CV. AMMONIA PRODUCTION BY ANIMAL TISSUES IN VITRO.

I. THE USE OF MIXED TISSUE EXTRACTS.

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(*Received June 14th, 1924.*)

One of the earliest theories of protein metabolism was based on the observation by Nencki and colleagues [1896, 2] that the portal blood contained considerably more ammonia than the systemic blood. To explain this fact, the theory was developed that the absorbed amino acids were immediately deaminised—probably in the intestinal wall. The work of Cohnheim [1909, 1912] on the deamination of amino acids by fish intestines in Ringer's solution, and the observation of Horodynski, Salaskin and Zaleski [1902] that even in starvation the ammonia content of the portal blood was greater than that of the systemic blood, considerably strengthened the theory of deamination by the intestine. The earlier weaknesses of the theory may be stated as follows:

(1) Cohnheim was not successful in demonstrating the deamination of amino acids by mammalian intestines (those of dogs and cats).

(2) The work of Horodynski, Salaskin and Zaleski [1902] mentioned above, is contradicted by Nencki's original observation.

The work of Folin and Denis [1912, 1, 2] gave to Nencki's observations a different interpretation than that of deamination by the intestinal wall. They found that the ammonia of the portal blood of cats came principally from the mesenteric vein of the large intestine and therefore concluded that the portal ammonia entered by absorption from the ammonia-rich faeces in the large intestine. Neither the amino acids of a pancreatic autolysate, nor glycine, nor asparagine were deaminised by the small intestine when injected into ligatured loops of that organ. Folin and Denis assumed that the blood rapidly transported the absorbed and undeaminised amino acids from the intestine to the tissues. Van Slyke and Meyer [1913] confirmed these results. By the use of Van Slyke's newly-developed method for the estimation of amino acid nitrogen, they were able to show [1913] that half an hour after the injection of totally hydrolysed caseinogen, 5 % of the amino nitrogen was still in the circulation, 11 % had been excreted in the urine, while the increased amino nitrogen content of the various tissues accounted for almost all of the remainder.

We are now concerned with the next step in amino acid catabolism. It has long been known that the ingestion of proteins is followed by an in-

creased excretion of urea. The early demonstration of v. Schroeder [1882] that the liver is able to synthesise urea from the ammonium salts of organic acids has been amply confirmed by much subsequent work. The Eck fistula experiments of Nencki and colleagues [1896, 1] demonstrated that in dogs, functional blocking of the liver decreased the urea excretion and increased the ammonia excretion. The normal ability of the animal to form urea from carbaminic acid (introduced *per os*) also disappeared. "Die Leber ist also der treue Wächter des Organismus, der die von dem Verdauungscanal kommende, für die anderen Organe giftigen Substanzen, in ungiftige verwandelt" [Nencki et al., 1896, 2].

The question then arose as to whether the liver could form urea from amino acids without the intermediate stage of ammonia liberation by deamination.

The suggestion had long been made that urea might be formed from amino acids by the intermediate formation of uramino acids [Salkowski, 1880].

$$R \cdot CH(COOH) \cdot NH_2 + CO_2 + NH_3 \longrightarrow R \cdot CH(COOH) \cdot NH \cdot CO \cdot NH_2$$
$$\longrightarrow R \cdot CH(COOH) \cdot OH + CO(NH_2)_2.$$

Recent work [Lewis, 1912; Rohde, 1918; Lewis and Root, 1921], however, has failed to demonstrate that such a mechanism exists *in vivo*. One might therefore conclude that in the liver, at least, urea may be formed from ammonium salts of organic acids, but that its direct formation from amino nitrogen is still a matter of doubt.

