

# Assessment of the lactulose – mannitol test in Crohn's disease

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**SUMMARY** The mannitol-lactulose intestinal permeability test was evaluated in 100 healthy controls and 47 patients with Crohn's disease. These patients were further separated into three subgroups of increased activity (Harvey-Bradshaw index) and in two subgroups, with elective colonic lesions and associated ileal and colonic lesions. Results were given as percentages of urinary recoveries for mannitol (M), lactulose (L), and L/M ratio. As a whole, patients with Crohn's disease have lower mean M and higher mean L and mean L/M ratios than controls. The magnitude of alterations in M, L, and L/M increased with activity. The sensitivity of the test, however, reached interesting figures (67%:L and 86%:L/M) only in subgroup III which was composed of relapsing patients. Mean M was lower in patients with associated ileal lesion but, whatever the criterion (M, L, or L/M), the test does not provide any clue for the detection of a possible infraclinical associated ileal localisation.

Since the introduction of simple and reliable tests, modifications in intestinal permeability have been observed in various diseases such as untreated coeliac disease,<sup>1-3</sup> dermatitis herpetiformis,<sup>1</sup> atopic eczema,<sup>3</sup> digestive allergy,<sup>1,3</sup> and during different types of chemotherapy.<sup>4</sup> The existence itself or the magnitude of these modifications have sometimes been questioned however; in treated coeliac disease for example.<sup>1,2,5,6</sup> Several investigators, using various probes of intermediate (MW 150-400) molecular size (<sup>51</sup>Cr EDTA, PEG 400, lactulose, mannitol, rhamnose), have reported either a lesser<sup>7</sup> or a greater<sup>8-11</sup> intestinal permeability in patients with Crohn's disease and their healthy relatives.<sup>12</sup> These discrepancies can be partly explained by large inter-individual variations even in healthy subjects and by the relatively small size of the investigated populations.

The aim of the present study was to examine the nature, the prevalence and the magnitude of such modifications by testing large groups of healthy people and patients with Crohn's disease of various activities and different localisations. For the sake of

safety and simplicity, lactulose and mannitol, used simultaneously in a combined test, were chosen as probes for unmediated intestinal permeation (reference in 13).

## Methods

### SUBJECTS

#### *Control subjects*

One hundred healthy volunteers (14–81 years), with no history of digestive, allergic or dermatologic diseases, and not under drug therapy at the time were included in the control group.

#### *Patients with Crohn's disease*

Forty seven subjects (16–77 years) with a clinical, biological, radiological, endoscopic, and histological diagnosis of Crohn's disease were included in the patients group (25 men and 22 women) and separated into three subgroups I, II, III according to the activity index of Harvey and Bradshaw (IHB)<sup>14</sup>; subgroup I: 15 patients (32%) with  $0 \leq \text{IHB} \leq 2$  (16–75 years) were considered to be in remission; subgroup II: 17 patients (36%, with  $2 < \text{IHB} \leq 4$  (19–77 years) were considered to be in a phase of mild activity;

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subgroup III: 15 patients (32%) with  $4 < \text{IHB}$  (17–55 years) were considered to be relapsing; among these, three patients had an IHB between 5 and 7 and 12 had an IHB greater than 7.

Furthermore, the whole group of 47 patients was divided into two subgroups A and B according to the localisation of their lesions: subgroup A: 13 patients (28%) (16–77 years) with one or many evolutive or quiescent colonic lesions but without any detectable lesion of the small bowel; subgroup B: 32 patients (68%) (19–77 years) with associated lesions of the large and the small bowel.

Finally, 14 patients had undergone intestinal resection, the length of which was less than 0.5 m in three patients, between 0.5 and 1 m in five patients and between 1 and 1.5 m in six patients.

#### PROCEDURE

After an overnight fast, patients and volunteers were asked to empty their bladders, and a sample of urine was taken in order to check for possible endogenous mannitol production. They then received by mouth 5 g of mannitol and 5 g of lactulose in 65 ml tapwater. Urine was then collected for the following five hours. During the two first hours the subjects remained without food and were allowed to drink water during the next three hours. At the end of the experiment, urinary lactulose and mannitol concentrations and outputs were determined by gas chromatography, as previously described.<sup>13</sup> Results were calculated as the percent recovery of the ingested dose (L and M) and as the ratio of lactulose and mannitol recovery (L/M). Unpaired, two-tailed Student's *t* tests were used for comparison of the means, and non-parametric tests for comparison of percentages.

