THE LIPID DISTRIBUTION IN NORMAL AND ABNORMAL LIVER TISSUES.

III. THE EFFECT OF DISEASE UPON THE LIPID DISTRIBUTION IN HUMAN LIVER TISSUE.

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In Papers I and II of this series, the lipid distribution in beef and rabbit liver tissue was discussed. It was shown that in normal liver tissue, of the total lipid material present in the tissue, there exists a certain definite relation between the phospholipid and the neutral fat. We have found that in perfectly normal tissue this ratio of phospholipid to neutral fat is constant. MacLean and Williams (1) in 1909 showed that the greater portion of fatty material extracted from liver tissue was present in the phospholipid form. These workers infer that the percentage of phospholipid with relation to the total lipid content may be 84 per cent. In our work, with livers of different species of animals, we have never been able to obtain much better than 75 per cent phospholipid. Mayer and Schaeffer (2) have examined various tissues and found the proportion of lipids in most tissues comparatively constant and even unchanged after fasting. These workers point out that it appears probable that the character of an organ may be conditioned as much by proportion of its constituents as by the specific properties of the constituents themselves. MacLean (3) writes as follows: "The constant presence of the lipins in every cell and their retention even under conditions of extreme emaciation makes it certain that they are essential for maintaining the vital processes of the cell."

Our work to date well supports the contentions of MacLean. We have found that normal liver tissue has a rather constant
lipid content, which for animals of the same species compares favorably within the experimental error. Under abnormal conditions this constancy usually maintains itself. However, there are other facts to be considered as well as the so called lipid constancy of the tissue. Not only is the total lipid material of the tissue, in the main, constant but the relation of the separate constituents to the total lipid material is also constant.

In the general lipid classification as outlined by Bloor (4), we find the lipids divided into three main groups, simple, compound, and derived lipids. For our purpose, it would seem necessary to discuss only the simple and compound lipid material; neutral fat is a representative of the former and phospholipid of the latter. It is possible to express the relation of the phospholipids to the neutral fat in an equilibrium equation: phospholipid ⇄ neutral fat. In normal liver tissues, this relation is apparently a constant. This ratio has been worked out for beef and rabbit liver material (5, 6) under both normal and abnormal conditions and may be expressed for the normal tissue as follows:

\[
\text{Phospholipid} \leftrightarrow \text{neutral fat} \\
\text{Beef liver} \quad \frac{55}{45} \\
\text{Rabbit liver} \quad \frac{55-65}{35-45}
\]

When, however, certain degenerative changes set in, or abnormal conditions arise, there may be either a change in total lipid content or more generally a shift to the right of the phospholipid-neutral fat ratio. Such an abnormal state as may occur in fatty degeneration may cause this ratio to change from 65:35 to 3.5:96.5 (5, 6). Injection of insulin into perfectly normal test rabbits, caused a marked change in the phospholipid-neutral fat ratio. The following tabulation shows the effect of various abnormal conditions upon the phospholipid-neutral fat ratio of rabbit liver tissue.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Phospholipid-neutral fat per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>65:35</td>
</tr>
<tr>
<td>Insulin-treated</td>
<td>4:96</td>
</tr>
<tr>
<td>Phosphorus-treated*</td>
<td>4:96</td>
</tr>
<tr>
<td>Castrated†</td>
<td>26:74</td>
</tr>
</tbody>
</table>

* Rabbits were given phosphorus in olive oil; animals killed in 36 hours; livers removed and examined.
† Series of males castrated and killed 24 hours later; livers removed and examined.
In the animals given phosphorus, we know that severe fatty degeneration of the liver tissue occurred and this is in accordance with our suggested theory that any condition which will tend to destroy the normalcy of the organ or tissue will cause the phospholipid-neutral fat ratio to change. In a former paper (6) we have pointed out two possible effects of insulin upon the animal economy: first, over a long period of time, insulin causes a decrease in the total lipid content of the liver; and secondly, over a short period, insulin causes a marked change in the phospholipid-neutral fat ratio.

Since it was found that abnormal conditions caused such great differences in the phospholipid-neutral fat ratio in beef and rabbit liver tissue, it seemed of interest to ascertain if such changes of ratio occurred with human liver tissue. It was our purpose to determine just what changes had taken place in the phospholipid-fat ratio of the human liver under the influence of disease. For working material we were dependent upon such cases as came to hand in the surrounding hospitals. In the work to follow, we have classed as normal liver tissue only such organs as appeared after postmortem operation to be perfectly healthy and free from all signs of degeneration of any kind. For diseased tissue, we used those that came to hand, paying particular attention to the organs which showed distinct signs of degeneration.

EXPERIMENTAL.

