# Faecal excretion products of cholic acid in man*†

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The two primary bile acids formed from cholesterol in man are cholic and cheno-deoxycholic acids. These bile acids are attacked by microbial enzymes in the intestine and excreted in the faeces as completely transformed metabolites. Hydrolysis of conjugates, oxidation of hydroxyl groups to ketones, removal of the $C_7$-hydroxyl group and inversion of the $3\alpha$-hydroxyl group to the $3\beta$ position are the main reactions performed by micro-organisms. The subject has been reviewed by Bergström, Danielsson & Samuelsson (1960). The main faecal bile acids in man are 3,12-dihydroxycholanic, 3-hydroxy-12-ketocholanic and 3-hydroxycholanic acids, appearing as a mixture of the $3\alpha$- and the $3\beta$-hydroxy isomers (Fischer, 1911; Carey & Watson, 1955; Heftmann, Weiss, Miller & Mosettig, 1959; Danielsson, Eneroth, Hellström, Lindstedt & Sjövall, 1963).

As faecal bile acid excretion is one of the measures of cholesterol degradation, interest in the factors controlling this excretion is increasing. However, the literature contains several contradictory reports on the bile acid excretion in man. Many of these discrepancies are probably due to the fact that certain methods used for measuring faecal bile acids are not completely satisfactory. As the investigation described here does not concern itself with the quantitative aspects of bile acid excretion, a discussion of those problems is omitted. A recent review is that of Danielsson (1964).

The turnover of cholic acid can be calculated by determining the rate of faecal isotope excretion after administration of $^{14}$C-labelled cholic acid. Such determinations in the rat have demonstrated that several factors influence the elimination of cholic acid. The slowest faecal excretion rate of labelled cholic acid has been observed in germ-free rats and in rats treated with phthalylsulphathiazole and oxytetracycline (Gustafsson, Bergström, Lindstedt & Norman, 1957; Lindstedt & Norman, 1956), the estimated biological half-life of cholic acid being at least three times that in conventional rats. Portman & Murphy (1958) have demonstrated that the type of diet has a pronounced influence on the turnover of cholic acid: with a commercial rat diet 'largely based on corn germ' (Portman, 1962), the half-life of cholic acid was 2·0 days, whereas with a standard low-residue, semi-purified diet with sucrose as the source of carbohydrate and with the same diet supplemented with 20% of cellulose the half-lives were 4·2 and 1·4 days, respectively.

Intestinal bacteria and dietary factors play important parts in the elimination of bile acids by influencing their reabsorption in the enterohepatic circulatory system. A common mechanism of action cannot be excluded, however, as changes in the composition of the diet induce changes in the intestinal flora. Decreased reabsorption of bile acids might be caused by changes in the transit time of the intestinal contents, formation of less absorbable derivatives or binding of bile acids to micro-organisms or indigestible food residues.

The physical state of the bile acids varies in different parts of the intestine (Gustafsson & Norman, 1962). After administration of [24-$^{14}$C]cholic acid to rats it was shown that the labelled bile acids in the small intestine were present in the supernatant fluid after centrifugation of the small intestinal contents at 25 000 $g$. In contrast, half of the labelled bile acids in the caecum were not readily soluble in water and could only be recovered by acetone extraction of the sediment. This difference has been ascribed to the action of intestinal micro-organisms, since the caecal bile acids of germ-free rats were found to occur mainly in a water-soluble form.

The experiments to be described form part of investigations on the influence of the intestinal flora on the metabolism and turnover of bile acids in man. The extractability and nature of the excretory products of [24-$^{14}$C]cholic acid are reported.

## EXPERIMENTAL AND RESULTS

### General

The efficiency of various extraction and dialysation procedures was tested by applying them to faecal homogenates to which bile acids had been added. These procedures were then applied to the faeces of patients to whom labelled cholic acid had been given. Bile acid metabolites in faeces were separated by diethyl ether extraction and chromatography. Finally, the chemical nature of metabolites separated by extraction procedure and by dialysis was determined.

[24-$^{14}$C]cholic acid (15 $\mu$c) was given as the sodium salt dissolved in water to three patients, who were in hospital during the experiment and received the ordinary hospital diet. The patients were suffering from neurological diseases and had no clinical or laboratory signs of disease of the liver, gall-bladder or gastro-intestinal tract.

### Fractionation of crude faeces extracts

Approximately 20 g faeces were homogenized in 40 ml saline with a Virtis Homogenizer (The Virtis Company Inc., Gardiner, New York) with propeller, for 1 min at 5000 rev/min and fractionated according to the scheme shown in Fig. 1. The faecal homogenate could be separated by centrifugation at 25 000 $g$ for 1 h into a top layer, a clear subnatant liquid and a sediment. The first two were combined in Extract A and the sediment was treated as shown in Fig. 1. All operations were done at 0–5°.

