THE ELECTROPHORETIC LIPOPROTEIN PATTERN IN DISORDERS OF THYROID FUNCTION

By

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It is generally recognized that the thyroid exerts a considerable influence on the serum lipids. The determination of the serum cholesterol has thus become an important aid in the diagnosis and treatment of both hyperthyroidism and hypothyroidism. The serum proteins also react in thyroid disorders, and the liver is probably largely responsible for these changes (Lamberg & Gräsbeck, 1955) as well as for the changes in the prothrombin level of the serum (Lamberg & Gordin, 1954, Gordin & Lamberg, 1955).

The serum lipids, however, do not seem to occur in the free form but combined with other serum lipids or with proteins to form complex aggregates, known as the lipoproteins. The considerable advances recently made in the field of lipoprotein research mainly inspired by investigations on atherosclerosis, have also thrown light on the relationship between the thyroid and the serum lipoproteins. The ultracentrifugal lipoprotein pattern in hypothyroidism is characterized by a high level of the $S_f 10-20$ fraction (Gofman et al., 1951). Kunkel & Slater (1952) studied one case of myxoedema by means of starch electrophoresis and observed that the bulk of the serum lipids migrate between the alpha$_1$ and beta globulins. Strisower et al. (1954) administered thyroid extract to schizophrenic patients and observed a significant lowering of the serum cholesterol and of the $S_f 0-20$ lipoproteins, the response in the ultracentrifugal fractions $S_f 20-400$ being of borderline significance.

In 1952 Swahn introduced a new method for staining the serum lipids on filter paper which he used for the quantitative determination of the total serum lipids and the electrophoretically separated serum lipoprotein fractions. Malmros & Swahn (1953) observed an increase in the beta lipoprotein in 13 cases of myxoedema whilst the alpha$_1$ lipoprotein was decreased or normal. No fraction with abnormal migration rate was seen in contrast to the findings of
Kunkel & Slater (1952). In all the cases treated with thyroid hormone the beta peak was considerably reduced and the lipoprotein pattern normalized. Mahaux & Köiw (1952) observed the same effect with thyroid hormone in a few cases of myxoedema.

We have been unable, however, to find any publication dealing with similar lipoprotein studies in hyperthyroidism. The explanation of this is probably that the study of lipoproteins is rendered more difficult by the diminution of the serum lipids in hyperthyroid states. The method described by Swahn (1952, 1953) seems, however, to be superior to other techniques in detecting small amounts of lipids. Further the technique can easily be combined with ordinary paper electrophoresis. We therefore undertook to perform parallel protein and lipid paper electrophoresis determinations in cases of thyrotoxicosis and hypothyroidism. This was done in order to get some insight into the behaviour of the lipoproteins in thyroid disorders and to evaluate the use of lipid electrophoresis according to Swahn (1952, 1953) in the diagnosis and regulation of these conditions. Along with these studies the changes in the level of the serum prothrombin were observed. In the following the results of the lipid fractionations are given. The results of the protein fractionations are reported elsewhere (Lamberg & Gräsbeck, 1955).

MATERIAL AND METHODS

The material consists of 22 cases of thyrotoxicosis, 7 cases of hypothyroidism and 22 control cases with no evidence of thyroid dysfunction or of other disturbances that might alter the lipoprotein level. Of the hypothyroid cases 2 were postoperative, one of them possibly with a hypopituitary component and 4 apparently of pituitary origin as evaluated from the response to treatment with thyroid stimulating hormone. The diagnosis was established on the findings of typical clinical symptoms together with various laboratory tests and the response to treatment. Cases in which the diagnosis seemed dubious or uncertain have been omitted. Lipoproteinograms were usually taken once a week until a definite response to treatment was established – at least a clinical euthyrotic state. The treatment of thyrotoxicosis was either preoperative or medical – usually a combination of thyrostatic drugs and potassium iodide, in a few operative cases potassium iodide only was given. Hypothyroidism was treated with thyroxin or thyroid preparations. No determinations were made after thyroidectomy.

The electrophoretic separation of the lipoprotein fractions was done by means of ordinary paper electrophoresis with subsequent staining of lipid with Sudan Black B and direct photometry of the dry strip (Swahn, 1952, 1953). The »total lipid« was determined according to Swahn (1953): The dye absorbed by serum spots on filter paper is determined and spots of triolein solution are used as reference. The »total lipid« values obtained with this method do not agree with the true lipid values because of the different staining capacities of the total lipid constituents as pointed out by Nikkilä & Gräsbeck (1954). Our values are also considerably lower than those obtained by Swahn (1953) though in our opinion we have followed the original description of the method in all details. This may be due to variations in the commercial Sudan Black.
The values obtained with the method are therefore to be considered more as "indices" of the true lipid content. The method is, however, easily adaptable for clinical work, and much more informative than the flocculation test of Kunkel, Ahrens & Eisenmenger (1948).