As regards the locus of ammonia formation arguments have already been advanced which render dubious the older theory that the intestinal mucosa is the seat of this change. Nash and Benedict [1921, 1922] have advanced the view that ammonia formation is a function of the kidney. They found the ammonia content of the blood drawn from the renal veins of anaesthetised dogs to be higher than that of arterial blood and of the blood from the inferior vena cava. This work was repeated by Henriques [1923], but the observations of Nash and Benedict were not confirmed. The valuable experiments of Neubauer and Fisher [1910] on liver perfusion demonstrated that this organ (the liver) was probably responsible for *in vivo* deamination. Subsequent workers [Fiske and Karsner, 1913] have had occasion to dispute the alleged deaminising power of the liver. Folin and Denis [1912, 1] state: "We soon discovered that while the liver does almost wholly abstract the ammonia from the portal blood and probably converts it into urea, it does not 'deaminise' the amino acids." These conclusions with fresh experimental evidence were restated at a recent date [Folin and Berglund, 1922]. Levene and Meyer [1913, 1, 2] were unsuccessful in demonstrating the deamination of various amino acids by the kidney, liver, or polymorphonuclear leucocytes. Similar results were reported by Griesbach and Oppenheimer [1913]. Levene and Meyer [1913, 2] conclude as follows: "These observations make it necessary to repeat all the older work on the deaminising action of tissues."

While investigating the amide nitrogen of animal proteins, the writer was attracted to the problem. It appeared desirable to make an extensive study of the deaminising activity of many tissue preparations on different substrates.

The use of mixed tissue extracts was suggested by Professor Hopkins, with the idea that a given organ may elaborate a hormone which stimulates deamination in other parts of the body. In addition to the estimation of free ammonia, the estimation of the urea production was also obviously desirable. The fact that all animal proteins contain amide nitrogen, and that these amides must have been synthesised by the animal body, suggested the estimation of amide nitrogen. The observation recorded in a previous paper [Luck, 1924] that a portion of the amide nitrogen of caseinogen was readily split off as ammonia by various tissues of the body, and the older observations on the ease of hydrolysis of amides by tissue extracts, suggested that one mechanism of ammonia production might be the synthesis of amides by certain organs and deamidation by others.

During the course of the first experiment certain observations of Nencki and of Van Slyke led to the use of the gastric mucosa in Exps. 2 and 3.

Nencki and his colleagues [1896, 2] by improved methods of analysis found that the gastric mucosa of dogs contained considerably more free ammonia than any other tissue investigated and ascribed this to a decomposition of proteins in the mucosa during the formation of the gastric juice.

Seventeen years later, Van Slyke [1913] observed that the stomach contained less amino acid nitrogen than any other tissue examined.

This feature of highest ammonia content and lowest amino nitrogen content suggested that the gastric mucosa might play a prominent part in ammonia production. That this is actually the case will be seen from the experimental results which follow.

Experimental.

The following technique was adopted. In the preparation of tissue extracts, the excised tissue was rinsed in cold, running, water, then minced and ground with washed sand, water and a small quantity of toluene until a fine suspension was obtained. Sufficient water was added to adjust the concentration of tissue material to approximately 10 %. In the case of the pituitary body, lymph glands, and submaxillary glands, the insufficiency of tissue necessitated a lower concentration. The bile and lymph were used without dilution. In Exp. 1 an animal was used which had fasted for 18 hours so that the lymph was practically free from fat and red cells. 55 cc. were collected in 4 hours and run into 10 cc. of 1 % potassium oxalate. The animal was killed by exsanguination from the external carotid. A portion of the blood was defibrinated as collected. The remainder was oxalated and centrifuged. The formed elements were twice washed with isotonic sodium chloride solution, and then laked by the addition of an equal volume of water.

In the preparation of the red bone marrow, the difficulty of separating the marrow from the bone was encountered. It was found convenient to use the ribs. These were carefully scraped free of adherent tissue, quartered by longitudinal section, cut into centimetre lengths, and used as such.

Three different substrates were investigated:

1. *Totally hydrolysed gelatin.* 280 g. of gelatin were hydrolysed by boiling with 1000 cc. of water and 135 cc. of concentrated sulphuric acid for 22 hours at atmospheric pressure. The sulphuric acid was removed with baryta, the filtrate adjusted to p_H 7·6 with soda, and diluted to an amino nitrogen concentration of 8·85 mg. per cc.

2. *Trypsin-hydrolysed caseinogen.* Caseinogen was hydrolysed in 15 % solution by an aqueous extract of the pancreas at p_H 7·8. The digestion was allowed to continue for 8 weeks with the weekly addition of fresh pancreas extract. The digest was then slightly acidified with acetic acid, filtered, boiled, and adjusted with soda to p_H 7·6. Water was added until the amino nitrogen concentration was 8·85 mg. per cc.

