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Effects of D- and L-triiodothyronine and of propylthiouracil on the production of bile acids in the rat*

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SUMMARY

The influence of D- and L-triiodothyronine (DT_3 and LT_3) and propylthiouracil (PTU) on turnover rate and pool size of bile acids in rats on a normal diet has been investigated. No significant difference was observed between half-lives of bile acids in normal, thyroid hormone-treated, or PTU-treated rats. All thyroid hormone-treated rats, however, had a chenodeoxycholic acid (CDC) pool that was 2–3 times greater (means ranging between 6.9 and 10.2 mg) than that of normal rats (mean 3.2 mg). The mean size of the cholic acid (C) pool in DT_3-treated (13.5 mg) and LT_3-treated (16.6 and 13.5 mg) rats was similar to that of normal rats (12.5 mg), which means that the total bile acid pool (C + CDC) tended to be increased. The size of the total bile acid pool in the PTU-treated rats (mean 16.3 mg) was very similar to that of untreated rats (mean 15.7 mg). When daily production of bile acids (C + CDC) was calculated from the values of turnover rate and pool size, it was found that normal rats had an average synthesis of 4.9 mg bile acids per day, while the production for the LT_3- and DT_3-treated rats averaged about 8.0 mg/day. This tendency towards increased total bile acid production in the thyroid hormone-treated rats was mainly due to a 2- to 3-fold increase in the daily synthesis of CDC. The PTU-treated rats, on the other hand, had a mean daily bile acid synthesis of 4.7 mg, which is very similar to that found in normal rats.

The thyroidal state is known to affect cholesterol metabolism in many species (review [1]). Thus, surgical or chemical thyroidectomy (I^{131} or thiouracil) in most animals is accompanied by an elevation of serum cholesterol level, while administration of thyroid hormones has the opposite effect. In recent years, it has been shown that different analogues of the natural iodothyronines (e.g., the optical isomers [D-isomers] of the natural L-thyroxine and L-triiodothyronine [LT_3][1]) have hypocholesterolemic action with only very minor calorigenic effects (review [2]). However, this dissociation has also been demonstrated in man when low doses of the natural thyroid hormones were used (3).

Furthermore, it has been repeatedly demonstrated that hyperthyroidism generally stimulates the rate of cholesterol biosynthesis both in vivo and in vitro, while the converse is true for the hypothyroid state (1). The lowered serum cholesterol and increased cholesterol synthesis in the blood-liver compartment generally has been explained by assuming a stimulation by thyroid hormones of the processes of cholesterol degradation and excretion. Studies by several workers (4, 5, 6) have shown that the output of biliary cholesterol is increased in hyperthyroid rats, while it is decreased in hypothyroid ones. Of the daily production of cholesterol in rats only about 20–30% is excreted as neutral sterols, while 70–80% is excreted as bile acids (7, 8), mainly cholic and chenodeoxycholic acid. Circumstantial evidence has been obtained indicating that thyroid hormones enhance the degradation of cholesterol to bile acids in intact animals. Thus, for example, Weiss and Marx (9), after intravenous administration of cholesterol-4-C^{14}, found an increased fecal excretion of both neutral and acidic labeled products in hyperthyroid mice when compared to normal ones. In bile fistula rats, administration of thyroid hormones (including D-triiodothyronine [DT_3][1]) reverses the normal ratio of about 4:1 between cholic (C) and

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[1] Abbreviations used are 3,5,3′-triiodo-L-thyronine, LT_3; 3,5,3′-triiodo-D-thyronine, DT_3; propylthiouracil, PTU. LT_3 (Cytomel Sodium) and DT_3 (S.K.F. No. D-2623) were generous gifts from Smith Kline and French Laboratories, Philadelphia, Pennsylvania.

