Faecal steroid profiles in ileal reservoir patients

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In the treatment of colonic disease such as ulcerative colitis, polyposis and colon cancer, an operation has been developed involving a restorative proctocolectomy with ileal reservoir (Nicholls, 1982). This restores intestinal continuity with the formation of a ‘pouch’ from the terminal ileum. Faecal samples were obtained from 30 individuals who had undergone the above operation, nine ileostomy patients and 20 healthy controls. The samples were immediately deep-frozen before being freeze-dried and homogenized. Faecal samples were obtained from 30 individuals who had undergone the above operation, nine ileostomy patients and 20 healthy controls. The samples were immediately deep-frozen before being freeze-dried and homogenized. Faecal steroids were extracted and fractionated according to the method of Owen et al. (1984) into neutral sterols, free bile acids, glycine-conjugated and taurine-conjugated bile acids. Neutral sterols and methyl esters of bile acids were analysed by g.l.c. (Owen et al., 1984), while conjugated bile acids where quantified using h.p.l.c. (Fadden et al., 1985).

The results (Table 1) show that both the pouch and ileostomy groups had far lower concentrations of neutral sterols than the controls. Degradation of cholesterol to its bacterial metabolite coprostanol was absent in the ileostomy group, whilst in the pouch group the level of degradation was only 12% compared with 68% for the controls. The pouch and ileostomy patients also had lower free bile acid concentrations than the control group, with the bacterial transformation of primary bile acids (cholic and chenodeoxycholic acids) into secondary bile acids (deoxycholic and lithocholic acids) via 7α-dehydroxylation showing marked differences between the groups. Primary bile acids were the principal free bile acids in the pouch and ileostomy groups, comprising 69% and 66% of the total respectively, whilst secondary bile acids were detected in the control group. This lack of bacterial metabolism of steroids in pouch and ileostomy patients was also highlighted by detection of high levels of conjugated bile acids. In the ileostomy group 56% of the total bile acid concentration was due to conjugated bile acids compared with 25% for the pouch and 6% for the control groups.

The results for the ileostomy patients are in agreement with the findings of Kay et al., (1979) and Fernandez et al., (1984) in that steroid metabolism is minimal in ileostomy effluent. However, in patients with an ileal reservoir cholesterol reduction and conjugate hydrolysis by the enteric flora are not so adversely affected, but the degree of 7α-dehydroxylation of primary bile acids is still minimal. This lack of steroid metabolism is probably due to a combination of the low counts of bacteria found in pouch effluent compared with faeces and the vast reduction in transit times compared with healthy controls. The high levels of primary bile acids found in pouch patients indicates that conditions within the pouch are not sufficiently anaerobic for the 7α-dehydroxylase enzyme to be elaborated by the enteric flora. A possible explanation for this lack of 7α-dehydroxylation may be shown by separating the pouch patients according to their design of rectal pouch construction. There are three types of pouch, namely the J (Utsunomiya et al., 1980), S (Parks et al., 1980) and W (Nicholls & Pezim, 1985) constructed from two, three and four loops of the terminal ileum respectively. It is the S pouch which exhibits the most 7α-dehydroxylation with the level of primary bile acids being only 56%, compared with 74% and 83% for the J and W pouches respectively. This difference may be due to the S pouch having a distal rectal segment attached to the anal canal, whereas the J and W pouches are attached directly to the anal canal which may make them less anaerobic.

In conclusion this study shows steroid metabolism is enhanced in a rectal pouch compared with an ileostomy pouch, but is considerably lower than controls, with the S pouch showing a closer approximation to the human colon.

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Table 1. Faecal steroid profiles of the three study groups and also for the pouch patients separated according to rectal pouch design

| Abbreviations: COP, coprostanol; XOL, cholesterol; FBA, free bile acid. |
|------------------|------------------|------------------|------------------|
|                  | Pouch patients   | Ileostomy patients | Control group |
| COP              | 1.1 ± 2.8        | 0                | 19.0 ± 9.3      | 0                | 1.9 ± 4.1        | 0.4 ± 1.0       |
| XOL              | 7.1 ± 5.4        | 6.7 ± 3.8        | 7.1 ± 5.2       | 5.8 ± 4.0        | 8.8 ± 4.9        | 6.3 ± 3.7       |
| Total steroids   | 8.3 ± 5.3        | 6.7 ± 3.8        | 26.1 ± 10.9     | 5.8 ± 4.0        | 10.7 ± 3.7       | 6.6 ± 3.7       |
| Total FBA        | 4.2 ± 3.2        | 2.5 ± 3.4        | 5.8 ± 3.5       | 3.5 ± 3.1        | 4.2 ± 2.9        | 5.9 ± 3.9       |
| New total conjugates | 1.9 ± 3.5 | 6.7 ± 4.5        | 0.4 ± 0.3       | 1.0 ± 1.1        | 0.1 ± 0.1        | 4.6 ± 5.5       |
| COP (%)          | 12 ± 25          | 0                | 12 ± 17         | 0                | 16 ± 31          | 7 ± 17          |
| Primary FBA (%)  | 69 ± 29          | 66 ± 33          | 0               | 74 ± 33          | 56 ± 33          | 83 ± 10         |
| Conjugates (%)   | 25 ± 26          | 56 ± 38          | 6 ± 7           | 19 ± 13          | 13 ± 23          | 40 ± 32         |