### Dialysis of faecal homogenate

Dialysis was performed in regenerated cellulose tubing (Visking Company). The faecal homogenate was dialysed with five volumes of 1/15 M-phosphate buffer, pH 7·4, which was renewed every 6 h.

### Separation of bile acids

Extracts A, B, C and the residue after evaporation of Extracts D and E were acidified to pH 1 with hydrochloric acid and extracted first with diethyl ether and then with butanol.

The labelled bile acids, which were almost entirely recovered in the ether extract, were separated by reversed-phase partition chromatography. The following phase systems were used:

| Phase system | | Moving phase | ml | Stationary phase | ml |
|---|---|---|---|---|---|
| C 1 | (Norman, 1953) | Methanol:water | 150:150 | Chloroform:isooctanol | 15:15 |
| F 1 | (Sjövall, 1953) | Methanol:water | 165:135 | Chloroform:heptane | 45:5 |
| F 2 | (Sjövall, 1953) | Methanol:water | 180:220 | Chloroform:heptane | 45:5 |
| G | (Norman, 1953) | Methanol:water | 255:45 | Heptane | 50 |
| HD | (Danielsson, 1958) | Isopropanol:water | 165:135 | Chloroform:heptane | 10:40 |

Columns for phase systems C 1, F 1, F 2 and G consisted of 4 ml stationary phase supported on 4·5 g hydrophobic Hyflo Super-Cel (Johns-Manville, New York).

Columns for phase system HD consisted of 3 ml stationary phase on 4·5 Hostalene (polyethylene powder; Farbwerke Hoechst, G.m.b.H., German Federal Republic).

A 4·5 g column was used when the amount of material to be analysed was less than 50 mg. For larger amounts, the columns were correspondingly increased. To facilitate comparison, the effluent volumes shown in the figures have been corrected to correspond to a 4·5 g column. The fractions collected from the columns were titrated with methanolic sodium hydroxide, and the isotope was determined in portions after plating in a Frieseke–Hoepfner methane gas-flow counter.

$3\alpha,12\alpha$-dihydroxycholanic and $3\beta,12\alpha$-dihydroxycholanic acids were separated on columns of Woelm aluminium oxide, grade V, as described by Danielsson *et al.* (1963). Thin-layer chromatography was performed as described by Eneroth (1963).

### Extraction of bile acids from faeces

[24-$^{14}$C]*cholic and deoxycholic acids added to faecal homogenate.* A mixture of $^{14}$C-labelled cholate and deoxycholate was added to portions of a faecal saline homogenate and, after further homogenization, extracted according to the scheme shown in Fig. 1. The amounts of labelled cholic and deoxycholic acids in the different extracts were determined after chromatographic separation. The distribution of isotope in the different extracts, shown in Table 1, indicates that both bile acids were adsorbed to the faecal residue. However, adsorption occurred to a much greater extent with deoxycholate than with cholate. For complete recovery of isotope, extraction with organic solvents was necessary. The acetone extracts contained the main part of labelled bile acids remaining after the phosphate buffer extraction, and the last small

quantity of isotope was recovered by extraction for 48 h with chloroform/methanol 1:1. The latter extraction method has been demonstrated to give quantitative recovery of bile acids from faeces (P. Eneroth, K. Hellström & J. Sjövall, 1964, to be published).
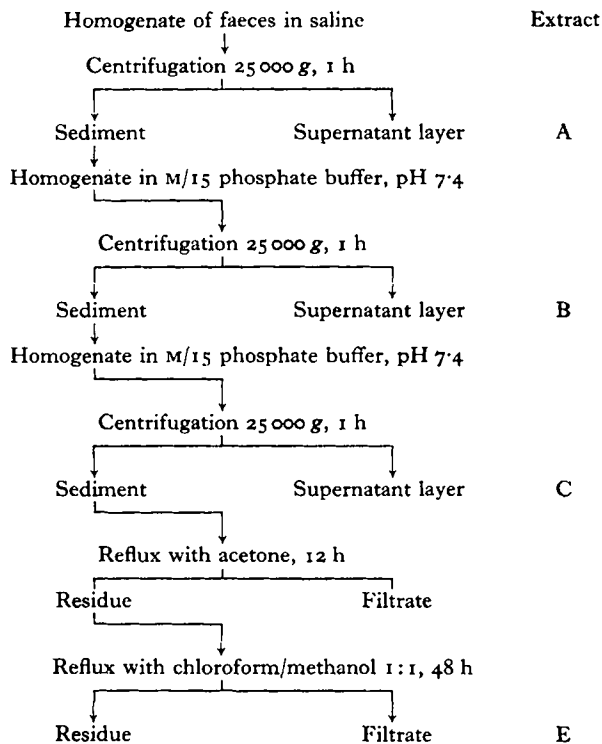


Fig. 1. Procedure for fractionation of faeces.

