trum exhibited by the human seminal plasma enzyme preparation is similar to that of the bovine seminal plasma enzyme preparation made by the same procedure(1).

Summary. Enzymes prepared from human seminal plasma were found to release phosphate from the 5-mononucleotides, cytidylic, uridylic, adenylic and guanylic acids. The preparations were not, or only slightly, active in hydrolyzing yeast cytidylic acid, glucose-l-phosphate, phosphorylcholine, flavine mononucleotide, and O-carboxyphenyl phosphate. Phosphate was also released from cytidine diphosphatecholine, uridine diphosphate glucose, oxidized and reduced diphosphopyridine nucleotide, and flavine adenine dinucleotide.

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Cholic Acid in Guinea Pig Bile.* (26103)

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During the investigation of formation of gall bladder precipitates produced by parenteral administration of a bacterial polysaccharide, we found that 3α, 7α, 12α-trihydroxycholanic (cholic) acid was not only present in guinea pig bile, but was the major bile acid. Further study disclosed that 3α, 7α, 12α-trihydroxycholanic acid was present in adult but not in immature guinea pigs, thereby offering an explanation for the previously reported absence(1,2) of this bile acid.

Methods. Non-inbred, albino and varicolored guinea pigs were obtained from commercial suppliers in Oregon, Utah, and Missouri. Eighteen adult guinea pigs of either sex, approximately 5 months of age and weighing 500-700 g, were fed with Purina rabbit chow and fresh green vegetables, carrots and apples *ad libitum*. Bile fistulae were performed on 6 animals from this group. Bile from the gall bladders of the remaining 12 animals was pooled in 2 samples. Seventy-two immature guinea pigs of either sex, 6-8 weeks of age, and weighing 250-350 g were divided into 3 equal groups. Each group of 24 animals was kept for 2 weeks on one of the following diets: a) Vit. C deficient diet of Rinehart and Mettier(3), rich in other vitamins, fats, and minerals; b) Purina rabbit chow heated for one hour at 100°C; c) Purina rabbit chow with green vegetables, carrots and apples as supplied to the group of adult guinea pigs. Bile from gall bladders was pooled in 3 samples per each dietary group. All gall bladders were aspirated by syringe within one minute after application of a neck clamp.

The bile fistulae were performed under ether anesthesia. One polyethylene catheter was inserted into the gall bladder and another one into the proximally ligated common bile duct. Both catheters were passed under the skin to the back where their free ends were pulled through a cutaneous wound and connected to allow enterohepatic cycling. To avoid operative and postoperative abnormalities, collection of the first bile samples was not begun until 48 hours after the surgical procedure. A volume of normal bile equal to that diverted was supplied through the duodenal fistulae. These samples obtained from the bile fistulae were not pooled but processed separately.

Except for small aliquots, the bile samples

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were acidified with hydrochloric acid, extracted with n-butyl alcohol, and submitted to partition chromatography on columns of kieselguhr treated with silane as described by Bergström and associates(4,5,6). The solvent system, isooctanol: chloroform (1:1) as stationary phase and 50% aqueous methanol as moving phase, was used for separation of the conjugated bile acids. For separation of the free bile acids the solvent system, chloroform: heptane (9:1) as stationary phase and 58% aqueous methanol as moving phase, was used. The free bile acids were obtained by hydrolysis of the conjugated bile acids in 1N NaOH for 5 hours at 110°C. The effluents were titrated with 0.01 N NaOH in methanol. Aliquots of original untreated bile, the butanolic extracts, and the conjugated and free bile acids obtained by column chromatography were submitted to paper chromatography in the systems introduced by Sjövall(7,8). After paper chromatography, the bile acids were detected by spraying the paper strips with phosphomolybdic acid(7). However, only 2 of the 3 bile acids separated by the chromatography on columns, gave a color reaction with this reagent. Therefore, the chromatograms were sprayed also with 15% phosphotungstic acid in absolute ethanol, as used by Martin(9) for detection of several sterols, but modified by heating at 80°C until development of pink spots within 3-5 minutes. All 3 bile acids separated on columns could be located by this procedure. After separation on the columns, the free bile acids were further purified as described by Bergström and Sjövall(6), and after several recrystallizations, were identified by infrared spectra, spectra in concentrated sulphuric acid(10), melting points, and specific rotations.

Results. Bile collected by fistula and from gall bladders of the mature guinea pigs contained 3α, 7α, 12α-trihydroxycholanic acid as the major bile acid, as well as 3α, 7α-dihydroxycholanic (chenodeoxycholic) and 3α-hydroxy, 7-ketocholanic (7-ketolithocholic) acid, with average ratio approximating 4:3:1. A typical separation of the conjugated and free bile acids by column chromatography is shown in Fig. 1.

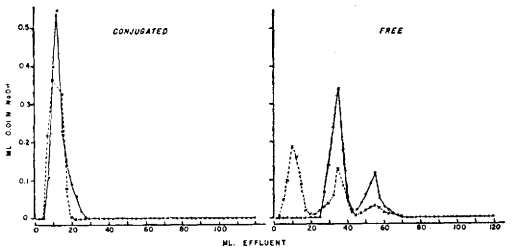

FIG. 1. Separation of bile acids by column chromatography. *----* adult animals. ●——● immature animals.

