Studies on Urea-Nitrogen Concentration of the Blood.

Part III.

The Effect of the Vegetative Nervous System upon the Blood Urea-Nitrogen Concentration.

By

KASANU TASHIRO.

(田代重)

*(From the Laboratory of Prof. T. Kato's Medical Clinic,
Tohoku Imperial University, Sendai, Japan.)*

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INTRODUCTION AND REFERENCES.

Our knowledge of the vegetative nervous system in relation to nitrogenous metabolism is not so clear as that of the carbohydrate exchange. Underhill and Closson¹⁾ and Eppinger, Falta and Rudinger²⁾ saw that if adrenaline is injected into fasting animals the elimination of nitrogenous substances increases, while Kraus and Hirsch³⁾ and Guerst⁴⁾ could observe no significant change in the excretion of nitrogenous substances by the adrenaline administration. Falta⁵⁾, Yanagawa⁶⁾, Pohl⁷⁾, and Strausky⁸⁾ noted an increase in the output of allantoin and uric acid after the adrenaline injection; according to Falta the increase of allantoin elimination is based on adrenaline leucocytosis. On the other hand, Michaelis⁹⁾, observing a significantly increased excretion of uric acid, when a diabetic puncture was made in a rabbit, called it "uric acid puncture." According to the experiments of Dresel and Ullmann¹⁰⁾, in rabbits caffeine causes glycosuria accompanied by increased allantoin elimination, while in splachnecotomized rabbits these phenomena can not be seen. Yanagawa also observed increased output of allantoin during the excitation of the nervous centres through caffeine, picture or asphyxia. Krauss and Österreich¹¹⁾ have asserted that at the same time that the blood pressure is raised through an adrenaline injection, the excretion of uric acid decreases while that of urea increases, the former being augmented after the fall of the blood pressure. Basing their opinion on these facts, they infer that the rise of blood pressure through adrenaline has some connection with the accumulation of uric acid in the blood. Contrary to this, Harpuder¹²⁾ pointed out an error in Krauss' experiments stating that the suprarenine influence upon purine exchange is very slight and indefinite, and that purine elimination is lessened by ergotamine while the influence of atropine and pilo-

- 1) Underhill and Closson, *Americ. Journ. Physiol.*, 1907, **2**, 119.
- 2) Eppinger, Falta and Rudinger, *Zeitschr. f. klin. Med.*, 1908, **66**, 1.
- 3) Kraus and Hirsch, *Berl. klin. Wochenschr.*, 1908, 38.
- 4) Guerst, *Zeitschr. f. exp. Pathol. u. Therap.*, 1908, **5**, 43.
- 5) Falta, *Zeitschr. f. exp. Pathol. u. Therap.*, 1914, **15**, 356.
- 6) Yanagawa, *Tokyo-Igakkwai-Zasshi*, 1916, **30**, 835 (Jap.).
- 7) Pohl, *Biochem. Zeitschr.*, 1917, **78**, 200.
- 8) Stausky, *ibid.*, 1923, **133**, 434.
- 9) Michaelis, *Zeitschr. f. exp. Pathol. u. Therap.*, 1913, **14**, 255.
- 10) Dresel and Ullmann, *Zeitschr. f. ges. exp. Med.*, 1921, **24**, 214.
- 11) Krauss and Österreich, *Verhandl. d. Deut. Gesellsch. f. inn. Med.*, 1922, **34**, 150.
- 12) Harpuder, *Zeitschr. f. ges. exp. Med.*, 1924, **42**, 1.

carpine is uncertain. According to Rosenbloom and Weinberg¹³⁾, the intraperitoneal injection of adrenaline has no effect upon the urea excretion. Further, Abl¹⁴⁾ observed a decrease of uric acid excretion through atropine, on the contrary, Marès¹⁵⁾ an increase through pilocarpine, while Strausky asserted that neither pilocarpine nor atropine give any definite change to a rabbit's excretion of allantoin.

Marshall and Davis¹⁶⁾ demonstrated a retention of urea in the blood after the removal of the suprarenals from a rabbit, and the experiments of Addis and others,¹⁷⁾ have shown that certain doses of adrenaline increase the urea excretion, but decrease the blood urea content. Quinquaud¹⁸⁾ noticed by electrically stimulating the splanchnics of a dog which was anesthetized with chloral, for a minute, once every ten minutes, extending over a period of an hour, an increase of the blood urea-nitrogen content of about 50%. Dubois and Polonovski¹⁹⁾ found in a dog fed on a large quantity of meat and bread for three days, that the urea-nitrogen in the blood obtained from its femoral artery showed an increase of over 50% two hours after an adrenaline injection, while in animals fed on bread alone the increase of urea was comparatively small. Similar results were obtained with rabbits.

We see thus, that the observations of the authors have very little in agreement and that their methods of experiment are also open to question. The experiments I intend to describe here below are to make clear what influence the excitation or paralysis of the vegetative nervous system would exercise on the urea and the residual nitrogen content of the blood. These experiments were carried out by electrically stimulating the vagus or sympathetics of, or by administering pilocarpine, choline, atropine and adrenaline to rabbits.

I. THE INFLUENCE OF THE PARASYMPATHETICS ON THE BLOOD UREA-NITROGEN CONCENTRATION.

1. The Effect of the Stimulation of the Peripheral Vagus Stump upon the Blood Urea-Nitrogen Concentration.

It was desirous first to investigate the effect of the vagus stimulation upon the blood urea or the residual nitrogen concentration, since there are

13) Rosenbloom and Weinberg, *Proc. Soc. Exp. Biol.*, New York, 1911, 81, 130.

14) Abl, *Schmiedeberg's Arch.*, 1913, 75, 117.

15) Marès, *Pflüger's Arch.*, 1910, 134, 59.

16) Marshall and Davis, *Journ. Pharmacol. & Exp. Ther.*, 1913, 8, 525.

17) Addis, Barnett and Shevsky, *Americ. Journ. Physiol.*, 1918, 46, 39 & 52.

18) Quinquaud, *Cpt. rend. d. soc. biol.*, 1923, 88, 1242.

19) Dubois and Polonovski, *Cpt. rend. d. soc. biol.*, 1924, 91, 293.

practically no experiments of this kind on record. As given in Part I, the rabbits were fastened tight on their backs on boards provided with electrical warming outfits. In Exps. I and II, the cervical vagi were cut on both sides; to the peripheral stump of one side a sling was attached, the exposed division of the nerve being protected with a piece of cotton cloth soaked in warmed Ringer's solution. The stimulation was made with an induction coil connected with a 2-volt accumulator.

Exp. I. October 25, 1922. Rabbit ♂. Body weight 1.9 kilos.

Time	Urea-N (mgrm. %)	Remarks
10 ⁵⁰ 11 ³⁵ 12 ³⁰	18.9	Tied up, operated. Operation finished.
12 ³⁶ -12 ⁴⁰		Peripheral vagus stimulation, coil distance 20 cms.
1 ⁰⁰	29.4	
1 ³⁰	25.9	
2 ⁰⁰	25.9	
2 ³⁰	22.4	
3 ⁰⁰	18.2	

Exp. II. September 21, 1923. Rabbit ♂. Body weight 1.6 kilos.

Time	Urea-N (mgrm. %)	Non-protein-N (mgrm. %)	Remarks
10 ⁴³ 10 ⁵¹ 11 ²⁵	21.7	37.1	Tied up, operated. Operation finished.
11 ³¹ -11 ³⁵			Peripheral vagus stimulation, coil distance 20 cms.
11 ⁵⁰	34.3	49.0	
12 ³⁵	35.0	49.7	
1 ³⁵	29.4	45.4	
2 ³⁵	26.6	42.0	

In Exp. I, when the cervical vagi were cut off on both sides and the right side peripheral stump was stimulated, the urea-nitrogen content of the arterial blood showed an increase of 56% in 20 minutes. Then gradually reducing, in 2 hours and 20 minutes after the stimulation it was restored to its original value. Exp. II showed an increase of 58% of urea-nitrogen concentration 10 minutes after the stimulation which remained at the same height for an hour and though commencing to decrease, did not attain its original value even when 3 hours had passed after the stimulation, also the non-protein nitrogen undergoing the same change as the urea-nitrogen, showing therefore no change in its amino-acid fraction. That is to say, that the greater part of the residual nitrogen increase was due to urea-nitrogen increase.

Thus, when the peripheral stump of the cervical vagus is stimulated, the urea-nitrogen content of the blood is strikingly increased, and with it the total non-protein nitrogen is also increased. Further, in order to see

the effect of stimulation of the peripheral division of the vagus, at the point where the thoracic vagi penetrate through the diaphragm, upon the blood urea-nitrogen content, I stimulated the abdominal vagi at the wall of the oesophagus direct below the diaphragm. The epigastric portion of the abdomen was incised and the vagi were severed along the wall of the oesophagus, a sling being attached to it. Since the vagus stimulation can be followed by augmented intestinal movement which may interrupt the stimulation at definite periods or give rise to some unpleasant incidents, I sutured the gastric wall in the portion of its large curvature to the abdominal wall so that the intestines might not come above the stomach. During the procedure the liver was carefully kept warm.

Exp. III. September 23, 1923. Rabbit ♀. Body weight 1.5 kilos.

Time	Urea-N (mgrm. %)	Non-protein-N (mgrm. %)	Remarks
9 ⁴² 10 ¹⁵ 11 ⁰⁰ 11 ¹⁶ -11 ²⁰	19.6	37.8	Tied up, operated. Operation finished. Peripheral vagus stimulation, coil distance 20 cms.
11 ³²	26.6	47.6	
12 ²⁰	30.8	54.6	
1 ²⁰	29.4	52.5	
2 ²⁰	27.2	50.4	

Exp. IV. October 27, 1922. Rabbit ♂. Body weight 1.6 kilos.

Time	Urea-N (mgrm. %)	Remarks
0 ⁴⁰ 1 ³⁰ 2 ³⁰ 2 ⁴¹ -2 ⁴⁵	20.3	Tied up, operated. Operation finished. Peripheral vagus stimulation, coil distance 20 cms.
3 ⁰⁰	28.7	
4 ⁰⁰	28.0	
5 ⁰⁰	22.4	

In Exp. III, 12 minutes after the stimulation, the blood urea-nitrogen content increased about 35%, and an hour later it further increased becoming about 57%. Then though it showed signs of gradual decrease it failed to come to its original value even in 3 hours. The increasing percentage of the total residual nitrogen went on parallel with that of the urea-nitrogen, thus the amino-acid fraction made only a slight change. In Exp. IV too, the blood urea-nitrogen, which increased about 41% in 15 minutes after the stimulation, did not return to its original value even after over 2 hours had passed.

In an another experiment similarly treated, the blood urea-nitrogen content showed an increase from 18.9 mgrm. % to 25.2 mgrm. % by five minutes' stimulation with a coil distance of 20 cms. after five minutes, and in still another experiment, in which the cervical

vagus was stimulated for 5 minutes with a coil distance of 30 cms., the urea-nitrogen content of the blood increased from its original value of 24.9 mgrm. % to 35.4 mgrm. % in 10 minutes after the stimulation, amounting to 41.3 mgrm. % 40 minutes later. The second stimulation (coil distance 20 cms., duration five minutes) applied 55 minutes after the 1st, lifted it to 44.1 mgrm. % after five minutes, and to 48.1 mgrm. % thirty five minutes later, an amount almost twice as great as the original value.

We see then that the blood urea-nitrogen content increases most strikingly when the peripheral stump of the vagus is stimulated at the cervical or abdominal division. Since the rate of increase is not much different whether the neck or abdomen division is stimulated, it may be presumed that the stimulation of the peripheral stump of the cervical vagus, when the abdominal vagus is previously severed, gives rise to no change in the blood urea-nitrogen content. This was really ascertained in the following experiments.

Exp. V. November 10, 1921. Rabbit ♀. Body weight 2.0 kilos.

Time	Urea-N (mgrm. %)	Remarks
2 ⁰⁰	30.1	Tied up, under slight ether anaesthesia, abdominal and cervical vagi on both sides severed, cannula inserted into femoral artery.
2 ⁵⁰		Operation finished.
3 ³⁰		Peripheral stump of cervical vagus stimulated, coil distance 20 cms.
3 ⁴¹ -3 ⁴⁵		
4 ⁰⁰		
4 ³⁰	29.4	
5 ³⁰	29.4	

Exp. VI. November 22, 1922. Rabbit ♂. Body weight 1.9 kilos.

Time	Urea-N (mgrm. %)	Remarks
11 ⁰⁰	25.9	Tied up, operated.
11 ³⁸		Operation finished.
12 ²⁰		Peripheral stump of cervical vagus stimulated, coil distance 20 cms.
12 ³⁶ -12 ⁴⁰		
12 ⁵⁵	26.6	
1 ³⁰	25.9	
2 ³⁰	25.9	
3 ³⁰	25.2	

In Exps. V & VI, in which the vagus was previously cut in the abdomen, the stimulation of the peripheral stump of the cervical vagus was followed by no change in the blood urea-nitrogen concentration,—if changed at all, it was within the limit of technical error. In another similar experiment the result was also the same.

Thus, even when the vagus is cut at the abdominal division and a

stimulation is given to its peripheral stump in the neck, the blood urea-nitrogen concentration does not increase. As we look over these experiments, on the whole, we come to the conclusion that the viscera that cause an increase of the blood urea-nitrogen content through the vagus stimulation must be someones innervated by the vagus in the abdomen. But, organs other than the abdominal, at least the organs in the thorax innervated by the vagus, are believed to have nothing to do directly in this case.