Table 1. *Extraction according to scheme shown in Fig. 1 of labelled cholate and deoxycholate added to a homogenate of human faeces*

| | | 0·05 mg cholate<br>0·05 mg deoxycholate<br>10 g faeces homogenate | | 5 mg cholate<br>5 mg deoxycholate<br>10 g faeces homogenate | |
| | | Percentage distribution of<br>recovered ¹⁴C-labelled: | | Percentage distribution of<br>recovered ¹⁴C-labelled: | |
| Extract | Lipids soluble<br>in ether<br>(mg) | Cholate | Deoxycholate | Cholate | Deoxycholate |
|---|---|---|---|---|---|
| A | 7·2 | 62·8 | 14·9 | 58·9 | 21·1 |
| B | 3·5 | 18·8 | 12·7 | 23·6 | 21·8 |
| C | 3·1 | 8·4 | 19·1 | 11·2 | 14·6 |
| D | 50·7 | 8·4 | 52·5 | 6·1 | 41·0 |
| E | 13·5 | 1·6 | 0·8 | 0·2 | 1·5 |

*Labelled metabolites of orally administered* [24-¹⁴C]*cholic acid.* Three patients were given [24-¹⁴C]cholic acid, and faeces were collected daily for 1 week. After homogenization in saline, portions of the homogenate were extracted according to the scheme shown in Fig. 1. Results of the differential extraction of labelled metabolites

from the faeces on the 5th day after the administration of [24-¹⁴C]cholic acid are shown in Fig. 2. Of the metabolites of cholic acid, 10–23% were extractable with saline (Extracts A). In addition, 10–22% of the metabolites were loosely bound to the faecal residue and could be extracted with phosphate buffer (Extracts B + C). Most of the isotope was found in the acetone extracts (D), but even with this method there was incomplete recovery, as 5–15% of the labelled compounds were obtained later in the chloroform/methanol Extracts (E).
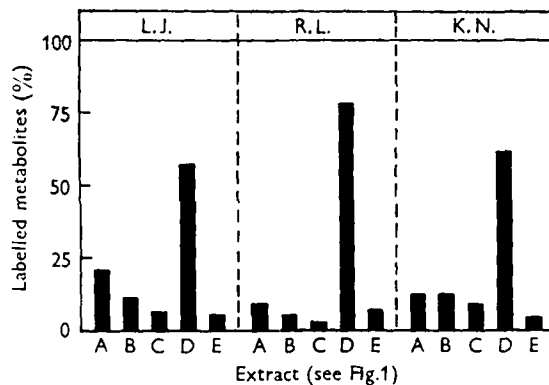


Fig. 2. Percentage distribution of recovered isotope after extraction according to scheme in Fig. 1 of samples of faeces 5 days after administration of [24-¹⁴C]cholic acid to patients L.S., R.L. and K.N.

Table 2. *Percentage distribution of the dialysable and non-dialysable labelled compounds recovered by dialysis of faecal homogenates against 1/15 M-phosphate buffer, pH 7·4. After dialysis, the contents of the dialysis sack were centrifuged for 60 min at 25 000 g*

| | Dialysable compounds | | Non-dialysable compounds | | | |
| | | | Supernatant layer | | Sediment | |
| Material | Lipids soluble in ether (mg) | Labelled compounds (%) | Lipids soluble in ether (mg) | Labelled compounds (%) | Lipids soluble in ether (mg) | Labelled compounds (%) |
|---|---|---|---|---|---|---|
| 10 g faeces homogenate and 0·05 mg [24-¹⁴C]cholate and 0·05 mg [24-¹⁴C]deoxycholate | 16 | 97 | 2 | 2 | 81 | 1 |
| 10 g faeces homogenate and 5·0 mg [24-¹⁴C]cholate and 5·0 mg [24-¹⁴C]deoxycholate | — | 96 | — | 2 | — | 2 |
| 10 g faeces homogenate from patient L.J. 3 days after administration of [24-¹⁴C]-cholate | 12 | 50 | 8 | 4 | 172 | 44 |
| 10 g faeces homogenate from patient K.N. 3 days after administration of [24-¹⁴C]-cholate | 12 | 61 | 2 | 2 | 107 | 37 |

*Dialysis of bile acids in faecal homogenates*

*[24-¹⁴C]cholic and deoxycholic acids added to a faecal homogenate.* ¹⁴C-labelled cholate and deoxycholate were added to faecal homogenates, which, after further homogenization, were transferred to dialysis bags. After dialysis, the contents were centrifuged for 1 h at 25 000 $g$ and the supernatant layer was separated from the sediment. The isotope was extracted from the dialysate and the supernatant layer by ether extraction after acidification, and from the sediment by chloroform/methanol extraction. Recoveries of added isotope in five similar experiments were between 94 and 98%. The upper half of Table 2 gives the percentage distribution of recovered labelled compounds, showing that practically all of the added labelled cholate and deoxycholate was separated from the faecal residue by dialysis.

