THE INFLUENCE OF THYROID ACTIVITY ON THE LIVER 
AND PLASMA LIPIDES OF CHOLINE- AND 
CYSTINE-DEFICIENT RATS

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The effects of choline deficiency on liver lipides have been well established 
and the variations in plasma lipides encountered in clinical hypo- and hyperthyroid states have been thoroughly studied. With the recent finding 
that hypothyroid animals do not exhibit the hepatic damage which results 
from chronic choline deficiency in normal rats (1) it was thought of interest 
to determine the influence of thyroid activity on the plasma and liver lipides 
of both choline- and cystine-deficient animals.

EXPERIMENTAL

The subjects of this study were male rats of the Vanderbilt strain (2). 
After weaning they were grown to 100 gm. on a stock ration and then housed 
in individual cages and fed the experimental diets. The basal diet for all 
groups was prepared as follows: casein 10, sucrose 59, cotton seed oil 10, 
lard 15, cod liver oil 2, salts (3) 3, cholesterol 0.5, and inositol 0.3. In addition, 
to each kilo of diet were added thiamine hydrochloride 3 mg., riboflavin 
5 mg., pyridoxine 3 mg., calcium pantothenate 50 mg., niacin 500 mg., 2-
methyl-1,4-naphthoquinone 3 mg., α-tocopherol 50 mg., and p-aminobenzoic acid 100 mg. All rats received 5 γ each of both folic acid and biotin 
twice weekly by pipette. The following materials were incorporated in 
the various diets, as specified in Tables I and II, at the expense of an equiva-

tent amount of sucrose: choline chloride 0.6, cystine 0.5, thiouracil 0.3, desic-
cated whole thyroid powder (Armour) 0.2, 0.3, and 0.4 per cent. Hyper-
thyroidism was produced by feeding thyroid powder and hypothyroidism 
both by thiouracil feeding and by total thyroidectomy performed under 
ether anesthesia when the rats weighed 80 gm. A number of thyroidectomy 
ized animals are not included in Tables I and II, as they never properly 
recovered from the surgical procedure and failed to eat or grow adequately.

At the conclusion of the experiments the animals were mildly anesthetized 
with nembutal given intraperitoneally, the jugular vein was exposed by 
proper dissection, and 0.1 ml. of heparin solution (Lederle) was administered 
intravenously. About 1 minute later, the animals were exsanguinated by

1 Handler, P., and Follis, R. H., Jr., in preparation.
bilateral carotid section and the blood collected in a small porcelain dish. By this procedure one may consistently obtain 6 to 8 ml. of blood from a 150 gm. rat and proportionately more from larger animals. The blood was immediately centrifuged and the plasma saved for analysis. The rats' livers were removed, weighed, and transferred to a bottle containing an alcohol-ether (3:1) mixture.

The analytical procedures were similar to those previously employed but with some modification. The livers were transferred to the container of a Waring blender and ground with 8 ml. of alcohol-ether for each gm. of liver. The suspension was transferred to a beaker and refluxed for 5 minutes. A round bottom flask with running water was used as a crude but efficient condenser. The suspension was filtered, the insoluble matter washed with ether, and the combined filtrates evaporated. The residue was extracted with warm petroleum ether, and the solution filtered through anhydrous sodium sulfate, evaporated, and weighed. This value was taken as the total lipide content of the liver. In a batch of control livers from normal stock rats, the results were virtually identical with values obtained by prolonged extraction of dried livers with chloroform in a Soxhlet apparatus. For further analysis the lipides were dissolved in petroleum ether and suitable aliquots removed. Total cholesterol was determined colorimetrically by the Liebermann-Burchard reaction with a Coleman spectrophotometer and lipide phosphorus determined by the Fiske-Subbarow procedure after perchloric acid digestion. Total lipides, cholesterol, and phospholipides were determined in the same manner on the alcohol-ether-soluble fraction of heparinized plasma. Phospholipides were calculated by assuming an average of 4 per cent as the phosphorus content of phospholipides.

