THE METABOLISM OF THE PHOSPHOLIPIDS.

I. THE INFLUENCE OF DIET ON THE AMOUNT AND COMPOSITION OF THE PHOSPHOLIPID FATTY ACIDS IN VARIOUS TISSUES OF THE CAT.*

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INTRODUCTION.

From the mass of information concerning the phospholipids of animal tissues several facts are outstanding: one is the marked constancy in the percentage amount present under widely different nutritional conditions (Mayer and Schaeffer, 1913); another is the apparent relationship between the functional activity of a tissue and its phospholipid content (Bloor, 1926, 1927, 1928); and still another is the high degree of unsaturation of their constituent fatty acids as compared with depot fat. The general conclusion has been that the phospholipids are undoubtedly essential constituents of protoplasm and fulfil a very important function in the vital reactions of the cell, but surprisingly little is known concerning the precise nature of this function and the purpose of the high degree of unsaturation.

As compared with the amount of work that has been done on the influence of ingested fat on the character of the fat deposited in the animal stores (see Anderson and Mendel, 1928; Eckstein, 1929), very little attention has been paid to the possible effect of food fat on the composition of the tissue phospholipids. Such investigations as have been made have been concerned, for the

* The substance of this paper was presented at The Thirteenth International Physiological Congress held at Boston in August, 1929 (Sinclair and Bloor (1929)).
most part, with the effect of food fat either on the degree of un-
saturation of the phospholipids in the liver (Joannovics and Pick,
1910; Shioji, 1924) or on the composition of the lecithin in the eggs
of laying hens (McCollum, Halpin, and Drescher, 1912-13). The
findings of these various workers agree in showing that the degree
of unsaturation of the phospholipids is influenced to a very
appreciable extent by the type of fat in the diet.

Recently Terroine and Belin (1927) have come to quite the
opposite conclusion. On the basis of a comprehensive study of
the fatty acids of the élément constant (which are, according to
these authors, the phospholipid fatty acids) of various animals
and animal tissues, they concluded that the composition as well
as the amount of the phospholipid in a tissue is a fixed characteris-
tic of that particular tissue and wholly independent both of the
species of the animal and of the nature of the fat in the diet.

In a recent paper (1929) the author presented evidence to show
that the fatty acids of the food fat are transformed into phospho-
lipid in the intestinal mucosa and in the liver within a few hours
after its ingestion, presumably as a stage in the resynthesis into
neutral fat. At the same time attention was called to the fact
that the degree of unsaturation of the phospholipid fatty acids in
the intestinal muscle is unmistakably higher in cats fed on beef
kidney than in those fed on meat scraps. Since the total fatty
acids of beef kidney are, on the average, more unsaturated than
those of beef muscle (Bloor, 1927, 1928) the obvious interpreta-
tion is that food fat in due time does exert an influence on the com-
position of the tissue phospholipids.

In view of the contradiction between our own observations and
those of Terroine and Belin, and, moreover, because of its great
importance for the proper understanding of the function of the
phospholipids, it was deemed advisable to obtain a definite answer
to the question of whether or not the composition of the phospho-
lipids is influenced by the character of the food fat.

In the present paper data are presented which show beyond any
doubt that the degree of unsaturation of the phospholipid fatty
acids of the liver, heart, kidneys, muscle, intestinal mucosa, and
probably the brain of the cat is dependent to a very consider-
able extent upon the type of diet fed.
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Procedure.

Since it had been found that the phospholipids of the intestinal and skeletal muscles of the cat are not affected by ingested fat during the period of absorption (Sinclair, 1929), it was concluded that the apparent effect of diet on the composition of the muscle phospholipids was probably a gradual process. Consequently all cats used in the present investigation were maintained on one or the other of the two diets for at least 2 weeks and usually longer before they were killed.

Diets.—In choosing diets the first requirement of course was that there should be a considerable difference in the degree of unsaturation of their total fatty acids. However, other factors had to be considered. Owing to the length of time over which the feeding had to be continued, it was necessary that the diets should remain reasonably constant in composition from day to day. Furthermore since cats are very particular about their food, the diets had to be of such a nature that they would be readily consumed. All of these requirements were nicely filled by beef kidney and lean beef muscle. From the work of Bloor (1927, 1928) it has been calculated that the total fatty acids in beef kidney have, on the average, an iodine number (I.N.) of 98, while those of beef muscle (in this case the round was used) have an average I.N. of 82. The difference between these average I.N. is not as great, nor is the composition of these tissues as uniform, as might be wished for, but these faults were compensated by the absence of any difficulty in getting the cats to eat their food regularly.

