LIV. THE PHYSIOLOGY OF GASTRIC UREASE.

By JAMES MURRAY LUCK

(1851 Exhibition Research Student)

AND TRILOK NATH SETH.

From the Biochemical Laboratory, Cambridge.

(Received March 12th, 1925.)

WE have recently shown that very active preparations of urease may be obtained from the stomachs of carnivora [Luck, 1924] and that the enzyme by its behaviour *in vitro* appears to be identical with soy bean urease [Luck and Seth, 1924].

As it was possible, in the absence of evidence to the contrary, that the enzyme might be a product of *post mortem* changes it was considered advisable to demonstrate if possible its presence *in vivo*. In proceeding to do so we have assumed that the observable effects would be either a catalysis of the hydrolysis of urea or the synthesis of that substance from ammonium carbonate. By this assumption we have not overlooked a view which theoretically and teleologically is more desirable. This is that both the synthetic and hydrolysing functions may be exercised by the enzyme, the direction of the reaction being controlled by the many physico-chemical factors which influence the equilibria within the cell.

Experimentally we have investigated the effect of altering the concentrations of urea and ammonium carbonate. In the one case the concentration of urea in the cells of the mucosa was raised by the gastric absorption of urea, or, as in one experiment, by the intravenous injection of that substance. The resulting effect on the cellular concentration of ammonia was observed by estimating at suitable intervals the ammonia content of the gastric venous blood. In the second case ammonium salts in aqueous solution were introduced into the stomach and urea estimations were made upon the blood flowing from that organ. In two experiments a third factor, the hydrogen ion concentration of the cell, was varied. This was done on the strength of that once popular idea that the formation of urea would be favoured by alkalosis and that of ammonia by acidosis.

The results suggest that the enzyme functions *in vivo* in the same way that it does *in vitro*. An increased concentration of urea in the cells of the gastric mucosa was invariably accompanied by an increase in the ammonia content of the gastric venous blood. On the other hand, we were unable to observe any indication of the gastric formation of urea under the conditions of our experiments.

EXPERIMENTAL.

All but the preliminary observations were made upon dogs which had fasted for 48 hours before use, to ensure freedom of the stomach from food. The animal was anaesthetised with ether and a tracheal cannula was inserted. The right femoral artery was exposed and a mid-ventral incision was made through the abdominal wall. The stomach was closed distally by a pyloric ligature. The mesenteric vessels were tied and the splenic vessels were ligatured close to the spleen. By this procedure the contents of the vena lienalis were limited to the blood which entered from the left gastro-epiploic vein, and the blood of the portal vein consisted principally of that from the stomach.

The blood samples of 6 cc. were drawn by syringe and were received in tubes containing 0.6 cc. of ammonia-free 2 % potassium oxalate. The syringe was washed with freshly distilled ammonia-free water before the drawing of a sample. The arterial punctures were made distal to the profunda artery. The gastric venous blood was drawn from the portal vein close to the porta hepatis. In general, six samples in all were collected, three portal and three arterial—the first pair serving as controls. After drawing the first pair of samples the experimental fluid was administered at 38°. The second and third pairs were drawn at approximately 1½ and 2½ hours after administration of the fluid. In some of the experiments modifications of this procedure were necessary. Where this has been so the experimental details have been given elsewhere in the text.

As we assumed the presence of a very labile ammonia-forming substance in blood [Medwedew, 1911; Henriques and Gottlieb, 1924; Parnas and Heller, 1924] the following method of ammonia estimation was designed to reduce autolytic changes to a minimum and to permit the simultaneous collection of several samples of blood. The principle of the method was the removal of the blood proteins with tungstic acid and the estimation of the ammonia in the centrifugate by distillation.

To 5 cc. of the oxalated blood were added immediately after collection 5 cc. each of ammonia-free water, 10 % sodium tungstate and 0.066 *N* sulphuric acid. The mixture was centrifuged. The centrifugates, which measured 11–13 cc. and were water-clear, were decanted into tubes and kept at – 2 to 0° until removed for estimation.