The main sequels of the experiments above described are summarized as follows: The peripheral stump of the vagus, if stimulated at the neck or abdomen, causes a marked increase in the blood urea and the residual nitrogen content, which reaches the maximum already in 5 to 20 minutes after the close of the stimulation. Some even go on increasing, reaching the highest value in about one hour. These increases return to their original values before the stimulation in one hour in some cases, while others keep up their high values, showing only a tendency to decrease, even after three hours. Non-protein nitrogen concentration increases in proportion to the urea-nitrogen value, so that the value of the amino-acid fraction seems to make little changes.

It was ascertained that the increase of the blood urea-nitrogen concentration coming through the vagus stimulation is chiefly originated in the increased formation of urea in the abdominal organs, and that it has no direct connection with the thoracic organs.

2. The Effect of the Stimulation of the Central Vagus Stump upon the Blood Urea-Nitrogen Concentration.*

It is often shown by experiment that in stimulating the central stump of the vagus an entirely different result is brought about from that of stimulating the peripheral stump of the nerve. Cl. Bernard²⁰⁾ showed that the stimulation of the central stump of the vagus gives rise to glycosuria. Starkenstein²¹⁾ demonstrated the occurrence of glycosuria by stimulating its central stump even in an animal whose adrenal body has been extirpated. This shows that the stimulation of the central stump of the vagus brings out almost the same result as when the peripheral stump of

*) It would be more appropriate to place this chapter after that of the chapter on the splanchnic stimulation. However, it is put here for the convenience of a comparison with the first chapter.

20) Cl. Bernard, *Système nerveux II*, Paris 1858, 435.

21) Starkenstein, *Zeitschr. f. exp. Pathol. u. Therap.*, 1912, 10, 78.

the splanchnics is stimulated. Griffith²²⁾ called hyperglycaemia resulting from the stimulation of the central stump of the vagus "reflex hyperglycaemia." He induced hyperglycaemia by stimulating, not only the vagus, but also the crural and sciatic nerves. Bayliss²³⁾ and others, observing the fall of the blood pressure after stimulating the central stump of the vagus, called it "depressor reflex," while Sollmann and Pilcher²⁴⁾ on the other hand, observed a rise of blood pressure and a constriction of the peripheral vessels, when they stimulated the central stump of the vagus and the sciatics.

Thus we see that the stimulation of the central stump of the vagus brings about glycosuria or hyperglycaemia, or a rise of the blood pressure as well as the constriction of the peripheral vessels, as in the case of the stimulation of the peripheral stump of the sympathetic nerves. In my experiments on the blood urea-nitrogen concentration, it rather decreased when the central vagus stump was stimulated, contrary to the cases in which the peripheral stump was stimulated. The experiments are as follow: (In those not specially mentioned here no anaesthesia was used).

Exp. VII. January 26, 1923. Rabbit ♂. Body weight 1.5 kilos.

Time	Urea-N (mgrm. %)	Remarks
2 ²⁵	28.0	Tied up, left cervical vagus severed, cannula inserted into femoral artery.
2 ⁴⁵		Operation finished.
3 ²⁸		Central stump of vagus stimulated, coil distance 20 cms.
3 ³⁶ -3 ⁴⁰		
3 ⁵⁰		
4 ⁰⁰	27.3	
5 ³⁰	22.4	
6 ³⁰	25.2	

Exp. VIII. February 6, 1923. Rabbit ♂. Body weight 1.5 kilos.

Time	Urea-N (mgrm. %)	Remarks
2 ⁰⁰	25.2	Tied up, operated, right cervical vagus severed, cannula inserted into femoral artery.
2 ¹⁵		Operation finished.
3 ⁰⁰		

22) Griffith, *Americ. Journ. Physiol.*, 1923, 66, 18.

23) Bayliss, *Journ. Physiol.*, 1908, 37, 264.

24) Sollmann and Pilcher, *Americ. Journ. Physiol.*, 1912, 29, 303.

Time	Urea-N (mgrm. %)	Remarks
3 ¹¹ -3 ¹⁵		Central stump of vagus stimulated, coil distance 20 cms.
3 ²⁵	18.2	
4 ⁰⁰	18.2	
5 ⁰⁰	19.6	
6 ⁰⁰	26.6	

Exp. IX. November 22, 1923. Rabbit ♂. Body weight 1.5 kilos.

Time	Urea-N (mgrm. %)	Remarks
12 ⁵⁵		Tied up, cervical vagi on both sides severed, cannula inserted into femoral artery. Operation finished.
1 ²⁰		
2 ²⁰	19.6	
2 ³¹ -2 ³⁵		Central stump of left vagus stimulated, coil distance 30 cms.
2 ⁵⁰	16.1	
3 ²⁰	14.7	
4 ²⁰	16.1	
5 ²⁰	18.9	

Exp. X. November 25, 1923. Rabbit ♀. Body weight 2.0 kilos.

Time	Urea-N (mgrm. %)	Remarks
9 ²⁰		Tied up, operated as in Exp. IX. Operation finished.
9 ⁴⁰		
10 ⁵⁰	17.5	
11 ⁰¹ -11 ⁰⁵		Central stump of right vagus stimulated, coil distance 20 cms.
11 ²⁰	15.4	
12 ⁰⁰	16.8	
1 ⁰⁰	17.5	
2 ³⁵	16.8	

In Exps. VII and VIII one side of the vagus was left untouched, while in Exps. IX and X both sides were divided. Exp. VII showed no remarkable change during the 10 minutes following the stimulation, but 20 minutes after it was seen to decrease about 20%, it not entirely recovering its original value even after one hour and 50 minutes. In Exp. VIII it was observed to decrease about 27% after the stimulation and to return to its former value in 2 hours and 45 minutes. In another experiment, in which the left vagus had not been cut, the central stump of the right vagus was stimulated for 3 minutes with a coil distance of 25 cms., causing a decrease in the blood urea-nitrogen concentration from 28.0 mgrm. % (original value) to 11.2 mgrm. %, which was followed by a gradual increase to 25.9 mgrm. % after 2 hours and 12 minutes and 25.2 mgrm. % after 3 hours and 25 minutes. Similar results were also obtained in Exps. IX & X.

From these experiments, we come to know that when the central stump of the vagus is stimulated, regardless as to whether the other vagus has been

severed or not, the blood urea-nitrogen concentration is seen to be remarkably decreased as the result.

The question is now, whether the afferent stimulation of the vagus results solely in the excitement of the nervous centres, producing change similar to that in cases when such stimulants as caffeine and strychnine are given, or to that obtained in cases of anger and other emotional excitement. If such is the case, similarly as the hyperglycaemia and the increase of the blood urea-nitrogen content through caffeine, observed in my experiment, are arrested by anaesthesia, a decrease of the urea-nitrogen concentration should not result even if the central stump of the vagus is stimulated during anaesthesia. With such presupposition I made an experiment similar to the above on an animal anaesthetized with paraldehyde.

Exp. XI. April 21, 1923. Rabbit ♂. Body weight 1.8 kilos.

Time	Urea-N (mgrm. %)	Remarks
12 ¹⁵ 12 ⁴⁵	13.3	1.8 c.c. of paraldehyde introduced into stomach. Tied up, operated, cervical vagi on both sides severed, cannula inserted into femoral artery. Operation finished.
1 ⁰⁰ 1 ¹⁰		
1 ¹⁶ -1 ²⁰		
1 ⁴⁰		
2 ²⁰	16.1	Central stump of vagus stimulated, coil distance 20 cms.
3 ²⁰	16.8	
	16.8	

Exp. XII. March 8, 1923. Rabbit ♂. Body weight 2.0 kilos.

Time	Urea-N (mgrm. %)	Remarks
10 ³⁰ 11 ⁰⁸ 11 ²⁰ 11 ⁵⁵	30.8	2.0 c.c. of paraldehyde introduced into stomach. Tied up, operated as in Exp. XI. Operation finished.
12 ⁰¹ -12 ⁰⁵		
12 ³⁰		
1 ⁰⁵		
2 ⁰⁵	31.5	Central stump of vagus stimulated, coil distance 20 cms.
	30.8	
	31.5	

Exp. XI showed that in the central vagus stimulation the blood urea-nitrogen did not only decrease but it rather increased. In Exp. VII there was almost no change manifest. In another one, here not described, no change also appeared as in Exp. XII. In still another a striking decrease was observed as in the case with no anaesthesia.

Thus we see that decrease in the blood urea-nitrogen content caused by the stimulation of the central stump of the vagus is generally arrested

by anaesthesia, though it is sometimes decreased, dependent upon the effectiveness of the anaesthesia.

According to the references, in spite of the central stump of the vagus being stimulated during anaesthesia Bayliss²³⁾ and others observed a fall of the blood pressure while Sollmann²⁴⁾ and others on the contrary observed a rise. Presumably all of these controversies may be attributed to the different effectiveness of anaesthesia in each experiment. Of these findings I shall give my experimental observations specially later.

Briefly, when the central stump of the vagus is stimulated the blood urea-nitrogen concentration shows a significant decrease. In an anaesthetized animal it sometimes decreases, but in most cases it does not only decrease but it increases.

3. The Effect of Pilocarpine, Atropine and Choline upon the Blood Urea-Nitrogen Concentration.

Since, as the foregoing experiments show, the stimulation of the peripheral vagus stump gives rise to an increase in the urea and the non-protein nitrogen content of the blood, it is very important in order to ascertain the relation of the vagus excitation to the urea formation, to investigate what effect would be brought about upon these nitrogen substances if such parasympathetic stimulants as pilocarpine and choline and such paralytics as atropine are given to the animals. The following experiments were performed to answer this question.

Pilocarpine. Okada²⁵⁾ and his co-worker reported that in dogs the amino-acid content of the blood was increased by the injection of pilocarpine. From the protocols of their experiments I find that through pilocarpine the urea-nitrogen value was increased, besides amino-acid. The subjects of my experiments described below were, of course, previously given all necessary preparations.

Exp. XIII. September 13, 1922. Rabbit ♂. Body weight 12 kilos.

Time	Urea-N (mgrm. %)	Remarks
Sept. 12, 1 ⁵⁰ P.M. 1 ³⁰ "	38.5	Subcutaneous injection of 0.005 grm. pilocarpine hydrochloride. Salivation, tachypnoea, diarrhoea, cyanosis and constriction of ear vessels significant.

25) Okada and Hayashi, Journ. Biol. Chem., 1922, 51, 121.

Time	Urea-N (mgrm. %)	Remarks
1 ⁴⁰ -1 ⁵¹ P.M.	53.2	Diarrhoea and salivation somewhat decreased.
2 ¹¹ -2 ²⁵ "	51.8	
3 ³⁰ "	78.4	
5 ³⁰ "	54.6	
7 ³⁰ "	49.5	
9 ³⁰ "	41.5	
Sept. 13, 8 ³⁰ A.M.	39.9	

Exp. XIV. May 3, 1923. Rabbit ♂. Body weight 1.8 kilos.

Time	Urea-N (mgrm. %)	Non-protein-N (mgrm. %)	Remarks
11 ⁰⁷ 11 ¹⁸	15.4	31.5	Subcutaneous injection of 0.003 gram. pilocarpine hydrochloride. Striking signs as in Exp. XIII.
11 ²⁰			
11 ³⁵ -11 ⁴⁵	25.2	40.6	
12 ¹⁸ -12 ²⁰	30.8	43.4	
1 ²⁴ -1 ³⁰	21.7	41.3	
2 ²⁰ -2 ²⁷	21.7	33.6	
3 ³⁰	21.0	32.9	

Exp. XIII shows that a rabbit with 0.004 gram. or more of pilocarpine per kilo body weight subcutaneously injected commenced a flow of saliva in a few minutes, its ears presenting cyanosis, followed by diarrhoea and tachypnoea. The condition appeared disquiet and serious. About an hour after the injection the cyanosis deepened and the blood vessels of the ears so contracted that the collection of blood was found very difficult. Showing signs of gradual recovery after then, the salivation and diarrhoea began to decrease in amount and the animal seemed to be somewhat revived in spirit. The blood urea-nitrogen concentration was seen increased about 32% from 10 to 20 minutes after the injection, in 50 minutes the same rate being still maintained, but when the general condition of the animal was recovered, that is 2 hours after the injection, the blood urea-nitrogen content showed a remarkable increase, even reaching 100% or more. Then it began to decrease gradually. In 8 hours after the injection it was still above the original value, which was only attained after 19 hours had passed.

In Exp. XIV 0.001 gram. pilocarpine per kilo body weight was administered into the vein of a rabbit, the general condition being the same as that shown in Exp. XIII, except that its recovery was a little faster. After 17-20 minutes after the injection of pilocarpine the blood urea-nitrogen concentration increased to 64 per cent, one hour afterwards reaching 100%, thence it gradually decreased, though it did not recover its original value within 4 hours. The residual nitrogen concentration of the blood showed a significant increase during that period, but this was not at all in parallel with the urea-nitrogen, though the amino-acid fraction had rather decreased. In another experiment, 0.005 gram. of pilocarpine was subcutaneously injected into a male rabbit of 2.2 kilos body weight with the result that the urea-nitrogen content increased to 37.8 mgrm. % from the original value of 23.7 mgrm. %. In still another case a subcutaneous injection of 0.003 gram. given a rabbit weighing 2.2 kilos, caused an increase of urea-nitrogen content from 23.8 mgrm. % to 34.3 mgrm. %.

