THE RATE OF TURNOVER OF PHOSPHOLIPIDS IN KIDNEY AND LIVER

BY ROBERT GORDON SINCLAIR

(From the Department of Biochemistry, Queen's University, Kingston, Canada)

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Within the past few years three new and quite distinct methods have been used for the study of the rate of turnover of the phospholipids in animal tissues. In one elaïdic acid, the trans isomer of oleic acid is employed (1), in another deuteriumated fatty acids are employed (2), and in the third radioactive phosphorus is used (3–6). All three methods are based on the same principle. One of the building stones of the phospholipid molecule is labeled so as to make it distinguishable from those normally present in the body; this labeled constituent is fed and the rate at which it becomes incorporated into the phospholipids in an organ is taken as a measure of their rate of turnover. Strictly speaking elaïdic acid and deuteriumated fatty acids can serve only as a means of measuring the rate of exchange of the fatty acids, while radioactive phosphorus, on the other hand, can serve only as a means of measuring the exchange of the phosphoric acid in the tissue phospholipids.

Now it is obvious that the exchange of the various constituents of the phospholipid molecule need not proceed at the same rate. When reduced to its simplest terms, the turnover of phospholipid in any given organ can be regarded as being due to one or both of two fundamentally different chemical processes. On the one hand, it is possible to conceive of the phospholipids as being in a state of dynamic equilibrium with each of the constituents of the molecule. The rate of exchange of each constituent would depend upon the rate of hydrolysis of each of the linkages in the phospholipid molecule. If these rates of hydrolysis should be markedly different, so too the rates of exchange would be different. On
the other hand, the phospholipids in the tissue cells may conceivably undergo an irreversible degradation, as by oxidation. Since the amount of phospholipid present is known to remain substantially constant, the degraded phospholipid must be continually replaced. This replacement might be due either to synthesis within each individual cell, or to diffusion of phospholipid from the blood stream. In either case the rate of appearance of new phospholipid would be proportional to the rate of degradation, and the rates of exchange of all the constituents of the phospholipid molecule would be the same.

In estimating the rates of turnover of the phospholipids or of any other tissue constituent by means of labeled molecules, it is obvious that the apparent rate of turnover will be a function, not only of the real rate, but also of the relative concentrations of labeled and non-labeled molecules. It is only when and if the relative concentrations remain constant over a reasonable period of time that the apparent and real rates of turnover are identical. Furthermore, a comparison of the rate of breakdown and replacement of the phospholipids in one organ with that in another, on the basis of the apparent rates of turnover, is valid only if the relative concentration of labeled and non-labeled molecules is the same in all cases.

The ultimate purpose of the study of the turnover of the phospholipids is to disclose their functions in the animal body. From what has been said, it is very evident that it would be of considerable importance to know the comparative rates of exchange of all the constituents of the tissue phospholipids.

Thus far the most extensive studies have been made with elaidic acid and with radioactive phosphorus. The results obtained by these two methods are, in some respects, in quite good agreement. Thus both show clearly that in the intestinal wall (3, 5–8), in the liver (1, 3, 5, 6), and in the blood plasma (9, 10), the phospholipids, taken as a whole, have a rapid turnover.

In the case of other organs, the rate of exchange of the phosphoric acid appears to be very definitely more rapid than that of the fatty acids. For instance, in the carcass of the rat the exchange of phosphoric acid is about two-thirds complete within 1 day (5). The uptake of elaidic acid in rat skeletal muscle, on the other hand, is only about one-half complete in 4 days (1). The most striking difference seemed to be in the kidney.
In the course of work done some years ago on the rate of turnover of the phospholipids of intestinal mucosa and blood of cats (7, 9), some analyses were also carried out on the livers, kidneys, and hearts. The data obtained are given in Table I. It is evident that in all three organs the iodine number of the solid fatty acids increased above the level found in the controls, even as early as the 8th hour after feeding elaidin. Elaidic acid had therefore entered into the phospholipids of these organs. However, except in the case of the liver, further uptake of elaidic acid on continued feeding of elaidin was rather slight. And even in the liver the maximum level of elaidic acid was not reached after 5 days.

