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STUDIES ON THE ULTRAFILTRATION OF SERUM CALCIUM

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For many years, Medicine has concerned itself with levels of calcium in and out of serum. Attempts have been made to define its level, to fractionate it and to use it diagnostically. The present communication deals with 166 determinations of serum ultrafilterable calcium levels in 150 individuals, with attempts to draw certain conclusions, regarding its value in clinical medicine. Numerous papers have appeared on the subject.1-26

Initially it was assumed that serum calcium was available in but a single form. However in 1871, based on evidence gathered by Pribram,1 it was suggested that more than a single form occurred. Using a semipermeable membrane Rona and Takahashi,2 in 1911 and in 1913, demonstrated the existence of two forms: A diffusible (ultrafilterable) and a nondiffusible (nonultrafilterable). They alluded to the presence of a third (supposedly a calcium soap).

MacLean and Hastings3-5 in 1934 developed a purely biological "frog-heart" method for determining the diffusible fraction. In so doing, they concluded that part of this fraction was not ionized. Estimates of the ultrafilterable calcium, comprising both ionized and non-ionized forms, have ranged from 40 to 75 per cent of the total serum calcium. One source, using an electrochemical method, estimated the value as low as 10 per cent.14

Several facts have emerged from our current knowledge of serum calcium.

I. Serum calcium exists in three fractions:

a. "The protein-bound, nondiffusible, nonultrafilterable calcium." This fraction constitutes 40 to 50 per cent of the total. It is largely bound to albumin. The beta globulins and cephalins bind calcium to a lesser extent.

b. "The ultrafilterable, ionized calcium." This fraction constitutes 50 to 60 per cent of the total.

c. "The ultrafilterable, nonionized calcium." This fraction, also known as the "complexed calcium", constitutes less than five per cent of the total. It occurs as the salt of the various organic acids such as calcium citrate.

From the Department of Medicine.
II. The ultrafilterable calciums are the physiologically active fractions.  

III. The complexed calciums appear to play a role in the renal clearance of calcium. 

In this report we are interested only in the role of ultra-filterable calcium as it relates to a group of diseases having disturbances in calcium metabolism or in diseases in which the serum ultrafilterable calcium has been reported to be altered, hoping to clarify its usefulness and place in clinical medicine. 

Methods for determination of ionized calcium, to the present at least, have been too cumbersome for routine clinical use. However, a newer method developed by Lumb and based on a modification of the technique of Ettori and Scoggan is less awkward and apparently not subject to the objections of time and error. For the future, values for ionized calcium in serum may be demonstrated to have greater clinical application than that for ultrafilterable calcium alone. 

METHODS 

Blood specimens were collected from fasting, healthy subjects including executives and technicians, and also patients having a variety of illnesses known to have calcium aberrations during some part of their course. The blood, collected under oil by the same technicians, was allowed to clot in Pyrex glassware. The specimens throughout were handled anaerobically. Ultrafiltrate was obtained using the centrifuge technique of Prasad and Flink. 

Prior equilibration of serum with five per cent CO₂ — 95 per cent O₂ was not done since, in reviewing the literature, significantly higher ultrafilterable values were encountered. Evidence confirmed in this laboratory suggests that an artefact is introduced whenever any type of CO₂ equilibration is used. 

Serum pH before and after ultrafiltration was determined using an anaerobic electrode with the Beckman Model G pH Meter. All serum transfers were done inside a plastic tent, ballooned up with the CO₂ — O₂ gas mixture. Final pH of the residue within the dialysis sac after ultrafiltration ranged from 7.2 to 7.7. 

Calcium determinations were run in duplicate using the method of Ferro and Ham. The insoluble calcium chloranilate, precipitated by the sodium salt of chloranilic acid after having been allowed to stand for at least two hours, was washed twice with isopropanol to remove excess reagent and to minimize the possibility of lipid turbidity in the final solution. 