Thus we see that if pilocarpine is injected into a rabbit the blood urea-nitrogen content makes a significant increase. What we wish to call attention to right here is, that the greatest increase of the blood urea-nitrogen content is manifested an hour or two after the injection. In an earlier stage of the injection when diarrhoea, salivation, cyanosis and the constriction of the ear vessels are considerable, blood urea-nitrogen value does not increase much; there is rather a tendency to increase when these symptoms are on the way to recovery. Of this relation I will describe in another chapter. At any rate, it is evident that the blood urea-nitrogen value is greatly increased by the injection of pilocarpine.

Pilocarpine possesses a paradoxical action of increasing, though slightly, the blood sugar which is augmented by the excitement of the sympathetics. Regarding this hyperglycaemia, some authors assert that this is due to the thickening concentration of blood caused by this alkaloid. I can say nothing about blood sugar, but the increase of the blood urea-nitrogen content is too evident to be explained by the increased blood concentration coming through anhydrosis. The increase of the blood urea-nitrogen concentration through pilocarpine must be, in short, based on the same reasons as in the case of abdominal vagus excited by electrical stimulation.

Atropine. Next the action of atropine, an antagonist of pilocarpine, upon the urea-nitrogen concentration of the blood was examined.

Exp. XV. June 1, 1923. Rabbit ♂. Body weight 1.9 kilos.

Time	Urea-N (mgrm. %)	Non-protein-N (mgrm. %)	Remarks
2 ¹⁰ 2 ¹⁵	23.8	51.8	0.0005 gram. of atropine sulphate subcutaneously injected.
2 ³⁰	16.1	46.9	
3 ¹⁵	18.9	49.0	
4 ¹⁵	23.8	52.5	
5 ¹⁵	23.8	51.8	

Exp. XVI. September 25, 1922. Rabbit ♂. Body weight 2.0 kilos.

Time	Urea-N (mgrm. %)	Remarks
11 ²⁰ 11 ³⁰	24.5	Subcutaneous injection of 0.0005 gram. atropine sulphate.
11 ⁵⁰	18.9	
12 ¹⁰	19.6	
12 ³⁰	18.2	
1 ³⁰	18.2	
3 ³⁰	26.6	

In both experiments, 0.00025 grm. per kilo body weight was subcutaneously injected. In Exp. XV the blood urea-nitrogen content was seen decreasing 32% or over in 15 minutes after the injection, gradually recovering and returning to the original value in two hours. During the process the non-protein nitrogen moved in but parallel with the urea-nitrogen value. In Exp. XVI it showed a decrease of 23% or less in 20 minutes after the injection, and did not recover the former value within 2 hours, only restoring it after four hours.

These experiments seem to prove that atropine injections greatly decrease the blood urea-nitrogen content, but in other experiments with almost the same amount of atropine injected, its decrease was quite slight, so slight that it could be said to be within the limits of experimental error. There was no instance where any increase of the urea-nitrogen was noticed.

The blood urea-nitrogen content seems to be decreased or to undergo little change through the injection of atropine in these experiments. It stands pharmacological reason that the urea-nitrogen content in the blood which is increased through pilocarpine or the vagus stimulation, is decreased or not changed by the injection of atropine. Atropine seems to possess the action of arresting the urea forming power. Owing to this action the blood urea-nitrogen content which is increased by caffeine is arrested by atropine.

We can not pass hasty judgement upon the fact that the decrease in the blood urea-nitrogen due to atropine are sometimes so limited that we can hardly notice any change, and say that it is caused by merely individual variation. Though the function of the urea production is arrested by atropine, if the elimination of urea is obstructed the blood urea-nitrogen will only slightly be decreased, while on the contrary when the urea elimination is normal or is augmented, it will decrease considerably according to the amount of urea eliminated. For instance, if caffeine and atropine are used at the same time the increase of the blood urea-nitrogen content through caffeine is arrested by atropine and on the other hand, the excretion of urine is augmented, thus decreasing the blood urea-nitrogen value very significantly and definitely. We must also take into consideration that in case the urea production is not arrested, if its excretion is abundant, it will cause a decrease of the urea-nitrogen in the blood. The fact that in paraldehyde anaesthesia, caffeine causes considerable decrease of the blood urea-nitrogen may be taken as a good example of the above fact. No change is brought about in the blood urea-nitrogen value during the paraldehyde anaesthesia of ordinary dosage, which inhibits the urea formation by arresting the central stimulation of caffeine so that caffeine diuresis gives rise to a decrease of the blood urea-nitrogen. In short, the variation in the decrease of the blood urea-nitrogen in rabbits is be-

lieved to depend upon the renal function at the time of the experiment.

As regards the effect of atropine on diuresis, studies are not lacking, but there seems to be nothing definite yet announced. Thompson²⁶⁾ saw the volume of urine decrease through atropine; Eichelberg²⁷⁾ asserted that there was a slight decrease sometimes brought about, but no effect was manifest; Loewi²⁸⁾ obtained no significant effect from it, while Cow²⁹⁾ inferred that atropine may have an indirect influence upon the secretion of urine through its effects upon the secretory functions of various organs and upon the ureter. Thus we see that atropine seems to bring no special change upon the renal function, and if there be any, the effect would be very slight indeed.

The Antagonistic Phenomenon of Pilocarpine and Atropine. From the experiments above described we have come to know that the blood urea-nitrogen concentration is subjected to entirely opposite effects by pilocarpine and atropine. Such are usually observed in the vegetative organs, and the increase of the blood urea-nitrogen concentration owing to administration of pilocarpine has to be arrested by atropine.

Exp. XVII. June 4, 1923. Rabbit ♂. Body weight 1.8 kilos.

Time	Urea-N (mgrm. %)	Non-protein-N (mgrm. %)	Remarks
9 ⁴⁰ 9 ⁴⁵	11.9	37.1	Subcutaneous injection of 0.005 gm. pilocarpine and 0.0005 gm. atropine.
10 ⁰⁰	12.6	37.8	
10 ⁴⁵	13.3	38.5	
11 ⁴⁵	12.6	37.5	
12 ⁴⁵	12.6	36.4	

Exp. XVIII. December 26, 1922. Rabbit ♂. Body weight 1.6 kilos.

Time	Urea-N (mgrm. %)	Remarks
2 ⁴⁰ 2 ⁵⁰	22.4	Subcutaneous injection of 0.003 gm. pilocarpine and 0.0008 gm. atropine.
3 ²⁰	21.0	
3 ⁵⁰	17.5	
4 ⁵⁰	20.3	
5 ⁵⁰	18.2	
6 ⁵⁰	19.6	

26) Thompson, Journ. Physiol., 1894, 15, 433.

27) Eichelberg, cited by Cushny in "Secretion of Urine," London 1917, 186.

28) Loewi, Schmiedeberg's Arch., 1903, 50, 326.

29) Cow, Journ. Physiol., 1914, 48, 1.

In Exp. XVII the simultaneous administration of 0.005 gm. pilocarpine and 0.0005 gm. atropine increased the blood urea-nitrogen content but slightly, compared with the case when pilocarpine alone was injected. From this we may easily perceive that pilocarpine and atropine will produce antagonistic results. In Exp. XVIII, 0.003 gm. pilocarpine and 0.0008 gm. atropine were at the same time injected, showing no signs of the action of pilocarpine. The blood urea-nitrogen concentration increase was, we can see from this, arrested by atropine.

Thus we can again demonstrate the antagonistic action of pilocarpine and atropine in the blood urea-nitrogen concentration.

Choline. Finally the action of choline was examined as another stimulant of the parasympathetics.

Exp. XIX. April 13, 1923. Rabbit ♂. Body weight 1.7 kilos.

Time	Urea-N (mgrm. %)	Non-protein-N (mgrm. %)	Remarks
1 ¹⁰ 1 ¹⁵	19.6	41.3	Subcutaneous injection of 0.03 gm. choline chloride.
1 ³⁰	22.4	43.4	
2 ¹⁵	22.4	43.4	
3 ¹⁵	21.7	42.8	
4 ¹⁵	21.7	42.8	

Exp. XX. July 2, 1923. Rabbit ♀. Body weight 1.9 kilos.

Time	Urea-N (mgrm. %)	Non-protein-N (mgrm. %)	Remarks
10 ³⁰ 10 ³⁵	16.8	36.4	Subcutaneous injection of 0.05 gm. choline chloride.
10 ⁵⁰	18.2	39.2	
11 ³⁵	19.6	38.0	
12 ³⁵	21.0	40.6	
1 ³⁵	21.7	40.6	

In these experiments, the salivation and diarrhoea as well as the increase in blood urea and residual nitrogen content were not so considerable as in the case of pilocarpine. The residual nitrogen value went almost parallel with that of the urea-nitrogen in its increase.

Thus, choline possesses also some action of increasing the blood urea-nitrogen.

II. THE INFLUENCE OF THE SYMPATHETICS ON THE BLOOD UREA-NITROGEN CONCENTRATION.

By the foregoing series of experiments, evidence was put forward that

the blood urea-nitrogen concentration is significantly increased when the vagus is electrically stimulated or when stimulated by administration of pilocarpine or choline and on the contrary decreased when the peripheral parasympathetics are paralysed by atropine; further that when the nerve centres are stimulated by the injection of caffeine or the binding of the animal, or by other treatments of similar significance, like anaesthesia, the blood urea-nitrogen content shows also an increase, which is absent, if the vagus has been previously or at the same time cut, or atropine is given, and sometimes it shows inversely a decrease, especially in case caffeine is applied. From these observations I have come to the conclusion that when the vagus, whether central or peripheral, is stimulated, the blood urea-nitrogen concentration is increased.

Authors do not agree in their observations on the effect of a splanchnic stimulation or an adrenaline injection upon the blood urea-nitrogen, so I thought it necessary to investigate what change the blood urea-nitrogen would undergo, when the sympathetics are stimulated. For this in the following series of experiments, I have estimated the change in the blood urea-nitrogen content after an adrenaline injection, the electrical stimulation of the splanchnics and the diabetic puncture.

1. The Effect of Adrenaline upon the Blood Urea-Nitrogen Concentration.

At first I examined the effect brought about by an adrenaline injection upon the urea-nitrogen content of the blood.

Exp. XXI. May 1, 1922. Rabbit ♂. Body weight 1.6 kilos.

Time	Urea-N (mgrm. %)	Remarks
1 ⁰⁰	23.8	0.3 c.c. adrenaline subcutaneously injected.
1 ¹⁰		
1 ²⁰ -1 ²⁸	15.4	
1 ⁴⁵ -1 ⁵⁰	22.4	
2 ²⁰	24.5	

Exp. XXII. March 16, 1923. Rabbit ♂. Body weight 2.3 kilos.

Time	Urea-N (mgrm. %)	Non-protein-N (mgrm. %)	Remarks
10 ¹⁵	16.8	42.0	0.5 c.c. adrenaline subcutaneously injected.
10 ²⁵			
10 ⁴⁵	13.3	42.0	
11 ²⁵	15.4	37.8	
12 ²⁵	15.4	39.8	
1 ²⁵	16.1	42.0	

In these experiments the contraction of the blood vessels caused by adrenaline was so great that much time was required to collect the blood sample from the ear vein, sometimes over 10 minutes being required. In all cases the urea-nitrogen value was observed diminished slightly but distinctly by the subcutaneous application of adrenaline. In Exp. XXI it recovered its original value in 30 to 40 minutes, while in Exp. XXII one hour after the injection it showed signs of recovery, only returning to its original value after 3 hours.

Also in other experiments, for instance, when 0.5 c.c. of adrenaline was subcutaneously injected into a male rabbit of 1.5 kilos body weight, it was found that the urea-nitrogen content in the blood decreased in 10 minutes from the original value of 30.1 mgrm. % to 25.2 mgrm. %, regaining its original value in 30 minutes. In another experiment with a male rabbit weighing 2.1 kilos, which had 0.5 c.c. of adrenaline injected, the blood urea-nitrogen content was seen to undergo almost no change from 25 minutes to one hour after the injection, showing only a very slight decrease of the residual nitrogen.

Briefly speaking, it was shown by these experiments that in rabbits the blood urea-nitrogen concentration, after the injection of adrenaline, only decreases for a time, and that the degree of that decrease is not definite. This indefiniteness seems to have some relation to diuresis at the time of the action of adrenaline, as discussed in the foregoing chapter. Through the injection of adrenaline there was no instance showing an increase of the urea-nitrogen value in the blood.

And again, adrenaline causes a slight reduction of the blood urea-nitrogen content, not only in the animal, but in the human body. Two experiments of this sort are given below (Exps. XXIII & XXIV).

Exp. XXIII. Patient ♂. Beri-beri. Fasting stage in the morning.

Time	Urea-N (mgrm. %)	Remarks
Before injection	21.7	1 c.c. adrenaline subcutaneously injected.
30 mins. after injection	15.4	
1 hour " "	14.0	
2 hours " "	—	
3 " " "	19.6	

Exp. XXIV. Patient ♂. Beri-beri. Fasting stage in the morning.

Time	Urea-N (mgrm. %)	Remarks
Before injection	23.1	1 c.c. adrenaline subcutaneously injected.
30 mins. after injection	17.5	
1 hour " "	14.7	
2 hours " "	12.7	
3 " " "	14.7	

In these experiments, the blood urea-nitrogen content made a marked decrease in 30 minutes after the subcutaneous injection of 1 c.c. of adrenaline, reaching the lowest point after one hour and beginning to restore after 3 hours, in the former instance, and reaching the minimum after two hours and tending rather to increase after 3 hours, in the latter instance.

