LXXXVI. THE PRODUCTION OF AMMONIA BY SURVIVING KIDNEY TISSUE.

PRELIMINARY PAPER.

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SINCE the publication of the work of Nash and Benedict [1921] it has been realised that the production of ammonia for the neutralisation of acid glomerular filtrates is one of the functions of normal kidney tissue. The precursor of this ammonia is not known, but it is certainly not urea. Failure of the ammonia-producing mechanism occurs in certain types of nephritis, and in the urine of such cases the ratio of acid to ammonia is very much higher than in normal urine [Henderson and Palmer, 1915].

The production of ammonia by excised kidney tissue, however, has so far received but little attention, and most of the work that has been done has been concerned with the autolytic production of ammonia, in experiments extending over several days.

It has been shown for other tissues [Warburg, Posener and Negelein, 1924; Meyerhof, Lohmann and Meyer, 1925; Loebel, 1925] that the presence of oxygen increases the rate of ammonia production, but in the absence of oxygen the production still continues to some extent. The effect of aerobic and anaerobic conditions upon the production of ammonia by kidney tissue was therefore first investigated.

Rat (or occasionally rabbit) kidneys were removed as soon as possible after death, chopped finely, washed twice in Ringer's solution, and then suspended in Ringer's solution and aerated with air for about 2 hours. More constant and satisfactory results were obtained after addition of glycine when the tissue had been aerated previously, and it was for this reason that preliminary aeration was carried out in all the experiments described in this paper. For estimating the anaerobic ammonia production the re-washed tissue, after filtration, was weighed (0.4 g. was usually taken) and tipped into a boiling-tube containing 1 cc. 0.1 *M* potassium phosphate (p_H 7.2) and 2 cc. Ringer's solution, or 1 cc. Ringer's solution and 1 cc. of substrate dissolved in Ringer's solution. The boiling-tube was fitted with a rubber stopper and an

outlet tube for evacuation, and small tubes containing alkaline hydrosulphite were suspended inside the boiling-tube to absorb any traces of oxygen. The rubber stopper was waxed thoroughly and the tube evacuated and incubated in the hot room at 37° for 3 hours.

The aerobic ammonia production was estimated after the tissue had been shaken in a Barcroft differential manometer at 37° for 3 hours, the amount of tissue taken and the media employed being similar to those described for the anaerobiosis experiments.

At the end of 3 hours 1 cc. of metaphosphoric acid (or 1 cc. 2 *N*/3 sulphuric acid and 1 cc. sodium tungstate solution) was added to the contents of the anaerobic tubes or Barcroft cups to precipitate proteins, and left overnight. The next day the contents of the tubes or cups were ground with further addition of metaphosphoric acid, and were filtered with repeated washing of the precipitates. The ammonia was estimated on a portion of the filtrate by Holmes and Watchorn's [1927] modification of Stanford's [1923] method.

The amounts of ammonia obtained were ample for estimation by this method, which was known to be a reliable one. All solutions used were free from ammonia.

Table I. *Ammonia production by kidney tissue anaerobically and aerobically.*

Resting	Values in mg. NH ₃ -N	
	Anaerobic	Aerobic
0.01	0.033	—
0.01	0.024	—
0.01	0.025	—
0.0069	0.038	0.115
0.0258	—	0.091
—	0.034	0.093
—	0.045	0.077
—	0.028	0.120

Duration of experiments—3 hours.

We always obtained a small production of ammonia anaerobically, and a larger production aerobically, as shown by the examples given in Table I. In unwashed and non-aerated tissue the aerobic production was larger than in the examples shown, and it is quite possible that the anaerobic production would also be larger under these circumstances.

When small amounts of glycine were added to the medium (final concentration 0.02 *M*) the ammonia production was much increased under aerobic conditions.

Table II. *Ammonia production in the presence of glycine.*

Values in mg. NH ₃ -N			
Anaerobic control	Anaerobic + glycine	Aerobic control	Aerobic + glycine
0.0275	0.0285	0.0800	0.200
0.0280	0.0302	0.1200	0.221
0.034	0.046	0.093	0.211
0.045	0.052	—	—
0.076	0.075	—	—
—	—	0.093	0.120
—	—	0.078	0.135

Under anaerobic conditions there was no increased ammonia production in the glycine-containing medium (Table II). The presence of oxygen was obviously necessary before the reaction could take place, and we were therefore inclined to assume that the increased ammonia formation in air represented an oxidative deamination of the glycine.

In order to obtain the ratio of the extra ammonia production from glycine to the extra oxygen required for deamination, measurements of oxygen uptake were made in the differential Barcroft apparatus. The ammonia was afterwards estimated in the contents of the Barcroft cups. We now found that the increased ammonia production was not necessarily accompanied by an increased oxygen uptake. Sometimes there was a slightly increased uptake, but this was in no way comparable with the extra amount of ammonia formed.

