In vivo evaluation of cholesterol 7α-hydroxylation in humans: effect of disease and drug treatment[1]

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Abstract 7α-Hydroxylation of cholesterol is a stereospecific reaction consisting of the replacement of the 7α-hydrogen with a hydroxyl group. When cholesterol labeled with tritium at the 7α position is administered, the hydroxylation of the substrate will result in the loss of tritium which in turn will label the body water. The rate of tritium enrichment of the body water could thus give a quantitative estimate of the hydroxylation rate. This study describes the validation of the procedure with some 21 studies performed on 15 subjects in different conditions. [7α-^{3}H]cholesterol was administered intravenously in 50 ml of plasma and thereafter blood was sampled at timed intervals for 4 to 5 days. The rate of the hydroxylation of cholesterol was calculated from the time course of the specific activities of plasma cholesterol and body water after tracer administration and was expressed as 7α-hydroxycholesterol formed/24 hr. Calculated values of hydroxylation in three control subjects (493 ± 206), five patients with hyperlipoproteinemia (539 ± 168), and seven cirrhotic patients (153 ± 136) are in good agreement with figures reported for bile acid synthesis determined with other techniques. Cholesterol 7α-hydroxylation rate is reduced in patients with cirrhosis, the impairment being related to the severity of the disease. Cholestyramine administered to one subject for 4 weeks produced a threefold increase of the hydroxylation. Administration of chenodeoxycholic acid resulted in a 50% decrease, whereas that of ursodeoxycholic did not produce consistent changes of the hydroxylation rate. The results support the current view that 7α-hydroxylation of cholesterol is rate-limiting in the synthesis of bile acids. The described procedure may constitute a useful tool for the study of bile acid synthesis regulation, especially in those situations where other techniques are not fully reliable.—**Bertolotti, M., N. Carulli, D. Menozzi, F. Zironi, A. Digrisolo, A. Pinetti, and M. G. Baldini.** In vivo evaluation of cholesterol 7α-hydroxylation in humans: effect of disease and drug treatment. *J. Lipid Res.* 1986. **27:** 1278–1286.

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Synthesis of bile acids, the main route of cholesterol removal from the body, is a complex process involving several changes of the steroid ring and of the side chain of the cholesterol molecule (1). Regulation of this process is ill-defined in humans mainly due to methodological problems. Indeed, techniques available for the study of bile acid synthesis under physiological conditions, either based on the isotope dilution principle (2) or on the balance technique (3) give only an indirect and overall estimation of synthesis. Attempts to evaluate the different steps of the pathway have usually been performed by studying the precursor–product relationship after administration of labeled precursors or by means of in vitro assay, where the responsible enzyme activity is measured in a specimen of liver tissue. These latter procedures give, at best, only relative estimates of the process which cannot "tout court" be extrapolated to the physiological situation. In this report we describe a new approach to evaluate in vivo the 7α-hydroxylation of cholesterol, a reaction that is considered to be the first and apparently rate-limiting step of the pathway of bile acid synthesis (4). The procedure is based on the principle first utilized by van Cantfort, Renson, and Gielen (5) to measure cholesterol 7α-hydroxylase activity in liver tissue. With the assumption that 7α-hydroxylation of cholesterol is a stereospecific reaction consisting in the replacement of the 7α-hydrogen with a hydroxyl group (6), the release of the hydrogen could give a measure of the rate of the reaction. Thus, by administering cholesterol tritiated at the 7α-position, tritium enrichment of the body water would give a quantitative estimate of the hydroxylation rate.

A similar approach has been already reported by Rosenfeld et al. (7) who employed [24,25-^{3}H]cholesterol to evaluate bile acid synthesis in vivo from the cleavage of the side chain of cholesterol.

The aim of the present study is to validate our procedure under some conditions known to be associated with changes of bile acid synthesis in order to assess the sensitivity of the method and to compare the results obtained

Abbreviations: CA, cholic acid; CDCA, chenodeoxycholic acid; HLP, hyperlipoproteinemia; TBW, total body water; UDCA, ursodeoxycholic acid.

