TABLE V. Comparative Effects of Intravenous Administration of 250 cc of Normal Plasma and 85 cc of Fraction IV* in PTC Deficiency.

	Coagulation time,† min.		Residual serum prothrombin,‡ units/cc	
	Plasma	Fraction IV	Plasma	Fraction IV
Before	>120	>120	260	260
15 min. after	15	11	85	82
4 days "	17	21	134	164
7 " "	16	22	220	260

* Containing 6.5% protein.
† Done in duplicate in 13 mm tubes at 37°C.
‡ Two-stage method of Ware and Seegers(4), after incubation of blood for 1 hr at 37°C.

about one-tenth of the AHF potency of plasma(6). Fraction IV is present in the plasma in a concentration of approximately 1 g per 100 cc, while fraction I is present in a concentration of only 0.38 g per 100 cc. It would, therefore, require almost twice the volume of an equivalent protein concentration of fraction I derived from 5 times the original quantity of plasma to produce similar results in hemophilia.

Conclusions. 1. The PTC factor is concentrated in fractions III and IV-1 of the plasma proteins. Small quantities are found in fractions I and IV-4. None is found in fractions II and V. 2. Since fraction III contains thrombin and fraction IV-1 is poorly soluble, neither is suitable for intravenous administration. 3. The intravenous administration of fraction IV produced marked shortening of the whole blood coagulation time and reduction in residual serum prothrombin in PTC deficiency. The results appear superior to those which can be achieved by the intravenous administration of fraction I in hemophilia.

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Metabolic Products of Cholesterol in Bile and Feces of Rat.* Steroids and Bile Acids. (20269)

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The transformation of cholesterol into cholic acid was first demonstrated in the dog with deuterated cholesterol(1). The same reaction has recently been shown to occur in the rat with cholesterol-4-C^{14}(2,3). In the latter work we further showed that cholic acid was the main excretory end product of cholesterol metabolism constituting more than 80% of the labelled acidic products of the bile. This agrees with observations(4,5) that most of the isotope of administered cholesterol-4-C^{14} that was excreted came via the bile and feces as acidic products and that only very small amounts appeared in the expired carbon dioxide. That the labelled acidic products in the bile and feces behaved like bile acids in fractionations has subsequently been shown in the same laboratory(6).

In this paper some further results will be presented on the fractionation of the acidic products in bile and feces of rats that had received cholesterol-4-C^{14} by intraperitoneal administration.

Experimental. One mg of cholesterol-4-C^{14}

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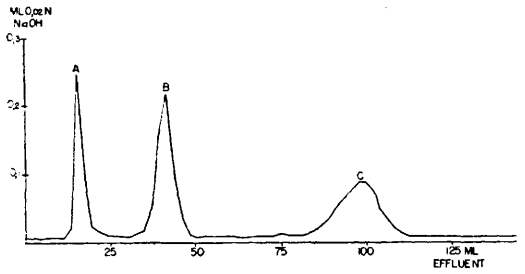

FIG. 1. Chromatography of taurocholic (A), glycocholic (B) and cholic acid (C). Stationary phase: 4 ml of octanolchloroform 50-50 on 4.5 g hydrophobic Supercel. Descending phase: 50% aqueous methanol.

containing 5 μc C^{14} was administered intraperitoneally to 200 g white rats in an aqueous colloidal solution. It was prepared by dissolving 1 mg cholesterol-4-C^{14} in 0.1 ml of hot ethanol. To this solution was added 2 ml of hot saline containing 0.12 mg of sodium palmitate; the mixture was heated at 90°C for about half a minute when a practically water clear solution was obtained. It has been found that cholesterol administered in this way was rapidly absorbed from the abdominal cavity and distributed in the body (10). After the injection the rats were kept in metabolic cages and the feces collected daily in ethanol. When the bile was to be collected, the rats were kept in a restraining cage after the introduction of a polythene cannula into the bile duct and the bile was collected directly into ethanol(3). The animals had free access to their normal stock diet and water or in the latter case 0.9% sodium chloride solution.

