# LEVELS OF CALCIUM, SODIUM AND POTASSIUM IN PLASMA AS INFLUENCED BY ANTICOAGULANTS

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**Abstract**

*Influence of anticoagulants in the levels of major electrolytes like calcium, sodium and potassium in plasma was studied and compared with their levels in serum. Forty two blood samples were collected from seven species namely human, elephant, cattle, sheep, goat, dog and rabbit. Heparin and Potassium Ethylene Diamine Tetra Acetate (EDTA) were used as anticoagulants. It was found that the levels of these minerals in the blood plasma got altered significantly by the addition of heparin and EDTA. However, less interference was rendered by heparin than EDTA, on comparison with the serum values.*

**Keywords**:- *EDTA, Heparin, Serum electrolytes, Plasma electrolytes.*

Anticoagulants are added in required quantity to inhibit the clotting of blood, thereby ensuring that the concentration of substance to be measured is changed as little as possible before analysis (Guder, 2001). Heparin and Ethylene Diamine Tetra Acetic acid (EDTA) exert their actions as anticoagulants by inhibiting thrombin in blood and chelating the calcium ions. Even though serum from coagulated blood is the most preferred specimen for clinical biochemistry, plasma obtained with an appropriate anticoagulant can also be served equally good. The most preferred anticoagulant meant for plasma clinical biochemistry is heparin, while for hematological examination is either sodium or potassium salt of EDTA. Since the harvest of serum requires a minimum of 30 min wait for the completion of coagulation before centrifugation, use of plasma is more specified in emergency situations. Furthermore, plasma yield from the given volume of whole blood is always greater than the yield of serum (Young and Bermes,1999).

The effects of various types of anticoagulants on plasma biochemistry have been studied in man and various animals. No comprehensive information is available with respect to levels of blood minerals like calcium, sodium and potassium in the serum and plasma collected using different anticoagulants of farm animals. Thus, the study was undertaken to compare the variations in the serum and plasma levels of calcium, sodium and potassium in various farm animal species and human and to determine and recommend the most suitable anticoagulant and its dose for separation of plasma from blood.

## Materials and Methods

About three ml of blood without anticoagulant was collected from clinically healthy subjects belonging to each species, viz. human, elephant, cattle, sheep, goat, dog and rabbit and serum was separated as per standard procedure. Anticoagulated blood samples (3ml) were collected simultaneously from each species with anticoagulant, dipotassium EDTA at concentrations of one, two, three and five mg per three ml blood as well as with lithium heparin, 60 units per three ml blood. All samples were chilled immediately after collection for 30 min, centrifuged at 1800 g for 10 min. The levels of calcium in the serum and anticoagulant added plasma were estimated spectrophotometrically using

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commercial kits following Arsenazo III method (Bauer, 1981). Sodium and potassium levels were estimated spectrophotometrically using commercial kits by the modified method described by Maruna and Trinder (1958). The results were analysed statistically using one-way ANOVA method.

## Results and Discussion

The serum and plasma levels of calcium, sodium and potassium of human and six domesticated species of animals viz. elephant, cattle, sheep, dog, goat and rabbit were estimated and compared with in group. It was found that the concentration of calcium in serum significantly ($p<0.05$) differed from that of plasma in all the species tested. The level of calcium was found to be significantly more in heparinised plasma compared to serum and other plasma samples (heparin and EDTA at varying concentrations). It was found that in cattle blood, addition of EDTA at the rate of 1 mg/ 3 ml completely chelated calcium in the blood. However in other species, this dose was not sufficient. Blood samples of human and sheep showed zero concentration of calcium when EDTA was added at the rate of 2mg/3ml. The blood from elephant and rabbit when collected with 5 mg EDTA/3ml, were left with trace amounts of free calcium in the plasma (Table 1).

Addition of heparin as anticoagulant for plasma separation resulted in significantly ($p<0.05$) high levels of sodium in the plasma samples when compared to serum in all species. Addition of EDTA to blood resulted significantly ($p<0.05$) higher levels of sodium in all the concentrations tested.. However, the variation seen in sodium level was not much relevant with different EDTA concentrations utilized (Table 2).

Heparinised plasma showed lesser degree of variations in potassium levels when compared to serum in all the species tested. However, significantly ($p<0.05$) higher increase in plasma potassium level was noticed when EDTA at different concentrations was used as anticoagulant (Table 3).