TABLE 1. PERCENTAGE DISTRIBUTION OF RADIOACTIVITY RECOVERED IN THE RAT BODY AFTER ADMINISTRATION OF A C^{14}-LABELED BILE ACID

Rat No.	Days After Administration	Percentage Distribution of Recovered C^{14} in Rat Body				Total Percentage Recovery of Administered C^{14}*
		Liver	Small Intestine	Cecum	Large Intestine	
N 1	9	5	74	8	13	89
N 3	8	6	78	10	6	100
LT_3I 4	6	4	78	16	2	85
LT_3II 2	6	3	86	8	3	85
LT_3II 5†	6	3	78	10	9	100
DT_3 1	6	2	83	9	6	86
PTU 2	10	6	71	16	7	90

* Includes the radioactivity excreted in feces plus that found in gastrointestinal tract and liver in the killed rats.
† Given C^{14}-chenodeoxycholic acid. In all other cases, distribution of C^{14}-cholic acid is shown.

chenodeoxycholic acid (CDC) (10, 11, 12). However, the total bile acid production is of the same magnitude in euthyroid and thyroid hormone-treated bile fistula rats. In hypothyroid bile fistula rats, the C/CDC ratio is about 9:1 and the total bile acid output is significantly decreased as compared to that of normal fistula rats.

Conclusions concerning the quantitative bile acid picture in intact rats should be drawn, however, with great caution from data obtained in bile fistula rats. In the latter animals, the enterohepatic circulation of bile acids is broken, leading to a highly unphysiological state with a 10- to 20-fold increase in the daily synthesis of bile acids (13). It seemed of interest, therefore, to make a detailed study of the rate of bile acid synthesis in *intact* rats treated with D- and L-triiodothyronine or propylthiouracil (PTU[1]). This has been done by means of an isotopic tracer technique in rats on a normal diet and assumed to be in a steady state with respect to cholesterol turnover.

MATERIALS AND METHODS

Animals. White male rats of the Sprague-Dawley strain weighing 180–250 g were used. They were kept in individual metabolic cages for 2 weeks before the hormone administration started. They were fed a commercial rat diet ad libitum (Anticimex, Stockholm, Sweden).

Five groups of rats were included: N (normal), LT_3I (40 μg LT_3/kg/day), LT_3II (200 μg LT_3/kg/day), DT_3 (40 μg/kg/day), and PTU (0.5% of diet as propylthiouracil). The hormones were solubilized in slightly alkaline saline and given in daily subcutaneous injections. Normal and PTU-treated rats were given daily injections of saline alone of the same alkalinity and volume as the injections given the thyroid hormone-treated rats. After 14 days of treatment for normal and thyroid hormone-treated rats and 30 days for PTU-treated rats, the oxygen consumption was measured according to Tomich and Woollett (14). The results are expressed as liters of oxygen consumed/kg body weight/hr.

Determination of Bile Acid Turnover. After measurement of O_2 consumption, about 1 mg of sodium cholate-24-C^{14} (4.6 μc/mg) or (in two rats) sodium chenodeoxycholate-24-C^{14} (4.0 μc/mg) (15) was given intraperitoneally in physiological saline. The feces then were collected in 24-hr fractions for 6–10 days, after which time the rats were killed by a blow on the neck and the small intestine, cecum, and large intestine, including their contents, and the liver were excised. The fecal samples were homogenized in water with an "Ultra Turrax" homogenizer (Janke and Kunkel, KG, Staufen i. Br., W. Germany) to a relatively thin liquid pulp, which was heated for 2–3 days in an oven (80°) until it was quite dry. It was then ground to a homogeneous dry powder, 100–200 mg of which was combusted in a modified Schöninger flask according to Kelly et al. (16) for determination of C^{14} as $C^{14}O_2$. The CO_2 was absorbed in ethanolamine-methylcellosolve as described by Jeffay and Alvarez (17) and measured in a Packard Tri-Carb scintillation spectrometer. Combustion of known amounts of cholic acid-C^{14} added to inactive fecal powder always gave 95–100% recovery of the radioactivity, showing the accuracy of this method for determination of C^{14}-labeled bile acids in the feces of rats. The excised small intestine, cecum, and large intestine, plus their contents, and the liver were homogenized and extracted three times with 80% ethanol by refluxing each time for 2 hr. The C^{14} content of an aliquot of the extracts was determined as above after evaporation of the solvent. Total recovery of administered radioactivity ranged from 75 to 100% (Table 1).