Fractionation of unhydrolyzed bile. The precipitated mucoids were filtered off and the filtrate taken to dryness *in vacuo*. The residue was dissolved in a small volume of water, acidified to pH 1 with hydrochloric acid and extracted 3 times with 2 volumes of butanol. The combined butanol extracts were then washed with distilled water until free of hydrochloric acid—all wash-waters passing a second butanol phase. The combined butanol phases contained about 95% of the total activity. After evaporation to dryness *in vacuo* the residue was subjected to reversed-phase partition chromatography(7,8). After titrating each chromatographic fraction it was plated on copper or aluminum planchets for determination of the isotope content. For reference a chromatographic separation of taurocholic, glycocholic and free cholic acid is illustrated in Fig. 1(8). The chromatography of material from the butanol extract of the bile from a rat that had received labelled cholesterol is shown in Fig. 2. The stationary phase that was washed out with chloroform contained about 15% of the original activity as neutral compounds. We have earlier shown that the influence of the taurine group is so pronounced that even the taurine conjugate of the monosubstituted lithocholic acid leaves the column before glycocholic acid under these conditions(8). It is therefore clear that practically all activity in acidic compounds was contained in compounds that behaved like taurine conjugates.

Fractionation after alkaline hydrolysis. The fractions containing the activity 10-25 effluent

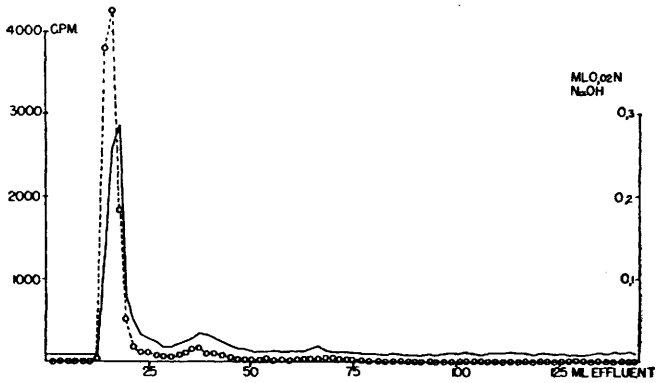

FIG. 2. Chromatography of butanol extract of unhydrolysed bile excreted on 8th day after intraperitoneal administration of cholesterol-4-C^{14}. Conditions: Fig. 1.

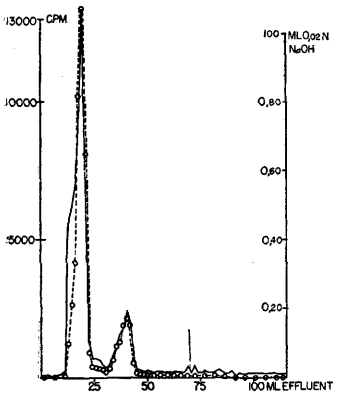

FIG. 3. Chromatography of hydrolysate of active fractions in Fig. 2 (10-25 ml effluent). Stationary phase: 4 ml of chloroform-heptane 9:1 on 4.5 g hydrophobic Supercel. Descending phase: 60% aqueous methanol.

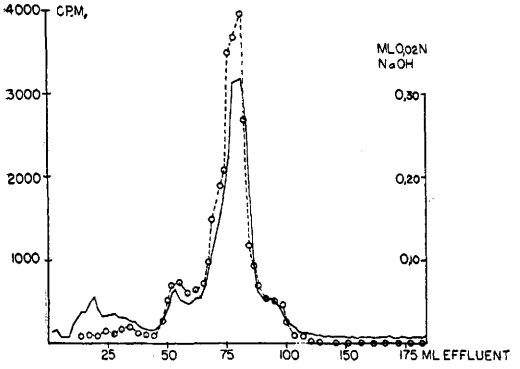

FIG. 4. Chromatography of material from cholic acid band (Fig. 3) (10-25 ml effluent). Stationary phase: 4 ml octanol-chloroform-heptane 45-45-10 on 4.5 g hydrophobic Supercel. Descending phase: 45% aqueous methanol.

(Fig. 2) were combined in an aqueous solution and hydrolyzed in one normal sodium hydroxide at 140° for 3 hours. The solution was then acidified with hydrochloric acid, extracted with ethyl ether and the extracts combined, washed with water and evaporated to dryness. Chromatography of these acids gave the results shown in Fig. 3, *i.e.*, a curve similar to normal rat bile with a large band in the cholic acid region followed by a smaller one in the region where dihydroxycholanic acids appear (7,9). The second band present in normal rat bile has been found to be chenodesoxycholic acid (11). The activity is also present as chenodesoxycholic acid as dilution with inactive chenodesoxycholic acid and recrystallization has shown that the specific activity remained constant. With isotope dilution it has already been shown that the main isotope-containing component of the first band is cholic acid (3). When this fraction is re-run with a more polar stationary phase a number of minor active fractions was found to appear, one just before and one just after the main part of the activity contained in cholic acid (Fig. 4). The labelled compounds in these bands have not yet been identified. It should be noted that bile secreted during the first day after the bile duct cannulation is not present in this material, *i.e.*, bile acids that have reached the intestine and have been in contact with the intestinal flora are not included.

