INTESTINAL AMMONIA IN URAEMIA: THE EFFECT OF A UREASE INHIBITOR, ACETOHYDROXAMIC ACID

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SUMMARY

1. The effects of intestinal ammonia in uraemia have been studied by administering a urease inhibitor, acetohydroxamic acid, to bilaterally nephrectomized rats.

2. Ammonia concentration was significantly decreased in the colon although it remained slightly higher than in normal animals. Caecal ammonia concentration was not altered. Total ammonia content was decreased in both colon and caecum.

3. A study in vitro indicated that ammonia production could be totally inhibited by acetohydroxamic acid. This suggests that the failure to suppress ammonia production in vivo is due to failure of the inhibitor to reach the site of urease production in sufficient concentration.

4. The animals treated with high doses of acetohydroxamic acid survived for a shorter time. Blood urea was higher and total serum protein lower in the treated group. The incidence of colitis was not affected.

Key words: ammonia, uraemia, urease inhibitor, acetohydroxamic acid, intestine, caecum, colon.

There is a raised ammonia concentration in the intestinal secretions of experimental uraemic animals (Bourke, Milne & Stokes, 1966) and of patients with renal failure (Bartels, 1876; Wilson, Ing, Metcalfe-Gibson & Wrong, 1968) and it has been suggested that ammonia may be involved in the development of uraemic colitis (Bourke et al., 1966; Harrison & Mason, 1937; Schreiner & Maher, 1961). Experimental uraemic colitis occurs only after the blood urea has been maintained at a high concentration for several days, suggesting that urea may act as a precursor of the toxic substance (Grollman & Grollman, 1959). Likewise, diarrhoea develops only slowly after the infusion of concentrated solutions of urea into dogs (Streicher, 1928). Germ-free nephrectomized animals, in which the ammonia content of the gastrointestinal tract is decreased, do not have colonic lesions but these appear when gastrointestinal organisms are

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introduced (Carter, Einheber, Bauer, Rosen & Burns, 1966). An alternative hypothesis is that colitis is secondary to local vascular lesions (Jaffe & Laing, 1934; Mason, 1952).

Urea hydrolysis is depressed in germ-free animals or after sterilization of the gastrointestinal tract by antibiotics (Wilson et al., 1968) but these models are not sufficiently specific for the investigation of the role of ammonia in uraemia. A specific urease inhibitor would have the advantage of selectively inhibiting the formation of ammonia in the gut. Acetohydroxamic acid is a powerful, non-competitive urease inhibitor (Fishbein, Carbone & Hochstein, 1965; Fishbein & Carbone, 1965), active against both bacterial and human mucosal urease (Aoyagi & Summerskill, 1966) but inactive against a wide range of other enzymes (Kobashi, Hase & Uehara, 1962). We have accordingly studied its effect on the biochemical and pathological features of uraemia in the intestine of bilaterally nephrectomized rats.

METHODS

Male albino Wistar rats, 250-300 g in weight, were fed on a pellet diet and given free access to water. Five normal rats were anaesthetized with ether, the abdomen of each was opened and the caecum and colon each ligated and excised. In four animals the contents of each organ were removed, transferred to filter paper and weighed. The colonic contents were immediately analysed for ammonia. The caecal contents were more bulky. They were diluted with twice their weight of normal saline, homogenized and the pH measured with a glass electrode pH meter. The ammonia concentration was measured on a 0.5 ml sample and corrected for dilution. The contents of the caecum and colon of the fifth animal were mixed with an approximately equal volume of phosphate-saline buffer, pH 7.2. Four 1 ml samples of this suspension were taken and to each was added 1 ml of urea solution (10 g/l). Three of the four urea-enriched samples were then made up to 3 ml with serial dilutions of an aqueous solution of acetohydroxamic acid (32.7 g/l) so that the mixtures contained respectively 10.9, 1.09, 0.109 mg of acetohydroxamic acid/ml. The inhibitor solution had previously been sterilized by passing through a bacteria-proof filter. The fourth sample was made up to 3 ml with sterile water and served as a control. The specimens were incubated at 37° for 6 h and ammonia concentrations measured at intervals on 0.2 ml portions.

