FATTY ACIDS OF LIVER LECITHIN

BY RUTH H. SNIDER AND W. R. BLOOR

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

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The phospholipids, along with cholesterol, are now recognized as essential elements of tissues, while the characteristic constituents of the phospholipids are the fatty acids. In any study of the metabolic activity of tissues the nature and proportions of the various fatty acids in the phospholipids therefore become important and have been the subject of increasing interest and speculation. Because of its many sided activity in metabolism, the liver has received a large share of attention and more work has been done on its lipid constituents than on those of any other organ.

In the following work an attempt is made to find out the nature of the fatty acids and the percentage distribution in liver lecithin with special reference to the unsaturated acids. Since these results were first reported (1) an outstanding piece of work by Klenk and Schoenebeck (2) has appeared dealing with the fatty acid distribution in the total ether-soluble phospholipid (lecithin plus cephalin) of liver, obtained by the method of fractional distillation, to which reference will be made.

The occurrence in liver of fatty acids not found in ordinary fat was first noted by Hartley (3) who described the 4 double bond C20 acid now known as arachidonic. Since that time the presence of this acid in various tissues has been established by many investigators. Levene and Rolf (4) in 1922 found it in egg yolk. Bloor (5, 6) showed its presence in varying amounts in liver, kidney, pancreas, lung, and in heart, jaw, diaphragm, neck, and round muscles. Brown (7) identified arachidonic acid in the thyroid, suprarenal glands, and spleen. In liver he believed that it was the only highly unsaturated acid, a finding which has since been disputed (2). It was found by Eckstein (8) and Ellis and Zeller (9) in
small amounts in the body fat. Wesson (10), on the basis of its occurrence and variations in tissues under changing conditions, gave it a leading place in the metabolism of fat. Hartley (3) found another acid in pig liver which has not been found elsewhere and which he thought was important in the consideration of the desaturating power of the liver, an acid isomeric with ordinary oleic acid.

The distribution of the fatty acids in the lipids of liver or any organ has not been closely examined. Hartley (3) showed the presence of palmitic, stearic, oleic, linoleic, and arachidonic acids in liver. Brown (11) found these acids plus more highly unsaturated ones in brain, as did Rudy and Page (12). Higher acids than C20 were not found in liver, however, until recently, when Klenk and Schoenebeck (13, 2) reported a C22 acid with five double bonds. Bloor (5, 6), Brown (14), and Theis (15) found no evidence of a C22 acid in the bromine addition products. Brain and liver apparently contain much larger amounts of the more highly unsaturated and long chain acids than any other organ. The fact that the liver has a large proportion of unsaturated acid could be accounted for in part by the theory of Leathes and Meyer-Wedell (16), according to which the fatty acids are desaturated by this organ. This theory, however, does not answer the question as to how the long chain acids originate. Hartley (3) stated that there is not enough arachidic acid in the body to account for the large amount of arachidonic acid found. Klenk and Schoenebeck (2) raise this point in their discussion of Leathes' hypothesis of fatty acid desaturation by the liver.

Hartley (3) had another idea, based on the data of Magnus-Levy (17), Leathes (18), and himself (3), that the highly unsaturated acids are formed directly from carbohydrates. He found that animals kept on a "fat-free" carbohydrate diet produced liver fatty acids with a high iodine number. Wesson (10), experimenting with normal, fasting, and phlorhizinized rats, found that his data did not support Hartley's idea (3). In fasting animals, where there would be an active fat but a subnormal sugar metabolism, the amounts of arachidonic acid were increased. He concluded that arachidonic acid was an intermediary product formed in the metabolism of fatty acids having less than 20 carbon atoms. Powell (19) fed
tricaprylin and trilaurin to rats and found in the body fats longer chain acids than those fed. This work indicated an ability of the organism to form longer chain acids from shorter ones.

**EXPERIMENTAL**

**Preparation of Lecithin**—Fresh liver tissue is freed from visible fat, finely ground in a meat chopper, and treated as described by Bloor (20), briefly as follows: 1000 gm. of finely ground tissue are dehydrated in 3 liters of 95 per cent alcohol for 1 to 2 hours at 36°, the alcohol is filtered off, and the tissue extracted for 3 hours with fresh alcohol. The combined alcohol is distilled under reduced pressure and the residue taken up in petroleum ether (Bloor used ordinary ether). The petroleum ether is concentrated to a syrup and poured into 100 cc. centrifuge tubes in 25 cc. portions. The phospholipid is precipitated by addition of 75 cc. of acetone to each tube, the precipitate is thoroughly kneaded with acetone, redissolved in petroleum ether, and the reprecipitation and kneading repeated three or four times. Sphingomyelin, being insoluble in petroleum ether, appears as a precipitate and is centrifuged out at the various times when the phospholipid is dissolved in petroleum ether. Cephalin is separated from the lecithin by precipitation with absolute alcohol. After the cephalin is removed the alcohol containing the lecithin is distilled under reduced pressure, the lecithin dissolved in petroleum ether, and aliquots taken for weight and iodine numbers.

