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Turnover of deoxycholic acid in the rabbit*

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SUMMARY

A method for studying the turnover of deoxycholic acid in the rabbit is described. The mean values for half-life, pool size, and daily production of deoxycholic acid were 6.8 days, 700 mg, and 73.4 mg, respectively, in 26 rabbits on a diet of conventional commercial food pellets. Of the bile acid pool, 97–98% was present in liver, gallbladder, and gastro-intestinal tract. A comparatively large amount (10%) was present in the stomach. Fecal excretion was the main excretory pathway for bile acids. An amount corresponding to 10% of the daily synthesis of deoxycholic acid was excreted in the urine. The concentration of bile acids in blood was calculated to be 0.26–3.10 mg/100 ml of whole blood.

It is well known that the serum cholesterol level of rabbits as compared to that of most other animals is particularly susceptible to changes in the diet. The reason for this difference between species is not known, nor have the exact mechanisms by which the serum cholesterol changes are brought about been elucidated. Cholesterol is eliminated from the body with the feces partly as neutral sterols and partly as bile acids. A small amount is excreted as hormone metabolites. In spite of the fact that the rabbit is one of the most commonly used animals for studies of the influence of diet and drugs on serum cholesterol levels, no detailed quantitative studies of sterol and bile acid excretion in this animal seem to have been published. Mosbach *et al.* (1), in a preliminary communication, reported that rabbits on a fat- and sterol-free diet excreted 50 mg of digitonin precipitable sterols and 60 mg of bile acids per day. To our knowledge, no detailed report of these findings has appeared.

In order to investigate the influence of diet on bile acid production, it was necessary to develop techniques permitting accurate determination of bile acid turnover in rabbits. In the present paper, the methods used and the results obtained with rabbits on a low-fat commercial pellet diet are described. The effects of different fats on the bile acid excretion are reported in the following paper (2).

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EXPERIMENTAL METHODS

Animals. Male albino rabbits weighing between 2 and 3.5 kg were used. At the beginning of the experimental period, they were full-grown. For the collection of bile samples, the animals were provided with a permanent stomach fistula. The fistula consisted of a polyvinyl chloride plastic tube (55 mm long, 9 mm o.d., 6.5 mm i.d.) introduced into the stomach 3–4 cm above the pylorus through the ventral wall close to the greater curvature. The tube had an end plate (19 mm diam) on the mucosal side, and was closed with a stopper that went through the lumen and completely occluded the mucosal end of the tube. No leakage of stomach fluid occurred. The tube was taken out through a hole to the left of the midline and kept in position with a plate fastened to the tube on the outer side of the abdominal wall (3). The rabbits were allowed to recover for 2 weeks after the operation; penicillin and streptomycin were given if minor infections developed. Most animals survived this period and then appeared to be completely normal and healthy. They took no notice of the tube and minor local infections occurred rarely. The fistula was closed except when bile samples were taken.

The animals were kept in single net hutches. Feces were collected on a fine-mesh net placed a few centimeters below the net floor. The urine flowed through this net into a metal tray. Feces were collected each day and stored at −15°. The urine was rinsed off the tray with ethanol each day.

Bile samples were taken every 4 to 7 days during the 1-month experimental period. This was done without anesthesia, with the rabbit lying on its back tied to a

TABLE 1. DIFFERENCES BETWEEN DUPLICATE VALUES FOR TOTAL RADIOACTIVITY AND TOTAL AMOUNT OF GLYCODEOXYCHOLIC ACID MEASURED AFTER PAPER CHROMATOGRAPHY OF DUODENAL BILE

Total Radioactivity	Number of Sets of Duplicates	Mean Difference	$\sqrt{\frac{\Sigma d^2}{N}}$	Range
cpm		%		%
10–20	17	13.3	12.8*	25.2
20–50	20	10.6	12.0	21.0
50–100	20	8.6	11.0	22.6
Above 100	20	7.7	9.4	15.4
Determination of Net Amounts Glycodeoxycholic Acid (4.9–88 μg)	20	2.6	3.3	5.5

* Standard error of individual differences within duplicates.

board. A piece of rubber tubing was introduced through the fistula into the duodenum and about 1 ml of bile was collected a few minutes after the intravenous injection of cholecystokinin (kindly supplied by Prof. E. Jorpes and Dr. V. Mutt, Karolinska Institutet). The samples were stored at −15° until analyzed. Blood samples were taken from the marginal ear vein.

