STUDIES OF THE SOLUBILITY OF CALCIUM SALTS.

I. THE SOLUBILITY OF CALCIUM CARBONATE IN SALT SOLUTIONS AND BIOLOGICAL FLUIDS.

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INTRODUCTION.

The biological importance of calcium has been recognized since the earliest times, but it has only been comparatively recently, through the stimulus afforded by the growing realization of the close association between some pathological conditions and disturbances of the calcium metabolism of the body, that the element calcium has claimed so much attention and study in biological fields. Being the only cation, except for small amounts of magnesium, capable of forming difficultly soluble salts with the common anions present, namely carbonate and phosphate, it carries unaided, the burden of furnishing the organism with its framework of ossified tissue, the skeleton. However, its function and purpose do not cease here. Its effect on nerve and muscle permeability and irritability alone would warrant more important consideration of the nature of its metabolism.

The work to be reported in this and subsequent papers does not represent an attempt to solve any one particular biological problem which is concerned with calcium metabolism, but is rather a quantitative investigation of certain biological systems in which calcium plays a rôle. Before work upon biological systems could be interpreted, however, much work was necessarily carried out on systems of simple composition. These will be given in the following order: First, studies of the solubility of calcium carbonate in salt solutions and biological fluids;
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second, studies of the solubility of tertiary calcium phosphate in the same systems; third, studies of the solubility of both salts in similar solutions.

Studies of equilibria in heterogeneous systems composed of the solid phases, calcium carbonate and calcium phosphate, the liquid phases, aqueous solutions of various electrolytes and non-electrolytes, and the gas phase, carbon dioxide, have long attracted the attention of theoretical, technical, and biological chemists. Interest in such a system may be centered upon the liquid phase in connection with such problems as the factors influencing the activity of the ions present in it, or upon the solid phase and the factors influencing the precipitation or solution of one or more of the components. To the biologist each of these points of view is of importance.

Ionic activity, particularly that of calcium ions, is believed to be intimately related to the problem of the functional activity of nerves and muscles, and hence may be considered an important factor in tetany.

Although there is present in serum far more calcium than one could dissolve in a salt solution of the same concentration, a diminution or increase in the calcium concentration is known to result in profound biological changes. Among the most striking of these, following a decrease in its concentration, is the condition of hyperexcitability which, in the intact animal, manifests itself in the symptom syndrome known as tetany. Conditions are known, however, as for example in nephrosis, in which low serum calcium concentrations are found without symptoms of hyperexcitability. It becomes of interest therefore to inquire first into the reason for the large amount of calcium in serum and the forms in which it is present, and second into the reason for the differences in biological response following changes in calcium concentrations.

A knowledge of the factors affecting the precipitation and solution of calcium carbonate and calcium phosphate underlies the solution of such physiological problems as bone and tooth formation and such pathological problems as rickets and arteriosclerosis.

Problems concerned primarily with the nutritional aspect of calcium metabolism, involving such questions as the amount of
elementary calcium necessary for cellular activity, maintenance, and growth, are not to be considered in this work.

Another point of view has for its purpose the understanding of the behavior of calcium in the organism. Before its functional activity can be understood, however, certain knowledge concerning the physicochemical system involved, is necessary. Such questions as the following occur to one taking this point of view.

What is the biological system of which calcium is an important constituent? Is this system in equilibrium, and if so, in what kind of an equilibrium? In what forms is calcium present in this system? What are the quantitative relationships between these forms? What factors influence these relationships? Our work has had for its purpose an attempt to answer some of these and similar questions. Although final answers have not been found in all cases, in certain of them a reasonable degree of success has been met, while in others evidence which may contribute to the final solution has been obtained.

Theoretical Considerations.

Much work has been done in recent years to show that the problem of the functional activity of calcium is really concerned with the various forms in which calcium may exist, and the relationships of the different forms involved. Apparently, calcification does not bear a simple relationship to the calcium of the blood. In other cases of calcium disturbance, the rise or fall of total calcium in serum is but a very poor index of what may really be happening. With the acquisition of new knowledge concerning the physiological behavior of calcium in the organism, it has become more and more apparent that we are concerned with several different forms of calcium, which may or may not be in equilibrium with each other, or exert influence upon each other.

The conception that the biological importance of calcium in cellular activity may be attributed to ionic calcium, is of fairly recent origin. This appreciation of the possible importance of the calcium ion in physiological systems has been the natural result of the development of the theory of solutions from theoretical considerations concerning the influence of the electronic environment upon the properties of ions in general. In recent years, various methods have been employed in an effort to accomplish a difficult task, the measurement of calcium ion concentrations in liquid systems in general, and in serum and biological fluids in particular. Many have attempted to obtain these values by the analysis of ultrafiltrates, some by electrometric measurements with amalgam electrodes (Neuhausen...
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and Marshall, 1922), some by colorimetric methods, and others by calculation from experiments with difficultly soluble salts (Rona and Takahashi, 1911), (Kugelmass and Shohl, 1923-24), (Holt and collaborators, 1925, a, b). The latter method is the one which, in connection with the theoretical considerations outlined in the following, has been adopted for these studies.

Within the last few years a conception of ionic behavior has entered biological investigations, which relates the environment about the ions to the activity of the ions. Since this conception seems to be fruitful in the interpretation of studies involving electrolyte behavior generally, a brief review of the more important features will be given.

The failure of strong electrolytes to conform to the behavior predicted by the mass law, led G. N. Lewis in 1907 to propose a new thermodynamic concept called the activity, related to fugacity as concentration is to pressure; namely,

\[ \text{fugacity} = RT \text{activity}. \]

The fugacity may be regarded as the vapor pressure of a solute corrected for all deviations from the gas law, and hence is an accurate measure of the tendency of a substance to escape from its surroundings. In a similar way, by defining the activity of a substance as the ratio between the fugacity \( f \) of a substance in some given state, and its fugacity \( f' \) in some other chosen standard state, we have a quantity which is an accurate measure of the escaping tendency of a substance expressed in terms of concentration units which have been corrected for all deviations from the gas laws. By using these new units corrected for deviations of the substance in the gaseous state, from the behavior of an ideal gas, one may apply them to any system, and yet obtain relationships which conform to those derived on the supposition that the gas laws are being obeyed. "Thus when stated in terms of fugacities or activities the law of mass action has a universal validity" (La Mer, 1925). However, since many substances are non-volatile, it has been more convenient to replace the absolute activity by the relative activity, for which the standard state of reference is defined in terms either of the pure liquid or of the pure solid state instead of the attenuated gaseous state.

In accordance with these principles, we have the relationship

\[ \gamma = \frac{\alpha}{c} \]

where \( c \) is the stoichiometric molal concentration, \( \alpha \) is the activity concentration, and \( \gamma \) is the activity coefficient, which, in dilute solutions, may be regarded as the thermodynamic degree of dissociation. In the case of solutions, the standard state is conveniently taken so that at infinite dilution of the solute, \( \gamma \) becomes unity, and all the gas laws are obeyed. Hence \( \alpha \) will be equal to \( c \) at infinite dilution. Although the fugacity and activity of a solute at infinite dilution are proportional by Henry's law to the mol fraction \( N \), one may still use molality units \( c \) as proportional to \( N \) in
dilute solutions. All of the data in these papers have been calculated on the molality basis; i.e., in mols per kilo of H₂O.

Using the concept of activity, one may write for a strong electrolyte such as NaCl,

\[
\frac{\alpha_+ \times \alpha_-}{\alpha_0} = K
\]

which is the mass law equation involving the thermodynamic concentrations or activities. The activity is related to the molal concentration so that

\[
\alpha_+ = \gamma_+ c_+ \\
\alpha_- = \gamma_- c_- \\
\alpha_0 = \gamma_0 c_0
\]

(3) therefore,

\[
\frac{\gamma_+ c_+ \times \gamma_- c_-}{\gamma_0 c_0} = K
\]

This is the thermodynamic mass law equation for dissociated electrolytes where subscripts +, −, and 0 denote positive ion, negative ion, and salt, respectively.

Lewis and Randall (1923), studying activity coefficients in mixtures of different valence types, made the generalization that "in dilute solutions, the activity coefficient of a given strong electrolyte is the same in all solutions of the same ionic strength." The ionic strength is related to the stoichiometric molality in the following manner:

Let \( \mu = \) ionic strength
and \( c_1, c_2, c_3, \ldots, c_n = \) molal concentration of ions present
\( z_1, z_2, z_3, \ldots, z_n = \) valence of the ions,

then

(4) \[
\mu = \frac{1}{2} (c_1 z_1^2 + c_2 z_2^2 + c_3 z_3^2 + \ldots + c_n z_n^2)
\]

Thus the concentration is related to the number and valence of ions present as they alter the electronic environment of a solution. It should be pointed out, however, that this is a limiting law, and serves only as a first approximation in solutions where individual ion properties are not significant.

The activity of a solute may be calculated from the vapor pressure of the solute, from the distribution of a solute between two solvents, from measurements of electromotive force, from freezing point data, and from solubility measurements. The solubility of slightly soluble salts in solutions of other salts has been utilized by many physical chemists, e.g., Lewis, Brönsted, Noyes, Bray, Harkins, and their collaborators, to calculate the activity coefficients of the slightly soluble salts in the solution. It is this method which has been utilized in these studies.
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At constant temperature, when a salt in solution is in equilibrium with the solid solute, it follows as a thermodynamic necessity that the activity of the salt in the solution is fixed and equal to the activity of the solid solute. No change in the solvent such as would be produced by addition of electrolytes or non-electrolytes can change that activity. If the activity coefficient of the salt in solution is decreased, due to a change in the solvent, more of the salt will go into solution until the activity in the solution and the solid state are equal. Hence

\[ \gamma_0 s_0 = \gamma_1 s_1 = \gamma_2 s_2 \ldots \ldots \ldots \ldots \]

where \( s_0 \) refers to solubility in pure water, and \( s_1 \) and \( s_2 \) refer to solubility in other solvents.

\[ \frac{\gamma_0}{\gamma_1} = \frac{s_1}{s_0} \quad \text{or} \quad -\log \gamma_1 = p\gamma_1 = \log \frac{s_1}{s_0} - \log \gamma_0 \]

Setting \( \gamma_0 \) arbitrarily equal to unity,

\[ -\log \gamma_1 = p\gamma_1 = \log \frac{s_1}{s_0} \]

If a salt \( A_{n1}B_{n2} \) dissociates so that \( A_{n1}B_{n2} \rightleftharpoons n_1 A + n_2 B \) at equilibrium, the following relationship must hold true:

\[ (\alpha A^+)^{n_1} \times (\alpha B^-)^{n_2} = K_{s.p.} \]

where \( \alpha A^+ \) and \( \alpha B^- \) denote the activities of the ions, \( n_1 \) and \( n_2 \) are the number of ions of \( A^+ \) and \( B^- \) respectively, \( + \) and \( - \) are used regardless of valence, and \( K_{s.p.} \) is the activity product or solubility product at ionic strength equal to zero. Substituting \( \gamma c \) for \( \alpha \), where \( \gamma \), \( c \), and \( \alpha \) refer to the ions, equation (8) becomes

\[ (\gamma A^+)^{n_1} \times (\gamma A^+)^{n_1} \times (\gamma B^-)^{n_2} \times (\gamma B^-)^{n_2} = K_{s.p.} \]

or using the conventional symbol \([ \cdot ] \) for concentration \( c \),

\[ [A^+]^{n_1} (\gamma A^+)^{n_1} \times [B^-]^{n_2} (\gamma B^-)^{n_2} = K_{s.p.} \]

By definition, the activity coefficient of a salt is the geometrical mean of the activity coefficients of its ions. Hence

\[ \gamma A_{n1}B_{n2} = \frac{\gamma_1^{n_1} \gamma_2^{n_2}}{\sqrt{(\gamma A^+)^{n_1} \times (\gamma B^-)^{n_2}}} \]

\[ \quad = \frac{\gamma_1^{n_1}}{(\gamma A^+)^{n_1 + n_2}} \times \frac{\gamma_2^{n_2}}{(\gamma B^-)^{n_1 + n_2}} \]

Logarithmically equation (11) becomes

\[ \log \gamma A_{n1}B_{n2} = \frac{n_1}{n_1 + n_2} \log \gamma A^+ + \frac{n_2}{n_1 + n_2} \log \gamma B^- \]
Equation (10) may be written in the form

\[ (13) \quad [A^+]^{n_1} \times [B^-]^{n_2} \times (\gamma A_{y_1} B_{y_2})^{n_1 + n_2} = K_{s.p.} \]

\[ (14) \quad [A^+]^{n_1} \times [B^-]^{n_2} \frac{K_{s.p.}}{(\gamma A_{y_1} B_{y_2})^{n_1 + n_2}} = K'_{s.p.} \]

We have found it convenient to transform equation (14) into its logarithmic form

\[ (15) \quad \log [A^+] + \log [B^-] = \log K_{s.p.} - (n_1 + n_2) \log \gamma A_{y_1} B_{y_2} = \log K'_{s.p.} \]

As a further simplification, we have uniformly employed the symbol \( \nu \chi \) to designate \( -\log [X^+] \). Equation (15) accordingly becomes

\[ (16) \quad \nu_1 \log [A^+] + \nu_2 \log [B^-] = \nu \chi A_{y_1} B_{y_2} = \nu \chi K_{s.p.} - (\nu_1 + \nu_2) \log \gamma A_{y_1} B_{y_2} = \nu \chi K'_{s.p.} \]

\[ (17) \quad \nu \chi A_{y_1} B_{y_2} = \nu \chi K_{s.p.} - \nu \chi K'_{s.p.} = (\nu_1 + \nu_2) \log \gamma A_{y_1} B_{y_2} = \Delta \nu \chi A_{y_1} B_{y_2} = \Delta \nu \chi K_{s.p.} \]

Equation (18) is thus identical with that derived by Brönsted and La Mer (1924) by substituting \( \sqrt{K_{s.p.}} \) for \( s \) in equations (5), (6), and (7).

Debye and Hückel (1923), from purely physical considerations, employing the theory of complete dissociation of strong electrolytes first advanced by Bjerrum (1909), have recently elaborated a theory which accounts for the behavior of these substances by taking into consideration the electrical forces at play between the ions. Their mathematical treatment has been further simplified and applied to salts of higher valence type by Brönsted and La Mer, who found that the activity coefficient was related to the ionic strength of the solution by the equation

\[ (19) \quad \log \gamma_{salt} = \alpha' z_1 z_2 \sqrt{\mu} \]

where \( \alpha' \) is a universal constant depending among other things on the dielectric constant of the medium, and \( z_1 \) and \( z_2 \) refer to the valences of the two ions of the salt. The addition of a correction term \( \beta \mu \) enables one to use the equation for higher ionic strengths. More recently, Hückel (1925), developing the theory advanced by Milner and by Debye and Hückel, has derived a general working equation of the form

\[ (20) \quad \log \gamma_{salt} = \frac{B z_1 z_2 \sqrt{\mu}}{1 + A \sqrt{\mu}} + C \mu \]

In this equation \( A, B, \) and \( C \) denote constants. This equation takes into consideration the effect of the ionic environment, the size of the ions, and the dielectric properties of the solution. Since the concent-
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Concentrations of the solutions used in our experiments were less than 0.2 molal, we have neglected the possible deviation in the dielectric properties of the solutions with changing ionic strength. The equation used in these papers to relate the activity coefficient of the salts studied to the ionic strength has been of the general form

\[ -\log \gamma_{salt} = \frac{B \sqrt{\mu}}{1 + A \sqrt{\mu}} \]

For convenience in calculation, BZ1 z2 has been used as if it were one constant B.

These theoretical considerations will be further elaborated and discussed in connection with the experimental work as it is presented in later sections and papers. These sections given in order will contain a presentation of the technique used in the work, the calculations used, and the constants involved. Studies of the solubility of calcium carbonate and tertiary calcium phosphate, singly and together, under various conditions, will then follow.

