7α-DEHYDROXYLATION OF CHOLIC ACID BY *CLOSTRIDIUM BIFERMENTANS* STRAIN ATCC 9714 AND *CLOSTRIDIUM SORDELLII* STRAIN NCIB 6929

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Received 19 November 1969

1. Introduction

It is well established that the primary bile acids, which are transformed from cholest-5-en-3β-ol (cholesterol) in the liver, are excreted in the intestine and then exposed to the 7α-dehydroxylation catalyzed by intestinal micro-organisms [1]. It has been considered, therefore, that studies on the biochemistry of 7α-dehydroxylating micro-organisms might provide significant information on the metabolism of cholesterol and bile acids.

Portman et al. [2] have at first isolated the pure culture of a micro-organism from rat faeces that is capable of converting 3α, 7α, 12α-trihydroxy-5β-cholanoic acid (cholic acid) into 3α, 12α-dihydroxy-5β-cholanoic acid (deoxycholic acid). However, their organism lost its ability after three transfers. Gustafsson et al. [3], Midtvedt [4], Hill and Drasar [5] and Bokkenheuser et al. [6] have also isolated several 7α-dehydroxylating bacteria from human or rat faeces and identified their isolates as the members of the tribe *Lactobacilleae* [3, 4], those of the genera *Bacteroides, Clostridium, Veillonella* and *Streptococcus* [5] and those of the genus *Bacteroides* [6], respectively. We have also isolated a bacterium from human faeces that is capable of 7α-dehydroxylating cholic acid and belongs to the genus *Bacteroides* [7]. The identification work of these isolated strains has been done in some detail and the results have demonstrated bacteriological differences among some of these strains [3, 4, 6, 7]. However, a definite identification of the organisms isolated so far within a specific species has not yet been performed, except for the findings by Cocucci and Ferrari [8, 9] who identified their isolate from human faeces as *Clostridium bifermentans*. Unfortunately, their organism also lost the 7α-dehydroxylating ability after serial transfers *in vitro* as well as that of Portman et al. [2]. Further, in spite of considerable efforts directed at the discovery of biochemical factors related to the loss of the specified ability in such transfer processes, Carini et al. [10] were unsuccessful in finding any factor. Our continued interest in studying the biochemistry of 7α-dehydroxylating micro-organisms has prompted us to investigate whether or not the strains of authentic type cultures, that belong to the members of several known genera isolated so far as 7α-dehydroxylating micro-organisms, have the 7α-dehydroxylating ability. A similar approach had been already adopted by Midtvedt and Norman [11] who tested 55 strains that belong within the genera often found in the intestinal tract of man and rat. However, none of the strains tested was capable of 7α-dehydroxylating cholic acid or 3α, 7α-dihydroxy-5β-cholanoic acid (chenodeoxycholic acid) [11].

In this communication we show that the type cultures, *Clostridium bifermentans* strain ATCC 9714 and *Clostridium sordellii* strain NCIB 6929, are responsible for the 7α-dehydroxylation of cholic acid *in vitro*.

2. Materials and methods

These were described in a previous paper [7], except for micro-organisms. The cultures of *C. bifermentans* ATCC 9714, *C. bifermentas* NCIB 506

and *C. sordellii* NCIB 6929 were purchased from the American Type Culture Collection, Rockville, Md., USA and the National Collection of Industrial Bacteria, Aberdeen, Scotland.

Anaerobic incubation was carried out with the use of Marcus and Talalay medium containing 0.025% (w/v) sodium cholate and each of the culture broth of the above organisms, grown in VF glucose broth for 2 days at 37°, was transfered into the medium in a ratio of 20% (v/v). After a 7-day incubation, each incubation mixture, resulted from the incubation with *C. bifermentans* ATCC 9714 and *C. sordellii* NCIB 6929, was treated in a manner similar to that described in a previous paper [7] and deoxycholic acid isolated as its methyl ester. Proof that the isolated material was methyl deoxycholate was provided by mixed m.p. and infrared comparison.

