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Importance of diet for reaction of bile acid pattern to altered thyroid function

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With 4 figures and 1 table

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Studies of the bile acid pattern of rats have shown that hyperthyroidism results in a marked shift from cholic-towards chenodeoxycholic acid. The results of studies in man were, however, not in accordance with these findings and in hamsters fed a gallstone inducing diet the exact opposite effect was observed. In order to elucidate these discrepancies we investigated the reaction of the bile acid pattern to altered thyroid function in hamsters under different dietary conditions.

Methods and Material

Young hamsters of both sexes with a mean weight of 60 g were used. The animals were partly from our own stock colony and partly purchased from Centraal Proefdierenbedrijf TNO, Zeist (Holland). They were divided in a strictly random fashion into 6 equal groups each consisting of 20 animals. Three groups were fed a vitaminized commercial chow chiefly consisting of protein, fat, cellulose and water[1]. One of these groups was fed this basic diet without supplement while the other two groups were fed the same diet but supplemented with 5 mg/100 g food l-thyroxine[2] and propylthiouracil (50 mg/100 g food) respectively. The three remaining groups were fed the fat free gallstone inducing diet 284 (1), the composition of which is shown in table 1. Again one group was fed this diet without supplement while similar doses of l-thyroxine and propylthiouracil respectively were added to the diets of the other two groups. All animals were individually caged and had free access to food and water.

After one month on these 6 respective diets the animals were operated upon in Nembutal narcosis. The common bile duct was ligated close to the duodenum in order to obtain complete filling of the gallbladder. This, as a rule, took 20–30 minutes. Afterwards the gallbladder was removed, the bile was collected for analysis and the animal was sacrificed. As the bile samples were as a rule rather small pooling was necessary. On the average 2–3 hamsters contributed to each analysed sample. In this way 8 samples of bile were collected in each dietary group. 0.050 ml of bile was extracted and hydrolysed according to the method described by WOLLENWEBER et al. (2), with the only modification that DC Fertigplatten, Kieselgel F 254[3] were used. The free bile acids were separated according to the same method together with cholic acid, chenodeoxycholic acid and deoxycholic acid standards. After spraying with 10 per cent phosphomolybdic acid solution in ethanol, the plates were heated to 110 °C for 10 minutes and the spots were quantified by means of a Vitatron Densitometer, U. F. D.[4]

[1] Harald Fors, Holmsund (Sweden)

[2] Generously supplied by Nyegaard AS Oslo (Norway)

[3] Merck, Darmstadt (Western Germany)

[4] Vitatron, Dieren (Holland)

equipped with a 578 mμ filter and compared with standard curves. For a discussion of the precision and accuracy of this method the reader is referred to JUUL & VAN DER LINDEN (3).

The presence or absence of gallstones was noted in each animal and the stones were classified as cholesterol stones, pigmented stones and mixed stones by two independent assessors.

The liver was weighed and together with the gallbladder, lungs, heart, spleen, kidneys, adrenals, thyroid and pituitary glands fixed in 10 per cent formalin solution. Paraffin-embedded sections were prepared and stained with heamatoxylin-eosin and VAN GIESONS stain.

In addition sections of the thyroid gland were stained with periodic acid Shiff (PAS). Frozen sections of the liver, heart, kidneys and adrenals were stained with Scharlach-Rot and Sudan Black B. The histological examinations were performed with the assessor being unaware of the group to which the animal belonged.

The statistical significance of group differences was tested with the Mann-Whitney U test as described by SIEGEL (4).

Table 1. Composition of gallstone inducing diet

	percentage
Glucose	74.3
Casein (crude)	20.0
Salt mixture (U. S. P. XIII No. 2)	5.0
Vitamin mixture	0.5
Choline chloride	0.2

Results

The occurrence of gallstones in the different groups was in accordance with the results of previous work. No gallstones were observed in the three groups with chow as the basic diet. In the three groups fed the gallstone inducing diet cholesterol stones were found in many animals while there also was an abundancy of pigmented and mixed stones in the group fed this basic diet supplemented with l-thyroxine.