In the immature guinea pigs 3α, 7α, 12α-trihydroxycholanic acid was not found in any of the bile samples, but the other 2 acids, 3α, 7α-dihydroxycholanic and 3α-hydroxy, 7-ketocholanic acid, were present in an approximate average ratio 2.5:1 (Fig. 1). The bile acid pattern in immature guinea pigs was the same in each of the 3 dietary groups.

In both mature and immature animals, the bile acids were found to be conjugated with taurine. No evidence was obtained by either column or paper chromatographic procedures that a portion of the bile acids was conjugated with glycine.

Bile acids were crystallized as follows: 3α, 7α, 12α-trihydroxycholanic acid: aqueous acetic acid (1×), aqueous methanol (2×); 3α, 7α-dihydroxycholanic acid: ethyl acetate (1×), ethyl acetate and petroleum ether (2×); 3α-hydroxy, 7-ketocholanic acid: aqueous acetic acid (1×), aqueous methanol (1×), ethyl acetate (2×). The infrared spectra corresponded to authentic samples of 3α, 7α, 12α-trihydroxycholanic (Fig. 2), 3α, 7α-dihydroxycholanic, and 3α-hydroxy, 7-ketocholanic acid. The values obtained for maximum absorption in concentrated sulphuric acid, melting points, and specific rotations (Table I) further identify these bile acids.

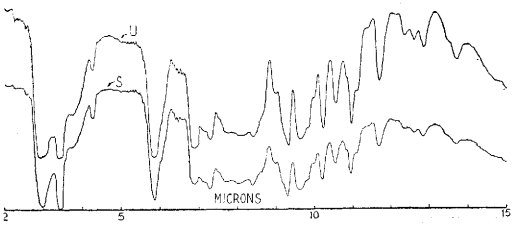

FIG. 2. Infrared spectrum of 3α,7α,12α-trihydroxycholanic acid isolated in this study (U) and of an authentic sample (S).

TABLE I. Absorption Maxima in Conc. H_2SO_4, Melting Points and Specific Rotations of the 3 Bile Acids Isolated in This Study Compared with References.

	3α,7α,12α-trihydroxy-cholanic acid		3α,7α-dihydroxy-cholanic acid		3α-hydroxy, 7-ketocholanic acid	
	Isolated	Reference	Isolated	Reference	Isolated	Reference
Absorb. max., mu	389	389(10)*	310	310(10)	301	301(10)
M. P., °C	199	199(15)	143	143(15)	198–201	201–203(1)
$[\alpha]_D^{25°}$ (Ethanol)	+37 ± 1.5	+37(15)	+11 ± 0.5	+11.5(15)	−27 ± 1.0	−27.27(1)

* Reference.

Discussion. There are 2 general aspects of bile acid formation: a) the characteristic pattern of bile acids of a species reflects phylogenetic development(11) and b) change in bile acids during enterohepatic cycling with conversion of trihydroxycholanic acids by intestinal microorganisms and with hepatic reconversion in some species(12). The abundance of 3α, 7α, 12α-trihydroxycholanic acid and the conjugation of all bile acids with taurine found in the present investigation, would characterize the guinea pig, at least the adult of this species, as less "modern" phylogenetically than has been thought(13).

The observation that 3α, 7α, 12α-trihydroxycholanic acid appears in the bile of the guinea pig only upon maturation is not explained through any known mechanism. It is possible that a similar effect of aging may be found in other species. Danielsson and Kazuno's(2) failure to find 3α, 7α, 12α-trihydroxycholanic acid in the guinea pig may be explained by the immaturity of their animals. Weights or age of the guinea pigs used by Imai(1) were not reported. It is of interest that Iwaki(14) observed cholic acid in the urine of guinea pigs given pentahydroxybufostane.

In our unpublished studies on conversion of cholesterol-4-C^{14} to bile acids in the guinea pig, labeled 3α, 7α, 12α-trihydroxycholanic acid appeared in the bile more slowly but attained higher concentration than 3α, 7α-dihydroxycholanic and 3α-hydroxy, 7-ketocholanic acid. These studies also suggest that the 3 bile acids form without action of enteric microorganisms in the guinea pig but that 3α, 7α, 12α-trihydroxycholanic acid may slowly accumulate in the adult animal by preferential conversion and recycling. However, such a mechanism would not explain the apparent absence of 3α, 7α, 12α-trihydroxycholanic acid in the bile of the immature guinea pig.

Summary. Cholic (3α, 7α, 12α-trihydroxycholanic) acid has been isolated from the guinea pig bile obtained from gall bladders as well as by biliary fistulae. However, this bile acid was found only in the bile of adult and not of immature guinea pigs. The other 2 bile acids isolated, chenodeoxycholic (3α, 7α-dihydroxycholanic) and 7-ketolithocholic (3α-hydroxy, 7-ketocholanic) acid, occurred in both immature and mature guinea pigs.

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