

# Gastrointestinal urease in man<sup>1</sup>

## Part I Activity of mucosal urease

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**EDITORIAL SYNOPSIS** Mucosal estimations of urease show that it is highest in the gastric mucosa and lowest in the large intestine. It seems that bacterial urease activity may be limited to the colon.

The relationship postulated between hepatic coma and hyperammonaemia implicates the gastrointestinal tract as a major source of the excess ammonia in the peripheral blood of some patients with cirrhosis (Butt and Summerskill, 1961; Chalmers, 1960; Sherlock, Summerskill, White, and Phear, 1954). Conventional therapy, comprising protein restriction, antibiotics, and enemas, is directed toward eliminating from the gut nitrogenous material and bacteria (Butt and Summerskill, 1961; Chalmers, 1960; Sherlock *et al.*, 1954) from which ammonia and other toxic substances may be produced. The metabolic processes involving ammonia in the gastrointestinal tract of man are poorly defined (Chalmers, 1960; McDermott, Adams, and Riddell, 1954); ammonia can be produced in the stomach (FitzGerald and Murphy, 1950), while both secretion and absorption have been quantitated in the small intestine (Ewe and Summerskill, 1965).

Several enzymes capable of liberating ammonia from various substrates occur in the bacterial flora of the gut and may also be present in the mucosa; they include oxidative deaminases, dehydrases, combined transaminase-deamidases, and urease (Bessman, 1956; White, Handler, and Smith, 1964). Urease, which produces ammonia by hydrolysis of urea, may originate from either the mucosa or the bacterial flora of the gut in animals (Conway, FitzGerald, McGeeney, and Geoghegan, 1959; Dintzis and Hastings, 1953; Kornberg and Davies, 1955; Kornberg, Davies, and Wood, 1954); findings in man pertaining to mucosal urease activity are scanty (FitzGerald and Murphy, 1950; Fossel, 1948)

or inferential (Rappoport and Kern, 1963) and liable to conflicting interpretations (FitzGerald and Murphy, 1950; Lieber and Lefèvre, 1959; Visek and Thomson, 1964). However, induction of immunity to urease (Thomson and Visek, 1963; Visek and Thomson, 1964), direct inhibition of urease with acetohydroxamic acid (Fishbein and Carbone, 1964), and colonization of the bowel with non-urease-producing bacilli (Macbeth, Kass, and McDermott, 1965) have recently been attempted in the treatment of the hepatic coma syndrome. The clinical importance of the enzyme implied by these trials prompted the present studies. Activity of urease in the mucosa and in washings was measured at various sites of the gastrointestinal tract of man, and the effect of antibiotics on urease concentrations was determined. The results from mucosa and washings of the colon were compared with faecal urease content.

### MATERIALS AND METHODS

Specific activity of urease was measured in gastrointestinal mucosa from 105 individuals who had fasted for at least 12 hours. Specimens were obtained by peroral biopsy from 23 healthy volunteers and, in the remaining instances, grossly normal tissue was acquired during abdominal operations for a variety of conditions. The material (Table) thus comprised: 39 specimens of stomach (19 from the fundus and 20 from the antrum) from healthy persons (10) and patients with gastric ulcer (10), duodenal ulcer (9), carcinoma of the stomach (8), or other conditions (2); 23 specimens of small intestine (16 from the jejunum and 7 from the ileum) from healthy individuals (13) and patients with regional enteritis (6) or with other diseases of the small intestine (4); and 43 specimens of colon (14 from the right side and 29 from the left side)

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from patients with diverticulitis (6), carcinoma (28), or other conditions (9). Sixty-four individuals were male and 41 were female; their ages ranged from 19 to 85 years, with a mean of 51.4 years. In addition, urease activity in the faeces of eight healthy individuals and that contained in the washings of 16 specimens of mucosa was also measured.

Tissue was placed in ice immediately. Surgical specimens were washed twice with 10 ml. of 0.9% sodium chloride, the washings later being assayed for urease activity; and the mucosa was then separated with the edge of a glass slide before homogenization. Peroral biopsy specimens were homogenized directly. Processing was performed as soon as possible, and always within 30 minutes. From duplicate specimens, it was established that the time lapse before processing had no significant effect on the enzyme activity up to a period of two hours. The mucosal and faecal preparations were weighed and homogenized in glass-distilled water with a Potter-Elvehjem-type homogenizer (Teflon pestle) at 45 r.p.m. for three minutes. The concentration of homogenate varied with the size and site of mucosal specimens, as well as with the enzyme activity anticipated for the tissue, and ranged from 1 to 10%. Faeces were prepared fresh as a 0.1% solution in distilled water.

