CLXXIII. PRODUCTION OF AMMONIA BY SURVIVING KIDNEY TISSUE.

II. STUDIES ON THE POSSIBLE PRECURSORS OF URINARY AMMONIA.

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IN a previous paper Patey and Holmes [1929] described three independent ammonia-forming systems which occur in washed kidney tissue (rat). One of these causes the appearance of ammonia under anaerobic conditions and one under aerobic conditions only. The precursors of the ammonia in both cases are still unknown. The third system, which for various reasons appears to be distinct from the second, is concerned with the deamination of glycine and probably various other amino-acids, and is also inactive in the absence of oxygen.

It is known that more ammonia is excreted by the kidney when the urine is acid than when it is neutral or slightly alkaline and, according to the theory first put forward by Nash and Benedict [1921] and supported by many other workers, most of the ammonia excreted is made in the kidney itself. We hoped that by testing the effect of variations in hydrogen ion concentration upon the three ammonia-producing systems we might be able to determine which, if any, of them is chiefly concerned in the physiological production of ammonia by the kidney.

The production of ammonia is an important mechanism for securing the neutralisation of an unduly acid urine where carnivorous animals are concerned. Other alkalis are doubtless also excreted (in rabbits and other herbivorous animals these are thought to play the chief part and the ammonia excretion to be unimportant), and when much acid is present the urine may also be very dilute. The power of the kidney to form the ammonia can be measured rather roughly by the ratio of titratable acid to ammonia in the urine. When the power of ammonia formation is much impaired, as in the cases of nephritis which are discussed later, the ratio acid/ammonia is higher than it should be, but the actual amount of acid per cc. is quite low because of the great dilution of the urine. When acid is added to the food of normal rats their urine becomes more than ordinarily dilute, but the amount of acid per cc. of urine may yet be three times as great, and the amount of ammonia per cc. ten times as great as in the case of the nephritic rats.

Henderson and Palmer [1915] consider that the greatest amount of ammonia is produced when the p_H of the urine is 5.6. Whether or no this is the case in man it does not seem to be true for rats. A few normal rats have been tested by feeding with acid. The urine of these rats, at least when fed upon a synthetic diet, is nearly always neutral or rather alkaline, and considerable amounts of acid have to be added to the food to lower the p_H . We have never succeeded in obtaining a reaction more acid than p_H 5.4, but up to this point there may be a steady increase in ammonia excreted and even a steady decrease of the ratio acid/ammonia. Each rat is considered individually, as there is considerable variation from one animal to another. There is no sign of maximum ammonia production or minimum acid/ammonia ratio at p_H 5.6.

In view of this, and also of the fact that the p_H as measured by Henderson and Palmer was the final one obtaining after ammonia and probably other alkalis had been added to the urine by the kidney, we had no particular reason for expecting to find an optimum p_H of exactly 5.6 for any system of ammonia production in the kidney tissue.

The technique used for the experiments with tissue (rat) was that described in the first paper. The required hydrogen ion concentration was obtained by the use of the Michaelis phosphate buffer mixtures, and it was found that in order to maintain the more acid reactions throughout the experiment it was necessary to have a phosphate concentration of $M/10$. This strength of phosphate solution was therefore used throughout. A preliminary experiment had shown that increase in phosphate concentration from $M/30$ to $M/3$ did not affect ammonia production.

A decrease of p_H from 7.3 to 5.2 had very little effect on the anaerobic ammonia production by washed kidney tissue (Fig. 1).

Fig. 1. Anaerobic system.

When determining the amount of ammonia formed by the aerobic system, it was, of course, necessary to estimate the anaerobic ammonia produced at each p_H and to subtract this from the total ammonia formation at each p_H .

