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METABOLISM OF UNSATURATED BILE ACIDS AND ANDROSTANES BY HUMAN FAECAL BACTERIA

R. W. OWEN* and R. F. BILTON
Department of Chemistry and Biochemistry, Liverpool Polytechnic, Byrom Street, Liverpool L3 3AF, U.K.

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Summary—The metabolism of unsaturated bile acids and androstanes by mixed human faecal cultures has been studied. The reactions observed were mainly reductive. Unsaturated 4-ene-3-oxo and 1,4-diene-3-oxo bile acids were reduced in Ring A. 5β-3-Oxo bile acids were reduced to 5β-3-hydroxy bile acids. 4-Ene, 1,4-diene and 4,6-diene-3,17-dioxo-androstanes were reduced in Ring A with concomitant reduction of oxo groups to hydroxyl groups. The Gram-negative facultative anaerobic faecal bacteria are implicated in the reductive process, whilst the genus *Clostridium* does not appear to be important. Inclusion of menadione, a synthetic form of vitamin K, retards the reductive process.

INTRODUCTION

Large bowel cancer (LBC) is common in Western Europe and North America and rare in black Africa, Asia and Central America and the Andean countries of South America [1]. Epidemiological studies have revealed that it is an environmental desease, with diet as a major contributing factor [2, 3]. There is a strong correlation between LBC and a high intake of dietary fat and meat [4, 5]. It has been shown that dietary fat determines to a large extent both the concentration of bile acid and neutral steroids in the large bowel [6] as well as influencing the composition of the microflora acting upon them [7]. Aries *et al.* have suggested that the intestinal flora is involved in the mechanism by which diet influences bowel cancer, because lecithinase-negative strains of Clostridia especially *Clostridium paraputrificum* spp (nuclear dehydrogenating Clostridia:NDC) are capable of introducing double bonds in 3-oxo-5β-androstanes [8]. Owen[9] has recently shown that NDC can also desaturate the bile acid nucleus. The oxidation reactions are carried out under anaerobic conditions and require menadione as terminal electron acceptor. The carriage rate of NDC correlates well with the incidence of LBC [10].

Whether or not unsaturated bile acids are produced in the colon has not been discovered, although the presence of 5α-orientated bile acids in faeces suggests that they are. It has been shown that bacteria are responsible for the inversion of 5β-bile acids to 5α-bile acids via unsaturated 4-en-3-one intermediates [11].

Conversely Schubert *et al.*[12] have shown that *Clostridium paraputrificum* hydrogenates unsaturated 3-oxo steroids at C_1, C_4 and C_6 *in vitro* under anaerobic conditions whilst the oxo group is reduced to a hydroxyl group. Furthermore it has been shown that mixed faecal bacteria hydrogenate both androstanes [13], oestrogens [14] and progestins [15]. A very common reaction carried out by colonic bacteria is the hydrogenation of sterols especially cholesterol via the oxidised intermediate cholest-4-ene-3-one to coprostanol (5β-cholestan-3β-ol). The genus *Eubacterium* has been implicated specifically in this reductive process [16, 17]. It appears that the metabolic activity of human faecal bacteria is mainly reductive especially in experiments involving a mixture of bacteria indigenous to the human intestinal tract.

Because unsaturated steroids especially bile acids are implicated in the aetiology of LBC [8] it is imperative to elucidate the fate of such compounds in a highly reductive environment such as the human intestine. This study was designed to examine the metabolism of unsaturated steroids when co-incubated with mixed faecal bacteria in simulated *in vitro* reductive environments. An attempt has also been made to categorise broadly the bacterial genera responsible for the reactions reported.

EXPERIMENTAL

Faecal extracts

Fresh faecal samples from a normal healthy subject were immediately placed on ice and were prepared within 30 min of defaecation. Extracts were made by mixing 200 mg faeces in 5.0 ml reduced medium. The mixture was centrifuged at 400 *g* for 10 min. Extracts were used immediately.

