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Relation Between the Isoelectric Range of Urease and the Reversibility of Its Action.

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The action of urease upon solutions of urea and ammonium carbonate was determined at various H^+ ion concentrations.

Methods. Into a series of Erlenmeyer flasks were placed in succession 5 cc. of a concentrated urea or ammonium carbonate solution, 10 cc. distilled water, 2 cc. of an indicator solution (phenol red brom-cresol purple or methyl red) and 2 cc. of an urease enzyme solution prepared according to the method of Folin and Youngburg.[1] The pH of successive flasks in a series was made to differ by 1 pH through addition of HCl or NaOH. The actual pH was determined by comparison with standard buffer mixtures to which the same indicators had been added. Any tendency for the pH to alter as a result of chemical interactions was compensated by adding small amounts of HCl or NaOH during the course of the experiment. The flasks so prepared for any set of observations were kept for a definite interval of time and at a constant temperature. In order to determine optimal conditions, the temperature in different sets of experiments was varied from that of the room to 45° C., and the reactions were allowed to continue from 15 min. to 2½ hours in different groups. It may be stated that the changes to be reported were obvious within 10-15 min. but usually became more pronounced after longer time intervals (45 min. to 1½ hours).

At the end of the time set, the amount of $(NH_4)_2CO_3$ formed in the urea solutions was determined by Nesslerization, compared with known standards and expressed in mg. ammonia nitrogen. Similarly, the amount of urea formed in the ammoniun carbonate solution was estimated by determining the *relative* quantities of unconverted $(NH_4)_2CO_3$ remaining in each flask. Unless it is assumed that $(NH_4)_2CO_3$ escaped as gas during the course of the experiment, which is not probable under the conditions of the experiment,* it is

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[1] Folin, O., and Youngburg, G. E., *J. Biol. Chem.*, 1919, xxxviii, 111.

* This source of error is improbable in view of the facts (1) that the greatest reduction in the NH_4 nitrogen occurred toward the acid side, while a greater tendency to liberate NH_4 would exist toward the alkaline side, and (2) that control flasks in which boiled urease had been placed showed no alterations in the NH_4 nitrogen, when the pH was similarly altered.

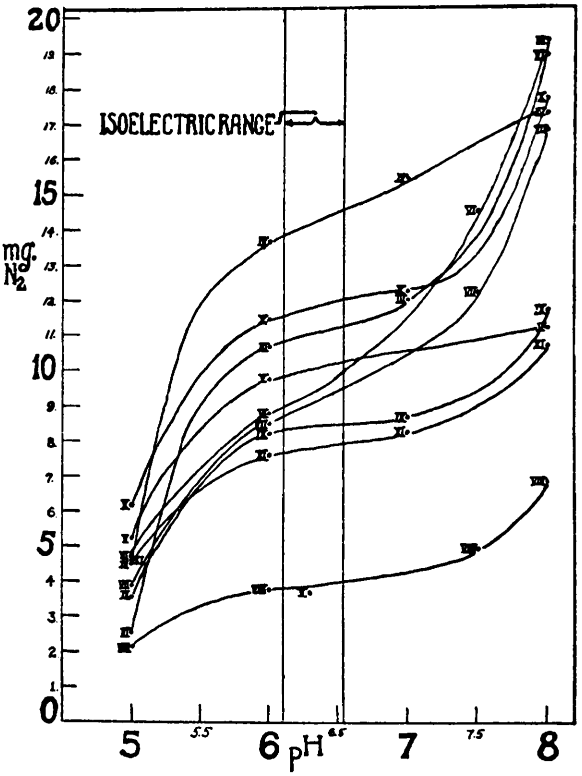

Fig. 1.

Curves showing mg. ammonia nitrogen (ordinates) obtained from action of urease on same initial quantities of urea at different pH (abscissae). Duration of action as follows: III, 75 min.; IV, 20 min.; VII, 15 min.; VIII, 6 min.; IX, 45 min.; X, 10 min.; XI, 5 min.

reasonable to assume that the relative losses of $(NH_4)_2CO_3$ are a criterion of the amount converted to urea. The actual presence of urea was demonstrated by use of the xanthydrol test. Thus, it was noted that the volume of precipitate formed agreed roughly with the loss of ammonia N. Quantitative estimates were not made however.

Results. The results of 9 experiments in which the effects of urease on urea solutions was tested are plotted in Fig. 1. They indicate that while $(NH_4)_2CO_3$ is formed from urea at all pH ranges, the conversion is slight toward the acid side of the isoelectric range and great toward the alkaline side. The curves of ammonia nitrogen so plotted also show the velocity of the reactions. They rise convex to the abscissae between pH 5-6, become horizontal during the isoelectric range (6.1-6.5), established by Sumner[2] for crystalline urease, but rise rapidly and concave to the abscissae beyond this range.