Preparation of Tissues for Extraction.—The cats were anesthetized with illuminating gas and then bled to death.

In order to obviate as much as possible postmortem changes, every effort was made to have the hashed tissues immersed in alcohol in the shortest time possible. Since four tissues from each cat were used and only two of them could be extracted at one time, it was necessary to preserve the other two immersed in cold alcohol in the dark for 6 to 8 hours before extraction. In order that preservation of the tissue in this way should not influence the comparative value of the results, the order in which the tissues were analyzed was alternated.

1 The author is indebted to Mrs. Jane Fagan for valuable assistance in preparing the tissues for extraction.
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Extraction and Separation of Lipids. The procedure followed in extracting and separating the lipids was essentially that used by Bloor (1926). Briefly, it involves extraction of the hashed tissue with hot alcohol in a continuous extractor, evaporation of the alcohol under reduced pressure, extraction of the residue with moist ether, and separation into an acetone-soluble (fat and cholesterol) and an acetone-insoluble fraction (phospholipids) by the addition of acetone. A small amount of MgCl₂ in alcoholic solution was used to aid complete precipitation of the acetone-insoluble substances. The acetone-insoluble fraction was freed from fat by scrubbing once with acetone and then reprecipitating with acetone from the ether solution. The total acetone-insoluble fraction—except in the case of brain, where only the ether-soluble phospholipids were taken—was saponified for 3 to 4 hours with NaOH in 50 per cent alcoholic solution. After acidification, the fatty acids were extracted with petroleum ether, dried on the steam bath in a current of CO₂, and weighed. The Hanus method was used for the determination of the iodine number.

It is essential to emphasize that—except for brain—the term "phospholipid fatty acids" is used to indicate the fatty acids obtained by the saponification of all the acetone-insoluble lipids.

DISCUSSION.

The data on the amount and I.N. of the phospholipid fatty acids in the various tissues of the cat are condensed in Table I. In every case the value given represents the average of seven cats, except for intestinal mucosa, intestinal muscle, and heart with the kidney diet, in which cases five, two, and six cats, respectively, were used. Since the purpose of the investagation was to compare the phospholipid fatty acids in the tissues of cats fed on a kidney diet with those of cats fed on beef muscle with respect to their amount and degree of unsaturation, it was deemed advisable to subject the data to statistical treatment. To this end, the standard deviation of the individual values from the mean has been calculated by the use of the formula $\sigma = \sqrt{\frac{\Sigma x^2}{n}}$. In addition, a mathematical expression of the significance of the differences between the average values for the two diets has been obtained by
The following is a table from the document:

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Weight of phospholipid fatty acids per 100 gm. moist tissue</th>
<th>Weight of phospholipid fatty acids per 100 gm. dry extracted tissue</th>
<th>Tissue No. of phospholipid fatty acids</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>gm. per cent</td>
<td>gm. per cent</td>
<td>gm. per cent</td>
</tr>
<tr>
<td>Beef kidney</td>
<td>4.82±0.256 5.00±0.218 58.9</td>
<td>43.0±2.93 44.2±2.09 74.1</td>
<td>105±2.4 100±1.8 9.5</td>
</tr>
<tr>
<td>Beef muscle</td>
<td>2.47±0.133 1.94±0.217 3.8</td>
<td>10.23±0.898 7.98±1.210 13.7</td>
<td>145±2.5 124±8.0 1.2</td>
</tr>
<tr>
<td>Brain</td>
<td>2.11±0.136 2.04±0.211 78.1</td>
<td>13.71±0.895 12.86±1.062 54.2</td>
<td>112±1.9 95±3.8 0.006</td>
</tr>
<tr>
<td>Liver</td>
<td>1.28±0.069 1.29±0.055 91.2</td>
<td>8.42±0.348 8.62±0.372 69.7</td>
<td>114±1.3 97±2.9 0.2×10^-8</td>
</tr>
<tr>
<td>Kidney</td>
<td>1.27±0.095 1.25±0.088 88.1</td>
<td>8.69±1.239 7.78±0.603 50.9</td>
<td>140±3.6 121±3.2 0.009</td>
</tr>
<tr>
<td>Mucosa</td>
<td>0.70±0.102 0.61±0.039 40.8</td>
<td>3.38±0.603 3.21±0.244 79.5</td>
<td>131±6.3 108±2.7 0.08</td>
</tr>
<tr>
<td>Heart</td>
<td>0.63 0.64±0.097</td>
<td>3.44 3.47±0.644</td>
<td>109±4.3* 92±4.0 0.37</td>
</tr>
</tbody>
</table>