**Saponification and Extraction of Fatty Acids**—The lecithin is saponified by boiling 5 or 6 hours with 16 to 20 gm. of stick sodium hydroxide dissolved in 200 cc. of 50 per cent alcohol. The fatty acids are extracted with petroleum ether after acidification with hydrochloric acid. Weight and iodine numbers are determined in aliquots. To the petroleum ether1 solution (200 cc.) are then added 3 or 4 volumes of acetone, which cause the formation of a flocculent precipitate which is centrifuged out (stoppers being needed, as the precipitate is so light that otherwise it will not settle). The petroleum ether and acetone are distilled under a vacuum and the fatty acids dissolved in 95 per cent alcohol.

**Lead Soap Separation of Liquid and Solid Fatty Acids** (Modified

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1 See "Notes on procedure."

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Fatty Acids of Liver Lecithin

Twitchell Method)—To the boiling alcoholic solution of fatty acids (100 cc.) are added 100 cc. of boiling alcohol containing lead acetate in excess over the amount calculated for the solid acids. The solution is allowed to cool slowly and then to stand at a temperature maintained constant at 15–20° overnight. The next morning the mixture is stirred and the solid lead soaps are centrifuged down. The solid lead soaps are thoroughly extracted with petroleum ether to remove the "solid unsaturated acid." The washings are evaporated and the residue is refluxed for 1/2 to 3/4 hour with about 20 cc. of 95 per cent alcohol containing 1 drop of glacial acetic for each 20 cc. of alcohol. The boiling alcohol, which contains any remnants of liquid unsaturated and solid saturated acids, is poured off, cooled slowly, and set away for 3 or more hours at about 17°, for the solid soaps to precipitate out, after which the standard procedure as described below is followed.

The residue (containing the so called solid unsaturated acid) which remains insoluble after refluxing with alcohol, is dissolved in ether, treated with 10 per cent hydrochloric acid (the precipitated lead chloride being thoroughly extracted with ether), and the ether extracts washed free from inorganic acid and lead. If emulsions form upon addition of water, these emulsions must be drawn off, warmed on a steam bath, and, upon melting, the fatty acid layer is extracted with ether; otherwise there is a considerable loss of fatty acids. After drying and weighing, iodine number and melting point are determined.

The lead soaps of the solid acids are suspended in ether and decomposed with 10 per cent hydrochloric acid, washed free from acid and lead, dried, weighed, and the iodine number and melting point determined. The liquid acids are freed in the usual manner (Twitchell (21)), dried over sodium sulfate, made up to a volume of 200 cc., and aliquots taken for weight and iodine number.

Bromination of Liquid Fatty Acids—If carefully purified petroleum ether has been used for the extraction of the liquid acids the bromination may proceed at once.

Approximately 5 to 6 gm. of fatty acids are dissolved in 200 cc. of petroleum ether. After cooling to a temperature of −5°, bromine is slowly added drop by drop from a burette (the flask being shaken constantly) until a dark orange or light red color is obtained. About 10 per cent excess of bromine is required. After standing
in the refrigerator for at least 15 hours the precipitate is centrifuged down and the excess bromine and the dibromide are washed out with petroleum ether.

**Separation of Dibromide**—The petroleum ether soluble fraction, which contains the dibromide and excess bromine, is immediately placed in a pressure flask (500 cc.) and the petroleum ether and excess bromine are distilled under reduced pressure with only heat enough to prevent the formation of ice on the flask. Additional petroleum ether is added once or twice to the residue, which is a light yellow oil, and the procedure repeated to insure complete removal of the excess bromine. The oil is redissolved in petroleum ether, transferred to a small weighed flask or beaker, and the petroleum ether concentrated to a volume of 15 cc. by distillation in a vacuum desiccator, in which a capillary tube, attached to a stopcock, reaches to the bottom of the liquid and serves to prevent foaming or bumping. The solution of dibromide is then allowed to stand in the refrigerator for several days. A white precipitate separates out in the petroleum ether which has been found to be tetrabromide. The precipitate is centrifuged out and the dibromide dried in the desiccator, weighed, and the bromine content determined according to the method of Brown and Beal (22).