Experimental Technique.

Analytical Methods.

Calcium.—Total calcium was determined by the method of Kramer and Tisdall (1921) slightly modified. It was found that washing the precipitated and centrifuged calcium oxalate three times with 3 cc. of 2 per cent ammonia water, without stirring, gave results within 2 per cent of theoretical on solutions of known calcium content. Titrations were made with N/100 or N/200 KMnO4 using 1 to 5 cc. samples according to the calcium content of the solution analyzed. The permanganate factor was redetermined in every experiment by means of standard sodium oxalate.

Clark and Collip (1925) have demonstrated that the conditions necessary for obtaining theoretical results with this and similar methods are purely empirical. Kramer and Tisdall, later Tisdall (1923) and Clark and Collip have all endeavored to work under

Some of the later determinations were checked by a gasometric method developed by Dr. Van Slyke, as yet unpublished. The washed precipitated calcium oxalate is dissolved in sulfuric acid as usual and transferred quantitatively into the Van Slyke manometric gas analyzer (Van Slyke and Neill, 1924). The oxalic acid is oxidized with an excess of permanganate under the proper conditions, and the yield of carbon dioxide, which is quantitative, is taken as a measure of the calcium in the precipitate.
conditions such that the combined error due to solubility of calcium oxalate, and the retention of ammonium oxalate after washing, might be a minimum. As pointed out by the latter authors, any deviation in an arbitrary procedure will cause corresponding differences in results. Clark and Collip's modification, although quite satisfactory, was abandoned because of loss of precipitate when tubes were used which were not narrow enough at the bottom. In agreement with their findings, the original Kramer-Tisdall method gave low results. Some comparative results obtained by using different methods in determining the calcium content of a known solution are given below. Frequent determinations on the above solution, alone and when added to serum, served as a check on the accuracy of the method and the technique of the individual performing the analysis.

**Determinations of Ca Content, by Different Methods, of an Aqueous Solution Containing 2.50 mEq Ca per Liter.**

<table>
<thead>
<tr>
<th>Clark and Collip.</th>
<th>Kramer and Tisdall.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>With stirring.</td>
</tr>
<tr>
<td>2.48</td>
<td>2.32</td>
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<tr>
<td>2.48</td>
<td>2.32</td>
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<tr>
<td>2.46</td>
<td>2.32</td>
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<tr>
<td>2.53</td>
<td>2.35</td>
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</tbody>
</table>

**Hydrogen Ion.**—The pH of salt solutions and biological fluids was determined in the various ways indicated in the tables of experimental results: e, electrometric; c, colorimetric; g, gasometric results. Electrometric determinations were made in a thermostat maintained at 38 ± 1°. The temperature of the reaction liquid was read to 38 ± 0.2° in the Clark-Cullen electrode vessel of 2 cc. capacity. A thermometer in the electrode vessel, as suggested by Cullen, permitted accurate temperature control of the liquid under investigation. The gas chain employed was the following.

<table>
<thead>
<tr>
<th>Hg</th>
<th>HgCl</th>
<th>Saturated KCl</th>
<th>Saturated KCl bridge</th>
<th>Unknown solution</th>
<th>H₂</th>
<th>Pt</th>
</tr>
</thead>
</table>
Pure mercury was prepared according to Hulett (1905) by redistilling five times. Calomel was prepared electrolytically from the purified mercury and redistilled hydrochloric acid. Merck's analytical KC1 was used in the saturated calomel electrode and salt bridge. The hydrogen used was found by analysis to be 99.5 per cent pure. Whenever CO2-containing solutions were being measured, the electrode was first flushed and filled with a mixture of hydrogen and CO2, the latter gas being at the same tension as that of the CO2 in the liquid. Usually the gas phase, with which the unknown had been equilibrated, was itself run from the large saturation tonometer into the electrode vessel, by displacement with mercury. Electrodes were made of heavy platinum foil. Before use in each experiment, they were freshly coated with platinum black. Platonic chloride for plating electrodes was prepared from pure platinum.

The standard used was N/10 HCl made from Hulett's (1909) constant boiling acid, twice redistilled. The strength of the N/10 HCl was confirmed by gravimetric analyses which indicated a chloride content within 0.1 per cent of theoretical. The pαH+ of this acid, on the basis of Lewis' (1923) value (p. 382) for the activity of H+ ion in a solution of this ionic strength, was assumed to be 1.08 at 38° and 20°. No correction was made for liquid junction potential.

Values of pαH+ of unknown solutions were calculated from the equation:

\[
p_{\alpha H^+} = \frac{E \text{ (corrected to 1 atmosphere of dry hydrogen)} - e_2}{0.0001983 T}
\]

It may be noted here, that pαH+, referred to in these papers is identical with pαH+, as used by Hastings and Sendroy (1925) and with the pH of Van Slyke, Hastings, Murray, and Sendroy (1925). However, this value is not identical with the pαH of Sørensen and Linderström-Lang (1924), which is approximately 0.04 higher than ours, but corresponds to their pH, which is obtained by using the Bjerrum extrapolation for liquid junction potential, and assigning to N/10 HCl a value based on conductivity measurements. No correction for liquid junction potentials between saturated KC1 and either N/10 HCl or our unknown solutions has been attempted. If in the future the difference between these diffusion potentials is established our pαH+ values are subject to a corresponding correction.
In many of the experiments, $p_{\text{H}^+}$ was determined colorimetrically on both salt solutions and serum, using bicolor standards and reading at 38°, according to the technique of Hastings and Sendroy (1924). From time to time, the color standards were checked against phosphate standards which in turn had been standardized electrometrically. Salt solutions were read immediately after sampling under oil for such solutions lose CO$_2$ and change their reaction very quickly. Colorimetric determinations on serum were accurate to $\pm 0.05$ $p_{\text{H}^+}$, salt solutions to $\pm 0.02$.3

Sometimes when a high degree of accuracy was required, or when the reaction was too acid or too alkaline to be read within the phenol red range, $p_{\text{H}^+}$ was indirectly determined from total CO$_2$ and CO$_2$ tension analyses, as outlined in the section on calculations.

CO$_2$ Tension.—The gas phase was analyzed in the Henderson-Haldane apparatus of 10 cc. capacity. The accuracy of these analyses was approximately 0.5 per cent.

CO$_2$ Content.—Total CO$_2$ was determined in the manometric gas apparatus described by Van Slyke and Neill (1924). These determinations were accurate to about 0.3 per cent.

$H_2O$ Content.—Moisture determinations for converting data from molarity to molality basis were obtained by drying 1 or 2 cc. samples for about 15 to 20 hours in a Freas oven at 120°C. Weighings were made to 1 mg., and the error involved was not more than 0.2 per cent.

3 The bicolor standards referred to, as described previously (Hastings and Sendroy, 1924), have values based on the comparison of such standards against electrometrically standardized M/15 Sörensen phosphate standards. However, experiments as yet unpublished indicate that the effect of ionic strength and valence type of salt on the activity of dyes is similar to their effect on other ions. In other words, the apparent dissociation constant of the dye changes with ionic strength and with different salts in the solution, thus behaving in conformity with the Debye-Hückel theory of strong electrolytes. In order to detect any possible difference in activity of phenol red in the M/15 phosphate solution and in artificial edema fluid, such as was usually employed, comparative results were obtained by electrometric and colorimetric determinations of the same solutions. Since the two methods agreed in all cases to within 0.01 $p_{\text{H}^+}$, it was decided that all readings on balanced salt solutions obtained with the bicolor standards could be considered reliable.
Phosphorus.—Determinations of the phosphorus in salt solutions and serum were made according to the method of Benedict and Theis (1924), an improvement on the Briggs (1922) modification of the original Bell and Doisy (1920) colorimetric method. Stanford and Wheatley (1925) have recently furnished proof of the validity of the principle upon which the method depends, and report that there is a direct proportionality between the color produced and the phosphate present, only when the method is done under certain conditions. Inasmuch as Benedict and Theis also confirm Buell's (1923) finding that there is no appreciable amount of acid-soluble organic phosphorus in serum which is hydrolyzed by concentrated sulfuric acid, a determination by this method, even in serum, can most probably be taken to represent only the inorganic phosphorus, hence the total phosphate. In our experiments we have calculated our phosphate results on this assumption. We have confirmed Stanford and Wheatley in that accurate results can only be obtained when total acidity, concentration of all reagents, and temperature are strictly uniform in standards and unknown solutions.

The reading of the solution and standard must not differ by more than 20 per cent in order to obtain a reading which is within 5 per cent of theoretical. In serum analyses, the precipitation of proteins in a solution diluted only five times may very well lead to still greater error. However, this is unavoidable in such cases where the extremely low phosphate content of the solution analyzed makes it necessary to have as concentrated a filtrate as possible, in order to be able to read the slight coloration produced.

Saturation Procedure.

Salt solutions approximating quantitatively and qualitatively the content of edema fluid and other body fluids as analyzed by Salvesen (1923) were made up as indicated in the protocols. Standard solutions of NaCl, KCl, MgCl₂, CaCl₂, NaHCO₃, K₂SO₄, and Na₂HPO₄·KH₂PO₄ of known composition were prepared. Quantities of these solutions required to give the desired final concentration of each of these salts were accurately pipetted into a 200 cc. volumetric flask. Before the CaCl₂ was introduced, 125 cc. of water saturated with CO₂ were added to the flask to
prevent premature precipitation of the calcium as carbonate or phosphate. The solution was then made up to 200 cc. and transferred to the saturating tonometer system previously described by Austin and collaborators (1922). About 50 cc. of this salt solution were introduced into either a small tonometer or a 50 cc. narrow mouth centrifuge tube, about 1 per cent in weight of solid added, and then connection made with rubber tubing to a larger tonometer of from 300 to 400 cc. capacity.

The liquid with solid was clamped off from the large tonometer, which was washed out three times with either air or hydrogen, depending on whether or not $p_{\text{H}^+}$ was to be measured electrometrically; the tension of $\text{CO}_2$ required to give the desired final reaction was introduced, and then air or hydrogen run in to 1 atmosphere pressure. The liquid phase was then allowed to run into the large tonometer, the whole being placed in a water bath maintained at $38 \pm 0.05^\circ$ and rotated for 20 minutes. This procedure was repeated three more times, in order to eliminate excess $\text{CO}_2$ and so approach the desired final tension. At the fourth saturation, air or hydrogen was run in only to within 80 mm. of atmospheric pressure. The next day, after rotation from 18 to 24 hours, the whole was equilibrated to the atmosphere, then liquid and solid were separated from the gas phase. When saturation was carried on for several days, the $\text{CO}_2$ tension was adjusted several times during the saturation period, and then, finally, about 20 hours or so before being analyzed.

When the $\text{CO}_2$ tension was desired, samples of gas were taken in small 70 cc. Barcroft tubes or else sampled directly from the large tonometer. When centrifuge tubes were used for the liquid and solid, these were quickly placed in a centrifuge after covering with oil and rubber stoppers, or else paraffin, to prevent loss of $\text{CO}_2$. After centrifugation, the clear supernatant fluid was transferred to small containers over mercury. When small tonometers were used directly, the solid phase was removed by filtration through small glass tubes containing cotton.

Early in the work, when solutions were kept in contact with the solid salt, a constant temperature of $38^\circ$ in centrifuging was maintained. However, it soon became apparent that the difference in solubility of calcium carbonate or phosphate between room and body temperature during the period between the end of
solubility and completion of all analyses, was within the limit of error of the methods of analysis. When absorbent cotton was used for filtration, it was thought that stray shreds of cotton might affect the determination of the calcium. To test this,

<table>
<thead>
<tr>
<th>p[H\text{+}]</th>
<th>[Ca\text{++}] M × 10^3 kg. H_2O</th>
<th>Total [CO_3] M × 10^3 kg. H_2O</th>
<th>Total [PO_4] M × 10^3 kg. H_2O</th>
<th>pCa\text{++}</th>
<th>pCO_3 =</th>
<th>pPO_4 =</th>
<th>pK'_{s.p.} CaCO_3</th>
<th>pK'_{s.p.} Ca_3(PO_4)_2</th>
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<tr>
<td>7.43</td>
<td>0.28</td>
<td>30.32</td>
<td>0.293</td>
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<td>3.90</td>
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<td>26.55</td>
</tr>
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<td>30.32</td>
<td>0.293</td>
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Summary.

| ±0.05 | ±5 per cent. | ±0.3 per cent. | ±5 per cent. | ±0.05 | ±0.12 | ±0.03 | ±0.09 | ±0.00 | ±0.04 | ±0.04 | ±0.08 | ±0.25 |

Average error. ........................................... ±0.04 ±0.08
Maximum “ ........................................... ±0.04 ±0.25
comparisons were made on clear solutions, some containing a few shreds, and others more cotton, during the heating with normal sulfuric acid and subsequent titration. The presence of cotton in these solutions within the time required to dissolve the calcium oxalate and to titrate, had no effect in reducing the permanganate.

The solid CaCO₃ and Ca₃(PO₄)₂ used were of the highest purity obtainable. Before use in these experiments, both were washed five times with distilled water, then dried at 110°.

Table I indicates the limit of accuracy attainable with these methods, and the effect of errors in determining the values pK'ₚₚ, CaCO₃ and pK'ₚₚ, Ca₃(PO₄)₂.

Symbols.

The symbols below have been used in this and the following papers.

\[ \alpha_{Ca^{++}} \] = activity of Ca⁺⁺ ion.
\[ \gamma_{Ca^{++}} \] = " coefficient of Ca⁺⁺ ion.
\[ [Ca^{++}] \] = concentration of Ca⁺⁺ ion in mols per kilo of H₂O.
\[ [Ca] \] = " total Ca " " " " " "
\[ [CaP] \] = " Ca bound by protein in mols per kilo of H₂O.
\[ [CaX] \] = concentration of Ca not in ionized form in mols per kilo of H₂O.
\[ \mu \] = ionic strength.
\[ \nu \] = number of ions of a salt.
\[ p\gamma_{Ca^{++}} \] = negative logarithm of [Ca⁺⁺].
\[ p\gammaCa^{++} \] = " " " " " γCa⁺⁺.
\[ K \] = activity dissociation constant of an acid.
\[ K' \] = stoichiometric dissociation constant of an acid.
\[ pK \] = negative logarithm of K.
\[ pK_{a,p}, \text{ CaCO}_3 \] = \[ p\alpha_{Ca^{++}} \times \alpha_{CO_2} \].
\[ pK'_{a,p}, \text{ CaCO}_3 \] = \[ p([Ca^{++}] \times [CO_3^{2-}]). \]
\[ K_{H_2O} \] = solubility coefficient of CO₂ in pure water.
\[ \alpha_{CO_2} \] = " " " " " " salt solution and serum.
\[ pCO_2 \] = CO₂ tension in mm. of Hg.
\[ f_1 \] = [CO₂⁻]:[CO₂].
\[ f_2 \] = [H₂CO₃]:[CO₂].
\[ f_1 \] = [HCO₃⁻]:[CO₂].
\[ f \] = [CO₃²⁻]:[CO₂].
\[ \text{mm} \] = millimols = \[ \mu \times 10^8 \].
Solubility of Calcium Carbonate

Calculations.

Calculation of \([CO_2=]\) Ion Concentration from Total \(CO_2\) Content and \(\rho H^+\).

