COMPOSITION OF CASEIN IN MILK

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The literature reveals numerous investigations (1) of the elementary composition of acid-precipitated casein but no recorded analyses of the percentage content of the elements of casein as it exists in the caseinate-phosphate complex in milk.

In this investigation a procedure for the separation of the casein complex from the other milk constituents by means of the Sharples supercentrifuge and the ultimate analysis of this complex is given. Also, experiments are described demonstrating the true chemical character of the calcium phosphate fraction of the complex. From the results of this investigation, the elemental composition of the calcium caseinate fraction and casein was then calculated.

Comparison of the results of the elemental composition of the casein obtained by this procedure with the values given in the literature for acid-precipitated casein should indicate whether the caseinate undergoes any change in elemental composition by acid precipitation other than substitution of hydrogen for calcium.

Preparation of Calcium Caseinate-Calcium Phosphate Complex

The bowl (approximately 300 ml. capacity) of the Sharples supercentrifuge was lined with a celluloid sheet and spun slowly until filled with distilled water. The speed of the bowl was then increased to 50,000 R.P.M. and 500 ml. of skim milk at 5° were run slowly into the bowl through a fine capillary, the rate of feed being such that about 20 minutes elapsed between the beginning and the end of the introduction of the skim milk. The skim milk was followed by 300 ml. of cold distilled water, with the speed of the bowl still at 50,000 R.P.M. The deposited caseinate was removed from the celluloid liner and ground sufficiently fine so that, when dispersed in 500 ml. of distilled water, it remained in suspension. This diluted suspension was then put through the supercentrifuge in a manner similar to that used in centrifuging the skim milk, by first filling the lined bowl with distilled water and finally by running in 300 ml. of additional water after the introduction of the emulsion. The washing of the complex was then repeated in the same manner. The twice washed deposit, which contained about 65 per cent of moisture, was allowed to dry in the air on
the celluloid sheet, the temperature being maintained at 20° so as to minimize fermentation. The dried material was then ground to pass a No. 60 sieve and stored in a securely stoppered glass bottle to prevent loss or gain in moisture. Samples for study were removed as desired. This material gave a negative test for lactose and chlorides, indicating freedom from serum components. However, ether extracted 0.015 per cent of fat; a correction was accordingly applied in the calculations of the results of the elementary composition.

Methods of Analysis

Nitrogen was determined by the semimicro-Kjeldahl method, with about 30 mg. of material and digestion for 8 hours as recommended by Chibnall, Rees, and Williams (2), in a Parnass-Wagner (3) digestion apparatus. The value reported is the average of six determinations. Calcium determinations were made on the ash obtained by incinerating in a muffle furnace and analyzing by the official macro volumetric permanganate method ((4) p. 127) and total phosphorus by the official gravimetric method ((4) p. 21). Inorganic phosphorus was obtained from the trichloroacetic acid filtrate and determined by the strychnine gravimetric method of Embden and Fetter (5). Values of four determinations did not differ by over 0.003 per cent. Organic phosphorus was calculated by determining the difference between total and inorganic phosphorus. Moisture was determined by heating in a vacuum oven to constant weight, while the temperature was not permitted to rise above 105°. Carbon and hydrogen determinations were made by the procedure outlined by Clark (6), with a semimicro combustion furnace described by this author. The official sodium peroxide fusion method ((4) p. 132) was used for the determination of sulfur.

Results of Analysis

The results of the ultimate analysis of the calcium caseinate-calcium phosphate complex are shown in the first and second lines of Table I.

Before the elementary composition of the calcium caseinate and of the casein could be calculated from the analysis of the complex, it was first necessary to establish the chemical formula of the calcium phosphate fraction so that the calcium could be correctly allocated between the protein and the inorganic phosphate.

Chemical Nature of Inorganic Phosphate Associated with Milk Caseinate

If the value 0.958 per cent obtained for the inorganic phosphorus is calculated as the tribasic phosphate, the complex will contain 4.80 per cent Ca₃P₂O₈ and 95.20 per cent calcium caseinate; or if it is calculated as the
dibasic phosphate, the complex will contain 4.21 per cent CaHPO$_4$ and 95.79 per cent calcium caseinate. As indicated in the reactions given below, addition of neutral potassium oxalate to the complex composed of the caseinate and the dibasic salt will not appreciably change the pH, while, on the other hand, if Ca$_3$P$_2$O$_8$ is present in the complex, the mixture will become more alkaline due to the formation of K$_3$PO$_4$.

