THE METABOLISM OF THE PHOSPHOLIPIDS.

II. THE INFLUENCE OF GROWTH ON THE PHOSPHOLIPID (AND CHOLESTEROL) CONTENT OF THE WHITE RAT.

BY ROBERT GORDON SINCLAIR.

(From the Department of Biochemistry and Pharmacology, The University of Rochester School of Medicine and Dentistry, Rochester, New York.)

(Received for publication, June 9, 1930.)

INTRODUCTION.

In the course of an investigation of the influence of the character and the amount of the fat of the food on the composition of the lipids, especially the phospholipids, of the white rat, data have been obtained on a series of rats ranging in weight from about 30 up to about 200 gm. It soon became apparent that the smaller the rat the higher was the percentage content of phospholipid fatty acids. A brief search of the literature having failed to reveal any such intimate and comprehensive investigation of the change in the phospholipid content of animal tissues with age, as the phenomenon would seem to justify, the original investigation was extended to include some analyses of suckling rats in order to cover the complete range from birth to adult age.

Mayer and Schaeffer (1914) were probably the first to observe the change in the phospholipid content of animals during growth. Their conclusion was that the percentage content of lipid phosphorus in rats increases sharply following birth and then remains practically constant throughout life. However, their data applied chiefly to rats during the first 3 weeks of postnatal life and, as will be pointed out later, it is altogether likely that the increase in the phospholipid content during this period is entirely due to the rapid decrease in the water content of the tissues. Recently, Javillier, Allaire, and Rousseau (1927) have observed an increase in the lipid phosphorus content of mice during the first 21 days of postnatal life. Furthermore, these authors determined the water content and, from the values obtained, calculated that the per-
Influence of Growth on Phospholipids

centage content of lipid phosphorus in the dry tissues also increases after birth. On the other hand, Robertson (1916) determined the content of alcohol-soluble phosphorus in mice of various ages and found a steady decrease, commencing at birth and continuing throughout life. In the light of our own results and those of Mayer and Schaeffer, and Javillier et al. it is difficult indeed to understand why Robertson did not find a lower content of alcohol-soluble phosphorus in mice at birth than at 14 or 35 days of age.

At best our own data are far from ideal for the purpose in hand but they are sufficiently consistent to permit some general conclusions concerning the influence of growth on the phospholipid content of animal tissues.

Graphical analysis of the data has revealed: (1) that there is a progressive decrease in the water content of the rat after birth, the most rapid period of dehydration being from birth to weaning age, in which time 50 per cent of the total loss in the first 100 days of life occurs; (2) that the percentage content of phospholipid fatty acids in the dry tissues decreases rapidly during the first 3 months of life; (3) that in consequence of the rapid decrease in water content during the first 3 weeks of postnatal life, the percentage content of phospholipid fatty acids in the moist tissue increases rapidly after birth, attains a maximum in about 3 weeks, and then declines throughout the following 13 weeks of life; (4) that the phospholipid : cholesterol ratio in the entire rat remains practically constant throughout the first 100 days of life.

EXPERIMENTAL.

The population of rats upon which the following data have been obtained was raised for the most part under quite varied circumstances. Some of the rats were raised in stock cages with half a dozen, more or less, other rats of about the same age; others were raised in individual metabolism cages made throughout of No. 2 mesh wire screen. The most important variant was the diet. Some of the rats were fed on a diet of kitchen scraps, while the great majority were raised, after weaning, on a ration consisting of alcohol-extracted casein, cane sugar, McCollum’s Salt Mixture 185, ether-extracted dried yeast, and Oscodal, generally supplemented

1 McCollum, E. V., and Simmonds, N., *J. Biol. Chem.*, 33, 63 (1918).
2 A cod liver oil concentrate containing vitamins A and D, kindly supplied by Dr. H. E. Dubin of the H. A. Metz Laboratories, Incorporated.
R. G. Sinclair

with different fats (cod liver oil, lard, olive oil, coconut oil, linseed oil) in amounts ranging from 2.5 to 40 per cent of the total calories. In all cases food and water were provided *ad libitum*.

