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AMMONIA FORMATION BY SURVIVING KIDNEY SLICES WITHOUT SPECIFIC SUBSTRATES

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The formation of ammonia by the kidney is physiologically important because it enables acid radicals to be excreted in the urine and the fixed base which accompanied them in the glomerular filtrate to be reabsorbed and conserved. Only acid urines contain considerable amounts of ammonia, and the general correlation between the acidity of the urine and the rate of excretion of ammonia in it (shown particularly clearly in man by Eggleton, 1947) suggests that the acidity may be the stimulus which leads to the production of ammonia (Pitts, 1948, 1950).

Patey & Holmes (1929) found that rats' kidneys chopped up and incubated in Ringer's solution produced ammonia, and that more ammonia was formed under aerobic than under anaerobic conditions. Subsequently, Holmes & Patey (1930) showed that the aerobic production of ammonia became more rapid when the pH was lowered to 5.2-5.8. The oxygen consumption was not measured. In the present experiments the ammonia production and the oxygen uptake of tissue slices were measured in isotonic (0.30 osmolar) media of different pH values at 38.5° C in Barcroft manometers.

EXPERIMENTAL

Choice of media

All media used were prepared exactly as was the medium 'A₂' described by Robinson (1949), except for the composition and the pH of the buffers. The capacity of phosphate buffers is low between pH's 5 and 6, and these buffers in the Clark and Lubs series, prepared according to Hawk, Oser & Summerson (1947), and added in the amount used in preparing the medium A₂, did not prevent the solutions becoming alkaline during the experiments. Phthalate buffers proved to be unsatisfactory because they inhibited both the uptake of oxygen and the production of ammonia. When the buffering capacity of the media was increased by adding more phosphate buffer (up to 20% of their volume) oxygen was taken up in the normal way, but the production of ammonia was inhibited. This inhibition was traced to the potassium added with the phosphate buffer, and accordingly a potassium-free phosphate buffer was prepared by adding sufficient 2M-phosphoric

acid to 75 ml. of 0.2 N-NaOH to give the required pH and diluting to 100 ml. with water. The acid media were buffered with 15% of their volume of this phosphate buffer. Table 1 shows the effect of adding potassium (as KCl) to the medium buffered in this way. pH rose by 0.2-0.4 unit during those experiments in which considerable amounts of ammonia were formed. Results have been expressed in terms of 'average pH', that is, the average of the values determined at the beginning and end of each experiment.

TABLE 1. Inhibition of ammonia formation by potassium in slices from kidneys of adult rats in acid media

Average pH	Concn. of K in medium (m.equiv/l.)	Oxygen uptake (μ l./hr/mg) Mean \pm s.e. (No.)	Ammonia production (μ g NH ₃ -N/hr/100 mg) Mean \pm s.e. (No.)
5.5-5.9 (from Table 2)	5	2.8 \pm 0.13 (10)	25 \pm 2 (11)
5.7	25	3.0 \pm 0.04 (6)	18 \pm 1 (6)

No significant effect of [K] on O₂ consumption. Significant effect of [K] on ammonia production; $t=3.02$, $P<0.01$.

Animals

Adult rats were 6-12-month-old males of the black and white hooded Lister strain maintained at the Department of Experimental Medicine, and were killed by a blow on the head and bled from the carotids. Infant rats were of either sex and were killed by decapitation. Most of these were used the day they were born, but the results of some experiments on kidneys from 5- and 13-day-old rats were not significantly different and were included in the averages. Cats were rapidly bled to death under ethyl chloride anaesthesia. Rabbits were killed by a blow on the head and dogs by intravenous magnesium sulphate or intraperitoneal Nembutal (pentobarbitone sodium). New-born kittens and puppies were decapitated.

Technique

Slices from the renal cortex were cut by Cohen's (1945) modification of the method of Deutsch (1936). They were incubated, usually for 1 hr at 38.5° C in the flasks of Barcroft manometers as described by Robinson (1949), except that the flasks were filled with oxygen at room temperature before they were placed in the thermostat. After incubation the tissue and its medium were removed from each flask to a centrifuge tube, a drop of the medium was taken for the determination of its pH by Capillator, and proteins were precipitated with 10% (w/v) trichloroacetic acid or tungstic acid. The clear supernatant fluid was made alkaline and distilled in steam at atmospheric pressure in the apparatus devised by Markham (1942), the distillate being caught in 4 ml. of 2% boric acid coloured with bromo-cresol green, and the ammonia estimated by direct titration with 0.0143 N-sulphuric acid from a micro-burette.

