

## *Progress report*

# Deoxycholic acid and the pathogenesis of gall stones

That deoxycholic acid (DCA) may have a role in cholesterol gall stone formation has been discussed on several occasions<sup>1-5</sup> but authoritative reviews of gall stone pathogenesis fail to mention this possibility.<sup>6-11</sup> This may be because the evidence has never been comprehensively reviewed and so its strength is not widely appreciated. Perhaps, too, it is hard to believe that events in the colon can influence the fate of the gall bladder. But the facts suggest that this is indeed the case.

### **Deoxycholic acid – basic facts**

Because of an efficient enterohepatic circulation, less than 10% of the circulating bile salt pool reaches the colon each day.<sup>12,13</sup> As bile salts pass down the ileum they are subject to bacterial attack, which may be extensive in the terminal ileum,<sup>14</sup> but this is usually limited to deconjugation which does not impair absorption. In the colon<sup>15,16</sup> any cholic acid which has escaped absorption in the terminal ileum is rapidly converted to DCA<sup>17,18</sup> by anaerobic 7  $\alpha$ -dehydroxylation,<sup>19-21</sup> so that only small amounts of cholic acid are detectable in the faeces.<sup>22,23</sup> Of the newly formed DCA one third to a half is absorbed by passive diffusion and recirculated.<sup>17,18,24</sup> In normal subjects this amounts to about 150 mg per day,<sup>25</sup> the remainder being precipitated or adsorbed to solid matter and lost in the faeces. The circulating pool of DCA is about 500 mg (1.25 mmol)<sup>25</sup> and DCA constitutes 10–40% of the bile acids in bile.

### **Epidemiological relationships**

The established risk factors (in the sense of personal characteristics) for cholesterol rich gall stones are increasing age, female sex, parity, obesity, and raised plasma triglycerides.<sup>26</sup> It is not widely appreciated that some of these risk factors are associated with increased concentrations of DCA in bile. With respect to age, the pool of circulating DCA was found to be significantly larger in a group of elderly Dutch people than in a group of young ones.<sup>27</sup> With respect to sex, the bile of healthy age matched men and women has not often been compared but in a Canadian study there was a trend towards more DCA in the bile of women.<sup>28</sup> In a small Australian study the women had 25% of DCA in their duodenal bile acids while men had only 9%<sup>29</sup> and, in Sweden, hyperlipoproteinaemic women had more DCA in their bile than men.<sup>30</sup> With respect to hypertriglyceridaemia, Swedish people with type IIB and type IV hyperlipoproteinemia had 39% and 41% DCA in their bile while healthy controls had only 23%.<sup>30</sup>

Vegetarian women have a lower than expected prevalence of gall stones (explained only in part by their tendency to be slim).<sup>31</sup> They have also been

reported to have small DCA pools – 18  $\mu\text{mol/kg}$  compared with 29  $\mu\text{mol/kg}$  in age matched meat eaters.<sup>32</sup>

Thus four risk factors for gall stones – age, female sex, hypertriglyceridaemia and not being a vegetarian – are associated with increased concentrations of DCA in bile. The other risk factors (parity and obesity) have not been looked at from this point of view.

### **Raised proportion of deoxycholic acid in the bile of gall stone patients**

In 1971 van der Linden<sup>33</sup> observed that there was an increase in the ratio of dihydroxy bile acids (deoxycholic and chenodeoxycholic acid) to trihydroxy bile acids (cholic) in subjects with gall stones. He suggested that the shift towards dihydroxy bile acids favoured gall stone formation. This shift is now known to be caused by an increase in DCA. In 19 publications there have been 20 comparisons of the bile acid composition of gall stone subjects and controls.<sup>28 34–51</sup> In 19 comparisons the proportion of DCA was higher in the gall stone subjects and in one there was no difference (Figure). In seven of the studies the difference was reported as statistically significant<sup>28 42 43 45 47 51</sup> and in another two<sup>35 36</sup> the difference was marked but tests of significance were not reported. In one of the latter studies<sup>36</sup> the data allow statistical

