CVI. AMMONIA PRODUCTION BY ANIMAL TISSUES *IN VITRO*.

II. THE DEMONSTRATION OF UREASE IN THE ANIMAL BODY.

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In the preceding paper [Luck, 1924] it was shown that a specific tissue mixture was capable of liberating a marked amount of ammonia when acting on totally hydrolysed gelatin. It was also shown that the "amide N" as commonly found in the reaction products of the liver preparations was absent from the mixed gastric mucosa-liver preparations. In continuing the investigation of the particular mechanism involved, three problems presented themselves:

- (1) The specificity of the liver factor.
- (2) The action of one factor on the reaction product of the second factor.
- (3) The presence or absence of urea in the reaction products of mixed gastric mucosa-liver preparations.

The technique, general procedure, and methods of analysis were the same as described in the first paper of this series [Luck, 1924].

Exp. 1. The tissues used were those of a male dog of 9 kilos. The results are recorded in Table I.

Table I.

Substrate totally hydrolysed gelatin	Nitrogen per 5 cc. (cc. N/10)		Substrate totally hydrolysed gelatin	Nitrogen per 5 cc. (cc. N/10)	
	Ammonia N (nett)	Amide N (nett)		Ammonia N (nett)	Urea N (nett)
1. Muscle (A)	0.07	0.08	25. 2 cc. M + 1 cc. B	1.99	- 0.03
2. " (A)	0.10	0.02	26. 2 cc. M + 1 cc. B	2.18	0.05
3. Liver (B)	0.30	—	27. 2 cc. M + 0.5 cc. B	1.70	- 0.02
4. " (B)	0.25	—	28. 2 cc. M + 0.5 cc. B	1.65	0.03
5. " (B)	—	—	29. 2 cc. M + 0.2 cc. B	1.65	0.07
6. " (B)	—	—	30. 2 cc. M + 0.2 cc. B	1.65	0.00
7. Intestinal mucosa (C)	0.05	—	31. 2 cc. M + 0.1 cc. B	1.80	—
8. " " (C)	0.07	—	32. 2 cc. M + 0.1 cc. B	1.99	—
9. Gastric mucosa (M)	0.10	0.00	33. 1 cc. M + 2 cc. B	2.30	0.00
10. " " (M)	0.08	0.05	34. 1 cc. M + 2 cc. B	2.18	0.02
11. " " (M)	—	—	35. 0.5 cc. M + 2 cc. B	2.18	—
12. " " (M)	—	—	36. 0.5 cc. M + 2 cc. B	2.20	—
13. Kidney (Q)	0.07	—	37. 0.2 cc. M + 2 cc. B	2.16	—
14. " " (Q)	0.05	—	38. 0.2 cc. M + 2 cc. B	2.20	—
15. M + A	0.10	—	39. 0.1 cc. M + 2 cc. B	2.26	0.01
16. M + A	0.08	—	40. 0.1 cc. M + 2 cc. B	2.16	0.02
17. M + B	2.28	—	41. B	0.30	2.26
18. M + B	2.33	—	42. B	0.25	2.20
19. M + B	2.25	0.13			
20. M + B	2.26	0.05			
21. M + C	0.64	—			
22. M + C	0.58	—			
23. M + Q	0.67	—			
24. M + Q	0.60	—			

From these results it may be observed that not only is ammonia liberated by mixtures of the gastric mucosa and liver extracts, but also in smaller but definite amounts by gastric mucosa + intestinal mucosa, and gastric mucosa + kidney extracts. It should also be noted that while urea is found in the reaction products of the liver preparations (tubes 41, 42) it is absent from the reaction products of the gastric mucosa + liver preparations (tubes 25-40). It may also be observed that the urea nitrogen in tubes 41, 42 is equal in quantity to the ammonia nitrogen in tubes 33-40. Either of two conclusions might therefore be drawn from the facts so far recorded:

- (1) The gastric mucosa of the dog contains urease.
- (2) The gastric mucosa of the dog contains a factor which inhibits the formation of urea (from ammonium salts).

These possibilities have been investigated in Exp. 2.

Exp. 2. A male dog of 11 kilos. was used for the preparation of the tissue extracts. After the addition of the substrates, the volumes were adjusted to 9 cc. and dilute soda added until the contents were neutral to phenol red. 0.5 cc. of toluene was added to each as an antiseptic and the tubes maintained at 37° for 5 days. Ammonia only was estimated—the entire contents of the tubes being washed into the aeration cylinders without previous heat coagulation and filtration. The results are recorded in Table II.

Table II.