From the mass law,

\[
\frac{\alpha_{H^+} \times \alpha_{HCO_3^-}}{\alpha_{H_2CO_3}} = K_1
\]

\[
\frac{\alpha_{H^+} \times \gamma_{HCO_3^-} \times [HCO_3^-]}{\gamma_{H_2CO_3} \times [H_2CO_3]} = K_1
\]

\[
\frac{\alpha_{H^+} \times [HCO_3^-]}{\gamma_{HCO_3^-} \times [H_2CO_3]} = K_1 = K_1'
\]

\[
\frac{\alpha_{H^+} \times [HCO_3^-]}{[H_2CO_3]} = \frac{K_1 \times \gamma_{H_2CO_3}}{\gamma_{HCO_3^-}} = K_1''
\]

However, \(K_1''\) is a constant only under conditions in which \(\gamma_{H_2CO_3}\) and \(\gamma_{HCO_3^-}\) are constant.

\[
\alpha_{H^+} \times [CO_3^-] = \frac{K_1 \times \gamma_{HCO_3^-}}{\gamma_{CO_3^-}} = K_1'
\]

\[
[CO_3^-] = \frac{\alpha_{H^+} \times [CO_3^-]}{K_1'}
\]

By equations (25) and (28),

\[
[CO_3^-] = \frac{\alpha_{H^+} \times [HCO_3^-]}{K_1'} = \frac{(\alpha_{H^+})^2 \times [CO_3^-]}{K_1'' K_1'}
\]

Therefore, equation (27) can be written

\[
\text{Total } [CO_2] = (\alpha_{H^+})^2 \times [CO_3^-] + \alpha_{H^+} \times [CO_2=] + \gamma_{CO_3^-} = K_1'' K_1' + [CO_2=]
\]

\[
\text{Total } [CO_3] = [CO_3=] \times \left[ \frac{(\alpha_{H^+})^2 + \alpha_{H^+} K_1'' + K_1'}{K_1'' K_1'} \right]
\]

\[
[CO_3=] = \frac{\text{Total } [CO_3] \times K_1'' K_1'}{(\alpha_{H^+})^2 + \alpha_{H^+} K_1'' + K_1'}
\]
### TABLE II.

Factors for the Calculation of $[\text{CO}_2^-]$ and $[\text{HCO}_3^-]$ from the Total $[\text{CO}_2]$ Content in Salt Solutions of $p = 0.156$ and in Serum.

$pK''_1 = 6.13$, $pK'_1 = 9.79$

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<tr>
<th>$p_{\text{H}^+}$</th>
<th>$f$ where $f \times [\text{CO}_2]$ = $[\text{CO}_2^-]$</th>
<th>$f_i$ where $f_i \times [\text{CO}_2]$ = $[\text{HCO}_3^-]$</th>
<th>$p_{\text{H}^+}$</th>
<th>$f$ where $f \times [\text{CO}_2]$ = $[\text{CO}_2^-]$</th>
<th>$f_i$ where $f_i \times [\text{CO}_2]$ = $[\text{HCO}_3^-]$</th>
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In a similar way
\[
\left[\text{HCO}_3^-\right] = \frac{\text{Total } [\text{CO}_3^-] \times \alpha_{H^+} K_s}{(\alpha_{H^+})^2 + \alpha_{H^+} K'_s + K'_s K_s}
\]

Knowing $K_s'$ and $K'_s$ for any particular solution, one may derive a set of values for each $p\alpha_{H^+}$, such that
\[
f = \frac{K_1' K_s'}{(\alpha_{H^+})^2 + \alpha_{H^+} K_1' + K_1' K_s'}
\]
\[
f_1 = \frac{\alpha_{H^+} K_1'}{(\alpha_{H^+})^2 + \alpha_{H^+} K_1' + K_1' K_s'}
\]

where $f$ and $f_1$ are really factors representing the percentage of total $[\text{CO}_3^-]$ in the form of $[\text{CO}_3^{2-}]$ and $[\text{HCO}_3^-]$ respectively. Values for use with salt solutions at $\mu = 0.152$ and with serum, between $p\alpha_{H^+} 6.0$ and $9.0$ at $0.1$ intervals, and for $p\alpha_{H^+}$ between $6.8$ and $7.8$ at $0.01$ intervals are given in Table II.
In experiments in which it was not possible to determine \( p\alpha_{\text{H}^+} \) directly, this value was obtained indirectly by calculation from analyses of the total CO\(_2\) content and the CO\(_2\) tension. Expressing H\(_2\)CO\(_3\) in terms of the CO\(_2\) tension, \( p\text{CO}_2 \),

\[
\alpha_{\text{H}_2\text{CO}_3} = \frac{\alpha_{\text{H}_2\text{O}}^0 \times p\text{CO}_2}{760 \times 0.0224} = \frac{0.557 \times p\text{CO}_2}{760 \times 0.0224} = 0.0327 \ p\text{CO}_2
\]

(36)

\[
[\text{H}_2\text{CO}_3] = \frac{\alpha_{\text{H}_2\text{O}}^0 \times p\text{CO}_2}{760 \times 0.0224} = \frac{(0.557 - 0.103 \mu + 0.36 \ P) \times p\text{CO}_2}{760 \times 0.0224}
\]

(37)

where, according to the notation in unpublished work of Van Slyke, Hastings, and Neill, \( \alpha_{\text{H}_2\text{O}}^0 \) and \( \alpha_{\text{CO}_2}^0 \) are the solubility coefficients of CO\(_2\) in pure water, and in a salt- and protein-containing solution. \( \mu \) has its usual significance and \( P \) indicates gm. of protein per gm. of H\(_2\)O in the equation. We can write equation (27) in the form

\[
\text{Total } [\text{CO}_2] = f \ [\text{CO}_2^-] + f_1 \ [\text{HCO}_3^-] + f_2 \ [\text{CO}_3^-]
\]

where \( f, f_1, \) and \( f_2 \) refer to the percentage factor of the total [CO\(_2\)] in the form of [CO\(_2^-\)], [HCO\(_3^-\)], and [H\(_2\)CO\(_3\)] respectively. It has been shown how \( f \) and \( f_1 \) may be derived in equations (34) and (35). Hence \( f_2 \) may be found by difference, by

\[
1.00 - f - f_1 = f_2
\]

(39)

By definition,

\[
f_2 \ [\text{CO}_2] = [\text{H}_2\text{CO}_3] = \frac{\alpha_{\text{CO}_2}^0 \times p\text{CO}_2}{760 \times 0.0224}
\]

(40)

For solutions of a constant composition, \( \alpha_{\text{CO}_2}^0 \) will be constant, and we may write

\[
p\text{CO}_2 = \frac{760 \times 0.0224 \times f_2 \ [\text{CO}_2]}{\alpha_{\text{CO}_2}^0} = f_2 \ [\text{CO}_2]
\]

(41)

where

\[
f_2 = \frac{f_2 \times 760 \times 0.0224}{\alpha_{\text{CO}_2}^0}
\]

(42)

By equations (39), (34), and (35)

\[
\frac{K_{\text{H}^+} K_{\text{H}^+} + \alpha_{\text{H}^+} K_{\text{H}^+}}{(\alpha_{\text{H}^+})^2 + \alpha_{\text{H}^+} K_{\text{H}^+} + K_{\text{H}^+} K_{\text{H}^+}} + f_2 = 1.00
\]

(43)
### TABLE III.

Factors for the Calculation of $p\alpha_{H^+}$ from Total CO$_2$ Content and CO$_2$ Tension for Use in Serum.

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\[ f_2 = 1 - \frac{K_1 K'_2 + \alpha_{H_2} K'_1}{(\alpha_{H_2})^2 + \alpha_{H_2} K'_1 + K'_2} \]

\[ f_s = \frac{(\alpha_{H_2})^2}{(\alpha_{H_2})^2 + \alpha_{H_2} K'_1 + K'_2} \]

Hence, \[ f_s = \frac{(\alpha_{H_2})^3 \times 760 \times 0.0224}{[\alpha_{CO_2} [(\alpha_{H_2})^2 + \alpha_{H_2} K'_1 + K'_1 K'_2]]} \]

\[ p_{CO_2} = \frac{\text{Total } [CO_2] \times (\alpha_{H_2})^3 \times 760 \times 0.0224}{\alpha_{CO_2} [(\alpha_{H_2})^2 + \alpha_{H_2} K'_1 + K'_1 K'_2]} \]

Equation (47) expresses the relationship between the CO\textsubscript{2} tension, total CO\textsubscript{2}, and \( \alpha_{H_2} \). In actual practice, no attempt was made to calculate \( p\alpha_{H_2} \) according to equation (47). Having calculated \( f \) and \( f_s \) at various \( p\alpha_{H_2} \)'s, \( f_2 \) was found by difference, and then \( f_s \), for the corresponding \( p\alpha_{H_2} \) values. Then, by plotting \( f_s \) against \( p\alpha_{H_2} \), a curve was obtained for the relationship \( f_s = \frac{p_{CO_2}}{\text{total } [CO_2]} \) at any desired \( p\alpha_{H_2} \). By determining the CO\textsubscript{2} tension and total CO\textsubscript{2}, \( f_s \) values obtained gave the corresponding \( p\alpha_{H_2} \). Corresponding values of \( f_s \) and \( p\alpha_{H_2} \) are given in Table III.

Inasmuch as \( \alpha_{CO_2} \) is a variable depending on the composition of a solution, the relationships given will not always hold for the factors \( f_2 \) and \( f_s \) as given in the foregoing. In order to calculate \( p\alpha_{H_2} \) from total \( [CO_2] \) and CO\textsubscript{2} tension, in solutions of changing composition, one proceeds as follows, using the \( \alpha_{CO_2} \) which is the appropriate one for that solution whose \( p\alpha_{H_2} \) is to be determined. Van Slyke, Hastings, and Neill have found (unpublished results) that \( \alpha_{CO_2} \) (cc. of CO\textsubscript{2} per gm. of H\textsubscript{2}O) varies according to the empirical formula

\[ \alpha_{CO_2}^0 = 0.557 - 0.103\mu + 0.36P^4 \]

where \( P \) = gm. of protein per gm. of H\textsubscript{2}O.

The solutions for which \( p\alpha_{H_2} \) was determined in the manner to be out-

\[ \alpha_{CO_2}^0 = 0.554 - 0.104\mu + 0.36P. \]

Our results would not be significantly altered by calculating according to the above equation.

\[ \alpha_{CO_2}^0 = 0.557 - 0.103\mu + 0.36P^4. \]
lined here, were never at a reaction higher than about 7.5. Hence the $[\text{CO}_2^-]$ ion concentration was so small that it could be neglected. Equation (25) therefore could be written

$$\frac{\alpha_{\text{H}^+} \times ([\text{CO}_2^-] - [\text{H}_2\text{CO}_3])}{[\text{H}_2\text{CO}_3]} = K_f$$

By using the proper value for $\alpha_{\text{CO}_2}$ in the solution under investigation, $[\text{H}_2\text{CO}_3]$ was calculated according to equation (37), and then $p\alpha_{\text{H}^+}$ found by converting equation (49) into the well known logarithmic form of the Henderson-Hasselbalch equation:

$$p\alpha_{\text{H}^+} = pK_1 + \log \frac{[\text{CO}_2^-] - [\text{H}_2\text{CO}_3]}{[\text{H}_2\text{CO}_3]}$$

We may derive the actual working equation corresponding to equation (47) as follows:

$$\gamma_{\text{H}_2\text{CO}_3} = \frac{\alpha_{\text{H}_2\text{CO}_3}}{[\text{H}_2\text{CO}_3]}$$

Substituting the values for $\alpha_{\text{H}_2\text{CO}_3}$ and $[\text{H}_2\text{CO}_3]$ given in equations (36) and (37)

$$\gamma_{\text{H}_2\text{CO}_3} = \frac{0.557}{0.557 - 0.103P + 0.36P} = \frac{1}{1 - 0.185P + 0.65P}$$

$$[\text{H}_2\text{CO}_3] = 0.0327 \ p\text{CO}_2 \ (1 - 0.185P + 0.65P)$$

Also by equation (36)

$$p\alpha_{\text{H}_2\text{CO}_3} = 1.486 + p \ p\text{CO}_2$$

$$p\alpha_{\text{HCO}_3^-} = p\gamma_{\text{HCO}_3^-} + p \ [\text{HCO}_3^-]$$

$$p\gamma_{\text{HCO}_3^-} = 0.5\sqrt{P}$$

Combining equations (27), (53), (54), (55), (56),

$$p\alpha_{\text{HCO}_3^-} = 0.5\sqrt{P} + p \{ [\text{CO}_2^-] - [\text{CO}_2^-] - 0.0327 \ p\text{CO}_2 (1 - 0.185P + 0.65P) \}$$

But since $pK_1 = 6.33$ at $38^\circ$, equation (22) may be written in the logarithmic form

$$p\alpha_{\text{H}^+} = 7.816 - 0.5\sqrt{P} + p \ p\text{CO}_2 - p \{ [\text{CO}_2^-] - [\text{CO}_2^-] - 0.0327 \ p\text{CO}_2 (1 - 0.185P + 0.65P) \}$$
In salt solutions containing no protein and in which the \([\text{CO}_3^-]\) concentration is negligible, equation (58) simplifies into

\[
(59) \quad p\alpha_{H^+} = 7.816 - 0.5\sqrt{\mu} + p\langle \text{CO}_2 \rangle - p\{ [\text{CO}_2] - p\text{CO}_2 (0.0327 - 0.00605\mu) \}
\]

In serum of average salt and protein composition where \(\mu = 0.16\) and \(P = 0.07\) gm. per gm. of \(H_2O\), equation (58) becomes

\[
(60) \quad p\alpha_{H^+} = 7.616 + p\langle \text{CO}_2 \rangle - p\{ [\text{CO}_2] - 0.0332 p\text{CO}_2 \}
\]

When the \([\text{CO}_3^-]\) is not negligible, i.e., when the \(p\alpha_{H^+}\) is greater than 8.0, the \(p\alpha_{H^+}\) is first approximated by equation (59) or (60), then the \([\text{CO}_3^-]\) is estimated by determining the value of \(f\) for the approximated \(p\alpha_{H^+}\). The \(\text{CO}_2\) content when multiplied by this value gives the \([\text{CO}_3^-]\) concentration. This value for \([\text{CO}_3^-]\) is then substituted in equation (58) and the \(p\alpha_{H^+}\) again calculated. Since most \(p\alpha_{H^+}\) values are less than 8.0, however, it is not often necessary to apply equation (58).

**Constants Used in CO\(_2\) Calculations.**

The values of the first and second dissociation constants and activity coefficients used in the calculations were taken from the determinations of Hastings and Sendroy (1925). The equations given by them for these constants are

\[
(61) \quad pK_1' = 6.33 - 0.5\sqrt{\mu} \\
(62) \quad pK_2' = 10.22 - 1.1\sqrt{\mu}
\]

For the balanced salt solutions used, the values calculated for an approximate ionic strength of \(\mu = 0.152\), were \(pK_1' = 6.13\), \(pK_2'' = 6.14\). Van Slyke, Hastings, Murray, and Sendroy (1925) found \(pK_1' = 6.13\), \(pK_2'' = 6.14\) for horse serum, while Hastings and Sendroy (unpublished results) have found \(pK_1' = 6.12\) and \(pK_2'' = 6.13\) in human serum. Therefore, in order to simplify the calculations, \(pK_2'' = 6.13\) was used in experiments with the serum of horse and humans and in salt solutions of approximate \(\mu = 0.152\). For the same ionic strength, \(pK_2' = 9.79\). Hence \(K_1'' = 7.41 \times 10^{-7}\) and \(K_2' = 1.62 \times 10^{-10}\). For \(\sigma_{\text{H}_2\text{O}}\) the value used was 0.557. In salt solutions and serum the variation of \(\sigma_{\text{CO}_2}\) was calculated according to equation (48). These solubility coefficients were derived without attempting to differentiate between the two reactions.
Solubility of Calcium Carbonate

Experimental Results.

Variation in Solubility of \( \text{CaCO}_3 \) with Varying Ionic Strength.

