Effects of Essential Fatty Acid Deficiency and X-Radiation on the Plasma Cholesteryl Ester Spectrum of the Rat

DAVID R. HOWTON, PH.D.* AND SAM HASHIMOTO, M.S.†

THE PRESENT study was motivated by curiosity concerning possible changes in the fatty acid spectrum of plasma cholesteryl esters accompanying such phenomena as the development of overt symptoms of deficiency in the mouse with chronic deficiency of fat following exposure to x-radiation.[1] It seemed reasonably certain that the animal's economy of polyunsaturated fatty acids is altered under such circumstances, and since the plasma cholesteryl esters are easily accessible, are known to be rich in acids of this type and are suspected of being involved in their transport, these lipids were an obvious choice for initial scrutiny.

METHODS

Adult male Sprague-Dawley rats were employed, pooled plasma from groups of fifteen to twenty-four animals was extracted with acetone, cholesteryl esters were isolated by an adaptation of the silicic acid adsorption chromatographic technic of Fillerup and Mead[2] and mixtures of fatty acids released from the esters by saponification were analyzed by reversed phase chromatography before and after catalytic hydrogenation.[3]

RESULTS

Table I presents a selection of analyses obtained from groups of animals with these histories: group I-C, reared on Rockland rat diet, maintained on synthetic fat free diet[1] supplemented with 4 per cent corn oil for fifteen days before sacrifice; group I-FF, the same diet except for omission of the corn oil supplement; group II-FF, raised on a fat free diet from weaning to cessation of growth, fasted two days and fed about 20 per cent of normal *ad libitum* intake on day before sacrifice; group II-FFX, the same diet as group II-FF, but exposed to 600 r, 250 kv. x-radiation of the whole body three days before sacrifice.

Seven different groups of animals yielded analyses, of which group I-C is typical, with arachidonic comprising about two-thirds of the total and the bulk roughly equal amounts of palmitic, oleic and linoleic acids. Under conditions employed in these studies, this fatty acid spectrum was not greatly altered except when ingestion of linoleic acid was restricted (see following paragraph). Although the normal rat spectrum reported here has been observed repeatedly and was checked in one instance by gas-liquid chromatography, other presumably comparable analyses which have appeared in the literature [4,5] are difficult to reconcile with it. The ratio of arachidonic to linoleic acid (5 ± 1) in the rat is much higher than in the dog (0.48),[6] in the cow (0.17)[7] or in man (0.17).[8]

From the Department of Nuclear Medicine and Radiation Biology, University of California School of Medicine, Los Angeles, California.

*Chief, Organic Chemistry Section, Biochemistry Division, Department of Nuclear Medicine and Radiation Biology, and Associate Professor of Physiological Chemistry. † Research Assistant, Department of Nuclear Medicine and Radiation Biology.

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Changes in the fatty acid composition of the lipids of animals maintained on diets deficient in linoleic acid (additional examples of which have been reported by Dr. Ralph Holman at this Conference) have been rationalized recently by Fulco and Mead[9] in terms of abortive transformations wrought on acids such as palmitoleic and oleic, which the animal can synthesize *de novo*, instead of on the normally utilized but unsynthesizable linoleic acid. A grotesque example of such changes is afforded by the plasma cholesteryl ester spectrum of the rat raised from weaning to cessation of growth on a diet free of linoleic acid (group II-FF): while palmitic and linoleic acids are essentially absent and arachidonic (or, more probably, an isomeric eicosatetraenoic derived from palmitoleic acid) occurs in very minor amount, oleic is now the major acid and two new components, palmitoleic and C_{20}-trienoic, are present in important quantities. Remarkably, a trend in this direction is readily discernible in the normal animal in as little as fifteen days after withholding linoleic acid from the diet (see group I-FF). (Here again, earlier analyses[4,5] are at odds with these observations.)

Except for a possibly significant slight increase in the proportion of arachidonic acid, x-irradiation of the whole body produces little change in the plasma cholesteryl ester spectrum of the animal (as reported earlier by Deuel and co-workers[10]) unless it be truly deficient in fat (as by rearing from weaning on a diet free of linoleic acid, group II-FFX), in which case a dramatic general increase in amounts of polyunsaturated fatty acids at the expense of the monoenoic components is observed.

TABLE I
Rat Plasma Cholesteryl Ester Compositions
(Per Cent of Total)

Acid	Group			
	I-C	I-FF	II-FF	II-FFX
Palmitic	6.2	8.9	0	0
Palmitoleic	0	11.4	13.4	5.4
Oleic	8.2	15.7	64.5	38.3
Linoleic	18.0	11.2	0	4.5
C_{20}-trienoic	0	7.1	18.2	32.5
Arachidonic	67.6	45.9	3.9	19.0

COMMENTS

These exploratory studies bear several interesting implications which would seem to warrent emphasis. First, the apparent constancy of the arachidonic-linoleic acid ratio even when supplies of the former are at a premium, suggests that linoleic may have some function in addition to that of serving as the obligate precursor of arachidonic acid. Second, the absence of evidence of stimulation of the production of pseudo essential fatty acids (i.e., those derived from oleic or palmitoleic acids) in the normal animal maintained post-radiation on a diet free of linoleic acid may evidence the availability for regenerative processes of true essential fatty acids released by disintegrating irreparable cells. Third, the appearance of eicosatrienoic acid in the plasma cholesteryl esters shortly after withdrawal of linoleic acid from the diet indicates that the linoleic-arachidonic transformation depends heavily, if not exclusively, on the dietary supply of linoleic acid, the rather substantial quantities of linoleic acid normally present in the tissues (including the blood) being not readily available for this presumably vital process.

SUMMARY

Exploratory studies of the serum cholesteryl ester spectrum of the rat indicate that when linoleic acid is withdrawn from the diet, the normally predominant arachidonate and linoleate components begin to be replaced rather quickly by other unsaturated fatty acid moieties which do not require linoleate as an obligatory precursor. Although exposure of the animal to 600 r x-radiation does not greatly effect the normal spectrum, such a physiologic insult dramatically aggrevates the abnormality of the spectrum of the animal with deficiency of fat.

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