The animals were kept on a commercial, low-fat food pellet diet (H. Fors Co., Stockholm, Sweden). According to the manufacturer, it had a total caloric value of 2.5 kcal/g; and the fat, protein, and carbohydrate made up 3.5, 18.5, and 49.3% of the weight, respectively. A ration was weighed and given to the animals in a dish each morning. Pellets spilled during the night were collected and added to the next day's ration. Water was given ad lib. The rabbits were weighed every other week after an initial period of adjustment. The ration was adjusted so as to keep a constant body weight.

Deoxycholic Acid-24-C^{14}, with a specific activity of 22.5 μc/mg, was synthesized according to Bergström *et al.* (4), and 0.6 mg was given intraperitoneally as the sodium salt in saline.

Determination of Specific Activity of Bile Acids. The bile sample was subjected directly to quantitative paper chromatography as described previously (5). Determinations were always carried out in duplicate. For determination of the radioactivity, the paper zone containing the labeled bile acid was cut out and placed directly in an ordinary scintillation vial (6) which was then counted for 10-min intervals five to six times in a Packard Tri-Carb scintillation spectrometer.

Extraction and Isolation of Bile Acids. Feces and various organs were homogenized in 96% ethanol with an Ultra-Turrax homogenizer (Janke and Kunkel KG, Staufen, Germany). They were then extracted continuously for 48 hr with hot ethanol in a 25 x 6-cm Soxhlet thimble. Pooled feces from 3–4 days were usually extracted at the same time. The isolation of the bile acid fraction in this extract was done as described by Eneroth, Hellström, and Sjövall.[1] The extract was passed through a strong cation exchanger, evaporated, and saponified. After acidification and ether extraction, the residue was put on the anion exchanger DEAE-Sephadex (Pharmacia, Uppsala, Sweden). The neutral fraction was eluted with benzene and ethanol. Acid material was eluted with 0.1 M ethanolic sodium hydroxide. After acidification with a cation exchanger in the H^+-form and evaporation of the ethanol, fatty acids were partly separated from bile acids on a silicic acid column. The bile acid fraction was subjected to gas-liquid chromatography (7) and/or paper chromatography.

The radioactivity of the various extracts was determined after evaporation of solvents by using the wet combustion method described by Jeffay and Alvarez (8).

Blood Bile Acids. Heparinized blood (3–5 ml) was added dropwise to 100 ml of ethanol. After heating for about an hour on a water bath, the extract was filtered and evaporated in the flasks where wet combustion was carried out.

RESULTS

Determination of Specific Activity. Because of the large number of samples to be analyzed, a reasonably rapid micro-method had to be developed. The procedure for determination of the specific activity of bile acids on paper chromatograms was tested in various ways. The specific activity determined was found to be independent of the amount of material applied to the chromatogram and directly proportional to the amount of radioactivity added to inactive bile acid. Table 1 shows the reproducibility of the scintillation counting procedure and the quantitative determination of glycodeoxycholic acid in randomly chosen bile samples. Results obtained with the paper chromatographic procedure were also compared with those obtained by wet combustion of the labeled deoxycholic acid crystallized after hydrolysis of the bile sample. As shown in Table 2, the two methods agree within experimental error.

Determination of Radioactivity by Wet Combustion. Of several methods tested for determination of radioactivity in the different extracts, the wet combustion method recently described (8) was found to be most suitable. Since this method was originally developed

[1] Eneroth, P., K. Hellström, and J. Sjövall. Unpublished results.

TABLE 2. COMPARISON BETWEEN DETERMINATIONS OF SPECIFIC ACTIVITY OF GLYCODEOXYCHOLIC ACID ISOLATED BY CHROMATOGRAPHY AND DETERMINATIONS OF DEOXYCHOLIC ACID ISOLATED BY CRYSTALLIZATION AND THEN COMBUSTED

	Specific Activity, cpm/mg	
Bile Sample	Paper Chromatography	Wet Combustion
KG	5,400	5,230
KR	5,430	6,440
K12	230	265
K8	2,060	2,280

for C^{14}-ascorbic acid, it was necessary to test its efficiency when employed to bile acids in biological extracts. Combustion of pure deoxycholic acid-24-C^{14}, as well as of evaporated biological extracts with known amounts of labeled deoxycholic acid, gave recoveries of 95–100% within the limits tested (<50 mg of organic material). The mean difference between single determinations of the same extract performed on different days was 7.4% in nine experiments.