The analysis of the lipids in the whole rats was carried out as follows: After the live weight had been obtained, the rat was stunned by a blow on the head and immediately the entire animal was hashed in a meat grinder. The hashed tissue remaining in the grinder was removed, the total lot thoroughly mixed, and again passed through the grinder. A 50 gm. sample of the well mixed hashed tissue was weighed out, and then stirred up in about 3 volumes of 95 per cent alcohol. After a few minutes standing the hashed tissue and alcohol were poured into the cloth sack of the hot alcohol extractor in use in this laboratory. The extraction was continued for 3 hours, the alcoholic extract being replaced by fresh alcohol and the tissue well stirred at the end of each hour. The combined alcoholic extracts were distilled practically to dryness on a water bath under reduced pressure. The pasty residue was taken up in moist ether, the ether extract (which in most cases was quite turbid) concentrated, and then washed with ether into acetone in a 100 cc. centrifuge tube. To facilitate complete precipitation of the phospholipids 2 cc. of a saturated solution of MgCl₂ in alcohol were added to the mixture of acetone and ether in the centrifuge tube. After centrifugalization the supernatant acetone and ether solution of the neutral fat and cholesterol was poured off into a flask, and the acetone-insoluble lipids, consisting mainly of the phospholipids, were rubbed up in fresh acetone. To insure against contamination of the phospholipids by neutral fat, the phospholipid fraction was redissolved in ether and again precipitated with acetone and MgCl₂. The phospholipid fraction was redissolved in ether—the ether solution was always quite turbid and contained a considerable amount of suspended material, probably non-lipid in nature—and transferred to a saponification flask. After evaporation of the ether, the phospholipids were saponified with NaOH in 50 per cent alcohol for 3 to 4 hours. The alcoholic solution was cooled and made acid to Congo red paper with concentrated HCl. The fatty acids were extracted with petroleum ether, transferred to a volumetric flask, and a suitable aliquot taken for weight and iodine number determination.²

² The data on the iodine numbers of the phospholipid and neutral fat fatty acids will be published in a separate paper.
The acetone-ether solution of the neutral fat and unsaponifiable substances was distilled to a small volume, rinsed into a separatory funnel with petroleum ether, and washed with water. The petroleum ether solution was concentrated and made up to volume in a volumetric flask. An aliquot of ⅔ or ⅔ was taken for weight and another was pipetted into a saponification flask, and, after evaporation of the petroleum ether, saponified with equal volumes of N sodium ethylate and absolute alcohol. After several hours of boiling under a reflux condenser, an equal volume of water was added and boiling continued for 2 to 3 hours longer. Then the soap solution was cooled, transferred to a separatory funnel, shaken up with petroleum ether, and allowed to stand overnight. The next day the soap solution was drawn off, the petroleum ether extract washed with 50 per cent alcohol, and again separated. The petroleum ether extract was distilled and the residue of unsaponifiable matter was dried and weighed. After acidification of the soap solution, the fatty acids were extracted with petroleum ether, made up to volume, and an aliquot taken for weight and iodine number determination.³

The tissue residue remaining after the 3 hours extraction with hot alcohol was dried in the air and weighed. This weight was found to be a fairly accurate measure of the water-free, lipid-free tissue solids.

Since neutral fat is probably to be regarded as inert deposit material, a calculation has been made of the fat-free moist weight of all rats. However, this weight is still an inaccurate measure of the active protoplasm since it includes the weight of the skeleton, teeth, hair, and the other relatively inert structural elements of the body. A correction for the weight of the bony skeleton could have been made by using the data given by Donaldson (1924, p. 188), but there seemed to be no particular advantage in doing so. A correction for the weight of the deposit fat was quite essential since the fat content of animals of the same age varied greatly because of the marked difference in the fat-producing quality of the various diets used.

From the data obtained, calculations have been made of the percentage content of phospholipid fatty acids, neutral fat, and unsaponifiable material in terms of the moist fat-free weight and the dry extracted weight of the tissue.
Fig. 1. Chart showing the change in the content of phospholipid fatty acids in the moist fat-free tissues of the rat with increase in body weight. Gm. of phospholipid fatty acids per 100 gm. of moist fat-free tissue are plotted on the ordinate and body weight on the abscissa. In this and the succeeding figures the curves have been placed purely by inspection.

Fig. 2. Chart showing the change in the content of phospholipid fatty acids in relation to the weight of the dry extracted tissues of the rat with increase in body weight. Gm. of phospholipid fatty acids per 100 gm. of dry extracted tissue are plotted on the ordinate, body weight on the abscissa.
**FIG. 3.** Chart showing the change in the content of phospholipid fatty acids in the moist fat-free tissue with increase in age. Gm. of phospholipid fatty acids per 100 gm. of moist fat-free tissue are plotted on the ordinate, postnatal age on the abscissa.