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by evaluating the 7α-hydroxylation step with the data reported in the literature for overall bile acid synthesis.

PATIENTS AND EXPERIMENTAL DESIGN

A total of 21 studies were carried out on 15 patients admitted to the Istituto di Clinica Medica of the University of Modena. Three subjects in whom hepatic, intestinal, or lipid disorders had been excluded, were taken as controls; five patients had hyperlipoproteinemia, associated with diabetes and/or obesity; seven patients were cirrhotics presenting with different degree of severity of the disease, assessed according to a clinical scoring system (8). **Table 1** shows relevant clinical and laboratory data. All the patients entering the study were informed about the aim and the design of the study and gave their oral consent. The protocol of this study was approved by the Ethical Committee of the University of Modena. From the admission and throughout the study, the patients were given a standard diet adequate to keep their weight constant; it contained approximately 400 mg of cholesterol. With the exception of three cirrhotic subjects, who were on diuretic treatment, no patient had been on medication for at least 15 days before entering the study. Seven to 10 days after admission, the patients, fasting overnight, were infused intravenously with 300 to 400 μCi of [7α-^{3}H]cholesterol. The tracer, in alcoholic solution, was passed through a Millex 0.22 μm filter and dissolved in 50 ml of homologous plasma. Blood samples were taken, in heparinized tubes, before the infusion and subsequently at timed intervals for 4 to 5 days in most of the studies. Two to 3 weeks after the end of the study all the patients were administered 50 μCi of tritiated water in order to evaluate total body water (TBW). All blood samples were centrifuged at 3000 rpm and, after separation of cells, the plasma was stored at −20°C and the erythrocyte fraction was stored at 4°C until assayed.

The whole body radiation exposure due to tritium administration, calculated according to the equation of Marinetti (9), was about 50 mRad.

Six subjects had the study repeated after one of the following treatments. Cholestyramine was given to one control subject, complaining of unexplained pruritus, at a dose of 12 g/day for 4 weeks; chenodeoxycholic acid (CDCA) was given to three subjects with HLP at a dose of 15 mg/kg per day for 4 weeks; ursodeoxycholic acid (UDCA) was given to one control and one HLP subject at a dose of 10 mg/kg per day for 4 weeks. In the subjects receiving bile acids, the compliance to the treatment was checked by evaluating the composition of bile acid pool at the end of the treatment period. After CDCA treatment, the percentage of the administered bile acid was nearly 80%; whereas in the case of UDCA, this bile acid enriched the pool by approximately 50%. No side effects were reported by the treated subjects and treatment was not discontinued during the period of the second study.

MATERIALS

[7α-^{3}H]Cholesterol (sp act 12 mCi/mmol) was prepared following the procedure of Corey and Gregoriou (10).

TABLE 1. Clinical data of the subjects

Subject	Sex/Age	Weight		Cholesterol[b]	Triglycerides[b]	Diagnosis
		Kg	% Ideal[a]			
				mg/dl		
1	M 59	72	105	251	145	Gastritis
2	F 45	73	129	249	151	Pruritus
3	M 43	60	91	166	160	Arthritis
4	M 38	132	168	520	750	HLP type III
5	M 41	70	107	304	620	HLP type IIb
6	M 47	71	114	405	585	HLP type IIb
7	M 46	86	130	235	725	HLP type IV
8	M 60	87	116	214	238	HLP type IV
9	M 57	87	120	210	105	Cirrhosis
10	M 53	56	82	183	97	Cirrhosis
11	M 67	49	86	124	98	Cirrhosis
12	M 60	53	98	132	126	Cirrhosis
13	M 57	70	114	118	48	Cirrhosis
14	M 57	80	112	132	58	Cirrhosis
15	M 49	54	88	104	69	Cirrhosis

[a] Calculated with the formula: {B.W.(kg)/[height (cm) − 100]} × 100. Such a value overestimated the weight of those cirrhotics who had ascites.
[b] Mean of three values obtained throughout the whole period of the study.