The precipitate was then dissolved in the tetrasodium salt of ethylene-diamine tetra acetic acid (EDTA) forming a pink-colored chelate whose optical density was determined at 520 millimicrons. The results are consistent with Beer’s Law in the range of 4 mg/100 ml to 40 mg/100 ml, with the accuracy and reproducibility of the oxalate precipitation method of Clark and Collip. 

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RESULTS

Group I — Normal.

Fifty-two determinations on 51 individuals yielded a mean total serum calcium of $9.7 \pm 0.11 \text{ mg/100 ml}$ with a mean ultrafilterable calcium of $5.3 \pm 0.22 \text{ mg/100 ml}$. The percentage of ultrafilterable calcium was $55 \pm 4$ percent. The distribution of the values was that of a normal bell-shaped curve with the lowest value 42 per cent and the highest 80 per cent. While the majority of values fell in the relative narrow range of $55 \pm 4$ per cent, the occasional marked deviation in the normal group is noted.

Of the group 27 were female, 24 male. The ages ranged from 14 to 66 years with an average of 45.6 years. There was no significant age or sex difference. Serum protein abnormalities were not present and all individuals were judged healthy. See Scattergraph I.

Group II — Malignancies.

Twenty-five determinations on 22 individuals having a variety of malignant diseases with or without osseous spread revealed a mean total calcium of $10.5 \text{ mg/100 ml}$

![Scattergraph I](image)

Note the diagonal placement for both groups indicating the direct relationship of the ultrafilterable calcium to the total calcium.
and an ultrafilterable calcium of 6.0 mg/100 ml, or 57 per cent. There was no obvious difference between those with or without bony metastasis or those individuals having sarcoma or carcinoma. The highest total calcium was 18.2 and the lowest 7.1 mg/100 ml. Sixteen of these individuals were female, reflecting the preponderance of breast carcinoma.

From Scattergraph I it can be appreciated that a high ultrafilterable calcium invariably accompanies a high total serum calcium. In those instances where increased ultrafilterability was noted serum albumin was found to be decreased.

Group III — Hyperparathyroidism.

Nineteen determinations on 11 individuals with surgically proved hyperparathyroidism yielded an average total calcium of 11.4 mg/100 ml and an ultrafilterable value of 6.1 mg/100 ml, or 58 per cent. The lowest total calcium was 10.7 mg/100 ml occurring in a patient who had polydipsia and polyuria. The ultrafilterable value in this instance was normal. There were seven females and four males. The average age was 53 years. See Scattergraph II.

\[\text{Scattergraph II}\]

The direct relationship between ultrafilterable calcium to total calcium in hyperparathyroidism and hypoparathyroidism is apparent.
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Group IV — Hypoparathyroidism.

Fourteen determinations were run on 11 individuals categorized as having either postoperative or idiopathic hypoparathyroidism. The average total calcium was 7.6 mg/100 ml with 4.6 mg/100 ml ultrafilterable, or 60 per cent. There were 10 females and one male reflecting the greater incidence of thyroid surgery in the female. The average age was 44.2 years. No member of this group had serum protein abnormalities.

Group V — Multiple Myeloma.

Eight determinations on eight individuals, all but one of whom had hyperglobulinemia, gave an average total calcium of 11.3 mg/100 ml and 6.2 mg/100 ml ultrafilterable, or 55 per cent. Three were normocalcemic. The average age was 60 years. See Scattergraph III.

Group VI — Uremia (characterized by serum urea nitrogen levels of at least 100 mg/100 ml).

Seven individual determinations yielded an average total calcium of 7.4 mg/100 ml and an ultrafilterable value of 4.2 mg/100 ml, or 53 per cent. The average age

![Scattergraph III](image)

The serum globulin abnormalities in myeloma and sarcoidosis do not affect the direct relationship between total and ultrafilterable calcium.
was 62 years. All were hyperphosphatemic and also acidotic with the CO₂ content below 20 mEq/l. See Scattergraph IV.

Scattergraph IV
The figures in parentheses are values for serum inorganic phosphorus in mg/100 ml. The percentage value for ultrafilterable calcium remained normal in those patients with uremia.