**FIG. 4.** Chart showing the change in the content of phospholipid fatty acids in relation to the weight of the dry extracted tissues with increase in age. Gm. of phospholipid fatty acids per 100 gm. of dry extracted tissue are plotted on the ordinate, postnatal age in days on the abscissa.
When the live weight of the rat taken was less than 50 gm., all of the hashed tissue was carefully removed from the grinder by rinsing with alcohol and included in the sample for extraction. Furthermore, in the cases of the very small rats weighing less than

**Fig. 5.** Chart showing the change in the water content of the tissues of the rat with increase in age. Gm. of dry extracted tissue per 100 gm. of moist fat-free tissue are plotted on the ordinate, age on the abscissa.

**Fig. 6.** Chart showing the change in the content of unsaponifiable material in relation to the weight of the dry extracted tissues of the rat with increase in age. Gm. of unsaponifiable material per 100 gm. of dry extracted tissue are plotted on the ordinate, age in days on the abscissa.
582 Influence of Growth on Phospholipids

20 gm., the hashed tissue was further ground in a mortar with a weighed amount of sand. The paste of sand and tissue, as well as the traces of tissue adhering to the grinder, were rinsed with alcohol into a weighed Schleicher and Schüll extraction thimble. The extraction was carried out in the ordinary hot alcohol extractor, the alcohol being changed and the tissue and sand rubbed up with alcohol in a mortar at the end of each hour. After the extraction was completed, the shell, tissue residue, and sand were dried and weighed; since the weight of both the shell and sand was known, the weight of dry extracted tissue could be calculated.

The completeness of the hot alcohol extraction of the lipids from the hashed rat tissue was tested in two ways; first, by extracting the tissue residues with ether in a Soxhlet apparatus for 6 hours; second, by saponifying the residues with strong alkali, extracting the acidified solution with ether, evaporating to dryness, and extracting the ether residue with petroleum ether. The residues of two batches of very young rats treated by the first method yielded less than 1 mg. of ether residue in either case, while the combined residues from several rats treated by the second method yielded fatty material amounting to, on the average, 0.56 per cent of the total lipids extracted.

Results.

The data obtained have been plotted in the form of graphs to show the change in the content of water, of phospholipid fatty acids, and of unsaponifiable material with increase in body weight and with increase in age. There is quite a considerable scattering of the points on the graphs (Figs. 1–6), a fact scarcely to be wondered at in view of the heterogeneity of the population with respect to diet and, therefore, the rate of growth. Nevertheless, the curves leave little room for doubt as to the general effect of growth on the content of phospholipid and of unsaponifiable material in the white rat.

Discussion.

Since the phospholipid content of such organs as the brain, heart, liver, and kidneys is notably higher than that of others, particularly the muscles, one is led to wonder if a shift in the relative proportions of the various organs of the body might not be
R. G. Sinclair

responsible for the apparent decrease in the phospholipid content of the rat during growth. Obviously an increase in the relative

TABLE I.

Percentage Weight of Brain and Viscera in Rats of Various Sizes.

<table>
<thead>
<tr>
<th>Body weight (gm.)</th>
<th>Brain: Per cent of body weight</th>
<th>Relative per cent</th>
<th>Viscera: Per cent of body weight</th>
<th>Relative per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.9</td>
<td>4.61</td>
<td>1.00</td>
<td>10.89</td>
<td>1.00</td>
</tr>
<tr>
<td>25.0</td>
<td>5.03</td>
<td>1.09</td>
<td>19.23</td>
<td>1.76</td>
</tr>
<tr>
<td>50.0</td>
<td>2.97</td>
<td>0.64</td>
<td>17.74</td>
<td>1.64</td>
</tr>
<tr>
<td>100.0</td>
<td>1.68</td>
<td>0.36</td>
<td>14.94</td>
<td>1.38</td>
</tr>
<tr>
<td>200.0</td>
<td>0.93</td>
<td>0.20</td>
<td>12.39</td>
<td>1.14</td>
</tr>
<tr>
<td>300.0</td>
<td>0.66</td>
<td>0.14</td>
<td>11.19</td>
<td>1.03</td>
</tr>
<tr>
<td>400.0</td>
<td>0.51</td>
<td>0.11</td>
<td>10.51</td>
<td>0.97</td>
</tr>
</tbody>
</table>

* Viscera includes heart, hypophysis, intestines, kidneys, liver, lungs, pancreas, spleen, stomach, submaxillaries, suprarenals, and thyroid.

