The role of the colon in urea metabolism in man

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Summary

1. The urea content of ileostomy effluent has been measured by the urease method as an indirect estimate of the urea concentration in the lumen of the normal ileum.

2. The plasma disappearance of intravenously administered \([^{14}\text{C}]\)urea was used to study intestinal urea breakdown. Normal subjects on high and low protein diets and patients with either excised (i.e. with ileostomies) or excluded colons were studied.

3. The 24 h intestinal urea breakdown was considerably greater than the quantity of urea estimated to be entering the colon from the ileum and across the colonic mucosa.

4. Intestinal urea breakdown increased with increase in dietary protein and decreased with, but was not abolished by, exclusion or excision of the colon.

5. Our results suggest that the colonic lumen is not the only site of intestinal ureolysis and that significant quantities of urea must be broken down either at a juxtamucosal site or in the ileum.

Key words: colon, urea metabolism.

Introduction

It is generally accepted that intestinal ammonia is produced by enzymic breakdown of protein metabolites and urea. As a result of the finding that about 130 mmol (7.8 g) of urea is broken down to ammonia in the intestine daily (Walser & Bodenlas, 1959; Jones, Smallwood, Craigie & Rosenoer, 1969) and the independent finding that an equivalent amount (260 mmol) of ammonia enters the portal circulation from the bowel daily (Summerskill & Wolpert, 1970), it has been suggested that urea is the major substrate for intestinal ammonia production (Summerskill & Wolpert, 1970; Wrong, 1971). Studies of urease activity in man (Aoyagi, Engstrom, Evans & Summerskill, 1966; Evans, Aoyagi & Summerskill, 1966) have shown that, apart from small quantities of mucosal urease in the stomach and small bowel, intestinal urease is bacterial in origin and is located in the colon.

We have undertaken the present study to elucidate further the role of the colon in urea hydrolysis and ammonia production. Urea excretion by patients with an ileostomy has been estimated as an indirect measure of the amount of urea entering the colonic lumen and urea turnover has been studied in control subjects and patients with either an excluded or absent colon to elucidate the quantitative role of the colon in urea metabolism.

Materials and methods

Patients

Twenty-nine ileostomy effluent specimens were obtained from twelve fit patients with normally functioning ileostomies. \([^{14}\text{C}]\)Urea-turnover studies were carried out in six normal subjects and six patients with non-functioning colons. The latter included four patients who had undergone colonic
exclusion with ileorectal anastomosis for chronic porto-systemic encephalopathy and two patients with ileostomy—one for Crohn’s disease and one for ulcerative colitis. Informed consent was obtained from all subjects.

**Ileostomy urea**

To minimize bacterial ureolysis before estimation, freshly produced ileostomy fluid was immediately cooled by placing it in a container packed in ice and transferred rapidly to the laboratory, where it was diluted with absolute ethanol (three parts fluid to one part ethanol) to inactivate bacteria. The liquid phase was separated by centrifugation through a Centriflow membrane filter (Amicon Corp., Lexington, Mass., U.S.A.) at 200 rev./min for 10 min. Urea concentration was estimated by the urease method. Ammonia concentration before and after incubation with urease was estimated by a modification of the method of Fenton & Williams (1968) with 5 ml of sodium hydroxide (100 mmol/l) for elution and Nessler’s reagent for colour development. The pH of samples incubated with urease was first adjusted to pH 6-6 with Sorensen’s phosphate buffer [KH$_2$PO$_4$ (0.067 mol/l) adjusted to pH 6-6 with Na$_2$HPO$_4$ (0.067 mol/l)] and 0.1 ml of jack-bean urease suspension (25 mg in 5 ml of ammonia-free water) was added. Incubation was continued for 30 min. The recovery of ammonia after urease incubation was found to be variable, but no systematic error could be found. The possibility that the ethanol used to inhibit bacteria might inhibit urease was considered but specimens diluted with water instead of ethanol showed similar recoveries. Chelating agents were also added in an attempt to remove possible metal inhibitors but these had no effect. Therefore the recovery of ammonia after urease incubation was checked in all specimens by incubating duplicate samples with known added amounts of urea. All results of ileostomy urea were then corrected for this recovery. The reproducibility of this method was assessed by performing serial estimations on two ileostomy specimens, one with a low and one with a high urea concentration. The mean urea concentrations were 0.86 mmol/l (SEM 0.23, n = 5) and 4.14 mmol/l (SEM 0.13, n = 8). Finally, bacteriological culture was carried out on a number of the alcoholic mixtures before filtration. These were always found to be sterile, indicating that spontaneous ureolysis had been effectively stopped.