3. *Trypsin-stable amide residue from caseinogen.* This was prepared by alcoholic precipitation and extraction as described in a previous paper [Luck, 1924]. The concentration of amino nitrogen was adjusted to 8·85 mg. per cc. and the hydrogen ion concentration to p_H 7·6.

In the text, these are designated as Substrate 1, 2 and 3 respectively. Substrate 1 contains no amide nitrogen. Substrate 2 contains both amide and amino nitrogen. Substrate 3 contains a relatively large amount of trypsin-stable amide nitrogen, while the amino nitrogen is limited to that of two amino acids, glutaminic acid and lysine.

The detailed procedure was as follows: 4 cc. portions of the substrate were placed in test tubes ($6'' \times \frac{5}{8}''$)—previously sterilised by steaming for 3 hours. 4 cc. of the tissue preparation were added. In those cases where mixed tissue suspensions were used, only 2 cc. of each were employed. To each tube were then added 1·5 cc. of water, 0·05 cc. of 5 % calcium chloride and 0·5 cc. of toluene. The tubes were thoroughly shaken, stoppered tightly with sterilised corks, and kept at 40°. While careful control of the hydrogen ion concentration throughout the period of incubation would have been a matter of great difficulty, the initial p_H of the substrate (7·6) was such that the final p_H was never found to be lower than 7·0. After five days the tubes were removed. Twelve tubes were put aside for immediate analysis, and the remainder placed in a refrigerator at 0–1°.

The contents of each daily lot of twelve were acidified with a single drop of glacial acetic acid and boiled for 15 minutes in a water-bath. The volume of each was then rapidly adjusted to 10 cc. in volumetric flasks and filtered. Of the filtrate, 3 cc. were used for the estimation of ammonia, 3 cc. for urea, and 2 cc. for amide nitrogen.

Ammonia was estimated by Van Slyke and Cullen's [1914] modification of Folin's aeration method. By the use of three pumps, six estimations could be made in 50 minutes.

In the estimation of urea, the 3 cc. samples were placed directly in the aeration tubes. Three drops of phenol red solution were added, and 0·5*N* soda added by drops until the indicator changed colour. 2 cc. of phosphate buffer

(p_H 7·4), 0·3 g. of soya bean meal, and four drops of caprylic alcohol were added to each tube. They were kept for 2 hours at 40° and the ammonia then estimated as above.

For the estimation of amide nitrogen, the 2 cc. samples were placed in 6″ combustion tubes with 3 cc. of water and 0·5 cc. of concentrated sulphuric acid. In the first experiment the tubes were then heated for 3 hours in an oil bath at 105–110°. It was soon found to be more satisfactory to heat for 2 hours in an autoclave at 5–10 lbs. pressure. Under such conditions urea is only slightly destroyed (Table VIII). The tubes were cooled, and the acid removed by the addition to each of 5 cc. of a warm baryta solution containing 2·95 g. of crystalline baryta per 5 cc. A few drops of bromocresol purple were added as an aid in this approximate neutralisation. The tubes were cooled and the ammonia content estimated as above without transfer of the contents.

The deaminising activity of the following tissues and fluids was investigated:

Muscle, *A*; liver, *B*; mucosa of the small intestine, *C*; adrenals, *D*; thyroid gland, *E*; spleen, *G*; pituitary body, *H*; parotid glands, *J*; submaxillary glands, *K*; gastric mucosa, *M*; pancreas, *N*; mesentery, *O*; bile, *P*; kidney, *Q*; ovaries, *R*; brain, *S*; heart, *T*; lymph, *U*; lymph glands, *V*; whole blood, *W*; defibrinated blood, *X*; formed elements, *Y*; red bone marrow, *Z*. The mixed diaphragm and pectoral muscles were used in the preparation of *A*. The letters following the name of the tissue or fluid have been used in the text as an abbreviated means of designation.

Exp. 1. The dog, a female of 10 kilos., was injected subcutaneously with 2·5 cc. of 2 % morphia. After one hour the animal was anaesthetised with alcohol and ether. The thoracic duct was approached through the subclavian triangle and the lymph collected over a period of 3 hours. It was then bled from the external carotid and the tissues and glands rapidly excised. Owing to the large amount of material that had to be prepared, a number of the organs were kept overnight at 0–1°. The kidneys, liver, intestine, stomach, bile, lymph, and blood were prepared and tubed at once. The results are expressed in Tables I–V which follow.

