

# Lactose tolerance in lambs with rotavirus diarrhoea

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**SUMMARY** It has been suggested that lactose malabsorption is an important factor in producing the diarrhoea of acute rotavirus infection. Accordingly, the lactose tolerance of gnotobiotic newborn lambs, infected with lamb rotavirus, has been investigated by clinical studies and tissue enzyme assays. Although lactase activity is low in affected areas of the small intestine, rotavirus infected lambs are not lactose intolerant as assessed by the measurement of reducing substances in the faeces, or by the clinical effects and blood glucose levels after a 5.8 mmol (2 g)/kg lactose load on the second day post-infection. Lactose intolerance could be demonstrated by using extremely high (29.2 mmol (10 g)/kg) doses of lactose, three or four times the normal dietary lactose intake. These experiments suggest that lactose-containing feeds (such as maternal milk) are not necessarily contraindicated in patients or animals with rotavirus diarrhoea.

Although gastrointestinal infection with rotavirus is an important cause of acute gastroenteritis in human children and in the young of many animal species, the pathogenesis of the diarrhoea has not yet been elucidated. Lactose malabsorption, with lactose intolerance and an osmotic diarrhoea, has been well documented in children with invasive bacterial gastroenteritis<sup>1,2</sup> and transient lactose intolerance is probably the commonest cause of diarrhoea with delayed recovery after acute gastroenteritis.<sup>3,4</sup> A possible role for lactose malabsorption in acute rotavirus diarrhoea has now been raised by the findings of lactase deficiency in duodenal biopsies from rotavirus infected children.<sup>5,6</sup> Disaccharidase deficiencies had previously been reported in small intestinal biopsies from adult volunteers infected with the Norwalk agent.<sup>7,8</sup> In a series of experiments in piglets infected with either rotavirus<sup>9</sup> or coronavirus (transmissible gastroenteritis virus (TGE))<sup>10-13</sup> Hamilton and his colleagues in Toronto have shown that during the diarrhoeal phase the enterocytes on the small intestinal villi migrate from the crypts at an accelerated rate and fail to differentiate fully. These immature, undifferentiated enterocytes are both disaccharidase deficient and deficient in Na<sup>+</sup>K<sup>+</sup>ATPase. This work provides the theoretical background to the postulates that sugar malab-

sorption<sup>14,15</sup> and/or defective sodium transport<sup>16</sup> are the primary mechanisms of diarrhoea.

Presence or absence of lactose malabsorption in acute rotavirus diarrhoea is of considerable practical importance in clinical management, not only in relation to recommendations as to whether or not disaccharides should be included in fluids for oral rehydration regimes<sup>17</sup> but also to allow for maintenance of nutrition by providing oral feeds which are not likely to be malabsorbed. For ethical as well as practical reasons this subject is difficult to investigate in human infants. However, a considerable amount of information relevant to the human disease has already accrued from work on the virology, pathology, and immunology of rotavirus infections in young domestic animals, and we are currently studying aspects of intestinal injury during and after rotavirus infection in newborn lambs. We have therefore extended our work to examine in detail a possible role of lactose intolerance in the acute diarrhoeal stage in this species. We have adapted a range of techniques which are used in human paediatric gastrointestinal practice to the investigation of these animals, and have supplemented *in vivo* methods by assay of lactase in tissues.

## Methods

### ANIMALS

Gnotobiotic lambs were delivered by hysterectomy and maintained in plastic isolators. Groups of lambs were infected when 2 days old with 2 ml of

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bacteria-free 20% faecal filtrate containing lamb rotavirus from the fourth or fifth gnotobiotic lamb passage.<sup>18,19</sup> Other lambs were kept as uninfected controls. The animals were hand fed using sterile, evaporated cows' milk reconstituted with sterile distilled water. The lactose content of the reconstituted milk was 123 mmol/l (4.2 g/100 ml).