Scattergraph V
Average ultrafilterable calcium for patients with scleroderma was depressed to 49 per cent, primarily due to the strikingly low value (34 per cent) observed in one case.
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Group VII — Connective Tissue Disorders.

Ten individuals determinations on patients with lupus and polymyositis yielded a mean total calcium of 9.4 mg/100 ml and an ultrafilterable calcium of 5.0 mg/100 ml, or 53 per cent.

A subdivision of systemic sclerosis or scleroderma was made and the results of six individual determinations yielded a mean total calcium of 9.4 mg/100 ml and an average ultrafilterable calcium of 4.6 mg/100 ml, or 49 per cent. These patients had normal serum albumin, but several had a modest elevation of the serum globulin fraction. See Scattergraph V.

Group VIII — Muscle Dystrophy.

Eight determinations on seven individuals with various forms of muscle dystrophy revealed a mean total calcium of 9.7 mg/100 ml, with an ultrafilterable value of 4.9, or 49 per cent. Of the seven individuals, five were male. The average age was 36 years. See Scattergraph VI.

Group IX — Vitamin D Resistant Rickets.

The five individuals considered to have vitamin D resistant rickets or phosphate diabetes had an average total calcium of 9.1 mg/100 ml with an ultrafilterable calcium of 5.3 mg/100 ml, or 58 per cent. The ages of the two males and three females were 10

A tendency to depressed values for ultrafilterable calcium in muscle dystrophy is noted, but, the number is too small for final conclusion.
ranged from five to 60 years. All had characteristically low serum inorganic phosphorus values of less than 2 mg/100 ml.

Group X — Osteoporosis.

There were seven patients in this category, six females and one male. The mean total calcium was 9.5 mg/100 ml, with an ultrafilterable calcium of 5.1 mg/100 ml, or 54 per cent. The average age was 64.5.

Group XI — Sarcoidosis.

Though hypercalcemia occurs in this entity, not one of the five cases considered had this finding. Their mean total calcium of 9.8 mg/100 ml with an ultrafilterable calcium of 5.6 mg/100 ml resulted in 57 per cent ultrafilterability. See Scattergraph III.

DISCUSSION

The total serum calcium in the normal group for this study varies little from the results of previous studies, except its range is more narrowly defined. If three S.D. include all but 0.5 per cent of the population, the range using our reported base is 9.7 ± 0.33 mg/100 ml. Comparing the normal total serum calcium values in this study with 100 age-matched normals from the routine hospital laboratory presented an interesting contrast. In the latter, the mean of 9.7 mg/100 ml had a S. D. ± 0.6 which perhaps reflects the greater volume in a central hospital laboratory and also the greater precision required in a research facility. Each laboratory responsible for determinations of serum calcium should define its range of normal values with accompanying standard deviations. Only then and when considered in conjunction with the serum albumin level will borderline hypo- and hypercalcemic states be uncovered. The frequently quoted range of serum calcium from 9 to 11 mg/100 ml is too wide and values from 10.5 to 11 mg/100 ml should be looked upon as suspicious of hypercalcemia.

Immediately apparent in the normal group is the direct relationship of the ultrafilterable calcium to the total calcium, maintaining a fairly constant 55 per cent. The occasional marked deviation from normal in this group should be taken into consideration when one considers isolated abnormal values in disease states. In comparison to previous reports, the values in this study confirm the observations of Prasad and Flink. Other observers though numerically close to the stated values in this paper, are so by virtue of basic techniques and not by statistics. A novel method depending on a coagulation system gives comparable results to those reported here. By studying Scattergraph I, a direct relationship in most instances between the two calcium fractions is apparent. This relationship has been emphasized by others.

All other constants remaining normal, it can be said that the ultrafilterable calcium is a function of the total serum calcium. This apparently is also true of the ionized calcium. Of the constants affecting calcium ultrafiltration, serum proteins give rise
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to the most frequent and most easily predictable abnormalities. Calcium is bound in the serum primarily to albumin, and changes in its level result in predictable calcium changes. In the malignant disease category, in each case with a depression in serum albumin, there was a corresponding increase in the ultrafilterable calcium value. The normogram of MacLean and Hastings reflects this change to some degree but where gross alterations are evident it is less reliable.