Extraction of Bile Acids. Continuous extraction with hot ethanol for 48 hr yielded 94–98% of the radioactivity in six fecal samples from rabbits given labeled deoxycholic acid. The amount of activity not extracted was determined by dry combustion of the residue (9). Twenty-four-hour extractions yielded only about 85% of the activity in the ethanol. The radioactivity in the residue after 48-hour extractions could be extracted by refluxing overnight in 0.1 M HCl in ethanol.

Analysis of Bile Acids in Bile. The main bile acid in rabbit bile is glycodeoxycholic acid. Paper chromatography showed that, in all bile samples analyzed (several hundred), glycodeoxycholic acid was by far the major component. As judged from the intensity of the spots, less than 5% of glycocholic acid was present. Analysis of several hydrolyzed bile samples from different rabbits by gas chromatography (7) confirmed these results and, in addition, showed the presence of small amounts of a compound with a retention time slightly longer than that of deoxycholic acid (Fig. 1). The nature of this compound is unknown; it is not any of the naturally occurring bile acids of known structure.

Half-Life, Pool Size, and Production of Deoxycholic Acid. Since the rabbits were to be used for further studies of the effect of diet on bile acid turnover, and since each rabbit had to be studied on the control diet, a large number of animals were investigated.

After the intraperitoneal administration of labeled deoxycholic acid, the specific activity of the glycodeoxycholic acid in the bile samples was followed for at least 1 month. By plotting the logarithm of the specific activity versus time, a straight line was always obtained and the half-life, pool size, and daily production could

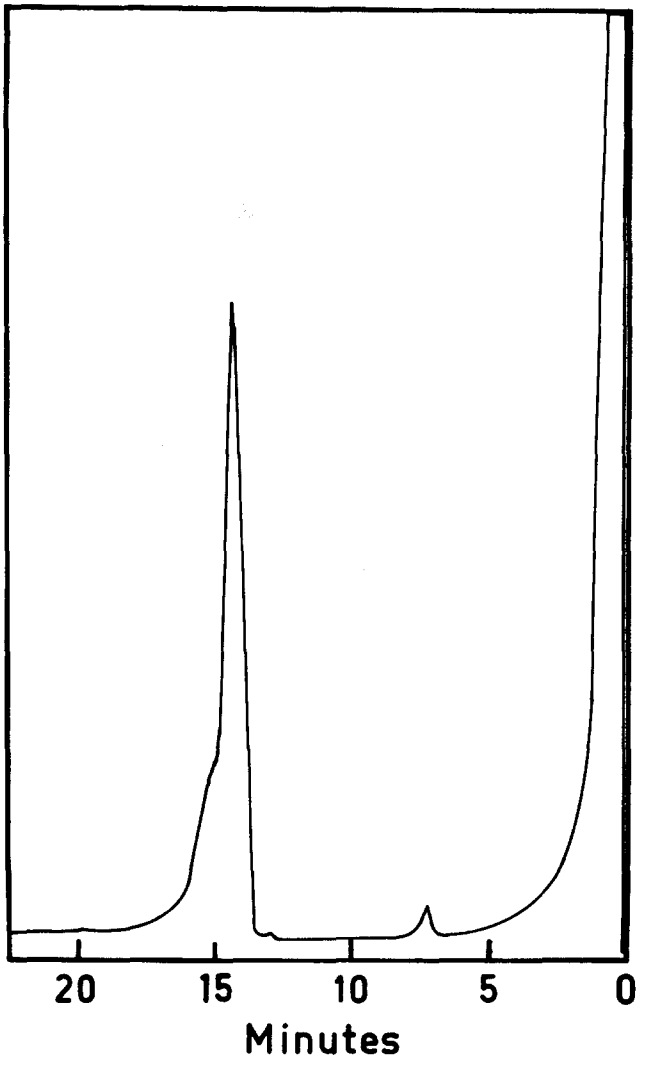

FIG. 1. Gas chromatography of methylated bile acids from hydrolyzed rabbit bile. Conditions: 6-ft x 4-mm glass column, 0.5% QF-1 on 100–140 mesh Gas-Chrom P, 221°, 1.0 kg/cm². The unknown compound (see text) emerges in the tail of methyl deoxycholate (main peak).