Table I. *Blanks with water.*

	cc. *N*/10 nitrogen per 3 cc.				cc. *N*/10 nitrogen per 3 cc.		
	Ammonia N	Amide N (nett)	Urea N (nett)		Ammonia N	Amide N (nett)	Urea N (nett)
Substrate 1	0·20	0·00	0·05	*O*	0·02	0·01	0·03
„ 2	0·55	0·35	0·05	*P*	0·02	0·00	0·05
A	0·02	0·01	0·01	*Q*	0·10	0·05	0·05
B	0·07	0·01	0·06	*R*	0·02	0·00	0·03
C	0·03	0·05	0·10	*S*	0·00	0·02	0·05
D	0·05	0·05	0·05	*T*	0·05	0·00	0·00
E	0·05	0·05	0·03	*U*	0·05	—	0·10
G	0·07	0·08	0·08	*V*	0·00	0·02	0·15
H	0·02	0·01	0·03	*W*	0·05	—	0·13
J	0·03	0·05	0·04	*X*	0·05	—	—
K	0·03	0·00	0·07	*Y*	0·10	—	0·00
M	0·13	0·07	0·05	*Z*	0·10	—	0·00
N	0·15	0·15	0·09				

In Tables II–V the blanks given above have been subtracted from the analytical figures in order to indicate the degree, if any, of ammonia liberation.

Table II. *Muscle mixtures.*

	Substrate 1 cc. $N/10$ nitrogen per 3 cc.			Substrate 2 cc. $N/10$ nitrogen per 3 cc.		
	Ammonia N (nett)	Amide N (nett)	Urea N (nett)	Ammonia N (nett)	Amide N (nett)	Urea N (nett)
A	−0·05	0·07	0·05	−0·04	0·32	0·02
A B	0·07	−0·02	0·95	0·40	−0·02	0·35
A C	0·03	0·02	0·56	0·20	0·20	0·15
A D	0·03	0·08	0·20	0·11	0·22	−0·03
A E	−0·03	−0·04	0·03	0·08	0·20	0·02
A G	0·04	−0·04	0·14	0·03	0·30	0·02
A H	0·00	−0·05	0·07	0·03	0·31	−0·03
A J	0·00	0·01	0·00	0·04	0·29	−0·01
A K	0·03	0·25	0·05	0·03	0·40	0·05
A N	0·03	2·28	−0·03	0·10	1·28	0·05
A O	0·05	−0·05	−0·05	0·08	0·28	0·00
A P	0·03	−0·04	0·02	0·05	0·68	0·00
A Q	0·05	−0·05	0·25	0·20	0·05	0·15
A R	0·00	0·00	0·00	0·05	1·13	−0·05
A S	−0·05	0·00	0·10	0·00	0·28	0·10
A U	0·10	−0·02	0·05	0·00	0·31	0·05
A X	−0·05	−0·05	0·13	−0·05	—	0·05
A Y	0·05	−0·04	0·05	0·15	0·06	0·00
A Z	0·45	0·05	−0·05	0·55	0·03	0·10

Table III. *Liver mixtures.*

	Substrate 1 cc. $N/10$ nitrogen per 3 cc.			Substrate 2 cc. $N/10$ nitrogen per 3 cc.		
	Ammonia N (nett)	Amide N (nett)	Urea N (nett)	Ammonia N (nett)	Amide N (nett)	Urea N (nett)
B	0·10	−0·05	0·95	0·50	−0·10	0·40
B A	0·07	−0·02	0·95	0·40	−0·02	0·35
B C	0·12	−0·02	1·05	0·55	0·38	0·35
B D	0·07	0·28	1·05	0·45	0·06	0·35
B E	0·07	−0·02	1·07	0·50	0·40	0·30
B G	0·10	0·00	1·07	0·51	0·75	0·37
B H	0·10	−0·05	1·05	0·58	0·60	0·25
B J	0·10	0·18	1·00	0·55	0·33	0·25
B K	0·09	−0·02	0·96	0·40	0·53	0·30
B N	0·10	0·03	1·27	0·35	0·30	0·40
B O	0·00	−0·03	0·67	0·45	0·43	0·30
B P	−0·03	—	1·08	0·25	0·56	0·30
B Q	0·02	−0·05	1·15	0·65	0·31	0·50
B R	0·10	0·00	1·07	0·50	0·61	0·25
B S	—	—	—	0·40	−0·07	0·28
B U	0·14	−0·04	0·93	0·43	−0·10	0·32
B X	0·07	—	0·87	0·25	0·18	0·37
B Y	0·22	0·00	0·85	0·70	0·30	0·45
B Z	0·62	−0·05	0·75	0·75	0·21	0·35

