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Ammonium Carbamate as the Intermediate Product in the Action of Urease on Urea.

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A method for quantitative estimation of ammonia nitrogen and carbamic acid nitrogen, in a solution containing ammonium salts, urea, and carbamates, has been developed. The method depends on the fact that ammonium carbamate is stable in Nessler solution so that one-half of the nitrogen in the ammonium carbamate does not affect the color of the Nessler solution. However, if the solution is acidified before Nesslerizing, the carbamic acid is almost instantly decomposed and Nesslerization gives the total of ammonia and carbamate nitrogen present. In other particulars analyses are made according to the well-known colorimetric method of determining ammonia. Each solution to be analyzed was Nesslerized under these 2 different conditions. The difference between the two values for nitrogen was equal to the carbamic acid nitrogen. Twice this figure was equal to the ammonium carbamate nitrogen. The method was shown to be quantitative within the limits of accuracy of reading a colorimeter.

A number of experiments have been carried out in which the enzyme was allowed to act on 100 cc. ice-cold 1% urea. The action was stopped after 5 to 7 minutes by adding a trace of potassio-mercuric iodide. During this time about 1 mg. per cc. of total combined nitrogen had been liberated from the urea. This concentration was found to be most convenient for analysis. After poisoning the enzyme, analyses were made immediately for ammonium carbamate and ammonium carbonate and then at intervals until the carbamate had fallen almost to zero. Figure 1 shows the curve for

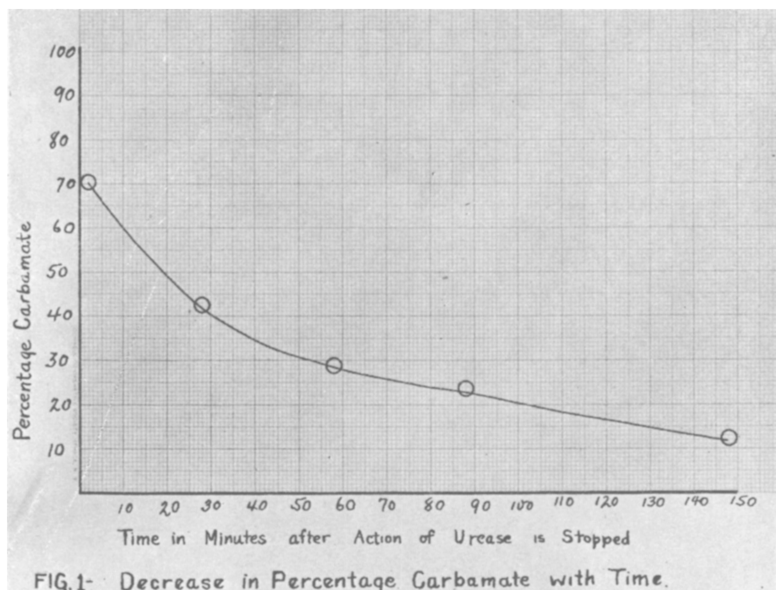

FIG. 1.

percentage ammonium carbamate nitrogen of the total nitrogen when plotted against time after the enzyme had been stopped.

The percentage of carbamate diminished very rapidly but at a measurable rate. Our method of analysis was not accurate enough to determine the point of equilibrium between the carbonate and the carbamate. An almost identical curve was obtained when 261 mg. of pure solid ammonium carbamate was added to ice-cold water and the percentages of carbamate nitrogen plotted against time of analysis. When the enzyme was allowed to act in acetate and phosphate buffers no carbamate could be detected. In sodium bicarbonate, carbon dioxide buffer at pH 9, the carbamate formed was detectable but disappeared very rapidly. It is known that ammonium carbamate decomposes quite slowly in presence of excess carbon dioxide. We accordingly allowed urease to act on urea in a solution kept acid with CO_2. 100 cc. of 0.2% urea were cooled with ice and CO_2 bubbled slowly through. Crystalline urease was added and allowed to act for 15 minutes and the reaction stopped with mercury. The first analysis was made 16 minutes after adding the enzyme and 84.5% of the nitrogen was in the form of ammonium carbamate.

These experiments we take to prove that an intermediate compound is formed by the action of urease which contains only half its nitrogen in the free ammonia form. The compound is identified as ammonium carbamate because its rate of hydrolysis is comparable

to ammonium carbamate and is affected in the same way by acid, alkali, ammonia and carbon dioxide. The proof rests on the fact that no cyanate is formed by the enzyme.

We conclude that carbamate is formed in large amounts during the action of urease on urea in alkaline solution and that after the urease has been inactivated the carbamate decomposes to carbonate at a measurable rate. Our experiments indicate that all of the ammonia liberated from the urea passes through the ammonium carbamate stage. We hope to make further analyses investigating this point. There is no evidence of any intermediate product other than ammonium carbamate.

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Phospholipid Content and Physiological Activity of Tissues.

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In tissues in differing states of physiological activity the phospholipid percentage was greater when the tissue was more active. In the corpus luteum of the pig the phospholipid content was about 3 times as great during its active state in ovulation and pregnancy as in the resting state. Cholesterol was not greatly different. Malignant tumors had about 3 times the content of phospholipid and twice the content of cholesterol as benign tumors. Mammary glands (rabbit) at the end of pregnancy had twice the phospholipid content of the resting gland, cholesterol remaining the same.

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The Polynuclear Count in the Rat, with Special Reference to Vaginal Smears.

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The presence of polynuclear leucocytes in vaginal smears of the rat has been noted by Long and Evans,[1] who observed the presence

[1] Long, J. A., and Evans, H. M., *Univ. of Calif. Pub.*, 1922, vi.