<table>
<thead>
<tr>
<th>Absorption time</th>
<th>Liver</th>
<th>Kidney</th>
<th>Heart</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Solid acids</td>
<td>Elaidic acid</td>
<td>Solid acids</td>
</tr>
<tr>
<td>Control</td>
<td>41.5</td>
<td>9.6</td>
<td>4.9</td>
</tr>
<tr>
<td>&quot;</td>
<td>41.7</td>
<td>9.0</td>
<td>4.2</td>
</tr>
<tr>
<td>&quot;</td>
<td>41.9</td>
<td>11.8</td>
<td>5.5</td>
</tr>
<tr>
<td>&quot;</td>
<td>38.4</td>
<td>8.8</td>
<td>3.8</td>
</tr>
<tr>
<td>&quot;</td>
<td>41.4</td>
<td>11.0</td>
<td>5.1</td>
</tr>
<tr>
<td>8 hrs.</td>
<td>40.0</td>
<td>19.6</td>
<td>8.7</td>
</tr>
<tr>
<td>18 &quot;</td>
<td>41.5</td>
<td>22.4</td>
<td>10.4</td>
</tr>
<tr>
<td>48 &quot;</td>
<td>44.0</td>
<td>41.4</td>
<td>20.2</td>
</tr>
<tr>
<td>120 &quot;</td>
<td>44.2</td>
<td>35.5</td>
<td>17.5</td>
</tr>
<tr>
<td>2 wks.</td>
<td>46.8</td>
<td>59.4</td>
<td>30.8</td>
</tr>
</tbody>
</table>

Meanwhile Artom and coworkers (3) and Perlman, Ruben, and Chaikoff (5) had presented evidence to show that the phospholipids in the kidneys of the rat have a high rate of turnover as measured by radioactive phosphorus. In view of the very ob-
Phospholipid Turnover

Previous importance which would be attached to an instance in which the rate of exchange of the phosphoric acid was quite definitely different from that of the fatty acids in the phospholipids, it was decided to determine the rate of exchange of the phospholipid fatty acids of rat kidney by means of elaidic acid. The results of that study have clearly shown that the fatty acids in kidney phospholipid undergo a turnover which is of a lower order than do those of the liver and intestinal mucosa. Furthermore the rate of turnover of the fatty acids seems to be much slower than that of the phosphoric acid in the kidney phospholipids.

**Experimental**

When elaidic acid is used as a means of measuring the rate of turnover of tissue phospholipids, it has seemed best that the intake of elaidic acid be continuously maintained at a sufficiently high level so that mobilization of previously stored fat would be inhibited and fat metabolism would be borne entirely by the inflowing food fat. Otherwise the mobilization and metabolism of non-labeled fatty acids will lower the uptake of labeled fatty acids, in this case elaidic acid. To this end, the rats used for the following experiments were fed melted elaidin at frequent intervals throughout the day and in addition were offered a diet containing a high percentage of elaidin (Diet 290 (11)). The amount of melted elaidin given was calculated to be about the maximum amount that would be absorbed. Actually some of the rats showed evidence of increased excretion of fat in the feces, indicating that the intake was probably in excess of the capacity of the small intestine to absorb elaidin.

Four separate experiments were carried out. Adult male rats of approximately the same weight and age were selected for each experiment. One group of rats weighed about 150 gm.; the other three groups weighed about 300 to 350 gm. Three of the groups were fed the melted elaidin dropwise. In spite of some slobbering of the fat, especially at first, this procedure was found to be preferable to the use of the stomach tube, which was used in one group. Many of the animals, even from the start, lapped down the melted fat as rapidly as it was administered. In the last two groups, about 1 to 1.5 gm. of elaidin was given to each rat every 4 hours over periods of 1, 2, 3, or 4 days.
At the end of each day, two rats were killed. The kidneys were pooled, weighed, ground with sand, and rinsed into a flask with 95 per cent ethyl alcohol. In some cases the pooled livers were also used. The total lipids were extracted and separated into acetone-soluble and acetone-insoluble fractions, MgCl₂ being used to aid precipitation. The acetone-insoluble lipids were saponified in a centrifuge tube, with 0.2 cc. of saturated KOH dissolved in 6 cc. of 50 per cent ethyl alcohol. During saponification the alcohol evaporated down to about 2 cc. Water was added to bring the volume up to 3 cc. After ½ hour's saponification (carried out in a gentle stream of N₂), 25 per cent H₂SO₄ was added and the fatty acids were extracted with peroxide-free ethyl ether. The combined ether extracts were evaporated to dryness, under a stream of N₂, and the residue was finally dried over H₂SO₄ in a vacuum desiccator. The solid residue was then extracted with hot acetone and centrifuged. The clear acetone solution of the fatty acids was evaporated to dryness under N₂; the flask was cooled in the vacuum desiccator and then weighed.

