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STUDIES ON IONIZED CALCIUM IN SERUM AND PLASMA FROM NORMAL COWS

ITS RELATION TO TOTAL SERUM CALCIUM AND THE EFFECTS OF SAMPLE STORING

By

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KVART, C. and L. LARSSON: *Studies on ionized calcium in serum and plasma from normal cows. Its relation to total serum calcium and the effects of sample storing.* Acta vet. scand. 1978, *19*, 487—496. — Ionized calcium has been determined with a new improved instrument on serum samples from 111 Swedish red-and-white cows. Simultaneous sampling of plasma and serum was performed in 32 cows for comparison of the ionized calcium level. Multiple sampling of plasma and serum from seven cows was performed to evaluate the effect of storage at 4°C and room temperature.

The normal range for ionized calcium found in this study implies that the ionized calcium fraction comprises for 43.4 ± 3.0 % (mean ± 2 s) of the total serum calcium. Simultaneous analyses on plasma and serum revealed that the plasma level of ionized calcium was generally 0.05 mmol/l lower than the serum value. pH changes in stored blood samples have a direct effect on the ionized calcium levels and is therefore to be avoided. Storing samples in vacutainers for five days at 4°C or for two days at room temperature was accompanied by only small decreases of serum or plasma ionized calcium.

The new instrument used in this study enables rapid analyses, and most of earlier drawbacks with calcium-ion-selective analyzers have been eliminated.

cow; Swedish red and white breed; ion-specific electrode; ionized calcium; total calcium; difference between plasma and serum; pH effect; effect of storage.

Calcium exists in blood in three forms, a free or ionized fraction (Ca_F), a fraction bound to plasma proteins and finally a fraction forming complex chemical compounds with different inorganic anions such as citrate and sulphate (*Fanconi & Rose* 1958). It is generally accepted that the physiological effects of calcium are exerted by the free calcium fraction (*McLean &*

Hastings 1934). Usually, however, calcium levels in blood are analyzed and expressed in terms of total plasma or serum calcium i.e. the sum of the three fractions. According to studies in normal sheep by *Belonje* (1973, 1976) the correlation between ionized and total calcium in blood is not good enough to allow reliable conclusions concerning plasma levels of free calcium from determinations of total calcium.

The sparsity of data on ionized calcium levels is mainly due to the methodological difficulties involved in such measurements with earlier available methods (*Ross* 1967, *Moore* 1970, *Rose* 1972). Lately, however, a new improved instrument for analysis of ionized calcium has been developed and become available (Orion Model SS-20). The versatility and applicability of this instrument has recently been evaluated (*Larsson & Öhman* 1978).

The aim of the present study was to establish normal data on the calcium homeostasis in the cow particularly with respect to the relationship between ionized calcium and total calcium. Comparisons of values for ionized calcium in bovine serum and plasma and the effects of sample storage at 4°C and at room temperature are also reported.

MATERIAL

Analyses were made on blood sampled from 111 cows of the Swedish red-and-white breed in different stages of lactation. None of the cows were in the week prior to or after parturition.

In 32 of the animals blood was simultaneously sampled for analyses on plasma. All sampling was done from the jugular vein with the Vacutainer® (Becton Dickinson) technique using 10 ml tubes. Non-heparinized tubes were used for the serum samples and heparinized tubes for the plasma samples. The samples were centrifuged at 2000 r.p.m. for 10 min. Most samples were analyzed within 24 hrs. after the blood collection. When this was not possible, samples were stored no longer than 4 days at 4°C.

METHOD AND RESULTS

Determination of ionized calcium

For this determination we used the new automatic calcium-ion analyzer Orion Model SS-20. This flow-through electrode system was used according to the instruction manual supplied by the manufacturer. Standard solutions and separate electrodes

were those delivered by the Orion Research Inc. In this machine analysis was performed automatically at 37°C and the results are visualized on a data panel 3 min. after the sample has been injected into the analyser. Every morning the analyser was routinely adjusted with three standards and the precision was tested with triple analysis. After every 20th unknown sample the electrode was controlled with a 1.0 mmol/l standard solution. The electrode was accepted if the deviation was less than ± 0.03 mmol/l.