The method of distillation was designed to minimise secondary ammonia formation by hydrolysis and to permit direct nesslerisation of low volume distillates. Stanford's apparatus [Stanford, 1923] was used. By immersing both the receiver and the trap in crushed ice and evacuating with a Geryk pump, rapid distillation could be obtained with a bath temperature of 25–30°. 5 cc. of 0.05 *N* sulphuric acid were placed in the receiver and a similar volume in the trap; 10 cc. of the centrifugate were placed in the distillation flask. When the apparatus was sufficiently evacuated 5 cc. of alkali (consisting of

4 cc. of saturated sodium carbonate and 1 cc. of saturated sodium bicarbonate) were added. Distillation was continued for 30–40 minutes, in which time 8–9 cc. of distillate were collected. A second fraction obtained by continued distillation into a fresh receiver gave no colour with Nessler's reagent. Recovery of the ammonia was therefore complete. The contents of the receiver were weighed, nesslerised with one-tenth their volume of the reagent and compared with ammonium sulphate standards.

In the estimation of blood urea the tungstic acid precipitation of Folin and Wu [1919] was employed for the removal of proteins. To 4 cc. of the filtrate were added 0.3 cc. of a molar phosphate solution of p_H 7.45 and 1 cc. of 0.5 % urease [Van Slyke and Cullen, 1914] filtered through glass wool. After standing for 30 minutes at 40° the ammonia was distilled into standard acid and estimated by nesslerisation.

All tubes, pipettes, and other pieces of apparatus were repeatedly washed with freshly prepared ammonia-free water which was obtained by the distillation of dilute sulphuric acid and was used for the preparation of all reagents. The potassium oxalate was thrice recrystallised from ammonia-free water. The sodium carbonate solution was well boiled and when cold was added to the saturated solution of sodium bicarbonate. The sulphuric acid was obtained from freshly opened supplies and was found to contain only a very small quantity of ammonia. The final quality of the reagents was controlled by blank estimations.

The ammonia content of whole blood in vitro at 0°.

The necessity of immediate treatment of the blood samples is shown by the rapid increase in the ammonia content of rabbit blood even at 0°. A rabbit under ether anaesthesia was bled by cannula from the abdominal aorta. 35 cc. of blood were run into 5 cc. of the oxalate solution. 5 cc. of the oxalated blood were precipitated immediately. The remainder was stored at –2 to 0° and precipitated in 5 cc. portions at varying intervals. The ammonia content of each centrifugate was estimated immediately.

Time after collection of blood	15 min.	1 h. 5 min.	2 h.	4 h. 5 min.	7 h.	24 h.
Mg. ammonia N per 100 cc. of blood	0.22	0.31	0.43	0.57	0.68	0.81

The ammonia content of the tungstic acid centrifugates at 0°.

By immediate precipitation of the blood samples it was found that the centrifugates could be stored at 0° without any increase in the ammonia content. It was concluded that either the labile ammonia precursor was precipitated by the tungstic acid or the acidity of the centrifugate inhibited ammonia formation. 30 cc. of blood were drawn from the abdominal aorta of a rabbit and run into 5 cc. of oxalate. Six portions of 5 cc. each were immediately precipitated and centrifuged. The centrifugates were stored at –2 to 0°.

Time after precipitation	25 min.	1 h. 40 min.	2 h. 35 min.	5 h. 40 min.	23 h. 30 min.
Mg. ammonia N per 100 cc. blood	0.11	0.08	0.09	0.11	0.10

The recovery of added ammonia.

40 cc. of rabbit arterial blood were collected in 5 cc. oxalate. To separate portions of 10 cc. each were added respectively, 10 cc. of water, 10 cc. aqueous solutions of ammonium sulphate. Sodium tungstate and sulphuric acid were added in the usual manner. The ammonia contents of the centrifugates were estimated by two different methods (a) by distillation, (b) by the Nash-Benedict apparatus [1921].

Mg. ammonia N per 100 cc. blood.				
Total ammonia found		Ammonia added	Ammonia recovered	
(a)	(b)		(a)	(b)
0.05	0.04	—	—	—
0.11	0.10	0.054	0.06	0.06
0.25	0.23	0.22	0.20	0.19
0.43	0.43	0.44	0.38	0.39

From these experiments it was concluded that if the blood samples were precipitated immediately after collection the ammonia content of the centrifugates could be estimated at leisure on the same day and that the values obtained would represent with sufficient accuracy the ammonia content of the shed blood.

