Effects on small-intestinal function and structure induced by feeding a deconjugated bile salt

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SUMMARY Feeding sodium deoxycholate orally to rats for three days caused inhibition of small-intestinal active sugar transport and ouabain-sensitive, sodium-potassium-dependent adenosine triphosphatase activity. At the same time, there was evidence of extensive ultrastructural damage to the microvillar and intracellular compartments of the small-intestinal epithelium while its light microscopic appearance was essentially normal. These functional and morphological changes reverted towards normal over the subsequent four days when a normal diet, without added bile salt, was reintroduced.

Chromatographically pure deconjugated bile salts inhibit intestinal sugar transport in vivo by an incompletely understood mechanism (Gracey, Burke, and Oshin, 1971a; Harries and Sladen, 1972). This seems pertinent to the production of diarrhoea in patients with bacterial overgrowth in the upper intestine, particularly in infancy, where monosaccharide malabsorption may be the major clinical problem in this situation (Gracey, Burke, Oshin, Barker, and Glasgow, 1971c). We report here a convenient experimental model in which the relationship of bile salts to intestinal sugar transport can be investigated in vitro or in the intact animal in vivo.

Materials and Methods

Animals and Feeding Procedure
Adult Wistar rats weighing 150-250 g were used throughout. They were bred from a long-established, closed colony in the University of Western Australia. Experimental animals each had 25 mg of chromatographically pure sodium deoxycholate added daily for 72 hours to the standard laboratory diet by sprinkling the bile salt powder over water-sodden feed cubes. After the feeding period, a normal diet, without added bile salt, was recommenced. Intestinal sugar absorption was studied in vitro and in vivo at daily intervals for up to four days from the completion of the feeding period; simultaneously, small-intestinal ouabain-sensitive, sodium-potassium-dependent adenosine triphosphatase (Na+ + K+ ATPase) activity was examined chemically and histochemically. Light and electron microscopy of the small intestine was also studied at these time intervals.

Uptake Studies in vitro
The uptake studies in vitro were done using the method described by Semenza and Mulhaupt (1969). This technique measures the uptake of substrate by pieces of tissue mounted in a plexiglass tissue-holding apparatus. The animals were killed in a jar saturated with anaesthetic ether. The abdominal cavity was then opened, the small intestine removed and washed through with Krebs-Henseleit (1932) bicarbonate buffer at 37°C and pH 7-4. The gut was then everted and segments 1-5 cm long not containing Peyer’s patches were mounted in a plexiglass tissue-holding apparatus. The substrate chosen was arbutin (p-hydroxyphenyl-β-glucoside); this is a synthetic analogue of D-glucose and is transported by the small intestine of the rat by the same mechanism as glucose (Alvarado and Crane, 1964). It has the advantage over glucose of not being metabolized by the small-intestinal epithelium and is a recognized material for investigation of the intestinal absorption.
active sugar transport pathway (Gracey et al, 1971a).

The incubation medium was based on Krebs-Henseleit (1932) bicarbonate buffer, at 37°C and pH 7.4, to which was added 5 mM arbutin; 3 mM 2-deoxy-D-glucose was included in the medium to assess the amount of sugar entering the tissue by passive diffusion (Bihler and Crane, 1962); this index was subtracted from the arbutin uptake figures. The medium was pregassed for 60 minutes immediately before and throughout the experiments with 95% O₂ and 5% CO₂.

**Absorption Studies in Vivo**

The substrate used was based on Krebs-Henseleit (1932) bicarbonate buffer to which was added 10 mM arbutin with 2 mg/ml of polyethylene glycol 4000 included as a non-absorbable marker. This solution, at 37°C and pH 7.4, was pregassed with 95% O₂ and 5% CO₂ for 60 minutes immediately before the experiments.

The animals were lightly anaesthetized with open ether and the abdomen opened with a mid-line incision. Two ml of the solution was introduced into a 20 cm segment of mid-jejunum which was then returned to the abdominal cavity and the abdominal wound closed with metal clamps. After 10 minutes, the segment was removed and its contents collected. The animals were killed by incising the heart.

**Analytical Methods**

Arbutin was determined as free phenol (Folin and Ciocalteu, 1927; Lowry, Rosebrough, Farr, and Randall, 1951); polyethylene glycol by a modification of Hyden’s method (1956); 2-deoxy-D-glucose was determined by the formation of malonic dialdehyde with peridate and on its condensation with barbituric acid (Waravdekar and Saslaw, 1957). (Na⁺ + K⁺) ATPase activities of tissues taken immediately before the uptake studies in vitro were assayed by a micro-modification of Hirschorn and Rosenberg’s (1968) procedure.