**Separation of Tetrabromide**—The petroleum ether-insoluble fraction, which contains the tetra- and octabromides, is treated with chilled, peroxide-free, dry ether to extract the tetrabromide which is ether-soluble. About 150 to 200 cc. of ether are used in 50 cc. portions. The ether is centrifuged until clear and placed in a weighed flask or beaker and the ether removed by vacuum distillation in the desiccator as previously described. It is allowed to remain in the desiccator for some time with the pump running to remove any excess bromine. It is then dissolved in 15 cc. of dry ether and set away in the refrigerator for a few days. In this fraction some octabromide is dissolved, which, upon chilling, precipitates out. The rest of the procedure is the same as for the dibromide.

**Separation of Octabromide**—The ether-insoluble fraction, containing the hexa- and octabromides, is treated with 50 cc. portions of boiling benzene. Better extraction is obtained if the bromides are powdered, vigorously boiled in the benzene, and centrifuged.
as quickly as possible, as the solubility in even boiling benzene is very small.

The benzene-insoluble fraction contains the octabromide (together with some bromides of more highly unsaturated acids). This fraction is dried and weighed and bromine content determined.

**Iodine Number**—Three methods were used to measure the degree of unsaturation of the fractions: the Hanus method, the Rosenmund-Kuhnmann pyridine-dibromide method (23), and the rhodanate or thiocyanogen method of Kaufmann (24).

**Notes on Procedure**

*Sphingomyelin*—It is well known that sphingomyelin is removed with difficulty from lecithin and cephalin although it is insoluble in ether and petroleum ether. A certain amount is held in solution by the soluble phospholipids. Although, as noted above, attempts are made throughout the separation of the phospholipid from neutral fat to remove the sphingomyelin, there is evidence that all is not taken out.

Following the procedure described by Sinclair (25) a precipitate was found to form upon addition of an excess of acetone to the mixed fatty acids. Like that found by Sinclair, it gave a red color with sulfuric acid, which is a property of cerebrosides. Further properties similar to those of sphingomyelin and cerebrosides were displayed by this substance. It is somewhat soluble in cold pyridine, more soluble in warm pyridine, partly soluble in ether and petroleum ether, and difficult to saponify with alkali. Not only has it been found in the mixed fatty acids but also in the liquid acid fraction and in the emulsions formed in the solid unsaturated acid fraction (see "Discussion").

*Lead Soap Separation of Liquid and Solid Acids*—Various workers have found in using the Twitchell lead soap method (21) for the separation of liquid and solid acids in animal tissue, that it is difficult to recover 100 per cent of the fatty acids. It has been observed that upon addition of boiling alcoholic lead acetate to a boiling alcoholic solution of fatty acids a sticky mass, commonly referred to as pitchy residue, is formed. After the lead soaps have stood in the cold overnight and the alcohol and solid lead soaps have been removed, the sticky residue has been found to remain in the bottom of the flask. It will not dissolve in boiling
alcohol, but is soluble in ether and can be decomposed with nitric or hydrochloric acid. The freed fatty acids are washed only with difficulty, because tenacious emulsions are formed upon addition of water. These emulsions are also encountered in both the liquid and solid acid fractions and their formation is apparently related to the presence of the pitchy residue.

Attempts were made to prevent the formation of this pitchy residue by varying the temperatures of the alcoholic solutions upon the addition of one to the other. At a temperature ranging between 50–55° it was found that no pitchy residue formed, but traces formed if the solid lead soaps were treated with boiling alcohol for their reprecipitation. When, however, there was no pitchy residue observed, the emulsion formation was increased tremendously in the liquid and solid fatty acid fractions. These emulsions, when drawn off and warmed on a steam bath, cleared fairly well. Upon extracting with ether it was found that between 10 and 15 per cent of the solid acids was recovered from them. An absolute recovery, however, seems almost impossible, for the acids have to be washed free of inorganic acid and the emulsions reform, carrying with them some fatty acid.

The method of Cocks, Christian, and Harding (26) for the extraction of solid, unsaturated acids was tried with the hope that it might help with the difficulties in the recovery of solid and liquid acids. The method, with modifications, has been described in detail above. It was found that if sufficient lead had been added to precipitate all the solid acids and if the solid lead soaps were thoroughly washed with petroleum ether that there would be no emulsions formed except in the solid, unsaturated fatty acid fraction. If the emulsions are carefully handled 93 to 95 per cent recovery can be made, apparently the greatest loss being incurred by failure thoroughly to extract the emulsions; at the best, it is a tedious task.