The values given in Fig. 2 were obtained in this way, and therefore represent the ammonia formed by the aerobic system (or systems) only. It will be seen that a very considerable increase in ammonia production by the aerobic system occurs with increase of hydrogen ion concentration to p_H 5.2, which suggests that it is at any rate one of the systems playing a significant part in the formation of urinary ammonia. The aerobic deamination of glycine, on the other hand, is very much less at p_H 5.2 than at p_H 7.3 (*e.g.* at p_H 7.3, 0.043 mg. and at p_H 5.2, 0.018 mg. from 0.3 g. of washed tissue in a medium containing glycine). Thus, although there may be a steady breakdown of amino-acids of this type by the kidney during life, it is very improbable that the extra ammonia excreted when the urine is acid is derived from such a reaction.

Fig. 2. Aerobic system.

Some observations were carried out with unwashed tissue in order that the conditions occurring in life might be more closely imitated than in the other experiments. It will be seen that there is once more (Fig. 2) a sharp increase of ammonia production at p_H 5.2, but that in this case there is also considerable production at p_H 7.3. In view of the results with glycine it seems reasonable to assume that part at least of this ammonia is derived from amino-acid deamination at p_H 7.3, and that these amino-acids are washed out in the other experiments. We did in fact find [Patey and Holmes, 1929] that we could not demonstrate the deamination of added glycine at all clearly unless we had first washed the tissue. The anaerobic system in unwashed tissue (Fig. 1) shows a definite maximum at p_H 5.8, and this was also shown very indefinitely in the curves obtained with washed tissue. To judge by results to be quoted later it seems possible that this maximum is due to the deamination of adenylic acid, which would in all probability be present. It should be remarked here that the "resting" value of the ammonia in the unwashed kidney was not known so that no base line can be drawn, and the actual magnitude of the figures obtained with washed and unwashed tissues cannot therefore be compared with one another.

Henderson and Palmer [1915] showed that the ammonia-producing mecha-

nism often failed in chronically nephritic kidneys, and that in these cases the amount of ammonia formed in response to the excretion of acid was small. They showed that the ratio acid/ammonia in the urine was thus greatly increased in nephritis of this type.

As a further method of determining which system is chiefly responsible for the urinary ammonia we decided to produce chronic nephritis in rats by uranium poisoning and to investigate the ammonia-producing systems in their kidneys after the greatly increased acid/ammonia ratio described by Henderson and Palmer had been obtained. The animals showed a remarkable power of recovery (at any rate temporary recovery) from uranium poisoning, and the very great decrease of sensitivity to uranium after the first few injections which workers with other animals have noted. It was therefore necessary to give increasing doses of uranium at intervals for 2-3 months to arrive at the desired condition. An increased ratio lasting only a day or so was obviously of no significance for us, since the urine was collected in 24-hour samples, and by the time the ratio had been determined improvement had often begun. Eventually however a permanent condition of severe chronic nephritis was produced and the animals were killed at a suitable interval after a final dose of uranium. They were then showing a very small excretion of ammonia, the acid:ammonia ratio was 2 or more, the urine was very dilute, and generally only small amounts of albumin were present.

The kidney tissue from the nephritic animals was washed and treated in exactly the same way as that from the normals, and the experiments were carried out at p_H 7.3 as usual.

The three ammonia-producing systems with which we were concerned were quite differently affected by the pathological state of the kidneys. It might well be expected that considerable amounts of dead tissue would be present in each sample used for the experiment, yet the anaerobic system was quite unaffected. The deamination of glycine was greatly reduced but still quite marked, and the degree to which it was reduced did not bear any clear relationship to the acid/ammonia ratio in the urine during life. The aerobic system on the other hand was entirely cut out at p_H 7.3, and no more ammonia was formed in air than in the vacuum tube. At p_H 5.2 very small amounts of ammonia were still produced by this system, showing that it had not been entirely destroyed. The urines during life were practically never as acid as this.

Sections of the kidneys of 11 of the rats were very kindly examined for us by Dr R. J. V. Pulvertaft of St Thomas' Hospital, who classified the material under the headings of calcification, fibrosis, cystic dilatation, round-celled infiltration and tubular degeneration. These conditions were present in all the kidneys to some extent, but calcification was particularly marked in 10 cases, fibrosis in 1, cystic dilatation in 7, round-celled infiltration in 4, and tubular degeneration in 2. The failure of ammonia production could not be associated with the presence of any particular lesion or group of lesions.

