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By

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(Received, May 11, 1962)

From 1928 when clupanodonic acid (1) was first characterized from sardine oil, the ethylene interrupted or divinyl ethane double bond arrangement for the highly unsaturated fatty acids (higher polyunsaturated fatty acids) of fish has been proposed and advocated by Japanese scientists. After the second World War, interest in the structure of fatty acids not only of mammals but also of fish was revived with the progress of methodology. Actually, it has reasonably been demonstrated by the following three groups that even in fish polyunsaturated fatty acids the divinyl methane arrangement, which is also called methylene interrupted or skipped type, does exist exclusively as in land animals. They are Klenk and his co-workers (2-6) on the studies of herring and cod liver oils; Silk and Hahn (7), and Whitcutt (8, 9) and Sutton (8) on south African pilchard oil; and Stoffel and Ahrens (10-12) dealing with menhaden body oil. Also Toyama *et al.* (13) isolated eicosatetraenoic, docosapentaenoic and docosahexaenoic acids with methylene interrupted structure from sardine and saury oils. At this point, one of the authors (14) and Takagi (15) reconsidered and criticized the previous views on the fish highly unsaturated acid structures which involve the ethylene interrupted double bond.

On the other hand, a very important behavior of polyunsaturated acid interconversion was observed almost simultaneously by Rieckehoff, Holman, and Burr (16), Widmer and Holman (17), and Reiser (18, 19) and later by Klein and Johnson (20). They noticed spectrophotometrically in the rat and chicken that three of the most characteristic signs were the increases of trienoic acid in the fat-deficient rat, and of tetraenoic acid in supplement of linoleic acid, and of pentaenoic and hexaenoic acids in linolenic acid addition to basal diet. Following these observations the systematic conversion studies of essential fatty acid using ^{14}C labeled acids were performed by Mead and his co-workers (21-29) in the rat. The metabolic pathway from linoleic (24) to arachidonic *via*

γ-linolenic (27) and homo-γ-linolenic (29) acids was definitely proved with labeling experiments in which the label in the proposed intermediate appears rapidly and efficiently in arachidonic acid as well as other evidence indicates that the following conversion scheme is the major one.

$CH_3-(CH_2)_4-CH=CH-CH_2-CH=CH-(CH_2)_7-COOH$

↓ Linoleic

$CH_3-(CH_2)_4-CH=CH-\overset{*}{C}H_2-CH=CH-CH_2-CH=CH-(CH_2)_4-COOH$

↓ γ-Lenolenic

$CH_3-(CH_2)_4-CH=CH-\overset{*}{C}H_2-CH=CH-CH_2-CH=CH-(CH_2)_4-CH_2-CH_2-COOH$

↓ Homo-γ-Linolenic

$CH_3-(CH_2)_4-CH=CH-\overset{*}{C}H_2-CH=CH-CH_2-CH=CH-CH_2-CH=CH-CH_2-CH_2-CH_2-COOH$

Arachidonic

In the previous labeling experiments the author *et al.* reported the conversions from linoleic to arachidonic acid (30) and from linolenic to eicosapentaenoic and docosahexaenoic acids (31) in fish. Here the possible conversion pathway or the biogenesis of polyunsaturated acids in fish is discussed, refering to the already defined fish polyunsaturated acid structure and proposing metabolic pathways.

Discussion for Conversion Pathway of Fish Polyunsaturated Acid

In Table 1 the polyunsaturated fatty acids isolated and identified from fish oils are listed.

It was noticed by the authors that very characteristic regularity does exist in the double bond position of fish polyunsaturated acids counting from the methyl group end (32, 36) of the acids as shown in the table. The unsaturated acids, longer than octadecadienoic acid, will be classified into three groups with first double bond starting at 3, 6, and 9 positions* respectively. The first group should be named linolenic (32-36), the second linoleic (32-36) and the third oleic families (35, 36) as follows.

$CH_3-CH_2-CH=$	Linolenic family
$CH_3-CH_2-CH_2-CH_2-CH_2-CH=$	Linoleic fomily
$CH_3-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH=$	Oleic family

From the evidence presented by Mead *et al.* it appears that the polyunsaturated acids are formed by alternative desaturation in divinyl methane relationship to the existing double bond and chain lengthening. It is logically

* All the positions of double bonds are counted from the terminal methyl group of the unsaturated acid in this rep ort.

Table 1. Characterized polyunsaturated acid from fish.