The amount of ammonia initially contained in the kidney slices from adult rats was not determined in some of the earlier experiments, and there was never enough tissue to determine it in the experiments upon new-born rats. Later, weighed collections of adult slices such as were placed in the manometric flasks were covered with distilled water in centrifuge tubes and placed in the refrigerator while the manometers were in the thermostat. These were treated in the same way as the slices and media from the manometric flasks when the latter were removed from the bath. The amounts of ammonia found per 100 mg of sliced tissue at the end of the manometric experiments were then corrected by subtracting the ammonia present before incubation. As the amount of ammonia initially present had not been determined on every batch of slices the average value of 9 μ g ammonia-N/100 mg moist tissue (s.d. \pm 2 μ g from sixteen rats) was used for all experiments upon adult rats. This figure is in good agreement with that of 9 μ l. ammonia/mg/hr (= 7 μ g ammonia-N/100 mg/hr) reported by Warburg, Posener & Negelein (1924). The figures for new-born rats have been given uncorrected, and this makes the rate at which they formed ammonia appear too high.

RESULTS

(1) *Kidney slices from adult and infant rats*

Fig. 1 shows the effect of the pH of the medium upon the oxygen consumption and the ammonia production of kidney slices from adult rats. Table 2 records the average values of oxygen consumption and ammonia formation shown by slices from the kidneys of adult and new-born rats, in media of pH between 7.0 and 7.4 and between 5.5 and 5.9.

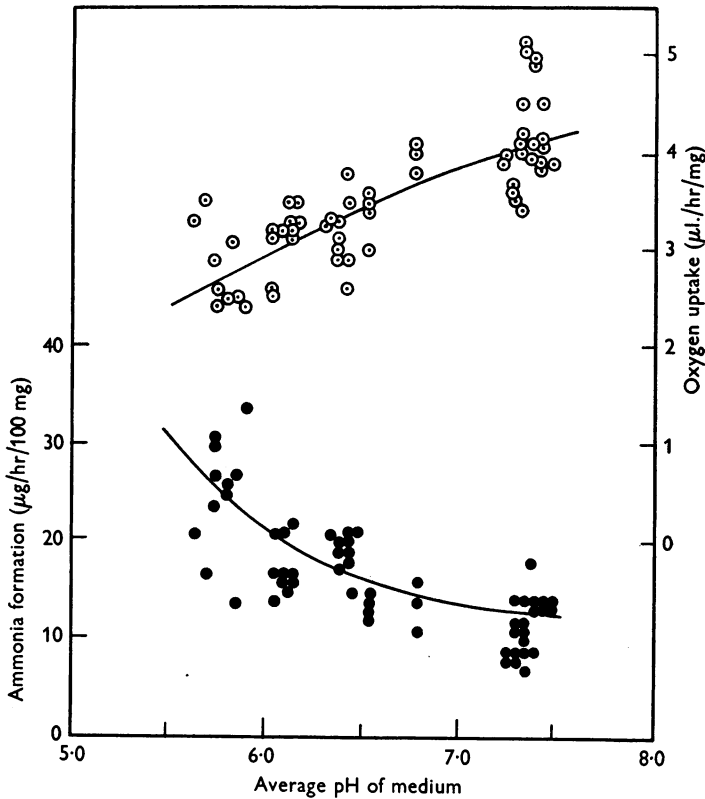


Fig. 1. Effect of pH of the medium upon respiration (⊙) and ammonia formation (●) by kidney slices from adult rats.

It will be seen from Fig. 1 and Table 2 that kidney slices from adult rats consumed more oxygen and produced more ammonia than slices from infant rats. In both, increasing acidity of the medium was accompanied by a diminished uptake of oxygen and a faster rate of formation of ammonia, but the adult tissues formed more ammonia in response to acidification than the infant tissues. All the differences were highly significant. The increased pro-

TABLE 2. Oxygen consumption and ammonia formation of isolated kidney slices from rats

All experiments at 38.5° C in 0.30 osmolar media containing glucose and buffered with phosphate; gas phase oxygen. Means with their standard errors. Numbers of experiments in parenthesis.