#### Results

##### COMPARISON BETWEEN CROHN'S DISEASE PATIENTS AND HEALTHY CONTROLS (Table 1)

Urinary mannitol recovery (M) was significantly ( $p < 0.01$ ) lower in each group and subgroup of patients than in controls, except in group A (13.65 (2.13) *v* 14.34 (0.33)%) which was composed of

patients without associated ileal localisations. In the same way, urinary lactulose recovery (L) and L/M ratio were significantly ( $p < 0.01$ ) higher in each group of patients than in controls. The prevalence of patients under the normal limits (mean  $\pm$  2 SD, calculated in the control population) for mannitol recovery (M) was 30%; that of patients above the normal limits – that is, for lactulose recovery (L) and L/M ratio was 40 and 47% respectively. These percentages, which were significantly different (Cochran test), provide an index for the sensitivity of these tests among the population of Crohn's disease patients, considered as a whole. It must be observed that intestinal resection, did not induce any significant ( $\chi^2$  test) change in these percentages whatever the criterion (L, M, or L/M) taken into account.

##### COMPARISON BETWEEN ACTIVITY SUBGROUPS I, II, AND III

Urinary mannitol recovery (M) was not significantly affected by the degree of activity calculated according to the HB index. Moreover, whatever the criterion (M, L, or L/M), no difference was found between subgroup I ( $0 \leq \text{IHB} \leq 2$ ) and subgroup II ( $2 < \text{IHB} \leq 4$ ). On the contrary, urinary lactulose recovery (L) was significantly greater in subgroup III than in subgroup I ( $p < 0.05$ ) and subgroup II ( $p < 0.01$ ). In the same way, the L/M ratio was significantly higher in subgroup III than in subgroup II (Table 2).

The prevalence of patients with an abnormally low urinary mannitol recovery (M) was no different ( $\chi^2$  test) among subgroups I, II, and III. On the contrary, the prevalence of patients with an abnormally high

Table 2 Comparison (two ways, unpaired *t* test) between subgroups I, II, and III (Cf. Table 1 for related data)

|           | I <i>v</i> II | I <i>v</i> III | II <i>v</i> III |
|-----------|---------------|----------------|-----------------|
| Mannitol  | NS            | NS             | NS              |
| Lactulose | NS            | $p < 0.05$     | $p < 0.01$      |
| L/M       | NS            | NS             | $p < 0.01$      |

Table 1 Urinary recovery (mean (SE)) for lactulose (L), mannitol (M), and L/M in controls and patients with Crohn's disease. The population of patients was separated into three subgroups I, II, III with increased activities and into two subgroups B and A with, or without an associated ileal lesion

|             | Control subjects<br><i>n</i> = 100 | Patients with Crohn's disease |                             |                              |                               |                             |                             |
|-------------|------------------------------------|-------------------------------|-----------------------------|------------------------------|-------------------------------|-----------------------------|-----------------------------|
|             |                                    | Whole group<br><i>n</i> = 47  | Subgroup I<br><i>n</i> = 15 | Subgroup II<br><i>n</i> = 17 | Subgroup III<br><i>n</i> = 15 | Subgroup A<br><i>n</i> = 13 | Subgroup B<br><i>n</i> = 32 |
| Mannitol %  | 14.34 (0.33)                       | 10.99 (0.85)                  | 10.21 (1.38)                | 10.78 (1.02)                 | 11.93 (1.88)                  | 13.65 (2.13)                | 9.68 (0.75)                 |
| Lactulose % | 0.30 (0.02)                        | 0.74 (0.12)                   | 0.51 (0.12)                 | 0.46 (0.13)                  | 1.25 (0.26)                   | 0.49 (0.12)                 | 0.77 (0.14)                 |
| L/M         | 0.021 (0.001)                      | 0.085 (0.015)                 | 0.074 (0.023)               | 0.054 (0.024)                | 0.132 (0.030)                 | 0.041 (0.009)               | 0.093 (0.017)               |