Atmospheric oxygen is therefore not directly used in the formation of the extra ammonia which appears when glycine is added, although the reaction does not proceed in its absence. It is possible that deamination of the glycine might occur as a secondary reaction, as the result, for instance, of the formation of hydrogen peroxide by the tissue, and without the uptake of further oxygen being necessary. Two experiments were made in which liver catalase was added to the preparations, but the ammonia formation was not inhibited thereby. The kidney tissue itself contains a great deal of catalase, so that it is possibly very difficult to alter the course of any reaction by the addition of more enzyme from outside sources. We cannot therefore draw any conclusions as regards the mechanism of the ammonia formation with glycine.

On the assumption that the increased production of ammonia was due to the deamination of the glycine, we have attempted to identify the deaminated products, but so far with complete lack of success. Comparison was made by Hagedorn and Jensen's [1923] method of the reducing values of the control preparations and of those containing glycine. The tissue was precipitated with tungstic acid, as this was found not to affect the estimations if neutralised before these were carried out. Ammonia was estimated in a portion of the same filtrate. It was found that ammonia formation was not accompanied by any increase in the reducing power of the filtrate. Control tests suggest that the method might fail to detect small amounts of acetaldehyde, and we have not yet made a more definite attempt to identify this possible product.

The possibility that a volatile acid, formic, acetic or glyoxylic acid, might be formed from the glycine was next considered. Larger amounts of tissue than usual were taken for these experiments, and were ground and precipitated with metaphosphoric acid after shaking at 37° for 3 hours in the appropriate media. Small portions of the filtrate were used for ammonia estimations.

To the main bulk of the filtrates excess of washed silver oxide suspended in water was added in order to remove the chlorides present. The solution was made strongly acid by the addition of H_2SO_4 , and filtered into a small

Claisen flask. A few drops of caprylic alcohol were added to prevent frothing, and a stream of air was sucked through for 20 minutes to get rid of any excess CO_2 that might be present in any of the samples. Finally, distillation *in vacuo* at about 60° was carried out, but we could never find any extra volatile acids in the preparations containing glycine. By the method used it was possible to estimate 1 cc. of 0.01 *N* acetic acid, and, judging by the ammonia produced in the glycine media, about twice this amount of extra volatile acid might have been expected.

We could conclude from these experiments that volatile acids other than CO_2 were not formed.

The estimation of CO_2 was carried out in the Barcroft cups by having two small glass tubes hooked on to the sodium hydroxide pots. One of these contained a small piece of filter-paper saturated with 10 % KOH to absorb the CO_2 produced and the other was half-filled with saturated oxalic acid. The tissue was shaken in the apparatus in the usual way for about an hour, the oxygen uptake being measured, and then the two small tubes were upset by gentle tapping and shaking was continued for 5 minutes. All the CO_2 absorbed by the KOH or dissolved in solution was driven off and the amount could be measured. In seven out of eight cases there was more CO_2 produced in the presence of glycine than by the tissue alone, but there was no good agreement between the extra CO_2 and the ammonia production, assuming that glycine is completely oxidised. These facts, which will be further investigated, suggest that part of the glycine may be broken down completely.

Effect of glucose and cyanide on ammonia production by kidney tissue.

Warburg, Posener and Negelein [1924] working with brain and carcinoma tissue, and Meyerhof, Lohmann and Meyer [1925] working with diaphragm, found that the aerobic production of ammonia was depressed by the addition of glucose. György and Röthler [1927] considered that the anaerobic production of ammonia by kidney tissue was also depressed by glucose, but they relied upon the addition of a little chloroform to maintain anaerobic conditions in their preparations. We tried the effect of added glucose upon the aerobic and anaerobic ammonia-producing systems of kidney tissue in order to discover whether they were both equally affected, or whether they could be distinguished from each other by the action of glucose upon them. The glucose was dissolved in Ringer's solution, various concentrations being used at different times, and 1 cc. of the solution was added to the Barcroft cup or anaerobic tube.

We found that glucose had an inhibiting action only on the aerobic production of ammonia. In some cases, where the concentration of glucose was high enough (0.02 *M*), the ammonia production was stopped entirely, so that the ammonia value for the aerobic tissue in the presence of glucose was exactly the same as that of the anaerobic control (Table III). The ammonia

production was never cut down below the anaerobic level and when added to an anaerobic tube glucose had no effect upon the ammonia content.

It would appear, therefore, that the type of reaction concerned in the production of ammonia anaerobically is not the same as that by which it is produced under aerobic circumstances, since the latter is inhibited by glucose, and the former is not. This result agreed with that obtained by Loebel [1925] when working with the grey matter of brain.