Uptake of arbutin in vitro is expressed as tissue concentration in μ mol/ml of tissue water after correction for passive diffusion indicated by the degree of entry of 2 deoxy-D-glucose while assuming a tissue water content of 80% (Crane and Mandelstam, 1960). The rate of absorption of arbutin in vivo is expressed as μ moles of substrate per cm of intestine per hour as measured by its disappearance from the lumen, after appropriate adjustment for changes in volume indicated by differences between polyethylene glycol concentrations before and after the experiments.

Standard mathematical methods were used to calculate means and standard deviations (SD). Levels of statistical significance were obtained by means of Student’s t test; p values of <0.05 are taken to be significant.

**Histology**

The ouabain-sensitive, sodium-potassium-dependent adenosine triphosphatase activity in control and experimental specimens was localized using the cytochemical technique of Ernst (1972). All specimens were maintained at –70°C then sectioned and placed in the same incubation medium, thus allowing direct comparison of the various specimens.

**Light and Electron Microscopy**

Specimens for light microscopy were fixed in formal saline and carefully orientated before mounting and staining with haematoxylin and eosin.

For electron microscopy, specimens were fixed in 3% glutaraldehyde in 0.1 M cacodylate buffer for 16 hours at pH 7.4. After washing in buffer, the tissues were postfixed in 1% osmium tetroxide in 0.1 M cacodylate buffer, at pH 7.4, for one hour. They were then dehydrated in graded solutions of ethanol and embedded in araldite. Sections were cut on an LKB ultramicrotome, stained with lead hydroxide, and examined on a JEM T6 or a Philips 300 electron microscope.

**Results**

**Sugar Uptake in Vitro (See Table I)**

With 5 mM as substrate, the mean uptake in normal animals ranged from 5.8 μ moles/ml tissue water at 10 minutes’ incubation to 7.5 μ moles/ml tissue water at 40 minutes. At the completion of the three-day bile salt feeding period the rate of uptake was significantly impaired throughout the incubation period. This effect persisted for 72 hours. Results at that time were 3.3 (±1SD, 1.4) at 10 minutes to 5.3 (±1SD, 0.8) at 40 minutes. However, after resumption of a normal diet for 96 hours, there was some recovery; after incubation for 30 and 40 minutes the results were 6.9 (±1SD, 1.1) and 7.3 (±1SD, 2.7) respectively. These results were not significantly different from control values.

**Sugar Absorption in Vivo (See Table II)**

Under control conditions the rate of absorption of arbutin was 3.17 μ moles per cm intestine per hour (±1SD, 1.02). Immediately after the bile salt feeding period this was significantly depressed (1.86; ±1SD, 0.9) and remained so for 48 hours (2.07, ±1SD, 0.75). By 72 hours after the feeding period the rate of absorption of arbutin had returned to within normal limits (2.99; ±1SD, 0.87).
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Table I  Arbutin uptake in vitro by normal rat small intestine and following a three-day period of feeding deoxycholate

<table>
<thead>
<tr>
<th>Incubation Time (mins)</th>
<th>Normal Rats</th>
<th>Hours after Bile Salt Feeding Period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>5.8 ± 0.9 (13)</td>
<td>3.6 ± 0.7 (17)</td>
</tr>
<tr>
<td>20</td>
<td>6.5 ± 1.1 (25)</td>
<td>4.5 ± 0.6* (16)</td>
</tr>
<tr>
<td>30</td>
<td>7.0 ± 1.3 (26)</td>
<td>5.6 ± 1.3* (18)</td>
</tr>
<tr>
<td>40</td>
<td>7.5 ± 1.9 (27)</td>
<td>5.6 ± 1.1* (16)</td>
</tr>
</tbody>
</table>

Table II  Arbutin absorption from a solution containing 10 mM arbutin in vivo by normal rat small intestine and following three-day period of feeding deoxycholate

<table>
<thead>
<tr>
<th>Normal Rats</th>
<th>Hours after Bile Salt Feeding Period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Number</td>
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<tr>
<td>P</td>
<td>&lt;0.005</td>
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</table>

Table III  Jejunal (Na⁺ + K⁺) ATPase activities in normal rats and following feeding sodium deoxycholate for three days

<table>
<thead>
<tr>
<th>Normal Rats</th>
<th>Hours after Bile Salt Feeding Period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Number</td>
<td>88 ± 25</td>
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<td>P</td>
<td>15</td>
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</table>

Jejunal (Na⁺ + K⁺) ATPase activity (see Table III)
In control animals the (Na⁺ + K⁺) ATPase activity of the mid-jejunum was 88 ± 25 μg phosphate per mg protein per hour. At the completion of the bile salt feeding period there was marked inhibition of (Na⁺ + K⁺) ATPase activity (17 ± 8; p < 0.001). There was gradual recovery of enzyme activity over the following 72 hours to within normal limits.