be calculated (10). The same result was obtained whether the labeled acid was given intraperitoneally or through the stomach fistula. The half-life did not seem to be influenced by the frequency of bile sampling, as illustrated in Figure 2. Even a slight diarrhea, however, caused a pronounced break in the line obtained; in such a case, the experiment had to be interrupted.

Table 3 shows the data obtained from 26 rabbits. In nine rabbits, two or three determinations were made and the mean values of these were used in calculating the figures of Table 3. There was a considerable individual variation of half-life, pool size, and daily production of deoxycholic acid. The rabbits examined several times showed little variation between the different experimental periods as shown in Table 4. The turnover data

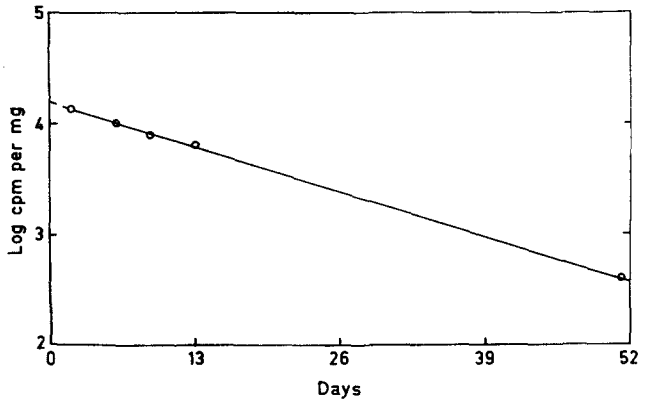

FIG. 2. Semilogarithmic plot of the specific activity of deoxycholic acid.

given in this table were obtained by giving the rabbits a new dose of labeled deoxycholic acid when the radioactivity from the previous period had been eliminated. In all cases, the semilogarithmic plot of the specific activity was a straight line. In some cases, there was a tendency for the half-life to become longer when the determinations were repeated. In most cases, the pool size also increased, resulting in a relatively constant daily production of deoxycholic acid.

Distribution of Labeled Bile Acids. The distribution of radioactivity in the body after the administration of labeled deoxycholic acid was studied in four rabbits (Table 5). Very small amounts of activity were found outside the liver and gastrointestinal tract. Rabbits K3 and K9 (Table 5) did not have a stomach fistula in contrast to rabbits KD and K12. Rabbits K3, KD, and K12 were kept in ordinary hutches, whereas K9 was kept in a plastic cage designed for the collection of CO_2. Rabbits KD and K12 had been given the coconut oil and corn oil diets, respectively, as described in the following paper (2). It is evident from Table 5 that large amounts of activity were present in the large intestine and that the stomach contained about 10% of the labeled bile acids. It was also shown that stomach contents collected through stomach fistulas invariably contained bile acids. The distribution will be influenced by the functional state of the gallbladder; thus, rabbit KD received cholecystokinin shortly before death, which occurred accidentally during bile sampling.

TABLE 3. TURNOVER OF DEOXYCHOLIC ACID-24-C^{14} IN 26 RABBITS ON A COMMERCIAL FOOD PELLET DIET

	Half-Life	Pool Size	Daily Production
	days	*mg*	*mg*
Mean value	6.8	700	73.4
Range	3.2–12.5	275–1,160	42.4–120.0

TABLE 4. VALUES OBTAINED FOR THE TURNOVER OF DEOXYCHOLIC ACID DETERMINED AT VARIOUS TIMES IN THE SAME ANIMAL

Rabbit No.	Months After First Determination	Pool Size	Half-Life	Daily Production
		mg	*days*	*mg*
K8	...	971	7.3	92.3
	2	971	8.2	82.2
	4	1152	7.5	106.0
K11	...	710	8.1	60.8
	2	790	9.1	60.1
	4	970	11.0	61.1
K30	...	706	7.3	67.0
	3	621	7.0	61.8
	4	906	10.1	62.8
K1	...	522	4.1	88.3
	4	556	5.1	75.6
K6	...	545	5.5	68.8
	2	658	7.1	64.3
	4	838	8.9	65.6
K14	...	622	7.2	59.9
	2	509	8.0	44.0
K15	...	559	8.8	44.2
	1	497	8.5	40.6
K18	...	679	5.8	81.5
	2	760	6.3	83.9
K19	...	279	3.4	56.9
	2	270	3.0	62.5

