Caecal pH and ammonia in experimental uraemia

E. BOURKE, M. D. MILNE, AND G. S. STOKES

From the Medical Unit, Westminster Medical School, London

EDITORIAL SYNOPSIS This paper describes the effect on the pH, ammonia, and urea content of the caecum in rabbits made uraemic by bilateral nephrectomy. The considerable quantities of free ammonia present are considered in relation to uraemic colitis in man.

Urea is freely diffusible from the blood into the lumen of the alimentary tract, where it is converted by the enzyme urease into ammonia and CO₂. Tracer studies indicate that at least 25% of the urea in the body undergoes hydrolysis within the gut (Walser and Bodenlos, 1959), but a proportion of the ammonia produced is absorbed and re-synthesized to urea in the liver. The practical importance of this metabolic cycle, therefore, lies in the concentration of ammonia within the gut and its effect on the pH of the gastric and intestinal fluids.

The possibility of some urease within the gastric mucosal cells is still an unsettled question (Kornberg and Davies, 1955; Conway, FitzGerald, McGeeney, and Geoghegan, 1959), but the greatest amount of the enzyme is bacterial in origin. The germ-free rat, injected with ¹⁴C-labelled urea, puts out only 1/100 the amount of ¹⁴CO₂ in the expired air within six hours as the normal animal (Levenson, Crowley, Horowitz, and Malm, 1959). Ammonia is probably produced in greatest amount in the caecum and ascending colon where bacterial action is maximal. This paper reports on the pH, ammonia, and urea content of the caecal fluid of normal and uraemic rabbits, and the effect of ingestion of large doses of neomycin.

METHODS

New Zealand white, male and female, rabbits weighing 2.3 kg. were used in these studies. The diet consisted of pellets with greens on alternate days.

In six control rabbits, a caecotomy was performed under pentobarbital anaesthesia and about 10 g. of caecal contents was removed. This was repeated in each animal four days later. In a further 11 rabbits, the first operation consisted of a similar caecotomy and bilateral nephrectomy. A second caecotomy was performed after an interval varying from 24 to 96 hours. Since the blood urea level was steadily rising after bilateral nephrectomy, this ensured that specimens were obtained at varying degrees of uraemia.

A third group of six rabbits was treated with neomycin 0.5 g. twice daily by stomach tube for two and a half days before caecotomy and bilateral nephrectomy. In two of these animals the same dose of neomycin was continued post-operatively, whereas in the other four, the dose was reduced to 0.25 g., b.d.

Blood for estimation of urea content was obtained from the lateral ear vein immediately before anaesthesia in all the animals. Caecal contents were promptly centrifuged for 15 minutes at 3,000 r.p.m., and the supernatant fluid analysed without delay for pH, urea, and ammonia content.

Chemical methods were as follows:—Blood urea, Nessler's method after conversion to ammonia by urease; caecal ammonia and urea, Conway's (1957) microdiffusion method; caecal fluid pH, glass electrode.

Incubation studies were carried out *in vitro* to supplement the information from the studies *in vivo*. These included incubations of suspensions of human faeces and rabbit caecal contents, and mixed intestinal bacteria from both sources obtained by the technique of Weissbach, King, Sjoerdsma, and Udenfriend (1959). A control incubation and one with the addition of 0.5% urea was carried out in each case. Volatile organic acids in the products of incubation were estimated semi-quantitatively by the method of Fink and Fink (1949).

RESULTS

Table I gives the results of the control experiment on six rabbits. There is no significant change in the blood urea, or in the pH, ammonia, or urea contents of the caecal fluid after the caecotomy. Changes in these parameters in the uraemic animals are, therefore, entirely due to the effects of nephrectomy and not of caecotomy.

Table II compares the results in 17 normal and 10 uraemic rabbits. One of the 11 nephrectomized rabbits died of uraemia before a second caecotomy was performed.