3. Results and discussion

Although the quantitative determination of formed deoxycholic acid and recovered cholic acid was not made, the present experiment clearly demonstrated that both type cultures of *C. bifermentans* ATCC 9714 and *C. sordellii* NCIB 6929 were capable of 7α-dehydroxylating cholic acid *in vitro*. However, when a 0.1% (w/v) cholate-containing Marcus and Talalay medium which was supplied in a previous experiment [7] was used, the ATCC strain did not produce a detectable amount of deoxycholic acid. It seems likely, therefore, that the 7α-dehydroxylating ability of the ATCC strain is more less intensive than that of our isolate, *Bacteroides* strain 28S [7]. Both ATCC and NCIB strains also produced 3α, 12α-dihydroxy-7-oxo-5β-cholanoic acid besides deoxycholic acid as well as the culture of *Bacteroides* strain 28S and the amounts of both acids were approximately equal. The demonstrated 7α-dehydroxylation of cholic acid by type cultures is the first instance in this field and it provides experimental material for biochemical studies of this reaction, which is unique in steroid metabolism. In contrast to both type cultures, the culture of *C. bifermentans* NCIB 506 had no such ability.

Concerning the 7α-dehydroxylating ability of micro-organisms, Hill and Drasar [5] have reported that, after exposure to bile, many oral strains belonging within the genera *Bacteroides* and *Veillonella* are capable of 7α-dehydroxylating cholic acid. However, the ability of both type cultures in this experiment was demonstrated without such pretreatment. Drs. Gherna and Winton informed us that the ATCC strain had been preserved as lyophilized cultures and Robertson's cooked meat medium used for maintenance of the NCIB strains. These evidences suggest that the ability of both type cultures had been present in their originally isolated strains and kept for many years.

According to the ATCC and NCIB catalogues published in 1968 and 1964 respectively, the cultures of *C. bifermentans* ATCC 9714 and *C. sordellii* NCIB 6929 had been at first deposited as *C. sordellii* and *C. bifermentans*, respectively. While the culture of *C. bifermentans* NCIB 506 had been deposited as the strain of this specified species. Stewart [12] has suggested that *C. sordellii* is a pathogenic variant of *C. bifermentans* and they should be regarded as a single species, *C. bifermentans*. However, it appears more likely that there are many discussions on this problem as described in the paper by Novotný [13] and many of the other literatures cited therein. In connection with this problem, it is of some interest to note that Brooks and Epps [14] proposed separation of *C. bifermentans* NCIB 506 and *C. sordellii* NCIB 6929 and that the latter culture only had the 7α-dehydroxylating ability in this experiment. Unfortunately the origin of the culture of *C. bifermentans* ATCC 9714 is unknown even after the perusal of the literature, and it seems likely that Cocucci and Ferrari [9] identified their strain as *C. bifermentans* according to Stewart's [12] opinion. Therefore, these strains might be excluded from our discussion on the possibility that separation of *C. bifermentans* and *C. sordellii* also is justifiable through their 7α-dehydroxylating abilities. However, more definitive conclusions about whether or not the 7α-dehydroxylating ability is one of the taxonomic characters in defining differences between both species must await further study.

Acknowledgements

We thank Dr. R.Gherna in the ATCC and Dr. J.E.P.Winton in the NCIB for providing us a

detailed information on maintenance of the type cultures used in this experiment.

References

[1] S.Bergström and H.Danielsson, in: Lipide Metabolism, ed. K.Bloch (John Wiley and Sons Inc. New York, 1960) p. 317.
[2] O.W.Portman, S.Shah, A.Antonis and B.Jorgensen, Proc. Soc. Exptl. Biol. Med. 109 (1962) 959.
[3] B.E.Gustafsson, T.Midtvedt and A.Norman, J. Exptl. Med. 123 (1966) 413.
[4] T.Midtvedt, Acta Pathol. Microbiol. Scand. 71 (1967) 147.
[5] M.J.Hill and B.S.Drasar, Gut 9 (1968) 22.
[6] V.Bokkenheuser, T.Hoshita and E.H.Mosbach, J. Lipid Res. 10 (1969) 421.
[7] T.Hattori and S.Hayakawa, Microbios. 3 (1969) 287.
[8] M.C.Cocucci and A.Ferrari, Rend. Ist. Lombardo Sci. Lettera B 97 (1963) 398.
[9] M.C.Cocucci and A.Ferrari, Lincei Rend. Sci. Fis. Mat. Nat. 36 (1964) 851.
[10] S.Carini, M.C.Cocucci and A.Ferrari, Progr. Biochem. Pharmacol. 2 (1967) 62.
[11] T.Midtvedt and A.Norman, Acta Pathol. Microbiol. Scand. 71 (1967) 629.
[12] S.E.Stewart, J. Bacteriol. 35 (1938) 13.
[13] P.Novotný, J. Med. Microbiol. 2 (1969) 81.
[14] M.E.Brooks and H.B.G.Epps, J. Gen. Microbiol. 21 (1959) 144.