Steroids

3-Oxo-5β-cholan-24-oic acid and 3,12-dioxo-5β-cholan-24-oic acid were synthesised from lithocholic

*Present address and to whom correspondence should be sent: PHLS Centre for Applied Microbiology and Research, Bacterial Metabolism Research Laboratory, Porton Down, Salisbury, Wiltshire SP4 OJG, U.K.

acid [3α-hydroxy-5β-cholan-24-oic acid (LA)] and deoxycholic acid [3α,12α-dihydroxy-5β-cholan-24-oic acid (DCA)] by Jones' chromic oxidation [18]. 3-Oxochol-4-ene-24-oic acid was obtained from the aerobic catabolism of cholesterol by *Pseudomonas* sp NCIB 10590 (Owen *et al.*, 1983)[19]. 12α-Hydroxy-3-oxo-1,4-pregnadiene-20-carboxylic acid and 12β-hydroxyandrosta-1,4-diene-3,17-dione were obtained from the metabolism of deoxycholic acid by *Pseudomonas* sp NCIB 10590 (Owen *et al.*, 1984)[20] whilst androsta-4,6-diene-3,17-dione was obtained from the anaerobic metabolism of chenodeoxycholic acid by *Pseudomas* sp NCIB 10590 (Owen *et al.*, 1983)[21]. The structures of the substrates are depicted in Fig. 1.

Androst-4-ene-3,17-dione, androsta-1,4-diene-3,17-dione, 5β-androstan-3,17-dione, 3β-hydroxy-5β-androstan-17-one, 3α-hydroxy-5β-androstan-17-one, 5β-androstan-3α,17β-diol, 5β-androstan-3β,17β-diol and 5α-cholestane were obtained from Koch Light Laboratories (Colnbrook, Bucks, England). The purity of these compounds was found to be greater than 99% by thin-layer chromatography (TLC) and gas–liquid chromatography (GLC).

Fermentation medium

The medium used was Brain–Heart Infusion (BHI) broth with added reducing agents [22]. The medium was dispensed in 100 ml aliquots into medical flats or 250 ml Erlenmeyer flasks (for aerobic experiments) and was autoclaved at 121°C for 15 min. Bile acids were maintained in the sodium salt form as sterile stock solutions (10%) in distilled water, whilst neutral steroids were maintained as sterile solutions (10%) in ethanol. Steroids were added to the fermentation medium to give a final concentration of 0.02%. Filter sterilised solutions of the antibiotics neomycin sulphate, kanamycin and vancomycin were dissolved in distilled water, filter sterilised and were added when relevant to a final concentration of 100, 4.5 and 7.5 μg/ml respectively.

The oxidising agent KNO_3 (0.1%) was added to the BHI prior to sterilisation whilst filter sterilised menadione (0.01%) was added to the BHI after sterilisation.

Prior to inoculation all media was steamed for 20 min to remove dissolved oxygen.

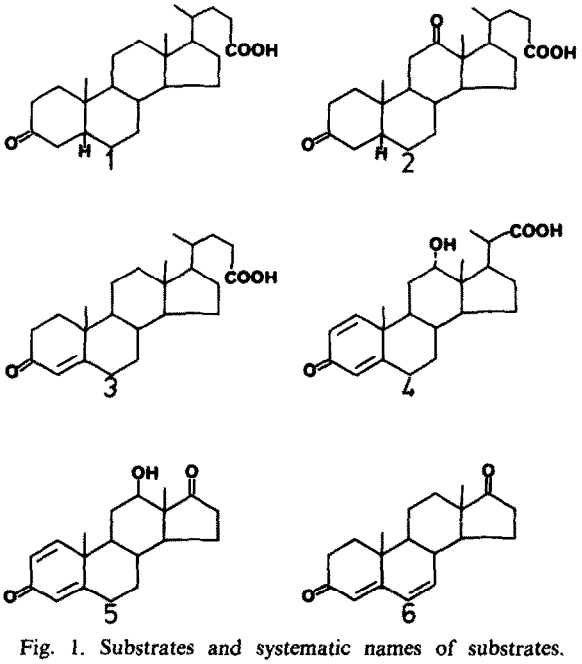

Fig. 1. Substrates and systematic names of substrates.
1. 3-Oxo-5β-cholan-24-oic acid.
2. 3,12-Dioxo-5β-cholan-24-oic acid.
3. 3-Oxochol-4-ene-24-oic acid.
4. 12β-Hydroxy-3-oxo-1,4-pregnadiene-20-carboxylic acid.
5. 12β-Hydroxyandrosta-1,4-diene-3,17-dione.
6. Androsta-4,6-diene-3,17-dione.