The assay of urease was designed to follow zero order of kinetics for a period of at least 20 minutes (Fig. 1) and to yield sufficient ammonia at 10 minutes at 37°C. for accurate measurement with a Bausch and Lomb Spectronic 20 colorimeter. To satisfy these requirements, 0.5 ml. of crude mucosal homogenate, tissue washings, or faecal suspension was added to 1.5 ml. of 10% buffered substrate (10 g. of urea in 100 ml. of 0.75 M phosphate buffer at pH 7 with 25 mg. of bovine albumin as a stabilizing agent) and incubated for 30 minutes at 37°C. Phosphate does not inhibit urease activity (Fasman and Niemann, 1951), and the buffer maintained the pH of the reaction mixture within 0.1 pH unit of optimum (pH 7) for the enzyme (Howell and Sumner, 1934). Under these circumstances, the amount of ammonia liberated was

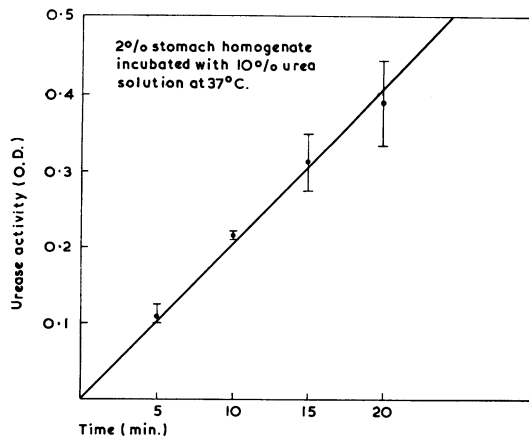


FIG. 1. Results of assays of urease activity.

linear with time and with enzyme concentration over a 20-minute period.

Ammonia was measured immediately (zero time) and from four to five duplicate aliquots each of 2 ml. were removed during the subsequent 20 minutes (Fig. 1). Results were expressed as micrograms of ammonia nitrogen per 10 minutes per milligram of protein. This value was obtained by adding the means of duplicate determinations from each specimen at five, 10, and 15 minutes and dividing by 3. The zero time (pre-formed) ammonia was subtracted from all subsequent determinations (Table). Control specimens comprised tissue, buffer, and albumin in the absence of added urea. These yielded 10-minute values not significantly different from, and mean results which were less than, the zero time values found under the conditions of the experiment.

Ammonia determinations were made with the modification of Reinhold and Chung (1961) of an earlier method (Seligson and Hirahara, 1957). Nesslerization was accomplished with 4 ml. of Nessler's reagent. Protein was determined by the method of Lowry and his associates (1951). The protein content of homogenates varied from 0.7 to 2.0 mg. of protein/ml., and those of tissue washings varied from 0.04 to 0.50 mg. of protein/ml. Results from duplicate specimens of tissue gave a standard error of  $\pm 9.3\%$  for urease activity by this method.

## RESULTS

**MUCOSAL UREASE ACTIVITY AND PRE-FORMED AMMONIA AT VARIOUS LEVELS OF THE GASTROINTESTINAL TRACT** There were no significant differences in mucosal urease activity derived by peroral biopsy and that of specimens taken from similar sites at operation (Fig. 2). Concentrations of the enzyme were comparable in health and in the various diseases included in the series; similarly, the age (below or above 40 years) and sex of the patient did not influence the results significantly. Urease activity of gastric mucosa was significantly higher than that of the small intestinal mucosa ( $p < 0.001$ ), and the latter was significantly greater than that of colonic mucosa ( $p < 0.01$ ) (Table). The range of activity was widest in the stomach (Fig. 2), but there was no difference between mucosa from the fundus and that from the antrum (Table). Jejunal mucosa contained significantly more urease than that of the ileum ( $p < 0.02$ ), but no significant difference was present when mucosal urease contents of the ileum, right hemicolon, and left hemicolon were compared (Table).

The mean value for pre-formed (zero time) ammonia was greatest in the stomach; in the small and large intestine, mean concentrations of pre-formed ammonia exceeded those liberated after 10 minutes of incubation during the enzyme assay procedure (Table). There was no correlation between pre-formed ammonia and mucosal urease activity in specimens obtained from any area.

