Applications of Serum Protein Electrophoresis in Exotic Pet Medicine

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KEYWORDS

• Electrophoresis • Exotic pets • Protein

KEY POINTS

- With exotic species, progress in the interpretation of the alteration of plasma proteins has been discontinuous and irregular.
- In several exotic species, especially those of small size, the need to work with small volumes of blood requires the clinician to collect the blood with an anticoagulant.
- In clinical practice, the normal plasma protein concentration is measured very simply by a refractometer.

Hundreds of proteins, having several different functions, circulate in the plasma that are, with the exception of immunoglobulins, largely synthesized in the liver. Plasma proteins in the complex are responsible for the colloidal osmotic effect necessary to maintain blood volume. Other important functions of plasma proteins are the buffer function of the blood pH (15%–20% of the total buffering capacity), transport of hormones, transport of drugs, and blood clotting. Different types of proteins are indispensable for the inflammatory reaction, the immune reaction, and for the process of healing and tissue repair. So, it is easily understood that the measurement of plasma proteins and the determination of their alterations may constitute an important method for assessing the health condition of a patient. Human medicine, such as the dog and cat's, has taken advantage for decades of this valuable diagnostic tool. With exotic species, progress in the interpretation of the alteration of plasma proteins has been more discontinuous and irregular. Despite this, the usefulness of this test is indisputable. This article analyzes the different applications of plasma protein electrophoresis in small mammals, birds, and reptiles.

METHOD OF ASSESSING PROTEINS

Traditionally in the medicine of dogs and cats, the measurement of protein is performed on the serum, the fluid that is obtained by centrifugation of a blood sample

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allowed to clot in a test tube for 15 to 20 minutes, the time needed for fibrinogen to be used in the coagulation cascade. In several exotic species, especially of small size, the need to work with small volumes of blood requires the clinician to collect the blood with an anticoagulant (frequently natrium heparin or lithium heparin, sometimes heparinizing the syringe to avoid the coagulation of the sample during collection); therefore, centrifugation is achieved in plasma. The main difference between the 2 products is the absence in the serum of fribrinogen, the protein involved in the processes of coagulation; the concentration of total solids of the plasma is thus slightly higher than that of serum (about 5%) and the electrophoretic pattern from it will result in a higher incidence of β-globulin fraction where the fibrinogen normally migrates. In clinical practice, the normal plasma protein concentration is measured very simply by using a refractometer: a few drops of blood are collected in a heparinized capillary whose centrifugation gives the value of the microhematocrit (percentage of blood occupied by cells compared with plasma) and a small amount of plasma. Refractometry of that plasma allows easy estimation of the concentration of total solids. This method is simple and intuitive; however, it is not reliable if the sample quality is poor. Among the factors that interfere with the measurement of total solids by refractometer include hemolysis, lipemia, hyperbilirubinemia, azotemia, severe hyperglycemia, hypernatremia, hyperchloremia, and administration of colloids, including synthetic hemoglobin–based oxygen carriers. In the absence of these factors, the amount of nonprotein solids present in plasma is relatively constant, therefore by subtracting the nonprotein component (1.5 g/dL) the value of total plasma proteins is obtained.

An increase in the concentration of total solids (hyperproteinemia) must always be evaluated in correlation with the microhematocrit value and reflects mainly dehydration or increased synthesis of globulins for various pathologic conditions. The decrease in total solids (hypoproteinemia), vice versa, may reflect overhydration, decreased synthesis of albumin or immunoglobulin, or protein loss associated with hemorrhage, vasculitis, nephropathy, or enteropathy. With the exception of bleeding, which causes a loss of balanced albumins and globulins, in all pathologies involving protein loss albumin concentration falls to a greater extent than that of the globulins because of lower dimensions of the molecule, which allow easier migration through vascular endothelia. The measurement of total protein is certainly very useful, but for the purposes of a diagnosis that is as accurate as possible, it becomes essential to know the alterations of each protein fraction. For this purpose is used the characteristic of plasma proteins to be separated based on their charge and the consequent mobility in an electric field specially created. This method defines electrophoresis of proteins. The distribution of protein migrants on strips of cellulose acetate or agarose gel is represented graphically by a curve in which the extension and the height of each peak is corresponding to the breadth and the density of each protein fraction on the substrate migration.

Normally, 5 protein fractions are identified in the mammalian plasma, ordered according to the electric charge: albumin (high negative charge and low molecular weight, migrates to the anode and conventionally to the left of the track), α1-globulin, α2-globulin, and β-globulins and γ-globulins that present the greatest molecular weight and the lowest negative charge and then migrate to the right end of the curve. A different protein fraction is present in most birds and reptiles, with an even more negative charge than albumin that is positioned at the left in the same electrophoretic pattern and is therefore referred to as prealbumin. Conventionally, you find a point halfway between the beginning and end of the curve as the limit between the α2-globulin and β-globulins, and a point halfway to the start of β-globulins and the end of the track