Effect of cyclodextrins and undigested starch on the loss of chenodeoxycholate in the faeces

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Starch that escapes digestion in the small intestine increases the elimination of chenodeoxycholate and its metabolites in the faeces of both mice and hamsters. In contrast, the elimination of cholate and its metabolites is not increased. *In vitro*, the affinity of starch for chenodeoxycholate is about 90-fold greater than for

cholate. β -Cyclodextrin, which approximates to one turn of the helical structures formed by the 1,4-linked glucose units of starch, shares these properties. It is proposed that these helical structures in starch act as binding sites for bile salts.

INTRODUCTION

Cardiovascular heart disease and also the occurrence of gallstones are believed to be associated with a Western lifestyle and to be aggravated by an inappropriate diet (Grundy et al., 1985; Pixley and Mann, 1988; Grundy, 1990). Cooking, farming and factory food processing have all contributed to making food more rapidly digestible, to reducing the bulk of food that has to be eaten for survival and, consequently, to reducing the quantity of digested and partially digested food that enters the large intestine (Cummings and Englyst, 1987). For example, the amount of undigested starch that reaches the large intestine is affected by its source and preparation. Starch from uncooked potatoes and bananas is almost completely undigestible (Walker and Hope, 1963; Englyst and Cummings, 1986) and, as cooked starch cools, its digestibility decreases (Englyst and Cummings, 1987). The fine milling of cereals, especially as opposed to stone grinding, produces a flour that is more rapidly and completely digested (O'Dea et al., 1980; Snow and O'Dea, 1981; Heaton et al., 1988; O'Donnell et al., 1989). Here, using trestatin to inhibit starch degradation (Yokose et al., 1983a; Eichler et al., 1984), we show that bile-salt metabolism, which may have a role in both cardiovascular heart disease and gallstone formation, is influenced by starch that escapes degradation in the small intestine.

MATERIALS AND METHODS

Chemicals

Taurochenodeoxycholate, taurodeoxycholate and glycodeoxycholate (purity > 96%) were from Calbiochem Corp.; other non-radiolabelled bile salts (purity > 97%) were from Sigma. [2,4-³H]Cholate (74 Gbq/mmol) and [24-¹⁴C]chenodeoxycholate (1.9 Gbq/mmol) were from NEN Research Products; the sodium salts of [3,12-³H]taurodeoxycholate (34 Gbq/mmol), [3,7-³H]-taurochenodeoxycholate (37 Gbq/mmol), [G-³H]glycodeoxycholate (26 Gbq/mmol), and [G-³H]glycochenodeoxycholate (30 Gbq/mmol) were synthesized on request by Amersham International. Cholestyramine, α - and γ -cyclodextrins were the purest available from Sigma; β -cyclodextrin (purity > 98%) from Wacker Chemie. Potato (*Solanum tuberosum*), cassava (also known as tapioca and manioc; *Manihot* sp.; Liliaceae) and maize (*Zea mays*) starch were from the local supermarket.

Trestatin was a gift from Dr. R. Labler; it had been purified as described by Yokose et al. (1983a) from a fermentation broth of *Streptomyces dimorphogenes*. Trestatin is a mixture of oligosaccharides (trestatins A, B and C) that, in addition to four to six units of glucose, contain one to three units of the pseudodisaccharide dehydro-oligobiosamine (Yokose et al., 1983b). Other reagents were of analytical quality or were the purest available.

Assay conditions for the determination of binding constants

The buffer used to prepare the incubation solutions contained 150 mM NaCl and 2 mM Bes, adjusted with NaOH to pH 7.2. Incubations were carried out overnight at room temperature (~ 22 °C) with vigorous shaking. The insoluble chelator was precipitated by centrifugation, and the radioactivity in the supernatant was measured.