Experiments are few, but we can ascertain from them that in the human body the urea-nitrogen content through adrenaline makes a marked and steady decrease. In the rabbit the decrease is rather sudden and for a time, recovering speedily, while in the human body it is observed that the decrease is even discernible after 3 hours.

Thus we see that when adrenaline is injected into either the rabbit or the human body the blood urea-nitrogen content is decreased, showing entirely opposite results from those obtained by Dubois and Polonovski¹⁹⁾ in their experiments on the dog and the rabbit.

2. The Effect of the Stimulation of the Splanchnics upon the Blood Urea-Nitrogen Concentration.

What we ought to be careful of in carrying out this experiment is the effect of binding. The blood urea-nitrogen content has a tendency to increase easily through the very act of binding an animal tight, and to prevent this to the least possible degree, it is necessary to cut the vagus at the neck or abdomen in the earliest stage of the operation. A rabbit was fastened on an animal board with a heating outfit, and laparotomized under a slight ether anaesthesia. First the vagi on the wall of the oesophagus were cut, and then the left splanchnics, a sling being put to their peripheral stump. A cannula was inserted into the carotid or femoral artery, preparatory to collect blood. Careful attention was paid to protect as much as possible the abdominal organs close to the wounds and the anaesthesia was removed directly after the operation was finished.

Exp. XXV. October 3, 1923. Rabbit ♂. Body weight 1.6 kilos.

Time	Urea-N (mgrm. %)	Non-protein-N (mgrm. %)	Remarks
10 ⁴⁵ 11 ¹⁰ 11 ⁴⁵ 12 ⁰¹ -12 ⁰⁵	25.9	53.2	Tied up and operated. Operation finished. Stimulation of splanchnics, coil distance 20 cms.
12 ²⁰	25.9	52.5	
1 ⁰⁵	22.4	49.7	
2 ⁰⁵	29.4	56.7	
3 ⁰⁵	30.8	58.8	

Exp. XXVI. February 24, 1923. Rabbit ♂. Body weight 1.8 kilos.

Time	Urea-N (mgrm. %)	Non-protein-N (mgrm. %)	Remarks
2 ⁴⁰ 2 ⁵⁰ 3 ¹⁵ 3 ¹⁰ -3 ²³	16.1	35.0	Tied up and operated. Operation finished. Stimulation of splanchnics, coil distance 20 cms.
3 ³²	10.5	28.0	
3 ⁵⁴	16.8	—	
5 ²⁷	19.6	32.2	

On looking over these experiments it is found that the blood urea-nitrogen content was decreased for a time after the splanchnic stimulation. In Exp. XXV, though no change was noticed 15 minutes after the stimulation, it showed a slight decrease after an hour, and then not only recovered its original value in two hours but even surpassed it, holding this state for 3 hours. The residual nitrogen went on in parallel with the urea-nitrogen content. In Exp. XXVI the decrease of the urea-nitrogen concentration was very marked 9 minutes after the stimulation, recovering its original value in an hour and a half and then showing a slight increase two hours later. The residual nitrogen content was also decreased by the stimulation of the splanchnics.

Thus the blood urea-nitrogen concentration brings about a slight decrease in from 10 minutes to one hour, owing to the splanchnic stimulation. This result is at one with that of adrenaline administration. Although it shows some increase in from 2 to 3 hours it is hard to believe this to be the direct effect of the splanchnic stimulation. When a rabbit with its vagi cut is fastened, a slight increase of blood urea-nitrogen content is sometimes observed. In this experiment its abdomen was cut and followed by various treatments, so that it is not unlikely that it had some unexpected incidental effects. That the blood urea-nitrogen concentration has increased in from 2 to 3 hours during the experiments might show these implications. The result of Quinquaud's¹⁸⁾ experiment of the splanchnic stimulation seems to be entirely opposed to that of mine, but when we consider the sensitive nature of the blood urea-nitrogen above stated, it is surely rather hasty to attribute the increase in the blood urea-nitrogen after the splanchnic stimulation directly to the nerve stimulation itself. In Dubois and Polonovski's¹⁹⁾ adrenaline experiment too it is regrettable to note that they did not measure the urea in the blood immediately after the adrenaline injection, but only that of two hours after. Even if adrenaline were injected it would be almost impossible to prevent the effect of binding and other things after two hours' time.

3. The Effect of Diabetic Puncture upon the Blood Urea-Nitrogen Concentration.

It remains still unsolved as to what part of the central nervous system has what effect upon the nitrogenous exchange. Michaelis,²⁹⁾ after experimenting on the uric acid elimination which is increased with sugar in the urine caused by a diabetic puncture, called it "uric acid puncture." He, seeing an increase of not only uric acid but also total nitrogen in the urine, inferred from it "protein puncture," but whether this inference is correct or not there is still much to be discussed. Yanagawa³⁰⁾ observed an increase of allantoin excretion by performing a piqûre on a rabbit. He also obtained the same result when a rabbit was given asphyxia instead of a piqûre. From this it may well be supposed that, as Yanagawa³¹⁾ thinks, the elimination of uric acid increases under the same condition as sugar, and the so-called "uric acid puncture" and diabetic puncture belong to the same rank. Leschke and Schneider³²⁾ observed that the nitrogenous exchange was arrested when the between-brain was punctured. Freund and Gräfe³¹⁾ also experimented that when the between-brain, especially the subthalamie region, was punctured at several points, the nitrogenous elimination was somewhat diminished. Freund and Laubender³²⁾ showed that, when the spinal cord in the neck was cut, the nitrogenous elimination was increased.

My experiments followed Eckhard's method of diabetic puncture. Since in carrying out these experiments long binding and other cruel treatments were found necessary, as the consequence, some unwelcome effects on the blood urea-nitrogen content were expected. No anaesthesia was purposely used and great pains were taken to shorten the time of operation as much as possible. According to Fujii's³³⁾ experiment, a marked glycosuria seems to show itself from 1 to 2 hours after applying piqûre. Hence to a rabbit with no sign of glycosuria, urine was collected with a catheter at different times, and examined by Trommer's test to see if glycosuria was produced or not, thus ascertaining the success or failure of my diabetic puncture. After the piqûre, the rabbit was at once released. Also in this experiment only rabbits were used whose large auricular nerve and cervical sympathetics had been previously cut.

30) Leschke and Schneider, *Zeitschr. f. exp. Pathol. u. Therap.*, 1917, 19, 58.

31) Freund and Gräfe, *Schmiedeberg's Arch.*, 1922, 93, 285.

32) Freund and Laubender, *Schmiedeberg's Arch.*, 1923, 99, 131.

33) Fujii, *Tohoku Journ. Exp. Med.*, 1920, 1, 39.

Exp. XXVII. December 24, 1923. Rabbit ♀. Body weight 1.9 kilos.

Time	Urea-N (mgrm. %)	Remarks
8 ³⁰	19.6	Urine sugar test —
9 ⁵⁰		
9 ⁵²		Tied up, operated.
10 ²³		Piqûre.
10 ²⁵	21.7	Untied.
11 ²⁰		Urine sugar test —
11 ³¹ –11 ⁴⁰		
12 ³⁰ –12 ⁴⁰		
12 ⁵⁰	18.9	Urine sugar test +
2 ³⁰ –2 ⁴⁰	20.3	
9 ⁴⁰		Found died.

Exp. XXVIII. November 25, 1923. Rabbit ♂. Body weight 1.5 kilos.

Time	Urea-N (mgrm. %)	Remarks
10 ³⁰	23.0	Urine sugar —
10 ³⁵		
10 ⁴⁰		Tied up and operated.
11 ¹⁸		Piqûre.
11 ²⁰	28.0	Untied.
1 ³⁰		Urine sugar weakly +
2 ³⁰		
2 ³⁵		Urine sugar +
5 ³⁰	22.4	
7 ²⁰		Found died.

In these experiments the binding lasted from 33 to 40 minutes. From my experience I expected an increase of the blood urea-nitrogen content during from 2 to 3 hours after the release from binding, but in Exp. XXVII, one hour and 15 minutes after the release it showed a slight increase over the original value, but not high enough to account for the effect of binding itself, especially as a cruel operation had been accorded to the animal in addition to the binding.

Exp. XXVII shows that in the urine 2 hours and 25 minutes after the piqûre, Trommer's test was strongly positive, showing that the diabetic puncture was successful. In 2 hours and 15 minutes after the diabetic puncture the blood urea-nitrogen content was noticed somewhat decreased, but not distinct. In Exp. XXVIII, the urine taken 3 hours and 10 minutes after the puncture, contained sugar. The blood urea-nitrogen value was the same as before the binding. In 6 hours and 10 minutes after the piqûre it seemed to indicate a sign of decrease, but whether this was due to the diabetic puncture or not is not certain.

By these experiments the blood urea-nitrogen content seems as if it would decrease a little when urine sugar is most apparent after the diabetic puncture, but it is not possible to prove a sure change. We must admit, at least, the blood urea-nitrogen does not increase by the diabetic puncture. But when we take into consideration the manipulations (binding and other

treatments) of the animals accompanying the diabetic puncture, which cause the blood urea-nitrogen content to increase after a time, and judging from the effect of adrenaline administration and splanchnic stimulation upon the blood urea-nitrogen content, the diabetic puncture might be expected also to show some signs of decreasing the blood urea-nitrogen concentration more or less.

That the elimination of nitrogen substances is increased by piqûre, might be explained by increased diuresis occurring often at the time of piqûre, together with the increase of the uric acid excretion, as Michaelis³² himself, besides Yanagawa³³ and others proved. For instance, Morris and Rees³⁴ have already proved that the output of uric acid and urea is increased also during the saline diuresis through sodium chloride and sodium sulphate.

What I desire to add here is that when the central stump of the vagus is stimulated, as mentioned before, there occurs what is called "reflex phenomenon." I observed a decrease of the blood urea-nitrogen content at cutting the vagus at the neck and stimulating its central stump. By the same treatment, Griffith²² obtained an increase in the blood sugar, while Sollmann and Pilcher²⁴ showed a rise of blood pressure. In my experiments it has also been demonstrated that this procedure has an arresting action on the movements of the stomach and intestine, though slight, and that when the splanchnics on both sides are previously severed, not only did the blood pressure fail to rise, but rather it fell. From this it might be inferred that when the central stump of the vagus is stimulated the same or a similar reaction is brought about as when the sympathetics, at least the splanchnics, are stimulated. It is not without reason that the urea-nitrogen concentration of the blood should be decreased.

As already stated, piqûre and adrenaline injection have the same effect upon the urea-nitrogen concentration of the blood as stimulation of the splanchnics, and as the organs, which are concerned in the increased urea formation when the vagus is stimulated, are those innervated by abdominal vagi, just so, the organs whose urea formation is inhibited by the stimulation of the sympathetics are supposed to exist within the district innervated by the splanchnics.

From the above experiments we ascertain without any inconsistency the nervous regulation of the blood urea-nitrogen concentration: the latter is augmented by the excitation of the parasympathetics, lessened by its

34) Morris and Rees, *Americ. Journ. Physiol.*, 1923, 66, 363.

paralysis or by the sympathetic stimulation. Now the question arises: In what part of the organism is it acted with an increase or with a decrease?

The liver is the first organ that attracts our attention as an urea forming organ. By the perfusion of the survived organ with ammonia mixtures or amino-acid mixtures (Schmiedeberg,³⁵⁾ Schröder,³⁶⁾ Fiske and Karsner,³⁷⁾ Jansen,³⁸⁾ Löffler,³⁹⁾ Felix and Tomita⁴⁰⁾ etc.) by comparing the blood urea content of the hepatic vein with that of the arterial blood or venous blood, collected from other organs (Herter⁴¹⁾ etc.) or by the methods of excluding the portal circulation by Eck's fistula or others or of destroying the liver (Schröder,⁴²⁾ Slosse,⁴³⁾ Lieblein,⁴⁴⁾ Nencki, Pawlow and Zaleski,⁴⁵⁾ Salaskin,⁴⁶⁾ Doyon and Dufourt,⁴⁷⁾ Van Slyke and Meyer⁴⁹⁾ etc.) it has been presumed that the urea formation is carried out exclusively or chiefly in a direct or indirect way in the liver. But Gscheidren⁵⁰⁾, Gréhant and Quinquaud,⁵¹⁾ Kaufmann,⁵²⁾ Biedl and Winterberg,⁵³⁾ Mathews and Nelson,⁵⁴⁾ Fiske and Summer,⁵⁵⁾ Taylor and Lewis⁵⁶⁾ and others could not assert that this function is monopolized by the liver alone.