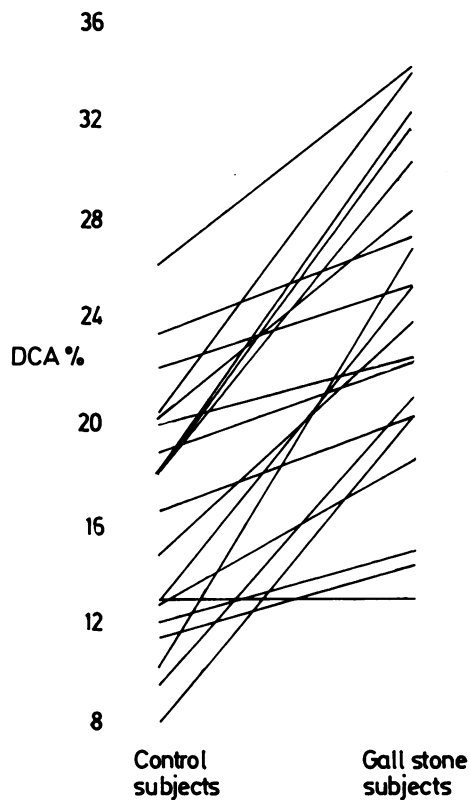


Figure Mean percentage of deoxycholic acid (DCA) in the duodenal bile acids of subjects with gall stones and of controls; results from 20 published studies.

testing and a significant difference is indeed present. In the remaining studies the difference was not significant but, with one exception,<sup>48</sup> the difference has always been in the same direction. Such conformity is unlikely to be due to chance.

### **Relationship between the proportion of DCA in bile and biliary saturation with cholesterol**

In several groups of subjects, including the 280 men in the US Cooperative Gallstone Study, a significant correlation has been shown between the proportion of DCA in duodenal bile acids and the moles % cholesterol or cholesterol saturation index (CSI) of gall bladder bile<sup>50-52-54</sup> (or hepatic bile<sup>55</sup>). Similarly, we found a correlation between artificially induced changes in these two parameters.<sup>25</sup> Subjects with type IIb and IV hyperlipoproteinaemia who, as noted, have a significantly higher proportion of DCA in their bile than healthy controls generally have bile supersaturated with cholesterol.<sup>30</sup>

These findings and the other associations between gall stone risk factors and raised % DCA in bile suggest that the excess of DCA in the bile of gall stone subjects is not secondary to their gall stones but, rather, that it antedates the gall stones and favours the formation of superstaturated bile, which is the metabolic precursor of cholesterol gall stone formation. If this is so, any agent or manoeuvre which causes a change in the DCA content of bile should also induce a change in CSI in the same direction.

### **Manoeuvres which reduce DCA in bile**

Wheat bran was the first agent shown to alter DCA metabolism<sup>56</sup> and it has been repeatedly confirmed that bran lowers % DCA in bile<sup>43-56-60</sup> (although, not surprisingly, the change is less marked or absent if the initial level of DCA is low<sup>29-61-62</sup>). At the same time, provided that bile is initially supersaturated, wheat bran causes a significant reduction in biliary cholesterol saturation (Table 1).<sup>29-43-57-60-62</sup> The effects of bran on bile have recently been reviewed.<sup>63</sup>

Other ways have been devised of lowering the amount of deoxycholic acid in bile in order to study how this would affect biliary CSI (Table 1). In the first such study, metronidazole was supplied to healthy men for 10 days in order to inhibit anaerobic bacterial activity in the colon and interfere with dehydroxylation of cholic acid. The percentage of DCA fell in all subjects and the CSI fell in all but one of them.<sup>64</sup> In another study, ampicillin was found to have similar effects on bile, probably for the same reasons, when given to subjects with or without gall stones.<sup>65-66</sup> In a different approach to the same problem, the synthetic, unabsorbed disaccharide lactulose was administered to healthy middle aged women. Lactulose acidifies the right side of the colon, thereby inhibiting bacterial 7  $\alpha$ -dehydroxylases which are inactive at pH <6. It lowered the percentage of DCA in duodenal bile acids in all the subjects and the CSI in all but one.<sup>67</sup> The ingestion of a preparation of live *Streptococcus faecium* had similar effects on bile, probably by displacing the anaerobic organisms in the colon.<sup>68</sup> When patients with hypothyroidism were treated with L-thyroxine, there was a significant fall in % DCA in the bile and in CSI.<sup>69</sup> At the time these findings were unexplained