Tritium labeling of the 7-position of the steroid nucleus was checked by subjecting [7α-^{3}H]cholesterol acetate to chromic oxidation in acetic acid (11); the 7-keto cholesterol acetate did not retain any radioactivity. Specificity of tritium labeling at the 7α position was subsequently controlled by subjecting the synthesized tritiated cholesterol to bromidation with N-bromo succinimide (12). The 7α-bromocholesterol retained 30–35% of the radioactivity, presumably at 7β, so that, by difference, tritium label at the 7α position was calculated to be 65 to 70%. This figure was taken into account when the specific activity of cholesterol was used to calculate the rate of its hydroxylation from the tritium that was released. Since about ⅓ of the radioactive label was not in the 7α position (and presumably was located in 7β) an appreciable amount of [7β-^{3}H]-labeled bile acid could be produced. To quantify the specific activity of the newly synthesized bile acid, a separate experiment was conducted on a healthy subject, who underwent naso-duodenal siphonage 72 hr after the injection of 390×10^6 cpm of [7α-^{3}H]cholesterol. The bile acids were extracted with diethyl ether and isolated by thin-layer chromatography; aliquots were then counted for radioactivity (see below) and assayed for bile acid concentration. Bile acid specific activity was calculated to be 1390 cpm/mg. After purification of the compound with chromatography on silicic acid and repeated crystallization in methanol, the radioactivity ratio 7α/7β did not change. Following the recommendation of Davidson et al. (13), radiochemical reliability of our tritiated cholesterol preparation was tested both in vitro and in vivo against [4-^{14}C]cholesterol. The ^{3}H/^{14}C ratios were between 0.92 and 0.95. Tritiated water (sp act 5 μCi/ml), [4-^{14}C]cholesterol (sp act 50–60 mCi/mmol) were purchased from Amersham, U.K. CDCA and UDCA were supplied by Gipharmex S.p.A. (Milano, Italy) as 300-mg capsules. Cholestyramine was a commercially available product (Questran, Bristol Co., Italy). All the reagents were analytical grade and purchased from Carlo Erba (Milano, Italy).

METHODS

Determination of plasma free and esterified cholesterol specific activity

Plasma samples were extracted as described by Folch, Lees, and Sloane Stanley (14) with 10 volumes of chloroform–methanol 2:1 (v/v) and the extracts were evaporated to dryness. An aliquot of the residue, dissolved in benzene, was then applied on a thin-layer silica gel plate and developed in hexane–diethylether–acetic acid 80:20:1 (v/v/v). The spots identified as free and esterified cholesterol were scraped off, eluted with chloroform–methanol and filtered. The extracts were again evaporated and processed as follows: the samples containing free cholesterol were dissolved in 3 ml of benzene, half of this was assayed for cholesterol according to Abell et al. (15) and half was counted for radioactivity. The samples containing esterified cholesterol, after adding alcoholic KOH, were incubated for 12 hr at 37°C in order to hydrolyze the esters and then were extracted with petroleum ether and evaporated to dryness. The residues were taken up in 3 ml of benzene and processed as for free cholesterol.

Evaluation of body water tritium

Erythrocyte samples were distilled with an apparatus similar to that described by Hutton, Tappel, and Udenfriend (16) and measured aliquots of distilled water were counted for radioactivity.

Counting

Water and cholesterol radioactivity was measured with a Packard 3320 counter using Picofluor 15 (Packard Instruments, Downers Grove, IL) as scintillation mixture. Quenching was corrected for by the external standard channel ratio method.

Calculation of cholesterol 7α-hydroxylation rate

Calculation of the rate of hydroxylation, based on the release of tritium, was essentially the same as that used by Rosenfeld et al. (7) to calculate bile acid synthesis from the tritium released during the cleavage of the side chain of [24,25-^{3}H]cholesterol. The rate was calculated as follows: increase of ^{3}H in body water (cpm)/specific activity of plasma cholesterol (cpm/mg) at a given time interval.

The increase in tritium was read off the curve of tritium enrichment as a function of time (**Fig. 1**). The time interval chosen to calculate the increase of tritium was that from 60 to 72 hr after the administration of cholesterol. Hydroxylation rate was expressed as the amount of 7α-hydroxycholesterol formed in 24 hr.

