

Deoxycholate depresses small-intestinal enzyme activity

MICHAEL GRACEY¹, MURRAY HOUGHTON, AND JENNIFER THOMAS

From the Gastroenterological Research Unit, Princess Margaret Children's Medical Research Foundation, Perth, Western Australia

SUMMARY Feeding sodium deoxycholate orally to rats for four days caused depression of the activity of the small intestinal enzymes lactase, sucrase, maltase, alkaline phosphatase, and N-acetyl- β -glucosaminidase. The first four are brush border enzymes, the last a lysosomal enzyme. Alkaline phosphatase activity recovered very rapidly and rebounded to above the normal level within 24 hours. The activity of the three disaccharidases returned to normal within seven days while no recovery was observed within 96 hours of the activity of the lysosomal enzyme, N-acetyl- β -glucosaminidase, after removing the bile salt from the diet.

It has recently been shown that extensive ultrastructural damage occurs in the upper intestinal epithelium in patients (Ament, Shimoda, Saunders, and Rubin, 1972) and experimental animals (Gracey, Burke, Oshin, Barker, and Glasgow, 1971; Gracey, Papadimitriou, and Bower, 1974) with bacterial overgrowth in the upper gut. The changes found include distortion and denudation of microvilli and swelling and vesiculation of mitochondria and other intracellular organelles and are accompanied by impaired intestinal active transport (Gracey *et al*, 1971) and depression of activity of brush border and lysosomal enzymes, effects, which, it has been suggested, may be due to the high intraluminal levels of deconjugated bile salts which occur as a consequence of bacterial overgrowth in the upper gut.

In the present study, the effect of the deconjugated bile salt, sodium deoxycholate, on the activity of several small-intestinal enzymes is examined in rats fed with this bile salt for some days. The results suggest that deconjugated bile salts may have harmful effects on enzymes in the small intestinal mucosa.

Materials and Methods

ANIMALS AND FEEDING PROCEDURE

Adult Wistar rats obtained from a long-established colony and weighing 150-250 g were used. Experi-

mental animals had 25 mg of sodium deoxycholate² added each day to their standard laboratory diet for four days. This was done by sprinkling the powdered bile salt over water-sodden feed cubes. At the end of the feeding period a normal diet, without added bile salt, was recommenced. Control animals were housed under identical conditions and had the same diet without the bile salt.

ENZYME ASSAYS

Enzyme assays were done in control animals and in experimental animals immediately on the completion of the bile salt feeding period and at 24-hour intervals up to seven days after recommencing a normal diet.

The enzymes studied were the disaccharidases, lactase, sucrase and maltase, as well as alkaline phosphatase and N-acetyl- β -glucosaminidase. The first four are brush border enzymes (Miller and Crane, 1961; Holt and Miller, 1962), the latter is a lysosomal enzyme (Peters, 1972).

Disaccharidase assays were done by Dahlqvist's method (1964) with minor modifications (Burke, Kerry, and Anderson, 1965). Glucose was determined by the glucose oxidase method of Huggett and Nixon (1957) and protein by the method of Lowry, Rosebrough, Farr, and Randall (1951). In expressing results, 1 disaccharidase unit means the amount of enzyme which hydrolyses 1 μ mole of substrate per minute. Alkaline phosphatase was assayed according to Fishman and Ghosh (1967); 1 unit is that amount

¹Adolph Basser research fellow of the Royal Australasian College of Physicians.

Received for publication 13 November 1974.

²Maybridge Chemical Company, Cornwall.

of enzyme which hydrolyses 1 μ mole of p-nitrophenyl phosphate per minute. N-acetyl- β -glucosaminidase was assayed using 6 mM 4-methylumbelliferyl-2-acetamido-2-deoxy- β -D-glucopyranoside in 1 M acetate buffer at pH 5.0 incubated at 37°C for 25 minutes; the reaction was stopped in 0.2 M glycine buffer at pH 10.7 and the fluorescence measured at an excitation wavelength of 370 nm and an emission wavelength of 448 nm using 10^{-4} M methylumbelliferone as the standard. One unit of enzyme activity is the amount of enzyme which hydrolyses 1 μ mole of the substrate per minute. All enzyme activities are expressed per g protein in the homogenate.

Results in control and experimental animals were compared using standard statistical methods; levels of statistical significance were obtained using Student's t test and P values of <0.05 are taken as being significant.

Results

Details of the results are given in tables I-III. All disaccharidase activities were depressed at the completion of the bile salt feeding period; with lactase and sucrase this effect was significant, with maltase it was not. Five days after the animals had been back on a normal diet this effect persisted to a very similar degree. After another two days, however, there was marked recovery in the activity of these three enzymes. With lactase and maltase, the enzyme activities at this time were significantly greater than

control levels; with sucrase the degree of elevation was outside the chosen level of statistical significance.

Alkaline phosphatase activity was very significantly depressed at the end of the four-day feeding experiment. However, within 24 hours of the animals resuming a normal diet the activity of this enzyme had increased very significantly above control levels; this effect persisted for a further 24 hours but then there was a return to normal levels 72 hours after the normal diet was recommenced.