The solubility of \( \text{CaCO}_3 \) has been determined in salt solutions, the ionic strength of which ranged from \( \mu = 0.024 \) to \( \mu = 0.208 \). Save for small variations in bicarbonate concentration, the ionic strength was increased by additions of NaCl. After 20 hours of saturation at 38° with solid \( \text{CaCO}_3 \), the liquid phase was analyzed for the CO₂ content, calcium content, and \( \text{pH}^+ \). In some instances the CO₂ tension in the gas phase was also determined. From the CO₂ content and \( \text{pH}^+ \) the \([\text{CO}_3^{2-}]\) concentration was calculated. From the \([\text{CO}_3^{2-}]\) concentration and the \([\text{Ca}^{++}]\) concentration

| Experiment No. | \( \mu \) | \( \nu \) | \( \text{pH}^+ \) | Total \([\text{CO}_3]_{\text{aq}}\) M X 10⁻³ | \([\text{Ca}^{++}]_{\text{aq}}\) M X 10⁻³ | \([\text{CO}_3^{2-}]_{\text{aq}}\) M X 10⁻³ | \([\text{HCO}_3^-]_{\text{aq}}\) M X 10⁻³ | \(\text{pK}_1\) & \(\text{pK}_2\) calculated | \(\text{pK}_1\) & \(\text{pK}_2\) calculated |
|----------------|--------|---------|----------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|
| 1              | 0.024  | 0.156   | 7.02 c         | 18.86                         | 0.0152                        | 0.764                         | 4.82                         | 3.12                          | 7.94                          | 7.96                          |
| 2              | 0.031  | 0.176   | 7.22 c         | 24.56                         | 0.0348                        | 0.372                         | 4.46                         | 3.43                          | 7.89                          | 7.90                          |
| 3              | 0.050  | 0.224   | 7.07 c         | 18.93                         | 0.0205                        | 0.865                         | 4.69                         | 3.06                          | 7.75                          | 7.76                          |
| 4              | 0.076  | 0.276   | 7.10 c         | 19.02                         | 0.0253                        | 0.916                         | 4.60                         | 3.04                          | 7.64                          | 7.64                          |
| 5              | 0.082  | 0.286   | 7.27 c         | 25.07                         | 0.0541                        | 0.423                         | 4.27                         | 3.37                          | 7.64                          | 7.61                          |
| 6              | 0.102  | 0.320   | 7.00 c         | 20.00                         | 0.0231                        | 1.208                         | 4.64                         | 2.92                          | 7.56                          | 7.54                          |
| 7              | 0.108  | 0.329   | 7.28 c         | 25.68                         | 0.0621                        | 0.474                         | 4.21                         | 3.32                          | 7.53                          | 7.52                          |
| 8              | 0.129  | 0.359   | 7.52 g         | 9.86                          | 0.0566                        | 0.706                         | 4.30                         | 3.15                          | 7.45                          | 7.46                          |
| 9              | 0.130  | 0.361   | 7.27 g         | 11.06                         | 0.0310                        | 1.134                         | 4.51                         | 2.95                          | 7.46                          | 7.46                          |
| 10             | 0.130  | 0.361   | 7.37 g         | 11.56                         | 0.0414                        | 0.968                         | 4.38                         | 3.02                          | 7.40                          | 7.45                          |
| 11             | 0.131  | 0.362   | 7.16 g         | 12.50                         | 0.0267                        | 1.482                         | 4.57                         | 2.83                          | 7.40                          | 7.45                          |
| 12             | 0.132  | 0.363   | 7.17 g         | 14.05                         | 0.0308                        | 1.330                         | 4.51                         | 2.88                          | 7.39                          | 7.45                          |
| 13             | 0.133  | 0.365   | 7.41 g         | 26.40                         | 0.0941                        | 0.494                         | 4.03                         | 3.31                          | 7.34                          | 7.44                          |
| 14             | 0.137  | 0.370   | 7.16 g         | 13.80                         | 0.0402                        | 1.008                         | 4.40                         | 3.00                          | 7.40                          | 7.44                          |
| 15             | 0.158  | 0.395   | 7.38 g         | 28.51                         | 0.0963                        | 0.494                         | 4.02                         | 3.31                          | 7.33                          | 7.38                          |
| 16             | 0.177  | 0.420   | 7.30 g         | 18.22                         | 0.0594                        | 1.040                         | 4.23                         | 2.98                          | 7.21                          | 7.34                          |
| 17             | 0.177  | 0.420   | 8.35 g         | 24.87                         | 0.0870                        | 0.050                         | 3.06                         | 4.30                          | 7.36                          | 7.34                          |
| 18             | 0.177  | 0.420   | 7.48 g         | 27.07                         | 0.126                         | 0.352                         | 3.90                         | 3.45                          | 7.35                          | 7.34                          |
| 19             | 0.202  | 0.450   | 7.32 c         | 18.44                         | 0.0683                        | 1.04                          | 4.17                         | 2.98                          | 7.15                          | 7.29                          |
| 20             | 0.208  | 0.455   | 7.46 c         | 23.70                         | 0.1243                        | 0.371                         | 3.91                         | 3.43                          | 7.34                          | 7.28                          |
the pK',., CaCO₃ was calculated. The ionic strength, \( \mu \), of the solution was calculated from the analyses of the solution at the end of the saturation period. The results of these experiments have been tabulated in Table IV and are graphically presented in Fig. 1. The smooth curve is the graphical representation of the equation

\[
\text{pK}',., \text{CaCO}_3 = 8.58 - \frac{4.94\sqrt{\mu}}{1 + 1.64\sqrt{\mu}}
\]

Fig. 1. The solubility of CaCO₃ in salt solutions of varying ionic strength saturated with CaCO₃ at 38°. Values of pK',., CaCO₃ are plotted as ordinates and \( \sqrt{\mu} \) as abscissae. The curved line has the equation pK',., CaCO₃ = 8.58 - \( \frac{4.94\sqrt{\mu}}{1 + 1.61\sqrt{\mu}} \).

Since it has been previously shown that \(-\log \gamma_{\text{CO}_3^-} = 1.6\sqrt{\mu}\) as a first approximation up to \( \mu = 0.160 \), \(-\log \gamma_{\text{Ca}^{2+}}\) can be calculated for any given ionic strength by means of the equation

\[
-\log \gamma_{\text{Ca}^{2+}} = \frac{4.94\sqrt{\mu}}{1 + 1.61\sqrt{\mu}} - 1.6\sqrt{\mu}
\]
Solubility of Calcium Carbonate

The manner in which the constants of equation (63) were obtained requires some explanation. The value 8.58 for $pK_{s,p.\text{CaCO}_3}$ at $\mu = 0$ was obtained in four ways. (1) By graphic extrapolation of a smooth curve through the experimental points given in this paper; (2) by calculation of $pK_{s,p.\text{CaCO}_3}$ at $\mu = 0$ from our experimental points at the two lowest ionic strength values where it may be assumed that the simple limiting relationship, $pK'_{s,p.\text{CaCO}_3} = pK_{s,p.\text{CaCO}_3} - 4 \sqrt{\mu}$ holds; (3) by extrapolation of the curve obtained from a study of the solubility of CaCO$_3$ in solutions saturated with Ca$_3$(PO$_4$)$_2$ as well as CaCO$_3$ (these data are to be presented in Paper III of this series); (4) by calculation of $pK_{s,p.\text{CaCO}_3}$ at $\mu = 0$ in the same manner as in method (2) from the data used in method (3). These methods of estimation led to the average value of 8.58 for $pK_{s,p.\text{CaCO}_3}$ at $\mu = 0$. Since the lowest salt concentration in which the solubility of CaCO$_3$ was determined was $\mu = 0.024$, the error involved in extrapolation is considerable and makes the value 8.58 correspondingly questionable. Considering the possible errors involved however, it is felt that this value is probably within 0.1 of the true $pK_{s,p.\text{CaCO}_3}$ at $\mu = 0.0$ and, as will be shown later, it agrees fairly well with previous determinations by other authors.

The manner in which the constants B and A were evaluated was as follows:

Equation (17) was converted to the form

$$pK_{s,p.\text{CaCO}_3} - pK'_{s,p.\text{CaCO}_3} = 2 \gamma_{\text{CaCO}_3}$$

But, by equation (21),

$$\gamma_{\text{CaCO}_3} = \frac{B \sqrt{\mu}}{1 + A \sqrt{\mu}}$$

Therefore

$$\frac{pK_{s,p.\text{CaCO}_3} - pK'_{s,p.\text{CaCO}_3}}{2} = \frac{B \sqrt{\mu}}{1 + A \sqrt{\mu}}$$

Rearranging and substituting 8.58 for $pK_{s,p.\text{CaCO}_3}$ equation (67) becomes

$$\frac{2}{8.58 - pK'_{s,p.\text{CaCO}_3}} = \frac{1 + A \sqrt{\mu}}{B \sqrt{\mu}} = \frac{1}{B} \left( \frac{1}{\sqrt{\mu}} \right) + \frac{A}{B}$$
By plotting values of \( \frac{2}{8.58 - \text{pK}_{s.p.} \text{CaCO}_3} \) as ordinates against \( \frac{1}{\sqrt{\mu}} \) as abscissae, one obtains a straight line, the slope of which equals \( \frac{1}{B} \) and the intercept of which on the \( y \)-axis equals \( \frac{A}{B} \). The values of \( A \) and \( B \) found in this way lead to the equation

\[
\text{pK}_{s.p.} \text{CaCO}_3 = 8.58 - \frac{4.94 \sqrt{\mu}}{1 + 1.61 \sqrt{\mu}}
\]

It is appreciated that several of our points above \( \sqrt{\mu} = 0.35 \) lie above the line which represents this equation. Whether these deviations are significant and are not due to errors involved in manipulation and analysis is not known at the present time. The point of significance seems to be that the solubility of \( \text{CaCO}_3 \) in salt solutions of \( \text{NaHCO}_3 \) and \( \text{NaCl} \) increases with increasing salt concentration in a manner which is not inconsistent with the predictions of the Debye-Hückel theory.

### TABLE V.

Activity Coefficients at 38° of \( \text{CaCO}_3 \), \( \text{Ca}^{++} \), and \( \text{CO}_3^{-} \) Calculated at Rounded Values of \( \mu \).

<table>
<thead>
<tr>
<th>( \mu )</th>
<th>( \gamma_{\text{CaCO}_3} )</th>
<th>( \gamma_{\text{CO}_3^{-}} )</th>
<th>( \gamma_{\text{Ca}^{++}} )</th>
<th>( \gamma_{\text{HCO}_3^{-}} )</th>
<th>( \gamma_{\text{Cl}^{-}} )</th>
<th>( \gamma_{\text{H}^{+}} )</th>
<th>( \gamma_{\text{Na}^{+}} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>0.05</td>
<td>0.99</td>
<td>0.44</td>
<td>0.35</td>
<td>0.77</td>
<td>0.84</td>
<td>0.88</td>
<td>0.84</td>
</tr>
<tr>
<td>0.10</td>
<td>0.31</td>
<td>0.31</td>
<td>0.31</td>
<td>0.69</td>
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<td>0.15</td>
<td>0.26</td>
<td>0.28</td>
<td>0.64</td>
<td>0.64</td>
<td>0.64</td>
<td>0.64</td>
<td>0.64</td>
</tr>
<tr>
<td>0.16*</td>
<td>0.25</td>
<td>0.23</td>
<td>0.27</td>
<td>0.63</td>
<td>0.63</td>
<td>0.63</td>
<td>0.63</td>
</tr>
</tbody>
</table>

* Approximately the ionic strength of biological fluids.

Since the activity coefficient of the carbonate ion in solutions of different ionic strength has been previously estimated, and since the activity coefficient of \( \text{CaCO}_3 \) has been determined, it is of some interest to estimate the activity coefficient of the calcium ion. This has been done by means of equation (64) and the results are tabulated in Table V for the activity coefficients at even ionic strengths.
For comparison we have given activity coefficients for the bicarbonate ion from our own data and for the H\(^+\), Na\(^+\), and Cl\(^-\) ions from Lewis and Randall’s “Thermodynamics.” It is particularly significant that the activity coefficients of the divalent ions CO\(_3^{2-}\) and Ca\(^{++}\) are much less than those of the monovalent ions HCO\(_3^-\) and Na\(^+\). In the terminology of the electrolytic dissociation theory one would say that the divalent salts were less completely dissociated. The activity coefficients of Ca\(^{++}\) and CO\(_3^{2-}\) at \(\mu = 0.160\) will be used to calculate the calcium activity in biological fluids.

It should be mentioned at this point that Bray (1925) in a classical investigation of HCO\(_3^-\) and CO\(_3^{2-}\) activity coefficients in salt solutions found a difference between the activity coefficient of the HCO\(_3^-\) ion as determined in systems composed of NaHCO\(_3\) - NaCO\(_3\) - NaCl and those composed of KHC\(_3\) - K\(_2\)CO\(_3\) - KCl. This suggests that one cannot use the same activity coefficient for the HCO\(_3^-\) ion in such biological systems as red blood cells which are rich in potassium, as in serum where sodium predominates.

Since in biological systems variations are encountered in \(p ([\text{Ca}] \times [\text{CO}_3^{2-}])\) other than those attributable to variations in ionic strength, certain conditions were varied in the salt solutions of the same ionic strength to determine what factors might there be significant.

The systems in which equilibrium with solid CaCO\(_3\) was established will first be discussed, followed by the presentation of experiments in which equilibrium was apparently not established. The criterion of equilibrium has been regarded as the attainment of the value 7.30 to 7.40 for \(pK'_{s,p}\). CaCO\(_3\) in solutions whose ionic strength was approximately \(\mu = 0.150\). This was the value found in salt solutions containing no ions which might form with calcium other insoluble or slightly ionized compounds. This figure, within the limits studied, was independent of the \(p\alpha_{H^+}\), the initial calcium concentration, and the duration of the saturation period beyond 16 hours. A shorter saturation period is undoubtedly adequate, although the minimum length of time necessary was not determined.
Salt solutions composed of all of the salts of serum except inorganic phosphate come rapidly into equilibrium with solid CaCO₃ whether there is no calcium initially in solution or whether there is an excess of calcium. Representative experiments illustrating this point are given in Table VI. These experiments show consistent values for pK's, CaCO₃ which agree with those

**Table VI.**

<table>
<thead>
<tr>
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</tr>
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<tbody>
<tr>
<td>1 (12)</td>
<td>44 hrs.</td>
<td>7.51</td>
<td>31.84</td>
<td>0.160</td>
<td>0.33</td>
<td>7.28</td>
<td>0.387</td>
</tr>
<tr>
<td>2 (17)</td>
<td>44 hrs.</td>
<td>7.39</td>
<td>27.30</td>
<td>0.103</td>
<td>0.46</td>
<td>7.33</td>
<td>0.374</td>
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<tr>
<td>3 (18')</td>
<td>64 hrs.</td>
<td>7.38</td>
<td>27.42</td>
<td>0.006</td>
<td>0.58</td>
<td>7.26</td>
<td>0.374</td>
</tr>
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</table>

**Table VII.**

<table>
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<tbody>
<tr>
<td>1 (9)</td>
<td>20 hrs.</td>
<td>7.43</td>
<td>31.04</td>
<td>0.129</td>
<td>0.31</td>
<td>7.40</td>
<td>0.383</td>
</tr>
<tr>
<td>2 (14)</td>
<td>8 days.</td>
<td>7.51</td>
<td>31.35</td>
<td>0.157</td>
<td>0.26</td>
<td>7.39</td>
<td>0.387</td>
</tr>
<tr>
<td>3 (8)</td>
<td>20 hrs.</td>
<td>7.20</td>
<td>19.20</td>
<td>0.045</td>
<td>1.33</td>
<td>7.23</td>
<td>0.378</td>
</tr>
<tr>
<td>4 (16)</td>
<td>44 hrs.</td>
<td>7.56</td>
<td>33.62</td>
<td>0.165</td>
<td>0.25</td>
<td>7.38</td>
<td>0.378</td>
</tr>
<tr>
<td>5 (24)</td>
<td>7 days.</td>
<td>7.57</td>
<td>33.67</td>
<td>0.195</td>
<td>0.23</td>
<td>7.37</td>
<td>0.380</td>
</tr>
<tr>
<td>6 (5)</td>
<td>20 hrs.</td>
<td>7.48</td>
<td>29.24</td>
<td>0.136</td>
<td>0.25</td>
<td>7.47</td>
<td>0.383</td>
</tr>
<tr>
<td>7 (20)</td>
<td>68 hrs.</td>
<td>7.37</td>
<td>27.14</td>
<td>0.097</td>
<td>0.03</td>
<td>7.39</td>
<td>0.401</td>
</tr>
</tbody>
</table>
Solubility of Calcium Carbonate

obtained in the study of the solubility of CaCO₃ in solutions of varying ionic strength.