Chain length	No. of double bond	Position of double bond		Occurence from fish
		Counted from carboxyl carbon	counted from methyl carbon	
Hexadeca-	dienoic	9,12	4,7	herring[6], menhaden[12]
		7,10	6,9	herring[6]
		6,9	7,10	herring[6], menhaden[12]
	trienoic	9,12,15	1,4,7	herring[6]
		7,10,13	3,6,9	herring[6], menhaden[12]
		6,9,12	4,7,10	herring[6], menhaden[12]
		4,7,10	6,9,12	herring[6]
	tetraenoic	6,9,12,15	1,4,7,10	herring[6], pilchard[7], menhaden[12]
		4,7,10,13	3,6,9,12	herring[6], menhaden[12]
Octadeca-	dienoic	9,12	6,9	herring[5], menhaden[12]
		6,9	9,12	menhaden[12]
	trienoic	9,12,15	3,6,9	herring[5], menhaden[12]
		6,9,12	6,9,12	menhaden[12]
	tetraenoic	6,9,12,15	3,6,9,12	herring[4], menhaden[12]
Eicosa-	dienoic	11,14	6,9	menhaden[12]
		8, 11	9,12	menhaden[12]
	trienoic	8,11,14	6,9,12	menhaden[12]
		5,8,11	9,12,15	menhaden[12]
	tetraenoic	8,11,14,17	3,6,9,12	menhaden[12]
		5,8,11,14	6,9,12,15	menhaden[12], sardine[13]
	pentaenoic	5,8,11,14,17	3,6,9,12,15	cod[3], pilchard[8], menhaden[12]
Docosa-	pentaenoic	7,10,13,16,19	3,6,9,12,15	cod[2], herring[5], menhaden[12]
		4,7,10,13,16	6,9,12,15,18	saury[13]
	hexaenoic	4,7,10,13,16,19	3,6,9,12,15,18	cod[2], herring[5], pilchard[9], menhaden[12], saury[13]

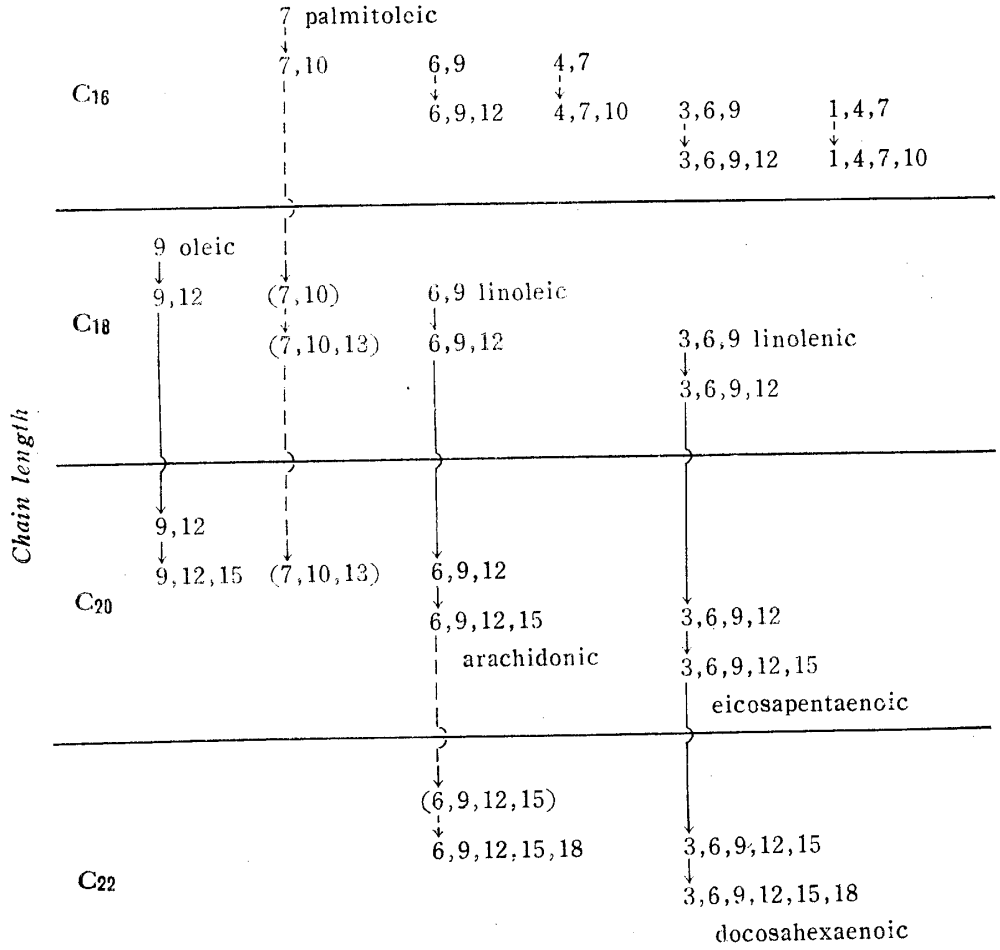
expected that starting from the existing double bond of the parent acid (in this case, linolenic, linoleic, and oleic acids) the additional double bonds are introduced in the 1-4 relationship toward the carboxyl group until the next double bond would be in the $\alpha\beta$ or $\beta\gamma$ position and the chain elongation then permits the more double bond addition.

When the authors tried to arrange the unsaturated acids listed in Table 1 according to their chain length along the row of C_{16}, C_{18} *etc.* and to connect with one another which have the same initial double bond position with vertical lines, the main part of Scheme 1 was otained.

