

CATION-EXCHANGE METHOD OF DETERMINING  
THE STATE OF MAGNESIUM, CALCIUM,  
AND STRONTIUM IN BLOOD PLASMA

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Adsorption of  $Mg^{++}$ ,  $Ca^{++}$ , and  $Sr^{++}$  ions from physiological solutions and from solutions containing canine blood plasma and physiological saline in different proportions was studied by a cation-exchange method on KU-2  $\times$  8 resin in the balanced salt form. Equilibrium constants were calculated from the experimental results and used to determine the molar fractions (or percentage content) of "free" and "bound" ions of the corresponding metals.

KEY WORDS: blood plasma; magnesium; calcium; strontium; cation-exchange method.

In normal human blood plasma 75-80% of  $Mg^{++}$  and 60-65% of  $Ca^{++}$  are in the ionized form [3] and the rest consists of protein-bound forms detectable by ultrafiltration and electrodialysis. These methods evidently do not give a complete picture, for complex particles with low-molecular-weight plasma ligands can evidently also pass through semipermeable membranes.

There is thus a need for the development of new methods of determining the content of substances in biological systems; the writers suggest the use of a cation-exchange method for this purpose, such as is widely used in the chemistry of unstable complex compounds which cannot be isolated from solutions by the methods of preparative chemistry [1, 2, 4]. The suggested method of determining the state of ions is based on the study of adsorption of the test substances on a cation-exchange resin during dilution of canine blood plasma with physiological saline. Under these circumstances the plasma is regarded as a "collective ligand," the composition of which is independent of dilution and, consequently, the character of the chemical reactions is unchanged.

EXPERIMENTAL METHOD

The cation-exchange resin KU-2  $\times$  8 ( $H^+$  form) (0.1-0.25 mm) was flushed to pH 7 with a solution of the following composition: NaCl 0.11 N, KCl  $5 \cdot 10^{-3}$  N,  $MgCl_2$   $2 \cdot 10^{-3}$  N,  $CaCl_2$   $5 \cdot 10^{-3}$  N,  $NH_4Cl$  0.0001%. A solution of the same composition was used to prepare the resin for adsorption of  $Sr^{90}$ , and in the experiments with Mg and Ca,  $MgCl_2$  and  $CaCl_2$  respectively were excluded from the solution. Weighed samples of the cation-exchange resin in the balanced salt form were treated with canine blood plasma and 0.85% NaCl solution in different proportions. A plasma concentration [PL] of 1.0 corresponds to whole plasma and 0.0 to pure physiological saline.

Known quantities of  $MgCl_2$ ,  $Ca^{45}Cl_2$ , and  $Sr^{90}Cl_2$  were added separately to the resin-plasma system, the vessels were shaken for 6 h at 25°C, the resin was then allowed to stand, and samples were taken from the solution for measurement of the magnesium concentration (by the atomic absorption method) or the radioactivity of  $Ca^{45}$  and  $Sr^{90}$ . The  $Sr^{90}$  was measured after radioactive equilibrium had been reached 15 days later, on the PP-8 apparatus with a  $\beta$ -counter.

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TABLE 1. Adsorption of Magnesium, Calcium, and Strontium from Canine Blood Plasma (mean results of three series of experiments);  $M \pm m$

[PL] (in fractions of normal)	Magnesium ( $MgCl_2$ )		Calcium ( $Ca^{45}Cl_2$ )		Strontium ( $Sr^{90}Cl_2$ )	
	$K$	$K_e$	$K$	$K_e$	$K$	$K_e$
0,00	83±4	—	130±10	—	2250±170	—
0,20	73±4	0,68	102±10	1,35	1580±130	2,10
0,40	69±4	0,50	85±6	1,32	1160±100	2,32
0,60	62±3	0,56	73±5	1,30	980±60	2,17
0,80	59±3	0,51	60±5	1,46	840±50	2,12
0,90	—	—	56±4	1,46	790±30	2,06
1,00	53±4	0,57	52±4	1,50	720±30	2,14
Mean value of $K_e$	—	0,56±0,5	—	1,40±0,04	—	2,16±0,05
Molar fraction of ionic form	0,64		0,42		0,32	
Molar fraction of bound form	0,36		0,58		0,68	

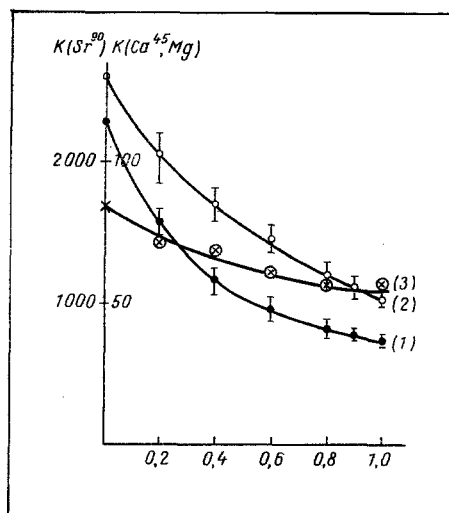


Fig. 1. Values of  $K$  for strontium (1), calcium (2), and magnesium (3) as functions of [PL]. Abscissa, [PL] (in fractions of normal); ordinate, value of  $K$ .

Adsorption of the substances was characterized by the partition coefficient

$$K = \frac{C_0 - C}{C} \cdot \frac{v}{m},$$

where  $C_0$  and  $C$  denote the initial and equilibrium concentrations or radioactivities of the solution respectively;  $v$  the volume of solution;  $m$  the mass of resin.

## EXPERIMENTAL RESULTS AND DISCUSSION

As Table 1 and Fig. 1 show, with an increase in [PL] the value of  $K$  fell steadily for all elements, which is correct for complex formation with the components of plasma. From the experimental results the equilibrium constant ( $K_e$ ) for complex formation with the components of plasma was calculated by means of the equation in [2, 4]:  $K = K_0 / (1 + K_e \cdot [PL])$ , or in a convenient form for the calculation:

$$K_0 / K - 1[PL] = K_e,$$

where  $K_0$  is the partition coefficient of the test substances in 0.85% NaCl at [PL]=0. The constancy of the left-hand side of this last equation points to the formation of complex particles of the same composition.

The molar fractions of free and bound forms of magnesium, calcium, and strontium for blood plasma, for which  $[PL] = 1$ , were calculated from  $K_e$ .

It follows from the results in Table 1 that the alkaline-earth elements in whole canine blood plasma are present as ions (from 64% for  $Mg^{++}$  to 32% for  $Sr^{++}$ ) and as complex particles of the general composition 1:1. The overwhelming majority of the latter are chemically bound with proteins of the blood plasma, forming the protein-bound forms.

It will be noted that the molar fraction of the ionic forms obtained in this investigation is rather lower than the results described in the literature [3], obtained by methods of dialysis and ultrafiltration. The reason is that the method used was based on ion exchange, its molecular mechanism is well known, and conclusions drawn from it are unambiguous. The low values of the conventional  $K_e$  show that equilibrium for binding of  $Mg^{++}$ ,  $Ca^{++}$ , and  $Sr^{++}$  ions may be easily shifted toward the lower side. For example, for a solution containing 70% plasma ( $[PL] = 0.7$ ) the molar fraction is 0.72 for  $Mg^{++}$ , 0.51 for  $Ca^{++}$ , and 0.40 for  $Sr^{++}$ .

The suggested method is universal: it can be used to determine the state of the ions studied in this case (and also many others) under normal and various pathological conditions.

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