The ability of other serum proteins, mainly those abnormal globulins associated with multiple myeloma or Boeck's sarcoidosis, to increase calcium binding to a significant degree seems doubtful. In those patients with hyperglobulinemia the ultrafilterable calcium was increased directly with the total calcium and not with the globulin level.

Contrary to what has been previously stated in the literature an increase in the ultrafilterable calcium value in our hands has not been of diagnostic use in hyperparathyroidism. Walser's recent studies support the concept that plasma protein binding of calcium is not affected by parathyroid hormone. This is true for percentage value as well as mean value of the ultrafilterable calcium. Hyperparathyroidism cannot be differentiated in this fashion from other hypercalcemic states.

In hypoparathyroidism low ultrafilterable calcium values have been reported. Again it is noted these are absolute values. The present series, in contrast, shows a tendency to increased ultrafilterability, thus further refuting the theory that parathyroid hormone decreases the ability of serum proteins to bind calcium.

In multiple myeloma and sarcoidosis there is substantial agreement, i.e., increased mean total and ultrafilterable calcium in many instances. However, as indicated, results are usually expressed in absolute terms where an increase in the ultrafilterable fraction as well as total calcium is not unexpected. The percent or relative value remains the same.

Uremia, with its attendant acidosis, presents a complicated and varied metabolic picture and has led to conflicting results not only in terminology of report but also in interpretation. With a decrease in serum albumin and a rise in organic acids seen in uremic states, an increase in ultrafilterable calcium might be expected. In the present series this percentage value remained normal. However, a rise in the complexed fraction at the expense of the total diffusible fraction cannot be ruled out and should be tested with the newer techniques for ionizable calcium.

In connective tissue disorders variable results for ultrafilterability of serum calcium have been reported. Previous authors have suggested that the ultrafilterable calcium is decreased in scleroderma. In the present series a single patient exhibited a markedly reduced value of 3.3 mg/100 cc or 34 per cent. Because of this single low value, the percentage of ultrafilterable calcium for the group was reduced below normal. The possibility that a reduced ultrafilterable value for serum calcium might help separate those individuals afflicted with pure scleroderma from the menage of other connective tissue disorders was not supported.

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Others have reported a decreased calcium ultrafilterability in patients with muscle dystrophy. The present study suggests a slight reduction in percentage of ultrafilterable in patients with muscle dystrophy, but the group is too small for final conclusion.

In vitamin D-resistant osteomalacia the occurrence of secondary hyperparathyroidism is a suggested feature of the disease. Since the total serum calcium is usually normal, a decrease in the ultrafilterable calcium at the expense of the non-ultrafilterable calcium could conceivably act as a stimulus for the secondary hyperparathyroidism. Evidence for this thesis was lacking in the patients so tested. However, an increase in the complexed calcium at the expense of ionic calcium as the necessary stimulus cannot be excluded. The absence of clinical tetany in these patients weighs against this possibility.

The current revival of interest in a metabolic defect for calcium as an etiologic role in osteoporosis led to the investigation of ultrafilterable calcium levels in this disease. While total serum calcium levels are usually found to be normal, it was theorized that decreased ultrafilterable levels or increased protein binding could theoretically be a factor in increased bone resorption that occurs in certain types of osteoporosis. In seven patients with postmenopausal and senile osteoporosis, aberrations of the ultrafilterable calcium level were not detected.

**SUMMARY**

One hundred sixty-six ultrafilterable calcium values on 150 individuals were analyzed. Normal values in this study were $55 \pm 4$ per cent. No constant deviations of diagnostic significance were observed in patients with malignancies, hyperparathyroidism, hypoparathyroidism, multiple myeloma, sarcoidosis, connective tissue disorders, muscle dystrophy, vitamin D-resistant rickets and osteoporosis.

Ultrafilterable calcium is a function of total serum calcium and as such is not generally of diagnostic usefulness in clinical medicine.

**REFERENCES**

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