N-acetyl- β -glucosaminidase activity was significantly depressed at the end of the bile salt feeding experiment. There was no evidence of recovery over the subsequent four days when a normal diet was given; in fact there was a further decline and the degree of depression remained highly significant ($P < 0.0005$).

Discussion

These results indicate that oral administration of the deconjugated bile salt, sodium deoxycholate, to rats caused depression of activity of small-intestinal mucosal enzymes examined under the experimental conditions described. After feeding sodium deoxycholate for four days activity of the three disaccharidases studied was depressed as was alkaline phosphatase, all brush border enzymes, and of N-acetyl- β -glucosaminidase, a lysosomal enzyme. After the animals resumed a normal diet, disaccharidase activity returned to normal over the next seven days,

Time after Bile Salt Feeding Period	Lactase	P	Sucrase	P	Maltase	P
Controls	4.5 \pm 0.8 (4) ²	—	25.4 \pm 6.3 (4)	—	164 \pm 39.9	—
0 hr	1.7 \pm 1.1 (4)	<0.005	16.8 \pm 4.7 (4)	<0.05	125.8 \pm 24.0 (4)	<0.1
120 hr	1.3 \pm 0.2 (3)	<0.0005	16.4 \pm 3.9 (3)	<0.05	125.3 \pm 32.2 (3)	<0.15
168 hr	8.5 \pm 3.9 (4)	<0.05	33.6 \pm 8.8 (4)	<0.1	271.4 \pm 94.9 (4)	<0.05

Table I Disaccharidase activity in jejunal epithelium of control rats and rats fed sodium deoxycholate for four days¹

¹Units per g protein

²Figures in parentheses indicate numbers of experiments

Time after Bile Salt Feeding Period	Alkaline Phosphatase Activity	P
Controls (20)	254.9 \pm 77.0	—
0 hr (12) ²	168.8 \pm 44.5	<0.0025
24 hr (16)	394.2 \pm 132.3	<0.0005
48 hr (16)	355.3 \pm 70.3	<0.0005
72 hr (16)	242.2 \pm 92.0	>0.35
96 hr (16)	303.9 \pm 93.0	>0.05

Table II Alkaline phosphatase activities in jejunal epithelium of control rats and rats fed sodium deoxycholate for four days¹

¹Units per g protein

²Figures in parentheses indicate numbers of experiments

Time after Bile Salt Feeding Period	N-Acetyl- β -Glucuronidase P	P
Controls (16)	151.7 \pm 45.2	—
0 hr (15) ²	124.5 \pm 31.2	<0.05
24 hr (16)	89.1 \pm 17.9	<0.0005
48 hr (16)	98.0 \pm 20.4	<0.0005
72 hr (16)	90.8 \pm 18.9	<0.0005
96 hr (16)	89.5 \pm 18.5	<0.0005

Table III N-acetyl- β -glucosaminidase activities in jejunal epithelium of control rats and rats fed sodium deoxycholate for four days¹

¹Units per g protein

²Figures in parentheses indicate numbers of experiments

alkaline phosphatase activity rapidly recovered to significantly above normal levels within 24 hours and then gradually returned to normal while the activity of N-acetyl- β -glucosaminidase remained inhibited for at least 96 hours, the extent of the period of observation.

The experimental model is a very simple but relatively crude one which, it has been suggested, can be applied to study the effects of deconjugated bile salts on the small intestine (Gracey, Papadimitriou, Burke, Thomas, and Bower, 1973). These substances are present in the lumen of the upper intestine when bacterial overgrowth occurs and the present model appears to provide a simple means of challenging the intestinal epithelium with large quantities of these materials.

The results show that sodium deoxycholate, when present in the lumen of the upper gut in excessive concentrations, may have harmful effects on intestinal digestive enzymes. Since severe ultrastructural damage to intestinal brush borders can be caused by inducing intestinal stasis and bile salt deconjugation (Gracey *et al.*, 1971 and 1974) or by feeding a deconjugated bile salt orally (Gracey *et al.*, 1973) it is not surprising that brush border enzyme activities suffer. This is supported in the present experiments by the finding of inhibition of activity of the brush border enzymes, lactase, sucrase, maltase, and alkaline phosphatase.

There were significant differences in the patterns of recovery of activity of the enzymes studied. Activity of the three disaccharidases studied returned to normal within seven days. This was probably due to regeneration of the small intestinal epithelium which, in rats, is known to occur within a few days (Leblond and Stevens, 1948). The pattern of recovery of alkaline phosphatase activity was, however, remarkably different. At the end of the experimental period this enzyme was significantly inhibited but it recovered and rebounded to well above the normal level within the next 24 hours. A return to normal levels did not occur for a further 48 hours. The normal role of intestinal alkaline phosphatase is obscure (Warnes, 1972) but, among other things, it is considered to be involved in intestinal transport of calcium. Furthermore, it is known that other organs such as bone and kidney (Henrichsen, 1958; Wackstein and Bradshaw, 1965) contain significant amounts of this enzyme and that there is a considerable interchange between intestinal and serum alkaline phosphatase (Langman, Leuthold, Robson, Harris, Luffman, and Harris, 1966). If alkaline phosphatase is required for essential intestinal transport processes and is interchangeable with pools of the enzyme from other sources, it seems possible that the acute, rebound increase in small

intestinal alkaline phosphatase seen in these experiments was due to a rapid attempt at regulation of an intestinal defect caused by a noxious insult.