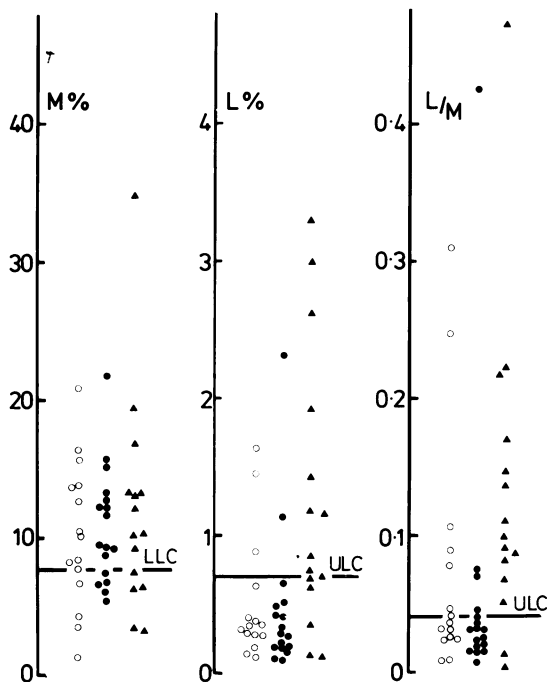


Fig. 1 Distribution of urinary recoveries for mannitol (M), lactulose (L), and for L/M in patients with Crohn's disease, and separated into three subgroups (I ○; II ●; III ▲) of increasing activity. LLC and ULC: lower and upper limits (mean  $\pm$  2 SD) in controls.

urinary lactulose output (L) rose significantly ( $p < 0.01$ ) with the degree of activity, reaching figures of 20 (I), 12 (II), and 67% (III). A parallel increase was observed for the L/M ratio with figures of 40 (I), 23 (II), and 86% (III) (Fig. 1). On the other hand, the sensitivity of the three indexes, M, L, and L/M ratio, expressed as percentages of results under (M) or above (L and L/M ratio) the normal limit was no different in activity subgroups I and II. On the contrary, in activity subgroup III, L and L/M ratio showed a higher sensitivity ( $p < 0.001$ ,  $\chi^2$  test) than M.

#### COMPARISON BETWEEN THE A AND B LOCALISATION SUBGROUPS

Mean urinary mannitol recovery (M) was significantly ( $p < 0.05$ ) lower in subgroup B, composed of patients with associated lesions in the small and in the large bowel, than in subgroup A, composed of patients with elective colonic localisation (Table 1). The prevalence of patients with values below (M), or above (L and L/M ratio), the normal limits, was not significantly different in subgroup with (B) or without (A) an associated small bowel lesion – that is, for subgroup B 37 (M), 44 (L), and 56% (L/M)

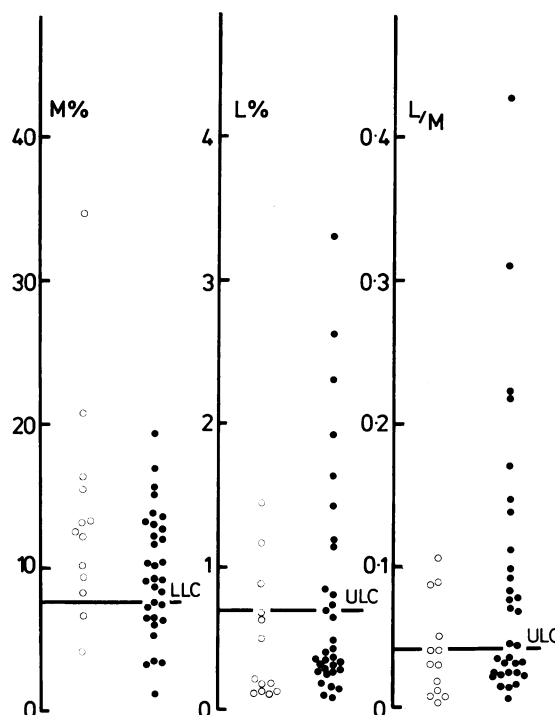


Fig. 2 Distribution of urinary recoveries for mannitol (M), lactulose (L), and L/M in patients with Crohn's disease and separated into two subgroups: A (○) patients with elective colonic localisation and B (●) patients with both ileal and colonic localisations.

and for subgroup A 15 (M), 38 (L), and 31% (L/M) (Fig. 2).

