[CONTRIBUTION FROM THE LABORATORY OF PHYSIOLOGICAL CHEMISTRY, COLLEGE OF MEDICINE, OHIO STATE UNIVERSITY]

THE OCCURRENCE OF ARACHIDONIC ACID IN LARD[1]

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RECEIVED OCTOBER 11, 1929 PUBLISHED MARCH 6, 1930

The fats and oils of warm-blooded animals are composed mainly of the glycerides of palmitic, stearic and oleic ($C_{18}H_{34}O_2$) acids. The presence of acids of lower molecular weight than palmitic is by no means unusual. Moreover, in a number of instances acids more unsaturated than oleic have been found, particularly linolic acid, $C_{18}H_{32}O_2$. The occurrence of linolenic ($C_{18}H_{30}O_2$) and clupanodonic ($C_{18}H_{28}O_2$) acids with three and four double bonds, respectively, has been rarely described, except in instances where these acids have been furnished the animal with the food. The fat of marine mammals such as the polar bear has been shown to contain considerable quantities of the characteristic highly unsaturated fish oil fatty acids, this no doubt being mainly a dietary effect.

Recent investigations have shown the presence of considerable quantities of arachidonic acid, $C_{20}H_{32}O_2$, in the lipids of the liver,[2] brain,[3] kidney, lung, pancreas,[3c] thyroid, suprarenal, spleen[4] and corpus luteum.[5] The lipids of these tissues differ markedly from the body fat in that they are more unsaturated and contain notable amounts of phospholipids together with other compounds of complex composition.

The writer in 1917 isolated from a specimen of woodchuck fat hexabromostearic acid corresponding to 3.0% of linolenic acid. The bromide was identified on the basis of melting point.[6] In an analysis of a specimen of human fat Eckstein found 0.33% of arachidonic acid.[7] Isbell and Ellis,[8] in studying the soft pork problem, found arachidonic acid in amounts varying from 0.02 to 0.21% in lards from hogs which had been fed on a number of experimental diets. In connection with their work, it is interesting to note that the largest amount of the acid occurred in lard from peanut-fed animals. The acid may have been formed therefore by desaturation of the arachidic acid in the diet.

[1] Presented at the fall meeting of the American Chemical Society, 1929, Minneapolis, Minnesota.

[2] P. Hartley, *J. Physiol.*, **38**, 353 (1909); P. A. Levene and H. S. Simms, *J. Biol. Chem.*, **48**, 185 (1921); J. B. Brown, *ibid.*, **80**, 455 (1928).

[3] (a) P. A. Levene and I. P. Rolf, *ibid.*, **54**, 91 (1922); **54**, 99 (1922); (b) L. G. Wesson, *ibid.*, **60**, 183 (1924); (c) W. R. Bloor, *ibid.*, **80**, 443 (1928); (d) J. B. Brown, *ibid.*, **83**, 783 (1929).

[4] J. B. Brown, *ibid.*, **83**, 777 (1929).

[5] G. F. Cartland and M. C. Hart, *ibid.*, **66**, 619 (1925).

[6] Unpublished research with Dr. G. D. Beal, University of Illinois (1917).

[7] H. C. Eckstein, *J. Biol. Chem.*, **64**, 797 (1925).

[8] N. R. Ellis and H. S. Isbell, *ibid.*, **69**, 219, 239 (1926).

The object of the present work was to find whether arachidonic acid occurs in commercial lard as it is marketed today. Four specimens of lard from different packing houses were converted into methyl esters and analyzed. Methyl octobromo-arachidate was isolated from each by bromination in cold ether. The yields corresponded to an arachidonic acid content of 0.31 to 0.40%. The bromide was identified on the basis of correct melting point and bromine content. One specimen of the methyl esters was distilled under reduced pressure into four fractions. The content of arachidonic acid increased with boiling point from 0.07 to 0.66%, which results are in agreement with the fact that it is the highest boiling of any of the acids present. The fatty acids of lard are stated by Lewkowitsch[9] to consist of lauric, myristic, palmitic, stearic, oleic and linolic acids (with possibly small amounts of linolenic acid).

Direct bromination of lard yielded small amounts of ether-insoluble material which contained from 7.13 to 10.56% of bromine. No definite evidence concerning the nature of this material was obtained. Assuming it to be a pure compound containing one molecule of octobromo-arachidic acid, it would have a total molecular weight of about 6000. However, there was evidence that the substance was a mixture of a brominated mixed glyceride of arachidonic acid with four parts of palmito-distearin.

The amount of arachidonic acid present in the four specimens examined was roughly proportional to the iodine number.

The results obtained on composite commercial samples of lard are in agreement with those of Ellis and Isbell on lard from hogs on special diets. The arachidonic acid content reported above is over three times that reported by the latter investigators, who found a variation from 0.02 to 0.21% with an average of 0.09%. This is largely due, however, to the method of calculation. Ellis and Isbell found the percentage of arachidonic acid by calculating the acid content of the ether-insoluble bromides. This result, as has been stated by the writer in a previous paper,[2] is probably far too low, since pure arachidonic acid gives a polybromide number of only 80, or a yield of ether-insoluble bromides about one-fourth the theoretical.

The finding of arachidonic acid in lard is contrary to the usual statement that warm-blooded animal fats do not contain acids more unsaturated than linolic.

Experimental Part

Lard.—Four specimens of lard were purchased in original containers on the market. Two of these were the best products of Chicago packers, one of a Columbus packer, and the fourth specimen was marketed under a trade name by a chain store in Columbus. These samples represent typical lard as prepared commercially in the United States today.

[9] Lewkowitsch, "Technology of Oils, Fats and Waxes," **1922,** 6th ed., Vol. II, p. 709.

