184°. A mixture of this sample with *p*-aminobenzoic acid resublimed to needles at 150° and melted at 187–188°.

Formylation of the Hydrolysis Product (II) (Resynthesis of Rhizopterin (I)).—Twenty-five milligrams of the hydrolysis product (II) was treated with a mixture of 2 ml. of formic acid (88%) and 0.7 ml. of acetic anhydride. The mixture was heated at reflux temperature until all dissolved (about twenty minutes). The solution was filtered and concentrated to dryness *in vacuo*. The solid residue was dissolved in 1 *N* ammonium hydroxide; the solution was filtered and diluted to about ten volumes with water. It was then warmed on a steam-bath and acidified slowly with acetic acid to about *p*H 4. The rhizopterin crystallized in yellow platelets. It was collected in a centrifuge tube, washed with water, and dried. Microbiological assays of the product, which was resynthesized in this manner, using *S. lactis* R. showed it to have the biological activity of rhizopterin. For analyses, samples were dried in a weighing-pig at 140°.

Anal. Calcd. for $C_{15}H_{12}N_6O_4$: C, 52.94; H, 3.56; N, 24.70. Found: C, 52.57; H, 3.24; N, 24.70.

Formylation of Pteroic Acid (II) (Synthesis of Rhizopterin (I)).—One hundred fifty milligrams of pteroic acid (II), prepared as described,[11] was treated with 10 ml. of formic acid (98%) and heated on a steam-bath. All solid dissolved after five minutes, but heating was continued for one hour. The solution was concentrated to dryness under reduced pressure. The residue was dissolved in 10 ml. of 1 *N* ammonium hydroxide. The solution was diluted to about 10 volumes, warmed to 70° and acidified with acetic acid to *p*H 4. Rhizopterin (I) crystallized from the solution in light yellow leaves. For analyses, samples were dried in a weighing-pig at 140°.

Anal. Calcd. for $C_{15}H_{12}N_6O_4$: C, 52.94; H, 3.56; N, 24.70. Found: C, 52.76; H, 3.85; N, 24.45.

The biological activity was determined using *S. lactis* R. and found to be the same as that of rhizopterin isolated from natural sources.

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Summary

The structure of rhizopterin, the S.L.R. factor, is *p*-[N-(2-amino-4-hydroxypyrimido[4,5-*b*]pyrazin-6-ylmethyl)-formamido]-benzoic acid (I). Rhizopterin has the characteristic ultraviolet absorption spectrum and solubility properties of pterins. It is biologically active for *S. lactis* R. but inactive for *Lactobacillus casei*.

Rhizopterin has been hydrolyzed under mild conditions to give *p*-[(2-amino-4-hydroxypyrimido[4,5-*b*]pyrazin-6-ylmethyl)-amino]-benzoic acid (II) and formic acid. Oxidation of this hydrolysis product (II) with a mixture of hydrochloric acid and potassium chlorate produced oxaloguanidine and chloranil. More drastic hydrolysis or pyrolysis of rhizopterin or its hydrolysis product (II) produced *p*-aminobenzoic acid. Oxidation of benzoylrhizopterin gave benzoylguanidine hydrochloride and chloranil. Desiminorhizopterin was obtained by the action of nitrous acid on rhizopterin.

Rhizopterin was obtained by formylation of the hydrolysis product (II) and also by formylation of the same compound prepared by synthesis.

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Calcium Ion Activities in Supersaturated Solutions Stabilized by Sodium Metaphosphate as Determined by Clay Membrane Electrodes

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Introduction

During the past decade, the retardation and prevention of precipitation from solutions presumably supersaturated with calcium carbonate has been accomplished by the addition of about 2 p. p. m. of glassy sodium hexametaphosphate. Systems thus stabilized include irrigation waters containing free ammonia,[3] alkaline soil solutions,[4] industrial and municipal water supplies,[5] and oilfield brines.[6] The efficacy of this minute concentration of hexametaphosphate in preventing the normal deposition of the excess calcium carbonate from irrigation waters was first demonstrated by Ayers in 1935 (which discovery was the basis for the first patent in this field[3]) and was an outgrowth of its use in stoichiometric proportions to soften hard water by formation of a soluble complex anion containing calcium.[7] Although the mechanism of the process has been investigated by various workers,[8,9,10] no completely satisfactory explanation has yet been proposed. A possible mechanism, which has not been directly investigated previously, is that the hexametaphosphate somehow reduces the activity of the calcium ions so that they cannot condense with carbonate

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ions to form calcium carbonate nuclei. This theory requires that calcium ions thus affected would have a reduced or negligible effect on a reversible calcium ion electrode. Calcium stabilized by metaphosphate does form less soluble salts than the carbonate, such as calcium oxalate and calcium fatty acid soaps, but this does not necessarily refute the postulated mechanism.

In recent years, Marshall and co-workers have developed clay membrane electrodes for measuring activities of alkali and alkaline earth cations in soils and other systems.[11,12,13] More recently, Marshall and Ayers[14] have investigated calcium systems in detail. The range of calcium ion activities in which these electrodes function satisfactorily includes that involved in these stabilized carbonate systems, namely, 0.0002–0.002 molal activity.