Table IV. *Intestinal mucosa mixtures.*

	Substrate 1 cc. $N/10$ nitrogen per 3 cc.			Substrate 2 cc. $N/10$ nitrogen per 3 cc.		
	Ammonia N (nett)	Amide N (nett)	Urea N (nett)	Ammonia N (nett)	Amide N (nett)	Urea N (nett)
C	0·04	−0·03	0·83	0·15	0·23	0·27
C A	0·03	0·02	0·56	0·20	0·20	0·15
C B	0·12	−0·02	1·05	0·55	0·38	0·35
C D	0·07	0·06	0·60	0·25	0·41	0·07
C E	0·04	−0·04	0·31	0·18	0·12	0·10
C G	0·02	−0·04	0·70	0·20	0·07	0·13
C H	0·02	−0·05	0·48	0·10	0·18	0·15
C J	0·00	−0·03	0·40	0·14	0·40	0·13
C K	0·00	0·01	0·45	0·12	0·21	0·26
C N	0·01	0·00	0·06	0·16	0·14	0·02
C O	−0·03	0·01	0·60	0·03	0·18	0·17
C P	0·01	−0·06	—	0·20	0·38	0·00
C Q	0·00	0·00	0·87	0·20	−0·04	0·42
C R	−0·03	0·00	0·47	0·23	0·05	0·07
C S	0·02	−0·05	0·45	0·10	0·15	0·20
C U	0·02	−0·06	0·63	0·20	0·38	0·10
C X	−0·03	0·05	—	0·02	0·11	0·08
C Y	0·02	−0·06	—	0·25	0·33	0·10
C Z	0·02	0·05	0·70	0·90	−0·15	0·10

Table V. *Single tissues and fluids.*

	Substrate 1 cc. $N/10$ nitrogen per 3 cc.			Substrate 2 cc. $N/10$ nitrogen per 3 cc.		
	Ammonia N (nett)	Amide N (nett)	Urea N (nett)	Ammonia N (nett)	Amide N (nett)	Urea N (nett)
A	−0·05	0·07	0·05	−0·04	0·32	0·02
B	0·10	−0·05	0·95	0·50	−0·10	0·40
C	0·04	−0·03	0·83	0·15	0·23	0·27
D	0·08	−0·05	0·10	0·08	0·23	0·00
E	0·03	0·65	0·02	0·03	0·25	0·05
G	0·03	−0·05	0·14	0·05	0·61	0·00
H	0·01	0·01	0·02	0·03	0·21	—
J	−0·03	0·01	0·03	0·05	0·43	0·55
K	0·03	−0·04	—	—	—	—
M	0·11	−0·02	0·14	0·23	0·70	−0·10
N	0·15	1·40	0·03	0·20	1·06	0·25
O	0·02	−0·02	—	—	—	—
P	−0·05	0·13	0·07	−0·05	0·48	0·05
Q	0·05	−0·07	0·67	0·40	0·02	0·30
R	−0·03	0·00	−0·02	−0·05	0·33	0·10
S	0·03	−0·05	0·09	0·10	0·30	−0·05
T	0·00	−0·10	0·00	0·20	0·28	−0·08
U	0·05	−0·03	0·03	0·05	0·25	−0·05
V	0·00	0·20	−0·05	0·05	0·78	0·02
W	0·02	—	0·08	−0·10	—	0·10
X	0·00	0·01	—	−0·05	—	0·15
Y	0·10	−0·05	—	0·20	0·23	0·05
Z	0·65	−0·07	0·05	0·65	0·31	0·03

CONCLUSIONS. *Exp.* 1. (1) Free ammonia from α-amino acids was liberated by the red bone marrow only, except in combination with the intestinal mucosa where no deamination was observed. The fact that in all the tubes but this one the ribs projected above the surface of the liquid, and might therefore become septic through lack of toluene, would suggest bacterial deamination. This impression is heightened by subsequent failure to obtain deamination by the red bone marrow (Exp. 3).

