THE ACTION OF RENNIN ON CASEIN.

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In order to determine if the change from casein to paracasein results in the cleavage of any of the elements contained in the casein molecule, it is imperative that pure casein be used as a standard of comparison, and that the rennin activity be positively differentiated from any further proteolytic activity of the enzyme under consideration; for it is quite evident that "Rennin action is probably a hydrolytic cleavage and may be considered the first step in the proteolysis of casein. It would follow from this that the action now attributed to rennin may be produced by any proteolytic enzyme."1

Pure casein and paracasein have been prepared according to the methods previously published.2 Pure paracasein was also prepared by allowing trypsin to act upon fat-free milk, after the addition of calcium chloride, and the curd produced was purified according to the method referred to. The use of an excess of ammonia as prescribed has been criticized by Harden and Macallum,3 who claim that preparations made in that way may have a low phosphorus content due to the cleavage of phosphorus from the casein molecule by the action of the ammonia. In a recent paper4 it has been shown that this criticism does not hold. The analyses of the preparations are given in the table.

These figures show that the composition of paracasein is the same irrespective of the enzyme used to produce it. The figures also show that casein and paracasein have the same percentage composition which excludes the possibility that cleavage of any of the elements of casein is a result of its transformation into paracasein by enzymes.

1 Bosworth: this Journal, xv, p. 236, 1913.
2 Van Slyke and Bosworth: ibid., xiv, p. 203, 1913.
Harden and Macallum, in their paper, conclude that "The conversion of caseinogen into casein by enzyme action is accompanied by the cleavage of nitrogen, phosphorus and calcium." It seems more probable to us that this cleavage follows, rather than accompanies, the conversion in question, and is to be attributed to a continuation of proteolytic activity by the enzyme beyond the point where casein has been changed to paracasem. This point was emphasized in my first paper on the action of rennin on casein.

The similarity between the composition of casein and paracasem, and the fact that casein has been shown to have a molecular weight of 8888 and a valency of 8, while paracasem has been shown to have a molecular weight of 4444 and a valency of 4, seems to be evidence enough for concluding that the transformation of casein into paracasem is a process of hydrolytic splitting, one molecule of casein yielding two molecules of paracasem, and that this splitting of casein is not accompanied by a cleavage of any of the elements contained in the original casein molecule.

5 The English caseinogen is equivalent to the American casein.

The English casein is equivalent to the American paracasem.

Harden and Macallum give the nitrogen-phosphorus ratio of casein as N: P = 100: 5.6. The high phosphorus content of their casein preparations (0.87-0.90 per cent) would seem to indicate the presence of considerable inorganic phosphorus. If our figures are correct for the nitrogen and phosphorus content of casein (15.80 per cent N and 0.71 per cent P), the ratio would be N: P = 100: 4.5. In only one of their experiments conducted to show the loss of phosphorus from the casein molecule was this ratio reduced to 4.5.

6 Bosworth: ibid., xv, p. 231, 1913.
7 Van Slyke and Bosworth: ibid., xiv, p. 227, 1913.