When we have the system (solid phase: CaCO₃ + Ca₃(PO₄)₂) (liquid phase: solution of NaCl + NaHCO₃, with and without

**TABLE VIII.**
Conditions Leading to Supersaturation with Respect to CaCO₃. Saturation in the Absence of a Solid Phase.

<table>
<thead>
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<td>7.38</td>
<td>28.01</td>
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<td>0.88</td>
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<td>6.34</td>
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</tr>
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<td>39.28</td>
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<td>0.88</td>
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<td>0.403</td>
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<td>6.35</td>
<td>0.403</td>
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</table>

**TABLE IX.**
Conditions Leading to Supersaturation with Respect to CaCO₃. Saturation with CaCO₃ in the Presence of Phosphate in Solution.

<table>
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<td>1.23</td>
<td>6.65</td>
<td>0.382</td>
<td>0</td>
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<td>31.39</td>
<td>0.136</td>
<td>1.26</td>
<td>6.77</td>
<td>0.387</td>
<td>4.65</td>
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<td>3 (13)</td>
<td>42</td>
<td>7.41</td>
<td>29.93</td>
<td>0.118</td>
<td>1.42</td>
<td>6.78</td>
<td>0.380</td>
<td>7.76</td>
</tr>
</tbody>
</table>

calcium, with and without phosphate) equilibrium is established with respect to CaCO₃ whether or not calcium is initially present in the liquid phase. This is also true whether or not inorganic phosphate is initially present. Some experiments illustrating these results are given in Table VII.
Conditions Leading to Apparent Supersaturation. Absence of Solid Phase. (Liquid Phase: Solution of NaHCO₃ + NaCl + CaCl₂.)

In systems composed initially of only the liquid phase NaHCO₃ – NaCl – CaCl₂ and the gas phase CO₂, at a definite tension, equilibration for 44 hours did not result in complete equilibrium with the solid phase CaCO₃. This, as was anticipated, was a condition of supersaturation in which equilibration was not continued until equilibrium was established. The details of this experiment are given in Experiments 1 and 2 of Table VIII.

Absence of Solid Phase. (Liquid Phase: Solution of NaHCO₃ + NaCl + CaCl₂ + \{Na₂HPO₄\}.)

Since our ultimate goal was the study of calcium activity in biological fluids the previous experiments were repeated with the addition of a mixture of Na₂HPO₄ and KH₂PO₄ at physiological pH so that the total phosphate approximately equalled the concentration in serum. No solid phase was present. The length of saturation varied from 18 to 68 hours. The protocols of three of these experiments are also given in Table VIII (Nos. 3 to 5). In each instance the solution was apparently markedly supersaturated with respect to CaCO₃.

(Solid Phase: CaCO₃) (Liquid Phase: Solution of NaHCO₃ + NaCl + CaCl₂ + \{Na₂HPO₄\}.)

If to the above aqueous phase, solid CaCO₃ is added, supersaturation with respect to CaCO₃ apparently still exists as is shown by the difference between pK′s, pCaCO₃ values of 6.7 under these conditions as compared with 7.3 under conditions of equilibrium in systems without phosphates in solution (Table IX). It was further found that this condition of apparent supersaturation existed even though no calcium was initially in solution. Whether this condition is one of delayed equilibrium or complex ion formation is at present unknown.
Solubility of Calcium Carbonate

Conditions Leading to Apparent Undersaturation. (Solid Phase: Ca$_3$(PO$_4$)$_2$) (Liquid Phase: Solution of NaHCO$_3$ + NaCl).

When the system composed initially of NaHCO$_3$ and NaCl was saturated with the solid phase Ca$_3$(PO$_4$)$_2$ at fixed CO$_2$ tensions, the liquid phase was unsaturated with respect to CaCO$_3$ even after 8 days (Table X). This was the case whether or not calcium or phosphate was initially present in the solution. The solubility product of CaCO$_3$ was not reached in this experiment.

**Table X.** Conditions Lending to Undersaturation. Saturation of Salt Solutions with Ca$_3$(PO$_4$)$_2$.

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Time of equilibration</th>
<th>$p$H$^+$</th>
<th>[CO$_3$]$^-$ M $\times$ 10$^2$ kg H$_2$O</th>
<th>[CO$_2$]$^-$/[HCO$_3$]$^-$ M $\times$ 10$^2$ kg H$_2$O</th>
<th>[Ca$^{++}$] M $\times$ 10$^3$ kg H$_2$O</th>
<th>$pK_a$ s.p.</th>
<th>$\sqrt{\mu}$</th>
<th>Initially in solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (7)</td>
<td>20 hrs.</td>
<td>7.37</td>
<td>27.14</td>
<td>0.097</td>
<td>0.05</td>
<td>8.31</td>
<td>0.381</td>
<td>0</td>
</tr>
<tr>
<td>2 (10)</td>
<td>8 days</td>
<td>7.47</td>
<td>28.53</td>
<td>0.130</td>
<td>0.03</td>
<td>8.41</td>
<td>0.386</td>
<td>0</td>
</tr>
<tr>
<td>3 (6)</td>
<td>20 hrs.</td>
<td>7.18</td>
<td>18.17</td>
<td>0.041</td>
<td>0.59</td>
<td>7.62</td>
<td>0.376</td>
<td>7.76</td>
</tr>
<tr>
<td>4 (4)</td>
<td>20 &quot;</td>
<td>7.38</td>
<td>25.66</td>
<td>0.004</td>
<td>0.04</td>
<td>8.43</td>
<td>0.379</td>
<td>1.21</td>
</tr>
<tr>
<td>5 (3)</td>
<td>20 &quot;</td>
<td>7.32</td>
<td>25.05</td>
<td>0.079</td>
<td>0.04</td>
<td>8.50</td>
<td>0.384</td>
<td>4.65</td>
</tr>
</tbody>
</table>

Discussion of Experiments upon Solubility of CaCO$_3$ in Salt Solutions.

It is a thermodynamic necessity that in all solutions in equilibrium with solid CaCO$_3$ the activity and the activity solubility product of CaCO$_3$ are the same. For 38° we have found this latter value (designated as $K_{s.p.}$) to be $1 \times 10^{-8.58}$. Since the activity coefficients of the calcium and carbonate ions decrease from unity as the ionic strength of the solutions increases from zero, the analytically determined values of Ca$^{++}$ and CO$_3^-$ will become progressively greater as will the product [Ca$^{++}$] $\times$ [CO$_3^-$].

In the foregoing experiments, it has been shown that salt solutions containing no phosphate may be brought into equilibrium with solid calcium carbonate and that the analytically determined product [Ca$^{++}$] $\times$ [CO$_3^-$] is a constant which varies with
the ionic strength of the solution in a manner which is in har-
mony with the Debye and Hückel theory of the behavior of
strong electrolytes.

From our data we have calculated activity coefficients for
Ca++, and CO3 ions in simple salt solutions of varying ionic
strength. Is one justified in applying these activity coefficients
to all solutions the ionic strengths of which are known, and which
have been equilibrated with solid CaCO3 until no further change
in the analytical product [Ca++] \times [CO3] occurs? Perhaps
not, because as Holt, La Mer, and Chown (1925, b) pointed out,
inhibiting influences may delay the attainment of equilibrium for
a long period of time. However, since this method of attack
seems at present the most fruitful, we have used it in subsequent
sections on serum.

It is further appreciated that there are specific ion effects of
considerable magnitude in solutions composed of salts other than
NaHCO3 and NaCl which will markedly affect the activity coeffi-
cients of the calcium and carbonate ions. In the absence of
information upon the magnitude of these specific influences, how-
ever, we have used the activity coefficients found in our experi-
ments on salt solutions.

A point of interest and importance for the interpretation of our
later work on biological fluids arises from our experiments upon
CaCO3 solubility in salt solutions containing inorganic phosphate
in solution. It will be recalled that the analytical product
[Ca] \times [CO3] was markedly greater than one found in entirely
comparable experiments but in which no phosphate was initially
present in solution. Furthermore, this product was practically
the same whether one started with calcium in solution or not.
Was the activity product \( \alpha_{Ca} \times \alpha_{CO3} \) greater in these solutions
than in others without phosphate? It is highly improbable
that CaCO3 had been formed but not precipitated in view of the
fact that an excess of solid salt was constantly present. Nor does
it seem possible that the presence of the small amount of phos-
phate was in itself sufficient to alter the activity coefficient of
the Ca++ and CO3 in the presence of phosphate when both solid
phases, CaCO3 and Ca3(PO4)2, were present.

Since solid Ca3(PO4)2 was absent in these experiments it is
possible that the calcium and the phosphate were combined
but not precipitated. If this is true, as will be explained later, we are justified in applying our activity coefficients for \( \text{Ca}^{++} \) and \( \text{CO}_3^{--} \) found for salt solutions containing no phosphate calculating then the calcium ion concentration, subtracting this from the total calcium analytically determined, and calling this value unionized calcium. This has been our procedure in the work now to be described and to which the Debye and Hückel theory did not seem applicable without some such hypothesis.

An alternative explanation of these results is that the reaction between \( \text{Ca}^{++} \) and \( \text{CO}_3^{--} \) was inhibited by the presence of the phosphate and a condition of delayed equilibrium resulted. If this were the true explanation, the thermodynamic method of treatment would be inapplicable to this experiment and the comparable condition found in serum. Realizing this, we must emphasize that our subsequent thermodynamic treatment serves as a method of attack which is applicable only if equilibrium exists. Whether it does or not has not as yet been rigidly determined.

*Solubility of \( \text{CaCO}_3 \) in Solutions of Sodium Citrate.*

Having determined the solubility of \( \text{CaCO}_3 \) in solutions of \( \text{NaHCO}_3 \) and \( \text{NaCl} \), and having studied some of the factors which seem to prevent the attainment of equilibrium with respect to \( \text{CaCO}_3 \), it was decided to study the effect of sodium citrate upon the solubility of \( \text{CaCO}_3 \). Complex calcium citrate ions are supposed to exist but we were unable to obtain accurate information regarding their exact nature. Since the citrate ion is a trivalent ion it was at first thought that its action in dissolving \( \text{CaCO}_3 \) might be explicable merely upon the basis of an increased ionic strength of the solution. This proved to be an inadequate explanation for several reasons. First, because the solubility product was not constant at varying \( p\alpha_{\text{H}^+} \) although the ionic strength was constant; second, because when values of \( pK'_{\text{s.p.}} \)

\( \text{CaCO}_3 \) chosen at constant \( p\alpha_{\text{H}^+} \), were plotted against the \( \sqrt{\mu} \) the curve did not conform to that predicted by the Debye and Hückel theory. To explain our results one would have to assume a large specific ion effect or the existence of a slightly dissociated calcium citrate compound. Our calculations have been made on the basis of the latter hypothesis.
In the simplest case, the liquid phase was initially composed only of a solution of sodium citrate, Na₃C₆H₅O₇. In some cases NaHCO₃ was also initially present. These solutions were equilibrated with solid CaCO₃ at definite CO₂ tensions which, however, changed during the course of the saturation. The technique and analytical procedures were those which have been already described. The results of our experiments are given in Table XI. At equilibrium the liquid phase contained the following: Na₃C₆H₅O₇, NaHCO₃, Ca₃(C₆H₅O₇)₂, Ca(HCO₃)₂, H₂CO₃. The reactions concerned with this change in composition are

\[ \text{CaCO₃} \rightleftharpoons \text{CaCO₃} + \text{H}_₂\text{CO₃} = \text{Ca(HCO₃)₂} \]

Solid. Solution.

\[ 2\text{Na₃C₆H₅O₇} + 3\text{Ca(HCO₃)₂} = \text{Ca₃(C₆H₅O₇)₂} + 6\text{NaHCO₃} \]

The p[H⁺] was sufficiently high so that forms of citrate other than the tertiary salt can be neglected.

The ionic strength of the solution at equilibrium being known and the [CO₃²⁻] concentration having been calculated, it is possible to calculate the [Ca⁺⁺] concentration which one would expect to find in a NaCl solution of the same ionic strength. It is appreciated that this is only an approximation because the specific ion effect in the case of the citrate ion is probably very different from that of the chloride ion. The total calcium content having been found by analysis, it is possible on the basis of the assumptions made with reference to \( \gamma_{\text{Ca}⁺⁺} \), to estimate the amount of non-ionized calcium (Column 8, Table XI).

Since Ca₃(C₂H₃O₇)₂ would be expected to ionize according to the equation

\[ \text{Ca₃(C₂H₃O₇)₂} \rightleftharpoons 3\text{Ca}⁺⁺ + 2\text{C₂H₃O₇}⁻⁻ \]

the following equation might be expected to prevail at equilibrium:

\[ \frac{[\text{Ca}⁺⁺]^3 \times [\text{C₂H₃O₇}⁻⁻]^2}{[\text{Ca₃(C₂H₃O₇)₂}]} = K'_{\text{Ca₃(C₂H₃O₇)₂}} \]

or

\[ 3p\text{Ca}⁺⁺ + 2p(C₂H₃O₇)⁻⁻ - p\text{Ca₃(C₂H₃O₇)₂} = pK'_{\text{Ca₃(C₂H₃O₇)₂}} \]
We have attempted to calculate $pK'_\text{Ca}(\text{Cit})$ in the experiments reported with the result shown in Column 9. There seems to be a correlation between the $p\alpha_{\text{H}^+}$ and the value $pK'_\text{Ca}(\text{Cit})$ as Fig. 2 indicates. Whether this means that at higher $p\alpha_{\text{H}^+}$ values the salt is of different composition from that at the lower values is unknown at present. A slow rearrangement from the salt $\text{CaH(Cit)_2}$ to the salt $\text{Ca}_2(\text{Cit}_2)^2$ has been described.

<table>
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<tr>
<th>Experiment No.</th>
<th>$\sqrt{\mu}$*</th>
<th>$p\alpha_{\text{H}^+}$ (g)</th>
<th>$[\text{CO}_3^-] \times 10^6$ kg. H$_2$O</th>
<th>$[\text{Cit}^-] \times 10^6$ kg. H$_2$O</th>
<th>Calculated $[\text{Ca}^{++}] \times 10^6$ kg. H$_2$O</th>
<th>Total $[\text{Ca}] \times 10^6$ kg. H$_2$O</th>
<th>$[\text{Ca}^2X] \times 10^6$ kg. H$_2$O</th>
<th>$pK'_\text{Ca}(\text{Cit})$</th>
<th>Initial concentration of $\text{Na}_2\text{Cit} \times 10^6$ kg. H$_2$O</th>
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<td>8.44</td>
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<td>11.47</td>
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<tr>
<td>17</td>
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<td>21.87</td>
<td>0.138</td>
<td>0.28</td>
<td>3.02</td>
<td>2.74</td>
<td>12.64</td>
<td>5</td>
</tr>
</tbody>
</table>

* Calculated from final composition of solutions.

(Pannozani, 1910), and it may be that some such phenomenon plays a rôle here.

The important points illustrated by the results of these experiments are:

1. The fact that the product $[\text{Ca}] \times [\text{CO}_3^-]$ is not a constant at constant ionic strength but varies with the $p\alpha_{\text{H}^+}$ of the solution and the concentration of citrate present.
2. That this cannot be explained on the basis of supersaturation or by the Debye-Hückel theory, without the assumption of an unusually large specific ion effect.