Bromination of Unsaturated Acids—The methods for the separation of the unsaturated acids by their bromination products are unsatisfactory. One difficulty is the lack of specific solvents for any one of the bromides. For instance, the solvent used for the separation of the hexabromide is not a good one. The hexabromide is only slightly soluble in warm benzene, while on the other hand some of the octabromide, which is slightly soluble in benzene, is carried along.
When fatty acids were brominated in ether difficulty was encountered in freeing the ether-soluble bromides from the excess bromine. The method of washing it out with sodium thiosulfate, as described by Ellis and Isbell (27) did not prove satisfactory, as a brown tarry mass resulted. In the present work removal of the solvent and excess bromine was accomplished by distillation under reduced pressure as described above, the bromination being carried out in petroleum ether instead of ether. Special care was found necessary in the preparation of the petroleum ether for use. Many samples contain unsaturated hydrocarbons, which, when brominated, interfere with the separation of the fatty acid bromide. The difficulty was finally solved by the finding of a sample of petroleum ether (Skelly Oil Company) which could be easily purified. The material was fractionally distilled and only that fraction used which boiled below 60°. This product had an iodine number of 4.6. It was allowed to stand over sulfuric acid, with occasional shaking, then distilled over barium hydroxide, and the iodine number was reduced to 1 or less.

The di- and tetrabromides, as first prepared, gave values for bromine of 10 to 14 per cent too high. This dibromide was redissolved in petroleum ether and allowed to stand in the refrigerator for a few days, when a precipitate separated out which proved to be a tetrabromide compound. The values of the soluble portion were still somewhat high for the dibromide but it is possible that there are isomers of the more highly unsaturated acids which are both ether- and petroleum ether-soluble and similar to those described by Rudy and Page (12). The tetrabromide fraction, after standing in ether in the cold for several days, gave a white precipitate. With this precipitate (which on analysis was found to be octabromide) removed, the percentages were greatly reduced. This procedure for both di- and tetrabromides is now routine.

The bromides, as finally obtained, are of a light yellow color, the dibromide being an oil and the tetrabromide generally a flaky mass which can be powdered. In some cases, however, the tetrabromide is a sticky, dark colored substance, which is difficult to handle in the determination of the bromine content.

Comparison of Hanus and Rosenmund-Kuhnhenn Methods for Iodine Number—The Hanus solution, containing bromine, iodine, and acetic acid, cannot be used for the determination of iodine
values of cholesterol, as it causes substitution as well as addition. Yasuda (28) examined the Rosenmund-Kuhnhenn solution (23), which consists of pyridine, bromine, and acetic and sulfuric acids, and found that it gave theoretical values for cholesterol and slightly lower values for ricinoleic and oleic acids than did the Hanus solution.

In our work values for each solution were determined for the various fractions of liver lipids and in every case the pyridine values were lower than the Hanus. The averages for intact lecithin, for example, were P 88, H 91, for mixed fatty acids P 121, H 126, for liquid fatty acids P 209, H 214 (Table I, Series I). Values obtained for oleic acid, Kahlbaum's K brand, were 90 for the pyridine method, while the Hanus method gave 93. Whether this higher value for the Hanus method is due to a slight tendency toward substitution cannot be definitely stated but the more unsaturated the acids the greater the difference in the two methods. Yasuda (28) found a difference of 2.8 for oleic acid, 1.7 for ricinoleic acid, and 6 for cod liver oil. In our work on lecithin there was an average difference of 3 for intact lecithin, 5 for mixed fatty acids, and 5 for unsaturated acids.

Thiocyanogen Number, Rhodanate Number—Kaufmann (24) found that thiocyanate dissolved in a solution of bromine in water-free acetic acid reacts with unsaturated fatty acids to a certain extent like bromine and iodine. Using various fatty acids and oils he obtained the following results, which he compared with the iodine values obtained on the same substances by converting the thiocyanogen or rhodanate number to the iodine equivalent.

<table>
<thead>
<tr>
<th>Fat</th>
<th>Iodine No.</th>
<th>Rhodanate Iodine No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oleic acid</td>
<td>89.9</td>
<td>89.9</td>
</tr>
<tr>
<td>Linoleic “</td>
<td>181.09</td>
<td>90.545</td>
</tr>
<tr>
<td>Palmitic “</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Olive oil</td>
<td>80.8</td>
<td>76.6</td>
</tr>
<tr>
<td>Peanut “</td>
<td>87.1</td>
<td>78.5</td>
</tr>
</tbody>
</table>

It is apparent from these results that only the bond in position \(\Delta^9-10\) adds thiocyanate. In later work, however, he found that it added to two bonds in linolenic acid (29).

