CLIX. GASTRIC UREASE.

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As one of us [Luck, 1924] has recently shown that the enzyme urease occurs in the gastric mucosa of the Carnivora and in specified portions of the stomachs of the Ruminantia, it was considered desirable to compare "animal" urease from the gastric mucosa of the dog, with "plant" urease from the soy bean. Two possibilities suggested themselves:

1. Unlike soy bean urease, gastric urease might be able to catalyse the dissociation and hydrolysis of ureides, and thus contribute to the processes of gastric digestion.

2. Gastric urease might be an endocellular enzyme, capable only of catalysing the dissociation of urea, and taking part in neutrality regulations within the body, by converting urea from the blood into ammonium carbonate, or by synthesising urea from absorbed ammonia.

The examination of the first of these hypotheses has been the object of this investigation. The action of gastric urease on many urea derivatives, and on several amides has been investigated. Most of the substrates employed have been examined by previous workers with soy bean urease, but were re-investigated in a parallel duplicate series. As uramino acids have at times been isolated from urine, and have been frequently considered as true metabolites, the uramino acids of glycine and leucine were prepared and subjected to the action of soy bean urease and gastric urease.

The results of this portion of the work indicate that gastric urease is identical with soy bean urease.

It has been shown [Van Slyke and Zacharias, 1914; Lövgren, 1921, 1923] that the optimum hydrogen ion concentration for the action of soy bean urease varies with the concentration of the substrate. Van Slyke and Zacharias found an optimum p_H of 7.7 for 0.02 molar urea and p_H 7.0 for 0.15 molar urea. Lövgren reported an optimum p_H of 7.70 for 0.01 molar urea, 7.45 for 0.1 molar and 7.20 for 1.6 molar. It was also shown by the latter author that the optimum hydrogen ion concentration for soy bean urease was not influenced by the phosphate concentration, the enzyme concentration, or the reaction time. As this dependence of the optimum p_H on

substrate concentration has not to the authors' knowledge been demonstrated with other enzymes, it appeared desirable to determine the optimum p_H for the action of gastric urease and observe its dependence, if any, on the substrate concentration. These results also demonstrated that like soy bean urease, the optimum p_H for the action of gastric urease increases with dilution of the substrate. It is therefore considered that gastric urease is identical with soy bean urease and that the first of the above hypotheses is untenable.

EXPERIMENTAL.

(1) *Preparation of the enzyme from soy bean meal and from the gastric mucosa of the dog.*

The method of Van Slyke and Cullen [1914], applied to aqueous extracts of the gastric mucosa of the dog, yielded preparations of low solubility and of only moderate activity.

Various modifications were tried and eventually a successful preparation was obtained by grinding the freshly stripped mucosa with much sand, desiccating the mixture *in vacuo* and extracting the dry powder by thorough grinding with water. The latter was added in the proportion of 10 parts of water to 1 part of freshly stripped mucosa. Assuming the water content of the fresh tissue to be 80 %, the treatment amounted to the extraction of 2 parts of dry tissue with 108 parts of water. The aqueous solution obtained on subsequent centrifuging was slightly opalescent and of high activity, being slightly more active than the solution obtained by grinding 2 parts of soy bean meal with 108 parts of water. The dried product retains its activity almost undiminished for many weeks. In the experiments which follow, fresh aqueous extracts were prepared from the dried product daily. The soy bean preparation was similarly obtained by grinding 2 parts of the meal with 108 parts of water, and centrifuging off the aqueous extract.

(2) *Technique.*

The amount of ammonia produced in 15 minutes at 38° by 1 cc. of the enzyme solution, acting on a fixed amount of urea in the presence of a fixed amount of phosphate solution was taken as an index of the activity of the enzyme.

Ammonia was estimated by Van Slyke and Cullen's [1914] aeration method.

Phosphate buffer solutions were prepared by mixing in requisite amounts molar solutions of dihydrogen potassium phosphate and monohydrogen potassium phosphate [Lövgren, 1923]. This series of solutions was then standardised against the hydrogen electrode.