The half-life of cholic and chenodeoxycholic acid was read from the plot of $-\log (1 - u_t/u_{max})$ vs time when $u_t/u_{max} = 0.5$ as described by Lindstedt and Norman (18); u_t = cumulative amount of activity excreted at time t, and u_{max} = total amount of activity recovered in liver, intestines, and feces.

Determination of Bile Acid Pool. When the rats were killed, they had excreted 70–90% of the injected C^{14}-labeled bile acid via the feces. The main part of the retained activity (70–90%) was confined to the small intestine (Table 1). The extract from the small intestine, after evaporation of solvent, was saponified in N KOH for 6 hr at 120° in sealed steel tubes. The free bile acids were extracted twice with ether after acidification of the saponification mixture with HCl. The ether extracts were washed with distilled water to neutrality and evaporated. They were then made up to constant volume with ethanol, and the isotopic recovery of the extraction was determined in aliquots by the above-mentioned dry combustion method after evaporation of solvent. The content of cholic acid (C) and chenodeoxycholic acid (CDC) was determined in the saponified extracts from small intestine by quantitative paper chromatography (19, 20). Determinations were carried out in triplicate and mean values calculated. The values for C and CDC were corrected for isotope losses during the extraction procedure to give the C and CDC pools within small intestine. As about 10–30% of the total cholic acid pool in the rat has been shown to reside within the cecum and to be absorbed via the portal circulation (21, 22), the approximate amounts of C and CDC plus their metabolites in cecum were calculated on the basis of isotopic distribution. Assuming that the specific radioactivity of the bile acids is the same in cecum and the small intestine by the time the rats are killed, and that the ratio CDC plus metabolites/C plus metabolites in the cecum is the same as the CDC/C ratio in the small intestine, one can calculate the amounts of C (or CDC if that is the labeled compound given) plus its metabolites in the cecum by simply dividing total cecal radioactivity by the specific radioactivity of small intestinal C. The amount of CDC plus its metabolites is then obtained by multiplying the value for the C pool thus obtained by the known CDC/C ratio of small intestine. The specific activity of C (or CDC) was never directly measured in this study. It is known, however, that only a minor fraction of the small intestinal bile acids are microbiological metabolites (21). Therefore, the approximation was made that all of the radioactivity in small intestine is accounted for by unchanged labeled bile acid (C or CDC), the quantity of which was measured. The values of specific activity so obtained will be too high to the extent that metabolites of C (or CDC) account for small intestinal radioactivity and should give minimal pools of C and CDC plus their metabolites in the cecum if the first two assumptions are correct.

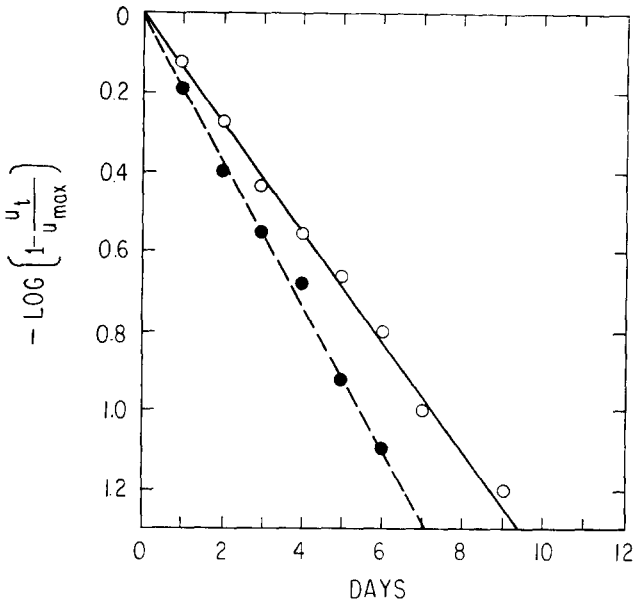

FIG. 1. Semilogarithmic plot of elimination of cholic acid in two representative experiments (see text for details of method). (O——O), normal rat No. 2; (●- - - -●), DT_3-treated rat No. 4.