RESULTS

In Fig. 1, depicting a typical experiment, is shown the time course of the specific activity of plasma free and esterified cholesterol and body water.

In the initial few hours after tracer, a sharp increase of water tritium was observed. The radioactivity of free cholesterol reached a peak (at about 12 hr) while that of cholesteryl esters rose more slowly. Thereafter the specific activity of free cholesterol decreased whereas that of esterified cholesterol increased in an almost mirror-image fashion until 36 to 48 hr when both fractions started to decline approximately with the same specific activity. From this time on there was an increase of water tritium

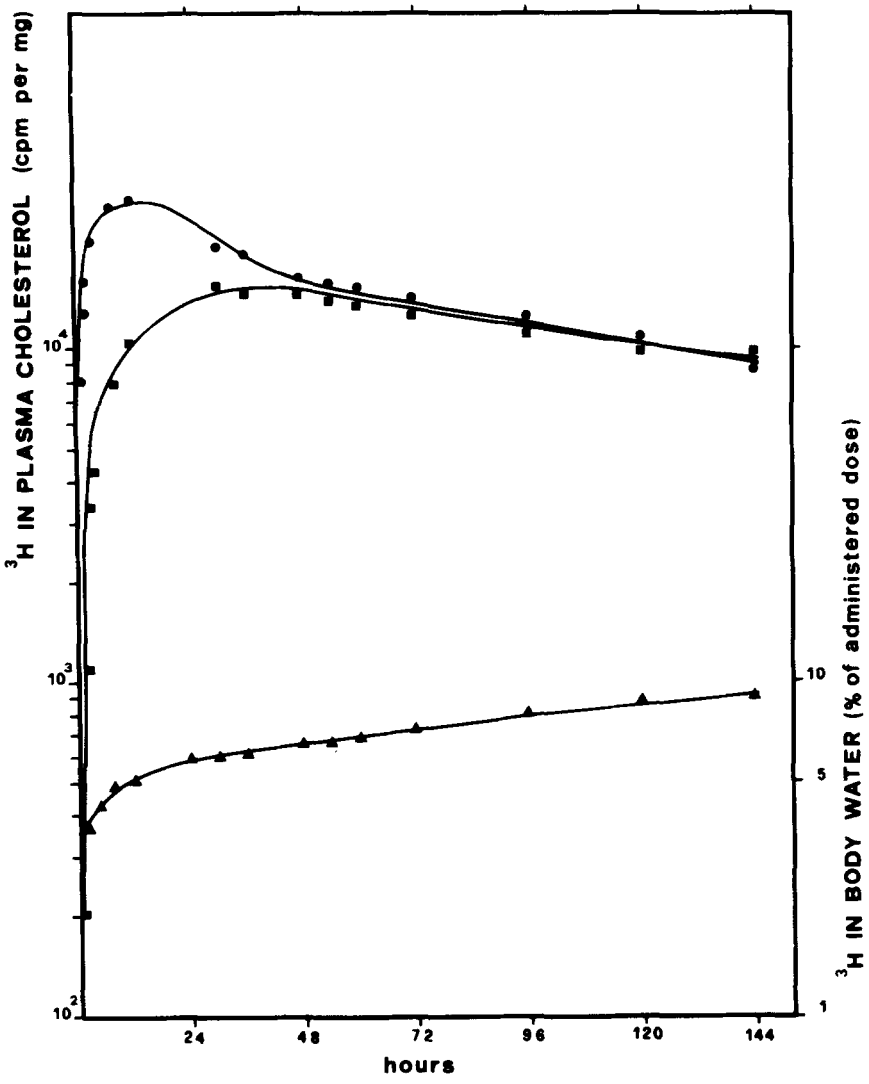

Fig. 1. Typical experiment (patient 6 after CDCA treatment) showing the time course of the specific activity of plasma free (●) and esterified (■) cholesterol and of body water (▲) after intravenous infusion of [7α-^{3}H]cholesterol.

for the duration of the experiment which in most of the cases was 96 hr.