**Urea-turnover studies**

*Diet.* Control subjects were studied on low (40 g) and high (100 g) protein diets. These diets were isoenergetic at 10.5 MJ per day, the change in dietary protein being balanced by changes in carbohydrate content. Subjects were stabilized on each diet for 7 days before the study, which was carried out on the eighth day. Of the six patients with non-functioning colons, two were studied on the 40 g diet only, with the protocol as above, and one was studied on both diets. Three patients who had recently undergone colonic exclusion for chronic encephalopathy were studied on normal ward diets containing approximately 70 g of protein.

**Materials.** [14C]Urea (The Radiochemical Centre, Amersham, Bucks, U.K.), specific radioactivity 30–50 mCi/mmol, was made up in sterile isotonic sodium chloride (150 mmol/l) at a concentration of 5 μCi/ml.

Radioactivity of the standard solution was estimated by adding 10 μl of [14C]urea solution with 0.5 ml Hyamine (500 mmol/l) to 15 ml of scintillation fluid and counting radioactivity for 5 min.

![Graph](image.png)

**FIG. 1.** Semilog regression of 14C concentration in plasma against time showing a straight-line relationship after the 90 min equilibration period.

**Experimental procedure**

Subjects were studied non-fasting, and regular meals were taken throughout the day of study. A steady state was confirmed both by the straight-line regression of log plasma [14C]urea radioactivity against time after at least 90 min had been allowed for equilibration of injected tracer (Walser & Bodenlos, 1959) (Fig. 1) and by the constancy of plasma urea concentration. The dose of [14C]urea injected for each experiment was approximately 5 μCi, the exact amount being calculated by comparing the weight of the syringe and needle before
and after injection. Immediately before intravenous injection of \(^{14}\text{C}\)urea the bladder was emptied. Thereafter, timed blood and urine samples were collected at intervals of between 1 and 2 h over a period of 10 h. Total urine volume was measured during each time-period and urine output maintained at at least 2 ml/min by ensuring that subjects drank at least 200 ml of fluid/h.

**Laboratory procedures**

Plasma and urine \(^{12}\text{C}\)urea was estimated by the diacetylmonoxime method on a Technicon 6/60 AutoAnalyzer (Technicon Instruments, New York, U.S.A.). In urine, the high concentration of urea necessitated a tenfold dilution before estimation. To minimize any error introduced by this dilution, both \(^{12}\text{C}\)urea and \(^{14}\text{C}\)urea were estimated in the same sample of diluted urine.

Estimation of \(^{14}\text{C}\) was carried out by a modification of the method of Walser & Bodenlos (1959). With an Oxford Sampler (Oxford Laboratories International Corp.) paired 1 ml aliquots of either urine or plasma were pipetted into the outer well of 25 ml centre-well flasks. The samples were then acidified with 1 ml of HCl (1 mol/l), a few drops of caprylic alcohol were added, and the flasks shaken to release carbon dioxide. Approximately 1 ml of NaOH (1 mol/l) was added to restore the pH to 7-0 and the solution was buffered to pH 7.2 with 1 ml of Sorensen's phosphate buffer \([\text{KH}_2\text{PO}_4 (0.067 \text{ mol/l})]\) adjusted to pH 7.2 with Na₂HPO₄ (0.067 mol/l)). Approximately 1 ml of Hyamine (500 mmol/l) was pipetted into each centre well. Finally, 1 ml of 3% \((w/v)\) urease solution (jack bean urease, Sigma Chemicals, London) was added to the outer well, and the flasks were stoppered with vaccine caps and incubated in a shaking water bath at 37°C for 20 min to activate the urease. The outer well was then acidified with approximately 1.5 ml of \(\text{H}_2\text{SO}_4 \) (1 mol/l) injected through the cap. Shaking was continued for a further hour to allow released \(^{14}\text{CO}_2\) to be trapped by the Hyamine in the centre well.

