APPLIED AND ENVIRONMENTAL MICROBIOLOGY, May 1984, p. 1167–1168
0099-2240/84/051167-02$02.00/0

Vol. 47, No. 5

Absence of Transformation of β-Muricholic Acid by Human Microflora Implanted in the Digestive Tracts of Germfree Male Rats

E. C. SACQUET,[1]* D. P. GADELLE,[1] M. J. RIOTTOT,[1] AND P. M. RAIBAUD[2]

Laboratoire des Animaux sans Germes du Centre National de la Recherche Scientifique[1] and Laboratoire d'Ecologie Microbienne de l'Institut National de la Recherche Agronomique[2] au Centre National de la Recherche Zoologique, Institut National de la Recherche Agronomique, 78350 Jouy-en-Josas, France

Received 22 July 1983/Accepted 1 February 1984

Germfree rats biosynthetize cholic and β-muricholic acids. The latter does not exist in humans. Germfree rats were given human fecal suspensions. These rats degraded cholic acid into deoxycholic acid but failed to metabolize β-muricholic acid.

In humans, the main bile acids biosynthetized by the liver are cholic acid (3α,7α,12α-trihydroxy-5β-cholan-24-oic acid) and chenodeoxycholic acid (3α,7α-dihydroxy-5β-cholan-24-oic acid). In male germfree (GF) rats the main bile acids are cholic acid and β-muricholic acid (3α,6β,7β-trihydroxy-5β-cholan-24-oic acid) (3). The microflora of the digestive tract transforms these bile acids into a large number of metabolites. We investigated whether the bacterial flora from human digestive tracts was capable of transforming β-muricholic acid, which does not exist in humans. To this end, we administered human fecal suspensions to male GF rats. We used this method because (i) according to Raibaud et al. (4), GF rodents receiving human fecal suspensions retained a large part of the human microflora in their digestive tracts and (ii) when GF rats received conventional (CV) rat fecal suspensions, their fecal bile acid pattern became similar to that of CV rats.

Feces of two human males and two human females were administered separately to two GF male rats kept in an isolator. After 2 weeks and 2 months, fecal bile acids of these rats, called M rats, were extracted by boiling ethanol, separated from neutral sterols by the method of Grundy et al. (2), and methylated by diazomethane; the trimethylsilyl ethers were formed and submitted to gas-liquid chromatography on 3 p 100 OV-17 fixed on Gas-Chrom Q (Applied Science Laboratory) by the method of Elliott et al. (1). The fecal bile acids of two GF and two CV rats were treated in the same way, and the gas-liquid chromatograms obtained from GF, M, and CV rats were compared.

Chromatograms from GF rats (Fig. 1A) exhibited two major peaks, 1 and 5, corresponding, respectively, to cholic and β-muricholic acids. Chromatograms from the M rats (Fig. 1B) were similar. They showed two major peaks, with 3 corresponding to deoxycholic acid (3α,12α-dihydroxy-5β-cholan-24-oic acid) and 5 corresponding to β-muricholic acid. These chromatograms were very different from those

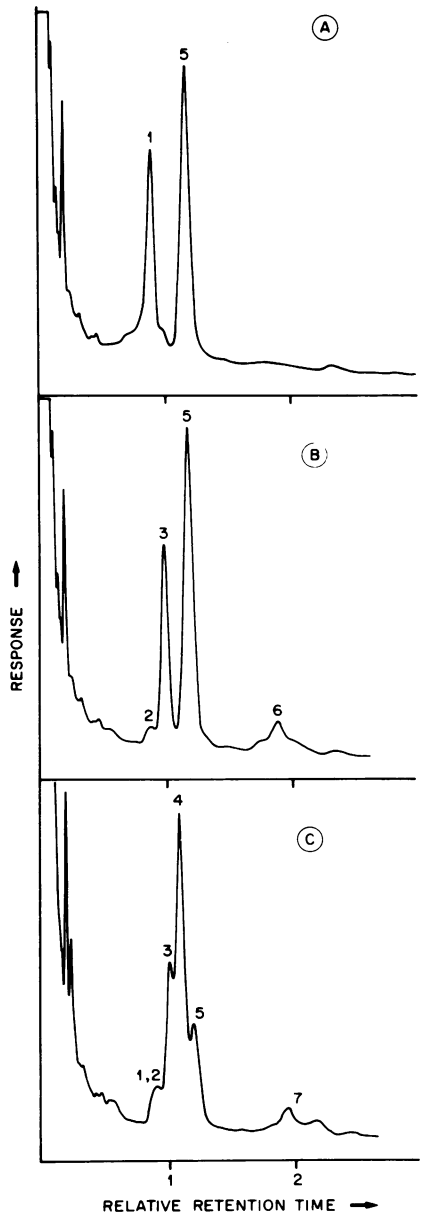

FIG. 1. Typical gas-liquid chromatograms of fecal bile acids obtained from: (A) GF rats, (B) M rats having received fecal suspensions from humans, and (C) CV rats. Retention times are relative to deoxycholic acid and indicated after each of the following bile acids: 1, cholic (0.90); 2, lithocholic (0.93); 3, deoxycholic (1.00); 4, hyodeoxycholic (1.08); 5, β-muricholic (1.18); 6 and 7, unseparated bile acids (1.65 to 2.25).

* Corresponding author.

obtained from CV rats (Fig. 1C). Peaks 3 and 5 were still present, but the latter was reduced; the major peak, 4, corresponded to hyodeoxycholic acid (3α,6α-dihydroxy-5β-cholan-24-oic acid).

These data suggest the absence of β-muricholic acid-transforming bacteria in human microflora. However, the hypothesis that β-muricholic acid-transforming bacteria were present in the human donors but did not thrive in the digestive tracts of rats cannot be rejected. Ingestion of β-muricholic acid by humans and a subsequent search for bacterial metabolites in the feces is the only way to prove the inability of human microflora to degrade this bile acid.

This research is in progress in our laboratory.

LITERATURE CITED

1. **Elliott, W. H., L. B. Walsh, M. M. Mui, M. A. Thorne, and C. M. Siegfried.** 1969. Bile acids. XXVIII. Gas chromatography of new bile acids and their derivates. J. Chromatogr. **44**:452–464.
2. **Grundy, S. M., E. H. Ahrens, and T. A. Miettinen.** 1965. Quantitative isolation and gas-liquid chromatographic analysis of total fecal acids. J. Lipid Res. **6**:397–410.
3. **Kellog, T. F., and B. S. Wostmann.** 1969. Fecal neutral steroids and bile acids from germfree rats. J. Lipid Res. **10**:495–503.
4. **Raibaud, P., R. Ducluzeau, F. Dubos, S. Hudault, H. Bewa, and M. C. Muller.** 1980. Implantation of bacteria from the digestive tract of man and various animals into gnotobiotic mice. Am. J. Clin. Nutr. **33**:2440–2447.