The CO_2 collected from rabbit K9 during two days contained less than 0.1% of the activity injected. A total of 88.5% and 95.3% of the activity given was recovered in the bodies and excreta of rabbits K3 and K9. Rabbits KD and K12 died accidentally during turnover studies. As the half-life was known, it was possible to calculate the amount of activity that should have been present in their bodies at the time of death (assuming the pool to be constant). The expected radioactivity was recovered in rabbits KD and K12–74% and 117%, respectively.

Chromatography of Bile Acids in the Stomach and Intestine. The nature of the bile acids present in the various parts of the gastrointestinal tract was studied qualitatively by paper chromatography and gas-liquid chromatography of purified bile acid fractions. In contrast to bile, the small intestine and stomach contained mainly free bile acids. In the large intestine and feces all bile acids were unconjugated. The same large amount of free deoxycholic acid in the small intestine was found in four rabbits of mixed stock eating green roughage and barley. In the rabbits studied to date, deoxycholic acid has always been the predominant bile acid in the stomach, small intestine, and large intestine.

TABLE 5. DISTRIBUTION OF RADIOACTIVITY RECOVERED IN THE BODY AFTER ADMINISTRATION OF C^{14}-LABELED DEOXYCHOLIC ACID

Rabbit No.	Days After Administration	Percentage of C^{14} Recovered in: Liver and Gall bladder	Stomach	Small Intestine	Large Intestine
K3*	3	26.1	12.8	31.0	27.9
K9	3	7.5	8.4	18.8	65.3
KD	30	2.7	14.7	17.5	65.1
K12	128	65.8	9.6	9.7	14.9

* Other organs and carcass (except skin) contained 2.2%.

Specific Activity of Deoxycholic Acid in the Stomach and Intestine. In order to study the equilibration of labeled deoxycholic acid within the pool, the specific activity of this bile acid was determined in different parts of the gastrointestinal tract in rabbits K3, KD, and K12. The paper chromatographic method was used for these determinations, and the results are shown in Table 6. The deoxycholic acid spot might contain small amounts of other compounds, which would increase the error of the method. Since there is no regular difference between the specific activities of the deoxycholic acid in the various organs, the variation between the values can, to a large extent, be ascribed to experimental error. Thus, equilibration of the administered isotope can be assumed to have occurred after 3 days. Unfortunately, it was not possible to determine the specific activity of the bile acids in the feces produced during one day.

Excretion of Bile Acids in Feces and Urine. The fecal excretion of deoxycholic acid and its metabolites was calculated, making the assumption that these acids had the same specific activity as that of the glycine-conjugated deoxycholic acid as isolated from bile samples. The total activity of extracts of feces collected during 3–4 consecutive days was divided by the mean specific activity of biliary deoxycholate during this period. To avoid errors due to incomplete equilibration of the isotope during the first few days, the values obtained during this time were not used. However, the amount of bile acids thus calculated was constant within the experimental error in samples collected intermittently during 1–2 months. A mean of 84% of the calculated daily production of deoxycholic acid was recovered in the feces as shown in Table 7.

TABLE 6. SPECIFIC ACTIVITY OF DEOXYCHOLIC ACID IN BILE AND IN DIFFERENT PARTS OF THE GASTROINTESTINAL TRACT AFTER INTRAPERITONEAL ADMINISTRATION OF LABELED DEOXYCHOLIC ACID

Rabbit No.	Days After Administration of Isotope	Specific Activity (cpm/mg) in: Bile*	Small Intestine	Large Intestine	Stomach
K3	3	5,430	5,100	6,350	6,740
KD	30	700	603	648	760
K12	128	230	242	182	166

* Calculated from the specific activity of glycodeoxycholic acid.