The interpretation of the results is rendered difficult by the same problem which is always encountered in lipoprotein and atherosclerosis research, i.e. the difficulty in determining the normal. Further the lipid-protein aggregates being labile structures, slight changes in the physico-chemical milieu or the mere physical "handling" of the samples might influence the results. Diet, various drugs such as for example iodide and heparin, estrogens and therefore probably the menstrual cycle, as well as innumerable other factors can influence the lipoprotein pattern. Electrophoretic serum lipoprotein fractionation possesses, however, many advantages among which are simplicity and cheapness, which makes its use possible in everyday clinical practice. One purpose of our investigation was, therefore, to assess the clinical value of this method in the diagnosis and regulation of the treatment of thyroid disorders.

The curve obtained by reading the dry filter paper strip in a special photometer was divided into three fractions: fraction 1, the "chylomicron« peak remaining at the starting point; fraction 2, the beta lipoprotein peak and fraction 3 consisting of the small peaks in the region of albumin and alpha globulins. The relative percentage of the total serum lipid and the absolute content in mg./100 ml. were calculated for every fraction. As the lagging phenomenon of the beta lipoprotein often makes it difficult to decide where the dividing perpendicular between fractions 1 and 2 should be found, the values for 1+2 were also calculated. Because of the low content of total lipids in the normal cases and the thyrotoxic cases no attempts were made to distinguish any further fractions, e.g. alpha\textsubscript{1} and alpha\textsubscript{2} lipoproteins. For further information on the technique, the reader is referred to the paper of Swahn (1953).

RESULTS

The results of the cholesterol and the total lipid determinations as well as of the electrophoretic lipoprotein fractionations are given in Table 1. A statistical evaluation is presented in Table 2.

The total lipid content (Fig. 1) was significantly increased in the untreated hypothyroid cases. During treatment the lipid content decreased. In the hyperthyroid cases the mean value did not differ from the normal mean, a significant augmentation being, however, noticeable during treatment.

The total cholesterol content (Fig. 1) showed the same changes as the total lipid values.

Fraction 1 (The "chylomicrons"). The non-migrating lipids were significantly increased in hypothyroidism but in hyperthyroid cases the values did not differ significantly from the normal. During the treatment the elevated hypothyroid values diminished, and the "chylomicron" fraction increased during the treatment of thyrotoxicosis.

Fraction 2 (The beta lipoprotein peak) was increased in hypothyroidism and diminished during treatment with thyroid preparations. In thyrotoxicosis this fraction did not differ from the normal, an increase being, however, evident
Table 1.
The cholesterol, the total lipid values and the lipoprotein fractions in normal, thyrotoxic and hypothyroid subjects.

<table>
<thead>
<tr>
<th>Lipids</th>
<th>Normal</th>
<th>Thyrotoxicosis*</th>
<th>Hypothyroidism*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>before</td>
<td>No.</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>156</td>
<td>16</td>
<td>132</td>
</tr>
<tr>
<td>Fraction 1 + 2</td>
<td>337</td>
<td>17</td>
<td>328</td>
</tr>
<tr>
<td>Fraction 1</td>
<td>69</td>
<td>17</td>
<td>74</td>
</tr>
<tr>
<td>Fraction 2</td>
<td>268</td>
<td>17</td>
<td>254</td>
</tr>
<tr>
<td>Fraction 3</td>
<td>103</td>
<td>17</td>
<td>108</td>
</tr>
<tr>
<td>Total lipid</td>
<td>452</td>
<td>20</td>
<td>452</td>
</tr>
</tbody>
</table>

*) Determinations were made before the start of treatment and after a euthyroid state had been reached.

Table 2.
Statistical evaluation of the data in Table 1.

