Highly Unsaturated Fatty Acids. I. A Survey of Possible Animal Sources[1]

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VERY little reliable information is available concerning the highly unsaturated fatty acids occuring in animal tissues. This is true partially because their isolation is very difficult by conventional methods and because they occur in low concentrations in the readily available tissue lipids. Isolation of these acids as polybromides has shown them to be present in a wide variety of tissues. Liver lecithin (1), brain phospholipids (2), corpus luteum (3), and egg yolk (4) have been reported to contain significant quantities of arachidonic acid. Bloor has surveyed many beef tissues for their polyunsaturated fatty acid content (5). Brown has isolated reasonably pure arachidonic acid from liver (6), and Ault and Brown (7) have used commercially available beef adrenal phosphatides as a source for its isolation. By use of chromatography White and Brown (8) and Herb *et al.* (9) have prepared pure natural arachidonate from this source. These and other reports of the occurrence of polyunsaturated fatty acids are based upon widely different means of isolation or detection, and the diversity of experimental methods makes comparison of the data very difficult.

Because it is becoming increasingly difficult to procure beef adrenal phosphatide from pharmaceutical houses for the preparation of arachidonate, it was thought advisable to survey possible sources of arachidonic acid and more highly unsaturated fatty acids. To do this it was first necessary to test various means of extraction of lipids or fatty acids to learn which method gave the best yield of polyunsaturated acids. Using dehydrated beef liver as the test substance, several methods of extraction were tested on 100 g. samples of dehydrated tissue. Repeated boiling ethanol extraction, saponification of the entire tissue with boiling 30% KOH for 4 hours, cold saponification of the entire tissue with 10% KOH over night, acid hydrolysis of the entire tissue in the presence of ethanol, and triple extraction of dehydrated tissue with boiling petroleum ether were employed. It was found that extraction by refluxing with 30% KOH gave the greatest apparent yield of arachidonic acid while extraction with boiling 95% ethanol was the simplest to perform and gave a high yield of lipid having a greater concentration of polyunsaturated fatty acids than lipid obtained by other procedures. These two methods were employed on all remaining samples which were tested. The subsequent tissue saponifications were made by refluxing the samples on a steam bath for 24 hours in 500 ml. of aqueous KOH solution per 100 g. tissue. Unsaponifiable material was removed by two extractions with petroleum ether, the soaps were acidified, and the fatty acids were recovered by four extractions with petroleum ether. The solutions were dried with sodium sulfate and filtered, and the solvent was removed to obtain the total free fatty acids. The ethanol extractions were made with three 500-ml. portions of boiling 95% ethanol, and the combined extracts were boiled down to about 200 ml. Water was then added and the lipids were extracted four times with petroleum ether. The solutions were dried with sodium sulfate and filtered, and the solvent was removed by vacuum to recover the total mixed lipids. In some cases the ethanol extractions were followed by two ether extractions, but the samples were handled in the same manner.

All samples were isomerized in duplicate (100 mg.), using 23.0% KOH in glycol for 8.0 minutes at 180° (10). These conditions of isomerization used in the author's laboratory for several years were designed to increase the sensitivity of measurement of polyunsaturated fatty acids. The extinction coefficients for

TABLE I

Polyunsaturated Fatty Acid Content of Tissue Lipids

Tissue	Extractive method	Lipid Yield g/100 g tissue	Percentage of Acid		
			Tetraenoic	Pentaenoic	Hexaenoic
Lamb					
Testes, fresh	Ethanol-ether	0.9	10.3	0.32	15.6
Testes, dehydrated	Ethanol	6.0	9.6	0.0	13.7
Testes, dehydrated	30% KOH	10.0	9.5	4.5	6.1
Hog					
Ovaries, dehydrated	Ethanol	11.0	9.2	1.6	0.42
Ovaries, dehydrated	30% KOH	10.5	11.1	2.4	—0.44
Testes, fresh	Ethanol-ether	2.8	8.8	2.6	1.52
Uterus, fresh	Ethanol-ether	1.4	7.8	1.3	0.14
Liver, dehydrated	Ethanol	11.2	4.6	1.9	1.0
Liver, dehydrated	30% KOH	16.0	4.3	1.6	—0.38
Liver, lipid (Armour Biopar)	?	?	8.2	2.1	—0.21
Brains, dehydrated	Ethanol	35.0	3.7	1.0	3.1
Brains, dehydrated	30% KOH	64.5	0.01	4.7	—0.96
Kidneys, dehydrated	Ethanol	12.0	3.6	0.96	0.09
Kidneys, dehydrated	30% KOH	18.5	4.3	0.62	0.05
Adrenals, dehydrated	Ethanol	36.1	3.5	0.12	0.72
Adrenals, dehydrated	30% KOH	37.0	3.7	0.18	0.01
Adrenals, lipid (Upjohn)	?	?	1.8	0.41	—0.21
Spleen, dehydrated	Ethanol	19.1	2.1	0.38	0.49
Spleen, dehydrated	30% KOH	20.5	1.9	0.65	—0.30
Heart, dehydrated	Ethanol	11.0	2.0	0.63	—0.07
Heart, dehydrated	30% KOH	26.5	1.8	0.32	—0.03
Spinal cord, fresh	Ethanol-ether	4.6	1.3	0.28	0.35
Pancreas, dehydrated	Ethanol	46.0	0.6	0.18	0.25
Pancreas, dehydrated	30% KOH	48.0	0.3	0.0	0.0
Spermatocords	Ethanol	1.1	0.0	0.0	0.0
Beef					
Testes, dehydrated	Ethanol	10.5	5.3	1.9	4.1
Testes, dehydrated	30% KOH	12.5	5.8	3.5	2.4
Liver, dehydrated	Ethanol	17.2	1.9	3.2	—0.63
Liver, dehydrated	30% KOH	23.5	2.9	4.4	—1.5
Liver, lipid (Armour Biopar)	?	?	2.6	3.2	—0.42
Pituitary, fresh	Ethanol-ether	0.84	2.0	0.79	0.26
Brain fatty acids (Armour)	?	?	3.9	0.64	0.65
Spinal cord, lipid (Armour)	?	?	0.6	0.0	0.91
Yellow bone marrow fatty acids (Armour)	?	?	0.0	0.0	0.0
Blood, dehydrated	Ethanol	0.0	....	....	....
Blood, dehydrated	30% KOH	0.0	....	....	....
Thyroid, fresh	Ethanol-ether	12.8	0.0	0.0	0.0
Parathyroid, fresh	Ethanol-ether	28.0	0.0	0.0	0.0
Thymus, fresh	Ethanol-ether	10.0	0.0	0.0	0.0