In two cases, rats which had been receiving the same treatment as the others,

Table I.

Rat	Duration of uranium treatment	Ammonia formation by tissue. Mg. per 0.3 g. tissue						Remarks
		Acid: NH ₃ ratio in urine	Resting	Anaerobic	Aerobic	With glycine	With adenylic acid	
I	4 months*	2	0.011	0.038	0.038	0.049	—	* Uranium always injected at intervals of a few days
III	4 months	3	0.015	0.043	0.045	0.074*	—	* Comparison of Rat I and Rat III illustrates the lack of relationship between rise of ratio and loss of glycine-deaminating power
IV	4 months	2.2	—	0.032	0.037	0.057	0.109*	* Adenylic deamination not affected
VII + X	2 months	1.6 3	} 0.014	0.031	0.053	0.053	—	This experiment gives the one exception to our usual results. The aerobic deamination is present, and glycine gives no extra NH ₃ . Rat VII has still a somewhat low ratio
IX + XI + XII	2 months	5 2 2						
I	4 months	1*	} 0.020	0.034	0.046†	0.082	—	* No high ratio. Used as controls † Aerobic deamination still present
VI	4 months	0.85*						
V	4 months (with longer intervals)	2.8	0.040	p _H 5.2 and unwashed 0.071	p _H 5.2 and unwashed 0.092	p _H 5.2 and unwashed 0.100	—	—
Normal rat		0.5	—	0.038	0.082	—	—	—

and which showed equally severe lesions (Rat II and Rat VI), failed to give a very high acid/ammonia ratio at the time of death. Strikingly enough, in these rats, in which ammonia formation had not failed to the same extent in life, there was still ammonia production by the aerobic system at p_H 7.3 *in vitro*. Although the results with nephritic kidneys alone do not prove that this system is an important one for the physiological production of ammonia, they give considerable support to the conclusions drawn from a study of the p_H curves. The ammonia which is formed by the kidney in response to the excretion of an acid urine is probably due, at least in part, to the activity of this aerobic system whose precursor is still unknown, but is not glycine or presumably any other simple amino-acid.

The behaviour of various substances which might possibly be the precursors of the ammonia formed aerobically was then studied. As the aerobic ammonia production was found to be much reduced or even entirely inhibited by glucose, no substance could be considered likely as a precursor unless deamination occurred more rapidly at p_H 5.2 than at p_H 7.3, and was to some extent hindered by the presence of glucose.

Benedict and Nash [1929] believe urea to be the precursor of the urinary ammonia. They argue in support of this that Mann and Bollman [1928] find that in hepatectomised dogs the ammonia excretion falls as the urea excretion falls,

and also that when urea is injected the ammonia excretion rises again. The latter argument by itself does not carry much weight, as it is known that urea is excreted into the intestine, whence ammonia produced by bacterial action may be absorbed. In the absence of the liver this ammonia would then be excreted. The former argument, on the other hand, certainly strongly supports the theory that urea is the precursor, and can only be explained on this assumption, or on the assumption that both the urea and ammonia are derived from substances, the supply of which fails at much the same time in the absence of the liver, or even that they are derived from the same substance. The suggestion of a common origin for the urea and ammonia formed by kidney tissue growing *in vitro* has been put forward by Holmes and Watchorn [1929].

On the other hand, urease (from all sources which have so far been investigated) has an optimum activity at a hydrogen ion concentration slightly on the alkaline side of neutrality. This is true of the only animal urease so far described in any detail, *i.e.* the gastric urease discovered by Luck and Seth [1924]. The kidney urease, whose existence has been postulated must have an optimum activity well on the acid side of neutrality (p_H 5.6 or less), and, if our conclusions are correct, must be active only under aerobic conditions, and in the absence of any large amounts of glucose. It has never to our knowledge been suggested that the activity of the known ureases is limited in this way. We have found it impossible to demonstrate urease action in our washed kidney tissue at p_H 7.3, since there is a considerable increase of urea as well as of ammonia during the course of the experiment (Table II). Less, or no, urea is formed at p_H 5.2, and it is always possible that any urea that is formed is broken down at this p_H and gives rise to the extra ammonia production already noted. If this is the case, further investigation should reveal a numerical relationship between the extra ammonia appearing and the urea which fails to appear.