The concentrations of total bile acids in bile (means and ranges) are shown in fig. 1. In the groups fed chow as the basic diet the highest concentration was found in the l-thyroxine-supplemented group and the lowest in the animals treated with propylthiouracil. Exactly the reverse was found in the three groups fed with the gallstone inducing diet 284 as the basic diet. With this basic diet the mean concentration was highest in the hamsters treated with propylthiouracil and lowest in those treated with l-thyroxine. The individual variations were, however, large in all six groups and when the differences were subjected to the Mann-Witney U test they were not statistically significant.

Fig. 2 shows the composition of the bile acids in the various groups, the amount of the different bile acids being given as a percentage of the total amount. The percentage composition is restricted to the three principal bile acids in hamster bile: cholic acid, chenodeoxycholic acid and deoxycholic acid. As seen in fig. 2, in hamsters fed the unsupplemented gallstone inducing diet 284 there was about as much chenodeoxycholic acid as cholic acid. With propylthiouracil as a dietary supplement there was a shift towards chenodeoxycholic acid. When subjected to the *Mann-Whitney* U test the difference in the cholic acid/chenodeoxycholic acid ratio was probably significant ($0.10 > P > 0.02$). In the animals treated with 1-thyroxine an opposite effect

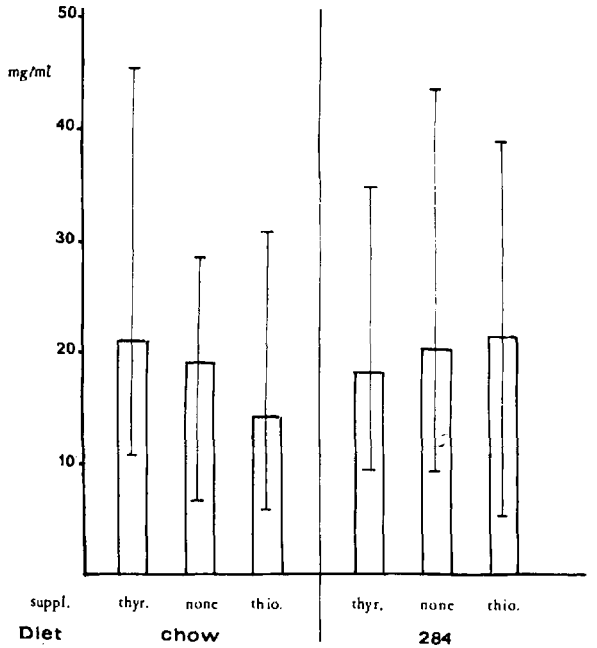

Fig. 1. Concentrations of bile acids in bile (means and ranges) in hamsters fed different diets. unsupplemented and with supplements of l-thyroxine (thyr.) and propylthiouracil (thio.),

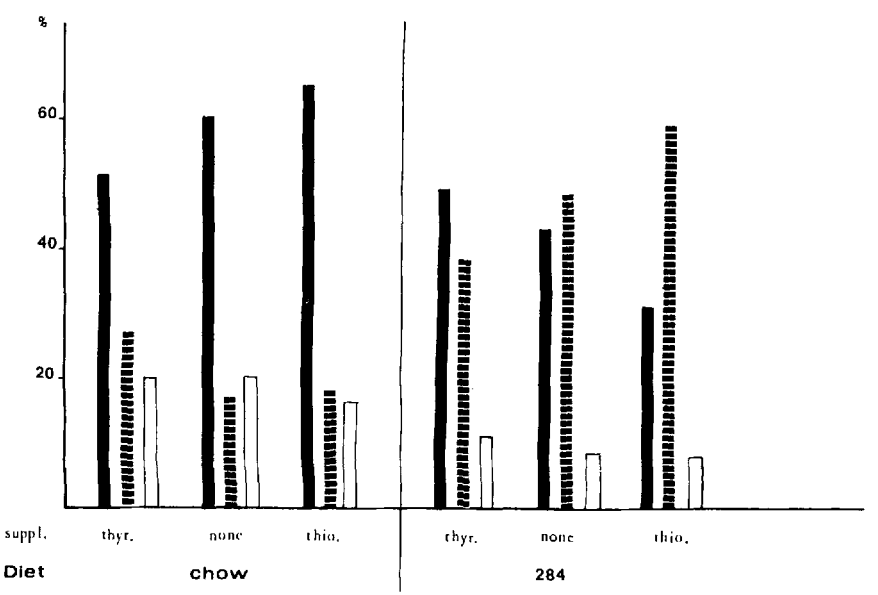

Fig. 2. Percentage composition (mg individual bile acid/100 gm total bile acids) in hamsters fed different diets, unsupplemented and with supplements of l-thyroxine (thyr.) and propylthiouracil (thio.).