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A reversible Ca\(^{2+}\)-dependent pore activated by oxidative stress in heart mitochondria

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It is well known that the energy-transducing and permeability properties of mitochondria are disrupted on accumulation of excess Ca\(^{2+}\), in particular in the presence of P. Recent studies of liver mitochondria in this laboratory (Al Nasser & Crompton, 1986a,b) indicate that the lesion may be due to a reversible Ca\(^{2+}\)-activated pore in the inner membrane that admits H\(^{+}\) and small solutes (e.g. sucrose, arsenazoIII) but not protein. The steady-state distribution between open and closed pore conformations appears to be very largely towards the closed form until a critical limit of about 10\(\mu\)M matrix free Ca\(^{2+}\) is exceeded, so that the pathological domain of Ca\(^{2+}\) action is clearly separated from the physiological domain of dehydrogenase control (around 1\(\mu\)M).

The present study extends these observations to heart mitochondria. Tissue Ca\(^{2+}\) overload is thought to be a critical factor in the progression of ischaemia/reperfusion injury in heart and other tissues. Since this form of injury is also associated with oxidative stress (e.g. Poole-Wilson et al., 1984) a related question is whether oxidative stress also affects the state of the hypothetical pore.

Pore opening was measured as an increase in sucrose permeability, i.e. decrease in sucrose-inaccessible space. Rat heart mitochondria (1.5 mg of protein/ml) were preincubated in basic medium (pH 7.0) containing 120 mM-KCl, 10 mM-Hepes, 1.5 \(\mu\)g of rotenone/ml, 5 \(\mu\)M-tetraphenylphosphonium ion (TPP\(^+\)), 5 mM-succinate and either 5 mM- or 0.2 mM-K\(_2\)H\(_2\)PO\(_4\), CaCl\(_2\) (100 nmol/mg of protein) was then added (zero time, Fig. 1a, and b). The sucrose-inaccessible space (\(\mathrm{H}_{2}\mathrm{O}\) minus \([^{14}\mathrm{C}]\mathrm{sucrose}\) and TPP\(^+\) uptake were determined as previously described (Al Nasser & Crompton, 1986b).

In Fig. 1(a), samples of the incubate were removed at intervals as indicated on the abscissa, labelled sucrose and H\(_2\)O were added, and the spaces determined. The data show that whereas excess Ca\(^{2+}\) produced a progressive decrease in the sucrose-inaccessible space, the space was restored on Ca\(^{2+}\) removal with EGTA, which is consistent with the presence of a reversible Ca\(^{2+}\)-activated pore. Of note is that pore closure displayed an initial rapid phase in which 65\% of maximal closure was complete in 5s.

In order to investigate the influence of oxidative stress (imposed by t-butylhydroperoxide), the [P] was decreased to 0.2 mM to slow the rate of permeabilization and facilitate measurement. In addition, labelled sucrose and H\(_2\)O were introduced to the whole incubate at zero time. Under these conditions, sucrose entry was very markedly increased by t-butylhydroperoxide (Fig. 1b). However, TPP\(^+\) uptake, an index of the inner membrane potential (\(\Delta\psi\)), was quite unaffected by t-butylhydroperoxide, at least in the early stages. These data extend our previous model of reversible Ca\(^{2+}\)-dependent permeabilization (Al Nasser & Crompton, 1986a) to heart mitochondria and oxidative stress; in simplified form:

\[ \text{Ca}^{2+}, \text{P}, \text{t-butylhydroperoxide} \rightarrow \text{ATP} \rightarrow \text{Open pore} \]

Abbreviation used: TPP\(^+\), tetraphenylphosphonium ion.

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**Fig. 1. Effects of Ca\(^{2+}\), P, and t-butylhydroperoxide on the sucrose-impermeable space and TPP\(^+\) uptake**

(a) 5 mM-P; sucrose-inaccessible space (D); Ca\(^{2+}\) was added at zero time and 10 mM-EGTA at 13 min. (b) 0.2 mM-P; residual sucrose-inaccessible space (\(\varphi, \Delta\)); TPP\(^+\) uptake (\(\bullet, \circ\)); Ca\(^{2+}\) was added at zero time in the presence (\(\Delta, \circ\)) or absence (\(\varphi, \bullet\)) of 50 \(\mu\)M-t-butylhydroperoxide.