The Effects Of 3,5-diiodothyroacetic And 3,3', 5-triiodothyroacetic Acids On The Time Course Of Steroid C14 Metabolism In The Rat

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THE EFFECTS OF 3,5-DIODO THYROACETIC AND
3,3', 5-TRIODO THYROACETIC ACIDS ON THE TIME
COURSE OF STERIOD C\textsuperscript{14} METABOLISM IN THE RAT\textsuperscript{*}\textsuperscript{†}

W. T. BEHER AND G. D. BAKER

It is well known that the thyroid hormones have important effects on steroid metabolism. Thus, elevated blood cholesterol concentrations are found in hypothyroidism. Conversely, the administration of thyroid hormones in cases of hypercholesteremia results in lowered blood cholesterol concentrations. Unfortunately, the use of thyroid hormones in euthyroid patients results in intolerable side effects, such as aggravated angina pectoris, tachycardia, and possible precipitation of myocardial infarction. A number of compounds (thyroid hormone analogs) similar in structure to L-thyroxine and L-triiodothyronine have been synthesized which lower blood cholesterol concentrations but have relatively small calorigenic effects. Although these substances may not be ideal, since they tend to produce side effects in certain patients, their mechanism of action is of interest and has not been clarified.

It has been assumed that the analogs act in a manner analogous to L-thyroxine and L-triiodothyronine which increase the output of fecal sterols and perhaps bile acids.\textsuperscript{1} The effects of thyroid hormones on bile acid excretion is controversial. Weiss and Marx\textsuperscript{2} observed that hyperthyroid mice excreted more radioactivity in the “acidic fraction” than hypothyroid animals, following cholesterol-4C\textsuperscript{14} administration. On the other hand, Erikson\textsuperscript{3} and Strand\textsuperscript{4} observed changes in the ratios of chenodeoxycholic and cholic acids excreted in hyperthyroid, euthyroid and hypothyroid bile fistula rats, during thyroid treatment, but the total amounts excreted in hyper- and euthyroid rats was about the same.

More recently, Strand\textsuperscript{5} has found an increase in the excretion of chenodeoxycholic acid in intact rats after administration of D or L-triiodothyronine but could detect no increase in cholic acid excretion. The data, unfortunately, are quite variable, so it is hard to assess the magnitude of the effect. Since no data were given on sterol excretion, its quantitative significance is unclear. A new approach to this problem was suggested by the investigations of Ruegamer and Silverman\textsuperscript{6} who showed that thyroid-analog-treated rats eliminated liver cholesterol much more rapidly than normal.

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\textsuperscript{†}A preliminary report was presented before the Council on Arteriosclerosis of the American Heart Association, October 1963.
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animals. We have utilized a variation of this approach in the experiments reported in this paper. Rats with accumulated tissue cholesterol-4C\(^{14}\) were treated with 3,5-Diiodothyroacetic (Diac) or 3,3',5-Triiodothyroacetic (Triac) acids and the rates and pathways of steroid C\(^{14}\) elimination determined and compared with the same parameters in normal animals.