(1) \[ \text{CaHPO}_4 + \text{K}_2\text{C}_2\text{O}_4 \rightarrow \text{CaC}_2\text{O}_4 + \text{K}_2\text{HPO}_4 \]

(2) \[ \text{Ca}_3\text{P}_2\text{O}_8 + 3\text{K}_2\text{C}_2\text{O}_4 \rightarrow 3\text{CaC}_2\text{O}_4 + 2\text{K}_3\text{PO}_4 \]

Consequently, increase in alkalinity will only be manifested if the tribasic salt is present in the complex.

The action of neutral oxalate on calcium phosphate has been studied by Van Slyke and Bosworth (7) and by Pyne (8) in their investigations of the nature of the insoluble inorganic phosphate in milk. They added the oxalate to milk and serum, however, and not to the isolated complex.

### Table I

| Per Cent Composition of Calcium Caseinate-Calcium Phosphate Complex |
|-------------------------|----------------|----------------|-------------|-------------|-------------|-------------|-------------|
|                        | Moisture       | Calcium        | Phosphorus   | Nitrogen    | Carbon      | Hydrogen    | Sulfur      | Oxygen, by difference |
| Complex                 | 10.06          | 2.680          | 0.667        | 0.861       | 13.13       | 44.76       | 5.923       | 0.653        |
| " dry basis*               | 2.982          | 0.742          | 0.958        | 14.60       | 49.78       | 6.587       | 0.726        |
| Calcium caseinate†        | 1.180          | 0.780          | 0.789        | 15.34       | 52.29       | 6.919       | 0.762       | 22.73        |
| Casein                   |                |                |              |             |             |             |             |              |

* Also ether extract-free.
† 95.20 per cent of complex.

To determine, by the application of this reaction, which compound, the dibasic or tribasic phosphate, is associated with the caseinate, the following experiment was undertaken.

Two 1 gm. samples of the complex were transferred to 200 ml. glass-stoppered bottles. To the first were added 50 ml. of distilled water and 2 ml. of toluene, and to the second 30 ml. of distilled water, 20 ml. of neutral 4 per cent potassium oxalate, and 2 ml. of toluene. Each mixture was then brought to a pH of 9.08 by adding, in small amounts and with occasional shaking, a total of 5.09 ml. of 0.05 N sodium hydroxide to the first and 0.48 ml. of 0.05 N hydrochloric acid to the second over a period of 3 days. The final pH of 9.08 was obtained only after holding the suspension for 20 hours following the final addition of the alkali or acid. Preliminary titrations indicated that at a pH of 9.08 the buffer effect was small and that any small addition of the standard alkali or acid yielded a distinct
change in pH. The sum of the alkali and acid added represented 0.2785 ml. of normal solution. This value, calculated to represent tricalcium phosphate, gave a concentration of 4.81 per cent Ca₃P₂O₈ in the complex, which is in good agreement with the value of 4.80 per cent Ca₃P₂O₈ calculated from the analysis. If the salt had been the dibasic phosphate, then the addition of neutral oxalate would not have changed the pH perceptibly from that of the sample without oxalate. We have isolated complexes that varied slightly in relative proportion of caseinate and phosphate from the values given in this paper. Variations that we found in percentage content of the two fractions of the complex are explained as due to different techniques used in washing the complexes, since difference in procedure might alter the relative proportions of the two components. These variations give credence to the assertion by some investigators (7, 9, 10) that the calcium phosphate is not bound chemically to the calcium caseinate fraction.

To obtain further evidence of the character of the inorganic phosphate fraction, so that the proper proportion of the calcium can be assigned to the protein fraction, a comparison was made of the pH of a water dispersion of the complex with pH values of a series of dispersions obtained from artificially prepared calcium caseinates of known calcium contents. The calcium caseinates were prepared from acid-washed and water-washed grain curd casein. The grain curd casein was obtained from fresh skim milk by precipitation with dilute hydrochloric acid, then washed thoroughly with acidulated distilled water, followed with distilled water, and was finally subjected to a pressure of 200 pounds for 20 hours to remove as much wash water as possible without actually drying the curd. The moist curd was then dispersed in distilled water and treated with highly diluted calcium hydroxide. During the period of reaction of the casein and calcium hydroxide, the mixture was agitated violently. Throughout, the reaction was kept below pH 8, and finally adjusted to the desired pH by the addition of more casein. The dispersions were then filtered through cheese-cloth and the calcium caseinate was freed from the mother liquor by passing the dispersions through the supercentrifuge, redispersing in distilled water, and recentrifuging. The moist caseinates were then air-dried, ground to pass a No. 60 sieve, and analyzed for calcium and inorganic phosphorus. The results of the analysis are given in Table II.