The first series of animals was maintained on the experimental rations for 3 weeks. The nature of the various groups and the results obtained are summarized in Table I. The animals of Groups A to D may be taken as the controls, from the nutritional standpoint, for this series. Groups E to H were choline-deficient, Groups J to L were cystine-deficient, and Groups M to O deficient in both choline and cystine. The groups in each nutritional category are then arranged in order of ascending thyroid activity.

With respect to the nutritional variables, in animals with no altered thyroid function, the results were in accord with previous findings. Thus, simple choline-deficient rats showed markedly fatty livers and increased liver cholesterol concentration. Neither liver fat nor cholesterol increased quite so much in animals deficient in both cystine and choline. The livers of rats receiving choline but not cystine contained less total lipide and cholesterol than any of the other groups. It should be noted that the level of choline administration employed under these conditions was just sufficient to maintain normal liver lipide and cholesterol concentrations. This pro-
vided a rather labile situation, so that the effects of varying thyroid activity would be more readily apparent.

The most dramatic effect of varying thyroid activity noted, under these conditions, was on the liver cholesterol fraction and is readily apparent in each nutritional category. Hypothyroidism invariably resulted in an elevated liver cholesterol concentration and hyperthyroidism in a marked decrease therein. In consequence, the highest cholesterol concentration in the series was observed in thyroidectomized, choline-deficient rats, while

| Table I |

**Effects of Thyroid Activity on Liver Lipides of Choline- and Cystine-Deficient Rats**

<table>
<thead>
<tr>
<th>Group</th>
<th>Dietary supplement</th>
<th>Level of thyroid activity</th>
<th>No. of rats</th>
<th>Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Food intake</td>
</tr>
<tr>
<td>A</td>
<td>Choline + cystine</td>
<td>Thyroidectomy</td>
<td>8</td>
<td>9.8</td>
</tr>
<tr>
<td>B</td>
<td>&quot; + &quot;</td>
<td>Thiouracil</td>
<td>8</td>
<td>8.8</td>
</tr>
<tr>
<td>C</td>
<td>&quot; + &quot;</td>
<td>Normal</td>
<td>16</td>
<td>10.7</td>
</tr>
<tr>
<td>D</td>
<td>&quot; + &quot;</td>
<td>Thyroid fed*</td>
<td>8</td>
<td>9.9</td>
</tr>
<tr>
<td>E</td>
<td>Cystine</td>
<td>Thyroidectomy</td>
<td>8</td>
<td>9.7</td>
</tr>
<tr>
<td>F</td>
<td>&quot;</td>
<td>Thiouracil</td>
<td>8</td>
<td>8.6</td>
</tr>
<tr>
<td>G</td>
<td>&quot;</td>
<td>Normal</td>
<td>16</td>
<td>10.3</td>
</tr>
<tr>
<td>H</td>
<td>&quot;</td>
<td>Thyroid fed*</td>
<td>8</td>
<td>10.4</td>
</tr>
<tr>
<td>J</td>
<td>Choline</td>
<td>Thiouracil</td>
<td>8</td>
<td>6.5</td>
</tr>
<tr>
<td>K</td>
<td>&quot;</td>
<td>Normal</td>
<td>16</td>
<td>7.1</td>
</tr>
<tr>
<td>L</td>
<td>&quot;</td>
<td>Thyroid fed*</td>
<td>8</td>
<td>7.5</td>
</tr>
<tr>
<td>M</td>
<td>None</td>
<td>Thiouracil</td>
<td>8</td>
<td>6.9</td>
</tr>
<tr>
<td>N</td>
<td>&quot;</td>
<td>Normal</td>
<td>16</td>
<td>6.8</td>
</tr>
<tr>
<td>O</td>
<td>&quot;</td>
<td>Thyroid fed*</td>
<td>8</td>
<td>7.4</td>
</tr>
</tbody>
</table>

* 0.3 per cent in diet.