METHODS

Thirty-two adult female albino Wistar strain rats were maintained for three weeks on a diet consisting of ground Rockland rat ration supplemented with 1% cholesterol-4-C\(^{14}\) (7.07 \(\mu\)c/100 g diet) and 0.5% cholic acid. At the end of the sterol-C\(^{14}\) build-up period, eight of the rats were killed to establish peak tissue sterol-C\(^{14}\) concentrations. The remaining rats were switched to unsupplemented Rockland rat ration for two days to clear the intestinal tract of unabsorbed cholesterol-4-C\(^{14}\). After this period the rats were divided into three equal groups, placed in individual metabolism cages, and fed the following diets ad lib: (a) rat ration, (b) rat ration supplemented with Diac (5 mg/100 g), and (c) rat ration supplemented with Triac (5mg/100 g). Feces, urine, and carbon dioxide were collected daily from each rat for two weeks. For tissue studies the rats were killed after the last collection and samples of serum, small intestine, kidney, lung, liver, and carcass removed for cholesterol and \(\beta\) sterol-C\(^{14}\) determinations. Fecal sterol-C\(^{14}\) and bile acid-C\(^{14}\) were assayed by a modification of the method of Siperstein. The feces were dried by lyophylization, ground and extracted continuously for 48 hours with boiling ethanol. The extract was evaporated to dryness and the residue autoclaved with 7N NaOH, at 15 lbs. pressure for 3 hours to effect hydrolysis of triglycerides, cholesterol esters, and bile acid conjugates. The alkaline hydrolysate was extracted 4 hours with petroleum ether (30-60\(^\circ\)) to remove \(\alpha\) and \(\beta\) sterols. The residue was then acidified and again extracted with petroleum ether to remove fatty acids. The acidic aqueous residue was then continuously extracted for 12 hours to isolate the “acidic fraction” which contains bile acids. The petroleum ether fractions containing \(\alpha\) and \(\beta\) sterols and the “acidic fraction” were plated on cup planchets and counted. The samples of small intestine, kidney, lung, liver, and whole carcasses were digested with KOH and the nonsaponifiable fraction extracted with petroleum ether. \(\beta\) sterols were isolated by precipitating with digitonin and cholesterol determined in the digitonide by application of the Lieberman-Burchard reaction. Tissue \(\beta\) sterol-C\(^{14}\) activity was assayed by plating the digitonide by the filtration tower technique followed by windowless gas flow counting. Serum cholesterol was determined according to Sperry and Webb. Serum \(\beta\) sterol-C\(^{14}\) was assayed as the digitonide. Urine samples were assayed for steroid-C\(^{14}\) by direct plating in cup planchets followed by counting. For carbon dioxide determinations, the animals were placed in all glass metabolism cages, and the expired carbon dioxide collected in carbonate-free NaOH solution (8 g/150 ml). The carbonate was precipitated with barium chloride, dried and weighed, or plated for counting.

RESULTS AND DISCUSSION

In Fig. 1 the various bar heights are proportional to cholesterol concentrations in the tissues. The black dots in the build-up bars represent control levels. It is obvious that, on a concentration basis, by far the largest accumulation of cholesterol was in
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Figure I

Tissue cholesterol concentrations of various tissues.

liver. Serum, kidney, and lung showed small, but significant increases, while small intestine and carcass remained essentially at control levels. The serum cholesterol concentration doubled, but in control rats had the lowest concentration observed in any of the tissues. It would seem that the bulk of the fecal steroids excreted during regression originated in the liver. There is, of course, evidence that some sterol may be synthesized and turned over in extrahepatic tissues in cholesterol fed animals. If the amounts of regression in controls and in Diac and Triac treated animals are compared, it is evident that after two weeks, both analogs completely reversed liver cholesterol accumulation, while controls still had elevated concentrations. In other tissues the cholesterol concentrations after two weeks were about equal in control and treated groups. It should be remembered that tissue sterol regression rates could have been quite different since only the terminal concentrations were determined. Figure II contains information about the total sterol-C\textsuperscript{14} content of several tissues at various points in the experiment. The sterol pools contained various total amounts of \( \beta \) sterol-C\textsuperscript{14} at the end of build-up. The liver, as expected, contained by far the largest amounts of activity. Small intestine and carcass, which accumulated little or no sterol during the build-up, were labeled showing that sterol exchange is active in these tissues. At the end of regression, total sterol-C\textsuperscript{14} was significantly lower in liver and serum of Diac and Triac treated rats than in controls. In other tissues the analogs had little or no effect on the final liver of sterol-C\textsuperscript{14} as is evidenced by comparison with controls.

Further information with respect to tissue sterol-C\textsuperscript{14} metabolism can be obtained by a study of the specific activities of the \( \beta \) sterol-C\textsuperscript{14} pools (Table I). The decreases in specific activity coupled with decreases in tissue cholesterol concentrations (Fig. 1)
can be interpreted to mean that newly synthesized \( \beta \) sterols are taking the place of sterol-C\(^{14} \), in a given tissue. Since such decreases are especially evident in small intestine, this must indicate that this tissue is synthesizing cholesterol very rapidly under conditions which block cholesterol synthesis in liver. Triac appears to increase the synthesis rates. On the other hand, the kidneys appear to have a very slow rate of cholesterol synthesis and exchange, since the decreases in sterol-C\(^{14} \) specific activities are not significant at the end of the regression intervals. Lung, carcass, and serum more or less follow the pattern of liver, indicating rapid exchange and a slow rate of synthesis.