Insoluble complexes

For these, two procedures were followed: in the first, a fixed concentration (50 or 100 μ M) of radiolabelled bile salt was incubated with different amounts of the insoluble polymer (1–16 mg cholestyramine and 10–160 mg of starch in 5 ml of the buffer); in the second, a fixed concentration of both the insoluble polymer (1.6 mg/ml) and [¹⁴C]chenodeoxycholate (~ 1 nM) were incubated with different concentrations of unlabelled chenodeoxycholate (the maximum concentration used did not exceed the critical micellar concentration).

Soluble complexes

For these, the first procedure described for insoluble complexes was followed, the bile salt being incubated with different amounts of cholestyramine in both the absence and presence of a fixed concentration (1 mM) of the soluble chelator.

Derivation of equations describing the binding of bile salts to chelators

Insoluble complexes

The following Mass Action equation describes the binding of a bile salt to an insoluble chelator:

$$(B_1 - B_1')(I - B_1') = B_1' \cdot K_{D1} \quad (1)$$

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where B_t and I are the total concentrations of the bile salt and insoluble chelator, B_i that of the insoluble bile-salt chelator complex, K_{DI} the dissociation constant of the complex. As $B_t - B_i = B_f$, where B_f is the concentration of the unbound bile salt, eqn. (1) can be rewritten as:

$$B_t(I - B_i) = B_i \cdot K_{DI} - B_i \cdot K_{DI} - B_i \cdot K_{DI}$$

and rearranged to give:

$$B_t/B_i = K_{DI}/(I - B_i + K_{DI}) \quad (2)$$

which, provided that $(I + K_{DI}) \gg B_i$, approximates to:

$$B_t/B_i = K_{DI}/(I + K_{DI}) \quad (3)$$

This equation was used to determine the K_{DI} from incubations of a fixed concentration of bile salt with different concentrations of starch (Figure 3a below) and cholestyramine (Figure 3c below).

Solving eqn. (1) for B_i gives a quadratic, of which the negative root, used in curve-fitting, is:

$$B_i = \{B_t + I + K_{DI} - [(B_t + I + K_{DI})^2 - 4 \cdot B_t \cdot I]^{1/2}\}/2$$

However, as B_f and not B_i was measured, and as $B_t/B_i = 1 - B_f/B_t$, the above equation was rearranged to give:

$$B_t/B_i = 1 - \{B_t + I + K_{DI} - [(B_t + I + K_{DI})^2 - 4 \cdot B_t \cdot I]^{1/2}\}/(2 \cdot B_t) \quad (4)$$

In order to allow for an experimental error in the determination of B_f , the theoretical value of unity in the above equation was replaced by the parameter B_{max} ; this gives:

$$B_t/B_i = B_{max} \cdot [1 - \{B_t + I + K_{DI} - [(B_t + I + K_{DI})^2 - 4 \cdot B_t \cdot I]^{1/2}\}/(2 \cdot B_t)] \quad (5)$$

Eqn. (5) was used to determine K_{DI} and I from incubations of a fixed concentration of starch with different concentrations of bile salt (Figure 3b below). As the derived values have the same units of concentration as those of the bile salt, it is possible, assuming a binding stoichiometry of 1:1, to calculate the number of binding sites per unit weight of the starch.

In a plot of B_t/B_i against B_t for which a fixed concentration of chelator has been incubated with different concentrations of bile salt (Figure 3b below), it can be seen from eqn. (2) that as B_f , and therefore B_i , tends to zero, B_t/B_i tends to $K_{DI}/(I + K_{DI})$.