Finally, the Hyamine was transferred to counting vials by a multiple washing technique using the scintillation mixture. Five washings of approximately 1 ml each were found to be adequate. The scintillation mixture used was 15 ml of 2,5-diphenyloxazole (0.3%) and 1,4-bis-(5-phenyloxazol-2-yl)-benzene (0.01%). Radioactivity of samples was counted for 20 min and at least 1500 counts using a Tracer-Lab Coromatic 200 Ambient Temperature liquid-scintillation counter. Results were recorded through a linked Diehl computer, programmed to correct for quenching, and results expressed in d.p.m.

**Calculation**

Calculation of urea dynamics was initially carried out by the method of Walser & Bodenlos (1959). It should be noted that in their studies urinary delay time varied from 1-6 h to zero. It is clear therefore that any error resulting in an underestimation of delay time could result in a negative value making further calculation invalid. Such values were obtained in some of our studies. A review of methodology showed that recovery of standard solution of \(^{14}\text{C}\)urea added to control plasma or diluted urine, and extracted as described, was over 99%. It is thought that any error was probably the result of incomplete bladder emptying or possibly in estimation of \(^{12}\text{C}\)urea in urine because of the high urinary urea concentration already mentioned. We have therefore used an alternative method of calculation (Deane, Desir & Umeda, 1968), which gives results comparable with those of other workers while avoiding the calculation of urinary delay time. In this method the urea volume of distribution \((V)\) is calculated at timed intervals after the injection of \(^{14}\text{C}\)urea from the formula:

\[
V_{\text{urea}} = \frac{\text{Injected dose of } ^{14}\text{C}\text{urea} - \text{cumulative urinary } ^{14}\text{C}\text{urea excretion up to time t}}{\text{time t}} \times \frac{\text{time t}}{\text{urea concentration in plasma at time t}}
\]

Plasma \(^{14}\text{C}\)urea concentration decreases faster than \(^{14}\text{C}\)urea is being excreted in the urine, indicating that urea is being lost by a second route, that is breakdown in the gut. Thus the apparent volume of distribution will increase with time. The reciprocal of \(V_{\text{urea}}\) plotted semilogarithmically against time gives a straight-line relationship with a negative slope if time is allowed for complete distribution of injected \(^{14}\text{C}\)urea in the native urea pool (Fig. 2). The slope of this line indicates the fraction of the residual \(^{14}\text{C}\)urea broken down in the gut each hour, which, after equilibration, will be equal to the fraction of the non-labelled urea pool broken down in the gut each hour. As stated, this method makes no specific allowance for the factors of excess excretion or delay of urinary
excretion. However, from the graph in Fig. 2 it can be seen that, at any time $t$:

$$\frac{1}{V_{\text{urea}}} = \frac{[^{14}\text{C}]\text{urea concentration in plasma at time } t}{\text{Injected dose of } [^{14}\text{C}]\text{urea} - \text{cumulative urinary excretion of } [^{14}\text{C}]\text{urea up to time } t}$$

Excess excretion will be contained in the cumulative urinary excretion of $^{14}\text{C}$ up to time $t$. Plasma concentrations of $[^{14}\text{C}]\text{urea}$ are only considered after equilibration, by which time excess excretion will have taken place. Excess of excretion will influence the intercept of the curve but the slope will be unaffected. Thus the fractional rate of urea breakdown will be valid, but because the urea space will be overestimated the absolute rate of breakdown may be rather too large. Urinary delay time implies that cumulative urinary excretion of $^{14}\text{C}$ will underestimate the total amount of $^{14}\text{C}$ which has left the circulation by time $t$. This could affect both slope and intercept and so introduce errors into the calculation of urea pool and fractional metabolism. The importance of this can be assessed by replotting the data, assuming a delay time of 1.0 h (the mean value derived by Walser & Bodenlos, 1959). In two studies reploting the curves increased the intercept by a mean of 7%, i.e. a 7% underestimation of the volume of distribution and hence urea pool. The slope was increased 8.5% on average. The resulting changes in urea production and gut urea breakdown were, however, relatively small (between 1% and 2%) and could be ignored.