the lowest was in the thyroid-fed, cystine-deficient group. The magnitude of this difference is, perhaps, more impressive when the total amounts of liver cholesterol in each group are compared. These may be readily calculated from the data of Table I. Thus, while the cholesterol concentration in Group E was 10 times that of Group L, the total liver cholesterol was 134.0 mg. and 8.6 mg., respectively, while food consumption was 9.7 and 7.5 gm. per day respectively. The effects of varying the level of thyroid activity on the neutral fat fraction of the liver lipides were not quite as striking. In fact, in each nutritional category, comparison of hyper- or hypothyroid animals with the normal controls of that category does not reveal differences which are more than barely statistically significant.
However, when the hyperthyroid group is compared with the hypothyroid group, it becomes obvious that qualitatively the neutral fat behaves in a pattern similar to that described for cholesterol; *viz.*, rising in the hypothyroid state and falling in the hyperthyroid state. In accord with previous findings (4, 5), the phospholipide fraction of liver under these circumstances is reduced in otherwise fatty livers. This fall, however, is deceptive, since it represents merely dilution by the other lipide components and actually the ratio of phospholipide protein N of the liver remains fairly constant. Neither the nutritional nor the thyroid variations produced any change in phospholipide concentration which was not accountable in these terms.

While the extent to which liver fat accumulates in choline-deficient rats is, in considerable measure, also a function of the growth rate (4, 6, 7), this variable does not seem to have materially affected the present results. It is patently impossible to obtain maximal growth under the dietary circumstances employed in the production of choline deficiency. Therefore, the animals of Group C must be taken as the “normal controls” in this series. When that is done, it can be seen that in this category the thyroid-fed and thyroidectomized rats grew at equal rates, about 75 per cent of that of the controls; yet the effects on liver lipides were entirely different. A similar situation was observed in the choline-deficient category. None of the rats in the two cystine-deficient categories grew well and the differences apparent in growth rate among the various groups are statistically insignificant. The one anomalous finding in the entire series was the greater neutral fat concentration in Group F than Group E. Considering the remainder of the series it appears doubtful that this had any real physiological significance.

In the second series, the experimental period was twice that of the first series; *viz.*, 6 weeks. The nature of the various groups and the results are summarized in Table II. While not indicated in Table II, cystine was added to all the diets of this series and so, nutritionally, there are presented only control and choline-deficient animals. While Table II indicates that twelve rats were used in each group, actually the entire experiment was performed twice, each time with six rats per group. The two experiments were in excellent agreement and the values shown in Table II were calculated as if a single large study had been made.

In most respects the results obtained in this series were in agreement with those found in the shorter trial. Again, the most prominent deviation from the basal conditions induced by alteration of the level of thyroid activity was in the cholesterol fraction of the liver lipides. In both nutritional categories, the livers of thyroidectomized animals contained about twice as much cholesterol as those of the basal animals, while the livers of the hyperthyroid rats contained somewhat less than half as much cholesterol.
as those of the basal animals. Moreover, when compared with the values of Table I, it can be seen that cholesterol continued to accumulate in the livers of thyroidectomized rats in the second 3 weeks. As in the first series, the effects of altered thyroid function on the neutral fat fraction were comparatively small, albeit, nevertheless, real and similar in direction to those shown in Table I. The failure of thiouracil to yield results comparable with those of thyroidectomy may, perhaps, be attributed to the toxicity of this compound. Thus, the rats of Groups B and G ate less and grew more slowly than did the basal controls or thyroidectomized rats. In the same manner, while the rats of Groups E and K ate more than those of Groups C and H for 5 weeks, their appetites dwindled in the last week, accompanied by a weight loss of about 4 gm., and it is difficult to determine the extent to which this influenced the results. Nevertheless, the over-all pattern seems clearly established. In evaluating these data it must be realized that the basal diet for this study contained 0.5 per cent cholesterol, which undoubtedly would exaggerate any physiological circumstance which would tend to permit an accumulation of hepatic cholesterol, although essentially normal values were obtained in the control rats of both series.