<table>
<thead>
<tr>
<th>Lipids</th>
<th>Untreated thyrotoxicosis vs.</th>
<th>Untreated hypothyroidism vs.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>normal</td>
<td>after treatment</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>&gt; 0.05</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Fraction 1 + 2</td>
<td>&gt; 0.05</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Fraction 1</td>
<td>&gt; 0.05</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Fraction 2</td>
<td>&gt; 0.05</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Fraction 3</td>
<td>&gt; 0.05</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Total lipids</td>
<td>&gt; 0.05</td>
<td>&lt; 0.01</td>
</tr>
</tbody>
</table>

during treatment. It must be mentioned that owing to the lagging phenomenon the best evaluation of the changes in fractions 1 and 2 is obtained by parallel examination as when for example they are put together (1 + 2). From Fig. 2 it is evident that there is a striking parallelism in the behaviour of the total lipid content, the cholesterol level and these two lipoprotein fractions. This parallelism is also shown in Figs. 3 and 4. Case no. 14 shown in Fig. 2 showed some
Distribution of the total lipid and the total cholesterol values in normal, thyrotoxic and hypothyroid subjects.

HT = hypothyroidism, N = normal, TT = thyrotoxicosis.

Changes in the total lipid, total cholesterol and lipoprotein fractions during treatment of thyrotoxicosis.

tl = total lipid, ch = cholesterol, 1, 2 and 3 lipoprotein fractions.

remarkable clinical peculiarities and was very resistant to treatment. The patient did not reach a euthyroid state during the period mentioned here whilst the other two cases in Fig. 2 showed an ordinary response to treatment.

Fraction 3 (The alpha lipoprotein) was not altered in the dysthyroid patients, nor were there any changes observed during treatment.

The migration velocity of the lipoprotein fractions was the same in the
Fig. 3.
Changes in the lipoproteinogram during treatment of thyrotoxicosis (Case 3).
Total lipid: 1 = 390 mg./%, 2 = 310 mg./%, 3 = 723 mg./%.

Fig. 4.
Changes in the lipoproteinogram during treatment of hypothyroidism (Case 1).
Total lipid: 1 = 811 mg./%, 2 = 433 mg./%, 3 = 243 mg./%.

Table 3.
The proportional distribution of the lipoprotein fractions in normal, thyrotoxic and hypothyroid subjects.

<table>
<thead>
<tr>
<th>Lipoprotein fraction</th>
<th>Mean values as percentage of total lipid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
</tr>
<tr>
<td></td>
<td>before</td>
</tr>
<tr>
<td>1 + 2</td>
<td>76.2</td>
</tr>
<tr>
<td>1</td>
<td>15.5</td>
</tr>
<tr>
<td>2</td>
<td>60.7</td>
</tr>
<tr>
<td>3</td>
<td>23.8</td>
</tr>
</tbody>
</table>

*) Determinations were made before the start of treatment and after a euthyroid state had been reached.

Number of determinations as in Table 1.
normal subjects, in hypothyroidism and in thyrotoxicosis. No changes were seen during the treatment of the dysthyroid states.

The proportional distribution of the fractions and the changes in the distribution are shown in Table 3. The most striking changes were seen in the hypothyroid cases, only smaller changes being observable in the thyrotoxic patients. The changes in the proportional distribution also illustrate the changes in the absolute level of the fractions 1 and 2 and in the constancy of fraction 3.

**DISCUSSION**

The results show that significant lipid changes are encountered in hypothyroid states and that normal values are often found in thyrotoxicosis. This corresponds well with the view that the cholesterol level is not a reliable index in the diagnosis of thyrotoxicosis, although it is very valuable in the diagnosis of hypothyroidism (Bartels, 1950). On the other hand, changes are encountered during appropriate treatment of both hyperthyroid and hypothyroid states, i.e., an increase during treatment of the former and a decrease in the latter case. Hence it is possible that a decrease in thyrotoxicosis might be observed if a sufficiently large material were collected and the precision of the electrophoretic separation method improved.

It is well known that the total lipid increases in hypothyroid and decreases in hyperthyroid states and that both neutral fat, phospholipid and cholesterol are involved in these changes. Our results are in full agreement with this view. The non-migrating fraction in paper electrophoresis consists of particles which are almost pure neutral fat (Swahn, 1953). This fraction also changes in the above mentioned way. On the other hand, there is neutral fat in other electrophoretic fractions, too, and the exact evaluation of the »chylomicron« peak is impaired by the lagging of the beta lipoprotein. The results are therefore not to be accepted uncritically. In some of the hypothyroid cases, however, the electrophoresis curve left no doubt about the increase of the »chylomicron« fractions. There seems therefore to be no reason to doubt that the non-migrating fraction reacts in the same way as the cholesterol and the beta lipoprotein.