Table 1. Total and ionized calcium, total protein, albumin and globulin in serum from 111 normal cows of the Swedish red-and-white breed. The values of total and ionized calcium are given in mmol/l and the concentration of the proteins in g/l. pH in the different samples ranged between 7.34 and 7.46. No correction of the Ca_F values was performed for pH differences.

	Mean	s	Range (mean ± 2 s)	
Total calcium (S-Ca_T)	2.51	0.16	(2.19—2.83)	mmol/l
Ionized calcium (S-Ca_F)	1.08	0.07	(0.94—1.22)	mmol/l
(Ca_F/Ca_T) · 100	43.4	3.0	(37.4—49.4)	%
S-albumin	39.8	3.0	(33.8—45.8)	g/l
S-protein	75.9	5.3	(65.3—86.5)	g/l
S-globulin	36.2	4.9	(26.4—46.0)	g/l
Albumin/Globulin	1.12	0.19	(0.74—1.50)	

Results from the 111 serum analyses are shown in Table 1. The distribution of serum total and ionized calcium can be seen in Fig. 1. Comparison of the Ca_F in plasma and serum from 32 cows gave a lower value for plasma with a mean difference of 0.05 mmol/l. A t-test for paired observations confirmed this difference as significant ($P < 0.001$).

Determination of pH

The blood pH was determined by a direct analysis with a thermostated glass electrode at 37°C (*Eisenman et al.* 1957).

Effects of storage and altered pH

The pH in the samples which were not stored or briefly stored at 4°C ranged between 7.34 and 7.46. Storage during five days at 4°C gave a decrease of about 5 % of ionized calcium in bovine

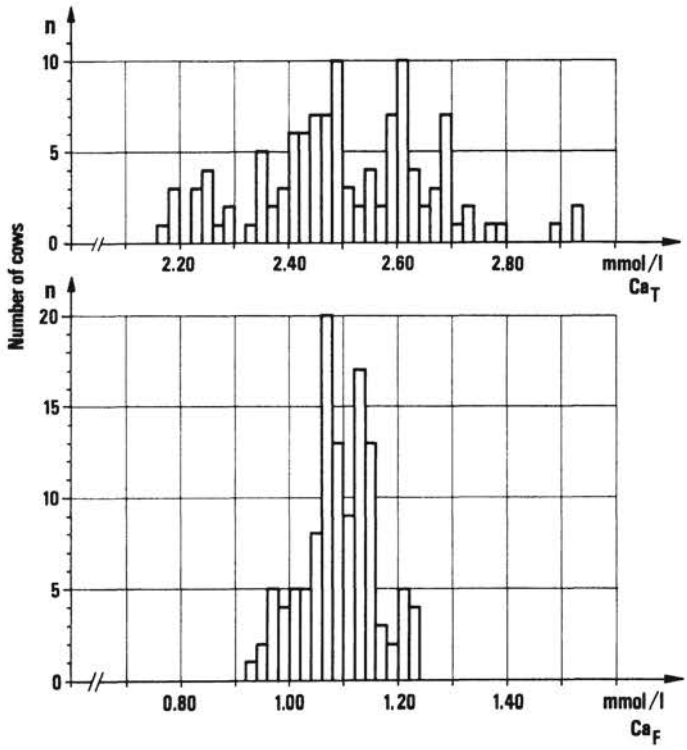

Figure 1. Distribution of serum total calcium (upper) and serum ionized calcium (lower) in 111 normal cows of the Swedish red-and-white breed. Values given in mmol/l.

serum and an increase of pH of on an average 0.13 units. There was a positive correlation between the pH increase and the decrease of ionized calcium in the samples so that a pH increase of 0.1 unit gave a decrease in S-Ca_F by about 4 % in the bovine serum. Similar effects have been found in human serum (*Larsson & Öhman* 1978). Simultaneous storage of plasma and serum under equal conditions from four cows gave an equivalent decrease of ionized calcium.

