur in the majority of the cases where the mother had been taking in a drug.

Mutagen risk. A drug can also cause damage of the genome through a mutation which can be with or without consequences. If the change affects the genome of the germ cells, it is transmissible to the following generations. The search for mutations, part of genetic toxicology, is performed using in vitro tests on mutant strains, for example *Salmonella typhymurium.*

Carcinogenic risk. To check if a product could increase the risk of cancers, it is administered daily for a long time, from one to three years, to mice or rats. The experiment must be performed on animals of both the two sexes. This research is especially important for the drugs used for long lengths of time. Immunosuppressive agents can induce cancers and have to be specially checked.

Techniques used for estimating efficacy and safety of a drug range from the informal methods of individual physicians to randomized clinical trials with complex methodological designs (Table 1-6). There are five techniques used in evaluating drug safety and efficacy. These are: preclinical, informal, epidemiological and statistical, controlled clinical trials, and formal consensus development. **Preclinical**. Before a medical technology or drug is put to human experimentation it is evaluated through biochemical and animal tests. Chemical analyses for purity, quantity, and quality of the active agents are typically undertaken. Other filler and stabilizing substances are evaluated for potential pharmacological activity. Animal testing provides a guide to potential therapeutic activity and toxicity and also determine the degree of toxicity, or safety. Safety tests are done to