(2) Free ammonia was liberated from trypsin-digested caseinogen by the liver, kidney, and very slightly by the intestinal mucosa. The failure of these organs to deaminise totally hydrolysed gelatin, as well as the fact that these organs liberate ammonia from Substrate 3, but not from acid-hydrolysed Substrate 3 [Luck, 1924], suggests that in this instance the mechanism is one of deamidation and not deamination.

(3) If this is so, the sum of free ammonia nitrogen and amide nitrogen in the experiments with Substrate 2 should be 0·35, viz. the amide nitrogen content of Substrate 2. Owing to the errors introduced by blanks and the number of manipulations required, this process of summation can only be expected to yield approximate results. However, Tables II, IV and V indicate by this method that the process of ammonia formation is one of deamidation. In Table II, Substrate 2, the sum of ammonia nitrogen and amide nitrogen is much greater in most cases than 0·35. This indicates either

(*a*) the liberation of ammonia from α-amino groups,

or (*b*) the synthesis of a material which by mild acid hydrolysis liberates ammonia.

The improbability of (*a*) has already been mentioned. That process (*b*) is more likely will appear from the results of Exps. 2 and 3 which follow.

(4) Urea was formed by preparations of the liver, intestinal mucosa and kidney.

It is probable that the urea has been formed from arginine by the action of arginase.

Exp. 2. A fresh supply of Substrate 2 was required. After filtration and boiling, the solution was made slightly alkaline with calcium hydroxide and distilled *in vacuo* for the removal of free ammonia. It was then filtered and adjusted to p_H 7·6 and an amino nitrogen concentration of 10·0 mg. per cc.

5 cc. of each substrate were used instead of 4 cc. as in Exp. 1. No prolonged period of anaesthesia as in Exp. 1 preceded the excision of the tissues. Duplicate mixtures of tissue and substrate were used throughout. 4 cc. of the filtrates were used in the ammonia estimations, and 5 cc. for the estimation of amide nitrogen. Urea was not estimated.

The dog, a healthy female of 10 kilos., was killed by exsanguination after 12 hours of fasting. The tissues were rapidly excised and prepared as previously described. The results are arranged in Tables VI, VII.

Table VI. *Single tissues and fluids.*

	Substrate 1 cc. $N/10$ nitrogen per 5 cc.		Substrate 2 cc. $N/10$ nitrogen per 5 cc.		Substrate 3 cc. $N/10$ nitrogen per 5 cc.	
	Ammonia N (nett)	Amide N (nett)	Ammonia N (nett)	Amide N (nett)	Ammonia N (nett)	Amide N (nett)
B	0·29	0·63	0·52	1·00	1·36	0·94
B	0·25	0·64	0·55	0·90	1·32	0·95
C	0·07	0·02	0·12	0·68	0·21	1·51
C	0·00	0·12	0·12	0·58	0·20	1·42
G	0·00	0·09	0·02	0·73	0·07	1·50
G	0·04	0·08	0·08	0·70	0·07	1·58
M	0·10	0·00	0·37	0·53	1·01	0·89
M	0·07	0·03	0·25	0·45	1·02	1·00
N	0·04	−0·03	0·03	0·42	0·07	1·35
N	0·04	−0·02	0·00	0·70	0·03	1·40
P	0·04	0·08	−0·04	0·83	−0·08	1·75
P	−0·02	0·10	0·02	0·70	−0·08	1·83
Q	0·11	0·03	0·11	0·71	0·22	1·55
Q	0·07	0·13	0·17	0·62	0·27	1·55
Water	0·00	0·06	0·00	0·70	0·00	1·62
„	0·00	0·00	0·00	0·73	0·00	1·60

