of the VMT, particularly when the lesions were located in the area surrounding the interpeduncular nucleus (figure, A). Lesions in this area led to irregular and mostly finely impregnated terminals in the whole LC with a higher concentration in the anterodorsal part of the 2 nuclei (figure, C, D). Destruction of the MRN (figure, B) led to a similar pattern of bilateral degenerations in the LC, but the degenerations were more abundant after MRN lesions than VMT lesions.

Discussion. Our results indicate that a ventral descending pathway originates in the ventral medial tegmentum and reaches the LC bilaterally. However these results do not definitely demonstrate a MRN-coeruleus pathway since the MRN lesion can destroy fibres coming from the anterior VMT and reaching the LC by a medial way. Nevertheless, the degenerations in the LC are more abundant after MRN than after VMT lesion and it is possible that some of the degenerations are due to the destruction of raphe cells per se. A large part of these cells are serotoninergic[18], and it is interesting to note in this respect that relatively high concentrations of serotonin were evidenced in the LC[19]. If subsequent work confirms a MRN-LC pathway, the existence of LC rostral raphe relations[20] will be completed in the sense of a LCN-MRN loop. Such a loop could improve our understanding of sleep mechanisms.

The VMT lesions made in animals that showed a high rate of self-stimulation were located in the area of the dopaminergic A10 group cells[18]. Dopamine had been evidenced in the LC at levels which suggest the existence of dopaminergic terminals[21], and one could suggest the existence of terminals issued from the A10 nucleus. More generally our results can explain the paradoxical biochemical data obtained after VMT self-stimulation[13,14] and the role of the dopaminergic systems in self-stimulation obtained from the LC demonstrated by some investigators[22].

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Reduced microbial transformation of bile acids in cystic fibrosis[1]

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Summary. The microbial transformation of bile acids by incubates of stool homogenates from children with cystic fibrosis is decreased.

The human gastrointestinal tract is endowed with a microecological system in which the bacterial population is relatively stable[2]. There are many factors responsible for maintaining the balance between microorganisms and host. The anatomical and functional integrity of the intestine is essential. Stasis of intestinal contents leads to abnormal microbial growth[3]. The 'acid barrier' provided by the stomach[4] and a normal ileocecal valve[5] insure that the upper and lower small bowel remain relatively sterile in order to fulfill its absorptive role. Other important factors which prevent the bacterial contamination of the small bowel include intestinal motility[6], mucus[7], immunoglobulins[8], bacterial metabolic products[9] and bile acids.

Bile acids exert considerable antibacterial activity. In vitro, free bile acids are particularly effective[10]. In vivo, the administration of bile acids leads to a decrease of the ileal anaerobic flora in man[11]. The intestinal flora in turn significantly affects the concentration and composition of intestinal bile acids[12]. We have shown large losses of bile acids in the feces of patients with cystic fibrosis (CF)[13]. Qualitative patterns of fecal bile acids were similar to those in children with an ileal resection. A significant prevalence of bile acids which had not undergone microbial transformation were found in CF and in ileal resections, as compared to controls in whom 86.5% are secondary bile acids[14]. Hence, it seemed interesting to study the capacity of the CF fecal flora to transform bile acids.

Material and methods. The capacity of fresh stool samples to conjugate and dehydroxylate bile acids was studied in 13 CF patients and compared to that of 9 children free of any hepatic, pancreatic or intestinal disorder. The mean age of the CF group was 7.9 years (2–15.9 years) while that of controls was 8.6 years (2–12 years). None of these children had received antibiotics during the previous week. Among the children with CF, 4 had no clinical evidence of pancreatic insufficiency, the 9 others were on

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Table 1. Deconjugation and dehydroxylation of taurine and glycine conjugated bile acids by incubated stool homogenates

	Controls (n = 9)	CF-1 (n = 4)	CF-2* (n = 9)
Tc	0.5 ± 0.07	1.0 ± 0.5	3.1 ± 1.1**
C	79.0 ± 4.3	68.3 ± 4.6	81.1 ± 3.8
Dc	20.5 ± 4.3	30.7 ± 4.6	15.8 ± 3.3
Gc	8.0 ± 2.1	7.4 ± 2.5	8.3 ± 1.4
C	70.8 ± 4.7	70.2 ± 6.4	73.8 ± 5.2
Dc	21.2 ± 5.2	22.4 ± 4.3	17.9 ± 5.6

Results (mean ± SE) shown are expressed as the percent of the sum of bile acids partitioned on T.L.C. plates. Tc, taurocholic; C, cholic; Dc, deoxycholic. *CF-2 and CF-1: Cystic fibrosis with and without clinical pancreatic insufficiency. **$p < 0.025$, CF-2 vs controls; $p < 0.05$, CF-2 vs CF-1.