These electrodes have been employed to measure the calcium activity of carbonate systems stabilized by metaphosphate with the view of testing the theory of reduction of calcium ion activity. The systems examined were calcium bicarbonate solutions in which the carbon dioxide content was reduced by (a) bubbling of air, and (b) boiling.

Previous experimental work by the senior author had established that metaphosphate stabilizes supersaturated solutions of calcium sulfate formed by dissolving $CaSO \cdot 0.5H_2O$ in water at ordinary temperatures. The increased solubility of this salt above that of $CaSO_4 \cdot 2H_2O$ is responsible for the establishment of this condition of supersaturation, which can be maintained for at least several days. Because this system represents another case of stabilization of a calcium salt its calcium ion activity has been measured likewise.

Materials and Methods

Doubly-distilled water prepared in Pyrex ware was used throughout these investigations.

The glassy molecularly dehydrated sodium phosphate was prepared by heating sodium dihydrogen orthophosphate in a platinum dish at 1000° for two hours and quenching the bottom of the dish in water. The composition and structure of this product have not been definitely established, but its early designation as a hexametaphosphate polymer, $(NaPO_3)_6$, has been retained. A stock solution containing 1000 p. p. m. was used.

A solution of calcium bicarbonate was prepared by passing carbon dioxide gas through a suspension of powdered calcium carbonate. After the excess carbonate had settled the clear supernatant liquid was removed by a siphon and the calcium concentration determined by a gravimetric calcium oxalate ignition method to be 0.0171 *N*. This solution was employed in two series of treatments designed to convert bicarbonate to carbonate by removal of carbon dioxide, namely: (a) bubbling of atmospheric air under a partial vacuum; (b) boiling.

(a) In seven 125-ml. Pyrex Erlenmeyer flasks, 100-ml. samples were prepared containing 100 p. p. m. of calcium as bicarbonate and the following concentrations of sodium hexametaphosphate: 0, 0, $^1/_2$, 1, 2, 3 and 5 p. p. m. Five of the flasks were connected to a vacuum-line manifold and air was bubbled through the solutions for six hours at room temperature. The remaining two flasks, containing 0 and 2 p. p. m. of hexametaphosphate, were stoppered and reserved for reference measurements. After six hours an appreciable quantity of precipitate had formed in the aerated 0 p. p. m. hexametaphosphate system but none was observed in the remainder of the series. Differential weighings indicated a loss of 1.5 ml. of water during aeration. *p*H values were determined by a glass electrode and calcium ion activities by 465° calcium bentonite clay membrane electrodes on separate subsamples pipetted from the seven flasks.

(b) A series of six similar bicarbonate solutions were prepared containing 0, 0, 1, 2, 4 and 6 p. p. m. of sodium hexametaphosphate. The solutions were heated separately for ten minutes over a bunsen burner, boiling vigorously during the second five-minute period. One of the 0 p. p. m. sodium hexametaphosphate solutions was boiled an additional twenty minutes; this flask lost 32 ml. of water. The loss of water from the other flasks ranged from 5.5 to 8 ml. The six solutions were restored to their initial weights by addition of water. Calcium ion activities and *p*H values were determined on separate subsamples.

Heating at 105° for twenty hours effected the loss of 19 g. of water from 100 g. of powdered $CaSO_4 \cdot 2H_2O$. Nine grams of the dried salt was added to 700 ml. of water in a flask and shaken for three minutes by hand. The mixture was filtered rapidly on a Buchner funnel under a partial vacuum. Immediately, 80 ml. of the clear filtrate was added to each of six 125-ml. Erlenmeyer flasks containing amounts of water and sodium hexametaphosphate required to provide sodium hexametaphosphate concentrations of 0, 1, 2, 3, 4 and 6 p. p. m. in a final volume of 100 ml. It was known from previous experiments that immediate dilution of the filtrate by 20% furnishes a calcium sulfate concentration that can be stabilized by 4 p. p. m. of sodium hexametaphosphate. After the solutions had stood four days at room temperature without further agitation samples were pipetted out for measurement of calcium ion activity.

The calcium ion activity of each system was determined from the potential difference across a clay membrane electrode between two saturated calomel electrodes as described in detail elsewhere.[12,13,14] The clay membranes were prepared from calcium-saturated bentonite heated at 465° for twenty-four hours. This material is not specific for calcium ions, but the systems studied intentionally contained no other cations except a negligible concentration of sodium ions from the sodium hexametaphosphate. The potential measured is attributed to calcium ion activity alone. Potentials were measured at 25° with a Leeds and Northrup type K potentiometer and a high resistance galvanometer. The solutions were sufficiently dilute that the Nernst equation was applicable. The activity values reported were calculated from the mean of the potentials indicated by duplicate clay electrodes.

Results

In Table I are listed the *p*H values and calcium ion activities of the carbonate systems and visual estimates of the relative extent of precipitation. The determined activities of the aerated carbonate systems have been reduced by 1.5% to correct for evaporation.