In **Table 2** are reported the individual figures of cholesterol specific activity, the increment in total body water radioactivity, and the calculated values of cholesterol 7α-hydroxylation rate in all the subjects. It can be seen that HLP patients had hydroxylation values similar to those of the controls.

In general, cirrhotics displayed a lower rate of hydroxylation, the mean value being about half that of noncirrhotic subjects. As illustrated in **Fig. 2,** some curves of tritium enrichment of the cirrhotic group overlapped the area of control values and this could be due to the different extent of liver impairment. In fact, when hydroxylation values are plotted against the severity of the disease, an inverse relation is evident between the two parameters (**Fig. 3**). Administration of cholestyramine to one subject resulted in a threefold increase of the hydroxylation rate, whereas treatment with different bile acids had disparate effects (**Table 3**). CDCA produced a significant reduction of 7α-hydroxylation in all three treated subjects, the mean posttreatment value being half the basal value, whereas the changes induced by UDCA were not consistent.

DISCUSSION

This study has shown first that, by using an adequate tritiated substrate, it is possible to obtain quantitative estimates of cholesterol 7α-hydroxylation in humans under physiological conditions; second, that the observed

TABLE 2. Calculated values of cholesterol 7α-hydroxylation rate in all subjects

Subject/Administered Radioactivity	Δ^3H_2O[a]	Cholesterol Sp Act[b]	7α-OH Cholesterol Formed[c]
cpm × 10⁻⁶	*cpm × 10⁻³*	*cpm/mg*	*mg/24 hr*
Controls			
1 408.29	1592	16097	295
2 391.02	1916	8096	706
3 416.81	2251	14075	477
			Mean ± SD 493 ± 206
Hyperlipoproteinemia			
4 333.44	734	3859	567
5 381.12	991	9149	323
6 419.40	2013	12916	465
7 349.73	1757	9466	554
8 408.02	2367	9000	785
			Mean ± SD 539 ± 168
Cirrhosis			
9 437.97	1518	13007	348
10 427.41	1487	14000	317
11 322.22	767	11539	198
12 337.46	344	9439	109
13 349.87	91	8424	32
14 431.91	95	8860	32
15 337.46	192	15076	38
			Mean ± SD 153 ± 136

[a] In this column is indicated the tritium increment in body water observed in a 12-hr interval (from 60 to 72 hr after injection of radioactivity).

[b] The values represent the mean of specific activity of cholesterol obtained at 60 and 72 hr. This figure must be multiplied by 0.67 (% of specific labeling at 7α position) for the calculation of 7α-OH cholesterol formed.

[c] This value is obtained multiplying by 2 the value observed in the 12-hr interval.

changes of the hydroxylation rate, either associated to disease or induced by drug treatment, are parallel to those reported to occur for total bile acid synthesis. This suggests that the rate-limiting step in bile acid biosynthesis cannot be subsequent to 7α-hydroxylation. If we assume from the literature that 7α-hydroxylation is the first committed step in the pathway, we can conclude that our results, even if indirectly so, are consistent with the rate-limiting role of 7α-hydroxylation in the synthesis of bile acids.

Major requisites to the use of our substrate for the evaluation of bile acid synthesis are that tritium label be located at the designated site of the cholesterol molecule with a high and known specific activity and that it be biologically stable. Our synthesis of [7α-^{3}H]cholesterol yielded a compound with nearly 70% of the label located at the 7α-position. Such a figure is similar to that obtained by others (5) and found suitable for the measure of cholesterol 7α-hydroxylase activity in vitro. In any event, a measurable amount of labeled bile acid, presumably with tritium in the 7β position, could be formed during the 7α-hydroxylation step. Subsequent bacterial degradation, such as dehydroxylation at the 7 position and 7-keto formation, could cause release of tritiated water thus leading to an overestimation of the production rate of bile acids. To quantitate this possible source of contamination, we calculated the specific activity of ^{3}H-labeled bile acid 72 hr after the infusion of [7α-^{3}H]cholesterol in a healthy subject (see Materials). The value obtained was 1390 cpm/mg of bile acid. In other words, assuming a bile acid pool size of 4 g, about 5.5×10^6 cpm of ^{3}H should be present in the total bile acid pool at that time point. Björkhem et al. (17) recently evaluated, in human subjects, the extent of oxidoreduction of different hydroxyl groups in bile acids, by contemporary administration of [^{14}C]carboxy compounds and [3β-^{3}H]-, [7β-^{3}H]-, or [12β-^{3}H]-labeled bile acid (17). In their experiments, the decrease of the $^3H/^{14}C$ ratio, after the first 12 hr, was rather slow; in the case of [7β-^{3}H]cholic acid, the fractional catabolic rate equalled 0.05 pools/day. When we apply that figure to our data, it comes out that only about 140,000 cpm of tritiated water would be released in the 12-hr time interval considered for our evaluation, that is, less than 8% of the increment observed in the radioactivity of total body water. Thus, we concluded that this potential cause of overestimation should not interfere significantly with the evaluation of 7α-hydroxylase activity.