Soluble complexes

The following Mass Action equations describe the binding of a bile salt to a soluble chelator in the presence of an insoluble chelator:

$$(B_t - B_s - B_i)(S - B_s) = B_s \cdot K_{DS}$$

$$(B_t - B_s - B_i)(I - B_i) = B_i \cdot K_{DI}$$

where S is the concentration of the soluble chelator, B_s that of the bile-salt soluble chelator complex and K_{DS} the dissociation constant of the complex. These equations can be combined to give:

$$(S - B_s)/(B_s \cdot K_{DS}) = (I - B_i)/(B_i \cdot K_{DI})$$

In order to eliminate B_s , which is not measurable, it was assumed that $B_f \ll B_s$, and therefore that B_s approximates to $B_t - B_i$, this gives:

$$(S - B_t + B_i)/[K_{DS}(B_t - B_i)] = (I - B_i)/(B_i \cdot K_{DI})$$

which, after rearrangement, results in the following quadratic:

$$B_i^2(K_{DI} - K_{DS}) - B_i(B_t \cdot K_{DI} - B_t \cdot K_{DS} - S \cdot K_{DI} - I \cdot K_{DS}) - B_t \cdot I \cdot K_{DS} = 0$$

of which the positive root, which is used in curve-fitting, is:

$$\begin{aligned} B_i = & \{ (B_t \cdot K_{DI} - B_t \cdot K_{DS} - S \cdot K_{DI} - I \cdot K_{DS}) \\ & + [(B_t \cdot K_{DI} - B_t \cdot K_{DS} - S \cdot K_{DI} \\ & - I \cdot K_{DS})^2 + 4 \cdot B_t \cdot I \cdot K_{DS}(K_{DI} - K_{DS})]^{1/2} \} / [2(K_{DI} - K_{DS})] \end{aligned}$$

However, as the concentration of the dissolved bile salt (B_d , where $B_d = B_t + B_s$) was measured and not that of B_i , and as $B_d/B_i = 1 - B_f/B_t$, the above equation was rearranged to give:

$$\begin{aligned} B_d/B_i = 1 - & \{ (B_t \cdot K_{DI} - B_t \cdot K_{DS} - S \cdot K_{DI} - I \cdot K_{DS}) \\ & - [(B_t \cdot K_{DI} - B_t \cdot K_{DS} - S \cdot K_{DI} - I \cdot K_{DS})^2 \\ & + 4 \cdot B_t \cdot I \cdot K_{DS}(K_{DI} - K_{DS})]^{1/2} \} / [2 \cdot B_t(K_{DI} - K_{DS})] \end{aligned} \quad (6)$$

Eqn. (6) was used to determine the dissociation constant (K_{DS}) of a soluble chelator–bile-salt complex. To do this, a fixed concentration of the soluble chelator (S) plus a fixed concentration of the bile salt (B_t) were incubated with different amounts (I) of an insoluble chelator with a known dissociation constant (K_{DI}) for the bile salt (see, e.g. Figure 3c below).

Analysis of binding data

At the end of the incubations, the radioactivity in the supernatant was measured and expressed as a fraction of the total radioactivity. These binding data were then analysed by a non-linear least-squares curve-fitting procedure. For insoluble polymers, the parameter K_{DI} in eqn. (3) was fitted to the data if the bile-salt concentration was fixed and the polymer concentration varied, and the parameters K_{DI} and I in eqn. (5) if the polymer concentration was fixed and the bile-salt concentration varied. For soluble polymers, the parameter K_{DS} in eqn. (6) was fitted to the data.

Determination of bile-salt loss in the faeces

Male mice (~20 g) were housed individually in cages with a wire-mesh floor. After an overnight fast, they were injected in a tail vein with a mixture of the [^3H]cholate (1 nmol) and [^{14}C]chenodeoxycholate (1.2 nmol) and were given a single meal (5 g of powdered chow plus 5 ml of water) containing the test substance. [The supplier of the chow had to be changed, with the result that the chow used for the experiment in Figure 1 (below) contained less non-digestible material than that used in the other experiments.] The faeces, which are not retained in the cages, were collected after 24 h. $^3\text{H}_2\text{O}$ and $^{14}\text{CO}_2$ were liberated from the faeces by oxidation in a Packard Oxidizer and were determined by scintillation counting.

