THE PHOSPHORUS CONTENT OF CASEIN.

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In a previous paper¹ from this laboratory, a method has been described for preparing casein practically ash-free, the last portion of calcium being removed by treating a solution of the casein in dilute NH₂OH with ammonium oxalate and excess of NH₂OH, and then allowing the mixture to stand about twelve hours. Casein thus prepared contains about 0.71 per cent of phosphorus. The accuracy of this figure has been questioned,² because it is considerably lower than that (about 0.85 per cent) hitherto commonly accepted as correct. The suggestion has been made that the lower figure is due to the splitting off of phosphorus from the casein molecule as the result of hydrolysis caused by prolonged contact with NH₂OH.

It is the purpose of this paper to present the results of an experimental study relating to the effects of partial hydrolysis of casein on the phosphorus content of casein preparations and also to offer an explanation as to why the higher figures that have been usually reported for the percentage of phosphorus in casein are not correct.

In connection with investigations recently carried on in this laboratory, the results of which have not been published yet, certain facts have been developed which appear to explain why the high figure usually accepted for the phosphorus content of casein is inevitably obtained in consequence of the method employed in making casein preparations. Two of the constituents of cow's milk are present in the form of colloidal solution, cal-

¹ This Journal, xiv, p. 203, 1913.
Phosphorus Content of Casein

cium caseinate and dicalcium phosphate. These two compounds appear to have a strong attraction for each other, as shown by the fact that, when casein is separated from milk by means of either centrifugal force or precipitation with a dilute acid, the casein always carries with it more or less dicalcium phosphate. It is evident, then, that in preparing casein by the usual method in which care is taken to avoid an excess of both acid and alkali, it is practically impossible to remove this phosphate completely. In order, therefore, to ascertain the true phosphorus content of casein, it is obviously necessary that the preparation be free from inorganic phosphorus and this can be accomplished only by removing all of the calcium. Several methods have been tried in this laboratory to effect this, and the one finally found to be the most satisfactory is that described in a previous paper, referred to above.

Further, a good reason for believing that the lower figure more closely approximates the truth than the higher one hitherto commonly accepted as correct is the relation of phosphorus to the molecular weight of casein. In a previous paper it was shown that the molecular weight of casein is approximately 8888. Now, if the casein molecule contains two atoms of phosphorus, the percentage of phosphorus is 0.698, while the phosphorus content would be 1.046 per cent if there were three atoms of phosphorus. The figure (0.85 per cent) heretofore regarded as correct represents, therefore, on account of the presence of impurities in the preparation, neither two atoms nor three atoms of phosphorus, while the lower figure (0.71 per cent) represents almost exactly two atoms.

Coming now to the criticism made that an excess of NH₄OH in contact with casein for twelve hours causes hydrolysis, resulting in the formation of inorganic phosphorus, there is reason to believe that, whatever hydrolysis takes place, it does not necessarily interfere with the composition of the final preparation, because as will be shown, the products of hydrolysis are not precipitated by dilute acetic acid and therefore form no part of the completed preparation, which is pure, unhydrolyzed casein.

* This Journal, xiv, p. 228, 1913.
EXPERIMENTAL.

After giving the ash and phosphorus content of several preparations of casein, we will present the results of a study of two special preparations of casein which were subjected to varying conditions in order to ascertain whether hydrolysis affects the phosphorus content of casein preparations.

Ash content and phosphorus content of casein. The percentage of ash and phosphorus in five samples of casein prepared in this laboratory during the past seven or eight years is as follows:

<table>
<thead>
<tr>
<th>SAMPLE</th>
<th>ASH PER CENT</th>
<th>PHOSPHORUS PER CENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.06</td>
<td>0.710</td>
</tr>
<tr>
<td>2</td>
<td>0.39</td>
<td>0.732</td>
</tr>
<tr>
<td>3</td>
<td>0.61</td>
<td>0.830</td>
</tr>
<tr>
<td>4</td>
<td>0.61</td>
<td>0.839</td>
</tr>
<tr>
<td>5</td>
<td>3.93</td>
<td>0.941</td>
</tr>
</tbody>
</table>

The results show that increase of ash is accompanied by an increase of phosphorus.