[1] Supported in part by grants from the National Livestock and Meat Board and the Office of Naval Research (contract N8onr-66218).
Hormel Institute publication No. 92.

pure acids (k) used in the calculation of hexaenoic, pentaenoic, and tetraenoic acids are the following:

	Extinction coefficient (k)		
	3750 Å	3475 Å	3000 Å
Docosahexaenoic acid (11)	27.1	43.9	45.4
Docosapentaenoic acid (11)	12.5	46.3	56.8
Arachidonic acid (10)	.....	.....	62.2

All extinction coefficients were corrected for background absorption. The calculation of hexaenoic and pentaenoic acids involves the use of simultaneous equations because end-absorption of docosapentaenoic acid at 3750 Å is very high. The data obtained by such calculation are still subject to some error but serve well for comparisons and where high precision is not required.

It should be pointed out that some of the constants used in the calculation were determined upon acids derived from fish oil, and these acids probably are not identical to the polyunsaturated acids found in mammalian tissue. For further discussion of the calculation and interpretation of spectrophotometric data, the reader is referred to current papers dealing with the limitations of the method (12, 13).

The results of our survey are summarized in Table I. From these data it is clear that gonadal tissue is highest in hexaenoic acid and arachidonic acid. As sources of arachidonic acid, lipids from hog testes, ovaries, uterus, liver, and brain are superior to adrenal lipid. Rich sources of hexaenoic acid are lipids of lamb, beef testes, and hog brain. The high content of polyunsaturated fatty acids in gonadal tissue may have some biochemical significance for it should be pointed out that depriving animals of dietary polyunsaturated acids results in impaired reproduction.

The method of extraction has an effect upon the yield of polyunsaturated acids. Saponification of the tissue and recovery of the fatty acids generally gave a yield of total fatty acids greater than the total lipid extracted by ethanol. The fatty acids obtained by tissue saponification generally had lower hexaenoic acid contents and higher apparent pentaenoic acid contents than did corresponding lipids obtained by ethanol extraction. Whether the differences in yield of the three types of acids are artifacts caused by treatment with KOH cannot be stated, but it appears that extraction with ethanol or ethanol-ether is preferred.

With two exceptions the negative values for hexaenoic acid content occurred in cases where the sample history was not known to us or in which the extraction was by saponification of the tissue. This also supports the preference for ethanol extraction. Attention should also be called to the several tissue lipids in which no polyunsaturated fatty acid was detected. It is particularly interesting that no lipid was extracted from dehydrated blood by either method in spite of the known existence of considerable lipid in blood. The inability to extract blood lipid after dehydration agrees with observations by Murphy (14) but remains unexplained.

The authors wish to acknowledge the generous cooperation of L. W. Murphy of George A. Hormel and Company in supplying the fresh and dehydrated tissues used in this survey. We are also grateful to M. A. Mitz of Armour and Company for supplying several samples of extracted lipids.

Summary

1. Lipids were extracted from various tissues of lambs, hogs, and cattle, and the content of tetraenoic, pentaenoic, and hexaenoic acids were determined upon the lipids.

2. Reproductive and glandular tissue lipids have the highest contents of polyunsaturated acids. Lamb testes lipid, containing 15.6% hexaenoic acid, was found to be the richest source of this acid. It also contains 10.3% arachidonic acid. The most practical sources for isolation of these acids from the point of view of availability as well as content are beef testes lipid and hog brain lipid for hexaenoic acid and hog liver lipid for arachidonic acid.

3. Extraction by saponification of the entire tissue generally increases yield of total fatty acids above the yield of lipid obtained by ethanol extraction, but the total acids obtained have generally lower content of hexaenoic acid. Ethanol extraction is preferred. No lipid was extractable from dehydrated blood.

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[Received July 21, 1953]