■ cholic acid ▤ chenodeoxycholic acid □ deoxycholic acid

was observed. In these animals the cholic acid/chenodeoxycholic acid ratio was significantly higher than in those fed the unsupplemented diet 284 ($P < 0.02$).

Fig. 2 also shows that with chow as the basic diet a much larger part of the total bile acids consisted of cholic acid while there was less chenodeoxycholic acid. With l-thyroxine as dietary supplement there was a marked shift towards chenodeoxycholic acid while a less clear shift towards cholic acid was observed in the hamsters treated with propylthiouracil. When subjected to the *Mann-Whitney* U test the cholic acid/chenodeoxycholic acid ratio was significantly lower in the thyroxine treated hamsters than in the unsupplemented controls ($P < 0.02$). With the same test the difference in this ratio between the animals fed thiouracil and the controls fed unsupplemented chow was not significant.

The histological findings were also in accordance with previous studies (5). With both basic diets marked liver steatosis was found in all the hamsters treated with thyroxine (fig. 3). In these animals the follicles in the thyroid gland were filled with homogenous eosinophilic, PAS-positive colloid and lined by flattened epithelium (fig. 4a). In the propylthiouracil treated hamsters, on the other hand, liver histology was normal while the thyroid glands showed marked hyperplasia and hyperemia. The follicles were irregular with infolding of their walls, the epithelium had a cuboidal form and the amount of colloid was severely reduced (fig. 4c).

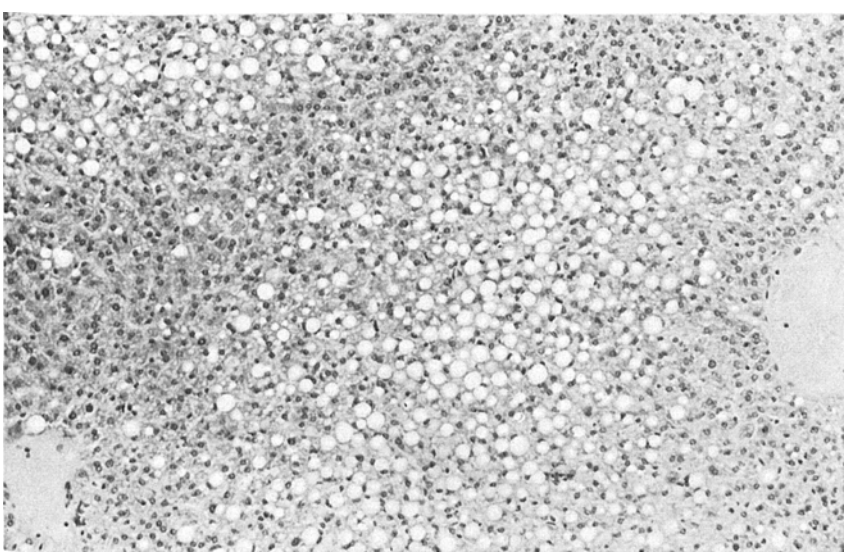

Fig. 3. Section of liver of hamster fed diet supplemented with 5 mg% l-thyroxine during 1 month. Marked fatty degeneration. Htx-eosin × 130.

