

## Distribution of Calcium and Strontium in the Blood of the Laying Hen.\* (31856)

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Some workers(1,2,3) have concluded that bone does not discriminate significantly against Sr in favor of Ca in passage from blood to bone, whereas others(4) reported that the blood-bone barrier discriminates by a factor of as much as 1.6 in favor of Ca and against Sr. On the other hand, numerous papers(5,6,7,8) devoted to the comparative study of the renal excretion of these 2 isotopes have revealed that Sr is more quickly eliminated by feces and urine than is Ca. This preferential elimination of Sr in urine may be due to the differences in their distribution pattern in blood serum. No successful attempt has been made to compare the behavior of Ca and Sr toward the binding effect of serum protein. Since Ca in blood serum is partially bound to serum protein(9), the same may be true for Sr. The exact distribution of Ca in blood serum protein has not been clearly elucidated. This study was undertaken to determine and compare quantitatively the distribution of Ca and Sr ions bound to serum protein.

*Experimental Procedure.* Two laying hens fed a practical laying ration (Texas A&M University Poultry Research Center) were used for this study. One hen received 25  $\mu\text{C}$  of  $\text{Sr}^{85}$  intramuscularly. The other was not injected and was used as a control. After 3 hours, 10 ml of blood were collected from each hen by heart puncture. The blood samples were allowed to clot and then carefully centrifuged to collect serum. Serum was stored in a refrigerator and after 24 hours Sr ions and Ca ions were separated from the serum proteins on Sephadex G-25 column. A small sample of serum (1 ml for Ca and 2 ml for  $\text{Sr}^{85}$ ) was applied to the column (76  $\times$

1.3 cm) which was equilibrated with 0.05 M tris (hydroxymethyl) aminomethane buffer (pH 7.4). When the serum was completely absorbed onto the column it was eluted with the tris buffer. Several fractions of definite volume (5 ml in case of  $\text{Sr}^{85}$  and 2.0 ml in case of Ca) were collected until the total eluted volume was at least 5 times that of the void volume of the column. This was done to ensure that all of the sample that was put on the column was filtered through the column.

Estimation of protein in the fractions was done by UV absorbance at 280  $\text{m}\mu$ . Calcium concentration in the fractions was determined on the Perkin Elmer atomic absorption spectrometer model 303, using an acetylene and compressed air burner by the method of Willis(10).  $\text{Sr}^{85}$  activity was measured by conventional NaI (T1) (1"  $\times$  1") crystal detector coupled to a single channel  $\gamma$ -ray spectrometer.  $\text{Sr}^{85}\text{Cl}$  was obtained from Union Carbide, Oak Ridge National Laboratories, Oak Ridge, Tenn.

*Results and discussion.* Sephadex has been widely used for the separation of free ions from proteins. Fig. 1a shows the elution pattern of serum obtained from a laying hen injected with 25  $\mu\text{C}$  of  $\text{Sr}^{85}\text{Cl}_2$ . The protein fraction did not contain any  $\text{Sr}^{85}$  activity, indicating a complete absence of protein bound  $\text{Sr}^{85}$ . Walser(9), however, using infusion techniques, observed that approximately 20% of the  $\text{Sr}^{85}$  was protein bound while there was 30% protein binding of Ca in human serum. The gel filtration pattern and Ca concentrations of normal serum are given in Fig. 1b. Unlike Sr, 35% of the Ca found in the serum was bound to protein.

The kidney has been shown to preferentially excrete  $\text{Sr}^{85}$  over  $\text{Ca}^{45}$ (5). Excretion through the kidney is the net result of glomerular filtration and tubular reabsorption. The glomeruli act as ultra filters and filter

\* Supported in part by U.S. Public Health Service Research Grant RH00354, National Center for Radiological Health.

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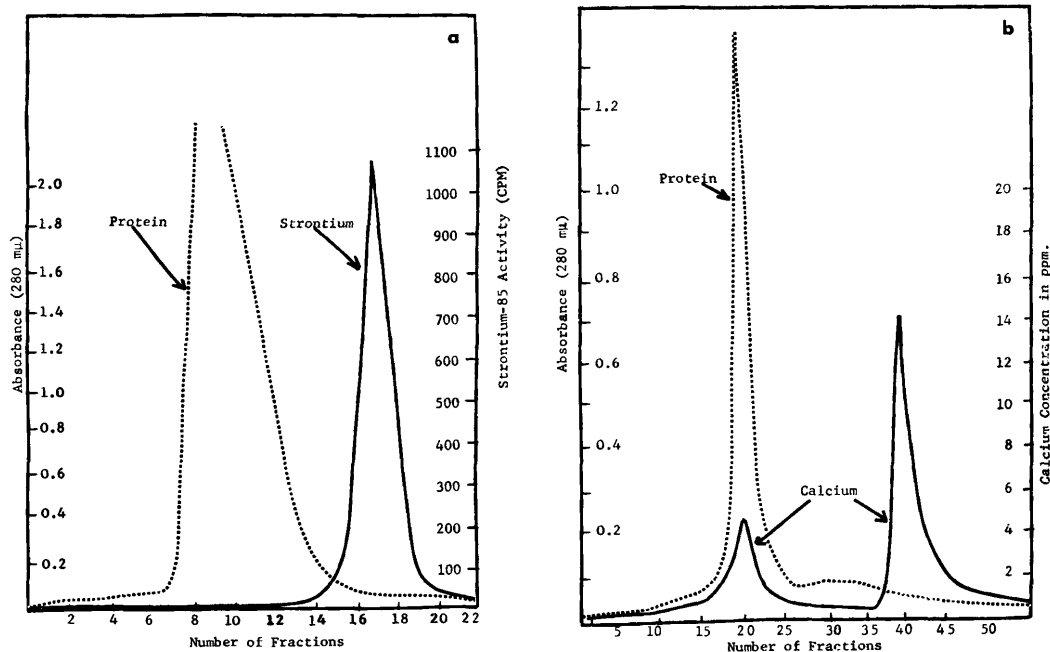


FIG. 1a. Separation of free Sr ions from blood serum proteins on Sephadex G-25 column. Experimental conditions: sample volume = 1 ml; bed dimensions =  $76 \times 1.3$  cm; flow rate 9 ml/h; eluting buffer = .05 M tris (hydroxymethyl) aminomethane; pH = 7.4; eluting volume of each fraction = 5 ml.

FIG. 1b. Separation of free Ca ions from blood serum proteins on Sephadex G-25 column. Experimental conditions: sample volume = 1 ml; bed dimensions =  $76 \times 1.3$  cm; flow rate 9 ml/hr; eluting buffer = .05 M tris (hydroxymethyl) aminomethane; pH = 7.4; eluting volume of each fraction = 5 ml.

all the constituents of plasma except the colloids (*i.e.*, proteins and fats). Ions that are bound to protein, therefore, will not be filtered. The fact that Sr behaves like a free ion in the plasma, while Ca, according to these data, is 35% bound to proteins, partly explains the preferential excretion of Sr<sup>85</sup> over Ca<sup>45</sup> by the kidney due to filtration, but does not provide information concerning tubular reabsorption.

**Summary.** Serum collected from a hen which had been given 25  $\mu$ c Sr<sup>85</sup> was separated on Sephadex G-25 column and found to contain Sr<sup>85</sup> activity only in the non-protein fractions. On the other hand, the serum of a control hen showed the Ca to be present in both protein and non-protein fractions. These data suggest that, unlike Ca, Sr is not bound to proteins in the blood.

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Received November 4, 1966. P.S.E.B.M., 1967, v124.