Table 3. *Combined Extracts A, B, C, D, and E from homogenates of faeces of patients 5 days after administration of [24-¹⁴C]cholic acid, extracted first with ether and then with butanol*

| | Ether extract | | Butanol extract | |
| --- | --- | --- | --- | --- |
| Patient | mg/g faeces | % of recovered isotope | mg/g faeces | % of recovered isotope |
| L. J. | 48 | 91 | 14 | 9 |
| R. L. | 53 | 98 | 12 | 2 |
| K. N. | 36 | 95 | 23 | 5 |

*Labelled metabolites of [24-¹⁴C]cholic acid.* The results of the dialysis of faecal homogenates from patients K. N. and L. J. 3 days after administration of [24-¹⁴C]cholic acid are shown in the lower half of Table 2. They indicate that at least a third of the excreted faecal metabolites of cholic acid are non-dialysable and are present in the sediment after centrifugation.

Dialysis of Extracts A, B and C of faeces from these patients showed that all the labelled metabolites in those extracts are dialysable. The residue after phosphate buffer extraction, however, also contained a large amount of labelled metabolites that could be separated from the residue by dialysis.

*Separation of metabolites of [24-¹⁴C]cholic acid according to extractability with ether*

Unconjugated bile acids and glycine conjugates of mono- and di-hydroxycholanic acids can be extracted with ether from an acidified aqueous solution, but glycine conjugates of trihydroxycholanic acids and all taurine conjugates must be extracted with butanol. Extracts A, B and C and the residue, after evaporation, of Extracts D and E were combined, acidified, and extracted first with ether and then with butanol. As shown in Table 3, most of the labelled material was recovered in the ether extracts, and only 2–9% of the isotope was present in the subsequent butanol extracts. Thus a maximum of 2–9% of cholic acid was excreted in the faeces as either taurine conjugates or glycocholic acid.

As the ether extracts contained almost all of the labelled metabolites, and less

impurities than the butanol extracts, they were used for the subsequent column chromatographic separations of labelled metabolites. No analysis of the labelled material in the butanol extracts was done.

### Column chromatographic separations of labelled metabolites of [24-$^{14}$C]cholic acid

Partition column chromatography was used to obtain group separations of [24-$^{14}$C]-cholic acid metabolites. In chromatography with phase system F 1, 3,7,12-trihy-droxycholanic and 3,12-dihydroxy-7-ketocholanic acids appear with or just after the front, and separated from 3,12-dihydroxycholanic and 3-hydroxy-12-ketocholanic acids which appear in effluent fractions 40–60 ml and 80–110 ml respectively. 3,12-diketocholanic acid is retained in the stationary phase. However, no separation of the 3$\alpha$- and 3$\beta$-hydroxy isomers is obtained.
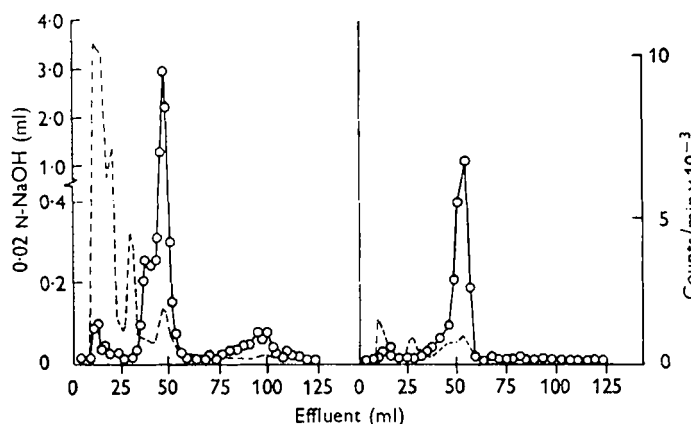


Fig. 3. Separation of labelled material from faeces 3 days after administration of [24-$^{14}$C]cholic acid to patient K. N. Phase system F 1 (see p. 175).
Left, combined ether extracts (A–E) (see Fig. 1); right, rechromatographed after saponification of labelled products retained in the column after the chromatograph shown in the left part; - - -, defined by left-hand legend to ordinate; o—o, defined by right-hand legend to ordinate.

The combined ether extracts of A, B, C, D and E were chromatographed with phase system F 1 and a representative chromatogram is shown on the left in Fig. 3. Labelled material representing four bile acid regions was obtained.

*Fraction (I)*. Minor amounts of labelled compounds appeared with or just after the front. Rechromatography of Fraction (I) with phase system C showed that it contained only small amounts of unchanged cholic acid, and instead several more-polar and less-polar metabolites of cholic acid were separated.

*Fraction (II)*. This fraction, which represents the deoxycholic acid band, contained most of the labelled products. It was methylated and further fractionated on a column of aluminium oxide, Woelm, grade V with increasing concentrations of ethyl acetate in benzene. The chromatographic fractions were analysed by subjecting small portions to thin-layer chromatography and by determination of isotope content (Fig. 4). Two

labelled compounds were isolated; the major one consisted of methyl 3α,12α-dihydroxycholanate and the minor one of methyl 3β,12α-dihydroxycholanate.