TABLE 7. COMPARISON BETWEEN THE DAILY PRODUCTION OF DEOXYCHOLIC ACID AND ITS METABOLITES CALCULATED FROM HALF-LIFE AND POOL SIZE AND THE MEASURED DAILY EXCRETION OF LABELED BILE ACIDS IN FECES

Rabbit No.	Calculated Production*	Calculated Fecal Excretion†	Percentage of Daily Production Recovered in Feces
	mg/day	*mg/day*	
KD	61.3	55.1	89.9
KL	54.5	50.6	92.8
K10	106.0	82.3	77.7
K14	89.9	80.5	89.6
K27	59.0	41.2	69.8

* Calculated from half-life of deoxycholic acid measured in bile samples and pool size, based on extrapolation of semilogarithmic plot back to zero time.

†Total daily radioactivity of fecal bile acid fraction divided by the specific radioactivity of deoxycholic acid isolated from bile.

After purification and methylation, the fecal bile acids were analyzed by gas chromatography. Several peaks were obtained, the relative proportions of which varied in different samples. The main peaks have been tentatively identified as the methyl esters of deoxycholic, 12-ketolithocholic, 3,12-diketocholanic, 3β,12α-dihydroxycholanic, lithocholic, and 3β-hydroxycholanic acids. A typical gas chromatogram of fecal bile acid methyl esters is shown in Figure 3. Further studies of these compounds will be the subject of future publications.

The excretion of bile acids in the urine was estimated in the same way as described for feces. The urinary excretion of isotope in four rabbits corresponded to 5.6, 7.0, 7.4, and 6.3 mg/day, respectively, of deoxycholic acid, representing 9.3, 10.0, 13.0, and 8.3%, respectively, of the daily production of deoxycholic acid in these rabbits.

A summary of the detailed findings in rabbit K27 is given in Figure 4, where the amount of bile acids removed by bile sampling is also shown. The reason for the unusually large discrepancy between the values for daily excretion and daily synthesis of deoxycholic acid in this animal is not known. The tendency for the calculated fecal bile acid excretion to decline with time is not representative for the rabbits studied, and may be

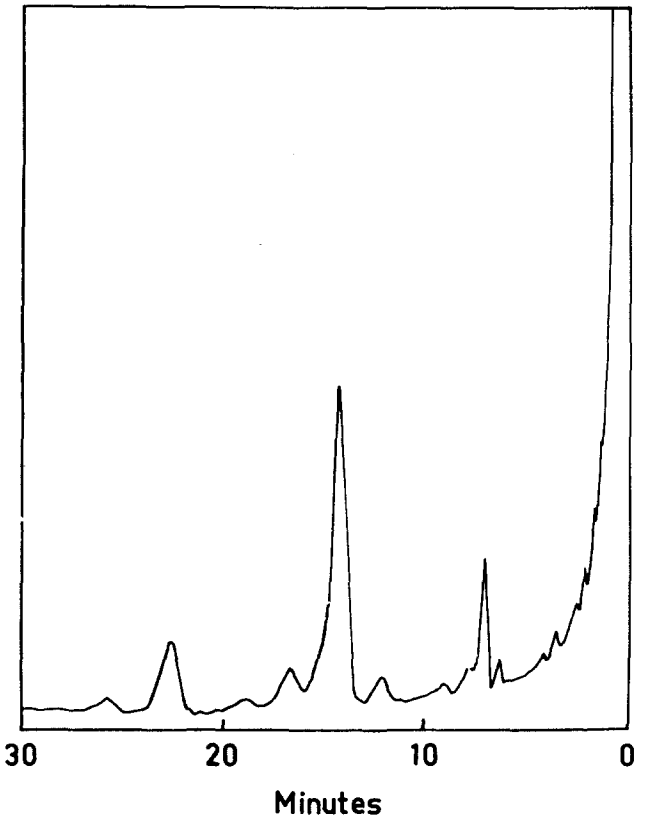

FIG. 3. Gas chromatography of methylated bile acids from a purified fecal extract. Conditions as in Figure 1. Main peak corresponds to methyl deoxycholate.

due to a slight systematic error in the determination of the turnover data of rabbit K27.