but it can now be postulated that they were due to speeding up of colonic transit as constipation is a feature of hypothyroidism and it has recently been observed that relief of constipation is associated with a fall in the concentration of DCA in bile as well as a fall in CSI.<sup>25</sup>

Thus, all seven agents which lower DCA in bile lower the CSI at the same time.

### Manoeuvres which increase DCA in bile (Table 2)

Impressed by the discovery (in their own laboratory<sup>57</sup>) that the fall in % DCA induced by bran is accompanied by a fall in the cholesterol content of bile, Low-Beer and Pomare decided to see what happens when % DCA is artificially increased. They fed physiological quantities of DCA (100–150 mg/day) to healthy volunteers and found a significant increase in moles % cholesterol in bile.<sup>1</sup> These findings aroused much interest and three other groups tried to reproduce them.<sup>66–72</sup> Only one succeeded in doing so.<sup>66</sup> This may be because in these further studies the DCA was administered in pharmacological doses (750–1000 mg/day) and not in the physiological amounts of the original experiment. In large doses, DCA inhibits

Table 1 Summary of published studies which report the cholesterol saturation index of 'gall bladder bile' (CSI) before and after a regimen that lowers the deoxycholic acid (DCA) content of bile. 'Gall bladder bile' was obtained by duodenal drainage after induced gall bladder contraction

City and reference	Subjects (male, female)	Age (mean or range) yr	Details of regimen			Mean % deoxycholic acid in duodenal bile acids			Mean cholesterol saturation index		
			Agent	Daily dose	Duration (weeks)	Before	After	p value	Before	After	p value
Bristol <sup>42</sup>	10 f Gall stones	33–53	Wheat bran	20–40 g	4–6	—*	—*	—	1.49	1.29	<0.05
Edmonton <sup>43</sup>	9 (?sex)	Not stated	Wheat bran	50 g	4	31.3	23.3	<0.05	1.43	0.76	<0.01
Adelaide <sup>29</sup>	5 f Healthy but supersatd bile	23	Wheat bran	30 g	8	29.8	21.9	SNA	1.36	0.95	SNA
Bristol <sup>60</sup>	4m, 16 f Constipated	49	Trifyba (80% wheat fibre)	10–32 g (mean 20 g)	6–12	26.6	23.0	<0.002	1.13†	1.07†	=0.049†
Birmingham <sup>64</sup>	11 m Healthy	21–46	Metronidazole	2 g	1.4	24.0	6.6	<0.001	1.0	0.83	<0.01
Modena <sup>65</sup>	3 m, 2 f Gall stones	57	Ampicillin	2 g	2–3	24.0‡	5.4‡	SNA	1.25	0.95	SNA
Modena <sup>66</sup>	3 m, 2 f 3 with Gall stones	50	Ampicillin	40 mg/kg	3	25.1	13.4	SNA	1.33	1.12	SNA
Bristol <sup>67</sup>	10 f Healthy	46	Lactulose	39–60 g (mean 56 g)	6	28.4	15.6	<0.002	1.40	1.19	<0.005
Modena <sup>68</sup>	5 m, 3 f Healthy	41–69	Strep. faecium	750 × 10 <sup>6</sup> bacteria	4	23.6	18.4	<0.01	1.05	0.98	<0.05
Stockholm <sup>69</sup>	2 m, 8 f Hypo-thyroidism	59	L-thyroxine	As required to normalise TSH levels	16–48	30.0	19.0	<0.01	1.35§	1.08§	NS§
Bristol <sup>25</sup>	2 m, 14 f Constipation	48 (38–69)	Senna laxative	15–60 mg	6	25.9	17.2	<0.001	1.20	0.99	=0.004

