

**Brief Communication**

**SIMPLE METHODS FOR DETERMINATION OF TOTAL AND ULTRAFILTRABLE CALCIUM AND MAGNESIUM IN SERUM**

There are several methods for determination of ultrafiltrable calcium, most being based on filtration of serum under constant pressure, temperature and pH. Efficient cone formed membrane filters were recently introduced and reported to be suitable for preparation of ultrafiltrate of serum (*Farese & Mayer 1970*). The primary aim of the present investigation was to study the usefulness of these cone filters for the determination of ultrafiltrable calcium and magnesium in bovine serum.

Automatic titration apparatuses for the determination of calcium in biological fluids are nowadays available, and the aim of the study was also to evaluate the usefulness of such an apparatus.

Serum samples were obtained from cows in various stages of lactation and from cows suffering from parturient paresis. Urine was sampled from cows treated for various disorders at the stationary clinic of the department. For the determination of ultrafiltrable calcium and magnesium, blood was drawn from the jugular vein of normal cattle by means of a  $2 \times 80$  mm injection needle and a 20 ml plastic syringe. The blood was immediately transferred to a tube containing about 1.5 ml liquid paraffin. After the blood was coagulated the tubes were centrifuged to separate serum under the paraffin layer. Amicon Centriflo filter cones (Amicon Corp. Lexington, USA) were used for preparation of ultrafiltrates of serum. The filter cones were soaked in bidistilled water over night, and were then placed in a special adapter and centrifuged until almost complete dryness. About 2 ml of liquid paraffin was then placed in the cones, and 5 ml of serum was aspirated through the paraffin layer in the serum tubes and transferred to the bottom of the cones. A portion of serum was used for determination of total calcium and magnesium. The cones were thereafter centrifuged at about  $1000 \times g$  for 30 min. The clear ultrafiltrate of serum was collected in the outer tube of the adapter.

Calcium was analyzed in a Marius Calcium Titrator (Canalco Europe, Vlaardingen, Netherlands). The apparatus consists of a fluorimeter, a motorized buret and an amplifier coupled with necessary circuitry and switching functions for controlling the rate of titration. EGTA (0.001 M) was used as calcium chelating agent.

The sample sizes were 20  $\mu$ l and 100  $\mu$ l for serum and urine and 100  $\mu$ l for ultrafiltrate of serum.

Magnesium in serum and ultrafiltrates was analyzed according to the xylydyle blue method (Merckotest Magnesium, E. Merck, Darmstadt, Germany).

The results of the calcium determinations in serum and urine are shown in Table 1. When a serum sample of 100  $\mu$ l was used, the error of the method was  $\pm 0.98$  %, which is quite comparable with the error of atomic absorption spectrophotometry.

The titration apparatus was very easy to handle. The titration of 100  $\mu$ l of serum with a calcium concentration within the nor-

Table 1. Calcium (mg/100 ml) in serum and urine determined by automatic fluorimetric titration.

Sample size	n	Mean $\pm$ s	Range	Error of the method
Serum				
100 $\mu$ l	174	9.3 $\pm$ 1.11	4.1—11.7	$\pm 0.98$ %
20 $\mu$ l	42	9.4 $\pm$ 0.81	6.7—10.8	$\pm 2.54$ %
Urine				
100 l	41	23.7 $\pm$ 31.24	0.7—133.2	$\pm 1.81$ %
20 l	40	43.1 $\pm$ 41.01	6.5—186.8	$\pm 3.22$ %

Table 2. Ultrafiltrable calcium and magnesium in bovine blood serum (mg/100 ml).

	n	Concentration					
		total		ultrafiltrable		% ultrafiltrable	
		mean $\pm$ s	range	mean $\pm$ s	range	mean $\pm$ s	range
Calcium	29	9.4 $\pm$ 0.81	8.0—11.5	6.0 $\pm$ 0.72*	5.0—8.2	64 $\pm$ 4	54—71
Magnesium	30	2.1 $\pm$ 0.26	1.5—2.5	1.5 $\pm$ 0.20**	1.1—1.8	69 $\pm$ 5	62—81

\* error  $\pm 2.41$  %

\*\* error  $\pm 5.23$  %

mal range took about 45 sec. When 100  $\mu$ l of urine with a very high calcium concentration (160—180 mg/100 ml) was titrated, the titration time was up to 3 min. Extremely careful pipetting was always necessary for good results.

The great advantages with the apparatus are that it works rapidly, and further that hyperphosphataemia, hyperbilirubinaemia and haemolysis do not interfere with the calcium analysis (Alexander 1971, Raman & Chong 1974). The values for ultrafiltrable calcium and magnesium in bovine serum are shown in Table 2. Similar values were reported in humans by Piemonte *et al.* (1973) and Farese *et al.* (1970). As long as simple methods for the determination of ionized calcium are missing, the determination of ultrafiltrable calcium may be helpful in the study of calcium metabolism. The method used in the present study seems to be easy and reliable.

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