The same procedure was used with male hamsters (~130 g), except that the hamsters were supplied with soft paper as nesting material and were deprived of food during the day; the meal (6 g of powdered chow plus 6 ml of water) was given in the late afternoon; the meal contained both the test substance and the

radioactive bile salts; the faeces below the cages and in the nesting material were collected after 48 h.

For both animals the collection period approximates to the intestinal transit time and was sufficient to recover ~90% of the radiolabel from [^3H]poly(ethylene glycol) when this non-absorbable marker was included in the test meal.

Figure 1 Effect of inhibiting starch degradation with trestatin on the elimination of bile salts in the faeces of mice

The [^{14}C]chenodeoxycholate (○, ●) and [^3H]cholate (△, ▲) were injected intravenously. The data are for individual mice; starch degradation was inhibited by including 0.5–10 mg of trestatin per mouse in the meal (○, △, controls; ●, ▲, treated). The curves are a visual guide. Experimental details are given in the Materials and methods section.

RESULTS

Effect of undigested starch, cyclodextrins and cholestyramine on bile-salt loss in the faeces

In mice, adding trestatin, an α -amylase inhibitor (Eichler et al., 1984), to the diet increases the weight of the faeces (see Figure 1). Moreover, although the radioactivity in the faeces from intravenously injected [^3H]cholate is independent of their weight, that from [^{14}C]chenodeoxycholate is directly proportional to it (Figure 1). Adding uncooked potato starch or cellulose to the diet also increases the weight of the faeces, but whereas potato starch mimics the effect of trestatin, cellulose has no effect on the loss of radioactivity from [^{14}C]chenodeoxycholate (Table 1). In contrast with potato starch, starch from maize and cassava increase neither the weight of the faeces nor the amount of radioactivity from [^{14}C]chenodeoxycholate that they contain (Table 1).

β -Cyclodextrin (cyclohepta-amylose) has the same effect in mice as trestatin and potato starch in that it increases the amount of radioactivity in the faeces from intravenously injected [^{14}C]chenodeoxycholate but not from [^3H]cholate (Figure 2a). However, in contrast with trestatin, it does not increase the weight of the faeces (Table 1). α -Cyclodextrin (cyclohexa-amylose) acts like cellulose: it increases the bulk of the faeces, but has no effect on the elimination of bile salts. γ -Cyclodextrin (cycloocta-amylose), like starch from maize and cassava, has no effect.

Unlike trestatin and β -cyclodextrin, cholestyramine, an anion-exchange resin that is used in man as a bile-salt chelator, increases the loss of radioactivity in the faeces of mice from both the intravenously injected [^{14}C]chenodeoxycholate and [^3H]cholate (Figure 2b).

The experiments with trestatin were not repeated in the hamster, but otherwise the data for the loss of radioactivity in the faeces from orally administered [^3H]cholate and [^{14}C]chenodeoxy-

Figure 2 Effect of β -cyclodextrin (a) and cholestyramine (b) on the elimination of bile salts in the faeces of mice

The [^{14}C]chenodeoxycholate (●) and [^3H]cholate (▲) were injected intravenously. The results are means $\pm$ S.E.M. for three to six mice; the curves are a visual guide. Experimental details are given in the Materials and methods section.

Table 1 Comparison of the effect of starch and cyclodextrins on the weight of the faeces and on the loss of radiolabel from intravenously injected [^3H]cholate and [^{14}C]chenodeoxycholate in the faeces of the mouse

Experimental details are given in the Materials and methods section. The mice weighed 20–22 g. Results are means $\pm$ S.E.M. The weight of the faeces from control mice was typically 1490 ± 39 mg.