TABLE II.

Content of Phospholipid Fatty Acids in the Carcasses* of Rats at Various Ages.

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>Body weight (gm.)</th>
<th>Weight of carcass in relation to body weight</th>
<th>Weight of phospholipid fatty acids per 100 gm. of:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Molar fat-free tissue</td>
<td>Dry extracted tissue</td>
</tr>
<tr>
<td>28</td>
<td>34</td>
<td>34.8</td>
<td>1.20</td>
</tr>
<tr>
<td>41</td>
<td>76</td>
<td>38.3</td>
<td>1.12</td>
</tr>
<tr>
<td>43</td>
<td>80</td>
<td>36.8</td>
<td>1.13</td>
</tr>
<tr>
<td>51</td>
<td>76</td>
<td>36.0</td>
<td>1.17</td>
</tr>
<tr>
<td>61</td>
<td>130</td>
<td>40.8</td>
<td>1.06</td>
</tr>
<tr>
<td>59</td>
<td>138</td>
<td>43.1</td>
<td>1.01</td>
</tr>
<tr>
<td>73</td>
<td>156</td>
<td>40.2</td>
<td>0.98</td>
</tr>
<tr>
<td>83</td>
<td>164</td>
<td>40.9</td>
<td>0.97</td>
</tr>
<tr>
<td>96</td>
<td>172</td>
<td>40.4</td>
<td>0.94</td>
</tr>
<tr>
<td>107</td>
<td>166</td>
<td>41.4</td>
<td>1.06</td>
</tr>
</tbody>
</table>

* The carcass comprises skeletal muscle and bone; the skin, head, tail, feet, and viscera being discarded.

proportion of muscle or bone would tend to decrease the phospholipid content of the entire animal.
The marked change in the percentage weight of the brain and viscera during the growth of the rat as shown by Table I (compiled from Donaldson (1924)) illustrates how important a factor the relative decrease in the weight of such phospholipid-rich tissues as the brain, heart, liver, and kidneys may be in causing the observed decrease in the phospholipid content of the entire rat. However, the data which have been obtained on the carcasses of rats of various ages (Table II) show that in the carcass as well as in the entire animal there is a decrease in the content of phospholipid fatty acids with increase in body weight. Unfortunately the interpretation of these data is also complicated in that the carcasses include the skeleton as well as the musculature and there is no saying how much of the phospholipid is contributed by the bone marrow; nevertheless it seems probable that these data indicate an actual decrease in the phospholipid content of the skeletal muscles during growth. Since the percentage weight of the skeleton decreases during growth (Donaldson, 1924), the inclusion of the bone with the soft tissues tends to diminish rather than to magnify the effect of growth on the phospholipid content of both the entire animal and the carcass. Koch and Koch (1913) have shown that the phospholipid content of rat brains, expressed in relation to the dry solids, increases from birth to about the age of 3 weeks and thereafter remains practically constant.

That the water content of animal tissues decreases throughout life has been observed by a number of investigators (Moulton, 1923). This fact is illustrated by Fig. 5 which shows the influence of age on the percentage content of dry lipid-free solids. The water content may be assumed to be approximately the difference between the moist fat-free weight and the dry extracted weight plus the phospholipid since the data calculated in this manner show good agreement with those obtained by Hatai (cited by Moulton (1923)).

The rapid decrease in the water content of the rat during the first 3 weeks of postnatal life is of especial interest in that it seems to be entirely responsible for the peculiar conformation of the curves in Figs. 1 and 3. These curves apparently show a rapid increase in the phospholipid content of the moist tissue during the first 3 weeks of life, although actually the phospholipid content of the tissue solids is decreasing as is shown by Figs. 2 and 4.
In Fig. 6 the weights of the unsaponifiable material per 100 gm. of dry extracted tissue have been plotted against the age of the rats. Despite the very considerable scattering of the points—for which there seems to be no satisfactory explanation—it is believed that the curve which has been drawn is reasonably representative and may well be taken as a measure of the influence of age on the content of unsaponifiable material in the white rat.