* The average of seven experiments, five of which were taken from earlier work (Sinclair, 1929).
using the formula \( \frac{\bar{x}}{\sigma} \) in which \( \bar{x} \) is the difference between the means and \( \sigma = \sqrt{(\sigma_1)^2 + (\sigma_2)^2} \), \( \sigma_1 \) and \( \sigma_2 \) being the standard deviations from each of the means (Dunn, 1929, pp. 310, 341).

**Influence of Diet on Amount of Phospholipid.**—The data in Table I show that the phospholipid content of all the tissues with the exception of the liver is the same in the cats fed beef kidney as in those fed on beef muscle. This is no more than is to be expected in the light of the observations of Mayer and Schaeffer (1913) that the diet has no influence on the content of ether-soluble phosphorus in tissues.

On the other hand, it is quite apparent that the livers of cats fed on beef kidney contain more (27 per cent) phospholipid fatty acids than those of cats fed on beef muscle. It seems probable that the explanation of this difference lies in the fact that the phospholipid content of beef kidney is about 7 times that of the round muscle, as shown by the work of Bloor (1927, 1928).

**Influence of Diet on Composition of Phospholipids.**—The data in the last three columns of Table I prove beyond a doubt that the degree of unsaturation of the phospholipids of the various tissues of the cat is influenced by the character of the fat in the diet. Only in the case of the brain is there any uncertainty as to the significance of the difference between the average I.N. These facts are brought out very clearly by the frequency curves given in Fig 1.

Attention has already been called to the fact that in the case of the brain only the ether-soluble substances (lecithin and cephalin) in the acetone-insoluble fraction were saponified. This was done because in the first three experiments, in which the regular procedure was followed, the very low values of 79, 84, and 83 for the I.N. were obtained. Removal of the substances insoluble in ether resulted in higher I.N., but even then the brain did not yield clear cut results as did the other tissues. This fact is hardly to be wondered at, since it is not improbable that the phospholipids of the brain fulfil a different—or an additional—function from that of the phospholipids in the parenchymatous and muscular tissues.

The fact that the fat in the diet governs to a very considerable extent the composition of the tissue phospholipids might be in-
terpreted in two ways: first, that it is evidence in favor of the belief that the phospholipids are intermediary in the metabolism of fat; second, that it shows that phospholipids, being essential constituents of the protoplasm, are subject to continual wear and tear and, just as ingested amino acids are used in the repair of tissue protein, so the fatty acids of food fat are used in the repair

**VARIATION IN IODINE NUMBERS OF PHOSPHOLIPID FATTY ACIDS OF THE CAT**

**FIG. 1.** Curves showing the frequency of distribution of the iodine numbers of the phospholipid fatty acids in various tissues of cats fed on beef kidney (continuous lines) and on beef muscle (dotted lines).
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of tissue phospholipids. Until more is known concerning the rate at which the composition of the phospholipids changes under the stimulus of a change in the character of the diet, it is considered inadvisable to attempt to decide as to which of the above interpretations is correct.

SUMMARY.

A study has been made of the question of whether or not the composition of the phospholipids in animal tissues is influenced by the character of the fat in the diet.

It has been found that the degree of unsaturation of the phospholipid fatty acids in the liver, heart, kidneys, smooth and skeletal muscle, intestinal mucosa, and probably the brain is consistently higher when cats are fed on an exclusive diet of beef kidney than when they are fed on hashed beef muscle.

This fact may indicate either that phospholipid is intermediary in the metabolism of fat or that the tissue phospholipids undergo continual wear and tear and replacement at the expense of food fat.

The amount of phospholipid fatty acids in all tissues except the liver is the same for both diets; in the liver there is a greater content of phospholipid when beef kidney is fed than when the diet consists of beef muscle.

The author takes pleasure in acknowledging his indebtedness to Professor Bloor for counsel and helpful criticism throughout this investigation.

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