OCCURRENCE OF A DOCOSATRIENOIC ACID IN THE CHOLESTEROL ESTERS OF ADRENALS OF RATS ON ESSENTIAL FATTY ACID DEFICIENT DIETS*

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Young rats reared on diets devoid of essential fatty acids develop a well defined deficiency syndrome (Aaes-Jørgensen, 1961). They also show significant alterations in the fatty acid composition of the lipids: reduced amounts of linoleic acid and longer chain acids derived from linoleic acid occur together with elevated proportions of monoenoic and trienoic acids.

In the present study we have determined the fatty acid composition of the adrenal cholesterol esters of rats raised on diets deficient in essential fatty acids. In esters of the adrenals of control animals there were 19% of monoenoic acids, 2% of trienoic acid, and 52% of other polyenoic acids. In the deficient rats there were about 50% of monoenoic acids, 18% of trienoic acids, and 4% of other polyenoic acids. This report is primarily concerned with the identification of a docosatrienoic acid. Small amounts of this acid have been found previously in liver mitochondria of certain fishes (Richardson et al., 1962), human serum phosphatides (Nelson, 1962), ox brain phosphatides (Klenk and Montag, 1958), and rat brain lipids (Mohrhauer and Holman, 1963), but it has not been noted in cholesterol esters. In the deficient rats, however, it comprises about 10% of the fatty acids of the adrenal cholesterol esters.

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Materials and Methods

Male Sprague-Dawley weanling rats were reared on a normal pellet diet (Rockland Farms Mouse Pellets) or on a fat free diet containing 0.4% cholesterol (General Biochemicals Co. Inc.). After 11 or 15 weeks the rats were killed and the adrenals removed. Pooled adrenals were homogenized in water and extracted with chloroform-methanol (2:1) (Folch et al., 1957). Cholesterol esters were isolated by column (Horning et al., 1960), or thin layer chromatography (Mangold, 1961). Methyl esters of the cholesterol ester fatty acids were prepared by the method of Stoffel et al., (1959), and purified by thin layer chromatography. Ester samples from two groups of adrenals pooled from animals on the control diet, and three groups of adrenals pooled from animals on the deficient diet were subjected to gas liquid chromatography on columns of ethylene glycol succinat (EGS) or Apiezon L (ApL) on Chromosorb W, 80 to 100 mesh.

Results

Of the nineteen different acids present in measurable amounts in the esters of control and/or deficient animals, all but six were identified by means of reference standards. On the basis of relative retention times on polar (EGS) or non-polar (ApL) columns, these acids were presumed to be 20:2,** 20:3 (5, 8, 11), 20:3 (8, 11, 14), 22:3, 22:4 and 22:5?. Table 1 shows the relative retention times of five of these six acids (all except 22:5?). Arachidonic acid (20:4) is included as a frame of reference. The various components on the polar and non-polar chromatograms were paired by comparing relative peak areas from the EGS columns with those from ApL columns. A log-log plot of relative retention times on EGS versus relative retention times on ApL was made for all components including the reference standards (James, 1959). From this type of plot, the identity of 20:2, 20:3 (5, 8, 11), 20:3 (8, 11, 14), 22:3, and 22:4 was established. The acid with a relative retention of 3.11 (present only in deficient animals) is probably 5, 8, 11 eicosatrienoic acid, while the other eicosatrienoic acid is probably the 8, 11, 14 isomer. The relationship of these two acids to each other on EGS columns is similar to that noted by Morin et al., (1962).

** The number before the colon refers to the number of carbon atoms, the number after the colon refers to the number of double bonds, and the numbers in parentheses refer to the positions of the double bonds numbering from the carboxyl carbon.