Table VII. *Mixed tissues and fluids.*

	Substrate 1 cc. $N/10$ nitrogen per 5 cc.		Substrate 2 cc. $N/10$ nitrogen per 5 cc.		Substrate 3 cc. $N/10$ nitrogen per 5 cc.	
	Ammonia N (nett)	Amide N (nett)	Ammonia N (nett)	Amide N (nett)	Ammonia N (nett)	Amide N (nett)
B C	0·28	0·94	0·50	0·73	1·07	1·20
B C	0·19	0·93	0·50	0·85	1·11	1·11
B G	0·29	0·88	0·38	1·02	1·01	1·36
B G	0·25	0·89	0·39	0·94	0·92	1·60
B M	2·29	−0·12	1·13	0·70	2·32	0·73
B M	2·44	−0·12	1·25	0·55	2·32	0·70
B N	0·20	0·92	0·09	1·19	0·76	1·51
B N	0·19	0·80	0·15	1·00	0·73	1·47
B P	0·07	0·63	0·19	0·76	0·60	1·17
B P	0·07	0·67	0·21	0·84	0·67	1·35
B Q	0·19	0·90	0·56	0·64	1·15	1·02
B Q	0·25	0·74	0·53	0·77	1·07	1·33
C M	0·54	−0·05	0·34	0·31	1·17	0·98
C M	0·48	−0·04	0·38	0·25	1·02	1·10
N M	0·04	0·08	0·03	0·78	0·10	1·62
N M	0·04	0·10	0·00	0·83	0·11	1·54
N P	0·02	−0·02	−0·01	0·66	0·05	1·68
N P	0·04	0·06	0·00	0·70	0·05	1·55
N C	0·04	−0·07	0·03	0·77	0·20	1·50

Exp. 3. A male dog of 8 kilos. which had fasted for 12 hours was used in this experiment. The substrate and details of procedure were the same as those of Exp. 2. The results are recorded in Table VIII.

Table VIII.

	Water cc. $N/10$ nitrogen per 5 cc.		Substrate 1 cc. $N/10$ nitrogen per 5 cc.		Substrate 2 cc. $N/10$ nitrogen per 5 cc.	
	Ammonia N (nett)	Amide N (nett)	Ammonia N (nett)	Amide N (nett)	Ammonia N (nett)	Amide N (nett)
B	—	—	0·27	0·55	0·63	0·70
B	—	—	0·32	0·60	0·67	0·66
M	—	—	0·32	0·02	0·40	0·45
M	—	—	0·32	−0·03	0·42	0·40
N	—	—	0·23	0·11	0·25	0·63
N	—	—	0·25	0·12	—	0·58
Y	—	—	0·23	—	—	—
Y	—	—	0·17	—	—	—
Z	—	—	0·04	0·18	—	—
Z	—	—	0·04	0·08	0·06	—
B M	0·23	−0·03	2·19	−0·17	1·25	0·60
B M	0·23	0·00	2·29	−0·17	1·25	0·50
B N	0·19	0·11	0·34	0·80	0·38	1·02
B N	0·23	0·08	0·36	0·86	0·34	0·99
N M	0·19	0·01	0·29	−0·02	0·21	0·64
N M	0·19	0·06	0·32	−0·02	0·19	0·71

	Ammonia N	Amide N	Urea N
Urea	0·00	0·18	2·00
„	0·01	0·12	2·05

CONCLUSIONS. *Exps.* 2 *and* 3. 1. Marked ammonia production was observed in mixtures of the gastric mucosa and liver preparations. Quantitative agreement of duplicate sets from a single animal and of preparations from both animals was also observed.

2. No appreciable deamination by either the gastric mucosa or liver, or other single tissue preparation could be demonstrated.

3. Definite deamidation was effected by the liver, gastric mucosa, kidney, intestinal mucosa, and most liver mixtures. (See Substrates 2 and 3, Tables VI–VIII.)

4. The synthesis by the liver and liver mixtures of an amide-like body (a substance yielding ammonia after mild acid hydrolysis) was also demonstrated. It is possible that the ammonia so liberated might have been formed by the destruction of urea, though a similar concentration of urea in 20 % sulphuric acid and under identical conditions of hydrolysis was only slightly hydrolysed.

5. Of all the liver mixtures investigated with totally hydrolysed gelatin, no residual amide nitrogen could be found in the gastric mucosa-liver experiments (Substrate 1, Tables VII, VIII).

To Professor F. G. Hopkins the writer is indebted for advice and helpful criticism. Dr L. E. Shore very kindly performed the thoracic duct operation, while the assistance of Dr C. S. Hicks in dissection is gratefully acknowledged. The writer would also express his thanks to the Royal Commission for the Exhibition of 1851 for the studentship that has been held during this investigation.

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