The beta lipoprotein consists to a large extent of cholesterol, 64 per cent of the serum cholesterol being found in this fraction in normal subjects (Nikkilä, 1953). As the beta fraction reacts in the same way as the cholesterol in thyroid disorders it seems justifiable to assume that cholesterol contained in this fraction is mainly responsible for these changes. The increase in the beta lipoprotein in hypothyroidism is in agreement with the findings of other workers (Gofman et al., 1951, Mahaux & Köiw, 1952, Malmros & Swahn, 1953, Swahn, 1953).
The failure to find a decrease in the lipids and especially in the beta lipoprotein in thyrotoxicosis is, of course, difficult to explain, though the same phenomenon is seen in the behaviour of the cholesterol. In some kinds of liver disorders an elevation of the beta lipoprotein and the cholesterol is seen (Nikkilä, 1953, Page, 1954). The protein component of the beta globulin is also elevated in thyrotoxicosis (Lamberg & Gräbeck, 1955) as well as in some liver diseases. Hence it is possible that simultaneous liver damage compensates the decreasing action of the thyroid hormone. Perhaps the primary rise is to be found in a »lipid carrier protein« which possessing a certain chemical or physical affinity for lipid, may bring about a rise in the lipid component.

In evaluating the factors that might be responsible for the increase of the cholesterol and the beta lipoprotein during the treatment of thyrotoxicosis it must be borne in mind that small doses of iodide might elicit this phenomenon in contrast to large doses (Page, 1954).

Our failure to detect significant changes in the alpha lipoprotein and to observe abnormal migration rates is in agreement with the results obtained by Swahn (1953). The former observation is not, however, very conclusive, as our fraction 3 consists of all the small and often irregular peaks which represent all the lipids which migrate faster than the beta lipoprotein. The electrophoresis curve often reaches the O-line well in front of the albumin, phospholipid or lipopolyptide lipid probably being stained. These lipid fractions therefore need more investigation before any valid deductions can be drawn.

What mechanism is responsible for the more or less general elevation of the lipids in hypothyroidism and the possible decrease in thyrotoxicosis remains, of course, obscure: This problem has been discussed in detail by Byers, Friedman & Rosenman (1952) and by Rosenman, Byers & Friedman (1952). Gofman et al. (1951) particularly have claimed that the serum lipoproteins represent a transport mechanism for the absorbed fat and that the fat migrates from the chylomicrons through the lipoprotein fractions to be finally metabolized by the tissues, and especially by the liver. Rosenman et al. (1952) have shown that the turnover of cholesterol in the liver is accelerated in hyperthyroidism, with a resulting fall in the cholesterol level of the blood and a rise in the cholesterol level in the bile, and that the position is reversed in hypothyroidism. This may also apply to the other constituents of the total lipids in the blood. Probably the liver is the organ most immediately concerned with the changes in the lipoprotein level in thyroid disorders. Further it might be mentioned that treatment with TSH will cause thyrotoxic changes in the lipid pattern when the thyroid gland responds to this treatment, but this is not the case in athyrotic subjects; this indicates a thyroid hormone action (unpubl. data).

As our results show, electrophoretic lipoprotein fractionation reveals changes which are to be expected, i.e. in hypothyroidism and during the treatment of hypothyroid and hyperthyroid states. The method seems to have been over-
estimated (Swahn, 1953) but provided that not too much is expected from the exactness of the method, it may be said that it might find clinical application especially as the ultracentrifugal method cannot be used in clinical laboratories.

SUMMARY

The total serum cholesterol, total serum lipid and the paper electrophorethetical lipoprotein fractions (according to Swahn) were determined in normal subjects and in cases of hypothyroidism and thyrotoxicosis before and during treatment. The following results were obtained:

There was a remarkable parallelism in the behaviour of the total lipid, the total cholesterol, the beta lipoprotein and the non-migrating «chylomicron» fractions. They were all elevated in hypothyroidism and decreased during the treatment of the hypothyroid state with thyroxin or thyroid. On the other hand, no significant decrease of these fractions could be detected in thyrotoxicosis but an augmentation nevertheless occurred during treatment. The alpha lipoprotein fraction did not exhibit any marked changes. No fraction with an abnormal migration rate could be observed either in hypothyroidism or in thyrotoxicosis.

The fractionation of the serum lipoprotein with paper electrophoresis seems to be of some value in the diagnosis of hypothyroidism and in the evaluation of the treatment of thyroid disorders.

REFERENCES

Byers, S. O., Friedman, M. & Rosenman, R. H.: Metabolism 1, 479, 1952.