\*DCA measured in only four subjects, it fell in all, by mean of 10.2%; †of 12 subjects with CSI initially >1.0, CSI fell in nine; mean fall 1.33 to 1.22, p=0.008; ‡calculated from published values for bile acid pool sizes; §of seven subjects with CSI initially >1.0, CSI fell in six, mean fall from 1.31 to 1.12; ||one 'rogue' CSI result excluded, of eight subjects with CSI initially >1.0, CSI fell in six; mean fall 1.40 to 1.20, p=0.02; SNA statistics not applied because of small numbers.

cholesterol absorption<sup>66</sup> and may even have direct toxic effects on the small intestine.<sup>73-75</sup> A toxic effect on the liver is also possible.<sup>70,76</sup>

Three studies of more appropriate design have recently been reported and all have supported the observation of Low-Beer and Pomare. Carulli *et al*<sup>65</sup> increased DCA input by physiological amounts when they administered cholic acid, the precursor of DCA, to 10 subjects with gall stones in doses which led to a rise in % DCA from 21.8 to 35.4. The CSI of bile also rose significantly. The same group has studied patients with cirrhosis, who tend to have a low concentration of DCA in their bile<sup>77</sup> and a low CSI.<sup>78</sup> When such patients were fed small quantities of DCA (about 200 mg/day), their cholesterol saturation index rose significantly, returning to the original value after DCA ingestion was stopped.<sup>79</sup> Finally, when we slowed down the intestinal transit of healthy volunteers with loperamide their circulating DCA pools expanded significantly and there was a rise in biliary CSI.<sup>25</sup>

Thus, a large number of studies indicate that any regimen which alters the amount of DCA in the bile acid pool (other than pharmacological doses of DCA) induces a change in the CSI of bile in the same direction. This strongly suggests that DCA directly affects the cholesterol saturation of bile. The relevant factor may not be % DCA as such but the size of the circulating DCA pool because, in our own study with loperamide,<sup>25</sup> a rise in CSI was associated with no change in % DCA even though the DCA pool had expanded (the total bile acid pool having expanded at the same time).

### Mechanism of the DCA effect on cholesterol saturation of bile

How DCA influences the CSI of bile remains to be established but there are two ways in which it could increase cholesterol secretion into bile. Increased

Table 2 Summary of published studies which report the cholesterol saturation of 'gall bladder bile' before and after a regimen that raises the deoxycholic acid (DCA) content of bile

City and reference	Subjects (male, female)	Mean age and/or range (yr)	Details of regimen			Mean % deoxycholic acid in duodenal bile acids			Mean cholesterol saturation index or moles % cholesterol		
			Agent	Daily dose	Duration (days)	Before	After	p value	Before	After	p value
Bristol <sup>1</sup>	9 m, 7 f Healthy	21-54	DCA	100-150 mg	14	21*	50*	<0.001	8.6%	9.9%	<0.01
Rochester <sup>71</sup>	7 f	22-34	DCA	750 mg	40±6	26	75	DNG	0.86	0.83	NS†
Stockholm <sup>70</sup>	8 f Healthy	37 (22-59)	DCA	750 mg	21-28	20	87	DNG	8.8%	8.6%	NS†
Modena <sup>72</sup>	4 m, 5 f	49	DCA	15 mg/kg	7-14	16	89	DNG	1.23	1.21	NS†
Modena <sup>66</sup>	6 m, 3 f 5 Gallstones	43	DCA	15 mg/kg	21	20.1	78.6	<0.01	1.41	1.82	<0.02†
Modena <sup>79</sup>	11 m Cirrhosis	56 (38-78)	DCA	180-250 mg	21-28	5.3	43.9	<0.001	0.92	1.34	<0.005
Modena <sup>65</sup>	10 (most f) Gallstones	51	Cholic acid	15 mg/kg	14-21	21.8	35.4	<0.01	1.07	1.42	<0.01
Bristol <sup>25</sup>	2 m, 10 f Healthy	45 (38-62)	Loperamide	4-20 mg Sufficient to cause constipation	42	0.40 g‡	0.57 g‡	=0.008	1.10	1.20	=0.01