3. That it does seem to be explicable on the hypothesis of the formation of a slightly ionized calcium compound.

4. This calcium citrate compound does not seem to be a simple substance such as Ca₃(C₆H₅O₇)₂.

Fig. 2. The apparent variation of pK'Ca₃(C₆H₅O₇)₂ with pαH⁺. The solutions containing different total citrate concentrations are designated by different symbols.

- ○ = 40 mm sodium citrate per kilo of H₂O.
- △ = 20 " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " 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" " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " " 

Precipitation of Calcium from a Citrate Solution in Equilibrium with Solid CaCO₃ by the Addition of Solid Ca₃(PO₄)₂.

In a later section it will be shown that serum, when equilibrated with CaCO₃ does not alter in its calcium concentration, but when it is equilibrated with Ca₃(PO₄)₂ calcium is precipitated. In order to determine whether solutions of citrate, in equilibrium with solid CaCO₃, showed the same behavior, the following experiment was performed. 40 cc. of a solution of sodium citrate (6.67 mm per liter) were saturated 20 hours at 38° with solid CaCO₃ at a CO₂ tension of approximately 25 mm. At the close of the saturation period the solution was separated from the solid.
Solubility of Calcium Carbonate

CaCO₃ and analyzed for CO₂, calcium, and pοH⁺. It was then saturated for 20 hours at 38° with Ca₃(PO₄)₂ and again analyzed. The results of this experiment are given in Table XII. That precipitation of calcium does occur is strikingly shown, thereby illustrating a property of citrate solutions which resembles that of serum.

Solubility of CaCO₃ in Serum.

The results of our study of the solubility of calcium salts in serum will now be presented. In this section only the experiments related to CaCO₃ solubility will be considered; in later sections the solubility of Ca₃(PO₄)₂ will be discussed.

**TABLE XII.**

Effect of Solid Ca₃(PO₄)₂ upon a Citrate Solution in Equilibrium with CaCO₃.

Initial concentration of Na₃C₆H₅O₇ = 0.00667 m. Saturation 20 hours with solid CaCO₃ at 38°.

<table>
<thead>
<tr>
<th>m</th>
<th>√ν</th>
<th>[Ca₃(PO₄)₂] × 10⁻⁵</th>
<th>[CO₃⁻]</th>
<th>[H⁺]</th>
<th>[HCO₃⁻]</th>
<th>[CO₃²⁻]</th>
<th>pκ⁺</th>
<th>pCaO⁻</th>
<th>pκCaO⁻</th>
<th>[Ca₄(PO₄)₂] × 10⁻⁵</th>
<th>[Ca₃(PO₄)₂] (calculated)</th>
<th>H₂O (calculated)</th>
<th>Ca₃(PO₄)₂/100 g.</th>
<th>Ca₃(PO₄)₂/100 g.</th>
<th>Ca₃(PO₄)₂/100 g.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.057</td>
<td>0.240</td>
<td>5.84</td>
<td>12.65</td>
<td>0.0273</td>
<td>7.72</td>
<td>4.564</td>
<td>3.16</td>
<td>0.092</td>
<td>5.15</td>
<td>0.094</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Same solution saturated 20 hours with solid Ca₃(PO₄)₂ at 38°.

| 0.057 | 0.240 | 3.78 | 8.63 | 0.00872 | 5.06 |

If one analyzes blood serum as drawn from the body without permitting a change in the gases of the blood to occur, one may obtain values of the pοH⁺ and CO₂ content from which [CO₃²⁻] ion concentration may be calculated.

The [CO₃²⁻] ion concentration has been calculated from the pοH⁺ and [CO₃⁻] content on the assumption that the activity coefficients of the [HCO₃⁻] ion and the [CO₃⁻] ion are identical in serum with the values which were found for them in a solution of NaHCO₃ and NaCl the ionic strength of which was 0.160. That this assumption is probably nearly correct for the HCO₃⁻ ion is indicated by the fact that the value for the activity coefficient of
the $\text{HCO}_3^-$ ion was found to be approximately the same in serum as in a salt solution of $\mu = 0.160$. We have no direct evidence that the activity coefficient of the $\text{CO}_3^{2-}$ ion is identical in serum and in a salt solution of the same ionic strength.

The $[\text{CO}_3^{2-}]$ ion concentration when multiplied by the total Ca concentration gives the product $[\text{Ca}] \times [\text{CO}_3^{2-}]$. The corresponding product in simple salt solutions containing no slightly ionized calcium and in equilibrium with solid CaCO$_3$ is the solubility product constant of calcium carbonate in this solution.

Analyses of serum show that the product $[\text{Ca}] \times [\text{CO}_3^{2-}]$ far

<table>
<thead>
<tr>
<th>TABLE XIII.</th>
<th>Determination of the $p([\text{Ca}] \times [\text{CO}_3^{2-}])$ in Human Serum.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment No</td>
<td>$p\text{H}^+$</td>
</tr>
<tr>
<td>-------------</td>
<td>----------------</td>
</tr>
<tr>
<td>1</td>
<td>7.47</td>
</tr>
<tr>
<td>2</td>
<td>7.42</td>
</tr>
<tr>
<td>3</td>
<td>7.44</td>
</tr>
<tr>
<td>4</td>
<td>7.42</td>
</tr>
<tr>
<td>5</td>
<td>7.33</td>
</tr>
<tr>
<td>6</td>
<td>7.49</td>
</tr>
<tr>
<td>7</td>
<td>7.34</td>
</tr>
</tbody>
</table>

Salt solution: $\mu = 0.160$. $\text{pK}_{s,p} \text{CaCO}_3 = 7.40$. 

exceeds that found for salt solutions of similar ionic strength. The ionic strength of serum has been estimated from the analysis of the constituents of serum to be 0.167 (Van Slyke and collaborators, 1925) where proteins are assumed to behave as univalent anions. In Table XIII have been collected the results of analyses of normal and pathological serum as drawn. The $\text{pK}_{s,p}$ of CaCO$_3$ for a salt solution of similar ionic strength has been given for comparison.

The great discrepancy in the values for $p([\text{Ca}] \times [\text{CO}_3^{2-}])$ between salt solutions not containing phosphate and in equi-
Solubility of Calcium Carbonate

librium with solid CaCO₃, on the one hand, and serum in vivo, on the other hand, gives rise to a question of great interest and importance; namely, the significance of this product in serum as drawn, and the factors which are responsible for it. Several possibilities suggest themselves as an explanation for the existence of calcium in the amounts in which it occurs in serum.

1. Serum in the body may not have direct access to the bone in the manner of a solution saturated with a solid salt. Hence not being in equilibrium with bone, serum in vivo may be supersaturated or undersaturated with respect to the calcium salts.
2. One or both of these salts may be present in finely divided form in suspension.
3. Whether in equilibrium with bone or not, calcium may be present in serum in an amount governed merely by the specific ion effect of the proteins or other substances to be found in serum, not present in our simple salt solutions.
4. Calcium in serum may be present as part of a complex ion or molecule of a substance, which is but slightly dissociated. Evidence will be offered in the following sections in support of our belief in the last named hypothesis.

To test the first possibility, horse serum was shaken at physiological CO₂ tensions in contact with solid CaCO₃ for varying lengths of time. At constant pCa⁺ no change occurred in the total calcium up to 22 hours. The value of p([Ca⁺] × [CO₃⁻²]) was greater at the end of the saturation period but the pCa⁺ was

### TABLE XIV.

*Effect of Saturating Normal Horse Serum with Solid CaCO₃ for Varying Lengths of Time at 38°.*

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Length of saturation period</th>
<th>pCa⁺</th>
<th>([\text{CO}_3^-] \times 10^6)</th>
<th>([\text{HCO}_3^-] \times 10^6)</th>
<th>([\text{Ca}^+ \times \text{kg. H}_2\text{O}] \times 10^6)</th>
<th>([\text{OH}^- \times \text{kg. H}_2\text{O}] \times 10^6)</th>
<th>(\text{H}_{2}\text{CO}_3) calculated</th>
<th>(\text{p}([\text{Ca}^+] \times [\text{CO}_3^-]) = \text{p}([\text{Ca}^+] \times [\text{OH}^-]))</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>7.47</td>
<td>31.07</td>
<td>29.68</td>
<td>0.141</td>
<td>3.37</td>
<td>0.28</td>
<td>3.09</td>
</tr>
<tr>
<td>2</td>
<td>5 min.</td>
<td>7.44</td>
<td>31.35</td>
<td>29.74</td>
<td>0.133</td>
<td>3.36</td>
<td>0.30</td>
<td>3.06</td>
</tr>
<tr>
<td>3</td>
<td>1 hr.</td>
<td>7.43</td>
<td>31.39</td>
<td>29.77</td>
<td>0.130</td>
<td>3.36</td>
<td>0.31</td>
<td>3.05</td>
</tr>
<tr>
<td>4</td>
<td>22 &quot;</td>
<td>7.17</td>
<td>19.07</td>
<td>17.44</td>
<td>0.042</td>
<td>3.46</td>
<td>0.96</td>
<td>2.50</td>
</tr>
</tbody>
</table>
lower, which, as later experiments show, practically accounts for the increase in this product (Table XIV).

This might suggest that the serum in vivo was already in equilibrium with solid CaCO$_3$ in the body. If this were indeed true, it seemed that equilibration of the serum with solid CaCO$_3$ after depletion of the calcium content of serum should result in the solution of CaCO$_3$, or augmentation of the calcium content should result in precipitation of calcium until the original product \([\text{Ca}] \times [\text{CO}_3^{2-}]\) was satisfied. This was not simply realized, however, as the following experiments show.

**TABLE XV.**

**Experiment Illustrating Failure of Serum to Dissolve CaCO$_3$ after Partial Precipitation of Calcium Originally Present by Na$_2$C$_2$O$_4$.**

Original calcium concentration = 3.01 mm per kilo of H$_2$O.

After precipitation with Na$_2$C$_2$O$_4$, Ca = 0.64 mm per kilo of H$_2$O.

Saturated with solid CaCO$_3$ at 38° for 24 hours.

<table>
<thead>
<tr>
<th>Sample</th>
<th>(\text{pH}^+)</th>
<th>[CO$_3^-$] $\times 10^5$ kg. H$_2$O</th>
<th>[CO$_2^-$] $\times 10^3$ kg. H$_2$O</th>
<th>[Ca] $\times 10^3$ kg. H$_2$O</th>
<th>pCO$_2^-$</th>
<th>pCa</th>
<th>(p([\text{Ca}] \times [\text{CO}_3^{2-}]))</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7.02</td>
<td>36.52</td>
<td>0.0548</td>
<td>0.596</td>
<td>4.26</td>
<td>3.22</td>
<td>7.48</td>
</tr>
<tr>
<td>2</td>
<td>7.18</td>
<td>33.53</td>
<td>0.0751</td>
<td>0.511</td>
<td>4.12</td>
<td>3.29</td>
<td>7.41</td>
</tr>
<tr>
<td>3</td>
<td>7.34</td>
<td>29.36</td>
<td>0.0978</td>
<td>0.404</td>
<td>4.01</td>
<td>3.39</td>
<td>7.40</td>
</tr>
</tbody>
</table>

**Solubility of CaCO$_3$ in Serum after Partial Precipitation of the Calcium as Calcium Oxalate.**

The calcium of serum was partially precipitated as calcium oxalate by the addition of a weighed amount of sodium oxalate. After allowing the serum to stand, the calcium oxalate was removed by centrifugation and the serum was equilibrated for 24 hours with solid CaCO$_3$. At the end of the saturation period the serum was analyzed for its \(\text{pH}^+\) and CO$_2$ and calcium content. The results of this experiment are given in Table XV. They show that CaCO$_3$ did not dissolve again after the calcium content had been depleted by precipitation as calcium oxalate.

That the serum had not lost its power to hold calcium in solution, however, is shown by Table XV.

After precipitation of the calcium as calcium oxalate, CaCl$_2$
was added to the serum in a quantity comparable to its original calcium content. This serum was then equilibrated with solid calcium carbonate and analyzed after 20 hours. The results of this experiment, given in Table XVI, show that the serum had not lost its power to hold calcium in solution.

**TABLE XVI.**

*Experiment Illustrating Ability of Serum to Hold CaCl₂ in Solution after Precipitation of the Original Calcium with Na₂CO₃.*

Original calcium concentration = 3.28 mm per kilo of H₂O.

After addition of Na₂CO₃, 

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>pH⁺</th>
<th>[CO₃]ₙ M X 10⁻⁶ kg H₂O</th>
<th>[CO₃]₂ M X 10⁻⁶ kg H₂O</th>
<th>[Ca] M X 10⁻⁶ kg H₂O</th>
<th>pCO₃⁻</th>
<th>pCa</th>
<th>p((Ca) x [CO₃]⁻²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.99</td>
<td>29.48</td>
<td>0.041</td>
<td>2.94</td>
<td>4.39</td>
<td>2.53</td>
<td>6.92</td>
</tr>
<tr>
<td>2</td>
<td>6.97</td>
<td>27.73</td>
<td>0.036</td>
<td>2.995</td>
<td>4.44</td>
<td>2.52</td>
<td>6.96</td>
</tr>
<tr>
<td>3</td>
<td>6.92</td>
<td>28.40</td>
<td>0.0336</td>
<td>2.93</td>
<td>4.48</td>
<td>2.53</td>
<td>7.01</td>
</tr>
</tbody>
</table>

**TABLE XVII.**

*Effect of Saturating Horse Serum with CaCO₃ after Increasing Original Calcium Concentration by Addition of Ca(HCO₃)₂.*

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Length of saturation period</th>
<th>pH⁺</th>
<th>[CO₃] M X 10⁻⁶ kg H₂O</th>
<th>[CO₃]² M X 10⁻⁶ kg H₂O</th>
<th>[Ca] M X 10⁻⁶ kg H₂O</th>
<th>pCO₃⁻</th>
<th>p((Ca × [CO₃]⁻²))</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0 min.</td>
<td>7.50</td>
<td>35.71</td>
<td>0.175</td>
<td>7.09</td>
<td>5.91</td>
<td>5.99</td>
</tr>
<tr>
<td>2</td>
<td>5 min.</td>
<td>7.40</td>
<td>37.03</td>
<td>0.143</td>
<td>7.02</td>
<td>6.00</td>
<td>6.00</td>
</tr>
<tr>
<td>3</td>
<td>1 hr.</td>
<td>7.59</td>
<td>35.24</td>
<td>0.214</td>
<td>7.02</td>
<td>5.82</td>
<td>5.97</td>
</tr>
<tr>
<td>4</td>
<td>3 hrs.</td>
<td>7.54</td>
<td>35.90</td>
<td>0.193</td>
<td>7.00</td>
<td>5.87</td>
<td>5.98</td>
</tr>
<tr>
<td>5</td>
<td>22 hrs.</td>
<td>7.39</td>
<td>34.58</td>
<td>0.131</td>
<td>7.09</td>
<td>6.03</td>
<td>6.02</td>
</tr>
</tbody>
</table>

**Solubility of CaCO₃ in Serum after Augmentation of the Original Calcium Concentration.**

To serum was added Ca(HCO₃)₂ in an amount sufficient to double the original calcium content of serum. This serum was then equilibrated with solid CaCO₃ and analyzed after varying
lengths of time. Apparently, none of the added calcium was precipitated, as shown by the results of this experiment (Table XVII). Furthermore, the log product, p([Ca] × [CO₃²⁻]) was markedly constant throughout the 22 hours of saturation.

