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Biological Monitoring III: Measurements in Blood

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Introduction

Blood represents the transport vehicle for chemicals and their metabolites in the body. Therefore, most determinants present in the body can be found in blood. The measurements represent the levels of determinants in peripheral blood during the short period of sampling.

The advantages of blood monitoring are the relatively small interindividual variation of those blood components which affect blood levels of most determinants, and a simple sampling technique which provides relatively little opportunity for contamination. The disadvantages are: 1) The sampling technique is invasive and can be performed only by medical personnel. 2) The samples deteriorate when not properly stored and transported. 3) Analytical procedures usually require a meticulous clean-up procedure.[1] Moreover, blood levels of determinants are significantly affected by the industrial environment and pharmacokinetic parameters.[2]

The determinant's level in blood tends to equilibrate with its level at the site of its entry in the body as well as with its levels in tissues and excreta. Therefore, the blood level of the determinant varies from one blood vessel to another.[3] The level at the site of entry can be given as the concentration in ambient air (in case of inhalation exposure), in a vehicle (in case of dermal exposure), or metabolite's concentration at metabolic sites (usually in liver). For the purpose of biological monitoring, the concentration differences in blood collected from different blood vessels can be significant on two occasions:

1. Blood collected from an arm which has been in direct contact with a chemical may have a higher concentration of the chemical than blood collected from another site.[1,4]
2. The pulmonary uptake or wash-out of volatile chemicals creates a concentration difference between arterial and venous blood.[5]

Therefore, measurements in capillary blood (mainly representing arterial blood levels) cannot be compared with biological exposure indices (BEIs) which are designed for venous blood.

Blood Circulation Function

Blood circulation has two functions: a) to supply oxygen, metabolic fuels, vitamins and hormones, and heat to every living cell of the organism; and b) to remove metabolic end products (carbon dioxide, water, urea, etc.) and excessive heat from every cell.[6] With exercise, the flow of blood to muscles increases enormously,[7] as it does to skin in a hot environment or the hepatoportal system after a meal.[6] Perfusion of other organs is relatively constant.

Blood flow carries the chemicals or their metabolites from sites of entry (usually lung or skin for parental chemicals and liver for metabolites) to other parts of the body. The transportation rate depends directly on perfusion of the organ. The rate of transfer of the chemicals or their metabolites from blood to tissues (i.e., tissue uptake rate) depends on the ratio of their solubility in tissue to that in blood.[3] For example, high lipid solubility prompts the accumulation of organic solvents in body fat.[8] The binding of the chemical or metabolite to a specific tissue constituent results in its accumulation at the binding site (e.g., binding causes accumulation of cadmium in kidneys and fluoride in bone).[9] Uptake of a chemical by the excretory organs is relatively high due to a large concentration gradient caused by removal of the chemical by excretion and metabolism.[10] After the exposure ceases, the determinant level in blood declines due to elimination. When the blood level drops below tissue levels, the transfer process reverses and the determinant is washed from the tissue into the blood.[3,11] The turning point does not appear in all tissues at the same time. Thus, determinants carried by blood from tissues with a higher saturation level are partly taken up by tissues with a lower saturation level (which is the redistribution process) and partly removed from the body by excretion or metabolism (which is the elimination process). Since levels of free (unbound) determinants in tissues and blood readily equilibrate, measurements of determinants in venous blood reflect the level of free determinant in the tissue perfused by the blood specimen. Since levels of the determinant in all tissues are not necessarily the same, the measurements in blood depend on the collection site and on the level of perfusion of the site. The mean level can be measured in arterial blood or in mixed venous blood. However, sampling of such blood specimens is not practicable in the field.

Blood Composition

Blood is a suspension of cellular elements in an aqueous solution of electrolytes and some nonelectrolytes and macromolecules.[12] Erythrocytes (red cells) are the main component of the cellular fraction. The straw-colored fluid in which the blood cells are suspended is plasma. Circulation maintains the cellular elements in suspension. Upon withdrawal of blood from circulation, the fluid and cells start to separate. Unless an anticoagulant is added, coagulation occurs and fibrinogen is removed from plasma into the clot. Plasma without fibrinogen is called serum. Fibrinogen can be retained in plasma if an anticoagulant is added to blood immediately after the sample is withdrawn. The anticoagulant can be a substance which binds calcium (oxalates, citrates or EDTA) or inactivates thrombin and thromboplastin (heparin). Coagulation can also be prevented by defibrination, i.e., by converting

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fibrinogen to fibrin using glass beads.[13]

The separation of fluids from cells can be achieved by sedimentation, i.e., allowing the blood to stand in a test tube, or by centrifuging. Centrifuging (at 3000 RPM) speeds up the separation process and makes the separation complete. The ratio of packed cell volume to total blood volume, measured in centrifuged blood, is the hematocrit. Normally cells occupy 47 percent in males (40–50%), and 42 percent in females (37–47%) of the total blood volume.[14]