\*% DCA values obtained in a subgroup of seven subjects given DCA for 6-16 days; †dose of DCA was pharmacological, not physiological, so data hard to interpret (see text); ‡DCA pool, measured by isotope dilution; DNG data not given but clearly significant; NS not significant (p>0.05).

cholesterol secretion is accepted as the major biochemical defect in most cases of cholesterol rich gall stones.<sup>6 10</sup>

Deoxycholic acid is very hydrophobic<sup>76</sup> and, of all the major bile acids, it has the strongest detergent property.<sup>2</sup> As bile acids pass into the biliary canaliculus they may leach out cholesterol from the cell membrane<sup>80 81</sup> as distinct from the cholesterol which is excreted in vesicles; if so DCA is likely to do this to a greater extent than the other bile acids. Increased cholesterol secretion has been shown in acute experiments when the natural bile acid pool was drained and replaced by DCA.<sup>76</sup> Whether the same thing happens with chronic changes in bile acid composition is less certain but seems likely from recent studies. Thus, when DCA 400 mg/day was fed to normal volunteers for four weeks, hepatic secretion of cholesterol (measured over an eight hour period) increased by 11% ( $0.05 < p < 0.10$ ).<sup>82</sup> Von Bergmann<sup>83</sup> has reported that the secretion of DCA is increased in gall stone patients and that it correlates with cholesterol secretion.

Another way DCA may act on the CSI is through displacement of the other dihydroxy bile acid chenodeoxycholic acid (CDCA) from the bile acid pool. Pomare and Low-Beer<sup>84</sup> were the first to notice that, when bile acid composition is examined in a large group of people with a wide range of values, there is a reciprocal relationship between % DCA and % CDCA, whereas there is little or no relationship between % DCA and % cholic acid. This has been confirmed in another very large study<sup>52 85</sup> though not in a third, smaller one.<sup>70</sup> In several studies an induced change in % DCA has been accompanied by a significant reciprocal change in % CDCA with little or no change in % cholic acid.<sup>25 56 64 67 84 86</sup> Orally administered CDCA is known to have an inhibitory effect on cholesterol secretion<sup>87</sup> and to lessen the cholesterol saturation of bile.<sup>38 88</sup> Therefore, high concentrations of DCA might reduce the curbing effect of endogenous CDCA on cholesterol secretion. This hypothesis<sup>84</sup> is attractive and, at first sight, plausible, but the evidence is weak. Published dose response relationships do not permit the conclusion that cholesterol secretion is influenced by changes in % CDCA as small as those which accompany changes in % DCA.<sup>89</sup>

As to how DCA displaces CDCA from the bile acid pool, one small study suggests that it is by inhibition of CDCA synthesis.<sup>84</sup> Another possibility is that DCA conjugates compete with CDCA conjugates for absorption from the ileum.<sup>85</sup>

### **Other physiological effects of DCA relevant to gall stone formation**

Besides its effect on cholesterol secretion DCA could influence cholesterol gall stone formation by an effect on nucleating mechanisms. Although supersaturated bile is a prerequisite for gall stone formation<sup>90 91</sup> nucleation of cholesterol crystals is also crucially important, at least when bile is only moderately supersaturated, that is, in the metastable labile zone.<sup>10 92-95</sup> Possible nucleating factors include mucin.<sup>10</sup> This seems a plausible candidate because many gall stones have a core of mucin. Hypersecretion of mucin by the gall bladder mucosa occurs when bile is supersaturated but the stimulus for this hypersecretion seems not to be excess biliary cholesterol as such<sup>96</sup> but rather an increase in the amount of phospholipid containing arachidonic acid.<sup>97 98</sup> Arachidonic acid is the precursor of prostaglandins. It has been suggested that prostaglandins stimulate mucin secretion from the gall