While choline deficiency and hypothyroidism exert similar influences on liver lipides, their effects on plasma lipides are opposite in direction. Choline deficiency resulted in a small, but real, decrease in all fractions of the

<table>
<thead>
<tr>
<th>Dietary supplement</th>
<th>Thyroid activity</th>
<th>Food intake</th>
<th>Liver</th>
<th>Plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Weight</td>
<td>Total</td>
</tr>
<tr>
<td>A</td>
<td>None</td>
<td>Thyroidectomy</td>
<td>7.8</td>
<td>68</td>
</tr>
<tr>
<td>B</td>
<td>&quot;</td>
<td>Thiouracil</td>
<td>6.5</td>
<td>33</td>
</tr>
<tr>
<td>C</td>
<td>&quot;</td>
<td>Normal</td>
<td>10.6</td>
<td>88</td>
</tr>
<tr>
<td>D</td>
<td>&quot;</td>
<td>Thyroid fed 0.2%</td>
<td>11.1</td>
<td>63</td>
</tr>
<tr>
<td>E</td>
<td>&quot;</td>
<td>&quot;</td>
<td>10.5</td>
<td>42</td>
</tr>
<tr>
<td>F</td>
<td>Choline</td>
<td>Thyroidectomy</td>
<td>8.3</td>
<td>73</td>
</tr>
<tr>
<td>G</td>
<td>&quot;</td>
<td>Thiouracil</td>
<td>6.3</td>
<td>37</td>
</tr>
<tr>
<td>H</td>
<td>&quot;</td>
<td>Normal</td>
<td>9.8</td>
<td>95</td>
</tr>
<tr>
<td>J</td>
<td>&quot;</td>
<td>Thyroid fed 0.2%</td>
<td>11.3</td>
<td>54</td>
</tr>
<tr>
<td>K</td>
<td>&quot;</td>
<td>&quot;</td>
<td>10.1</td>
<td>31</td>
</tr>
</tbody>
</table>

* Each group consisted of twelve rats.
plasma lipides, while hypothyroidism, in both nutritional categories, resulted in a marked rise in the plasma lipides which was somewhat more striking in the choline-fed than in the choline-deficient rats. Hyperthyroidism occasioned a comparatively slight decrease in the plasma lipides. These findings, in direction, are all in keeping with clinical experience with alterations in human thyroid function. In the main, total lipides, cholesterol, and phospholipides behaved in a parallel manner, rising and falling under the same circumstances and in a roughly proportional fashion. Of these, the phospholipide fraction was perhaps the least consistent. It should be remarked that the values for plasma phospholipide found in these rats were considerably lower than those usually seen in human subjects. The mean value for plasma lipide phosphorus concentration for the entire series was 2.4 mg. per 100 ml. with a range between 1.5 and 3.3. Normal human plasma contains 8 to 11 mg. of lipide phosphorus per 100 ml. with considerably higher values found in the hyperlipemic plasma of hypothyroid individuals. Similar low values for lipide phosphorus in rat plasma have recently been observed by others.²

DISCUSSION

From the data presented in Tables I and II it would seem established that the concentration of cholesterol in the livers of both normal and choline-deficient rats varies inversely with the level of thyroid activity. These observations are in accord with the findings of Forbes (8) that thyroxine administration augmented the lipotropic action of choline at moderate levels of choline feeding. It is unfortunate that this author's data did not permit an evaluation of the effects of thyroxine in choline-deficient rats. In another paper from the same laboratory (9) it was found that thiourea feeding was without effect on liver fat or cholesterol concentration. In view of the present findings this may, perhaps, indicate merely that thiourea is not as effective as thiouracil or thyroidectomy in the production of an experimental hypothyroid state. No definitive statement concerning the influence of thyroid activity on the neutral fat of the liver can be made. According to the present data hyperthyroidism depresses the accumulation of neutral fat in the livers of both control and choline-deficient animals, while hypothyroid function operates in opposite fashion. However, the differences were small and, while statistically significant, there was some overlapping of the various groups. Since the extent of neutral fat accumulation in choline deficiency is, in a large measure, dependent also upon such factors as food consumption, growth rate, toxic agents, and the supply of other dietary essentials (4, 6), these relatively small differences found at

² Artom, C., personal communication.
various levels of thyroid activity are difficult to evaluate. Nevertheless, comparison of the lipide content of hyperthyroid and hypothyroid livers indicates that the behavior of neutral fat parallels that of cholesterol under these circumstances.

In the present study, the effects of cystine feeding were equally pronounced on the cholesterol and neutral fat fractions of the liver lipides. This was readily apparent at all levels of thyroid activity. Since it has been stated that the increased liver fat content which results from feeding cystine to choline-deficient rats is the result of an augmented rate of fatty acid synthesis (10), this would suggest that cystine feeding may accelerate the production of some common precursor of both fatty acids and cholesterol, perhaps at the 2-carbon stage.