Analytical Methods.—The usual methods of analysis for iodine and saponification numbers were employed.

The polybromide number, *i. e.*, the percentage yield of bromides, was found by brominating weighed samples in cold ether, allowing to stand several hours, removing the ether by centrifugation and washing four times more with fresh cold ether, stirring each time. The white product was dried for several hours in a warm oven (not over 50°). The polybromide numbers of the esters from lard were so small that it was necessary to brominate 500-g. samples in one liter of ether to get large enough specimens for the bromine determination. The product was transferred to a 250-cc. centrifuge bottle for the first washing, and to a 50-cc. tube for final washings and weighing.

Bromine analyses were made by the Parr peroxide bomb method as modified by Brown and Beal.[10]

Arachidonic acid was determined according to the following formula

$$\% \text{ arachidonic acid} = \frac{\text{polybromide number of ester}}{77.6} \times 100$$

where 77.6 is the polybromide number of pure methyl arachidonate. This method of calculation has been discussed previously.[4]

Preparation of Methyl Esters.—One kilo of lard was transferred to a 5-liter pyrex flask and refluxed for eighteen hours with 1200 cc. of dry methyl alcohol containing 1 to 2% of hydrogen chloride gas. The esters and alcohol were poured into 2 liters of cold water. Separation was facilitated by the addition of a handful or two of salt. The ester layer was removed and transferred to a 2-liter Claisen flask. The esters were warmed under reduced pressure. After small amounts of water and methyl alcohol had passed over, the esters were distilled: yield, 968, 961, 923, and 976 g. of water-white esters from 1-kilo lots of the four lards, respectively. Analyses of the samples of lard and methyl esters are given in Table I.

TABLE I

ANALYSIS OF FOUR SPECIMENS OF LARD AND RESULTANT METHYL ESTERS

Lard no.	B. p., °C.	Iodine number	Saponification number	Polybromide number	Br, %	Arachidonic acid, %
			Lard			
1	.....	58.55	195.7	..	...	..
2	.....	64.55	197.4	1.5	10.56	..
3	.....	59.78	199.7	..	...	..
4	.....	65.33	197.9	3.0	7.13	..
			Methyl Esters			
1	170.210 (4 mm.)	56.39	195.4	0.24	66.70	0.31
2	170.220 (6 mm.)	62.34	193.5	.28	66.30	.35
3	165.220 (6 mm.)	58.53	192.7	.25	66.46	.32
4	170.220 (6 mm.)	63.56	194.4	.31	66.44	.40

The bromide from Lard 3 in Table I, containing 10.56% of bromine, was further studied. Most of it melted at 60–70°, forming a distinctly turbid melt. This turbidity disappeared at about 180°. The material was soluble in chloroform, but sparingly soluble in ether and *n*-butyl alcohol at ordinary temperature. It was freely soluble in boiling butyl alcohol and toluene, and was crystallized from the former, giving tufts of needles. The product of crystallization contained 12.73% of bromine. It is probable that the material was a mixture, therefore, of 4 to 5 parts of α-palmito-distearin,

[10] J. B. Brown and G. D. Beal, THIS JOURNAL, **45**, 1289 (1923).

which melts at 68° and is sparingly soluble in ether, with one part of brominated mixed glyceride, containing one molecule of octobromo-arachidic acid. α-Octobromo-arachido-distearin should contain 41.3% of bromine. α-Palmito-distearin has been isolated from lard by Bömer.[11]

Fractionation of Methyl Esters.—Six hundred and twenty-five g. of the methyl esters from Lard 1 was distilled slowly from a 1-liter Claisen flask, four fractions being cut. The results of analysis of these fractions are given in Table II.

TABLE II

RESULTS OF FRACTIONATION OF METHYL ESTERS FROM LARD NO. 1

B. p., °C.	Wt., g.	Iodine number	Sapon. number	Polybromide number	Arachidonic acid, %
180–194	114	42.73	203.3	0.05	0.07
194–199	181	49.98	199.0	.07	.09
199–210	222	62.03	195.4	.13	.17
210–220	105	72.82	190.9	.51	.66

The identity of the methyl octobromo-arachidate isolated from the methyl esters from each specimen of lard is proved by the bromine analyses, which agree satisfactorily with the theoretical, 66.78%, and by the melting point, which in each case was 228–230°, the same as that found for this compound isolated from the glandular lipids.

Summary

Four specimens of typical commercial lard have been found to contain arachidonic acid in amounts varying from 0.31 to 0.40%. Two of the samples of lard yielded ether-insoluble bromine addition products which were shown to be a mixture, probably of α-palmito-distearin with a glyceride of octobromo-arachidic acid.

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ALLENE AND METHYLACETYLENE TETRABROMIDES[1]

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RECEIVED OCTOBER 14, 1929 PUBLISHED MARCH 6, 1930

Methylacetylene tetrabromide was studied by Oppenheim[4] in 1864, and by Gustavson and Demjanov[5] in 1888. The more recent and more

[11] Bömer, *Z. Nahr. Genussm.*, **17**, 393 (1909); **25**, 354 (1913).

[1] This paper contains results obtained in an investigation on "The Non-Catalytic Thermal Decomposition of Pure Hydrocarbons and Related Compounds," listed as Project No. 18 of American Petroleum Institute Research. Financial assistance in this work has been received from a research fund of the American Petroleum Institute donated by the Universal Oil Products Company. This fund is being administered by the Institute with the coöperation of the Central Petroleum Committee of the National Research Council.

[2] Director, Project No. 18.

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[4] Oppenheim, *Ann.*, **132**, 124 (1864).

[5] Gustavson and Demjanov, *J. prakt. Chem.*, [2] **38**, 201 (1888).