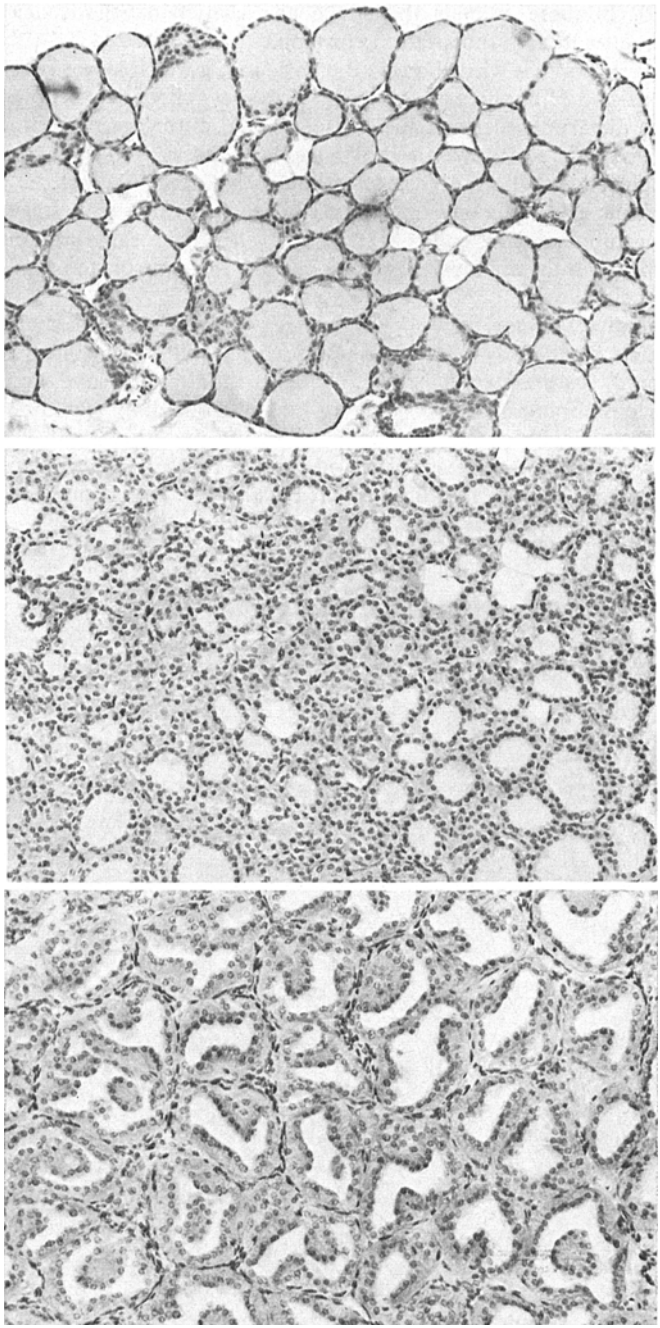

Fig. 4. Thyroid gland of hamster fed different diets, unsupplemented (b) and with supplements of l-thyroxine (a) and propylthiouracil (c). Htx-eosin × 100.

Discussion

It has long been clear from an abundancy of clinical observations that a relationship of some sort exists between thyroid activity and cholesterol metabolism. The discovery that bile acids are the main endproducts of cholesterol led to studies on the influence of thyroid hormones on the excretion of these acids. These studies were originally performed in bile fistula rats and they showed among other things that administration of thyroid hormones reversed the normal ratio of about 4:1 between cholic- and chenodeoxycholic acid. Hypothyreoidism on the other hand resulted in a shift towards chenodeoxycholic acid. These marked changes originally discovered by ERIKSSON (6) were confirmed by STRAND (7). However, the enterohepatic circulation was interrupted in the studied animals. This interruption leads to a highly unphysiological state with a large increase in the daily synthesis of bile acids. Therefore caution was needed in the interpretation of the results obtained. This uncertainty was dispersed by a detailed study in intact rats of STRAND (8), who used an isotopic tracer technique. These studies showed intact hypothyroid rats to have about the same bile acid pool size and cholic acid/chenodeoxycholic acid ratio as normal rats. On the other hand in intact hyperthyroid rats there was a marked shift towards chenodeoxycholic acid; the bile acid pool was found to be greatly increased mainly due to a 2–3 fold enlargement of the chenodeoxycholic acid pool. It seems therefore that whether the enterohepatic circulation of the rat is interrupted or not, the administration of thyroid hormones results in a shift from cholic-towards chenodeoxycholic acid. When, on the other hand, the effect of thyroid hormone was recently studied in the intact hamster (9) the reverse was found – i. e. a shift towards cholic acid – and a species difference in the reaction to thyroxine was suspected. However, in the hamster studies the animals were fed a variant of a gallstone inducing diet whereas the rats had been fed with commercial diets; a mixture of pig feed and barley meal (6) and commercial rat chow (6, 7).

