An Alternative Pathway for the Degradation of Cholic Acid by Micro-organisms

SINCE the publication of the first report[1] on the microbiological degradation of cholic acid (I) to 7α-hydroxy-3 : 12-dioxo-Δ^4-*bis*-norcholenic acid (VII) by *Streptomyces gelaticus* 1164, additional investigations have been continued and two possible pathways[2] for cholic acid degradation have been proposed.

S. gelaticus 1164 can grow on a medium containing the *bis*-nor acid (VII) as the sole source of carbon. On the other hand, *Streptomyces rubescens*[3] is able to utilize cholic acid as the sole source of carbon as well as *S. gelaticus* 1164, but is unable to utilize this *bis*-nor acid.

Though interpretation of such experiments is not simple, we suggest that cholic acid degradation by *S. rubescens* proceeds through an alternative pathway different from that found with *S. gelaticus* 1164.

Recently, it was found[4] that *S. rubescens*, cultured in a medium containing cholic acid as the sole source of carbon, produced various intermediates such as 7α : 12α-dihydroxy-3-oxo-Δ^4-cholenic acid (III), 7α-hydroxy-3 : 12-dioxo-Δ^4-cholenic acid (IV), 12α-hydroxy-3-oxo-$\Delta^{4,6}$-choladienic acid (V), 3 : 12-dioxo-$\Delta^{4,6}$-choladienic acid (VI) and some unidentified compounds different from the *bis*-nor acid (VII).

Considering the structures of these intermediates, an oxidative pathway of cholic acid by *S. rubescens* may be deduced as follows (Fig. 1), though the acid II has not yet been isolated.

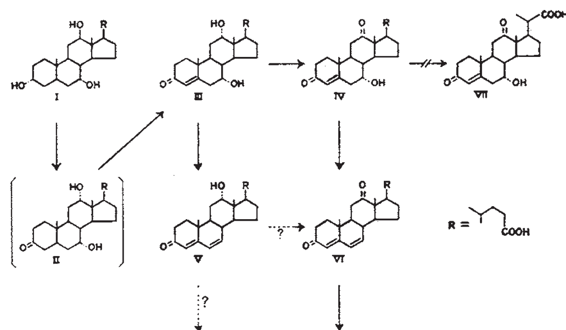

Fig. 1

S. rubescens cultures in a cholate medium give a light absorption peak at 246 mμ at the earlier incubation period, and a parallelism between an increase of the optical density and the incubation period is observed. After the optical density at 246 mμ reaches its maximum, it decreases in parallel with continuation of the incubation, and, simultaneously, a new peak at 290 mμ appears ; finally, both the peaks at 246 and 290 mμ disappear at an almost equal rate at the end of the incubation period.

These findings may be interpreted as follows : an appearance of a peak at 246 mμ in the cholate culture begins with the conversions I → II → III → IV ; a decrease of the optical density at 246 mμ and an appearance of a peak at 290 mμ are due to the conversion III → V or IV → VI ; and further degradation of V or VI accelerates the conversion III → V or IV → VI. This process will finally lead to the disappearance of both the peaks at 246 and 290 mμ.

Much of the representation of our proposed pathway for cholic acid degradation by *S. rubescens* is still largely hypothetical. However, the isolation and identification of the acids V and VI in this experiment demonstrate for the first time a microbiological introduction of the $\Delta^{4,6}$-3-ketone structure into the cholane nucleus of cholic acid.

Further study on the proposed pathway is in progress and will be reported in detail elsewhere.

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Uridine Diphosphate Amino-sugar Compounds from *Staphylococcus aureus* inhibited by Penicillin

IN 1952, Park[1] showed that three uridine diphosphate amino-sugar compounds were accumulated in *Staphylococcus aureus* inhibited by penicillin. Compound I contained no amino-acid residue. Compound II contained one alanine residue and compound III contained a peptide which was composed of one glutamic acid, one lysine and three alanine residues. Recently, Strominger indicated that, even though in much smaller amount, these compounds are contained also in normal cells[2], and that the amino-sugar component is 3-O-carboxyethyl glucosamine[3], which is known as one of the main components of cell walls[4].

We have found a variety of similar uridine diphosphate amino-sugar compounds, some of which contained aspartic acid and glycine. Cells of *S. aureus* strain 209 *P* treated with penicillin were extracted with trichloracetic acid. After removal of trichloracetic acid, barium hydroxide and sufficient sodium hydroxide were added to the extract to adjust the *p*H to 9, and the precipitate was removed. To the concentrated supernatant, ethanol was added to 50 per cent by volume and the precipitate was separated. More ethanol was added to the supernatant to make it 90 per cent and the precipitate was separated. Each precipitate was fractionated by ion exchange chromatography with 'Dowex 1' in the chloride form. The fractions with the same peak of optical density at 260 mμ were pooled, concentrated by adsorption on 'Norit' and subsequent elution with ammoniacal ethanol, and were analysed for components. After hydrolysis with 6 *N* hydrochloric acid at 120° for 6 hr. in a sealed tube, amino-acids were separated and estimated by two-dimensional paper chromatography with butanol/acetic acid/water (4 : 1 : 5) and phenol/*m*-cresol/borate buffer *p*H 9·3 (25 : 25 : 7) as solvent systems[5]. The result of paper chromatography on fractions containing appreciable amounts of all the three components, uridine, N-acetyl-amino-sugar and amino-

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