	Ammonia nitrogen (cc. N/10)
1. 5 cc. urea (0.02 molar) + water	0.00
2. 5 cc. urea + water	-0.02
3. 5 cc. urea + 2 cc. gastric mucosa (<i>M</i>)	1.90
4. 5 cc. urea + 2 cc. gastric mucosa (<i>M</i>)	1.85
5. 5 cc. urea + 2 cc. gastric mucosa + 2 cc. liver	1.95
6. 5 cc. urea + 2 cc. gastric mucosa + 2 cc. liver	1.98
7. 8 cc. filtered, boiled, reaction product (tube 5, Exp. 1) + 2 cc. <i>M</i>	4.23
8. 8 cc. filtered, boiled, reaction product (tube 6, Exp. 1) + 2 cc. <i>M</i>	4.00
9. 8 cc. filtered, boiled, reaction product (tube 11, Exp. 1) + 2 cc. liver	1.08
10. 8 cc. filtered, boiled, reaction product (tube 12, Exp. 1) + 2 cc. liver	0.98
11. 5 cc. arginine dinitrate (0.03 molar) + 2 cc. <i>M</i> + 2 cc. liver	3.10
12. 5 cc. arginine dinitrate + 2 cc. <i>M</i> + 2 cc. liver	2.98

From the results of this and preceding experiments it is concluded that the gastric mucosa of the dog contains urease. From Exp. 1 it may be seen that even 0.1 cc. of a 10 % suspension of the gastric mucosa contains sufficient urease to catalyse the hydrolysis of 5 cc. of 0.02 *M* urea. The ammonia formation that was observed in the gastric mucosa + intestinal mucosa and gastric mucosa + kidney mixtures arises from the hydrolysis of the urea which the intestinal mucosa and kidney are capable of producing. Inasmuch as the intestinal mucosa and kidney contain arginase (though in smaller quantity than the liver), it is suggested that the mechanism investigated and recorded in these papers is as follows.

The arginase contained in the liver catalyses the formation of urea and ornithine from the arginine contained in the substrate. The urea thus formed is broken down to ammonium carbonate by the urease in the gastric mucosa.

As the finding of urease in the dog was quite unexpected, it was a matter of some interest to examine the stomachs of other animals for urease. The procedure adopted was as follows.

As soon as possible after the excision of the stomach, the organ was opened and thoroughly washed with cold running water. The stomachs of the sheep and bullock, owing to the papillary nature of the surfaces, were thoroughly washed and brushed. A portion was then scraped with a knife, the tissue thus removed being used for the urease tests. In the dog and cat, the material was then ground with washed sand and the required quantity of phosphate buffer of p_H 7.45. The material prepared from the sheep and bullock was so finely divided that grinding with sand was not considered necessary.

For the qualitative tests, the material to be examined was suspended in 2 cc. of water in approximately 10 % suspension. 3 cc. of 0.02 *M* urea were added with a few drops of phenol red and very dilute sodium hydroxide till neutral. Subsequent reddening of the solutions through the production of ammonium carbonate was considered to be a positive indication of the presence of urease.

For comparative quantitative estimations, the tissue material was weighed and suspended in 10 parts of phosphate buffer solution of p_H 7.45 at a temperature of 42°. The suspension was then kept at 42–43° for 20 minutes. 5 cc. were then added by pipette to 6 cc. of 0.02 *M* urea in aeration tubes, the whole being at a temperature of 42°. At the end of 30 minutes, caprylic alcohol and potassium carbonate were added and the ammonia estimated by 20 minutes' aeration into standard acid.

The results are recorded in Table III. Duplicate estimations were made throughout.

It may therefore be concluded that urease is contained in the animal body. Of the animals examined, the gastric mucosa of the Carnivora gave the most active preparations. The distribution of the enzyme in the stomachs of the Ruminantia is puzzling but quite definite. It should be noted that no urease could be demonstrated in that portion of the stomach of the Ruminantia in which peptic digestion takes place (the abomasum). No urease could be found in the gastric mucosa of the Rodentia.

Little can be said regarding the possible physiological significance of urease in the animal body. The increased ammonia excretion that has been observed in acidosis in animals (though not in the rabbit) and the corresponding quantitative decrease in the urea excretion could be explained if we assumed that the ammonia came from urea through the carefully regulated action of urease.

The finding of urease in the gastric mucosa explains the results recorded in the preceding paper, but also demonstrates that ammonia formation from α -amino nitrogen was not observed in any of the tissues examined under the conditions of those experiments. The suggestion is that the true deamination of α -amino acids by physiological means requires the survival or growth of the tissue under examination.

Table III.

Tissue examined				Urease tests	
				Quantitative	
				Qualitative	cc. N/10 ammonia after 30 minutes % urea decomposed
RAT STOMACH					
Oesophageal area	...	...	...	—	.
Pyloric area	...	...	...	—	.
RABBIT STOMACH					
Oesophageal area	...	...	...	—	.
Pyloric area	...	...	...	—	.
GUINEA-PIG STOMACH					
SHEEP STOMACH				.	{ 0.10 4
Rumen	...	...	...	+	{ 0.10 4
Psalterium	...	...	...	+	.
Reticulum	...	...	...	+++	{ 0.83 34
Abomasum	...	...	...	—	{ 0.80 33
BULLOCK STOMACH					{ 0.78 32
Rumen—anterior extremity	...	...	...	+++	{ 0.80 33
Rumen—posterior extremity	...	...	...	+++	{ 0.80 33
Psalterium	...	...	...	+	{ 0.75 31
Reticulum	...	...	...	+	.
Abomasum	...	...	...	—	{ 0.13 5
CAT STOMACH				+++	{ 0.16 6
DOG STOMACH				+++	{ 1.58 66
GOAT STOMACH				+++	{ 1.90 79
Rumen	...	...	...	+++	{ 2.05 85
Reticulum	...	...	...	+++	{ 2.15 90

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REFERENCE.

Luck (1924). *Biochem. J.* **18**, 814.