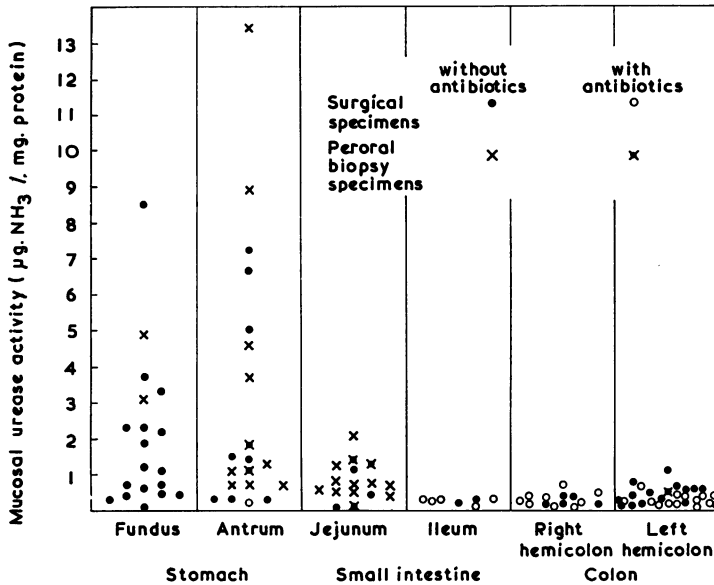


FIG. 2. Histogram showing the urease activity of the mucosa of the stomach, small intestine, and colon; values are also shown in relation to the action of antibiotics upon the mucosa.

TABLE  
SPECIFIC ACTIVITY OF UREASE IN THE GASTROINTESTINAL TRACT

	Number of Specimens	Urease Concentration (10-minute value)		Pre-formed Ammonia (zero time)	
		Mean	S.E.	Mean	S.E.
<i>Mucosa</i>					
<i>Stomach</i>					
Fundus	19	2.04	0.47	—	—
Antrum	20	3.04	0.80	—	—
Total	39	2.56	0.47	2.33	0.68
<i>Small intestine</i>					
Jejunum	16	0.81	0.13	—	—
Ileum	7	0.26	0.03	—	—
Total	23	0.64	0.10	1.12	0.14
<i>Colon</i>					
Right hemicolon	14	0.26	0.04	—	—
Left hemicolon	29	0.39	0.04	—	—
Total	43	0.35	0.03	1.16	0.10
<i>Tissue washings</i>					
Stomach	4	2.51	0.53	—	—
Colon and ileum	12	8.26	2.58	—	—
<i>Faeces</i>					
	8	26.10	3.87	—	—

EFFECTS OF ANTIBIOTICS ON COLONIC MUCOSAL UREASE ACTIVITY AND PRE-FORMED AMMONIA Mean urease activity of colonic mucosa from 24 patients who had been prepared for surgery by the oral administration of antibiotics (neomycin, streptomycin, tetracycline, chlortetracycline, and penicillin, alone or in various combinations) was 0.29 (S.E.  $\pm$  0.03)  $\mu\text{g.}$  of ammonia nitrogen per milligram of protein and less ( $p < 0.05$ ) than the value from 19 patients whose preoperative management had not included antibiotics. The value in this group was 0.45 (S.E.  $\pm$  0.06)  $\mu\text{g.}$  ammonia nitrogen per milligram of pro-

tein. No difference in gastric or small intestinal mucosal urease activity in relation to antibiotic administration was found (Fig. 2).

Pre-formed (zero time) ammonia was also significantly lower ( $p < 0.01$ ) in colonic mucosa from 22 patients who had been given antibiotics. The mean value was 0.92 (S.E.  $\pm$  0.03)  $\mu\text{g.}$  ammonia nitrogen per milligram of protein, whereas specimens from 18 patients who had not received antibiotics yielded 1.42 (S.E.  $\pm$  0.07)  $\mu\text{g.}$  No differences in pre-formed ammonia were present in gastric or small bowel mucosa in relation to the prior administration of antibiotics.

UREASE SPECIFIC ACTIVITY OF MUCOSAL WASHINGS AND FAECES Urease specific activity was present in all the washings from 16 specimens of gastric, ileal, and colonic mucosa. Activity in washings from the stomach was comparable to the mucosal enzyme content; the urease concentration in washings from the colon and ileum did not differ, but was higher than that in the mucosa ( $p < 0.001$ ) in every instance (Table). Faecal urease activity, although widely variable, was yet greater, being significantly higher than that in colonic washings ( $p < 0.001$ ). There were no correlations among urease activity of washing solutions, mucosal urease activity, and pre-formed ammonia concentrations.

#### DISCUSSION

Urease specific activity was found throughout the gut mucosa, the amount declining in a gradient from

the upper to the lower part of the gastrointestinal tract. Since Luck (1924) reported the presence of mucosal urease in the canine stomach, extensive studies (Kornberg and Davies, 1955) in animals have shown variation in the location and amount of the enzyme in the gut of different species as well as within the same species. Our results in humans showed less inconsistency, except for the relatively wide range of gastric mucosal urease activity.