Incubations

The faecal cultures were prepared by the addition of fresh faecal extracts to 100 ml of presteamed BHI broth giving a 10^{-3} dilution of faeces. Anaerobic fermentations were carried out in 100 ml medical flats under an atmosphere of 90% H_2:10% CO_2 in anaerobic jars. Palladium catalyst was used to remove any residual oxygen. Aerobic fermentations were conducted in 250 ml Erlenmeyer flasks on an LH Engineering orbital shaker. Aeration was achieved by shaking at 200 rpm. Incubations were conducted at 37°C for 72 h.

Bacteriology

Culture flora analyses were performed as described elsewhere [22] at 0 and 72 h in an attempt to correlate metabolism with the presence of the major genera of faecal bacteria.

Culture extraction

After incubation the reactions were stopped by direct extraction into redistilled ethyl acetate (2 vol). Residual aqueous phase was removed by drying over anhydrous $MgSO_4$. The solvent was removed by evaporation *in vacuo* on a Büchi rotary evaporator, leaving an analytical sample. Based on starting material, recovery of substrate + products was 80–90% efficient by this method. Quantification of neutral and acidic steroids was achieved by gas–liquid chromatography with reference to the internal standards 5α-cholestane and methyl hyodeoxycholate respectively.

Thin-layer chromatography

Silica gel 60 GF_{254} precoated (0.25 mm) plastic backed plates (E. Merck, Darmstadt, W. Germany) were used for TLC. These were developed in the solvent systems methanol–dichloromethane (1:19, v/v) or 2,2,4-trimethylpentane–ethyl acetate–acetic acid (4.5:4.5:1, by vol). Developed plates were studied under a ultraviolet lamp (254 nm) for the detection of unsaturated steroids and all spots were finally visual-

ised by spraying the plates with freshly prepared anisaldehyde reagent [23] followed by heating at 110–120°C for 10 min.

Gas–liquid chromatography

Routine analyses were performed isothermally at 260°C on a Hewlett–Packard HP 5470 gas chromatograph fitted with a 1.5 m × 2 mm silanised glass column packed with 3% OV-17 on 80/100 acid washed Supelcoport (Queensferry, U.K.). Acid steroids were analysed as their methyl esters. More complex mixtures of steroids were analysed on a Pye 304 chromatograph fitted with a 3.0 m × 4 mm silanised glass column packed with 3% OV-1 on 100/200 acid washed Supelcoport. Neutral steroids were analysed as their trifluoroacetate (TFA) derivatives whilst acidic steroids were analysed as their methyl ester TFA derivatives. Separation was effected by temperature programming the column oven from 160–260°C at 4°C/min. The flow-rate of nitrogen through the columns was 30 ml min^{-1} for each instrument.

Identification of steroids

After incubation of mixed faecal bacterial (MFB) cultures for 72 h, duplicate (500 μl) samples were removed for bacteriology and the remainder was extracted with ethyl acetate and analysed by TLC and GLC. Retention times and mobilities of metabolites were compared where possible with authentic or previously characterised compounds; otherwise metabolites were identified by gas chromatography–mass spectrometry (GC–MS) and proton magnetic resonance spectroscopy (PMR) as previously described [24].

The TLC and GLC profiles of reaction mixtures were compared to those for zero time-points, 72 h cultures not containing steroids and uninoculated media so that only products of bacterial transformation and not artifacts were identified. Relative retention times by GLC were determined against the internal standard 5α-cholestane whilst TLC R_f values were measured relative to androsta-1,4-diene-3,17-dione.

RESULTS

3-oxo-5β-cholan-24-oic acid (3KLA)

The results showed that MFB incubated under anaerobic conditions reduced 3 KLA to lithocholic acid (LA) in the presence (56% conversion) and absence (45%) of the oxidising agent KNO_3. When menadione (100 μg/ml) was included in the medium the reaction observed was oxidative in that 3 KLA was oxidised to 3-oxochol-4-ene-24-oic acid (2%). This result was similar to aerobic experiments in which 20% conversion of 3 KLA to 3-oxochol-4-ene-24-oic acid occurred.