(3) *The optimum p_H for the action of gastric urease.*

(i) 1 cc. of enzyme solution at 38° was added to 9 cc. of a solution at 38° consisting of 5 cc. of urea (0.04 molar), 1 cc. water, 3 cc. molar phosphate solution of given p_H . The tubes were corked. After exactly 15 minutes, the

reaction was stopped by the addition of caprylic alcohol and potassium carbonate in the usual manner. This series of experiments was performed in duplicate on the same day with the same enzyme solution. The duplicates were always observed to be in close agreement. The average values have been plotted in Fig. 1, Curve (a). The optimum p_H for gastric urease in 0.02 molar urea was observed to be about 8.1.

(ii) On a second day the observations were repeated with 5 cc. of 2*M* urea. The results are presented in Fig. 1, Curve (b). The optimum p_H for gastric urease in molar urea was observed to be about 7.5.

Fig. 1

These observations regarding the dependence of the optimum p_H for gastric urease on the substrate concentration are in entire agreement with the observations of Van Slyke and Zacharias [1914] and Lövgren [1923] on soy bean urease. While the optima observed by us are in general higher than those reported for similar concentrations of urea with soy bean urease, it is suggested that the vast differences in the composition of the extraneous matter associated with the enzyme in each case may cause this apparent difference. The fundamental fact is that the optimum p_H of both gastric urease and soy bean urease varies with the concentration of the substrate.

(4) *The specificity of gastric urease.*

The "specificity" of soy bean urease is one of its most distinctive features and in order to determine the identity or otherwise of gastric urease and soy

bean urease the action of the two preparations on a large number of substrates allied in composition to urea was studied under comparable conditions.

The enzyme solutions were prepared as described in section (1).

Duplicate experiments with suitable controls were carried out with each enzyme preparation, using in each case:

- 5 cc. of 0.04 molar substrate.
- 3 cc. of molar phosphate (p_H 7.99).
- 1 cc. of water.
- 1 cc. of enzyme solution.

The reaction in each case was allowed to proceed for two hours at 39–40°, after which ammonia was estimated in the usual manner. The following substrates were investigated:

Urea, monomethyl urea, *unsym.* dimethyl urea, *sym.* dimethyl urea, monoethyl urea, *unsym.* diethyl urea, *sym.* diethyl urea, normal butyl urea, phenyl urea, monoacetyl urea, thio-urea, biuret, urethane, creatine, acetamide, propionamide, benzamide, succinamide, succinimide, asparagine, oxamic acid, oxamide, adenine sulphate, guanine chloride, uramino-acetic acid and α -uramino-isobutylacetic acid. The last two were prepared by the method of Lippich [1908] from glycine and leucine respectively.

All the above substrates except urea and normal butyl urea gave negative results. The observations of Armstrong and Horton [1912] (negative results), Werner [1923] (negative), Takeuchi [1909] (negative), Wester [1921] (negative), and Fearon [see Werner, 1923, p. 110] (positive results with normal butyl urea) are confirmed. The positive results of Takeuchi with biuret, and of Pin Yin Yi [1920] with *unsym.* dimethyl urea, and *unsym.* diethyl urea (using Robinia urease) could not be reproduced. The results obtained with urea and butyl urea follow:

Substrate	Enzyme	Time	Nett cc. N/50 NH ₃
Urea	Gastric urease	15 min.	4.45
			4.55
"	Soy bean urease	15 "	3.90
			3.95
Butyl urea	Gastric urease	2 hours	0.90
			0.95
"	Soy bean urease	2 "	0.82
			0.85

These results suggest that the two enzymes are identical.

Urea is probably the sole metabolite which is capable of functioning as substrate for the gastric enzyme. The ammonia of the gastric juice, 2 mg. per 100 cc. [Huber, 1917], is 50 to 100 times more concentrated than the ammonia of the arterial blood. We would suggest that the gastric juice ammonia arises at least in part from the localised action of gastric urease on urea from the blood.

The second hypothesis is now under investigation.

SUMMARY.

1. Gastric urease from the mucosa of the stomach of the dog has been compared with soy bean urease.

2. The common dependence of the optimum p_H on substrate concentration, the common specificity of the two enzymes, and their similar action on butyl urea, suggest that gastric urease and soy bean urease are identical.

3. A partial explanation of the origin of the gastric juice ammonia is proposed.

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