*Fraction (III).* After the deoxycholic acid band minor radioactive bands appeared, the quantitatively most important one corresponding to 3-hydroxy-12-ketocholanic acid (80–110 ml).
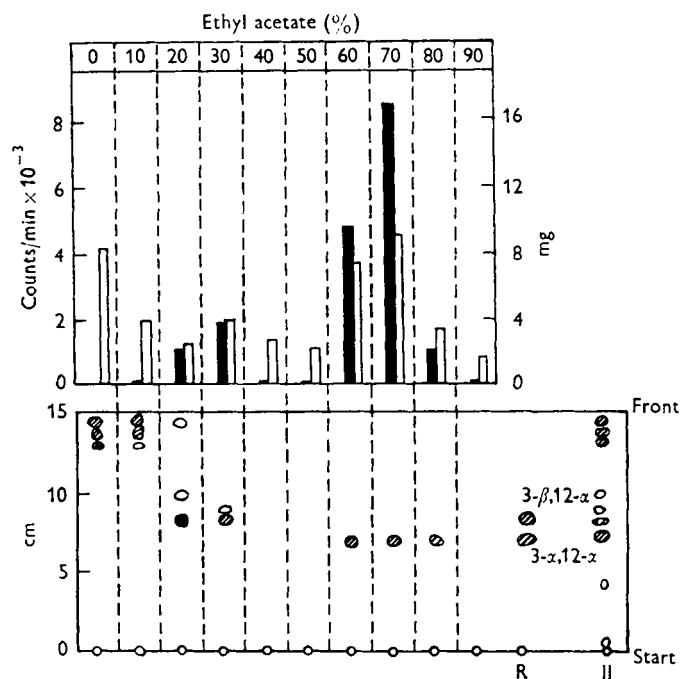


Fig. 4. Alumina fractionation of the 'deoxycholic acid band' from the chromatograph shown in Fig. 3, left. Column, 10 g aluminium oxide; fractions, 100 ml. The composition of the eluate is given as the percentage of ethyl acetate in benzene. □, amount of eluted solids (mg); ■, amount of isotope (counts/min × 10⁻³).

The lower part of the figure shows the thin-layer silica gel chromatograph of the different fractions. R, reference substances: methyl 3α,12α-dihydroxycholanate and 3β,12α-dihydroxycholanate; II, portion of Fraction II before chromatography. Moving phase: trimethylpentane/ethyl acetate/acetic acid 10:20:0·2. Spots revealed by spraying with sulphuric acid and heating. Intensity of spots shown by differences in cross-hatching.

*Fraction (IV).* This fraction represents the labelled material retained in the stationary phase after chromatography with phase system F 1. It contained 25–50 % of the labelled compounds and more than 90 % of the lipids in the extracts used for chromatography. Rechromatography with phase system F 2 showed that only trace amounts of isotope were eluted at the place of 3,12-diketocholanic acid and almost all of the isotope remained in the stationary phase. By means of the phase system G, with which cholanic acid is eluted at 60–100 ml (Fig. 5A), part of the labelled compounds was eluted in the front fractions and part as a peak at 100–130 ml (Fig. 5C). As shown by the titration curve, the main part of the acidic products, consisting of fatty acids, was eluted with this phase system. The stationary phase, however, still contained the majority of the labelled products. For their separation, phase system HD, described

by Danielsson (1958) and used for the separation of cholesterol and related neutral steroids, was used. With phase system HD, as shown in Fig. 5$D$, the remaining labelled products were eluted in effluent fractions 70–150 ml just after cholesterol (Fig. 5$B$). No radioactive material was retained in the stationary phase after elution of the column with 150 ml of moving phase.

Thus Fraction (IV) consisted of at least three different labelled compounds. After heating in M-methanolic sodium hydroxide at 37° for 2 h, and chromatography with phase system F 1, all the isotope in this fraction could be eluted as a single peak at the place of 3,12-dihydroxycholanic acid as illustrated on the right in Fig. 3. Chromatography of the deoxycholic acid band on a column of aluminium oxide showed that the main labelled compound consisted of 3$\beta$,12$\alpha$-dihydroxycholanic acid. Hence, Fraction (IV) consisted of saponifiable derivatives of mainly 3$\beta$,12$\alpha$-dihydroxycholanic acid.
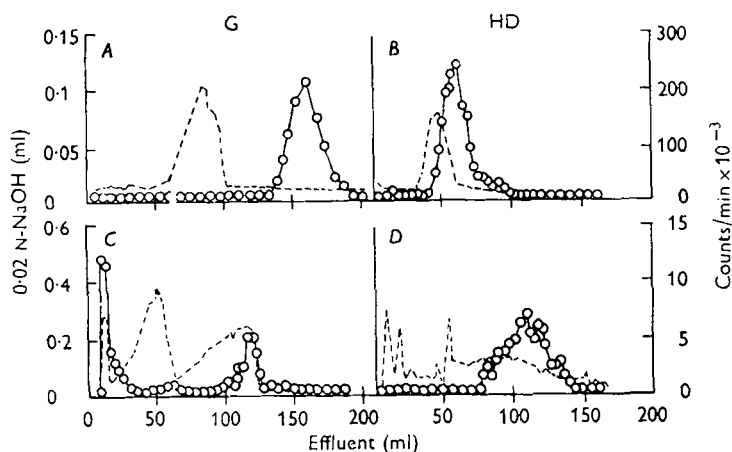