When carbon dioxide was not removed, metaphosphate affected neither the *p*H nor the calcium activity. The average activity of the controls, 0.00164, is also the maximum activity of the aeration and boiling series. In the aeration series, metaphosphate over the range 0.5–5 p.p.m. caused

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(12) Marshall and Bergman, THIS JOURNAL, **63**, 1911 (1941); *J. Phys. Chem.*, **46**, 52, 325 (1942).

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(14) Marshall and Ayers, *Soil Sci. Soc. Amer. Proc.*, **11**, 171 (1947).

TABLE I

PRECIPITATION, *p*H VALUES, AND CALCIUM ION ACTIVITIES OF CARBONATE SYSTEMS

$(NaPO_3)_6$ concn., p. p. m.	No treatment			Aerated 6 hours			Boiled 5 minutes		
	Precipitation	*p*H	Ca activity, $a_{Ca^{++}}$	Precipitation	*p*H	Ca activity, $a_{Ca^{++}}$	Precipitation	*p*H	Ca activity, $a_{Ca^{++}}$
0[a]	0	6.60	0.00158	+++	8.60	0.00109	+++	8.22	0.00109
1/2	..	..	.....	0	8.80	.00155	....	..	.....
1	..	..	.....	0	8.85	.00161	++	8.68	.00125
2	0	6.60	.00169	...	..	....	+	8.76	.00135
3	..	..	....	0	8.87	.00165	...	..	.....
4	..	..	....	...	..	.....	0	8.68	.00161
5	..	.	.....	0	8.87	.00165	....	..	.....
6	..	..	.....	....	..	.....	[b]0	8.70	.00149

[a] Results of twenty-five minute boiling: Precipitation, ++++, *p*H 8.62; Ca activity, 0.00026. [b] Slight turbidity, attributed to reaction of calcium with a component of the sodium hexametaphosphate glass or with orthophosphate formed during boiling.

no reduction in calcium activity, even though these systems were supersaturated, as shown by precipitation and lower *p*H of the 0 p.p.m. sodium hexametaphosphate sample.

Under the five-minute boiling treatment, 4 and 6 p. p. m. of sodium hexametaphosphate prevented precipitation of carbonate and maintained the maximum calcium activity. With decreasing metaphosphate concentration, precipitation increased accompanied by a corresponding reduction in calcium activity. The twenty-five-minute boiling indicates that a substantial period at 100° is necessary to reduce the calcium content markedly.

Corresponding results on the sulfate systems are presented in Table II. The same general relationships occurred in this series. Only those samples in which precipitation occurred showed reduced calcium activity, and the reduction increased with the extent of precipitation. The 2 p. p. m. sodium hexametaphosphate sample showed the same activity as the 3, 4 and 6 p. p. m. samples because the surface deposition was too slight to affect the activity measurement.

TABLE II

PRECIPITATION AND CALCIUM ION ACTIVITIES OF SULFATE SYSTEMS

$(NaPO_3)_6$ concentration, p. p. m	Precipitation	Ca activity, $a_{Ca^{++}}$
0	++++	0.00586
1	++	.00757
2	[a]0	.01031
3	0	.01031
4	0	.01007
6	0	.01020

[a] Barely noticeable slight surface deposition.

In previous work by the senior author, a specific conductance value of 0.00436 at 25° was obtained on a similar hemihydrate solution stabilized by 4 p.p.m. of sodium hexametaphosphate five days after preparation, and a value of 0.00223 sixty-seven days later, when the solution was at equilibrium with the precipitated $CaSO_4 \cdot 2H_2O$. This increased conductivity is further evidence of dissociation of the stabilized fraction of the calcium sulfate.

The results obtained with both the carbonate and sulfate systems demonstrate that the addition of the metaphosphate does not reduce the thermodynamic activity of the calcium ions by any appreciable amount. The action of the small amounts of metaphosphate in stabilizing the supersaturation of the calcium carbonate and calcium sulfate solutions therefore cannot be attributed to any reduction in the calcium activity of the systems. Furthermore, this evidence supports previous work refuting the possibility of a colloidal system stabilized by the glassy phosphate.[5,9] The condensation of any appreciable proportion of calcium and carbonate ions, or calcium and sulfate ions, to form either molecular-sized, colloidal, or larger nuclei of calcium carbonate or sulfate would be accompanied by a reduction in the calcium ion activity. The possible separation of *traces* of the two salts is not precluded by these activity measurements.

Summary

1. Clay membrane electrodes were used to measure the calcium ion activity of supersaturated solutions of calcium carbonate and calcium sulfate stabilized by minute concentrations of sodium hexametaphosphate.
2. In completely stabilized systems the action of the metaphosphate does not involve a reduction of the calcium activity, and the activity is lower only in those incompletely stabilized systems in which precipitation occurs.
3. Although the results do not constitute positive evidence as to the stabilization mechanism, they eliminate the theory of reduction of calcium activity within a single phase system and the theory of stabilization of calcium carbonate in colloidal form.

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