RESULTS

Oxygen Consumption. As demonstrated in Table 2, the normal rats (N) had a total body oxygen consumption of about 1.50 liters/kg/hr. Administration of LT_3 increased the consumption according to the dose given. Rats in group LT_3I (40 μg/kg/day) showed an increment in oxygen consumption of about 20% while LT_3II rats (200 μg/kg/day) showed an increase of 60–70%. DT_3 given in doses of 40 μg/kg/day did not lead to any significant elevation of oxygen consumption as determined by this method. The PTU-treated rats showed a decrease in oxygen consumption of 30–40% as compared to the normal rats.

Half-Life of C and CDC. In Fig. 1 are shown two representative semi-logarithmic plots of the elimination of cholic acid showing a straight-line relationship with time. Only minor variations in turnover rate of cholic acid were found among rats of the different groups (see Table 2). The untreated rats showed a half-life for cholic acid of 1.7–2.9 days, with a mean of 2.3 days. The rats treated with LT_3 had mean half-lives of almost the same magnitude as the control animals (mean $t_{1/2}$ = 2.1 and 2.2 days for LT_3I and LT_3II, respectively), while the DT_3-treated rats had a somewhat

TABLE 2. TURNOVER RATE, POOL SIZE, AND DAILY PRODUCTION OF BILE ACIDS IN RATS

Group		Oxygen Consumption	Half-Life[a]	Pool Size of C[b]			Pool Size of CDC[b]			Daily Production of C[f]	Daily Production of CDC[f]	Total Daily Production (C + CDC)
				Small Intestine[c]	Cecum[d]	Total[e]	Small Intestine[c]	Cecum[d]	Total[e]			
		liter/kg/hr	*days*	*mg*	*mg*	*mg*	*mg*	*mg*	*mg*	*mg*	*mg*	*mg*
Rat No.	1	1.43	2.9	13.9	1.5	15.4	4.2	0.4	4.6	3.7	1.1	4.8
	2	1.41	2.3	5.1	2.8	7.9	1.2	0.6	1.8	2.4	0.5	2.9
	3	1.58	1.7	11.5	1.5	13.0	0.9	0.1	1.0	5.3	0.4	5.7
N	4	1.56	2.0	11.7	2.4	14.1	3.5	0.7	4.2	4.2	1.5	6.4
	5	1.44	2.5	10.7	1.6	12.3	4.0	0.6	4.6	3.4	1.3	4.7
Mean		1.48	2.3	10.6	1.9	12.5	2.8	0.5	3.2	3.9	1.0	4.9
	1	2.13	2.2	15.0	1.4	16.4	7.7	0.7	8.4	5.2	2.6	7.8
	2	1.50	2.0	17.0	0.9	17.9	4.8	0.2	5.0	6.2	1.7	7.9
LT_3I	3	1.75	2.0	20.6	1.5	22.1	4.8	0.7	5.5	7.7	1.9	9.6
	4	1.75	2.2	8.7	1.1	9.8	8.2	1.0	7.2	3.1	2.9	6.0
Mean		1.78	2.1	15.3	1.3	16.6	6.4	0.6	7.0	5.6	2.3	7.9
	1	2.53	2.0	7.5	0.4	7.9	9.4	0.5	9.9	2.7	3.4	6.1
	2	2.23	2.3	9.0	0.4	9.4	10.5	0.5	10.9	2.9	3.3	6.2
	3	2.27	2.2	6.6	0.1	6.7	13.1	0.2	13.3	2.1	4.2	6.3
LT_3II	4	2.70	2.2	15.0	1.2	16.2	7.0	0.6	7.6	5.1	2.4	7.5
	5	2.61	1.8	21.0	2.5	23.5	10.0	1.2	11.2	9.0	4.3	13.3
	6	2.60	2.0	15.9	1.6	17.5	7.8	0.8	8.6	6.1	3.0	9.1
Mean		2.49	2.1	12.5	1.0	13.5	9.6	0.7	10.3	4.7	3.4	8.1
	1	1.51	1.7	14.0	1.4	15.4	6.6	0.7	7.3	6.3	3.0	9.3
	2	1.41	1.8	15.2	2.6	17.8	3.3	0.6	3.9	6.8	1.5	8.3
DT_3	3	1.58	1.7	10.1	1.5	11.6	6.6	1.0	7.6	4.7	3.1	7.8
	4	1.56	1.6	7.7	1.5	9.3	7.5	1.4	8.9	4.0	3.9	7.9
Mean		1.52	1.7	11.8	1.7	13.5	6.0	0.9	6.9	5.5	2.9	8.4
	1	1.07	2.1	7.8	3.6	11.4	4.4	0.4	4.1	3.6	1.3	4.9
PTU	2	1.07	2.3	9.3	1.5	10.8	2.8	1.3	2.1	3.2	0.6	3.8
	3	0.98	2.8	14.1	1.4	15.6	1.8	0.3	4.8	3.9	1.2	5.1
Mean		1.04	2.4	10.4		12.6	3.0	0.7	3.7	3.6	1.0	4.6