There was a rise of caecal fluid pH (mean rise 1.0 pH unit), and the total ammonia content was approximately 3.5 times the control value in the uraemic

TABLE I
BLOOD UREA AND ANALYSES OF CAECAL FLUID IN SIX CONTROL RABBITS¹

	Blood Urea (mg./100 ml.)	Caecal pH	Caecal Ammonia (mEq./l.)	Caecal Urea (mg./100 ml.)
First caecotomy	37.7 (24-70)	6.07 (5.84-6.59)	10.8 (2.5-23.4)	0.05 (0.0-3)
Second caecotomy	37.5 (27-75)	6.18 (6.04-6.28)	10.4 (6.0-19.5)	0.03 (0.0-2)
t	0.04	0.89	0.11	0.33
P	> 0.9	> 0.3	> 0.9	> 0.7

Mean and range

TABLE II
EFFECT OF URAEMIA ON COMPOSITION OF RABBIT CAECAL FLUID

	No. of Animals	Blood Urea (mg./100 ml.)	Caecal pH	Caecal Ammonia (total) (mEq./l.)	Caecal Free Ammonia (mEq./l.)	Caecal Urea (mg./100 ml.)
Controls	17	41.6 (24-90)	6.07 (5.81-6.59)	12.9 (2.5-31.4)	0.012 (0.001-0.047)	0.3 (0.2-0)
Uraemic animals	10	510 (186-734)	7.05 (6.38-7.90)	44.6 (14.3-72.0)	0.55 (0.04-1.73)	0.6 (0.2-3)
t		44.7	3.52	3.27	3.31	1.06
P		< 0.001	< 0.01	< 0.01	< 0.01	< 0.3

animals. The combined increase in pH and total ammonia caused a very large rise in free unionized ammonia, as calculated from the Henderson-Hasselbalch equation. The urea content of the caecal fluid was very low, both in the controls and in the uraemic animals, and there was no significant difference between the two groups. The results suggest that the speed of metabolic destruction of urea is greater than that of its diffusion into caecal fluid.

Figures 1, 2, and 3 give the relationship between the pH, total ammonia and free ammonia in caecal fluid, and the blood urea. With increasing severity of uraemia there is a progressive increase in the concentration of both total and free ammonia in caecal fluid, but the pH only rises in the range of blood urea from normal to about 300 mg./100 ml. At higher levels there is no further increase. The results are highly significant statistically, the correlation coefficient between total ammonia and blood urea being 0.90 ($P < 0.001$), and between free ammonia and blood urea 0.75 ($P < 0.001$).

After ingestion of neomycin the pH of caecal fluid was significantly increased from a control mean value of 6.07 to a mean of 6.66 ($t = 2.67$; $P < 0.02$) but there was no change in ammonia content. After nephrectomy, at the higher dosage of neomycin (0.5 g., b.d.), there was evidence of systematic toxicity from absorption of the antibiotic producing neuromuscular block and respiratory paralysis. At the lower dosage (0.25 g., b.d.), the symptoms were less severe, but the animals were obviously ill and it was impossible to attain the severe degrees of uraemia produced in rabbits not treated with neomycin. Two

of the animals died before a second caecotomy was performed. Neomycin did not modify the pH and ammonia content of caecal fluid from the uraemic animals (Figs. 1, 2, and 3), indicating that inhibition of bacterial degradation of urea was only slightly affected by the drug. There was, however, a significant rise in the urea content of the caecal fluid in the

FIG. 1. The pH of caecal fluid in normal and uraemic rabbits in relation to blood urea. There is a rise of pH up to values of blood urea of 300 mg./100 ml. but no further rise in more severe degrees of uraemia. Open circles: normal animals. Closed circles: uraemic rabbits. Crosses: uraemic rabbits treated with large doses of neomycin by mouth.

FIG. 2. Total ammonia content of caecal fluid in normal and uraemic rabbits in relation to blood urea, with calculated regression line. Symbols as in Figure 1.

FIG. 3. Free un-ionized ammonia content of caecal fluid in normal and uraemic rabbits in relation to blood urea, with calculated regression line. Symbols as in Figure 1. Results in the 17 normal rabbits all fall within the small hatched rectangle.

neomycin-treated animals, but this was still extremely low when compared with the concentration in the blood. The mean concentrations of urea in caecal fluid were 1.2 mg./100 ml. before nephrectomy and 7.6 mg./100 ml. in the uraemic animals.