Inclusion of vancomycin (V) in the medium gave a slightly different pattern in that 3 KLA was reduced to LA (15%) and isolithocholic acid (ILA, 6%) in the absence and to LA (20%) and ILA (10%) in the presence of KNO_3. Metabolism of 3 KLA was not detected in the presence of menadione. Inclusion of vancomycin and kanamycin (V + K) in the medium together prevented metabolism completely under all the prescribed conditions. Similar results were obtained by heat shocking + neomycin (HS + N).

3,12-Dioxo-5β-cholan-24-oic acid (Dioxo-DCA)

Incubation of Dioxo-DCA with MFB gave reduction products in the presence and absence of KNO_3. In both instances the major product ($\sim$95%) was deoxycholic acid (3α,12α-dihydroxy-5β-cholan-24-oic acid) with small amounts of another metabolite which had identical TLC and GLC mobilities (Table 2) to authentic 3β,12α-dihydroxy-5β-cholan-24-oic acid. In addition the mass spectrum of the trimethylsilyl–ether (TMS–ether) of the methyl ester derivative showed the following characteristic major ions; M^+ 550 (2%), *m/e* 535 (M^+-15, CH_3, 18%), *m/e* 460 (M^+-90, TMS, 22%), *m/e* 370 (M^+-180, 2TMS, 50%) and base peak at *m/e* 255 (M^+-295, 6C side-chain + 2TMS, 100%) which was identical to that of the authentic compound. In the presence of menadione, only one product was observed namely 12α-hydroxy-3-oxo-5β-cholan-24-oic acid (10%) indicating only partial reduction of the substrate. When V was added to the medium, similar results were obtained except that in the presence of menadione, metabolism was undetectable. Addition of V + K and HS + N also prevented metabolism completely.

3-Oxochol-4-ene-24-oic acid (Δ^4-LA)

Incubation of Δ^4-LA with MFB resulted in the production of LA (100%), in the presence and absence of KNO_3. Inclusion of menadione in the medium prevented metabolism completely. Inclusion of V in the medium resulted in the production of 3KLA (5%) and LA (95%) in the absence and LA (100%) in the presence of KNO_3. Metabolism was again not observed in the presence of menadione. Inclusion of V + K and HS + N prevented metabolism completely under the prescribed conditions.

12α-Hydroxy-3-oxo-1,4-pregnadiene-20-carboxylic acid (T2)

Incubation of T2 with MFB gave one major ($\sim$95%) and two minor metabolites ($\sim$5%) in the presence and absence of KNO_3, whilst metabolism was undetectable in the presence of menadione. Analysis of the major product (methyl ester-TMS-ether) by GC–MS revealed the following characteristic major ions; M^+ 522 (3%), base peak *m/e* 507 (M^+-15, CH_3, 100%), *m/e* 432 (M^+-90, TMS, 15%), *m/e* 345 (M^+-177, 4C side-chain + TMS, 85%), *m/e* 342 (M^+-180, 2TMS, 4%) and *m/e* 255 (M^+-267, 4C side-chain + 2TMS, 73%). This spectrum being consistent with a fully reduced bisnor acid carrying two hydroxyl groups. The position and stereochemistry of

Table 1. Major metabolites formed upon incubation of 12β-hydroxyandrosta-1,4-dien-3,17-dione (S2) with MFB