It will be realized that the conversion steps from linoleic to arachidonic acid presented by Mead *et al.* (21, 24, 27, 29) are placed in the scheme just as their original. Moreover, this pathway has been shown to occur in the fish,

Scheme 1. Possible conversion pathways of polyunsaturated acids in fish.

Double bond position counted from methyl carbon



Tilapia mosambica (30). Also it can be assumed that the linoleic acid is essential in the true meaning even in fish, because its activity isolated after injection of acetate-1-^{14}C was almost negligible (30). For linolenic acid it can be seen that the similar process is formulated in the scheme. This conversion pathway from linolenic to eicosapentaenoic (25) and docosahexaenoic acids has been demonstrated in the kelp bass, *Paralabrax clathratus* (31). In this experiment the result proved that the injected linolenic acid was converted to eicosapentaenoic acid and the latter incorporated into docosahexaenoic acid. And as the probable metabolic route from linolenic acid to eicosapentaenoic and docosahexaenoic acids the same conversion pathway as the scheme was proposed. The intermediates involved in this system were logically expected with great confidence to be these acids in the scheme. Although rather high activity was observed in margaric acid resulted from the degradation of arachidic (hydrogenated eicosapentaenoic) and behenic (hydrogenated docosahexaenoic) acids after injection of methyl linolenate-1-^{14}C, presumably the activity was

induced from partial degradation and resynthesis process and thus the linolenic acid synthesis or conversion from a certain parent acid is of vrey small order if any. Pathways from 6, 9-C_{16} to 6, 9, 12-C_{16} and from 3, 6, 9-C_{16} to 3, 6, 9, 12-C_{16} may be formulated as shown in the scheme, hawever, chain lengthening will be difficult, because the last double bond of 12 position is in the $\gamma\delta$ position and the next double bond must be placed before the carboxyl group. Still more the connecting steps from 6, 9, 12-C_{16} and 3, 6, 9, 12-C_{16} to linoleic and linolenic acids are very doubtful respectively refering to the report (37) which demonstrated the difficulty of biohydrogenation of palmitoleic to palmitic acid.

Similar processes are assumed to occurr in oleic and palmitoleic acids of fish as illustrated in the scheme. The observation (16, 20, 26) that had noticed a characteristic increase of trienoic acid was proved by Fulco and Mead (28). The 9, 12, 15-C_{20} was indeed derived from oleic acid in the fat-deficient rat and this conversion pathway was formulated just as shown in the scheme. In the same experiment it was also discussed that since eicosatrienoic acid, derived from the fat-deficient rat, contained about 7 per cent of 7, 10, 13-C_{20} isomer, this isomer must have been derived from palmitoleic acid. Fish may have such a period of starvation or fat-deficient state in life due to environmental condition. Also to the other acids of C_{16} chain length which have the last double bond at 10 position, chain elongation should be considered; however, the products to be derived are not characterized from fish oil source even for the palmitoleic family.

In the scheme the arrow signs within the same chain length represent the desaturation or one double bond addition steps, and the long arrows which are across the chain length border line stand for chain elongation or two carbon atoms addition steps to the precursor acids' carboxyl end. Although some successive processes with one of the two kinds of steps would be considered for these conversions as postulated by Klenk and Mohrhauer (38), it seems that the alternative desaturation and chain lengthening are major and more probable in nature. Acids which are not separated from fish oils at present are parenthesized in the scheme. Also the broken lines mean actual assumption for conversion pathways. These pathways are not interconvertible nor are the paths of formation of the higher members reversible.

Concerning the biogenesis of the polyunsaturated acids of linoleic and linolenic families, the authors assume the linoleic and linolenic acids of phytoplanktons (39, 40) in the aquatic field. Phytoplankton is to the sea what plant is to the land— the basic food. Since we are now proceeding a model experiment of aquatic food chain with special significance of fatty acid conversion, a general consideration of fish fatty acids will be reported later.

Conclusion

It may thus be concluded that there are three major conversion pathways of polyunsaturated acids in fish derived from oleic, linoleic and linolenic acids. The last metabolic route seems to be a main one in fish, since the linolenic family, such as eicosapentaenoic and docosahexaenoic acids, is commonly found and contained in fairly large amount.

In addition to these major courses, other minor pathways for the complicated hexadecapolyunsaturated acids, which are assumed to be rather peculiar in fish oil, as well as for palmitoleic family, are tentatively formulated in the scheme.

Acknowledgment

It was the authors' great pleasure to have had the opportunity of valuable discussion with Professor James F. Mead, Laboratory of Nuclear Medicine and Radiation Biology of the Department of Biophysics and Nuclear Medicine, School of Medicine, University of California at Los Angeles, and Professor Raymond Reiser, Department of Biochemistry and Nutrition, Texas Agricultural and Mechanical College, to whom the authors are deeply indebted.

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