The formation of ammonia in shed blood.

Having confirmed the findings of Parnas and Heller on the rapid formation of ammonia in rabbit blood *in vitro*, we were interested in the results obtained by Nash and Benedict [1921]. The evidence advanced by these authors indicated that the ammonia content of the shed blood of dogs did not increase during a post-collection period of several hours. On repeating these experiments we found the very marked specific differences shown in Fig. 1. For a comparison of methods, parallel estimations on the same blood were made

Fig. 1. Ammonia formation in blood at 2 to 0°.

1. Rabbit blood—Distillation method.
2. Rabbit blood—Nash-Benedict method.
3. Dog blood—Nash-Benedict Method.
4. Dog blood—Distillation method.

by our method and by that of Nash and Benedict. In each case the animal was bled from an artery during ether anaesthesia. The blood was collected in oxalate and was kept at -2 to 0° .

The differences in behaviour between rabbit and dog blood are so striking as to suggest that the latter does not contain a labile ammonia-forming substance or that some necessary enzyme is absent. Incidentally the results also show that by the application of two very different methods of estimation essentially the same values for the ammonia content of the blood have been obtained. This indicates either that the two methods give accurate and absolute values or that both are subject to a common error. Of the two possibilities the latter is the more improbable.

Experiments with urea.

In Exps. 1-5 which follow we have studied the effect of an increased urea concentration in the cells of the gastric mucosa on the ammonia content of the gastric venous blood. The results have been summarised in Table I.

From these experiments it was concluded that if the urea concentration in the cells of the gastric mucosa be increased, either by the gastric absorption of urea or by diffusion from the blood, part of the urea is converted into an ammonium salt.

The administration of the urea in dilute hydrochloric acid and the positive results following intravenous injection of urea show that the observed effects cannot be due to the action of ureolytic organisms which might be in the stomach. The original experiments, which were all done *in vitro* in the presence of toluene, and in which it was found that the hydrolysis of urea was effected only by preparations of the gastric mucosa, also prove that the enzyme is a constituent of the gastric cells in the carnivora.

The results of two control experiments are presented in Table II.

The results presented in Table II, Exp. 6, proved that for the increased ammonia production in the gastric mucosa, as recorded in Table I, the presence of urea was necessary. In Exp. 7 the ammonia content of the mesenteric blood prior to the urea absorption was already so high that the results are little more than suggestive. The absence of any increase in the ammonia content, and in fact its very noticeable decrease, do at least suggest that the intestinal absorption of urea is not accompanied by an increased ammonia production. This view is supported by the results of experimentation *in vitro* by which it was shown that urea is not hydrolysed by preparations of the intestinal mucosa [Luck, 1924]. One should also refer to the persistently low values of the arterial ammonia, for they indicate the absence of any general systemic production of ammonia.

It might therefore be concluded that under the conditions of these experiments urea was converted into ammonia by the gastric mucosa only, and that this hydrolysis of urea was catalysed by gastric urease.

Table I.