Pooled samples of urine were analyzed for deoxycholic acid. About 5–10 vol of ethanol were added and, after heating, the extract was filtered. The solvents were evaporated *in vacuo*, and the residue was dissolved in 2 N KOH and hydrolyzed at 110° for 6 hr. The hydrolysate was acidified and extracted with ether. After evaporation of the ether, the residue was subjected to reversed phase partition chromatography by using 55% methanol as moving phase and 10% heptane in chloroform as stationary phase (11). Most of the radioactivity appeared as a single peak with the elution volume of deoxycholic acid.

Analyses by paper chromatography showed a compound with the mobility of deoxycholic acid; after methylation, gas chromatography gave a peak with the retention time of methyl deoxycholate. In addition to deoxycholic acid, column and gas chromatography indicated the presence in the urine extract of 12-ketolithocholic acid. Further studies of the urinary bile acids have not been carried out.

Concentration of Bile Acids in Blood. The large urinary excretion of isotope prompted us to study the

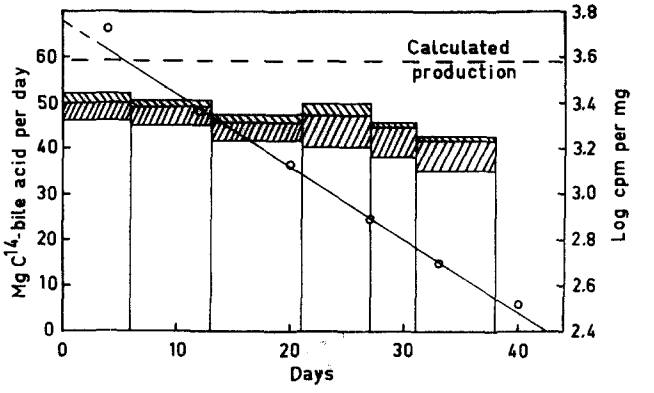

FIG. 4. Semilogarithmic plot of the specific activity of deoxycholic acid (O—O) and recovery in feces (□), urine (▨), and bile samples (▧) of isotope given to rabbit K27. Isotope excretion is calculated in equivalents of milligrams deoxycholic acid per day (see text).

bile acid concentration in blood. This concentration was calculated by dividing the total radioactivity in a blood sample by the specific activity of the deoxycholic acid in bile. The results are shown in Table 8. A mean value of 1.21 mg/100 ml of whole blood was obtained in seven rabbits.

DISCUSSION

Several methods have been described for the determination of bile acid excretion. These include titrimetric determination of partly purified fecal extracts (12, 13), determination of ultraviolet absorption in sulfuric acid before or after chromatographic purification (14, 15) and various isotope techniques (10, 16, 17).

In our opinion, the isotope techniques give the most reliable results. The quantitative isolation of the very complex mixture of partly unknown bile acids in feces is

TABLE 8. CONCENTRATION OF LABELED BILE ACIDS IN BLOOD EXPRESSED AS MILLIGRAMS OF DEOXYCHOLIC ACID PER 100 ML OF WHOLE BLOOD*

Rabbit	No. of Analyses	Mean Blood Concentration of Bile Acids	Range of Concentrations	Days Between Samples
		mg/100 ml	*mg/100 ml*	
KA	1	0.44	...	...
KF	1	1.94	...	...
KG	1	0.44	...	...
KL	1	2.24	...	...
K1	3	0.64	0.46–0.84	7–8
K2	3	0.80	0.26–1.72	6–8
K27	2	1.99	3.10–0.87	2

* Total radioactivity per 100 ml of blood divided by specific radioactivity of deoxycholic acid in bile determined at the same time.

extremely difficult. Furthermore, the qualitative bile acid composition is variable and depends on the intestinal flora and dietary conditions. At the present time, therefore, it is difficult to envisage an accurate method for quantitative determinations of the bile acids in feces. The use of labeled bile acids makes it possible to study the urinary excretion and to follow the efficiency of various extraction procedures. This is important, since Norman has shown that intestinal microorganisms can bind certain bile acids firmly (18). For this reason, the extraction of fecal bile acids cannot be properly estimated in conventional recovery experiments.