Fractionation of activity in feces. After intraperitoneal administration of the colloidal solution of cholesterol-4-C^{14} the feces were collected in ethanol. The feces from every 24 hours were homogenized and extracted 3 times with boiling 90% ethanol. The isotope content was determined on a small aliquot of the combined filtrates. The remainder was evaporated to dryness *in vacuo*. The residue was dissolved in 25 ml of 0.2 M sodium carbonate and this solution was extracted 3 times with a large volume of ether. The ether extracts were combined, washed with water and evaporated to dryness.

The aqueous phase was then acidified to pH 1 with hydrochloric acid and extracted with butanol. The combined butanol extracts were washed with water until free of hydrochloric acid. The residue from the butanol extracts was distributed between equal volumes (30 ml) of 70% ethanol and light petroleum by a 3-stage counter-current extraction. The residue from the combined aqueous phases was then chromatographed as shown in Fig. 5 (7,8) with a solvent system suitable for separation of conjugated bile acids (Fig. 1 and 2). The material that stayed in the stationary phase was eluted with chloroform and then subjected to a new separation, as shown in Fig. 6, in a system suitable for the separation of unconjugated bile acids.

From a comparison of Fig. 5 and 6 with Fig. 1 and 2 it is noticed that whereas in the bile practically all the labelled acidic com-

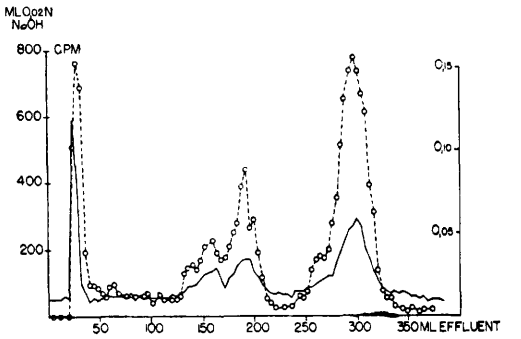

FIG. 5. Fecal acids from 7th day after intraperitoneal administration of cholesterol-4-C^{14}. Conditions: phases equal to Fig. 2. Column: 9 g hydrophobic Supercel.

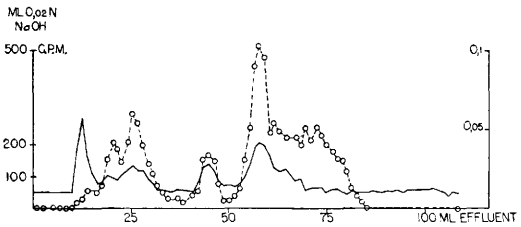

FIG. 6. Fecal acids: Material remaining in stationary phase Fig. 5 eluted with chloroform and re-run under conditions identical with those in Fig. 3.

pounds are present as taurine conjugates only a small part is present in this form in the feces.

However, it is not only a simple splitting of the peptide bond of the conjugates present in the bile that has taken place in the intestine. No activity is found at the place where free cholic acid appears (100 ml effluent). Instead a number of new bands are present of which 3 major ones that have been observed regularly are seen in Fig. 5—two eluted earlier and the largest one later than cholic acid. The less hydrophilic acids that stay in the stationary phase in the run shown in Fig. 5 were eluted with chloroform and rechromatographed as shown in Fig. 6. They apparently also consist of a complicated mixture of presumably unconjugated bile acids.

Thus the taurocholic acid that is the main labelled excretory product in the rat bile has not only been split but the cholic acid has been further modified in various ways by the intestinal microorganisms.

This is of course in accord with a number of old observations that the bile acids present in bile cannot be found in the feces. As expected some of the compounds have been found to contain ketonic groups.

This is also in accord with the findings of Schmidt, Hughes, *et al.*(12) on the metabolism of cholic acid in the guinea pig and their demonstration that this bile acid was transformed into dehydrocholic acid by *Alcaligenes faecalis.*

Summary. 1. After administration of cholesterol-4-C^{14} to rats the main excretory products in the bile are cholic and chenodesoxycholic acid present as taurine conjugates. 2. The labelled material present in feces has been fractionated. It has been found that most of the conjugate has been split. No unchanged cholic acid seems to be left in the feces. A series of different labelled products have been separated some at least of which contain ketonic groups.

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