		Mg. N per 100 cc. blood.				
		Portal vein		Femoral artery		
	Time	NH ₃	Urea	NH ₃	Urea	Remarks
Exp. 1.	Male dog of 12 kg.					
	10.30	0.17	—	—	—	
	10.36	—	—	0.08	—	
	10.40	—	—	—	—	3 g. urea in 50 cc. water administered by stomach tube
	11.12	—	—	0.10	—	
	11.30	0.27	—	—	—	
	12.10	0.52	—	—	—	
	12.15	—	—	0.12	—	
Exp. 2.	Male dog of 23 kg.					
	11.00	—	—	—	7.5	
	11.04	0.13	5.9	—	—	
	11.07	—	—	—	—	5 g. urea in 100 cc. water administered by stomach tube
	11.35	—	—	0.12	8.8	
	11.40	0.39	10.0	—	—	
	12.41	—	—	0.22	11.0	
	12.43	1.60	14.2	—	—	
	12.50	—	—	—	—	Stomach excised. Urease present in mucosa
Exp. 3.	Female dog of 9 kg. Animal pregnant. Uterus excised.					
	10.42	—	—	0.24	5.6	
	10.46	0.25	5.8	—	—	
	10.50	—	—	—	—	3.7 g. urea in 75 cc. 0.05 N HCl by stomach tube
	11.42	0.54	13.6	—	—	
	11.45	—	—	0.21	12.3	
Exp. 4.	Male dog of 13 kg.					
	10.55	—	—	0.07	12.5	
	11.00	0.07	12.3	—	—	
	11.04	—	—	—	—	5 g. urea in 100 cc. 0.05 N HCl by stomach tube
	11.47	—	—	—	17.8	
	11.50	0.12	17.0	—	—	
	1.04	—	—	0.05	18.1	
	1.06	0.28	26.2	—	—	
Exp. 5.	Male dog of 15 kg.					
	10.31	—	—	0.07	—	
	10.40	0.13	—	—	—	
	10.50-11.10	—	—	—	—	4 g. urea in 100 cc. 0.085 % NaCl injected in the left femoral vein
	11.50	0.14	—	—	—	
	12.53	—	—	0.11	—	
	12.56	0.32	—	—	—	
	1.10	—	—	—	—	Stomach excised. Urease present in the mucosa

(Note to Exp. 5.—To induce acidosis, the animal received on each of the two fasting days 4-8 g. anhydrous calcium chloride in milk.)

Experiments with ammonium salts.

Though we have shown that gastric urease functions *in vivo* by the hydrolysis of urea it is also apparent that it may be capable of urea synthesis. In Exps. 8 and 9 we have followed the urea content of arterial and gastric venous blood during the gastric absorption of ammonium salts.

Table II.

		Mg. ammonia N per 100 cc. blood.		
	Time	Portal vein	Femoral artery	Remarks
Exp. 6.	Male dog of 13 kg.			
	10.52	—	0.19	100 cc. 0.05 <i>N</i> HCl by stomach tube
	10.55	0.18	—	
	11.00	—	—	
	12.04	0.15	—	
	12.56	—	0.14	
	1.03	0.18	—	
Exp. 7.	Female dog of 18 kg.			
	10.20	—	0.02	4 g. urea in 100 cc. 0.05 <i>N</i> HCl were injected into a loop of the small intestine (jejunum and ileum)
	10.30	1.39	—	
	10.45	—	—	
	11.23	—	0.06	
	11.28	0.49	—	
	12.26	0.46	—	

(Note to Exp. 7.—The spleen was tied off and the splenic vein was ligatured close to the portal vein. The mesenteric vessels were not tied.)

Table III.

	Time	Mg. N per 100 cc. blood.							
		Portal v.		L. gastro-epiploic v.		Femoral a.		Femoral v.	
		NH ₃	Urea	NH ₃	Urea	NH ₃	Urea	NH ₃	Urea
Exp. 8. Male dog of 12 kg.	10.30	—	—	0.20	8.5	—	—	—	—
	11.05	—	—	9.8	8.2	—	—	—	—
	11.13	—	—	—	—	—	—	—	10.6
	11.16	—	—	—	—	2.10	—	—	—
	12.03	—	—	2.0	14.9	—	—	—	—
	12.09	—	—	—	—	—	—	0.98	13.1
	12.10	—	—	—	—	1.02	17.3	—	—
	12.15	2.29	16.7	—	—	—	—	—	—
	12.47	—	—	3.2	19.4	—	—	—	—
	12.52	—	—	—	—	—	—	1.1	12.8
	12.54	—	—	—	—	1.01	17.9	—	—
	12.57	3.4	19.4	—	—	—	—	—	—

(Note.—10.34: 5 g. ammonium chloride in 100 cc. water by stomach tube.)

Exp. 9. Male dog of 11 kg.	10.59	—	—	—	—	0.11	—	—	—
	11.02	0.21	13.4	—	—	—	—	—	—
	11.50	—	—	—	—	0.36	19.4	—	—
	11.55	3.9	19.2	—	—	—	—	—	—
	12.43	—	—	—	—	0.35	23.1	—	—
	12.48	2.5	21.5	—	—	—	—	—	—

(Note.—On each of the two fasting days the animal received 10 g. sodium bicarbonate in milk.
11.08: 4 g. ammonium carbonate in 100 cc. water by stomach tube.)