Radiochemical reliability of our compound as compared to ^{14}C-labeled cholesterol was shown to be satisfactorily adequate for the study.

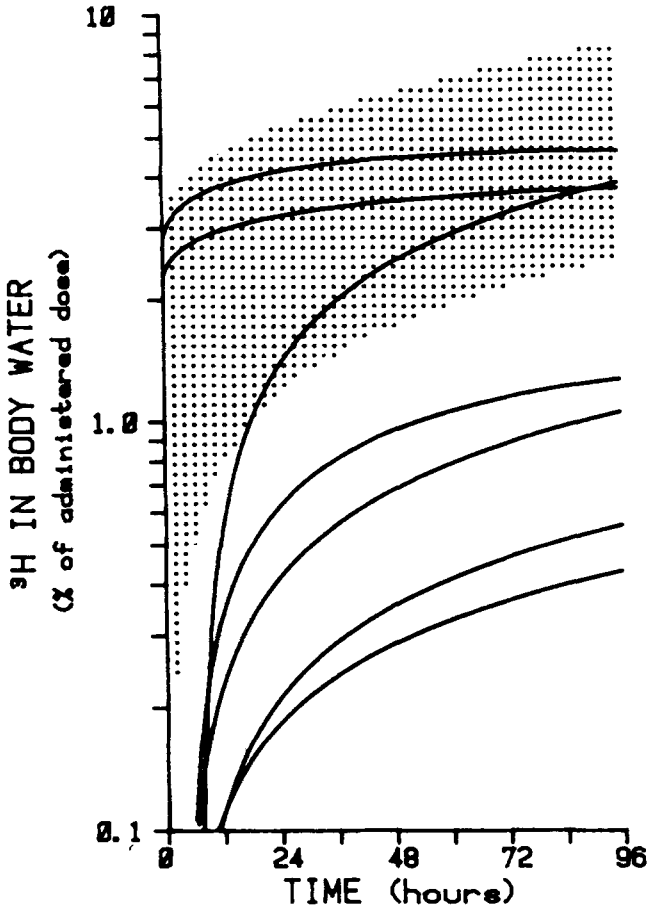

Fig. 2. Individual curves of tritium enrichment of body water in the cirrhotic patients, after infusion of [7α-^{3}H]cholesterol. The shaded area is derived from the curves of noncirrhotic subjects. Some curves from cirrhotic patients are within the limits of the control area.

Two more sources of error should be considered when calculating bile acid synthesis with our method. First, the released tritium could be recycled and thus contribute to the de novo synthesis of cholesterol. This would slow down the decay of the specific activity of the administered tritiated compound, thus leading to an underestimation of 7α-hydroxylation rate. Second, part of the administered substrate could be diverted for the synthesis of compounds (steroid hormones) other than bile acids, contributing to enhance the decay of the specific activity of plasma cholesterol. This would in turn lead to an overestimation of bile acid synthesis. As discussed by Rosenfeld et al. (7) in their report on the use of cholesterol labeled on the side chain for the evaluation of bile acid synthesis, the order of magnitude of such errors was so low that they could be neglected in the calculation.

Moreover, our technique employs cholesterol labeled in position 7, a position that is not involved in the several hydroxylation–dehydrogenation steps of the metabolic pathway of steroid hormones (18). Thus, in our experimental conditions, we can safely assume that tritium enrichment of body water does reflect the rate of 7α-hydroxylation of the administered substrate.