	Oxygen consumption ($\mu\text{l./hr/mg}$)		Ammonia formation ($\mu\text{g NH}_3\text{-N/hr/100 mg}$)	
	pH 7.0-7.4 Mean \pm s.e. (24)	pH 5.5-5.9 Mean \pm s.e. (10)	pH 7.0-7.4 Mean \pm s.e. (24)	pH 5.5-5.9 Mean \pm s.e. (11)
Adult rat	4.1 \pm 0.1 (24)	2.8 \pm 0.1 (10)	13 \pm 0.6 (24)	25 \pm 1.75 (11)
Infant rat	2.3 \pm 0.1 (8)	1.75 \pm 0.06 (7)	8 \pm 0.6 (8)	12 \pm 0.8 (8)

Significances of differences of means:

Adult rat	Effect of pH on oxygen consumption	$t=2.42$	$P \div 0.02$
Adult rat	Effect of pH on ammonia formation	$t=8.18$	$P \div 0$
Infant rat	Effect of pH on oxygen consumption	$t=5.16$	$P < 0.001$
Infant rat	Effect of pH on ammonia formation	$t=5.1$	$P < 0.001$
	Effect of age on oxygen consumption at pH 7.0-7.4	$t=3.17$	$P < 0.002$
	Effect of age on oxygen consumption at pH 5.5-5.9	$t=6.9$	$P \div 0$
	Effect of age on ammonia formation at pH 7.0-7.4	$t=4.59$	$P < 0.0001$
	Effect of age on ammonia formation at pH 5.5-5.9	$t=6.1$	$P \div 0$

duction of ammonia did not appear to be a direct consequence of the impaired oxygen consumption of the slices in acid media, because when the respiration of slices from the kidneys of adult rats was inhibited by incorporating $m/200$ cyanide in the medium or by filling the manometric flasks with hydrogen, ammonia production was reduced whether the medium was neutral or acid and averaged only $6 \mu\text{g}$ ammonia-N/hr/100 mg (range from 4 to $11 \mu\text{g}$ in eleven experiments). This appeared to correspond with the observation of Nicholson (1949) that cyanide introduced into the renal artery stopped the excretion of ammonia by the dog's kidney *in situ*. Negligible ammonia production was observed in a small number of experiments with rat liver slices or diaphragm under aerobic conditions. The source of the ammonia released by kidney slices has been investigated in this Department by Hines & McCance (1954).

(2) Kidney slices from other animals

Slices from the kidneys of dogs, cats, rabbits, puppies and a kitten were studied only in two ranges 7.0-7.4 and 5.5-6.0. The results have been set out in Table 3. It will be seen that oxygen consumption was diminished and ammonia production was increased by acidifying the medium in all these species at both ages, and that slices from the kidneys of adult animals made more ammonia than those from the corresponding infants in the three species in which they were compared. All the differences demonstrated by the experiments on kidneys from dogs and puppies were highly significant. Moreover, the isolated kidney tissue of adults of each species showed a greater increase in ammonia formation when the medium was made acid than did that of infants of the

TABLE 3. Oxygen consumption and ammonia formation of isolated kidney slices

All experiments at 38.5° C in 0.30 osmolar media containing glucose and buffered with phosphate; gas phase oxygen. Means with standard errors or ranges (x). Individual values for kitten. Numbers of experiments in parentheses.

	Oxygen consumption ($\mu\text{l./hr/mg}$)		Ammonia formation ($\mu\text{g NH}_3\text{-N/hr/100 mg}$)	
	pH 7.0-7.4	pH 5.5-5.9	pH 7.0-7.4	pH 5.5-5.9
Adult dog	2.75, s.e. \pm 0.12 (13)	1.75, s.e. \pm 0.06 (15)	8, s.e. \pm 1.0 (11)	12, s.e. \pm 0.3 (15)
Puppy	1.6, s.e. \pm 0.1 (7)	1.15, s.e. \pm 0.03 (10)	3, s.e. \pm 0.6 (7)	5, s.e. \pm 0.5 (10)
Adult cat	1.7, r. 1.6-1.7 (3)	1.3, r. 1.2-1.4 (6)	8, r. 7-9 (3)	10, r. 7-15 (6)
Kitten	1.7 (1)	1.1, 1.2 (2)	6 (1)	6, 8 (2)
Adult rabbit	2.7, r. 2.5-2.8 (4)	1.3, r. 1.2-1.4 (6)	0.3, r. 1-1.5 (4)	4, r. 1-6 (8)