#### Discussion

To assess intestinal permeability in clinical practice, measurement of urinary recovery of a test sugar is a safe and simple tool. Mannitol and lactulose are non-metabolisable, hydrophilic and lipophobic, with negligible affinity for the monosaccharide transport system, and are absorbed passively by non-mediated means. Recovery in urine is almost total and renal clearance is high. Endogenous mannitol production is negligible and lactulose is not hydrolysed by lactase (reference in 9). The simultaneous use of two sugars such as mannitol (MW 182 d, radius 0.40 nm) and lactulose (MW 342 d, radius 0.52 nm) or different MW and thus of different molecular volume ( $206 \times 10^{-3} \text{ nm}^3$ : mannitol and  $362 \times 10^{-2} \text{ nm}^3$ : lactulose), is supposed to allow a differential estimation of transcellular pathways through small size channels, and paracellular pathways through large size channels.<sup>15,16</sup> Furthermore, the simultaneous use

of these two sugars and the calculation of L/M makes the test independent of the degree of completion of the urine collection. Finally, it is known that intestinal permeability to mannitol is close to that of rhamnose<sup>16</sup> and permeability to lactulose does not differ from that of EDTA <sup>51</sup>Cr, and the temporal pattern of absorption of these three markers has been recently described.<sup>17</sup> This second point suggests that, at last in healthy subjects, errors caused by microbial catabolism of the sugar test are unlikely.<sup>15</sup>

Several experiments either with <sup>51</sup>Cr EDTA given orally<sup>7,18</sup> and rectally<sup>18</sup> or sugars<sup>3,9,11,19</sup> have already shown modifications in intestinal permeability. The present study was carried out by comparing a population of one hundred healthy controls and a group of 47 patients – that is, a group far larger than that already examined and made up of 32<sup>8</sup>, 8<sup>9</sup>, 7<sup>3</sup>, 14<sup>11</sup> and 20<sup>10</sup> patients. The results are, however, rather closely similar. Permeation of mannitol is generally low except in patients free from localisation of Crohn's disease in the small bowel. This is in agreement with the concept that mannitol absorption is mainly trans-cellular and located in the small bowel. The low permeability in Crohn's disease could be a consequence of either intestinal hurry or of a smaller surface of absorption possibly related to a reduction in the mean cellular pore size.

As already reported with <sup>51</sup>Cr EDTA in 11 patients and with lactulose-mannitol in seven patients, our results showed an increased permeation of lactulose in 13 patients free of conspicuous ileal lesions. Two hypotheses can be offered to explain these results. It is often considered that permeation tests can disclose slight lesions which cannot be detected by radiology, endoscopy, or histology. The consequence is that, in Crohn's disease, the lesions are probably wider spread than is generally suspected on morphological examination. This opinion is confirmed by Hollander *et al*<sup>12</sup> who recently observed a greater permeation of PEG 400 in healthy relatives of Crohn's disease patients, suggesting that the modification in permeability is not caused by inflammation but is rather a primary defect. As already noted, however,<sup>20</sup> this opinion needs to be confirmed by additional studies, all the more so as recent data show that, in children suffering from Crohn's disease, the increased lactulose permeability is returned to normal by elemental diet.<sup>11</sup> The other hypothesis<sup>21</sup> is based on an increased colonic porosity to large molecular size sugar such as lactulose.

From a practical point of view, the use of a combined lactulose-mannitol test is of small interest in patients in the remission phase, as sensitivity reaches interesting percentages only in patients with IHB>4.

In conclusion, comparisons made between large

populations show, as a whole, that permeation of mannitol is reduced and permeation of lactulose increased in patients suffering from Crohn's disease as compared with healthy people. From a diagnostic point of view, however, the sensitivity of the combined lactulose – mannitol test is low except in relapsing patients. Furthermore, this test does not provide any clue for the detection of an associated infraclinal ileal localisation of the disease.

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