The results of the present studies show that the basic diet is of fundamental importance for the reaction of the bile acid pattern to altered thyroid function. When the hamsters were fed with commercial chow the administration of l-thyroxine resulted in a marked shift towards chenodeoxycholic acid. In hypothyroid animals there was somewhat more cholic acid. In other words the cholic acid/chenodeoxycholic acid ratio changed in a similar way as previously described in another rodent i. e. the rat when fed with commercial chow.

When on the other hand the hamsters were fed with a fat free gallstone inducing diet we met with a very different picture. Just as in an earlier study wih another kind of gallstone provoking diet (9) we found with this diet more chenodeoxycholic acid and less cholic acid than with normal chow. In spite of this difference in bile acid composition the mean total bile acid concentrations were about the same, large individual differences being noted in both groups. When l-thyroxine was added to the gallstone inducing diet a clear rise instead of a fall of the cholic acid/chenodeoxycholic acid ratio was noted. With thiouracil on the other hand this ratio fell whereas we noted a slight rise when chow was the basic diet.

The interpretation of these findings is difficult especially as we have no information as to how bile acid pool size and turnover rate are affected by altered thyroid function under different dietary conditions. Still the results show clearly that when the effect of thyroid hormones on bile acid metabolism is studied, the influence of the diet should be taken into account.

Studies of the bile acid pattern of humans with different degrees of thyroid function were not in accordance with the results obtained in rats. HELLSTRÖM & LINDSTEDT (10) observed no consistent influence of thyroid hormone on the relative proportions of the different bile acids. FAILEY et al. (11) on the other hand found in the intact person a significant rise of the cholic acid/chenodeoxycholic acid ratio following administration of thyroid hormone instead of the decrease they had expected. The present results indicate that dietary factors may be of importance in explaining these discrepancies between results obtained in man and in experimental animals.

Summary

Hamsters were fed with commercial chow and with a gallstone inducing diet. These two basic diets were given both unsupplemented and with supplements of l-thyroxine and propylthiouracil respectively. No significant differences in the total biliary bile acid concentration were observed between the different groups. In the groups fed chow the administration of thyroxine induced a decrease of the cholic acid/chenodeoxycholic acid ratio. With propylthiouracil this ratio was slightly higher than in controls. In the groups fed the gallstone inducing diet an opposite effect was observed: a shift towards cholic acid in the hyperthyroid animals and a shift towards chenodeoxycholic acid in those fed with propylthiouracil. The diet may be a factor of importance in explaining the differences in the reaction of the bile acid pattern to altered thyroid function which have been observed between man and the experimental animal.

Zusammenfassung

Hamster wurden mit Standardfutter und einer gallensteinerzeugenden Diät gefüttert. Diese beiden Grunddiäten wurden sowohl ohne als auch mit Zusatz von l-Thyroxin und Propylthiouracil gegeben. Zwischen den verschiedenen Gruppen wurden keine bedeutsamen Unterschiede in der Gesamtkonzentration der Gallensäuren in der Galle beobachtet. In den mit Standardfutter gefütterten Gruppen verursachte der Zusatz von Thyroxin eine Abnahme von Cholsäure/Chenodeoxycholsäure Quote. Mit Propylthiouracil war diese Quote wenig höher als die der Kontrolltierchen. Bei den mit gallensteinerzeugender Diät gefütterten Gruppen wurde ein gegensätzlicher Effekt beobachtet: eine Verschiebung zu Cholsäure bei den hyperthyroiden Tierchen und eine Verschiebung zu Chenodeoxycholsäure bei den mit Propylthiouracil gefütterten. Die Diät könnte ein bedeutsamer Faktor sein, um die Unterschiede in der Reaktion des Gallensäuremusters zur veränderten Schilddrüsenfunktion, die man zwischen Menschen und Experimenttier beobachtet hat, zu erklären.

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