Significance of differences of means (experiments in dog and puppy):

Adult dog	Effect of pH on oxygen consumption	$t=2.48$	$P<0.02$
Adult dog	Effect of pH on ammonia formation	$t=4.67$	$P<0.0002$
Puppy	Effect of pH on oxygen consumption	$t=3.94$	$P<0.002$
Puppy	Effect of pH on ammonia formation	$t=2.55$	$P=0.02$
	Effect of age on oxygen consumption at pH 7.0-7.4	$t=6.15$	$P<0.0001$
	Effect of age on oxygen consumption at pH 5.5-5.9	$t=9.4$	$P<0.0001$
	Effect of age on ammonia formation at pH 7.0-7.4	$t=4.09$	$P<0.001$
	Effect of age on ammonia formation at pH 5.5-5.9	$t=11.9$	$P\div 0$

same species. Although 5- and 13-day-old rats had behaved as new-born, one 3-day-old puppy gave results intermediate between those shown in Table 3 for adult dogs and those for younger puppies. The rabbit is habitually herbivorous, and its urine is alkaline. It was therefore interesting to find practically no ammonia formation in neutral media, although the increase in ammonia formation in response to acidity was not obviously less than in the cat.

DISCUSSION

Ferguson (1951) found that the rate of ammonia excretion by intact adult rats of the same strain as those used in the present experiments ranged from about 10 $\mu\text{g/hr/100 mg}$ of renal cortical tissue when the pH of the urine was 8.0 to about 40 $\mu\text{g/hr/100 mg}$ when it was 5.8. These rates are of the same order as those shown in Fig. 1 for kidney slices, although the intact kidneys produced rather more ammonia than the sliced ones. The comparison may have favoured the slices because all their cells were exposed to acid media in the manometer flasks, and not merely those of the distal tubules as in the body (Smith, 1951); on the other hand, the cells were better supplied with substrates in Ferguson's experiments. The similarity between the responses of the slices and of the intact kidneys to acidity is remarkable because the conditions of a manometric experiment exclude nervous or hormonal controls which might operate in the body. The fact that kidney slices from a number of animals reproduced the

behaviour of the intact kidney when the pH was varied within physiological limits supports the suggestion that the pH of the fluid in contact with the tubular epithelium determines the rate at which ammonia is produced *in vivo*.

The resemblance of the responses of the slices to those of intact kidneys extended to what may be characteristic difference between adults and infants within a species. McCance (1950) suggested that the mechanism which produces ammonia might be relatively undeveloped in human infants, and Cort & McCance (1954) recently found that young puppies excreted far less ammonia than adult dogs in response to a comparable acidosis, although they could lower the pH of their urine to the same extent. Incidentally, Cort also found that the rate at which acidotic puppies excreted ammonia increased measurably during the first 2 weeks of life, and the ammonia production of kidney slices from puppies appeared to behave in the same way (p. 5).

SUMMARY

1. Kidney slices from adult rats under aerobic conditions produced ammonia at a rate similar to that for intact kidneys *in situ*.

2. The ammonia production of the slices increased with increased acidity of the buffered medium (pH 7.4-5.5). Under anaerobic conditions it was markedly depressed and ceased to be dependent on the pH change.

3. Slices from the kidneys of new-born rats, dogs and probably cats consumed less oxygen and produced less ammonia at any pH than did slices from the kidneys of the corresponding adults, and the increased ammonia production resulting from lowered pH was smaller for the slices of the infant kidney.

4. The relation of ammonia production by slices under aerobic conditions to the pH of the medium was similar to the physiological relation of excretion of urinary ammonia to the urinary pH. This is consistent with the suggestion that the acidity of the fluid in the tubules is the physiological stimulus to ammonia formation by the kidney.

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