Coste⁵⁷⁾ succeeded recently in extirpating the liver of a dog by three

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- 35) Schmiedeberg, Schmiedeberg's Arch., 1878, 8, 1.
 - 36) Schroeder, Schmiedberg's Arch., 1882, 15, 383 & 1885, 19, 373.
 - 37) Fiske and Karsner, Journ. Biol. Chem., 1914, 18, 285.
 - 38) Jansen, Journ. Biol. Chem., 1915, 21, 557.
 - 39) Löffler, Physiol. Abstr., 1916-1917, 1, 23.
 - 40) Felix and Tomita, Zeitschr. f. physiol. Chem., 1923, 128, 40.
 - 41) Herter, Johns Hopkins' Hosp. Rept., 1900, 9, 69.
 - 42) Schroeder, Schmiedeberg's Arch., 1882, 15, 364.
 - 43) Slosse, Arch. f. Physiol., 1890, 57, 482.
 - 44) Lieblein, Schmiedeberg's Arch., 1893-1894, 33, 318.
 - 45) Nencki, Pawlow and Zaleski, Schmiedeberg's Arch., 1896, 37, 26.
 - 46) Salaskin, Zeitschr. f. physiol. Chem., 1898, 25, 128.
 - 47) Doyon and Dufourt, Arch. de physiol. norm. et pathol., 1898, (S) V, 10, 522.
 - 48) Horodynski, Salaskin and Salaeski, Zeitschr. f. physiol. Chem., 1902, 35, 246.
 - 49) Van Slyke and Meyer, Journ. Biol. Chem., 1913-14, 16, 213.
 - 50) Gscheidren, cited in Maly's Jahresberichte, 1871, I, 141 & 209.
 - 51) Gréhant and Quinquaud, Cpt. rend. l'acad. de Science, 1884, 98, 1312.
 - 52) Kaufmann, Arch. de physiol. norm. et pathol., 1894, (S) V, 6, 531.
 - 53) Biedl and Winterberg, Pflüger's Arch., 1902, 88, 140.
 - 54) Mathews and Nelson, Journ. Biol. Chem., 1914, 18, 229.
 - 55) Fiske and Summer, Journ. Biol. Chem., 1914, 18, 2.
 - 56) Taylor and Lewis, Journ. Biol. Chem., 1915, 22, 77.
 - 57) Coste, Presse méd., 1923, 21, 1039.

times operation. In such a dog, nitrogen elimination was noticed remarkably decreased, while ammonia and amine made a great increase in excretion. Mann⁵⁸⁾ removed the liver by the same method, but since the blood urea content showed no decrease he did not believe that the urea synthetic function belonged to the liver alone.

Again, Gréhant and Quinquaud⁵¹⁾ and Kaufmann⁵²⁾ asserted that as the urea-nitrogen value of the venous blood coming from the spleen, similar to that of the hepatic vein, was of much higher value than that of blood coming through other various organs, urea was also formed in the spleen.

Addis and Watanabe⁵⁹⁾ observing that the blood urea content in the renal vein was of higher value than that of the arterial blood when urine excretion did not take place, concluded that the kidney had a urea forming function. Quinquaud¹⁸⁾ also supposed that urea was synthesized in the kidney, and that this function was accelerated when the splanchnics were stimulated.

But Folin⁶⁰⁾ and their scholars and Gottschalk and Nonnenbruch,⁶¹⁾ seem to believe that the urea forming function is carried out in the ubiquitous tissues. Kotake⁶²⁾ and his co-workers think that the desamination is conducted in the reticulo-endothelial system.

Thus we see that respecting the organs of urea formation various opinions are made public, but by what process the materials for forming urea are supplied must be another question of great importance.

Evidence that urea is formed from ammonia has been often proved by perfusing the survived liver (Schmiedeberg,³⁵⁾ Schröder³⁶⁾ Fiske and Karsner³⁷⁾). According to Schröder's experiment the kidney and muscles possess no such function, but Jansen,³⁸⁾ Löffler³⁹⁾ and Felix and Tomita⁴⁰⁾ showed that the survived liver seem to be capable of forming urea not only from ammonia, but also directly from amino-acid. According to Löffler³⁹⁾ some of the amino-acids are not yet changed into urea in the liver.

Van Slyke and Meyer⁴⁹⁾ noticed in their experiment that the liver absorbs amino-acid quicker and in a greater quantity than the muscle, and that when the amino-acid content in the liver diminishes the blood

58) Mann, Arch. Intern. Med., 1923, 31, 797.

59) Addis and Watanabe, Americ. Journ. Physiol., 1917, 43, 363.

60) Folin, Journ. Biol. Chem., 1922, 51, 395.

61) Gottschalk and Nonnenbruch, Schmiedeberg's Arch., 1923, 99, 270.

62) Kotake, Zeitschr. f. physiol. Chem., 1922, 122, 211.

urea concentration increases. Contrary to this Folin⁶⁰⁾ observed after eating gelatine, first the increase of amino-acid, and a few hours afterwards the increase of urea, in the systemic blood.

It is an acknowledged fact that ammonia is very important as a precursor of urea, but if all amino-acids are directly reformed into urea, it leaves much room for further study and discussion.

Where is the desamination then carried on? That the ammonia content of the systemic venous blood is more abundant than that of the arterial blood is already ascertained (Salaskin and Zalaeski⁶³⁾), but that the ammonia value of the portal blood is higher than any of the venous or arterial blood is also a known fact (Folin and Denis⁶⁴⁾). Nencki and his co-workers⁴⁵⁾ particularly experimented that the mucous membrane of the stomach contains a high value of ammonia, and Biedl and Winterberg,⁵³⁾ Somogyi⁶⁵⁾ and Barnett and Addis⁶⁶⁾ maintained that the digestive canal served as an important station in the desamination in the animal body.

Again, Nash and Benedict⁶⁷⁾ saw ammonia formed also in the kidney.

In what organ then is the blood urea content increased by the vagus stimulation, as definitely confirmed by my own experiments, synthetized? On going over the above summarized references and in view of my experiments, in which the urea-nitrogen was seen increased when the abdominal vagus was stimulated, while the stimulation of the cervical vagus trunk can bring about a change in the blood urea-concentration when the vagus is cut in its abdominal part, I am compelled to pay special attention, first to the abdominal organs, especially to the liver.

III. THE EFFECT OF THE VAGUS STIMULATION UPON THE BLOOD UREA-NITROGEN CONCENTRATION IN ANIMAL, WHOSE PORTAL SYSTEM HAS BEEN EXCLUDED FROM SYSTEMIC CIRCULATION.

I, therefore, first experimented to find out how the blood urea- and non-protein nitrogen concentrations would be affected when the portal system is removed from the systemic circulation, and then what changes would be

63) Salaskin and Zalaeski, *Zeitschr. f. physiol. Chem.*, 1898, **25**, 449.

64) Folin and Denis, *Journ. Biol. Chem.*, 1911, **10**, 407.

65) Somogyi, *Zeitschr. f. physiol. Chem.*, 1911, **71**, 125.

66) Barnett and Addis, *Journ. Biol. Chem.*, 1917, **30**, 41.

67) Nash and Benedict, *Journ. Biol. Chem.*, 1921, **48**, 463.

brought about to these substance if the vagus was stimulated under such conditions. For this purpose a rabbit was slightly anaesthetized with ether and its portal circulation removed, after ligating the mesenteric and hepatic arteries and then tying up the portal vein. A cannula was inserted into the carotid or femoral artery for collecting blood, and as soon as the operation was completed the animal was released from ether anaesthesia.

Exp. XXIX. November 29, 1923. Rabbit ♂. Body weight 1.4 kilos.

Time	Urea-N (mgrm. %)	Non-protein-N (mgrm. %)	Remarks
10 ⁴⁰ 11 ³⁰ 11 ³³ -11 ⁴⁵	39.9	58.8	Tied up, laparotomized. Ligation of mesenterial and hepatic arteries and portal vein.
12 ⁰³	39.9	58.8	
12 ³⁰	30.1	65.1	
1 ⁰⁰	28.7	68.6	
2 ⁰⁰	27.3	71.4	

Exp. XXX. January 22, 1925. Rabbit ♀. Body weight 1.6 kilos.

Time	Urea-N (mgrm. %)	Non-protein-N (mgrm. %)	Remarks
9 ¹⁰ 10 ⁰⁰ 10 ⁰⁵ -10 ¹³	30.8	50.4	Tied up and laparotomized. Ligation of vessels as in Exp. XXIX.
10 ³⁰	30.8	51.1	
10 ¹⁰	29.4	50.4	
1 ¹⁰	28.0	53.2	
2 ¹⁰	28.0	54.6	

Exp. XXXI. November 5, 1923. Rabbit ♀. Body weight 1.6 kilos.

Time	Urea-N (mgrm. %)	Non-protein-N (mgrm. %)	Remarks
1 ¹⁰			Tied up, laparotomized, ligating of mesenterial and hepatic arteries and portal vein.
1 ⁴⁰			Operation finished.
2 ⁰⁰	25.2	50.4	
2 ³⁰	25.9	50.4	
3 ⁰⁰	28.0	50.4	
3 ³⁰	28.0	50.4	
4 ⁰⁰	28.7	50.4	

Exp. XXXII. November 12, 1923. Rabbit ♀. Body weight 1.8 kilos.

Time	Urea-N (mgrm. %)	Non-protein-N (mgrm. %)	Remarks
10 ¹⁰			Tied up, operated as in Exp. XXXI. Operation finished.
10 ⁵⁰			
11 ³⁰	27.3	57.4	
12 ⁰⁰	27.3	59.1	
12 ³⁰	26.6	59.1	
1 ⁰⁰	27.3	59.1	
1 ³⁰	26.6	60.2	

Exp. XXXIII. November 24, 1923. Rabbit ♂. Body weight 1.7 kilos.

Time	Urea-N (mgrm. %)	Non-protein-N (mgrm. %)	Remarks
10 ⁰⁰			Tied up, operated as in Exp. XXXII. Operation finished.
10 ³⁰			
11 ¹⁰	28.0	55.3	
11 ³¹ -11 ³⁵			
11 ⁴⁰	28.0	54.6	Stimulation of abdominal va- gus, coil distance 20 cms.
12 ¹⁰	28.0	49.0	
12 ⁴⁰	28.0	48.3	
1 ¹⁰	25.8	48.3	

Exp. XXXIV. November 15, 1923. Rabbit ♀. Body weight 1.8 kilos.

Time	Urea-N (mgrm. %)	Non-protein-N (mgrm. %)	Remarks
9 ⁵⁰			Tied up, operated as in Exp. XXXII. Operation finished.
10 ²⁵			
11 ⁰⁰	23.8	53.2	
11 ¹⁰ -11 ¹⁵			
11 ³⁰	23.8	52.5	Stimulation of abdominal va- gus, coil distance 20 cms.
12 ⁰⁰	23.8	53.9	
12 ³⁰	23.8	53.9	
1 ⁰⁰	23.8	54.6	

From these experiments we confirm as regards the changes in the blood urea- and non-protein nitrogen contents after ligating the blood vessels of the abdominal viscera, that the urea-nitrogen concentration appeared to have decreased very slightly, though not distinctly, when the blood supply in the portal system was excluded, a fact agreeing with the result of Mann's experiment, in which he extirpated the liver from a dog. The residual nitrogen value of the blood seemed to increase gradually in a very small degree.

But in another experiment, when the blood circulation of the abdominal organs was excluded, the urea- and residual nitrogen concentrations were found very slightly decreased. In Exp. XXXI, the portal circulation being excluded, the residual nitrogen did not show the least change, while the urea-nitrogen indicated, on the contrary, a tendency towards a slight and gradual increase. Thus the results were various. Under such circumstances, the vagus stimulation brings about no definite effects (Exps. XXXIII & XXXIV).

It is too hasty to decide that the viscera of the portal system, especially the liver, possesses no urea synthetic function because the blood urea-nitrogen content does not show any signs of decrease when the blood circulation of the portal system is excluded. The change in the blood urea-nitrogen content is affected by the functional condition of its forming organ as well as its eliminating apparatus. Contrary to the anticipation that, after the blood supply of the portal organs is removed, the urea-nitrogen value of the blood would decrease, there was in reality no decrease—if any, very insignificant—and sometimes even causing a suspicion that there was not increase at all. These, I believe, are swayed chiefly by the diuretic condition. This is what we always learn through our experiences, that after an abdominal operation, especially after such a complex operation as subjected to in this experiment, the diuretic function of the kidney is easily disordered and when the urine excretion of the kidney is retarded or stopped, in spite of the removal of the urea-synthetic organ, it is no wonder that the blood urea-nitrogen value is seen not decreased. Mann is not justified, when he judges the urea forming function of the liver simply from the fact that he observed no decrease of the blood urea-nitrogen concentration in a dog whose liver was plucked out. Much more so when opposition is raised by Magnus⁶⁸⁾ and Rich⁶⁹⁾ against tying up the liver vessels or applying an Eck's fistula for the purpose of excluding the liver function.

On the other hand if, as Folin⁶⁹⁾ and his co-workers as well as other investigators maintain, the muscles and other organs possess any possibility of synthesizing urea, however little, the blood urea-nitrogen concentration, depending on the renal function, does not only decrease but in some cases would rather increase, even though the portal system were perfectly removed. Fiske and Summer⁷⁰⁾ experimented on a cat and discovered that

68) Magnus, *Ergebn. d. Physiol.*, 1920, 18, 51.

69) Rich, *Bull. Johns Hopkins Hosp.*, 1923, 34, 321.

70) Fiske and Summer, *Journ. Biol. Chem.*, 1914, 18, 2.

if the vessels in the splanchnic area and the renal vessels were tied up, the urea content in the blood and muscles would be increased. One of my own experiments (Exp. XXXI) actually indicated a tendency of increasing, though indistinctly, as time went on.

Now to turn to the change of the total non-protein nitrogen value of the blood. In Exps. XXIX, XXX & XXXII it showed a gradual increase; in Exp. XXXIII and one other it rather decreased, while in Exps. XXXI and XXXIV no distinct change was noticed.