On the other hand, there are certain drawbacks to the isotope technique in the present investigation. Thus it was necessary to collect small bile samples at regular intervals, which was done by intubation through a stomach fistula. Judging from the results, the bile sampling and the presence of a closed gastric fistula do not seem to influence the metabolism of deoxycholic acid. Furthermore, certain assumptions regarding the bile acid turnover have to be made, as discussed by Lindstedt (10). We have tried, therefore, to evaluate the correctness of these assumptions by comparing the values for deoxycholic acid synthesis obtained by isotope dilution with the directly measured daily excretion of labeled bile acids. In most cases, the recovery of isotope in feces and urine corresponded to 80–90% of the daily synthesis of deoxycholic acid. In some cases (e.g., rabbit K27 [Fig. 4]), somewhat less activity was recovered. This may be due to several factors. The calculation of daily synthesis is based on the assumption that all of the injected isotope is absorbed and completely mixed with the pool; if this is not the case, the figures for pool size and daily synthesis will be too high. Some of the losses can be ascribed to incomplete sampling of feces and urine. Losses may also occur during extractions and isotope determinations (see Results). Small amounts of radioactivity are removed in the bile samples and it was difficult to avoid some leakage of stomach fluid containing bile acids during the bile sampling. No loss of activity through formation of labeled carbon dioxide was observed in the one rabbit examined.

The main bile acid in rabbits is deoxycholic acid, formed by the action of intestinal microorganisms on the cholic acid synthesized in the liver (19). This intestinal conversion takes place rapidly; normally, only small amounts of cholic acid (conjugated with glycine) are present in the bile. We have therefore chosen deoxycholic acid to study the bile acid turnover in the rabbit. One experiment was also carried out with cholic acid-4-C^{14} biosynthesized from cholesterol-4-C^{14} in bile fistula rats. The cholic acid was rapidly transformed into deoxycholic acid and the isotope was excreted at the same rate as when deoxycholic acid-24-C^{14} was used.

Small amounts of chenodeoxycholic acid might be formed from cholesterol in the rabbit (S. Lindstedt, private communication). This acid is rapidly transformed into other metabolites in the intestine, mainly lithocholic acid (20). Gas chromatograms of the methyl esters of fecal bile acids showed a peak with the retention time of methyl lithocholate. If this peak is methyl lithocholate, it would correspond roughly to 10–20% of the total fecal bile acids, and it would not be included in the bile acid production as studied with labeled deoxycholic acid.

The present investigation has shown considerable differences between the bile acid turnover in rabbits compared to that in rats and man. The size of the bile acid pool of the rabbit is about twice that of the rat (21) and about 4 times that of man (10) when calculated per kilogram of body weight. Using the same basis for calculation, the daily bile acid production is about the same in rats and rabbits and 2–3 times that in man. The mean value of the half-life of deoxycholic acid in rabbits was found to be 6.8 days, which is 2–3 times longer than the half-life of bile acids in normal rats and of cholic acid in healthy human subjects.

The distribution of bile acids in the gastrointestinal tract depends largely on the amount of bile acids present in the gallbladder. It is interesting to note that when the liver and gallbladder contain little bile acids most of the pool is present in the large intestine, and vice versa. In contrast to what is found in the rat, rabbits have about 10% of the bile acid pool in the stomach. Some glycine-conjugated deoxycholic acid was present, but the main part consisted of free deoxycholic acid. The presence of free bile acids in the stomach could possibly be due to the fact that rabbits eat part of their feces directly from the anus (22). In the small intestine, however, the bile acids were mainly unconjugated; part of the bile acids in the stomach might therefore come from regurgitated duodenal contents.

Rabbits excrete significant amounts of radioactivity in the urine after administration of labeled deoxycholic acid. The excretion corresponded to about 10% of the total daily deoxycholic acid production. The high urinary excretion might be explained by the fairly high concentration of bile acids in the blood. Twelve analyses of blood bile acids in seven rabbits showed concentrations between 0.26 and 3.10 mg/100 ml of whole blood. Using similar methods, we have found the concentration of cholic acid and its metabolites to be 0.055–0.128 mg/100 ml of whole blood in the rat (23), and Portman and Shah have found 0.16–0.53 mg of

cholic and chenodeoxycholic acids in 100 ml of monkey serum (24).

It is thus evident from the present investigation that the metabolism of bile acids in the rabbit differs in many respects from that of other species so far investigated. The possible relationship between these findings and the susceptibility of the serum cholesterol to dietary changes cannot yet be assessed.

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