Bilateral nephrectomies were performed under ether anaesthesia on 120 rats. Alternate rats were given a daily subcutaneous injection of 0.2 ml of a sterile aqueous solution of acetohydroxamic acid (250 mg/kg, fifty rats; 500 mg/kg, ten rats), starting at the time of operation. Control nephrectomized animals were given subcutaneous injections of 0.2 ml of sterile glucose solution (50 g/l). Animals were housed four to a cage (two controls, two treated animals) and were given 5 % (w/v) glucose to drink, but no food post-operatively.

Survival times (in hours) were determined for sixty animals, thirty untreated and thirty treated with acetohydroxamic acid (twenty, with doses 250 mg/kg; ten 500 mg/kg). Twenty animals (ten controls; ten treated with acetohydroxamic acid, 250 mg/kg) were anaesthetized with ether 72 h after bilateral nephrectomy. Blood (10 ml) was obtained from aortic puncture and the serum analysed for urea and protein. The caecum and colon were separately ligated and excised, their contents removed and ammonia content and pH determined after weighing as in the untreated animals.

Forty nephrectomized animals (twenty controls, twenty treated with 250 mg of acetohydroxamic acid/kg) were reserved for histological studies. They were observed until moribund,
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when they were killed by ether anaesthesia. The caecum and colon were removed, fixed in 15% (v/v) formol in saline and processed for light microscopy.

Ammonia was measured by the method of Conway (1962). Trace quantities of volatile bases in the faeces do not significantly affect the titration which has been shown to be specific for faecal ammonia (Wilson et al., 1968). Serum urea was measured by the diacetyl monoxime method on a Technicon autoanalyser; acetohydroxamic acid in the concentrations injected gave a zero value. Serum protein was measured by the biuret method.

RESULTS

In the normal rats ammonia concentration in the caecum ranged between 8.0 and 15.2 mEq/kg and the total ammonia content between 0.024 and 0.033 mEq. The corresponding values for the colon were 7.4-10.2 mEq/kg and 0.006-0.007 mEq respectively. In high concentration (10.9 mg/ml) acetohydroxamic acid completely inhibited the formation of ammonia by the preparation of caecal and colonic contents (Fig. 1). At one-tenth of this concentration there was 80% inhibition of ammonia formation after 6 h, but at 1/100 of this concentration the inhibitor was almost ineffective.

One animal in the treated group died at 20 h with a large intra-abdominal abscess and was discarded. With one exception (36 h, treated) all other animals survived for 48 h or more. The mean survival time was less in the treated groups (Table 1). The difference in survival between animals treated with the higher dose of acetohydroxamic acid (500 mg/kg) was statistically significant, whether they were compared with their own controls or the control nephrectomized group as a whole (P<0.05; Student's t test). The difference in survival between animals

![Fig. 1. Effect of acetohydroxamic acid (AHA) on the formation of ammonia by rat colonic contents in vitro.](image-url)
treated with the lower dose of acetohydroxamic acid and control animals was not statistically significant ($P < 0.05$).