Assuming that all the synthesized 7α-hydroxycholesterol is committed to the synthesis of bile acids, the calculated values of the compound formed in 24 hr in the control and HLP subjects altogether are within the range of value reported for bile acid synthesis in man (19). Variations of these values could also be due to the technique used; thus it has been reported that fecal acidic steroid output overestimates, whereas the isotope dilution underestimates, bile acid synthesis (20). Our values of 7α-hydroxycholesterol are in the higher region of the range of bile acid synthesis in man, but whether or not our procedure tends to overestimate synthesis cannot be stated from the present study. Work is in progress to obtain comparative figures of synthesis by using our method and the more standardized techniques.

However, our procedure has been shown to be sensitive enough to detect small changes of the hydroxylation rate in different situations. Thus, in the group of cirrhotics, a good correlation was observed between the reduction of 7α-hydroxylation of cholesterol and the impairment of liver function as assessed by a scoring technique. These results are in keeping with the data reported, in patients with cirrhosis, on the synthesis of cholic acid evaluated by means of the isotope dilution technique (8).

Of the many abnormalities of bile acid metabolism observed in patients with cirrhosis, the one that has attracted more attention is the defective synthesis of primary bile acids, mainly attributable to cholic acid since chenodeoxycholic acid is less affected (21). Our data demonstrate

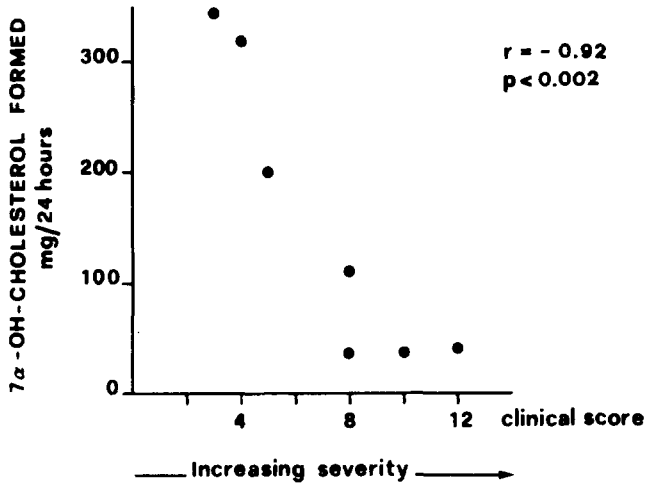

Fig. 3. Cholesterol 7α-hydroxylation in cirrhosis. Relation between severity of the disease and the rate of hydroxylation. The points represent individual patients.

TABLE 3. Effect of drug treatment on cholesterol 7α-hydroxylation

Treatment	Subject/Administered Radioactivity	Δ^3H_2O[a]	Cholesterol Sp Act[b]	7α-OH Cholesterol Formed[c]	% Changes
	cpm × 10⁻⁶	*cpm × 10⁻³*	*cpm/mg*	*mg/24 hr*	
Cholestyramine	2 Before 391.02	1916	8096	706	
	After 400.20	5283	7592	2077	+193
Chenodeoxycholic acid	6 Before 419.40	2013	12916	465	
	After 403.51	1412	14452	291	−37
Chenodeoxycholic acid	7 Before 349.73	1757	9466	554	
	After 374.54	805	9672	248	−55
Chenodeoxycholic acid	8 Before 408.02	2367	9000	785	
	After 410.63	903	10192	264	−66
Ursodeoxycholic acid	1 Before 408.29	1592	16097	295	
	After 339.17	1390	13025	318	+8
Ursodeoxycholic acid	5 Before 381.12	991	9149	323	
	After 395.98	776	7913	292	−9

[a,b,c] See legend to Table 2.

that the impairment of 7α-hydroxylation of cholesterol is correlated with, and presumably due to, the reduced liver cell mass.