The time needed for this reaction to take place is much greater
than for the addition of iodine or bromine as by the Hanus procedure. Kaufmann found that 5 hours was a sufficient length of time for a value of 90 to be obtained for oleic acid. However, at the end of 5 hours the highest value obtained in this laboratory was 84, for an oleic acid which with the pyridine and Hanus solutions gave values of 90 and 93 respectively. After allowing a sample to stand 84 hours Dr. H. C. Hodge of this laboratory obtained a value of 90. After 5 hours linoleic acid gave a value of 108, cod liver oil 99, and linseed oil 102. Zeleny and Bailey (30), working with the fatty acids of lard, found that it was necessary to let the sample stand for 17 hours before titrating.

**DISCUSSION**

**Fatty Acids**—Table I, Series I, shows the averages of several analyses of three different lots of lecithin (kindly sent to us by Dr. H. Gregg Smith, State University of Iowa), used primarily for the perfection of the procedure. In Series II of Table I are given the data obtained from seven fresh beef livers. On the whole the values are not very different in the two series. In Series II the weight of lecithin is per 1000 gm. of moist liver. The recovery of mixed fatty acids is 66 to 67 per cent respectively of the intact lecithin, which is 93 per cent of the theoretical value for the fatty acids in an oleyl-stearyl lecithin and about three points higher for palmityl-oleyl lecithin. The iodine values in both series are considerably higher than those found previously by Bloor (5). The average for the intact lecithin in his work was 83 as compared with 90 and 91 in the present—the Hanus figures being used in both cases. The difference may probably be referred to diet since Sinclair (25, 31) has shown that diet influences the unsaturation of the phospholipids. Correspondingly higher values are also found for the liquid acids, 213 as compared with 160 in the earlier work, while the percentage of liquid acids in this work is somewhat higher—averaging 49 as compared with 40. These differences are due in part, at least, to the improvement in technique in the separation of liquid and solid acids.

Calculated from the figures of liquid acids in per cent of mixed acids the iodine numbers of the liquid acids are about 30 per cent too low. Two possible causes present themselves: (1) The low percentage of recovery of the solid, liquid, and solid unsaturated
<table>
<thead>
<tr>
<th>Series</th>
<th>Sample</th>
<th>Lecithin</th>
<th>Mixed fatty acids</th>
<th>Liquid fatty acids</th>
<th>Solid fatty acids</th>
<th>Solid unsaturated acid</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Lecithin</td>
<td>Peroxide</td>
<td>Diene</td>
<td>Total</td>
<td>Peroxide</td>
<td>Diene</td>
</tr>
<tr>
<td>Series I</td>
<td>Sample 1 (2)</td>
<td>88</td>
<td>87</td>
<td>65</td>
<td>65</td>
<td>124</td>
<td>125</td>
</tr>
<tr>
<td></td>
<td>2* (5)</td>
<td>93</td>
<td>87</td>
<td>68</td>
<td>66</td>
<td>126</td>
<td>120</td>
</tr>
<tr>
<td></td>
<td>3 (6)</td>
<td>91</td>
<td>89</td>
<td>64</td>
<td>67</td>
<td>128</td>
<td>118</td>
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<tr>
<td></td>
<td>Average</td>
<td>91</td>
<td>88</td>
<td>66</td>
<td>66</td>
<td>126</td>
<td>121</td>
</tr>
<tr>
<td>Series II</td>
<td>Sample 1</td>
<td>24.1</td>
<td>93</td>
<td>87</td>
<td>58</td>
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<td></td>
<td>2</td>
<td>21.6</td>
<td>88</td>
<td>88</td>
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<td>68</td>
<td>126</td>
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<tr>
<td></td>
<td>3</td>
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<td>94</td>
<td>92</td>
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<td>126</td>
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<tr>
<td></td>
<td>5</td>
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<td>82</td>
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<td>130</td>
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<td>6</td>
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<td>96</td>
<td>93</td>
<td>70</td>
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<td>84</td>
<td>69</td>
<td>111</td>
<td>109</td>
</tr>
<tr>
<td></td>
<td>Average</td>
<td>22.3</td>
<td>90</td>
<td>86</td>
<td>67</td>
<td>67</td>
<td>121</td>
</tr>
</tbody>
</table>

The figures in parentheses show the number of analyses made of each sample.