To separate the fatty acids into solids and liquids, they were dissolved in 95 per cent ethyl alcohol in a 15 cc. centrifuge tube, boiled, and brought to a volume of about 6 cc., 0.6 mg. of lead acetate in alcohol for every mg. of fatty acid was added, and the alcohol was centrifuged hot to remove the slight precipitate which formed. The volume of alcohol was adjusted so that the fatty acid concentration was always 10 mg. per cc. The tube was then put away at 15–16° for at least 4 hours. The insoluble lead soaps were centrifuged out, redissolved in six-tenths of the original volume of alcohol, and again set away at 15–16°. The insoluble soaps were stirred up with 5 per cent HCl and the fatty acids were extracted thoroughly with ether. This ether solution was washed with water and transferred to a weighed, glass-stoppered 125 cc. flask. The ether was evaporated under N₂. The alcohol solution of the soluble lead soaps was evaporated under N₂ until just barely dry. Then 5 per cent HCl and ether were added and the flask shaken repeatedly. The contents of the flask were transferred to a 15 cc. centrifuge tube and centrifuged. The clear or very slightly milky ether solution was aspirated into a stoppered centrifuge tube and shaken up with water. On centrifuging, the clear ether solution separated sharply. This
Phospholipid Turnover

was aspirated into a weighed 125 cc. glass-stoppered flask and evaporated under N₂. After evaporation of the ether, the flasks containing the solid and liquid fatty acids were cooled in a vacuum desiccator and then weighed. The iodine numbers of the fatty acids were then determined immediately by the Rosenmund-Kuhnenn method (12). The elaidic acid content was calculated from the iodine number and percentage of solid acids (1).

Results and Comments

The data obtained from the study of the turnover of the phospholipids in rat kidneys are given in Table II. It will be observed (Column 4) that there is no indication of any change in the amount of phospholipid in the kidney as a result of the continuous ingestion and metabolism of large amounts of elaidin. On the other hand, the iodine numbers of the solid acids (Column 6) and the calculated percentage of elaidic acid in the phospholipid fatty acids (Column 8) clearly show that there is a progressive exchange of the fatty acids in the kidney phospholipids. However, if the percentages of elaidic acid after 3 days are compared with those found in rats which have been fed elaidin throughout their lifetime, it is equally clear that the rate of turnover is such that it reaches completion only after several days. By extrapolation of the rough curve which may be fitted to the data given in Table II, it may be estimated that the turnover in the kidney will be about 90 per cent complete at the end of a week.

The data in Column 5 (Table II) show that there is no clear cut change in the percentage of solid acids coincident with the incorporation of elaidic acid into the phospholipids of the kidney. It is true that the values seem to be somewhat higher in the rats fed elaidic acid than in the controls. This applies especially to those rats fed elaidin over a long period of time. Possibly a greater number of data would show that the differences are really significant. Nevertheless it is quite clear that most of the elaidic acid that is built into the phospholipids of the kidney takes the place of the fully saturated fatty acids. There is no evidence of any consistent change in the iodine numbers of the unsaturated fatty acids as the percentage of elaidic acid increases.

In Table III are presented some data showing the rate of increase in the elaidic acid content of the livers of rats. Those in
Group A were secured on a group of rats which were given melted elaidin by mouth every 6 hours over periods ranging from 12 to 26 hours. The rats in Group III are the same as those used for the study of the rate of turnover of kidney phospholipid.