In the present study it was found that the levels of minerals like calcium, sodium and potassium in the blood was altered significantly by addition of natural anticoagulant heparin and EDTA. Changes in electrolyte contents induced by EDTA addition were described earlier in man ,dog, cattle, horse and sheep (Morris *et al.*, 2002; Mohri *et al.*, 2007 a, b; Ceron *et al.*, 2004). They have reported an increase in sodium level in

**Table 1.** Levels of calcium (mg/dl) in the serum and plasma.

| Species | Serum | Heparinised plasma | EDTA chelated blood plasma | | | |
|---|---|---|---|---|---|---|
| | | | 1 mg | 2 mg | 3 mg | 5 mg |
| Human | 7.77±0.049$^{c}$ | 8.62±0.060$^{d}$ | 2.33±0.049$^{b}$ | 0.0$^{a}$ | 0.0$^{a}$ | 0.0$^{a}$ |
| Elephant | 11.72±0.048$^{d}$ | 14.93±0.085$^{e}$ | 4.50±0.058$^{c}$ | 0.77±0.049$^{b}$ | 0.62±0.060$^{ab}$ | 0.50±0.05$^{a}$ |
| Cattle | 8.58±0.060$^{b}$ | 13.23±0.067$^{c}$ | 0.0$^{a}$ | 0.0$^{a}$ | 0.0$^{a}$ | 0.0$^{a}$ |
| Sheep | 7.67±0.049$^{c}$ | 9.18±0.060$^{d}$ | 0.82±0.060$^{b}$ | 0.0$^{a}$ | 0.0$^{a}$ | 0.0$^{a}$ |
| Goat | 6.88±0.060$^{d}$ | 7.62±0.060$^{e}$ | 1.52±0.060$^{c}$ | 1.02±0.060$^{b}$ | 1.02±0.060$^{b}$ | 0.0$^{a}$ |
| Dog | 6.90±0.097$^{d}$ | 11.63±0.073$^{e}$ | 1.10±0.068$^{c}$ | 1.02±0.060$^{c}$ | 0.62±0.048$^{b}$ | 0.0$^{a}$ |
| Rabbit | 11.73±0.067$^{e}$ | 13.50±0.058$^{f}$ | 6.62±0.048$^{d}$ | 3.35±0.076$^{c}$ | 1.78±0.095$^{b}$ | 0.62±0.087$^{a}$ |

*Means with the same superscripts in a row did not differ significantly (P<0.05)*

**Table 2.** Levels of sodium (mmol/l) in the serum and plasma.

| Species | Serum | Heparinised plasma | EDTA chelated blood plasma | | | |
|---|---|---|---|---|---|---|
| | | | 1 mg | 2 mg | 3 mg | 5 mg |
| Human | 146.43±0.071$^{a}$ | 181.32±0.060$^{c}$ | 186.73±0.076$^{e}$ | 191.22±0.060$^{f}$ | 184.33±0.067$^{d}$ | 162.40±0.073$^{b}$ |
| Elephant | 91.65±0.076$^{a}$ | 99.65±0.076$^{b}$ | 104.05±0.118$^{c}$ | 114.52±0.079$^{e}$ | 112.20±0.058$^{d}$ | 114.40±0.097$^{e}$ |
| Cattle | 160.67±0.067$^{a}$ | 163.77±0.067$^{b}$ | 167.50±0.073$^{c}$ | 177.42±0.060$^{d}$ | 178.75±0.076$^{e}$ | 180.72±0.048$^{f}$ |
| Sheep | 168.77±0.067$^{a}$ | 173.15±0.076$^{c}$ | 173.15±0.076$^{c}$ | 179.72±0.087$^{e}$ | 172.02±0.060$^{b}$ | 179.05±0.076$^{d}$ |
| Goat | 161.50±0.107$^{a}$ | 171.22±0.079$^{c}$ | 192.00±0.107$^{f}$ | 174.10±0.093$^{d}$ | 163.22±0.060$^{b}$ | 184.70±0.058$^{e}$ |
| Dog | 125.22±0.060$^{a}$ | 130.57±0.076$^{b}$ | 130.52±0.060$^{b}$ | 132.38±0.060$^{c}$ | 158.78±0.060$^{d}$ | 176.02±0.060$^{e}$ |
| Rabbit | 141.48±0.060$^{a}$ | 141.40±0.073$^{a}$ | 145.05±0.076$^{c}$ | 142.07±0.088$^{b}$ | 151.87±0.049$^{d}$ | 142.42±0.060$^{b}$ |