#### REDUCING SUBSTANCES IN FAECES

Specimens of faeces were diluted one in two with water and to 15 drops of diluted faeces a Clinitest tablet was added. The amount of reducing substances was estimated according to the colour resulting, ranging from 0%–2%.<sup>20</sup>

#### LACTOSE TOLERANCE TESTS

Lactose was dissolved in distilled water at a concentration of 292 mmol/l (10 g/100 ml), and sterilised by filtration (0.22 µm). Lambs were weighed within the isolator, and after an overnight fast lactose was administered orally in the animals given a dose of 5.8 mmol/kg and by stomach tube in the animals receiving 29.2 mmol/kg. Lambs were bled at 15 minute intervals for two hours after dosing. Heparinised plasma was separated and stored at -20°C and batches assayed for glucose by the method of Trinder.<sup>21</sup> Results were converted to values for whole blood by a correction factor using the packed cell volume.

#### LACTASE ASSAY

This was carried out in specimens of proximal jejunum, mid-small intestine, and terminal ileum from six infected animals one, two, four, eight, 11, and 15 days post-infection, and six uninfected age-matched controls. The animal was removed from the isolator, anaesthetised, laparotomy carried out, and the biopsies of three small intestinal sites obtained. Thereafter the animal was killed. Specimens were weighed, homogenised, and lactase was assayed by the method of Dahlqvist.<sup>22</sup> Results are expressed as units per gram of tissue wet weight.

## Results

#### CLINICAL AND VIROLOGICAL

Examination of faeces confirmed the presence of rotavirus infection in all of the infected animals and in none of the controls. The clinical course was as previously described, with recovery by four days after infection.<sup>18,19</sup> Infected animals drank less milk than controls on the first and second days after infection, their mean daily intake being 650 and 880 ml compared with 900 and 1080 in uninfected age-matched controls. The difference between

groups was significant, being  $P < 0.01$  on the day after infection. Thereafter infected and noninfected animals had milk intake from 1–1.3 l/day.

#### FAECES REDUCING SUBSTANCES

Samples of faeces were examined at intervals from two to 12 days after birth, in eight uninfected and nine infected animals. Faeces from four of the eight uninfected lambs contained reducing substances at up to 1% concentration as did faeces from seven of the nine infected animals. In both groups positive specimens were obtained from animals aged between 5 and 8 days. The diarrhoeal faeces from infected lambs did not contain reducing substances. The difference between these two groups is not significant (Fisher's test).<sup>23</sup>

#### LACTOSE TOLERANCE TESTS (5.8 mmol (2 g)/kg)

Lactose tolerance tests were carried out at 48 hours post-infection. Four uninfected and seven infected lambs were studied. There was no significant difference between the groups in blood glucose levels after the lactose load (Fig. 1). The animals remained clinically stable: in the infected lambs, there was no change in the character of the faeces, which were semiliquid due to rotavirus infection, and which did not contain reducing substances.

#### TISSUE LACTASE ACTIVITY

In the six uninfected control lambs there was no effect of age on the tissue lactase activity and so these six animals have been grouped and individual values from infected animals compared with the group, by

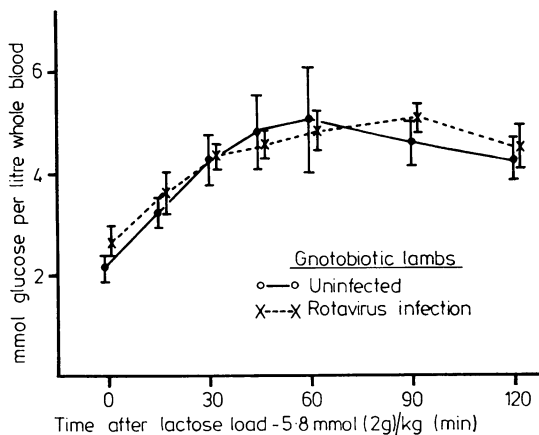


Fig. 1 Blood glucose values (mean ± SE) after 5.8 mmol (2 g)/kg lactose load in groups of gnotobiotic lambs uninfected, or infected 48 hours previously with lamb rotavirus.