Generally speaking, the portal organs and especially the stomach and the intestine are not only the importing channel of these nitrogenous substances, but also perform a mass of many important functions. Among them is the pancreas, whose functional disturbance, together with certain endocrine disequilibrium, called forth by itself may exercise some influence upon the protein or the residual nitrogen metabolism, a fact which must be taken into consideration in this respect. Okada and Hayashi²⁵⁾ have pointed out that the hypo-function of the pancreas leads to an increase of the amino-acids content of the blood. My own experiment showed that an injection of insuline into a rabbit caused a remarkable decrease in the urea- and the residual nitrogen content of the blood (Part IV), while also Paulesco⁷¹⁾ observed a decrease of the urea content of the blood after the administration of pancreatic hormone. Marshall and Davis¹⁶⁾ demonstrated by extirpating the adrenal bodies an increase of the blood urea-nitrogen, while Addis and his co-worker⁵⁹⁾ observed, that if a proper amount of adrenaline be given, the urea-nitrogen concentration of the blood decreases and the urea excretion increases, whereas, pituitrine causes a decrease of the urea elimination. Also in my own experiment the blood urea-nitrogen concentration was lessened by giving adrenaline. It is also a well known fact that when the function of the thyroid gland, which has a close functional relation with the pancreas and suprarenal body, is either excited or inhibited, some change is brought on the nitrogenous exchange. Thus if the endocrine organs have each some influence on nitrogenous metabolism, the effect brought upon the nitrogen exchange by the functional disturbances of some of these organs with internal secretion, would not be insignificant.

71) Paulesco, cited by Strans in "Insulin," Berlin 1925, 62.

IV. THE EFFECT OF THE VAGUS STIMULATION UPON THE BLOOD UREA-NITROGEN CONCENTRATION IN SPLENECTOMIZED ANIMALS.

There are some who have discovered certain relations existing between the spleen and nitrogenous metabolism. In the early days Gréhan and Quinquaud⁵¹⁾ and Kaufmann⁵²⁾ found that the blood urea content of the splenic vein, together with that of the hepatic vein, was higher in amount compared with that of other venous blood. Nencki, Powlow and Zaleski⁴⁵⁾ believed that the ammonia content of the portal blood was lowest in the splenic vein, and that the ammonia content of blood in the pancreatic, mesenteric and gastric veins could be diluted by the blood of the splenic vein. Of late Nakayama⁷²⁾ observed that a normal dog given meat showed a remarkable increase of the basal metabolic rate, while in a spleenless dog given the same diet the rate of increase was much smaller. This means, he argued, that the spleen has something to do with the liver in the function of protein metabolism in an augmentative way. That the spleen and the liver are closely related can be comprehended from the opinion which Silvestrini⁷³⁾ expressed, when he saw some portion of the liver transformed into the lymphatic tissues after removing the spleen, declaring that this was a partial splenization of the liver.

If, as Nakayama⁷²⁾ supposed, the proteolytic function of the liver in a splenectomized animal would be reduced, the animal might react on the vagus stimulation with no increase of the blood urea-nitrogen concentration, as in case of a normal animal. To decide this question, the vagus stimulation experiment was performed on spleenless rabbits, the urea- and residual nitrogen content of the arterial blood being estimated.

Exp. XXXV. August 14, 1923. Rabbit ♀. Body weight 1.4 kilos.

Time	Urea-N (mgrm. %)	Non-protein-N (mgrm. %)	Amino-N (mgrm. %)	Remarks
11 ¹⁵				Tied up, extirpation of spleen.
11 ²⁵				Untied.
12 ⁵⁷				Tied up, cervical vagi on both sides severed, cannula inserted into carotid.
1 ¹²				Operation finished.
1 ³⁰	42.7	59.5	16.8	
2 ³⁰	42.7	59.5	16.8	
3 ³⁰	43.4	59.5	16.1	
4 ³⁰	42.7	59.5	16.8	

72) Nakayama, Biochem. Zeitschr., 1924, 149, 491.

73) Silvertrini, cited in Berichte ü. d. ges. Physiol., 1921, 5, 236.

Exp. XXXVI. October 14, 1923. Rabbit ♂. Body weight 1.6 kilos.

Time	Urea-N (mgrm. %)	Non-protein-N (mgrm. %)	Amino-N (mgrm. %)	Remarks
1 ⁴⁰				Tied up, splenectomized, abdominal vagi on the both sides severed, cannula inserted into carotid. Operation finished.
2 ⁰³				
2 ³⁰	28.7	58.1	29.4	
3 ³⁰	29.4	58.1	28.7	
4 ³⁰	28.7	58.1	29.4	
5 ³⁰	29.4	57.4	27.4	

Exp. XXXVII. July 13, 1923. Rabbit ♀. Body weight 1.3 kilos.

Time	Urea-N (mgrm. %)	Non-protein-N (mgrm. %)	Amino-N (mgrm. %)	Remarks
10 ⁰⁰				Tied up, operated as in Exp. XXXV. Operation finished.
10 ³⁵				
11 ³⁰	21.0	45.4	24.4	
11 ³⁶ -11 ⁴⁰				Stimulation of servical vagus, coil distance 20 cms.
11 ⁵⁰	23.8	46.2	22.4	
12 ⁴⁰	33.6	46.2	12.6	
1 ⁴⁰	30.1	46.8	16.7	
2 ⁴⁰	27.3	47.6	20.3	

Exp. XXXVIII. July 7, 1923. Rabbit ♀. Body weight 1.8 kilos.

Time	Urea-N (mgrm. %)	Non-protein-N (mgrm. %)	Amino-N (mgrm. %)	Remarks
8 ³⁵				Tied up, operated as in Exp. XXXVII. Operation finished.
10 ⁰⁵				
10 ²⁰	14.7	51.8	37.1	
10 ³⁶ -10 ⁴⁰				Stimulation of cervical vagus, coil distance 20 cms.
11 ⁰⁰	21.7	46.2	24.5	
11 ⁴⁰	22.4	31.5	9.1	
12 ⁴⁰	22.4	35.7	13.3	
1 ⁴⁰	20.5	35.7	15.2	

Exp. XXXIX. August 17, 1923. Rabbit ♀. Body weight 1.7 kilos.

Time	Urea-N (mgrm. %)	Non-protein-N (mgrm. %)	Amino-N (mgrm. %)	Remarks
10 ²⁵				Tied up, splenectomy. Untied. Tied up, cervical vagi on both sides severed, cannula inserted into carotid.
10 ⁴⁵				
11 ⁵⁰				

Time	Urea-N (mgrm. %)	Non-protein-N (mgrm. %)	Amino-N (mgrm. %)	Remarks
12 ⁵⁰ 12 ⁵⁶⁻¹⁰⁰	30.8	79.8	49.0	Stimulation of cervical vagus, coil distance 20 cms.
1 ¹⁰	39.2	79.8	40.6	
2 ⁰⁰	45.5	79.1	33.6	
3 ⁰⁰	45.5	81.2	35.7	
4 ⁰⁰	36.4	82.6	36.2	

In the first two experiments, the vagus was cut after the removal of the spleen, and during about 3 hours of binding the blood urea- and residual nitrogen content did not indicate any marked change, being within the limit of technical error. With these experiments as a control in the last three experiments the vagus was stimulated. In Exp. XXXVII the blood urea-nitrogen concentration was noted slightly increased after 10 minutes of the vagus stimulation, but not significantly. After one hour the increase was quite marked (the rate being 60%), afterwards gradually decreasing or beginning to recover, not restoring its original value, however, even after 3 hours had passed. On the other hand, the residual nitrogen concentration appeared to have slightly increased by the vagus stimulation, though not distinctly, going only a little over the limit of methodic error. The amino-acids fraction decreased as the urea-nitrogen concentration increased; the decrease marking about half in one hour after the vagus stimulation. In Exps. XXXVIII and XXXIX the course of those changes was also the same.

From this we know that a spleenless animal showed not only no increase of the non-protein nitrogen content by the vagus stimulation, but in some cases even a decrease, while the urea-nitrogen content indicated a remarkable increase to the same extent as in a spleen-healthy animal, thus suggesting a significant decrease of the amino-acids fraction. In a spleen-healthy animal the stimulation of the vagus caused an increase of the non-protein nitrogen concentration almost in parallel with that of the urea-nitrogen concentration.

This finding seems to show that the spleen-healthy animal is capable of supplementing the loss of the amino-acids fraction formed into urea, when the urea-nitrogen content is increased, while in a spleenless animal such ability is disturbed. In other words, the spleen possesses a certain function of making up the amino-acids fraction, lost by the increased formation of the urea coming through the vagus stimulation.

But the view that the spleen has an action of accelerating the proteolytic function of the liver as Nakayama⁷²⁾ thought, is worth considering. On taking meat the oxygen consumption should be augmented, when amino-acids absorbed through the intestinal wall leave the amino-radicles in the liver. As factors causing no or only slight increase in the basal metabolic rate in Nakayama's⁷²⁾ experiment with a spleenless animal when it is fed

on proteinic food, I can mention that the amino-acid would not reach the liver or even when it reaches the liver, it would pass out without desamination; or that the liver would detain the amino-acid that has reached there without decomposing it. If the sluice of the liver should allow these nitrogenous substances to pass without undergoing any change, splenectomy would probably bring about the same result as Eck's fistula. In my experiment a spleenless animal, as in case of a spleen-healthy one, had the urea-forming power accelerated when the vagus was stimulated. This means that the existence or non-existence of the spleen seems to have nothing to do with the urea-forming power. Probably the liver of a spleenless animal possesses the power of desaminating and forming urea on adequate stimulation as normal. Thus, we arrive at the conclusion that the spleen has no direct concern with the liver in these functions. If so, it follows that the supposition of the amino-acids being detained in the liver without any change must be denied.

From these considerations it is suggested that a spleenless animal is normal in its function of forming urea from amino-acids, but in it the process with which the protein introduced into the digestive canal is digested into amino-acids and absorbed, is disturbed, thus giving rise to an injured or retarded supply of amino-acid fraction.

In short, it has been demonstrated that in a spleenless animal, the vagus stimulation increases the blood urea-nitrogen content, as in a normal animal, but the increase of non-protein nitrogen does not go with it. Moreover, it can be concluded from the results of these experiments that even in case of the non-urea-residual nitrogen not increased under the vagus stimulation the urea formation is increased.

V. THE EFFECT OF THE VAGUS STIMULATION UPON THE BLOOD UREA-NITROGEN CONCENTRATION IN NEPHRECTOMIZED ANIMALS.

Besides being the eliminating exit of urea the kidney has sometime been considered as an organ forming urea. Addis and Watanabe⁵⁹⁾ have stated that at the time when the kidney is not excreting urine the blood urea content of the renal vein is much higher than that of the arterial blood. Nash and Benedict⁶⁷⁾ finding a large amount of ammonia contained in the renal vein, supposed that ammonia was formed in the kidney. Quinquaud,¹⁸⁾ from his observation of late that the blood urea content was increased when the splanchnics supplying the kidney were stimulated,

arrived at the conclusion, as stated before, that urea is formed in the kidneys and thus the urea-synthetic function is accelerated by the splanchnic stimulation. If urea is formed in the kidneys, after the nephrectomy is carried out, the increase of the blood urea concentration caused by the vagus stimulation must be greatly affected. The following experiments were performed in order to see what changes would be brought upon the blood urea- and the non-protein nitrogen concentration in a nephrectomized rabbit, when its vagus was stimulated. The nephrectomy was carried out with the extraperitoneal method. I examined beforehand what would be the rate of changes in the blood urea- and non-protein nitrogen concentration after bilateral nephrectomy.

Exp. XL. August 6, 1923. Rabbit ♀. Body weight 1.6 kilos.

Time	Urea-N (mgrm. %)	Non-protein-N (mgrm. %)	Amino-N (mgrm. %)	Remarks
9 ¹⁵				Tied up, nephrectomized. Released. Tied up, cervical vagi on both sides severed, cannula in- serted into carotid artery. Operation finished.
9 ⁴⁵				
11 ³⁰				
11 ³⁵				
1 ⁰⁰	39.2	61.8	22.6	
2 ⁰⁰	44.8	68.6	23.8	
3 ⁰⁰	48.3	71.4	23.2	
4 ⁰⁰	55.3	75.6	20.3	
Average changes per hour	+ 5.4	+ 4.6	- 0.77	

Exp. XLI. August 9, 1923. Rabbit ♀. Body weight 1.4 kilos.

Time	Urea-N (mgrm. %)	Non-protein-N (mgrm. %)	Amino-N (mgrm. %)	Remarks
10 ³⁰				Tied up, nephrectomized. Released. Tied up, cervical vagi on both sides severed, cannula in- serted into carotid artery. Operation finished.
11 ⁰⁰				
1 ¹⁵				
1 ²²				
1 ⁵⁰	30.8	66.5	35.7	
2 ⁵⁰	37.8	68.6	30.8	
3 ⁵⁰	47.6	67.9	20.3	
4 ⁵⁰	53.2	77.0	23.8	
Average changes per hour	+ 7.5	+ 3.5	- 4.0	

Exp. XLII. March 18, 1925. Rabbit ♂. Body weight 1.5 kilos.

Time	Urea-N (mgrm. %)	Non-protein-N (mgrm. %)	Amino-N (mgrm. %)	Remarks
8 ¹⁶				Tied up, cervical vagi on both sides severed, cannula inserted into carotid artery, laparotomized.
8 ³⁵				Operation finished.
8 ³⁵	25.9	61.6	35.7	
8 ⁵⁷ -9 ⁰⁵				Both kidneys extirpated.
9 ³⁰	39.4	61.8	25.4	
10 ⁰⁰	46.2	70.7	24.5	
11 ⁰⁰	51.8	78.4	26.6	
Average changes per hour	+ 10.2	+ 11.0	+ 0.6	

Exp. XLIII. June 23, 1923. Rabbit ♂. Body weight 1.5 kilos.