As the urea content of the blood leaving the stomach is practically the same as that of the inflowing arterial blood, there is no indication of any gastric synthesis of urea from the absorbed ammonia. The available evidence therefore favours the view that gastric urease functions *in vivo* by the conversion of urea into ammonium carbonate and that to no measurable degree does it manifest any synthetic function.

DISCUSSION.

As a first consideration it is apparent that gastric urease cannot be associated with the production of the urinary or blood ammonia. Any ammonia which it may produce under the most favourable conditions would be promptly converted into urea by the liver. Even if some general significance could be attached to the portal ammonia, most of it enters from the mesenteric veins and not from the gastric veins.

As a second conclusion, it would therefore follow that gastric urease could have only a local function if any. This consideration limits the function of the enzyme to the production of ammonia for secretion into the stomach or in response to some local intracellular requirement. Without becoming involved in too much speculation it is sufficient at present to enumerate the various possible functions of the enzyme that have been suggested to us, viz., the secretion of alkali into the stomach for the control of gastric hyperacidity [McBaird, Campbell and Hern, 1924; Huber, 1917], an intracellular mechanism for the protection of the cells of the mucosa against the highly acid gastric juice with which they are in contact, a mechanism for the local regulation of the movements of the stomach wall either by the direct effect of the base (ammonium carbonate) on the muscle fibres [Kupaloff, 1924; Goldenberg, 1924] or as a specific property of ammonium ions [Práwdicz-Neminski, 1924]. A final suggestion would be to class the enzyme as a vestigial structure. Without the support of further experimental evidence we are not prepared however to ascribe any specific function to the enzyme.

SUMMARY.

(1) Under normal physiological conditions urea constantly diffuses into those cells of the gastric mucosa which contain urease and is converted into ammonia. In dogs under ether anaesthesia an increased diffusion of urea caused either by the gastric absorption or intravenous injection of that substance is accompanied by an increase in the ammonia content of the gastric venous blood.

(2) The gastric absorption of ammonium salts is not accompanied by an increase in the urea content of the gastric venous blood.

(3) The enzyme appears to function only by the conversion of urea into ammonia in response to local conditions or requirements.

(4) The shed blood of dogs shows no increased ammonia content in 24 hours at -2 to 0° . That of rabbits rapidly increases.

We would conclude by expressing our indebtedness to Sir F. G. Hopkins for much helpful criticism, to Dr J. B. S. Haldane for many valuable suggestions, and to the Medical Research Council for the assistance that has been extended to one of us (J. M. L.).

Note. Since preparing these results for publication an interesting paper by Parnas [1925] has appeared in which are reported the blood ammonia values for several different species. His observations on the specific differences in the behaviour of rabbit and dog blood after long standing are in entire agreement with the results which we have recorded.

REFERENCES.

- Folin and Wu (1919). *J. Biol. Chem.* **38**, 81.
Goldenberg (1924). *Pflüger's Arch.* **204**, 487.
Henriques and Gottlieb (1924). *Z. physiol. Chem.* **138**, 254.
Huber (1917). *Amer. J. Physiol.* **42**, 404.
Kupaloff (1924). *Pflüger's Arch.* **204**, 42, 356.
Luck (1924). *Biochem. J.* **18**, 825.
Luck and Seth (1924). *Biochem. J.* **18**, 1227.
McBaird, Campbell and Hern (1924). *J. Physiol.* **58**, Proc. xx-xxi.
Medwedew (1911). *Z. physiol. Chem.* **72**, 410.
Nash and Benedict (1921). *J. Biol. Chem.* **48**, 463.
Parnas (1925). *Biochem. Z.* **155**, 247.
Parnas and Heller (1924). *Biochem. Z.* **152**, 1.
Práwdicz-Neminski (1924). *Biochem. Z.* **152**, 388.
Stanford (1923). *Biochem. J.* **17**, 847.
Van Slyke and Cullen (1914). *J. Biol. Chem.* **19**, 211.