From this method the urea pool is the product of $V_{\text{urea}}$ and plasma urea concentration, and the breakdown of urea in the gut is calculated from the product of the urea pool and the slope of the line in Fig. 2. Urinary urea excretion is calculated from the excretion of $[^{14}\text{C}]\text{urea}$ in the urine. In the steady state it is assumed that:

Urea production = urea excretion in urine + urea breakdown in the gut

Statistical comparison of mean values in studies within and between individuals was carried out with Student's $t$-test.

Results

Ileostomy urea

Ileostomy urea concentration in the twenty-nine specimens varied between 0.58 and 9.17 mmol/l. In twenty-six studies the blood urea was estimated at the time of ileostomy sampling and the ratio of

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![Graph](image-url)

**Fig. 2.** Semilog regression of $10^2/V_{\text{urea}}$ against time in one control subject (J.A.G.) on the low (a: 40 g/day) and high (b: 100 g/day) protein diets.

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![Graph](image-url)

**Fig. 3.** Ratio of urea concentration in ileostomy fluid and blood. Blood was taken at the time of each ileostomy fluid sampling and the ratio of ileostomy urea to blood urea estimated.
### Table 1. Urea metabolism in control subjects

<table>
<thead>
<tr>
<th>Subject</th>
<th>Weight (kg)</th>
<th>Volume of distribution (l)</th>
<th>Blood urea (mmol/l)</th>
<th>Urea pool (mmol)</th>
<th>Production of urea (mmol/h)</th>
<th>Excretion of urea (mmol/h)</th>
<th>Gut breakdown of urea (mmol/h)</th>
<th>Gut breakdown as percentage of production</th>
</tr>
</thead>
<tbody>
<tr>
<td>J.M.R.</td>
<td>57.0</td>
<td>40.6 36.9</td>
<td>2.8 4.7</td>
<td>108 173</td>
<td>10.0 20.6</td>
<td>6.2 16.3</td>
<td>3.8 4.3</td>
<td>38 21</td>
</tr>
<tr>
<td>R.L.B.</td>
<td>80.7</td>
<td>47.6 44.7</td>
<td>3.3 5.4</td>
<td>157 240</td>
<td>12.7 31.6</td>
<td>9.7 24.8</td>
<td>3.0 6.8</td>
<td>24 22</td>
</tr>
<tr>
<td>R.A.</td>
<td>68.0</td>
<td>43.2 41.0</td>
<td>4.1 7.0</td>
<td>172 288</td>
<td>18.5 29.3</td>
<td>12.0 20.0</td>
<td>6.5 9.3</td>
<td>25 32</td>
</tr>
<tr>
<td>G.B.</td>
<td>90.5</td>
<td>44.5 49.6</td>
<td>3.4 6.4</td>
<td>148 317</td>
<td>15.7 26.2</td>
<td>11.2 20.0</td>
<td>4.5 6.2</td>
<td>29 24</td>
</tr>
<tr>
<td>H.T.</td>
<td>60.4</td>
<td>37.4 34.1</td>
<td>3.4 5.6</td>
<td>127 190</td>
<td>14.1 23.8</td>
<td>10.3 14.3</td>
<td>3.8 9.5</td>
<td>27 40</td>
</tr>
<tr>
<td>J.A.G.</td>
<td>85.6</td>
<td>51.7 53.6</td>
<td>2.6 6.3</td>
<td>137 337</td>
<td>17.5 37.3</td>
<td>13.5 27.8</td>
<td>4.0 9.5</td>
<td>23 27</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>44.2 43.3</td>
<td>3.3 5.9</td>
<td>142 258</td>
<td>14.8 28.2</td>
<td>10.5 20.5</td>
<td>4.3 7.6</td>
<td>29 28</td>
</tr>
<tr>
<td>S.D.</td>
<td></td>
<td>2.1 3.1</td>
<td>0.2 0.3</td>
<td>9.2 2.8</td>
<td>1.3 2.4</td>
<td>1.0 2.1</td>
<td>0.5 0.9</td>
<td>2.5 3.0</td>
</tr>
</tbody>
</table>

(1) Dietary protein (g/day).
ileostomy urea to blood urea calculated. This varied between 0·13 and 1·90, mean 0·715 (so 0·40, n = 26) (Fig. 3).