[a] Half-life estimated for C except for LT_3II rats, No. 5 and 6, where half-life of CDC was measured.
[b] C = cholic acid; CDC = chenodeoxycholic acid.
[c] Determined by quantitative paper chromatography.
[d] Includes metabolites of C and CDC, respectively. Calculated as described under Methods.
[e] Sum of pools in small intestine and cecum.
[f] Calculated as described in Results. Assumed that half-life for C and CDC are the same.

more rapid turnover of cholic acid, with a mean value for $t_{1/2}$ of 1.7 days, the values ranging between 1.6 and 1.8 days. The PTU-rats showed a mean $t_{1/2}$ value of 2.4 days. In two rats of the LT_3II group (see Table 2), the turnover rate of chenodeoxycholic acid was determined. Figure 2 shows the semi-logarithmic elimination plot for chenodeoxycholic acid in one of these rats. Values for $t_{1/2}$ of 1.8 and 2.0 days were obtained, confirming the previous observation (18) that the turnover rate of cholic and chenodeoxycholic acid are of about the same magnitude.

Pool Size and Distribution of C and CDC. In Table 1 is shown the distribution of radioactivity in liver and gastrointestinal tract at the time of killing in some typical rats. It also gives the total recovery of injected radioactivity, which in all rats was in the range 75–100%.

In agreement with previous reports on normal rats (22), about 70–90% of the total bile acid pool is found in the small intestine and 10–30% in the cecum, the small remainder being distributed between large intestine and liver. This was true for all groups of rats and applied to both C and CDC pools.

In Table 2 are given values of pool size for C and CDC in the small intestine and cecum plus their metabolites in cecum using the methods of determination and calculation already described. The total bile acid pool in this study constitutes the sum of the C and CDC pools in small intestine and cecum. In the normal rats, a mean value for the total C pool of 12.5 mg and for the CDC pool of 3.2 mg was found. In the rats treated with LT_3 and DT_3, the mean values for the total C pool were similar to those for normal rats. There was, however, great individual variation within both the normal and the treated groups. The average total CDC pool of the thyroid hormone-treated rats was in all cases higher than that of normal rats. Thus DT_3 and LT_3I rats had an average total CDC pool of 6.9 and 7.0 mg, respectively, while the LT_3II rats had a mean pool size of 10.2 mg as compared to 3.2 mg in the normal rats.

Thus, it was found that treatment with LT_3 or DT_3 tended to increase the size of the bile acid pool (C + CDC) over that found in normal rats (average increase of 40–50% in this study). This enlargement was mainly due to a 2- to 3-fold increase of the CDC pool.

On the other hand, the three PTU-treated rats had

C and CDC pools of about the same size as those found in normal rats, as is evident from Table 2.

Daily Production of C and CDC. With knowledge of the pool size and half-life for C, one can calculate the daily synthesis of this compound by dividing the value for the size of the C pool by the turnover time, $\bar{T}$. The turnover time (i.e., the time for renewal of the pool) is calculated through the relation $\bar{T} = t_{1/2}/\ln 2$ (cf. 23). The values for production of C so determined give an average daily synthesis of 3.9 mg for normal rats, which is in agreement with previous determinations in normal rats (24). The thyroid hormone-treated rats had a mean daily production of about the same magnitude (see Table 2).