Total ammonia and ammonia concentration in the ascending colon of the treated animals were lower than in untreated nephrectomized animals (Table 2); the weight of the contents did not differ in the two groups. Caecal and colonic ammonia concentrations were still significantly greater in treated nephrectomized animals than in normal animals ($P < 0.05$). The concentration of ammonia in the caecal contents did not differ in treated and untreated nephrectomized animals (Table 2), although the total ammonia content was significantly less in the treated animals ($P < 0.05$), as the weight of caecal content was less in these animals. The values were higher than the corresponding values in normal animals. There was a significant correlation between ammonia concentration and pH in the caecal contents ($r = +0.679$; Fig. 2). The mean serum urea concentration was higher in the treated group (Table 3), although the difference was not significant when examined by Student’s $t$ test. However, if treated and untreated animals nephrectomized on the same occasion are studied by using a paired $t$ test, the difference is statistically significant ($P < 0.05$). Total serum protein was lower in the treated group ($P < 0.05$) whether animals were compared as groups or as individual pairs (Table 3).
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Inflammatory lesions were found on microscopy in the large bowel in seven animals from the untreated and nine animals from the treated group (Table 4). Colitis was not seen in treated or untreated animals which had survived for less than 65 h after bilateral nephrectomy but there was no relation between survival time and the occurrence of large bowel lesions in animals who died more than 65 h after nephrectomy. In both groups the lesions appeared to be more severe in the caecum than in the colon. The lesions predominantly affected the mucosal layer, but when the inflammatory process was severe, it spread to the submucosa. Mucosal lesions were present as small, well-demarcated foci (Fig. 3) or as larger foci extending over relatively large areas (Fig. 4). In well-developed lesions there was abundant mucus and a heavy

![Graph](image)

**Fig. 2.** Relation between pH and ammonia concentration of rat caecum in control and treated animals. The line represents the calculated regression equation which is $x = 0.016y + 6.75$, where $x$ is pH and $y$ is ammonia concentration (mEq/l).

**Table 3.** Effect of treatment (250 mg of acetohydroxamic acid/kg) on serum urea and protein; results are expressed as means ± standard deviation

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<thead>
<tr>
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<th>Serum urea (mg/100 ml)</th>
<th>Serum protein (g/100 ml)</th>
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<tbody>
<tr>
<td>Control</td>
<td>648 ± 103</td>
<td>6.09 ± 0.65</td>
</tr>
<tr>
<td>Treated</td>
<td>698 ± 86</td>
<td>5.38 ± 0.51</td>
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<td>$P$ value</td>
<td>$&gt; 0.05$</td>
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polymorphonuclear exudate in the lumen of the bowel with masses of organisms, either cocci or bacilli close to the surface of the epithelium in places (Fig. 5). The epithelium was frequently shed and some of the adjoining crypts were dilated. The mucosa contained a heavy inflammatory infiltrate and there were foci of coagulative necrosis often superficial but sometimes deep, extending to the muscularis (Fig. 5). Superficial capillaries were dilated and occasionally a deeper venule was distended by thrombus. There was conspicuous oedema of the submucosal layers (Fig. 3) but inflammatory infiltrates were usually sparse and, where evident, quite focal (Fig. 6). The muscle coat was normal.

### DISCUSSION

Colonic ammonia is formed by the breakdown of nitrogenous compounds (Folin & Denis, 1912), especially the hydrolysis of urea by the enzyme urease (Walser & Bodenlos, 1959). Urease inhibition should therefore prevent the formation of significant amounts of ammonia.

The doses of acetohydroxamic acid which we gave to uraemic rats were much greater than those used in previous experiments on normal animals (Fishbein et al., 1965) or in clinical trials (Summerskill, Thorsell, Feinberg & Aldrete, 1968) and were chosen to induce complete inhibition of urease activity, assuming free diffusion through body fluids. Since acetohydroxamic acid is excreted unchanged in the urine (Fishbein et al., 1965) the problem of cumulative toxicity arises. As the LD<sub>50</sub> in mice for acetohydroxamic acid is 2.5 g/kg by intraperitoneal injection (Summerskill et al., 1968) and 900 mg/kg has been given daily to rats for 1 month (Fishbein et al., 1965), this contingency is unlikely but the possibility that the higher doses used in these experiments (3 x 500 mg/kg) had a toxic effect unrelated to urease inhibition cannot be entirely eliminated, as in the absence of renal excretion high serum concentrations probably occur.