OA	At	Major metabolites	% Conversion	TLC R_f	GLC R_f
—	—	12β-Hydroxy-5β-androstan-3,17-dione	5	0.90	1.40
		12α-Hydroxy-5β-androstan-3,17-dione	4	0.74	1.37
		3β,12β-Dihydroxy-5β-androstan-17-one	20	0.50	1.30
		3α,12β-Dihydroxy-5β-androstan-17-one	34	0.48	1.25
		3β,12α-Dihydroxy-5β-androstan-17-one	17	0.43	1.10
		3α,12α-Dihydroxy-5β-androstan-17-one	20	0.36	1.18
KNO_3	—	3β,12β-Dihydroxy-5β-androstan-17-one	30	0.50	1.20
		3α,12β-Dihydroxy-5β-androstan-17-one	18	0.48	1.25
		3β,12α-Dihydroxy-5β-androstan-17-one	24	0.43	1.10
		3α,12α-Dihydroxy-5β-androstant-17-one	28	0.36	1.18
Men	—	12β,17β-Dihydroxyandrosta-1,4-diene-3-one	2	0.24	2.2
—	V	12β-Hydroxyandrost-4-ene-3,17-dione	32	0.80	2.2
		12β,17β-Dihydroxyandrost-4-ene-3-one	3	0.30	2.6
		12β,17β-Dihydroxyandrosta-1,4-diene-3-one	2	0.24	2.8
KNO_3	V	12β-Hydroxyandrost-4-ene-3,17-dione	29	0.80	2.2
		12β,17β-Dihydroxyandrost-4-ene-3-one	2	0.30	2.6
		12β,17β-Dihydroxyandrosta-1,4-diene-3-one	1	0.24	2.8
Men	V	None	—	—	—
—	VK	12β-Hydroxyandrost-4-ene-3,17-dione	26	0.80	2.2
		12β,17β-Dihydroxyandrost-4-ene-3-one	2	0.30	2.6
		12β,17β-Dihydroxyandrosta-1,4-diene-3-one	2	0.24	2.8
KNO_3	Vk	12β-Hydroxyandrost-4-ene-3,17-dione	21	0.80	2.2
		12β,17β-Dihydroxyandrost-4-ene-3-one	3	0.30	2.6
		12β,17β-Dihydroxyandrosta-1,4-diene-3-one	1	0.24	2.8
Men	VK	None	—	—	—
—		9α-Hydroxyandrost-1-ene-3,17-dione	100		
KNO_3	HS + N	9α-Hydroxyandrost-1-ene-3,17-dione	100	0.91	1.75
Men		9α-Hydroxyandrost-1-ene-3,17-dione	100		

OA: oxidising agent. VK: vancomycin and kanamycin. At: antibiotic. HS + N: heat shock and neomycin V: vancomycin.

the hydroxyl groups was deduced by pmr δ 0.80, 1.20 (6H, s, 18-CH_3 and 19-CH_3), 1.24 (3H, s, 21-CH_3), 3.60 (1H, m, 3-H), 3.65 (3H, s, 22-OCH_3) and 3.98 (1H, t, J = 3H_3). The pmr spectrum corroborated the saturated nature of this compound by the absence of vinylic protons. Also present was a well defined triplet at 3.98δ indicating the presence of a 12α-hydroxyl group along with a multiplet centred at 3.60δ which is typical for a 3β-proton attached to an α-orientated hydroxyl group [25]. On the basis of the above data the major reduction product of T2 has been assigned the structure 3α,12α-dihydroxy-5β-pregnane-20-carboxylic acid. The two minor components also gave mass spectra very similar to 3α,12α-dihydroxy-5β-pregnane-20-carboxylic acid but sufficient material was not available for further structural analyses. Similar results were obtained when V was included in the medium, whilst the addition of V + K and HS + N prevented metabolism completely.

12β-Hydroxyandrosta-1,4-diene-3,17-dione (S2)

Incubation of S2 with MFB resulted in the production (Table 1) of 3β,12β-dihydroxy-5β-androstane-17-one, 3α,12β-dihydroxy-5β-androstane-17-one, 3β,12α-dihydroxy-5β-androstane-17-one and 3α,12α-dihydroxy-5β-androstane-17-one in the absence of oxidising agents. The above compounds have been described in detail elsewhere [26]. Inclusion of KNO_3 in the medium inferred that metabolism had been retarded somewhat as indicated by the presence also of 12β,17β-dihydroxyandrosta-1,4-diene-3-one [20] which was the only detectable product in the presence of menadione.

Inclusion of V in the medium also retarded the reductive process because the products in this instance were 12β-hydroxyandrost-4-ene-3,17-dione, 12β,17β-dihydroxyandrost-4-ene-3-one and 12β,17β-dihydroxyandrosta-1,4-diene-3-one in the presence and absence of KNO_3. Inclusion of menadione in this system prevented metabolism completely. Similar results were obtained when V + K were included in the medium.