**Solubility of CaCO₃ in Serum after Removal of Phosphates and Augmentation of the Calcium.**

Having found in later experiments that equilibration of serum with augmented calcium at pOH above 8.0, in the presence of

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>pH*</th>
<th>[CO₃²⁻] x 10⁶ M</th>
<th>[Ca] x 10⁶ M</th>
<th>p(Ca) x (CO₃⁻)</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7.40</td>
<td>3.48</td>
<td></td>
<td></td>
<td>Initial condition.</td>
</tr>
<tr>
<td>2</td>
<td>&gt;8.5</td>
<td>6.55</td>
<td>2.51</td>
<td></td>
<td>After addition of Ca(HCO₃)₂.</td>
</tr>
<tr>
<td>3</td>
<td>&gt;8.5</td>
<td>6.20</td>
<td>1.95</td>
<td></td>
<td>After addition of CaCl₂.</td>
</tr>
<tr>
<td>4</td>
<td>&gt;8.5</td>
<td>13.80 ± 0.028</td>
<td>2.56</td>
<td>7.13</td>
<td>After equilibration with CaCO₃, alone, at 25 mm. CO₂ tension.</td>
</tr>
</tbody>
</table>

* Assumed.

solid CaCO₃ and Ca₃(PO₄)₂, reduces the amount of inorganic phosphate in solution, and believing that the presence of the inorganic phosphate of serum may have prevented the precipitation of CaCO₃ in the previous experiment, serum was treated in this manner until no determinable amount of inorganic phosphate remained in solution; i.e., the concentration was less than 0.01 mm per kilo of H₂O. The calcium concentration at this time was 2.51 mm. Calcium chloride was then added until the calcium concentration was 6.20 mm, and this serum was saturated with...
solid CaCO₃ at pαH⁺ = 8.5. After 18 hours the calcium concentration was 1.95 mM, showing that 4 mM of CaCO₃ had been precipitated by saturation with solid CaCO₃. It should be noted, however, that phosphates were now absent from the serum. Continued saturation of the serum with CaCO₃ at 25 mM CO₂ tension at a pαH⁺ of 7.15 resulted in the solution of 0.6 mM of calcium, the amount which one would expect to dissolve in a salt solution of serum ionic strength and CO₂⁻ concentration, as one passed from pαH⁺ 8.5 to 7.15. These results are shown in Table XVIII. The total amount of calcium in solution finally, 2.56 mM, was still about 1 mM more than one can account for simply by the solubility of CaCO₃ and the effect of the proteins, and this in the absence of phosphates. This experiment seems to indicate, therefore, that in the absence of phosphates, calcium is precipitated by saturation with CaCO₃ from serum even though it has been greatly increased in concentration.

Taken in conjunction with the previous experiment, it would appear that to the presence of phosphates in the serum may be assigned, in part at least, the rôle of delaying or preventing the precipitation of calcium after augmentation of the calcium. That the presence of the phosphates is not necessary for the maintenance of the calcium level above that accounted for by the salts and the proteins is also shown. It is recognized, however, that with the precipitation of the phosphates, the precipitation of some other factor which is involved in the maintenance of the normal calcium level is not precluded.

Effect of Varying pαH⁺ upon the Solubility of CaCO₃.

The pαH⁺ of the serum was varied from approximately 6.0 to 8.0 by varying the CO₂ tension, and the serum rotated in contact with solid CaCO₃. The results of one such experiment are given in Table XIX and graphically presented in Fig. 3. It will be seen that only at the highest pαH⁺ was there a significant drop in calcium. This was accompanied by a drop in phosphate. Calculating the precipitated salt as Ca₃(PO₄)₂ the decrease in phosphate corresponded to a decrease in calcium of 0.43 mM as compared with an observed fall of 0.58 mM. It is of more importance, however, that the total calcium concentration did not
TABLE XIX.
Solubility of CaCO₃ in Horse Serum Saturated with Solid CaCO₃ at 38° and Varying pα⁺ Values.

Length of saturation, 16 hours.

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>pα⁺</th>
<th>[Ca] × 10⁶</th>
<th>[CO₃⁻] × 10⁶</th>
<th>[Ca] / kg H₂O</th>
<th>[CO₃⁻] / kg H₂O</th>
<th>Total [PO₄] / kg H₂O</th>
<th>Calculated [Ca⁺] / kg H₂O</th>
<th>(1 - X)CO₃ / kg H₂O</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.30</td>
<td>58.03</td>
<td>0.011</td>
<td>3.23</td>
<td>0.76</td>
<td>3.63</td>
<td>7.45</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>6.91</td>
<td>39.61</td>
<td>0.045</td>
<td>3.23</td>
<td>0.75</td>
<td>0.89</td>
<td>2.34</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>7.51</td>
<td>31.60</td>
<td>0.158</td>
<td>3.21</td>
<td>0.74</td>
<td>0.25</td>
<td>2.96</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>7.97</td>
<td>28.36</td>
<td>0.397</td>
<td>3.14</td>
<td>0.73</td>
<td>0.10</td>
<td>3.04</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>8.46</td>
<td>24.37</td>
<td>1.084</td>
<td>2.65</td>
<td>0.48</td>
<td>0.096</td>
<td>2.61</td>
<td></td>
</tr>
</tbody>
</table>

Length of saturation, 16 hours.

FIG. 3. The negative log product of [Ca] × [CO₃⁻] in serum under varying conditions plotted against pα⁺.

change from pα⁺ = 7.5 to pα⁺ = 6.3. Because of the change which occurs in CO₃⁻ ion concentration with changing pα⁺, the negative log product pK_{CaCO₃} varies progressively with
the $p\alpha_{\text{H}^+}$. At $p\alpha_{\text{H}^+}$ 6.3 to 6.4, $p([\text{Ca}] \times [\text{CO}_3^{2-}])$ equals that obtained in salt solutions of the same ionic strength.

Assuming that each of these solutions was in equilibrium with solid CaCO$_3$ it is possible to calculate $[\text{Ca}^{++}]$ and $[\text{CaX}]$ at each $p\alpha_{\text{H}^+}$. The results of such a calculation are given in Table XIX.

These experiments have been repeated several times with the same result. They show the variation of CaX with changing $p\alpha_{\text{H}^+}$ and suggest the hypothesis that up to a certain point CaX exists in greater amount at more alkaline reactions. Were it a calcium proteinate compound which were concerned, this phenomenon would be understandable, because it is well established that serum proteins bind more base at higher $p\alpha_{\text{H}^+}$ values.

It may be felt that in a solution containing such a preponderance of sodium salts, one would not expect a significant amount of calcium proteinate to be formed. However, as the citrate experiments presented in the previous section indicate, when a slightly ionized compound is formed, one may reasonably look for an increased amount of calcium.

Influence of Serum Concentration upon CaCO$_3$ Solubility.

To determine whether the $[\text{CaX}]$ concentration varied proportionally to the amount of serum in the solution equilibrated with CaCO$_3$, horse serum was diluted with known proportions of a salt solution of the same inorganic salts of serum and of the same ionic strength. These solutions were equilibrated with solid CaCO$_3$ at definite CO$_2$ tensions. The results of such an experiment are given in Table XX and the amount of CaX has been calculated for each concentration of serum. This is approximately proportional to the concentration of serum in the solution equilibrated although the fact that the $p\alpha_{\text{H}^+}$ values are not identical makes it impossible to compare strictly the results with each other.

Influence of the Proteins of Serum upon CaCO$_3$ Solubility.

Serum was dialyzed in collodion bags until chloride-free, then NaCl and NaHCO$_3$ were added until a solution of serum ionic strength was obtained. Calcium was also added in the form of CaCl$_2$ so that its initial concentration was 5 mM. This solution
was equilibrated with solid CaCO₃ at varying p[H +], and analyzed for total Ca, CO₂, and p[H +]. The results of these experiments are given in Table XXI. The amount of unionized calcium per

### Table XX.

**Solubility of CaCO₃ in Serum Diluted with Varying Amounts of Salt Solution of Same Ionic Strength.**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Serum, 100%</th>
<th>Salt solution, 0%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.0</td>
<td>40</td>
</tr>
<tr>
<td>2</td>
<td>3.0</td>
<td>70</td>
</tr>
<tr>
<td>3</td>
<td>4.0</td>
<td>90</td>
</tr>
</tbody>
</table>

Each equilibrated 20 hours with solid CaCO₃.

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>pH</th>
<th>[CO₃]⁻ [m × 10⁻⁶ kg⁻¹ H₂O]</th>
<th>[HCO₃]⁻ [m × 10⁻⁶ kg⁻¹ H₂O]</th>
<th>[Ca] [m × 10⁻⁶ kg⁻¹ H₂O]</th>
<th>pCO₂</th>
<th>pCa</th>
<th>p[H+]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.97</td>
<td>0.0276</td>
<td>3.15</td>
<td>4.56</td>
<td>2.50</td>
<td>1.82</td>
<td>7.06</td>
</tr>
<tr>
<td>2</td>
<td>7.08</td>
<td>0.0387</td>
<td>1.84</td>
<td>4.41</td>
<td>2.74</td>
<td>0.94</td>
<td>7.15</td>
</tr>
<tr>
<td>3</td>
<td>7.14</td>
<td>0.0545</td>
<td>0.95</td>
<td>4.26</td>
<td>3.02</td>
<td>0.35</td>
<td>7.28</td>
</tr>
<tr>
<td>4</td>
<td>7.30</td>
<td>0.0863</td>
<td>0.58</td>
<td>4.06</td>
<td>3.24</td>
<td>0.00</td>
<td>7.30</td>
</tr>
</tbody>
</table>

### Table XXI.

**Solubility of CaCO₃ in Dialyzed Horse Serum, μ = 0.150. Total Protein = 67 Gm. per Kilo of H₂O.**

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>pH</th>
<th>[CO₃]⁻ [m × 10⁻⁶ kg⁻¹ H₂O]</th>
<th>[HCO₃]⁻ [m × 10⁻⁶ kg⁻¹ H₂O]</th>
<th>[Ca] [m × 10⁻⁶ kg⁻¹ H₂O]</th>
<th>(CaXCO₃ = 1) [m × 10⁻⁶ kg⁻¹ H₂O]</th>
<th>[Ca] [m × 10⁻⁶ kg⁻¹ H₂O]</th>
<th>[Ca] [m × 10⁻⁶ kg⁻¹ H₂O]</th>
<th>[Ca] [m × 10⁻⁶ kg⁻¹ H₂O]</th>
<th>[Ca] [m × 10⁻⁶ kg⁻¹ H₂O]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7.67</td>
<td>32.60</td>
<td>0.2380</td>
<td>1.075</td>
<td>6.59</td>
<td>0.03</td>
<td>0.0163</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>7.48</td>
<td>30.38</td>
<td>0.1415</td>
<td>1.075</td>
<td>6.82</td>
<td>0.83</td>
<td>0.0145</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The amount of calcium bound by serum proteins may be evaluated also in the following way. By plotting the amount of calcium found in serum and transudates of serum containing amounts of protein varying from 0.0 to 80 gm. per kilo of water against the protein concentration, one obtains the curve given in Fig. 4. The data from which this figure is made are taken partly from data published by Salvesen and Linder, and partly from data obtained in connection with this investigation. These data may be considered to be those obtaining in solutions at \( p_{\alpha H^+} = 7.4 \) and of a constant ionic strength, approximately 0.165. The equation for the line relating the calcium to the protein concentration is

\[
[\text{Ca}] = 0.014 \, [P] - 1.4
\]

where \([\text{Ca}]\) = total calcium concentration in \( \text{mm} \) per kilo of \( \text{H}_2\text{O} \), and \([P]\) = gm. of protein per kilo of \( \text{H}_2\text{O} \). These data taken from human serum and transudates as drawn from the body indicate, therefore, that the calcium bound per gm. of protein = 0.014 \( \text{mm} \) at \( p_{\alpha H^+} = 7.4 \). This compares well with the value of 0.0137 \( \text{mm} \) estimated for horse serum proteins.

Although our data are admittedly scanty, it is of interest to use them to derive a relationship between the calcium bound by protein, the protein concentration, and the \( p_{\alpha H^+} \). Such an approximate expression fitting our results obtained upon solutions of dialyzed serum protein is
The above relationship is purely empirical and holds only for the conditions in which the salt concentrations are those found in serum. The question may be raised as to whether the increase in the ionic strength of the serum due to the proteins may not be sufficient to account for the increase in calcium due to their presence. This we do not believe to be the case for reasons given previously and in the following. Assuming that the increase is due to an increased ionic strength of the solution, and that the following formula holds

\[ \Delta \gamma_{\text{Ca}^{++}} = 0.5 z^2 (\sqrt{\mu_1} - \sqrt{\mu_2}) \]

In a salt solution of \( \mu = 0.170 \) without protein, the concentration of calcium at \( p\alpha_{H^+} = 7.4 \) is about 0.4 mM. In serum under similar conditions, it is about 1.4 mM. Now \( \frac{0.4}{1.4} \) may be considered to be equal to the change in the activity coefficient in passing from a solution without protein to serum. For simplicity we shall calculate the increase in \( \mu \) as if we passed from 0.0 to \( \mu \) ionic strength. Therefore

\[ \Delta \gamma_{\text{Ca}^{++}} = - \log \frac{0.4}{1.4} = 0.5 \times 2 \times 2 \sqrt{\mu} = 2 \sqrt{\mu} \]

Solving for \( \mu \), one finds it equal to 0.073. If the ionic strength of serum were 0.073 more than the value of 0.167 calculated from inorganic salt analyses, then the activity coefficient of a univalent ion such as \( \text{HCO}_3^- \) would be 0.57 instead of 0.63, the value actually found and which is the value found for a salt solution of ionic strength \( \mu = 0.167 \). That it may play some rôle is not denied, but, in the absence of more exact knowledge of the effective valence type of the serum proteins, it does not seem profitable to attempt to evaluate the portion of calcium which is bound to the proteins in the non-ionized form, and the portion which represents the reduction in activity due to the ionic strength of the added protein.
In a few instances analyses were made of edema fluid as drawn from the body. These results are given in Table XXII. They show that when allowance is made for the rôle played by the small amount of protein present, there is still a large discrepancy between the amount of calcium which is found and the value calculated for a salt solution which is in equilibrium with solid CaCO₃. These figures are given in the column headed \([CaX] - [CaP]\).

**TABLE XXII.**

*Calculation of \(p([Ca] \times [CO₃]⁺)\) and \([CaX]\) in Transudates, as Drawn.*

<table>
<thead>
<tr>
<th>Date</th>
<th>(pH)</th>
<th>([CO₃]⁺ \times 10^6)</th>
<th>([Ca] \times 10^6)</th>
<th>Calculated ([Ca⁺] \times 10^6)</th>
<th>([CaX] \times 10^6)</th>
<th>Protein (kg) H₂O</th>
<th>Calculated Ca proteinate (kg) H₂O</th>
<th>([CaX] - [CaP]) (kg) H₂O</th>
<th>Total ([PO₄] \times 10^6) (kg) H₂O</th>
<th>(p([Ca] \times [CO₃]⁺))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mar. 29, 1923</td>
<td>7.46</td>
<td>31.8</td>
<td>0.141</td>
<td>1.6</td>
<td>0.28</td>
<td>1.32</td>
<td>2.97</td>
<td>0.04</td>
<td>1.26</td>
<td>6.65</td>
</tr>
<tr>
<td>&quot; 29, 1923</td>
<td>7.41</td>
<td>31.8</td>
<td>0.125</td>
<td>2.0</td>
<td>0.32</td>
<td>1.68</td>
<td>26.6</td>
<td>0.38</td>
<td>1.26</td>
<td>6.60</td>
</tr>
<tr>
<td>May 8, 1923</td>
<td>7.46</td>
<td>25.3</td>
<td>0.110</td>
<td>1.5</td>
<td>0.30</td>
<td>1.14</td>
<td>2.4</td>
<td>0.03</td>
<td>1.26</td>
<td>7.58</td>
</tr>
<tr>
<td>Sept. 21, 1923</td>
<td>7.47</td>
<td>38.20</td>
<td>0.174</td>
<td>1.41</td>
<td>0.23</td>
<td>1.18</td>
<td>3.0</td>
<td>0.04</td>
<td>1.26</td>
<td>6.61</td>
</tr>
<tr>
<td>Dec. 1, 1925</td>
<td>7.46</td>
<td>32.20</td>
<td>0.143</td>
<td>2.30</td>
<td>0.28</td>
<td>2.02</td>
<td>33.0</td>
<td>0.48</td>
<td>1.26</td>
<td>6.49</td>
</tr>
<tr>
<td>Sept. 29, 1924</td>
<td>7.12</td>
<td>31.03</td>
<td>0.0617</td>
<td>1.98</td>
<td>0.65</td>
<td>1.33</td>
<td>15.5</td>
<td>0.17</td>
<td>1.26</td>
<td>6.61</td>
</tr>
</tbody>
</table>

*Ca proteinate calculated from the equation Ca proteinate = 0.01 protein (p\(H⁺\) - 6.0).