Compound	n	Dose (mg)	Increase in weight of faeces (mg)	Radiolabel in faeces (%) from:	
				Chenodeoxycholate	Cholate
Control	38	—	—	20 ± 1	18 ± 1
Cellulose	3	1500	1310 ± 40	25 ± 2	20 ± 2
Potato starch	3	1500	1040 ± 50	82 ± 3	19 ± 2
Maize starch	3	1500	40 ± 10	13 ± 1	14 ± 1
Cassava starch	3	1500	60 ± 4	16 ± 2	11 ± 1
α -Cyclodextrin	5	1500	1520 ± 310	24 ± 5	18 ± 3
β -Cyclodextrin	3	150	—	83 ± 7	11 ± 1
	10	500	72 ± 36	—	—
γ -Cyclodextrin	5	1500	84 ± 80	17 ± 1	15 ± 1

Table 2 Comparison of the effect of starch and cyclodextrins on the loss of radiolabel from orally administered [^3H]cholate and [^{14}C]chenodeoxycholate in the faeces of the hamster

Experimental details are given in the Materials and methods section. The hamsters weighed ~ 130 g. Results are means $\pm$ S.E.M. ($n = 5$).

Compound	Dose (mg)	Radiolabel in faeces (%) from:	
		Chenodeoxycholate	Cholate
Control	—	36 ± 2	17 ± 1
Potato starch	1500	50 ± 2	17 ± 4
Maize starch	1500	35 ± 2	18 ± 2
Cassava starch	1500	44 ± 2	14 ± 1
α -Cyclodextrin	1500	39 ± 2	23 ± 6
β -Cyclodextrin	1200	50 ± 5	19 ± 1
γ -Cyclodextrin	1500	35 ± 3	13 ± 1
Cholestyramine	150	70 ± 2	50 ± 3

cholate are similar to those described above in the mouse (Table 2).

Binding of bile salts to starch, cyclodextrins and cholestyramine *in vitro*

In vitro, potato, maize and cassava starch all bind the taurine conjugate of chenodeoxycholate, deoxycholate and cholate. The stability of the bile-salt–starch complex derived from incubations of a fixed concentration of bile salt with different concentrations of starch is, within the accuracy of the determinations, independent of the source of the starch, but highly dependent on the bile salt (Figure 3a and Table 3). Similarly, within the

accuracy of the determinations, the stability of the complex between potato starch and bile salts is independent of whether the bile salt is unconjugated or conjugated with either glycine or taurine (Table 3). The dissociation constant of the bile-salt–starch complex decreases from cholate (370 mg/ml) to deoxycholate (39 mg/ml) to chenodeoxycholate (4 mg/ml; averages from data in Table 3). The accuracy of the dissociation constants determined for the cholate complexes is very low, because, at the highest starch concentration used, only about 10% of the bile salt is bound (Figure 3a). As expected from the data in Figure 3a, dissociation constants derived from incubations of a fixed concentration of potato and cassava starch with different concentrations of taurochenodeoxycholate are similar: $128 \mu\text{M}$ with potato and $152 \mu\text{M}$ with cassava starch (see Figure 3 and Table 4).

Figure 3 Binding of bile salts to starch (a, b) and to cholestyramine and β -cyclodextrin (c) *in vitro*

In (a) starch from maize ($\circ$), cassava ($\triangle$) and potato ($\square$) was incubated with [^3H]taurochenodeoxycholate (—), [^3H]taurodeoxycholate (-----) and [^3H]taurocholate (.....); the bile salts were at $50 \mu\text{M}$. Each datum point represents a duplicate determination, and the curves result from fitting the parameter K_D in eqn. (3) to the data. In (b) potato starch, at 1.6 mg/ml , was incubated with different concentrations of [^3H]taurochenodeoxycholate; the results are means $\pm$ S.E.M. from three duplicate determinations, and the curves result from fitting the parameters K_D and I in eqn. (5) to the data. In (c) cholestyramine was incubated with $100 \mu\text{M}$ [^3H]taurochenodeoxycholate in the presence ($\circ$, $\bullet$), and absence ($\square$) of 1 mM β -cyclodextrin. The data for cholestyramine in the absence of β -cyclodextrin are averages from eight duplicate determinations (the S.E.M. is smaller than the symbol), and in the presence of β -cyclodextrin each datum point is the average of duplicate determinations. In the absence of β -cyclodextrin the curves result from fitting the parameter K_D in eqn. (3) to the data, and in the presence of β -cyclodextrin from fitting the parameter K_{DS} in eqn. (6) to the data. Values for the parameters derived from the above and similar data are given in Tables 3 and 4. Experimental details are given in the Materials and methods section.