Time	Urea-N (mgrm. %)	Non-protein-N (mgrm. %)	Amino-N (mgrm. %)	Remarks
9 ⁴⁵				Tied up, nephrectomized.
10 ¹⁵				Released.
11 ¹⁵				Tied up, cervical vagi on both sides severed, cannula inserted into carotid artery.
11 ²⁵				Operation finished.
11 ⁵⁵	27.3	63.0	35.7	
12 ⁰¹ -12 ⁰⁵				Stimulation of cervical vagus, coil distance 20 cms.
12 ²⁰	27.3	72.1	44.8	
1 ⁰⁵	31.5	74.1	42.6	
2 ⁰⁵	45.5	77.7	32.2	

Exp. XLIV. June 21, 1923. Rabbit ♀. Body weight 1.7 kilos.

Time	Urea-N (mgrm. %)	Non-protein-N (mgrm. %)	Amino-N (mgrm. %)	Remarks
9 ³⁰				Tied up, nephrectomized, cervical vagi on both sides severed, cannula inserted into carotid artery.
10 ¹⁰				Operation finished.
10 ³⁰	23.8	44.8	21.0	
10 ³⁶ -10 ⁴⁰				Stimulation of cervical vagus, coil distance 20 cms.
11 ⁰⁰	23.8	53.9	30.1	
11 ³⁵	23.8	54.6	30.6	
12 ²⁵	25.2	56.7	31.5	

Exp. XLV. June 15, 1923. Rabbit ♂. Body weight 1.9 kilos.

Time	Urea-N (mgrm. %)	Non-protein-N (mgrm. %)	Amino-N (mgrm. %)	Remarks
10 ³⁰ 11 ⁰⁵ 12 ¹⁰				Tied up, nephrectomized. Released. Tied up, cervical vagi on both sides severed, cannula in- serted into carotid artery.
12 ⁴⁰ 12 ⁴⁶ -12 ⁵⁰	36.1	40.6	4.5	Stimulation of cervical vagus, coil distance 20 cms.
1 ⁰⁰ 1 ⁵⁰ 2 ⁵⁰ 3 ⁵⁰	36.1 37.1 37.1 36.4	40.6 56.0 61.8 60.4	4.5 18.9 24.7 24.0	

In Exp. XL after the bilateral nephrectomy the blood urea-nitrogen content commenced to increase gradually, and between 2 hours and 45 minutes and 5 hours and 45 minutes, its average increase per hour was 5.4 mgrm. %, and also the non-protein nitrogen showed, on an average, an increase of 4.6 mgrm. % per hour, indicating in consequence a very slight decrease of amino-acids fraction. In Exp. XLI, between the 3rd hour and the 6th hour after the removal of both kidneys the blood urea-nitrogen content increased on an average by 7.5 mgrm. % per hour and the non-protein nitrogen about 3.5 mgrm. %, the amino-acids fraction value decreasing significantly, averaging 4.0 mgrm. % per hour. Thus we see that in such a stage after the nephrectomy, the increasing rate of the urea-nitrogen content is much greater than that of the non-protein nitrogen, while the amino-acids fraction showed a tendency rather to decrease. In Exp. XLII, soon after the nephrectomy what had made a speedy increase was urea-nitrogen content, non-protein nitrogen value showing no change; the amino-acids fraction decreased greatly during this period. During the course of another hour and 30 minutes the urea-nitrogen content increased with an average rate of 10.2 mgrm. % per hour and the non-protein nitrogen with a rate of 11.0 mgrm. %. The amino-acids fraction showed no distinct changes.

In Exp. XLIII the urea-nitrogen content kept the same value 15 minutes after the vagus stimulation; after one hour the increase was no more than 4.2 mgrm %, in another hour an increase of 14.0 mgrm. % was seen. The amino-acids fraction increased 9.1 mgrm. % 15 minutes after the stimulation, and after an hour a slight decrease was noted, but still higher than the original value; in 2 hours, when the urea-nitrogen value made a sudden increase, it began for the first time to decrease lower than the original value. In Exp. XLIV the urea-nitrogen content did not increase even 55 minutes after the vagus stimulation; in another hour only a slight increase was noticed, while the amino-acids fraction increased 9.1 mgrm. % 20 minutes after the stimulation of the vagus. In Exp. XLV the urea-nitrogen content showed a very slight increase, or no change. The amino-acids fraction, however, was seen to increase very distinctly.

In comparing these results with those of Exps. XLI & XLII we find that the urea-nitrogen value does not only increase under the vagus stimulation, but that even the natural increase observed in the control experiments seems to have been checked. On the other hand, the amino-acids frac-

tion makes a significant increase. In a kidney-healthy animal, when the vagus is stimulated, the increase of the non-protein nitrogen chiefly depends on the increase of the urea-nitrogen content, but in a kidneyless animal the former is not due to the increase of the urea-nitrogen, but to that of the amino-acids fraction. At the time when the effect of the vagus stimulation is thought weakened, the once increased amino-acids fraction seems to make a sudden and rapid decrease, while the urea-nitrogen rapidly increases. That is to say, that in case of a nephrectomized animal, the amino-acids fraction increased by the vagus stimulation is not immediately decomposed into urea, but even the natural increase of urea due to the absence of the kidneys is for a time suspended, or in other words, the vagus stimulation rather seems to act inhibitorily upon the urea increasing process. In a normal animal, if the vagus is stimulated, the amino-acids fraction is also increased, and this increased amino-acids fraction is at once desaminated and synthetized into urea. In a nephrectomized animal the amino-acids fraction, increased by the vagus stimulation is not so rapidly decomposed into urea as in a normal animal. The fact that in a kidney-less animal the increase of the urea-nitrogen is for a time arrested by the vagus stimulation, may be explained by the supposition here that the vagus stimulation brings an increased formation of the amino-acids fraction, but at the same time brings directly an inhibition of the urea formation in the liver, or that indirectly the function of a certain unknown organ, which inhibits the urea formation, is acting in a way antagonistic to the hypothetical function of the kidney to augment its desaminating power. But this is only a hypothesis which requires further study.

VI. THE UREA-NITROGEN CONCENTRATION OF THE AFFERENT AND EFFERENT BLOOD OF THE LIVER.

The view that the liver is the most important organ for the formation of urea is generally accepted. On going over the experimental proofs of those authors who do not recognize this fact, Taylor and Lewis,⁵⁰⁾ Löffler,³⁹⁾ Gottschalk and Nonnenbruch⁶¹⁾ and others seem to base their views on their observation that the blood urea-nitrogen concentration in the hepatic vein, compared with that of other venous and arterial blood shows no difference or at most a very slight increase. But Gréhant and Quinquaud,⁵¹⁾ Kaufmann⁵²⁾ and others find the former much greater than the latters. How is it that the observations are so different in spite of the same sort of experiments carried out?

I have also had similar experience. If the liver were an urea-forming organ superior to the other organs, the urea-nitrogen content of the blood of the liver vein must be greater than that of any other veins, and after the vagus stimulation it must greatly increase. Upon this assumption I estimated the urea- and residual nitrogen content in the blood of the hepatic and the portal vein as well as in the arterial. But I found that it was very variable and unsettled in those blood vessels changing even by mild treatments, so to my great regret it was utterly impossible to carry on the experiments to observe the effect of vagus stimulation upon them.

A rabbit was slightly anaesthetized with ether, and after its epigastrium had been cut open, the abdominal organs, especially the liver, were covered with cloths soaked in body-warm Ringer's solution, thus seeing that they got all possible protection. In collecting blood the liver was pressed lightly downward, and first the liver vein, then the portal vein were punctured with clean injection-syringes. (Unless the punctured aperture of the portal vein is cleverly closed with a clamp it might cause a haemorrhage of large quantity and cause the experiment to end in failure.) After the puncture, the liver and other abdominal organs were protected as before. Lastly a sample of the arterial blood was taken from a cannula inserted into the femoral or carotid artery. All the blood samples can be obtained, if fortunately carried out, in from 2 to 5 minutes.

Of the results of the experiments, described in Table I, I do not include those which had large haemorrhages from the puncture of the liver or the portal vein. After an interval of from 10 to 70 minutes the second blood collection was made.

Table I.

Exp. No.		I.			II.		
Rabbit: sex, body weight (kilos)		♀ 1.7			♀ 1.7		
		Urea-N (mgrm. %)	Non-pro- tein-N (mgrm. %)	Amino-N (mgrm. %)	Urea-N (mgrm. %)	Non-pro- tein-N (mgrm. %)	Amino-N (mgrm. %)
First blood samples	Hepatic vein	19.6	—	—	15.4	26.2	10.8
	Portal vein	19.6	—	—	14.0	33.8	19.8
	Artery	—	—	—	—	—	—
Intervals (minutes)		40			52		
Second blood samples	Hepatic vein	19.6	—	—	14.0	33.8	19.8
	Portal vein	19.6	—	—	14.0	39.6	25.6
	Artery	—	—	—	—	—	—

Exp. No.		III.			IV.		
Rabbit: sex, body weight (kilos)		♂ 1.5			♂ 1.8		
		Urea-N (mgrm. %)	Non-pro- tein-N (mgrm. %)	Amino-N (mgrm. %)	Urea-N (mgrm. %)	Non-pro- tein-N (mgrm. %)	Amino-N (mgrm. %)
First blood samples	Hepatic vein	14.2	32.2	18.0	30.8	35.0	4.2
	Portal vein	14.2	49.6	35.4	18.2	23.8	5.6
	Artery	14.2	32.2	18.0	—	—	—
Intervals (minutes)		70			60		
Second blood samples	Hepatic vein	25.2	46.2	21.0	21.0	46.2	25.2
	Portal vein	25.2	61.2	26.0	21.0	35.0	14.0
	Artery	25.2	42.0	16.8	22.4 (clott)	28.0 (clott)	5.6

Exp. No.		V.			VI.		
Rabbit: sex, body weight (kilos)		♂ 1.6			♂ 1.5		
		Urea-N (mgrm. %)	Non-pro- tein-N (mgrm. %)	Amino-N (mgrm. %)	Urea-N (mgrm. %)	Non-pro- tein-N (mgrm. %)	Amino-N (mgrm. %)
First blood samples	Hepatic vein	28.0	42.0	14.0	19.6	28.0	8.4
	Portal vein	16.8	35.0	18.2	11.2	25.2	13.0
	Artery	21.0	25.2	4.2	14.0	26.6	12.6
Intervals (minutes)		31			10		
Second blood samples	Hepatic vein	21.0	35.0	14.0	14.0	30.8	16.8
	Portal vein	19.4	40.2	20.8	11.2	32.2	21.0
	Artery	14.0	32.2	18.2	15.4	32.6	17.2

Exp. No.		VII.			VIII.		
Rabbit: sex, body weight (kilos)		♀ 1.6			♀ 1.6		
		Urea-N (mgrm. %)	Non-pro- tein-N (mgrm. %)	Amino-N (mgrm. %)	Urea-N (mgrm. %)	Non-pro- tein-N (mgrm. %)	Amino-N (mgrm. %)
First blood samples	Hepatic vein	26.6	37.8	11.2	19.6	33.6	14.0
	Portal vein	16.8	32.2	15.4	11.2	36.4	25.2
	Artery	16.8	28.0	11.2	11.2	28.0	16.8
Intervals (minutes)		32			42		
Second blood samples	Hepatic vein	20.8	42.0	21.2	12.6	37.8	25.1
	Portal vein	20.8	42.0	21.2	12.6	39.2	26.6
	Artery	20.8	32.2	11.4	12.6	39.2	26.6

In comparing the urea-nitrogen concentration and the amino-acids fraction in the blood obtained from the hepatic vein, the portal vein and the artery, it was found that of the first collections of blood Exps. I and III showed no difference in the urea-nitrogen content, in Exps. II, IV, V, VI, VII and VIII, the urea-nitrogen concentration of the hepatic vein was seen more or less greater than that of the portal vein and the artery, and that of the portal vein blood was almost equal to that of the arterial blood or little more. In the first blood samples from the three vessels, the four experiments (Exps. V, VI, VII & VIII) showed more or less an increase of the urea-nitrogen content, giving an average value of the urea-nitrogen and the non-protein nitrogen as follows:

Table II.

Source of Blood	Hepatic vein	Portal vein	Artery
Urea-N (mgrm. %)	23.45	14.0	15.75
Non-protein-N (mgrm. %)	35.35	32.2	26.95
Amino-N (mgrm. %)	11.90	18.2	11.2

The blood urea-nitrogen content of the hepatic vein showed about 49% greater than that of the portal vein, while that of the portal vein was found a little less than that of the artery or almost the same as it.

Nencki, Pawlow and Zaleski,⁴⁵⁾ Biedl and Winterberg,⁵³⁾ Folin and Denis⁶⁴⁾ observed that the ammonia content of the blood of the portal vein was much greater than that of any other. Barnett and Addis⁶⁶⁾ agreed, seeing the increase of the blood ammonia much retarded when the urea was intravenously injected into the animal with its circulation in the intestinal tract excluded, that the intestinal tract plays a very important rôle in decomposing urea into ammonia. It is a fact beyond any doubt that the blood is more or less diluted in the portal system, and this evidence forms also a base to explain that the urea content of the arterial blood is reduced in the portal vein.

Now turning to the second collection of blood we find that only in Exps. VI & VII the blood urea-nitrogen content in the hepatic vein was greater than that of the portal vein; in all of the other experiments there was no difference demonstrable. In all experiments, except Exps. VI & VII, the blood urea-nitrogen concentration in the artery, portal and hepatic veins are nearly the same, as in the first blood collection of Exps. I, II & III.

