TABLE III. Recoveries of Standard Glycine-Glutamic Acid Mixtures (S) and Amino Acids of Casein Hydrolysate (C) Added to Urine.

Urine specimen No.	Volume of urine diluted to 100 ml, ml	Amino N in urine, mg %	Amino N added to urine, γ	Amino N in urine containing added N, mg %	Recovery of added amino N, %
1	2	24.2	250 (S)	40	98
2	1.50	18.5	258 (S)	34.8	95
3	1.50	34.8	258 (S)	52.7	103
4	1.50	43	250 (S)	59.1	99
5	.50	30	270 (S)	82.5	98
6	1	12.2	245 (S)	37	101
7	1	43.3	377 (C)	79.5	98
8	1	48.3	377 (C)	87.5	102
9	1	19.5	237 (S)	83.3	100
10	1	37	313 (C)	69.3	101
11	1	38.2	313 (C)	68.3	98
12	1	16.3	285 (C)	43	96
13	2	8.8	260 (S)	21.9	100
14	1.50	11	238 (S)	26.7	100
15	1	9.5	260 (S)	35	99
16	1	27	260 (S)	52.5	100
				Mean recovery	99%

No. 1-12, casual and fasting specimens; No. 13-16, 24 hr specimens.

these complicating substances, and it is possible that the procedure might be adapted to the needs of the clinical chemical laboratory.

Conclusion. 1. Russell's colorimetric procedure for the determination of blood amino nitrogen has been adapted for urine. 2. A simple method has been described for removing the interfering substances, ammonia and uric acid, without altering the precision and accuracy of the colorimetric technic. 3. Recoveries of urinary amino nitrogen were attained with a mean value of 99%.

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Calcification. IV. Influence of Strontium and Magnesium Ions on Calcification *in vitro*.* (19193)

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It was reported earlier by one of the authors that strontium in small amounts inhibits the calcification of rachitic cartilage *in vitro* (1,2). In attempting to repeat this experiment, Marks and Shorr(3) were unable to detect such inhibition. In order to resolve this discrepancy, the subject was further investigated. As will be shown below, the results indicate the importance of magnesium ions in the inhibition of the calcifying

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TABLE I. The Influence of Sr and Mg Ions on Calcification *In Vitro*.*

Sr, mM/L	Mg, mM/L	Degree of calcification (mean)† Ca × P = 40‡	Ca × P = 50‡	Ca × P = 70‡
0	0	1.5 (4+)	1.5 (4+)	2.7 (4+)
1	0		1 (4+)	
0	1		1.5 (4+)§	
.30	.70		Trace	
1.33	0			1.5 (4+)
0	1.33			2.3 (4+)
.58	.75	0		0
.58	0	1.1 (4+)		
0	.75	1.1 (4+)		

* In expressing the Ca × P product, Ca is expressed in mg % and inorganic phosphate is expressed as P in mg %. Ca was 10 mg % in the above experiments.

† The degree of calcification as evaluated in the low power microscope is as follows: 1 (4+) = complete thin line across the provisional zone; 2 (4+) = heavy line across provisional zone including primary tongues of cartilage; 3 (4+) = heavy line across provisional zone including primary and secondary tongues of cartilage; 4 (4+) = practically complete calcification of metaphysis(11).

‡ Calcifying solution, in addition to components in table, contains per liter 70 mM of NaCl, 5 mM of KCl, 22 mM of $NaHCO_3$; pH = 7.3, temperature = 36.5°, and incubation time = 18-20 hr (4 slices from 2 tibiae split among experiments; 4 to 6 rats used for each study).

§ Calcification is as extensive but not as dense as control sections.

mechanism by strontium. It is worth pointing out that a better understanding of the role of strontium in modifying the calcifying mechanism is of interest not only from the academic point of view, but also in its application in the treatment of humans. Strontium salts have been employed in an apparently contradictory manner, (a) as a means of softening bones prior to straightening them without surgical interference(4) and (b) as an adjuvant to cause more rapid mineralization of the skeleton in osteoporosis than does administration of calcium alone(5,6).