In order to determine whether edema fluid is in equilibrium with solid CaCO₃ it was equilibrated with solid CaCO₃. The calculated amount of \([CaX] - [CaP]\) was 1.16 mm, showing that there had been no measurable loss in this moiety of the calcium due to the saturation. This fraction of the calcium, as we have stated before, is considered to be calcium in the non-ionic form, but whether it is unprecipitated Ca₃(PO₄)₂ or CaHPO₄, or is an un-
dissociated calcium organic compound, it would be impossible to decide from these experiments. It should be pointed out in connection with the above experiments, that the values of $[\text{CaX}] - [\text{CaP}]$ are approximately equimolar with the concentration of phosphate.

**Effect of Parathormone on CaCO₃ Solubility.**

Since extirpation of the parathyroid glands results in such marked reduction in the calcium concentration of serum, it was obvious that one should attempt to demonstrate an influence of the parathyroid hormone prepared by Collip upon the solubility of CaCO₃ in salt solutions and in serum.

**TABLE XXIII.**

**Effect of Paralhormone upon Solubility of CaCO₃ in Salt Solutions, $\mu = 0.175$.**

Solutions saturated 20 hours with solid CaCO₃ at 38°.

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Concentration of parathormone, m per 100</th>
<th>Initial calcium concentration, kg. H₂O</th>
<th>pH</th>
<th>$\text{CO}_3^-$, mg</th>
<th>$\text{Ca} \times [\text{CO}_3^-] = \text{mg}$</th>
<th>$\text{Ca} \times [\text{CO}_3^-] = \text{mg}$</th>
<th>$\text{Ca} \times [\text{CO}_3^-] = \text{mg}$</th>
<th>$\text{Ca} \times [\text{CO}_3^-] = \text{mg}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.0</td>
<td>3.25</td>
<td>7.33c</td>
<td>19.77</td>
<td>0.0642</td>
<td>0.93</td>
<td>7.22</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>2.0</td>
<td>3.25</td>
<td>7.47c</td>
<td>29.80</td>
<td>0.1356</td>
<td>2.18</td>
<td>6.53</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.0</td>
<td>0.0</td>
<td>7.53c</td>
<td>31.84</td>
<td>0.1672</td>
<td>0.56</td>
<td>7.03</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>2.0</td>
<td>0.0</td>
<td>7.36c</td>
<td>22.09</td>
<td>0.0771</td>
<td>0.62</td>
<td>7.33</td>
<td></td>
</tr>
</tbody>
</table>

This has been attempted, but, although an effect of the parathyroid hormone upon the solvent power of serum for CaCO₃, has apparently been demonstrated, it is appreciated that the concentration of extract in our solutions was many times the concentration which is effective in raising the calcium of the blood *in vivo*. Therefore, in spite of the fact that the following experiments seem to indicate that this substance aids in the retention of calcium in solution, it is not felt that it, and it alone, constitutes the unknown organic substance which combines with calcium in the blood.

In the first experiments, salt solutions of serum ionic strength containing no calcium initially, but containing some of Collip's
Solubility of Calcium Carbonate

preparation, were equilibrated with solid CaCO₃. No more CaCO₃ was dissolved than would have been dissolved by the salt solutions alone, as Table XXIII shows. When the same salt-hormone solutions contained calcium in solution initially, saturation with solid CaCO₃ did not result in precipitation of CaCO₃ as it did in the case of salt solutions containing no hormone. Whether this effect can be attributed entirely to the hormone or to the presence of some impurity, it is impossible to say. Nor do we know what is the limiting amount of calcium which it would be possible for the extract to hold in solution. These are questions upon which further work must be done.

An experiment similar to the above, except in two respects, namely that a small amount of phosphate as well as calcium was initially in solution, and the saturating phase consisted of Ca₃(PO₄)₂ as well as CaCO₃, was also performed. In this case the calcium was precipitated to the same level in the presence as in the absence of the extract.

Although these experiments do not prove that the parathyroid hormone plays a significant part in maintaining the non-ionized moiety of calcium in the body normally, they do constitute evidence that a substance of biological origin may act like citric acid in leading to what appears to be supersaturation with respect to calcium carbonate under conditions in which supersaturation is apparently precluded as a possibility.

Solubility of CaCO₃ in Serum of Parathyroidectomized Dogs.

Another argument against the hypothesis that the internal secretion of the parathyroid glands is the X factor in the blood which is combined with calcium in the unionized form, is found in the following series of experiments.

After removing a sample of blood for control analyses, thyreoparathyroidectomy was performed on a dog under ether anesthesia and with aseptic technique. The operation was performed by Dr. Douglas Boyd to whom we are greatly indebted. Samples of blood were withdrawn for analysis of the CO₂, pOH⁺, and Ca at various times subsequent to the operation. Well marked tetany developed on the 2nd day following the operation, which was relieved by the intravenous injection of a 20 per cent solution
of CaCl₂. An attack of tetany recurred on the following day and was again relieved by the intravenous injection of CaCl₂. This was repeated several times during the course of 2 weeks.

The point of particular interest to us was to determine whether CaCO₃ could be held in solution by the blood serum when the dog was in tetany and its calcium, as is well known, was markedly below normal. Three experiments upon this point, all of which showed the same result, were performed. The protocol of one such experiment is given in Table XXIV. It illustrates that although the serum calcium had fallen to 1.28 mM of calcium per kilo of water, nevertheless, even after saturation with CaCO₃, the serum was fully capable of holding calcium in solution if it was added as CaCl₂.

**DISCUSSION.**

Our experiments upon CaCO₃ solubility in sodium chloride solutions provided us with the data necessary for the calculation
Solubility of Calcium Carbonate

of the activity coefficient of calcium in salt solutions of varying ionic strength. In the case of citrate solutions it was found that either the presence of the citrate ion markedly affected the activity coefficient of the calcium ion or a slightly ionized calcium citrate compound was formed. The latter hypothesis seemed better substantiated by our data than the former.

In the above experiments, the same final state was reached whether one started with a higher or lower initial concentration of calcium than was required for equilibrium conditions. In experiments with serum these criteria of equilibria could not be successfully applied. Serum rotated with solid CaCO$_3$ showed no change in calcium concentration up to 24 hours whether one started with a normal, high, or low initial calcium concentration. The amount of CaX$^6$ in serum, i.e. the amount present in excess

6 The calcium of serum drops after 48 hours but concomitantly the inorganic phosphate rises indicating that certain splitting of organic phosphate compounds is occurring. This may account for the drop in serum calcium found by Irving (1926) after shaking serum with CaCO$_3$.

Although the behavior of strong electrolytes has been satisfactorily accounted for by the theory of complete dissociation and the mathematical treatment accorded this theory by Debye and Hückel, the conformity of weak electrolytes to this theory has not yet been as thoroughly demonstrated. If one is dealing with a substance in solution which is completely dissociated and if one can measure the thermodynamic activity of its ions and their stoichiometric concentration, then the activity divided by the concentration equals the activity coefficient; e.g.,

$$\frac{\alpha_{Ag^+}}{[Ag^+]} = \gamma_{Ag^+} \text{ and } \frac{\alpha_{Cl^-}}{[Cl^-]} = \gamma_{Cl^-}$$

In this case the silver ion concentration and total silver concentration are identical.

If, on the other hand, one has a solution of the complex potassium silver cyanide, KAg(CN)$_2$, which dissociates but slightly into Ag$^+$ ions how should one define the activity coefficient of the silver ion?

$$KAg \text{(CN)}_2 \rightleftharpoons K^+ + Ag(CN)_2^-$$

$$\uparrow$$

$$Ag^+ + 2(CN)^-$$
Hastings, Murray, and Sendroy

of that accounted for by the salts of serum, is only partially the result of the serum proteins. At \( p\alpha_{H^+} 7.40 \) approximately 0.5 mM is due to the salts and 1.0 mM due to the proteins, leaving a residuum of 1.0 mM unaccounted for. These facts might be interpreted either on the hypothesis \( (a) \) that a portion of the calcium exists in serum in non-ionic form, or \( (b) \) that there is a delayed equilibrium between the calcium and carbonate in the serum.

According to the first hypothesis the calcium could conceivably exist as unprecipitated \( \text{CaCO}_3 \), \( \text{CaHPO}_4 \), or \( \text{Ca}_3(\text{PO}_4)_2 \), or as a non-ionized calcium compound. The possibility that it is unprecipitated \( \text{CaCO}_3 \) seems precluded by the fact that the same values of \( pK'_I \) of \( \text{H}_2\text{CO}_3 \) are obtained in normal serum and serum from which the calcium has been removed. This would not be the case if unprecipitated \( \text{CaCO}_3 \) were present. Nor does the

\[
 Will it be \\
(a) \frac{\alpha_{Ag^+}}{[Ag^+]^2} = \gamma_{Ag^+} \quad \text{or} \quad \frac{\alpha_{Ag^+}}{[Ag]} = \gamma_{Ag^+} \\
\]

where \([Ag^+]\) represents the molal concentration of the silver ions and \([Ag]\) equals the molal concentration of total silver in the solution? In this example the silver ion concentration is only a fraction of the total silver concentration. In the treatment of the calcium systems with which we are dealing we have used the activity coefficient as defined by expression \( (a) \); namely,

\[
\frac{\alpha_{Ca^{++}}}{[Ca^{++}]} = \gamma_{Ca^{++}}
\]

For the numerical value of \( \gamma_{Ca^{++}} \) in serum we have used that value found in a salt solution of the same ionic strength (the proteins being regarded as monovalent). From \( \gamma_{Ca^{++}} \) and the value calculated for \( \alpha_{Ca^{++}} \), the value of \([Ca^{++}] \) has been calculated. This, when subtracted from the total Ca gives what we have termed the unionized calcium, \([CaX]\). It should be noted that \( CaX \) does not represent a single chemical individual however. If it should be subsequently proven that the calcium compounds in serum are completely ionized, then this treatment would be incorrect and the activity coefficient of calcium in serum should be defined as \( \alpha_{Ca^{++}} \) divided by \([Ca]\), the total molal concentration of calcium in serum.
existence of unprecipitated $\text{CaHPO}_4$ or $\text{Ca}_3(\text{PO}_4)_2$ seem to explain the facts because of the observation that serum shaken with $\text{Ca}_3(\text{PO}_4)_2$ results, not in the precipitation of calcium phosphate, but of calcium carbonate. The calculated amount of non-ionized calcium compound varies with the $p\alpha_{\text{H}^+}$ and the concentration of serum, but is independent of the length of time that serum is equilibrated with $\text{CaCO}_3$, providing no other change occurs in the serum.

The amount of residual calcium is as high in protein-free transudates as it is in serum. This suggests that the residual calcium is diffusible.

Against the first hypothesis is the fact that serum does not change its concentration of calcium when equilibrated with $\text{CaCO}_3$ after depletion or augmentation of its calcium concentration. It is realized that this is a real difficulty and at present is not understood.

According to the second hypothesis, a condition of delayed equilibrium exists in serum normally, and some factors are present which prevent the precipitation of $\text{CaCO}_3$. The fact that serum from which most of the inorganic phosphate has been removed becomes reversible with respect to $\text{CaCO}_3$ suggests that the phosphate of serum might be such an inhibiting factor. This experiment shows furthermore that there is no physical reason, such as coating of the particles, why $\text{CaCO}_3$ should not precipitate from serum. If, however, phosphate brings about a condition of apparent supersaturation of $\text{CaCO}_3$ in serum, it is difficult to understand why serum from which the calcium has been removed does not return to the initial condition of supersaturation, as it does in salt solutions containing phosphate. Additional evidence that there is no physical reason why $\text{CaCO}_3$ does not precipitate from serum will be given in the succeeding paper where it will be shown that shaking serum with solid $\text{Ca}_3(\text{PO}_4)_2$ causes the rapid precipitation of $\text{CaCO}_3$.

Further discussion will be postponed until the data of the succeeding papers have been given. It may be anticipated, however, that our work tends to illustrate the complexity of the system serum plus calcium salts and to throw doubt upon the possibility of describing the system in terms of any one factor.
SUMMARY.

1. A method has been described whereby the solubility product constant of calcium carbonate may be determined.

2. The method of calculating \( [CO_3^-] \) ion concentration from analyses of \( p\alpha_{H^+} \) and total \([CO_3]\) and of calculating \( p\alpha_{H^+} \) from analyses of total \([CO_3]\) and \( CO_2 \) tension have been presented.

3. The stoichiometric solubility product of calcium carbonate has been found to vary with the ionic strength, according to the equation:

\[
pK'_{s,p} \text{CaCO}_3 = 8.58 - \frac{4.94 \sqrt{\mu}}{1 + 1.61 \sqrt{\mu}}.
\]

4. The activity coefficient of Ca\(^{++}\) ion has been calculated from the above data, and found to vary with the ionic strength according to the equation:

\[
p\gamma_{Ca^{++}} = \frac{4.94 \sqrt{\mu}}{1 + 1.61 \sqrt{\mu}} - 1.6 \sqrt{\mu}.
\]

5. The solubility of \text{CaCO}_3 in balanced salt solutions under various conditions has been studied, and the conditions for equilibrium determined.

6. Such solutions, when containing phosphate in solution, even in the presence of solid \text{CaCO}_3, indicated an apparent state of supersaturation with regard to that salt.

7. The solubility of \text{CaCO}_3 in solutions of sodium citrate was studied and was found to vary with the \( p\alpha_{H^+} \) and the concentration of citrate. The formation of a slightly ionized calcium citrate compound seems indicated. The precipitation of calcium from a citrate solution saturated with \text{CaCO}_3, by equilibration with \( \text{Ca}_3(\text{PO}_4)_2 \) was demonstrated.

8. Analyses of serum as drawn indicate a stoichiometric solubility product for \text{CaCO}_3 of \( 10^{-6.40} \) compared with \( 10^{-7.40} \) in salt solutions of comparable ionic strength.

9. This value is unchanged by saturation of serum with solid \text{CaCO}_3 up to 22 hours.

10. Calcium carbonate did not dissolve in serum from which much of the calcium had been removed by oxalate.

11. Calcium carbonate did not precipitate from serum whose calcium concentration had been augmented by the addition of calcium chloride, even in the presence of solid \text{CaCO}_3.
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12. Serum appears to be reversible with respect to calcium salts following removal of the inorganic phosphates.
13. The effect of varying $p\text{H}^+$ on the stoichiometric product, $p([\text{Ca}] \times [\text{CO}_3^{2-}])$, has been studied.
14. The influence of varying the serum protein concentration on the product $p([\text{Ca}] \times [\text{CO}_3^{2-}])$ was determined.
15. The solubility of CaCO$_3$ is transudates has been studied.
16. Parathormone was found to influence the solubility of CaCO$_3$ in salt solutions.
17. No evidence was found that the serum of parathyroidectomized dogs was unable to hold as much calcium in solution as normal serum.

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STUDIES OF THE SOLUBILITY OF CALCIUM SALTS: I. THE SOLUBILITY OF CALCIUM CARBONATE IN SALT SOLUTIONS AND BIOLOGICAL FLUIDS
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