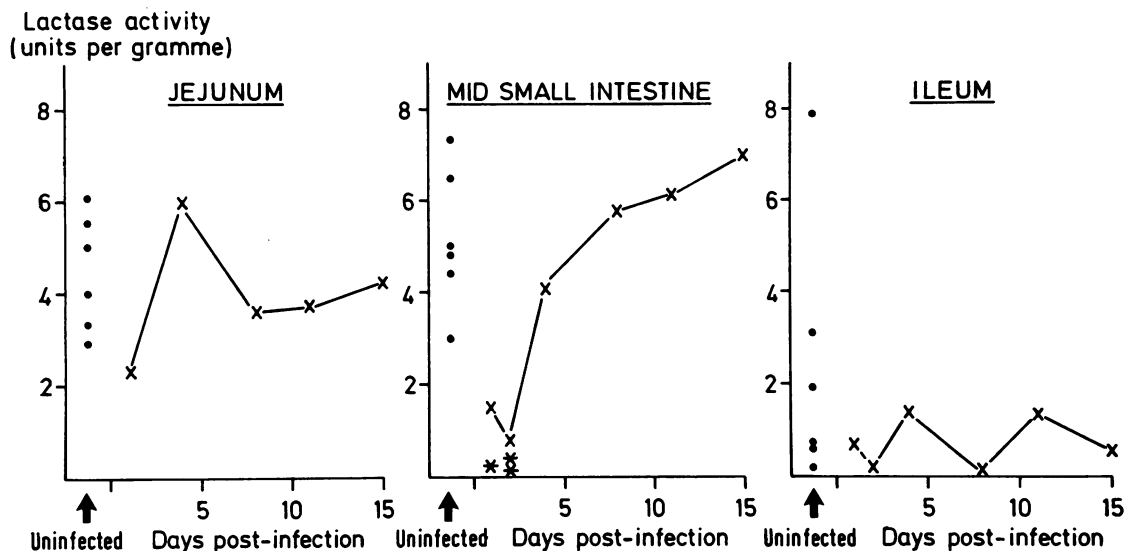


Fig. 2 Lactase activity in small intestine of gnotobiotic lambs, uninfected, or one to 15 days after infection with lamb rotavirus infection (significance of difference from uninfected group \* $P < 0.05$  † $P < 0.01$ ).

using the Zm test.<sup>23</sup> The results are illustrated in Fig. 2. Lactase levels were abnormally low in the specimens taken from mid-intestine at one and two days post-infection, but had returned to normal values by the fourth day. Values in proximal and distal intestine were within the normal range.

**LACTOSE TOLERANCE TESTS (29.2 mmol (10 g)/kg)**  
In view of the apparent discrepancy between the findings of lactase deficiency in biopsy specimens, but apparently normal lactose tolerance by *in vivo* test, we proceeded to use a very large, totally unphysiological lactose load in an attempt to confirm that disaccharide intolerance could indeed be demonstrated in this experimental system. Lactose tolerance tests at 29.2 mmol/kg were carried out in two infected lambs at 48 hours post-infection and in two uninfected controls. Blood glucose levels are illustrated in Fig. 3. In the uninfected lambs the large dose of lactose produced a higher and more sustained rise in blood glucose than the 5.8 mmol/kg dose. In both of the infected lambs blood glucose also rose but the levels were lower than in the control animals. The control animals remained clinically well throughout the procedure, although their faeces were positive at  $\frac{1}{2}$ % and 1% when tested for reducing substances. The two rotavirus infected lambs appeared well for some 90 minutes, but thereafter profuse watery diarrhoea which contained 2% reducing substances was superimposed on the rotavirus diarrhoea. This diarrhoea continued until

the animals were killed some six hours later, at which time the ileum and colon were found to be grossly distended with watery yellow faeces.