The liver tissue was removed as soon after death as practical and immediately cut into very small pieces. This material was then dehydrated with absolute alcohol at a temperature of 35°. After preliminary dehydration, the total lipid material was extracted from the tissue with boiling absolute alcohol. After extraction, the alcoholic extract was concentrated in vacuo at a low temperature. The extract remaining after concentration was treated with ether and the lipid material removed. The ether solution was allowed to stand for several hours in order that any water might separate. The ether solution was then made up to 500 ml. with anhydrous ether. An aliquot portion of the ether solution was used for the determination of total lipid material. The remaining solution was used for the separation of the following fractions: (1) total phospholipids, (2) solid and liquid fatty material. Each of these fractions was then subjected to the
following analysis: percentage of mixed fatty acids; percentage of solid and liquid fatty acids; percentage of brominated acids

**TABLE I.**

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total lipid extracted</td>
<td>3.45</td>
<td>3.04</td>
<td>2.88</td>
<td>2.00</td>
<td>3.94</td>
<td>5.41</td>
<td>5.16</td>
<td></td>
</tr>
<tr>
<td>Phospholipid</td>
<td>49.80</td>
<td>58.90</td>
<td>38.80</td>
<td>44.50</td>
<td>16.60</td>
<td>31.10</td>
<td>39.93</td>
<td>33.20</td>
</tr>
<tr>
<td>Solid fatty material</td>
<td>2.97</td>
<td>3.39</td>
<td>5.94</td>
<td>6.20</td>
<td>1.00</td>
<td>2.30</td>
<td>9.92</td>
<td>12.80</td>
</tr>
<tr>
<td>Liquid fatty material</td>
<td>48.00</td>
<td>40.50</td>
<td>53.60</td>
<td>51.20</td>
<td>82.40</td>
<td>66.60</td>
<td>57.13</td>
<td>54.00</td>
</tr>
</tbody>
</table>

* The sample numbers refer to samples cited in the text.

**TABLE II.**

<table>
<thead>
<tr>
<th>Phospholipid fraction</th>
<th>Sample 1</th>
<th>Sample 2</th>
<th>Sample 3</th>
<th>Sample 4</th>
<th>Sample 5</th>
<th>Sample 6</th>
<th>Sample 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iodine No. of total lipid</td>
<td>85.6</td>
<td>73.7</td>
<td>71.3</td>
<td>84.2</td>
<td>94.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&quot; &quot; &quot; mixed fatty acids</td>
<td>121.0</td>
<td>101.0</td>
<td>105.0</td>
<td>114.0</td>
<td>103.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&quot; &quot; &quot; liquid fatty acids</td>
<td>199.0</td>
<td>187.5</td>
<td>151.0</td>
<td>185.0</td>
<td>148.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Per cent of mixed fatty acids</td>
<td>63.4</td>
<td>60.0</td>
<td>51.0</td>
<td>68.0</td>
<td>68.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Solid fatty acids, per cent of mixed fatty acids</td>
<td>34.4</td>
<td>41.0</td>
<td>31.3</td>
<td>40.4</td>
<td>26.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liquid fatty acids, per cent of mixed fatty acids</td>
<td>65.6</td>
<td>59.0</td>
<td>68.7</td>
<td>59.6</td>
<td>74.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4-Bond acids, per cent of liquid fatty acids</td>
<td>14.0</td>
<td>14.2</td>
<td>18.3</td>
<td>25.2</td>
<td>12.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Liquid fatty fraction</th>
<th>Sample 1</th>
<th>Sample 2</th>
<th>Sample 3</th>
<th>Sample 4</th>
<th>Sample 5</th>
<th>Sample 6</th>
<th>Sample 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iodine No. of total lipid</td>
<td>103.7</td>
<td>103.0</td>
<td>112.0</td>
<td>81.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&quot; &quot; &quot; mixed fatty acids</td>
<td>119.0</td>
<td>115.0</td>
<td>130.4</td>
<td>83.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&quot; &quot; &quot; liquid fatty acids</td>
<td>176.0</td>
<td>151.0</td>
<td>145.0</td>
<td>153.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Per cent of mixed fatty acids</td>
<td>76.0</td>
<td>60.5</td>
<td>78.0</td>
<td>81.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Solid fatty acids, per cent of mixed fatty acids</td>
<td>29.8</td>
<td>21.0</td>
<td>38.0</td>
<td>34.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liquid fatty acids, per cent of mixed fatty acids</td>
<td>70.2</td>
<td>79.0</td>
<td>62.0</td>
<td>66.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4-Bond acids, per cent of liquid fatty acids</td>
<td>14.4</td>
<td>11.4</td>
<td>14.4</td>
<td>10.0</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* The numbers refer to samples cited previously.

(insoluble in ether) in the liquid fatty acids; iodine values of the fraction, mixed acids, and liquid fatty acids.
The specimens examined are listed as follows:

**Sample 1.**—Female, age 35 years. The liver appeared healthy; histological examination showed no degeneration. This was taken as a normal specimen.

**Sample 2.**—Female, age 22 years; general peritonitis. The liver appeared to be in healthy condition, histological examination showed no degeneration.

**Sample 3.**—Male, age 37 years. The liver showed distinct signs of fatty degeneration; macroscopically it appeared yellow, streaked, and spotted.

**Sample 4.**—Male, age 35 years; cerebral thrombosis associated with cerebral edema and chronic adhesive pleurisy. The liver showed signs of fatty degeneration.