Urea metabolism

Normal subjects. Results of studies in six normal subjects are shown in Table 1. The mean breakdown of urea in the gut increased from 4·3 mmol/h (SEM 0·49, n = 6) on a low protein diet to 7·6 mmol/h (SEM 0·89, n = 6) on a high protein diet. Paired testing showed that this increase is statistically significant (t 3.938, P < 0.025). This increase in dietary protein intake also resulted in an increase in urea production from a mean value of 14·2 mmol/h (SEM 1.03, n = 6) to a mean value of 28.2 mmol/h (SEM 2.4, n = 6). Thus, if urea metabolism is expressed as a percentage of urea synthesis in each study, as shown in the last column of Table 1, this percentage is unaffected by variations in dietary protein. From paired testing there is no significant difference (t 0.502, 0.7 > P > 0.6). That is to say, in the subjects studied with normal renal function, a constant proportion of synthesized urea is broken down in the gut.

Patients without functional colons. The results of urea-turnover studies in subjects with non-functioning colons is shown in Table 2. The variable dietary protein intake in these patients makes it impossible to compare gut urea breakdown in absolute amounts with breakdown in normal control subjects. However, we have already shown in the control group that gut urea breakdown is a constant percentage of urea production unaffected by dietary protein. If this percentage is compared in the two groups it will be seen that removal of the colon results in a fall from a mean value of 28.3% (SEM 1·87, n = 6) in control subjects to 19·7% (SEM 2·6, n = 6) in patients with non-functioning colons. By unpaired t-testing this difference is significant at the 2·5% level (t 2-693, P < 0.025). (In patients studied on two diets the mean of the percentages on the two diets was used in this comparison.)

Discussion

The studies of a number of workers indicate decreasing intestinal permeability to urea from the jejunum to the ileum (Fordtran, Rector, Ewton, Soter & Kinney, 1965; Fordtran, Rector, Locklear & Ewton, 1967; Sraer, Rambaud, Bernier & Richet, 1971). Perfusion studies on the intact human colon (Wolpert, Phillips & Summerskill, 1971) suggested that up to 16.6 mmol of urea could cross the intestinal mucosa every 24 h. This is much more than the 1·7 mmol/24 h found by Bown, Gibson, Fenton Snedden, Clark & Sladen (1975) perfusing the excluded colon. The latter workers have suggested that the high figure of Wolpert et al. (1971) could result from contamination of the perfusion segment by ileal contents. Studies of mucosal pore size (Billich & Levitan, 1969) also indicated that urea molecules do not pass readily through the human colonic mucosa; thus, as colonic permeability to urea is very limited, most of the urea entering the colonic lumen must come from the ileum.

Table 2. Urea metabolism in patients without functioning colons

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Dietary protein (g/day)</th>
<th>Weight (kg)</th>
<th>Volume of distribution (l)</th>
<th>Blood urea (mmol/l)</th>
<th>Urea pool (mmol)</th>
<th>Production of urea (mmol/h)</th>
<th>Excretion of urea (mmol/h)</th>
<th>Gut breakdown of urea (mmol/h)</th>
<th>Gut breakdown percentage of production</th>
</tr>
</thead>
<tbody>
<tr>
<td>R.W.(1)</td>
<td>70 (approx.)</td>
<td>85.4</td>
<td>43.0</td>
<td>1·8</td>
<td>77</td>
<td>9·2</td>
<td>8·2</td>
<td>1·0</td>
<td>11</td>
</tr>
<tr>
<td>C.M.(1)</td>
<td>70 (approx.)</td>
<td>85.0</td>
<td>38.2</td>
<td>3·6</td>
<td>138</td>
<td>20·0</td>
<td>16·0</td>
<td>4·0</td>
<td>20</td>
</tr>
<tr>
<td>G.D.(1)</td>
<td>70 (approx.)</td>
<td>62.5</td>
<td>35.3</td>
<td>8·3</td>
<td>292</td>
<td>17·3</td>
<td>12·5</td>
<td>4·8</td>
<td>28</td>
</tr>
<tr>
<td>J.H.(2)</td>
<td>40</td>
<td>59·5</td>
<td>35·7</td>
<td>3·2</td>
<td>115</td>
<td>8·5</td>
<td>6·3</td>
<td>2·2</td>
<td>25</td>
</tr>
<tr>
<td>G.P.(2)</td>
<td>40</td>
<td>58·2</td>
<td>37·9</td>
<td>4·0</td>
<td>153</td>
<td>8·5</td>
<td>6·8</td>
<td>1·7</td>
<td>20</td>
</tr>
<tr>
<td>E.W.(2)</td>
<td>57·0</td>
<td>26·3</td>
<td>3·0</td>
<td>6·5</td>
<td>170</td>
<td>22·8</td>
<td>18·0</td>
<td>4·8</td>
<td>21</td>
</tr>
</tbody>
</table>