Fig. 5. *A* and *B*: separation of 4 mg cholanic acid and a tracer dose of [4-$^{14}$C]cholesterol with phase system G and HD (see p. 175). *C* and *D*: subsequent separation of labelled material retained in the column after the chromatograph shown in Fig. 3, left. - - -, defined by left-hand legend to ordinate; o—o, defined by right-hand legend to ordinate.

### Correlation between chemical nature of metabolites of [24-$^{14}$C]cholic acid and their extractability

The ether extracts of (A), (B + C) and (D + E) from faeces 2, 3 and 4 days after administration of [24-$^{14}$C]cholic acid, were subjected to chromatography with phase system F 1 and HD. Representative chromatograms of labelled metabolites of Extracts (A) and (D + E) are shown in Figs. 6 and 7. The percentage distribution of isotope in each of Fractions (I), (II), (III) and (IV) for the three patients was determined. The results, summarized in Fig. 8, showed that the composition of the labelled bile acids in the saline (A) and phosphate buffer (B + C) extracts was quite different from that in the acetone and chloroform–methanol extracts (D + E). The extractability of Fractions I–IV was as follows:

Fraction (I) and (III) were mainly present in Extracts (A) and (B + C).

Fraction (IV) was not extracted by saline or phosphate buffer.

12-2

Fraction (II) was mainly recovered in Extracts (D + E) but was also present in the aqueous Extracts (A + (B + C)). The labelled $3\alpha,12\alpha$-dihydroxycholanic and $3\beta$-, $12\alpha$-dihydroxycholanic acids in Fraction (II) from samples 4 days after administration of [24-$^{14}$C]cholic acid were separated on columns of aluminium oxide. As shown in
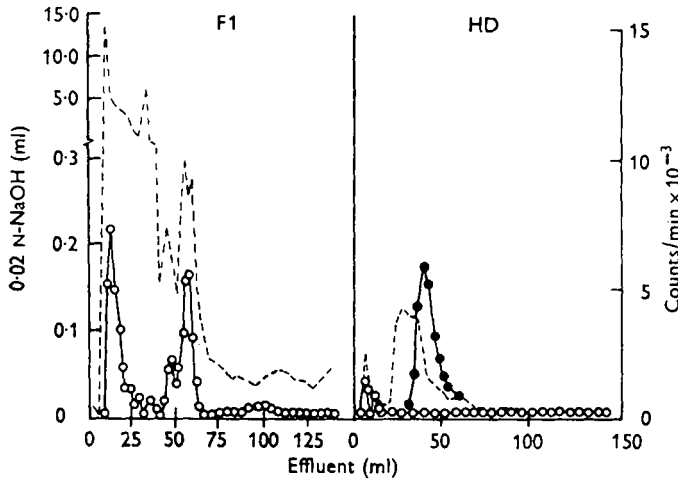


Fig. 6. Separation of unhydrolysed labelled material in Extract A (see Fig. 1) from faeces 3 days after administration of [24-$^{14}$C]cholic acid to patient E. L. Extract A was first chromatographed with phase system F 1 (see p. 175). The material remaining in the stationary phase was rechromatographed with phase system HD. - - -, titration values (defined by left-hand legend to ordinate); o—o radioactivity (defined by right-hand legend to ordinate); ●—●, extinction values in the Tschugaeff colour reaction. Carrier: 2 mg cholesterol.
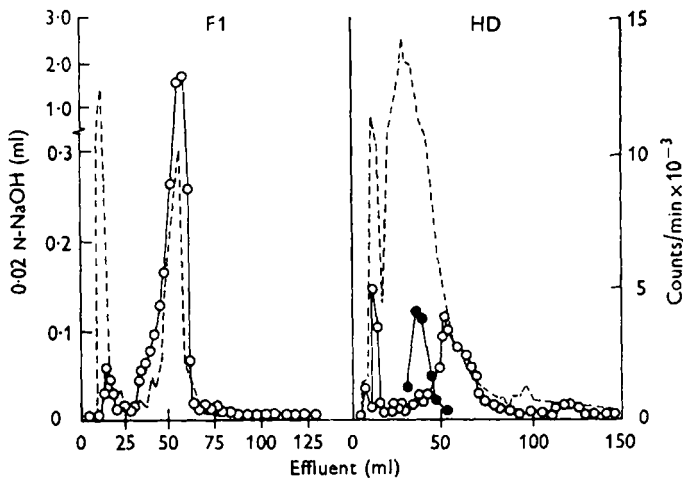


Fig. 7. Separation of unhydrolysed labelled material in Extract (D + E) (see Fig. 1) from faeces 3 days after administration of [24-$^{14}$C]cholic acid to patient E. L. For explanation see legend to Fig. 6.