**Sample 5.**—Male, age 21 years; bilateral pulmonary tuberculosis with extensive caseation, necrosis, and cavitation. Further examination showed early tuberculous enteritis, peritonitis, and hepatitis. The liver was yellow and spotted and in very poor condition and indicated extensive fatty degeneration.

**Sample 6.**—Female, age 35 years; chronic peritonitis; hepatitis with toxic degeneration. Histological examination showed the liver to be in poor condition.

**Sample 7.**—Male, age 30 years; pneumonia. The liver showed distinct signs of fatty degeneration.

**Sample 8.**—Child, male, died at birth. No nourishment was received and since the liver had not functioned in the normal sense, it cannot be classed as a normal organ.

Table I shows the lipid distribution of the various human liver tissues examined.

Table II shows the fatty acid distribution of the lipids obtained from some of the liver tissue examined.

**DISCUSSION.**

From an examination of Table I, the following conclusions can be drawn.

(a) Samples 1 and 2 represent as nearly normal liver tissue as could be expected from the cases available for analysis. Taking these specimens as representative of a possible normal condition, we can express the equilibrium equation

\[ \text{Phospholipid} \rightleftharpoons \text{neutral fat} \\
60\text{ per cent} \quad 40\text{ per cent} \]

This ratio is practically identical with the ratio obtained for normal beef and rabbit liver tissue.
Samples 3 and 4 may be said to represent average fatty degeneration conditions, and it is seen that the phospholipid-neutral fat ratio has been shifted markedly to the right, being shown in Sample 3 as 38.8 to 61.2 and in Sample 4 as 44.5 to 55.5. Sample 5 showed histologically severe fatty degeneration and in our examination gave a phospholipid-fat relation of 16.6 to 83.4, which is in line with what should occur if this equilibrium relation is of value.

Sample 7 shows the effect of pneumonia upon the lipid distribution of the liver, and it is seen that the toxic poisons have caused a distinct change in the lipid material of this organ. Sample 8 cannot be taken as representative of any one class and is given only for general interest. This liver can only be taken as embryonic tissue, and it may well be that the lipid distribution in it is representative of the embryonic state.

The total lipid content is of fair constancy in both the normal and abnormal tissue, especially in consideration of the variety of diseased organs. In most of the abnormal cases it is also to be noted that the percentage of solid fatty material is greater than in the normal states.

The chemical examination checked well with the histological examinations conducted in the hospital at the time of the post-mortem operation.

**Fatty Acid Distribution.**

We find that the fatty acid distribution in human liver tissue is for all practical purposes approximately the same as that for beef and rabbit tissue. The unsaturation of the liquid fatty acids is of the same order as that of other liver material examined to date. The 4-bond acids, which represent arachidonic acid, are distributed throughout both the phospholipid and liquid fatty fractions to about the same degree. Leathes and Meyer-Wedell (7) brought forward evidence that desaturation of the fatty acids takes place in the liver. Later MacLean pointed out that if the fatty acids were built up into phospholipids before desaturation, it would be expected that the highly unsaturated acids would be confined to the phospholipid fraction. Our investigations up to the present time have shown that the unsaturation of the fatty acids is not confined to the phospholipid fraction but that in most...
cases the neutral fat fraction contains but little less unsaturated fatty acids. The 4-bond acids are distributed throughout both the phospholipid and the neutral fat fractions.

The iodine values of the fatty acids of the neutral fat fraction are somewhat lower than those of the phospholipid fraction, but sufficiently high to indicate strongly that the unsaturated fatty acids are by no means confined to any one fraction.

As pointed out by Bloor and also in our previous papers, liver lipids apparently contain no 3-bond acids but do contain certain amounts of 2-bond acids. From the percentage of 4-bond acids and the iodine value of the liquid fatty acids, the amount of linoleic or 2-bond acids can be readily calculated. The 2-bond acids in the phospholipid fractions show a value of approximately 70 per cent.

**Unsaponifiable Matter.**

After saponification of the fraction there was usually a certain amount which was unsaponifiable under the conditions maintained. As a general thing, the amount of the unsaponifiable material was less in the phospholipid fraction than in the neutral fat fraction. In all the cases examined the phospholipid fraction showed an average percentage of unsaponifiable matter of about 5, while the neutral fat fraction showed about 10 per cent. On further examination of this material, it was found that the cholesterol was practically all in the free state.

There are many indications that the lipids play an important rôle in fat metabolism, but just what is that rôle? It has been suggested that both phospholipids and neutral fat are stored in the tissue, but the writer believes only the neutral fat to be stored material and the phospholipids to be active in the actual metabolism of the organ. This contention is supported by all our work. In every case in which the actual functioning of the organ is disturbed, we find the lipid distribution changed to the disadvantage of the phospholipid content.

**CONCLUSION.**

The lipid distribution and fatty acid distribution of human liver tissue under normal and abnormal conditions have been investigated. The data obtained support the work with beef and rabbit
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liver tissue. Pneumonia, tuberculosis, and fatty degeneration alter the phospholipid-fat ratio of the normal tissue, the phospholipids decreasing proportionally with the stage of the disease.

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BIBLIOGRAPHY.

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