Since 7α-hydroxycholesterol is precursor to both cholic and chenodeoxycholic acid, the reduction of its synthesis, were this the sole defect, should affect both primary bile acids equally. Since this is not the case, it seems that possibly multiple defects, including the 7α-hydroxylation step, at different levels of the pathway could be responsible of the preferential defect of cholic acid synthesis (22–24).

Work on laboratory animals such as the rat has led to the presently accepted view that the 7α-hydroxylation of cholesterol is the rate-limiting step in the pathway from cholesterol to bile acids and that it is feed-back-inhibited by bile acids returning to the liver (4, 25). This undoubtedly may hold true also for humans (26), but information on the relation between overall bile acid synthesis and 7α-hydroxylation of cholesterol is very limited in man.

In this study the administration of cholestyramine to one subject produced a threefold increase of the hydroxylation rate. A possible source of error should be considered in conditions of stimulated sterol synthesis. As observed by Redinger, Chow, and Grace (27) in bile fistula primates, bile acid synthesis evaluated with $^{14}CO_2$ breath analysis was greatly underestimated. This is probably due to the preferential utilization, especially in conditions of stimulated bile acid synthesis, of newly synthesized microsomal cholesterol with which the tracer could mix incompletely. Even when our results are in close agreement with data obtained with the isotope dilution method (19), we cannot exclude that our technique could have underestimated, to some extent, the rate of 7α-hydroxylation under these conditions. It is apparent anyway that the interruption of the enterohepatic circulation does derepress the synthesis of bile acids. However, the mechanism controlling the responsible enzyme, the microsomal cholesterol 7α-hydroxylase, is poorly defined. This enzyme activity, when assayed on microsomal preparations from liver of rats or humans fed bile acids, has been shown to be either depressed (28–30) or unchanged (31, 32). In addition, recent work, by several groups, on isolated cells has failed to demonstrate inhibition of bile acid synthesis by naturally occurring bile acids (33–35), thus casting some doubt that bile acids themselves are the ultimate effectors of the feed-back inhibition and suggesting that other regulatory mechanisms, including availability of microsomal cholesterol (36), might well be of importance. Whatever the responsible molecular mechanism of the feed-back control, it seems that not only the size but also the composition of the bile acid pool influences bile acid synthetic rate. Our results, although derived from a limited number of subjects, would suggest that enrichment of the bile acid pool with chenodeoxycholic acid depresses the 7α-hydroxylation of cholesterol whereas enrichment with UDCA has no effect.

These data are in line with some recent reports on the different effects of CDCA and UDCA feeding on bile acid synthesis evaluated with different procedures.

Thus Nilsell et al. (37) using the isotope dilution technique reported that UDCA feeding increases the synthesis of CA and CDCA, respectively, by 80% and 40%.

Hardison and Grundy (38) by means of balance technique found that the administration of UDCA does not influence bile acid synthesis. Finally, von Bergmann, Epple-Gutsfeldt, and Leiss (39) reported that UDCA depresses bile acid synthesis at a lesser extent than CDCA.

More in accord are the results showing a significant reduction of CA synthesis during CDCA administration (37, 39–41). Differences between studies might be due to the different procedures used to investigate bile acid synthesis, but the overall trend seems to point to a major

effect of CDCA, as compared to UDCA, on the bile acid synthetic rate. Certainly more data are needed to define whether bile acid structure, in particular the α or β conformation of the hydroxyl groups, has any direct bearing on the regulation of bile acid synthesis or whether bile acid structure affects synthesis by changing hepatocyte cholesterol metabolism.

The use of an in vivo technique to evaluate the 7α-hydroxylation of cholesterol, such as the one described here, should overcome the limitation shared by all the in vitro measurements and provide useful information on the regulation of bile acid synthesis, especially in those conditions, e.g., bile acid feeding, where other techniques seem to be not fully reliable. Moreover, the figures of total bile acid synthesis calculated with our procedure are comparable to those obtained with major methods such as isotope dilution or fecal acidic sterol balance. The figure of merit of our method as compared to others is difficult to assess. Certainly the number of assumptions to be made or the technical difficulties are not greater than in other methods. As for the discomfort to the patient, repeated blood sampling seems to be better tolerated than duodenal intubation.

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