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Remarks</th>
<th>Time on elaidin</th>
<th>Content of phospholipid fatty acids</th>
<th>Per cent of solid acids</th>
<th>I No.</th>
<th>Elaidic acid per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>Purina Fox Chow diet</td>
<td>days</td>
<td>per cent</td>
<td>1.87</td>
<td>33.8</td>
<td>6.5</td>
</tr>
<tr>
<td>I</td>
<td>Fed melted elaidin dropwise at 9 a.m. and 1, 5, and 9 p.m.</td>
<td>1</td>
<td>2.00</td>
<td>33.7</td>
<td>19.7</td>
<td>202</td>
</tr>
<tr>
<td>II</td>
<td>Slightly anesthetized with ether; fed 1.5-2.5 cc. melted elaidin at 8 hr. intervals</td>
<td>2</td>
<td>1.98</td>
<td>39.3</td>
<td>35.6</td>
<td>220</td>
</tr>
<tr>
<td>III</td>
<td>Fed 0.9-1.1 gm. melted elaidin dropwise every 4 hrs.</td>
<td>3</td>
<td>1.94</td>
<td>37.8</td>
<td>32.2</td>
<td>206</td>
</tr>
<tr>
<td>IV</td>
<td>Fed 1-1.5 gm. melted elaidin dropwise every 4 hrs.</td>
<td>1</td>
<td>1.90</td>
<td>36.1</td>
<td>26.7</td>
<td>204</td>
</tr>
<tr>
<td></td>
<td>Fed Diet 290-C, high in elaidin (11) from weaning age for at least 10 wks.</td>
<td>2</td>
<td>1.82</td>
<td>38.1</td>
<td>39.0</td>
<td>210</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>1.82</td>
<td>38.1</td>
<td>33.3</td>
<td>218</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>1.87</td>
<td>39.5</td>
<td>35.7</td>
<td>221</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>1.87</td>
<td>38.8</td>
<td>36.3</td>
<td>223</td>
</tr>
</tbody>
</table>

On comparing the percentages of elaidic acid in the liver phospholipids of Groups A and III with those found in rats which had been raised on a high elaidin diet, one will see at once that as early as 18 hours after the first dose of elaidin, maximal values are reached. Unfortunately there is a rather large variation from one animal to another, even in those which had been on a
standard diet for many weeks.\footnote{As duplicate analyses give reasonably consistent results, there seems to be no reason to suspect that the analytical procedure is responsible for the variation from one animal to another.} In spite of this variation, the results in Table III bear out the earlier observation \footnote{As duplicate analyses give reasonably consistent results, there seems to be no reason to suspect that the analytical procedure is responsible for the variation from one animal to another.} that the turnover of liver phospholipid, as measured by the elaidic acid method, is substantially complete within 1 day. Certainly the contrast in the rates of turnover of liver and kidney phospholipid is very striking.

**Table III**

*Turnover of Liver Phospholipid in Rats*

<table>
<thead>
<tr>
<th>Group</th>
<th>Remarks</th>
<th>Time on elaidin</th>
<th>Content of phospholipid fatty acids</th>
<th>1 No.</th>
<th>Elaidic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>hrs.</td>
<td>per cent</td>
<td>Solid acids</td>
<td>Liquid acids</td>
</tr>
<tr>
<td>Controls</td>
<td>Average of 8 analyses given in Table II (1)</td>
<td>12</td>
<td>2.84</td>
<td>37.8</td>
<td>28.2</td>
</tr>
<tr>
<td>A</td>
<td>Elaidin dropwise every 6 hrs.</td>
<td>12</td>
<td>2.67</td>
<td>37.8</td>
<td>45.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>18</td>
<td>2.57</td>
<td>34.8</td>
<td>48.3</td>
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<tr>
<td></td>
<td></td>
<td>18</td>
<td>2.71</td>
<td>42.7</td>
<td>53.9</td>
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<td></td>
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<td>18</td>
<td>2.83</td>
<td>41.3</td>
<td>51.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>24</td>
<td>2.59</td>
<td>41.5</td>
<td>44.9</td>
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<tr>
<td></td>
<td></td>
<td>26</td>
<td>40.3</td>
<td>38.8</td>
<td>228</td>
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<tr>
<td></td>
<td></td>
<td>26</td>
<td>42.3</td>
<td>49.2</td>
<td>23.1</td>
</tr>
<tr>
<td>III</td>
<td>Elaidin every 4 hrs.</td>
<td>48</td>
<td>2.85</td>
<td>39.8</td>
<td>46.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>72</td>
<td>2.63</td>
<td>43.3</td>
<td>47.2</td>
</tr>
<tr>
<td>Fed high elaidin Diet 290-C (11) from weaning until at least 13 wks. of age</td>
<td></td>
<td>2.74</td>
<td>43.9</td>
<td>56.0</td>
<td>225</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.60</td>
<td>47.8</td>
<td>60.5</td>
<td>235</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.64</td>
<td>43.9</td>
<td>52.9</td>
<td>25.8</td>
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<td></td>
<td></td>
<td>2.74</td>
<td>44.2</td>
<td>52.9</td>
<td>26.0</td>
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<tr>
<td></td>
<td></td>
<td>2.98</td>
<td>41.4</td>
<td>51.5</td>
<td>240</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.80</td>
<td>41.6</td>
<td>50.3</td>
<td>238</td>
</tr>
</tbody>
</table>