Fig. 9, the percentage of labelled $3\beta$ isomer is about the same in Fractions (II) of Extracts (A), (B + C) and (D + E), but is less than half of that in the deoxycholic acid band isolated after hydrolysis of the saponifiable labelled products (Fraction (IV)).

As also is seen in Fig. 8, the distribution of the isotope between the different extracts showed that the percentage of isotope in Extracts (D + E) increases from the 2nd to the 4th day after the administration of [24-¹⁴C]cholic acid. Parallel with these changes, the percentage of labelled bile acids in Fraction (I) decreased and the percentage of labelled saponifiable derivatives of 3,12-dihydroxycholanic acid increased.
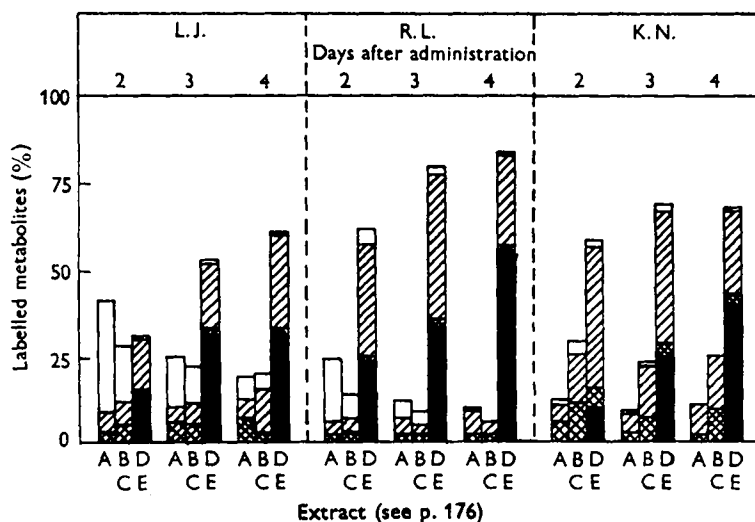


Fig. 8. Distribution, according to extractability and chromatographic behaviour, of labelled metabolites in Fractions I, II, III and IV (see p. 179) of extracts of faeces of patients L.J., R.L. and K.N. after administration of [24-¹⁴C]cholic acid. □, Fraction I; ◪, Fraction II; ▨, Fraction III; ■, Fraction IV.
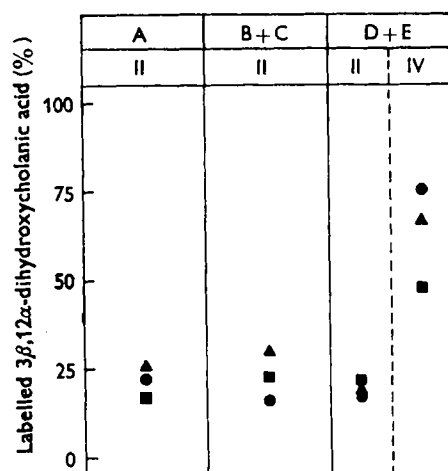


Fig. 9. Percentage of 3β,12α-dihydroxycholanic acid in Fractions II and IV (see p. 179) after chromatographic separation of Extracts A, (B + C) and (D + E) (see Fig. 1) of faeces of patients L.J. (▲), R.L. (●) and K.N. (■) 4 days after administration of [24-¹⁴C]cholic acid.

*Composition of dialysable and non-dialysable metabolites of [24-$^{14}$C]cholic acid*

The dialysable and non-dialysable labelled products from the experiment illustrated in Table 2 were each chromatographed with phase system F 1. The results showed that all the labelled products in Fractions (I) and (III) and more than 90% of the labelled 3,12-dihydroxycholanic acid in Fraction (II) could be separated from the faecal residue by dialysis. The non-dialysable bile acids consisted of the saponifiable derivates of 3,12-dihydroxycholanic acid and small amounts of 3,12-dihydroxy-cholanic acid.

## DISCUSSION

### Extraction methods

Various extraction procedures for faecal bile acids have been described: 80% aqueous ethanol, ethanol, chloroform, methylal/methanol 4:1 and chloroform/methanol 1:1 have been proposed as extraction solvents. The subject has been reviewed by Danielsson (1964). P. Eneroth, K. Hellström & J. Sjövall (1964, to be published) have shown that chloroform/methanol 1:1, but not ethanol, gave complete recovery of isotope from human faeces after administration of labelled bile acids. The main metabolites of cholic acid after hydrolysis of faecal extracts, deoxycholic and 3-hydroxy-12-ketocholanic acids, are as sodium salts very soluble in water. It therefore seemed peculiar that those metabolites had to be extracted with chloroform–methanol.