In view of the importance which is frequently attached to the phospholipid:cholesterol ratio it is of interest to determine whether or not the process of growth, which has a marked influence on the content of phospholipid and unsaponifiable material in the entire rat, also has an effect upon the ratio of these substances to one another. Accordingly the ratios phospholipid fatty acids: unsaponifiable material have been calculated for every 10 day interval from birth to 100 days of age, from the values given by the curves in Figs. 4 and 6. Since the ratios thus calculated vary, quite independently of age, between the rather narrow limits of 3.0 to 3.3, it seems probable that growth influences the content of phospholipid and unsaponifiable material in the rat to about the same extent, the ratio of the two substances thereby remaining constant.

Since the total unsaponifiable material includes, besides cholesterol, other substances about which very little is known, it seemed desirable to determine the percentage content of cholesterol in the unsaponifiable material. This can easily be done by the Bloor colorimetric method (1916) which is based upon the Liebermann-Burchard reaction for cholesterol. The cholesterol content of the unsaponifiable material from twenty-four rats, ranging in age from newborn to 105 days, fell between 57.1 and 75.5 per cent, with an average value of 67.1 ± 5.6 per cent. With the use of this average value of 67.1 per cent of cholesterol in the unsaponifiable material, and the value 66 per cent for the percentage weight of fatty acids in the phospholipid, the average phospholipid:cholesterol ratio for the entire body of the white rat was found to be 7.0.

At the present time it would seem to be rather difficult to arrive at any satisfactory conclusion as to the physiological significance of the marked decrease in the phospholipid content of the rat with increase in body weight. An extensive study of the phospholipid content of various organs of the beef led Bloor (1926, 1927, 1928)
to conclude that the phospholipid content of a tissue is a function of its physiological activity. On this basis, the very considerable decrease in the phospholipid content of rats during the first 3 months of postnatal life would indicate a progressive decline in the physiological activity during this period. However, in so far as muscular activity is concerned, there seems to be a lack of agreement between the phospholipid content of the skeletal muscles of the rat and their degree of activity since, according to Slonaker (1907), the activity of rats increases with age up to 87 to 120 days whereas during this period the phospholipid content is steadily decreasing (Table II).

With respect to the cause of the decrease in phospholipid content with increase in body weight, the shape of the curves in Figs. 1 to 4 indicates that the period of most rapid decline in phospholipid content coincides with the period of most rapid growth. This fact suggests that there may be a certain measure of independence in the rates of synthesis of phospholipid and of the other solid constituents of the tissues. In this connection the case of a young rat which grew at an unusually rapid rate may be of some significance; the phospholipid content of this young rat was found to be much below the normal value for rats of the same age or of the same weight whereas the actual weight of phospholipid was approximately normal for its age. Of interest also are the data on a rat which for some unknown reason maintained a constant weight of around 36 gm. for 8 weeks. The data for this abnormal rat in the charts have been encircled by a line. It may be seen that the content of phospholipid fatty acids corresponds quite closely to that of normal rats of the same size but much younger. This fact is especially interesting since, according to Mendel and Judson (1916), the water content of rats stunted by deficient diets is typical of their age rather than of their body weight.

**SUMMARY.**

A study of the content of phospholipid fatty acids and unsaponifiable material in the entire bodies of white rats during the first 4 months of life has shown:

1. That the phospholipid content, when expressed in relation to the tissue solids, decreases rapidly after birth, the period of most rapid decline in the phospholipid content coinciding with the period of most rapid growth.
2. On account of the rapid decrease in the water content of the tissues of the rat as a whole during the first few weeks of postnatal life, the phospholipid content of the moist fat-free tissues increases rapidly after birth, reaches a maximum in about 3 weeks, and then declines throughout the remainder of the period studied.

3. The ratio phospholipid fatty acids: unsaponifiable material remains practically constant during the first 3 months of life.

The author is indebted to Professor W. R. Bloor for advice and criticism throughout the progress of this work.

**BIBLIOGRAPHY.**

Bloor, W. R., *J. Biol. Chem.*, 24, 227 (1916); 68, 33 (1926); 72, 327 (1927); 80, 443 (1928).


Robert Gordon Sinclair


Access the most updated version of this article at http://www.jbc.org/content/88/2/575.citation

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/88/2/575.citation.full.html#ref-list-1