In contrast to all other experiments, metabolism of a steroid substrate was detected when MFB (HS + N) were incubated in the presence of S2. Metabolism was evident in the presence and absence of both KNO_3 and menadione. Under all conditions the sole product (100% conversion) was 9α-hydroxyandrost-1-ene-3,17-dione [20] as determined by GC–MS and pmr. The product showed the following characteristics after isolation by preparative TLC and crystallization from methanol–dichloromethane (1:9); ν_{max} 3460 (12-OH), 1740 (17-ketone), 1690 and 1615 cm^{-1} (1-ene-3-one); λ_{max} 231 nm; M^+ 302 (82%), *m/e* 122 (1-ene-3-one, 69%) and *m/e* 284 (M^+-H_2O, 6%); δ 0.97, 1.22 (6H, s, 18-CH_3 and 19-CH_3), 5.90 (1H, d, J = 10H_3, 2-H) and 6.76 (1H, d, J = 10H_3, 1-H).

Androsta-4,6-diene-3,17-dione (Δ^{46}AD)

Incubation of Δ^{46}AD with MFB resulted in the production of 5β-androstane-3,17-dione,

3β-hydroxy-5β-androstane-17-one, 3α-hydroxy-5β-androstane-17-one, 5β-androstane-3α,17β-diol and 5β-androstane-3β,17β-diol in the absence of oxidising agents. In the presence of KNO_3 the products were 5β-androstan-3,17-dione, 3β-hydroxy-5β-androstan-17-one and 3α-hydroxy-5β-androstan-17-one. Inclusion of menadione in the medium prevented metabolism completely as did the addition of V, V + K and HS + N.

DISCUSSION

Incubation of bile acids carrying oxo groups with MFB under anaerobic conditions resulted in the reduction of the ketone groups to hydroxy groups. Under suitable incubation conditions, conversion to the fully reduced products was approx. 75%, with the stereochemistry of the hydroxy group being in the predominantly α-orientation. Reduction of oxo-bile acids was not observed under aerobic conditions indicating that a low Eh is a prerequisite for the equilibrium to favour reduction. Under anaerobic conditions, reduction of oxo-bile acids was almost completely inhibited in the presence of the oxidising agent menadione (100 μg/ml). In contrast, reduction was not retarded in the presence of KNO_3.

The addition of vancomycin to the incubation mixtures did not adversely affect reduction of the oxo-bile acids indicating that Gram-positive organisms were not involved in reductive metabolism. Addition of vancomycin + kanamycin together to the incubation mixtures resulted in the absence of oxo-bile acid metabolism, indicating (and supported by bacteriological studies) that in mixed cultures the Gram-negative facultative anaerobic bacteria are more important than the strict anaerobes in the reductive process.

Further studies using selective methods (heat shock + neomycin) for *Clostridium* spores revealed that the faecal flora were unable to metabolise oxo-bile acids, indicating specifically that the faecal Clostridia are not involved in reduction of bile acid type molecules.

When unsaturated bile acid A-ring and A,B-ring intermediates (C_{24}, C_{22} and C_{19}) were incubated in the presence and absence of KNO_3, 4-en-3-one, 1,4-diene-3-one and 4,6-diene-3-one steroids were hydrogenated to 3-oxo steroids. In the majority of cases the oxo group was further reduced to an α- or β-orientated hydroxy group (Fig. 2). In the presence of menadione, hydrogenation was again completely inhibited except when S2 was presented to HS + N cultures. The use of antibiotics revealed that the hydrogenation process was prevalent amongst the Gram-negative facultative anerobic bacteria.

Although previous studies have shown that pure cultures of bacteria (a poor representation of the *in vivo* situation) especially NDC can desaturate bile acids [9] and androstanes [8], the results of the present study are in agreement with those of Lombardi *et al.*[13] and indicate that hydrogenation is the prominent reaction carried out by MFB under anaerobic conditions. Lombardi *et al.*[13] have suggested therefore that hydrogenation is the prominent reaction carried out by the gut flora *in vivo*. This conclusion is vindicated by the fact that faecal sterols are comprised almost entirely of coprostanol, the reduced form of cholesterol.

