Paper Electrophoretic Study of Ca^{45} Binding in Sera of Normal, Parathyroidectomized and Estrogen Treated Rats.* (23089)

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Maintenance of a normal level of ionic calcium in body fluids is of great biological importance. Blood calcium exists in a diffusible and non-diffusible state, the former being biologically active and the latter bound to the plasma proteins. Equilibrium between ionized and non-ionized calcium is influenced by pH, diffusible calcium decreases with increased pH(1,2). The parathyroid hormone tends to increase the ionized calcium(3), whereas, estrogen increases the protein-bound calcium(4). These hormones may act to influence the calcium binding properties of the different plasma proteins. Visek *et al.*(5) presented evidence that Ca^{45} added to serum was readily interchangeable with the Ca^{40} present, and within a few minutes equilibrium was attained. Some investigators have used this technic to study electrophoretically the components responsible for calcium binding. Electrophoresis of cattle blood to which had been added Ca^{45} failed to show the existence of a blood fraction of higher than average specific activity(6). Clegg *et al.*(7) added Ca^{45} to the buffer solution used to dilute the serum before dialysis in an effort to increase the Ca-proteinate. In normal animals they found Ca^{45} bound to albumin; in the sera of diethylstilbestrol-treated cockerels addition of Ca^{45} reduced mobility of the leading component (albumin) which migrated to the area considered to represent the β-globulin fraction. This behavior is not clear. If addition of Ca^{45} results only in an interchange of Ca^{40} and Ca^{45}(5), and the addition of calcium scarcely increases the amount of Ca-proteinate(8), then the results of Clegg *et al.*(7) may be an artifact attributable to the procedure. This report concerns the same problem modified by *in vivo* injection of Ca^{45} prior to paper electrophoresis of blood sera of normal, parathyroidectomized and/or estrogen-treated rats.

Materials and methods. Twenty-nine adult male albino rats were divided into 4 groups and subjected to the following treatments: The control group of 8 rats received a total dosage of 2.65 μc of Ca^{45} in the form of $CaCl_2$ (specific activity>0.2 mc/g Ca)/100 g body weight, intraperitoneally for 4 days and sacrificed on the sixth day. The second group of 8 rats were injected subcutaneously for 7 days with estradiol benzoate, at daily dosage of 82.5 μg in 0.25 ml of sesame oil. The third group of 6 rats were parathyroidectomized (chloroform anesthesia). Group four (7 rats) were parathyroidectomized and treated similar to Group 2 with estradiol benzoate. The animals in Groups 2 to 4 received Ca^{45} during 3d to 7th days in the same dosage as controls before being sacrificed on eighth day. All animals were bled to death (chloroform anesthesia) from the left carotid artery. The sandwich technic of paper electrophoresis was employed on samples of 0.5 ml of fresh, undiluted sera using filter paper (Whatman No. 4) with a cross section of 2.5 inches and an electric field length of 11 inches. The D.C. applied was 7 milliamperes for 12 hours. Veronal solution with pH of 8.6 and molarity of 0.015 for the acid and 0.07 for the salt, was the buffer. After electrophoresis the strip was rapidly dried on horizontal rack for 30 minutes at temperature of 120° to 130°C. Seven disks 1 inch in diameter were cut in series from the paper strip for measuring radioactivity. The remainder of paper strip was stained in the normal manner (bromphenol blue) for observation of protein fractions. Sera samples were not dialyzed since the migration rates between calcium ions and protein were sufficient to separate one from the other. It was necessary to measure ra-

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dioactivity before staining because the procedure (in acid medium) dissolved the calcium from the paper strip. Radioactivity was determined by scintillation detector employing a cylindrical anthracene crystal 2.5 cm in diameter, coupled to a photomultiplier tube and decade scaler. The distance between the anthracene crystal and paper disk samples was less than 1 mm.

Results. The sandwich technic of paper electrophoresis imposes definite limitations, nevertheless it is valid to conclude from the results obtained that the ionized calcium migrated at a greater rate than the albumin. In the sera of normal animals the various protein fractions contained different amounts of Ca^{45}; the highest amounts were found in albumin and α-globulin. After parathyroidectomy there was a tendency for the amount of Ca^{45} bound to the albumin to decrease and the amount in the α-globulin to increase. In the group of estrogen-treated rats most of the Ca^{45} was in the β- and gamma-globulin components. Variations occurred in the parathyroidectomized - estrogen - treated group; highest amounts of Ca^{45} were found in either the albumin, α- or β-globulin fractions.

The present results differ from those of previous investigators. This may be due to the technic used or to species difference. Concerning the relationship between ionized and non-ionized calcium, McLean and Hastings (9) believe that Ca-proteinate behaves as a weak electrolyte and that a simple mass law equation expresses the relationship between these forms. If this is true for a single Ca-proteinate, it may not be valid for calcium binding in a complex system of proteins with different affinities and changing ratios in the sera of normal and experimental animals.

Summary. (1) In the normal rat most of the Ca^{45} is bound to albumin and α-globulin. (2) After parathyroidectomy the Ca^{45} decreases in the albumin and increases in the α-globulin fraction. (3) In animals treated with estrogen most of the Ca^{45} was present in the β and gamma-globulin components. (4) In the parathyroidectomized-estrogen-treated animals highest amounts of Ca^{45} were found in either albumin, α- or β-globulin fractions.

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