We conclude from our results that formation of ammonia from urea in the colon does not impair survival in uraemia. By hydrolysing urea, urease plays an important part in the recycling of bowel urea nitrogen (Walser & Bodenlos, 1959; Jones, Smallwood, Craigie & Rosenoer, 1969) and in the first stage of the resynthesis of urea nitrogen into protein (Foster, Schoenheimer & Rittenberg, 1939; Richards, Metcalfe-Gibson, Ward, Wrong & Houghton, 1967). This phenomenon has been demonstrated in rats which were, like ours, deprived of dietary protein (Rose & Dekker, 1956). The lower serum protein and higher serum urea in the treated group may equally be attributed to decreased calorific intake and a greater catabolism of endogenous protein in these animals. No such effect could be demonstrated in normal animals.
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Fig. 3. Photomicrograph of rat cecum showing focal lesions of the mucosa. There is conspicuous submucosal oedema and a sparse inflammatory exudate. (H. & E. x 40).

Fig. 4. Photomicrograph of rat cecum showing a confluent lesion involving a large area of mucosa. There is a heavy inflammatory infiltrate in the muscularis. (H. & E. x 150).

(Facing p. 110)
**Fig. 5**

Photomicrograph of rat caecum. Portion of the lesion shown in Fig. 3. There is a superficial zone of coagulative necrosis with heavy inflammatory exudate, small masses of organisms, erythrocytes and mucus in the lumen. (H. & E. × 250).

**Fig. 6**

Photomicrograph of rat colon. A focal lesion with heavy inflammatory exudate extending to the lumen and focally to submucosa. (H. & E. × 250).
given greater doses of acetohydroxamic acid than were used in our experiments, but such results cannot be confidently extrapolated to uraemic animals.

Since lesions were found with the same frequency in both caecum and colon of treated and untreated nephrectomized animals despite a 64% decrease in mean colonic ammonia concentration our results provide no support for the suggestion that ammonia is responsible for large bowel lesions in uraemia (Harrison & Mason, 1937; Schreiner & Maher, 1961). Although the mean colonic ammonia concentration was slightly elevated even in treated animals this concentration is similar to that obtained in only mildly uraemic animals since blood urea and large-intestinal ammonia concentration are positively correlated (Bourke et al., 1966). In all our experiments colitis occurred only in rats that survived at least 60 h after nephrectomy, and had blood urea concentrations of more than 400 mg/100 ml. If ammonia was aetiologically important in uraemic colitis, the decrease in ammonia concentration to values corresponding to a comparatively low blood urea concentration should have had a profound effect on the incidence of colitis. Our results also suggest that the prolonged survival and freedom of germ-free rats from colitis (Carter et al., 1966) is not due to the suppression of gastrointestinal ammonia formation.

Caecal ammonia concentration in uraemic animals, as opposed to total caecal ammonia, was not influenced by acetohydroxamic acid. Studies in vitro showed that caecal ammonia formation could be completely inhibited by acetohydroxamic acid. This finding excludes possible alternate sources of caecal ammonia (Bourke et al., 1966) and suggests that acetohydroxamic acid did not penetrate to sites of urease action within the caecal lumen. In the rat the caecum is a large blind sac with copious contents. The ascending colon contains a smaller quantity of material with a relatively greater absorptive area. A substance which diffuses throughout the tissue fluids could readily reach the colonic contents, but the volume of caecal contents, the relatively small mucosal surface in relation to this volume and the stagnant nature of this sac would produce an obstacle to the inhibitor reaching the site of urease activity. Moreover, at sites of profuse bacterial multiplication the local concentration of urease may be high enough for inhibitor activity to be incomplete (Fishbein et al., 1965). The persistence of hyperammonaemia in some patients under treatment with acetohydroxamic acid (Summerskill et al., 1968) and the failure of rectal administration of acetohydroxamic acid to influence colonic ammonia production (Wolpert, Phillips & Summerskill, 1970) may also be due to the compound not reaching the site of urea hydrolysis in sufficient concentration.

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