*Means with the same superscripts in a row did not differ significantly (P<0.05)*

**Table 3.** Levels of potassium (mmol/L) in the serum and plasma.

| Species | Serum | Heparinised plasma | EDTA chelated blood plasma | | | |
|---|---|---|---|---|---|---|
| | | | 1 mg | 2 mg | 3 mg | 5 mg |
| Human | 3.97±0.088$^{a}$ | 4.10±0.058$^{ab}$ | 4.32±0.060$^{b}$ | 4.67±0.088$^{c}$ | 4.55±0.076$^{c}$ | 4.23±0.067$^{b}$ |
| Elephant | 5.52±0.060$^{a}$ | 5.52±0.060$^{a}$ | 6.03±0.088$^{b}$ | 6.23±0.067$^{c}$ | 5.92±0.060$^{b}$ | 6.32±0.060$^{d}$ |
| Cattle | 6.38±0.108$^{a}$ | 6.42±0.070$^{b}$ | 6.52±0.095$^{bc}$ | 6.73±0.067$^{c}$ | 6.62±0.060$^{bc}$ | 7.42±0.060$^{d}$ |
| Sheep | 6.12±0.060$^{a}$ | 6.23±0.088$^{a}$ | 6.23±0.067$^{a}$ | 6.22±0.060$^{a}$ | 6.42±0.070$^{b}$ | 6.42±0.070$^{b}$ |
| Goat | 6.22±0.060$^{a}$ | 6.52±0.095$^{b}$ | 6.62±0.060$^{b}$ | 6.83±0.049$^{c}$ | 6.42±0.070$^{b}$ | 6.92±0.060$^{c}$ |
| Dog | 5.12±0.060$^{a}$ | 5.52±0.060$^{b}$ | 6.22±0.060$^{c}$ | 8.62±0.060$^{d}$ | 11.62±0.060$^{e}$ | 6.52±0.095$^{f}$ |
| Rabbit | 3.92±0.060$^{a}$ | 4.03±0.088$^{a}$ | 4.98±0.060$^{c}$ | 5.62±0.060$^{d}$ | 4.52±0.095$^{b}$ | 6.12±0.060$^{e}$ |

*Means with the same superscripts in a row did not differ significantly (P<0.05)*

heparinised plasma, while a decrease in potassium level as compared to the serum values. In the present study sodium as well potassium levels were on the higher side in the plasma samples collected with EDTA. Serum and heparinised plasma yielded similar results for sodium and potassium levels, while the calcium level in EDTA chelated blood plasma was significantly low. The reason for increased potassium level in heparinised and EDTA chelated blood plasma could be attributed to the activation of platelets.

EDTA acts as a chelating agent forming complexes with calcium which is essential for coagulation, is recommended for routine haematology, since it provides a very good staining quality of blood cells. It can be used as disodium, dipotassium or tripotassium salts. The latter two being preferred as they are more soluble.

The use of serum or plasma in clinical pathology remains debatable. Serum is preferred by many laboratories for clinical biochemical tests, since it avoids the addition of anticoagulants which can interfere with some analytical methods or change the concentration of parameters being measured. Using EDTA as anticoagulant caused a significant difference for concentrations of urea, creatinine, total protein, calcium, and magnesium and the activity of aspartate aminotransferase and alkaline phosphatase, in plasma comparing with serum (Mohri and Rezapoor, 2008).

In the serum stored at low temperature, the chance of formation of fibrin strands is lower when compared to plasma and thus there is less risk of occlusion of automated analyzers. However the use of plasma is preferred in some centres as it saves time because there is no necessity to wait until coagulation is completed and also 15 to 20 per cent more plasma can be obtained from the same volume of blood.

With the results of the study, it is recommended that the relative amounts of anticoagulants that can be used for separating plasma using heparin is 20U/ml of blood for all species, while that of dipotassium EDTA for cattle blood is 1mg/3ml, for sheep and human blood is 2mg/3ml, for goat and dog blood is 5mg/3ml and for rabbit and elephant blood is a minimum of 5mg/3ml or slightly above. Excess addition of EDTA and other anticoagulants would result in changes in the levels of other biochemical parameters. Results of the present study is also suggestive of the less interference rendered by heparin in the plasma electrolytes level.

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