As mentioned above, the half-life of CDC in two LT_3II rats was determined and proved to be of the same order of magnitude as that of C, which is in agreement with earlier reports on normal rats (18). In order to evaluate the daily production of CDC, it seemed justifiable, therefore, to assume that the half-life of CDC is the same as that of C in the same rat. Knowing the pool size of CDC, its daily synthesis can then be calculated. The figures so obtained give an average daily production for CDC of 1.0 mg in normal and PTU-treated rats, 2.3 mg in LT_3I rats, and 3.4 and 2.9 mg in LT_3II and DT_3 rats, respectively. Thus, treatment with LT_3 and DT_3 increases the production of CDC in rats 2- to 3-fold as compared to normal rats.

The total daily bile acid production in rats is the sum of the daily synthesis of C and CDC. For normal rats, this production was found to have a mean value of 4.9 mg, while all the thyroid hormone-treated rats had mean values of about 8.0 mg (see Table 2). There is, however, a considerable variation within each group for values of daily bile acid production and the number of animals in each group is small. However, the tendency of the thyroid hormones to increase the bile acid synthesis is apparent in all groups given LT_3 or DT_3. The three PTU-treated rats had a mean daily bile acid synthesis of 4.6 mg or almost the same production as normal rats.

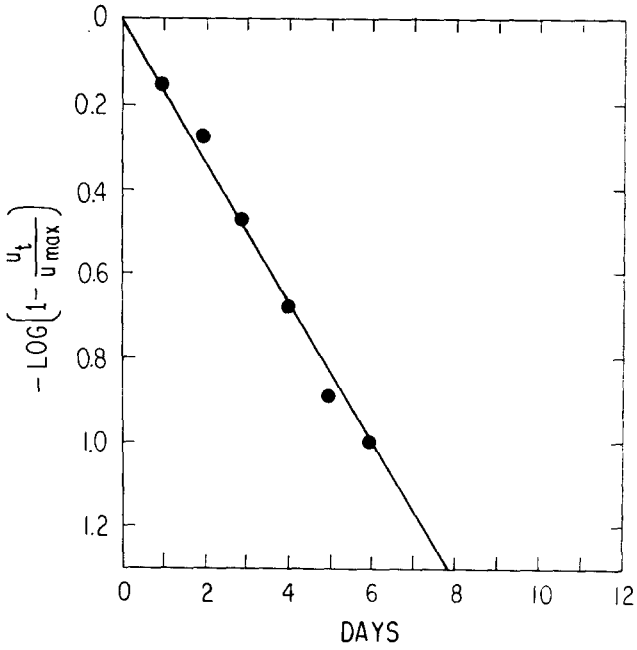

FIG. 2. Semilogarithmic plot of elimination of chenodeoxycholic acid in rat No. 5 of group LT_3II.

DISCUSSION

The aim of the present investigation was to evaluate the daily production of cholic and chenodeoxycholic acid in the intact rat under the influence of LT_3, DT_3, and PTU treatment. It has previously been demonstrated that practically all of the bile acids in rats are excreted via the feces. The bile acids are profoundly altered by fecal microorganisms as they pass through the cecum and large intestine, giving rise to a very complex mixture of bile acids, some as yet not identified (21). It is, therefore, an extremely difficult task to find a method that allows a specific and quantitative determination of the fecal bile acids. Until such a method has been developed, some type of isotope technique to determine bile acid production probably gives the most reliable results. In this study, both half-life and pool size of bile acids were determined by injecting a tracer dose of C^{14}-labeled acid after 14 days of hormone treatment. The turnover rate was estimated by following the rate of fecal excretion of labeled bile acids. By killing the rats after about 80% of the labeled bile acids had been excreted, the remaining radioactivity could be used for determination of the bile acid pool by means of an isotopic dilution technique. Knowing the turnover rates and pool sizes of the bile acids, their daily synthesis could then be calculated. The total circulating bile acid pool as determined in this study was the sum of C and CDC in small intestine and cecum plus their cecal metabolites. No absorption of bile acids has been shown to take place from the remaining part of the large intestine, and the bile acids in that part should therefore not be included in the circulating bile acid pool, which is assumed to be dynamically homogeneous. The part of the circulating bile acid pool not included in this study is that in the liver and bile ducts, which constitutes only a small percentage of the total pool (see Table 1). The present method for determining the cecal bile acid pool rests on the assumption that the specific radioactivity of the injected bile acid and its metabolites in the cecum is the same as that for the un-