The present data do not permit a categorical statement concerning the mechanism by which the thyroid regulates fat metabolism. Hypothyroidism results in a relative hyperlipemia and an increased liver fat content, while hyperthyroidism has the opposite effect. Since the influence of the thyroid is most marked on the liver cholesterol fraction, it seems possible that the thyroid specifically controls cholesterol metabolism. That the increase in the neutral fat of liver and plasma and of plasma phospholipides which results from hypothyroidism may all be secondary to the behavior of cholesterol under these conditions appears quite likely in view of the work of Popjak (11), who has shown that feeding emulsified free cholesterol results in a marked rise in the concentration of all plasma lipides at the expense of depot neutral fat. Since no measurements of extrahepatic tissue fat were made in the present study, it is not possible to state whether the thyroid regulates the rate of cholesterol synthesis and utilization or its transport and distribution. This question can best be answered with the aid of the isotope tracer technique.

Hypothyroidism induced by thyroidectomy or by feeding thiouracil (1), \( p \)-aminobenzoic acid,\(^1\) or sulfonamides\(^1\) protects rats against the cirrhosis which usually results from the ingestion of choline-deficient diets. In the present study it has been found that, with all other conditions being held constant, hypothyroid activity actually augments the usual accumulation of liver lipides in choline-deficient rats. It appears, therefore, that unless hepatic metabolism is proceeding at the rate dictated by at least normal thyroid function, the severe hepatic necrosis and fibrosis of choline deficiency do not occur, despite the fact that the parenchymatous liver cells are engorged with masses of lipide material. This situation is analogous to the previous finding that the extremely fatty livers which result from the addition of nicotinamide to a diet containing 18 per cent casein do not undergo the usual necrosis and fibrosis observed in the fatty livers resulting from choline deficiency on a low protein diet (7). It recalls also the fact
that liver damage due to experimental hyperthyroidism has only been observed when accompanied by some other toxic agent such as carbon tetrachloride (12), anoxia (13), staphylococcus toxin (14), or rabbit papilloma virus (15), although none of these are, of themselves, hepatotoxic under the conditions employed.

The alterations in the levels of plasma lipides which were induced by choline deficiency and by varying levels of thyroid activity were in accord with all previous findings. The diminution in plasma lipides is consistent with the concept that the fatty liver of choline-deficient rats is due to a failure of phospholipide synthesis and consequently of fat transport from the liver. Hyperthyroidism did not diminish the plasma lipides as markedly as is sometimes encountered in Grave's disease. Hypothyroidism, however, resulted in an elevation of plasma lipides which was more pronounced in choline-fed than in choline-deficient animals. Experimentally, the hyperlipemia of hypothyroidism has been observed in dogs (16) and rabbits (17), but not monkeys (18). In the dog, the degree of the lipemia was determined by the animal's appetite. It may well be that the failure to find an appreciable drop in the plasma lipide concentration of thyroid-fed rats and the exaggerated hyperlipemic response to thyroidectomy and thiouracil feeding was the result of the use of a basal diet high in fat and cholesterol in the present study.

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SUMMARY

1. Hypothyroidism, produced both by thyroidectomy and by thiouracil feeding, resulted in a marked increase in the cholesterol concentration and a relatively small increase in the neutral fat content of the livers of both control and choline-deficient rats.

2. Thyroid feeding resulted in a pronounced decrease in the cholesterol concentration and a slight decrease in the neutral fat concentration of the livers of both control and choline-deficient rats.

3. Cystine deficiency partially prevented the accumulation of neutral fat and cholesterol in the livers of normal and choline-deficient rats. The effects of cystine deficiency and thyroid feeding were cumulative and cystine deficiency partially offset the accumulation of liver lipides in hypothyroid rats.

4. The plasma lipide concentrations of choline-deficient rats were some-
what reduced below those of normal rats. Thyroid feeding slightly diminished plasma lipides of both series, while in hypothyroidism the plasma lipide concentrations of both series were greatly increased.

5. The significance of these findings is discussed.

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