* In Sample 2, solid acid was found in the liquid acid fraction.

† Not included in average. ‡ "Substance X" removed. § Oxidation occurred.
acids—which might indicate the loss of a highly unsaturated acid during the separation. On the basis of the discrepancy between the theoretical and actual iodine number (Hanus) the percentage of loss would be 12, which is near the 10 per cent deficiency found. However, when the recovery is higher than 90 per cent there is still a discrepancy in the iodine number. (2) Oxidation during separation is the other and probably more likely cause for the low value. The iodine number of the solid unsaturated acid (which averages 5 per cent of the mixed fatty acids) is much lower than that of the liquid acids. As this fraction is removed, the liquid acids should have an iodine number higher than calculated, but actually it is lower, which again points to oxidation. Isomeric change in the fatty acid molecules with change in the readiness with which they absorb iodine is another possibility.

The averaged mean molecular weight of the liquid acid is 319, a figure which is close to the 312 obtained by Hartley. This high figure indicates three possibilities: (1) the presence of high molecular fatty acids, (2) oxidation, and (3) association compounds of some kind.

The solid acids as separated, average 36 per cent of the mixed fatty acids, have an iodine number of 5, a melting point of 63°, and a mean molecular weight of 276, which again agrees with that of Hartley, who obtained a molecular weight of 275 for the saturated acids. On the basis of the mean molecular weight, there would be a percentage proportion of stearic to palmitic acids of 71 to 29, which indicates that the saturated as well as the unsaturated fraction of phospholipid has longer chain acids than does the neutral fat.

Analysis of Bromides. Fatty Acid Recovery—The method most commonly used for the separation of the unsaturated acids has been that making use of the solubilities of their bromine addition products. In the present work, however, a great deal of difficulty was encountered in fractionating the bromides, especially in the ether and petroleum ether fractions, the bromine content of the material separated being far too high for the bromides of linoleic and oleic acids respectively (Table III). It was obvious that bromides of the more unsaturated acids must be present, probably as more soluble isomers, a conclusion which has been confirmed by the recent work of Rudy (32), who found that bromination does result in isomeric bromides of different solubility.
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After considerable experimenting with calculations on different assumptions it seemed simplest and probably most correct (especially after the appearance of Rudy's work) to assume that the high percentage of bromine in the petroleum ether-soluble fraction is due to isomeric tetrabromides and the high value in the ether-soluble fraction to isomeric octabromides. The values in Table II, which show the distribution of the fatty acids, were obtained by calculation on this basis and under these conditions the total recovery of the various acids gave a value of 100 per cent or close to it in nearly every sample. The distribution of acids differed somewhat from that of Klenk and Schoenebeck (2) on the mixed phos-

TABLE II

Distribution of Unsaturated Fatty Acids

<table>
<thead>
<tr>
<th>Type of acid*</th>
<th>Per cent of liquid acids</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Series I</td>
</tr>
<tr>
<td></td>
<td>Sample 1</td>
</tr>
<tr>
<td>Arachidonic</td>
<td>32.0</td>
</tr>
<tr>
<td>Linoleic</td>
<td>44.3</td>
</tr>
<tr>
<td>Oleic</td>
<td>25.0</td>
</tr>
<tr>
<td>Total recovery</td>
<td>101.3</td>
</tr>
</tbody>
</table>

* These terms represent fractions, the exact composition of which is unknown. See discussion.
† Not included in average.

...phatides of beef liver, obtained by a distillation procedure. Klenk and Schoenebeck found 28 per cent of the mixed fatty acids or 47 per cent of the liquid acids to be highly unsaturated C₁₉ and C₂₁ acids, and the rest to be C₁₆ and C₁₈ acids. Our data gave a different distribution; 30 per cent of the liquid acids was highly unsaturated, 45 per cent was linoleic acid, and 20 per cent oleic acid.

Theis (15), using the bromination method, obtained a value of 34 per cent for arachidonic acid, which is in close agreement with our figure.