Histochemical demonstration of (Na⁺ + K⁺) ATPase (fig 1)
In the intestines from normal controls, heavy deposits of reaction product were present on the brush border of enterocytes. At the end of the three-day bile salt feeding period a marked reduction in the histochemically demonstrable enzymatic activity on the luminal surface of the jejunal epithelium was observed. The loss of activity was not uniform and some enterocytes showed a greater reduction than others.

One day after the completion of the feeding period a slight increase in enzymatic activity occurred. During the ensuing two days the amount of reaction product gradually increased but was still less than in control tissues. Enterocytes in the basal regions of crypts displayed more enzymatic activity than surface cells.

By the end of the fourth day after the feeding period the appearances of experimental and control tissues were similar although a few cells still exhibited low enzymatic activity. These changes show a remarkable resemblance, in quantitative terms, to the rate of recovery of (Na⁺ + K⁺) ATPase activity, as assessed chemically (see table III).

Light and electron microscopy (figs 2-7)
Light microscopy revealed no significant changes in...
Fig 1  Histochemical demonstration of (Na⁺ + K⁺) ATPase on rat intestine. (a) Normal control, × 35. (b) After feeding deoxycholate for three days; × 40 (c) After feeding deoxycholate for three days and then a normal diet for two days. Enterocytes lining the base of the crypts show more reaction product. × 140. (d) After feeding deoxycholate for three days and then a normal diet for four days; recovery of enzymatic activity is almost complete. × 35.
Fig 2  Normal rat intestine. Note regular, long slender microvilli. × 15 000.

Fig 3  Enterocyte after feeding deoxycholate for three days. The surface shows prominent blebbing (arrows) and microvillar denudation. The remaining microvilli are swollen. × 19 500.
Fig 4  Enterocyte after feeding deoxycholate for three days. Microvilli are swollen and detached. The terminal web is disrupted, mitochondria are swollen, and endoplasmic reticulum is vesicular. × 35,000.

Fig 5  Enterocyte after feeding deoxycholate for three days. Some microvilli are irregular (arrows), mitochondria are swollen, and the ergastoplasm is vesicular. × 35,000.
Fig 6  Enterocytes after feeding deoxycholate for three days. Some enterocytes show fewer and shorter microvilli while others show complete microvillar denudation (arrow) with disruption of the terminal web. Micropinocytosis is absent. \( \times 29000 \).

Fig 7  Enterocytes after feeding deoxycholate for three days and then with normal diet for another three days. The microvillar pattern is normal but the contents of goblet cells protrude into the lumen (arrow). \( \times 11500 \).
the integrity of the small-intestinal epithelium after
the end of the bile salt feeding period. The only
detectable abnormality was a minor degree of
separation of the submucosal tissues and com-
ponents of the lamina propria.

However, at the end of the feeding period there
were numerous enterocyte abnormalities when
examined by electron microscopy. These structural
lesions were more pronounced on the luminal
surface of the epithelial cells but abnormalities were
present in other parts of the enterocytic cytoplasm.

Some enterocytes were normal, while adjoining
cells had shortened and blunted microvilli, often with
a reduction in the concentration of microvilli; in
some areas microvilli were virtually absent, present-
ing a flat, featureless contour.

Some microvilli were swollen and rounded, others
were elliptical or fused together at their bases. The
terminal web apparatus in these damaged cells was
distorted and disorganized, often reduced in amount
and sometimes almost completely absent. Micro-
pinocytotic activity was reduced in these areas. In
some cells the terminal web was replaced by a mass
of scattered fibrils embedded in an electron-dense
hyaloplasm. Cells with only few microvilli often
showed rarefaction of the apical part of the cytoplasm
which in addition lacked the normal concentration
of organelles. The rest of the cytoplasm displayed
various degrees of mitochondrial swelling, fragmenta-
tion of the rough endoplasmic reticulum, and
degranulation of the cisternae. Goblet cells were
prominent and their mucous contents often pro-
truded into the lumen.

During the subsequent four days there was a
gradual recovery of ultrastructural integrity. After
24 hours improvement was slight; by 48 hours fewer
damaged cells were visible, rarefaction of the apical
part of the cytoplasm was not found but abnormal
microvilli and enterocytes with microvillar denu-
mation were still seen in a few cells. By the end of
the fourth day very few damaged cells were found but
occasional enterocytes with all the described
changes, except rarefaction of the apical cytoplasm,
were still present. Large mucus-containing goblet
cells remained prominent.

Discussion

The present study indicates that important, deleteri-
ous effects on small-intestinal function and structure
can be induced experimentally by feeding a deconju-
gated bile salt orally. In these experiments there was
impairment of intestinal active sugar transport and
\((Na^+ + K^+)\) ATPase activity while extensive damage
to the microvillar and cytoplasmic parts of entero-
cytes was evident by electron microscopy. All these
changes recovered significantly within four days of
returning the animals to a normal diet. The exper-
imental model used is a convenient one and could
be used to examine further the interrelationships of
bile salt metabolism and intestinal sugar trans-
port.