The apparent contradiction between the findings of Marks and Shorr(3) and Sobel *et al.*(2) was resolved when we employed a calcifying medium similar to that used in the experiments of Sobel *et al.*(2) which contained 0.75 millimol of magnesium ions per liter as part of the artificial serum, following the original technic of Shipley *et al.*(7). This magnesium was eliminated by many of the later investigators of *in vitro* calcification, since satisfactory calcification *in vitro* can be obtained in its absence. As seen in Table I, when strontium is used in the presence of magnesium, the inhibition of the calcifying mechanism is practically complete. In contrast, there is excellent calcification when strontium alone or magnesium alone is employed as the inhibitory ion in concentrations as high as the sum total of strontium and magnesium when both ions are employed. Thus it appears that the *intense inhibition of the calcifying mechanism by strontium observed in* in vitro *calcification depends on the presence of magnesium ions.* Strontium indeed has a mild inhibitory function when tested in a calcifying solution which does not give maximal *in vitro* calcification. This is evident in Table I. The magnitude of this inhibition appears to be of the same order as that of magnesium. However, one cannot detect the inhibitory power of the same amount of strontium or that of magnesium when tested in a calcifying solution which gives more marked healing *in vitro*. This finding with magnesium is similar to that obtained by Shelling *et al.* (see Table III in ref. 8). Marks and Shorr(3) obtained excessive calcification in their control sections. Under these conditions the relatively mild inhibition due to strontium alone is difficult to detect.

These results prompted us to reinvestigate mineralization *in vitro* of bones from animals suffering from rickets due to strontium. These bones are characterized by a markedly diminished calcifiability *in vitro*(1,2), although their histological appearance is similar to that encountered in the usual type of rickets induced by a high calcium diet(9). As seen in Table II, strontium-rachitic sections placed in

TABLE II. Influence of Mg^{++} Ions on the Calcification *In Vitro* of Strontium Rachitic Bones.

Mg, in mM/L	Degree of calcification (mean)* Sr rickets	Control (Ca rickets)
0	2 (4+)	2 (4+)
1	0 to trace	2+ (4+)
	Bone sections shaken 2 hr with sol. containing 75 mM/L $CaCl_2$ prior to incubation with calcifying sol.	
0	3.5 (4+)	3.5 (4+)
1	3.5 (4+)	3.5 (4+)

* As evaluated in the low power microscope.

a calcifying medium devoid of magnesium calcify to the same extent as do the control calcium-rachitic sections. When, however, magnesium is placed in the solution, no calcification *in vitro* takes place, whereas in the same solution with the control rickets, one observes excellent calcification *in vitro*. Thus, again it appears that the *injury to the calcifying mechanism in rickets due to strontium is associated with the presence of magnesium ions* in the calcifying medium.

In other experiments, strontium-rachitic bone sections were shaken with calcium chloride in a manner previously described (10,11) for the reactivation of the calcifying mechanism, and calcified *in vitro* both in the presence and absence of magnesium. As seen in Table II, magnesium no longer had a marked injurious effect on the calcifying mechanism. From this it may be deduced that injury to the calcifying mechanism in strontium rickets is due to the interaction of strontium and magnesium ions.

The results obtained with strontium in the presence of magnesium are probably more indicative of the *in vivo* mechanism than experiments in the absence of magnesium, since magnesium is always present, both in intracellular and extracellular fluids.

These findings indicate that in the treatment of humans with strontium salts(3-6) the role of magnesium should be carefully evaluated. Moreover, these findings indicate the importance of studying the influence of various ions on calcification *in vitro*, both in the presence and absence of magnesium. Studies of the calcifying mechanism, to be published elsewhere, indicate an entirely different behavior of ions like cyanide and fluoride in the presence and absence of magnesium ions.

Summary. The intense inhibition of calcification *in vitro* by strontium ions requires the presence of magnesium ions. The injury to the calcifying mechanism in rickets due to strontium is associated with the presence of magnesium ions. The results suggest the importance of studying the influence of various ions on the calcifying mechanism, both in the presence and absence of magnesium.

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