(1) Colonic exclusion with ileorectal anastomosis.
(2) Ileostomy.
The only report of ileal urea concentration in the intact subject is that of Billich & Levitan (1969). They found a urea concentration of 2.7 mmol/l with a blood urea of 4 mmol/l, giving a luminal to blood ratio of 0.67. This compares quite closely with our results in which the mean ileostomy urea concentration was 3.6 mmol/l with a mean luminal to blood ratio of 0.72, despite the differing ileostomy bacterial flora (Gorbach, Nahas, Weinstein, Levitan & Patterson, 1967) and ileal adaptation (Phillips & Giller, 1973), which tends to concentrate the ileal fluid. Thus we think that ileostomy urea concentration may be taken as a reasonable estimate of the urea content of normal ileal fluid.

In calculating the [14C]urea kinetics, we have made the simplified assumption that urea is distributed in a single pool. As stated by Jones et al. (1969) this will underestimate results by less than 7%, and, furthermore, in this study data from controls and subjects was handled in the same fashion so that comparison of data is valid. The results in this study are comparable with those of other workers; thus the mean urea volume of distribution expressed as a percentage of body weight in control subjects is 60-0% (SD 6.0, n = 12), which agrees satisfactorily with the data of Walser & Bodenlos (1959), 61.9% (SD 5.3, n = 6), and Jones et al. (1969) 64.7% (SD 13.6, n = 6). These figures, together with the urea pool size and urea production, urinary excretion and gut breakdown, are shown on Table 3. Although there is satisfactory agreement between these variables in the three studies they are not strictly comparable because of varying experimental design regarding diet and physical activity. However, we have already shown that when gut breakdown is expressed as a percentage of urea production, the relationship is unaffected by dietary protein intake and we have therefore compared this percentage in the three studies in the final column of Table 3. There is close agreement between the present study and that of Jones et al. (1969). The percentage is somewhat lower (22%) in the study of Walser & Bodenlos (1959), but Walser has recently reported studies (Walser & Dlabl, 1974) in six further normal control subjects from which may be derived a figure of 29% for gut urea breakdown as a percentage of production. Thus the overall results of other workers are in close agreement with those of our study.

Studies in germ-free rats (Levenson, Crowley, Horowitz & Malm, 1959) and eviscerated rats (Chao & Tarver, 1953) indicate that ureolysis is bacterial in origin and confined to the gut. Studies in man with antibiotics confirm that urease is largely bacterial in origin but are more specific in localizing its site to the large intestine (Aoyagi et al., 1966; Evans et al., 1966). This suggests that urea

<table>
<thead>
<tr>
<th>Table 3. Comparison of results for urea metabolism in control subjects in the present study with results derived from data of previous workers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume of distribution as percentage of body wt.</td>
</tr>
<tr>
<td>--------------------------------------------------</td>
</tr>
<tr>
<td>Present study (Mean, n = 6 on each diet) 40(4) 100(4)</td>
</tr>
<tr>
<td>Walser &amp; Bodenlos (1959)(1) 61.9(3) (n = 6)</td>
</tr>
<tr>
<td>Walser &amp; Dlabl (1974)(1)</td>
</tr>
<tr>
<td>Jones et al. (1969)(2) 64.7(3) (n = 6)</td>
</tr>
</tbody>
</table>

(1) All subjects were studied on uncontrolled normal diets.
(2) All subjects were on a low protein diet on the day before and during the study.
(3) Values for volume of distribution expressed as a percentage of body weight represent the mean of all patients studied whereas values for all other parameters represent the mean of results in normal subjects only.
(4) Dietary protein (g/day).
breakdown occurs mainly within the lumen of the colon, a suggestion which is not supported by the present work.