The other enzyme examined was N-acetyl- β -glucosaminidase, a lysosomal enzyme. Feeding sodium deoxycholate significantly inhibited its activity and it showed no evidence of recovery over the subsequent 96 hours. This suggests that significant quantities of the deoxycholate entered the lysosomal compartments of enterocytes and this is compatible with electron-microscopic studies in the same experimental model which have shown extensive damage to intracellular organelles (Gracey *et al.*, 1973). The lack of recovery of this enzyme activity over the period of observation suggests that this lysosomal enzyme does not regenerate as rapidly as brush border enzymes or that concentrations of sodium deoxycholate remain elevated for longer in this part of enterocytes than in the brush borders.

The relevance of these findings to clinical situations is not yet known. However, the demonstration of ultrastructural damage following intestinal stasis (Ament *et al.*, 1972) and its reproduction in experimentally induced stasis (Gracey *et al.*, 1974), with subsequent impaired intestinal transport and enzyme activity suggest that there is a significant but previously unrecognized mucosal lesion in the 'contaminated small-bowel syndrome'. The findings of the present study suggest that these deleterious effects may be caused by excessive intraluminal concentrations of deconjugated bile salts.

We are very grateful to Mrs A. Malajczuk for her expert technical assistance.

References

- Ament, M. E., Shimoda, S. S., Saunders, D. R., and Rubin, C. E. (1972). Pathogenesis of steatorrhea in three cases of small intestinal stasis syndrome. *Gastroenterology*, **63**, 728-747.
- Burke, V., Kerry, K. R., and Anderson, C. M. (1965). The relationship of dietary lactose to refractory diarrhoea in infancy. *Aust. paediat. J.*, **1**, 147-160.
- Dahlqvist, A. (1964). Method for assay of intestinal disaccharidases. *Analyt. Biochem.*, **7**, 18-25.
- Fishman, W. H., and Ghosh, N. K. (1967). Isoenzymes of human alkaline phosphatase. *Advanc. clin. Chem.*, **10**, 255-370.
- Gracey, M., Burke, V., Oshin, A., Barker, J., and Glasgow, E. F. (1971). Bacteria, bile salts and intestinal monosaccharide malabsorption. *Gut*, **12**, 683-692.
- Gracey, M., Papadimitriou, J., and Bower, G. (1974). Ultrastructural changes in the intestines of rats with self-filling blind loops. *Gastroenterology*, **67**, 646-651.
- Gracey, M., Papadimitriou, J., Burke, V., Thomas, J., and Bower, G. (1973). Effects on small-intestinal function and structure induced by feeding a deconjugated bile salt. *Gut*, **14**, 519-528.
- Henrichsen, E. (1958). Alkaline phosphatase and calcification: histochemical investigations on the relationship between alkaline phosphatase and calcification. *Acta orthopaed. scand.*, Suppl. **34**.
- Holt, J. H., and Miller, D. (1962). The localization of phosphom-esterase and aminopeptidase in brush borders isolated from intestinal epithelial cells. *Biochim. biophys. Acta (Amst.)*, **58**, 239-243.

- Huggett, A. St. G., and Nixon, D. A. (1957). Use of glucose oxidase, peroxidase and O-dianisidine in determination of blood and urinary glucose. *Lancet*, **2**, 368-370.
- Langman, M. J. S., Leuthold, E., Robson, E. B., Harris, J., Luffman, J. E., and Harris, H. (1966). Influence of diet on the 'intestinal' component of serum alkaline phosphatase in people of different ABO blood groups and secretor status. *Nature (Lond.)*, **212**, 41-43.
- Leblond, C. P., and Stevens, C. E. (1948). The constant renewal of the intestinal epithelium in the albino rat. *Anat. Rec. (Lond.)*, **100**, 357-377.
- Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *J. biol. Chem.*, **193**, 265-275.
- Miller, D., and Crane, R. K. (1961). The digestive function of the epithelium of the small intestine. II. Localisation of disaccharide hydrolysis in the isolated brush border portion of intestinal epithelial cells. *Biochim. biophys. Acta (Amst.)*, **52**, 293-298.
- Peters, T. J. (1972). Subcellular fractionation of the enterocyte with special reference to peptic hydrolases. In *Peptide Transport in Bacteria and Mammalian Gut: A Ciba Foundation Symposium*, edited by K. Elliott and M O'Connor, pp. 107-122. Associated Scientific Publishers, Amsterdam.
- Wackstein, M., and Bradshaw, M. (1965). Histochemical localization of enzyme activity in the kidneys of three mammalian species during their postnatal development. *J. Histochem. Cytochem.*, **13**, 44-56.
- Warnes, T. W. (1972). Alkaline phosphatase. *Gut*, **13**, 926-937.