pancreatic supplements at the time of the study. The technique used was essentially that of Samuel et al.[15]. Complete bowel movements evacuated within 2 h of being processed were diluted with H_2O (1:3 w/v) and homogenized. 3 g of homogenate calculated to be equivalent to 200 mg or more of dry stool weight was placed in incubation tubes. After adding either 0.5 μCi of ^{3}H-taurocholic (3 Ci/mM), 0.25 μCi of ^{3}H-glycocholic (3 Ci/mM), 0.25 μCi of ^{14}C-cholic acid (0.04 Ci/mM) obtained from New England Nuclear Corp. or 0.2 μCi of ^{14}C-chenodeoxycholic acid (Calatomic L.A., 0.003 Ci/mM) to each tube, 30 ml of an incubation medium made up of NaCl (118.3 mM), KCl (4.69 mM), $CaCl_2$ (2.51 mM), KH_2PO_4 (1.18 mM), $MgSO_4$ (2.41 mM) and $NaHCO_3$ (25 mM) was mixed with the homogenate. Nitrogen was bubbled through the incubation mixture for 60 sec. At the end of an incubation period of 30 min at 37 °C, 3 ml of 33% KOH and 36 ml of warm ethanol were added to stop the reaction.

After cooling the incubation mixture at 4 °C, centrifugation was followed by collection of the supernatant. The residue was washed with 5 ml of methanol, centrifuged and transferred to the original supernate. In an ice waterbath, the pH was adjusted to 2 with 6 N HCl. The volume was then reduced to 15 ml before 5 extractions with diethyl ether. The ether extract was evaporated to dryness and reconstituted with chloroform and 95% ethanol (1:1 v/v) before application on 2 mm silica gel G thin layer chromatography plates. Appropriate bile acid standards (Steraloids, Wilton, N. H.) were applied to each plate. Hofman I and II running solvents were used for free and conjugated bile acids. The bile acid standard spots were visualized by a spray of 15% phosphomolybdic acid to facilitate identification. Bile acid spots were scraped from the plates and extracted 4 times with methanol. Radioactivity was counted in a NCS-toluene solution and corrected for quenching.

More than 95% of labeled bile acids added to stool homogenates were recovered from thin layer plates. Heating the homogenates to 80 °C before adding the radioactive substrates for deconjugation and dehydroxylation led to the recovery of 96% of the labelled bile acid. When meconium from 5 newborns was tested 76.8, 93.1 and 83.0% of the radioactivity was found in the spots corresponding to the labeled bile acids, glycocholic, chenodeoxycholic and cholic added to the stool homogenates. There was no attempt at maintaining anaerobic conditions during the incubation, since it made little difference. When glycocholic and taurocholic were used as substrates, little radioactivity was found outside the cholic and deoxycholic acid spots. The same observations were made in the case of cholic and chenodeoxycholic experiments with respect to deoxycholic and lithocholic acid. Therefore the sum of these spots was taken as 100% and results for individual bile acids were expressed as a percent of this sum. Analysis of variance and the standard t-test were used for statistical analysis of the data.

Results. The extent of deconjugation and dehydroxylation of labeled taurocholic and glycocholic is shown in table 1. A larger percent of radioactivity was recovered from the spot corresponding to taurocholic acid in CF with pancreatic insufficiency (CF-2) than in those without (CF-1) and in controls. In experiments carried out with labeled glycocholic, there was no difference between the 3 groups. It is apparent that the taurine amide bond was much more vulnerable ($p<0.01$) to hydrolysis than the glycine bond for the 3 groups. Although in studies with both taurocholic and glycocholic acid, the percent of deoxycholic acid generated by CF-2 homogenates was lower than in the 2 other groups, wide standard deviations preempted the demonstration of a statistical difference. Results of incubations, carried out with the 2 free primary bile acids, cholic and chenodeoxycholic acid (table 2), show a significant decrease of dehydroxylation to deoxycholic and lithocholic in CF-2 as compared to controls. In addition, the capacity of CF-1 stools for the 7α-dehydroxylation of chenodeoxycholic to lithocholic was lower than that of control stools.

Discussion. This study shows that the microbial transformation of bile acids by stool homogenates of patients with CF is decreased. Despite the crudeness of the method[15], the fecal constituents responsible for the findings are likely of bacterial origin. Heating inhibited the appearance of degraded bile acids. Incubates of relatively sterile meconium collected during the first 3 days of life showed very little activity as a very high percent of the added labeled bile acids were recovered unchanged. Since degradation of bile acids is mainly accomplished by the anaerobic flora[16], it was surprising that anaerobic conditions failed to improve reproducibility of the method as well as the extent of microbial transformation.