The striking feature of the present study is that the inclusion of menadione in the culture medium at concentrations of 100 μg/ml and even as low as

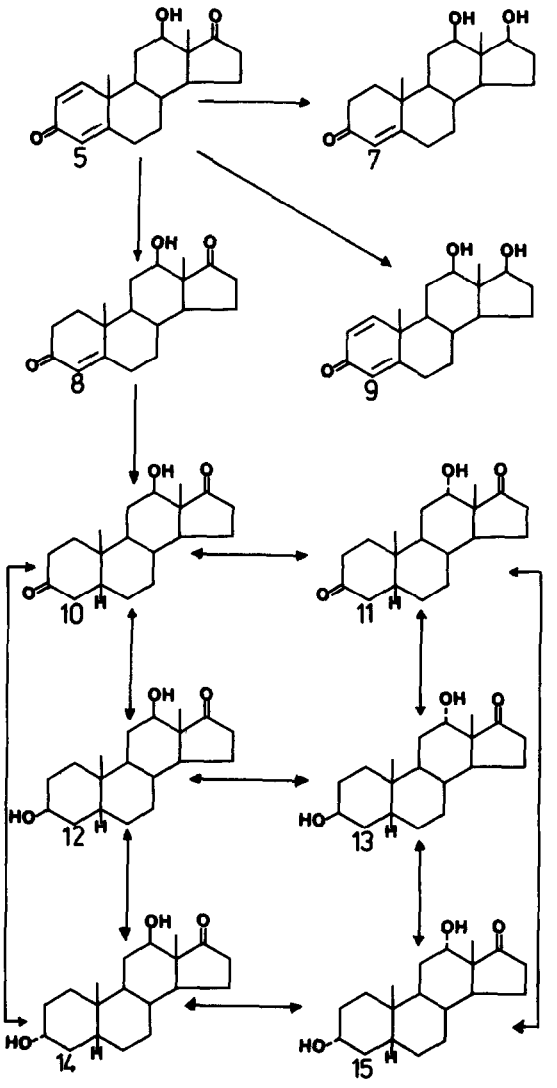

Fig. 2. Proposed reductive pathway of 12β-hydroxy-androsta-1,4-diene-3,17-dione by MFB.

5. 12β-Hydroxyandrosta-1,4-diene-3,17-dione.
7. 12β,17β-Dihydroxyandrost-4-ene-3-one.
8. 12β-Hydroxy-androst-4-ene-3,17-dione.
9. 12β,17β-Dihydroxyandrosta-1,4-diene-3-one.
10. 12β-Hydroxy-5β-androstane-3,17-dione.
11. 12α-Hydroxy-5β-androstane-3,17-dione.
12. 3β,12β-Dihydroxy-5β-androstane-17-one.
13. 3β,12α-Dihydroxy-5β-androstane-17-one.
14. 3α,12β-Dihydroxy-5β-androstane-17-one.
15. 3α,12α-Dihydroxy-5-androstane-17-one.

50 μg/ml inhibited hydrogenation by MFB. Menadione is a synthetic form of Vitamin K_2 which is synthesised by the bowel flora and thus it is likely that the presence of Vitamin K may inhibit hydrogenation in the gut.

Lombardi *et al.*[13] also showed that in the presence of menadione, MFB were able to dehydrogenate androstanes. Similar results were obtained by us with 3-oxo-5β-cholanoic acid as substrate and shows that mixed cultures as well as pure cultures can desaturate bile acids. Menadione therefore can promote the desaturation of bile acids by bacteria and also inhibit hydrogenation of unsaturated bile acids.

Individuals with a high carriage rate of NDC may therefore contain appreciable levels of unsaturated bile acids in bowel contents, especially those with high concentrations of vitamin K_2. However faecal analyses have never revealed the presence of unsaturated bile acids, and indicates that unsaturated bile acids have a short half-life in gut contents. Whether or not they are hydrogenated when they move into areas of low vitamin K_2 concentrations or absorbed or further metabolised remains to be elucidated.

In conclusion this study has shown that the concentration of vitamin K_2 in the bowel may be an important factor in the unsaturated bile acid/bowel cancer hypothesis of Aries *et al.*[8]. It is difficult to evaluate the precise role of vitamin K in the gut situation since no studies give conclusive values for the *in vivo* concentration of this vitamin and related quinones.

It is recommended that the role vitamin K may play in colon cancer be investigated more fully.

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