Malabsorption of carbohydrate occurs in patients
with bacterial contamination of the upper gut
(Paulk and Farrar, 1964; Donaldson, 1967), parti-
cularly infants (Gracey, Burke, and Anderson,
1969), presumably because of the presence of excess-
ous amounts of deconjugated bile salts (Gracey
et al, 1971c) produced in the lumen of the gut by
bacterial degradation. This suggested mechanism
seems supported by recent experiments in which
impaired intestinal sugar transport was produced in
rats in vivo by surgically inducing stasis and
bacterial overgrowth in the upper gut (Gracey et al,
1971c) or by perfusing the intact normal upper gut
with solutions containing deconjugated bile salts
(Gracey, Burke, and Oshin, 1971b; Harries and
Sladen, 1972). The effect appears to be a direct
inhibition of the intestinal active sugar transport
pathway by these substances since it is not modified
by the presence of normal amounts of conjugated
bile salt (Gracey et al, 1971b). In this way, it differs
from the pathogenesis of steatorrhoea in the ‘con-
taminated small-bowel syndrome’ where the most
important factor is reduction of the amount of
conjugated bile salts present to below the critical
micellar concentration rather than the presence of
excessive amounts of deconjugated bile salt (Cheney,
Burke, Clark, and Senior, 1970).

In the present studies, temporary depression of
\((Na^+ + K^+)\) ATPase activity was demonstrated,
chemically and histochemically, and recovered
gradually as did the ability of the gut to transport
arbutin, an analogue of D-glucose. Linkage between
transport of sodium and sugars has been shown
consistently in vivo (Csáky and Zollicoffer, 1960)
and in vitro (Bihler and Crane, 1962), and it is often
considered that \((Na^+ + K^+)\) ATPase is closely
associated with these phenomena (Skou, 1965). The
present findings would appear to support this
postulate. However, recent studies in vitro have
demonstrated that intestinal active sugar transport
can continue in the virtual absence of this enzyme
activity (Gracey, Burke, Storrie, and Oshin, 1972).

Of what significance are the electron microscopic
findings of the present investigation? It has been
widely held that bacterial contamination in the
upper gut does not influence the appearances of the
intestinal epithelium. Others have presented the
opposite view (Paulley, 1969) and recent electron
microscopy studies (Gracey et al, 1971c; Ament,
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Shimoda, Saunders, and Rubin, 1972) have shown extensive damage to enterocytes in patients and experimental animals with an abnormal small intestinal flora. Morphological changes have also been induced experimentally by using deconjugated bile salts. Fry and Staffeldt (1964) added 2% sodium deoxycholate to the diet of mice and subsequently observed submucosal oedema and separation in the duodenum with some later denudation of ileal enterocytes; these changes were temporary and attributed to the surface action of the bile salt on the cells. Shiner (1969) instilled deoxycholate intraduodenally in rats and, by adjusting the concentration of the bile salt, was able to induce significant ultrastructural changes while the appearance of the epithelium on light microscopy was normal. In the present study the integrity of the intestinal epithelium was maintained to light microscopic appearances yet there was extensive and irregularly distributed damage to the epithelial cells evident on electron microscopy. The interpretation of the significance of these findings is difficult. They show close similarities to those found in experimental animals in which deoxycholate is instilled intraduodenally (Shiner, 1969) and in rats with a self-filling jejunal blind loop (Gracey et al., 1971c). They are therefore, probably, of more relevance than the more marked histological findings reported previously in experiments conducted in vitro with deoxycholate or by perfusing the deconjugated bile salt into isolated loops of gut in vivo (Low-Beer, Schneider, and Dobbins, 1970; Tomkin and Love, 1972). These latter experiments involve a considerable amount of inevitable experimental interference under somewhat unphysiological conditions. Apart from these criticisms, the present method may have more relevance than acute experiments, such as those mentioned above, to clinical situations in which deconjugated bile salts affect small intestinal function over days, weeks, or months, rather than for minutes or a couple of hours as in acute experiments. Furthermore, it is much more convenient than another alternative technique in which prolonged bacterial contamination and deconjugation of bile salts are produced surgically by the construction of a self-filling jejunal blind loop (Cameron, Watson, and Witts, 1949).

The technique used in these experiments appears to be a convenient and acceptable one in which the interrelationships of bile salts and intestinal sugar transport can be pursued further. It results in temporary impairment of sugar transport and (Na+ + K+) ATPase activity as well as ultrastructural damage to the small-intestinal epithelium. These abnormalities return towards normal within a few days, probably because of regeneration of the epithelial cells (Leblond and Stevens, 1948).

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References