The results of 7 experiments in which the effects of urease on $(NH_4)_2CO_3$ solutions were tested are graphically presented in Fig. 2. The curves showing the mg. of unconverted ammonia nitrogen and by inference the amount of urea formed at various pH points, demonstrate that a reversal of the curve contours from that of Fig. 1 obtains. They show also that urea formation gradually diminishes during the acid range; that it tends to become stabilized during the isoelectric range, but rapidly declines toward the alkaline side. The change in the inflection of the curves again occurs during the isoelectric range.

Conclusion. The results strongly support the probability that changes in pH can determine the directional action of the reversible

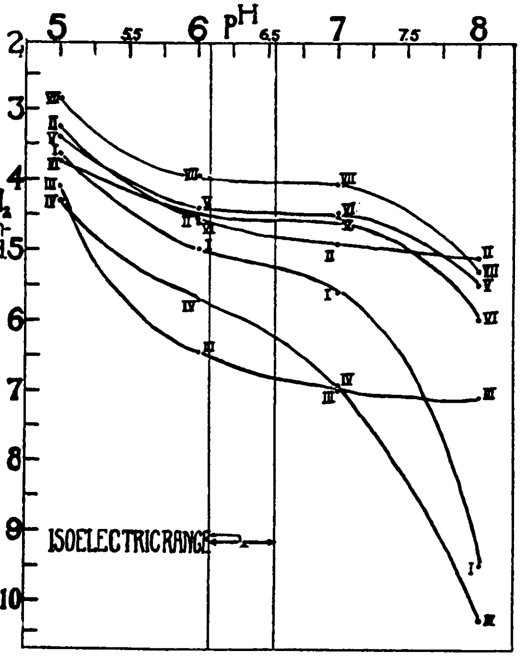

FIG. 2.

Curves showing mg. ammonia nitrogen unconverted to urea and by inference amount of urea formed (ordinates) after action of urease on same initial quantities of $(NH_4)_2 CO_3$ at different pH (abscissae). Duration of action as follows: I, 47 min.; II, 120 min.; III, 60 min.; IV, 45 min.; V, 35 min.; VI, 70 min.; VII, 135 min.

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[2] Sumner, J. B., and Hand, D. B., *J. Biol. Chem.*, 1928, lxxvi, 149.

ferment, urease. Toward the alkaline side of the isoelectric range the tendency to convert urea into $(NH_4)_2CO_3$ predominates; toward the acid side the tendency to synthesize urea is more pronounced.

In conclusion the writer wishes to express his gratitude to Dr. M. Garcia Banus for his advice and supervision of the work.

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Exogenous Arginine as the Precursor of Creatine in the Dog.

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Of the substances normally present in the diet, structural relationships point to arginine as the most logical precursor of creatine and creatinine. In view of the recent work of Benedict and Osterberg,[1] who fed creatine over long periods of time, and the confirmation of their work in man by Rose, Helming and Ellis,[2] it seemed possible that earlier attempts to demonstrate that creatine may originate from exogenous arginine failed because the amino acid was administered over too short a period to observe any significant change in the excretion of creatine or creatinine. Therefore it was decided to study the effect of arginine on the creatine-creatinine elimination, when the arginine was fed daily to a dog over a period of several weeks. After the completion of the experiment, Hyde and Rose[3] published the results of a similar study of prolonged administration of arginine to a normal man and woman. Although their results showed no evidence of the conversion of exogenous arginine to creatine or creatinine, they have suggested that other species, particularly the pig,[4] may differ in their response to arginine feeding. Our results presented in Table I, with another species, the dog, confirm those of Hyde and Rose, in that oral administration of arginine for a period of 35 days failed to influence the excretion of urinary creatine or creatinine, although exogenous creatine (Periods XIII and XIV) in small amounts resulted in prompt increases in both these catabolites, thus confirming the earlier work of Benedict and Osterberg.

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[1] Benedict, S. R., and Osterberg, E., *J. Biol. Chem.*, 1923, lvi, 229.

[2] Rose, W. C., Ellis, R. H., and Helming, O. C., *J. Biol. Chem.*, 1928, lxvii, 171.

[3] Hyde, E. C., and Rose, W. C., *J. Biol. Chem.*, 1929, lxxxiv, 535.

[4] Gross, E. G., and Steenbock, H., *J. Biol. Chem.*, 1921, xlvii, 33.