Phosphorus content of casein preparations treated in different ways. In order to study the effect of treating casein in different ways upon the content of phosphorus, and especially to ascertain what effect partial hydrolysis may have upon the phosphorus content of casein preparations, two preparations of casein were made and each of these was treated in the manner described below.

Preparation A was made in the usual way, treating alternately with dilute acetic acid and ammonia, avoiding an excess of each reagent. This preparation contained 0.857 per cent of phosphorus.

Preparation B was made according to the method given in a previous paper,4 the distinctive feature of which is treatment of a solution of casein in dilute alkali with ammonium oxalate and excess of alkali. This preparation contained 0.711 per cent of phosphorus.

(1) Treatment with excess of ammonia. Each of preparations A and B (20 grams) was dissolved in dilute NH₄OH and an excess

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4 This Journal, xiv, p. 203, 1913.
of the same reagent was added; after standing twelve hours at 
37°C., the solution was centrifugalized and filtered, the casein 
in the filtrate being then precipitated with dilute acetic acid. 
This precipitated casein was washed, redissolved, reprecipitated 
and finally washed with water, alcohol and ether.

In the case of preparation A, the yield was 14 grams, contain-
ing 0.841 per cent of phosphorus; in the case of preparation B, 
the yield was 15 grams and the phosphorus content 0.713 per 
cent.

The decreased yield in each case was due in part to hydrolysis 
of casein and in part to mechanical losses. It is evident that 
partial hydrolysis of casein preparations has no effect on the per-
centage of phosphorus in the unhydrolyzed casein that is recovered.

(2) Treatment with ammonium oxalate and excess of ammonia. 
Each of preparations A and B (20 grams) was dissolved in dilute 
NH₂OH and then ammonium oxalate and an excess of NH₂OH 
added, the mixture being allowed to stand twelve hours at 37°C. 
The casein was separated as before.

In the case of preparation A, the yield was 14 grams, contain-
ing 0.723 per cent of phosphorus; in the case of preparation B, 
the yield was 14.5 grams, containing 0.71 per cent of phosphorus.

In these two experiments, hydrolysis of casein by alkali has no 
effect upon the percentage of phosphorus in the casein finally 
recovered. In the case of preparation A, the phosphorus con-
tent is reduced from 0.857 to 0.723 per cent, as a result of the 
removal of calcium phosphate from the casein preparation. In 
the case of preparation B, the phosphorus content remains the 
same as in the original preparation, because the casein used had 
already been subjected to treatment with ammonium oxalate and 
excess of NH₂OH, the calcium phosphate having been removed 
as completely as practicable.

(3) Treatment as in (2) but prolonged. Preparation B (20 
grams) was treated as in the preceding experiment, except that 
the mixture was allowed to stand seventy-two hours (instead of 
twelve) at 37°C. The amount of casein recovered was 12.4 grams 
containing 0.721 per cent of phosphorus. The prolonged treat-
ment giving opportunity for increased hydrolysis of casein, did 
not change the percentage of phosphorus in the casein recovered.
SUMMARY.

The amount of phosphorus in casein has been commonly given as about 0.85 per cent. By treating a solution of casein in dilute \( \text{NH}_4\text{OH} \) with ammonium oxalate and an excess of \( \text{NH}_4\text{OH} \) and letting stand 12 hours the phosphorus content is reduced to about 0.70 per cent. This lower percentage can not be explained as being due to hydrolysis of casein and splitting off of phosphorus. While some of the casein is hydrolyzed, this portion does not enter into the final preparation and does not affect its composition, because the hydrolyzed portion is not precipitated by acetic acid while the unhydrolyzed part is. The higher figure ordinarily given is due to the presence of inorganic phosphorus (dicalcium phosphate) carried from the milk into the precipitated casein and not entirely removed under the usual conditions of preparation. The lower figure corresponds very closely to two atoms of phosphorus (0.698 per cent) in the casein molecule. Analyses of various preparations of casein containing varying amounts of ash show a general correspondence between the ash and phosphorus content.
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