A quantitative and qualitative study of the intestinal flora in CF has never been done. It could be different from that of normal children. Although diet has little effect on the flora, metabolic products secondary to maldigestion and

Table 2. Dehydroxylation of cholic and chenodeoxycholic acid by incubated stool homogenates

	Controls (n = 9)	CF-1 (n = 4)	CF-2* (n = 9)
C	76.5 ± 4.3	86.2 ± 7.0	97.3 ± 1.0
Dc	23.5 ± 4.3	13.8 ± 7.0	2.7 ± 1.0**
CDC	60.7 ± 4.8	79.2 ± 4.5	83.9 ± 1.4
L	39.3 ± 4.8	20.8 ± 4.5***	16.1 ± 1.4**

Results (mean ± SE) shown are expressed as the percent of the sum of bile acids partitioned on T.L.C. plates. C, cholic; Dc, deoxycholic, CDC, chenodeoxycholic; L, lithocholic. *CF-2 and CF-1: Cystic fibrosis with and without clinical pancreatic insufficiency. **$p <$ 0.005, CF-2 vs controls; ***$p < 0.025$, CF-1 vs controls.

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malabsorption of nutrients could produce floral changes or inhibit certain bacterial enzymes. In this regard, 7 α-dehydroxylase, the bacterial enzyme responsible for the anaerobic and irreversible dehydroxylation, which affects the bulk of intestinal bile acids, is inhibited by dehydroxylated fatty acids[17]. However, results in the 4 CF children without pancreatic insufficiency (CF-1) do not favour that hypothesis. Although the present studies were carried out while patients were off antibiotics, it is possible that the intestinal flora could be chronically altered because of intermittent but prolonged periods of antibiotic therapy. This would best account for the observation that the percent lithocholic acid generated by CF-1 stools incubated with chenodeoxycholic was lower than the percent obtained in controls.

Bile acids, especially in their unconjugated form, are inhibitory to anaerobic intestinal microorganisms[18]. Because of the large fecal losses of bile acids in CF with pancreatic insufficiency[14], it is reasonable to suppose that the anaerobic flora responsible for the 7 α-dehydroxylation of cholic and chenodeoxycholic acid[18] is reduced. In view of the reported inhibition of 7 α-dehydroxylase by an excess of substrate[19], the possibility that the decreased dehydroxylation could be due to large concentrations of free primary bile acids in CF stool homogenates cannot be dismissed. However, the concentrations necessary to achieve substrate inhibition[15] are far in excess of concentrations we have found in CF.

The pathogenesis of bile acid malabsorption in CF remains obscure. Although free bile acids are absorbed faster than conjugated ones[20], the present findings do not suggest that the hydroxylase bacterial enzymes could be a limiting factor. The impaired capacity of CF stool homogenates to dehydroxylate bile acids is not likely to be responsible for the large fecal sequestration of bile acids in CF, since free and dehydroxylated bile acids are probably equally well absorbed by the ileum[20]. In fact, impaired dehydroxylation would favour bile acid absorption in the colon since deoxycholic acid, readily bound to dietary residues and bacteria, and lithocholic, precipitated out of solution, are largely unavailable for absorption[21].

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Olfactory discrimination between glycine and deuterated glycine by fish

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Summary. Electrophysiological and behavioural experiments showed that whitefish (Coregonus clupeaformis) were able to discriminate between glycine and fully deuterated glycine by olfaction, while both chemicals stimulated the olfactory receptors to induce bulbar responses of similar magnitude.

Fish may detect various chemical stimuli and respond by changing their behavioural patterns. These may be characterized as either avoidance or preference depending upon the stimuli perceived. These chemical stimuli are detected through at least 2 different sensory channels, olfaction and taste, as in terrestrial vertebrates. In fish, both olfaction and taste take place entirely in the aquatic environment. The carrier of stimulant molecules is water, therefore chemicals that are detected olfactorily need not be volatile. Thus the spectra of chemicals detected by fish could be entirely different from those detected by terrestrial animals. One of such chemical classes is the amino acid. Electrophysiological studies indicate that certain amino acids, which are normally non-odourous to humans, are extremely effective olfactory stimuli and may play an important role, such as acting as chemical signals, in olfactory communication in fishes[2–5]. Recent investigation of the specificity of olfactory stimulation by amino acids and analogues has led to the establishment of definite structure-activity relationships, and further suggested the nature of a possible receptor membrane binding site which involves 2 charged subsites, one cationic, one anionic, capable of interacting with ionized α-amino and α-carboxyl groups of amino acid molecules[6,7]. The substitution of an isotopic atom such as deuterium for protium in a molecule containing hydrogen should leave essentially unchanged those molecular properties that are associated with electronic structure and force fields. In contrast, molecular properties dependent upon mass are changed[8]. If the olfactory receptor for amino acid detection involves an electric field that fits the receptor site mentioned, the replacement of hydrogen with deuterium should not change the stimulatory effectiveness of a molecule. If it involves molecular motions or dipole moments[9,10], the isotopic effects should cause

1 The author thanks Dr D. Tucker, Department of Biological Science, Florida State University, for critical reading of the manuscript, and Bruce Thompson and Ryo Hara for technical assistance.
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