**Ether-Insoluble Bromides**—It is in this fraction that our two series differ most (see Table III). In Series I the bromine content averages 67.6 per cent before treatment with benzene and 68.4 after. In Series II the values are lower—64.8 and 66.3, respec-
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tively. The higher percentage in Series I suggests the possibility of a C\textsubscript{22} 5-bond acid which has a theoretical bromine content of 70.8 per cent. Klenk and Schoenebeck (13) found that the ether-insoluble bromide from beef liver gave the following analysis: C 24.7 per cent, H 3.5 per cent, and Br 68.2 per cent, which is too high for a C\textsubscript{20} 4-bond acid. The theoretical figure for a mixture containing 25 per cent of the bromine derivative of a C\textsubscript{22} 5-bond acid and 75 per cent of the bromine derivative of arachidonic acid would be 68.5 per cent, which is very close to the above value found and indicates the presence of a C\textsubscript{22} 5-bond acid in this series of livers. In a more recent paper Klenk and Schoenebeck (2) isolated a C\textsubscript{22} 5-bond acid by means of distillation of the methyl esters.

**Benzene-Soluble Bromide**—Since the percentage of bromine of the benzene-insoluble fraction was higher than that of the total ether-insoluble bromide it indicates that the benzene removes a substance with a lower bromine content. The percentage of bromine in the benzene-soluble fraction is 65 to 66, which is too high for

### Table III

<table>
<thead>
<tr>
<th>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>
<th>Average</th>
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<tr>
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hexabromide. It is probable then that this fraction is composed of octabromide and a small amount of tetrabromide. The possible presence of octabromide of higher molecular acids is not excluded.

**Solid Unsaturated Acid**—In the method of Cocks, Christian, and Harding (26) the solid lead soaps are extracted with petroleum ether to remove any solid or liquid unsaturated acids clinging to this fraction. The residue from the petroleum ether extraction is refluxed in boiling alcohol and the whole is normally soluble in hot alcohol. Following this procedure we found a fraction, insoluble in boiling alcohol, and it was separated from the rest and analyzed. It was similar in appearance to the pitchy residue found in earlier work (5) and was also ether-soluble. Upon acidification with 10 per cent hydrochloric acid, a brown, clear, semisolid substance was isolated. Its iodine number was 98, very close to that of elaidic acid and the substance was consequently referred to as a solid unsaturated acid. A melting point determination was attempted but the material did not behave like pure fatty acid. It sintered at 45°, turned black between 50–60°, and at no time formed a true liquid.

After this fraction had been isolated from several samples of lecithin the separation of substance X by acetone from a petroleum ether solution of the mixed fatty acids, as described by Sinclair (25), was made and it was found that when a large amount of substance X precipitated out, the alcohol-insoluble fraction was very much decreased. The two substances as isolated were very different in appearance, the acetone precipitate (substance X) being a white or pale yellow flaky material, while the other was similar to a solid, unsaturated acid. Positive qualitative tests for nitrogen and phosphorous had been found for substance X and so quantitative analyses were made on the solid unsaturated acid. There was found a high percentage of nitrogen, averaging 7.6, while the phosphorous values averaged 2.8 per cent, making a P:N ratio of 1:6.

Some of substance X was then treated with hot alcohol and part was soluble and part remained insoluble. The soluble fraction contained 9.2 per cent nitrogen, while the insoluble part contained but a mere trace. Another sample was treated with lead acetate

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2 The term solid unsaturated acid is used as a convenience rather than with a specific meaning. The composition of the substance is unknown.
and the fraction corresponding to the solid unsaturated acid yielded an acid identical in appearance with it, but of a lower degree of unsaturation. Sinclair (personal communication) has found a similar material in his substance X.

In many respects the properties of the solid unsaturated acid resembled those of the pitchy residue acids. In appearance they were unlike, because the latter were contaminated with solid acids and for the same reason the iodine number was lower. The lead soaps of both were sticky, insoluble in cold and boiling alcohol, and soluble in ether. As acids, both formed thick emulsions with water; in fact their affinity for water was so great as to suggest hydroxyl groups and the material was accordingly acetylated (33). The pitchy residue acids gave values ranging from 140 to 149, while the solid unsaturated acid gave more divergent and lower values. Before the presence of nitrogen was found it was concluded that the acetyl value might be due to the presence of a hydroxyl group, but as the amino group also forms an acetyl derivative one cannot draw that conclusion, without further analysis.

Klenk and Schoenebeck (2) in their work on liver have reported a substance with a nitrogen content of 2.19 per cent and a phosphorous percentage of 3.34, giving the following ratio, P:N 1:1.49. This substance has a melting point of 156–157° and begins to sinter at 80°. This material was recrystallized from alcohol and pyridine and so undoubtedly was much purer than ours. They concluded that their product was lignoceryl sphingomyelin and split-products.