bladder mucosa as the anti-prostaglandin agent aspirin inhibits mucin release and gall stone formation in the prairie dog.<sup>96</sup> Similarly, indomethacin is said to inhibit prostaglandin release and gall bladder mucin secretion.<sup>99</sup> In a recent study of healthy subjects, a significant correlation ( $r=0.71$ ) was found between % DCA in the bile acid pool and the amount of arachidonic acid in the phospholipids of bile.<sup>100</sup> The mechanism of this relationship is unclear but it raises the possibility that bile with a high DCA concentration is prone to gall stone formation because it favours nucleation through increased biliary mucin. Nevertheless, it is far from certain that mucus glycoprotein is an important nucleating agent.<sup>101</sup>

Deoxycholic acid may influence cholesterol crystallisation in a more direct way. In artificial bile, nucleation time is reported to be shorter if the predominant bile acid is the taurine conjugate of DCA than if it is the taurine conjugate of chenodeoxycholic or cholic acid.<sup>100</sup> If this can also be shown to be true of native bile the implications in terms of gall stone formation are apparent.

### Problems and areas of ignorance

Female villagers in rural Zimbabwe have Western concentrations of DCA in their bile yet their gall bladder bile remains unsaturated.<sup>102</sup> Tongan islanders actually have more DCA in their bile than New Zealand Tongans,<sup>103</sup> yet they are thought rarely to develop gall stones. These findings are paradoxical but suggest that, in non-Westernised communities, there are unidentified protective factors against supersaturated bile and gall stones.

In the 264 women in the US National Cooperative Gallstone Study the correlation between % DCA and moles % cholesterol in bile was not significant.<sup>52</sup> It was, however, in the same direction as in the men. Deoxycholic acid is only one of the variables affecting bile. Women have more such variables than men, namely, parity and oestrogenic activity.

In one bran feeding study<sup>62</sup> there was a fall in biliary CSI from initially supersaturated concentrations despite no change in % DCA. The initial level of DCA, however, was low. The fact that, despite this, bran lowered the CSI is hard to explain but wheat bran is a complex and heterogeneous product. In our own study with a concentrate of bran<sup>60</sup> we found no correlation between change in CSI and change in % DCA suggesting that wheat fibre may affect bile in more than one way.

Patients with colonic adenomas tend to have large DCA pools;<sup>104</sup> it would be interesting to know whether they have an increased prevalence of gall stones.

Little is known of the physiological variables which determine the size of the circulating DCA pool and the proportion of DCA in bile other than intestinal transit time<sup>25</sup> and the intake of dietary fibre.<sup>56 57 60 86</sup> Older people have a larger DCA pool,<sup>27</sup> more saturated bile<sup>105 106</sup> and a higher prevalence of gall stones than younger people. Their increased DCA pools could simply be because of the slowing of colonic transit which tends to occur with age. Research is, however, needed on other physiological determinants of DCA formation and absorption. The pH and bacterial flora of the colonic contents are likely candidates because there is a relationship between DCA input and the ratio of anaerobes to aerobes in the faeces.<sup>107</sup>

Finally, attention should be given to the frequency of enterohepatic

circulation of the bile salt pool. More frequent circulation has been suggested as the cause of the small pool in gall stone subjects.<sup>108-110</sup> By analogy with the situation after cholecystectomy it could perhaps also explain the increased proportion of DCA in the bile of gall stone subjects, as more frequent circulation results in more exposure of bile salts to intestinal bacteria and so greater dehydroxylation of cholic acid.<sup>41 111-113</sup>

### Conclusion

Much evidence suggests that high concentrations of deoxycholic acid in the circulating bile salt pool promote the hepatic secretion of cholesterol and hence supersaturation of bile. They may also promote the nucleation of cholesterol crystals in bile. Deoxycholic acid deserves recognition as a factor in the pathogenesis of cholesterol rich gall stones which helps to explain the increasing prevalence of gall stones with age and their lower prevalence in vegetarians.

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