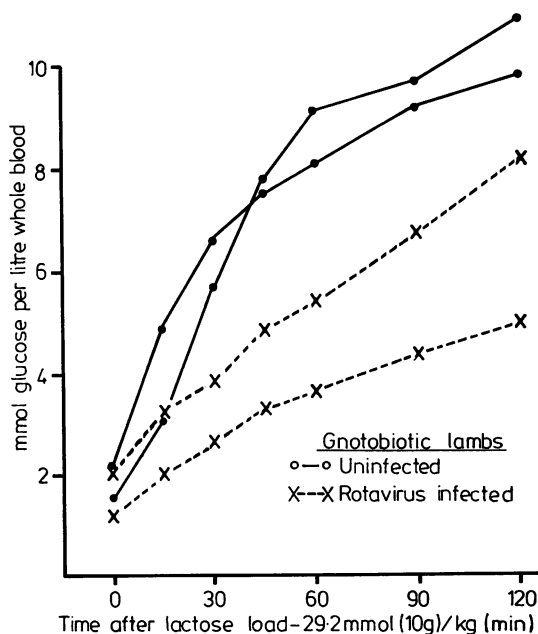


Fig. 3 Blood glucose values after 29.2 mmol (10 g)/kg lactose load in two uninfected gnotobiotic lambs, and in two lambs infected 48 hours previously with lamb rotavirus.

## Discussion

Rotavirus infection of newborn gnotobiotic lambs is typical of the group of acute viral diarrhoeas in neonates. There is a brief acute illness with a low but significant mortality. Infected animals are lethargic and anorectic, diarrhoea lasts up to three or four days, and is accompanied by transient villous atrophy and crypt hyperplasia.<sup>18 19 24</sup> Clinical recovery, and histological recovery as assessed subjectively, are complete by four days post-infection, although measurements of epithelial cell kinetics have shown that crypt hyperplasia persists for at least two weeks.<sup>24</sup>

The Toronto group have been the main proponents of the theory that, from the second day after rotavirus or coronavirus infection, villi are clothed by immature enterocytes, the majority of which do not contain virus, and that the presence of these undifferentiated cells on the villi results in transient malabsorption and net water secretion—that is, diarrhoea. Among the evidence supporting their hypothesis and illustrating the differences between the viral diarrhoeas and the toxigenic bacterial diarrhoeas, are the findings of defective glucose stimulated sodium and chloride transport; low values for  $\text{Na}^+\text{K}^+\text{ATPase}$ ; normal tissue levels of cyclic AMP; reduced sucrase activity and increased thymidine kinase activity in suspensions of enterocytes separated by a vibration technique from the villi.<sup>9-13</sup> Theil has reported similar results in rotavirus-infected piglets,<sup>25</sup> and we have recently reported that the basic assumption underlying this hypothesis, that there is substantially accelerated production of immature enterocytes from the crypts, is correct.<sup>24</sup> The overall crypt cell production rate in uninfected gnotobiotic lambs was 5.8 cells/crypt/hour, and values were found to be significantly increased above this level from day two post-infection, with a peak of 21.2 cells/crypt/hour on the eighth day—four days after apparent return to complete health in these lambs. This large and sustained increase in crypt cell production rate during and after rotavirus infection was surprising, for although an abnormality of cell kinetics with the production of immature undifferentiated enterocytes might explain the acute diarrhoea, cell kinetic changes have been even more profound after clinical recovery. Furthermore, our working hypothesis as to the reason for lactase deficiency was based on the suggestion by Rey and his colleagues some years ago,<sup>26</sup> that rapidly proliferating villus enterocytes will be deficient in lactase because of inadequate time for this disaccharidase to be synthesised by immature cells. Clearly, comparison of our results for lactase activity with the previously published cell

kinetic measurements (which were performed in the same animals) show that changes in cell kinetics have not produced lactase deficiency, for tissue lactase levels were entirely normal in animals from the fourth day after infection.