Table III. *Effect of glucose on ammonia production.*

Glucose	Resting	Values in mg. $\text{NH}_3\text{-N}$.			
		Anaerobic + glucose	Anaerobic control	Aerobic + glucose	Aerobic control
<i>M</i> /50	0.01	—	0.033	0.033	—
<i>M</i> /50	0.0107	—	0.0279	0.0276	—
<i>M</i> /150	—	0.0477	0.0449	0.0565	0.077
<i>M</i> /75	—	0.043	0.045	0.048	0.075

Moreover, it was next found that glucose did not inhibit the extra ammonia formation with added glycine, so that the system responsible for this, although an aerobic one, is apparently not identical with that by which ammonia is produced from the tissue in the absence of glycine.

Watchorn and Holmes [1927] found that the formation of ammonia and urea by embryo kidney tissue growing *in vitro* could be completely inhibited by the presence of glucose.

Table IV. *Effect of glucose on ammonia production with glycine present.*

Exp.	Values in mg. $\text{NH}_3\text{-N}$. All experiments aerobic.					
	Without glucose			With glucose		
	Control	Glycine	Difference	Control	Glycine	Difference
1	0.089	0.205	0.116	0.053	0.190	0.137
2	0.077	0.189	0.112	0.052	0.148	0.096
3	0.093	0.211	0.118	0.060	0.190	0.130
	Av. 0.1153			Av. 0.121		

Judging by the results obtained with anaerobiosis and by the addition of glucose, we are concerned with three types of ammonia-producing systems. One of these is an anaerobic system, whilst the other two are aerobic, and of the two aerobic systems, one is affected by glucose and the other is not. The distinction between these two latter systems might, of course, only be apparent and might be due to a difference in configuration of the substrate. We are ignorant of the nature of the substrate from which ammonia is formed in the absence of glycine. The results obtained when cyanide is added, do, however, once more suggest a distinction between the type of reaction by which ammonia is formed in the presence of glycine and that which causes the appearance of ammonia in the aerobic controls. The former is very much more sensitive to cyanide than the latter (Table V). The addition of enough cyanide (p_{H} 7.2) to bring the final concentration up to *M*/50 prevents any extra ammonia formation with glycine, and *M*/150 only allows a slight production.

Table V. *Effect of cyanide (p_H 7.2) on the formation of ammonia.*

	NH ₃ -N in mg.				
	Anaerobic control	Aerobic control	Aerobic + KCN	Aerobic + glycine + KCN	Aerobic + glycine
<i>M</i> /50 KCN	0.038	0.115	0.078	0.085	0.243
<i>M</i> /50 KCN	0.028	0.120	0.0708	0.0748	0.221
<i>M</i> /150 KCN	0.0275	0.080	0.0456	0.063	0.200

It can be seen from Table V that even *M*/50 cyanide, although diminishing the difference between the aerobic and anaerobic production of ammonia, does not prevent extra ammonia being formed in air by the tissue alone. On the other hand the ammonia production due to the presence of glycine is entirely inhibited, and the preparation containing glycine and cyanide gives the same ammonia values as that which contains no glycine (columns 4 and 5, Table V). The effect of cyanide on the anaerobic production of ammonia was tried only once, but as might be expected it did not alter this in any way.

It is not possible to say from these experiments which of these systems is responsible for the production of ammonia by the intact kidney for the neutralisation of acid glomerular filtrates. The greatest amount of ammonia is produced by the tissue in the presence of glycine but this may only be due to the high concentration of the substrate. It is hoped that a study of the effect of p_H upon the different systems will help to decide which of them is responsible for this function of the kidney.

SUMMARY.

1. Washed kidney tissue produces some ammonia under anaerobic conditions, and more under aerobic conditions.

2. Glucose inhibits the aerobic formation of ammonia; the ammonia production is thus cut down to the anaerobic level in the presence of glucose. The anaerobic production is not affected.

3. In the presence of glycine extra ammonia is formed. This occurs only in air and not anaerobically, and yet no extra atmospheric oxygen is taken up by the tissue. The production of this extra ammonia is not inhibited by glucose.

4. Cyanide (0.02 *M*) diminishes but does not entirely inhibit the aerobic ammonia formation by the tissue alone. On the other hand, the production of extra ammonia which is due to the presence of glycine is entirely inhibited by 0.02 *M* cyanide.

5. Apparently three systems at least may be concerned in the ammonia production by kidney tissue, one anaerobic system and two aerobic. One of the aerobic systems is completely inhibited by glucose but not completely by 0.02 *M* cyanide, while the other is completely inhibited by cyanide and not at all by glucose.

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