Comparison of our findings with earlier work is limited by differences in methods. The highest concentrations of the enzyme were found in the stomach, thus according with the observations of Fossel (1948); in contrast to previous reports from man (FitzGerald and Murphy, 1950; Fossel, 1948) and animals (Linderstrøm-Lang and Søeberg-Ohlsen, 1936; FitzGerald and Murphy, 1950), no difference between the fundic and antral areas was apparent. Small intestinal mucosal urease activity was less than that in the stomach, but it could be quantitatively as important because of the relatively larger mucosal surface. Urease activity has been identified in the duodenal mucosa of cats (FitzGerald and Murphy, 1950), but the only previous investigation in man revealed none in the small intestine (Fossel, 1948). In proportion to gastric urease activity, our values for colonic mucosa were higher than those reported by Fossel (1948).

The sources of ureas in the gastrointestinal tract of man have not been established. Both bacteria (Kornberg and Davies, 1955) and mucosal cells (Conway *et al.*, 1959) have been proposed as the site of gut urease activity in animals. Conway *et al.* (1959) presented extensive evidence that gastric urease in the mouse is located in the epithelial cells, and the enzyme has been identified in the mitochondrial and soluble subcellular fractions of the mucosa of the mammalian stomach (Lind and Martinson, 1964). The enzyme has also been isolated from presumably sterile stomachs of human fetuses (Cardin, 1933). Recently, Fleshler and Gabuzda (1965) have accumulated indirect evidence of mucosal urease activity in the adult human stomach. However, others have interpreted evidence from several species, including man, to indicate only a bacterial origin of urease in the gut (Kornberg and Davies, 1955; Thomson and Visek, 1963). The results of the present studies and those from a concurrent investigation (Evans, Aoyagi, and Summerskill, 1966) are consistent with contributions from both bacteria and the mucosa to gastrointestinal tract urease activity in man. Values from mucosal homogenates were highest in the stomach and upper part of the small intestine and presumably reflected largely mucosal enzyme, since bacteria are believed normally to be scanty or absent (Cregan, Dunlop, and Hayward, 1953; Cregan and

Hayward, 1953; Goldstein, Wirts, and Josephs, 1962; Shiner, Waters, and Gray, 1963) in these areas. Mucosal activity was lowest in the large intestine, despite the presence there of abundant urease-producing bacteria (Donaldson, 1964), as shown also by the high faecal urease content in the present study. The barely significant reduction in mucosal urease activity of the large bowel in patients who had received antibiotics suggests that contaminating bacteria may have contributed to ammonia formation during incubation of the mucosal homogenates from this organ. Alternatively, inhibition of enzyme activity by the chemotherapeutic agents independent of their antibiotic effect (Belding and Kern, 1963) may have occurred, but this is less likely since the effect was not observed in the small intestine. Unfortunately, the pre-operative circumstances precluded standardization of the drugs and doses used.

Additional evidence of bacterial urease activity was indirect and was confined to the large intestine. Thus, the urease content of tissue washings from the colon exceeded that of washings from other organs and was also much higher than that in colonic mucosa, though not nearly as great as faecal urease activity. In addition, the use of antibiotics reduced the amount of pre-formed ammonia measured in the large bowel, but not that in other tissues. A more direct comparison of bacterial and mucosal urease activity in the hydrolysis of urea within the lumen of the upper and lower regions of the intestinal tract of man is reported in Part II.

#### SUMMARY

A method of measuring urease specific activity was applied to specimens of mucosa taken from the human gastrointestinal tract. The enzyme was present throughout the gut, concentrations being highest in the stomach and lowest in the large intestine and thus contrasting with the location of the gut flora, which may also contain urease. Evidence of bacterial urease activity was limited to the large intestine; the enzyme content of tissue washings was greatest in the large bowel, and antibiotics reduced the amount of pre-formed ammonia measured in homogenates of colon.

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## Part II Urea hydrolysis and ammonia absorption in upper and lower gut lumen and the effect of neomycin

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The purposes of the present study in patients with cirrhosis are to investigate and compare *in vivo* urease activity in the upper and lower portions of the gastrointestinal tract, as reflected by the hydrolysis of urea to ammonia in the gut lumen and the subsequent change in blood ammonium concentrations. The antibiotic effect of neomycin on these processes was assessed to evaluate relative contributions and locations of mucosal and bacterial urease activity. In addition, transfer of ammonia to the circulation from the upper and lower parts of the gastrointestinal tract was compared, both before and after treatment with the antibiotic.

### MATERIAL AND METHODS

Patients with cirrhosis of the liver were studied, since in this condition measurable changes in the ammonium concentration of the peripheral blood follow administration of ammonium salts or urea into the gastrointestinal tract. The diagnosis was confirmed by hepatic biopsy in all of the 17 participants. Fifteen were chronic alcoholics and two had a previous history consistent with hepatitis; they comprised 10 males and seven females whose ages ranged from 39 to 62 years (mean 52 years). All had moderately severely impaired hepatic function. Six were jaundiced, four had ascites, six gave a history of previous coma, and eight had earlier experienced massive gastro-