Lactose malabsorption, if present, would contribute to the diarrhoea and illness of rotavirus infected animals and children by the production of an osmotic diarrhoea, by the loss of a substantial number of calories in the faeces, and possibly also by predisposing to hypoglycaemia. Since gnotobiotic lambs have no intestinal bacteria, measurement of faeces pH and of breath hydrogen excretion after a lactose load could not be used in the evaluation of lactose malabsorption in this system. However, we have used lactose tolerance tests, examination of faeces for reducing substances, and assays of tissue lactase levels to investigate this matter. Our results indicate that, although lactase levels are very low in the mid-intestine, where the viral induced damage is maximal, lactose intolerance does not appear to be present, at least at the doses of lactose which are taken by the animals spontaneously in their ingested milk or with tolerance tests using a dose of 5.2 mmol (2 g)/kg. Standard lactose tolerance tests had no adverse effects in infected lambs, and their blood glucose measurements showed normal absorption. Furthermore, there was no worsening of diarrhoea during or after the lactose load in infected animals. We have been able, by using a greatly increased lactose load, to produce severe watery diarrhoea in rotavirus infected animals but the character of the post-lactose diarrhoeal faeces was quite different from the faeces in rotavirus diarrhoea; and the amounts of lactose used were, in any event, three to four times more than would be ingested spontaneously either by an animal or by a human infant. In the healthy lambs, higher and more sustained levels of blood glucose after the 29.2 mmol (10 g)/kg lactose load, indicate that these animals have considerable reserve capacity for lactose absorption, when tested by a conventional lactose load, and when balanced against their normal milk intake. After rotavirus infection, the intestinal damage has caused a reduction in this reserve of lactase activity, though the animals are still tolerant of the amount of dietary lactose in milk.

It could be argued that lactose intolerance may not be present in those rotavirus infections which spare the jejunum, but will occur in rotavirus infection with a more proximal distribution—for example, in the calf.<sup>27</sup> However, this is not the case. In our studies of rotavirus infected calves (Snodgrass, unpublished) we have again failed to detect reducing substances in diarrhoeal faeces. The enzymes present in relatively undamaged areas of the small intestine

(jejunum in lamb rotavirus infection, ileum in calf rotavirus infection) appear to be adequate for absorption of dietary lactose in these species.

In human children, discrepancy between a state of lactose tolerance or intolerance, and the lactase content of a single jejunal biopsy has been recognised.<sup>28</sup> Again, the explanation is almost certainly that many enteropathies are patchy, and lactose is absorbed by the relatively undamaged parts of the intestine. It should be emphasised that, although pathological changes have been demonstrated in duodenal biopsies from rotavirus infected children, there is no information as to the relative severity of mucosal damage in proximal, mid and distal small bowel in humans. It is likely that some parts of the human small intestine are spared in rotavirus infection, for the reports of clinical aspects of acute rotavirus diarrhoea do not highlight malabsorption as a significant clinical problem.<sup>29-31</sup>

In children who are slow to recover after an acute gastroenteritis, lactose intolerance is not infrequently present. From our work it would seem that, as lactose intolerance is not a feature of the acute rotavirus diarrhoea, some mechanism other than a delayed recovery from rotavirus associated enteropathy should be sought to explain lactose intolerance. Milk intolerance in cows' milk protein hypersensitivity can itself be associated with lactose intolerance,<sup>32</sup> and hypersensitivity to cows' milk is regularly demonstrable in patients with the postenteritis syndrome.<sup>33</sup> This hypersensitivity mechanism producing disaccharide malabsorption deserves additional investigation, in animal models as well as in human clinical practice.

Although one must be cautious in extrapolating the results of experiments such as these to clinical practice in man, our findings suggest that, in the management of acute rotavirus diarrhoea, lactose-containing fluids (such as breast milk) are not necessarily contraindicated as agents for the maintenance of nutrition and hydration.

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