Table 3 Dissociation constants (K_D) of complexes of bile salts with starch, cyclodextrins and cholestyramine

The K_D of the complex between the insoluble polymers and the bile salts was determined by incubating a fixed concentration of the bile salt with different concentrations of the polymer (see, e.g., Figure 3a). The K_D , therefore, has the same units of concentration as the polymer. The K_D values for α -, β - and γ -cyclodextrins are also given as a weight concentration (M , 972, 1135 and 1297 respectively). The values are given as $K_D \pm$ S.E.M. (or S.E.) (n) where n is the number of determinations; for $n = 1$ the S.E. of the fit is given, and for $n > 1$ the S.E.M. Each fit was made to six duplicate data points (in Figures 3a and 3c the averages of these are shown). Experimental details are given in the Materials and methods section.

Bile salt	K_D							
	Polymer ...	Potato starch (mg/ml)	Maize starch (mg/ml)	Cassava starch (mg/ml)	α -Cyclodextrin (μ g/ml)	β -Cyclodextrin (μ g/ml)	γ -Cyclodextrin (μ g/ml)	Cholestyramine (μ g/ml)
Cholate		338 $\pm$ 28 (1)				401 $\pm$ 27 (4)		1047 $\pm$ 45 (3)
Deoxycholate		28 $\pm$ 1 (1)				150 $\pm$ 22 (4)		139 $\pm$ 7 (3)
Chenodeoxycholate		3 $\pm$ 0 (1)				14 $\pm$ 2 (4)		101 $\pm$ 16 (2)
Glycocholate		658 $\pm$ 82 (1)				485 $\pm$ 27 (2)		1012 $\pm$ 114 (3)
Glycodeoxycholate		50 $\pm$ 3 (1)				222 $\pm$ 26 (2)		163 $\pm$ 13 (2)
Glychenodeoxycholate		6 $\pm$ 0 (1)				22 $\pm$ 5 (2)		145 $\pm$ 16 (2)
Taurocholate		328 $\pm$ 96 (2)	314 $\pm$ 27 (1)	239 $\pm$ 22 (1)	∞	558 $\pm$ 18 (3)	366 $\pm$ 12 (1)	419 $\pm$ 25 (12)
Taurodeoxycholate		44 $\pm$ 8 (2)	39 $\pm$ 1 (1)	30 $\pm$ 1 (1)	∞	171 $\pm$ 9 (3)	80 $\pm$ 3 (1)	62 $\pm$ 5 (9)
Taurochenodeoxycholate		4 $\pm$ 2 (2)	4 $\pm$ 0 (1)	3 $\pm$ 0 (1)	∞	14 $\pm$ 1 (3)	22 $\pm$ 0 (1)	56 $\pm$ 6 (8)

Table 4 Dissociation constants (K_D) and binding-site concentration of complexes of taurochenodeoxycholate with starch

The K_D of the complex was determined by incubating a fixed concentration of starch with taurochenodeoxycholate (as in Figure 3b). The parameters K_D and I (binding-site concentration) in eqn. (5) were fitted to the data. Each fit was made to nine duplicate data points. Results are means $\pm$ S.E.M. (n), where n is the number of determinations.

Starch	n	K_D (μ M)	Binding-site concn.		Glucose units per binding site
			(μ M)	(nmol/mg)	
Potato	4	128 $\pm$ 12	33 $\pm$ 5	20 $\pm$ 3	320 $\pm$ 44
Cassava	2	152 $\pm$ 9	38 $\pm$ 3	23 $\pm$ 2	265 $\pm$ 18

The affinity of β -cyclodextrin for the various bile salts parallels that of starch, except that the discrimination between cholate and chenodeoxycholate is smaller (30- versus 100-fold); however, on a weight basis, the affinity of β -cyclodextrin is at least 200-fold greater than that of starch (Table 3). The affinity of γ -cyclodextrin for taurine-conjugated bile salts is similar to that of β -cyclodextrin, whereas the binding of bile salts to α -cyclodextrin could not be detected (Table 3).