Hemoglobin is an important component of red cells which facilitates oxygen transport. When the cellular membrane of red cells is ruptured, hemoglobin is released from cells and appears in plasma. This process is called hemolysis. In rare disease states, such as hemolytic anemia, hemolysis can appear *in vivo*. However, hemolysis of blood specimens during sampling, transportation, and storage is a common event which may affect the measurements. Hemolysis can be caused by some chemicals (saponin), by change of osmolar strength (adding water), by changes of temperature, and by agitation. Hemolysis is manifested by red coloration of plasma or serum.[13]

Chemicals and their metabolites can either be distributed equally or unequally in cellular and plasma water or they can be present exclusively in one fraction. The distribution depends on the ability of the chemical to cross the biomembrane. Hemolysis of a specimen allows the content of the red blood cells to leak into plasma or serum, which can compromise the measurements associated with one fraction of blood. For example, red cell cholinesterase and plasma cholinesterase are two different enzymes, the activity of which can be differentiated only in nonhemolyzed samples. Hexavalent chromium readily crosses the cellular membrane, but trivalent chromium does not. Benzene is equally distributed between plasma and cells, but its metabolite, phenol, is found in plasma and not in cells.[9]

Sampling, Storage, Analysis

In order to use a BEI as a reference value, the determinant must be analyzed in whole blood, plasma, or serum as specified in the BEI table.[15] Collection of venous blood is most common, but collection of capillary blood from fingers or ear lobes is also feasible in field conditions.[16] Capillary blood resembles arterial blood. Collection of capillary blood is not permissible under three conditions:

1. When more than 0.5 ml of blood is needed for analysis.
2. When samples collected in the workplace are to be analyzed for a chemical present in the working environment (because of an increased risk for external contamination of the specimen).
3. When a specimen is analyzed for volatile chemicals (because of loss by evaporation).

The appropriate anticoagulant must be selected after consultation with the laboratory since the choice may depend on the analytical method. For less than 10 ml of unclotted blood, the container should contain one of the following anticoagulants: 20 mg of potassium or sodium oxalate, 50 mg of sodium citrate, 15 mg of Na_2-EDTA, or 2 mg of heparin. The anticoagulant is dispersed in a concentrated solution along the bottom wall of the tube and then desiccated.[17] To prevent coagulation, the sample should be mixed by rotation of the specimen containers. Containers with the appropriate anticoagulants are commercially available. However, they should be used cautiously since the rubber stoppers may introduce biases by increasing or decreasing the determinant levels by contamination or absorption, respectively.

To obtain a good separation of serum from clotted cells, the sample should be cut around the wall of the container by a spatula or wire about ten minutes after withdrawal. This procedure, however, can be a source of contamination.

Hemolysis can create difficulties whenever the sample is analyzed by a spectrophotometric method. Hemolysis can be prevented by avoiding agitation and temperature changes during transportation. Samples of whole blood stored frozen are always hemolyzed. To prevent hemolysis, the samples should be centrifuged and plasma stored separately. For overnight storage, refrigeration at 4°C is usually satisfactory.[18]

Additional precautions to be taken in blood monitoring are as follows:

1. The skin is washed prior to sampling, usually with isopropyl alcohol; however, other cleaning disinfectants can be used. Precaution must be taken that the cleaning liquid does not contain the determinant and that the sampling site is not contaminated.
2. Heavy metals are usually present in blood in very small concentrations. Therefore, a significant contamination of the sample can occur. Blood for determination of heavy metal should be collected directly into an acid-washed, "heavy metal-free" glass container in order to minimize possible contamination.
3. To avoid errors induced by sedimentation, the samples must be well mixed before an aliquot is taken for analysis. Vigorous shaking should be avoided.
4. In the selection of analytical methods, consideration must be given to the fact that some determinants can be present in blood in three forms: free, conjugated, or bound to protein. For determination of the free fraction, specimens must be treated so that the bond is not disrupted. Samples, analyzed for "total determinant," require that the determinant is released from the binding site by acid or enzymatic hydrolysis. The yield of acid and enzymatic hydrolysis can be different when the determinant is bound to more than one species. For example; determinants such as phenol, pentachlorophenol, p-nitrophenol, p-aminophenol, and trichloroethanol are partly conjugated with glucuronic acid and partly with another conjugate, such as sulfate.[19] The measurements in acid hydrolysate are then higher than when β-glucuronidase is used for hydrolysis. To apply a BEI to such a determinant, the hydrolysis must be compatible with the procedure indicated in the BEI documentation.[9] Polar determinants usually bind to proteins. Therefore, the results of analysis may be pH-dependent. Chelating of metals by blood components or by anticoagulants makes the measurement dependent on the analytical procedure. The documentations to specific BEIs give advice as to which procedure should be used.[9]

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