Table II.

Exp.	Mg. of N formed by 0.3 g. tissue			
	Ammonia-N		Urea-N	
	Resting	Aerobic	Resting	Aerobic
1	0.048	0.096	0.032	0.054
2	0.036	0.085	0.030	0.077
3	0.037	0.079	0.026	0.054

Holmes and Watchorn [1929] have not found any suggestion of urease activity in embryonic kidney tissue, although their experiments were carried out under such conditions that any break-down of urea at a neutral reaction would have been apparent. Embden and Schumacher [1929] state that minced kidney does not make ammonia from urea.

Another possible precursor is adenylic acid, as this is undoubtedly present in the blood and has been shown to be the source of a great deal of the ammonia formed by tissues. There is however general agreement that adenylic acid can

be completely deaminated under anaerobic conditions. Therefore, although it may be the source of some of the ammonia in the urine it cannot be the substrate of the particular aerobic system, and the p_H optimum curves do not suggest that it is the substrate for extra ammonia formation with an acid urine.

Professor Parnas was kind enough to send us some samples of adenylic acid prepared from muscle and from pig's blood. In a recent paper Embden and Deuticke [1930] have shown that the adenylic acid present in muscle is the same as that in kidney. Using these samples, some nucleic acid and also some of the calcium salt of adenylyl pyrophosphoric acid that we had prepared ourselves, we found that a change of reaction from p_H 7.3 to 5.2 had very little effect upon the deamination of these substances, but that there was a rather faintly marked optimum at p_H 5.8. This is in fairly reasonable agreement with the value Schmidt [1928] obtained for muscle, but the optimum in his case was much more definitely marked. There is rather less deamination of adenylic acid at p_H 5.2 than at p_H 7.3; when yeast adenosine or adenylic acid are used the optimum is definitely at p_H 7.3. It will be seen that the p_H curve of the anaerobic system in unwashed kidney tissue has also an optimum at p_H 5.8, which suggests that there is some adenylic acid left in it which is undergoing deamination anaerobically. We have also found that severely nephritic kidney tissue, which showed no increase of ammonia in air at p_H 7.3, was still capable of deaminating adenylic acid. Therefore, if this acid is the precursor, the failure in this type of nephritis must be in the supply of adenylic acid and not in the power to deaminate it. This seems somewhat improbable, but Embden and Schumacher [1929] have suggested that there is much less adenylic acid present in the kidney in nephritis than under normal conditions.

Histidine and asparagine do not give rise to any ammonia at all when added to surviving kidney tissue, and tyramine gives a higher yield in the presence of glucose than in its absence. Aspartic acid and lysine both show considerable deamination at p_H 7.3, and the deamination of lysine is much reduced by glucose. It is very probable that adenylic acid is one of the sources of ammonia appearing in the urine, but we have to conclude that the precursor of the aerobic ammonia-producing system and of the extra ammonia appearing in the urine at an acid reaction is still unknown.

CONCLUSIONS.

1. It has been shown that an ammonia-producing system which acts only under aerobic conditions is present in rat-kidney tissue, and that this system has an optimum activity at p_H 5.2 or lower.
2. It is suggested that this system is concerned in the physiological production of ammonia by the kidney, as this is known to be greatest when the urine is acid.

3. This idea is confirmed by the fact that ammonia production by this system is much decreased in uranium nephritis, when ammonia excretion is abnormally low.

4. The precursor of the ammonia produced is unknown. The possibility that it may be adenylic acid or urea is discussed. The deamination of other possible precursors has also been investigated.

5. Urea is also formed by kidney tissue in air at p_H 7.3.

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