The purpose of this report is to present the deconjugation of bile acids by numbers of strains of bacteria in the small intestine and feces. The small intestinal juice was aseptically aspirated by a double lumen tube with a rubber cover on the tip devised by us (“Fukushima Type 1”). Bile acids were analyzed with thin layer chromatography. The results: 1) Among aerobic bacteria, species of which all of the strains split conjugated bile acids was enterococcus, and most of the strains split were Staphylococcus (S.) epidermidis and Lactobacillus (L.) bifidus. Species of which none of the strains split were Escherichia (E.) coli, E. communior, L. plantarum, L. acidophilus, L. buchneri, L. cellobiosus, L. bulgaricus, S. aureus, Aerobacter aerogenes, Pseudomonas aeruginosa, candida, proteus, serratia, and almost none of the species split was Intermediate coliform bacilli. 2) Among anaerobic bacteria, species of which all of the strains split were Bacteroides (B.) vulgatus, B. thetaitaomicron, B. uniformis, Corynebacterium (C.) granulosum, C. avidum, Peptostreptococcus (Peptostrept.) putridus, Eubacterium (Eubact.) lentum, Peptococcus (Pept.) grigoroffii, Pept. anaerobius, Veillonella (V.) oribiculus, and most of the strains split were Coryne. diphtheroides, Eubact. parvum, Peptostrept. intermedius. Species of which none of the strains split were Coryne. parvum, Peptostrept. micros, V. alcalescens, V. parvula, Catenabacterium (Catena.) cateniforme, and Catena. filamentosum. 3) All or none, or almost all or none, of the strains of each species tested split conjugated bile acids, and it seems probable that the presence or absence of this ability would be a proper character of each species.

Key Words: human intestinal bacteria, bile acids metabolism, deconjugation of bile acids.

Introduction

The blind loop syndrome and Crohn’s disease\textsuperscript{1,2} are often associated with steatorrhea. According to a current hypothesis\textsuperscript{3}, the steatorrhea is caused by excessive proliferation of bacteria in the lumen of the small bowel.

Conjugated bile acids synthesized from cholesterol at the liver are secreted into the duodenal lumen. In the above mentioned diseases, they were then converted into free bile acids by bacteria in the lumen of the upper small bowel and consequently the concentration of conjugated bile acids is reduced to the levels below the critical micellar concentration. Since anaerobes are so active in deconjugating bile acids, it is presumed that the anaerobes in the small bowel may play an important role in the pathogenesis of the steatorrhea\textsuperscript{4,5,6}.

On the other hand, in patients with hepatic diseases, especially in hepatic cirrhosis, bacterial proliferation was found by us in the lumen of their small bowels. It is pronounced
by the overgrowth of aerobic gram negative bacilli and anaerobes. But, the causal relation between the hepatic disease and the enterohepatic circulation of bile acids under the influence of bacterial overgrowth had not been elucidated.

The several literatures on deconjugation of bile acids by various bacteria have been reported since the evidence of bacterial splitting was first presented by Norman and Grubb (1955) who isolated bacteria from rat faeces capable of hydrolyzing conjugated bile acids. But many reports are rather fragmental and we can not get a general view of bacterial deconjugation.

The purpose of this report is to present the deconjugation of bile acids by numbers of strains of bacteria found in the small intestine.

**Material and Methods**

Materials: The majority of strains tested were isolated from the small intestinal juice obtained from patients with hepatic diseases admitted to the Yokohama City University hospital. Some of the bacteria were isolated from the feces and intestinal juices of patients without hepatic diseases. Anaerobes and aerobic gram-negative bacilli except lactobacillus and enterococcus were identified according to Bergey’s Manual of determinative bacteriology in 1957 and Schaub’s diagnostic bacteriology respectively. Enterococcus was identified by SF medium “Eiken” and lactobacillus was identified according to Modern Media and Bergey’s Manual of determinative bacteriology in 1957. The small intestinal juice was aseptically aspirated by a double lumen tube with a rubber cover on the tip devised by us (“Fukushima Type 1”). This tube was the best suited for aspirating the fluid aseptically from the small intestine. The rubber cover was blasted off by instillation with the saline just before aspiration at the desired site in the small intestine. The kinds of bacteria tested were strains of Escherichia coli, Esch. communior, Esch. freundii, lactobacillus (Lact.), Staphylococcus (Staph.) epidermidis, Staph. aureus, Intermediate coliform bacilli, Aerobacter aerogenes, Pseudomonas aeruginosa, candida, enterococcus, proteus, serratia, bacteroides (Bact.), corynebacterium (Coryne.), peptostreptococcus (Peptostrept.), eubacterium (Eu-bact.), peptococcus (Pept.), veillonella (V.), catenabacterium (Catena.).

Methods: A loopful of organisms from a plate culture was inoculated into 10 ml of broth containing 0.5 ml of sterilized ox gall (Fig. 1). All organisms except strictly anaerobic bacteria were grown in glucose broth containing 0.5 ml of sterilized ox gall. The anaerobic bacteria were grown in Thioglycollate liquid medium containing 0.5 ml of sterilized ox gall. Cultures were incubated at 37°C for 48 hours. The broth was acidified to pH 1 with hydrochloric acid and extracted three times with double volume of butanol. The butanol phases were washed free of hydrochloric acid and evaporated to dryness. The residues were dissolved in 2 ml of methanol.

A loopful of bacteria was inoculated into broth containing 0.5 ml of sterilized ox gall

```
Incubation 37c, 48 hrs
Acidify to pH 1 with HCl
Extract with butanol
Evaporate to dryness
Dissolve in 2 ml of methanol
Thin layer chromatogram
Fig. 1. Preparation.
```