Determination of total S-Ca (Ca_T) and serum proteins

Total serum calcium was analyzed by an atomic absorption method (Perkin-Elmer). Total protein concentrations were estimated by a Biuret method. Albumin was determined by a spectrophotometric method with bromcresol green (*Doumas et al.* 1971). The results of these analyses are shown in Table 1.

Correlation between variables

The Ca_F in serum was correlated significantly to the Ca_T ($r = 0.36$, $P < 0.01$). Ca_T was weakly correlated to total protein ($r = 0.21$, $P < 0.01$) but somewhat stronger to albumin ($r = 0.33$, $P < 0.01$), while Ca_F was not at all correlated to these variables ($r = -0.04$ and $r = 0.08$, respectively). All correlations can be seen in Table 2. The correlation were calculated according to principles given by *Snedecor & Cochran* (1967).

Table 2. Correlations between ionized calcium or total calcium to protein, albumin and globulin in serum. The calculations were performed according to principles given by *Snedecor & Cochran* (1967).

Correlation between	Correlation coefficient
Ca_F and Ca_T	0.36
Ca_F and protein	—0.04
Ca_F and albumin	0.08
Ca_F and globulin	—0.09
Ca_T and protein	0.21
Ca_T and albumin	0.33
Ca_T and globulin	—0.04

DISCUSSION

The present method for measurement of ionized calcium has improved the possibilities to investigate different problems with regard to disturbances in the calcium homeostasis. Earlier methods used e.g. the murexid method (*Karlström* 1955, *Rose* 1957), different types of ion-selective electrodes (*Belonje* 1973) and ultra filtration technique (*Luthman & Persson* 1975) were, from all points of view, more demanding. The method used in this study has proved to have a high degree of precision, sensitivity and reliability (*Husdan et al.* 1977). It has also been shown that changes in the sodium balance within the physiological range do not interfere with the measurements. This is also the case with interference of potassium and magnesium (*Ladenson & Bowers* 1973).

The normal values for S-Ca_F for cows amounting to 43.4 ± 3.0 % (mean $\pm$ 2 s) of total calcium agree well with the data from other species (e.g. *Moore* 1969, *Sachs et al.* 1969, *Hattner et*

al. 1970, *Orrell* 1971, *Lindgärde & Zettervall* 1971, *Belonje* 1973, 1976, and *Fuchs et al.* 1976). For ewes and suckling lambs the P-Ca_F is reported to amount to 41.3 ± 4.4 % and 41.7 ± 5.7 % (mean ± 2 s), respectively, of the total calcium (*Belonje* 1976). In the non-pregnant ewes or ewes in early pregnancy the percentage of ionized calcium amounts to 47.0 ± 4.4 % (mean ± 2 s) (*Belonje* 1973). In man, finally, mean values between 41.4 % and 53.0 % have been reported (*Moore* 1969, *Sachs et al.* 1969, *Li & Piechocki* 1971, *Lindgärde & Zettervall* 1971).

No simultaneous analysis of ionized calcium in whole blood has been performed in this study, but good agreement between ionized calcium in human plasma and whole blood has recently been reported (*Fuchs et al.* 1976). In man, however, 0.05 mmol/l higher Ca_F values were reported for whole blood than for serum (*Larsson & Öhman* 1978). In the present study simultaneous analysis of Ca_F in serum and plasma was performed in 32 cows and a significantly lower value was found in plasma ($P < 0.001$). This is in agreement with earlier reports. *Li & Piechocki* and *Ladenson & Bowers* found that plasma values were about 0.03 mmol/l lower than serum values.