As to the amino-acids fraction, in all of the experiments but Exp. V it is found to be the highest in the blood of the portal vein. It is of interest that in Exp. VIII, at the second collection of blood, there was no difference in the amino-acids fraction noted in all the bloods.

Thus, the analysis of the first blood sample in Exps. I, II & III and the second ones in almost all experiments did not yield evidence that the formation of urea takes place in the liver, based on the blood urea-nitrogen concentration. It is proper to think, from this fact that at the time of the second collection of blood the urea-forming function in the liver was already lost, so also at the first collection in Exps. I, II & III, showing that even under our careful treatment this function of the liver is easily disturbed. None of the urea- and the non-protein nitrogen contents in all the blood

samples agrees with one another at the time of the first and second collection of blood; sometimes (second blood samples of Exp. VIII) even showing no difference either in the urea- nor in the non-protein nitrogen contents between the three blood samples.

Discrepancy of the views with regard to the urea formation in the liver may be partly attributed to the delicate character of the liver function, at least so far as the urea synthesis is concerned, which seems to be easily disturbed by even mild experimental treatments. But from the experimental data it seems probable that the liver is one of the most important organs for the formation of urea.

As to the vegetative nerve supply in the liver there are many reports besides those of sympathetic accelerating and parasympathetic inhibiting innervations in relation to glycogen decomposition. Eigar⁷⁴⁾ saw the increase of the gall secretion when the vagus was stimulated in the thoracic cavity and Heidenhain⁷⁵⁾ noticed that atropine arrested the secretion of gall. Junkersdorf⁷⁶⁾ observing the existence of protein in a muddy mass in the parenchyma cells of the liver called it a "protein-depot" in the organ. This protein-mud was lost sight of when adrenaline was given to the animal (Stübel⁷⁷⁾). Rossi⁷⁸⁾ inferred that when atropine is injected into an animal or the vagus cut, the liver undergoes regular changes microscopically seen; the liver cells decreasing in volume and Benda's granules increasing in number. Thus the liver seems to be doubly innervated by the sympathetic and parasympathetic nerves, but how does it undergo the nervous control towards desamination and urea synthesis, remains still unknown, and is a problem very difficult to investigate.

On the other hand, it is a question whether the liver is a depot for urea, or its antecedent amino-acid or ammonia. It has already often been described that the urea content in the liver tissue is not so much increased as that in the tissues of the other organs or the blood (Gscheidlen,⁵⁰⁾ Marshall and Davis,¹⁶⁾ Inada⁷⁹⁾). It is questionable, whether we can draw an inference that an organ is neither an important urea synthetic organ nor a depot for urea, because of the fact that the urea content in its tissue is not seen to increase. Van Slyke and Meyer⁴⁹⁾ observed that

74) Eigar, *Zeitschr. f. Biol.*, 1915, **66**, 229.

75) Heidenhain, cited by Greving in Müller's "Die Lebensnerven," Berlin 1924, 284.

76) Junkersdorf, *Pflüger's Arch.*, 1921, **186**, 238.

77) Stübel, *Pflüger's Arch.*, 1920, **185**, 84.

78) Rossi, cited in *Berichte u. d. ges. Physiol.*, 1922, **9**, 72.

79) Inada, *Fukuoka-Ikadaigaku-Zasshi*, 1918, **11**, 437 (Jap.).

after the administration of amino-acid, the liver takes in the amino-acid more quickly and in a larger amount than the muscles, and that when the amino-acid content decreased in the liver the urea content of the blood increased. Against this view Folin⁶⁰⁾ asserted that the liver's taking in of a great quantity of amino-acid never constitutes a proof of its being an urea-forming organ. Inada⁷⁹⁾ saw in a normal rabbit after examining the non-protein nitrogen content in its various organs and its blood, that it was least in blood, next came the brain, and that the muscles contained the largest quantity. Besides these organs, little difference was noted in the liver, spleen, kidney, intestine and lung.

Considered from my comparison of the urea- and the non-protein nitrogen contents in the blood going in or out of the liver, experiments in which the blood urea content of the hepatic vein was found much more increased than that of the portal vein, or in other words, that which could prove the energetical urea formation in the liver showed the total nonprotein nitrogen content of the hepatic vein much higher in value than that of the portal vein. Exps. IV, V, VI and VII belonged to this class. Of these Exp. IV showed that the non-protein nitrogen of the hepatic vein was 35.0 mgrm. % and the urea-nitrogen 30.8 mgrm. %, while the portal vein showed only 23.8 mgrm. % of non-protein nitrogen and 18.2 mgrm. % of urea-nitrogen, indicating that the urea-nitrogen content of the blood in the hepatic vein was much higher in value than the non-protein nitrogen of the blood in the portal vein. In the second blood sample, in spite of the urea-nitrogen content being almost of the same value in all the vessels, and of the amino-acids fraction value being increased in the portal vein, the non-protein nitrogen in the hepatic vein so increased that it stood 46.2 mgrm. % against 35.0 mgrm. % of the portal vein. In this experiment as well as in Exps. V, VI & VII, the water detaining function in the liver is not sufficient to explain the high value of the non-protein nitrogen in the hepatic vein. In the experiments where the blood urea-nitrogen content in the hepatic vein neither showed any distinct increase nor increased at all, compared with that of the portal vein, that is, the liver did not indicate any evidence of urea formation process, the non-protein nitrogen content of the portal vein was found to be of higher value than that of the hepatic vein, or similar to it. In all of those in the second collection of the blood it was noted that the non-protein nitrogen content of the portal vein was much higher than that of the hepatic vein. In Exp. VIII the first blood sample showed a significantly greater content (75% greater) of the urea-nitrogen in the hepatic vein in comparison with that in the portal

vein, but the non-protein nitrogen in the portal vein higher than that in the hepatic vein. Some showed the amino-acids fraction in the arterial blood greater than that in the hepatic vein (the first and second blood samples of Exps. VI & VIII and the second blood samples of Exp. V), while there is no instance in which the urea-nitrogen content of arterial blood was higher than that of hepatic venous blood.

These mutual relations being considered, it must be recognized that the liver occupies a very important position in urea formation. It appears, moreover, to possess the function of detaining the amino-acids fraction or at the same time the urea-nitrogen. It is understood that urea is sometimes formed directly from the amino-acids fraction of the portal vein and sent to the hepatic vein.

A greater part of the amino-acid fraction imported into the liver is absorbed through the portal system, probably by the digestive tract, and the amino-acids which form some part of the amino-acids fraction will be partly reformed into protein bodies in the liver, must be contained. With regard to the position of the organs other than the liver and the portal system, for instance, like the muscles, as the source of non-urea-residual nitrogen, it is demonstrated in Exps. VI & VIII that the amino-acids fraction of the arterial blood is greater than that of the hepatic vein. But its difference is so meagre that it hardly goes over the limit of technical error. When we recollect that Inada⁷⁹⁾ observed that a large quantity of non-protein nitrogen is contained in the muscular tissues, and that according to Van Slyke and Meyer's⁴⁹⁾ experiments, the amino-acid is taken up first by the liver quickly and in a large quantity, and then by the muscular tissue, it appears we can not think lightly of the muscle as a depot for non-protein nitrogen. But evidence brought out by my experiments that the arterial blood is very low not only in its urea-nitrogen content but also in its total non-protein nitrogen content, compared with those of the hepatic vein, seems to show that the muscle plays no big rôle in the normal nitrogen metabolism.

It would be interesting to assume that the amino-acids or similar nitrogenous substances having passed through the gate-way of the liver are taken in and embraced by the muscle tissue. Some of my experiments and also those of Van Slyke and Meyer's⁴⁹⁾ stand in accordance with this hypothesis.

The regulation of the absorption in the digestive tract, especially in the intestine, by the vegetative nervous system, lacks clearness. Reid⁸⁰⁾

80) Reid, Journ. Physiol., 1896, 20, 298.

infers that atropine has an inhibitory action upon the amino-acid absorption of the intestines, an argument supported by Nakamura.⁸¹⁾ But then they could detect no effect from pilocarpine and adrenaline. Concerning the absorptive function of the intestines, Oshima⁸²⁾ insists that pilocarpine and vagus stimulation augment the absorption of water and salt in the intestines, and that atropine and splanchnic stimulation depress it. Thus it seems that the absorption in the intestine is increased by the excitement of the parasympathetics, while it is inhibited by their paralysis or by the excitement of the sympathetics.

On the other hand the secretion of the pancreatic juice is depressed by atropine (Pawlow,⁸³⁾ Mett,⁸⁴⁾ Kudrewetzki,⁸⁵⁾ Modraskowski,⁸⁶⁾ Bylina,⁸⁷⁾ Smirnow⁸⁸⁾ and Wertheimer and Lepage⁸⁹⁾ and is increased by the vagus stimulation and pilocarpine (Babkin and Sawitsch,⁹⁰⁾ Camus and Gley⁹¹⁾). We must consider at this junction the effect given by pilocarpine or vagus stimulation upon the vessels in the intestinal wall. Contrary to Dixon and Brodie's⁹²⁾ observation that vasoconstriction was brought out in the isolated intestine by pilocarpine, Koennecke⁹³⁾ showed that the blood vessels of the intestines were dilated by vagus stimulation and contracted by sympathetic excitement.

Briefly speaking, several intestinal functions are accelerated by parasympathetic excitement and the sympathetics stand against them. An inference may be drawn out of this that under the vagus stimulation the residual nitrogen, especially the non-urea-residual nitrogen content of the blood in the portal vein is first increased, that of the arterial blood is then increased.

Another source thought to affect the blood urea-nitrogen content is the

81) Nakamura, *Tohoku Journ. Exp. Med.*, 1924, 5, 29.

82) Oshima, *Hokkaido-Igaku-Zasshi*, 1925, 3, 111 (Jap.).

83) Pawlow, *Pflüger's Arch.*, 1878, 13; cited in Müller's "Die Lebensnerven," Berlin 1924, 271.

84) Mett, cited in Müller's "Die Lebensnerven," Berlin 1924, 271.

85) Kudrewetzki, cited in Müller's "Die Lebensnerven," Berlin 1924, 271.

86) Modraskowski, *Pflüger's Arch.*, 1906, 114, 487.

87) Bylina, cited by Gloss in "Die Erkrankungen d. Pankreas," Berlin 1924, 17.

88) Smirnow, *Pflüger's Arch.*, 1912, 147, 234.

89) Wertheimer and Lepage, cited by Gloss in "Die Erkrankungen d. Pankreas," Berlin 1924, 17.

90) Babkin and Sawitsch, *Zeitschr. f. physiol. Chem.*, 1908, 56, 321.

91) Camus and Gley, *Arch. intern. de Physiol.*, 1913, 13, 102.

92) Dixon and Brodie, *Journ. Physiol.*, 1904, 30, 485.

93) Koennecke, *Klin. Wochenschr.*, 1922, 1262.

urea eliminating organ, which is very important. Urea is excreted not only in the kidney but it is often contained thickly in many other secrets, so when we discuss the change of blood urea-nitrogen content as a subject we must take gastro-intestinal juice, tear, sweat and saliva into consideration. It is hardly believed that in the rabbit the changes in diuresis and in other secretions caused by the stimulation and paralysis of the vegetative innervation have any marked effect upon the blood urea-nitrogen concentration.

It may be thus presumed that the increase of the blood residual nitrogen content brought about by the vagus stimulation is due partly to the increase of the nitrogenous substances in the portal blood owing to the accelerated digestive and absorptive function of the intestinal tract.

As seen in my starvation test, as well as others, the blood urea-nitrogen content gradually decreases from the day of repletion and on the second or third starvation day it reaches the height of its decrease. This stage seems to indicate the famine of the residual nitrogen depot in the liver and portal system, and the gradual increase afterwards is probably due to the mobilization of amino-acid stored up in the muscle, or to the decomposition of the endogenous protein. This is a production of so-called starvation protein decomposition. According to Eppinger and his co-workers the starvation protein decomposition seems to be accelerated by the thyroid and adrenals. Of adrenaline, I am in great doubt from my own experiments as to whether it possesses the function of accelerating the mobilization of nitrogen substances stored up in the muscle and other organs or endogenous protein substances even in a normal rabbit. There remains much room for study concerning these nitrogenous substances.

CONCLUSIONS.

1. When the peripheral stump of the vagus is stimulated or the parasympathetic stimulants, such as pilocarpine and choline, are administered the blood urea- and residual nitrogen contents are increased. On the contrary, if atropine is given, these nitrogen substances decrease more or less. If atropine is used together with pilocarpine the augmentative action of the latter is arrested. The stimulation of the central stump of the vagus lessens the blood urea- and residual nitrogen contents, in opposition to the stimulation of the peripheral stump of the nerve.

2. After the injection of adrenaline, the stimulation of the splanchnics or piqure, the blood urea-nitrogen and the residual nitrogen contents

rather show a tendency to decrease.

3. When a rabbit with its spleen extirpated is stimulated in the vagus, the blood urea-nitrogen increases while the blood residual nitrogen content does not increase, or rather decreases.

4. When the vagus of nephrectomized animals is stimulated, the residual nitrogen content of the blood increases, but the urea-nitrogen content does not, and the amino-acids fraction increases.

5. The blood of the liver vein contains apparently more urea-nitrogen than that of the portal vein or the artery. In opposition to this, the blood of the portal vein contains a much greater quantity of amino-acids fraction than that of the liver vein or the artery.