In contrast with starch and β -cyclodextrin, the affinity of the anion-exchange resin cholestyramine for chenodeoxycholate and deoxycholate is similar, and its affinity for chenodeoxycholate is less than 10-fold larger than for cholate. Also, in contrast with starch and β -cyclodextrin the affinity of taurine-conjugated bile salts is higher than that of either unconjugated or glycine-conjugated bile salts (Table 3).

DISCUSSION

Trestatin inhibits the digestion of starch in the intestine (Eichler et al., 1984), and, consequently, increases the weight of the faeces. The amount of radioactivity, from intravenously injected [14 C]chenodeoxycholate, that appears in the faeces is linearly related to the increase in their weight, whereas that from [3 H]cholate is not (Figure 1). This increased loss of radioactivity

could be caused by trestatin itself, the increased weight of the faeces or the amount of starch in the faeces. A direct action of trestatin is unlikely as, in its absence, uncooked potato starch, which is resistant to degradation in the intestine (Walker and Hope, 1963), also increases the loss of radioactivity. The increase in the weight of the faeces can be ruled out, because adding cellulose or α -cyclodextrin to the diet increases their weight without increasing the loss of radioactivity (Table 1). This leaves starch as the probable causative agent and therefore its interaction with bile salts was studied *in vitro*.

The stability of the bile-salt-starch complex *in vitro* is independent of the source of the starch (potato, maize or cassava; Figure 3a), dependent on the bile salt (cholate, deoxycholate or chenodeoxycholate; Figure 3a) and independent of whether the bile salt is unconjugated or conjugated with taurine or glycocholate (Table 3). The most likely binding site for an amphiphilic ligand in the starch molecule is in the hydrophobic interior of the helical structures that are formed by the 1,4-linked glucose units of amylose and amylopectin (Hinrichs et al., 1987, and references therein). α -, β - and γ -cyclodextrin, which are produced by bacterial degradation of starch, are cyclic molecules that contain six, seven and eight glucose units respectively, and approximate to one turn of these helical structures (Saenger, 1980). They have a hydrophobic central cavity, with a diameter of 0.47, 0.6 and 0.75 nm respectively, and form inclusion complexes with a large variety of hydrophobic and amphiphilic molecules (Saenger, 1980). In contrast with β - and γ -cyclodextrin, α -cyclodextrin does not form complexes with taurine-conjugated bile salts (Table 3), presumably because its central cavity is too small. The affinity of β - and γ -cyclodextrin, like that of starch, increases from taurocholate to taurodeoxycholate to taurochenodeoxycholate, but by a smaller factor (~ 1 to 10 to 100 for starch, 1 to 11 to 30 for β -cyclodextrin, and 1 to 4 to 17 for γ -cyclodextrin; Table 3).

On a weight basis the affinity of β - and γ -cyclodextrin for the taurine-conjugated bile salts is similar, whereas that of starch is at least 200 times weaker (Table 3). This may reflect a difference between the absolute stability of the starch and β -cyclodextrin complexes, a difference in the number of binding sites per unit weight or both. The dissociation constant derived from incubations of a fixed concentration of starch with different concentrations of taurochenodeoxycholate is 128 μ M for potato and

152 μM for cassava starch (Table 4). These values are ~ 10 times higher than that for β -cyclodextrin (14 $\mu\text{g}/\text{ml}$ or 12 μM ; Table 3). From the same incubations, the concentration of the binding sites in the incubation medium was found to be 33 μM for potato and 38 μM for cassava starch and, as both starches were at 1.6 mg/ml , this gives one binding site per 320 and 265 glucose units respectively (Table 4). These values are ~ 40 times larger than that for β -cyclodextrin. Assuming that the helical structures within starch are the binding sites for bile salts, then either their number is restricted to 1 per ~ 300 glucose units or those in excess of this do not bind bile salts. The reason could be that they are physically inaccessible to bile salts or that, by analogy to α -cyclodextrin, their central cavity is too small.