The values obtained for the additional bovine blood parameters determined were well within the normal ranges reported by e.g. *Hewett* (1974). Serum albumin and serum total protein have been reported to influence the total calcium concentration (*Orrell, Sachs et al.* 1971) but not the ionized calcium fraction. This was also evident in this study (Table 2). Competitive binding of free calcium and magnesium to albumin has been discussed by among others *MacIntyre* (1969). This competition is difficult to study as no sufficiently sensitive, direct method for determination of magnesium ion activity is available at present. Imbalances between these two ions as an explanation to different disturbances in the calcium homoestasis can thus, at present, not be studied in detail.

In agreement with earlier reports, a positive correlation between ionized calcium and total calcium was found in the present study ($P < 0.01$). This correlation ($r = 0.36$) is, however, not sufficiently high to allow for a prediction of the level of ionized calcium from the total calcium levels. Similar results have earlier been reported for both non-pregnant and early pregnant ewes (*Belonje* 1973) and for humans (*Hattner et al., Schwartz et al.* 1971 and *Ladenson & Bowers*). Data in the literature indicate

that the correlation between Ca_F and Ca_T values are, however, still reported to be unreliable in individual cases because of a large scatter around the calculated regression line (*Ladenson & Bowers*).

The importance of pH measurement at the same time as measurement of ionized calcium has been discussed by *Fuchs et al.* (1977) as well as by *Schwartz et al.* Previous investigators (*Moore* 1970, *Pedersen* 1970) have found a close correlation between pH and ionized calcium both in vivo and in vitro studies. The present study also established a correlation between these two variables. This fact suggests that the decrease of ionized calcium observed during storage is mainly due to the concomittant increase of pH. This pH increase is probably due to an unavoidable diffusion of CO_2 from the blood sample into the gas phase in the vacutainer.

In the present study reliable values for ionized calcium were obtained even after storage of the samples for five days at 4°C or for two days at room temperature. This shows that blood sampling under field conditions in bovine clinical cases is apparently no problem.

CONCLUSIONS

The new ionized calcium analyzer was found to be a convenient and reproducible instrument.

The reference range established was well in agreement with earlier reports both on different animals and human beings.

The correlation between total calcium and ionized calcium was not high enough in the cow to permit conclusions about ionized calcium levels from total calcium concentrations.

The effect of storage was analyzed, and in clinical practice it seems possible to use both serum and plasma for analysis of ionized calcium if the samples are transported and handled properly.

The difference between serum and plasma ionized calcium was found to be about 0.05 mmol, the plasma analyses showing the lower values.

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SAMMANFATTNING

Studier över joniserat calcium i serum och plasma från normala kor och dess relation till serumcalcium och effekten av lagring av proven.

Hos 111 SRB kor bestämdes fritt calcium i serum med en ny förbättrad calcium-jon-selektiv elektrod (Orion Model SS-20). Hos 32 av dessa togs prov för samtidig plasma- och serumbestämning av fritt calcium. Hos 7 av djuren utvärderades effekten av lagring av plasma och serum i såväl 4°C som i rumstemperatur.

Referensområdet för joniserat calcium definierat i denna studie stämde väl överens med i litteraturen tidigare angivna data. Joniserat calcium visade sig omfatta 43,4 ± 3,0 % (± 2 s) av total calcium. Analys av joniserat calcium i plasma och serum visade 0,05 mmol/l lägre värden i plasma än i serum. pH-förändringar i lagrat prov har en direkt effekt på joniserat calcium och bör därför undvikas. Vi fann det möjligt att lagra prover under åtminstone 5 dagar vid 4°C eller under åtminstone 2 dagar i rumstemperatur utan medföljande påtagliga förändringar av halten joniserat calcium.

Vi fann att det här använda instrumentet gav klart bättre möjlighet till snabba och mer tillförlitliga analyser utan de stora metodologiska svårigheter som varit förenade med tidigare använda metoder.

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