In the case of the liver there is a significant decrease in the percentage of liquid acids with the increase in the elaidic acid content. As there is no indication that a high percentage of solid acids coincides with an increased amount of phospholipid.
in the liver, the decrease in liquid acids must be due to partial replacement by elaidic acid. However, in the liver as in the kidney, most of the elaidic acid that is built into the phospholipids replaces fully saturated fatty acids. It can be calculated that when the elaidic acid has reached its apparent maximal percentage, the fully saturated fatty acids are reduced from 37 to 18 per cent and the unsaturated acids from 63 to 56 per cent, on the average. At the present time there does not seem to be any purpose in further speculation (1, 7) about the mechanism underlying the replacement of the natural fatty acids by elaidic acid. The study of the problem is being continued.

DISCUSSION

The results of this investigation have clearly shown that, as measured by the elaidic acid method, the turnover of fatty acids in the phospholipids in the liver may be substantially complete within 1 day, while in the kidney it is only about 60 per cent complete in 3 days. This rate of exchange of the fatty acids is in sharp contrast to the apparent rate of exchange of the phosphoric acid in the phospholipids of both organs (5). The peak in radioactive phosphorus content is reached in 5 to 10 hours in the case of the liver and in about 24 hours in the case of the kidney.

It is obvious that in making a comparison of the rates of exchange of the fatty acids and the phosphoric acid in the phospholipids it would be best to carry out the determinations on the same animals under exactly the same conditions. Indeed, the conditions in Perlman and coworker’s experiments (5) are so different from those in the present study that it may be doubtful that the results should be compared. However, the apparent rate of turnover of the fatty acids is so distinctly different from that of the phosphoric acid that it seems very unlikely that the two really proceed at the same rate and are always mutually dependent on one another.

According to the hypothesis that the phospholipids act as intermediaries in fatty acid transport and catabolism, one must visualize a rapid turnover of the fatty acids in such metabolic phospholipids. For the purpose of testing that hypothesis, the direct methods of estimating fatty acid exchange, such as the elaidic acid method, would seem to be most suitable. In the
case of the intestinal mucosa, the liver, and the blood plasma
the fatty acids in the phospholipids do undergo a rapid turnover.
It has seemed best therefore to conclude that, in these organs,
phospholipids do act as intermediaries in fatty acid metabolism.
On the other hand, the rate of turnover of the fatty acids in the
phospholipids of the muscles (1) and, as the present results show,
of the kidneys as well is considerably slower than the assumed
rate of fatty acid catabolism in these organs. If one is correct
in assuming that fatty acids enter the muscles and kidneys, either
free or combined, before combustion begins, and also that fatty
acids serve as a predominant source of energy to these cells when
the diet is rich in fat, then the slow turnover of phospholipid
fatty acids in muscles and kidneys must be related, not to fatty
acid catabolism, but to those rather indefinite reactions which
are summed up under the term wear and tear. And if wear and
tear are responsible for the fatty acid exchange in kidney phos-
pholipid, then it is easy to imagine why the rate of exchange of
the phosphoric acid in the phospholipids can be quite different.

At this time it perhaps should be pointed out that, although
a rapid turnover of fatty acids in the phospholipids of an organ is
certainly consistent with the hypothesis that phospholipids in
that organ are acting as intermediaries in fat metabolism, it need
not be regarded as proof that they do so. It is possible to imagine
that the turnover of the fatty acids in the phospholipids of all
the organs of the body is fundamentally due to the same process
which goes on at different rates from one organ to another.
Whether or not one wishes to attach a physiological function to
the turnover of fatty acids or, as Weissberger has done (13), to
that of phosphoric acid in the tissue phospholipids is mainly a
matter of personal predilection.

SUMMARY

The rate of turnover of the fatty acids in the phospholipids of
rat kidneys, as measured by the elaidic acid method, has been
found to be comparatively slow. After 3 days of continuous
elaidin ingestion, the uptake of elaidic acid by kidney phos-
lipids is only about 60 per cent complete. It may be estimated
that the turnover would be about 90 per cent complete at the
end of 1 week.
R. G. Sinclair

The rate of turnover of liver phospholipids, as in previous work, has been found to be quite rapid. The maximal uptake of elaidic acid by liver phospholipids occurs as early as 18 hours after its ingestion.

The interpretation of the rates of turnover of the various constituents of the phospholipid molecule is discussed.

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