The pH values of 3 per cent water suspensions of these four calcium caseinates are plotted against their calcium percentages in Fig. 1. The pH determinations were made with the Beckman glass electrode. For purposes of comparison that part of the Palmer and Richardson (11) titration curve within similar pH range is included. These authors constructed their curve from data obtained by titrating potentiometrically 1 per cent casein-water dispersions with standard calcium hydroxide solution.
If the inorganic phosphate exists as a tribasic salt, the calcium in excess of the requirement of this salt would give a caseinate of only 1.18 per cent calcium and, according to the graph, give a pH of 6.40, a value very near to that of the milk itself.

Lack of precise agreement between Palmer and Richardson’s curve and ours is undoubtedly due to the marked differences in the methods by which the curves were obtained. These authors used a highly purified casein,

### Table II

<table>
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<tr>
<th>Experiment No.</th>
<th>pH of 3 per cent dispersion</th>
<th>Phosphorus, inorganic</th>
<th>Calcium, total</th>
<th>Calcium, corrected for Ca in Ca₃P₂O₈</th>
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</table>

![Graph](http://example.com/graph.png)

**Fig. 1.** Relationship between pH and calcium percentage of calcium caseinate. Curve A, 3 per cent calcium caseinate emulsion; Curve B, 1 per cent calcium caseinate emulsion, "Palmer and Richardson."

following, in its preparation, the procedure outlined by Van Slyke (12). That differences in base-binding capacity of caseins prepared by different methods do exist has been pointed out by Cohn and Berggren (13).

Thus, having established the existence of only Ca₃P₂O₈, and not CaHPO₄, in the complex, we then calculated the ultimate analysis of the calcium phosphate fraction and casein from the analytical data of the complex. The results are incorporated in Table I (third and fourth lines).

The value of 1.18 per cent calcium is somewhat higher than the values
given in the literature. Söldner (10) reports 1.07 to 1.11 per cent, Lehmann (14) 1.04 per cent, and Ling (15) 1.00 per cent. We have isolated samples of the complex with a calcium content as low as 1.02 per cent calcium after correcting the total calcium found by the calcium bound to the inorganic phosphorus calculated as Ca₃P₂O₈. These variations in calcium bound by the protein are evidence of differences in milks. Small differences could arise also from experimental errors of the methods of analysis; i.e., errors within the limits of accuracy of the methods.

In the literature there is no analysis of the elementary composition of casein obtained by our procedure, and that the results check, as a whole, surprisingly well with analyses of casein obtained by acid precipitation is interesting evidence that the changes in physical character through the action of acid are not accompanied by a pronounced alteration in its percentage elemental composition. However, the fact that the percentages we found for sulfur and phosphorus are somewhat higher than those obtained for casein repeatedly dissolved by alkali and precipitated by acid indicates that such treatment removes a portion of these elements from casein.

SUMMARY

A new procedure for obtaining the elementary composition of casein is described. The technique is to isolate the calcium caseinate-calcium phosphate complex from milk by means of the supercentrifuge, analyze the complex, and from the results calculate the percentage content of the elements of the casein. Such a procedure avoids separation and alteration of the casein by chemical means and, in consequence, prevents loss of phosphorus and sulfur. Otherwise, the percentage composition of casein as determined by this procedure does not differ appreciably from values given in the literature.

Increase in alkalinity of the complex by the addition of neutral potassium oxalate indicates the presence of tricalcium phosphate rather than dicalcium phosphate in the complex.

On this basis the complex was found to contain 4.80 per cent Ca₃P₂O₈ and 95.20 per cent calcium caseinate.

The calcium caseinate fraction of the complex was found to contain 1.18 per cent calcium.

A series of calcium caseinates was prepared from grain curd casein and the percentages of calcium were plotted against the pH of 3 per cent dispersions prepared from them. The curve indicates that a caseinate of 1.18 per cent calcium content would yield a pH of 6.40, which is only 0.07 pH more acid than the pH of the milk from which the complex was obtained.

Note—Since the preparation of this paper, an article by de Kadt and
van Minnen (16) has come to our attention. These authors described the partial fractionation of the complex by means of the Sharples super-centrifuge and found that the inorganic phosphate exists as the tribasic calcium phosphate and that the ratio between the caseinate and inorganic phosphate is not always constant. They also found that slightly more phosphate is centrifuged out with the first fraction than with the following fractions.

BIBLIOGRAPHY