Table 1

RELATIVE RETENTION TIMES OF EICOSAENOIC AND DOCOSAENOIC ACIDS

Retention Time (Methyl Stearate =100)		Acid
EGS	ApL	
2.87	1.74	20:2
3.11	1.57	20:3 (5, 8, 11)
3.45	1.57	20:3 (8, 11, 14)
3.99	1.48	20:4
5.93	3.38	22:3
7.55	3.20	22:4

Table 2 summarizes the composition of the cholesterol esters in the control and deficient groups. The control adrenals contain principally arachidonic acid and docosatetraenoic acid esters; together they comprise 37.5% of the total acids; other major components are palmitate (15%) and oleate (12.8%). The deficient rats show both qualitative and quantitative differences from the controls. There is a striking reduction in the C_{20} and C_{22} tetraenoic acids: only 2.8% in deficient animals as compared to 37.5% in the controls; there are also lower percentages of 18:2, 22:5?, and 22:6. On the other hand the proportion of monoenoic acids is significantly higher (more than double the controls), and these acids, especially 18:1, now constitute the major fatty acid fraction. The most important change, however, is the appearance of two trienoic acids with twenty and twenty-two carbons. These acids, not present in the control animals, comprise 17.6% of the total acids in the cholesterol esters of the adrenals of the deficient rats.

Discussion

Other investigators have also reported high proportions of arachidonic and docosatetraenoic acids in the cholesterol esters of rat adrenals (Dailey et al., 1960)(Goodman, 1962). It has been established that arachidonic acid is

derived from linoleic acid (Mead, 1961), and recently Chang and Sweeley (1962) have isolated from canine adrenal cholesterol esters a docosatetraenoic with double bonds at positions 7, 10, 13 and 16. This sequence indicates that this acid is also derived from linoleic acid. The virtual disappearance of this docosatetraenoic acid in the animals on the fat-free diet also suggests that it is the 7, 10, 13, 16 isomer.

Table 2

FATTY ACID COMPOSITION OF ADRENAL CHOLESTEROL ESTERS FROM CONTROL AND ESSENTIAL FATTY ACID DEFICIENT RATS

Acid	Control	Deficient
	*Relative amount of fatty acid (% of total)	
16:0	15.0	18.2
16:1	2.1	7.4
18:0	3.2	2.3
18:1	12.8	33.7
18:2	5.0	1.0
20:1	2.6	6.5
20:2	0.6	0.8
20:3 (5, 8, 11)	-	7.9
20:3 (8, 11, 14)	2.2	Trace
20:4	17.9	1.1
22:3	-	9.7
22:4	19.6	1.7
22:5?	3.7	0.3
22:6	6.0	0.3

* 14:0, 15:0, 20:0, 22:1, and 22:5 were present in measurable amounts (<2.5%) in all the samples. 12:0, 13:0, 16:?, 17:0, 20:5, and 21:0 were present in trace amounts (<0.3%) in some samples.

An eicosatrienoic acid with double bonds at positions 5, 8, 11 from the carboxyl end is characteristically found in rats deficient in essential fatty

acids (Aaes-Jørgensen, 1961). This acid which is derived from oleic acid would seem to be a "substitute" for arachidonic acid in essential fatty acid deficient animals since it is usually found in highest proportion in those lipids which in control animals have a high content of arachidonic acid. The docosatrienoic acid has not been previously noted in cholesterol esters from either control or from essential fatty acid deficient rats. A number of isomers of this acid are possible, but the only one that has been described is the 7, 10, 13 isomer (Klenk and Montag, 1958), a double bond sequence that would indicate that the acid is derived from oleic acid (Mead, 1961). Thus, the docosatrienoic acid is analogous to the 5, 8, 11 eicosatrienoic acid in fat deficient animals and would appear to be a "substitute" for 7, 10, 13, 16 docosatrienoic acid. These trienoic acids are the result of a normal sequence of biosynthetic reactions on an "abnormal" substrate, i.e. oleic acid. The lack of these trienoic acids in control animals, in which both linoleic and oleic acids are available as substrates, suggest that the enzymes responsible for the desaturation and elongation reactions have a greater affinity for linoleic acid.

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