metabolized labeled bile acid in the small intestine at the time the rats are killed. Since the pool has been estimated to circulate about 10 times/24 hr (25), this should certainly be the case after 6–10 days of enterohepatic circulation. The specific activity of intestinal bile acids was not directly measured, and the values for specific activity used to calculate the cecal pools are probably too high by about 10–20%, representing the fraction of small intestinal metabolites of C and CDC (21), thus giving minimal values for the cecal bile acids. The calculation of this pool rests on several assumptions. As the cecal bile acids, however, represent only about 10–30% of the total bile acid pool, even an error in their determination of, for example, ±20% will give a maximal error of only ±6% in the value of the total bile acid pool, and it is felt that the determination of the small intestinal bile acid pool (C and CDC) is accurate.

The main difference with regard to bile acid formation between normal and thyroid hormone-treated rats found in this study was a 2- to 3-fold increase of the CDC pool of the latter animals, tending to increase the total bile acid pool. This was true for all thyroid hormone-treated rats, including the group given DT_3 in an amount that gave no significant increase of total body oxygen consumption. As there were no great differences between normal and treated rats in the turnover rate of bile acids, this means that the daily production of CDC is increased in the thyroid hormone-treated rats as compared to untreated rats. Although there was overlapping between the normal and thyroid hormone-treated groups, this increase in CDC production gave an average increase in total bile acid synthesis of 50–60% in the latter groups. It should be stressed, however, that the values given for total daily production of bile acids are dependent upon the validity of certain assumptions and approximations. This, together with the variation found between individual rats regarding pool sizes and total daily production of bile acids, necessitates cautious interpretation of differences in these parameters between the groups.

An increased CDC synthesis has been observed earlier in bile fistula rats given thyroxine, LT_3, or DT_3 (10, 11, 12). In these rats, the normal ratio between C and CDC of about 4:1 is almost reversed, while there is no change in total bile acid production as compared to normal rats. This means that the synthesis of C is actually substantially diminished in the bile fistula rats while that of CDC is correspondingly increased.

In the intact thyroid hormone-treated rats of the present study, there was a mean C/CDC ratio of about 3:2 as compared to 4:1 in the normal rats, and the mean synthesis of C was unchanged or slightly increased in all the treated groups in contrast to what happens in thyroid hormone-treated bile fistula rats.

The hypothyroid PTU-treated rats showed about the same half-life, pool size, and daily production of bile acids as the normal rats. This is in marked contrast to the condition in bile fistula rats, where the total daily output of bile acids drastically drops in PTU-treated rats and the C/CDC ratio increases somewhat (10, 11). However, the output of bile acids in hypothyroid bile fistula rats during the first 6–12 hr after the fistula operation, when the endogenous bile acid pool is excreted, is of about the same magnitude and has about the same C/CDC ratio as that found in the normal fistula rats. This finding is thus compatible with the unchanged bile acid pool found in the intact PTU rats. The pronounced difference between normal and propylthiouracil-treated bile fistula rats is obvious as early as the second day after fistula operation and onwards, when the hypothyroid rats only produce about 15–30 mg bile acids per day as compared to 70–80 mg per day in the normal fistula rats. The data of the present investigation thus further stress that results obtained from bile fistula rats should be applied with great caution to intact rats with a normal enterohepatic bile acid circulation.

Whether the changes of bile acid metabolism brought about by the thyroid hormones are of importance for the effect of these hormones on the serum cholesterol level cannot yet be assessed. Further studies of the intimate mechanisms whereby this level is regulated are required in order to evaluate the influence of cholesterol degradation on these processes. It should be pointed out, however, that most of the studies of the influence of thyroid hormones on the serum cholesterol level in rats have used animals given an atherogenic diet. In this study, the rats were given a normal diet in order to study the action of thyroid hormones *per se* on bile acid metabolism; the results are not necessarily relevant to the situation in rats on an atherogenic diet where a mixed hormonal-dietary influence on the bile acid metabolism is conceivable. Supporting this idea is a recent report(26) indicating that dietary cholesterol stimulates the rate of bile acid excretion in rats.

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