Similar results were obtained by incubation of homogenates of human faeces, rabbit caecal fluid, and mixed intestinal bacteria prepared from these

sources, with and without the addition of 0.5% urea. Without urea there was a fall of pH from a basal value of 6.8-7.0 to 6.2-6.3 after 24 hours' incubation. Simultaneously, there was an increase of organic acid which was chiefly acetic and propionic acids. Incubation with added urea caused a rapid rise of pH to values of 8.9-9.0 with the production of large amounts of ammonium carbonate. Studies of the pH of solutions of ammonium carbonate showed that there was a gradual rise of pH from 7.0 to 8.9 as the concentration increased from zero to 0.02 N, but with greater concentration there was virtually no change in pH despite steadily increasing ammonia concentration.

DISCUSSION

Urease hydrolyses urea to one molecule of CO_2 and two molecules of ammonia, which may be regarded as combining to form ammonium carbonate.

The net result is an increase of pH up to a possible maximum value of 9.0, at concentrations of ammonium carbonate above 0.02 N. The results show that in the rabbit caecum, total ammonia (ionized NH_4^+ and free NH_3) and also unionized NH_3 concentrations are linearly correlated with the blood urea. The pH, however, only increases in the blood urea range of normal to 300 mg./100 ml. without there being any further rise with more severe degrees or uraemia. This would be expected from the properties of solutions of ammonium carbonate where there is increase of pH with rising concentrations up to maximal levels of pH 9.0 in solutions stronger than 0.02 N. The fact that lower pH values were obtained for caecal fluid from uraemic rabbits (maximum pH = 7.9) than from the studies *in vitro* is probably due to the buffering properties of caecal proteins.

The ammonia found in the caecal contents of the normal animals was probably derived both from the action of urease on the small amounts of urea diffusing in from plasma, and also from bacterial deamination of unabsorbed amino-acids. The mean value of 12.9 mEq./l. (range 2.5 to 31.0 mEq./l.) agrees well with that reported by Marty and Raynaud (1964). They found the mean value was 11.0 mEq./l. (range 6 to 15 mEq./l.) and conclusively proved that ammonia was freely absorbed from the rabbit caecum. Blood in the veins draining the caecum contained considerably more ammonia than arterial blood, and ammonium chloride introduced into the caecal cavity decreased in concentration with time.

Administration of large doses of neomycin had only slight effects on the pH of caecal contents, and

caused no change in caecal ammonia concentration. This may have been due to neomycin-resistant strains of bacteria in this species, but this aspect was not investigated. The rise of the basal pH of caecal contents after neomycin was probably due to organic acid production being inhibited, but the antibiotic did not prevent the formation of ammonia and rise of caecal pH in the uraemic animals. Without neomycin, metabolism of urea within the caecal contents was virtually complete, whereas after the antibiotic easily detectable amounts of urea were present both in the controls and uraemic animals, although these were very low compared with plasma levels.

Free ammonia in uraemic stools is usually considered to play a part in the production of uraemic colitis (Schreiner and Maher, 1961). These experiments show that considerable quantities of free ammonia are present in caecal fluid from uraemic rabbits, and increase proportionately with the blood urea. The results are probably applicable to uraemia in man. Change in faecal pH will alter the activity of bacterial enzymes, alkalinity being unfavourable to most bacterial decarboxylases (Gale, 1940). We have found that metabolic products from decarboxylation of many amino-acids are considerably reduced in amount in human uraemia. This strongly suggests that the contents of the caecum and ascending colon become alkaline in uraemia in man, similar to that proved in the uraemic rabbit.

SUMMARY

This paper reports the effects of uraemia in rabbits on the pH, total and free ammonia, and the urea concentration, of the caecal contents. The mean caecal pH was estimated as 6.07 in normal rabbits

and 7.05 in uraemic rabbits. Caecal pH increases with rising blood urea values in the range from 30 to 300 mg. urea/100 ml. blood, but does not increase further in more severe degrees of uraemia. Total and free ammonia show a significant positive correlation with blood urea concentrations up to values of 750 mg. urea/100 ml. blood. The urea content of caecal fluid was extremely low both in normal and uraemic animals. Oral neomycin at high dosage had surprisingly little effect in this species, but caused a rise in the pH of caecal fluid in normal rabbits, and a slight increase in the urea content of the fluid in the uraemic animals.

Reasons are advanced that some of the results may be applicable to uraemia in man.

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