The critical micellar concentrations (11, 4 and 3 mM ; Hofmann and Roda, 1984) and the relative retention times on reversed-phase h.p.l.c. (1, 1.5 and 1.6; Ruben and van Berge-Henegouwen, 1982; 1, 1.8 and 1.9, Carducci et al., 1985) indicate that there is an increase in hydrophobicity from cholate to chenodeoxycholate to deoxycholate. In contrast, their affinity for starch and β -cyclodextrin increases from cholate (3,7,12-trihydroxycholanolic acid) to deoxycholate (3,12-dihydroxycholanolic acid) to chenodeoxycholate (3,7-dihydroxycholanolic acid; see Table 3). Evidently, the presence of the 7-hydroxy or, perhaps more probably, the absence of the 12-hydroxy group plays an important role in stabilizing the binding of bile salts to starch and cyclodextrins.

In both mice and hamsters, β -cyclodextrin, like starch, increases the loss of radioactivity in the faeces from [^{14}C]chenodeoxycholate, but not from [^3H]cholate (Figures 1 and 2a and Tables 1 and 2). The much larger affinity of starch and β -cyclodextrin for chenodeoxycholate than for cholate (Table 3) apparently explains this difference. However, although this explanation may be relevant to the hamster, which has cholate, deoxycholate and chenodeoxycholate as its major bile salts, it is not directly relevant to the mouse, which converts chenodeoxycholate into α -muricholate (3,6,7-trihydroxycholanolic acid). The affinity of this bile salt for starch and cyclodextrins is not known. *In vivo*, the situation is further complicated in that the bile salts that escape absorption in the small intestine are degraded by colonic bacteria, deoxycholate being the major degradation product of cholate and lithocholate of both α -muricholate and chenodeoxycholate.

There is an apparent discrepancy between the *in vitro* and *in vivo* data, in that, although β - and γ -cyclodextrin have similar affinities for taurochenodeoxycholate (Table 3) *in vitro*, only β -cyclodextrin increases the elimination of radioactivity from [^{14}C]chenodeoxycholate *in vivo* (Tables 1 and 2). Further, they are both degraded in the intestine, as neither causes an increase in the weight of the faeces (Table 1). However, their site of degradation differs: γ -cyclodextrin is digested by the α -amylases in the small intestine, whereas β -cyclodextrin is resistant (Mar-

shall and Miwa, 1981) and is fermented in the large intestine (Szejtli et al., 1980). Apparently, it is sufficient to increase the quantity of chenodeoxycholate or α -muricholate entering the large intestine to ensure its increased elimination in the faeces. By analogy, starch that resists degradation in the small intestine will, even if it is fermented in the large intestine, increase the elimination of chenodeoxycholate and this could have an effect on cholesterol metabolism. Although they did not indicate the mode of action, Amelsvoort et al. (1992) have patented the use of resistant starch to lower plasma cholesterol. However, its use in man seems unlikely, as it is ~ 35 times less effective at eliminating chenodeoxycholate than cholestyramine (i.e. 23 g as opposed to 0.7 g/kg increase chenodeoxycholate elimination to 50% in mice; cf. Figures 1 and 2b), which is used at a dose of 12–30 g/day (Miettenin, 1979). In contrast, β -cyclodextrin, which has been shown to decrease plasma cholesterol in both hamsters and genetically hypercholesterolaemic rats (Riottot et al., 1993), is only three times less effective than cholestyramine (cf. Figures 2a and b) at eliminating chenodeoxycholate in mice.

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