The amount of urea entering the colon may be calculated from the ileostomy urea concentration and the ileal effluent volume of approximately 1500 ml/day in normal subjects (Phillips & Giller, 1973). Taking the highest urea concentration, this would give only 13·8 mmol/day. As ammonia as well as urea concentration was measured in ileostomy fluid, it is possible to calculate potential colonic urea input assuming that the ammonia resulted from ureolysis. Even using this assumption, the largest result would be only 25·0 mmol/day. If the 1·7 mmol of urea/day found to cross the colonic mucosa (Bown et al., 1975) are added to this figure it will be seen that total colonic urea input will be only 26·7 mmol/day at the very most. If this is compared with the urea breakdown on the low (40 g) protein diet (mean 103 mmol/day) it can be seen that at the very most 25·0% of urea breakdown occurs in the colonic lumen.

Our metabolic studies show that removal or exclusion of the colon results in a significant reduction in gut urea breakdown, expressed as a percentage of urea production. In three subjects with excluded or excised colons (J.H., G.P. and E.W.) studied on a 40 g protein diet, direct comparison with control subjects on the same diet is possible. This shows that removal or exclusion of the colon results in an overall reduction of urea metabolism, production being reduced by 28% and excretion by 14%. However, the effect is far greater on gut breakdown with a 66% reduction, suggesting that removal or exclusion of the colon particularly affects this aspect of urea metabolism. Thus we see a marked reduction of urea breakdown with removal or exclusion of the colon in the face of evidence that only a small amount of urea enters the colon to be broken down in the lumen. This would suggest that urea is being broken down in the colon at a non-luminal site. Houpt & Houpt (1968) first described juxtamucosal ureolysis in ruminants, where urea was broken down in the rumen wall by urease derived from luminal bacteria. A similar mechanism has since been postulated in man in studies on the intact colon (Summerskill & Wolpert, 1970; Wolpert, Phillips & Summerskill, 1970, 1971).

The inhibition of such a mechanism would account for the reduction in ureolysis observed after colonic exclusion or removal. However, if this were the only site of ureolysis, removal or exclusion of the colon should reduce the apparent urea breakdown by this method to a figure equivalent to the mean daily ileal output of 13·8 mmol/day. Thus it is probable that ureolysis in these subjects also occurs in the ileum. This is not surprising in view of the current evidence that the normal ileum contains urea-splitting bacteria (Phear & Ruebner, 1956; Hamilton, Dyer, Dawson, O’Grady, Vince, Fenton & Mollin, 1970; Vince, Dyer, O’Grady & Dawson, 1972). In this study the ileum could account for up to 38% of urea breakdown, a figure derived from the average gut urea breakdown after colon excision or exclusion, 67·0 mmol/day (less 13·8 mmol/day lost into the ileostomy bag) expressed as a percentage of the mean overall gut urea breakdown in the control group (143 mmol/day). It is accepted that this percentage is derived from ileostomy data and must be applied with caution to the normal ileum. Nevertheless, it is probable that ureolysis occurs in the ileum in intact subjects.

It is interesting that colonic exclusion and excision inhibit juxtamucosal ureolysis to a comparable extent. A similar situation was noted by Bown et al. (1975) in their perfusion studies in the excluded colon. The colonic effluent in the perfusion studies contained high counts of faecal-type organisms capable of hydrolysing urea in vitro (Vince, Bown, O’Grady & Dawson, 1973). The absence of non-luminal ureolysis by the excluded colon is not readily explained. It may be that the deeply seated crypt bacterial flora, which may differ from luminal flora (Vince et al., 1973), or the mucosal blood supply are greatly altered by the exclusion procedure or by the underlying disturbance of the portal circulation in these patients.

From the present study we would suggest that the role of the colonic lumen in gut urea breakdown is not as great as has been previously suggested. Ureolysis must take place either in the colon at a non-luminal (juxtamucosal) site or in the ileum or both, but the relative role of each in health cannot be deduced from this study. Inhibition of colonic juxtamucosal ureolysis by colonic exclusion is noted, but the exact mechanism is not fully understood.

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