Rhodanate Numbers—Until more data have been accumulated concerning the action of rhodanate on the higher fatty acids, nothing can be said in regard to the significance of the rhodanate numbers obtained for liver lecithin. If this radical adds only to the one bond, then the values should not be higher than 90 for the liquid fatty acids; actually they average 111. These figures obtained for the mixed fatty acids (average 77) are not what would be expected by calculation from the liquid acids, nor are those of the intact lecithin (average 67) in accordance with calculations based on the mixed fatty acid values, both sets of values being too high.

The solid fatty acids of liver lecithin are essentially those of the fat stores but in a different proportion, the proportion as calculated
from the molecular weight being 30 for palmitic and 70 for stearic while in the fat of the stores and in the neutral fat of the liver palmitic is found to predominate. Of the liquid fatty acids oleic and linoleic are found in the stored fat and food fat, but ordinarily the proportion of linoleic is very much less than is found in the present work in liver lecithin. Arachidonic acid and other highly unsaturated long chain acids are found only in traces in stored fat and in slightly larger amount in the neutral fat of liver. The presence of the large proportion of linoleic acid, an 18-carbon acid, would fit in with Leathes' hypothesis of desaturation by the liver, originating either from oleic or from the stearic acid. As was noted by Klenk and Schoenebeck (2) the arachidonic acid and the clupandonic acid (C_{18}) found by them in liver do not easily fit into Leathes' scheme of desaturation because in these cases there is an increase in the length of chain. The preponderance of stearic over palmitic in the lecithin acids might be another example of lengthening of the chain by the liver.

The absence of linolenic acid in this fatty acid mixture and apparently in most animal lipids, as has been commented on several times in reports from this laboratory, is worthy of note as compared with its wide distribution in plant fats. When fed in not too large amount it is largely destroyed (Ellis et al.) and the presumption is that if formed in the animal organism it is promptly destroyed. However, it is not difficult to get it deposited in the depot fat as Rosenfeld showed long ago. Whether it can be introduced into the phospholipid molecule has not been shown.

The questions of whether the fatty acids in the phospholipids are there as the result of introduction bodily from the transported fatty acids or whether their nature can be changed in situ are unanswered. It appears certain that during fat absorption some of the phospholipids of the intestinal mucosa of the liver and blood plasma do undergo these substitutions and it seems likely that all phospholipids may do so. Sinclair has found definitely (25) that the nature of the fatty acids in tissue phospholipids may be changed by food fat and the time relations of the change and the fact that relatively small amounts of certain fats bring about the change strongly suggest a selection of the more highly unsaturated acids by the phospholipids. The factor of selection was mentioned in a previous discussion of Leathes' hypothesis of
fatty acid desaturation by the liver, in connection with the neutral fat of liver (34). However, it is unlikely that selection could account for the large proportion of arachidonic acid found in liver phospholipid. There appears to be no explanation except that of formation on the spot. In this connection the fact was pointed out by André (35) that it is usual for naturally occurring fatty acids to have in their molecule one group of 9 carbon atoms (generally at the carboxyl end) free from double bonds, while the first bond occurs in position $\Delta^{9-10}$ and the others are spaced so that double bonds come at every 3rd carbon atom. Because of this peculiarity an 18-carbon acid would have a maximum of three double bonds and when four or more bonds are present the chain is longer by 2 carbon atoms for each extra double bond. Thus the commonly occurring fatty acids with the higher degree of unsaturation, arachidonic, etc., are 20- or more carbon acids.

The lengthening of the chain proceeds according to the characteristic rule for changing the length of the chain of the fatty acids, 2 carbon atoms at a time and presumably involves the same hypothetical 2-carbon fragment as is formed when the fatty acids are broken down by $\beta$ oxidation, but since the extra double bond appears at the end opposite the carboxyl group the change can hardly be a reversal of the $\beta$ oxidation unless it is assumed that the whole molecule is rearranged.

SUMMARY

In the lecithin of beef liver the proportion of liquid (unsaturated) to solid (saturated) fatty acids was found to be about 55:40. The saturated acid of lecithin consisted of about 71 parts of stearic to 29 parts of palmitic acid as compared with the neutral fat of liver which contains more palmitic acid than stearic acid.

The distribution of the unsaturated acids was found to be linoleic 45 per cent, arachidonic 31 per cent, and oleic 21 per cent. Acids with three double bonds were not found. Acids of larger molecule than C$_{20}$ were present in small amount in one series.

BIBLIOGRAPHY

FATTY ACIDS OF LIVER LECITHIN
Ruth H. Snider and W. R. Bloor


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