**Table I**

Tissue \( \beta \) Sterol Specific Activities

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Build-up c/min/mg ( \beta ) sterol</th>
<th>Control Regression c/min/mg ( \beta ) sterol</th>
<th>Triac Regression c/min/mg ( \beta ) sterol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>2091±77</td>
<td>1773±120</td>
<td>1416±252</td>
</tr>
<tr>
<td>Carcass</td>
<td>1179±94</td>
<td>880±100</td>
<td>655±154</td>
</tr>
<tr>
<td>Small intestine</td>
<td>1700±106</td>
<td>990±156</td>
<td>662±205</td>
</tr>
<tr>
<td>Kidney</td>
<td>1632±43</td>
<td>1535±150</td>
<td>1281±246</td>
</tr>
<tr>
<td>Lung</td>
<td>2152±76</td>
<td>1561±98</td>
<td>1000±400</td>
</tr>
<tr>
<td>Serum</td>
<td>2300±200</td>
<td>1530±150</td>
<td>1015±403</td>
</tr>
</tbody>
</table>
These findings confirm and extend those of Lindsey and Wilson\(^\text{16}\) to intact rats. The data presented thus far indicate that Diac and Triac in some way accelerate the mobilization of accumulated \(\beta\) sterol in certain tissues. The major effect is limited to the liver pool.

Two major and several minor pathways account for final elimination of tissue sterols in mammals. Fecal sterols and bile acids are major, while urinary steroid and sebum lipids represent the main minor pathways. We have investigated the effects of Triac and Diac on the rates of elimination of fecal sterol-C\(^{14}\), urinary steroid-C\(^{14}\), and respiratory C\(^{14}\)O\(_2\) during the two weeks regression. The curves in Fig. 3 show that the rate of elimination of fecal sterol-C\(^{14}\) was accelerated by Diac and Triac. On the other hand, the rate of elimination of Bile Acid-C\(^{14}\) was much the same in the control and treated animals. It could be argued that Diac and Triac accelerated the rate of liver cholesterol synthesis and thus diluted the Bile Acid-C\(^{14}\) pool with nonlabelled bile acid, thus masking any accelerating effect of Diac and Triac on bile acid-C\(^{14}\) excretion. However, the data in Table I show that even after two weeks the liver cholesterol C\(^{14}\) specific activity was only moderately lower than that at build-up. This indicates that very little cholesterol synthesis took place in this organ during regression. Since liver cholesterol concentrations were elevated during most of the regression period, this could be expected.\(^9\) The effects of Diac and Triac on the excretion of urinary steroid-C\(^{14}\) are shown in Figure 4. Here the two thyroid analogs caused increased amounts of C\(^{14}\) activity to be excreted via this pathway. From a quantitative point of view this is a minor effect. No C\(^{14}\)O\(_2\) could be detected in the expired carbon dioxide in any of the groups during the entire experiment. If the data are considered \textit{in toto}, it is obvious that both Diac and Triac accelerated
the rate of elimination of accumulated cholesterol by increasing the rate of its elimination via fecal sterols and urinary steroids. Quantitatively, the largest effect is on fecal sterol elimination. One must be cautious in applying these data as an explanation of the effects of the analogs in normal animals, since metabolism of accumulated sterol may differ from sterol metabolism at normal concentrations. Further experiments will be necessary to establish whether any differences do occur, and whether the rate changes account for the effects on blood cholesterol concentrations.

**SUMMARY**

The effects of 3,5-Diiodothyroacetic and 3,3',5-Triiodoacetic acids (Diac and Triac) on the mobilization of accumulated tissue $\beta$ sterol in rats were studied. Diac and Triac had similar effects: rapid increases in rates of hepatic $\beta$ sterol-$C^{14}$ mobilization. The effects on extrahepatic mobilization rates varied from tissue to tissue. Both thyroid analogs double the excretion rate of fecal $\alpha$ and $\beta$ sterol-$C^{14}$, but neither affected fecal bile acid-$C^{14}$ excretion. Urinary $C^{14}$ excretion was moderately elevated in the treated rats. No detectable $C^{14}$ dioxide was expired by any of the rats at any time.
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