The investigation now described showed that only 20–30% of the labelled metabolites of cholic acid were extractable with saline and phosphate buffer. Most of the remaining isotope was recovered by acetone extraction, but extraction with chloroform–methanol 1:1 was found necessary for quantitative recovery. One explanation of why faecal bile acids have to be extracted by organic solvents is that 3,12-dihydroxycholanic acid is adsorbed to the residue in a saline homogenate of faeces. Another explanation was found when the different unhydrolysed extracts were analysed by chromatography. The main parts of the labelled products left after the extraction with saline and phosphate buffer were saponifiable derivatives of 3,12-dihydroxycholanic acid. These compounds were found to be less soluble in water.

An intracellular localization of bile acids also could explain the need to extract bile acids with organic solvents. However, deoxycholic acid formed in vitro in subcultures of anaerobic intestinal micro-organisms is present extracellularly (Norman & Shorb, 1962). In addition, 3,12-dihydroxycholanic acid in human faeces is mainly present in a dialysable form, a fact that makes an intracellular localization less likely.

### Metabolites of cholic acid

Owing to the limited number of subjects it is impossible to draw conclusions about variations from one individual to another. In all patients studied, deoxycholic acid is the main microbial metabolite of cholic acid. Small amounts of other metabolites were also isolated. These results are in accordance with the findings of other investigators who have studied the nature of the metabolites of cholic acid after hydrolysis of the

primary extracts. There are, however, no reports on the presence of saponifiable derivates of 3,12-dihydroxycholanic acid in human faeces. These compounds are probably formed by the action of microbial enzymes. Labelled products with the same chromatographic behaviour are formed in cultures of human faecal micro-organisms from [24-$^{14}$C]deoxycholic acid (Norman, 1964, to be published). Investigations on the chemical structure of these compounds are in progress.

### General

Human bile contains the conjugates of cholic, deoxycholic and chenodeoxycholic acids. Deoxycholic acid is formed in the intestine by microbial enzymes in the process of enterohepatic circulation. However, other metabolites of cholic and chenodeoxycholic acids, i.e. different 3$\beta$-hydroxy isomers and lithocholic acid, do not appear in bile in appreciable amounts. These findings could be explained by differences in absorbability of different bile acid metabolites themselves or by their formation at more distal levels in the intestine where the intestinal capacity to absorb them is reduced.

Borgström (1960) has separated human intestinal contents from the proximal part of the small intestine by centrifugation at high speed (above 45 000 $g$) into an oily top layer, a clear subnatant layer and a sediment. The clear subnatant layer contained many different lipids together with the bile acids, which were present as conjugates. In more distal parts of the small intestine and in the colon the microbial transformations of the bile acids change their physical state by the formation of bile acid derivatives which are either poorly soluble in water or easily adsorbed to the residue. Assuming that the bile acids are absorbed only in a water-soluble form, such alterations can influence the absorption of the bile acids. Thus, for an elucidation of factors influencing the removal of bile acids from the enterohepatic circulation, the chemical and physical state of the bile acids in the intestinal contents as well as the ability of the intestinal wall to absorb these compounds should be investigated at various levels in the intestine.

### SUMMARY

1. The faecal excretory products of cholic acid have been studied in man by analysing the labelled products after oral administration of [24-$^{14}$C]cholic acid.

2. Sequential extractions of faeces with saline, phosphate buffer (pH 7·4), acetone and chloroform/methanol 1:1 were performed. Of the labelled metabolites, 20–45 % appeared in the aqueous extracts. Most of the remaining isotope was recovered in the acetone extracts, but the last 5–15 % required extraction with chloroform/methanol.

3. More than half of the labelled compounds in faecal homogenates were dialysable.

4. Column chromatographic separations of the labelled unhydrolysed metabolites of cholic acid showed that only small amounts of conjugated bile acids and unchanged cholic acid were left. The main metabolites were 3,12-dihydroxycholanic acid and 3-hydroxy-12-ketocholanic acid. At least 25 % of the labelled metabolites were present as saponifiable derivatives of 3,12-dihydroxycholanic acid, mainly the 3$\beta$ isomer.

5. The bile acid composition of the different extracts varied. Labelled compounds more polar than 3,12-dihydroxycholanic acid and 3-hydroxy-12-ketocholanic acids were recovered in the aqueous extracts. 3,12-dihydroxycholanic acid was about equally distributed between the different extracts. The saponifiable derivatives of 3,12-dihydroxycholanic acid were present only in the organic solvent extracts.

6. The non-dialysable bile acids mainly consisted of saponifiable derivatives of 3,12-dihydroxycholanic acid. More than 90% of the 3,12-dihydroxycholanic acid was dialysable.

7. The results show that chemical transformations of cholic acid by intestinal micro-organisms also change the physical state of the bile acids in the intestinal contents by forming derivatives which are either poorly soluble or easily adsorbed to the residue.

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