CC. THE HEAT COAGULATION OF CASEINOGEN.

II. THE RATE OF PHOSPHORUS CLEAVAGE.

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In a previous paper [Howat & Wright, 1934] it was shown that drastic heat treatment of neutral solutions of calcium caseinogenate resulted in a gradual dephosphorylation of the caseinogen molecule and ultimately in coagulation of the protein. There was also a slow liberation of acid-soluble nitrogen.

In the original experiments the protein solutions were heated in closed tubes at 120°. It appeared desirable to carry through a further series of experiments using a lower range of temperatures. It was hoped by this means (*a*) to determine the temperature coefficients of the reactions, and (*b*) to secure further evidence regarding the relationship between phosphorus cleavage and the coagulation of the protein.

TECHNIQUE.

Solutions of calcium caseinogenate were prepared by the technique described in the earlier paper, but were made up to contain 3.0% in place of 3.5% caseinogen. The amount of $\text{Ca}(\text{OH})_2$ used was 0.065 g. per 100 ml., which gave a pH of roughly 6.9. 5 ml. portions of the solution were heated in closed tubes in a thermostatically controlled glycerol bath. The temperatures employed ranged from 90 to 115°. The periods of heating varied from 1 hour up to 45 hours, the time necessary to produce dephosphorylation and coagulation of the protein being found to increase progressively as the temperature of heating was lowered.

At suitable time intervals duplicate tubes were removed from the heating bath. One tube was used for estimating acid-soluble phosphorus and nitrogen and the second tube for determining the extent of protein coagulation. The latter figure was ascertained by centrifuging the solution for 5 min. at 3000 r.p.m. and determining the percentage of uncoagulated protein in the supernatant liquid.

RESULTS.

The results are shown in Figs. 1–5. It will be seen that at all the temperatures employed the curves show a close similarity to those obtained in the previous work. There is a slow liberation of acid-soluble nitrogen and a relatively rapid liberation of acid-soluble phosphorus. The proportion of phosphorus liberated does not exceed 60%. As regards coagulation of the protein, this takes place slowly at first, and then more rapidly. A final slowing-up of the reaction gives a typical “S” shape to the curve. It may be noted that visible coagulation usually occurs when 50–60% of the protein has been rendered insoluble, namely on the steep gradient of the curve.

In order to determine the temperature coefficients of dephosphorylation and heat coagulation, a diagram has been constructed (Fig. 6) in which the temperature of heating is plotted against the time of heating (expressed logarithmically)

Figs. 1 to 5. Effect of various temperatures of heating. (Continuous line, acid-soluble phosphorus; dotted line, acid-soluble nitrogen; broken line, protein coagulated. Time in hours shown below each figure.)

Fig. 6. Time-temperature relationships. (White circles, visible coagulation; black circles, 50% protein coagulated; crosses, 45% dephosphorylation.)

in hours) required to produce (a) 45 % dephosphorylation, (b) visible coagulation and (c) 50 % coagulation of the protein. It will be seen that all the points fall

roughly on a straight line. This fact provides strong presumptive evidence that dephosphorylation and heat coagulation are related reactions, coagulation probably being preceded by liberation of phosphorus from the caseinogen molecule.

It may be noted that the slope of the curve shows that, for each rise in temperature of 10° , the reaction velocity increases threefold. This fact has one interesting application. For various reasons it is necessary to store milk products, such as evaporated milk, for relatively long periods. It appeared possible that during such prolonged storage dephosphorylation of the caseinogen might occur, with consequent coagulation of the protein. In order to obtain some idea of the rate of dephosphorylation and coagulation at storage temperatures the curve in Fig. 6 was extrapolated to 20° . It was found that at this temperature dephosphorylation would take well over 7 years—a storage period completely outside the range of commercial practice. To confirm this point samples of caseinogen were prepared from freshly manufactured evaporated milk and from evaporated milk which had been stored for 6–9 months. The N/P ratios of the caseinogen were found to be as follows:

Freshly manufactured samples	18-91, 21-31, 21-32, 19-62	Mean 20-29
Stored samples	19-14, 21-20, 20-90, 20-90, 18-50, 19-90	Mean 20-09

These figures show that during 6–9 months' storage at ordinary temperatures there was no detectable liberation of phosphorus from the caseinogen molecule.

SUMMARY.

1. A study has been made of the rates of liberation of nitrogen and phosphorus from neutral solutions of calcium caseinogenate at temperatures between 90 and 115° . Determinations of the rate of coagulation of the protein have been made simultaneously.
2. The results confirm the conclusions reached in previous work, namely that the dephosphorylation and heat coagulation of caseinogen are related reactions.
3. It is shown that for each rise in temperature of 10° the reaction velocity of dephosphorylation and coagulation increases three-fold.
4. No detectable liberation of phosphorus takes place as a result of storing evaporated milk at ordinary temperatures for periods of 6–9 months.

REFERENCE.

Howat & Wright (1934). *Biochem. J.* **28**, 1336.